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Inhibition of Neuronal Nitric Oxide Synthase by 4-Amino Pteridine Derivatives: Structure–Activity Relationship of Antagonists of (6*R*)-5,6,7,8-Tetrahydrobiopterin Cofactor

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The family of nitric oxide synthases (NOS) catalyzes the conversion of L-arginine to L-citrulline and nitric oxide (NO), an important cellular messenger molecule which has been implicated in the pathophysiology of septic shock and inflammatory and neurodegenerative disease states. NOS can be maximally activated by the ubiquitous cofactor, (6*R*)-5,6,7,8-tetrahydrobiopterin (H₄Bip), and antagonists of H₄Bip may be of therapeutic importance to inhibit pathologically high NO formation. The 4-amino substituted analogue of H₄Bip was reported to be a potent NOS inhibitor. Therefore, we developed a series of novel 4-amino pteridine derivatives, anti-pterins, to pharmacologically target the neuronal isoform of nitric oxide synthase (NOS-I). To functionally characterize the pterin/anti-pterin interaction and establish a structure–activity relationship (SAR), we systematically altered the substituents in the 2-, 4-, 5-, 6-, and 7-position of the pteridine nucleus. Varying the substitution pattern in the 2-, 5-, and 7-position resulted in no significant inhibitory effect on enzyme activity. In contrast, bulky substituents in the 6-position, such as phenyl, markedly increased the inhibitory potency of the reduced 4-amino-5,6,7,8-tetrahydropteridines, possibly as a consequence of hydrophobic interactions within NOS-I. However, this was not the case for the aromatic 4-amino pteridines. Interestingly, chemical modification of the 4-amino substituent by dialkyl/diaralkylation together with 6-arylation of the aromatic 2,4-diamino pteridine resulted in potent and efficacious inhibitors of NOS-I, suggesting possible hydrophilic and hydrophobic interactions within NOS-I. This SAR agrees with (a) the recently published crystal structure of the oxygenase domain of the inducible NOS isoform (NOS-II) and (b) the comparative molecular field analysis of selected NOS-I inhibitors, which resulted in a 3D-QSAR model of the pterin binding site interactions. Further optimization should be possible when the full length structure of NOS-I becomes available.

Introduction

For over a decade the biomolecule nitric oxide (NO) has been of great interest to medicinal chemists from designing more effective nitrovasodilator drugs to modulating endogenous NO biosynthesis. NO is a ubiquitous modulator of both physiological and pathophysiological functions¹ that is generated endogenously² by the enzyme nitric oxide synthase (NOS),³ which metabolizes L-arginine⁴ substrate to L-citrulline and NO or a related *N*-oxide.⁵ To date, three structurally distinct NOS isoforms have been identified.⁶ The two constitutively expressed NOS are localized either in the vascular endothelium⁷ (NOS-III or eNOS) and involved in the regulation of vascular tone and antithrombosis, or in neuronal tissue⁸ (NOS-I or nNOS) and involved in neuromodulation.⁹ The so-called inducible isoform (NOS-II or iNOS) is expressed in macrophages and many other

cells upon exposure to cytokines and plays a key role in early immune responses by functioning as a cytotoxic agent.^{10,11} Importantly, there is an increasing appreciation of the role of NO in contributing to the etiology of pathophysiological conditions including septic shock, inflammatory, and neurodegenerative disease states.¹² Therefore, the use of NOS inhibitors to downregulate pathologically elevated synthesis of NO has great therapeutic potential.

At the biochemical level a number of cofactors of NOS have been identified that are uniformly required for catalysis by all isoforms including NADPH and flavins (FAD, FMN).^{13,14} In addition, the cofactor (6*R*)-5,6,7,8-tetrahydrobiopterin (H₄Bip) markedly stimulates NOS catalytic activity by a unique and as yet to be fully elucidated mechanism, possibly involving enzyme stabilization¹⁵ and protection.¹⁶ Thus, the H₄Bip binding site of NOS may represent an ideal target for selective pharmacological intervention. Prototypical NOS inhibitors were mainly analogues of their endogenous substrate, L-arginine,⁴ e.g., *N*^ω-methyl-L-arginine (L-NMA), *N*^ω-methyl-L-argininemethylester (L-NMMA), *N*^ω-nitro-L-arginine (L-NNA), its methylester (L-NAME), and *N*^δ-

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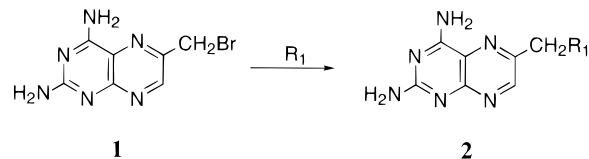
Table 1. 6-Substituted 2,4-Diamino Pteridines **2**

entry	R ₁	UV/VIS; λ _{MAX} in nm (log ε), [] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
2a	OMe	pH 1: 242 (4.18); 285 (3.65); 337 (3.97); [350 (3.92)]	C ₈ H ₁₀ N ₆ O (206.1): C 46.60 (46.44), H 4.85 (4.69), N 40.77 (40.38)
2b	OC ₂ H ₅	pH 1: 242 (4.19); 284 (3.67); 337 (3.98); [348 (3.92)]	C ₉ H ₁₂ N ₆ O (220.2): C 49.11 (48.86), H 5.45 (5.24), N 38.16 (37.76)
2c	OC ₆ H ₅	pH 1: 243 (4.13); 287 (3.61); 336 (3.90); 350 (3.82)	C ₁₃ H ₁₂ N ₆ O (268.3): C 58.19 (57.64), H 4.51 (4.36), N 31.36 (31.40)
2d	O(CH ₂) ₉ CH ₃	pH 1.2: 221 (4.18); 288 (3.69); 335 (3.99); [348 (3.92)]	C ₁₇ H ₂₈ N ₆ O (332.3): C 61.46 (61.53), H 8.42 (8.70), N 25.28 (25.13)
2e	OCOCH ₂ CH ₂ -C ₆ H ₄ -4CO-C ₆ H ₅	MeOH: 203 (4.56); 260 (4.60); 372 (3.90)	C ₂₃ H ₂₀ N ₆ O ₃ (428.5): C 64.48 (64.01), H 4.70 (4.94), N 19.61 (18.87)
2f	NH ₂	pH 1: 244 (4.15); 290 (3.96); [346 (3.91)]	C ₇ H ₉ N ₇ (191.1): C 43.98 (44.12), H 4.71 (5.19), N 51.29 (50.50)
2g	N(CH ₃) ₂	pH 1: 245 (4.20); 294 (3.71); 336 (3.99); [348 (3.94)]	C ₉ H ₁₃ N ₇ × 0.25 H ₂ O (223.6): C 48.34 (48.21), H 6.03 (6.10), N 43.83 (43.58)
2h	SMe	pH 2: 245 (4.22); 286 (3.96); 342 (3.98); [350 (3.95)]	C ₈ H ₁₀ N ₆ S (222.2): C 43.26 (43.36), H 4.50 (4.36), N 37.82 (38.04)
2i	NHCOCH(CH ₃) ₂	pH 2: 245 (4.22); 285 (3.69); 337 (4.02); [348 (3.96)]	C ₁₁ H ₁₅ N ₇ O (261.2): C 50.59 (50.46), H 5.47 (5.73), N 37.53 (37.29)

iminoethyl-L-ornithine (L-NIO).^{17–19} Although the NOS coproduct, L-citrulline, does not autoregulate NOS function, various analogues were found to be potent NOS inhibitors,¹⁹ e.g., L-thiocitrulline (L-TC), L-homothiocitrulline (L-HTC), and S-methyl-L-thiocitrulline (L-SMTC). Further modifications of the guanidino moiety of L-arginine led to the development of cyclic and acyclic amidines, guanidines, and isothioureas as active site inhibitors^{20–22} with reversible and irreversible modes of action. Finally, it is a short conceptual step from a cyclic amidine to pyridyl precursors, such as 2-aminopyridines,²³ and from there to aniline derivatives²⁴ which show some isoform selectivity but are otherwise less potent.

Apart from the L-arginine-based NOS inhibitors, other binding sites of NOS have been pharmacologically targeted. Compounds which are thought to interfere with the flavin cofactor binding site within the reductase domain include diphenyleneiodonium (DPI)¹⁹ and some quinazolines.²⁵ Within the oxygenase domain of NOS, various indazoles¹⁹ or imidazoles¹⁹ bind as ligands to the heme prosthetic group to inhibit catalysis while other aromatic hydroxamic acid derivatives²⁶ antagonize H₄Bip function. However, only a few of the NOS inhibitors that have been previously described appear to be acting exclusively at one binding site of NOS. Moreover, most of these agents also have failed to display any appreciable isoform selectivity.

We have previously shown that a novel group of pterin-derived compounds, anti-pterins, are effective inhibitors of NOS activity in intact cells and are, therefore, novel pharmacological tools to downregulate pathologically high NO formation.²⁷ These compounds appear to specifically interact with the pterin binding site of NOS and do not interfere with any other known cofactor/substrate binding sites.²⁷ In the present study we synthesized a second generation of refined H₄Bip-based NOS inhibitors with a 4-amino pteridine nucleus^{27,28} and systematically varied the substitution patterns in the 2-, 4-, 5-, 6-, and 7-position. The effect of these compounds on NOS activity was taken as a biological indicator of efficacy and potency; a structure–activity relationship (SAR) was then determined. The implications of the present finding for chemical interac-

Scheme 1. Synthesis of 6-Substituted 2,4-Diamino Pteridines **2**

tions within the pterin binding pocket of NOS are discussed.

Chemistry

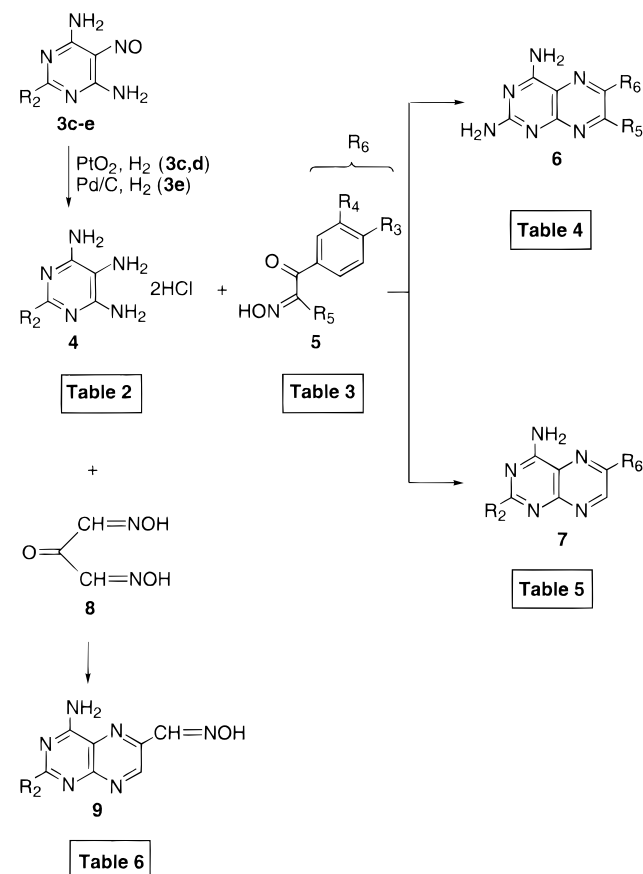
The 6-substituted 2,4-diamino pteridines **2a–i**^{30–32} were synthesized by reacting the 2,4-diamino-6-bromomethylpteridine × HBr × 2-hydroxy-propane²⁹ (**1**) with the nucleophile R₁ by stirring at room temperature (Scheme 1; Table 1).

