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Analogs of Tetrahydrofolic acid. XXXIX (1,2).

Selective Bromoacylation of Polyfunctional Molecules for Synthesis of Active-site-directed Irreversible Enzyme Inhibitors

B. R. Baker, Daniel V. Santi, James K. Coward, Howard S. Shapiro and Johannes H. Jordaan (3)

A number of methods for selective bromoacylation of side-chain amino groups on 2-amino-4-pyrimidinols or 2,4,6-triaminopyrimidines have been developed for these extremely sensitive products. The choice of method depends upon whether (a) the aminopyrimidine is a stronger base than the amine to be bromoacylated, (b) as weak a base as the amine to be bromoacylated but the amine is more reactive, (c) the amine group to be bromoacylated is a strong aliphatic amine. In case (a) the aminopyrimidine can be protonated to protect it from acylation with an anhydride; in case (b), the reaction with an anhydride is controlled by temperature, stoichiometry, and time of reaction; in case (c), the reaction is selectively controlled by use of the less reactive *p*-nitrophenyl esters. Other difficulties were solved such as (a) proper characterization of the products when combustion analyses were unsatisfactory due to polymerization; in these cases a combination of thin layer chromatography, color reactions, and derivatization were employed; and (b) insolubility leading to overreaction on the aminopyrimidine which was solved with partial aqueous systems.

One of the major programs in this laboratory is the design, synthesis and enzymic evaluation of active-site-directed irreversible inhibitors (4) for dihydrofolic reductase; the design of such an irreversible inhibitor is based on the principle that the inhibitor can form a reversible complex with the enzyme, then within the enzyme-inhibitor complex undergo a rapid neighboring group reaction between an enzymic nucleophilic group and a properly juxtaposed leaving group on the inhibitor (4). For example, 4-(iodoacetamido)salicylic acid (I) forms a complex with glutamic dehydrogenase -- presumably at the active-site -- then undergoes a rapid neighboring group reaction within the complex which results in inactivation of the enzyme (5,6). Similarly, 4-(bromoacetamido)salicylic acid (II) (7) will form just as good a reversible complex with glutamic dehydrogenase and inactivate the enzyme at about the same rate as I.

The bromoacetyl group is preferred over iodoacetyl since the necessary bromoacetic precursors are more readily available and the products are more stable and easier to purify. From the enzymic point of view the bromoacetyl group is preferred for initial exploration since it has the greatest breadth of reaction with enzymic nucleophilic groups, being capable of reacting with about eight out of the possible fifteen enzymic amino acids having a third functional group (4).

The preparation of the haloacetamido salicylic acids (I, II) and related compounds (8) is relatively simple since bromoacetyl bromide will react only with the amino group of 4-aminosalicylic acid in aqueous sodium bicarbonate solution. However, the type of carrier for the amino group can have tremendous variation in structure depending upon the structure necessary for formation of a reversible complex with a given enzyme; at times selective reaction of the requisite amino group is difficult since other reactive groups may be present or the products may be unstable or difficult to purify. Many of these problems were encountered in the synthesis of candidate activated-site-directed irreversible inhibitors for dihydrofolic reductase; their solutions are the subject of this paper.

The basic structure for pyrimidine-type reversible inhibitors of dihydrofolic reductase can be represented by III (4b) where R_5 is a three to five-atom bridge (9-14) or a direct attachment of the aryl group to the heterocycle (15-19) and (a), R_4 = OH or NH_2 and R_6 = alkyl (9,13,16,17,20), aryl (13,20-23), or aralkyl (24); (b) R_4 = OH, R_6 = NH_2 (14,25); (c) R_4 = R_6 = NH_2 (14); and (d), R_4 = SH, R_6 = CH_3 (26,27). The design of active-site-directed irreversible inhibitors of dihydrofolic reductase called for the placement of a bromoacetamido group either directly on the phenyl or bridged to the phenyl as indicated in structure IV, by bromoacylation of the

corresponding amine (IV, R_B = H). The following difficulties were encountered:

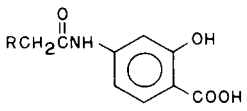
(1) Problems of insolubility led to slow reaction and lack of selectivity since the bromoacetyl derivatives that formed were usually more soluble and thus subject to further reaction even though a 1:1 stoichiometry was used.

(2) Bromoacetylation on the nuclear amino groups of the heterocycles followed by ring closure to a non-cleavable group was observed in certain cases.

(3) The instability of the compounds to excessive handling for purification led to solvolysis of the bromo group or polymerization by intermolecular alkylation of the pyrimidine; it was therefore imperative to get minimal formation of by-products by increasing selectivity.

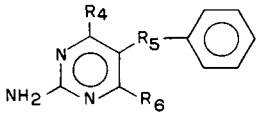
(4) Polymerization during combustion could lead to quite variable combustion analyses on the same sample.

All of the above problems can be surmounted if the bromine atom could be introduced under acid conditions after the acylation step, since in acid solution the aminopyrimidines are protonated and therefore not subject to intermolecular alkylation;

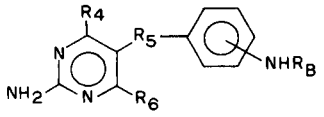


I, R = Iodo

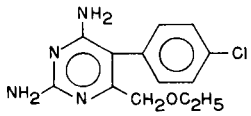
II, R = Bromo



III



IV



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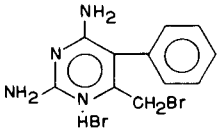

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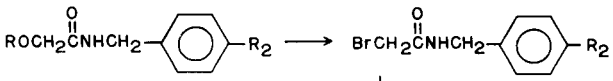
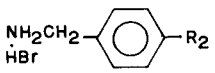


VII, R = C_2H_5-

VIII, R = CH_3CO-

IX, R = H

X



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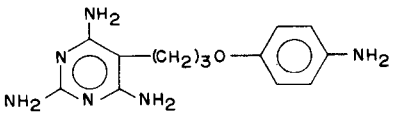

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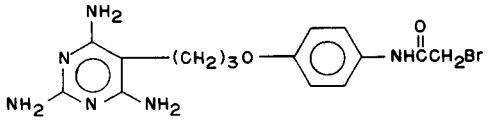

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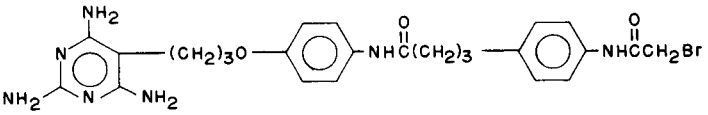

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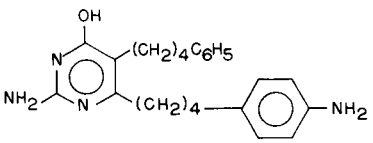

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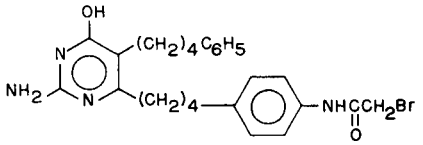
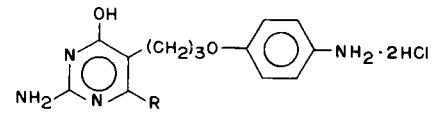


XVI

for example, cleavage of the ether group of V by anhydrous hydrogen bromide in acetic acid proceeded smoothly to the bromomethyl pyrimidine (VI) which has an extremely reactive halogen (16); the hydrobromide salt stabilized the molecule so that neither intra- nor inter-molecular alkylation could occur.

