resulting suspension was heated for 1 hr on a steam bath to ensure hydrolysis of the ketimine. The product was then extracted (Et_2O), carried through an acid–base extraction, and finally purified by vacuum distillation.

3-Substituted 2-Pyrrolidinones (Table II).—To 1.5 l. of liquid NH_3 was added 1.5 moles of Li in small pieces followed by a catalytic amount of $FeCl_3$. Stirring was commenced and when the blue color had changed to gray, 1 mole of the 1-substituted 2-pyrrolidinone was added slowly. Stirring was continued for 1 hr and then an ether solution of 1 mole of the ketone was added slowly. The mixture was stirred for 1 hr and then treated with 1.5 moles of solid NH_4Cl. Ether was added to replace the evaporated NH_3. The ether solution was washed (H_2O), dried ($MgSO_4$), and evaporated. The product which crystallized on standing was purified by recrystallization.

Pyrrolidinecarbinols (Table III). Procedure I. By Reaction of 3-Aroylpyrrolidine with Alkylmagnesium Halide.—To a stirred solution of 0.3 mole of alkylmagnesium halide in 200 ml of ether maintained at 10° was added slowly a solution of 0.15 mole of 1-substituted 3-aroylpyrrolidine in 50 ml of dry ether. After the addition was complete, the mixture was stirred for 1 hr with no external cooling and then treated slowly with a solution of 0.3 mole of NH_4Cl in 300 ml of H_2O. The ether layer was separated and the aqueous suspension was extracted (Et_2O). The combined extracts were washed (H_2O) and the solvent was evaporated. The crude product was purified by distillation or recrystallization. In some cases where solid free bases were obtained the isomers were separated by fractional crystallization.

Procedure II. By Reduction of 3-Substituted 2-Pyrrolidinones.—To a suspension of $LiAlH_4$ (0.90 mole) in 500 ml of THF at gentle reflux was added a solution of 0.60 mole of the 3-substituted 2-pyrrolidinone in 200 ml of THF. The mixture was stirred at gentle reflux for 2 hr, cooled, and poured slowly onto a stirred 25% NaOH (1 l.). The solution was separated and the solvent was evaporated at reduced pressure. The crude product was purified by recrystallization.

Procedure III. By $LiAlH_4$ Reduction of 1-Substituted 3-Aroylpyrrolidine.—To a stirred suspension of 0.20 mole of $LiAlH_4$ in 300 ml of ether was added slowly at a rate which maintained gentle refluxing 0.19 mole of 1-substituted 3-aroylpyrrolidine in 100 ml of ether. Stirring and refluxing were continued for 1 hr after the addition was complete. The mixture was cooled and treated successively with 25 ml of H_2O and 200 ml of 25% NaOH. The ether layer was separated and the aqueous layer was extracted with ether. The combined extracts were washed (H_2O) and evaporated. The carbinol was purified by distillation or recrystallization.

Procedure IV. By Catalytic Reduction of the N-Benzylcarbinol.—A solution of 0.46 mole of the N-benzylcarbinol in 150 ml of 95% EtOH was reduced catalytically with 6 g of 10% Pd–C. The mixture was heated at 70° and shaken with H_2 until 1 equiv of H_2 was absorbed (about 2 hr). After cooling, the suspension was filtered and the solvent evaporated. The residual oil was purified by distillation or recrystallization.

Procedure V. By Alkylation of 3-Substituted Pyrrolidines.—A mixture of 0.09 mole of 3-substituted pyrrolidine, 0.10 mole of arylalkyl bromide, 40 g of K_2CO_3, and 200 ml of toluene was heated at reflux for 16 hr, cooled, and treated with 100 ml of H_2O. The organic layer was separated and washed (cold H_2O), and the solvent was evaporated at reduced pressure. The residual oil was purified by distillation or converted to a solid salt.

Pyrrolidinecarbinol Esters (Tables IV and V).—A mixture of 0.05 mole of the pyrrolidinemethanol, 0.07 mole of propionic or acetic anhydride, 5 ml of pyridine, and 150 ml of C_6H_6 was heated at reflux for 2–5 days under N_2. After cooling, the solution was washed (10% $NaHCO_3$, H_2O). The solvent was evaporated and the crude product was purified by distillation or conversion to a salt.

Acknowledgment.—The authors thank E. K. Rose, John Eyler, and Dr. William J. Welstead, Jr., for technical assistance.

Irreversible Enzyme Inhibitors. CXVI.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 6-Substituted 2,4-Diamino-5-phenylpyrimidines. III[3]

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Received January 27, 1968

Ten candidate irreversible inhibitors derived from 5-(*p*-chlorophenyl)-2,4-diaminopyrimidine bearing a leaving group on a chain at the 6 position have been evaluated on dihydrofolic reductase from Walker 256 rat tumor and L1210/FR8 mouse leukemia; three had a chloromethyl, four had a sulfonyl fluoride, and three had a bromoacetamido leaving group. Strong evidence was obtained that the diaminopyrimidine could complex as one of two rotomers depending upon the hydrophobicity of the group at the 6 position; 6-phenoxymethyl- and 6-phenethylpyrimidines were bound in a conformation giving a hydrophobic interaction of the 6 group with the enzyme, but the more polar 6-anilinomethylpyrimidines were bound in a "flipped-over" conformation. Three of the sulfonyl fluorides, 6-[*m*-(*m*-fluorosulfonylphenylureido)phenoxymethyl]-2,4-diamino-5-(*p*-chlorophenyl)-pyrimidine (**8**), the 5-(3,4-dichlorophenyl) analog (**10**) of **8**, and the phenethyl analog (**9**) of **8** were good active-site-directed irreversible inhibitors of dihydrofolic reductase.

Recently[3b] the 6-(*p*-chloroacetylanilinomethyl)pyrimidine (**1**) was found to be an active-site-directed irreversible inhibitor[4,5] of dihydrofolic reductase[6] from several sources; that **1** probably was an irreversible inhibitor of the endo type[7] was indicated by the fact that the inactivation was slowed in the presence of the coenzyme, TPNH. The rate of inactivation of an enzyme by an active-site-directed irreversible inhibitor is dependent first upon the concentration of reversible complex between enzyme and inhibitor, which in turn is dependent upon the concentration of inhibitor and the dissociation constant of the reversible enzyme–

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous papers of this series see B. R. Baker and E. H Erickson, *J. Med. Chem.*, **11**, 245 (1968).

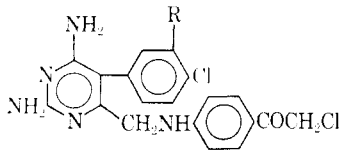
(3) For the previous papers on this type of irreversible inhibitor see (a) B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, **56**, 660 (1967), paper LXXXVIII of this series; (b) B. R. Baker, P. C. Huang, and A. L. Pogolotti, Jr., *J. Med. Chem.*, **10**, 1134 (1967), paper CVIII of this series.

(4) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

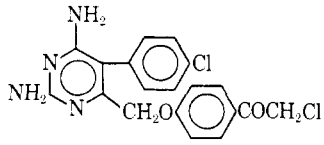
(5) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964), a review.

(6) For a review on the mode of binding of inhibitors to dihydrofolic reductase see ref 4, Chapter X.