The general principle for the synthesis of 2,4-diamino-6/7-aryl pteridines **6**³⁴ and the 2-substituted 4-amino-6-aryl pteridines **7** was based on the condensation of 2-substituted 4,5,6-triaminopyrimidine dihydrochloride derivatives **4** with different substituted phenylglyoxalmonoximes **5**^{35–38} by heating in MeOH under reflux.³⁴ Modification of the pteridines **7b–e** in the 2-position was achieved by varying the 2-position of the pyrimidine adducts **4b**^{39–e}, which were obtained by reduction of the nitroso-precursor **3c–e**⁴⁰ (Scheme 2). The corresponding 2-substituted 4-aminopteridine-6-carboxal-doximes **9b–d**⁴³ were prepared by reacting the 4,5,6-triaminopyrimidine dichlorides **4b–d** with the mesoxalal-carbaldoxime **8** (Scheme 2; Tables 2–6).

Another synthetic approach involved using initially 2,6-diamino-4-chloro-5-*p*-chlorophenylazopyrimidine **12**⁴⁴ which was prepared from *p*-chloroaniline **10** by diazotization and subsequent coupling with 2,6-diamino-4-chloropyrimidine **11**. In **12**, the chlorine atom is sufficiently activated to be nucleophilically displaced by acyclic or cyclic secondary amines **13b–f** to form the 2,6-diamino-4-dialkylamino-5-*p*-chlorophenylazopyrimidines **14b–f**, which on reduction with Raney nickel catalyst and H₂ generated the 2,5,6-triamino-4-dialkylaminopyrimidines **16b–f**. For the 4-(dimethylamino)-substituted 2,5,6-triaminopyrimidine **16a**, the direct route of PtO₂/H₂ catalyzed reduction of 2,6-diamino-4-(dimethylamino)-5-nitrosopyrimidine **15a**⁴¹ was chosen.

Table 2. 2-Substituted 4,5,6-Triaminopyrimidines **4**^a

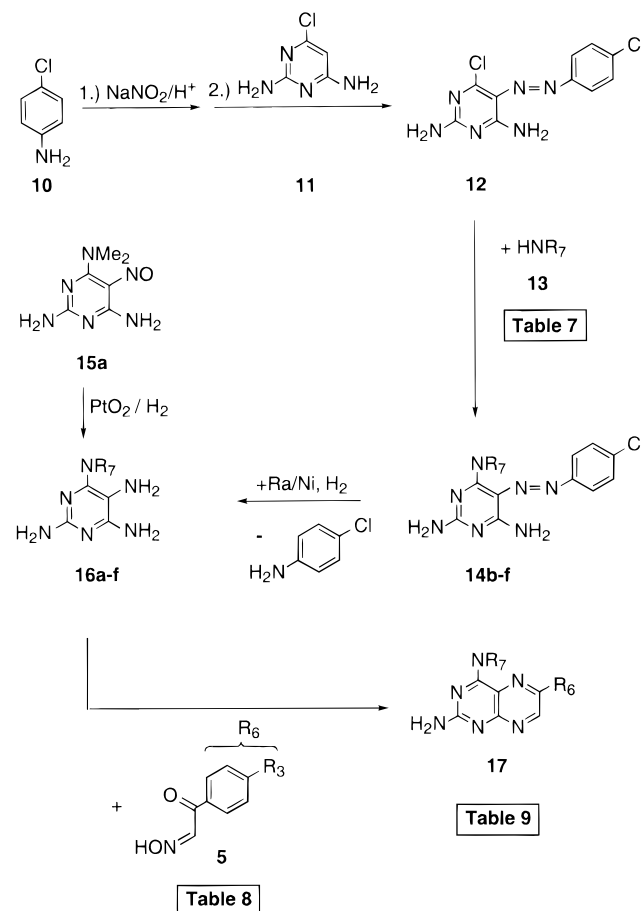
entry	R ₂	UV/VIS; λ _{MAX} in nm (log e), [] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
4a ³⁴	NH ₂		
4b ³⁹	H		
4c	SMe	H ₂ O: [231 (4.15)]; 286 (3.99)	C ₅ H ₁₀ N ₅ S × 2 HCl (244.1): C 24.60 (24.73), H 4.54 (4.58), N 28.68 (29.01)
4d	C ₆ H ₅	H ₂ O: 292 (4.01); [334 (3.80)]	C ₁₀ H ₁₃ Cl ₂ N ₅ × 0.5 H ₂ O (283.2): C 42.42 (42.36), H 4.98 (5.00), N 24.73 (24.37)
4e	NHCH ₂ CH ₂ OH	H ₂ O: 286 (4.07)	C ₆ H ₁₅ Cl ₃ N ₆ O (282.3): C 24.55 (25.04), H 5.15 (5.18), N 28.63 (28.66)

^a Compounds **4a** and **4b** were prepared according to literature.^{34,39}**Scheme 2.** Synthesis of 4-Amino-6/7-aryl Pteridines **6** and **7****Table 3.** Derivatives of Phenylglyoxalmonoxime **5**

entry	R ₃	R ₄	R ₅
5a	H	H	H
5b	F	H	H
5c	Cl	H	H
5d	Br	H	H
5e	Me	H	H
5f	OMe	H	H
5g	OMe	OMe	H
5h	H	H	Me
5i	H	H	C ₆ H ₅

Finally, boiling a solution of the 2,5,6-triamino-4-dialkylaminopyrimidines **16** with the arylglyoxalmonoximes **5** yielded the 2-amino-4-dialkylamino-6-aryl pteridines **17** with either C₆H₅- (**17aa-fa**) or *p*-MeOC₆H₄- (**17af-fh**) substitution in the R₆-position of the 2-amino-4-dialkylaminopteridine (Scheme 3; Tables 7–9).

4-Amino-2-methylaminopteridine (**20c**)⁴² and its 6,7-dimethyl (**20a**) and 6,7-diphenyl derivative (**20b**) were prepared by condensation of the 4,5,6-triamino-2-me-

Scheme 3. Synthesis of 2-Amino-4-dialkylamino-6-aryl Pteridines **17**

thylaminopyrimidine **18** with the 1,2-dicarbonyl compounds **19** (19a: diacetyl; 19b: benzil; 19c: glyoxal) and then catalytically reduced with PtO₂ catalyst under H₂ atmosphere to generate the corresponding 5,6,7,8-tetrahydropteridines **21a-c**.⁴² In an analogous manner, various 6-substituted 2,4-diaminopteridines (**2b**, **6aa**³⁴ or **20d**^{33,g⁴⁵,h³⁴}) were converted by catalytic reduction into their 5,6,7,8-tetrahydro-derivatives **21d-h**. Mild acylation led to monosubstitution in the 5-position to give **22da** and **22db** (Scheme 4; Tables 10–13).

2,4-Diamino-7-benzylpteridine (**24**) was prepared by condensation of 2,4,5,6-tetraaminopyrimidine (**4a**) with benzylglyoxal (**23**)⁴⁶. Bromination in the side chain and subsequent hydrolysis generated 2,4-diamino-7-benzoylpteridine (**25**) (Scheme 5).

2,4-Diamino-7-aminomercaptopteridine (**27**) was obtained by treatment of 2,4-diamino-7-mercaptopteridine (**26**)⁴⁷ with chloramine (Scheme 6).

Table 4. 2,4-Diamino-6/7-substituted Pteridines **6**^a

entry	R ₆	R ₅	UV/VIS; λ _{MAX} in nm (log ε), [] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
6aa ³⁴	C ₆ H ₅	H	pH 2: 266 (4.48); 364 (4.11)	
6ab	<i>p</i> -FC ₆ H ₄	H	pH 1: 264 (4.41); 365 (4.05)	C ₁₂ H ₉ FN ₆ × 2 H ₂ O (292.2): C 49.31 (49.05), H 4.48 (3.88), N 28.75 (28.85)
6ac	<i>p</i> -ClC ₆ H ₄	H	pH 2: 268 (4.42); [294 (4.32)]; 365 (4.02)	C ₁₃ H ₁₂ ClN ₆ (272.6): C 52.85 (52.73), H 3.33 (3.42), N 30.83 (29.98)
6ad	<i>p</i> -BrC ₆ H ₄	H	DMF: 288 (4.50); [321 (4.20)]; 400 (4.10)	C ₁₂ H ₉ BrN ₆ × 2 H ₂ O (353.1): C 40.81 (41.02), H 3.71 (3.93), N 23.80 (23.91)
6ae	<i>p</i> -MeC ₆ H ₄	H	pH 2: 268 (4.48); 369 (4.10)	C ₁₃ H ₁₂ N ₆ (252.2): C 61.89 (61.98), H 4.79 (4.95), N 33.31 (32.55)
6af	<i>p</i> -MeOC ₆ H ₄	H	pH 2: 273 (4.42); [291 (4.35)]; 379 (4.07)	C ₁₃ H ₁₂ N ₆ O (277.2): C 56.31 (56.63), H 4.73 (4.61), N 30.31 (29.98)
6ag	3,4-Di-MeOC ₆ H ₃	H	DMF: 285 (4.38); 317 (4.35); 408 (4.08)	C ₁₄ H ₁₄ N ₆ O ₂ × 2 H ₂ O (334.3): C 50.30 (49.90), H 5.43 (5.05), N 25.14 (25.18)
6ah	C ₆ H ₅	Me	pH 1: 253 (4.32); 348 (4.09)	C ₁₃ H ₁₂ N ₆ × 1.5 H ₂ O (279.3): C 55.90 (55.85), H 5.41 (5.05), N 30.09 (30.31)
6ai ³⁴	C ₆ H ₅	C ₆ H ₅		

^a Compounds **6aa** and **6ai** were prepared according to literature.³⁴**Table 5.** Varying the Substituents in the 2- and 6-Position of the 4-Amino Pteridines **7**

entry	R ₂	R ₆	UV/VIS; λ _{MAX} in nm (log ε), [] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
7ba	H	C ₆ H ₅	pH 1: 240 (4.26); 275 (4.23); 362 (4.09)	C ₁₂ H ₉ N ₅ (223.2): C 64.56 (64.49), H 4.06 (3.98), N 31.37 (30.80)
7be	H	<i>p</i> -MeC ₆ H ₄	pH 1: 244 (4.25); 284 (4.27); 371 (4.10)	C ₁₃ H ₁₁ N ₅ (273.3): C 65.81 (65.99), H 4.67 (4.70), N 29.52 (30.17)
7bf	H	<i>p</i> -MeOC ₆ H ₄	pH 1: 251 (4.15); 297 (4.30); 385 (4.08)	C ₁₃ H ₁₁ N ₅ O (253.3): C 61.64 (61.61), H 4.38 (4.29), N 27.65 (27.35)
7ca	SMe	C ₆ H ₅	DMF: 289 (4.52); 314 (4.06); 380 (4.10); [394 (4.06)]	C ₁₃ H ₁₁ N ₅ S (269.3): C 57.97 (58.03), H 4.12 (4.14), N 26.00 (26.49)
7ce	SMe	<i>p</i> -MeC ₆ H ₄	DMF: 291 (4.57); [320 (4.10)]; 383 (4.16); [401 (4.06)]	C ₁₄ H ₁₃ N ₅ S (283.4): C 59.34 (59.51), H 4.62 (4.65), N 24.72 (25.14)
7cf	SMe	<i>p</i> -MeOC ₆ H ₄	DMF: 296 (4.45); [320 (4.20)]; 389 (4.10); [404 (4.05)]	C ₁₄ H ₁₃ N ₅ OS (299.4): C 56.17 (56.35), H 4.38 (4.39), N 23.39 (23.88)
7da	C ₆ H ₅	C ₆ H ₅	DMF: 259 (4.32); 293 (4.65); 377 (4.24); [397 (4.11)]	C ₁₈ H ₁₃ N ₅ (299.3): C 72.23 (72.13), H 4.38 (4.45), N 23.40 (23.50)
7df	C ₆ H ₅	<i>p</i> -MeC ₆ H ₄	DMF: 259 (4.33); 305 (4.53); 390 (4.31); [409 (4.21)]	C ₁₉ H ₁₅ N ₅ O (329.4): C 69.29 (69.25), H 4.59 (4.63), N 21.26 (21.55)
7ea	NHCH ₂ CH ₂ OH	C ₆ H ₅	pH 1: 269 (4.49); 386 (4.13)	C ₁₄ H ₁₄ N ₆ O (282.3): C 59.56 (59.81), H 5.00 (4.96), N 29.77 (29.48)
7ee	NHCH ₂ CH ₂ OH	<i>p</i> -MeC ₆ H ₄	pH 1: 269 (4.49); 368 (4.13)	C ₁₅ H ₁₆ N ₆ O (296.3): C 60.80 (60.84), H 5.44 (5.49), N 28.36 (28.56)
7ef	NHCH ₂ CH ₂ OH	<i>p</i> -MeOC ₆ H ₄	pH 1: 278 (4.50); 384 (4.12)	C ₁₅ H ₁₆ N ₆ O ₂ (312.3): C 57.68 (57.64), H 5.16 (5.21), N 26.91 (26.83)

