# Synthesis of 2,4-Diaminopyrido[2,3-*d*]pyrimidines and 2,4-Diaminoquinazolines With Bulky Dibenz[*b,f*]azepine and Dibenzo[*a,d*]cycloheptene Substituents at the 6-Position as Inhibitors of Dihydrofolate Reductases from *Pneumocystis carinii*, *Toxoplasma gondii*, and *Mycobacterium avium* †



Andre Rosowsky* [a], Hongning Fu [a], and Sherry F. Queener [b]

[a] Dana-Farber Cancer Institute and the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115
[b] Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202

Received February 21, 2000

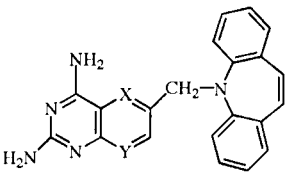
**†This paper is dedicated to the memory of Professor Raymond N. Castle, the Founding Editor of the Journal of Heterocyclic Chemistry, who passed away on August 11, 1999. Professor Castle will be missed by many as a distinguished scientist, respected teacher, and friend.**

The synthesis of four previously undescribed 2,4-diaminopyrido[2,3-*d*]pyrimidines (**3**,**4**) and 2,4-diaminoquinazolines (**5**,**6**) with a bulky tricyclic aromatic group at the 6-position is described. Condensation of dibenz[*b,f*]azepine with 2,4-diamino-6-bromomethylpyrido[2,3-*d*]pyrimidine (**8**) and 2,4-diamino-6-bromomethylquinazoline (**17**) in the presence of sodium hydride afforded *N*-[(2,4-diaminopyrido[2,3-*d*]-pyrimidin-6-yl)methyl]dibenz[*b,f*]azepine (**3**) and *N*-[(2,4-diaminoquinazolin-6-yl)methyl]dibenz[*b,f*]-azepine (**4**), respectively. Condensation of 5-chlorodibenzo[*a,d*]cycloheptene (**19**) and 5-chloro-10,11-dihydrodibenzo[*a,d*]cycloheptene (**20**) with 2,4,6-triaminoquinazoline (**13**) afforded 5-[(2,4-diaminoquinazolin-6-yl)amino]-5*H*-dibenzo[*a,d*]cycloheptene (**5**) and the corresponding 10,11-dihydro derivative (**6**), respectively. The bromides **8** and **17**, as hydrobromic acid salts, were obtained from the corresponding nitriles according to a standard three-step sequence consisting of treatment with Raney nickel in formic acid followed by reduction with sodium borohydride and bromination with dry hydrogen bromide in glacial acetic acid. Compounds **3-6** were evaluated *in vitro* for the ability to inhibit dihydrofolate reductase from *Pneumocystis carinii*, *Toxoplasma gondii*, *Mycobacterium avium*, and rat liver. Compounds **3** and **4** were potent inhibitors of all four enzymes, with $IC_{50}$ values in the 0.03-0.1 $\mu M$ range, whereas **5** was less potent. However the selectivity of all four compounds for the parasite enzymes relative to the rat enzyme was <10-fold, whereas the recently reported lead compound in this series, *N*-[(2,4-diaminopteridin-6-yl)methyl]dibenz[*b,f*]azepine (**1**) has >100-fold selectivity for the *T. gondii* and *M. avium* enzyme and 21-fold selectivity for the *P. carinii* enzyme.

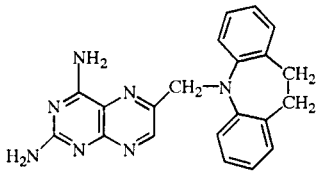
*J. Heterocyclic Chem.*, **37**, 921 (2000).

We have been engaged for several years [1-7] in the synthesis and biological evaluation of 2,4-diaminopyrimidine heterocycles as potential inhibitors of the parasitic organisms *Pneumocystis carinii*, *Toxoplasma gondii*, and *Mycobacterium avium*, all of which can produce life-threatening illness in individuals whose immune system has become compromised as a result of infection by the AIDS virus [8], or who have been subjected to immunosuppressive chemotherapy for cancer or other diseases [9]. 2,4-Diaminopyrimidines are potent inhibitors of the enzyme dihydrofolate reductase, which plays a pivotal role in the biosynthesis of purine and pyrimidine nucleotide precursors of DNA by these or other pathogenic organisms [10], and thus in the replication of such organisms in an infected host. Although it does not necessarily guarantee success *in vivo*, tight binding of a 2,4-diaminopyrimidine derivative to the enzyme of the pathogen as compared to that of the host allows potential drugs against these opportunistic pathogens to be identified rapidly and at relatively low cost [11,12]. During a recent study of 2,4-diaminopteridine analogues with a bridged diarylamine side chain [5] we chanced upon a compound (**1**) whose favorable potency and selectivity profile suggested that a selected number of structural variants of the molecule ought to be synthesized and tested. The 9,10-dihydro derivative **2** was also studied, but was less potent as well as less selective, suggesting that the nearly planar seven-membered ring of **1** may hold the key to its unusual combination of potency and target selectivity. Accordingly we set out to examine (a) whether the diaminopteridine moiety could be replaced by another 2,4-diamino heterocycle, and (b) whether the diazepinomethyl moiety could be replaced by the approximately isosteric dibenzocycloheptenylamino moiety. We thus synthesized the previously unknown 2,4-diaminopyrido[2,3-*d*]pyrimidine derivative **3** and the 2,4-diaminoquinazolines **4-6**, and evaluated their ability to inhibit dihydrofolate reductases. Compounds **3-5** may be viewed as simple analogues of **1** in which the B-ring is modified by replacement of N by

