

Aleem Gangjee* [a], Nauzer P. Dubash [a] and Sherry F. Queener [b]

[a] Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282. [b] Department of Pharmacology and Toxicology, Indiana School of Medicine, Indiana University, Indianapolis, IN 46202
Received April 4, 2000

Dedicated to the memory of Professor Raymond N. Castle

Nonclassical 2,4-diamino-5-substituted furo[2,3-*d*]pyrimidines **4a-i**, **5a-b** and **7a-f** were synthesized as extended aromatic ring appended analogs of previously reported antifolates **1a-b**. The extended aromatic system was designed to better interact with a phenylalanine residue (Phe69) of dihydrofolate reductase from the opportunistic pathogen *Pneumocystis carinii* to afford potent and selective inhibitors of *Pneumocystis carinii* dihydrofolate reductase. The target compounds were synthesized by nucleophilic displacement of 2,4-diamino-5-(chloromethyl)furo[2,3-*d*]pyrimidine **3** with the appropriate aromatic amine or thiol. The compounds were evaluated as inhibitors of dihydrofolate reductase from *Pneumocystis carinii* and *Toxoplasma gondii*, and their selectivity was determined using rat liver dihydrofolate reductase as the mammalian reference. In the C8-N9 bridged series, compound **4e**, with a 3-(2-methoxydibenzofuran)- side chain, exhibited greatest potency and was more than 3 times as selective for *Pneumocystis carinii* dihydrofolate reductase compared to rat liver dihydrofolate reductase. Compounds **4b** and **4c** also exhibited selectivity. Compounds in the C8-S9 bridged series showed comparable potencies, and each showed higher selectivity for *Pneumocystis carinii* dihydrofolate reductase compared to rat liver dihydrofolate reductase.

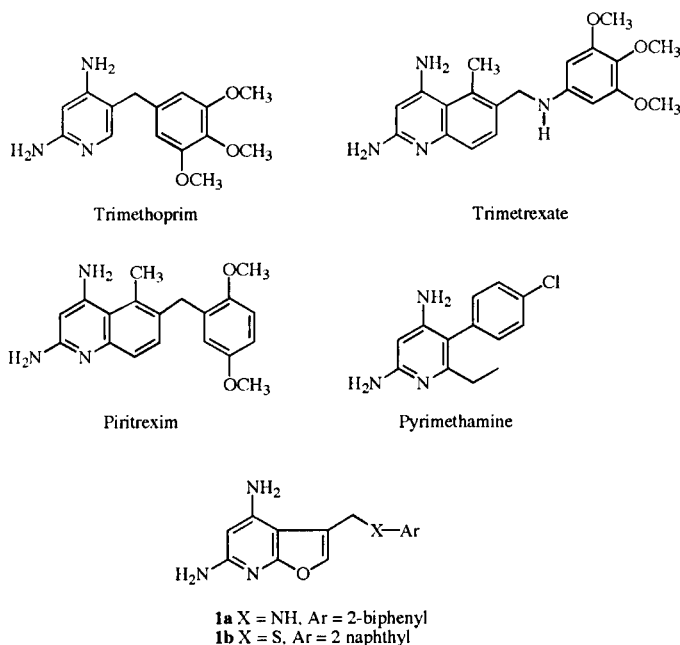
J. Heterocyclic Chem., **37**, 935 (2000).

Opportunistic infections caused by *Pneumocystis carinii* and *Toxoplasma gondii* are responsible for significant morbidity and mortality in individuals with compromised immune systems, such as those with Acquired Immunodeficiency Syndrome [2]. The enzyme dihydrofolate reductase of these opportunistic pathogens represents an attractive target for the development of inhibitory agents. A desirable goal is to obtain inhibitors that are

potent and selective for *Pneumocystis carinii* dihydrofolate reductase and/or *Toxoplasma gondii* dihydrofolate reductase as compared to mammalian dihydrofolate reductase, such as rat liver dihydrofolate reductase. Currently used dihydrofolate reductase inhibitors include monocyclic agents such as trimethoprim and pyrimethamine, and bicyclic agents exemplified by trimetrexate. Both trimethoprim and pyrimethamine are weakly inhibitory and require augmentation with sulfonamides for clinical utility. Trimetrexate is a highly potent dihydrofolate reductase inhibitor, but has significant toxicity and must be used in conjunction with leucovorin to rescue host cells [3]. Thus, the development of dihydrofolate reductase inhibitors with sufficient potency and selectivity for *Pneumocystis carinii* dihydrofolate reductase and/or *Toxoplasma gondii* dihydrofolate reductase versus mammalian dihydrofolate reductase is of considerable significance.

Several 6-6 ring fused analogs, which include pteridines [4], quinazolines [5] and pyridopyrimidines [6,7], and 6-5 ring fused analogs, which include thienopyrimidines [8] and pyrrolopyrimidines [9], have shown reasonable selectivity for *Toxoplasma gondii* dihydrofolate reductase versus rat liver dihydrofolate reductase, but demonstrate significantly lower or complete absence of selectivity for *Pneumocystis carinii* dihydrofolate reductase.

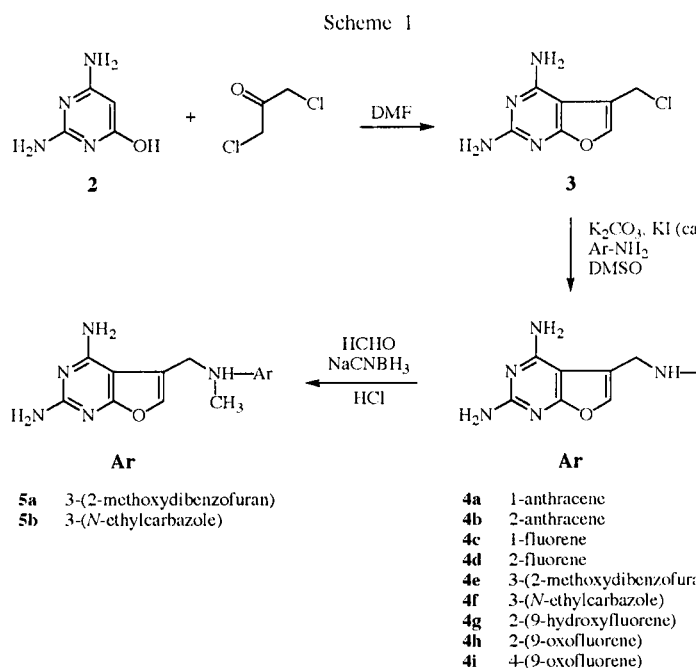
Gangjee *et al.*, [10] reported the inhibitory effects of classical and nonclassical 2,4-diamino-5-substituted-furo[2,3-*d*]pyrimidine antifolates, which indicated that some furo[2,3-*d*]pyrimidines demonstrate selectivity for



Pneumocystis carinii dihydrofolate reductase over rat liver dihydrofolate reductase, but lack selectivity for *Toxoplasma gondii* dihydrofolate reductase. A more recent report [11] shows that compound **1a**, with a methylaminobiphenyl side chain, has a selectivity ratio (IC₅₀ rat liver dihydrofolate reductase / IC₅₀ *Pneumocystis carinii* dihydrofolate reductase) of 18 and compound **1b**, with a methylthio-2-naphthyl side chain, has a selectivity ratio of 19.