Table 6. Varying the Substituents in the 2-Position of the 4-Aminopteridine-6-carbox-aldoxime **9**^a

entry	R ₂	UV/VIS; λ _{MAX} in nm (log ε), [] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
9b	H	pH 12: 249 (4.05); 294 (4.27); [320 (4.04)]; 376 (4.01)	C ₇ H ₆ N ₆ O (190.2): C 43.39 (43.49), H 3.33 (3.12), N 43.37 (43.18)
9c ⁴³	SMe	pH 12: [227 (3.84)]; 292 (4.31); [316 (4.18)]; 390 (3.99)	
9d	C ₆ H ₅	pH 12: 294 (4.43); [329 (4.19)]; 387 (4.20)	C ₁₃ H ₁₀ N ₆ O (266.3): C 58.64 (58.49), H 3.79 (3.65), N 31.56 (31.17)

^a Compound **9c** was prepared according to literature.⁴³**Table 7.** Dialkylamines **13**

entry	R ₇
13b	(C ₂ H ₅) ₂
13c	(CH ₂ C ₆ H ₅) ₂
13d	(C ₂ H ₄) ₂ O
13e	(CH ₂) ₅
13f	(C ₂ H ₄) ₂ NCH ₃

Table 8. Phenyl- or Anisyl-Substituted Glyoxalmonoximes **5**

entry	R ₃
5aa	H
5fb	OMe

cally varied. This yielded several new, potent derivatives as inhibitors of NOS-I activity. Indeed, some inhibitors were found to be very effective over a narrow concentration range and inhibited total enzyme activity whereas others, despite being potent, were incomplete enzyme inhibitors.

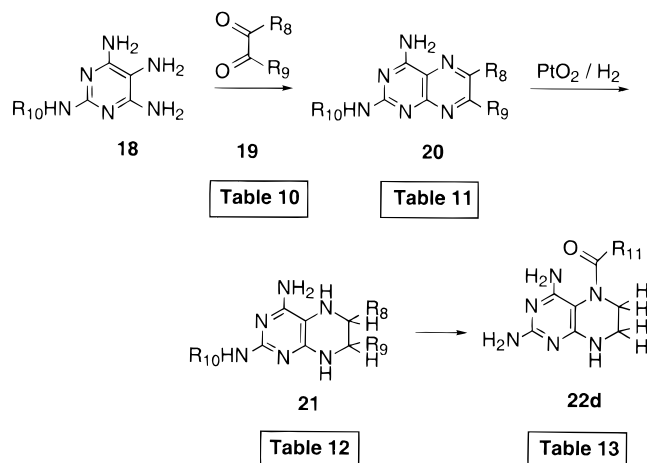
Methyl substitution (**21c**) at the 2-amino group of the 2,4-diamino-5,6,7,8-tetrahydropteridine derivative (**21d** of **I**; see Table 14, ranked according to degree of enzyme

Structure–Activity Relationship

To establish a structure–activity relationship (SAR) of the NOS pterin/anti-pterin binding site, the pteridine substitution pattern and functionalities within the scaffolds of general formula **I–IV** (4-amino substituted pteridine derivatives, Tables 14–17) were systemati-

Table 9. 2-Amino-4-dialkylamino-6-aryl Pteridines **17**

entry	R ₇	R ₆	UV/VIS; λ _{MAX} in nm (log ε), [] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
17aa	(Me) ₂	C ₆ H ₅	pH 1: [227 (4.08)]; 275 (4.36); 366 (4.08)	C ₁₄ H ₁₄ N ₆ × 0.5 H ₂ O (275.3): C 61.08 (61.38), H 5.49 (5.43), N 30.53 (30.27)
17af	(Me) ₂	<i>p</i> -MeOC ₆ H ₄	pH 1: 289 (4.44); 381 (4.44)	C ₁₅ H ₁₆ N ₆ O (296.3): C 60.80 (60.79), H 5.44 (5.53), N 28.36 (28.62)
17ba	(C ₂ H ₅) ₂	C ₆ H ₅	MeOH: [209 (4.42); 236 (4.18)]; 291 (4.44); [317 (3.97)]; 395 (4.02); [411 (3.97)]	C ₁₆ H ₁₈ N ₆ × H ₂ O (312.4): C 61.52 (61.44), H 6.45 (6.07), N 26.90 (26.62)
17bf	(C ₂ H ₅) ₂	<i>p</i> -MeOC ₆ H ₄	MeOH: 208 (4.49); [240 (4.11)]; 298 (4.51); [321 (4.15)]; 401 (4.07)	C ₁₇ H ₂₀ N ₆ O × H ₂ O (342.4): C 59.63 (59.91), H 6.48 (6.22), N 24.54 (25.16)
17ca	(CH ₂ C ₆ H ₅) ₂	C ₆ H ₅	MeOH: 207 (4.62); [246 (4.14)]; 288 (4.46); [317 (3.98)]; 388 (4.06); [415 (3.88)]	C ₂₆ H ₂₂ N ₆ × 2 H ₂ O (454.4): C 68.71 (68.15), H 5.77 (5.01), N 18.49 (18.51)
17cf	(CH ₂ C ₆ H ₅) ₂	<i>p</i> -MeOC ₆ H ₄	MeOH: 207 (4.63); [246 (4.09)]; 298 (4.44); [322 (4.11)]; 406 (4.02)	C ₂₇ H ₂₄ N ₆ O (448.4): C 72.30 (71.89), H 5.39 (4.83), N 18.74 (18.84)
17da	(C ₂ H ₄) ₂ O	C ₆ H ₅	MeOH: [212 (4.40)]; [237 (4.18)]; 291 (4.50); 399 (4.07)	C ₁₆ H ₁₆ N ₆ O × H ₂ O (326.4): C 58.89 (59.03), H 5.56 (5.30), N 25.75 (26.29)
17df	(C ₂ H ₄) ₂ O	<i>p</i> -MeOC ₆ H ₄	MeOH: 210 (4.41); [241 (4.10)]; 299 (4.49); 404 (4.05)	C ₁₇ H ₁₈ N ₆ O ₂ × HCl × H ₂ O (392.8): C 51.98 (51.69), H 5.39 (5.70), N 21.39 (22.08)
17ea	(CH ₂) ₅	C ₆ H ₅	MeOH: 204 (4.50); [245 (4.24)]; 293 (4.48); [318 (4.04)]; 399 (4.13); [418 (4.00)]	C ₁₇ H ₁₈ N ₆ × 2 H ₂ O (342.4): C 59.63 (59.11), H 6.48 (5.98), N 24.54 (25.96)
17ef	(CH ₂) ₅	<i>p</i> -MeOC ₆ H ₄	MeOH: 209 (4.49); [240 (4.18)]; 300 (4.50); [322 (4.15)]; 406 (4.04)	C ₁₈ H ₂₀ N ₆ O × 0.5 H ₂ O (345.4): C 62.59 (62.13), H 6.12 (5.84), N 24.33 (25.50)
17fa	(C ₂ H ₄) ₂ NCH ₃	C ₆ H ₅	MeOH: 209 (4.46); [242 (4.15)]; 288 (4.48); [318 (3.97)]; 393 (4.04); [413 (3.97)]	C ₁₇ H ₁₉ N ₇ × 0.5 H ₂ O (330.4): C 61.80 (61.80), H 6.10 (5.94), N 29.67 (29.69)
17ff	(C ₂ H ₄) ₂ NCH ₃	<i>p</i> -MeOC ₆ H ₄	MeOH: [212 (4.55)]; [245 (4.16)]; 299 (4.52); 407 (4.10)	C ₁₈ H ₂₁ N ₇ O × 0.5 H ₂ O (360.4): C 59.98 (59.68), H 6.15 (6.27), N 27.20 (26.73)

Scheme 4. Synthesis of 2,4-Diamino- and 4-Amino-2-methylamino-5,6,7,8-tetrahydropteridines **21** and Formylation/Benzoylation Products **22d****Table 10.** 2,3-Diketones **19**

entry	R ₈ = R ₉
19a	Me
19b	C ₆ H ₅
19c	H

inhibition observed with 100 μM anti-pterin) was without effect on NOS-I activity. In contrast, additional 6,7-diphenyl (**21b**) or 6,7-dimethyl substitution (**21a**) resulted in compounds that began to inhibit NOS activity (down to 86 ± 23 and 57 ± 5%, respectively) at the tested concentration. A similar degree of enzyme inhibition was achieved by N-5 acylations alone (**22da,db**; Table 14).

Interestingly, 4-amino-5,6,7,8-tetrahydrobiopterin (**21g**), which differs from the naturally occurring H₄-Bip only in the 4-amino group, was found to be a potent and effective inhibitor (down to 38 ± 2% of *V*_{max}; IC₅₀ = 6 μM). Further inhibition was observed by substituting the dihydroxypropyl side chain in the 6-position, with either a phenyl (**21f**, inhibition down to 17 ± 1% of *V*_{max};

IC₅₀ = 6 μM) or an ether substituent (**21e**, inhibition down to 3 ± 1% of *V*_{max}; IC₅₀ = 30 μM). This resulted in some of our most potent NOS inhibitors based on a reduced 4-amino-5,6,7,8-tetrahydropteridine scaffold **I**. Noteworthy, monophenyl substitution in the 7-position (**21h**) resulted in a weaker NOS inhibitor (39 ± 1% of *V*_{max}; IC₅₀ = 24 μM) compared to the 6-substituted analogue (**21f**, inhibition down to 17 ± 1% of *V*_{max}; IC₅₀ = 6 μM).

In contrast, in the heteroaromatic series, the 7-(**24**, **25**, **27**) and 6-mono-(**6aa**)³⁴ and 6,7-disubstituted-(**6ah**, **6ai**)³⁴ 2,4-diamino pteridines (general formula **II**, Table 15) were only slightly better NOS inhibitors compared to the inactive 6,7-unsubstituted analogue (**20d**). Furthermore, varying the substitution pattern in the para-position of the 6-phenyl group with either electron-withdrawing (**6ab-ad**) or electron-donating (**6ae-ag**) substituents did not influence the degree of enzyme inhibition. Similar results were obtained for substituents in the ortho- and meta-position (data not shown). However, a significant decrease in NOS activity (16 ± 1% of *V*_{max}; IC₅₀ = 30 μM) could be obtained by using a long-chain aliphatic substituent in the 6-position such as the decyloxymethyl ether **2d** compared, for example, to the ethyloxymethyl ether **2b**, which was otherwise an effective substituent in the 4-amino-5,6,7,8-tetrahydropteridine-series (**I**; Table 14; e.g., **21e**).