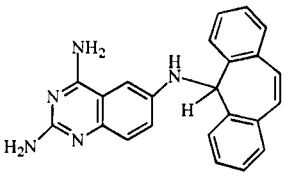
CH, whereas **6** may be viewed as an analog of **2** in which both the B-ring and the bridge are modified. To our knowledge, **1-4** are the only dibenz[*b,f*]azepine derivatives with antifolate activity thus far reported in the literature.



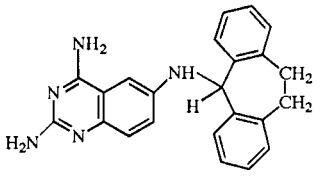
1: X = Y = N
3: X = CH, Y = N
4: X = Y = CH



2



5

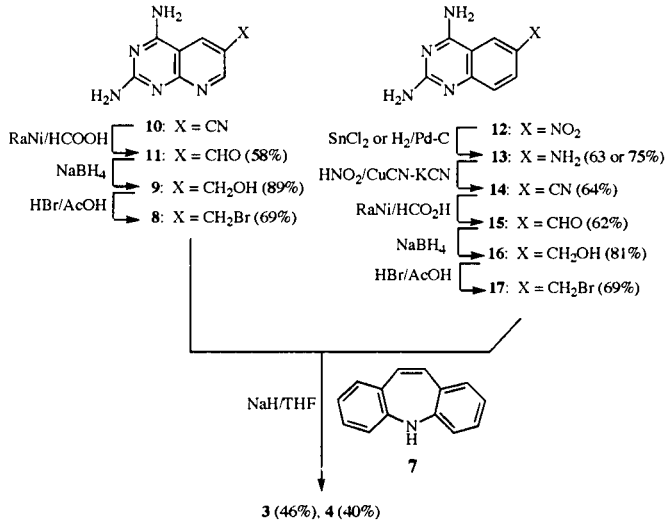


6

The synthesis of **3** was accomplished by condensing dibenz[*b,f*]azepine (iminostilbene, **7**) with 2,4-diamino-6-bromomethylpyrido[2,3-*d*]pyrimidine (**8**), whose hydrobromide salt was obtained in 82% yield from 2,4-diamino-6-hydroxymethylpyrido[2,3-*d*]pyrimidine (**9**) by treatment with dry hydrogen bromide in glacial acetic acid as described by Piper and coworkers [13]. When gaseous hydrogen bromide in this reaction was replaced by 30% aqueous hydrogen bromide the yield remained approximately the same, but when the bromination was performed in dioxane [14] instead of acetic acid, or by the older method employing triphenylphosphine-bromine in *N,N*-dimethylacetamide [15], the yield was lower (50-55%). As shown in Scheme 1, **9** was obtained *via* the published two-stage procedure [15] involving nickel-catalyzed reduction of nitrile **10** to aldehyde **11** (58% yield), followed by reduction of the aldehyde with sodium borohydride (89% yield). Nitrile **10** was prepared in four easy steps from ethyl orthoformate and malononitrile [13]. Because of their extremely low solubility, **9-11** were used as crude mixtures after confirming that each compound had the expected infrared and $^1$H-nmr spectral characteristics and showed the expected parent peak by mass spectrometry. After chromatography on flash-grade silica gel, using a methanol-chloroform gradient mixture (0 to 20% methanol) as the eluent, the yield of **3** from **8** was 46%. The product was a light-yellow solid and its $^1$H-nmr spectrum, determined in $d_6$-dimethylsulfoxide solution, showed the requisite features, including the $CH_2$ protons of the $C^9$-$N^{10}$ bridge as a singlet at δ 4.94 and the C-5 and C-7 protons of the pyrido[2,3-*d*]pyrimidine moiety as singlets at δ 8.44 and 8.68, respectively. The ten iminostilbene protons gave rise to a complex multiplet in the δ 6.98-7.26 region. Support for the structure was also provided by the mass spectrum, which featured a prominent peak with the expected mass of 367.