The X-ray crystal structure of **1b** with *Pneumocystis carinii* dihydrofolate reductase [11] shows that **1b** binds to *P. carinii* dihydrofolate reductase in a similar fashion as other 6-6 bicyclic antifolates such as trimetrexate and piritrexim. While most of the amino acid residues of dihydrofolate reductase that interact with the bicyclic system are either maintained or conserved in *Pneumocystis carinii* dihydrofolate reductase, as is evident from the crystal structures of trimethoprim and piritrexim with *Pneumocystis carinii* dihydrofolate reductase, some are varied [13]. Of particular interest is the replacement of an asparagine residue (Asn64) in human dihydrofolate reductase by a phenylalanine residue (Phe69) in *Pneumocystis carinii* dihydrofolate reductase. This residue lies just outside the diaminopyrimidine binding site and is in the vicinity of the side chain substituent at the 5-position of furo[2,3-*d*]pyrimidines. The naphthyl side chain of compound **1b** is shown from X-ray crystal structure with *Pneumocystis carinii* dihydrofolate reductase to be involved with this Phe69 in a hydrophobic interaction, which could, in part, provide the selectivity of **1b** for *P. carinii* dihydrofolate reductase versus mammalian dihydrofolate reductase.

Molecular modeling studies with SYBYL 6.5 [14] and its minimization program indicate that replacement of the biphenyl group in **1a** with a tricyclic aromatic ring system should result in a much better hydrophobic interaction with Phe69 of *Pneumocystis carinii* dihydrofolate reductase, which could afford compounds with better potency and selectivity than **1a** against *Pneumocystis carinii* dihydrofolate reductase. In addition, molecular modeling also indicated that substitution of the naphthyl group of **1b** with flexible aromatic ring systems such as biphenyls or phenoxyphenyls might allow for better "edge-on" hydrophobic interaction of the aromatic rings with Phe69 of *Pneumocystis carinii* dihydrofolate reductase, resulting in compounds with improved potency and/or selectivity. On the basis of molecular modeling, we designed compounds **4a-i** as side chain analogs of **1a**, in which the biphenyl ring is extended to a tricyclic aromatic system, and compounds **7a-f** as flexible analogs of **1b**, in order to obtain compounds which might be more potent and more selective for *Pneumocystis carinii* dihydrofolate reductase than either **1a** or **1b**. In addition, we also synthesized compounds **5a-b** to determine the effect of *N*-methylation of the side chain nitrogen on selectivity and potency. A



recent report by Rosowsky *et al.*, [15] which shows that tricyclic substituents on 2,4-diaminopteridine systems provides selective inhibitors lends further credence to our design of compounds **4a-i**.

The key intermediate for the synthesis of target compounds **4a-i** and **7a-f** was 2,4-diamino-5-chloromethyl-furo[2,3-*d*]pyrimidine **3** (Scheme 1), which was first reported by Secrist and Liu in 1978 [16]. The chloromethyl function of **3** could be conveniently transformed by nucleophilic displacements with appropriate tricyclic aromatic amines or aromatic thiols to afford target molecules **4a-i** and **7a-f** in a convergent fashion.

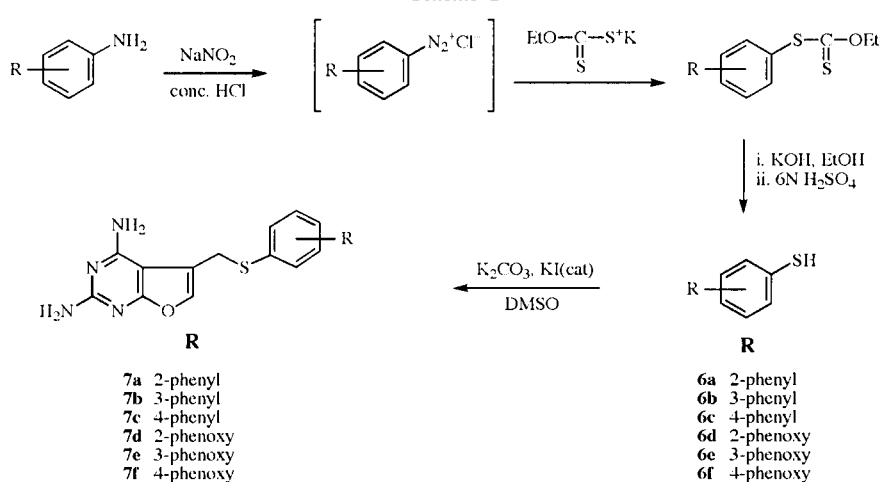
Compound **3** was prepared from 2,4-diamino-6-hydroxypyrimidine **2** and 1,3-dichloroacetone. Thin layer chromatographic analysis of the reaction mixture after 24 hours showed complete disappearance of the starting material, and the formation of a mobile spot (*R*_f=0.30 in 9:1 chloroform:methanol), along with by-products which remained at the origin. Vacuum filtration of the reaction mixture afforded an off-white solid in 78% yield. Nucleophilic displacement reactions on this crude compound resulted in the target compounds in extremely poor yields (<2%). Purification of **3** was accomplished chromatographically using a silica gel plug. The pure compound, obtained in 55% yield, was unstable at room temperature and decomposed on prolonged exposure to air. It was therefore necessary to seal the dried compound and store it at low temperature (<-20 °C).

Nucleophilic displacement of purified **3** with the appropriate tricyclic aromatic amine afforded compounds **4a-i** in yields ranging from 1.2% to 41%. These reactions were

carried out in anhydrous dimethylsulfoxide, in the presence of anhydrous potassium carbonate and a catalytic amount of potassium iodide; temperatures were maintained between 55-60 °C, and the reactions were com-

pleted in about 8 hours. All chromatographic purifications of the products were performed by loading a dry silica gel plug of the crude material on top of a wet silica gel column, packed with silica gel slurry in either chloroform or ethyl acetate.