The above findings consequently prompted us to change further the chemical substituent in the 6-position. Introduction of an ester substituent containing a long side chain (**2e**) generated a NOS inhibitor that was slightly less effective (29 ± 6% of *V*_{max}) and potent (IC₅₀: 48 μM; *p* < 0.05) whereas substitution with either a phenoxymethyl (**2c**), an aminomethyl (**2f**), a dimethylaminomethyl (**2g**), an isobutyroylaminomethyl (**2i**), or a methylthiomethyl (**2h**) was without effect on NOS-I activity (Table 15). These findings apparently argue against any hydrophilic interaction between a substituent in the 6-position of the aromatic 2,4-diamino pteridine nucleus (**II**) and the pterin binding pocket. Hydro-

Table 11. Aromatic 4-Amino-6/7-alkyl-(aryl-) Pteridines^a

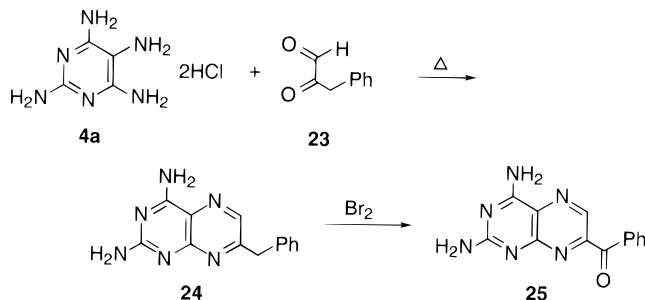
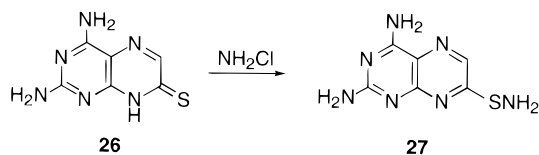
entry	R ₈	R ₉	R ₁₀	UV/VIS; λ _{MAX} in nm (log ε), [] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
20a	Me	Me	Me	pH 1: 203 (4.28); 224 (4.21); 245 (4.19); 282 (3.68); 341 (4.08)	C ₉ H ₁₂ N ₆ (204.2): C 52.93 (52.37), H 5.92 (5.92), N 41.16 (41.17)
20b	C ₆ H ₅	C ₆ H ₅	Me	pH 1: 204 (4.68); [228 (4.43)]; 269 (4.36); 374 (4.26)	C ₁₉ H ₁₆ N ₆ × 0.5 H ₂ O (337.4): C 67.63 (68.10), H 5.08 (5.01), N 24.91 (24.64)
20c ⁴²	H	H	Me		
20d ³³	H	H	H		
2b	CH ₂ OC ₂ H ₅	H	H	pH 1: 242 (4.19); 284 (3.67); 337 (3.98); [348 (3.92)]	C ₉ H ₁₂ N ₆ O (220.2): C 49.11 (48.86), H 5.45 (5.24), N 38.16 (37.76)
6aa ³⁴	C ₆ H ₅	H	H		
20g ⁴⁵	CHOHCHOHCH ₃	H	H		
20h ³⁴	H	C ₆ H ₅	H		

^a Compounds **6aa** and **20c,d,g,h** were prepared according to literature.^{34,42,33,45,34}**Table 12.** Reduced 4-Amino-6/7-alkyl-(aryl)-5,6,7,8-tetrahydropteridines **21**

entry	R ₈	R ₉	R ₁₀	UV/VIS; λ _{MAX} in nm (log ε), [] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
21a	Me	Me	Me	pH 0: 277 (4.11)	C ₉ H ₁₆ N ₆ × 2 HCl (281.1): C 38.45 (38.39), H 6.46 (6.42), N 29.90 (29.44)
21b	C ₆ H ₅	C ₆ H ₅	Me	pH 0: 277 (4.13)	C ₁₉ H ₂₀ N ₆ × 2 HCl × H ₂ O (423.3): C 53.90 (53.68), H 5.67 (5.54), N 19.86 (20.02)
21c	H	H	Me	pH 0: 277 (4.07)	C ₇ H ₁₂ N ₆ × 2 HCl × H ₂ O (271.2): C 30.90 (31.02), H 5.20 (5.48), N 30.90 (30.68)
21d	H	H	H	MeOH: [209 (4.15)]; 224 (4.25); 299 (3.95)	C ₆ H ₁₀ N ₆ × 2 HCl (239.1): C 30.14 (30.41), H 5.06 (5.20), N 35.15 (34.95)
21e	CH ₂ OC ₂ H ₅	H	H	pH 0: 220 (4.72); 275 (4.09)	C ₉ H ₁₆ N ₆ O × 3 HCl × H ₂ O (351.4): C 30.75 (30.70), H 5.97 (5.75), N 23.90 (23.96)
21f	C ₆ H ₅	H	H	pH 0: 209 (4.39); 218 (4.38); 273 (4.15)	C ₁₂ H ₁₄ N ₆ × 2 HCl × H ₂ O (333.2): C 43.25 (42.89), H 4.84 (5.31), N 25.22 (24.81)
21g	CHOHCHOHCH ₃	H	H	MeOH: 219 (4.37); 290 (4.02)	C ₉ H ₁₆ N ₆ O ₂ × 2 HCl × H ₂ O (331.2): C 32.64 (32.50), H 6.09 (6.07), N 25.37 (25.10)
21h	H	C ₆ H ₅	H	pH 0: 216 (4.38); 273 (4.17)	C ₁₂ H ₁₄ N ₆ × 2 HCl × 0.5 H ₂ O (324.2): C 44.45 (44.76), H 5.29 (5.25), N 25.92 (25.87)

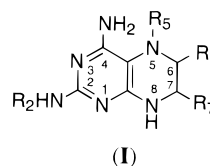
Table 13. Substitution in 5-Position of **21d**

entry	R ₁₁
22da	H
22db	C ₆ H ₅

Scheme 5. Synthesis of the 2,4-Diamino-7-benzyl (**24**) and 7-benzoylpteridines (**25**)**Scheme 6.** Synthesis of 2,4-Diamino-7-aminomercaptopteridine (**27**)

phobic interactions, due to a long aliphatic side chain like in **2d,e**, are thus most likely responsible for NOS-I inhibition at this 6-position.

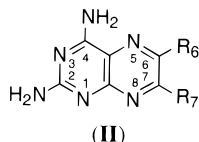
In the case of inactive compounds of the aromatic 4-amino pteridine scaffold (general formula **II**, Table 15: **6aa,ae,af**), additional changes in the 2-position of

Table 14. Inhibition of NOS-I by 4-Amino-5,6,7,8-tetrahydropteridine Derivatives **I**

compd (entry)	R ₂	R ₅	R ₆	R ₇	NOS activity (% of V _{max}) ^a	IC ₅₀ (μM) ^b
21d	H	H	H	H	102 ± 8	
21c	CH ₃	H	H	H	108 ± 23	
21b	CH ₃	H	C ₆ H ₅	C ₆ H ₅	86 ± 23	
21a	CH ₃	H	CH ₃	CH ₃	57 ± 5	
22da	H	CHO	H	H	61 ± 1	300
22db	H	COC ₆ H ₅	H	H	59 ± 6	
21g	H	H	CHOHCHOHCH ₃	H	38 ± 2	6
21h	H	H	H	C ₆ H ₅	39 ± 1	24
21f	H	H	C ₆ H ₅	H	17 ± 1	6
21e	H	H	CH ₂ OCH ₂ CH ₃	H	3 ± 1	30

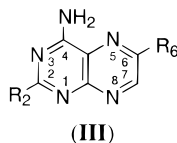
^a Inhibition of H₄Bip (2 μM) stimulated NOS total activity at an inhibitor concentration of 100 μM. ^b The SEM of the IC₅₀ was generally lower than 5% and omitted in the table.

the pyrimidine moiety failed to produce NOS inhibitors (general formula **III**, Table 16). These changes included the replacement of the 2-amino group with either a hydrogen (**7ba,be,bf, 9b**), a methylthio (**7ca,ce,cf, 9c**), a phenyl (**7da,df, 9d**), or a 2-hydroxyethylamino substituent (**7ea,ee,ef**). However, modification at the 4-amino substituent by dialkyl/diaralkylation of the heteroaromatic 2,4-diamino pteridine nucleus (general structure **IV**; Table 17), e.g., dimethylation (**17a**), combined with an anisyl (**17af**, IC₅₀: 74 μM) but not a phenyl (**17aa**)

Table 15. Effect of Substituents in the 6- and the 7-Position on Inhibition of NOS-I by Aromatic 2,4-Diamino Pteridine Derivatives **II**

compd (entry)	R ₆	R ₇	NOS activity (% of V _{max}) ^a	IC ₅₀ (μM)
20d	H	H	104 ± 4	
24	H	CH ₂ C ₆ H ₅	89 ± 6	
25	H	COC ₆ H ₅	90 ± 7	
27	H	SNH ₂	71 ± 1	
6aa	C ₆ H ₅	H	85 ± 24	
6ab	4-FC ₆ H ₄	H	87 ± 11	
6ac	4-ClC ₆ H ₄	H	94 ± 5	
6ad	4-BrC ₆ H ₄	H	67 ± 11	
6ae	4-CH ₃ C ₆ H ₄	H	113 ± 12	
6af	4-CH ₃ OC ₆ H ₄	H	109 ± 19	
6ag	3,4-(CH ₃ O) ₂ C ₆ H ₃	H	78 ± 10	
6ah	C ₆ H ₅	CH ₃	79 ± 11	
6ai	C ₆ H ₅	C ₆ H ₅	62 ± 4	
2b	CH ₂ OCH ₂ CH ₃	H	115 ± 5	
2c	CH ₂ OC ₆ H ₅	H	87 ± 28	180
2d	CH ₂ O(CH ₂) ₉ CH ₃	H	16 ± 1	30
2e	CH ₂ OCO(CH ₂) ₂ C ₆ H ₄ COC ₆ H ₄	H	29 ± 6	48
2f	CH ₂ NH ₂	H	94 ± 3	
2g	CH ₂ N(CH ₃) ₂	H	111 ± 3	
2h	CH ₂ SC ₆ H ₅	H	103 ± 4	
2i	CH ₂ NHCOCH(CH ₃) ₂	H	101 ± 0.3	

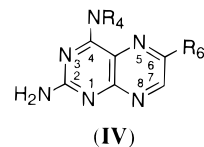
^a Inhibition of H₄Bip (2 μM) stimulated NOS total activity at an inhibitor concentration of 100 μM.