For the synthesis of **4** (Scheme 1), we used previously well described reactions [17,18] to reduce 2,4-diamino-6-nitroquinazoline (**12**) to amine **13** with stannous chloride (63% yield) or, more conveniently by catalytic hydrogenation in the presence of 5% palladium-carbon (75% yield), and thereupon to nitrile **14** by diazotization in the presence of potassium cyanide and copper cyanide (64% yield). Then, in a sequence that had until then been described for the corresponding quinazolines only in a meeting abstract [19], **14** was converted stepwise to aldehyde **15** (Raney nickel in 95-97% formic acid at 80°, 62% yield), alcohol **16** (sodium borohydride, 81% yield), and bromide **17** (dry hydrogen bromide in glacial acetic acid at room temperature, 69% yield). As in the case of **9-11**, conventional techniques of recrystallization or column chromatography could not be used for the purification of **15** and **16**, and we therefore relied on infrared and $^1$H-nmr spectroscopy to determine that these compounds were pure enough for direct use in the next step. The $^1$H-nmr spectrum of **17** showed the amino groups as broad singlets at δ 8.22 and 9.25, whereas the nonprotonated amino groups in alcohol **16** produced broad singlets at δ 5.52 and 7.61 in the same solvent. Not surprisingly, **17** proved to be rather unstable, even as a salt, and thus was used as soon as possible in the next step. After column chromatography on flash-grade silica gel with a methanol-chloroform gradient mixture (0 to 20% methanol) as the eluent, the isolated yield of analytically pure **4** was 40%. The $^1$H-nmr spectrum showed the expected features, including the bridge $CH_2$ as a singlet at δ 4.95, the C-5 proton of the quinazoline as a singlet at δ 8.17, and the C-7 and C-8 protons of the quinazoline as

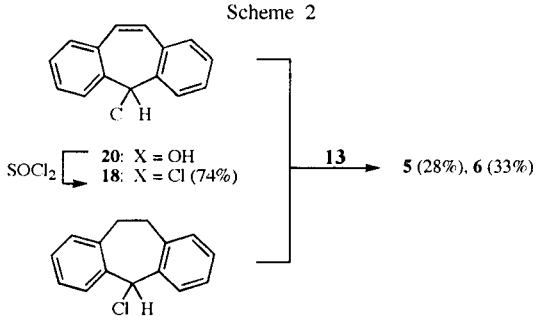
Scheme 1



doublets at δ 7.19 and 7.73 respectively. As in the spectrum of **3**, the ten iminostilbene protons produced a complex multiplet in the δ 6.83-7.24 region. Support for the structure was also provided by the mass spectrum, which featured a prominent peak with the expected mass of 366.

Initial efforts to synthesize **5** and **6** sought to start with the formation of a Schiff's base between 2,4,6-triaminoquinazoline (**13**) and 5*H*-dibenzo[*a*,*d*]cycloheptene-5-one (dibenzosuberenone) or 10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cycloheptene-5-one (dibenzosuberanone), followed by selective reduction of the CH=N bond. However a number of reaction conditions for acid-catalyzed imine formation under non-aqueous conditions were found to yield only unchanged starting materials in the case of these particular ketones, presumably because of an unfavorable combination of steric and electronic factors. Likewise unsuccessful were attempts to effect reductive coupling between the amine and the ketones in the presence of sodium cyanoborohydride. Faced with this setback, we therefore turned to an alternative approach (Scheme 2) utilizing 5-chloro-5*H*-dibenzo[*a*,*d*]cycloheptene (**18**) and 5-chloro-10,11-dihydro-5*H*-benzo[*a*,*d*]cycloheptene (**19**), the latter of which is commercially available under the name 5-chlorobenzosuberane. Chloride **18** is not commercially available, but was readily obtained in 74% yield from the commercially available alcohol **20** by reaction with thionyl chloride in refluxing toluene [20]. Treatment of **13** with

Scheme 2



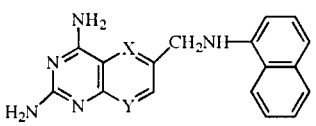

**19**

either **18** or **19** and excess triethylamine in dry tetrahydrofuran under reflux for 24 hours produced very dark solutions, which on evaporation yielded dark-brown solids. Column chromatography on silica gel with 95:5 chloroform:methanol as the eluent, followed by reprecipitation of the purified product from isopropyl alcohol or chloroform solution with ether, afforded **5** as a bright-yellow solid in 28% yield and **6** as an off-white solid in 33% yield, respectively. The $^1$H-nmr spectrum of **5**, taken in $d_6$-dimethylsulfoxide solution, showed the tertiary proton at the 5-position of the dibenzo[*a*,*d*]cycloheptene as a multiplet at δ 5.18, the C-5 proton of the quinazoline as a singlet at δ 7.44, the C-7 and C-8 protons of the quinazoline as a pair of closely spaced doublets at δ 7.71 and 7.73, the other ten protons of the 5*H*-dibenzo[*a*,*d*]cycloheptene as a complex pattern at δ 7.22-7.42, and the NH proton as a broad signal at δ 8.56. The $^1$H-nmr spectrum of **6** was similar to that of **5** except that the $CH_2CH_2$ group of the 10,11-dihydrodibenzo[*a*,*d*]cycloheptene was visible as a multiplet centered at δ 3.32, the C-5' proton was shifted upfield to δ 6.18, the integrated area for the complex aromatic multiplet at δ 7.05-7.44 was consistent with eight rather than ten protons, and the NH proton was shifted downfield to δ 8.70. An interesting feature of the spectrum of **6** was the upfield shift of the tertiary dibenzo[*a*,*d*]cycloheptene proton and the downfield shift of the NH proton in comparison with the corresponding protons in **5**.