Scheme 2



pleted in about 8 hours. All chromatographic purifications of the products were performed by loading a dry silica gel plug of the crude material on top of a wet silica gel column, packed with silica gel slurry in either chloroform or ethyl acetate.

The *N*-methyl analogs of **4e** and **4f** were synthesized by reductive methylation of the 9-nitrogen using formaldehyde and sodium cyanoborohydride in an acidic medium. These reactions yielded multiple products, which afforded low yields of the *N*-methylated products **5a** (32%) and **5b** (21%).

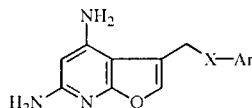
Nucleophilic displacement of compound **3** with appropriate aromatic thiols afforded the target compounds **7a-f**. Since none of the desired thiols were commercially available, they were synthesized from the corresponding aromatic amines using a literature method [17]. This was accomplished by diazotization of the amine with sodium nitrite in aqueous acid, followed by displacement of the diazo moiety with potassium ethyl xanthate and hydrolysis of the xanthate ester (Scheme 2). Acidification of the xanthic acid so obtained resulted in the evolution of carbon oxysulfide and the formation of an oil, which was extracted in ether. The crude oil obtained on evaporation of the ether contained the desired aromatic thiols **6a-f**, and was directly used, without purification, for the nucleophilic displacement reaction with **3** in the presence of potassium carbonate and catalytic amounts of potassium iodide in anhydrous dimethyl sulfoxide. Chromatographic purification afforded target compounds **7a-f**.

Compounds **4a-i**, **5a-b** and **7a-f** were evaluated as inhibitors of dihydrofolate reductase from *Pneumocystis carinii*, *Toxoplasma gondii* and rat liver, which served

as the mammalian reference. The results are listed in Table 1. Inhibitory concentrations are expressed as IC₅₀ values; in cases where the curve could not be completed, percent inhibition at the concentration used is included. Selectivity ratios are expressed as IC₅₀ rat liver dihydrofolate reductase/IC₅₀ *Pneumocystis carinii* dihydrofolate reductase or *Toxoplasma gondii* dihydrofolate reductase. In the series of C8-N9 bridged compounds, the IC₅₀ values could not be determined in most instances due to extremely poor solubility of these compounds. The percent inhibition values, however, show encouraging results in some instances. Compound **4b**, which has a 2-anthracene side chain, shows 30% inhibition of *Pneumocystis carinii* dihydrofolate reductase at a concentration of 14 μM and does not inhibit rat liver dihydrofolate reductase at that concentration, which points to *Pneumocystis carinii* selectivity. Similarly, compound **4c**, with a 1-fluorene side chain, shows greater inhibition of *Pneumocystis carinii* dihydrofolate reductase than rat liver dihydrofolate reductase at 29 μM concentration, again indicating that the compound is selective. Compound **4e** exhibited the greatest potency in this series with an IC₅₀ of 10.3 μM. Moreover, **4e** inhibits only 20% of rat liver dihydrofolate reductase at 32 μM, which translates into a *Pneumocystis carinii* selectivity ratio of greater than 3.2. Similarly, compound **4i** has an IC₅₀ of 19.3 μM, and shows only 12% inhibition of rat liver dihydrofolate reductase at 38 μM, indicating greater than 2-fold selectivity for *Pneumocystis carinii* dihydrofolate reductase over rat liver dihydrofolate reductase.

The most potent compound in the C8-S9 bridged series is **7d**, which has a 2-phenoxyphenyl appendage. It has an IC₅₀ of 14 μM and a selectivity ratio of 1.29. The most favorable result was obtained for compound **7c**, which has a 4-biphenyl side chain. Although **7c** was not a very

Table 1
Inhibitory Concentrations (IC_{50} , mM) and Selectivity Ratios against Dihydrofolate Reductase (DHFR) from
Pneumocystis carinii (pc), *Toxoplasma gondii* (tg) and rat liver (rl)



Compound	X	Ar	DHFR				
			pc	rl	rl/pc	tg	rl/tg
4a	NH	1-anthracene	50% @ 51mM	37% @ 51mM	ND	15.4	>3.3
4b	NH	2-anthracene	30% @ 14mM	0% @ 14mM	ND	15% @ 14mM	ND
4c	NH	1-fluorene	38% @ 29mM	10% @ 29mM	ND	38% @ 29mM	ND
4d	NH	2-fluorene	36.2	40% @ 10mM	ND	27.7	ND
4e	NH	3-(2-methoxy-dibenzofuran)	10.3	20% @ 32mM	>3.2	38% @ 32mM	ND
4f	NH	3-(N-ethyl-carbazole)	16.2	12.6	0.78	4.5	2.80
4g	NH	2-(9-hydroxy-fluorene)	8% @ 13mM	34% @ 13mM	ND	63	ND
4h	NH	2-(9-oxofluorene)	57% @ 63mM	34% @ 63mM	N	29% @ 63mM	ND
4i	NH	4-(9-oxofluorene)	19.3	12% @ 38mM	>2	31% @ 38mM	ND
5a	N(CH ₃)	3-(2-methoxy-dibenzofuran)	15% @ 54mM	241	ND	392	0.61
5b	N(CH ₃)	3-(N-ethyl-carbazole)	18.7	24.7	1.32	22.6	1.09
7a	S	2-biphenyl	46	49	1.07	105	0.47
7b	S	3-biphenyl	22	31	1.41	23	1.35
7c	S	4-biphenyl	41	351	8.56	79	4.44
7d	S	2-phenoxyPh	14	18	1.29	38	0.47
7e	S	3-phenoxyPh	33	39	1.18	19	2.05
7f	S	4-phenoxyPh	25	65	2.60	259	0.25

DHFR data includes % inhibition when curves cannot be completed.

potent inhibitor of *Pneumocystis carinii* dihydrofolate reductase ($IC_{50} = 41\mu M$), it is a poor inhibitor of rat liver dihydrofolate reductase, with potency in the millimolar range, which translates into a selectivity ratio of 8.56. It is interesting to note that all the compounds in the C8-S9

bridged series demonstrate selectivity for *Pneumocystis carinii* dihydrofolate reductase over rat liver dihydrofolate reductase. This observation lends support to the hypothesis underlying the design of this series of compounds, which is that a flexible, extended aromatic side chain