(7) The endo type of irreversible inhibitor is defined as one that forms a covalent bond within the enzymic active-site, whereas the exo type forms a covalent bond outside of the active site;[5] see also ref 4, Chapter I.



1, R = H
2, R = Cl



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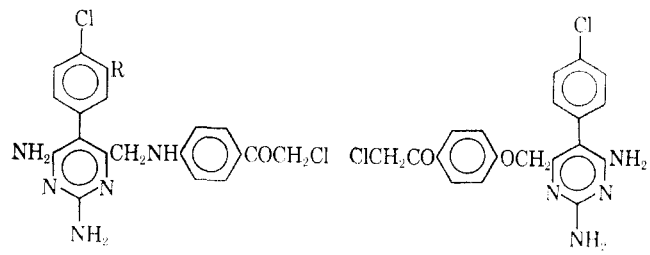
inhibitor complex;[8] and second, the rate of inactivation is dependent upon how close the electrophilic leaving group on the inhibitor is juxtaposed in the reversible complex to an enzymic nucleophilic group and upon the ability of the nucleophile and electrophile to interact.[8] Compound **1** met these criteria in that it could rapidly inactivate the enzyme when more than 50% of the enzyme was reversibily complexed with the inhibitor; a concentration of about 10^{-6} *M* of **1** was necessary to convert 50% of the enzyme into a reversible complex.

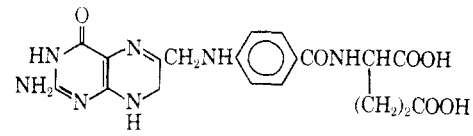
Once a potent active-site-directed irreversible inhibitor for an enzyme is found, the next phase of research is to make the irreversible inhibitor tissue specific by utilization of the bridge principle of specificity.[5,9] Before starting such a study with **1**, two structural variants of **1** were synthesized for evaluation. The 3,4-dichlorophenyl derivative (**2**) was synthesized to lower the concentration of inhibitor necessary to complex reversibly 50% of the enzyme; this structural change ordinarily gives a tenfold or greater increment in binding.[6,10] The second structural change was to replace the 6-NH group by 6-oxygen (**3**); such a structural change could give added flexibility in modification of the bridge between the diaminopyrimidine moiety and the leaving group for application of the bridge principle of specificity.[5,9] As expected, **2** was complexed 7–20-fold better to the dihydrofolic reductases from Walker 256 rat tumor and L1210/FR8 mouse leukemia and **3** was complexed about the same as **1** within a factor of three (Table I). Completely unexpectedly, irreversible inhibition by **2** and **3** was lost. Such unexpected results could hardly go unchallenged if the design of active-site-directed irreversible inhibitors of enzymes in general and dihydrofolic reductase in particular is to be put on a sound scientific basis. Additional studies on these surprising results are the subject of this paper.

The fact that **1** inactivates dihydrofolic reductase, but **3** does not, clearly indicates that **1** and **3** are complexed to the enzyme in different ways. As a working hypothesis it was assumed that **1** was complexed to the enzyme in configuration **1a** and **3** in configuration **3a** with respect to the conformation of the pteridine ring of the substrate, dihydrofolate (**4**), when its ring is arbitrarily assigned conformation **4**[6] in the complex.



1a, R = H
2a, R = Cl

3a



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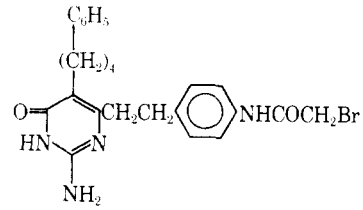

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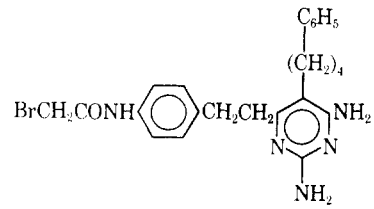

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This working hypothesis was based on the following information.

(1) The 4-pyrimidone, **5**, is an active-site-directed irreversible inhibitor of dihydrofolic reductase,[11] but its 4-amino analog (**6**) is not;[12] these results were rationalized on the basis[12] that the bromoacetamido group of **5** projected into the active site, since protection against inactivation was observed with TPNH, whereas **6** projected the bromoacetamido group in the opposite direction.

(2) The 6-CH_2NH group of **1a** is considerably more polar than the 6-CH_2O group of **3a** with Hansch π constants calculated to be −0.4 and 0.0, respectively.[13]

(3) Since the 3-chloro group on the 5-phenyl moiety of **2a** is complexed to the enzyme, it must be complexed either to the right as shown in **2a** or to the left. If it is complexed on the same side of the pyrimidine as the 6 side chain bearing the leaving group, the 3-chloro group could interfere with the 6 side chain of **2a** approaching juxtaposition to the enzymic nucleophilic group attacked by **1a**. Therefore, additional evidence for this assignment of the binding position of this 3-chloro group was sought.

The major clue to additional compounds that could give further evidence supporting the **1a–3a** conformational assignments was based on the observation that

(8) For the kinetics of irreversible inhibition see (a) B. R. Baker, W. W. Lee, and E. Tong, *J. Theoret. Biol.*, **3**, 459 (1962); (b) ref 4, Chapter VIII.

(9) (a) B. R. Baker, *Biochem. Pharmacol.*, **12**, 293 (1963); (b) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, **53**, 714 (1964); (c) ref 4, Chapter IX.

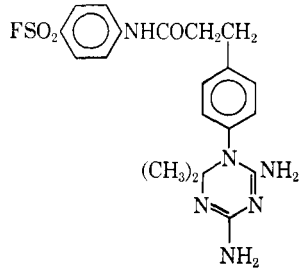
(10) (a) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, **53**, 1457 (1964); (b) B. R. Baker, B.-T. Ho, and D. V. Santi, *ibid.*, **54**, 1415 (1965); (c) B. R. Baker and B.-T. Ho, *J. Heterocycl. Chem.*, **2**, 335 (1965).

(11) B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, **55**, 1417 (1966), paper LXVII of this series.

(12) B. R. Baker and J. H. Jordaan, *J. Heterocycl. Chem.*, **4**, 31 (1967), paper LXXXIII of this series.

(13) T. Fugita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

the dihydro-*s*-triazine (**7**) bearing a sulfonyl fluoride leaving group was an extremely rapid irreversible inhibitor of dihydrofolic reductase from three different species.[14] The sulfonyl fluoride **8** was synthesized and found to be an effective irreversible inhibitor of dihydrofolic reductase. If **1a** and **3a** complex to dihydrofolic



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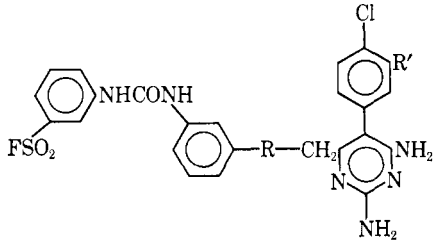
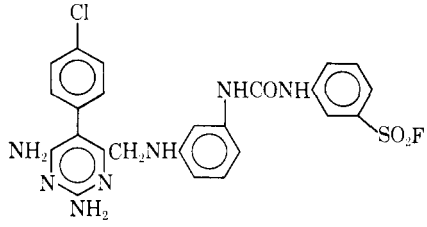


8, R = O; R′ = H
9, R = CH_2; R′ = H
10, R = O; R′ = Cl



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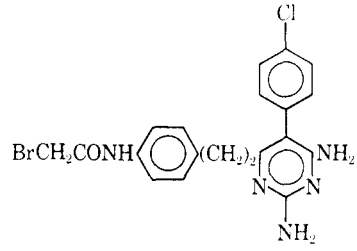
reductase in the conformations shown, then **8** should complex to the enzyme with the leaving group projected to the left as shown.

