HETEROCYCLES, Vol. 77, No. 2, 2009, pp. 953 - 970. © The Japan Institute of Heterocyclic Chemistry
Received, 28th July, 2008, Accepted, 18th September, 2008 Published online, 22nd September, 2008
DOI: 10.3987/COM-08-S(F)70

PTERIDINES CXX.[1] SYNTHESIS AND PROPERTIES OF TETRAHYDRO- PTERINS COUPLED TO 1,4-DIHYDROPYRIDINES

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Dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75th birthday

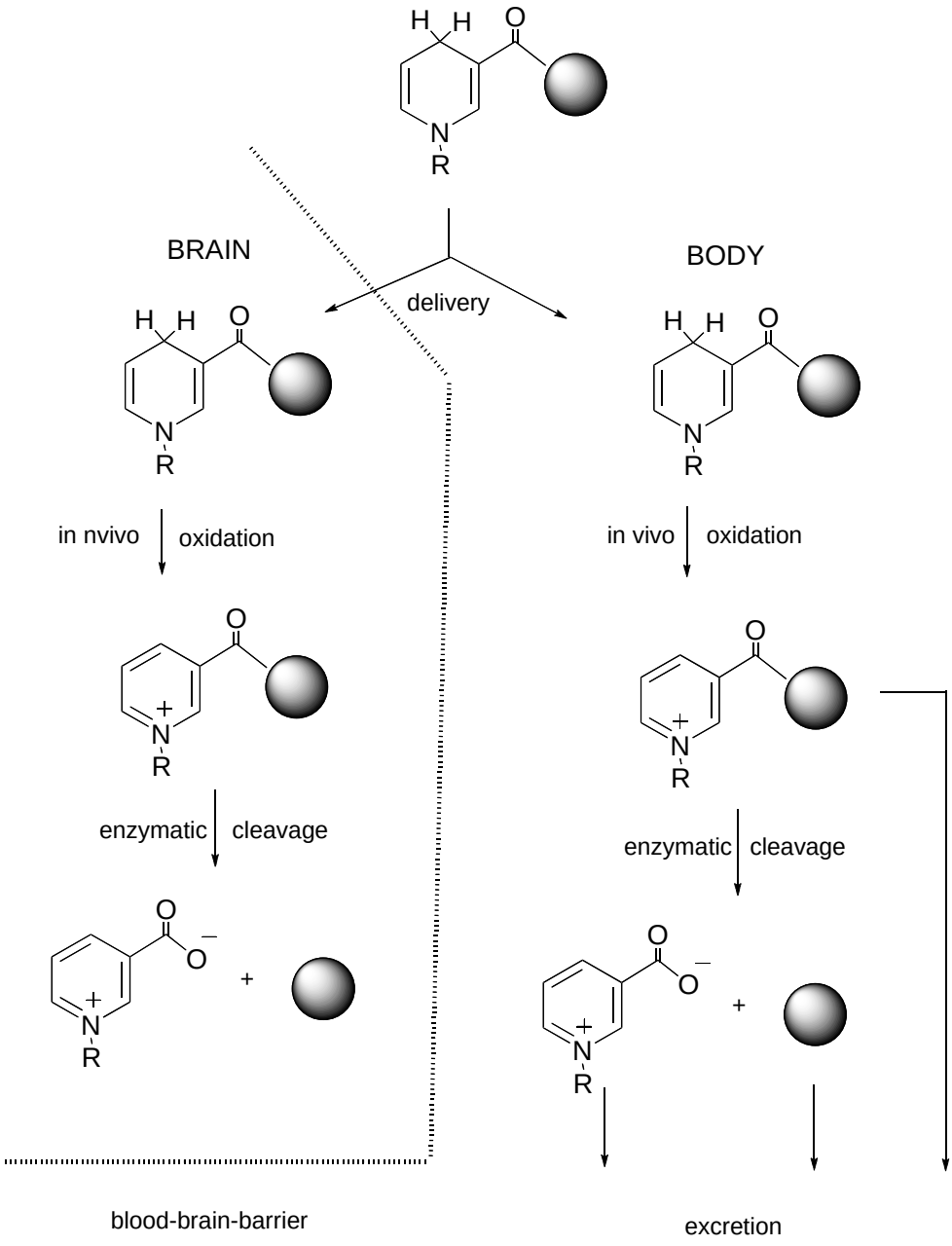
Abstract - N^2-Isobutyroyl-5,6,7,8-tetrahydropterins (**6-10**) have been coupled with nicotinic acid to form the N^5-nicotinoyl derivatives **11-15**. Quaternization at the pyridine moiety led to **19-31** which can be reduced to the corresponding *N*-substituted 1,4-dihydropyridine dertivatives **35-39**. Partial deacylation of the isobutyroyl group afforded the various types of terahydropterins **16-18**, **32-34** and **40-42**. The newly synthesized 5,6,7,8-tetrahydropterin derivatives have been characterized by pK_a-determinations, UV- and NMR-spectra as well as elemental analyses.

INTRODUCTION

Tetrahydrobiopterin (BH_4) is an important cofactor for some aromatic monooxygenases which participate in the synthesis of tyrosine, L-Dopa and 5-hydroxytryptophan. A lack of this cofactor causes several neurological diseases such as Parkinson disease and atypical phenylketonuria. Since the potential clinical agent, tetrahydrobiopterin, does not penetrate the blood-brain-barrier very effectively[2] this problem may be overcome by addition of a lipophilic substituent[3-5] or by the attachment of a carrier moiety.[6,7] The Bodor-system[8-10] offers with the dihydropyridine $\rightleftharpoons$ pyridinium salt carrier an interesting alternative and we decided to investigate this possibility in the tetrahydropterin series.

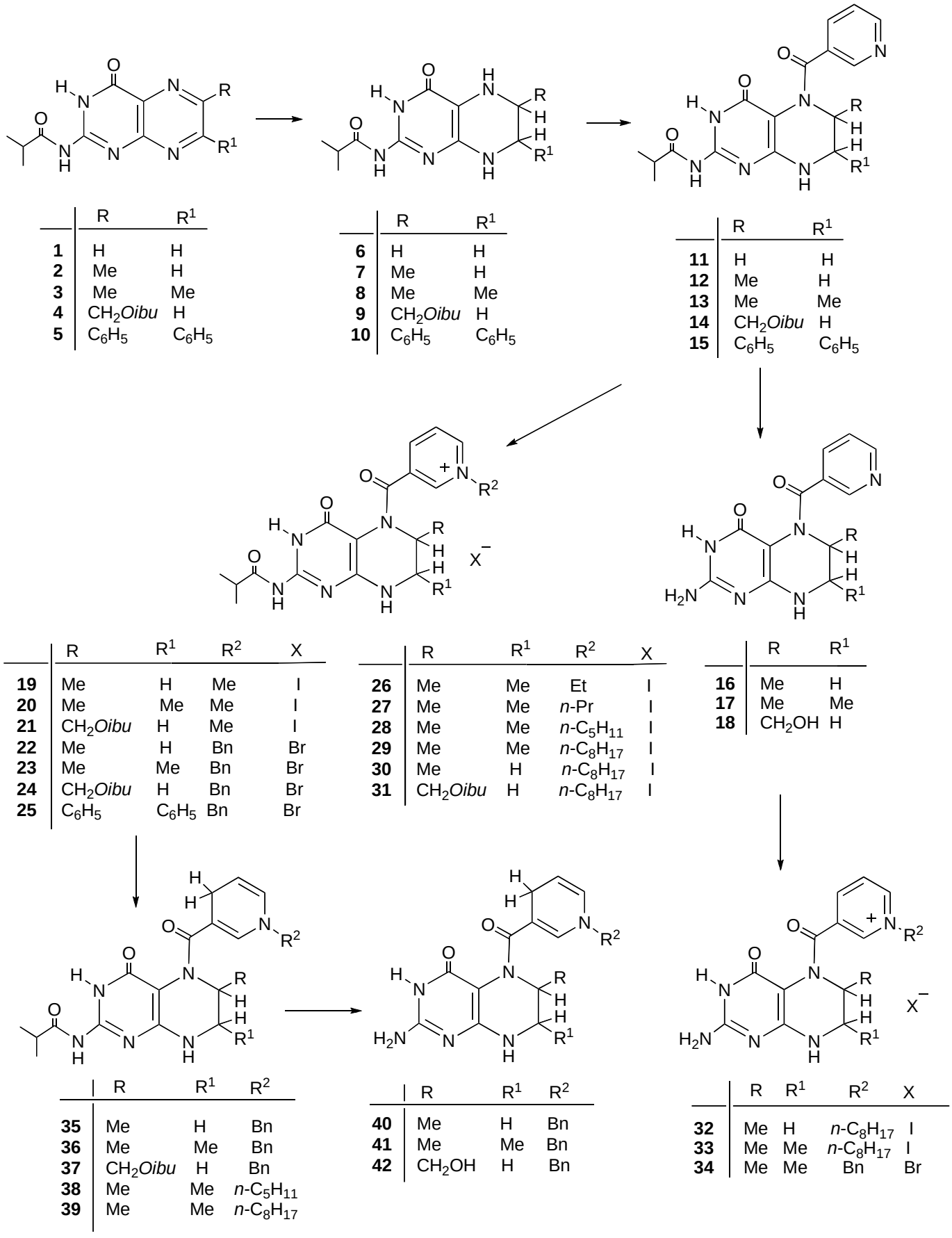
The basic concept of this approach is illustrated by the coupling of nicotinic acid to a biologically active molecule, quaternization with alkylating agents at the pyridine moiety and followed by reduction of the pyridinium salt to the corresponding 1,4-dihydropyridine compound. Such a compound will be distributed on dispension in the body including the brain. The dihydropyridine moiety will be oxidized to

form the quaternary salt which due to its ionic character should be eliminated fast from the body while the blood-brain-barrier should prevent its elimination from the brain. After cleavage of the nicotinoyl residue the biologically active compound is regenerated in the brain in free form. A properly selected carrier should either be eliminated or should be pharmacologically harmless. The overall result is a brain specific sustained release of the active molecule.



