94. 5-(*N*-Arylnortropan-3-yl)- and 5-(*N*-Arylpiperidin-4-yl)-2,4-diaminopyrimidines. Novel Inhibitors of Dihydrofolate Reductase

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(28.IV.86)

Based on a computer-assisted analysis of the three-dimensional structure of the binary complex of *E. coli* dihydrofolate reductase (DHFR) with methotrexate, 5-(*N*-arylnortropan-3-yl)- and 5-(*N*-arylpiperidin-4-yl)-2,4-diaminopyrimidines **2** and **4** were designed as inhibitors of DHFR. Syntheses of the designed compounds have been carried out. The most potent compound **2a** inhibited *E. coli* DHFR with $K_i = 0.49 \cdot 10^{-9}$M. The activities within the series of compounds synthesized could be rationalized by molecular-modelling experiments which served as the basis of this work. Several compounds within the presented series exhibit antimalarial activities *in vitro* and *in vivo*.

1. Introduction. – Dihydrofolate reductase (DHFR E.C. 1.5.1.3), an essential enzyme in all living organisms, has been studied extensively [1]. Especially in the last few years, more detailed insight into the structure and the mode of action of this enzyme has been gained [2], in particular through the X-ray determination of the structures of the enzymes isolated from *E. coli* [3], *L. casei* [3], and most recently from chicken liver [4].

The best known inhibitors of DHFR's include methotrexate (MTX, **1**), trimethoprim, and pyrimethamine [1], which to a different extent discriminate between DHFR's from eucaryotic and procaryotic organisms. Thus, MTX is used in cancer chemotherapy, trimethoprim is a broad-spectrum antibacterial, whereas pyrimethamine is useful in prophylaxis and treatment of malaria.

The three-dimensional structure of the enzymes from *E. coli* and *L. casei* became available at the same time as molecular graphics emerged as a new tool in drug design. Together they allowed a new approach to the design of novel inhibitors of dihydrofolate reductase [5]. An other example of such an approach leading to new inhibitors and their biological testing is discussed below.

2. Design of the Target Compounds. – Both the single-crystal X-ray data sets of the binary complex (*E. coli* DHFR)-MTX [6] and the ternary complex (*L. casei* DHFR)-MTX-NADPH [4] were available from the *Protein Data Bank* at Brookhaven National Laboratory [7]. Although data from both complexes were used throughout this work, the discussion below is restricted to the analysis of the modelled complexes of the designed inhibitors with *E. coli* DHFR.

Scheme 1

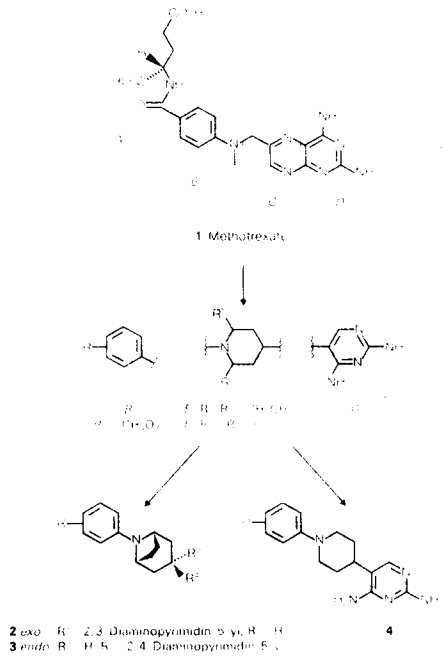


Fig. 1. *Stereoview of* **1** *in the active site of* E. coli *DHFR*

As starting point for the design of new DHFR inhibitors, we chose the structure of MTX (**1**; *Scheme 1*) and information on its conformation when bound in the active site of the enzyme in the above-mentioned complexes (*Fig. 1*). Being aware of the crucial contribution of the pyrimidine portion *D* of the pteridine moiety of MTX (**1**) to the DHFR binding, we decided to integrate it into the compounds to be designed. Further,

we chose to preserve the phenyl ring *B* of MTX and to delete the pyrazine moiety *C*. Though aware of its importance for the MTX binding [1], we disregarded the glutamate side-chain *A* and, instead, varied substitution of the phenyl ring *B*. The two structural segments *B'* and *D'* were then to be joined by an appropriately designed 'spacer'. It was our objective that this spacer should allow the rings *B'* and *D'* to occupy the same positions in the active site of the enzyme studied as are taken by the corresponding structural elements of MTX in the complexed state. From among the many possibilities, nortropane and piperidine spacers *E* and *F*, respectively, were chosen as linking units, leading to compounds **2–4** (*Scheme 1*).