Table 16. Effect of Additional Substituents in the 2-Position on Inhibition of NOS-I by Aromatic 4-Amino-6-Substituted Pteridine Derivatives **III**

compd (entry)	R ₂	R ₆	activity (% of V _{max}) ^a
7ba	H	C ₆ H ₅	79 ± 6
7be	H	4-CH ₃ C ₆ H ₄	104 ± 4
7bf	H	4-CH ₃ OC ₆ H ₄	101 ± 8
9b	H	CH=NOH	81 ± 10
7ca	SCH ₃	C ₆ H ₅	104 ± 18
7ce	SCH ₃	4-CH ₃ C ₆ H ₄	96 ± 13
7cf	SCH ₃	4-CH ₃ OC ₆ H ₄	104 ± 14
9c	SCH ₃	CH=NOH	79 ± 11
7da	C ₆ H ₅	C ₆ H ₅	86 ± 11
7df	C ₆ H ₅	4-CH ₃ OC ₆ H ₄	105 ± 13
9d	C ₆ H ₅	CH=NOH	93 ± 6
7ea	NHCH ₂ CH ₂ OH	C ₆ H ₅	100 ± 27
7ee	NHCH ₂ CH ₂ OH	4-CH ₃ C ₆ H ₄	114 ± 9
7ef	NHCH ₂ CH ₂ OH	4-CH ₃ OC ₆ H ₄	112 ± 5

^a Inhibition of H₄Bip (2 μM) stimulated NOS total activity at an inhibitor concentration of 100 μM. Since the degree of NOS inhibition with 100 μM anti-pterin was small, no IC₅₀ values were determined.

group in 6-position, led to inhibitors with moderate potency, whereas diethylation (**17b**) at the 4-amino group, combined with an anisyl group in the 6-position (**17bf**, IC₅₀: 45 μM) increased inhibitory effectiveness further down to 2% of V_{max}. Dibenzoylation at the 4-amino substituent together with either a phenyl group (**17ca**, IC₅₀: 3 μM) or an anisyl group (**17cf**, IC₅₀: 5 μM) in the 6-position of **IV** led to complete inhibition of

Table 17. Effect of Additional Disubstitution at the 4-Amino Position on Inhibition of NOS-I by Aromatic 2,4-Diamino-6-aryl Pteridine Derivatives **IV**

compd (entry)	R ₄	R ₆	NOS activity (% of V _{max}) ^a	IC ₅₀ (μM) ^b
17aa	(CH ₃) ₂	C ₆ H ₅	92 ± 11	
17af	(CH ₃) ₂	4-CH ₃ OC ₆ H ₄	13 ± 4	74
17ba	(C ₂ H ₅) ₂	C ₆ H ₅	75 ± 3	
17bf	(C ₂ H ₅) ₂	4-CH ₃ OC ₆ H ₄	2 ± 0.1	45
17ca	(CH ₂ C ₆ H ₅) ₂	C ₆ H ₅	0 ± 0.05	3
17cf	(CH ₂ C ₆ H ₅) ₂	4-CH ₃ OC ₆ H ₄	0 ± 0.05	5
17da	(C ₂ H ₄) ₂ O	C ₆ H ₅	41 ± 8	82
17df	(C ₂ H ₄) ₂ O	4-CH ₃ OC ₆ H ₄	5 ± 0.1	34
17ea	(CH ₂) ₅	C ₆ H ₅	0 ± 0.05	62
17ef	(CH ₂) ₅	4-CH ₃ OC ₆ H ₄	7 ± 0.2	50
17fa	(C ₂ H ₄) ₂ NCH ₃	C ₆ H ₅	83 ± 1	
17ff	(C ₂ H ₄) ₂ NCH ₃	4-CH ₃ OC ₆ H ₄	84 ± 5	

^a Inhibition of H₄Bip (2 μM) stimulated NOS total activity at an inhibitor concentration of 100 μM. ^b IC₅₀ values were only determined for those compounds which inhibited NOS total activity at least by 50%.

NOS activity (to 0% of V_{max}) with IC₅₀ values in the very low micromolar range. The integration of the 4-amino group into an alicyclic ring such as a morpholine (**17da,df**), piperidine (**17ea,ef**) or 4-methyl piperazine ring (**17fa,ff**) system, combined with a phenyl- (**17da,ea,fa**) or an anisyl- (**17df,ef,ff**) group in 6-position, respectively, was successful and generated, in the case of the first two heterocyclic substituents, highly effective inhibitors with complete enzyme inhibition to 0% of V_{max} (**17df**, IC₅₀: 34 μM; **17ef**, IC₅₀: 62 μM; **17ef**, IC₅₀: 50 μM). In contrast, introducing a second basic group, as in the 4-methyl piperazine derivative (**17fa,ff**: still ~80% of V_{max}), decreased the NOS inhibitory potency of **IV**.

Thus, given the lower IC₅₀ values of the nucleus **IV** (down to 3 μM, see Table 17; with a disubstituted 4-amino group) compared to **II** (Table 15; **6aa,ae,af** with an unsubstituted 4-amino group), this suggested an increased interaction between enzyme and inhibitor, possibly due to an improved fit within the pterin binding pocket. Furthermore, it became apparent that in nearly all cases, substitution at the 6-position of **IV** by an anisyl, contrary to a phenyl ring, led to an increase in potency with respect to NOS-I inhibition.

Discussion

We systematically developed, screened, and refined a novel class of 4-amino pteridine-based NOS inhibitors as possible therapeutic agents in NOS-mediated pathophysiology, e.g., stroke, septic shock, and inflammatory and neurodegenerative disease states. Previous studies from other investigators²⁸ and our laboratory²⁷ suggested that the 4-position of H₄Bip may be an important functional target for developing potent inhibitors of NOS. Indeed, a 4-amino substituted H₄Bip derivative was taken as a prototypical inhibitor^{27,28} and, therefore, as a lead compound on which this investigation is based.

Initial investigations focused on varying the chemical substitution pattern in the 6-position of the aromatic

that the hydrophobic structural feature at this part of the molecule is crucial for NOS-I inhibition potential.

In conclusion, the addition of either a bulky aromatic substituent or an aliphatic side chain in the 6-position of the reduced 2,4-diamino-5,6,7,8-tetrahydropteridine derivatives **I** resulted in potent NOS inhibitors, possibly due to hydrophobic interactions, e.g., with Phe⁴⁷⁰, unlike the aromatic 2,4-diamino-6-substituted pteridine **II**. In contrast to **II**, the ability of the aromatic 2-amino-4-dialkyl/diaralkyl-6-substituted pteridine compounds **IV** to inhibit NOS-I activity appears to be due to increased hydrophilic and hydrophobic interactions of the 4-substituent within the binding pocket compared to the naturally occurring (6*R*)-5,6,7,8-tetrahydrobiopterin cofactor. Interestingly, anisylation at C-6 markedly potentiated the inhibitory effectiveness of **IV**. The present findings using the 4-amino pteridine derivatives **I–IV**, support an intimate localization of the pterin binding pocket adjacent to the heme and the L-arginine binding site in the crystal structure of NOS-II. From our structure–activity relationship study, we suggest that the upper portion of the pterin molecule (position 4, 5, and 6 of H₄Bip) is responsible for efficient insertion and binding of H₄Bip into the pterin binding pocket of NOS. In addition, the present SAR agrees with comparative molecular field analysis (CoMFA) of a data set of selected NOS-I inhibitors, which resulted in a statistically significant and predictive 3D-QSAR model of the pterin binding site interactions (unpublished observation). Thus, further chemical alterations of H₄Bip at these positions may represent an approach to novel, pterin-based NOS antagonists with increased potency. Importantly, highly species- and isoform-specific effects were observed (unpublished observation), raising the possibility to specifically target a particular isoform of NOS by using the anti-pterin approach.

Experimental Section

Chemistry. Thin-layer chromatography (TLC): precoated silica gel thin-layer sheets F 1500LS254 and cellulose thin-layer sheets F 1440LS254 from Schleicher and Schüll (Dassel, Germany). Detection by UV light λ : 254 and 366 nm. Flash chromatography (FC): silica gel (Baker, 30–60 μ m), 0.3–0.4 bar. High-performance liquid chromatography (HPLC): Merck-Hitachi (Darmstadt, Germany) L-6200, D-2000 chromatointegrator. UV/VIS: Perkin-Elmer (Überlingen, Germany), Lambda 15; IMAX in nm (log ϵ); [] shoulder. Products were dried either at room temperature under high vacuum or in an oven at 100 °C. Mp: Gallenkamp MFB 595 (England) or Büchi 510 (Switzerland) melting point apparatus; no corrections.

Synthesis. General Procedure A: 6-Substituted 2,4-Diamino Pteridines (2). 2,4-Diamino-6-bromomethylpteridine \times HBr \times 2-hydroxypropane (**1**)²⁹ (1.00 g, 2.5 mmol) was reacted with the appropriate nucleophile by stirring at room temperature. The resulting precipitate was collected and purified by recrystallization.

2,4-Diamino-6-methoxymethylpteridine (2a).³⁰ Sodium (0.69 g, 3 mmol) was dissolved in absolute MeOH (50 mL) before compound **1** (1 g) was added, and the mixture stirred for 6 h. The solution was then evaporated to one-third of the volume, and the precipitate (0.394 g) was collected and recrystallized from DMF/H₂O with charcoal. Yield: 0.19 g (36%). Mp: 256–257 °C.

2,4-Diamino-6-ethoxymethylpteridine (2b). Method was analogous to the preceding procedure, using absolute EtOH (50 mL) and sodium with stirring for 1 h and recrystallization from DMF (15 mL). Yield: 0.18 g (32%). Mp: 240–241 °C.

2,4-Diamino-6-phenoxyethylpteridine (2c).³¹ Phenol (0.56 g, 6 mmol) and potassium *tert*-butoxide (0.67 g, 6 mmol)

were dissolved in dimethylacetamide (DMA) (30 mL), compound **1** was added, and the mixture was stirred for 5 days. The precipitate (0.31 g) was then collected and recrystallized from DMF (10 mL). Yield: 0.21 g (31%). Mp: 287–288 °C.

2,4-Diamino-6-*n*-decyloxymethylpteridine (2d). Sodium hydride (1.00 g, 80%) was added over a period of 1 h to a mixture of DMA (30 mL) and *n*-decanol (5 mL) with continuous stirring before compound **1** (1 g) was added. Stirring was continued at room temperature for 6 h and then diluted with H₂O (100 mL) and kept cool (0–4 °C) for 2 days. The precipitate was collected and recrystallized from EtOH/concentrated NH₃ (16:1). Yield: 0.40 g (50%). Mp: 231–232 °C.

6-[3-(4-Benzoylphenyl)propionyloxymethyl]-2,4-diamino-pteridine (2e). 3-(4-Benzoylphenyl)propionic acid (0.50 g, 2 mmol)³² was dissolved in DMF (10 mL) and then triethylamine (0.4 mL), and compound **1** (0.40 g, 1 mmol) was added with stirring. After 4 h it was filtered from a small precipitate, the filtrate was evaporated with silica gel (1.00 g), and then the crude product was purified on silica gel with a CH₂Cl₂/MeOH gradient (0–4% MeOH). The main fraction was evaporated, the residue treated with H₂O/MeOH (2:3) and filtered again, and the residue was dried to give a yellow powder. Yield: 0.03 g (8%). Mp: 208–210 °C (decomp.).

2,4-Diamino-6-aminomethylpteridine (2f). A solution of compound **1** (1.00 g) in DMF (20 mL) was cooled to –50 °C and then a slow stream of gaseous NH₃ bubbled through this solution for 2 h. Slow warming to room temperature was achieved by removing the cooling. Stirring was continued overnight, and the solution was then evaporated to dryness in high vacuum. The residue was treated with MeOH (20 mL) to give a yellow powder. The crude product was dissolved in hot 0.1 N AcOH (45 mL), treated with charcoal, and filtered, and the filtrate was adjusted to pH 12 by 2 N NaOH. On cooling, a yellow precipitate was obtained which, upon washing with EtOH and drying at 100 °C, gave a yellow powder. Yield: 0.29 g (60%). Mp: >350 °C.

2,4-Diamino-6-dimethylaminomethylpteridine (2g). Method was analogous to the preceding procedure, but in 2-methoxyethanol (20 mL) with dimethylamine gas. The crude product was further purified by dissolving in 1 N AcOH (20 mL), and a precipitate was obtained at pH 12 with 2 N NaOH. Yield: 0.25 g (60%). Mp: 276–277 °C.

2,4-Diamino-6-methylthiomethylpteridine (2h). Sodium methylthiolate (0.50 g), compound **1** (1.00 g), and potassium *tert*-butoxide (0.05 g) were added to DMA (10 mL), and the mixture was stirred at room temperature for 2 h. It was neutralized with 1 N HCl to pH 7, diluted with H₂O (20 mL), and chilled overnight on ice. The precipitate was collected and purified by dissolving in hot 1 N AcOH (40 mL), treated with charcoal, and precipitated by neutralization to pH 9 with ammonia, which gave upon filtration a yellow crystal powder. Yield: 0.38 g (67%). Mp: 290–295 °C (decomp.).