If **8** has its phenoxymethyl group (to the left) in a relatively hydrophobic region when complexed to the enzyme, two predictions should follow. First, replacement of the R = O group by R = CH_2 as in **9** should give a better reversible inhibitor and **9** should still be an irreversible inhibitor; this was indeed the case. Second, if the 3-chloro group (R′) of **2a** and **10** is complexed to the enzyme to the right as shown, then **10** should still be an irreversible inhibitor was well as a better reversible inhibitor than **2a**. This was also the case.

The sulfonyl fluoride derived from the 6-anilinomethylpyrimidine (**11**) should have its sulfonyl group projected to the right as shown; if such were the case, **11** would either not be an irreversible inhibitor or would attack a different amino acid than that attacked by **8**. Unfortunately, this supporting evidence could not be obtained due to failure of several routes to synthesize **11**.

One of the earlier candidate irreversible inhibitors of dihydrofolic reductase of the 2,4-diaminopyrimidine type synthesized in this laboratory was **12**; although it was an excellent reversible inhibitor of the dihydrofolic reductase from pigeon liver, **12** showed no irreversible inhibition of the enzyme from pigeon liver,[12] Walker 256 or L1210 leukemia (Table I). These negative results were attributed to the binding conformation as shown in **12** (see discussion of **5** *vs.* **6**). The bromoacetamide (**13**) was synthesized as an analog of **1** before it



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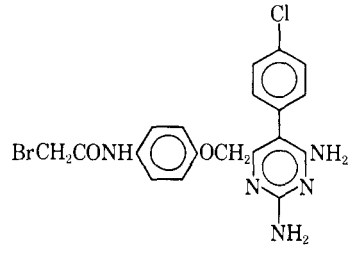

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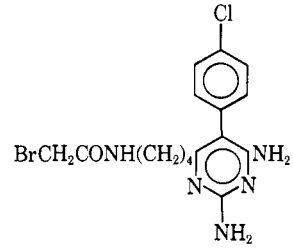

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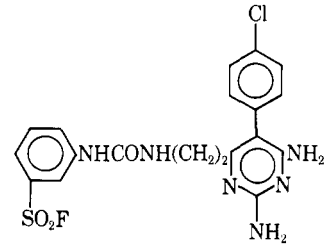

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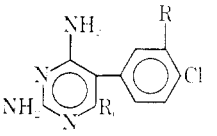
was appreciated that **1** and **3** could have different binding conformations and before **3** was synthesized. *A posteriori*, it can now be expected rationally that **13** would bind to dihydrofolic reductase in the same conformation as **12** and therefore should not be an irreversible inhibitor; this was indeed the case (Table I).

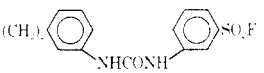
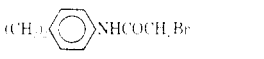
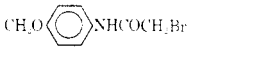
Similarly, **14** would bind in the conformation shown and is not an irreversible inhibitor, as would be expected (Table I). The final compound synthesized (**15**) was an analog of **9** where the benzene ring on the 6 side chain was removed. Although this would be expected to have conformation **15**, it failed to inactivate dihydrofolic reductase indicating that its sulfonyl fluoride failed to juxtapose to an appropriate enzymic nucleophilic group.

Although the sulfonyl fluorides **8**–**10** were active-site-directed irreversible inhibitors of the dihydrofolic reductase from L12101/FR8 mouse leukemia and Walker 256 rat tumor, these compounds were more effective on the mouse leukemia enzyme. Not only did 16 μM **8** inactivate the L1210 enzyme about three times as rapidly as the Walker 256 enzyme, but the extent of inactivation was greater on the L1210 enzyme. The extent of inactivation with a given concentration of a sulfonyl fluoride is dependent upon the relative rate of covalent bond formation within the reversible enzyme–inhibitor complex *vs.* the enzyme-catalyzed hydrolysis

(14) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of this series.

Table I
Inhibition[a] of Dihydrofolic Reductase by



No.	R	R_6	Enzyme source[b]	Reversible: I_{50},[c] μM	Reversible: Estd K_i $\times 10^6$ M[d]	Irreversible: Inhib, μM	Irreversible: TPNH, μM	Irreversible: EI,[e] %	Time, min	% inactn
1[f]	H	$CH_2NHC_6H_4COCH_2Cl$-*p*	L1210/FR8	5.8	0.96	1.8	30	65	15	84
						6.0	30	87	2[g]	50
			W256	5.0	0.83	1.8	0		<2	80
						1.8	30	69	3[g]	50
2	Cl	$CH_2NHC_6H_4COCH_2Cl$-*p*	L1210/FR8	0.94	0.16	5.0	30	97	60	0
			W256	0.22	0.036	1.0	60	95	60	0
						1.0	0		60	0
3	H	$CH_2OC_6H_4COCH_2Cl$-*p*	L1210/FR8	13	2.2	25	30		60	0
			W256	1.1	0.18	5.5	30	97	60	0
						5.5	0		60	0
8	H		L1210/FR8	3.3	0.55	16	30	96	60	90
						16	30	96	13[g]	50
			W256	3.5	0.58	16	60	95	120	68
						16	60	95	40[g]	50
9	H		L1210/FR8	1.6	0.27	8.0	60	97	60	>95
						1.6	60	87	4[g]	50
			W256	0.77	0.13	3.8	60	97	60	95
						3.8	60	97	5[g]	50
10	Cl		L1210/FR8	1.4	0.23	7.0	60	97	60	100
						7.0	60	97	6[g]	50
						1.4	60	87	6[g]	50
			W256	0.56	0.093	2.8	60	97	60	30
12[h]	H		L1210/FR8	0.24	0.040	1.2	30	97	120	0
			W256	0.24	0.040	1.2	30	97	120	0
						1.2	0		120	0
13	H		L1210/FR8	13	2.2	25	30	92	60	0
			W256	1.4	0.23	7	0		60	0
						7	60	97	60	0
14	H	$(CH_2)_4NHCOCH_2Br$	L1210/FR8	8.7	1.4	25	30	90	60	0
			W256	0.66	0.11	3.3	60	97	60	0
						3.3	0		60	0
15	H	$(CH_2)_2NHCONHC_6H_4SO_2F$-*m*	L1210/FR8	3.6	0.60	18	30	97	60	0
			W256	0.80	0.13	4.1	60	97	60	0
						4.1	0		60	0

[a] The technical assistance of Barbara Baine and Jean Reeder with these assays is acknowledged. [b] W256 = Walker 256 rat tumor. [c] Inhibitor concentration necessary for 50% inhibition of the enzyme in the presence of 6 μM dihydrofolate and 30 μM TPNH at pH 7.4 by the methods previously described.[14] [d] Calculated from $K_i = K_m[I]_{50}/[S]$ where $K_m = 1 \times 10^{-6}$ M and $[S] = 6 \times 10^{-6}$ M; this equation is valid when $K_m > 4[S]$.[6,10b] [e] Per cent of enzyme reversibly complexed as calculated from $[EI] = [E_t]/(1 + K_i/[I])$. [f] Data from ref 3b. [g] Half-time for inactivation.[14] [h] See ref 3a for synthesis.

of the sulfonyl fluoride within this complex;[3,15] apparently the Walker 256 enzyme forms a less favorable complex with **8** for inactivation than does the L1210 enzyme. This difference was even more pronounced with **10**.