The ability of **3-6** to inhibit the reduction of dihydrofolate to tetrahydrofolate by dihydrofolate reductase from *P. carinii*, *T. gondii*, *M. avium*, and rat liver was measured spectrophotometrically as previously described [11,12]. The concentration of each compound required to inhibit the catalytic reaction by 50%, defined as the $IC_{50}$, is shown in Table 1, along with the $IC_{50}$ values obtained previously [5] for **1**, **2**, and trimethoprim, which, when combined with a sulfa drug, is a widely prescribed lipophilic antifolate for the therapy and prophylaxis of opportunistic parasitic infections [21].

Comparison of the $IC_{50}$ values of **3** and **4** relative to **1** reveals that replacement of nitrogen by carbon at the 5- and 8-position of the diaminoheterocyclic moiety produces several fold increases in binding to *P. carinii* and rat liver dihydrofolate reductase, with much less change in binding to the *T. gondii* or *M. avium* enzyme. Thus, while they were less potent than **1**, the deaza analogs **3** and **4** are still considerably more potent than trimethoprim.

It was of interest to compare our results for the dibenz-[*b*,*f*]azepine derivatives **1**, **3**, and **4** with those reported for the corresponding 6-(1-naphthylamino)methyl derivatives **21-23** by Piper and coworkers [22]. In the latter series, the 5-deaza analog **22** was a better inhibitor of the rat enzyme than the pteridine **21**, but was a less potent inhibitor of both the *P. carinii* and *T. gondii* enzyme. In contrast, the quinazoline analog **22** was more potent than **21** against the *T. gondii* enzyme. Thus the effect of carbon for nitrogen replacement appears to be somewhat different depending on whether the side chain is a 1-naphthylamine or a bulkier dibenz[*b*,*f*]azepine moiety.




**21**: X = Y = N
**22**: X = CH, Y = N
**23**: X = Y = CH

As can be seen in Table 1, the favorable selectivity index of **1** reflects the relatively weak binding of this compound to the rat enzyme versus the *P. carinii*, *T. gondii*, or *M.*

Table 1

Dihydrofolate Reductase Inhibition by Compounds **1-6** and Other Lipophilic 2,4-Diaminopyrimidines

| | $IC_{50}$ (μM) [a] | | | | Selectivity Index [b] | | |
|---|---|---|---|---|---|---|---|
| Compound | *P. carinii* | *T. gondii* | *M. avium* | rat liver | *P. carinii* | *T. gondii* | *M. avium* |
| **1** [c] | 0.21 | 0.043 | 0.012 | 4.4 | 21 | 102 | 370 |
| **2** [c] | 1.4 | 0.91 | [d] | 5.1 | 3.6 | 5.6 | [d] |
| **3** | 0.043 | 0.040 | 0.027 | 0.19 | 4.4 | 4.8 | 7.0 |
| **4** | 0.037 | 0.034 | 0.053 | 0.053 | 1.5 | 1.6 | 1.0 |
| **5** | 13 | 5.8 | 16 | 1.5 | 0.12 | 0.26 | 0.094 |
| **6** | 0.51 | 0.13 | 2.0 | 0.019 | 0.037 | 0.15 | 0.0095 |
| Trimethoprim [c] | 12 | 2.7 | 0.19 | 130 | 11 | 48 | 680 |

[a] Enzyme activity was determined according to a standardized method which has been reliably in use in this program for a number of years. Each concentration of drug was tested in triplicate. As an example of the reproducibility of the assay, the $IC_{50}$ values (mean ± standard error) in Dr. Queener's laboratory over a five-year period using pyrimethamine and partially purified dihydrofolate reductase from *P. carinii*, *T. gondii*, and rat liver have been 2.39 ± 0.42, 0.50 ± 0.23, and 1.52 ± 0.32 μM, respectively. [b] Selectivity Index = $IC_{50}$ (rat liver)/$IC_{50}$ (*P. carinii*, *T. gondii*, or *M. avium*). [c] Data from reference 5. [d] Not determined.

*avium* enzyme. Unfortunately, however, it was also the rat enzyme against which the greatest increase in *potency* was observed upon carbon for nitrogen replacement in the B-ring. As a result, whereas the selectivity index of **1** against *P. carinii*, *T. gondii*, and *M. avium* dihydrofolate reductase was 21, 102, and 370, respectively, these values decreased to 4.4, 4.8, and 7.0 in the case of **3**, and decreased to <2 in the case of **4**. A decrease in selectivity was similarly reported by Piper and coworkers for various pteridine, 5-deazapteridine, and 5,8-dideazapteridine derivatives containing a bulky 6-(1-naphthylaminomethyl), 6-(1-naphthylthio), or 6-[2-(1-naphthyl)ethyl] side chain [13]. Thus, our results support their conclusion that carbon for nitrogen replacement at the 5- and 8-position in dihydrofolate reductase inhibitors by a bulky arylaminomethyl group at the 6-position tends to enhance potency but has the opposite effect on selectivity against the non-mammalian enzymes.

Comparison of the $IC_{50}$ values of **5** versus those of **1** revealed that the structural modification embodied in **5** is unfavorable in terms of both potency and selectivity. Moreover, when the results for **5** and **6** are compared (Table 1), it can be seen that the potency of the latter is greater against all four reductases, but is particularly enhanced against the rat enzyme. As a result, **6** is the least selective of the four compounds in this group. The unfavorable selectivity index of **6** may be due to the shortness of the bridge between the two halves of the molecule and the fact that the phenyl rings in the dibenzosuberane moiety exists in a twisted configuration that does not allow the molecule to make optimal hydrophobic contacts to residues lining the active site of the enzyme from the three parasites. The data we have obtained to date with compounds **1-6** suggests that the synthesis of analogs in which the pteridine moiety is retained but the dibenz[*b,f*]azepine moiety is substituted in one or both of the phenyl rings would be of interest.