2,4-Diamino-6-isobutyrylaminoethylpteridine (2i). 2,4-Diamino-6-aminomethylpteridine (**2e**) (0.80 g, 4.2 mmol) was suspended in ethyl acetate (50 mL), and then isobutyric anhydride (10 mL) and 4-(dimethylamino)pyridine (0.05 g) were added. After it was heated for 2 h at 80 °C with stirring, the solution was evaporated in a vacuum. The residue was dissolved in EtOAc and purified on silica gel following elution with EtOAc. The main fraction was collected and evaporated, and the product was recrystallized from EtOAc/ether (2:1) to give 0.88 g (52%) of yellow crystals of 2,4-bis(isobutyrylamino)-6-isobutyryl-aminomethylpteridine. Mp: 192–193 °C. This intermediate (0.40 g, 1 mmol) was treated in MeOH (12 mL) with concentrated NH₃ (12 mL) by heating to 60 °C for 2 days. After cooling overnight, the yellow crystals were collected and dried at 60 °C. Yield: 0.14 g (77%). Mp: >215 °C (decomp.).

General Procedure B: 2,4-Diamino-6-phenylpteridines (6). 2,4,5,6-Tetraaminopyrimidine dihydrochloride (**4a**) (1.00 g, 4.7 mmol) was suspended in MeOH (50 mL) and heated to boiling, and then a solution of substituted phenylglyoxalmonoxime **5** (7 mmol) in MeOH (10 mL) was added dropwise over a 10 min period. After heating for 2 h and cooling to room

temperature, concentrated ammonia was added to pH 9 and stirred for 15 min. The usually chromatographically pure precipitate was collected to give 80–95% of a yellow solid. Further purification can be achieved by recrystallization from DMF.

2,4-Diamino-6-phenylpteridine (6aa)³⁴ was obtained from **4a** and phenylglyoxalmonoxime (**5a**)³⁵ (1.04 g). Yield: 93%. Mp: 345 °C. Lit. mp: 347–348 °C.

2,4-Diamino-6-(4-fluorophenyl)pteridine (6ab) was obtained from **4a** and *p*-fluorophenyl-glyoxalmonoxime (**5b**) (1.17 g). Yield: 1.19 g (87%). Mp: >300 °C.

2,4-Diamino-6-(4-chlorophenyl)pteridine (6ac) was obtained from **4a** and *p*-chlorophenyl-glyoxalmonoxime (**5c**)³⁸ (1.29 g). Yield: 1.15 g (90%). Mp: >300 °C.

2,4-Diamino-6-(4-bromophenyl)pteridine (6ad) was obtained from **4a** and *p*-bromophenyl-glyoxalmonoxime (**5d**) (1.59 g). Yield: 1.48 g (89%). Mp: >300 °C.

2,4-Diamino-6-(4-tolyl)pteridine (6ae) was obtained from **4a** and *p*-tolylglyoxalmonoxime (**5e**)³⁶ (1.14 g). Yield: 1.04 g (88%). Mp: >300 °C.

2,4-Diamino-6-(4-methoxyphenyl)pteridine (6af) was obtained from **4a** and *p*-anisylglyoxalmonoxime (**5f**)³⁷ (1.25 g). Yield: 1.15 g (91%). Mp: >300 °C.

2,4-Diamino-6-(3,4-dimethoxyphenyl)pteridine (6ag) was obtained from **4a** and 3,4-dimethoxyphenylglyoxalmonoxime (**5g**) (1.46 g). Yield: 1.32 g (87%). Mp: >280 °C (decomp.).

2,4-Diamino-7-methyl-6-phenylpteridine (6ah) was obtained from **4a** and 3-phenylmethylglyoxal-2-monoxime (**5h**) (1.15 g). Yield: 0.86 g (66%). Mp: >235 °C (decomp.).

2,4-Diamino-6,7-diphenylpteridine (6ai). See ref 34.

4-Amino-6-phenylpteridine (7ba). 4,5,6-Triaminopyrimidine dihydrochloride (**4b**)³⁹ (1.00 g, 5 mmol) was suspended in MeOH (30 mL) and then heated to reflux, and a solution of phenylglyoxalmonoxime (**5a**) (1.13 g, 7.6 mmol) in MeOH (10 mL) was added dropwise over a 10 min period. The mixture was heated for 2 h under reflux. After cooling it was neutralized by ammonia to pH 8 and stirred for 30 min. The yellow precipitate was collected and dried to give a chromatographically pure product. Yield: 0.99 g (88%). Mp: 265–268 °C (decomp.). The substance can be recrystallized from a large volume of MeOH.

4-Amino-6-(4-tolyl)pteridine (7be). Method was analogous to the preceding procedure, using instead **4b** (1.00 g) and **5e** (1.23 g). Yield: 1.08 g (90%). Mp: 285–287 °C (decomp.).

4-Amino-6-(4-methoxyphenyl)pteridine (7bf). Method was analogous to the preceding procedure, using instead **4b** (1.00 g) and **5f** (1.35 g). Yield: 1.26 g (99%). Mp: 270–272 °C.

4,5,6-Triamino-2-methylthiopyrimidine Dihydrochloride (4c). A suspension of 4,6-diamino-2-methylthio-5-nitrosopyrimidine (**3c**)⁴⁰ (5.00 g, 27 mmol) in MeOH (125 mL) was catalytically reduced with PtO₂/H₂ in a shaking apparatus until the theoretical amount of hydrogen was consumed. The clear solution was filtered from the catalyst and evaporated to dryness, and the residue was treated with saturated methanolic HCl (120 mL) with stirring for 3 h. The precipitate was collected and washed with MeOH and ether before being dried in a desiccator over P₄O₁₀ to give a colorless crystal powder. Yield: 6.12 g (93%). Mp: 252–257 °C (decomp.).

4-Amino-2-methylthio-6-phenylpteridine (7ca). Method was analogous to the procedure for **7ba**, using instead **4c** (1.00 g, 4.1 mmol) and **5a** (0.95 g, 6.15 mmol). Yield: 1.07 g (97%). Mp: 293–295 °C (decomp.). Recrystallization from DMF/H₂O (10:1) gave yellow needles.

4-Amino-2-methylthio-6-(4-tolyl)pteridine (7ce). Method was analogous to the procedure for **7ba**, using instead **4c** (1.00 g, 4.1 mmol) and **5e** (1.23 g, 6.15 mmol). Yield: 1.12 g (97%). Mp: 292–293 °C (decomp.). Recrystallization was done from DMF/H₂O (10:1).

4-Amino-2-methylthio-6-(4-methoxyphenyl)pteridine (7cf). Method was analogous to the procedure for **7ba**, using instead **4c** (1.00 g, 4.1 mmol) and **5f** (1.35 g). Yield: 1.18 g (96%). Mp: 291–293 °C.

4,5,6-Triamino-2-phenylpyrimidine Dihydrochloride (4d). Analogous to the procedure for **4c**, a suspension of 4,6-diamino-5-nitroso-2-phenylpyrimidine **3d**⁴⁰ (3.10 g, 14 mmol) was catalytically reduced and gave on workup a colorless powder. Yield: 3.40 g (88%). Mp: >220 °C (decomp.).

4-Amino-2,6-diphenylpteridine (7da). Method was analogous to the procedure for **4c**, using instead **4d** (1.00 g, 3.65 mmol) and **5a** (0.82 g, 5.5 mmol). Yield: 0.96 g (88%). Recrystallization from DMF/H₂O gave yellow needles. Mp: 287–290 °C.

4-Amino-6-(4-methoxyphenyl)-2-phenylpteridine (7df). Method was analogous to the procedure for **4c**, using instead **4d** (1.00 g, 3.65 mmol) and **5f** (0.98 g, 5.5 mmol). Yield: 1.07 g (89%). Recrystallization from DMF/H₂O gave yellow needles. Mp: 292–295 °C.

4,6-Diamino-2-(2-hydroxyethyl)amino-5-nitrosopyrimidine (3e). A mixture of 4,6-diamino-2-methylthio-5-nitrosopyrimidine **3d**⁴⁰ (2.00 g, 10.8 mmol) and ethanolamine (10 mL) in H₂O (50 mL) was heated with stirring for 3 h to 60 °C. After cooling, it was evaporated to half of the volume, and the precipitate was collected, washed, and dried in a desiccator. Recrystallization from EtOH/H₂O (2:1) gave violet crystals. Yield: 1.18 g (55%). Mp: 235–238 °C. UV (H₂O): [231 (3.50)]; [274 (3.59)]; 333 (4.35). Anal. Calcd for C₆H₁₀N₆O₂ (198.2): C, 36.36; H, 5.09; N, 42.41. Found: C, 36.43; H, 5.14; N, 42.34.

4,5,6-Triamino-2-(2-hydroxyethyl)aminopyrimidine Trihydrochloride (4e). Analogous to the procedure for **4c**, a suspension of 4,6-diamino-2-(2-hydroxyethylamino)-5-nitrosopyrimidine **3e** (5.00 g, 25.3 mmol) was catalytically reduced with Pd/C (0.20 g) under H₂ atm. Yield: 1.36 g (92%). Mp: >205 °C (decomp.).

4-Amino-2-(2-hydroxyethylamino)-6-phenylpteridine (7ea). Method was analogous to the procedure for **7ba**, using instead **4e** (1.00 g, 3.4 mmol) and **5a** (0.76 g, 5.1 mmol). Yield: 0.87 g (91%). Mp: 232–239 °C.

4-Amino-2-(2-hydroxyethylamino)-6-(4-tolyl)pteridine (7ee). Method was analogous to the procedure for **7ba**, using instead **4e** (1.00 g, 3.4 mmol) and **5e** (0.83 g, 5.1 mmol). Yield: 0.56 g (56%). Mp: 259–263 °C (decomp.).

4-Amino-2-(2-hydroxyethylamino)-6-(4-methoxyphenyl)pteridine (7ef). Method was analogous to the procedure for **7ba**, using instead **4e** (1.00 g, 3.4 mmol) and **5f** (0.92 g, 5.1 mmol). Yield: 0.93 g (87%). Mp: 270–275 °C.

4-Aminopteridine-6-carboxaldoxime (9b). A suspension of 4,5,6-triaminopyrimidine dihydrochloride (**4b**) (0.80 g, 4 mmol) in MeOH (20 mL) was heated under reflux. A solution of mesoxalaldicarboxaldoxime (**8**) (0.70 g, 6 mmol) in MeOH (10 mL) was added slowly dropwise, and then the reaction mixture was heated for 2 h. After cooling, it was neutralized with concentrated ammonia to pH 7 and stirred for 30 min, and then the precipitate was collected, washed with MeOH, and dried at 100 °C. Yield: 0.74 g (96%). Mp: >250 °C (decomp.).

4-Amino-2-methylthiopyrimidine-6-carboxaldoxime (9c)⁴³. Method was analogous to the preceding procedure, using instead 4,5,6-triamino-2-methylthiopyrimidine dihydrochloride (**4c**) (1.00 g, 4.1 mmol) and **8** (0.70 g, 6 mmol). Yield: 0.96 g (99%). Mp: >250 °C (decomp.). Lit. mp: 250 °C.

4-Amino-2-phenylpteridine-6-carboxaldoxime (9d). Method was analogous to the preceding procedure, using instead 4,5,6-triamino-2-phenylpyrimidine dihydrochloride (**4d**) (1.00 g, 3.7 mmol) and **8** (0.65 g, 5.5 mmol). Yield: 0.81 g (83%). Mp: >249 °C (decomp.).

2,6-Diamino-4-chloro-5-*p*-chlorophenylazopyrimidine (12)⁴⁴. A solution of *p*-chloroaniline **10** (25.5 g, 0.2 mol) in 6 N HCl (100 mL) was cooled to 0–5 °C, and then NaNO₂ (13.8 g, 0.2 mol) in water (40 mL) was added dropwise with stirring. After the addition was completed, the solution was stirred for another 15 min and checked by iodine-starch-paper to give a blue color. Urea (5.00 g) was added to destroy the excess of HNO₂. The diazonium salt solution was then poured into a solution of 2,6-diamino-4-chloropyrimidine **11** (26.0 g, 0.18 mol) in water (500 mL) and stirred for 30 min. Potassium acetate (70.0 g) was then added, and the mixture was stirred for 16 h at room temperature. The resulting precipitate was

collected by suction, washed with H₂O and dried in a vacuum desiccator over P₂O₁₀ to give a yellow solid. Yield: 44.0 g (81%). Recrystallization was done from DMF/H₂O. Mp: 268 °C. Lit. mp: 270 °C. Anal. Calcd for C₁₀H₈Cl₂N₆ × H₂O (301.1): C, 39.89; H, 3.35; N, 27.91. Found: C, 40.14; H, 2.95; N, 27.64.