Additional studies with **8**–**10** and appropriate analogs have shown that the L1210/FR8 mouse leukemia enzyme can be inactivated by some members of this series with little effect on the mouse liver enzyme.[16]

Chemistry.—The 5-(3,4-dichlorophenyl)pyrimidine diethyl acetal (**17**) was synthesized in three steps from 3,4-dichlorophenylacetonitrile and ethyl diethoxyacetate as previously described for **16**,[12] then hydrolyzed to the pyrimidine-6-carboxaldehyde (**19**) with dilute HCl. This aldehyde (**19**) was converted to the candidate irreversible inhibitor **3** by the same route previously used for **1**.[3b] Condensation of **19** with 2-(*p*-aminophenyl)-2-chloromethyldioxolane[17] followed by reduction with $NaBH_4$ gave **22**, which was hydrolyzed to **2** (Scheme I).

Condensation of **18** with *m*-nitroaniline was sluggish; reduction of the resultant anil with $NaBH_4$ gave **20**. Catalytic reduction of the nitro group of **20** to give **23** followed by reaction with *m*-fluorosulfonylphenyl isocyanate to the candidate irreversible inhibitor (**11**) gave a mixture which could not be purified. Alternately, catalytic reductive condensation of **24a** or **24b** with **18** with PtO_2 or Ni gave a mixture from which pure **11** could not be isolated; although the reductive condensation of **18** with *m*-acetamidoaniline with $NaBH_4$ in MeOH was successful, these alkaline conditions were not compatible with the sulfonyl fluoride group of **24b**.

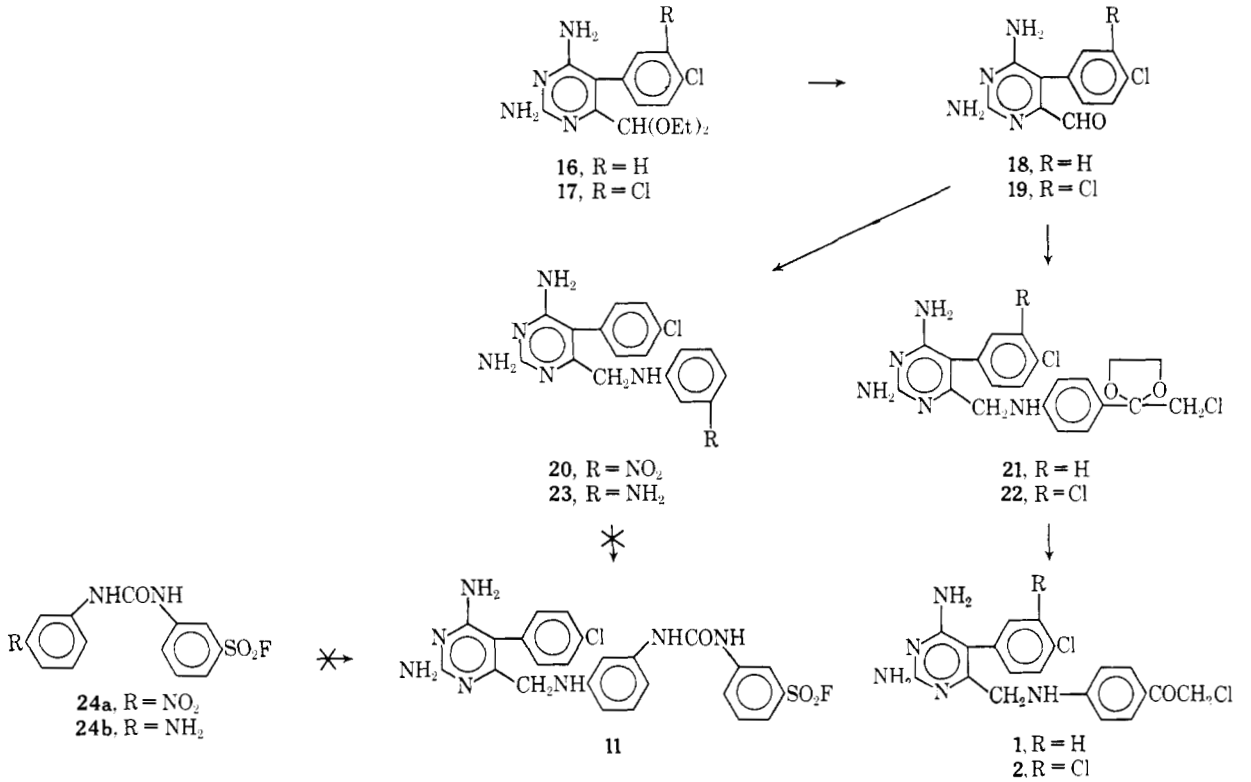
Attempts to condense the pyrimidine-6-aldehyde (**18**) with *m*-nitrobenzyltriphenylphosphonium bromide

(15) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 233 (1968), paper CXIII of this series.

(16) B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968), paper CXX of this series.

(17) B. R. Baker and J. H. Jordaan, *ibid.*, **8**, 35 (1965).

SCHEME I



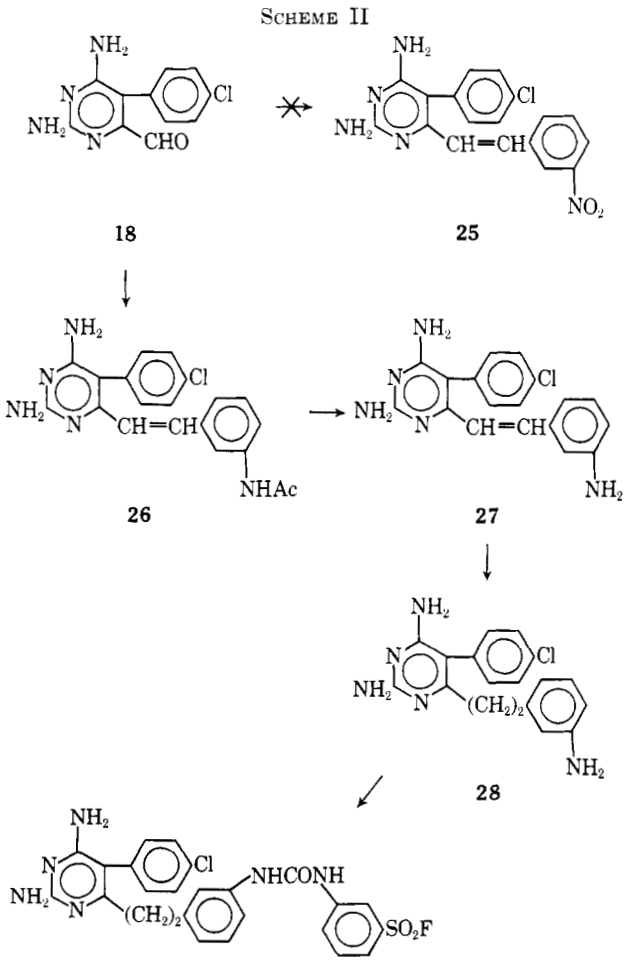
to give **25**, as described for the *para* isomer,[12] gave a mixture of products from which **25** could not be isolated. The condensation of **18** with triphenyl *m*-acetylamidobenzylphosphonium bromide[18] proceeded more smoothly; although analytically pure **26** could not be isolated, **26** was obtained sufficiently pure for further reactions. Acid hydrolysis to **27**, catalytic reduction to **28**, and reaction with *m*-fluorosulfonylphenyl isocyanate afforded the irreversible inhibitor **9** (Scheme II).

