Pteridines

Part CXI[1])

Pteridine-Based Photoaffinity Probes for Nitric Oxide Synthase and Aromatic Amino Acid Hydroxylases

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Various 6-substituted pteridines and 5,6,7,8-tetrahydropterins carrying photolabile functions at the side chain (see **7**, **20**–**22**, **34**–**36**, **38**, and **39**) as well as at the 5-position (see **27**–**29**) were synthesized from pterin and from 6-phenylpterin (**1**) and 6-(hydroxymethyl)pterin (**10**). Attachment of the photoaffinity labels *via* ester bonds required a special protecting-group strategy based upon acid-labile (see **30**–**33**) and β-eliminating blocking groups (see **17**–**19**). The 6-(4-azidophenyl)pterin (**7**) was obtained from 6-phenylpterin (**1**) *via* intermediates **2** and **4**–**6**, due to the low solubility of simple pterins in general. The pteridine derivatives **21**, **22**, **25**, **26**, **28**, **29**, **32**, **33**, **35**, **36**, **38**, and **39** were screened as inhibitors of neuronal (type I) NO synthase (see *Table*) from porcine cerebellum, of which **22**, **35**, **36**, and **38** showed interesting inhibitory activity with similar potency and effectiveness.

1. Introduction. – The technique of photoaffinity labeling has a high potential in characterizing the function of enzyme cofactors and substrate binding sites [2][3]. One such binding site which has recently gained considerable interest is located in tetrahydrobiopterin-requiring enzymes such as aromatic amino acid hydroxylases (tyrosine hydroxylase, phenylalanine hydroxylase) and NO synthases. Photolabile pteridines would be ideally suited to elucidate the tetrahydrobiopterin binding site and pterin-based inhibitors of these enzymes. To ensure successful labeling, the photoaffinity label must fullfil several criteria, such as specific binding with high affinity to the natural cofactor binding site, formation of a highly reactive species on irradiation to bind specifically to amino-acid moieties belonging to the binding site, and photochemical features which allow irradiation at wavelengths >300 nm to avoid enzyme damage. At the same time, these compounds must be chemically stable enough to survive normal synthetic and biological manipulations. Finally, the synthetic approach should include the possibility to synthesize also a tritiated derivative so that radioactivity labeling could be applied as an additional tool in such investigations.

Thus, three established, chemically distinct photolabile functions were introduced at the 6-position of the pteridine skeleton to meet as closely as possible the structural requirements of the natural cofactor: the azidophenyl group, which releases N_2 and forms nitrenes [4], the 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl group [5][6]

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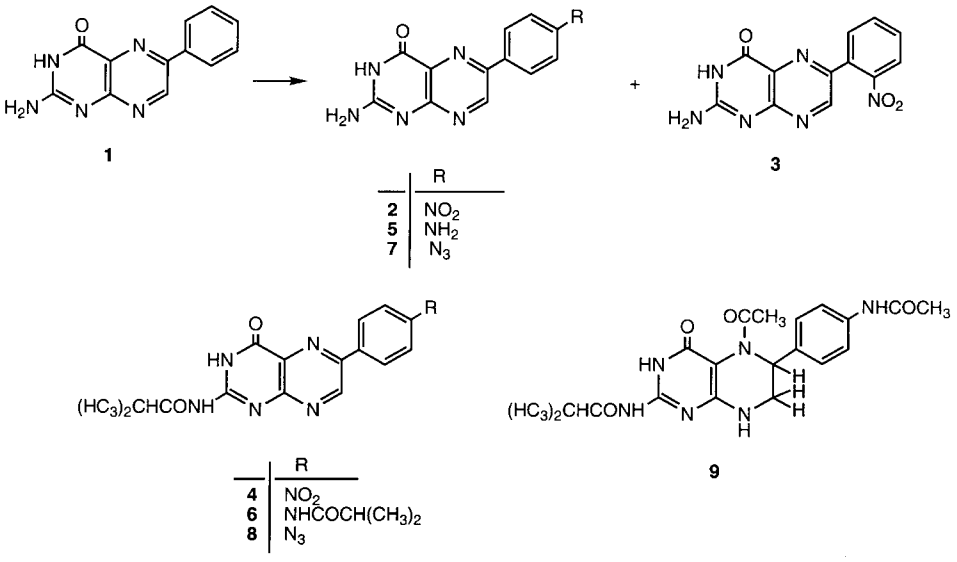
which is a carbene precursor [7], and the benzoylphenethyl group [8], which generates radicals [9].

The inhibitory effects of the modified pteridines and their competitiveness with respect to 5,6,7,8-tetrahydrobiopterin (H_4Bip) were taken as evidence for their interaction with the respective enzyme cofactor binding site. While NO synthases can be inhibited by pteridines of the common oxidation states (aromatic, 7,8-dihydro, and 5,6,7,8-tetrahydro forms), the aromatic amino acid hydroxylases bind only the fully reduced pterins, which must contain an unblocked amino function at the N(5) position of the pterin moiety.

2. Synthesis. – Our first approach was directed towards the synthesis of 6-(azidophenyl)pterins as potential inhibitors of NO synthases. The synthesis was initiated by direct-nitration studies of 6-phenylpterin (**1**) [10][11] by means of various types of nitric acid under different reaction conditions, which, however, always led to mixtures of (*ortho*-(*o*)- and *para*(*p*)-nitrophenyl)pterins (**2**/**3**), indicating that the pterin moiety served in this case as a first-order substituent (*Scheme 1*). This may be explained by the interaction of the 2-amino function, which is in conjugation with the phenyl ring and provides by mesomerism electrons to the *o*- and *p*-positions of the phenyl ring. All attempts to separate the *o*- and *p*-isomers failed so far, due to solubility reasons. To overcome this problem, the 6-(*o*/*p*-nitrophenyl)pterin mixture was acylated by reflux in isobutyric anhydride/pyridine leading to the corresponding N^2-isobutyryl derivatives. Of these compounds, N^2-isobutyryl-6-(4-nitrophenyl)pterin (**4**), fortunately, precipitated from the reaction solution by crystallization upon cooling. Reduction of the nitro group was achieved by reflux in 20% aqueous ammonium sulfide to form 6-(4-aminophenyl)pterin (**5**). Due to the loss of the isobutyryl moiety, this derivative was

Scheme 1

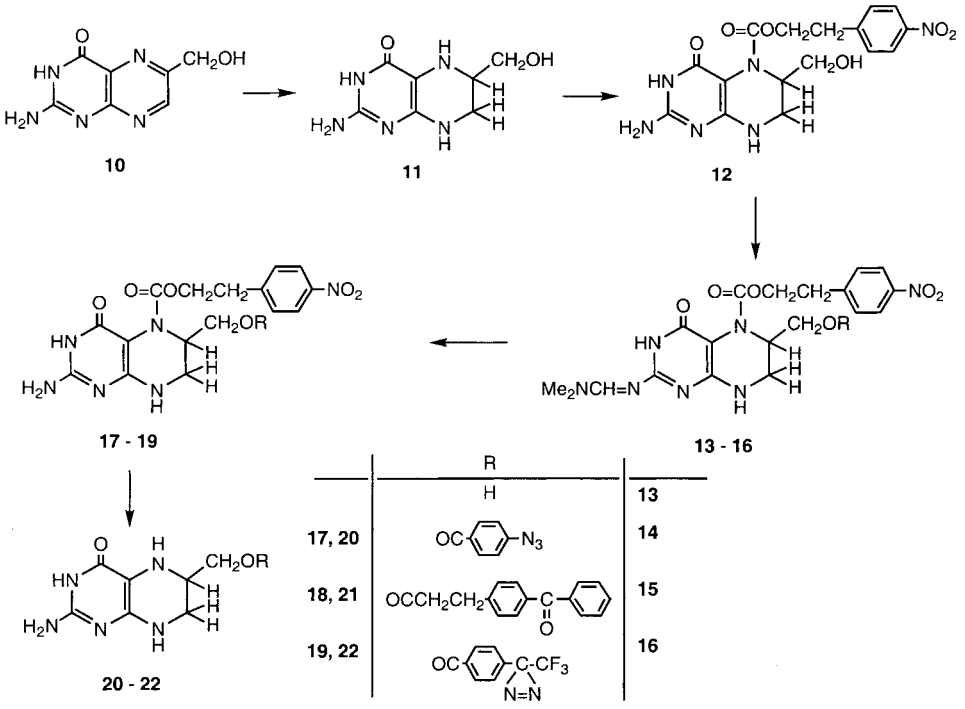


again extremely insoluble and could not be purified at this stage. It was characterized in form of the N^2-isobutyryl-6-[4-(isobutyrylamino)phenyl]pterin (**6**). Diazotization of crude **5** and subsequent addition of NaN_3 led to 6-(4-azidophenyl)pterin (**7**). Its purification required again isobutyrylation to 6-(4-azidophenyl)-N^2-isobutyrylpterin (**8**), which could be recrystallized from MeOH/AcOEt. Then the isobutyryl group of **8** was removed by treatment with NH_3/MeOH ($\rightarrow$ **7**).