The latter molecules were then matched to MTX in the conformation found in the complex with *E. coli* DHFR. The matching was accomplished by superimposing the pyrimidine rings of compounds **2–4** to that of MTX, and the best match for the phenyl ring was looked for. Whereas a reasonable match of the phenyl rings of MTX and the *endo*-compounds **3** could not be achieved, almost perfect match was readily accomplished with the *exo*-isomers **2**, in which the diaminopyrimidine ring is in an equatorial orientation and the phenyl ring adopts an axial conformation (see *Fig. 2* for **1** and **2a**).

The designed compounds **2** in the conformation deduced from the aforementioned matching experiment were then docked into the active site of the *E. coli* DHFR in such a way, that the 2,4-diaminopyrimidine ring of **2** occupied the same position as the 2,4-diaminopyrimidine portion of the pteridine moiety of MTX in the same complex. Then, the interactions with the surrounding protein were examined both with the parent compound

Fig. 2. *Methotrexate* (**1**) (red) *in conformation found in the active site of* E. coli [3] *matched to* **2a** (black)

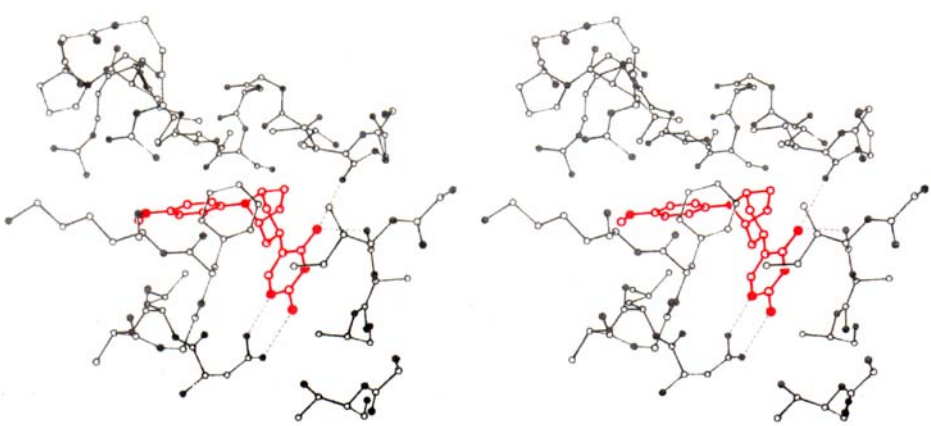


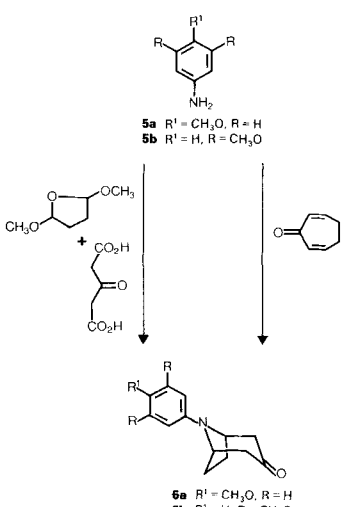
Fig. 3. ***Stereoview of* 2a *in the active site of* E. coli *DHFR***

2 (R = H) and its methoxy-substituted derivatives[1]) in order to identify any potential non-bonding interactions with the protein. The designed target compounds **2** could be accommodated in the active site of the enzyme without any serious collision with the protein. Only two minor contacts with the enzyme were observed, that of the benzene ring with the side chain of Leu-28 and another of the CH_2 group in the pyrrolidine portion of the nortropane moiety with Ile-50 *(Fig. 3)*. The piperidines **4** being conformationally flexible can adopt, like the *exo*-nortropanes **2**, a conformation which mimics the position of the diaminopyrimidine and phenyl rings in MTX (**1**), but due to lack of the ethylene bridge as compared with **2**, they are devoid of any collision with the protein.

Considering the outcome of our modelling, matching, and docking studies, we anticipated that the *endo*-nortropanes **3** would be essentially devoid of inhibitory activities on DHFR's. Their *exo*-isomers **2**, however, should fulfill the requirement for being potent DHFR inhibitors, although it was rather difficult to predict the impact of the minor interactions with the protein on their binding strength. On the other hand, the binding of the piperidines **4**, while avoiding unwanted contacts with the enzyme, may suffer from loss of entropy due to their conformational flexibility, resulting in a weaker binding to the enzyme. The importance of these parameters for the binding can be conveniently quantified by determining the IC_{50} and K_i values of the compounds in question on different DHFR's. These data could contribute to our knowledge of the steric requirements for DHFR inhibition and establish the predictive value of molecular-modelling experiments. Toward this end, the synthesis of the nortropanes **2** and **3** as well as of the piperidines **4** was carried out and the inhibitory activity determined.