## EXPERIMENTAL

Infrared spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer, but for the sake of brevity only peaks with wave numbers greater than 1300 $cm^{-1}$ are reported. The $^1$H-nmr spectra were recorded at 60 MHz on a Varian EM360 instrument with tetramethylsilane as the reference or at 500 MHz on a Varian VX500 instrument. Mass spectra were obtained by the Molecular Biology Core Facility, Dana-Farber Cancer Institute, in the electron impact (EI) or fast atom bombardment (FAB) mode. Analytical thin layer chromatography (tlc) was on Whatman MK6F silica gel slides (60 μm layer, 1.5 x 4.5 cm, with fluorescent indicator), using 254-nm illumination to visualize the spots. Preparative separations were on Aldrich silica gel plates (1000 μm layer, 20 x 20 cm, with fluorescent indicator). Column chromatography was on Baker 7024 flash silica gel (40 μm particle size). The hydrobromic acid salt of 2,4-diamino-6-bromomethylpyrido[2,3-*d*]pyrimidine (**8**) was synthesized from nitrile **10** *via* aldehyde **11** and alcohol **9** as described [16]. 2,4-Diamino-6-quinazoline-6-carbonitrile (**14**) was synthesized from 2,4-diamino-6-nitroquinazoline (**12**) *via* the triamine **13** as described [17-19]. To simplify stoichiometric calculations in their reactions with **7**, **18**, or **19**, the 6-bromomethyl derivatives **8** and **17** were assumed to be dihydrobromide salts, though excess sodium hydride was used in order to take into account the high probability of residual acetic acid in the sample [16,22]. Because of their sensitivity to moisture, the bromides were used as soon as possible in the next step. 5-Chloro-5*H*-dibenzo[*a,d*]cycloheptene (**18**) was obtained in 74% yield by adding excess thionyl chloride dropwise to a stirred solution of the alcohol **20** in dry toluene, and refluxing overnight [20]. After recrystallization from ether, **18** had a melting point of 120-122° (lit. 123-125°) [20]. Sodium hydride (dry powder, 95%) was purchased from Aldrich (Milwaukee, WI), and used without delay. Other chemicals, as well as solvents ('Sure-Seal' grade) for moisture sensitive reactions, were likewise from Aldrich. Melting points were determined in Pyrex capillary tubes using a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Elemental analyses were performed by QTI Laboratories, Whitehouse, NJ, or Robertson Laboratories, Madison, NJ, and were within ±0.4% of theoretical values unless

otherwise indicated. Despite being dried overnight under vacuum in an Abderhalden apparatus at 60°, analytical samples of the products consistently retained small amounts of chloroform and methanol as judged from their microanalytical data and $^1$H-nmr spectra. Tenacious retention of organic solvents has previously been observed by us in other lipophilic compounds of the general type reported here [23].

*N*-[(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]dibenz[*b*,*f*]-azepine (**3**).

Sodium hydride powder (55 mg, 2.3 mmol) was added to a stirred solution of 114 mg (0.59 mmol) of dibenz[*b*,*f*]azepine (**7**) in dry tetrahydrofuran (10 mL) at 5°. After 20 minutes, a solution of the hydrobromic acid salt of **8** (245 mg, 0.59 mmol, based on assumed dihydrobromide formula) in dry *N*,*N*-dimethylacetamide (5 mL) was added to the mixture, and the reaction flask was sealed under a nitrogen balloon and stirred at room temperature for 2 days. Methanol (3 mL) containing 3 drops of glacial acetic acid was then added, and the mixture was concentrated to dryness by rotary evaporation. The residue was suspended in water (40 mL) and the product was extracted with 85:15 chloroform:methanol (5 x 100 mL). The combined extracts were dried over magnesium sulfate and evaporated under reduced pressure to obtain a yellow solid, which was purified by chromatography on a column of flash grade silica gel (60 g). The column was eluted with chloroform containing a gradually increasing amount of methanol until the final concentration of the latter was 20%. Fractions giving a single tlc spot with $R_f$ 0.24 (silica gel, 4:1 chloroform-methanol) were pooled and evaporated to obtain a yellow solid (110 mg, 48%), mp 238° dec; ir (potassium bromide) ν 3420, 3180, 1630, 1610, 1570, 1545, 1475, 1440, 1390, 1350 cm$^{-1}$; ms: theory for $C_{22}H_{19}N_6$ ($MH^+$), m/z 367.167; found, 367.436; $^1$H nmr ($d_6$-dimethylsulfoxide) δ 4.94 (s, 2H, $CH_2$), 6.82 (s, 2H, CH=CH), 6.98 (t, 2H, aromatic 3'-H), 7.10 (d, 2H, aromatic 1'-H), 7.21 (d, 2H, aromatic 4'-H), 7.26 (t, 2H, aromatic 2'-H), 8.44 (s, 1H, 5-H), 8.68 (s, 1H, 7-H).