For preparation of structures such as IV, it would be necessary that a glycollic amide derivative such as VII be converted to the bromoacetamide (X) faster than it is cleaved to the amide (XI); a feasibility study was performed with *N*-benzyl-α-bromoacetamide (X), *N*-(*p*-nitrobenzyl)-α-bromoacetamide and α-bromoacetanilide.

Since these bromoacetamides have no further functional groups such as those present in IV, it is a simple matter to bromoacetylate the respective amine; these were then used as a thin-layer-chromatography (TLC) reference in the study of conversion of an ethoxyacetamide such as VII to the bromoacetamide (X). Anhydrous hydrogen bromide in acetic acid resulted in acetolysis to the acetoxy derivative (VIII); when the reaction was then forced, cleavage to the benzylamine hydrobromide (XI) occurred faster than formation of X. With lithium bromide in methanesulfonic acid, the main product appeared to be the glycollic amide, IX, even though



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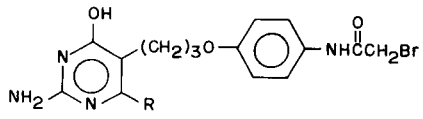

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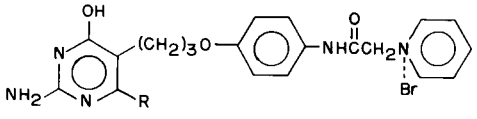

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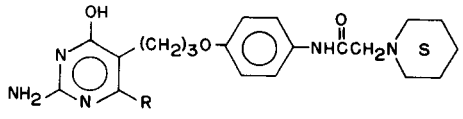
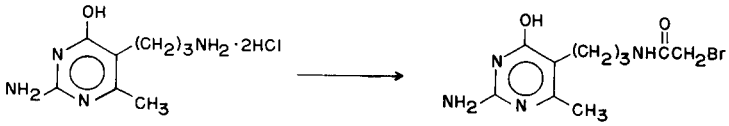
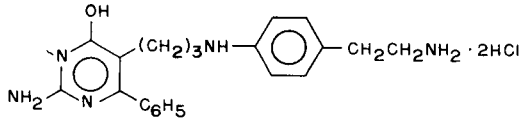


XX

a series, R = C_6H_5 ; b series, R = CH_3



XXI XXII



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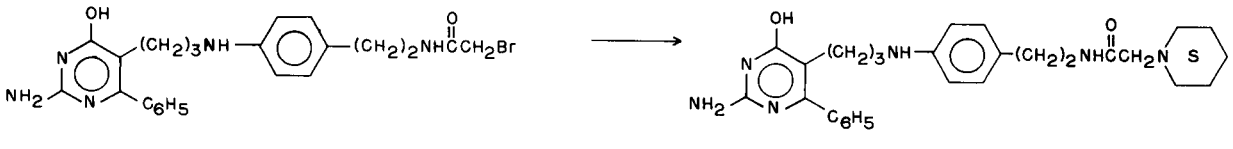
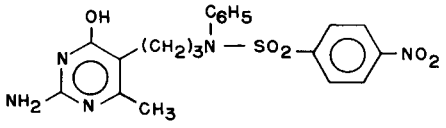


XXIV

efforts were made to keep the reaction mixture anhydrous. Similarly, no suitable conditions for conversion of IX to X could be found that would not cause cleavage to the benzylamine (XI); similar results were obtained with α-ethoxyacetanilide. Attention was therefore directed to selective bromoacylation reactions in order to find conditions that could avoid the solubility and instability problems listed earlier.

In almost all cases with pyrimidines such as IV, selective bromoacylation is required since other groups capable of undergoing bromoacylation are present. Therefore, a 1:1 stoichiometric ratio of acylating agent to IV would be ideal; in order to avoid competing hydrolysis of the bromoacylating agent, anhydrous conditions would be preferable - otherwise the stoichiometry might be difficult to adjust for duplicable results. A bromoacyl anhydride would be preferred over a bromoacyl halide since with the former no acid acceptor is needed during reaction with an aryl amine and the anhydride is less sensitive to reaction with traces of water.



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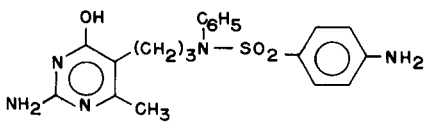

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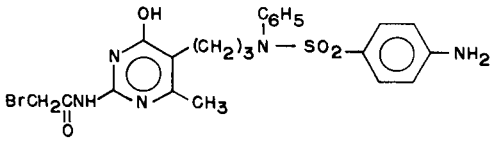

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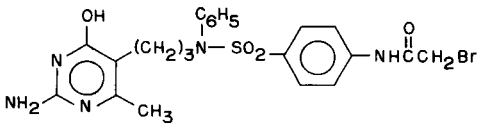

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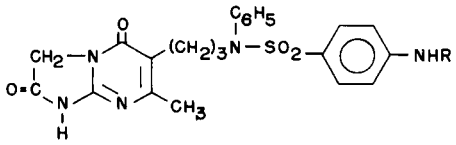
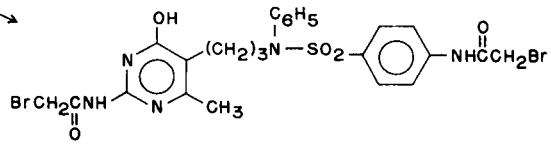


XXX, R = H

XXXI, R = -COCH$_2$Br



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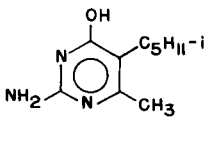