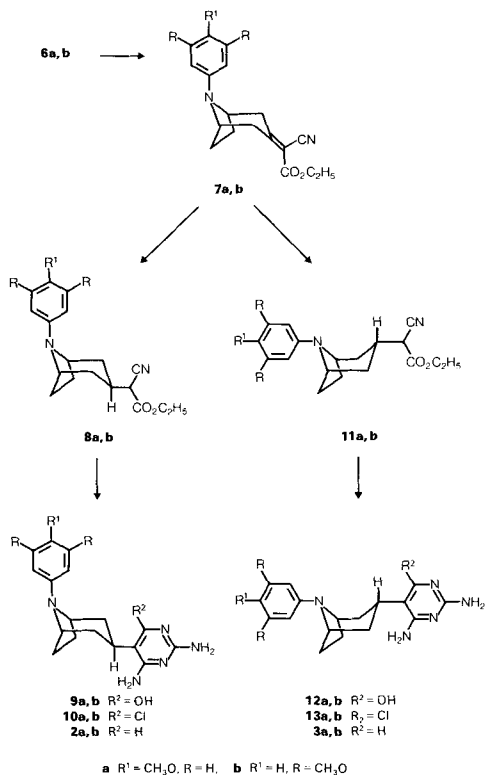
3. Synthesis of 2 and 3. – The nortropanones **6** can be prepared either from cyclohepta-2,6-dien-1-one [8] and the corresponding anilines **5** [9], or, more efficiently, by a

Scheme 2



[1]) A variety of other substituents and substitution patterns on the phenyl ring was scrutinized in the same way and, subsequently, synthesized. In this paper, we have restricted our discussion to the 4-methoxy and 3,5-dimethoxy derivatives.

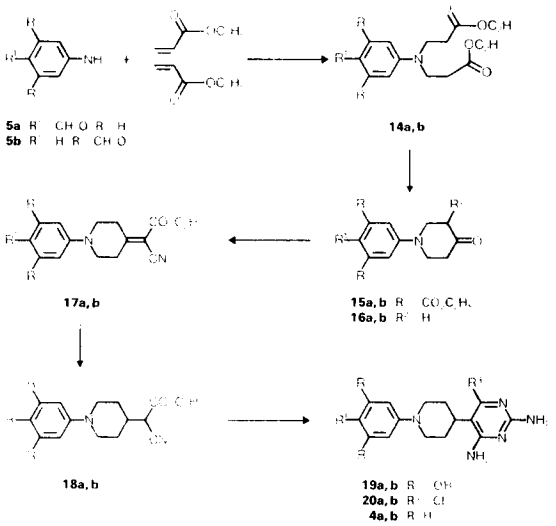
Scheme 3



Mannich reaction of **5**, 3-oxoglutaric acid, and 2,5-dimethoxytetrahydrofuran in dioxane/H_2O *(Scheme 2)*. By this convenient one-pot procedure, the ketone **6b** was prepared in up to 40% yield from readily available starting materials. *Knoevenagel* condensation of the ketones **6** with ethyl cyanoacetate afforded the olefins **7** *(Scheme 3;* for yields, see *Exper. Part, Table 3)*, which, on catalytic hydrogenation over Pd/C, yielded almost exclusively the *endo*-isomers **11**. Li in liquid NH_3 with EtOH as a proton source reduced **7** in a highly stereoselective manner to the desired *exo*-derivatives **8** in *ca.* 30–35% yield, while starting material was regenerated after usual workup. When phenol was added as a proton donor, **8** was obtained in 71% yield.

The condensation of **8** and **11** with guanidine yielded the diaminopyrimidinones **9** and **12**, respectively. Chlorination of the latter with $POCl_3$ in the presence of *N,N*-dimethylaniline afforded the chloro derivatives **10** and **13**, which were catalytically reduced to the target compounds **2** and **3**, respectively. The structures of **2a** and **3a** (R = H, $R^1 = CH_3O$, $R^2 = H$) were unambiguously assigned by single-crystal X-ray analyses (see below, *Fig. 4* and *5*). The *exo*-isomer **2a** has – at least in the solid state – the conformation (phenyl ring in an axial orientation) anticipated and used in the matching experiment at the outset of our study.

Scheme 4



4. Synthesis of 4. The required *N*-phenyl-4-piperidones **16** were prepared from anilines **5** and ethyl acrylate (→**14**), followed by *Dieckmann* condensation and decarboxylation (*Scheme 4*). Basic ester hydrolysis of **15** resulted in smooth decarboxylation leading to **16**. The subsequent reaction steps from **16** comprising *Knoevenagel* condensation (→**17**), catalytic hydrogenation (→**18**), condensation with guanidine (→**19**), chlorination (→**20**), and removal of the Cl-atom were carried out in close analogy to the same reactions in the nortropane series, yielding the target compounds **4** (for yields, see *Exper. Part, Table 4*).