A condensation reaction between **18** and the phthalimidopropyl Wittig reagent (**29**)[19] afforded **30** as a mixture of *cis* and *trans* isomers which was reduced catalytically to a single 6-phthalimidobutylpyrimidine (**32**). Hydrazinolysis to **31** followed by bromoacetylation[20] gave the candidate irreversible inhibitor **14** (Scheme III).

The 6-bromomethyl-5-(3,4-dichlorophenyl)pyrimidine (**36**) was synthesized from 3,4-dichlorophenylacetonitrile in four steps *via* **34**, as previously described for the 4-chlorophenyl analog (**35**).[21] Alkylation of *m*- and *p*-nitrophenol with **35** or **36** in DMF afforded the nitrophenyl ethers (**38**–**40**). These were reduced catalytically to the amines (**42**–**44**). Bromoacetylation[20] of **44** afforded the candidate irreversible inhibitor **13** and reaction of **42** and **43** with *m*-fluorosulfonylphenyl isocyanate afforded the irreversible inhibitors **8** and **10** (Scheme IV).

Similarly, condensation of the 6-bromomethylpyrimidine (**35**) with the *p*-hydroxyphenyldioxolane (**37**) afforded **41** which was hydrolyzed to the candidate irreversible inhibitor (**3**) with dilute HCl.

SCHEME II



Reaction of the 6-bromomethylpyrimidine (**35**) with NaCN in DMF afforded the nitrile (**45**), which was

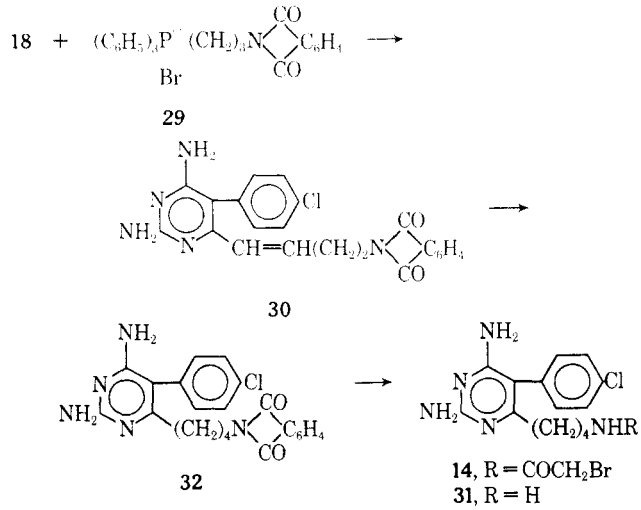
(18) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 26 (1968), paper CIX of this series.

(19) B. R. Baker and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 319 (1966).

(20) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *ibid.*, **3**, 425 (1966).

(21) B. R. Baker and J. H. Jordaan, *ibid.*, **2**, 21 (1965).

Scheme III



reduced catalytically to **46** and converted to the candidate irreversible inhibitor (**15**) with *m*-fluorosulfonylphenyl isocyanate (Scheme V).

Experimental Section[22]

3,4-Dichloro-α-diethoxyacetylphenylacetonitrile.—To a stirred solution of 14.6 g (83 mmoles) of ethyl diethoxyacetate and 15.1 g (83 mmoles) of 3,4-dichlorophenylacetonitrile in 50 ml of C_6H_6 was added in portions 4.1 g (83 mmoles) of 50% dispersion of NaH in mineral oil; the temperature was maintained at 7–10°. Ten minutes after the addition was complete, an additional 2.1 g (40 mmoles) of NaH was added. The mixture was stirred 30 min at 10°, 30 min at ambient temperature, then at reflux for 1 1 hr. The cooled mixture was acidified with 15 ml of HOAc, then diluted with 200 ml of H_2O. The separated organic layer was washed (H_2O), dried ($MgSO_4$), then evaporated *in vacuo*. Three recrystallizations from EtOAc–petroleum ether (bp 60–110°) gave 15 g (53%) of white crystals, mp 85–86°. *Anal.* ($C_{14}H_{15}Cl_2NO_3$) C, H, N.

2,4-Diamino-5-(3,4-dichlorophenyl)-6-(diethoxymethyl)pyrimidine (17).—A solution of 12.6 g (40 mmoles) of the preceding compound in 40 ml of triethyl orthopropionate was refluxed for 1 hr, then slowly distilled to a thin syrup. The remainder of the volatile material was evaporated *in vacuo*. To the residual enol ether was added a filtered solution of 3.8 g (40 mmoles) of guanidine·HCl and 2.3 g (40 mmoles) of NaOMe in 100 ml of absolute EtOH. The solution was refluxed for 4 hr, then concentrated *in vacuo* to about 50 ml and diluted with 100 ml of H_2O. The cooled mixture was filtered and the product was washed with H_2O. Two recrystallizations from EtOH–H_2O gave 10 g (70%) of white crystals, mp 193–195°. *Anal.* ($C_{15}H_{18}Cl_2N_4O_2$) C, H, N.

2,4-Diamino-5-(3,4-dichlorophenyl)pyrimidine-6-carboxaldehyde (19).—A mixture of 5.0 g (14 mmoles) of **17** and 80 ml of 0.4 *N* HCl was refluxed with stirring for 1 hr, treated with charcoal, and filtered. The filtrate was cooled in an ice bath, then the pH was adjusted to about 9 with cold 1 *N* NaOH. The product was collected on a filter and washed with H_2O; yield 3.6 g (90%) of a yellow powder, mp >300°. *Anal.* ($C_{11}H_8Cl_2N_4O$) C, H, N.

2-Chloromethyl-2-{N-[2,4-diamino-5-(3,4-dichlorophenyl)-pyrimidine-6-methyl]-*p*-aminophenyl}-1,3-dioxolane (22) was synthesized from **19** and 2-(*p*-aminophenyl)-2-chloromethyl-1,3-dioxolane[17] as previously described[3b] for **21**; two recrystallizations from EtOH–H_2O gave 194 mg (40%) of white crystals, mp 188–190°. *Anal.* ($C_{21}H_{20}Cl_3N_5O_2$) N.

6-(*p*-Chloroacetylanilinomethyl)-2,4-diamino-5-(3,4-dichlorophenyl)pyrimidine (2) Hydrochloride.—A solution of 150 mg (0.31 mmole) of **22** in 10 ml of 0.1 *N* HCl and 1 ml of EtOH was refluxed for 1 hr, then cooled in an ice bath. The product was collected on a filter, washed with a small amount of ice water, then recrystallized from EtOH–0.1 *N* HCl; yield 135 mg (89%), mp 188–189 dec. *Anal.* ($C_{11}H_{15}Cl_3N_5O\cdot HCl\cdot H_2O$) C, H, N.