Next the selective reduction of **8** to the corresponding 5,6,7,8-tetrahydropterin derivative by various types of catalytic hydrogenations and chemical reductions with $NaBH_4$ or sodium dithionite was studied, but none of these methods worked successfully since the azido function was attacked much more readily than the pyrazine ring. After Pt-catalyzed hydrogenation of **8** and workup with Ac_2O, 5-acetyl-6-[4-(acetylamino)phenyl]-5,6,7,8-tetrahydro-N^2-isobutyrylpterin (**9**) was isolated in 55% yield (*Scheme 1*).

Another approach to photoactive 5,6,7,8-tetrahydropterins was based on 6-[(acyloxy)methyl] derivatives first synthesized by *Traub* [12][13] from 6-(bromomethyl)pterin by reaction with carboxylates and subsequent reduction of the (pterin-6-yl)methyl esters. Our strategy, however, started from 6-(hydroxymethyl)pterin (**10**) [14][15], which was first reduced to 5,6,7,8-tetrahydro-6-(hydroxymethyl)pterin (**11**) and then stabilized by protection of the N^5-position with 2-(4-nitrophenyl)ethyl carbonochloridate [16] as acylating agent (*Scheme 2*). The obtained 5,6,7,8-tetrahydro-

Scheme 2

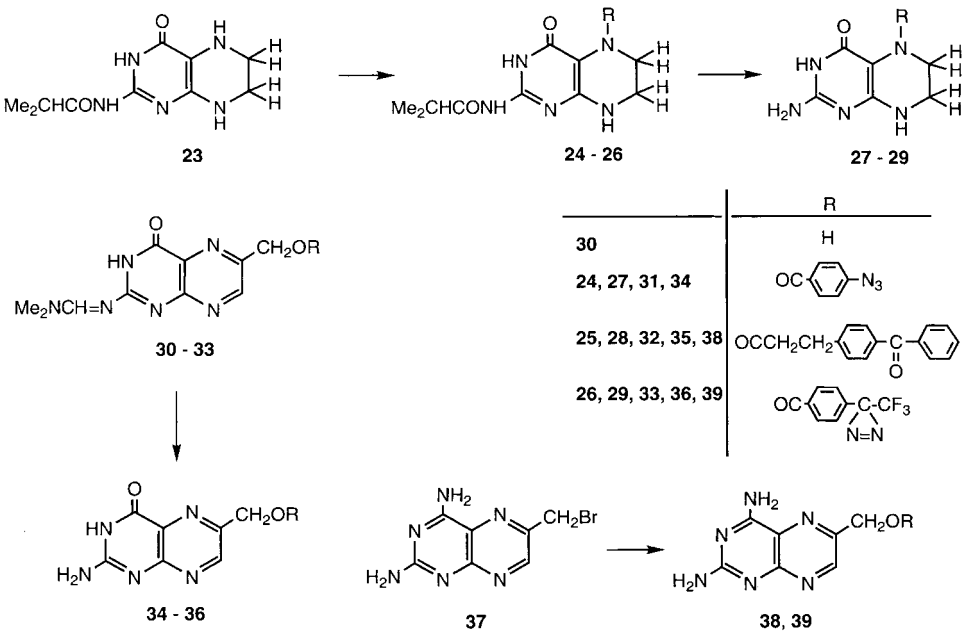


6-(hydroxymethyl)-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}pterin (**12**) required further protection at the 2-amino group, which was selectively converted by *N*,*N*-dimethylformamide diethyl acetal [17] to the corresponding N^2-[(dimethylamino)-methylene] derivative **13**.

The terminal OH group of **13** was then prone to acylation by 4-azidobenzoic acid [18], 3-(4-benzoylphenyl)propanoic acid [19], and 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid [5] to give **14**–**16**, respectively, in good yields in the presence of *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*,*N*-dimethylpyridin-4-amine (DMAP) (*Scheme 2*). The 4-azidobenzoic anhydride [20], but not 4-azidobenzoyl chloride [21], also worked successfully furnishing **14** in 63% yield. The removal of the blocking groups of **14**–**16** was achieved stepwise, first by cleavage of the (dimethylamino)methylene group with MeOH/HCl to give **17**–**19**, respectively, and finally by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) under aprotic conditions to remove the [2-(4-nitrophenyl)ethoxy]carbonyl group in a β-elimination process affording the 6-substituted 5,6,7,8-tetrahydropterin derivatives **20**–**22**, respectively, in the form of their tosylate salts. These turned out to be stable against air oxidation, even during recrystallization from H_2O/MeOH.

A further extension of these investigations was achieved by the introduction of the photoactive functions at N(5), since various 5-acyl-5,6,7,8-tetrahydropterins turned out to be interesting NO-synthase inhibitors. For this purpose, 5,6,7,8-tetrahydro-N^2-isobutyrylpterin (**23**) was treated with 4-azidobenzoyl chloride [21] to give 5-(4-azidobenzoyl)-5,6,7,8-tetrahydro-N^2-isobutyrylpterin (**24**) (*Scheme 3*). Acylation of **23** with 3-(4-benzoylphenyl)propanoic acid or 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-

Scheme 3



benzoic acid in the presence of EDC and DMAP led in good yields to the 5-acyl derivatives **25** and **26**, respectively. In this series, the isobutyryl group was much more labile towards hydrolysis than the substituents at N(5) and could, therefore, easily be cleaved by NH_3/MeOH to give the stable 5-acyl-5,6,7,8-tetrahydropterins **27**–**29** in high yields.

Acylations of 6-(hydroxymethyl)pterin (**10**) at the side chain were successful only when starting from N^2-[(dimethylamino)methylene]-6-(hydroxymethyl)pterin (**30**) and with activation of the three photoactive acids by EDC and DMAP in the usual manner leading to **31**–**33**. The N^2-protective group was then cleaved in almost quantitative yield to give the anticipated 6-[(acyloxy)methyl]pterins **34**–**36**, respectively.

Analogous pteridine-2,4-diamine derivatives were synthesized from 6-(bromomethyl)pteridine-2,4-diamine (**37**) [1][22] in a nucleophilic displacement reaction with triethylammonium 3-(4-benzoylphenyl)propanoate and 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoate in DMF to give **38** and **39**, respectively; however, the yields were very low, despite the fact that all starting material disappeared during this reaction.

3. Physical Data. – The newly synthesized compounds were characterized by their UV and ^{1}H-NMR data (see *Exper. Part*) and their composition further established by the elemental analyses.

4. Biological Data. – Preliminary investigation of the newly synthetized photoreactive pteridines in bioassays indicated that some compounds are suitable for inhibition of type-I NO synthase [23] purified from porcine cerebellum (*Table*). None of the various pterin derivatives serve as a substitute for tetrahydrobiopterin (H_4Bip) with respect to stimulation of NO-synthase activity which was measured under standard assay conditions as previously described [23]. The H_4Bip concentration was 2 μM, and the inhibitor concentration 50 times higher. The activity measured in presence of inhibitor was referred to a control assay without inhibitor present. All other activity measurements were expressed as % of this control activity. Among the H_4Bip

Table. *Inhibition of Type-I NO-Synthase Activity by Different Pteridine Derivatives*

	Conc. [μM]	H_4Bip conc. [μM]	NO-Synthase activity [% of control]	IC_{50} [μM]
–	–	2	–	100
21	100	2	57	
22	100	2	28	42
25	100	2	62	190
26	100	2	76	
28	100	2	75	
29	100	2	93	
32	100	2	95	
33	100	2	63	
35	100	2	2	60
36	100	2	18	44
38	100	2	29	48
39	100	2	55	140

derivatives, **22** was the only potential inhibitor of NO synthase recognized. The maximal inhibition observed with all other compounds was below 50% of control. However, two very potent inhibitors of NO synthase were found among the aromatic 6-substituted pterins, of which **35**, **36**, and **38** inhibited NO-synthase activity down to 2, 18, and 29% of control, respectively, making two of these derivatives promising candidates for photoaffinity-labeling experiments.

Monooxygenases, on the other hand, bind only tetrahydropteridines bearing a free NH function at the 5-position. While the 6-{[(4-azidobenzoyl)oxy]methyl}- and the 6-[({4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}oxy)methyl]-substituted 5,6,7,8-tetrahydropterins **20** and **22** showed almost the same cofactor activity as H_4Bip, surprisingly, the benzophenone derivative **21** seems to be a strong inhibitor of human tyrosine hydroxylase (hTH1) with high affinity for the enzyme, as indicated by the rather low K_i value [24].

Experimental Part

General. TLC: Precoated cellulose thin-layer sheets *F 1440 LS 254* and silica-gel thin-layer sheets *F 1500 LS 254* from *Schleicher & Schüll*. Flash chromatography (FC): silica gel (*Baker*, 30–60 mm); 0.2–0.3 bar. M.p.: *Büchi* apparatus, model *Dr. Tottoli*; no corrections. UV/VIS: *Uvikon 820*, *Kontron*, and *Lambda 5* (*Perin-Elmer*); λ_{max} (log ε). IR: in cm^{-1}. ^{1}H-NMR: *Bruker-WN-250*; δ in ppm rel. to Me_4Si.