*Anal.* Calcd. for $C_{22}H_{18}N_6 \cdot 0.5CH_3OH \cdot 0.2CHCl_3$: C, 67.10; H, 5.01; N, 20.68. Found: C, 66.81; H, 5.08; N, 20.44.

*N*-[(2,4-Diaminoquinazolin-6-yl)methyl]dibenz[*b*,*f*]azepine (**4**).

Step 1. A solution of 1.1 g (6.0 mmol) of **14** in 25 mL of 95-97% formic acid was added in a thin stream with mechanical stirring to 7 g of wet Raney nickel (50% content), and the mixture was stirred for 1.5 hours in a heating bath at 80°. The catalyst was filtered and washed with several portions (15 mL each) of warm formic acid until all the color was removed. The combined filtrate and washes were concentrated to dryness on a rotary evaporator, and final traces of formic acid were removed by repetitive co-evaporation with 5 mL of ethanol. The residue was dissolved in hot water (100 mL), the solution was decolorized with charcoal (Norit) and filtered through Celite. The orange filtrate was neutralized to pH 7 with 2 *N* sodium hydroxide to obtain 2,4-diaminoquinazoline-6-carboxaldehyde (**15**) as a yellowish-brown solid which was used without additional manipulation for the next step (680 mg, 62%), mp 239° dec; ms: theory for $C_9H_9N_4O$ ($MH^+$), m/z 189.085; found, 189.304; $^1$H-nmr ($d_6$-dimethylsulfoxide) δ 6.68 (s, 2H, 2-$NH_2$), 7.30 (d, 1H, 8-H), 7.75 (s, 2H, 4-$NH_2$), 7.96 (d, 1H, 7-H), 8.67 (s, 1H, 5-H), 9.92 (s, 1H, CH=O).

Step 2. A stirred suspension of 1.2 g (6.4 mmol) of **15** in 220 mL of methanol was treated at room temperature with 240 mg (6.3 mmol) sodium borohydride in small portions over a period of 10 minutes. Stirring was continued for 2 hours, after which a second identical portion of sodium borohydride was added, and the mixture was left to stir at room temperature overnight. A third identical portion of sodium borohydride was then added, and stirring was resumed for a final 2 hours, after which the solvent was evaporated and the residue was stirred with 60 mL of water and adjusted from pH 11 to pH 8 by dropwise addition of 2 *N* hydrochloric acid. The mixture was then stirred in a heating bath at 75° for 20 minutes, and the solid was filtered and washed with 15 mL of ethanol followed by 30 mL of ether to obtain **16** as a yellow solid which was used directly in the next step (976 mg, 81%), mp 266° dec; ms: theory for $C_9H_{10}N_4O$ ($MH^+$), m/e 190.085; found, 190.240; $^1$H-nmr ($d_6$-dimethylsulfoxide) δ 4.63 (s, 2H, $CH_2O$), 5.52 (s, 2H, 2-$NH_2$), 7.45 (d, 1H, 7- or 8-H), 7.61 (s, 2H, 4-$NH_2$),7.70 (s, 1H, 5-H), 8.25 (d, 1H, 7- or 8-H).

Step 3. Method A. A suspension of 1.4 g (7.4 mmol) of **16** (previously dried *in vacuo* overnight at 100° over $P_2O_5$) in 67 mL of glacial acetic acid was kept at 95° until all the solid dissolved, and was then cooled to room temperature and stirred while a 30% solution of hydrogen bromide in glacial acetic acid (133 mL) was added dropwise. When addition was complete, a clear yellow solution remained. The flask was tightly closed and left to stand at room temperature for 48 hours. The reaction mixture was then added dropwise with stirring to ice-cold ether (733 mL). The precipitate was collected by suction filtration under a gentle stream of nitrogen, and the solid was washed with ether and dried *in vacuo* to obtain the hydrobromic acid salt of 2,4-diamino-6-bromomethylquinazoline (**17**) as a yellowish-brown solid which was used as soon as possible for the next reaction (2.14 g, 69% based on dihydrobromide structure), mp 183° dec; ms: theory for $C_9H_9BrN_4$ ($MH^+$) m/z 253.009; found, 253.103, 254.227; $^1$H-nmr ($d_6$-dimethylsulfoxide) δ 4.91 (s, 2H, $CH_2Br$), 8.22 (s, 2H, 2-$NH_2$), 8.92 (d, 1H, 7- or 8-H), 8.98 (s, 1H, 5-H), 9.03 (d, 1H, 7- or 8-H), 9.25 (s, 2H, $NH_2$). Protonation of the amino groups could not be discerned because of rapid exchange.

Method B. Dry hydrogen bromide gas was bubbled slowly for 30 minutes into a stirred suspension of 1.3 g (6.8 mmol) of **16** in 100 mL of dry dioxane kept at 25° by means of a cold water bath. The flask was tightly closed, and the mixture was stirred at room temperature overnight. A small amount of insoluble material was removed by filtration, and the filtrate was added dropwise into 500 ml of ether to obtain a brilliant-yellow solid. After being kept overnight at 4°, the mixture was filtered to obtain a brownish-yellow solid suitable for use for the next reaction (1.6 g, 55% yield).