N-(*m*-Fluorosulfonylphenyl)-N′-(*m*-nitrophenyl)urea (24a).—To a solution of 1.58 g (10 mmoles) of *m*-nitroaniline in 30 ml of $CHCl_3$ and 5 ml of DMF was added a solution of 1.99 g (9.5 mmoles) of *m*-fluorosulfonylphenyl isocyanate in 5 ml of $CHCl_3$. After 30 min the solution was evaporated *in vacuo* and the residue was crystallized from EtOH–H_2O. The product was collected on a filter, washed with 10% HOAc, then recrystallized twice more from EtOH–H_2O; yield 1.4 g (44%), 219–220°. *Anal.* ($C_{13}H_{10}FN_3O_5S\cdot 0.5H_2O$) C, H, N.

N-(*m*-Aminophenyl)-N′-(*m*-fluorosulfonylphenyl)urea (24b).—A mixture of 2.75 g (7.9 mmoles) of **24a**, 100 ml of absolute EtOH, and 100 mg of PtO_2 was shaken with H_2 at 2–3 atm for 45 min when hydrogenation was complete. The filtered solution was evaporated *in vacuo*. Recrystallization from EtOAc–petroleum ether (bp 60–110°) gave 1.45 g (59%) of white crystals, mp 170–172°. The compound was too unstable to purify.

6-(*m*-Aminostyryl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (27).—To a stirred mixture of 1.90 g (4 mmoles) of *m*-acetamidobenzyl triphenylphosphonium bromide,[18] 1.00 g (4 mmoles) of **18**,[12] and 10 ml of DMF was added 0.45 g (4 mmoles) of KOBu-*t* over a period of about 5 min. After being stirred at ambient temperature for 1 hr and 60° for 2 hr protected from moisture, the mixture was diluted with 10 ml of H_2O, then cooled. The solid was collected on a filter and washed with 10 ml of H_2O and 10 ml of benzene. Recrystallization from THF–H_2O gave 0.78 g (52%) of **26**, mp 156–158°, that was not quite analytically pure.

A stirred solution of 0.55 g (1.5 mmoles) of **26** in 5 ml of EtOH and 5 ml of 6 *N* HCl was refluxed for 1 hr, then cooled. The hydrochloride salt was collected on a filter and dissolved in 5 ml of MeOEtOH. The solution was added to 30 ml of stirred 7% $NaHCO_3$. The product was collected on a filter and washed with H_2O. Recrystallization from EtOH–H_2O gave 0.41 g (84%) of yellow crystals, mp 169–170°. *Anal.* ($C_{18}H_{16}ClN_5$) N.

6-(*m*-Aminophenethyl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (28).—A solution of 450 mg (1.3 mmoles) of **27** in 100 ml of MeOEtOH was shaken with H_2 at 2–3 atm in the presence of 40 mg of PtO_2 for 16 hr when 1.3 mmoles of H_2 had been absorbed. The filtered solution was evaporated *in vacuo*. Recrystallization of the residue from EtOH gave 400 mg (86%) of white crystals, mp 196–198 dec. Analysis indicated the compound was not quite pure. *Anal.* Calcd for $C_{18}H_{18}ClN_5$: C, 63.6; H, 5.34; N, 20.6. Found: C, 63.0; H, 6.10; N, 20.0.

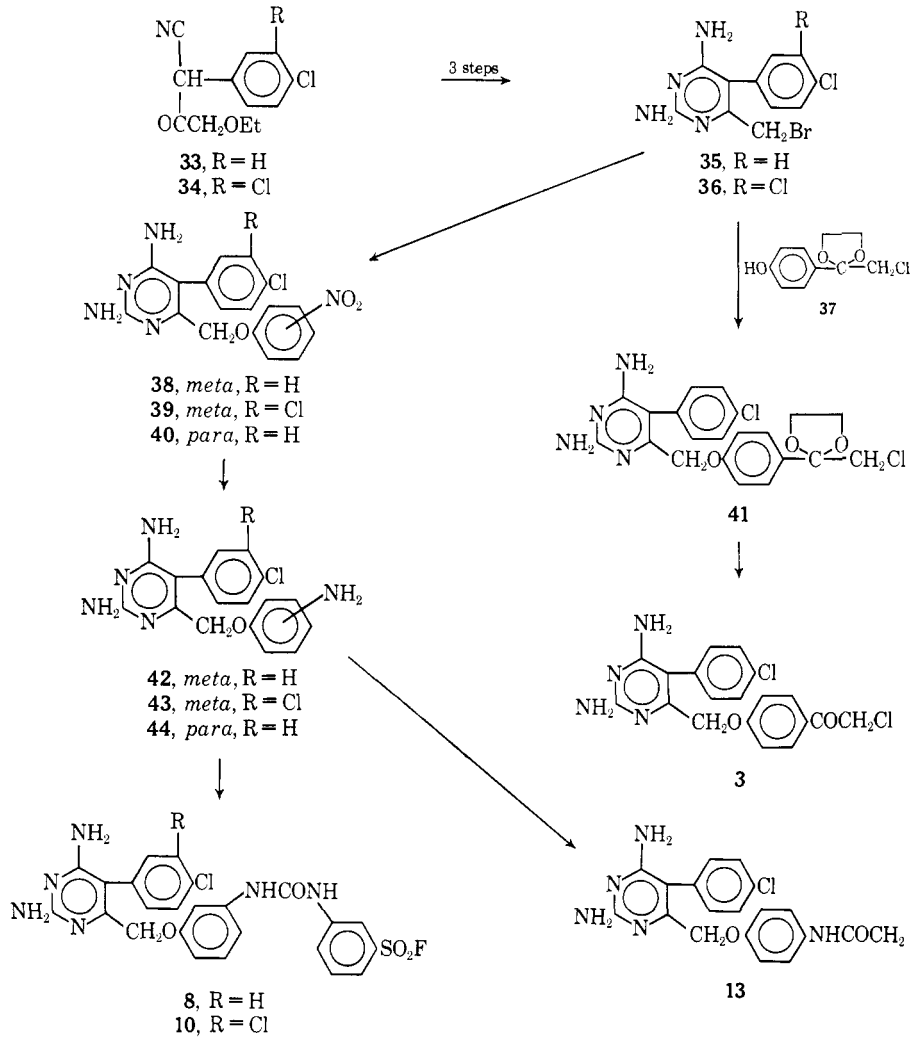
5-(*p*-Chlorophenyl)-2,4-diamino-6-(4-phthalimido-1-butenyl)-pyrimidine (30) Hemisulfate.—A solution of 80 mg (3.3 mmoles) of NaH (48% dispersion in mineral oil) in 10 ml of DMSO (dried with molecular sieves) was prepared at 50° protected from moisture. Then 1.62 g (3 mmoles) of **29**[19] and 0.75 g (3 mmoles) of **18** were added at ambient temperature. After 3 hr the solution was heated at 80° for 24 hr, then poured into a stirred mixture of 40 ml of C_6H_6 and 75 ml of 20% H_2SO_4. The mixture was filtered through a Celite pad. The filter cake was extracted with 40 ml of hot MeOEtOH. The extract was evaporated *in vacuo*. The amorphous residue was dissolved in MeOH and the product precipitated with H_2O. Recrystallization from MeOEtOH–H_2O gave 0.46 g (31%) of white crystals, mp 168–173°, which showed two close-moving spots of *cis-trans* isomers on tlc. *Anal.* ($C_{22}H_{18}ClN_5O_2\cdot 0.5H_2SO_4\cdot H_2O$) C, H, N.