5. X-Ray Structure Analysis of the *exo*- and *endo*-Configurated 2,4-Diamino-5-[8-(4-methoxyphenyl)-8-azabicyclo[3.2.1]oct-3-yl]pyrimidines 2a and 3a, Respectively. The crystal and molecular structures of **2a** and of **3a** have been determined by single-crystal X-ray structure analysis. All calculations were carried out with the

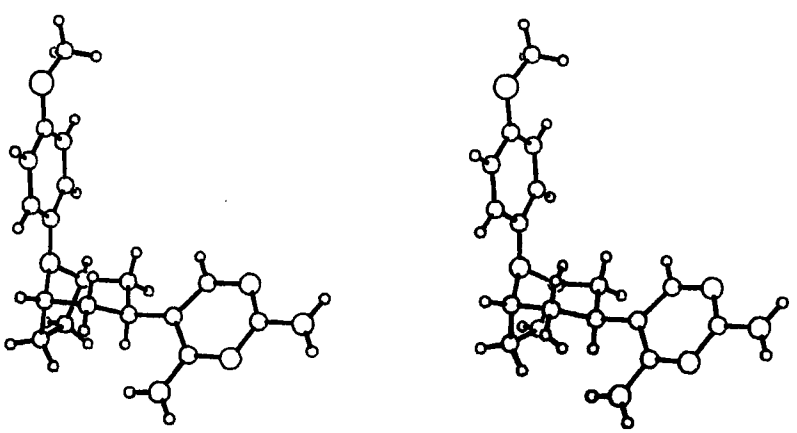


Fig. 4. *Stereoscopic drawing of* **2a**

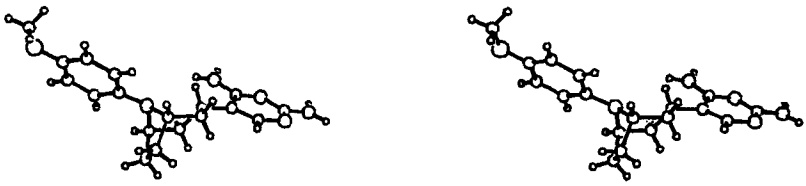


Fig. 5. *Stereoscopic drawing of* **3a**

SHELXTL [10] package of the *Nicolet R3m* diffractometer system. Stereoscopic drawings of **2a** and **3a** are shown in *Figs. 4* and 5[2]).

6. Biological Results and Discussion. – The target compounds **2**–**4** and the reference compounds MTX(**1**) and pyrimethamine were tested for their inhibitory activities on DHFR's isolated from *L. casei*, *E. coli*, and rat liver *(Table 1)*. The purification of DHFR's and the determination of IC_{50} and K_i were carried out as described previously [11].

Table 1. *Inhibition of Dihydrofolate Reductase* (DHFR)

	Dihydrofolate reductase from						
	E. coli		*L. casei*		Rat liver		Q^a)
	IC_{50} [nM]	K_i [nM]	IC_{50} [nM]	K_i [nM]	IC_{50} [nM]	K_i [nM]	
2a	0.095	4.99	3.8	210.8	0.09	3.90	0.95
2b	0.0046	0.49	3.3	142.4	0.04	1.21	8.70
3a	74	n.d.[b])	< 100	n.d.[b])	9.5	n.d.[b])	0.13
3b	19	n.d.[b])	< 100	n.d.[b])	4.0	n.d.[b])	0.21
4a	6.6	1128.9	8.6	439.22	0.86	23.78	0.13
4b	0.79	128.7	1.85	111.59	0.23	8.14	0.29
MTX (**1**)	0.0024	0.013	0.004	0.005	0.006	0.022	2.5
Pyrimethamine	1.4	285	15	2.0	n.d.[b])	n.d.[b])	–

[a]) Defined as ratio IC_{50} rat-liver DHFR/IC_{50} *E. coli* DHFR.
[b]) n.d. = not determined.