Table 2
Elemental Analysis of compounds 4a-i, 5a and 7a-f

Compound	Molecular Formula	Found	Calculated
4a	C ₂₁ H ₁₇ N ₅ O•0.30 CH ₃ CO ₂ C ₂ H ₅	C, 69.59; H, 4.96; N, 18.55	C, 69.83; H, 5.12; N, 18.34
4b	C ₂₁ H ₁₇ N ₅ O•0.20 H ₂ O	C, 70.26; H, 4.87; N, 19.21	C, 70.26; H, 4.89; N, 19.51
4c	C ₂₀ H ₁₇ N ₅ O•0.60 C ₂ H ₆ SO	C, 64.92; H, 5.21; N, 17.67	C, 65.25; H, 5.32; N, 17.94
4d	C ₂₀ H ₁₇ N ₅ O•0.60 H ₂ O	C, 65.45; H, 4.74; N, 19.32	C, 65.77; H, 4.86; N, 19.17
4e	C ₂₀ H ₁₇ N ₅ O ₃ •0.30 H ₂ O	C, 63.16; H, 4.58; N, 18.02	C, 63.08; H, 4.66; N, 18.39
4f	C ₂₁ H ₂₀ N ₆ O•0.30 CH ₃ CO ₂ C ₂ H ₅	C, 67.90; H, 5.53; N, 22.14	C, 67.60; H, 5.45; N, 22.35
4g	C ₂₀ H ₁₇ N ₅ O ₂ •0.80 H ₂ O	C, 64.45; H, 4.67; N, 18.39	C, 64.26; H, 5.02; N, 18.74
4h	C ₂₀ H ₁₅ N ₅ O ₂ •0.40 CH ₃ OH	C, 66.33; H, 4.32; N, 18.65	C, 66.19; H, 4.52; N, 18.92
4i	C ₂₀ H ₁₅ N ₅ O ₂ •0.40 C ₂ H ₆ SO	C, 64.33; H, 4.18; N, 18.14	C, 64.29; H, 4.51; N, 18.02
5a	C ₂₁ H ₁₉ N ₅ O ₃ •0.40 H ₂ O	C, 63.54; H, 4.87; N, 17.29	C, 63.60; H, 5.03; N, 17.66
7a	C ₁₉ H ₁₆ N ₄ OS•0.20 H ₂ O	C, 64.89; H, 4.67; N, 15.76; S, 9.47	C, 64.83; H, 4.70; N, 15.92; S, 9.11
7b	C ₁₉ H ₁₆ N ₄ OS•0.20 C ₂ H ₆ SO	C, 64.39; H, 4.70; N, 15.74; S, 10.81	C, 64.01; H, 4.76; N, 15.39; S, 10.57
7c	C ₁₉ H ₁₆ N ₄ OS•0.10 C ₂ H ₆ SO	C, 64.67; H, 4.63; N, 15.64; S, 9.88	C, 64.73; H, 4.70; N, 15.73; S, 9.90
7d	C ₁₉ H ₁₆ N ₄ O ₂ S•0.10 H ₂ O	C, 62.14; H, 4.23; N, 15.05; S, 8.37	C, 62.31; H, 4.46; N, 15.30; S, 8.76
7e	C ₁₉ H ₁₆ N ₄ O ₂ S•0.15 C ₂ H ₆ SO	C, 61.34; H, 4.15; N, 15.03; S, 9.44	C, 61.63; H, 4.53; N, 14.89; S, 9.80
7f	C ₁₉ H ₁₆ N ₄ O ₂ S	C, 62.41; H, 4.35; N, 15.31; S, 9.05	C, 62.62; H, 4.43; N, 15.37; S, 8.80

interacts better with Phe69 of *Pneumocystis carinii* dihydrofolate reductase than with rat liver dihydrofolate reductase. Comparing the selectivity ratios of compounds **7c** and **7f** with the other compounds in this series suggests that substitution at the 4-position of the aromatic ring directly attached to S9 is more conducive to *Pneumocystis carinii* dihydrofolate reductase selectivity as opposed to substitution at either the 2- or 3-position.

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Melting points were determined on a Mel-Temp II melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Bruker WH-300 (300MHz) spectrometer. Chemical shifts are expressed as δ values (parts per million) relative to tetramethylsilane as an internal standard: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Thin layer chromatography was performed on silica gel plates with fluorescent indicator, and visualized with light at 254nm and 366nm. Column chromatography was performed on 230-400 mesh silica gel purchased from Aldrich Chemical Co., Milwaukee, WI. Solvents routinely used for reactions and purification were purchased from Aldrich or Fisher Scientific Co., Pittsburgh, PA and used without further purification. Proportions of solvents used for thin layer chromatography and column chromatography are by volume. Samples for elemental analysis were dried *in vacuo* in an Abderhalden drying apparatus over phosphorous pentoxide and refluxing ethanol. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Elemental compositions are within $\pm 0.4\%$ of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates could not be prevented despite drying *in vacuo* at 80 °C and were confirmed, where possible, by their presence in the ^1H NMR spectra.

2,4-Diamino-5-chloromethylfuro[2,3-*d*]pyrimidine (**3**).

2,4-Diamino-6-hydroxypyrimidine (12.13 g, 96.17 mmol) and 1,3-dichloroacetone (12.21 g, 96.17 mmol) were stirred in anhydrous dimethylformamide (75 ml) at room temperature under an inert nitrogen atmosphere for 24 hours. Silica gel (10 g) was added directly to the reaction mixture and dimethylformamide was removed under vacuum to afford a plug, which was dried, loaded on top of a tightly-packed, dry silica gel column (15 cm x 7 cm) and eluted using a 7:1 mixture of chloroform:methanol. Fractions containing product were pooled and solvent was evaporated to afford 10.12 g (53%) of a white solid: mp 179-182 °C; ^1H NMR (DMSO- d_6) δ 4.93 (s, 2H, $-\text{CH}_2\text{Cl}$), 6.37 (bs, 2H, 4-NH₂), 6.82 (bs, 2H, 2-NH₂), 7.52 (s, 1H, 6-H).

General procedure for the synthesis of compounds **4a-i**.

An equimolar mixture of **3** and the appropriate aromatic amine was dissolved in anhydrous dimethylsulfoxide (1 ml/25 mg **3**). Anhydrous potassium carbonate (1.5 molar excess) and a catalytic quantity of potassium iodide (2 mole percent) were added and the mixture was heated under an inert nitrogen atmosphere at 55-60 °C for 8 hours. The reaction mixture was cooled

to room temperature and poured into cold water (50-100 ml). The precipitate obtained was filtered, washed with water (2x25 ml) and dried. The solid was dispersed in acetone, silica gel was added and the solvent evaporated to afford a plug, which was dried, loaded on top of a silica gel column (20 cm x 2.5 cm) and eluted using either ethyl acetate, or a gradient of 2-5% methanol in chloroform. Fractions containing product were pooled and solvent evaporated to afford the product.