1. N^2-*Isobutyryl-6-(4-nitrophenyl)pterin* (= N-*[3,4-Dihydro-6-(4-nitrophenyl)-4-oxopteridin-2-yl]-2-methylpropanamide*; **4**). Powdered 6-phenylpterin (**1**) [10][11] (12 g, 50.16 mmol) was added in small portions to conc. H_2SO_4 soln. (72 ml) at 0° with stirring. The resulting red soln. was treated with fuming HNO_3 (24 ml) by dropwise addition, keeping the temp. below 10°. After stirring for 2 h at r.t., the mixture was poured on ice. The yellow precipitate was immediately collected, washed with small amounts of H_2O, and dried over KOH *in vacuo:* 11.67 g (82%) of 6-(nitrophenyl)pterins of **2**/**3** (by NMR) as a yellow powder. This mixture (8.32 g) was suspended in dry pyridine (110 ml), then isobutyric anhydride (28 ml) added, and the mixture refluxed for 4 h. The resulting dark brown soln. was cooled to r.t. and kept refrigerated overnight to give a yellowish precipitate. The latter was then suspended in H_2O, the suspension stirred for 10 min, and the solid filtered off, washed with EtOH/H_2O, and dried at 40°: 7.1 g (40%) of **4**. Recrystallization from EtOH/H_2O. M.p. 310° (darkening), 321° (dec.). TLC (cellulose, 1% NH_3/PrOH 1:2): R_f 0.71. UV (MeOH): 363 (4.36), 319 (4.36), 261 (4.07), 233 (sh, 4.18), 203 (4.39). ^{1}H-NMR ((D_6)DMSO): 12.37 (br. *s*, H–N(3)); 12.06 (br. *s*, CONH–C(2)); 7.60 (*s*, H–C(7)); 8.46–8.38 (2*d*, arom. H); 2.72–2.88 (*sept.*, Me_2CH); 1.16 (*d*, Me_2CH). Anal. calc. for $C_{16}H_{14}N_6O_4$ (354.3): C 54.24, H 3.98, N 23.72; found: C 53.91, H 4.00, N 23.18.

2. *6-(4-Aminophenyl)pterin* (=*2-Amino-6-(4-aminophenyl)pteridin-4-(3*H*)-one*; **5**). A suspension of **4** (2 g, 5.64 mmol) in 20% aq. $(NH_4)_2S$ soln. (30 ml) was heated to 100° for 1 h. The starting material dissolved, and subsequently an orange solid precipitated. After cooling, the solid was collected, treated with CS_2 to remove sulfur, filtered again, washed with EtOH, and dried at 100°: 1.4 g (98%) of **5**. The crude material was not purified but was sufficiently pure for transformations into **6**–**8**.

3. N^2-*Isobutyryl-6-[4-(isobutyrylamino)phenyl]pterin* (= N-*(3,4-Dihydro-6-{4-[(2-methyl-1-oxopropyl)-amino]phenyl}-4-oxopteridin-2-yl)-2-methylpropanamide*; **6**). A suspension of **5** (1.02 g, 4 mmol) in dry pyridine (10 ml) was treated with isobutyric anhydride (3 ml) and heated under reflux for 2 h. On cooling, colorless crystals precipitated. The solid was suspended in H_2O, stirred, again filtered, and washed with EtOH: 0.8 g (50% of **6**). M.p. >340°. UV (MeOH): 372 (4.22), 316 (4.51), 225 (sh, 4.24), 202 (4.44). ^{1}H-NMR ((D_6)DMSO): 12.32 (br. *s*, H–N(3)); 11.93 (br. *s*, CONH–C(2)); 10.05 (br. *s*, CON*H*C_6H_4); 9.45 (br. *s*, H–C(7)); 8.15 (*d*, 2 arom. H); 7.79 (*d*, 2 arom. H); 2.78 (*sept.*, 1 H, Me_2CH); 2.63 (*sept.*, 1 H, Me_2CH); 1.13 (*m*, 4 Me). Anal. calc. for $C_{20}H_{22}N_6O_3 \cdot 0.5\ H_2O$ (403.4): C 59.54, H 5.75, N 20.38; found: C 59.88, H 5.73, N 20.53.

4. *6-(4-Azidophenyl)pterin* (=*2-Amino-6-(4-azidophenyl)pteridin-4-(3*H*)-one*; **7**). At r.t., **8** (see below; 0.2 g, 0.57 mmol) was stirred in NH_3/MeOH (16 ml) overnight. The precipitate was dried at 40°: 148 mg (93%) of **7**. Yellowish powder. M.p. >300°. UV (pH 14): 386 (4.10), 301 (4.34), 278 (sh, 4.31), 216 (4.21). IR (KBr): 2122 (N_3). ^{1}H-NMR (CF_3COOD): 9.37 (*s*, H–C(7)); 8.20 (*d*, 2 arom. H); 7.34 (*d*, 2 arom. H). Anal. calc. for $C_{12}H_8N_8O \cdot 0.5\ H_2O$ (289.3): C 49.83, H 3.14, N 38.74; found: C 50.24, H 3.33, N 37.95.

5. *6-(4-Azidophenyl)-*N^2*-isobutyrylpterin* (= N-*[6-(4-Azidophenyl)-3,4-dihydro-4-oxopteridin-2-yl]-2-methylpropanamide*; **8**). Crude **5** (1.44 g, 5.7 mmol) was suspended in 5N HCl (200 ml). Insoluble sulfur was removed by filtration, and the filtrate was evaporated. The residue was dissolved in H_2O (80 ml) and conc. HCl soln. (20 ml). After cooling to 0°, a soln. of $NaNO_2$ (0.83 g, 12 mmol) in H_2O (3 ml) was added dropwise under vigorous stirring. After 30 min at 0°, a soln. of NaN_3 (0.74 g, 11.4 mmol) in H_2O (2 ml) was added dropwise. The mixture was stirred for 3 h at 0° and for 2 h at r.t. The precipitate was collected, washed with H_2O, and dried over KOH in a vacuum desiccator: 0.9 g of crude **7**. The crude **7** was suspended in dry pyridine (12 ml) and treated with isobutyric anhydride (3 ml) under reflux for 2 h and then filtered while hot. The filtrate was evaporated and the partly crystalline residue suspended in MeOH. After filtration, recrystallization from EtOH/AcOEt 2:1 gave 0.67 g (34%) of **8**. M.p. >360°. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.53. UV (MeOH): 368 (4.22), 314 (4.48), 226 (sh, 4.22), 202 (4.40). IR (KBr): 2122. ^{1}H-NMR ((D$_6$)DMSO): 12.32 (br. *s*, H–N(3)); 11.97 (br. *s*, CONH–C(2)); 9.47 (*s*, H–C(7)); 8.22 (*d*, 2 arom. H); 7.27 (*d*, 2 arom. H); 2.78 (*sept.*, Me_2CH); 1.14 (*d*, Me_2CH). Anal. calc. for $C_{16}H_{14}N_8O_2$ (350.3): C 54.85, H 4.03, N 31.98; found: C 54.61, H 4.03, N 31.21.

6. *5-Acetyl-6-[4-(acetylamino)phenyl]-5,6,7,8-tetrahydro-*N^2*-isobutyrylpterin* (= N-*{5-Acetyl-6-[4-(acetylamino)phenyl]-3,4,5,6,7,8-hexahydro-4-oxopteridin-2-yl}-2-methylpropanamide*; **9**). PtO_2 (70 mg) was stirred in a mixture of abs. MeOH (15 ml) and CF_3COOH (0.8 ml) for 1 h under H_2 at r.t. After addition of **8** (0.1 g, 0.3 mmol), hydrogenation was continued for 2.5 h at r.t. and normal pressure. The catalyst was filtered off under an inert gas and the filtrate evaporated. The oily dark brown residue was treated with Ac_2O (5 ml), and after stirring for 12 h, the mixture was evaporated. The yellowish residue was treated with MeOH (3 ml) and cooled in the refrigerator. Then the precipitate was collected: 66 mg (55%) of crystalline **9**. M.p. 289–290°. TLC (CH_2Cl_2/MeOH 5:1): R_f 0.66. UV (MeOH): 301 (sh, 3.99), 234 (4.64), 204 (4.55). ^{1}H-NMR ((D$_6$)DMSO): 11.33 (br. *s*, NH); 11.23 (br. *s*, NH); 9.88 (br. *s*, NH); 7.43 (*m*, 3 H, arom. H, H–N(8)); 7.14 (*d*, 2 arom. H); 5.79 (*m*, H–C(6)); 3.98 (*dd*, 1 H–C(7)); 3.35 (*m*, 1 H–C(7)); 2.66 (*sept.*, Me_2CH); 2.12 (*s*, MeCO); 1.98 (*s*, MeCO); 1.03 (*d*, Me_2CH). Anal. calc. for $C_{12}H_{24}N_6O_4$ (412.5)·0.5 H_2O: C 57.00, H 5.98, N 19.94; found: C 57.32, H 6.00, N 19.73.