The inhibitory activities of the compounds synthesized are in line with the predictions based on molecular-modelling experiments as described in *Chap. 2*. Thus, the *exo*-nortropanes **2a** and **2b** whose 2,4-diaminopyrimidine portion *D'* and substituted phenyl ring *B' (Scheme 1)* could be well matched to MTX(**1**) in a conformation as found in the binary complex with *E. coli* DHFR *(Fig. 2)* and could be accomodated in the active site of this enzyme almost free of steric constraints and collisions with the surrounding protein, are the most active compounds in this series. The rather small interaction (*ca.* 0.25 Å overlap of the corresponding *van der Waals* radii) of the ethylene bridge of *exo*-nortropanes **2** with the side chain of Ile-50 is apparently not reflected by the IC_{50} values as judged by the much higher activities of these compounds compared to piperidines **4**. The dimethoxy derivatives **2b**, **3b**, and **4b** are better inhibitors than the corresponding monomethoxy compounds. As no steric conflict of any of the MeO groups with the protein could be observed, we assume that this difference is rather due to the electronic effect of the

[2]) Coordinates and thermal parameters for all compounds have been deposited with the *Crystallographic Data Centre*, Cambridge University, University Chemical Laboratory, Cambridge CB2 1EW, England.

substitution than to the steric one. The inhibitory concentrations of **2a** and **2b** are comparable with those of MTX (**1**) and lower than those of the corresponding antimalarial drug pyrimethamine. On the other hand, the *endo*-nortropanes **3a** and **3b** could neither be – using the same premises as in the matching of **2a** and **2b** – reasonably matched to **1**, nor accommodated in the active site of *E. coli* DHFR. In accord with these findings, their inhibitory potency is by 2–3 orders of magnitude inferior to those of **2a** and **2b**, respectively.

The compounds **4a** and **4b** can both be matched to **1** and accommodated in the active site of the enzymes as good as the conformationally more restricted *exo*-nortropanes **2a** and **2b**. In spite of this fact, though still respectable DHFR inhibitors, their activity is by an order of magnitude lower than that of the *exo*-nortropanes. This can be attributed to the conformational flexibility resulting in a weaker binding to the enzyme *(Table 1)*.

An important question addressed in the design of DHFR inhibitors is the one of their specificity toward enzymes isolated from different sources. Structural features of DHFR inhibitors which govern their selectivity are subject of permanent interest [1] [2]. In our previous work, we were able to show on derivatives of an antibacterial agent brodimoprim that utilisation of additional binding sites for the glutamate side-chain of MTX (*i.e.* Arg-57 in *E. coli* DHFR) enhances the binding both toward DHFR's from *E. coli* and rat liver, but do not alter the specificity of the parent compound [5].

The results of the present study shows that waiving of the glutamate side-chain of MTX and the use of a different spacer in compounds **2** and **4** (*E* and *F* respectively, *vs. C* in MTX, *Scheme 1*) do not greatly affect the specificity of binding of this compounds in comparison with MTX. The so far available data suggest that the specificity of binding of DHFR inhibitors depends at least in part on the relative position of the phenyl ring and the 2,4-diaminopyrimidine moiety in such compounds.

There are no information on the tertiary structure of DHFR from *Plasmodia* which could serve as a base for a rational design of new antimalarials. A bonus of the performed work has been that some of the compounds synthesized exhibited pronounced antimalarial activity, probably by inhibiting the DHFR of the parasites.

Compounds **2b** and **4b**, in contrary to MTX, obviously do penetrate the protozoal cells. They were shown to be by a factor of 20 more potent in *in-vitro* tests against *Plasmodium falciparum* [12] than an antimalarial drug pyrimethamine and are potential candidates for development as chemotherapeutic agents against malaria. Their good *in-vitro* activity was confirmed in *in-vivo* tests as well. Whereas the monomethoxy compounds **2a** and **4a** are almost equipotent with **2b** and **4b**, respectively, the *endo*-nortropanes **3a** and **3b** were found to be essentially inactive *(Table 2)*.

Table 2. In-vitro *Activity against* Plasmodium falciparum

	ID_{50} (μg/l) *P. falciparum*	
	Strain T9CL96	Strain 13
2b	0.177	0.136
4b	0.547	0.376
Pyrimethamine	32.9	22.1

Our thanks are due to Mr. *S. Bürli*, *G. Humer*, and *W. Müller* for their valuable technical assistance, colleagues from *Roche Central Laboratories* for spectral data and elemental analysis, Drs. *A. Kroehn* and *K. Müller* for molecular-modelling studies, and Dr. *Then* and Mrs. *R. Reber* for biological data.

Experimental Part

General. Experimental details are given for the dimethoxy compounds. *The monomethoxy derivatives were synthesized in an analogous manner.* Compound **6a** was prepared according to a published procedure [9]. Usual workup means: The reaction mixture is diluted with the solvent indicated. The org. phase is washed with H_2O, dried over Na_2SO_4, and after filtration, evaporated under reduced pressure. TLC: silica gel *60 F_{254} (Merck)*. Column chromatography: silica gel *60 (Merck,* 0.040–0.063 mm, 230–400 mesh). M.p.: *Büchi-510* apparatus; uncorrected. ^{1}H-NMR: *Brucker WP-80 CW* or *HX-270;* chemical shifts in ppm with respect to TMS (= 0 ppm) as internal standard; coupling constants *J* in Hz.