2,4-Diamino-5-[*N*-(anthran-1-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4a**).

Compound **4a** was synthesized from **3** (2.0 g, 10 mmol), 1-aminoanthracene (1.95 g, 10 mmol), potassium iodide (33 mg, 0.20 mmol) and anhydrous potassium carbonate (2.08 g, 15 mmol). Column chromatography using ethyl acetate as eluant afforded 65 mg (1.8%) **4a** as 65 mg (1.8%) of a yellow solid: mp >275 °C (dec); ^1H NMR (DMSO- d_6) δ 4.47 (s, 2H, $-\text{CH}_2\text{NH}-$), 6.03 (bs, 2H, 4-NH₂), 6.67 (bs, 2H, 2-NH₂), 6.69 (bs, 1H, $-\text{CH}_2\text{NH}-$), 7.52 (s, 1H, 6-H), 6.90-8.84 (m, 9H, anthracene).

Anal. Calcd. for C₂₁H₁₇N₅O•0.30 CH₃CO₂CH₂CH₃: C, 69.83; H, 5.12; N, 18.34. Found: C, 69.59; H, 4.96; N, 18.55.

2,4-Diamino-5-[*N*-(anthran-2-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4b**).

Compound **4b** was synthesized from **3** (2.0 g, 10 mmol), 2-aminoanthracene (1.95 g, 10 mmol), potassium iodide (33 mg, 0.20 mmol) and anhydrous potassium carbonate (2.08 g, 15 mmol). Column chromatography using ethyl acetate as eluant afforded 45 mg (1.2%) **4b** as a pale green solid: mp >250 °C (dec); ^1H NMR (DMSO- d_6) δ 4.37 (d, 2H, $-\text{CH}_2\text{NH}-$), 6.07 (bs, 2H, 4-NH₂), 6.55 (bs, 2H, 2-NH₂), 6.57 (t, 1H, $-\text{CH}_2\text{NH}-$), 7.54 (s, 1H, 6-H), 7.00-8.31 (m, 9H, anthracene).

Anal. Calcd. for C₂₁H₁₇N₅O•0.20 H₂O: C, 70.26; H, 4.89; N, 19.51. Found: C, 70.26; H, 4.87; N, 19.21.

2,4-Diamino-5-[*N*-(fluoren-1-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4c**).

Compound **4c** was synthesized from **3** (218 mg, 1.10 mmol), 1-aminofluorene (200 mg, 1.10 mmol), potassium iodide (4 mg, 0.024 mmol) and anhydrous potassium carbonate (230 mg, 1.65 mmol). Column chromatography using ethyl acetate as eluant afforded 40 mg (11%) **4c** as a pale yellow solid: mp >195 °C (dec); ^1H NMR (DMSO- d_6) δ 3.73 (s, 2H, 9'-CH₂), 4.35 (s, 2H, $-\text{CH}_2\text{NH}-$), 6.02 (bs, 3H, 4-NH₂ & $-\text{CH}_2\text{NH}-$), 6.69 (bs, 2H, 2-NH₂), 7.42 (s, 1H, 6-H), 6.73-7.82 (m, 7H, fluorene).

Anal. Calcd. for C₂₀H₁₇N₅O•0.60 C₂H₆SO: C, 65.25; H, 5.32; N, 17.94. Found: C, 64.92; H, 5.21; N, 17.67.

2,4-Diamino-5-[*N*-(fluoren-2-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4d**).

Compound **4d** was synthesized from **3** (218 mg, 1.10 mmol), 2-aminofluorene (200 mg, 1.10 mmol), potassium iodide (4 mg, 0.024 mmol) and anhydrous potassium carbonate (230 mg, 1.65 mmol). Column chromatography using ethyl acetate as eluant afforded 49 mg (13%) **4d** as a buff colored solid: mp >275 °C (dec); ^1H NMR (DMSO- d_6) δ 3.78 (s, 2H, 9'-CH₂), 4.26 (d, 2H, $-\text{CH}_2\text{NH}-$), 6.05 (bs, 2H, 4-NH₂), 6.23 (t, 1H, $-\text{CH}_2\text{NH}-$), 6.60 (bs, 2H, 2-NH₂), 7.40 (s, 1H, 6-H), 6.78-7.66 (m, 7H, fluorene).

Anal. Calcd. for C₂₀H₁₇N₅O•0.60 H₂O: C, 65.77; H, 4.86; N, 19.17. Found: C, 65.45; H, 4.74; N, 19.32.

2,4-Diamino-5-[*N*-(2-methoxydibenzofuran-3-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4e**).

Compound **4e** was synthesized from **3** (500 mg, 2.51 mmol), 2-methoxy-3-aminodibenzofuran (537 mg, 2.51 mmol), potassium iodide (9 mg, 0.054 mmol) and anhydrous potassium carbonate (522 mg, 3.77 mmol). Column chromatography using ethyl acetate as eluant afforded 190 mg (20%) **4e** as an off white solid: mp >290 °C (dec); ¹H NMR (DMSO-*d*₆) δ 3.91 (s, 3H, 2'-OCH₃), 4.38 (d, 2H, -CH₂-NH-), 6.01 (bs, 3H, 4-NH₂ & -CH₂-NH-), 6.65 (bs, 2H, 2-NH₂), 7.45 (s, 1H, 6-H), 7.25-7.90 (m, 6H, dibenzofuran).

Anal. Calcd. for C₂₀H₁₇N₅O₃•0.30 H₂O: C, 63.08; H, 4.66; N, 18.39. Found: C, 63.16; H, 4.58; N, 18.02.

2,4-Diamino-5-[*N*-(9-ethylcarbazol-3-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4f**).

Compound **4f** was synthesized from **3** (500 mg, 2.51 mmol), 3-amino-9-ethylcarbazole (530 mg, 2.51 mmol), potassium iodide (9 mg, 0.054 mmol) and anhydrous potassium carbonate (522 mg, 3.77 mmol). Column chromatography using ethyl acetate as eluant afforded 390 mg (41%) **4f** as a buff colored solid: mp >275 °C (dec); ¹H NMR (DMSO-*d*₆) δ 1.26 (t, 3H, -CH₂-CH₃), 4.27 (d, 2H, -CH₂-NH-), 4.35 (q, 2H, -CH₂-CH₃), 5.84 (t, 1H, -CH₂-NH-), 6.03 (bs, 2H, 4-NH₂), 6.81 (bs, 2H, 2-NH₂), 7.54 (s, 1H, 6-H), 7.01-8.03 (m, 7H, carbazole).

Anal. Calcd. for C₂₁H₂₀N₆O•0.30 CH₃CO₂CH₂CH₃: C, 67.60; H, 5.45; N, 22.35. Found: C, 67.90; H, 5.53; N, 22.14.