7. *5,6,7,8-Tetrahydro-6-(hydroxymethyl)-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}pterin* (=*2-(4-Nitrophenyl)-ethyl 2-Amino-3,4,5,6,7,8-hexahydro-6-(hydroxymethyl)-4-oxopteridine-5-carboxylate*; **12**). In CF_3COOH (50 ml), 6-(hydroxymethyl)pterin (**10**) [14][15] (2 g, 10.4 mmol) was reduced under H_2 in the presence of PtO_2 (0.4 g) at r.t. under normal pressure. After 4 h, the catalyst was filtered off under an inert gas atmosphere and the solvent evaporated. The residue (**11**) was dissolved under an inert gas atmosphere in dry pyridine (100 ml) and treated with 2-(4-nitrophenyl)ethyl carbonochloridate [16] (3.1 g, 26.9 mmol). After stirring for 12 h at r.t., the soln. was evaporated and the residue co-evaporated with toluene (3×) and then repeatedly evaporated with MeOH. Finally, the partially crystalline residue was sonicated, the mixture cooled for 12 h, and the crystalline solid dried at 40°. Workup of the filtrate by evaporation and treatment with little MeOH gave a second crop. Total yield: 3.51 g (86%) of **12**. M.p. 223–224° (dec.). TLC (H_2O/^{i}PrOH/AcOEt 1:5:3): R_f 0.64. UV (MeOH): 279 (4.37), 219 (4.51), 205 (sh, 4.35). ^{1}H-NMR ((D$_6$)DMSO): 8.13 (*d*, arom. H); 7.68 (br. *s*, NH_2); 7.51 (*d*, arom. H); 4.26 (*m*, 3 H, H–C(6), CH_2); 3.47 (*d*, 1 H–C(7)); 3.12 (*m*, 5 H, H–C(7), 2 CH_2). Anal. calc. for $C_{16}H_{18}N_6O_6 \cdot 2\ H_2O$ (426.4): C 45.07, H 4.69, N 19.80; found: C 45.19, H 5.20, N 19.32.

8. N^2*-[(Dimethylamino)methylene]-5,6,7,8-tetrahydro-6-(hydroxymethyl)-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}pterin* (=*2-(4-Nitrophenyl)ethyl 2-{[(Dimethylamino)methylene]amino}-3,4,5,6,7,8-hexahydro-6-(hydroxymethyl)-4-oxopteridine-5-carboxylate*; **13**). A suspension of **12** (0.5 g, 1.28 mmol) in dry DMF (25 ml) was treated with dimethylformamide diethyl acetal (1.25 ml) at r.t., yielding immediately a clear yellowish soln. After 2.5 h stirring (TLC: no **12** left), the mixture was evaporated under high vacuum, and the residue dissolved in $CHCl_3$ and purified by FC (silica gel (10 g), gradient 0 → 6% MeOH/CH_2Cl_2). The product was treated with MeOH and sonicated to give a crystalline precipitate, which was dried at 30°: 0.33 g (57%) of **13**. M.p. 221° (dec.). TLC (CH_2Cl_2/MeOH 5:1): R_f 0.62. UV (MeOH): 299 (sh, 4.36), 281 (sh, 4.42), 258 (4.50), 217 (4.48). ^{1}H-NMR ((D$_6$)DMSO): 10.71 (br. *s*, H–N(3)); 8.38 (*s*, H–C=N); 8.09 (*d*, 2 arom. H); 7.52 (*d*, 2 arom. H); 6.78 (*d*, H–N(8)); 4.79 (br. *s*, OH); 4.09–4.31 (*m*, 3 H, CH_2, H–C(6)); 3.41 (*dd*, 1 H–C(7)); 3.19 (*m*, 1 H–C(7)); 3.08 (*s*, MeN); 2.99 (*m*, 9 H, MeN, 3 CH_2). Anal. calc. for $C_{19}H_{23}N_7O_6 \cdot 0.5\ H_2O$ (454.4): C 50.22, H 5.32, N 21.58; found: C 50.60, H 5.26, N 21.25.

9. *6-{[(4-Azidobenzoyl)oxy]methyl}-*N^2*-[(dimethylamino)methylene]-5,6,7,8-tetrahydro-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}pterin* (=*2-(4-Nitrophenyl)ethyl 6-{[(4-Azidobenzoyl)oxy]methyl}-2-{[(dimethylamino)methylene]amino}-3,4,5,6,7,8-hexahydro-4-oxopteridine-5-carboxylate*; **14**). *a*) A mixture of 4-azidobenzoic acid [18] (0.22 g, 1.35 mmol), EDC (0.26 g, 1.35 mmol), and DMAP (0.17 g, 1.35 mmol) in dry pyridine (10 ml) was stirred for 2 h at r.t. Then **13** (0.15 g, 0.34 mmol) was added and stirring continued at r.t. for 16 h. After evaporation and co-evaporation with toluene (3×), the oily residue was dissolved in a small amount of $CHCl_3$

and purified by FC (silica gel (8 g), gradient 0–6% MeOH/$CHCl_3$). The oily product was crystallized by treatment with AcOEt and sonication. The precipitate was collected after cooling for 2 h and dried at 40°: 109 mg (56%) of **14**. Yellowish powder. M.p. 198–200°.

b) A mixture of 4,4′-azidobenzoic anhydride [20] (0.33 g, 1.1 mmol) and DMAP (0.13 g, 1.1 mmol) in dry pyridine (10 ml) was stirred at r.t. for 1 h. Then compound **13** (0.12 g, 0.27 mmol) was added and stirring continued for 24 h. Evaporation under high vacuum and workup analogous to *a*) gave 98 mg (63%) of **14**. M.p. 198–200°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.68. UV (MeOH): 318 (sh, 4.18), 271 (4.63), 265 (4.63), 213 (4.58). ^{1}H-NMR ((D$_6$)DMSO): 10.78 (br. *s*, H–N(3)); 8.40 (*s*, H–C=N); 8.06 (*d*, 2 arom. H); 7.94 (*d*, 2 arom. H); 7.52 (*d*, 2 arom. H); 7.21 (*d*, 2 arom. H); 6.87 (*d*, H–N(8)); 4.62 (br. *s*, H–C(6)); 3.98–4.32 (*m*, 4 H, 2 CH_2); 3.39 (*m*, 1 H–C(7)); 3.17 (*m*, 1 H–C(7)); 3.09 (*s*, MeN); 3.00 (*m*, 5 H, MeN, CH_2). Anal. calc. for $C_{26}H_{26}N_{10}O_7$ (590.6): C 52.88, H 4.44, N 23.72; found: C 53.06, H 4.49, N 23.92.

10. *6-{[3-(4-Benzoylphenyl)-1-oxopropoxy]methyl}-*N^2*-[(dimethylamino)methylene]-5,6,7,8-tetrahydro-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}pterin* (=*2-(4-Nitrophenyl)ethyl 6-{[3-(4-Benzoylphenyl)-1-oxopropoxy]-methyl}-2-{[(dimethylamino)methylene]amino}-3,4,5,6,7,8-hexahydro-4-oxopteridine-5-carboxylate*; **15**). At r.t., **13** (0.5 g, 1.12 mmol), 3-(4-benzoylphenyl)propanoic acid [19] (0.57 g, 2.24 mmol), EDC (0.43 g, 2.24 mmol), and DMAP (0.27 g, 2.24 mmol) were dissolved in dry pyridine (20 ml) and stirred for 14 h. The mixture was evaporated to dryness and co-evaporated with toluene (3×) and the residue purified by FC (silica gel (12 g), gradient 0–2% MeOH/CH_2Cl_2). A second purification by FC (up to 4% MeOH/CH_2Cl_2) was necessary. Evaporation gave a yellowish foam, which was treated with Et_2O and sonicated. The resulting yellowish solid was washed with Et_2O and dried at 40°: 0.61 g (80%) of **15**. M.p. 85° (evolution of gas), 130° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.52. UV (MeOH): 311 (sh, 4.30), 281 (sh, 4.53), 258 (4.69), 206 (4.65). ^{1}H-NMR ((D$_6$)DMSO): 10.79 (br. *s*, H–N(3)); 8.39 (*s*, H–C=N); 8.08 (*d*, 2 arom. H); 7.63–7.72 (*m*, PhCO); 7.50–7.57 (*m*, 4 arom. H); 7.41 (*d*, 2 arom. H); 6.84 (*d*, H–N(8)); 4.57 (br. *s*, H–C(6)); 4.13–4.45 (*m*, CH_2); 3.80 (*q*, 2 H, CH_2); 3.24 (*m*, 2 H–C(7)); 3.08 (*s*, MeN); 2.91–3.04 (*m*, 7 H, MeN, 2 CH_2); 2.67 (*t*, CH_2). Anal. calc. for $C_{35}H_{34}N_7O_8$ (680.7): C 61.76, H 5.03, N 14.40; found: C 62.25, H 5.41, N 13.98.