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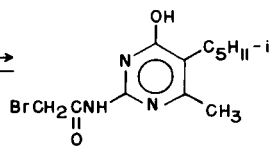
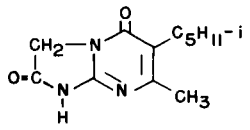


XXXIV



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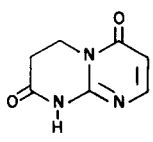

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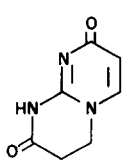

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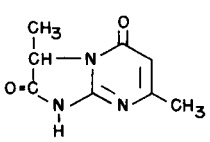
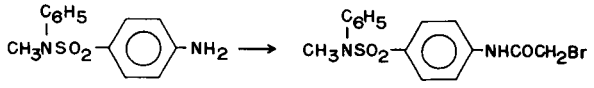


XXXVIII



XXXIX XL

Minimum reaction conditions should be used in order to avoid excessive contact time of the product with the solvent; usually about 20 minutes at 0° in a solvent such as anhydrous acetone, *N,N* dimethylformamide, or acetic acid is sufficient with aryl amines of normal reactivity. If the product did not ideally precipitate from the reaction mixture, it was precipitated by flooding with water, ether or petroleum ether, then collected immediately by centrifugation or filtration and dried. When neutral solutions of the bromoacyl derivatives (IV) were allowed to stand or are concentrated even *in vacuo* polymerization and other reactions often occurred.

In some cases, highly polar amine salts cannot be dissolved in a non-aqueous system even by adding triethylamine. Then a partial aqueous system such as 70% acetone or *N,N*-dimethylformamide was used; with these conditions it was difficult to adjust the stoichiometry to avoid under or over reaction. However, when the amine in IV is aliphatic, the less reactive *p*-nitrophenyl bromoacetate could then be used since it hydrolyzed more slowly in water than the anhydride; furthermore the nitrophenyl ester is practically inert towards a nuclear amino group on a pyrimidine and therefore the ester can be used in excess.

Our operating experience showed that combustion analyses on bromoacylamido pyrimidines such as IV are quite variable on the free base; this difficulty appears to be due to polymerization during the combustion analysis. Whenever possible, the bromoacylamido pyrimidine (IV) was converted to a salt such as the hydrobromide or sulfate since these salts gave satisfactory combustion analysis; apparently the protonated bromoacylamidopyrimidines (IV) did not polymerize during combustion. In some cases no suitable salts could be prepared; in these cases, the following criteria were used:

(1) The bromoacylamido pyrimidine moved primarily at one spot on TLC and should be greater than 90% pure.

(2) The major spot gave a positive test for active halogen with 4-(*p*-nitrobenzyl) pyridine (16).

(3) Starting amine was absent as shown by a negative Bratton-Marshall test with the aromatic amines or by TLC with the aliphatic amines.

(4) The bromoacylamido pyrimidine (IV) was further characterized by conversion to a product by a reaction dependent upon the bromoacyl group such as displacement of the halogen by a nucleophile; the latter type of product then gave satisfactory combustion analyses. The nucleophiles chosen were pyridine and piperidine where the reaction was run in the nucleophile as a solvent in high dilution to avoid polymerization as much as possible.

The following examples are illustrative of the different methods devised for selective bromoacylation:

CLASS I; Strong heterocyclic base that is a stronger base than an arylamine to be bromoacylated.

This class is usually composed of 2,4-diamino heterocycles which have pK_a's greater than 6. As an example, the 2,4,6-triamino pyrimidine system of XII has a pK_a near 6.8, but the pK_a of the aniline moiety is near 5. The triamino system can therefore be protonated by dilute acetic acid, whereas the aniline moiety will remain mainly dissociated; since the protonated species will not bromoacylate, an excess of the bromoacyl anhydride could be employed safely without over-reaction. The reaction was run in 3:1 acetone: 10% aqueous acetic acid with a 70% excess of bromoacetic anhydride; the bromoacetate salt of the product XIII separated from solution and could then be converted to the hydrobromide or sulfate salt for further purification.

5-(*p*-Bromoacetamidophenoxypropyl)-2,4,6-triaminopyrimidine (XIII) Hydrobromide.

To an ice-cooled solution of 274 mg. (1 mmole) of XII (28) in 6 ml. of acetone and 2 ml. of 10% aqueous acetic acid was added 450 mg. (1.7 mmole) of bromoacetic anhydride. After being magnetically stirred at 0° for 1 hour during which time the mixed bromoacetate and acetate salts of XIII separated, the mixture was filtered and the product washed with acetone. The product was dissolved in 10 ml. of warm glacial acetic acid, then the solution was treated with 2 ml. of 30% anhydrous hydrogen bromide in acetic acid. After cooling, the mixture was filtered and the product washed with acetic acid, then ethyl acetate; yield, 335 mg. of dihydrobromide, m.p. 220-222° dec. Recrystallization from aqueous ethanol afforded 172 mg. (31%) of monohydrobromide as white crystals, m.p. 170-172°. The compound gave a positive 4-(*p*-nitrobenzyl)-pyridine test, but a negative Bratton-Marshall test; ν max, 3450-3150 (NH); 1675-1600 (NH, C=C, C=N, C=O); 1250 (ether C-O-C); 825 cm^{-1} (*p*-C_6H_4); λ max (*p*H 1): 283 mμ; (*p*H 13): 275 mμ.

Anal. Calcd. for $C_{15}H_{19}BrN_6O_2 \cdot HBr$: C, 37.9; H, 4.20; Br, 33.6. Found: C, 37.8; H, 4.46; Br, 33.2.

p-Bromoacetamidophenylbutyric acid.

To a vigorously stirred solution of 15.1 g. of potassium bicarbonate (0.15 mole) and 3.58 g. (0.02 mole) of 4-(*p*-aminophenyl)butyric acid in 100 ml. of water cooled in an ice-bath was added 3.5 ml. (0.04 mole) bromoacetyl bromide in 3 portions over 15 minutes. After being stirred for an additional 1 hour, the solution was clarified by filtration, then acidified to *p*H 2 with aqueous hydrochloric acid. The product was collected and washed with 25 ml. of 0.1 *N* hydrochloric acid, then water. Recrystallization from ethyl acetate, then benzene gave 2.4 g. (37%) of analytical product, m.p. 135-136°; ν max (Nujol), 3260 (NH); 1675, 1650 (C=O); 845, 790 cm^{-1} (*p*-C_6H_4).

Anal. Calcd. for $C_{12}H_{14}BrNO_3$: C, 48.0; H, 4.70; N, 4.67. Found: C, 48.3; H, 4.90; N, 4.60.