2,4-Diamino-5-[*N*-(9-hydroxyfluoren-2-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4g**).

Compound **4g** was synthesized from **3** (200 mg, 1.01 mmol), 2-amino-9-fluorenol (200 mg, 1.01 mmol), potassium iodide (4 mg, 0.024 mmol) and anhydrous potassium carbonate (210 mg, 1.52 mmol). Column chromatography using a gradient of 2-5% methanol in chloroform as eluant afforded 40 mg (11%) **4g** as a pale orange solid: mp >245 °C (dec); ¹H NMR (DMSO-*d*₆) δ 4.26 (d, 2H, -CH₂-NH-), 5.32 (d, 1H, 9'-H), 5.68 (d, 1H, 9'-OH), 6.07 (bs, 2H, 4-NH₂), 6.30 (t, 1H, -CH₂-NH-), 6.62 (bs, 2H, 2-NH₂), 7.38 (s, 1H, 6-H), 6.74-7.54 (m, 7H, fluorene).

Anal. Calcd. for C₂₀H₁₇N₅O₂•0.80 H₂O: C, 64.26; H, 5.02; N, 18.74. Found: C, 64.45; H, 4.67; N, 18.39.

2,4-Diamino-5-[*N*-(9-oxofluoren-2-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4h**).

Compound **4h** was synthesized from **3** (500 mg, 2.51 mmol), 2-amino-9-fluorenone (491 mg, 2.51 mmol), potassium iodide (9 mg, 0.054 mmol) and anhydrous potassium carbonate (522 mg, 3.77 mmol). Column chromatography using a gradient of 2-5% methanol in chloroform as eluant afforded 105 mg (12%) **4h** as a pale orange solid: mp >260 °C (dec); ¹H NMR (DMSO-*d*₆) δ 4.32 (d, 2H, -CH₂-NH-), 6.05 (bs, 2H, 4-NH₂), 6.50 (bs, 2H, 2-NH₂), 6.60 (t, 1H, -CH₂-NH-), 6.84-7.53 (m, 8H, 6-H & fluorene).

Anal. Calcd. for C₂₀H₁₅N₅O₂•0.40 CH₃OH: C, 66.19; H, 4.52; N, 18.92. Found: C, 66.33; H, 4.32; N, 18.65.

2,4-Diamino-5-[*N*-(9-oxofluoren-4-yl)amino]methylfuro[2,3-*d*]pyrimidine (**4i**).

Compound **4i** was synthesized from **3** (605 mg, 3.05 mmol), 4-amino-9-fluorenone (595 mg, 3.05 mmol), potassium iodide (11 mg, 0.066 mmol) and anhydrous potassium carbonate (631

mg, 4.57 mmol). Column chromatography using a gradient of 2-5% methanol in chloroform as eluant afforded 36 mg (4%) **4i** as a bright orange solid: mp >250 °C (dec); ¹H NMR (DMSO-*d*₆) δ 4.73 (d, 2H, -CH₂-NH-), 6.02 (bs, 2H, 4-NH₂), 6.14 (t, 1H, -CH₂-NH-), 6.70 (bs, 2H, 2-NH₂), 7.43 (s, 1H, 6-H), 6.99-7.94 (m, 7H, fluorene).

Anal. Calcd. for C₂₀H₁₅N₅O₂•0.40 C₂H₆SO: C, 64.29; H, 4.51; N, 18.02. Found: C, 64.33; H, 4.18; N, 18.14.

2,4-Diamino-5-[*N*-(2-methoxydibenzofuran-3-yl)-*N*-methyl]amino]methylfuro[2,3-*d*]pyrimidine (**5a**).

To a suspension of **4e** (100 mg, 0.26 mmol) in acetonitrile (10 ml) was added formaldehyde (0.13 ml) followed by sodium cyanoborohydride (52 mg, 0.78 mmol). Concentrated hydrochloric acid was added dropwise until a clear solution was obtained (pH 2.0-3.0), after which the reaction mixture was stirred at room temperature for 5 minutes. The solvent was evaporated under vacuum. The residue was dissolved in water (5 ml) and neutralized (pH 7) with concentrated ammonium hydroxide. The resulting precipitate was washed with water, dispersed in methanol, silica gel (250 mg) was added and the solvent was evaporated to form a plug which was dried, loaded on top of a silica gel column (20 cm x 2.5 cm) and eluted using 2% methanol in chloroform. Fractions containing the product were pooled and solvent evaporated to afford 33 mg (32%) of **5a** as a pale yellow solid: mp 211-213 °C; ¹H NMR (DMSO-*d*₆) δ 2.63 (s, 3H, N-CH₃), 3.97 (s, 3H, 2'-OCH₃), 4.07 (s, 2H, -CH₂-N), 6.01 (bs, 2H, 4-NH₂), 6.55 (bs, 2H, 2-NH₂), 7.52 (s, 1H, 6-H), 7.29-8.08 (m, 6H, dibenzofuran).

Anal. Calcd. for C₂₁H₁₉N₅O₃•0.40 H₂O: C, 63.60; H, 5.03; N, 17.66. Found: C, 63.54; H, 4.87; N, 17.29.

2,4-Diamino-5-[*N*-(9-ethylcarbazol-3-yl)-*N*-methyl]amino]methylfuro[2,3-*d*]pyrimidine (**5b**).

To a suspension of **4f** (200 mg, 0.53 mmol) in acetonitrile (20 ml) was added formaldehyde (0.25 ml) followed by sodium cyanoborohydride (104 mg, 1.65 mmol). Concentrated hydrochloric acid was added dropwise until a clear solution was obtained (pH 2.0-3.0), after which the reaction mixture was stirred at room temperature for 5 minutes. The solvent was evaporated under vacuum. The residue was dissolved in water (5 ml) and neutralized (pH 7) with concentrated ammonium hydroxide. The resulting precipitate was washed with water, dispersed in methanol, silica gel (250 mg) was added and the solvent was evaporated to form a plug which was dried, loaded on top of a silica gel column (20 cm x 2.5 cm) and eluted using 2% methanol in chloroform. Fractions containing the product were pooled and the solvent evaporated to afford 45 mg (21%) of **5b** as a buff colored solid: mp >200 °C (dec); ¹H NMR (DMSO-*d*₆) δ 1.27 (t, 3H, -CH₂-CH₃), 2.81 (s, 3H, N-CH₃), 4.26 (s, 2H, -CH₂-N), 4.39 (q, 2H, -CH₂-CH₃), 6.06 (bs, 2H, 4-NH₂), 6.98 (bs, 2H, 2-NH₂), 7.38 (s, 1H, 6-H), 7.12-8.14 (m, 7H, carbazole).