M.p. and b.p. are listed in *Tables 3* and *4*. IR, MS, and ^{1}H-NMR data as well as elemental analyses compatible with the given structure were obtained for all compounds (available from the authors upon request).

Table 3. *Yields and M.P. of* **2**, **3**, **7–9**, **11**, *and* **12**

	2a	**2b**	**3a**	**3b**	**7a**	**7b**	**8a**	**8b**	**9a**	**9b**	**11a**	**11b**	**12a**	**12b**
M.p. [°]	242	262	172	197	121–122	112	oil	92–93	> 250	> 250	62–63	73–74	> 250	> 280
Yield [%]	70	60	22	15	28	81	34[a])	71	50	92	73	53	52	60

[a]) Compound **8a** was prepared by reduction of **7a** with $LiAlH_4$ followed by separation from **11a** by chromatography.

Table 4. *Yields, M.P., and B.P. of* **4**, *and* **14–19**

	4a	**4b**	**14a**	**14b**	**15a**	**15b**	**16a**	**16b**	**17a**	**17b**	**18a**	**18b**	**19a**	**19b**
M.p. [°]	224–225	220–221	–	–	–	60–61	69	–	87	115	65	–	308	> 250
B.p. [°/0.1 Torr)	–	–	142	139	oil	–	–	oil	–	–	–	oil	–	–
Yield [%]	45	62	80	62	85	68	71	50	75	78	98	97	84	74

8-(3,5-Dimethoxyphenyl)-8-azabicyclo[3.2.1]octan-3-one (**6b**) [9]. To a soln. of 3,5-dimethoxyaniline (**5b**; 45.9 g, 300 mmol) in 1,4-dioxane (3 l) and distilled H_2O (2 l) was added 3-oxoglutaric acid (43.8 g, 300 mmol) followed by 2,5-dimethoxytetrahydrofuran (39.6 g, 300 mmol) in 0.1N HCl[3]) (600 ml) and NaOAc (102 g, 1.2 mol). The resulting mixture was stirred at r.t. for 36 h and then treated with 2,5-dimethoxytetrahydrofuran (19.8 g, 150 mmol) in 0.1N HCl[3]) (300 ml), 3-oxoglutaric acid (21.9 g, 150 mmol), and NaOAc (51 g, 600 mmol). The mixture was again stirred for 36 h, treated with conc. HCl (75 ml) and extracted with a total of 8 l of hexane/Et_2O 3:1. After drying over Na_2SO_4, evaporation yielded 45 g of crude product, which was purified by column chromatography on silica gel with hexane/AcOEt 3:1 yielding 18.3 g of **6b**, m.p. 93–94° ([9]: 96–97°) and 2.9 g of 1-(3,5-dimethoxyphenyl)pyrrole as by-product.

Ethyl Cyano[8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]oct-3-ylidene]acetate (**7b**). To a soln. of **6b** (22.5 g, 86.1 mmol) in ethyl cyanoacetate (97.9 g, 861 mmol) were added AcOH (2.1 g, 34.4 mmol) and NH_4OAc (1.3 g, 16.9 mmol). The mixture was stirred at 60° for 24 h. Usual workup and crystallization from $(i\text{-}Pr)_2O$ gave 24.9 g of **7b** as white crystals.

*Ethyl α-Cyano-8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]octane-3-*exo*-acetate* (**8b**). In a 1.5-l flask (dry-ice cooler, magnetic stirring) were condensed 600 ml of NH_3, previously dried in another connected flask over 400 mg of Li. The apparatus was put under Ar, Li (3.2 g, 461 mmol) was added in small portions, and the resulting deep blue soln. was treated at –78° with **7b** (40 g, 112 mmol) and phenol (10.5 g, 112 mmol) dissolved in dry THF (200 ml). The mixture was stirred for 15 min whereupon excess Li was destroyed by addition of solid NH_4Cl (20 g, 374 mmol). The NH_3 was distilled off and the mixture was partitioned between H_2O and AcOEt. The org. phase was washed with 5% NaOH soln. and H_2O, dried over $MgSO_4$ and evaporated. Chromatography of the resulting yellow oil on 1.5 kg of silica gel with hexane/AcOEt 4:1 gave, in addition to **11b**, 28.5 g of pure **7b**, which crystallized upon standing.