RESULTS AND DISCUSSION

We applied the Bodor concept first to three synthetic analogs of BH_4, the 6-methyl-, the 6,7-dimethyl-, the 6-hydroxymethyl- and the 6,7-diphenyl-5,6,7,8-tetrahydropterin,[11-13] respectively, all of which showing some cofactor activity *in vitro*.



	R	R^1
1	H	H
2	Me	H
3	Me	Me
4	CH_2*Oibu*	H
5	C_6H_5	C_6H_5

	R	R^1
6	H	H
7	Me	H
8	Me	Me
9	CH_2*Oibu*	H
10	C_6H_5	C_6H_5

	R	R^1
11	H	H
12	Me	H
13	Me	Me
14	CH_2*Oibu*	H
15	C_6H_5	C_6H_5

	R	R^1	R^2	X
19	Me	H	Me	I
20	Me	Me	Me	I
21	CH_2*Oibu*	H	Me	I
22	Me	H	Bn	Br
23	Me	Me	Bn	Br
24	CH_2*Oibu*	H	Bn	Br
25	C_6H_5	C_6H_5	Bn	Br

	R	R^1	R^2	X
26	Me	Me	Et	I
27	Me	Me	*n*-Pr	I
28	Me	Me	*n*-C_5H_{11}	I
29	Me	Me	*n*-C_8H_{17}	I
30	Me	H	*n*-C_8H_{17}	I
31	CH_2*Oibu*	H	*n*-C_8H_{17}	I

	R	R^1
16	Me	H
17	Me	Me
18	CH_2OH	H

	R	R^1	R^2
35	Me	H	Bn
36	Me	Me	Bn
37	CH_2*Oibu*	H	Bn
38	Me	Me	*n*-C_5H_{11}
39	Me	Me	*n*-C_8H_{17}

	R	R^1	R^2
40	Me	H	Bn
41	Me	Me	Bn
42	CH_2OH	H	Bn

	R	R^1	R^2	X
32	Me	H	*n*-C_8H_{17}	I
33	Me	Me	*n*-C_8H_{17}	I
34	Me	Me	Bn	Br

Scheme 1. Interonversions of pterins into 5-nicotinoyl-5,6,7,8-tetrahydropterin derivatives

The starting N^2-isobutyroylpterins (**1-5**) have first been reduced catalytically with Pt under H_2-atmosphere in a shaken apparatus to their corresponding 5,6,7,8-tetrahydro derivatives (**6-10**) which were then subject to acylation with nicotinoyl chloride in pyridine at rt to form selectively the N^5-nicotinoyl derivatives (**11-15**). These substances were isolated as racemic mixtures due to the new chiral centers in 6- and 7 position, respectively. 5,6,7,8-Tetrahydropterins possess in N^5-position their most nucleophilic center in form of a secundary amine type whereas the HN^8 -side is part of a vinylogous amide moiety and is therefore less reactive under the chosen reaction conditions. The high hydrolytic stability of the N^5-acyl bond is seen from the fact that treatment of **12-15** with sodium methoxide in MeOH at rt cleaves selectively only the N^2-isopropionyl group to give **16-18**. Reactions of **12-15** with various alkylating agents such as methyl, ethyl, *n*-propyl, *n*-pentyl and *n*-octyl iodide in MeOH or DMF as well as with benzyl bromide in DMSO solution led under mild conditions at rt for 1- 5 days in high yields to the *N*-alkylated pyridinium salts (**19-31**). Quarternization of the pyridine moiety takes place also selectively with **17** and **18** to yield **32-34**. Reduction of several pyridinium salts under mild basic conditions with sodium dithionite to the corresponding 1,4-dihydronicotinoyl derivatives (**35-39**) worked only in a few cases (**22-24**, **28** and **29)**, especially in the *N*-benzyl series. Selective removal of the isobutyroyl group by ammonia treatment at rt resulted in the carrier molecules **40-42**. The characterization of the newly synthesized pterin derivatives was achieved by elemental analyses, ^{1}H-NMR spectra (experimental part) and the determination of the pK_a values by the spectrophotometric method[14] combined with UV-spectra (Tables 1, 2 and 3). On the basis of the *pKa*-values the defined UV-spectra of the monocation, neutral form and monoanion as well as the dication, monocation and zwitter iononic form have been determined to compare the equivalent molecular species as further proof of the structural assignments.

Table 1. UV-Spectra of *N*5-Nicotinoyl- and *N*5-(1-alkylnicotinoyl)-5,6,7,8-tetrahydropterins

-5-nicotinoyl-5,6,7,8-tetrahydropterin	pK_a in H_2O	λ_{max}*	log *e*	pH	molecular species
*N*2-isobutyroyl- (**11**)	3.16 9.44	233 (260) 314 235 270 310 214 (244) (268) 304	4.45 (4.03) 3.81 4.50 3.92 3.92 4.51 (4.18) (3.95) 3.82	1 7 12	+ o -
*N*2-isobutyroyl-6-methyl-(**12**)	3.19 9.68	204 234 (262) 316 202 235 268 309 214 (242) (268) 306	4.33 4.47 (4.06) 3.84 4.33 4.50 3.92 3.92 4.51 (4.21) (3.97) 3.84	1 7 12	+ o -
*N*2-isobutyroyl-6,7-dimethyl-(**13**)	3.16 9.59	2046 235 (264) 317 237 (268) 310 207 (2414 (268) 300	4.31 4.46 (4.05) 3.82 4.51 (3.93) 3.93 4.52 (4.50) (3.96) 3.83	1 7 12	+ o -
*N*2-isobutyroyl-6-isobutyroyloxy-methyl-(**14**)	3.00 9.47	206 233 (264) 316 235 266 310 213(246) (266) 300	4.34 4.45 (4.01) 3.76 4.51 3.91 3.93 4.51 (4.20 (3.98) 3.84	1 7 12	+ o -
*N*2-isobutyroyl-6,7-diphenyl-(**15**)	3.06 9.34	230 (262) 320 203 227 (268) 314 212(268) (288) 318	4.54 (4.16) 3.83 4.63 4.55 (4.10) 3.91 4.66 (4.06) (3.89) 3.78	1 7 12	+ o -

6-methyl- (**16**)	3.48 10.46	217 270 (316) 222 (276) 286 207 267 (310)	4.31 4.06 (3.68) 4.37 (3.99) 4.00 4.44 3.97 (3.60)	2.3 7 12	+ o -
6,7-dimethyl-(**17**)	3.55 10.33	(206) 218 270 (320) 222 (276) 287 209 267 (328)	(4.29) 4.32 4.02 (3.65) 4.35 (3.96) 3.98 4.40 3.94 (3.53)	2.3 7 12	+ o -
6-hydroxymethyl-**(18)**	3.38 10.30	216 270 (320) 223 (278) 287 206 (224) 268 (308)	4.36 4.10 (3.71) 4.41 (4.03) 4.05 4.57 (4.27) 4.00 (3.69)	2 6 12	+ o -
-5,6,7,8-tetrahydro-pterin					
6-methyl-5-(1-*n*-octyl-nicotinoyl)- iodide (**32**)	1.09 9.62	217 275 (312) 220 273 334 215 266 360	4.41 4.13 (3.76) 4.56 4.03 3.55 4.52 4.05 3.40	- 1 5 12	+ + + + -
6,7-dimethyl-5-(1-*n*-octyl-nicotinoyl)- iodide (**33**)	1.12 9.46	218 276 (316) 222 273 334 215 266 363	4.44 4.14 (3.76) 4.57 4.05 3.57 4.52 4.07 3.43	- 1 5 12	+ + + + -
6,7-dimethyl-5-(1-benzyl-nicotinoyl)- bromide (**34**)	1.07 9.81	278 334 209 (220) 274 346 206 (254) 266 372	4.10 3.59 4.47 (4.41) 4.03 3.47 4.64 (4.05) 4.07 3.35	- 1 5 12	+ + + + -

*( ) shoulder

Table 2. UV-Spectra of *N*2-Isobutyroyl-5,6,7,8-tetrahydropterin-5-carbonyl-1-alkylpyridinium Salts

*N*2-Isobutyroyl-5,6,7,8-tetra-hydropterin-5-carbonyl-	pK_a in H_2O	λ_{max}*	log *e*	pH	molecular species
6-methyl-(1-methyl-pyridinium) iodide (**19**)	- 1.35 8.77	214 274 (316) 227 264 320 219 (264) 324	4.58 4.09 (3.90) 4.60 4.02 3.80 4.64 (4.05) 3.63	- 3 5 11	++ + + -
6,7-dimethyl-(1-methyl-pyridinium) iodide (**20**)	- 1.23 8.97	214 273 (308) 226 (268) 319 219 (266) 318	4.58 4.09 (3.95) 4.59 (4.02) 3.81 4.64 (4.06) 3.68	- 3 5 11	++ + + -
6-isobutyroyloxymethyl (1-methylpyridinium) iodide (**21**)	- 1.18 8.83	214 274 (304) 226 266 320 219 (263) 338	4.57 4.07 (3.94) 4.58 3.99 3.78 4.64 (4.05) 3.62	- 3 5 11	++ + + -
6-methyl-(1-benzylyl-pyridinium) bromide (**22**)	- 1.23 8.97	212 276 324 230 (268) 335 205 (262) 351	4.49 4.03 3.78 4.39 (3.95) 3.59 4.57 (4.03) 3.43	-4 5 11	++ + + -
6,7-dimethyl-(1-benzyl-pyridinium) bromide (**23**)	- 1.66 9.09	(209) 276 (320) 231 (272) 334 (226) (264) 351	(4.59) 4.09 (3.90) 4.47 (3.97) 3.62 (4.45) (4.07) 3.48	- 4 5 11	++ + + -
6-isobutyroyloxymethyl-(1-benzyl-pyridinium) bromide (**24**)	- 2.28 9.14	(212) 276 (316) 230 (268) 335 220 (264) 347	(4.56) 4.06 (3.89) 4.44 (3.96) 3.61 4.63 (4.11) 3.48	- 4 5 11	++ + + -
6,7-diphenyl-(1-benzyl-pyridinium) bromide (**25**)	- 2.46 9.09	279 320 232 (268) 336 (228) (264) 360	4.09 3.88 4.53 (4.05) 3.61 (4.50) (4.11) 3.47	- 4.3 5 11	++ + + -
6,7-dimethyl-(1-ethyl-pyridinium) iodide (**26**)	- 1.23 8.97	213 274 (316) 226 (266) 318 219 (264) 340	4.60 4.10 (3.90) 4.60 (4.02) 3.79 4.65 (4.07) 3.57	- 4 5 11	++ + + -