11. N^2*-[(Dimethylamino)methylene]-5,6,7,8-tetrahydro-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}-6-[({4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}oxy)methyl]pterin* (=*2-(4-Nitrophenyl)ethyl 2-{[(Dimethylamino)-methylene]amino}-3,4,5,6,7,8-hexahydro-4-oxo-6-[({4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}oxy)methyl]pteridine-5-carboxylate*; **16**). A mixture of **13** (0.2 g, 0.45 mmol), 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid [5] (0.21 g, 0.9 mmol), DMAP (0.11 g, 0.9 mmol), and EDC (0.17 g, 0.9 mmol) was stirred for 14 h in dry pyridine (20 ml). The clear soln. was evaporated and the residue co-evaporated with toluene (3×) and then purified by FC (silica gel (8 g), gradient 0–3% MeOH/CH_2Cl_2). The product was treated with AcOEt, and sonicated to give a crystalline material, which was washed with Et_2O, and dried at 40°: 0.23 g (75%) of **16**. M.p. 235° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.54. UV (MeOH): 305 (sh, 4.38), 279 (sh, 4.48), 255 (4.61), 221 (4.61). ^{1}H-NMR ((D$_6$)DMSO): 10.77 (br. *s*, H–N(3)); 8.39 (*s*, H–C=N); 8.04 (*m*, 4 arom. H); 7.51 (*d*, 2 arom. H); 7.38 (*d*, 2 arom. H); 6.87 (*d*, H–N(8)); 4.63 (br. *s*, H–C(6)); 3.99–4.31 (*m*, 4 H, 2 CH_2); 3.39 (*m*, 1 H–C(7)); 3.17 (*m*, 1 H–C(7)); 3.09 (*s*, MeN); 3.00 (*m*, 5 H, MeN, CH_2). Anal. calc. for $C_{28}H_{26}N_9O_7F_3 \cdot 0.5\ H_2O$ (666.6): C 50.45, H 4.08, N 18.92; found: C 50.30, H 3.92, N 18.54.

12. *6-{[(4-Azidobenzoyl)oxy]methyl}-5,6,7,8-tetrahydro-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}pterin Hydrochloride* (=*2-(4-Nitrophenyl)ethyl 2-Amino-6-{[(4-azidobenzoyl)oxy]methyl}-3,4,5,6,7,8-hexahydro-4-oxo-pteridine-5-carboxylate Hydrochloride*; **17**·HCl). Overnight, **14** (0.8 g, 1.49 mmol) was stirred in MeOH (160 ml) and 1M HCl (16 ml). The resulting precipitate was washed with small amounts of MeOH and dried at 40°: 0.7 g of **17**·HCl. Workup of the mother liquor gave a second crop of 90 mg. Total yield: 0.79 g (91%). Colorless crystals. M.p. 199–200°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.44. UV (MeOH): 274 (4.64), 215 (4.65). ^{1}H-NMR ((D$_6$)DMSO): 11.30 (br. *s*, H–N(3)); 8.08 (*d*, 2 arom. H); 7.93 (*d*, 2 arom. H); 7.50 (*m*, H–N(8), 2 arom. H); 7.23 (*d*, 2 arom. H); 4.64 (br. *s*, H–C(6)); 4.08–4.33 (*m*, 4 H, 2 CH_2); 3.49 (*m*, 1 H–C(7)); 3.20 (*m*, 1 H–C(7)); 2.99 (*t*, CH_2). Anal. calc. for $C_{23}H_{21}N_9O_7 \cdot HCl \cdot 0.5\ H_2O$ (580.9): C 47.55, H 3.99, N 21.70; found: C 47.67, H 3.92, N 21.38.

13. *6-{[3-(4-Benzoylphenyl)-1-oxopropoxy]methyl}-5,6,7,8-tetrahydro-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}pterin* (=*2-(4-Nitrophenyl)ethyl 2-Amino-6-{[3-(4-benzoylphenyl)-1-oxopropoxy]methyl}-3,4,5,6,7,8-hexahydro-4-oxopteridine-5-carboxylate*; **18**). At r.t., **15** (0.3 g, 0.44 mmol) was stirred in MeOH (12 ml) and 1M HCl (2 ml) for 24 h. The precipitate was washed with Et_2O and dried at 40°: 0.22 g (80%) of colorless **18**. M.p. 239° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.46. UV (MeOH): 338 (sh, 3.51), 266 (4.55), 214 (sh, 4.64), 206 (4.64). ^{1}H-NMR ((D$_6$)DMSO): 9.99 (br. *s*, H–N(3)); 8.09 (*d*, 2 arom. H); 7.64–7.72 (*m*, PhCO); 7.51–7.57 (*m*, 4 arom. H); 7.41 (*d*, 2 arom. H); 6.76 (*d*, H–N(8)); 6.16 (br. *s*, NH_2); 4.43 (br. *s*, H–C(6)); 4.16–4.32 (2*m*, 2 H,

CH_2); 3.81 (*m*, 2 H, CH_2); 3.40–3.48 (*m*, 1 H–C(7)); 3.20–3.72 (*m*, 1 H–C(7)); 2.91–3.08 (*m*, 6 H, 3 CH_2); 2.67 (*t*, CH_2). Anal. calc. for $C_{32}H_{30}N_6O_7 \cdot H_2O$ (628.6): C 61.14, H 5.13, N 13.37; C 60.84, H 4.82, N 13.30.

14. *5,6,7,8-Tetrahydro-5-{[2-(4-nitrophenyl)ethoxy]carbonyl}-6-[({4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-benzoyl}oxy)methyl]pterin Hydrochloride* (=*2-(4-Nitrophenyl)ethyl 2-Amino-3,4,5,6,7,8-hexahydro-4-oxo-6-[({4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}oxy)methyl]pteridine-5-carboxylate Hydrochloride*; **19**·HCl). At r.t., **16** (0.18 g, 0.27 mmol) was stirred in MeOH (10 ml) and conc. HCl soln. (1.5 ml) for 24 h. The resulting precipitate was washed with a small amount of MeOH and Et_2O and dried at 30° *in vacuo:* 135 mg (78%) of colorless **19**·HCl. M.p. 239° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.63. UV (MeOH): 348 (sh, 3.50), 278 (4.39), 245 (sh, 4.41), 220 (4.63). ^{1}H-NMR ((D$_6$)DMSO): 11.26 (br. *s*, H–N(3)); 8.07 (*d*, 2 arom. H); 8.01 (*d*, 2 arom. H); 7.49 (*m*, 5 H, H–N(8), NH_2, arom. H); 7.40 (*d*, 2 arom. H); 4.64 (br. *s*, H–C(6)); 4.08–4.34 (*m*, 4 H, 2 CH_2); 3.48 (*m*, 1 H–C(7)); 3.20 (*dd*, 1 H–C(7)); 2.98 (*t*, CH_2). Anal. calc. for $C_{25}H_{21}N_8O_7F_3 \cdot HCl$ (639.0): C 47.00, H 3.47, N 17.54; found: C 46.84, H 3.36, N 17.11.

15. *6-{[(4-Azidobenzoyl)oxy]methyl}-5,6,7,8-tetrahydropterin 4-Toluenesulfonate* (=*(2-Amino-3,4,5,6,7,8-hexahydro-4-oxopteridin-6-yl)methyl 4-Azidobenzoate 4-Methylbenzenesulfonate*; **20**·TsOH). To a suspension of **17** (0.4 g, 0.69 mmol) in dry DMF (7 ml), DBU (574 µl) was added under N_2. After stirring overnight at r.t., the soln. was acidified with AcOH (2 ml) and evaporated under high vacuum. The oily residue was treated with TsOH (1.0 g, 5.35 mmol) in EtOH (10 ml) at 0°. The precipitate was washed with EtOH and dried at 40° under high vacuum. The crude material (0.3 g) was recrystallized from H_2O/MeOH under N_2, the insoluble material filtered off the hot mixture, and the filtrate kept in the refrigerator overnight: 99 mg (28%) of colorless **20**·TsOH. M.p. 203–204° (dec.). TLC (CH_2Cl_2/MeOH 5:1): R_f 0.56. UV (MeOH): 292 (sh, 4.29), 268 (4.55), 219 (sh, 4.29), 206 (4.55). IR (KBr): 2124. ^{1}H-NMR ((D$_6$)DMSO): 10.62 (br. *s*, H–N(3)); 8.10 (*d*, 2 arom. H); 7.54 (br. *s*, H–N(8)); 7.47 (*d*, 2 arom. H); 7.27 (*d*, 2 arom. H); 7.10 (*d*, 2 arom. H); 6.65 (br. *s*, NH_2); 4.46–4.64 (*m*, CH_2O); 3.73 (br. *s*, H–C(6)); 3.57 (*m*, 1 H–C(7)); 3.26–3.46 (*m*, 1 H–C(7)); 2.28 (*s*, Me). Anal. calc. for $C_{14}H_{14}N_8O_3 \cdot CH_3C_6H_4SO_3H$ (514.5): C 49.02, H 4.31, N 21.78; found: C 48.76, H 4.35, N 21.76.