5-[*p*-(*p*-Bromoacetamidophenylbutyramido)phenoxypropyl]-2,4,6-triaminopyrimidine (XIV) Sulfate.

To an ice-cold solution of 330 mg. (1.1 mmoles) of *p*-bromoacetamidophenylbutyric acid in 5 ml. of reagent tetrahydrofuran was added 124 mg. (0.6 mmole) of dicyclohexylcarbodiimide. After 2 hours protected from moisture, the mixture was cooled to 0° and the dicyclohexylurea was removed by filtration and washed with 5 ml. of tetrahydrofuran. The combined filtrate and washings were poured into about 70 ml. of petroleum ether (b.p. 30-60°). The anhydride was collected on a filter and washed with petroleum ether; yield, 240 mg. (78%); ν max (Nujol), 1795, 1740 cm^{-1} (anhydride C=O).

To a stirred solution of 548 mg. (2 mmoles) of XII (28) in 10 ml. of acetone and 4 ml. of 10% aqueous acetic acid cooled in ice-bath was added 1.20 g. (2.06 mmoles) of *p*-bromoacetamidophenylbutyric anhydride. After 1 hour at 0°, the mixture was filtered and the product washed with 20 ml. of ethyl acetate. The acetate salt was dissolved in warm aqueous 2-methoxyethanol, then 5 ml. of 2 *N* sulfuric acid was added to the solution. After about 18 hours at 3°, the mixture was filtered and the sulfate salt washed with 5 ml. of ethanol and 20 ml. of ethyl acetate; yield, 696 mg. (53%) of white crystals, m.p. 174-176° dec., that gave a positive 4-(*p*-nitrobenzyl)pyridine test. The compound had ν max, 3400-3150, (NH); 1675-1600 (NH, C=C, C=N, C=O); 1250 (ether C-O-C); 830 (broad), 790 cm^{-1} (*p*-C_6H_4); λ max (*p*H 1): 283 mμ; (*p*H 13): 275 mμ.

Anal. Calcd. for $C_{25}H_{30}BrN_7O_3 \cdot H_2SO_4$: C, 45.8; H, 4.90; Br, 12.2. Found: C, 45.6; N, 5.14; Br, 12.1.

CLASS II; Weak heterocyclic base equal or weaker base than arylamine, but arylamine more reactive.

In this case, protonation cannot be used to protect the aminopyrimidine from bromoacylation; therefore the reaction is clean only when the arylamine is more reactive; only a slight excess of anhydride is used and a short reaction time is employed. Two types are described below, one in which a salt of the product separates from solution and the other that does not. Since XV (29) is readily soluble in acetone, the bromoacetylation was run in this solvent at 0° for 30 minutes; the bromoacetate salt of XVI separated from solution and was then converted to its more stable sulfate salt which gave good combustion analyses. The free base of XVI was uniform on TLC, gave the proper colors tests, but variable combustion analyses.

2-Amino-6-(*p*-bromoacetamidophenylbutyl)-5-phenylbutyl-4-pyrimidinol Sulfate (XVI).

To a solution of 200 mg. (0.50 mmole) or XV (29) in 1 ml. of reagent acetone was added with ice-cooling and magnetic stirring a solution of 146 mg. (0.55 mmole) of bromoacetic anhydride in 1 ml. of acetone. After 20 minutes at 0°, during which time the bromoacetate salt of XVI separated, the mixture was filtered and the product washed with 1 ml. of acetone, then petroleum ether; yield, 200 mg. (69%), of white crystals that had no definite melting point. The compound had λ max (*p*H 1): 261 mμ; (*p*H 13): 275 mμ, showing that bromoacetylation of the 2-amino group on the pyrimidine had not taken place; it moved as a single spot on TLC in chloroform-ethanol (5:1), the spot giving a positive test with 4-(*p*-nitrobenzyl)pyridine. The analysis varied depending upon the extent of drying which led to partial loss of bromoacetic acid.

Anal. Calcd. for $C_{26}H_{31}BrN_4O_2 \cdot {}^1/_2BrCH_2COOH$: C, 55.8; H, 5.63; N, 9.64. Found: C, 55.9; H, 5.93; N, 9.35.

The more stable sulfate salt of XVI was prepared by solution of 100 mg. of the bromoacetate salt in ethanol, then treatment with 0.5 *N* aqueous sulfuric acid until no more precipitate formed. Recrystallization of the precipitate by the same procedure gave 86 mg. (88%) of white crystals with no definite m.p.; ν max, 3400, 3340, 3230 (NH); 1700, 1680-1650, 1600, 1540-1500 (NH, C=O, C=C, C=N, $C=NH^+$); 1100 ($SO_4^=$); 733, 697 cm^{-1} (C_6H_5).

Anal. Calcd. for $C_{26}H_{31}BrN_4O_2 \cdot {}^1/_2H_2SO_4$: C, 55.7; H, 5.75; N, 10.0. Found: C, 55.8; H, 5.93; N, 9.94.

Similarly, bromoacetylation of 100 mg. (0.28 mmole) of 2-amino-6-(*p*-aminophenethyl)-5-phenylbutyl-4-pyrimidinol (29) gave 109 mg. (72%) of 2-amino-6-(*p*-bromoacetamidophenethyl)-5-phenylbutyl-4-pyrimidinol sulfate, m.p. 122-124°.

Anal. Calcd. for $C_{24}H_{27}BrN_4O_2 \cdot {}^1/_2H_2SO_4 \cdot {}^1/_4H_2O$: C, 53.7; H, 5.38; N, 10.4. Found: C, 53.6; H, 5.71; N, 10.2.

In contrast to XV, the dihydrochloride of the 6-phenylpyrimidine, XVIIa, could not be dissolved in acetone even in the presence of triethylamine; therefore it was necessary to use *N,N*-dimethylformamide as a solvent which added the difficulty that the product did not separate from solution. After a 20 minute reaction at 0°, the product (XVIIIa) was isolated by flooding the solution with water. Although XVIIIa moved as a single spot on TLC and gave the proper color reactions, no suitable salt could be prepared; furthermore, since XVIIIa gave erratic combustion results, it was converted to the crystalline piperidide (XXa) for combustion analysis. A crystalline pyridinium derivative (XIXa) could not be isolated by reaction of XVIIIa with pyridine as both nucleophilic and solvent.