6,7-dimethyl-(1-propyl-pyridinium) iodide (**27**)	- 1.47 8.66	213 274 (314) 226 (268) 319 220 (266) 338	4.61 4.09 (3.93) 4.60 (4.01) 3.75 4.64 (4.05) 3.53	- 4 5 11	++ + + -
6,7-dimethyl-(1-*n*-pentyl-pyridinium) iodide (**28**)	- 1.61 8.68	213 274 (316) 226 (268) 314 220 (264) 343	4.62 4.09 (3.93) 4.62 (4.00) 3.73 4.65 (4.06) 3.54	- 4 5 11	++ + + -
6,7-dimethyl-(1-*n*-octyl-pyridinium) iodide (**29**)	- 1.71 9.09	213 274 (316) 226 (268) 314 220 (264) 346	4.62 4.09 (3.92) 4.62 (3.97) 3.72 4.66 (4.07) 3.52	- 4 5 11	++ + + -
6-methyl-(1-*n*-octyl-pyridinium) iodide (**30**)	- 2.06 8.92	213 274 (308) 226 (268) 320 220 (262) 343	4.63 4.09 (3.94) 4.62 (4.00) 3.73 4.64 (4.05) 3.52	- 4 5 11	++ + + -
6-isobutyroyloxymethyl-(1-*n*-octylpyridinium iodide (**31**)	- 1.90 8.95	214 274 (304) 226 (266) 316 220 (264) 347	4.61 4.06 (3.94) 4.61 (3.96) 3.71 4.63 (4.02) 3.50	- 3 3 11	++ + + -

* ( ) shoulder

Table 3. UV-Spectra of *N*2-Isobutyroyl-5-(1-alkyl-1,4-dihydropyridine-3-carbonyl)-5,6,7,8-tetrahydropterin

*N*2-Isobutyroyl- -5,6,7,8-tetrahydropterin	λ_{max}*	log *e*	pH	molecular species
-6,7-dimethyl-5-(1-benzyl-1,4-dihydropyridine-3-carbonyl)- (**35**)	204 234 278 316 356	4.46 4.46 3.94 3.89 3.81	MeOH	o
-6-methyl-5-(1-benzyl-1,4-dihydropyridine-3-carbonyl)- (**36**)	203 233 282 (312) 356	4.43 4.46 3.98 (3.88) 3.81	MeOH	o
-6-isobutyroyloxymethyl-5-(1-benzyl-1,4-dihydro-pyridine-3-carbonyl)- (**37**)	204 232 276 313 353	4.46 4.44 3.98 3.92 3.84	MeOH	o
-6,7-dimethyl-5-(1-*n*-pentyl-1,4-dihydropyridine-3-carbonyl)- (**38**)	207 235 278 320 359	4.33 4.47 3.93 3.90 3.86	MeOH	o
-6,7-dimethyl-5-(1-*n*-octyl-1,4-dihydropyridine-3-carbonyl)- (**39**)	205 234 276 320 354	4.33 4.46 3.92 3.89 3.83	MeOH	o
-5,6,7,8-tetrahydropterin				
-6,7-dimethyl-5-(1-benzyl-1,4-dihydropyridine-3-carbonyl)- (**40**)	209 (217) 289 359	4.45 (4.44) 4.02 3.76	MeOH	o
-6,7-dimethyl-5-(1-benzyl-1,4-dihydropyridine-3-carbonyl)- (**41**)	207 (218) 282 358	4.44 (4.40) 4.04 3.73	MeOH	o
-6,7-dimethyl-5-(1-benzyl-1,4-dihydropyridine-3-carbonyl)- (**42**)	211 (220) 288 360	4.46 (4.43) 4.07 3.82	MeOH	o

* ( ) shoulder

EXPERIMENTAL

General. TLC: precoated cellulose thin-layer sheets F 1440b LS 254 and silica gel thin-layer sheets F 1500 LS 254 from *Schleicher and Schüll*. Column chromatography: silica gel 60 from *Merck*. M.p.: *Büchi* Melting Point B-545; no corrections. The pK_a measurements were performed by the spectrophotometric method.[14] UV: Lambda 15 recording spectrometer from Perkin-Elmer: λ_{max} (nm); log ε; (sh) shoulder. ^{1}H-NMR: *Bruker* WM-250 spectrometer in δ (ppm) relative to TMS. Mass spectra: Firma *Finnigan*, model MAT 312. Elemental analyses were performed in the microanalytical laboratory of Konstanz University.

***N*^{2}-Isobutyroylpterin (1):** A suspenion of pterin (1.0 g, 6.1 mmol) in abs. pyridine (40 mL) was treated with isobutyric anhydride (10 mL) under reflux for 4 h. It was evaporated and the residue recrystallized from MeOH (180 mL) with charcoal to give 1.06 g (75%) colorless crystals, mp >290 °C (decomp.). pK_a: 7.39. UV (pH 5): 214 (sh 4.13), 230 (4.16), 276 (4.14), 326 (3.87), 334 (sh 3.85); (pH 10): 224 (3.95), 252 (4.42), 282 (3.63), 339 (3.83). ^{1}H-NMR (DMSO-d_6): 1.15 (d, 6H, Me_2C), 2.72 (sept, 1H, H-CMe_2), 8.71 (d, 1H, H-C(6)), 8.92 (d, 1H, H-C(7)), 11.90 (s, 1H, H-N(3)), 12.32 (s, 1H, H-N). Anal. Calcd for $C_{10}H_{11}N_5O_2$ (233.2): C, 51.50; H, 4.75; N, 30.03. Found: C, 51.60; H, 4.82; N, 30.01.

***N*^{2}-Isobutyroyl-6-methylpterin (2):** To a suspension of 6-methylpterin[15,16] (2.0 g, 0.011 mmol) in abs. pyridine (20 mL) was treated with isobutyric anhydride (5 mL) under reflux for 4.5 h. Charcoal was added to the hot solution, filtered and after cooling evaporated to dryness. The residue was treated with MeOH and the solid collected to give after drying in the oven at 100 °C 1.59 g (57%). Recrystallization from MeOH/H_2O gave 1.02 g (37%) colorless needles, mp 285 °C. pK_a: 7.64. UV (pH 5): 228 (4.12), 278 (4.20), 335 (3.88); (pH 10): 224 (sh 3.97), 253 (4.42), 282 (sh 3.73), 344 (3.84). ^{1}H-NMR, (DMSO-d_6): 1.12 (d, 6H, $C(Me_2)$, 2.59 (s, 3H, H_3C-C(6)), 2.76 (sept, 1H, H-CMe_2), 8.79 (s, 1H, H-C(7)), 11.89 (s, H-N(3)), 12.3 (bs, 1H, H-N). Anal. Calcd for $C_{11}H_{13}N_5O_2$ (247,3): C, 53.43; H, 5.30; N, 28.32. Found: C, 53.23; H, 5.31; N, 27.88.

***N*^{2}-Isobutyroyl-6-isobutyroyloxymethylpterin (4):** A suspension of 6-hydroxymethylpterin[17] (4,0 g, 20.4 mmol) in abs. pyridine was treated with isobutyric anhydride (30 mL) under reflux for 5 h. After cooling MeOH (20 mL) was added, stirred for 30 min, then evaporated, and coevaporated with toluene. The residue was treated with EtOAc, the resulting precipitate collected, then dissolved in $CHCl_3$ and purified by silica-gel column chromatography with $CHCl_3$/MeOH 30:1. The main fraction was separated, evaporated and the residue recrystallized from *n*-hexane/$CHCl_3$ to give 4.32 g (62 %) colorless crystals. mp 183 °C. pK_a: 7.24. UV (pH 5): 222 (sh 4.12), 233 (4.15), 281 (4.33), 335 (3.92); (pH 10): 224 (sh

3.98), 256 (4.47), 284 (sh 3.74), 346 (3.89). ^{1}H-NMR (DMSO-d_6): 1.10-1.20 (2d, 12H, 2 Me_2C), 2.52 (sept, 1H, H-CMe_2), 2.72 (sept, 1H, H-CMe_2), 5.33 (s, 2H, CH_2), 8.91 (s, 1H, H-C(7)), 11.80 (s, 1H, H-N(3)), 12.22 (s, 1H, H-N). Anal. Calcd for $C_{15}H_{19}N_5O_4$ (333.4): C, 54.05; H, 5.75; N, 21.01. Found: C, 53.67; H, 5.75; N, 20.90.