2,6-Diamino-4-diethylamino-5-*p*-chlorophenylazopyrimidine (14b). A solution of 2,6-diamino-4-chloro-5-*p*-chlorophenylazopyrimidine (**12**) (5.00 g, 16.6 mmol) in DMF (50 mL) and diethylamine **13b** (10.0 g) was heated in an oil bath to 70 °C for 5 h. Then water (50 mL) was added, the solution cooled, and the yellow precipitate was collected, washed with water, and dried. Recrystallization was done from EtOH. Yield: 3.04 g (56%). Mp: 145–148 °C. UV (60% H₂SO₄): 213 (4.28); 266 (4.22); [334 (3.93)]; 389 (4.38); [420 (4.29)]. Anal. Calcd for C₁₄H₁₈ClN₇ × 0.5 H₂O (328.8): C, 51.14; H, 5.82; N, 29.82. Found: C, 51.36; H, 5.54; N, 29.29.

2,6-Diamino-4-dibenzylamino-5-*p*-chlorophenylazopyrimidine (14c). Method was analogous to the preceding procedure, using instead **12** (5.00 g) and dibenzylamine **13c** (10.0 g). Recrystallization from acetone/H₂O gave yellow crystals. Yield: 5.22 g (67%). Mp: 185–186 °C. Anal. Calcd for C₂₄H₂₆ClN₇ × H₂O (465.9): C, 61.88; H, 6.06; N, 21.04. Found: C, 61.53; H, 5.83; N, 21.88.

2,6-Diamino-4-morpholino-5-*p*-chlorophenylazopyrimidine (14d). Method was analogous to the preceding procedure, using instead **12** (5.00 g) and morpholine **13d** (10.0 g). Recrystallization was done from EtOH. Yield: 5.00 g (90%). Mp: 219–221 °C. UV (60% H₂SO₄): 213 (4.30); 271 (4.17); [333 (3.86)]; 389 (4.35); [414 (4.24)]. Anal. Calcd for C₁₄H₁₆ClN₇O (333.8): C, 50.38; H, 4.83; N, 29.37. Found: C, 50.55; H, 4.83; N, 29.37.

2,6-Diamino-4-piperidino-5-*p*-chlorophenylazopyrimidine (14e). Method was analogous to the preceding procedure, using instead **12** (5.00 g) and piperidine **13e** (10.0 g). Recrystallization from EtOH gave yellow crystals. Yield: 4.85 g (88%). Mp: 199–201 °C. UV (60% H₂SO₄): 213 (4.20); 271 (4.15); [339 (3.86)]; 396 (4.29); [421 (4.22)]. Anal. Calcd for C₁₅H₁₈ClN₇ (331.8): C, 54.30; H, 5.47; N, 26.08. Found: C, 54.10; H, 5.27; N, 28.81.

2,6-Diamino-4-*N*-methylpiperazino-5-*p*-chlorophenylazopyrimidine (14f). Method was analogous to the preceding procedure, using instead **12** (5.00 g) and *N*-methylpiperazine **13f** (10.0 g). Yield: 5.50 g (91%). Mp: 218–220 °C. UV (60% H₂SO₄): 212 (4.38); 270 (4.24); [331 (3.85)]; 389 (4.38); [416 (4.28)]. Anal. Calcd for C₁₅H₁₉ClN₈ × H₂O (364.8): C, 49.38; H, 5.80; N, 30.71. Found: C, 49.02; H, 5.64; N, 30.42.

2,5,6-Triamino-4-dimethylaminopyrimidine Dihydrochloride (16a). Method was analogous to the procedure for **4c**, using instead 2,6-diamino-4-(dimethylamino)-5-nitrosopyrimidine **15a**⁴¹ (5.00 g, 27.5 mmol) with PtO₂ (0.50 g) as catalyst under H₂ atm. Yield: 2.68 g (56%). Mp: 210–212 °C (decomp.). UV (H₂O): [231 (4.12)]; 300 (4.08). Anal. Calcd for C₆H₁₄Cl₂N₆ (241.0): C, 29.89; H, 5.85; N, 34.85. Found: C, 29.73; H, 5.77; N, 34.81.

2,5,6-Triamino-4-diethylaminopyrimidine Dihydrochloride (16b). A suspension of 2,6-diamino-4-diethylamino-5-*p*-chlorophenylazopyrimidine (**14b**) (3.28 g, 10 mmol) in MeOH (70 mL) and concentrated ammonia (10 mL) was reduced in a shaking apparatus under H₂ atm in the presence of Raney nickel catalyst (3.50 g) for 2 days. The catalyst was filtered off under argon atmosphere, and then the filtrate was evaporated in a vacuum to dryness. The residue was treated with ether to remove the *p*-chloroaniline and filtered, and then the solid was stirred in methanolic HCl (10%, 50 mL) overnight. The dihydrochloride salt was collected and dried in a vacuum desiccator over KOH. Yield: 2.63 g (89%). Mp: 138–142 °C. UV (MeOH): 213 (4.19); 233 (4.22); 294 (4.10); [323 (3.54)]. Anal. Calcd for C₈H₁₆N₆ × 2 HCl × 1.5 H₂O (296.2): C, 32.44; H, 6.46; N, 28.37. Found: C, 32.37; H, 6.37; N, 29.35.

2,5,6-Triamino-4-dibenzylaminopyrimidine Dihydrochloride (16c). Method was analogous to the preceding procedure, using instead **14c** (4.65 g, 10 mmol). Yield: 3.82 g (93%). Mp: 165–167 °C. UV (MeOH): 206 (4.41); 233 (4.26); 295 (4.10); [332 (3.62)]. Anal. Calcd for C₁₈H₂₀N₆ × 2 HCl ×

H₂O (411.3): C, 52.56; H, 5.88; N, 20.43. Found: C, 52.54; H, 5.94; N, 20.49.

2,5,6-Triamino-4-morpholinopyrimidine Dihydrochloride (16d). Method was analogous to the preceding procedure, using instead **14d** (3.34 g, 10 mmol). Yield: 1.81 g (64%). Mp: 215–218 °C (decomp.). UV (MeOH): 208 (4.04); 237 (4.26); 301 (4.09); [332 (3.89)]. Anal. Calcd for C₈H₁₄N₆ × 2 HCl (283.2): C, 33.93; H, 5.70; N, 29.68. Found: C, 34.04; H, 5.42; N, 29.47.

2,5,6-Triamino-4-piperidinopyrimidine Dihydrochloride (16e). Method was analogous to the preceding procedure, using instead **14e** (3.32 g, 100 mmol). Yield: 2.11 g (75%). Mp: 238–242 °C. UV (MeOH): 219 (4.03); 234 (4.01); 300 (3.77); [328 (3.61)]. Anal. Calcd for C₈H₁₆N₆ × 2 HCl (281.2): C, 38.44; H, 6.45; N, 29.89. Found: C, 38.11; H, 5.98; N, 29.53.

2,5,6-Triamino-4-*N*-methylpiperazinopyrimidine trihydrochloride (16f). Method was analogous to the preceding procedure, using instead **14f** (3.65 g, 100 mmol). Yield: 3.07 g (88%). Mp: 226–230 °C. (decomp.). UV (MeOH): [205 (3.88)]; 236 (4.27); 300 (3.92); [336 (3.75)]. Anal. Calcd for C₉H₁₇N₇ × 3 HCl × H₂O (350.7): C, 30.83; H, 6.32; N, 27.96. Found: C, 30.99; H, 6.29; N, 27.29.

General Procedure C: 2-Amino-4-dialkylamino-6-aryl Pteridines 17. A solution of arylglyoxalmonoxime **5** (7.5 mmol) in MeOH (10 mL) was added dropwise to a boiling solution of 2,5,6-triamino-4-dialkylaminopyrimidine dihydrochloride salt **16** (5 mmol) in MeOH (20 mL), and then the mixture was heated under reflux for 3 h. After cooling, the suspension or solution was made alkaline by concentrated ammonia to pH 9–10, and the resulting precipitate was filtered off, washed with water, and dried. Recrystallization was done from EtOH and DMF/H₂O, respectively, to give a yellow solid.

2-Amino-4-dimethylamino-6-phenylpteridine (17aa). Method was analogous to the general procedure, using instead **16a** (0.80 g, 3.3 mmol) and **5a** (0.74 g, 5 mmol). Yield: 0.76 g (86%). Mp: 247–250 °C.

2-Amino-4-dimethylamino-6-(4-methoxyphenyl)pteridine (17af). Method was analogous to the general procedure, using instead **16a** (0.80 g) and **5f** (0.89 g, 5 mmol). Yield: 0.80 g (81%). Mp: 280–284 °C (decomp.).

2-Amino-4-diethylamino-6-phenylpteridine (17ba). According to the general procedure, **17ba** was obtained from **16b** (1.48 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 0.87 g (56%). Mp: 203–205 °C.

2-Amino-4-diethylamino-6-(4-methoxyphenyl)pteridine (17bf). According to the general procedure, **17bf** was obtained from **16b** (1.48 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 0.79 g (46%). Mp: 220–222 °C.

2-Amino-4-dibenzylamino-6-phenylpteridine (17ca). According to the general procedure, **17ca** was obtained from **16c** (2.06 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 1.57 g (69%). Mp: 225–227 °C.

2-Amino-4-dibenzylamino-6-(4-methoxyphenyl)pteridine (17cf). According to the general procedure, **17cf** was obtained from **16c** (2.06 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 1.57 g (70%). Mp: 245–247 °C.

2-Amino-4-morpholino-6-phenylpteridine (17da). According to the general procedure, **17da** was obtained from **16d** (1.42 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 1.37 g (84%). Mp: 224–227 °C.

2-Amino-4-morpholino-6-(4-methoxyphenyl)pteridine (17df). According to the general procedure, **17df** was obtained from **16d** (1.42 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 1.53 g (78%). Mp: 238–240 °C.

2-Amino-4-piperidino-6-phenylpteridine (17ea). According to the general procedure, **17ea** was obtained from **16e** (1.41 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 1.37 g (80%). Mp: 209–211 °C.

2-Amino-4-piperidino-6-(4-methoxyphenyl)pteridine (17ef). According to the general procedure, **17ef** was obtained from **16e** (1.41 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 1.40 g (81%). Mp: 211–214 °C.

2-Amino-4-*N*-methylpiperazino-6-phenylpteridine (17fa). According to the general procedure, **17fa** was obtained from

16f (1.75 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 0.96 g (58%). Mp: 245–247 °C.

2-Amino-4-N-methylpiperazino-6-(4-methoxyphenyl)-pteridine (17ff). According to the general procedure, **17ff** was obtained from **16f** (1.75 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 1.55 g (83%). Mp: 228–230 °C.

4-Amino-6,7-dimethyl-2-methylaminopteridine (20a). A solution of 4,5,6-triamino-2-methylaminopyrimidine dihydrochloride (**18**) (2.25 g, 0.01 mol) in water (50 mL) was treated with diacetyl **19a** (1.50 g) in dioxane (10 mL) and then heated under reflux for 1 h. The warm solution was neutralized with concentrated ammonia to pH 9, and the precipitate was collected after cooling. Yield: 1.75 g (86%). Mp: 268 °C (decomp.).