The related 6-methyl series (XVIIb) also was bromoacetylated in *N,N*-dimethylformamide; the bromoacetamide (XVIIIb) was obtained in 90% yield as an amorphous white powder that gave a negative Bratton-Marshall test for aromatic amine, and moved as a single spot on TLC, the spot giving a positive 4-(*p*-nitrobenzyl)pyridine test. Since XVIIIb gave erratic combustion analyses and could not be converted to a suitable salt, it was converted to its crystalline pyridinium derivative, XIXb, which gave good combustion values.

2-Amino-5-(*p*-bromoacetamidophenoxypropyl)-6-phenyl-4-pyrimidinol (XVIIIa).

To an ice-cooled and magnetically stirred solution of 49.5 mg. (0.49 mmole) of triethylamine in 3 ml. of reagent *N,N*-dimethylformamide protected from moisture was added 100 mg. (0.245 mmole) of XVIIa (30). When solution was complete, 64 mg. (0.245 mmole) of bromoacetic anhydride was added in one portion. After being stirred in the ice-bath for 20 minutes, the solution was poured into about 25 ml. of ice-cold water containing 41 mg. (0.49 mmole) of sodium bicarbonate with good stirring. The crude product was collected by centrifugation and washed several times with ice-water by resuspension and centrifugation; after being dried over phosphorus pentoxide in high vacuum the yield of product was 95 mg. (85%). The dry material was further purified by solution in the least amount of *N,N*-dimethylformamide at room temperature, clarification by centrifugation, then precipitation with a large volume of water; the product was collected by centrifugation as before; yield 85 mg. (76%) that had no definite melting point. The compound gave a negative Bratton-Marshall test and moved as a single spot in 3:1 benzene-methanol, the spot giving a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen; ν max, 3400, 3300 (NH); 1650, 1590, 1500 (NH, C=C, C=N, C=O); 1240 (ether C-O-C); 825 (*p*-C_6H_4); 695 cm^{-1} (C_6H_5).

2-Amino-6-phenyl-5-[*p*-(piperidinoacetamido)phenoxypropyl]-4-pyrimidinol (XXa).

To 50 ml. of freshly distilled piperidine was added 100 mg. (0.218 mmole) of XVIIIa with magnetic stirring. After standing at room temperature for 24 hours protected from moisture, the solution was spin-evaporated *in vacuo*. The residue was dissolved in warm 2-methoxyethanol, the *p*H was adjusted to about *p*H 8 with 3 *N* aqueous hydrochloric acid, then water was added to the hot solution to turbidity. After being cooled in an ice-bath, the mixture was filtered and the product was recrystallized from 2-methoxyethanol-water; yield, 51 mg. (50%) of white crystals, m.p. 226-228° dec.; λ max, 3470 (NH); 1680, 1660, 1640, 1600, 1530 (C=O, C=C, C=N, NH); 1230 (ether C-O-C); 825, 815 (*p*-C_6H_4); 705 cm^{-1} (C_6H_5).

Anal. Calcd. for $C_{26}H_{31}N_5O_3 \cdot H_2O$: C, 65.1; H, 6.90; N, 14.6. Found: C, 65.4; H, 6.74; N, 14.4.

2-Amino-5-(*p*-bromoacetamidophenoxypropyl)-6-methyl-4-pyrimidinol (XVIIIb).

This compound was prepared as described for XVIIIa; yield, 103 mg. (90%) of a white powder that gave a negative Bratton-Marshall test and moved as a single spot in 3:1 benzene-methanol, the spot giving a positive 4-(*p*-nitrobenzyl)pyridine test. Since erratic combustion values were obtained, XVIIIb was characterized as follows:

2-Amino-6-methyl-5-[*p*-(pyridiniumacetamido)phenoxypropyl]-4-pyrimidinol Bromide (XIXb).

To 50 ml. of reagent pyridine was added 25 mg. (0.063 mmole) of XVIIIb with magnetic stirring. The clear solution was allowed to stand for 24 hours protected from moisture, during which time the product crystallized from solution. The product was collected on a filter and washed with ether; yield, 11 mg. (37%) of white crystals that were uniform on TLC in 4:1 methanol-water.

Anal. Calcd. for $C_{21}H_{24}BrN_3O_3$: C, 53.3; H, 5.08; N, 14.8. Found: C, 53.1; H, 5.09; N, 14.6.

Additional, less pure product could be isolated from the filtrate.

CLASS III; Heterocyclic base weaker than an aliphatic amino group.

Since the heterocyclic base is weaker than the aliphatic amino group in a side-chain, the heterocyclic base cannot be protonated for protection else the simultaneously protonated aliphatic amine will not acylate. Selectivity in bromoacetylation is readily achieved with *p*-nitrophenyl bromoacetate (31) since the latter does not react with nuclear amino groups on a pyrimidine at any significant rate, slowly reacts with water, but readily reacts with an aliphatic amine. Two examples are given demonstrating different techniques dependent upon solubility properties.

The free base of XXI in anhydrous solvent could not be prepared due to the insolubility of XXI in organic solvents (12). However, a solution of XXI base in 70% aqueous acetone can be prepared with sodium hydroxide (12); reaction with *p*-nitrophenyl bromoacetate proceeded rapidly at room temperature and the product (XXII) separated from solution. Since erratic combustion results were obtained, it was characterized by reaction with pyridine.

The less polar XXIII was treated similarly in 70% aqueous *N,N*-dimethylformamide to give XXIV in 88% yield; although XXIV was uniform on TLC and gave the proper color test, again erratic combustion analyses were obtained. Therefore XXIV was further characterized by conversion to its piperidino derivative, XXV. Note that the secondary arylamino group of XXIII was not acylated by *p*-nitrophenyl bromoacetate, as could be anticipated, since arylamines are known to react with *p*-nitrophenyl esters with difficulty.

2-Amino-5-(bromoacetamidopropyl)-6-methyl-4-pyrimidinol (XXII).

To a magnetically stirred solution of 255 mg. (1 mmole) of XXI (12) in 0.67 ml. of 3 *N* aqueous sodium hydroxide and 0.5 ml. of water was added 3.5 ml. acetone (12). To the resulting solution was added 260 mg. (1 mmole) of *p*-nitrophenyl bromoacetate (31); an immediate yellow color developed and within 5 minutes the product began to separate. After 30 minutes, the mixture was filtered and the product washed with water, then dried; any residual *p*-nitrophenol was removed by leaching with hot ethyl acetate; yield, 170 mg. (56%). Recrystallization from ethanol-petroleum ether gave white crystals, m.p. 205-215° dec. with softening at 195°;

λ max (*p*H 1): 268 mμ; λ max (*p*H 13): 282 mμ; ν max (KBr), 3400, 3350, 3100 (NH); 1650, 1640, 1600, 1550 cm^{-1} (C=O, C=C, C=N, NH). The compound was uniform on TLC in ethanol:2-methoxyethanol (3:1) and gave a positive 4-(*p*-nitrobenzyl)-pyridine test for active halogen. Since combustion results were erratic, XXII was reacted with pyridine.