16. *6-{[3-(4-Benzoylphenyl)-1-oxopropoxy]methyl}-5,6,7,8-tetrahydropterin 4-Toluenesulfonate* (=*(2-Amino-3,4,5,6,7,8-hexahydro-4-oxopteridin-6-yl)methyl 4-Benzoylbenzenepropanoate 4-Methylbenzenesulfonate*; **21**·TsOH). To a suspension of **18** (0.15 g, 0.25 mmol) under an inert gas in dry DMF (1.6 ml), DBU (183 µl, 1.23 mmol) was added and the soln. stirred for 14 h at r.t. The mixture was acidified with AcOH (1.1 ml) to bring the pH to 5–6 and then evaporated under high vacuum. The brown oily residue was treated at 0° with TsOH (0.37 g, 1.92 mmol) in EtOH (10 ml) and sonicated to convert all material to a solid. After 30 min at 0°, the precipitate was washed with EtOH and dried. The crude product was recrystallized under an inert gas from H_2O to which MeOH was added dropwise until a clear hot soln. resulted. After cooling and standing refrigerated for 3 h, the collected solid was dried at 30° *in vacuo*: 95 mg (63%) of colorless **21**·TsOH. M.p. 183°. TLC (CH_2Cl_2/MeOH 5:1): R_f 0.43. UV (MeOH): 312 (sh, 4.02), 259 (4.47), 218 (4.69), 206 (sh, 4.66). ^{1}H-NMR ((D$_6$)DMSO): 10.62 (br. *s*, H–N(3)); 7.64–7.72 (*m*, PhCO); 7.41–7.58 (*m*, 9 arom. H, H–N(8)); 7.10 (*d*, 2 arom. H); 6.65 (br. *s*, NH_2); 4.31 (*m*, CH_2O); 3.43–3.57 (*m*, H–C(6), 1 H–C(7)); 3.16 (*m*, 1 H–C(7)); 2.98 (*t*, CH_2); 2.76 (*t*, CH_2); 2.27 (*s*, Me). Anal. calc. for $C_{23}H_{23}N_5O_3 \cdot CH_3C_6H_4SO_3H$ (605.7): C 59.49, H 5.16, N 11.56; found: C 59.52, H 5.15, N 11.57.

17. *6-[({4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}oxy)methyl]-5,6,7,8-tetrahydropterin 4-Toluenesulfonate* (=*(2-Amino-3,4,5,6,7,8-hexahydro-4-oxopteridin-6-yl)methyl 4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoate 4-Methylbenzenesulfonate*; **22**·TsOH). As described for **21**, with **19** (80 mg, 0.13 mmol), DMF (1.3 ml), DBU (94 µl, 0.65 mmol), AcOH (0.5 ml), TsOH (0.19 g, 0.99 mmol), and EtOH (3 ml). After 45 min. at 0°, the solid was washed with EtOH and Et_2O and dried: 84 mg of crude **22**·TsOH. The material was recrystallized under an inert gas from H_2O (3 ml) by dropwise addition of MeOH until a clear soln. resulted on boiling. After cooling in the refrigerator for 2 h, the collected solid was washed with small amounts of MeOH and Et_2O and dried at 30°: 50 mg (66%) of colorless **22**·TsOH. M.p. 259° (dec.). TLC (CH_2Cl_2/MeOH 5:1): R_f 0.58. UV (1N HCl): 333 (sh, 3.54), 255 (4.43), 244 (sh, 4.42), 228 (sh, 4.45), 223 (4.47), 205 (4.54). UV (MeOH): 329 (sh, 3.72), 279 (sh, 4.01), 235 (sh, 4.52), 224 (4.58). ^{1}H-NMR ((D$_6$)DMSO): 10.63 (br. *s*, H–N(3)); 8.17 (*d*, 2 H, Ts); 7.43–7.56 (*m*, 5 H, arom. H, H–N(8)); 7.10 (*d*, 2 H, Ts); 6.66 (br. *s*, NH_2); 4.49–4.69 (*m*, CH_2O); 3.73 (*m*, H–C(6)); 3.57 (*m*, 1 H–C(7)); 3.27–3.46 (*m*,1 H–C(7)); 2.27 (*s*, Me). Anal. calc. for $C_{16}H_{14}F_3N_7O_3 \cdot CH_3C_6H_4SO_3H$ (581.5): C 47.50, H 3.81, N 16.86; found: C 47.57, H 3.93, N 16.98.

18. *5,6,7,8-Tetrahydro-*N^2*-isobutyrylpterin* (=*2-Methyl-*N*-(3,4,5,6,7,8-hexahydro-4-oxopteridin-2-yl)propanamide Hydrochloride*; **23**·HCl). A mixture of *N*^{2}-isobutyrylpterin [25] (4.0 g, 17.15 mmol) and PtO_2 (0.4 g) in MeOH (200 ml) was treated in a shaking apparatus under H_2 for 24 h until the uptake of H_2 stopped. Then conc. HCl soln. (7 ml) was added to dissolve the precipitate. The catalyst was filtered off and the filtrate concentrated to 50 ml and then added dropwise to Et_2O (500 ml) with stirring. After cooling in the refrigerator for 2 h, the

colorless precipitate was washed with Et_2O and dried in a vacuum desiccator. From the filtrate, a second crop of **23** · HCl was obtained after standing overnight in the refrigerator. Total yield: 315 g (67%). M.p. 235° (dec.). UV (pH 0): 265 (4.02), 229 (4.57). ^{1}H-NMR ((D_6)DMSO): 11.59 (br. *s*, NH); 11.54 (br. *s*, NH); 7.87 (br. *s*, H–N(8)); 3.42 (*m*, CH_2(6)); 3.22 (*m*, CH_2(7)); 2.75 (*sept.*, Me_2CH); 1.07 (*d*, Me_2CH). Anal. calc. for $C_{10}H_{15}N_5O_2 \cdot HCl \cdot 0.5\ H_2O$ (282.7): C 42.48, H 6.06, N 24.77; found: C 42.40, H 6.41, N 25.24.

19. *5-(4-Azidobenzoyl)-5,6,7,8-tetrahydro-*N^2*-isobutyrylpterin* (= N-*[5-(4-Azidobenzoyl)-3,4,5,6,7,8-hexahydro-4-oxopteridin-2-yl]-2-methylpropanamide*; **24**). PtO_2 (70 mg) was hydrogenated with molecular H_2 in MeOH (50 ml) in a shaking apparatus. After addition of N^2-isobutyrylpterin [25] (0.5 g, 2.14 mmol), the mixture was stirred under H_2 for 20 h. The solvent was evaporated, and the residue dried under high vacuum and then treated with 4-azidobenzoyl chloride [21] (0.53 g, 2.9 mmol) in dry pyridine (15 ml) for 24 h at r.t. with stirring. The mixture was evaporated and co-evaporated with toluene, the residue heated in $CHCl_3$ (150 ml) and MeOH (20 ml) to reflux, the catalyst filtered off, and the filtrate concentrated to a small volume. After mixing with silica gel (1.5 g), the mixture was evaporated to a powder, which was put on top of a column (silica gel) for separation. FC (gradient 1–8% MeOH/CH_2Cl_2) gave a product which was suspended in CH_2Cl_2, heated, and treated dropwise with MeOH to give a clear soln. After cooling to r.t. the soln. was concentrated until the product began to crystallize. After cooling overnight in the refrigerator, the colorless crystalline solid was collected and dried: 0.23 g (28%) of **24**. M.p. 271–275°. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.3. UV (MeOH): 318 (4.01), 268 (4.30), 236 (4.48), 205 (4.47). ^{1}H-NMR ((D_6)DMSO): 11.29 (br. *s*, H–N(3)); 11.08 (br. CONH–C(2)); 7.47 (*d*, 2 arom. H); 7.32 (br. *s*, H–N(8)); 7.03 (*d*, 2 arom. H); 4.46 (br. *s*, CH_2(6)); 3.44 (br. *s*, CH_2(7)); 2.69 (*sept.*, Me_2CH); 1.04 (*d*, Me_2CH). Anal. calc. for $C_{17}H_{18}N_8O_3$(382.4): C 53.40, H 4.74, N 29.30; found: C 52.85, H 4.75, N 28.72.

20. *5-[3-(4-Benzoylphenyl)-1-oxopropyl]-5,6,7,8-tetrahydro-*N^2*-isobutyrylpterin* (= N-*[5-[3-(4-Benzoylphenyl)-1-oxopropyl]-3,4,5,6,7,8-hexahydro-4-oxopteridin-2-yl}-2-methylpropanamide*; **25**). At r.t., **23** (0.4 g, 1.46 mmol), 3-(4-benzoylphenyl)propanoic acid [19] (0.74 g, 2.92 mmol), EDC (0.56 g, 2.92 mmol), and DMAP (0.36 g, 2.92 mmol) were stirred in dry pyridine (20 m) for 24 h. After evaporation, the residue was co-evaporated with toluene (3×) and then purified by FC (silica gel (18 g), gradient 0–6% MeOH/CH_2Cl_2). The product fractions were purified by a second FC. The pure substance recrystallized from hot AcOEt by dropwise addition of MeOH until a clear soln. was obtained on heating. After cooling to r.t., the soln. was concentrated under reduced pressure to a small volume and kept refrigerated for 3 h. The collected crystals were dried at 40° under high vacuum: 0.23 g (33%) of **25**. M.p. 234°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.49. UV (MeOH): 313 (sh, 3.94), 260 (4.44), 235 (4.63), 204 (4.58). ^{1}H-NMR ((D_6)DMSO): 11.38 (br. *s*, NH); 11.32 (br. *s*, NH); 7.40–7.71 (*m*, 8 H, arom. H, H–N(8)); 7.34 (*d*, 2 arom. H); 4.61 (*d*, 1 H–C(6)); 3.38 (*m*, 1 H–C(7)); 2.61–2.96 (*m*, 6 H, 1 H–C(6), 1 H–C(7), 2 CH_2); 2.72–2.80 (*m*, Me_2CH); 2.40 (br. *s*, H–C(6)); 1.06 (*d*, Me_2CH). Anal. calc. for $C_{26}H_{27}N_5O_4$ (473.5): C 65.95, H 5.75, N 14.79; found: C 66.34, H 5.76, N 14.32.