5-(*p*-Chlorophenyl)-2,4-diamino-6-(4-phthalimidobutyl)pyrimidine (32) Hemisulfate.—A solution of 0.95 g (1.95 mmoles) of **30** in 100 ml of MeOEtOH was shaken with H_2 at 2–3 atm in the presence of 20 mg of PtO_2 for 2 hr when reduction was complete. The filtered solution was evaporated *in vacuo*. Three recrystallizations from MeOEtOH–H_2O gave 0.55 g (58%) of white crystals, mp 230–234°. *Anal.* ($C_{22}H_{20}ClN_5O_2\cdot 0.5H_2SO_4\cdot H_2O$) C, H, N.

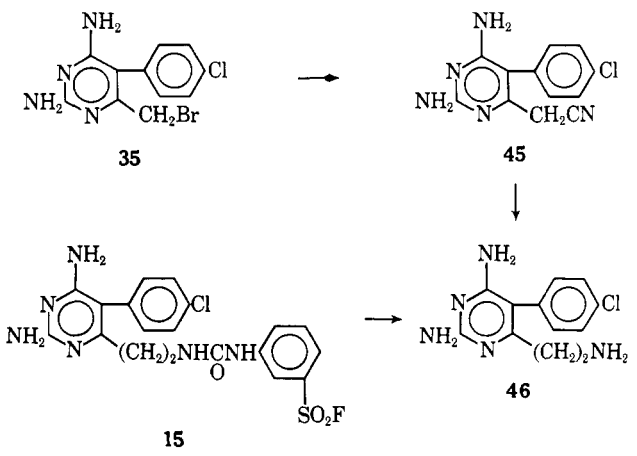
6-(4-Bromoacetamidobutyl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (14) Picrate.—A mixture of 450 mg (0.92 mmole) of **32** hemisulfate, 25 ml of EtOH, and 3 ml of 85% $N_2H_4\cdot H_2O$ was refluxed for 24 hr, then evaporatd *in vacuo*. The residue was extracted with 20 ml of 1 *N* HCl, then clarified with decolorizing carbon. The solution was adjusted to pH 11–12 with 1 *N* NaOH. The product (**31**) was collected on a filter and washed with H_2O, yielding 230 mg (86%) of white crystals that had mp 198–199° and moved as a single spot on tlc in BuOH–HOAc–H_2O (10:5:1).

(22) All analytical samples moved as a single spot on tlc, had ir and uv spectra in agreement with their assigned structure, and gave combustion analyses within 0.4% of theoretical (unless otherwise indicated). Melting points were determined in capillary tubes on a Mel-Temp block and those below 230° are corrected.

SCHEME IV



SCHEME V



To a stirred solution of 70 mg (0.24 mmole) of **31** in 1 ml of DMF was added 80 mg of *p*-nitrophenyl α-bromoacetate.[20] After 45 min, the solution was poured into 5 ml of 4 *N* H_2SO_4; a gum separated which did not solidify. The gum was redissolved by warming the mixture; the solution was treated with 1.5 ml of picric acid in EtOH, then cooled at 5°. The picrate was collected by centrifugation. Recrystallization from MeOEtOH–H_2O gave 120 mg (78%) of yellow crystals, mp 212–215°, that moved as a single spot on tlc in EtOH–$CHCl_3$ (1:10) (with picric acid remaining at the origin) and gave a positive 4-(*p*-nitrobenzyl)-pyridine test for activated halogen.[20] *Anal.* ($C_{16}H_{19}BrClN_5O \cdot C_6H_3N_3O_7$) C, H, N.

3,4-Dichloro-α-ethoxyacetylphenylacetonitrile (**34**).—The reaction of 29 g (0.15 mole) of 3,4-dichlorophenylacetonitrile with 20 g (0.15 mole) of ethyl ethoxyacetate in EtOH containing 9.2 g (0.17 mole) of NaOMe, as described for the preparation of **33**,[21] and recrystallization from CH_2Cl_2–petroleum ether (bp 30–60°) gave 22.5 g (55%) of white crystals, mp 106–108°. *Anal.* ($C_{12}H_{11}Cl_2NO_2$) C, H, N.

2,4-Diamino-5-(3,4-dichlorophenyl)-6-ethoxymethylpyrimidine.—A solution of 13.6 g (50 mmoles) of **34** in 40 ml of ethyl orthoformate was refluxed for 30 min, then distilled to a thin syrup. The resultant 2-(3,4-dichlorophenyl)-3,4-diethoxycrotononitrile was dissolved in 100 ml of absolute EtOH, then 5.7 g (60 mmoles) of guanidine·HCl and 3.3 g (60 mmoles) of NaOMe were added. The stirred mixture was refluxed for 2 hr, then poured into 500 ml of H_2O. The product was collected on a filter and washed with H_2O. Recrystallization from EtOH gave 8.7 g (55%) of white crystals, mp 207–208°. *Anal.* ($C_{13}H_{14}Cl_2N_4O$) C, H, N.

6-Bromomethyl-2,4-diamino-5-(3,4-dichlorophenyl)pyrimidine (**36**).—A mixture of 5.5 g (18 mmoles) of 2,4-diamino-5-(3,4-dichlorophenyl)-6-ethoxymethylpyrimidine and 100 ml of 15% anhydrous HBr in HOAc was refluxed for 8 hr. The solution was concentrated *in vacuo* to about 20 ml, then cooled. The hydrobromide salt was collected on a filter, washed with Me_2CO, then dissolved in 20 ml of DMSO. The filtrered solution was added dropwise to 200 ml of stirred 7% $NaHCO_3$. The product was collected on a filter and washed with H_2O; yield 4.7 g (75%), mp >300°. *Anal.* ($C_{11}H_9BrCl_2N_4$) H, N; C: calcd, 38.0; found, 37.5.

2-Chloromethyl-2-(*p*-hydroxyphenyl)-1,3-dioxolane (**37**).—A mixture of 1.70 g (10 mmoles) of α-chloro-*p*-hydroxyacetophenone, 1.2 g of ethylene glycol, and 70 ml of C_6H_6 was refluxed stirring under a Dean–Stark trap for 10 hr when H_2O separation was complete. The solution was clarified with decolorizing carbon,

then evaporated *in vacuo*. Recrystallization of the residue from MeOH–H_2O gave 1.60 g (75%) of white crystals, mp 118–119°. *Anal.* ($C_{10}H_{11}ClO_3$) C, H.

6-(*p*-Chloroacetylphenoxymethyl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (3) Hydrochloride.—To a solution of 626 mg (2 mmoles) of **35**[21] and 450 mg (2.1 mmoles) of **37** in 40 ml of Me_2CO was added 290 mg (2.1 mmoles) of K_2CO_3. The mixture was refluxed with stirring for 16 hr, then spin evaporated *in vacuo*. Trituration of the residue with 15 ml of H_2O gave a solid that was collected on a filter and washed with H_2O. Two recrystallizations from EtOH–H_2O gave 450 mg (55%) of **41**, mp 205–207°, that did not quite give acceptable combustion analyses.