N^2-Isobutyroyl-6.7-diphenylpterin (5): A suspension of 6.7-diphenylpterin[18] (5.3 g, 16.8 mmol) in abs. pyridine (60 mL) was treated with isobutyric anhydride (15 mL) under reflux for 2.5 h. After cooling down to 80 °C EtOH was added to the clear yellowish solution. On cooling a colorless precipitate separated, was collected, washed with MeOH and dried at 100 °C to give 6.0 g (92%), mp 269 °C. *pK_a*: 7.63. UV (pH 5): 225 (4.44), 256 (4.26), 296 (4.30), 365 (4.19); (pH 10): 221 (sh 4.37), 252 (sh 4.37), 258 (4.38), 371 (4.15). ^{1}H-NMR, (DMSO-d_6): 1.13 (d, 6H, $C(Me_2)$, 2.80 (m, 1H, H-CMe_2), 7.26-7.53 (m, 10H, 2 ph), 9.82 (s, H-N(3)), 12.5 (bs, 1H, H-N). Anal. Calcd for $C_{22}H_{19}N_5O_2$ (385.4): C, 68.56; H, 4.97; N, 18.17. Found: C, 67.94; H, 5.05; N, 17.97.

N^2-Isobutyroyl-5-nicotinoyl-5,6,7,8-tetrahydropterin (11): In a shaking apparatus a suspension of N^2-isobutyroylpterin (**1**) (1.0 g, 4.3 mmol) in MeOH (100 mL) was reduced under H_2-atmosphere in presence of PtO_2 (100 mg) as catalyst. After 24 h 2 equivalents of H_2 were consumed. The suspension was evaporated in vacuum to dryness and the residue treated with abs. pyridine (30 mL) and nicotinoyl chloride hydrochloride (1.03 g, 5.8 mmol) with stirring overnight. EtOH (20 mL) was added, stirred for 1 h and then the precipitate filtered off. Recrystallization from EtOH/H_2O (1:1) with charcoal gave 0.385 g (26%) of a yellowish crystal powder, mp 297-299 °C (decomp.). *pK*: 3.16, 9.44. ^{1}H-NMR, (DMSO-d_6): 1.01 (d, 6H, $C(Me_2)$, 2.69 (sept, 1H, H-CMe_2), 3.31 (m, 2H, H-C(7)), 3.48 (m, 2H, H-C(6)), 7.47 (s, 1H, H-N(8)), 7.38 (dd, 1H, nic-5), 7.79 (d, 1H, nic-4), 8.49 (d, 1H, nic-6), 8.59 (s, 1H, nic-2), 11.06 (s, H-N(3)), 11.38 (bs, 1H, H-N). Anal. Calcd for $C_{16}H_{18}N_6O_3$ x 0.25 H_2O (346.9): C, 55.40; H, 5.38; N, 24.23. Found: C, 55.37; H, 5.54; N, 23.83.

N^2-Isobutyroyl-6-methyl-5-nicotinoyl-5,6,7,8-tetrahydropterin (12): Analogous to the preceding procedure with **2** (1.0 g, 4.05 mmol). The crude naterial was purified by recrystallization from MeOH (20 mL) with little charcoal to give 0.65 g (45%) colorless needles, mp 310 °C. *pK_a*: 3.19, 9.68. ^{1}H-NMR, (DMSO-d_6): 1.03 (d, 6H, $C(Me_2)$, 1.08 (d, 3H, Me-C(6)), 2.69 (sept, 1H, H-CMe_2), 3.29-3.49 (m, 2H, H-C(7)), 4.81 (m, 1H, H-C(6)), 7.43 (s, 1H, H-N(8)), 7.31 (dd, 1H, nic-5), 7.79 (d, 1H, nic-4), 8.49 (d, 1H, nic-6), 8.58 (s, 1H, nic-2), 11.06 (s, H-N(3)), 11.32 (bs, 1H, H-N). Anal. Calcd for $C_{17}H_{20}N_6O_3$ (356.4): C, 57.29; H, 5.66; N, 23.58. Found: C, 57.10; H, 5.77; N, 23.45.

***N*^{2}-Isobutyroyl-6,7-dimethyl-5-nicotinoyl-5,6,7,8-tetrahydropterin (13):** A solution of *N*^{2}-isobutyroyl-6,7-dimethyl-5,6,7,8-tetrahydropterin **(8)**[5] **(**10.78 g, 33.7 mmol) in abs. pyridine (200 mL) was cooled to 0°C and under N_2-atmosphere nicotinoyl chloride hydrochloride (8,5 g, 47 mmol) added with stirring. After stirring for 2 days under rt MeOH (10 ml) was added and the reaction mixture kept overnight in the icebox. The precipitate was collected, washed with acetone and ether and dried in the vacuum desiccator to give 7.3 g (58%) colorless powder, mp > 300 °C, From the filtrate were isolated another 1.46 g (12%). pK_a: 3.16, 9.59. ^{1}H-NMR, (DMSO-d_6): 0.85 (d, 3H, Me-C(7)), 1.03 (d, 6H, C(Me_2), 1.17 (d, 3H, Me-C(6)), 2.72 (sept, 1H, H-CMe_2), 3.80 (m, 1H, H-C(7)), 4.59 (m, 1H, H-C(6)), 7.33 (bs, 1H, H-N(8)), 7.34 (dd, 1H, nic-5), 7.79 (d, 1H, nic-4), 8.48 (d, 1H, nic-6), 8.57 (s, 1H, nic-2), 11.08 (s, H-N(3)), 11.18 (bs, 1H, H-N). Anal. Calcd for $C_{18}H_{22}N_6O_3$ (370.4): C, 57.29; H, 5.66; N, 23.58. Found: C, 57.10; H, 5.77; N, 23.45.

***N*^{2}-Isobutyroyl-6-isobutyroyloxymethyl-5-nicotinoyl-5,6,7,8-tetrahydropterin (14):** Analogous to procedure **11** with **4** (2.0, g, 6 mmol) in MeOH (180 mL) and PtO_2 (0.36 g). The catalyst was filtered off and the filtrate evaporated to dryness. The residue was dissolved in abs. pyridine (80 mL) and nicotinoyl chloride hydrochloride (1.75 g, 7.75 mmol) added with stirring overnight. EtOH (10 mL) was added, stirred for 30 min, evaporated and twice coevaporated with toluene/EtOH. The residue was recrystallized from EtOH (30 mL) to give 1.18 g (44%) of colorless crystals, mp 300 °C (decomp.). pK_a: 3.00, 9.47. ^{1}H-NMR, (DMSO-d_6): 0.99 (d, 6H, CMe_2)), 1.04 (d, 6H, C(Me_2), 2.49 (sept, 1H, H-CMe_2), 2.71 (sept, 1H, H-CMe_2), 3.35-3.51 (m, 2H, H-C(7)), 3.97 (m, 2H, O-CH_2), 4.98 (bs, 1H, H-C(6)), 7.47 (bs, 1H, H-N(8)), 7.37 (dd, 1H, nic-5), 7.81 (d, 1H, nic-4), 8.51 (d, 1H, nic-6), 8.61 (s, 1H, nic-2), 11.02 (s, H-N(3)), 11.32 (bs, 1H, H-N). Anal. Calcd for $C_{21}H_{26}N_6O_5$ (442.5): C, 57.00; H, 5.42; N, 18.99. Found: C, 56.58; H, 5.89; N, 18.94.

***N*^{2}-Isobutyroyl-6,7-diphenyl-5-nicotinoyl-5,6,7,8-tetrahydropterin (15):** Analogous to the preceding procedure with **5** (5.48 g, 14.2 mmol) in MeOH (350 mL) and PtO_2 (0.5 g). The resulting suspension was evaporated, the residue dissolved in abs. pyridine (200 mL) and nicotinoyl chloride hydrochloride (3.4 g, 19.2 mmol) added. After stirring for 18 h the catalyst was filtered off and the filtrate evaporated to half of its volume. EtOH (50 mL) was added, the precipitate collected and recrystallized from little EtOH/DMF (1:1) to give 2.1 g (29%) of yellowish crystal powder, mp 289 °C. pK_a: 3.06, 9.34. ^{1}H-NMR, (DMSO-d_6): 1.05 (d, 6H, C(Me_2), 2.76 (sept, 1H, H-CMe_2), 5.40 (m, 2H, H-C(7)), 5.65 (bs, 1H, H-C(6)), 6.80-7.20 (m, 10H, ph), 7.35 (dd, 1H, nic-5), 7.91 (d, 1H, nic-4), 8.17 (bs, 1H, H-N(8)), 8.53 (d, 1H, nic-6), 8.70 (s, 1H, nic-2), 11.02 (s, H-N(3)), 11.32 (bs, 1H, H-N). Anal. Calcd for $C_{28}H_{26}N_6O_3$ x 0.5 H_2O (503.6): C, 66.78; H, 5.40; N, 16.68. Found: C, 66.64; H, 5.47; N, 16.56.

6-Methyl-5-nicotinoyl-5,6,7,8-tetrahydropterin (16): In abs. MeOH (180 mL) was dissolved Na (0.234 g, 10 mmol) and then under stirring **12** (3.0 g, 8.4 mmol) added. After stirring for 12 h the yellowish solution was evaporated, the residue dissolved in H_2O (80 mL), filtered, extracted with ether (30 mL) and then neutralized with AcOH to pH 7. After cooling overnight the precipitate was collected and dried in a vacuum desiccator to give 1.8 g (70%) colorless needles, mp 265-270 °C. pK_a: 3.48, 10.46. ^{1}H-NMR, (DMSO-d_6): 0.98 (d, 3H, Me-C(6)), 3.22 (m, 1H, H-C(7)), 3.45 (m, 1H, H-C(7)), 4.80 (m, 1H, H-C(6)), 6.08 (bs, 2H, NH_2), 7.02 (s, 1H, H-N(8)), 7.30 (dd, 1H, nic-5), 7.81 (d, 1H, nic-4), 8.40 (d, 1H, nic-6), 8.54 (s, 1H, nic-2), 9.71 (s, H-N(3)). Anal. Calcd for $C_{13}H_{14}N_6O_2$ x H_2O (304.3): C, 51.31; H, 5.30; N, 27.62. Found: C, 51.75; H, 4.90; N, 27.74.