*2,6-Diamino-5-[8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]oct-3-*exo*-yl]pyrimidin-4(3H)-one* (**9b**). To a soln. of Na (18.9 g, 823 mmol) in abs. EtOH (800 ml), guanidine hydrochloride (78.6 g, 823 mmol) was added and the mixture then treated with **8b** (118 g, 329 mmol). After 3 h at reflux, the mixture was cooled to 0° and neutralized

[3]) The soln. of 2,5-dimethoxytetrahydrofuran in 0.1N HCl was heated at 65–70° for ½ h prior to its addition.

with AcOH (40 ml). The precipitated product was filtered off, washed with H_2O, EtOH, and $(i\text{-}Pr)_2O$, and dried in a vacuum oven at 100° yielding 113 g of **9b**.

*2,4-Diamino-6-chloro-5-[8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]oct-3-*exo*-yl]pyrimidine* (**10b**). To a suspension of **9b** (113 g, 304.2 mmol) in $POCl_3$ (713.8 g, 426 ml, 4.65 mol), *N,N*-dimethylaniline (81 g, 81.6 ml, 668.4 mmol) was added and the mixture heated at reflux for 5 h. About ½ of the $POCl_3$ was removed at reduced pressure, and the residue was poured into 4 l of ice/H_2O and left hydrolyzing at r.t. for 4 days. The pH of the mixture was adjusted to 9–10 by the addition of a 25% aq. NH_4OH soln., and the *N,N*-dimethylaniline was removed by steam destillation. The mixture was cooled to r.t., filtered, and the solids were washed with EtOH and $(i\text{-}Pr)_2O$: 113.3 g of crude **10b** which was used directly in the next step. A sample was further purified by filtration over silica gel and recrystallized from MeOH, m.p. 214–215°.

*2,4-Diamino-5-[8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]oct-3-*exo*-yl]pyrimidine* (**2b**). A soln. of crude **10b** (105 g, 269.3 mmol) in AcOH (1.5 l) distilled H_2O (0.63 l) was decolorized with *Norit SX-1*, filtered, and the filtrate hydrogenated over 20 g of 10% Pd/C. Usual workup yielded 83 g of crude product, which was purified by chromatography on 1 kg of silica gel with CH_2Cl_2/MeOH 9:1 resulting in 57.2 g of pure **2b** which was recrystallized from MeOH, m.p. 262° (dec.). ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.25 (*s*, 1 H); 6.11 (*s*, 2 H); 5.95 (*d*, *J* = 2, 2 H); 5.82 (*t*, *J* = 2, 1 H); 5.62 (*s*, 2 H); 4.21 (*m*, 2 H); 3.69 (*s*, 6 H); 3.09–2.91 (*m*, 1 H); 2.12–1.85 (*m*, 4 H); 1.80–1.64 (*m*, 2 H); 1.51–1.37 (*m*, 2 H).

*Ethyl α-Cyano-8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]octane-3-*endo*-acetate* (**11b**). A soln. of **7b** (700 mg, 1.96 mmol) in EtOH (100 ml) was hydrogenated over 500 mg of 10% Pd/C (50% in H_2O) at r.t. The mixture was filtered through *Celite* and the residue, after evaporation, chromatographed on silica gel with AcOEt/hexane 1:4 giving 480 mg of an oil which solidified on standing. Recrystallization from MeOH gave 370 mg **11b**.

*2,6-Diamino-5-[8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]oct-3-*endo*-yl]pyrimidin-4(3H)-one* (**12b**). From **11b** (1.6 g, 4.46 mmol) were obtained 1.0 g of **12b** as described for **9b**. A sample was recrystallized from DMF/H_2O.

*2,4-Diamino-6-chloro-5-[8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]oct-3-*endo*-yl]pyrimidine* (**13b**). From 1 g (2.69 mmol) of **12b** were obtained 750 mg of **13b** as described for **10b**. A sample was recrystallized from EtOH/H_2O, m.p. 197–198°.

*2,4-Diamino-5-[8-(3,5-dimethoxyphenyl)-8-azabicyclo[3.2.1]oct-3-*endo*-yl]pyrimidine* (**3b**). A soln. of 350 mg (0.9 mmol) of **13b** in 15 ml of AcOH/H_2O was hydrogenated over 500 mg of catalyst as described for **2b**. Chromatography and crystallization from MeOH gave 47 mg of **3b**, m.p. 197°. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.74 (*s*, 1 H); 6.01 (*d*, *J* = 2, 2 H); 5.82 (*t*, *J* = 2, 1 H); 5.70 (br. *s*, 2 H); 5.64 (br. *s*, 2 H); 4.22 (*m*, 2 H); 3.69 (*s*, 6 H); *ca.* 2.49–2.27 (*m*, 3 H); 1.98–1.82 (*m*, 2 H); 1.76–1.64 (*m*, 2 H); 1.50–1.39 (*m*, 2 H).