2-Amino-6-methyl-5-(pyridiniumacetamidopropyl)-4-pyrimidinol Bromide.

A mixture of 55 mg. (0.18 mmole) of XXII and 25 ml. of reagent pyridine protected from moisture was magnetically stirred at ambient temperature until solution took place (2 hours). After an additional 6 hours, during which time the product separated, the mixture was filtered and the white product washed with benzene; yield, 47 mg. (68%), m.p. 225-229° dec. Recrystallization from 80% aqueous ethanol-ether gave a hydrate as white crystals, m.p. 213-215° dec. with softening at 180°. The compound had λ max (*p*H 1): 263 mμ; (*p*H 13): 270, 283 mμ (shoulder); ν max, 3300 (broad) (H_2O, NH); 3050 (NH); 1660, 1590, 1550 cm^{-1} (C=O, C=C, C=N, NH).

Anal. Calcd. for $C_{15}H_{20}BrN_5O_2 \cdot H_2O$: C, 45.0; H, 5.54; N, 17.5. Found: C, 45.0; H, 5.52; N, 17.2.

2-Amino-5-[*p*-(bromoacetamidoethyl)anilinopropyl]-6-phenyl-4-pyrimidinol (XXIV).

To a solution of 150 mg. (0.37 mmole) of XXIII (30) in 0.38 ml. of 1 *N* aqueous sodium hydroxide, 0.82 ml. of water and 2.8 ml. of *N,N*-dimethylformamide was added with magnetic stirring 98 mg. (0.37 mmole) of *p*-nitrophenyl bromoacetate (31). After being stirred at ambient temperature for 30 minutes, the reaction mixture was diluted with 4 ml. of water, cooled to 0°, then filtered. The product was washed with water, then was triturated with ether to remove traces of *p*-nitrophenol; yield, 160 mg. (88%) of a nearly white solid that gradually decomposed over 200°, but moved as a single spot in 3:1 benzene-methanol and gave a positive 4-(*p*-nitrobenzyl)pyridine test; ν max, 3440, 3310 (NH); 1660, 1610, 1590 (NH, C=O, C=C, C=N); 813 (*p*-C_6H_4); 697 cm^{-1} (C_6H_5). Since erratic combustion values were obtained, it was characterized as follows:

2-Amino-5-[*p*-(piperidinoacetamidoethyl)anilinopropyl]-6-phenyl-4-pyrimidinol (XXV).

By reaction of 85 mg. (0.18 mmole) of XXIV with 50 ml. of freshly distilled piperidine as described for the preparation of XXa was obtained, after recrystallization, 21 mg. (25%) of analytically pure material. The compound had m.p. 126-128° dec.; ν max, 3430, 3340 (NH); 1660-1630 (broad), 1510 (NH, C=O, C=C, C=N); 810 (*p*-C_6H_4), 703 cm^{-1} (C_6H_5).

Anal. Calcd. for $C_{28}H_{36}N_6O_2$: C, 68.8; H, 7.38; N, 17.2. Found: C, 68.9; H, 7.49; N, 17.2.

CLASS IV; Strong heterocyclic base with an aliphatic amino group.

No specific example is yet available; from the studies in class III it is highly probable that reaction of the free base with *p*-nitrophenyl bromoacetate will proceed selectively on the aliphatic amino group since nuclear amino groups on a pyrimidine do not react with this reagent for an aliphatic amine under conditions used.

CLASS V; Weak heterocyclic base stronger than arylamine, but arylarime only competitively reactive.

In the case of the *p*-aminobenzenesulfonamidopyrimidine (XXVII) (32), the arylamine is deactivated by the strongly electron-withdrawing *p*-sulfonamido group; as a result, this *p*-amino group reacts sluggish enough with bromoacetic anhydride that reaction of the 2-amino group becomes competitive. Thus with an equimolar ratio of bromoacetic anhydride, four types of molecules could be formed; subsequently, the presence of two more were indicated.

If the reaction of the *p*-amino and 2-amino were equally competitive, then at completion, the two monobromoacetyl compounds, XXVIII and XXIX, will be present along with some bis-bromoacetyl derivative (XXXII) and some starting material (XXVII). Furthermore, the 2-bromoacetamido group of both XXVIII and XXXII spontaneously slowly cyclized to the bicyclic structure, XXX, and XXXI, respectively; cyclization is instantaneous in 0.1 *N* base and is readily detectable by the u.v. peak of bicyclic system near 300 mμ. Furthermore, the presence of XXVIII and XXXII are indicated by the high frequency carbonyl band at 1700 cm^{-1} which shifts to the still higher frequency of 1750 cm^{-1} on cyclization to XXX and XXXI; thus, no less than five products and starting material were detected in this reaction of equimolar amounts of bromoacetic anhydride and XXVII. Although these products were not separated, the spectrophotometric assignments were verified by bromoacetylation of the isoamyl pyrimidine, XXXIII, which does not have the complexity of the extra side-chain functional groups.

Bromoacetylation of the isoamyl-2-aminopyrimidine (XXXIII) (33) with excess bromoacetic anhydride in *N,N*-dimethylformamide gave 63% of the pure bromoacetamido pyrimidine (XXXIV); the compound gave a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen and showed an amide carbonyl at 1700 cm^{-1}; XXXIV had the proper u.v. spectrum in neutral and acid solution, namely, peaks at 255 mμ at *p*H 1 and 254 and 282 (sh) at *p*H 5, the same as 2-acetamido-4-pyrimidinol (26); however, in 0.1 *N* base the maximum was near 300 mμ compared to 245 and 276 mμ for a 2-acetamido-4-pyrimidinol (26). On acidification of the basic solution, the resulting precipitate had a carbonyl shifted to 1750 cm^{-1} and now contained no halogen; this product identified as the imidazo-pyrimidine (XXXV) by spectral and combustion analyses. The bicyclic compound had u.v. peaks in 0.1 *N* acid at 247 and

280 and u.v. peaks in 0.1 *N* base at 249 and 303 mμ. That cyclization had occurred at N_3 as shown in structure XXXV and not at N_1 was verified by comparison with the u.v. spectra of authentic XXXVI and XXXVII (34); XXXVI had u.v. peaks in 0.1 *N* acid at 231 and 282 and in 0.1 *N* base at 249 and 304 mμ, whereas XXXVII had peaks in acid at 220 and 260 and peaks in base at 233 and 260. An analogous cyclization has been observed by Prokof'ev *et al.* (35), who prepared XXXVIII by cyclization of 2-(α-bromopropionamido)-6-methyl-4-pyrimidinol.