6,7-Dimethyl-5-nicotinoyl-5,6,7,8-tetrahydropterin (17): A solution of 6,7-dimethyl-5,6,7,8-tetrahydropterin dihydrochloride[5] (11.3 g, 42 mmol) in abs. pyridine (600 mL) was cooled to 5 °C and then under stirring and N_2-atmosphere nicotinoyl chloride hydrochloride (9.5 g, 53.4 mmol) added. It was stirred at rt over night, evaporated and coevaporated with toluene/EtOH. The residue was treated with EtOH (100 mL), after cooling the precipitate collected and dried to give 10.4 g (74%) of the monohydrochloride salt. It was dissolved in H_2O (300 mL), $NaHCO_3$ (2.4 g) added, stirred for 1 h and then the precipitate collected to give 9.0 g (61%) of a yellowish crystal powder, mp 295 °C (decomp.). pK_a: 3.55, 10.30. ^{1}H-NMR, (DMSO-d_6): 0.98 (d, 3H, Me-C(6)), 1.05 (d, 3H, Me-C(7)), 3.65 (m, 1H, H-C(7)), 4.51 (m, 1H, H-C(6)), 6.08 (bs, 2H, NH_2), 7.05 (s, 1H, H-N(8)), 7.30 (dd, 1H, nic-5), 7.75 (d, 1H, nic-4), 8.40 (d, 1H, nic-6), 8.60(s, 1H, nic-2), 9.70 (bs, H-N(3)). Anal. Calcd for $C_{14}H_{16}N_6O_2$ (300.3): C, 55.99; H, 5.37; N, 27.98. Found: C, 55.52; H, 5.47; N, 27.53.

6-Hydroxymethyl-5-nicotinoyl-5,6,7,8-tetrahydropterin (18): Analogous to procedure **16** with **14** (2.0 g, 4.5 mmol) in MeOH (100 mL) and Na (0.125 g). The resulting solid was recrystallized from EtOH/H_2O to give 0.55 g (40%) of a yellowish crystal powder, mp 220-224 °C. pK_a: 3.38, 10.30. ^{1}H-NMR, (DMSO-d_6): 3.12 (m, 2H, CH_2), 3.35 (m, 1H, H-C(7)), 3.55 (m, 1H, H-C(7)), 4.59 (m, 1H, H-C(6)), 4.95 (t, 1H, OH), 6.08 (bs, 2H, NH_2), 7.04 (s, 1H, H-N(8)), 7.28 (dd, 1H, nic-5), 7.75 (d, 1H, nic-4), 8.44 (d, 1H, nic-6), 8.55 (s, 1H, nic-2), 9.70 (bs, H-N(3)).; EtOH: 1.04 (t, 3H, CH_3), 3.40 (m, 2H, CH_2), 4.36 (1H, OH), Anal. Calcd for $C_{13}H_{14}N_6O_3 \cdot$ EtOH (348.4): C, 51.72; H, 5.79; N, 24.12. Found: C, 51.67; H, 5.79; N, 24.01.

***N*^{2}-Isobutyroyl-6-methyl-5-(1-methylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (19):** A solution of **12** (2.06 g, 5.77 mmol) in abs. MeOH (200 mL) was treated with CH_3I (20 mL) under stirring at rt for 4 days. The yellow solution was evaporated and the residue recrystallized from MeOH/H_2O to give 1.88 g

(55%). Further recrystallization from H_2O (20 mL) yielded 1.3 g (45%) of yellowish crystals, mp 293 °C (decomp.). pK_a: 8.77. ^{1}H-NMR, (DMSO-d_6): 1.02 (d, 6H, C(Me_2), 1.08 (d, 3H, Me-C(6)), 2.72 (sept, 1H, H-CMe_2), 3.32 (dd, 1H, H-C(7)), 3.62 (m. 1H, H-C(7)), 4.35 (s, 3H-Me-N), 4.85 (m, 1H, H-C(6)), 7.69 (s, 1H, H-N(8)), 7.99 (dd, 1H, nic-5), 8.57 (d, 1H, nic-4), 8.93 (d, 1H, nic-6), 9.36 (s, 1H, nic-2), 11.09 (s, H-N(3)), 11.43 (bs, 1H, H-N). Anal. Calcd for $C_{18}H_{23}N_6O_3I$ (498.4): C, 43.39; H, 4.65; N, 16.86. Found: C, 43.09; H, 4.83; N, 16.66.

***N*^{2}-Isobutyroyl-6,7-dimethyl-5-(1-methylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (20):** Analogous to the preceding procedure with **13** (6.05 g, 16,3 mmol) in MeOH (700 mL) with CH_3I (25 mL). After 5 days was concentrated to 100 mL, cooled and the precipitate collected to give after drying in a vacuum desiccator 5.65 g (66%) pure material, mp >300 °C. Recrystallization from H_2O gave yellowish crystals. *pK*: 8.97. ^{1}H-NMR, (DMSO-d_6): 0.86 (d, 3H, Me-C(7)), 1.04 (d, 6H, C(Me_2), 1.23 (d, 3H, Me-C(6)), 2.72 (sept, 1H, H-CMe_2), 3.89 (m, 2H, H-C(7)), 4.31 (s, 3H, Me-N), 4.59 (m, 1H, H-C(6)), 7.18 (s, 1H, H-N(8)), 7.97 (dd, 1H, nic-5), 8.57 (d, 1H, nic-4), 8.86 (d, 1H, nic-6), 9.21 (s, 1H, nic-2), 11.15 (s, H-N(3)), 11.23 (bs, 1H, H-N). Anal. Calcd for $C_{19}H_{25}N_6O_3I \cdot 0.5\ H_2O$ (521.4): C, 43.77; H, 5.03; N, 16.12. Found: C, 43.65; H, 4.93; N, 16.33.

***N*^{2}-Isobutyroyl-6-isobutyroyloxymethyl-5-(1-methylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (21):** Analogous to the preceding procedure with **14** (0.975 g, 2.2 mmol) in abs. MeOH (80 mL) with CH_3I (10 mL) for 2 days. After evaporation the oily residue was treated with little EtOAc to form a solid which was recrystallized from MeOH/EtOAc (1:4, 50 mL) to give 0.665 g (50 %) yellow needles and from the mother liquid 0.51 g (39%), mp 200 °C. pK_a: 8.83. ^{1}H-NMR, (DMSO-d_6): 1.02 (d, 6H, C(Me_2), 1.08 (d, 6H, C(Me_2), 2.69 (sept, 1H, H-CMe_2), 2.49 (sept, 1H, H-CMe_2), 3.53 (dd, 1H, H-C(7)), 3.67 (dd, 1H, H-C(7)), 3.89 (dd, 1H, OCH_2), 4.01 (dd, 1H, OCH_2), 4.35 (s, 3H-Me-N), 4.96 (m, 1H, H-C(6)), 7.66 (s, 1H, H-N(8)), 7.96 (dd, 1H, nic-5), 8.57 (d, 1H, nic-4), 8.88 (d, 1H, nic-6), 9.22 (s, 1H, nic-2), 11.12 (s, H-N(3)), 11.39 (bs, 1H, H-N). Anal. Calcd for $C_{22}H_{29}N_6O_5I \cdot 0.75\ H_2O$ (597.9): C, 44.19; H, 5.14; N, 14.05. Found: C, 44.26; H, 5.14; N, 14.05.

***N*^{2}-Isobutyroyl-6-methyl-5-(1-benzylnicotinoylium)-5,6,7,8-tetrahydropterin bromide (22):** A solution of **12** (0.52 g, 1.46 mmol) in DMSO (15 mL) was treated with benzyl bromide (0.35 g, 2.04 mmol) for 18 h at rt. Evaporation at high vacuum to an orange oil, which was dissolved in MeOH (20 ml) and added dropwise with vigorous stirring into Et_2O (80 mL). The precipitate was collected, washed with Et_2O and recrystallized from little *i*PrOH to give 0.41 g (52%) yellow crystals, mp 235 °C. pK_a: 8.87. ^{1}H-NMR, (DMSO-d_6): 0.98-1.12 (m, 9H, Me-C(6), C(Me_2), 1.15 (d, 3H, Me-C(6)), 2.74 (sept, 1H, H-CMe_2),

3.35 (m, 1H, H-C(7)), 3.62 (dd. 1H, H-C(7)), 4.82 (m, 1H, H-C(6)), 5.80 (dd, 2H, N-CH_2), 7.36 (m, 5H, arom. H), 7.72 (s, 1H, H-N(8)), 8.08 (dd, 1H, nic-5), 8.69 (d, 1H, nic-4), 9.13 (d, 1H, nic-6), 9.47 (s, 1H, nic-2), 11.09 (s, H-N(3)), 11.43 (bs, 1H, H-N). Anal. Calcd for $C_{24}H_{27}N_6O_3Br \cdot 0.5\ H_2O$ (536.4): C, 53.74; H, 5.26; N, 15.67. Found: C, 53.66; H, 5.50; N, 15.27.

***N*^{2}-Isobutyroyl-6,7-dimethyl-5-(1-benzylnicotinoylium)-5,6,7,8-tetrahydropterin bromide (23):** Analogous to the preceding procedure with **13** (5.0 g, 13.5 mmol) and benzyl bromide (2.3 mL, 19.5 mmol) in DMSO (125 mL) for 12 h at rt. The crude product was recrystallized from MeOH (150 mL) to give 6.55 g (88%) yellowish crystals, mp 251 °C. *pK*$_a$: 9.09. ^{1}H-NMR, (DMSO-*d*$_6$): 0.85 (d, 3H, Me-C(7)), 1.07 (d, 6H, Me_2C), 1.22 (d, 3H, Me-C(6)), 2.78 (sept, 1H, H-CMe_2), 3.93 (m, 1H, H-C(7)), 4.59 (m, 1H, H-C(6)), 5.81 (dd, 2H, N-CH_2), 7.35 (m, 5H, arom. H), 7.65 (s, 1H, H-N(8)), 8.09 (dd, 1H, nic-5), 8.70 (d, 1H, nic-4), 9.15 (d, 1H, nic-6), 9.51 (s, 1H, nic-2), 10.99 (s, H-N(3)), 11.28 (bs, 1H, H-N). Anal. Calcd for $C_{25}H_{29}N_6O_3Br \cdot 0.5\ H_2O$ (550.4): C, 54.55; H, 5.49; N, 15.27. Found: C, 54.66; H, 5.63; N, 15.37.