A solution of 224 mg (0.5 mmole) of **41** in 15 ml of EtOH and 10 ml of 0.25 *N* HCl was refluxed for 1 hr, then cooled to 0°. The product was collected on a filter and washed with cold EtOH. Recrystallization from EtOH gave 150 mg (68%) of white crystals, mp 290–292 dec. *Anal.* ($C_{19}H_{16}Cl_2N_4O_2 \cdot HCl$) C, H, N.

2,4-Diamino-5-(3,4-dichlorophenyl)-6-(*m*-nitrophenoxymethyl)pyrimidine (39).—A mixture of 280 mg (2 mmoles) of *m*-nitrophenol, 110 mg (2 mmoles) of NaOMe, 700 mg (2 mmoles) of **36**, and 3.3 ml of DMF was stirred at ambient temperature for 1 hr and 60° for 5 hr. The cooled mixture was diluted with 15 ml of H_2O. The solid was collected on a filter and washed with H_2O. Recrystallization from MeOEtOH–H_2O gave 620 mg (76%) of nearly white crystals, mp 189–190°. *Anal.* ($C_{17}H_{13}Cl_2N_5O_3$) C, H, N.

The 4-chlorophenyl analog (**38**) was obtained in 48% yield, mp 141–143°, after recrystallization from EtOH–THF but was not analytically pure. Similarly, the *para* isomer (**40**) was obtained in 56% yield after recrystallization from EtOH–THF, mp 208–209°, but was not analytically pure.

6-(*p*-Aminophenoxymethyl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (44).—To a solution of 640 mg (2 mmoles) of **40** in 100 ml of THF was shaken with H_2 at 2–3 atm in the presence of 110 mg of 5% Pd–C for 2 hr when reduction was complete. The filtered solution was evaporated *in vacuo*. Recrystallization of the residue from EtOH–H_2O gave 580 mg (85%) of product, mp 240–241° dec. *Anal.* ($C_{17}H_{16}ClN_5O_2$) C, H, N.

The *meta* isomer (**42**) was obtained in 80% yield, mp 170–171°, after recrystallization from EtOH–H_2O. These crystals appeared solvated and suitable combutions analyses were not obtainable.

The dichlorophenyl analog (**43**) was prepared similarly and had mp 216–218 dec. Recrystallization from EtOH–1 *N* HCl gave white crystals of the dihydrochloride, mp 287–289° dec. *Anal.* ($C_{17}H_{15}Cl_2N_5O \cdot 2HCl \cdot 2H_2O$) C, H, N.

6-(*p*-Bromoacetamidophenoxymethyl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (13) Hemisulfate.—To a stirred solution of 170 mg (0.5 mmole) of **44** in 1 ml of DMF and 40 μl of HOAc cooled in an ice bath was added a cold solution of 145 mg (0.55 mmole) of bromoacetic anhydride in 1 ml of DMF.[20] After 20 min the solution was diluted dropwise with 0.5 *N* H_2SO_4 until no more precipitate formed. The product was collected on a filter and washed with H_2O. Reprecipitation from DMF with 0.5 *N* H_2SO_4 gave 190 mg (77%) of a white powder, mp 180–182° dec, that gave a negative Bratton–Marshall test for aromatic amine and positive 4-(*p*-nitrobenzyl)pyridine test for activated halogen.[20] *Anal.* ($C_{19}H_{17}BrClN_5O_2 \cdot 0.5H_2SO_4$) C, H, N.

5-(*p*-Chlorophenyl)-2,4-diamino-6-[*m*-(*m*-fluorosulfonylphenylureido)phenoxymethyl]pyrimidine (8).—To a stirred solution of 171 mg (0.5 mmole) of **42** in 1.5 ml of reagent dioxane cooled in an ice bath was added a solution of 101 mg (0.5 mmole) of *m*-fluorosulfonylphenyl isocyanate in 0.5 ml of dioxane. The product soon began to separate. After addition of 2 ml more of dioxane, the mixture was stirred 15 min at 0° and 20 min at ambient temperature. The mixture was cooled to 0°, then filtered. The product was washed with petroleum ether (bp 30–60°), then recrystallized from EtOH–H_2O; yield, 170 mg (62%) of white crystals, mp 201–203° dec. *Anal.* ($C_{24}H_{20}FClN_6SO_4$) C, H, N.

5-(*p*-Chlorophenyl)-2,4-diamino-6-[*m*-(*m*-fluorosulfonylphenylureido)phenethyl]pyrimidine (9) Hemisulfate.—To a stirred solution of 136 mg (0.4 mmole) of **28** in 1 ml of DMF was added a solution of 80 mg (0.4 mmole) of *m*-fluorosulfonylphenyl isocyanate in 0.5 ml of DMF. After 30 min at ambient temperature, the solution was treated dropwise with 4 ml of 0.5 *N* H_2SO_4. The product was collected on a filter and washed with H_2O. Recrystallization from MeOEtOH–H_2O gave 150 mg (63%) of white crystals, mp 190–192° dec. *Anal.* ($C_{25}H_{22}ClF_6O_5S \cdot 0.5H_2SO_4 \cdot H_2O$) C, H, N.

Similarly, **10** was prepared from **43** except that the reaction was run at 0°: yield 115 mg (63%), mp 186–188° dec, after recrystallization from MeOEtOH–H_2O. *Anal.* ($C_{24}H_{18}Cl_2FN_5O_4S \cdot 0.5H_2SO_4 \cdot H_2O$) C, H, N.

5-(*p*-Chlorophenyl)-6-cyanomethyl-2,4-diaminopyrimidine (45).—A mixture of 1.52 g (4 mmoles) of **35**·HBr,[21] 10 ml of DMF, and 1.00 g of NaCN was stirred at ambient temperature for 3 hr, then poured into 150 ml of H_2O and adjusted to about pH 11 with 1 *N* NaOH. The product was collected on a filter and washed with H_2O. Recrystallizations from MeOEtOH–H_2O gave 0.60 g (60%) of nearly white crystals, mp 250–260°. *Anal.* ($C_{12}H_{10}ClN_5$) C, H, N.

5-(*p*-Chlorophenyl)-2,4-diamino-6-(*m*-fluorosulfonylphenylureidoethyl)pyrimidine (15).—A mixture of 750 mg (2.9 mmoles) of **45**, 85 ml of EtOH, 15 ml of 1 *N* HCl, and 100 mg of PtO_2 was shaken with H_2 at 2–3 atm for 24 hr when hydrogenation was complete. The filtered solution was evaporated *in vacuo* to 5–10 ml, then adjusted to pH 11–12 with cold 1 *N* NaOH. The crude **46** was collected on a filter and washed with H_2O; yield, 400 mg (53%) of an amorphous powder, mp 154–156°, that moved on tlc in BuOH–HOAc–H_2O (10:5:1) as one major spot with several minor impurities.

To a solution of 140 mg (0.53 mmole) of **46** in 1 ml of dioxane was added 100 mg (0.05 mmole) of *m*-fluorosulfonylphenyl isocyanate. After 30 min, during which time the product began to crystallize from the solution, the mixture was cooled to 0°. The product was collected by centrifugation and washed with $CHCl_3$. Recrystallization from EtOH–H_2O gave 110 mg (47%) of white crystals that gradually decomposed over 146°. *Anal.* ($C_{19}H_{15}ClFN_6O_5S$) C, H, N.