Method C. A solution of 7.2 g (27.4 mmol) of triphenylphosphine in 20 mL of anhydrous *N*,*N*-dimethylacetamide was cooled to 0-5° and stirred while bromine (4.4 g, 27.4 mmol) was added dropwise over 10 minutes. This was followed by addition of 1.3 g (6.8 mmol) of **16** and stirring at room temperature for 4 hours. The clear red solution was treated with 70 mL of benzene to produce a reddish-yellow precipitate. The clear liquid phase was decanted, and the solid was stirred with 100 mL of benzene followed by 100 mL of ether. The crude solid was dissolved in 4 mL of 48% hydrobromic acid at room temperature, and the clear red solution was added dropwise with stirring to 300 mL of ether. The mixture was stored at 0-4° overnight and filtered to obtain a yellowish-brown solid (1.2 g, 51%). Although the product gradually softened and darkened when exposed to air, it was suitable for use in the next step.

Step 4. The same procedure as in the synthesis of **3** was carried out, using 192 mg (0.96 mmol) of **7**, 110 mg (4.6 mmol) of sodium hydride powder, 15 mL of tetrahydrofuran, and the crude hydrobromic acid salt of **17** (390 mg, 0.94 mmol based on

assumed dihydrobromide formula) in 5 mL of *N,N*-dimethylacetamide. The product after flash chromatography on silica gel (60 g) was a yellow solid (185 mg, 40%), mp 221° dec; tlc: $R_f$ 0.21 (silica gel, 4:1 chloroform:methanol); ir (potassium bromide) ν 3410, 3190, 1635, 1610, 1590, 1525, 1480, 1465, 1380 $cm^{-1}$; ms: theory for $C_{23}H_{20}N_5$ ($MH^+$) m/z 366.1712; found, 366.376; $^1H$ nmr ($d_6$-dimethylsulfoxide) δ 4.95 (s, 2H, $CH_2$), 6.83 (s, 2H, CH=CH), 6.97 (t, 2H, aromatic 3'-H), 7.11 (d, 2H, aromatic 1'-H), 7.17 (d, 2H, aromatic 4'-H), 7.19 (d, 1H, 7- or 8-H), 7.24 (t, 1H, aromatic 2'-H), 7.73 (d, 1H, 7- or 8-H), 8.17 (s, 1H, 5-H).

*Anal.* Calcd. for $C_{23}H_{19}N_5 \cdot CH_3OH \cdot 0.8CHCl_3$: C, 60.42; H, 4.87; N, 14.21. Found: C, 60.15; H, 5.23; N, 14.20.

5-[(2,4-Diaminoquinazolin-6-yl)amino]-5*H*-dibenzo[*a,d*]-cycloheptene (**5**).

A mixture of 1.0 g (4.4 mmoles) of 5-chloro-5*H*-dibenzo[*a,d*]-cycloheptene (**18**), 0.85 g (5.0 mmol) of **13**, and 1.5 mL (0.89 g, 8.8 mmol) of triethylamine in dry tetrahydrofuran (15 mL) was refluxed in a 90° bath for 24 hours. Evaporation under reduced pressure afforded a dark-brown solid, which was chromatographed on silica gel (40 g, 9:1 chloroform-methanol) followed by successive reprecipitation from isopropanol-ether and chloroform-ether mixtures afforded bright-yellow powder (0.56 g, 28%), mp 235° dec; tlc: $R_f$ 0.17 (silica gel, 4:1 chloroform-methanol); ms: theory for $C_{23}H_{20}N_5$ ($MH^+$) m/z 366.449; found 366.376; ir (potassium bromide) ν 3420, 3320, 3190, 3140, 1665, 1640, 1630, 1625, 1585, 1530, 1490, 1410, 1355 $cm^{-1}$; $^1H$-nmr ($d_6$-dimethylsulfoxide): δ 5.18 (d, 1H, dibenzocycloheptene 5-H), 7.22 (s, 2H, CH=CH), 7.25-7.42 (m, 8H, dibenzocycloheptene aromatic protons), 7.44 (s, 1H, quinazoline 5-H), 7.71 (d, 1H, quinazoline 7-H or 8-H), 7.73 (d, 1H, quinazoline 8-H), 8.56 (broad signal, 1H, NH).

*Anal.* Calcd. for $C_{23}H_{19}N_5 \cdot 0.4CHCl_3 \cdot 0.5CH_3OH$: C, 66.88; H, 5.03; N, 16.32. Found: C, 66.69; H, 5.06; N, 16.59.

5-[(2,4-Diaminoquinazolin-6-yl)amino]-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene (**6**).