***N*^{2}-Isobutyroyl-6-isobutyroyloxymethyl)-5-(1-benzylnicotinoylium)-5,6,7,8-tetrahydropterin bromide (24):** Analogous to the preceding prucedure with **14** (0.74 g, 1,67 mmol) and benzyl bromide (0.4 mL, 2.34 mmol) in DMSO (20 mL) for 18 h at rt. The crude product was recrystallized from EtOAc/MeOH to give 0.61 g (58 %) yellow crystals, mp 263 °C. *pK*$_a$: 9.14. ^{1}H-NMR, (DMSO-*d*$_6$): 1.01-1.11 (m, 12H, 2 Me_2C), 2.49 (sept, 1H, H-CMe_2), 2.75 (sept, 1H, H-CMe_2), 3.47 (m, 1H, H-C(7)), 3.61 (m, 1H, H-C(7)), 3.85 (dd, 1H, H_2C-C(6)), 4.00 (dd, 1H, H_2C-C(6)), 5.92 (dd, 2H, N-CH_2), 7.33 (m, 5H, arom. H), 7.72 (s, 1H, H-N(8)), 8.10 (dd, 1H, nic-5), 8.67 (d, 1H, nic-4), 9.15 (d, 1H, nic-6), 9.45 (s, 1H, nic-2), 10.95 (s, H-N(3)), 11.44 (bs, 1H, H-N). Anal. Calcd for $C_{28}H_{33}N_6O_5Br \cdot 0.5\ H_2O$ (622.5): C, 54.02; H, 5.50; N, 13.50. Found: C, 54.07; H, 5.48; N, 13.51.

***N*^{2}-Isobutyroyl-6,7-diphenyl-5-(1-benzylnicotinoylium)-5,6,7,8-tetrahydropterin bromide (25):** Analogous to the preceding procedure with **15** (1.0 g, 2.02 mmol) and benzyl bromide (0.46 g, 2.7 mmol) in DMSO (20 mL) at 60 °C for 5 h. The crude product was recrystallized from MeOH/H_2O (1:1, 150 mL) to give 1.1 g (79%) yellow needles, mp 269 °C. *pK*$_a$: 9.09. ^{1}H-NMR, (DMSO-*d*$_6$): 1.10 (m, 6H, Me_2C), 2.82 (sept, 1H, H-CMe_2), 5.51 (d, 1H, H-C(7)), 3.88 (d, 1H, H-C(6)), 5.88 (dd, 2H, N-CH_2), 7.03-7.44 (m, 10H, arom. H), 8.12 (dd, 1H, nic-5), 8.47 (s, 1H, H-N(8)), 8.78 (d, 1H, nic-4), 9.18 (d, 1H, nic-6), 9.66 (s, 1H, nic-2), 11.16 (s, H-N(3)), 11.47 (bs, 1H, H-N). Anal. Calcd for $C_{35}H_{33}N_6O_5Br \cdot H_2O$ (683.6): C, 61.49; H, 5.16; N, 12.29. Found: C, 61.37; H, 5.61; N, 12.05.

N^2-Isobutyroyl-6,7-dimethyl-5-(1-ethylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (26): Analogous to the preceding procedure with **13** (0.82 g, 2.2 mmol) and C_2H_5I (2 mL) in DMSO (10 mL) at rt for 2 days. The crude product was recrystallized from EtOH to give 0.8 g (67%) yellow crystals, mp 290 °C. *pK*$_a$: 8.97. ^{1}H-NMR, (DMSO-*d*$_6$): 0.85 (d, 3H, Me-C(7)), 1.02 (d, 6H, C(Me$_2$), 1.21 (d, 3H, Me-C(6)), 1.44 (t, 3H, *H*$_3$C-CH$_2$), 2.72 (sept, 1H, H-CMe$_2$), 3.91 (m, 1H, H-C(7)), 4.62 (m, 3H, H$_2$C-N, H-C(6)), 7.62 (s, 1H, H-N(8)), 8.00 (dd, 1H, nic-5), 8.60 (d, 1H, nic-4), 8.99 (d, 1H, nic-6), 9.39 (s, 1H, nic-2), 11.13 (s, H-N(3)), 11.23 (bs, 1H, H-N). Anal. Calcd for $C_{20}H_{27}N_6O_3I \cdot 0.5\ H_2O$ (535.4): C, 44.87; H, 5.27; N, 15.70. Found: C, 44.72; H, 5.32; N, 15.46.

N^2-Isobutyroyl-6,7-dimethyl-5-(1-n-propylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (27): Analogous to the preceding procedure with **13** (1.0 g, 2.7 mmol) and *n*-C_3H_7I (2 mL) in DMF (40 mL) at 100°C for 8 h. The reaction solution was evaporated, twice coevaporated with EtOH. The crude product was recrystallized from EtOH (30 mL) to give 1.1 g (72%) orange crystals, mp 288-290 °C (decomp.). *pK*$_a$: 8.66. ^{1}H-NMR, (DMSO-*d*$_6$): 0.79 (t, 3H, *H*$_3$C-CH$_2$CH$_2$), 0.91 (d, 3H, Me-C(7)), 1.01 (d, 6H, C(Me$_2$), 1.21 (d, 3H, Me-C(6)), 1.44 (t, 3H, *H*$_3$C-CH$_2$), 1.81 (m, 2H, CH$_3$C*H*$_2$CH$_2$), 2.72 (sept, 1H, H-CMe$_2$), 3.91 (m, 1H, H-C(7)), 4.54 (m, 3H, H$_2$C-N, H-C(6)), 7.64 (s, 1H, H-N(8)), 8.02 (dd, 1H, nic-5), 8.65 (d, 1H, nic-4), 8.98 (d, 1H, nic-6), 9.37 (s, 1H, nic-2), 11.15 (s, H-N(3)), 11.30 (bs, 1H, H-N). Anal. Calcd for $C_{21}H_{29}N_6O_3I$ (540.4): C, 46.47; H, 5.41; N, 15.55. Found: C, 46.41; H, 5.46; N, 15.09.

N^2-Isobutyroyl-6,7-dimethyl-5-(1-n-pentylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (28): Analogous to the preceding procedure with **13** (1.04 g, 2.8 mmol) and *n*-$C_5H_{11}I$ (0.7 g, 3.53 mmol) in DMF (40 mL) at 150 °C for 4 h. The reaction solution was evaporated, twice coevaporated with EtOH. The crude product was dissolved in little EtOH and dropwise added to stirring cold *n*-hexane (200 mL). The precipitate was collected and recrystallized from acetone to give 1.4 g (88 %) orange crystals, mp 255-257 °C. *pK*$_a$: 8.68. ^{1}H-NMR, (DMSO-*d*$_6$): 0.78-1.01 (m, 10H, *H*$_3$C-C*H*$_2$C*H*$_2$, Me-C(7)), 1.02 (d, 6H, C(Me$_2$), 1.21 (d, 3H, Me-C(6)), 1.78 (t, 2H, H$_3$C-CH$_2$CH$_2$C*H*$_2$), 2.73 (sept, 1H, H-CMe$_2$), 3.91 (m, 1H, H-C(7)), 4.56 (m, 3H, H$_2$C-N, H-C(6)), 7.64 (s, 1H, H-N(8)), 8.04 (dd, 1H, nic-5), 8.64 (d, 1H, nic-4), 8.98 (d, 1H, nic-6), 9.33 (s, 1H, nic-2), 11.14 (s, H-N(3)), 11.27 (bs, 1H, H-N). Anal. Calcd for $C_{23}H_{33}N_6O_3I$ (568.5): C, 48.60; H, 5.85; N, 14.78. Found: C, 48.11; H, 5.86; N, 14.77.

N^2-Isobutyroyl-6,7-dimethyl-5-(1-n-octylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (29): Analogous to the preceding procedure with **13** (1.0 g, 2.7 mmol) and *n*-$C_8H_{17}I$ (1.0 g, 4.16 mmol) in DMF (40 mL) at 150 °C for 3 h. The reaction solution was evaporated, twice coevaporated with EtOH. The crude product was dissolved in little EtOH and dropwise added to stirring cold *n*-hexane (200 mL). The

crude product was recrystallized from EtOAc/EtOH to give 1.15 g (70 %) orange crystals, mp 245-247 °C. pK_a: 8.77. ^{1}H-NMR, (DMSO-d_6): 0.81-1.23 (m, 25H, H_3C-(C$H_{2,}$)$_5$ Me-C(6), Me$_2$C, Me-C(7)), 1.78 (m. 2H, H_2C-H$_2$C-N), 2.78 (sept, 1H, H-CMe$_2$), 4.56 (m, 4H, H$_2$C-N, H-C(7), H-C(6)), 7.63 (s, 1H, H-N(8)), 8.03 (dd, 1H, nic-5), 8.63 (d, 1H, nic-4), 8.98 (d, 1H, nic-6), 9.34 (s, 1H, nic-2), 11.14 (s, H-N(3)), 11.26 (bs, 1H, H-N). Anal. Calcd for $C_{26}H_{39}N_6O_3I$ (610.6): C, 51.15; H, 6.44; N, 13.77. Found: C, 50.88; H, 6.34; N, 13.50.