21. *5,6,7,8-Tetrahydro-*N^2*-isobutyryl-5-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}pterin* (= *2-Methyl-*N*-(3,4,5,6,7,8-hexahydro-4-oxo-5-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}pteridin-2-yl)propanamide*; **26**). As described for **25**, with **23** (0.3 g, 1.1 mmol), 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid [5] (0.5 g, 2.19 mmol), EDC (0.42 g, 2.19 mmol), DMAP (0.27 g, 2.19 mmol), and pyridine (15 ml) (14 h under N_2). FC (silica gel (9 g), gradient 0–4% MeOH/CH_2Cl_2) was performed twice. The product was then treated with AcOEt and sonicated to give a solid, which was washed with Et_2O and dried: 83 mg (17%) of **26**. M.p. 284° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.53. UV (MeOH): 312 (4.03), 275 (4.05), 234 (4.61), 204 (4.47). ^{1}H-NMR ((D_6)DMSO): 11.27 (br. *s*, NH); 11.09 (br. *s*, NH); 7.55 (*d*, 2 arom. H); 7.38 (br. *s*, H–N(8)); 7.19 (*d*, 2 arom. H); 4.57 (br. *s*, 1 H–C(6)); 3.44 (*m*, CH_2(7)); 2.69 (*m*, 1 H–C(6), Me_2CH); 1.04 (*d*, Me_2CH). Anal. calc. for $C_{19}H_{18}F_3N_7O_2 \cdot H_2O$ (451.4): C 50.55, H 4.47, N 21.72; found: C 50.48, H 4.11, N 21.64.

22. *5-(4-Azidobenzoyl)-5,6,7,8-tetrahydropterin* (= *2-Amino-5-(4-azidobenzoyl)-5,6,7,8-tetrahydropteridin-4(3*H*)-one*; **27**). At r.t., **24** (80 mg, 0.2 mmol) was dissolved in NH_3/MeOH (4 ml) and stirred for 6 h. After 1 h, a colorless solid started to separate, which was washed with MeOH and dried: 55 mg (88%) of colorless **27**. M.p. 310° (dec.). TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.21. UV (pH 1): 278 (4.41), 202 (4.52). UV (MeOH): 280 (sh, 4.25), 269 (4.29), 212 (4.47). ^{1}H-NMR ((D_6)DMSO): 9.71 (br. *s*, H–N(3)); 7.45 (*d*, 2 arom. H); 7.02 (*d*, 2 arom. H); 6.94 (*s*, H–N(8)); 6.10 (br. *s*, NH_2); 4.50 (br. *s*, CH_2(6)); 2.68 (br. *s*, CH_2(7)). Anal. calc. for $C_{13}H_{12}N_8O_2$ (312.3): C 50.00, H 3.87, N 35.88; found: C 49.79, H 3.89, N 35.23.

23. *5-[3-(4-Benzoylphenyl)-1-oxopropyl]-5,6,7,8-tetrahydropterin* (= *2-Amino-5-[3-(4-benzoylphenyl)-1-oxopropyl]-5,6,7,8-tetrahydropteridin-4(3*H*)-one*; **28**). At r.t., **25** (0.1 g, 0.21 mmol) was stirred in NH_3/MeOH overnight. The soln. was evaporated and the residue co-evaporated several times with MeOH, treated again with MeOH, and sonicated. The resulting solid was washed with Et_2O and dried: 50 mg (60%) of colorless **28**. M.p. 263–264°. TLC (CH_2Cl_2/MeOH 5:1): R_f 0.55. UV (MeOH): 280 (sh, 4.35), 260 (4.40), 220 (4.50), 205 (4.50). ^{1}H-NMR ((D_6)DMSO): 10.02 (br. *s*, H–N(3)); 7.72–7.44 (*m*, 7 arom. H); 7.34 (*d*, 2 arom. H); 6.97

(*d*, H–N(8)); 6.20 (br. *s*, NH_2); 4.57 (*m*, 1 H–C(6)); 3.35–3.26 (*m*, 1 H–C(7)); 2.98–2.84 (*m*, 2 CH_2); 2.79–2.55 (*m*, 1 H–C(6)); 2.41–2.32 (*m*, 1 H–C(7)). Anal. calc. for $C_{22}H_{21}N_5O_3$ (403.4): C 65.50, H 5.25, N 17.36; found: C 65.01, H 5.22, N 17.66.

24. *5,6,7,8-Tetrahydro-5-[4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl]pterin* (=*2-Amino-5,6,7,8-tetrahydro-5-[4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl]pteridin-4-(3*H*)-one*; **29**). At r.t., **26** (0.1 g, 0.23 mmol) was stirred in NH_3/MeOH for 24 h. The precipitate was washed with Et_2O, and dried: 75 mg (86%) of colorless **29**. M.p. 315° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.29. UV (MeOH): 280 (4.12), 224 (4.53), 203 (4.43). ^{1}H-NMR ((D_6)DMSO): 9.78 (br. *s*, H–N(3)); 7.52 (*d*, 2 arom. H); 7.17 (*d*, 2 arom. H); 7.01 (br. *s*, H–N(8)); 6.09 (br. *s*, NH_2); 4.59 (br. *s*, 1 H–C(6)); 3.50–3.33 (*m*, 1 H–C(7)); 2.65 (br. *s*, 1 H–C(6), 1 H–C(7)). Anal. calc. for $C_{15}H_{12}F_3N_7O_2$ (379.3): C 47.50, H 3.19, N 25.85; found: C 47.65, H 3.24, N 25.39.

25. *6-[[(4-Azidobenzoyl)oxy]methyl]-*N^2*-[(dimethylamino)methylene]pterin* (=*(2-[[(Dimethylamino)methylene]amino]-3,4-dihydro-4-oxopteridin-6-yl)methyl 4-Azidobenzoate*; **31**). Compound **30** [1] (0.2 g, 0.81 mmol) was co-evaporated twice with dry pyridine (10 ml) and subsequently dissolved in dry pyridine/CH_2Cl_2 1:1 (16 ml). After addition of 4-azidobenzoic chloride [21] (0.22 g, 1.21 mmol), the mixture was stirred at r.t. for 2 h. Solvents were evaporated, and the residue was co-evaporated with toluene (3×). The oily residue was treated with EtOH (10 ml) and sonicated. The resulting precipitate was dissolved in $CHCl_3$/MeOH, silica gel (1.5 g) added, the mixture evaporated, and the residue put on top of a column (silica gel (10 g)) for purification. FC (gradient 0–5% MeOH/CH_2Cl_2) yielded a product, which was dried under high vacuum: 96 mg (30%) of **31**. Yellowish powder. M.p. 183°. TLC (CH_2Cl_2/MeOH 5:1): R_f 0.82. UV (MeOH): 352 (4.16), 304 (4.56), 295 (4.56), 286 (4.55), 278 (4.54), 203 (4.42). ^{1}H-NMR ((D_6)DMSO): 12.06 (br. *s*, H–N(3)); 8.91 (*s*, CH=N); 8.81 (*s*, 1 H–C(7)); 8.03 (*d*, 2 arom. H); 7.22 (*d*, 2 arom. H); 5.47 (*s*, CH_2O); 3.22 (*s*, MeN); 3.09 (*s*, MeN). Anal. calc. for $C_{17}H_{15}N_9O_3 \cdot 0.5\ H_2O$ (402.4): C 50.75, H 4.01, N 31.33; found: C 51.17, H 3.93, N 30.82.

26. *6-[[3-(4-Benzoylphenyl)-1-oxopropoxy]methyl]-*N^2*-[(dimethylamino)methylene]pterin* (=*(2-[[(Dimethylamino)methylene]amino]-3,4-dihydro-4-oxopteridin-6-yl)methyl 4-Benzoylbenzenepropanoate*; **32**). A soln. of **30** [1] (0.3 g, 1.21 mmol), 3-(4-benzoylphenyl)propanoic acid [19] (0.62 g. 2.42 mmol), EDC (0.46 g, 2.42 mmol), and DMAP (0.3 g, 2.42 mmol) in dry pyridine (15 ml) was stirred overnight. After evaporation, the residue was co-evaporated with toluene (3×) and purified by FC (silica gel (12 g), gradient 0–2% MeOH/CH_2Cl_2). The product was purified by a second FC. The resulting yellowish foam was sonicated in Et_2O and the collected solid dried: 0.39 g (67%) of **32**. M.p. 123°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.70. UV (MeOH): 353 (4.07), 306 (4.50), 259 (4.45), 203 (4.55). ^{1}H-NMR ((D_6)DMSO): 12.06 (br. *s*, H–N(3)); 8.80 (*s*, H–C(7)); 8.78 (*s*, CH=N); 7.72–7.52 (*m*, 7 arom. H); 7.41 (*d*, 2 arom. H); 5.24 (*s*, CH_2O); 3.21 (*s*, MeN); 3.09 (*s*, MeN); 2.99 (*t*, CH_2); 2.82 (*t*, CH_2). Anal. calc. for $C_{26}H_{24}N_6O_4$ (484.5): C 64.45, H 4.99, N 17.35; found: C 64.43, H 5.09, N 17.00.