4-Amino-2-methylamino-6,7-diphenylpteridine (20b). Analogous to the preceding procedure, **20b** was obtained from **18** (2.25 g, 0.01 mol) and benzil **19b** (2.50 g, 0.01 mol) in EtOH (50 mL). Yield: 2.88 g (88%). Mp: 303 °C.

4-Amino-2-methylaminopteridine (20c). See ref 42.

2,4-Diaminopteridine (20d). See ref 33.

2,4-Diamino-6-(1,2-dihydroxypropyl)pteridine (20g). See ref 45.

2,4-Diamino-7-phenylpteridine (20h). See ref 34.

4-Amino-2-methylamino-6,7-dimethyl-5,6,7,8-tetrahydropteridine Dihydrochloride (21a). A solution of **20a** (0.61 g, 3 mmol) in TFA (25 mL) was reduced catalytically with PtO₂ (0.10 g)/H₂ in a shaking apparatus until the uptake of hydrogen ceased. The catalyst was filtered off, the filtrate was evaporated to dryness, and the residue was treated with methanolic HCl (10%, 20 mL) by stirring for several hours. The colorless crystal powder was collected, washed with ether, and dried in a vacuum desiccator. Yield: 0.59 g (68%). Mp: 265 °C.

4-Amino-2-methylamino-6,7-diphenyl-5,6,7,8-tetrahydropteridine Dihydrochloride (21b). Analogous to the preceding procedure, **21b** was obtained from **20b** (0.98 g, 3 mmol). Yield: 0.88 g (69%). Mp: 245 °C.

4-Amino-2-methylamino-5,6,7,8-tetrahydropteridine Dihydrochloride (21c). Analogous to the preceding procedure, **21c** was obtained from **20c** (0.54 g, 3 mmol). Yield: 0.50 g (61%). Mp: 275 °C.

2,4-Diamino-5,6,7,8-tetrahydropteridine Dihydrochloride (21d).³³ A suspension of 2,4-diaminopteridine **20d**³³ (3.00 g, 18.5 mmol) in MeOH (700 mL) was reduced catalytically with PtO₂ (0.90 g)/H₂ in a shaking apparatus for 3 days. The suspension was filtered under argon atmosphere, concentrated HCl (10 mL) was added to the filtrate, and the mixture was concentrated to 100 mL whereby a precipitate was formed, then diluted with ether (100 mL), and cooled for 3 h. Finally, the solid was collected, washed with ether, and dried in a vacuum desiccator. Yield: 3.93 g (89%). Mp: 253 °C (decomp.).

2,4-Diamino-6-ethoxymethyl-5,6,7,8-tetrahydropteridine Trihydrochloride (21e). A solution of 2,4-diamino-6-ethoxymethylpteridine (**2b**) (0.91 g, 4.1 mmol) in trifluoroacetic acid (100 mL) was reduced catalytically with PtO₂ (0.10 g)/H₂ in a shaking apparatus for 16 h. The catalyst was filtered off, the filtrate evaporated, and the residue was treated with methanolic HCl (20 mL). The precipitate was collected, recrystallized from MeOH/concentrated HCl with charcoal to give a colorless powder. Yield: 0.47 g (38%). Mp: > 130 °C (decomp.).

2,4-Diamino-6-phenyl-5,6,7,8-tetrahydropteridine Dihydrochloride (21f). A solution of 2,4-diamino-6-phenylpteridine (**6aa**)³⁴ (4.28 g, 0.02 mol) in TFA (50 mL) was reduced catalytically with PtO₂ (0.20 g)/H₂ in a shaking apparatus until the uptake of hydrogen ceased. The catalyst was filtered off, the filtrate evaporated to dryness, and then the residue was treated with methanolic HCl (10%, 50 mL) by stirring for 3 h. The resulting precipitate was collected, dissolved in MeOH, and then diluted with the same volume of ether. A colorless precipitate was formed. After cooling, the solid was collected and dried in a vacuum desiccator. Yield: 3.85 g (58%). Mp: 248 °C.

2,4-Diamino-6-(1,2-dihydroxypropyl)-5,6,7,8-tetrahydropteridine Dihydrochloride (21g). A suspension of 2,4-diamino-6-(1,2-dihydroxypropyl)pteridine (**20g**)⁴⁵ (0.40 g, 1.7 mmol) in MeOH (50 mL) was reduced catalytically with PtO₂ (0.05 g)/H₂ in a shaking apparatus until 2 mol of H₂ were consumed (2 days). Methanolic HCl (20%, 5 mL) was added, then the catalyst was filtered off and evaporated to dryness, and the residue was treated again with methanolic HCl (5 mL) for 3 h with stirring and cooling. The colorless precipitate was collected, washed with ether, and dried in a vacuum desiccator over KOH. Yield: 0.26 g (47%). Mp: >300 °C.

2,4-Diamino-7-phenyl-5,6,7,8-tetrahydropteridine Dihydrochloride (21h). According to the preceding procedure, **21h** was obtained from 2,4-diamino-7-phenylpteridine (**20h**)³⁴ (4.28 g, 0.02 mol). Yield: 5.00 g (75%). Mp: 240–242 °C.

2,4-Diamino-5-formyl-5,6,7,8-tetrahydropteridine (22da). See ref 33.

2,4-Diamino-5-benzoyl-5,6,7,8-tetrahydropteridine (22db). See ref 33.

2,4-Diamino-7-benzylpteridine (24). A solution of 2,4,5,6-tetraaminopyrimidine dihydrochloride (**4a**) (5.00 g, 23 mmol) in H₂O (250 mL) was adjusted with concentrated ammonia to pH 9. Benzylglyoxal (**23**)⁴⁶ (3.85 g, 25 mmol) in EtOH (100 mL) was added, and then the mixture was heated under reflux for 1 h. The precipitate was collected after cooling, washed with H₂O and acetone, and then dried at 100 °C to give a yellow powder. Yield: 3.65 g (60%). Mp: 260–264 °C (decomp.). UV (pH 8): 217 (4.15); 255 (4.32); [279 (3.72)]; 366 (3.97). Anal. Calcd for C₁₃H₁₂N₆ (252.3): C, 61.89; H, 4.79; N, 33.31. Found: C, 61.87; H, 4.98; N, 32.95.

2,4-Diamino-7-benzoylpteridine (25). 2,4-Diamino-7-benzylpteridine (**24**) (4.03 g, 16 mmol) in AcOH (150 mL) was heated to 70 °C in an oil bath, and then bromine (10.2 g, 64 mmol) was added dropwise with stirring. The mixture was heated under reflux for 1 h whereby a precipitate began to separate out after 15 min. After cooling, the solid was collected and dried (3.70 g). The filtrate was concentrated to 50 mL and allowed to stand overnight before a second crop was collected (1.70 g). The combined crude reaction product was then heated in a mixture of H₂O (80 mL) and dioxane (120 mL) for 1 h. The solution was cooled to 50 °C and then neutralized by NaOH (5%) to pH 9 to give a yellow precipitate (2.53 g). Recrystallization was done from dioxane/H₂O. Yield: 2.28 g (54%). Mp: 288–290 °C. UV (pH 7): 226 (4.33); 249 (4.31); [272 (4.22)]; 394 (3.89). Anal. Calcd for C₁₃H₁₀N₆O (266.3): C, 58.64; H, 3.79; N, 31.56. Found: C, 58.29; H, 3.82; N, 31.72.

2,4-Diamino-7-aminomercaptopteridine (27). A solution of 2,4-diamino-7-mercaptopteridine (**26**)⁴⁷ (0.98 g, 5 mmol) in 1 N NaOH (25 mL) was cooled to 0 °C, and then a cold solution of chloramine (prepared from concentrated ammonia (18 mL) and NaOCl (9 mL, 13%)) was added slowly with stirring. After 15 min, the resulting precipitate was collected, washed with H₂O and EtOH, and dried to give a yellowish crystal powder. Yield: 0.52 g (55%). Mp: >300 °C (decomp.). UV (pH 1): 205 (4.26); 224 (4.16); 250 (3.94); [356 (4.09)]; 369 (4.10). Anal. Calcd for C₆H₇N₇S × H₂O (227.2): C, 31.72; H, 3.99; N, 43.16. Found: C, 31.99; H, 3.53; N, 43.06.

Biology. [2,3,4,5-³H]L-Arginine hydrochloride (2.85 Tbq mmol⁻¹) was purchased from Amersham (Freiburg, Germany); H₄Bip, from Dr. B. Schircks Laboratories (Jona, Switzerland); β-nicotinamide adenine dinucleotide phosphate tetrasodium-salt tetrahydrate (NADPH), from Applchem GmbH (Darmstadt, Germany); flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and glutathione (GSH), from Boehringer-Mannheim (Mannheim, Germany); L-arginine hydrochloride, from Fluka (Buchs, Germany); 2',5'-adenosine diphosphate (ADP)-sepharose and calmodulin (CaM)-sepharose 4 B, from Pharmacia (Freiburg, Germany). All other chemicals were of the highest purity grade available and obtained from Sigma Chemicals (Deisenhofen, Germany). Water was deionized to 18 MΩ cm (Milli-Q; Millipore, Eschborn, Germany), deoxygenated by gassing with argon.

Enzyme Purification. Native NOS-I (nNOS) was isolated from porcine brain cerebellum by ammonium sulfate precipita-

tion and 2',5'-ADP-sepharose affinity chromatography and eluted with excess 2'-adenosine monophosphate (AMP) as previously described.⁴⁹ To exclude the possibility that the purification procedure per se influenced the inhibitory profile of the anti-pterins, native NOS-I was additionally purified by diethyl aminoethyl (DEAE) ion exchange chromatography (200 mM, NaCl elution buffer; 20–25 mL min⁻¹, flow rate) instead of ammonium sulfate precipitation and subsequent 2',5'-ADP-sepharose affinity chromatography. Finally, the enzyme was further purified in the presence of Ca²⁺ using CaM-sepharose 4 B and eluted by chelating Ca²⁺ with excess EGTA.⁷ The yield of this purification method was 0.50 mg of enzyme from 1.00 kg of tissue with greater than 90% purity and a specific activity of up to 670 nmol L-citrulline mg⁻¹ min⁻¹. Protein concentrations were determined spectrophotometrically according to a modified Bradford method⁵⁰ using bovine serum albumin as a standard and a Microplate reader (Molecular Devices; Sunnyvale, CA). The purity was established from densitometric scanning of Coomassie-stained SDS-PAGE gels using NIH Image software (National Institutes of Health; Bethesda, MD). NOS-I immunoreactive bands were examined by Western blot analysis using an ECL enhanced chemiluminescence kit (Amersham; Braunschweig, Germany) and a NOS-I specific antibody (Transduction Laboratories; Hamburg, Germany).

Enzyme Activity. NOS-I enzyme activity was determined from the calcium/calmodulin-dependent conversion of [³H]-L-arginine to [³H]-L-citrulline.^{3,49} NOS-I was incubated for 15 min at pH = 7.2 and 37 °C in a mixture containing 2 μM H₄Bip, 50 nM CaM, 1 mM CaCl₂, 5 μM FAD, 10 μM FMN, 250 μM 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonate (Chapso), 50 mM triethanolamine (TEA), 1 mM NADPH, 7 mM GSH, and 50 μM L-arginine + [2,3,4,5-³H]-L-arginine, and the reaction was stopped by adding ice-cold acetate buffer (pH = 5.5). [³H]-L-Citrulline was separated from the mixture by cation exchange chromatography, and the amount of radioactivity was determined by liquid scintillation counting. The effect of the anti-pterins, at an initial inhibitor concentration of 100 μM, on NOS-I stimulated total activity in the presence of 2 μM H₄Bip was examined. Dose-response curves of the most effective inhibitors (>50% inhibition of stimulated NOS activity) in the range from 0 to 1000 μM were constructed, and the corresponding IC₅₀ values were determined by nonlinear regression analysis (UltraFit software Biosoft, Cambridge, U.K., or GraphPad software, Prism, Version 2.0a, San Diego, CA).

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