N^2-Isobutyroyl-6-methyl-5-(1-n-octylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (30):
Analogous to the preceding procedure with **12** (2.0 g, 5.6 mmol) and *n*-$C_8H_{17}I$ (1.12 mL) in DMF (60 mL) at 150 °C for 6 h. The reaction solution was evaporated, twice coevaporated with EtOH. The crude product was heated with H_2O (50 mL) and EtOH added till solution took place. Charcoal was added, filtered and the filtrate put in the icebox to get 1.52 g (46 %) yellow crystals, mp 203-205 °C. pK_a: 8.92. ^{1}H-NMR, (DMSO-d_6): 0.83 (t, 3H, H_3C(CH$_2$)$_7$), 1.00-1.23 (m, 19H, H$_3$C-(C$H_{2,}$)$_5$ Me-C(6), Me$_2$C), 1.77 (m. 2H, H_2C-H$_2$C-N), 2.69 (sept, 1H, H-CMe$_2$), 3.34 (m, 1H, H-C(7)), 3.62 (m. 1H, H-C(7)), 4.56 (t, 2H, H$_2$C-N), 4.83 (m, 1H, H-C(6)), 7.70 (s, 1H, H-N(8)), 8.03 (dd, 1H, nic-5), 8.62 (d, 1H, nic-4), 8.99 (d, 1H, nic-6), 9.34 (s, 1H, nic-2), 11.10 (s, H-N(3)), 11.41 (bs, 1H, H-N). Anal. Calcd for $C_{25}H_{37}N_6O_3I \cdot 0.5\ H_2O$ (605.6): C, 49.58; H, 6.32; N, 13.87. Found: C, 49.73; H, 6.31; N, 13.70.

N^2-Isobutyroyl-6-isobutyroyloxymethyl-5-(1-*n*-octylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (31): Analogous to the preceding procedure with **14** (3.0 g, 6.78 mmol) and *n*-$C_8H_{17}I$ (1.75 mL) in DMF (90 mL) at 150 °C for 4 h. The reaction solution was evaporated, twice coevaporated with EtOH and the residue dissolved in EtOAc (100 mL). Cooling overnight yielded 3.54 g (77%) crude material. Recrystallization from EtOAc/EtOH (5:4, 90 mL) gave 2.67 g (58%) yellow crystals, mp 235-236 °C. pK_a: 8.95. ^{1}H-NMR, (DMSO-d_6): 0.83 (t, 3H, H_3C(CH$_2$)$_7$), 1.00-1.23 (m, 16H, H$_3$C-(C$H_{2,}$)$_5$, Me$_2$C), 1.77 (m. 2H, H_2C-H$_2$C-N), 2.69 (sept, 1H, H-CMe$_2$), 3.52 (m, 1H, H-C(7)), 3.65 (m. 1H, H-C(7)), 3.89 (dd, 1H, H$_2$C-C(6)); 3.99 (dd, 1H, H$_2$C-C(6)); 4.52 (t, 2H, H$_2$C-N), 4.97 (m, 1H, H-C(6)), 7.69 (s, 1H, H-N(8)), 8.04 (dd, 1H, nic-5), 8.61 (d, 1H, nic-4), 8.98 (d, 1H, nic-6), 9.32 (s, 1H, nic-2), 11.08 (s, H-N(3)), 11.41 (bs, 1H, H-N). Anal. Calcd for $C_{29}H_{34}N_6O_3I$ (682.6): C, 51.03; H, 6.35; N, 12.31. Found: C, 50.96; H, 6.39; N, 12.08.

6-Methyl-5-(1-*n*-octylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (32): A solution of **16** (0.5 g, 1.64 mmol) in DMF (10 mL) was treated with *n*-octyl iodide (0.38 mL) at 100 °C for 6 h. It was evaporated, coevaporated with EtOH and the residue recrystallized from EtOH/MeOH to give 0.6 g (67%) yellowish crystals, mp 206-208 °C. pK_a: 1.09, 9.62. ^{1}H-NMR, (DMSO-d_6): 0.84 (t, 3H, H_3C-(CH$_2$)$_7$), 0.97 (d, 3H, Me-C(6)), 1.01-1.24 (m, 10H, H$_3$C-(CH_2)$_5$), 1.79 (m, 2H, N-CH$_2$CH_2), 3.23 (m, 1H,

H-C(7)), 3.55 (m, 1H, H-C(7)), 4.57 (t, 2H, N-CH_2), 4.77 (m, 1H, H-C(6)), 6.25 (bs, 2H, NH_2), 7.33 (s, 1H, H-N(8)), 8.01 (dd, 1H, nic-5), 8.57 (d, 1H, nic-4), 8.97 (d, 1H, nic-6), 9.30 (s, 1H, nic-2), 9.80 (s, H-N(3)). Anal. Calcd for $C_{21}H_{31}N_6O_2I$ (526.4): C, 47.92; H, 5.96; N, 15.96. Found: C, 47.76; H, 5.99; N, 15.94.

6,7-Dimethyl-5-(1-*n*-octylnicotinoylium)-5,6,7,8-tetrahydropterin iodide (33): Analogous to the preceding procedure with **17** (2.5 g, 8.3 mmol) in DMF (70 mL) and *n*-octyl iodide (1.85 mL) at 150 °C for 3 h. Recrystallization from EtOH (25 mL) yielded 3.94 g (79%) yellowish plates, mp 180 °C. pK_a: 1.07, 9.81. ^{1}H-NMR, (DMSO-d_6): 0.83 (t, 3H, H_3C-$(CH_2)_7$), 0.98 (d, 3H, Me-C(6)), 1.15 (d, 3H, Me-C(7)), 1.24 (m, 10H, H_3C-(C$H_2)_5$), 1.79 (m, 2H, N-CH_2CH_2), 3.81 (m, 1H, H-C(7)), 4.56 (t, 2H, N-CH_2), 4.57 (m, 1H, H-C(6)), 6.17 (bs, 2H, NH_2), 7.32 (s, 1H, H-N(8)), 8.01 (dd, 1H, nic-5), 8.58 (d, 1H, nic-4), 8.95 (d, 1H, nic-6), 9.31 (s, 1H, nic-2), 9.80 (s, H-N(3)). Anal. Calcd for $C_{22}H_{33}N_6O_2I \cdot 0.5\ H_2O$ (549.6): C, 48.09; H, 6.24; N, 15.30. Found: C, 47.84; H, 6.43; N, 15.08.

6,7-Dimethyl-5-(1-benzylnicotinoylium)-5,6,7,8-tetrahydropterin bromide (34): Analogous to the preceding procedure with **17** (0.253 g, 0.84 mmol) in DMSO (10 mL) and benzyl bromide (0.1 g, 1.18 mmoL) at rt for 18 h. After evaporation in high vacuum the residue was recrystallized from EtOH (15 mL) to give 0.294 g (74%) of yellowish crystals, mp 275 °C (decomp.). pK_a: 1.07, 9.81. ^{1}H-NMR, (DMSO-d_6): 0.83 (d, 3H, Me-C(7)), 1.15 (d, 3H, Me-C(6)), 3.85 (m, 1H, H-C(7)), 4.50 (m, 1H, H-C(6)), 5.84 (s, 2H, N-CH_2), 6.31 (bs, 2H, NH_2), 7.35 (m, 6H, arom. H, H-N(8)), 8.03 (dd, 1H, nic-5), 8.65 (d, 1H, nic-4), 9.06 (d, 1H, nic-6), 9.51 (s, 1H, nic-2), 9.82 (s, H-N(3)). Anal. Calcd for $C_{21}H_{23}N_6O_2Br \cdot 0.5\ H_2O$ (480.4): C, 52.51; H, 5.04; N, 17.50. Found: C, 52.53; H, 5.24; N, 16.84.

N^2-Isobutyroyl-6-methyl-5-(1-benzyl-1,4-dihydronicotinoyl)-5,6,7,8-tetrahydropterin (35): A solution of **22** (0.2 g, 0.37 mmol) and $NaHCO_3$ (0.125 g) under N_2-atmosphere was treated with EtOAc (6 mL). Under vigorous stirring $Na_2S_2O_4$ (0.26 g, 1.5 mmol) was added in small portions and after 1 h the precipitate collected. The solid was washed with H_2O, EtOAc and ether and dried in a vacuum desiccator to give 0.145 g (87%) crude material. Recrystallization from little EtOH yielded 0.086 g (52%) of a colorless powder, mp 210 °C. ^{1}H-NMR, (DMSO-d_6): 0.88 (m, 3H, Me-C(6)), 1.14 (d, 6H, Me_2C), 2.74 (sept, 1H, H-CMe_2), 2.93 (dd, 1H, nic-4'), 3.15 (dd, 1H, nic-4''), 3.19 (m, 2H, H-C(7)), 4.19 (s, 2H, N-CH_2), 4.51 (m, 2H, H-C(6), nic-5), 5.84 (dd, 1H, nic-6), 6.30 (s, 1H, nic-2), 6.98 (s, 1H, H-N(8)), 7.15 (m, 5H, arom. H), 11.22 (s, H-N(3)), 11.24 (bs, 1H, H-N). Anal. Calcd for $C_{24}H_{28}N_6O_3$ (448.5): C, 64.27; H, 6.29; N, 18.74. Found: C, 63.83; H, 6,23; N, 18.58.