HRMS (EI): Observed Mass 386.18551 (Calculated Mass 386.184653).

General Method for the Synthesis of Compounds **7a-f**.

The appropriate aromatic amine was slowly added to a cold solution of concentrated hydrochloric acid in crushed ice. After stirring for 5 minutes, a cold solution of sodium nitrite in water was added dropwise. The cold diazonium solution was transferred dropwise to a solution of potassium ethyl xanthate in

water kept at 40–50 °C, over a period of 30 minutes. The mixture was heated for an additional 30 minutes and cooled to room temperature. Ether (50 ml) was added and the ether layer was separated. The aqueous layer was washed with ether (2 x 25 ml), and the combined ether extracts were washed with 10% sodium hydroxide, then with water, and dried over anhydrous sodium sulfate. Ether was evaporated, and ethanol (25 ml) was added to the residue. The solution was heated to reflux, and potassium hydroxide pellets were slowly added. The mixture was heated under reflux for 8 hours. The solution was concentrated under vacuum, water (75 ml) was added, and the solution was extracted with ether (2 x 25 ml); the ether extracts were discarded. The aqueous layer was cooled in an ice bath and acidified to pH 1–2 with 6*N* sulfuric acid. The oil that separated was extracted with ether (2 x 25 ml). The ether layer was washed with water (2 x 50 ml) and dried over anhydrous sodium sulfate. Ether was removed under vacuum to afford a crude oil that contained the aromatic thiol **6a-f**.

A solution of **3**, the crude oil **6** and anhydrous potassium carbonate in anhydrous dimethylsulfoxide (1 ml/25 mg **3**) was stirred at room temperature for 2–4 hours in presence of a catalytic amount of potassium iodide (2 mole percent) under an inert nitrogen atmosphere. The reaction mixture was poured into crushed ice (2–5 g) and the resulting precipitate was filtered, washed with cold water (2 x 25 ml) and dried. The solid was dispersed in acetone, silica gel added, and the solvent evaporated to form a plug which was dried, loaded on top of a silica gel column (20 cm x 2.8 cm) and eluted using a gradient of 0–1% methanol in chloroform. Fractions containing the product were pooled and solvent evaporated to afford the products.

2,4-Diamino-5-[*S*-(biphen-2-ylthio)]methylfuro[2,3-*d*]pyrimidine (**7a**).

Compound **7a** was synthesized using 2-aminobiphenyl (5.0 g, 29.5 mmol), sodium nitrite (2.07 g, 30 mmol), potassium ethyl xanthate (7.2 g, 45 mmol) and potassium hydroxide (6.6 g, 117 mmol). The crude oil obtained (1.16 g) was reacted with **3** (500 mg, 2.51 mmol), potassium iodide (9 mg, 0.054 mmol) and anhydrous potassium carbonate (522 mg, 3.77 mmol) to afford 120 mg **7a** (14% from **3**) as a white solid: mp 186–188 °C; ¹H NMR (DMSO-*d*₆) δ 3.96 (s, 2H, -CH₂S-), 6.00 (bs, 2H, 4-NH₂), 6.57 (bs, 2H, 2-NH₂), 6.98 (s, 1H, 6-H), 7.14–7.66 (m, 9H, biphenyl).

Anal. Calcd. for C₁₉H₁₆N₄OS•0.20 H₂O: C, 64.83; H, 4.70; N, 15.92; S, 9.11. Found: C, 64.89; H, 4.67; N, 15.76; S, 9.47.

2,4-Diamino-5-[*S*-(biphen-3-ylthio)]methylfuro[2,3-*d*]pyrimidine (**7b**).

Compound **7b** was synthesized using 3-aminobiphenyl (3.0 g, 17.7 mmol), sodium nitrite (1.25 g, 18 mmol), potassium ethyl xanthate (4.32 g, 27 mmol) and potassium hydroxide (3.96 g, 70 mmol). The crude oil obtained (1.8 g) was reacted with **3** (400 mg, 2.01 mmol), potassium iodide (7 mg, 0.042 mmol) and anhydrous potassium carbonate (417 mg, 3.02 mmol) to afford 175 mg **7b** (25% from **3**) as a white solid: mp 201–202 °C; ¹H NMR (DMSO-*d*₆) δ 4.35 (s, 2H, -CH₂S-), 6.05 (bs, 2H, 4-NH₂), 6.59 (bs, 2H, 2-NH₂), 7.19 (s, 1H, 6-H), 7.30–7.62 (m, 9H, biphenyl).

Anal. Calcd. for C₁₉H₁₆N₄OS•0.20 C₂H₆SO: C, 64.01; H, 4.76; N, 15.39; S, 10.57. Found: C, 64.39; H, 4.70; N, 15.74; S, 10.81.

2,4-Diamino-5-[*S*-(biphen-4-ylthio)]methylfuro[2,3-*d*]pyrimidine (**7c**).

Compound **7c** was synthesized using 4-aminobiphenyl (1.0 g, 5.9 mmol), sodium nitrite (0.414 g, 6 mmol), potassium ethyl xanthate (1.44 g, 9 mmol) and potassium hydroxide (1.32 g, 24 mmol). The crude oil obtained (160 mg) was reacted with **3** (155 mg, 0.78 mmol), potassium iodide (3 mg, 0.018 mmol) and anhydrous potassium carbonate (162 mg, 1.17 mmol) to afford 150 mg **7c** (55% from **3**) as a white solid: mp >290 °C; ¹H NMR (DMSO-*d*₆) δ 4.33 (s, 2H, -CH₂S-), 6.05 (bs, 2H, 4-NH₂), 6.59 (bs, 2H, 2-NH₂), 7.20 (s, 1H, 6-H), 7.32–7.66 (m, 9H, biphenyl).

Anal. Calcd. for C₁₉H₁₆N₄OS•0.10 C₂H₆SO: C, 64.73; H, 4.70; N, 15.73; S, 9.90. Found: C, 64.67; H, 4.63; N, 15.64; S, 9.88.

2,4-Diamino-5-[*S*-(phenoxyphen-2-ylthio)]methylfuro[2,3-*d*]pyrimidine (**7d**).