27. N^2*-[(Dimethylamino)methylene]-6-[([4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}oxy)methyl]-pterin* (=*[2-[[(Dimethylamino)methylene]amino]-3,4-dihydro-4-oxopteridin-6-yl]methyl 4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoate*; **33**). A soln. of **30** [1] (0.2 g, 0.81 mmol), 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid [5] (0.37 g, 1.6 mmol), EDC (0.31 g, 1.6 mmol), and DMAP (0.20 g, 1.6 mmol) in dry pyridine (20 ml) was stirred at r.t. for 14 h. After evaporation, the oily residue was co-evaporated with toluene (3×) and then purified by FC (silica gel (16 g), gradient 0–4% MeOH/CH_2Cl_2). The product was treated with AcOEt and sonicated to form a crystalline solid. Drying under high vacuum gave 0.18 g (49%) of yellowish **33**. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.61. M.p. 227–230° (dec.). UV (MeOH): 351 (4.17), 307 (4.54), 238 (4.48). ^{1}H-NMR ((D_6)DMSO): 12.07 (br. *s*, H–N(3)); 8.92 (*s*, CH=N); 8.82 (*s*, H–C(7)); 8.11 (*d*, 2 arom. H); 7.42 (*d*, arom. H); 5.51 (*s*, CH_2O); 3.23 (*s*, MeN); 3.09 (*s*, MeN). Anal. calc. for $C_{19}H_{15}F_3N_8O_3 \cdot 0.5\ H_2O$ (460.4): C 49.57, H 3.28, N 24.34; found: C 49.47, H 3.51, N 24.11.

28. *6-[[(4-Azidobenzoyl)oxy]methyl}pterin* (=*(2-Amino-3,4-dihydro-4-oxopteridin-6-yl)methyl 4-Azidobenzoate*; **34**). At r.t., **31** (0.2 g, 0.51 mmol) in MeOH (10 ml) and 1M HCl (4 ml) was stirred for 36 h. The resulting precipitate was washed with H_2O and dried: 0.16 g (93%) of yellowish **34**. M.p. >350°. TLC (CH_2Cl_2/MeOH 5:1): R_f 0.64. UV (1M HCl): 321 (sh, 3.85), 276 (4.18), 209 (4.23). ^{1}H-NMR ((D_6)DMSO): 11.46 (br. *s*, , H–N(3)); 8.83 (*s*, H–C(7)); 8.02 (*d*, 2 arom. H); 7.25 (*d*, 2 arom. H); 6.97 (br. *s*, NH_2); 5.44 (*s*, CH_2O). Anal. calc. for $C_{14}H_{10}N_8O_3$ (338.3): C 49.71, H 2.98, N 33.12; found: C 49.56, H 3.14, N 33.14.

29. *6-[[3-(4-Benzoylphenyl)-1-oxopropoxy]methyl]pterin* (=*(2-Amino-3,4-dihydro-4-oxopteridin-6-yl)-methyl 4-Benzoylbenzenepropanoate*; **35**). At r.t., **32** (0.17 g, 0.35 mmol) in MeOH (4 ml) and 1M HCl (0.5 ml) was stirred for 16 h. The resulting solid was washed with Et_2O and dried: 124 mg (83%) of colorless **35**. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.35. M.p. 255° (dec.). UV (MeOH): 344 (3.82), 266 (4.39), 205 (4.47). ^{1}H-NMR ((D_6)DMSO): 11.48 (br. *s*, H–N(3)); 8.65 (*s*, H–C(7)); 7.71–7.46 (*m*, 7 arom. H); 7.00 (br. *s*, NH_2); 5.20

(*s*, CH_2O); 2.98 (*t*, CH_2); 2.80 (*t*, CH_2). Anal. calc. for $C_{23}H_{19}N_5O_4$ (429.4): C 64.33, H 4.46, N 16.31; found: C 64.79, H 4.77, N 16.43.

30. *6-[([4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}oxy)methyl]pterin* (= *(2-Amino-3,4-dihydro-4-oxopteridin-6-yl)methyl 4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoate*; **36**). At r.t., **33** (80 mg, 0.17 mmol) in MeOH (3 ml) and 1M HCl (0.5 ml) was stirred overnight. After 3 h, a solid started to precipitate. It was washed with Et_2O and dried: 70 mg (99%) of colorless **36**. M.p. 295° (dec.). TLC (CH_2Cl_2/MeOH 5:1): R_f 0.58. UV (MeOH): 347 (3.87), 277 (4.29), 236 (4.42), 202 (4.39). ^{1}H-NMR ((D_6)DMSO): 11.48 (br. *s*, H–N(3)); 8.83 (*s*, H–C(7)); 8.10 (*d*, 2 arom. H); 7.43 (*d*, 2 arom. H); 6.97 (br. *s*, NH_2); 5.47 (*s*, CH_2O). Anal. calc. for $C_{16}H_{10}F_3N_7O_3$ (405.3): C 47.42, H 2.49, N 24.19; found: C 47.53, H 2.65, N 23.77.

31. *6-[[3-(4-Benzoylphenyl)-1-oxopropoxy]methyl]pteridine-2,4-diamine* (= *(2,4-Diaminopteridin-6-yl)-methyl 4-Benzoylbenzenepropanoate*; **38**). To a soln. of 3-(4-benzoylphenyl)propanoic acid [19] (0.30 g, 1.18 mmol) in DMF (10 ml), dry Et_3N (0.12 g; 1.18 mmol) and 6-(bromomethyl)pteridine-2,4-diamine (**37**) [1][22] (0.2 g, 0.78 mmol) were added. Then the suspension was stirred for 2 h at r.t. (TLC: no **37** left). The soln. was filtered, the red filtrate mixed with silica gel (0.3 g), the mixture evaporated under high vacuum, and the residue put on top of a column (silica gel (11 g)) for purification. FC (gradient 0–4% MeOH/CH_2Cl_2) gave a product, which was suspended in Et_2O. The collected solid was suspended in H_2O/MeOH 2:3 (removal of (Et_3NH)Br) and dried 26 mg (8%) of **38**. Yellowish powder. M.p. 208–210° (dec.). TLC (CH_2Cl_2/MeOH 5:1): R_f 0.52. UV (MeOH): 372 (3.90), 260 (4.60), 203 (4.56). ^{1}H-NMR ((D_6)DMSO): 8.68 (*s*, H–C(7)); 7.62 (*m*, 9 arom. H, NH_2); 7.42 (*d*, 2 arom. H); 6.76 (br. *s*, NH_2); 5.20 (*s*, CH_2O); 2.98 (*m*, CH_2); 2.81 (*m*, CH_2). Anal. calc. for $C_{23}H_{20}N_6O_3$ (428.5): C 64.48, H 4.70, N 19.61; found: C 64.01, H 4.94, N 18.87.

32. *6-[([4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl}oxy)methyl]pteridine-2,4-diamine* (= *(2,4-Diaminopteridin-6-yl)methyl 4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoate*; **39**). To a soln. of 4-[(3-trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid [19] (0.23 g, 1.02 mmol) in DMF (15 ml), dry Et_3N (0.10 g, 1.02 mmol) and **37** (0.2 g, 0.78 mmol) were added. The suspension was stirred at r.t. for 2 h. (TLC: no **37** left) and then filtered. The red filtrate was evaporated under high vacuum, the residue dissolved in a small volume of DMF/MeOH (silica gel (3 g)) added, the mixture again evaporated, and the residue put on top of a column (silica gel (11 g)) for purification. FC (gradient 0–6% MeOH/CH_2Cl_2) gave a product, which was suspended in Et_2O, filtered off, then again suspended in H_2O/MeOH 2:3 (removal of (Et_3NH)Br), filtered off, and dried: 17 mg (5%) of **39**. Yellowish powder. M.p. 220–225° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.43. UV (MeOH): 370 (3.93), 260 (4.50), 236 (4.45), 202 (4.40). ^{1}H-NMR ((D_6)DMSO): 8.87 (*s*, H–C(7)); 8.11 (*d*, 2 arom. H); 7.67 (br. *d*, NH_2); 7.43 (*d*, 2 arom. H); 6.75 (br. *s*, NH_2); 5.46 (*s*, CH_2O). Anal. calc. for $C_{16}H_{11}F_3N_8O_2$ (404.3): C 47.53, H 2.74, N 27.71; found: C 47.10, H 2.96, N 26.60.

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