Diethyl 3,3'-[(3,5-Dimethoxyphenyl)imino]dipropionate (**14b**). A mixture of **5b** (50.4 g, 322 mmol), CuCl (6.0 g, 60 mmol), AcOH (46.0 g, 600 mmol), and ethyl acrylate (100 g, 1.0 mol) was refluxed for 19 h. After cooling to r.t., the mixture was diluted with CH_2Cl_2 (*ca.* 30 ml) and washed consecutively with H_2O, 10% aq. NH_3 soln. and H_2O. The org. phase was dried over Na_2SO_4 and evaporated. The residue was chromatographed on 1.5 kg of silica gel with hexane/AcOEt 4:1. Destillation of the crude product gave 70.9 g of **14b** as a yellow oil.

Ethyl 1-(3,5-Dimethoxyphenyl)-4-oxo-piperidine-3-carboxylate (**15b**). To a freshly prepared NaOEt soln. in abs. EtOH (4.1 g, 178 mmol) of Na in 70 ml of EtOH) was added under Ar **14b** (62.9 g, 178 mmol) in xylene (100 ml). The mixture was heated slowly to *ca.* 110° while EtOH was distilled off. The heating was continued for 2 h and the mixture cooled to r.t. and poured onto ice. Neutralization with conc. HCl and usual workup with Et_2O gave 37.4 g of **15b** as an oil, which solidified upon standing. For anal. purpose, a sample was recrystallized from EtOH.

1-(3,5-Dimethoxyphenyl)-4-piperidone (**16b**). A mixture of 12.52 g of **15b** in 81.6 ml of 1N NaOH was heated with vigorous stirring for 2 h on a steam bath. Usual workup with Et_2O, chromatography on silica gel with hexane/AcOEt 4:1 gave 4.8 g of **16b** as a yellow oil.

Ethyl Cyano[1-(3,5-dimethoxyphenyl)piperidin-4-ylidene]acetate (**17b**). To a soln. of **16b** (4.9 g, 20.8 mmol) in benzene (50 ml) and AcOH (0.25 g, 4.1 mmol) were added piperidine (0.18 ml, 2.08 mmol) and ethyl cyanoacetate (3.0 g, 26.7 mmol). After heating at reflux for 3 h using a *Dean-Stark* trap, the mixture was cooled to r.t. and diluted with Et_2O. The Et_2O soln. was washed with 2N $NaHCO_3$, dried, and distilled off. The oily residue was triturated with $(i\text{-}Pr)_2O$ yielding 5.4 g of cristalline **17b**. A sample was recrystallized from MeOH.

Ethyl α-Cyano-1-(3,5-dimethoxyphenyl)piperidine-4-acetate (**18b**). A soln. of **17b** (19.2 g, 58.1 mmol) in AcOEt (400 ml) was hydrogenated over 6 g of 10% Pd/C (50% in H_2O) as described for **11b**. Yield: 18.7 g **18b** as an oil.

2,6-Diamino-5-[1-(3,5-dimethoxyphenyl)piperidin-4-yl]pyrimidin-4(3H)-one (**19b**). From **18b** (6.5 g, 19.6 mmol) and guanidine hydrochloride (3.7 g, 39 mmol) were obtained 5.0 g of **19b** as described for **9b**. A sample was recrystallized from DMF/H_2O.

2,4-Diamino-6-chloro-5-[1-(3,5-dimethoxyphenyl)piperidin-4-yl]pyrimidine (**20b**). From **19b** (5 g, 14.5 mmol), $POCl_3$ (36.8 g, 240 mmol), and *N,N*-dimethylaniline (3.7 g, 30.4 mmol) were obtained 4.3 g of **20b** as described for **10b**. A sample was recrystallized from MeOH/$CHCl_3$, m.p. 241–242°.

2,4-Diamino-5-[1-(3,5-dimethoxyphenyl)piperidin-4-yl]pyrimidine (**4b**). A soln. of **20b** (5.7 g, 15.7 mmol) was hydrogenated over 1.8 g of 10% Pd/C as described for **2b**, furnishing, after chromatography and crystallization from MeOH, 3.2 g of **4b**. ^{1}H-NMR (90 MHz, (D_6)DMSO): 7.54 (*s*, 1 H); 6.18 (br. *s*, 2 H); 6.07 (*d*, *J* = 2); 5.94 (*t*, *J* = 2, 1 H); 5.61 (br. *s*, 2 H); 3.89–*ca.* 3.64 (*m*, 2 H); 3.70 (*s*, 6 H); 2.92–*ca.* 2.52 (*m*, 3 H); 1.94–1.40 (*m*, 4 H).

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