In order to verify that reaction of the sulfanilamide amino group with bromoacetic anhydride was slow, bromoacylation of a sulfanilamide fragment, XXXIX, was studied. In contrast to a normal arylamine such as XV that required no more than 20 minutes at 0° for complete reaction, XXXIX required 4 hours at 25° to be converted to XL when followed by the Bratton-Marshall test (36).

When the sulfonamidopyrimidine, XXVII, was allowed to react with 3 equivalents of bromoacetic anhydride in *N,N*-dimethylformamide until reaction with the arylamine was complete (negative Bratton-Marshall test (36)) the product isolated contained significant amounts of the bicyclic structure, XXXI, as shown by the presence of a band at 1750 cm^{-1}; therefore it was not possible to reconvert XXXII back to XXIX by selective acid cleavage, even though such a reaction was feasible with the isoamylpyrimidine, XXXIV ⟶ XXXIII.

One possible solution was similar to the solution of the Class I selective bromoacetylation, except the whole pK_a scale is moved to higher values. With Class I inhibitors, the heterocyclic base (XII) had a pK_a near 6.8 and the aniline moiety of pK_a near 5.3 - a difference of about 1.5 pK_a units; thus acetic acid was sufficiently strong to protonate the pyrimidine, but not the arylamine. In the case of the aminobenzenesulfonamido pyrimidine (XXVII), the pyrimidine moiety has a pK_a near 4.0 and the arylamine a pK_a near 2.3 - a difference of 1.7 pK_a units; therefore if an acid about 1.7 pK_a units stronger than acetic acid were used, then the 2-amino-4-pyrimidinol should be protonated, but the weak arylamine should be considerably less protonated. Such an acid is bromoacetic acid (pK_a = 2.9).

When the sulfonamide, XXVII, was treated with 1.5 mole-equivalents bromoacetic anhydride in *N,N*-dimethylformamide in the presence of 2 mole-equivalents of bromoacetic acid, bromoacetylation was quite slow. After 24 hours at room temperature the Bratton-Marshall test was still positive. Thin layer chromatography indicated considerable polymeric material was present that failed to move from the original. A check with the sulfanilamide fragment, XXXIX, showed that the presence of bromoacetic acid considerably slowed an already slow reaction. An attempt to use the more reactive bromoacetyl bromide with XXVII was still too slow and polymeric material appeared to be obtained.

It seems clear that selective bromoacylation of a weakly reactive amine in a polyfunctional molecule such as XXVII is an awkward problem that is not solvable by usual means. The bromine atom of XXIX must be introduced last, but such a sequence has not been easy to devise. An alternate solution is to use other groups in place of the bromoacetamido that could be useful irreversible enzyme inhibitors; this has been accomplished by synthesis of bromomethyl ketone and bromoacetamidomethyl groups *para* to the sulfonamide group (32).

2-Bromoacetamido-5-isoamyl-6-methyl 4-pyrimidinol (XXXIV).

To a suspension of 100 mg. (0.5 mmole) of XXXIII (33) in 5 ml. of *N,N*-dimethylformamide was added 200 mg. (0.77 mmole) of bromoacetic anhydride. The mixture was stirred at ambient temperature for 3 hours, solution being complete in 30 minutes. The solution was poured in 25 ml. of water and the product was collected on a filter; yield, 150 mg. (63%), m.p. 136-140°. Recrystallization from ethanol gave white plates, m.p. 142-143°; ν max, 3240 (NH); 1710 (amide C=O); 1650, 1625, 1590 cm^{-1} (NH, C=O, C=C, C=N); λ max (*p*H 1): 255 mμ; (*p*H 13): 295 mμ; (*p*H 5): 254, 282 mμ (sh).

Anal. Calcd. for $C_{12}H_{18}BrN_3O_2$: C, 45.6; H, 5.72; Br, 25.3. Found: C, 45.9; H, 5.80; Br, 25.1.

If the reaction was allowed to proceed for 2 days, then XXXIV cyclized to XXXV which was isolated in 50% yield. When a solution of XXXIV in 10 ml. of 1 *N* methanolic hydrogen chloride was allowed to stand at ambient temperature, the shift in ultraviolet peak from 255 mμ to 268 mμ was complete in 90 minutes; XXXIII was isolated by addition of 1 *N* aqueous sodium hydroxide to *p*H 8.

6-Isoamyl-7-methylimidazo[1,2-a]pyrimidine-2,5-(1*H*,3*H*)-dione (XXIV).

A solution of 50 mg. (0.16 mmole) of XXXIII in 2 ml. of ethanol and 3 ml. of 0.1 *N* aqueous sodium hydroxide was allowed to stand at room temperature for 2 hours. Acidification gave 21 mg. (60%) of product, m.p. 261-264° dec. Recrystallization from ethyl acetate afforded white crystals, m.p. 264-265° dec.; λ max (*p*H 1): 247, 280 mμ (sh); (*p*H 13): 249, 303 mμ; ν max, 3300 (NH); 1750 (amide C=O); 1680, 1625, 1580 cm^{-1} (NH, C=O, C=C, C=N).

Anal. Calcd. for $C_{12}H_{17}N_3O_2$: C, 61.3; H, 7.28; N, 17.9. Found: C, 61.5; H, 7.40; N, 18.0.

N^4-Bromoacetyl-N^1-methyl-N^1-phenylsulfanilamide (XL).

The appropriate amine (XXXIX) was synthesized in two steps: (a) by reaction of *p*-nitrobenzenesulfonanilide with methyl iodide in dimethylsulfoxide in the presence of potassium carbonate gave *N*-methyl-*p*-nitrobenzenesulfonanilide in 91% yield, m.p. 128-129° (lit. m.p. 127-128°) (37); (b) catalytic reduction in ethanol in the presence of platinum oxide gave 70% of XXXIX, m.p. 137-140° (lit. m.p.

138-140°) (38).

To a solution of 131 mg. (0.5 mmole) of XXXIX in 3 ml. of *N,N*-dimethylformamide was added 200 mg. (0.75 mmole) of bromoacetic anhydride. After 4 hours at ambient temperature protected from moisture, the solution showed an essentially negative Bratton-Marshall test (36); the reaction mixture was poured into 10 ml. of water. The product was collected on a filter and washed with water; yield, 151 mg. (80%), m.p. 139-141°. Recrystallization from aqueous ethanol afforded white needles, m.p. 143-144°.