***N*^{2}-Isobutyroyl-6,7-dimethyl-5-(1-benzyl-1,4-dihydronicotinoyl)-5,6,7,8-tetrahydropterin (36):** A solution of **23** (3.9 g, 7.2 mmol) and $NaHCO_3$ (2.4 g, 28.8 mmol) in H_2O (225 mL) was kept under N_2-atmosphere. Under stirring $Na_2S_2O_4$ (2.4 g, 28.8 mmol) was added in small portion within 15 min. and the resulting precipitate collected after 3 h. The crude material was recrystallized from abs. EtOH (200 mL) under nitrogen to give 1.41 g (42 %) of yellowish needles, mp 230 °C. ^{1}H-NMR, (DMSO-d_6): 0.75 (d, 3H, Me-C(7)), 1.12 (d, 6H, Me_2C), 1.15 (d, 3H, Me-C(6)), 2.77 (sept, 1H, H-CMe_2), 2.93 (dd, 1H, nic-4'), 3.15 (dd, 1H, nic-4"), 3.45 (m, 1H, H-C(7)), 4.14 (m, 3H, N-CH_2, H-C(6)), 4.49 (m, 1H, nic-5), 5.84 (dd, 1H, nic-6), 6.31 (s, 1H, nic-2), 6.83 (s, 1H, H-N(8)), 7.15 (m, 5H, arom. H), 11.18 (m, H-N(3), H-N). Anal. Calcd for $C_{25}H_{30}N_6O_3 \cdot 0.5\ H_2O$ (471.6): C, 63.68; H, 6.63; N, 17.82. Found: C, 64.01; H, 6,48; N, 17.78.

***N*^{2}-Isobutyroyl-6-isobutyroyloxymethyl-5-(1-benzyl-1,4-dihydronicotinoyl)-5,6,7,8-tetrahydropterin (37):** A solution of **24** (0.2 g, 0.32 mmol) and $NaHCO_3$ (0.11 g, 1.3 mmol) in H_2O (10 mL) was treated under N_2-atmosphere and stirring with $Na_2S_2O_4$ (0.226 g, 1.3 mmol) for 1 h at rt. The reaction solution was kept overnight in the icebox, the resulting precipitate was collected and recrystallized from little MeOH to yield 0.1 g (42%) of colorless crystals, mp 208 °C. ^{1}H-NMR, (DMSO-d_6): 1.02 (d, 6H, Me_2C), 1.12 (d, 6H, Me_2C), 2.48 (sept, 1H, H-CMe_2), 2.74 (sept, 1H, H-CMe_2), 2.94 (dd, 1H, nic-4'), 3.17 (dd, 1H, nic-4"), 3.76 (m, 2H, H-C(7)), 3.85 (dd, 2H, H_2C-C(6)), 4.20 dd, 2H, N-CH_2), 4.1 (m, 1H, nic-5), 4.55 (m, 1H, H-C(6)), 5.83 (dd, 1H, nic-6), 6.30 (s, 1H, nic-2), 7.00 (d, 1H, H-N(8)), 7.16 (m, 5H, arom. H), 11.26 (s, 1H, H-N(3)), 11.28 (s, 1H, H-N). Anal. Calcd for $C_{28}H_{34}N_6O_5 \cdot 0.5\ H_2O$ (543.6): C, 61.86; H, 6.49; N, 15.46. Found: C, 61.54; H, 6,22; N, 15.25.

***N*^{2}-Isobutyroyl-6,7-dimethyl-5-(1-*n*-pentyl-1,4-dihydronicotinoyl)-5,6,7,8-tetrahydropterin (38):** Analogous to the preceding procedure with **28** (0.25 g, 0.44 mmol) and $NaHCO_3$ (0.15 g, 1.76 mmol) in H_2O (10 mL) under N_2-atmosophere. After successive addition of $Na_2S_2O_4$ (0.306 g, 1.76 mmol) was stirred for 1 h, then cooled in the icebox and the precipitate collected after 6 h to give 62 mg (32%) of yellowish crystals, mp >200 °C (decomp.). ^{1}H-NMR, (DMSO-d_6): 0.84 (m. 6H, $H_3C(CH_2)_5$, Me-C(7)), 1.11-1.19 (m, 15H, Me_2C, Me-C(6), $H_3C(CH_2)_3$), 2.74 (sept, 1H, H-CMe_2), 2.90 (m, 3H, N-CH_2, nic-4'), 3.11 (dd, 1H, nic-4"), 3.47 (m, 1H, H-C(7)), 4.20 (dd, 2H, H_2C-C(6)), 4.45 (m, 1H, nic-5), 5.76 (dd, 1H, nic-6), 6.20 (s, 1H, nic-2), 6.89 (d, 1H, H-N(8)), 11.20 (bs, 2H, H-N(3), H-N). Anal. Calcd for $C_{23}H_{34}N_6O_3 \cdot 0.5\ H_2O$ (543.6): C, 61.86; H, 6.49; N, 15.46. Found: C, 61.54; H, 6,22; N, 15.25.

***N*^{2}-Isobutyroyl-6,7-dimethyl-5-(1-*n*-octyl-1,4-dihydronicotinoyl)-5,6,7,8-tetrahydropterin (39):** Analogous to the preceding procedure with **29** (0.25 g, 0.41 mmol) and $NaHCO_3$ (0.15 g, 1.76 mmol) in

H_2O (10 mL) under N_2-atmosphere. After successive addition of $Na_2S_2O_4$ (0.285 g, 1.64 mmol) was stirred for 1 h, then cooled in the icebox and the precipitate collected after 4 h to give 0.10 g (50%) of yellowish crystals, mp > 200 °C (decomp.). ^{1}H-NMR, (DMSO-*d*$_6$): 0.79 (m. 6H, $H_3C(CH_2)_7$, Me-C(7)), 1.02-1.26 (m, 21H, Me_2C, Me-C(6), $H_3C(CH_2)_6$), 2.74 (sept, 1H, H-CMe_2), 2.90 (m, 3H, N-CH_2, nic-4'), 3.07 (dd, 1H, nic-4''), 3.47 (m, 1H, H-C(7)), 4.18 (dd, 2H, H_2C-C(6)), 4.45 (m, 1H, nic-5), 5.76 (dd, 1H, nic-6), 6.22 (s, 1H, nic-2), 6.88 (d, 1H, H-N(8)), 11.20 (bs, 2H, H-N(3), H-N). Anal. Calcd for $C_{26}H_{40}N_6O_3 \cdot 0.5\ H_2O$ (493.7): C, 63.26; H, 8.37; N, 17.02. Found: C, 62.94; H, 8.06; N, 17.12.

6-Methyl-5-(1-benzyl-1,4-dihydronicotinoyl)-5,6,7,8-tetrahydropterin (40): A suspension of **35** (0.751 g, 1.67 mmol) in MeOH (40 mL) was treated under N_2-atmosphere with K_2CO_3 (0.255 g, 1.84 mmol) at rt and stirring overnight. It was evaporated and the resulting oil recrystallized from H_2O (15 mL) to give after drying in a vacuum desiccator 0.393 g (60%) of yellowish crystal powder, mp 232 °C. ^{1}H-NMR, (DMSO-*d*$_6$): 0.88 (d, 3H, Me-C(6)), 1.14 (d, 6H, Me_2C), 2.88 (dd, 1H, nic-4'), 3.07 (dd, 1H, nic-4''), 3.09 (m, 2H, H-C(7)), 4.18 (s, 2H, N-CH_2), 4.45 (m, 2H, H-C(6)), nic-5), 5.82 (d, 1H, nic-6), 6.28 (bs, 2H, NH_2), 6.32 (s, 1H, nic-2), 6.57 (s, 1H, H-N(8)), 7.25 (m, 5H, arom. H), 10.5 (bs, H-N(3)). Anal. Calcd for $C_{20}H_{22}N_6O_2 \cdot H_2O$ (396.5): C, 60.59; H, 6.10; N, 21.20. Found: C, 60.34; H, 6,12; N, 20.93.

6,7-Dimethyl-5-(1-benzyl-1,4-dihydronicotinoyl)-5,6,7,8-tetrahydropterin (41): Analogous to the preceding procedure with **36** (2.51 g, 4,55 mmol) in MeOH (110 mL) under N_2-atmosphere with K_2CO_3 (0.6.85 g, 0.49 mmol) at rt and stirring overnight. The resulting precipitate gave after drying in a vacuum desiccator 1.42 g (72%) analytically pure yellowish crystals, mp 255 °C. ^{1}H-NMR, (DMSO-*d*$_6$): 0.75 (d, 3H, Me-C(6)), 1.02 (d, 3H, Me-C(6)), 2.90 (dd, 1H, nic-4'), 3.10 (dd, 1H, nic-4''), 3.35 (1H, H-C(7)), 4.13 (m. 1H, H-C(6)), 4.16 (dd, 2H, N-CH_2), 4.45 (m, 1H, nic-5), 5.84 (d, 1H, nic-6), 6.04 (bs, 2H, NH_2), 6.31 (s, 1H, nic-2), 6.52 (s, 1H, H-N(8)), 7.25 (m, 5H, arom. H), 9.80 (bs, H-N(3)). Anal. Calcd for $C_{21}H_{24}N_6O_2$ (392.5): C, 64.27; H, 6.16; N, 21.41. Found: C, 63.95; H, 6,123; N, 21.33.

6-Hydroxymethyl-5-(1-benzyl-1,4-dihydronicotinoyl)-5,6,7,8-tetrahydropterin (42): Analogous to the preceding procedure with **37** (0.22 g, 0.41 mmol) and K_2CO_3 (66 mg) in MeOH (12 mL). It was evaporated and the residue recrystallized from H_2O (5 mL) to give 94 mg (53%) of yellowish crystals, mp 210 °C. ^{1}H-NMR, (DMSO-*d*$_6$): 2.89 (dd, 1H, nic-4'), 3.09 (m, 2H, H-C(7)), 3.12 (dd, 1H, nic-4''), 3.49 (m, 2H, H_2C-C(6)), 4.17 (s, 2H, N-CH_2), 4.25 (m, 2H, H-C(6)), 4.45 (dt, 1H, nic-5), 5.80 (d, 1H, nic-6), 6.04 (bs, 2H, NH_2), 6.44 (s, 1H, nic-2), 6.61 (s, 1H, H-N(8)), 7.25 (m, 5H, arom. H), 10.1 (bs, H-N(3)). Anal. Calcd for $C_{20}H_{22}N_6O_3 \cdot 2\ H_2O$ (430.5): C, 55.81; H, 6.09; N, 19.52. Found: C, 55.64; H, 5.86; N, 18.92.

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