Compound **7d** was synthesized using 2-phenoxyaniline (5.0 g, 27 mmol), sodium nitrite (1.86 g, 27 mmol), potassium ethyl xanthate (6.49 g, 40 mmol) and potassium hydroxide (6.05 g, 108 mmol). The crude oil obtained (2.9 g) was reacted with **3** (300 mg, 1.5 mmol), potassium iodide (5 mg, 0.030 mmol) and anhydrous potassium carbonate (310 mg, 2.26 mmol) to afford 105 mg **7d** (19% from **3**) as a pale yellow solid: mp 195–197 °C; ¹H NMR (DMSO-*d*₆) δ 4.24 (s, 2H, -CH₂S-), 6.04 (bs, 2H, 4-NH₂), 6.53 (bs, 2H, 2-NH₂), 7.19 (s, 1H, 6-H), 6.85–7.47 (m, 9H, phenoxyphenyl).

Anal. Calcd. for C₁₉H₁₆N₄O₂S•0.10 H₂O: C, 62.31; H, 4.46; N, 15.30; S, 8.76. Found: C, 62.14; H, 4.23; N, 15.05; S, 8.37.

2,4-Diamino-5-[*S*-(phenoxyphen-3-ylthio)]methylfuro[2,3-*d*]pyrimidine (**7e**).

Compound **7e** was synthesized using 3-phenoxyaniline (3.0 g, 16.2 mmol), sodium nitrite (1.12 g, 16.2 mmol), potassium ethyl xanthate (3.89 g, 24.2 mmol) and potassium hydroxide (3.63 g, 64.7 mmol). The crude oil obtained (1.8 g) was reacted with **3** (300 mg, 1.5 mmol), potassium iodide (5 mg, 0.030 mmol) and anhydrous potassium carbonate (310 mg, 2.25 mmol) to afford 31 mg **7e** (6% from **3**) as a pale orange solid: mp 182–183 °C; ¹H NMR (DMSO-*d*₆) δ 4.27 (s, 2H, -CH₂S-), 6.05 (bs, 2H, 4-NH₂), 6.55 (bs, 2H, 2-NH₂), 7.10 (s, 1H, 6-H), 6.81–7.40 (m, 9H, phenoxyphenyl).

Anal. Calcd. for C₁₉H₁₆N₄O₂S•0.15 C₂H₆SO: C, 61.63; H, 4.53; N, 14.89; S, 9.80. Found: C, 61.34; H, 4.15; N, 15.03; S, 9.44.

2,4-Diamino-5-[*S*-(phenoxyphen-4-ylthio)]methylfuro[2,3-*d*]pyrimidine (**7f**).

Compound **7f** was synthesized using 4-phenoxyaniline (5.0 g, 27 mmol), sodium nitrite (1.86 g, 27 mmol), potassium ethyl xanthate (6.49 g, 40.4 mmol) and potassium hydroxide (6.05 g, 108 mmol). The crude oil obtained (2.91 g) was reacted with **3** (400 mg, 2.01 mmol), potassium iodide (7 mg, 0.042 mmol) and anhydrous potassium carbonate (417 mg, 3.02 mmol) to afford 190 mg **7f** (26% from **3**) as a buff colored solid: mp 218–220 °C; ¹H NMR (DMSO-*d*₆) δ 4.21 (s, 2H, -CH₂S-), 6.03 (bs, 2H, 4-NH₂), 6.50 (bs, 2H, 2-NH₂), 7.08 (s, 1H, 6-H), 6.90–7.41 (m, 9H, phenoxyphenyl).

Anal. Calcd. for C₁₉H₁₆N₄O₂S: C, 62.62; H, 4.43; N, 15.37; S, 8.80. Found: C, 62.41; H, 4.35; N, 15.31; S, 9.05.

Acknowledgement.

This work was supported, in part, by a NIH grant from the National Institute of Allergy and Infectious Diseases AI 41743 (AG).

REFERENCES AND NOTES

- [1a] Presented in part at the 216th American Chemical Society National Meeting, Boston, MA, August 23-27, 1998; Abstr: MEDI 333.
[b] Presented in part at the 217th American Chemical Society National Meeting, Anaheim, CA, March 21-25, 1999; Abstr: MEDI 048.
- [2] H. Masur, *J. Infect. Dis.*, **161**, 858 (1990).
- [3] H. Masur, M. A. Polis, C. U. Tuazon, D. Ogata-Arakaki, J. A. Kovacs, D. Katz, D. Hilt, T Simmons, I. Feuerstein, B. Lundgren, H. C. Lane, B. A. Chabner and C. J. Allegra, *J. Infect. Dis.*, **167**, 1422 (1992).
- [4] J. R. Piper, C. A. Krauth, R. L. Carter, C. A. Hosmer, S. F. Queener, S. E. Borotz and E. R. Pfefferkorn, *J. Med. Chem.*, **39**, 1271 (1996).
- [5] A. Rosowsky, C. E. Mota, J. E. Wright and S. F. Queener, *J. Med. Chem.*, **37**, 4522 (1994).
- [6] A. Gangjee, J. Shi, S. F. Queener and R. L. Kisliuk, *J. Med. Chem.*, **36**, 3437 (1993).
- [7] A. Gangjee, A. Vasudevan, S. F. Queener and R. L. Kisliuk, *J. Med. Chem.*, **38**, 1778 (1995).
- [8] A. Rosowsky, C. E. Mota, J. E. Wright, J. E. Freisheim, J. J. Heusner, J. J. McCormack and S. F. Queener, *J. Med. Chem.*, **36**, 3103 (1993).
- [9] A. Gangjee, F. Mavandadi, S. F. Queener and J. McGuire, *J. Med. Chem.*, **38**, 2158 (1995).
- [10] A. Gangjee, R. Devraj, J. McGuire, R. L. Kisliuk, S. F. Queener and L. R. Barrows, *J. Med. Chem.*, **37**, 1169 (1994).
- [11] A. Gangjee, X. Guo, S. F. Queener, V. Cody, N. Galitsky, J. R. Luft and W. Pangborn, *J. Med. Chem.*, **41**, 1263 (1998).
- [12] J. C. Edman, U. Edman, M. Cao, B. Lundgren, J. Kovacs and D. V. Santi, *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 8625 (1989).
- [13] J. N. Champness, A. Achari, S. P. Ballantine, P. K. Bryant, C. J. Delves and D. K. Stammers, *Structure*, **2**, 915 (1994).
- [14] Tripos Associates, Inc., 1699 South Hanley Road, Suite 303, St. Louis, MO 63144.
- [15] A. Rosowsky, V. Cody, N. Galitsky, H. Fu, A. T. Papoulis and S. F. Queener, *J. Med. Chem.*, **42**, 4853 (1999).
- [16] J. A. Secrist, III and P. S. Liu, *J. Org. Chem.*, **43**, 3937 (1978).
- [17] D. S. Tarbell and K. D. Fukushima, *Org. Synth.*, **27**, 81 (1947).