Anal. Calcd. for $C_{15}H_{15}BrN_2O_3S$: C, 47.0; H, 3.95; Br, 20.8. Found: C, 47.2; H, 3.86; Br, 20.8.

Identification procedures.

Thin layer chromatograms (TLC) were run on Brinkmann silica gel GF_{254} and spots were detected by visual examination under ultraviolet light. Spots with active halogen were detected by spraying the chromatograms with 0.05 *M* aqueous potassium biphthalate (*p*H 4.2) followed immediately by 2% 4-(*p*-nitrobenzyl)pyridine in acetone; the plate was heated at about 90° for 10-15 minutes, then sprayed with 0.5 *N* aqueous potassium hydroxide which gave dark blue spots that faded in 3-10 minutes. The Bratton-Marshall test (36) for aromatic amines was performed on the total sample either in a solution or as a spot test on filter paper; the test was much less sensitive when performed on silica gel plates.

Infrared spectra were run in potassium bromide pellet, unless other wise indicated, on a Perkin-Elmer 137B spectrophotometer; ultraviolet spectra were determined in 10% alcohol with a Perkin-Elmer 202 spectrophotometer. Melting points were taken in capillary tubes on a Mel-temp block and those below 230° are corrected.

REFERENCES

(1) This work was generously supported by the National Cancer Institute, U. S. Public Health Service by grants CA 05867, CA-06624, and CA-08695.

(2) For the previous paper in this series see B. R. Baker and J. H. Jordaan, *J. Heterocyclic Chem.*, 3, 324 (1966).

(3) J. H. Jordaan wishes to thank the Atomic Energy Board, Republic of South Africa, for a fellowship.

(4) For reviews on the design of active-site-directed irreversible enzyme inhibitors see (a) B. R. Baker, *J. Pharm. Sci.*, 53, 347 (1964); (b) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site." John Wiley and Sons, New York, N. Y., in press.

(5) B. R. Baker, W. W. Lee, E. Tong, and L. O. Ross, *J. Am. Chem. Soc.*, 83, 3713 (1961).

(6) B. R. Baker, W. W. Lee, and E. Tong, *J. Theoret. Biol.*, 3, 459 (1962).

(7) B. R. Baker and H. S. Shapiro, unpublished.

(8) B. R. Baker, W. W. Lee, A. P. Martinez, L. O. Ross, and L. Goodman, *J. Org. Chem.*, 27, 3283 (1962).

(9) B. R. Baker, D. V. Santi, P. I. Almaula, and W. C. Werkheiser, *J. Med. Chem.*, 7, 24 (1964); paper X of this series.

(10) B. R. Baker, B.-T. Ho, and G. B. Chheda, *J. Heterocyclic Chem.*, 1, 88 (1964); paper XIII of this series.

(11) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, 54, 1187 (1965); paper XX of this series.

(12) B. R. Baker and J. K. Coward, *ibid.*, 54, 714 (1965); paper XXII of this series.

(13) B. R. Baker and B.-T. Ho, *J. Heterocyclic Chem.*, 2, 72 (1965); paper XXIII of this series.

(14) B. R. Baker and D. V. Santi, *J. Pharm. Sci.*, 54, 1252 (1965); paper XXIV of this series.

(15) B. R. Baker and B.-T. Ho, *ibid.*, 53, 1137 (1964); paper XVI of this series.

(16) B. R. Baker and J. H. Jordaan, *J. Heterocyclic Chem.*, 2, 21 (1965); paper XXI of this series.

(17) B. R. Baker and J. H. Jordaan, *ibid.*, 2, 162 (1965); paper XXVI of this series.

(18) B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, 54, 1740 (1965); paper XXVIII of this series.

(19) B. R. Baker and B.-T. Ho, *J. Heterocyclic Chem.*, 2, 335 (1965); paper XXIX of this series.

(20) B. R. Baker and H. S. Shapiro, *J. Pharm. Sci.*, 55, 308 (1966); paper XXXIV of this series.

(21) B. R. Baker and H. S. Shapiro, *J. Med. Chem.*, 6, 664 (1963); paper IX of this series.

(22) B. R. Baker, D. V. Santi, and H. S. Shapiro, *J. Pharm. Sci.*, 53, 1317 (1964); paper XIV of this series.

(23) B. R. Baker, H. S. Shapiro, and W. C. Werkheiser, *J. Med. Chem.*, 8, 283 (1965); paper XVIII of this series.

(24) B. R. Baker and J. H. Jordaan, *J. Heterocyclic Chem.*, 3, 315 (1966); paper XXXVI of this series.

(25) B. R. Baker and G. J. Lourens, *ibid.*, 2, 344 (1965); paper XXXI of this series.

(26) B. R. Baker, C. E. Morreal and B.-T. Ho, *J. Med. Chem.*, 6, 658 (1963); paper VIII of this series.

(27) B. R. Baker, B.-T. Ho, and T. Neilson, *J. Heterocyclic Chem.*, 1, 79 (1964); paper XII of this series.

(28) B. R. Baker and D. V. Santi, *J. Pharm. Sci.*, in press.

(29) B. R. Baker and J. H. Jordaan, *J. Heterocyclic Chem.*, 3, 319 (1966); paper XXXVII of this series.

(30) B. R. Baker and H. S. Shapiro, *J. Pharm. Sci.*, in press.

(31) D. W. Russell, *Biochem. J.*, 78, 696 (1961).

(32) B. R. Baker and J. K. Coward, to be published.

(33) B. R. Baker, B.-T. Ho, and D. V. Santi, *J. Pharm. Sci.*, 54, 1415 (1965); paper XXVII of this series.

(34) R. B. Angier and W. V. Curran, *J. Org. Chem.*, 26, 1891 (1961).

(35) M. A. Prokof'ev, E. G. Antonovich and Yu. P. Shvachkin, *Dokl. Akad. Nauk, S.S.S.R.*, 87, 783 (1952); *Chem. Abstr.*, 48, 169f (1954).

(36) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, 128, 537 (1939).

(37) R. J. Irani, *Current Sci.*, 14, 126 (1945); *Chem. Abstr.*, 39, 4597 (1945).

(38) S. I. Lur'e and T. E. Rostkovskaya, *J. Gen. Chem. U.S.S.R.*, 14, 137 (1944); *Chem. Abstr.*, 39, 2228 (1945).

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