The same procedure as in the synthesis of **5** was carried out, using 1.0 g (4.4 mmol) of 5-chloro-10,11-dihydro-5*H*-dibenzo-[*a,d*]cycloheptene (**19**), 0.85 g (5.0 mmol) of **13**, and 1.5 mL (0.89 g, 8.8 mmol) of triethylamine in tetrahydrofuran (15 mL). Evaporation under reduced pressure afforded a dark-brown solid, which was chromatographed on silica gel (45 g) with 4:1 chloroform-methanol as the eluent. Appropriately pooled fractions were evaporated, the residue was dissolved in warm chloroform (75 mL), and the solution was added dropwise to ether to obtain an off-white solid (0.64 g, 33%), mp 233° dec; tlc: $R_f$ 0.21 (silica, 4:1 chloroform-methanol); ms: theory for $C_{23}H_{21}N_5$ ($M^+$) and $C_{23}H_{22}N_5$ ($MH^+$) m/z 367.457 and 368.443; found, 367.433 and 368.443; ir (potassium bromide) ν 3420, 3320, 3190, 2925, 1655, 1650, 1630, 1585, 1535, 1480, 1410, 1310 $cm^{-1}$; $^1H$-nmr ($d_6$-dimethylsulfoxide): δ 3.21-3.43 (m, 4H, $CH_2CH_2$), 6.18 (d, 1H, dibenzocycloheptene 5-H), 7.05-7.44 (m, 8H, dibenzocycloheptene aromatic protons), 7.52 (s, 1H, quinazoline 5-H), 7.54 (d, 1H, quinazoline 7- or 8-H), 7.56 (d, 1H, quinazoline 7- or 8-H), 8.70 (broad signal, 1H, NH).

*Anal.* Calcd. for $C_{23}H_{21}N_5 \cdot 0.7CH_3OH \cdot 0.3CHCl_3$: C, 67.72; H, 5.71; N, 16.45. Found: C, 67.44; H, 5.42; N, 16.60.

Acknowledgement.

This work was supported in part by grant RO1-AI29904 from the National Institute of Allergy and Infectious Diseases, Department of Health and Human Services. Helpful discussions with Drs. Vivian Cody and Nikolai Galitsky, Hauptman-Woodward Institute, Buffalo, NY, are grateful acknowledged.

REFERENCES AND NOTES

[1] A. Rosowsky, C. E. Mota, and S. F. Queener, *J. Heterocyclic Chem.*, **33**, 1959 (1996); see also earlier papers cited in this reference.

[2] A. Rosowsky, A. T. Papoulis, and S. F. Queener, *J. Med. Chem.*, **40**, 3694 (1997).

[3] A. Rosowsky, R. A. Forsch, and S. F. Queener, *Pteridines*, **8**, 173 (1997).

[4] A. Rosowsky, A. T. Papoulis, and S. F. Queener, *J. Med. Chem.*, **41**, 913 (1998).

[5] A. Rosowsky, A. T. Papoulis, R. A. Forsch, and R. F. Queener, *J. Med. Chem.*, **42**, 1007 (1999).

[6] A. Rosowsky, A. T. Papoulis, and S. F. Queener, *J. Heterocyclic Chem.*, **36**, 723 (1999).

[7] A. Rosowsky, V. Cody, N. Galitsky, H. Fu, A. T. Papoulis, and S. F. Queener, *J. Med. Chem.*, **42**, 4853 (1999).

[8] For a recent review of this major clinical problem in AIDS management, see: M. E. Klepser and T. B. Klepser, *Drugs*, **53**, 40 (1997).

[9] I. Vaarthalitis and F. Meunier, *Cancer Treatment Revs.*, **19**, 387 (1993).

[10] B. I. Schweitzer, Dicker, A. P., and J. R. Bertino, *FASEB J.*, **4**, 2441 (1990).

[11] M. C. Broughton and S. F. Queener, *Antimicrob. Agents Chemother.*, **35**, 1348 (1991).

[12] L.-C. Chio and S. F. Queener, *Antimicrob. Agents Chemother.*, **37**, 1914 (1993).

[13] J. R. Piper, C. A. Johnson, J. A. Maddry, N. D. Malik, J. J. McGuire, G. M. Otter, and F. M. Sirotnak, *J. Med. Chem.*, **36**, 4161 (1993).

[14] J. R. Piper, N. D. Malik, M. S. Rhee, J. Galivan, and F. M. Sirotnak, *J. Med. Chem.*, **35**, 332 (1992).

[15] J. R. Piper and J. A. Montgomery, *J. Org. Chem.*, **42**, 208 (1977).

[16] J. R. Piper, G. S. McCaleb, J. A. Montgomery, R. L. Kisliuk, Y. Gaumont, and F. M. Sirotnak, *J. Med. Chem.*, **29**, 1080 (1986).

[17] J. Davoll, and A. M. Johnson, *J. Chem. Soc.* (C), 997 (1970).

[18] E. F. Elslager and J. Davoll, Lectures in Heterocyclic Chemistry, R. N. Castle and L. B. Townsend, eds, Hetero Corporation, Orem, Utah, 1974, S-97.

[19] M. Graffner-Nordberg, K. Sjödin, A. Tunek, and A. Hallberg, Chemistry and Biology of Pteridines and Folates 1997, W. Pfleiderer and H. Rokos, eds, Blackwell Science, Berlin, 1997, p. 225.

[20] G. Berti, *J. Org. Chem.*, **22**, 230 (1957).

[21] J A. Fishman, *Antimicrob. Agents Chemother.*, **42**, 1309-1314.

[22] J. R. Piper, C. A. Johnson, 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).

[23] A. Rosowsky, C. E. Mota, and S. F. Queener, *J. Heterocyclic Chem.*, **32**, 335 (1995).