

Synthesis of 6 β -N(5)-Methyl-5,6,7,8-tetrahydro-L-biopterin*

Rene Bosshard, Claus W. Heizmann, and Max Viscontini[#]

Division of Clinical Chemistry, Department of Pediatrics, University of Zurich, Steinwiesstr. 75, CH-8032 Zurich, Switzerland

[#]Institute of Organic Chemistry, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland

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Summary

6 β -N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl, a new derivate of biological interest, was synthesized from 6 β -5,6,7,8-tetrahydro-L-biopterin·2 HCl and characterized. The latter compound has been used therapeutically in atypical phenylketonuria for many years. The catalytic reductive methylation with PtO₂/H₂ and formaldehyde allowed selective methylation at the N(5) position. The chemical properties and structures of the new compound are compared with N(5)-methyl-tetrahydropterin and N(5)-methyl-tetrahydrofolate.

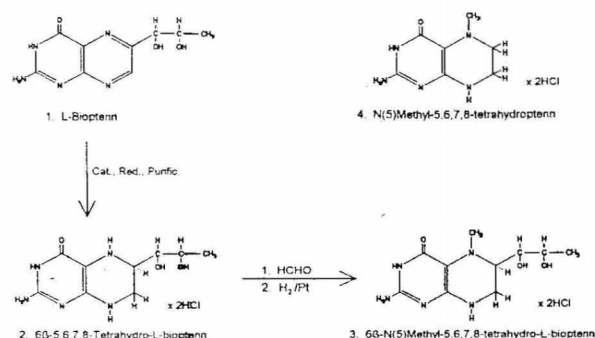
Key words: 6 β -N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin, Catalytic reductive methylation, 6 β -5,6,7,8-tetrahydro-L-biopterin, 6 β -5,6,7,8-tetrahydro-L-biopterin, L-biopterin, N(5)-methyl-5,6,7,8-tetrahydro-pterin, Hyperphenylalaninemia, Phenylketonuria.

Introduction

6 β -5,6,7,8-Tetrahydro-L-biopterin (BH₄, **2**) is the cofactor of aromatic amino acid monooxygenases, enzymes that control the biosynthesis of several neurotransmitters (1, 2). Patients with BH₄ deficiency may suffer from mental retardation and other symptoms. Treatment of these patients requires high doses of this cofactor (10-20 mg/kg, per os), possibly due to its limited penetration through the blood-brain barrier.

We synthesized various BH₄ analoges with the aim to obtain compounds with better penetration properties that could be applied in lower concentrations. In the course of these studies, we synthesized for the first time 6 β -N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin (**3**) (Scheme 1). Its synthesis and chemical properties are described in this paper. In future experiments we will now be able to test the *in vivo* cofactor activities and brain penetration abilities of **3**.

*This work is dedicated to Prof. Hans-Christoph Curtius on the occasion of his 70th birthday.



The regiospecific L-biopterin synthesis from L-rhamnose or 5-deoxy-L-arabinose with 4-hydroxy-2,5,6-triaminopyrimidine was reported earlier (3, 4). For the nomenclature α, β of the two C(6)-diastereoisomers of 5,6,7,8-tetrahydro-L-biopterin see Ref. (5). The natural form is 6 β -(earlier called 6R-); according to IUPAC rules the configuration is 1'R, 2'S, 6R. The coenzyme function of BH₄ is related to the redox system: 6 β -5,6,7,8-tetrahydro-quinonoid-6,7-dihydro[8H]-L-biopterin. The title compound **3** may be

as interesting as the homologous N(5)-methyl-tetrahydrofolate (6).

The synthesis of 6 β -N(5)-methyl-tetrahydro-L-biopterin is illustrated in Scheme 1. L-Biopterin is converted to BH₄ (**2**) by catalytic hydrogenation in hydrochloric acid solution. Pure 6 β -diastereoisomer was used for further synthesis. The catalytic reductive methylation at N(5), as the strongest basic NH-group of the molecule, is described in detail (modified general method) in the Experimental section. In an earlier publication by Matsuura and Sugimoto (7) the methylation method developed by Rylander (8) was applied to tetrahydropterin (**4**) and its 6-alkyl derivatives. In a later electrochemical oxidation study 7,8-dihydropterin was formed from **4** under release of the 5-methyl group (9).

Results and Discussion

N(5)-methyl-tetrahydro-L-biopterin (**3**) is more stable to oxidation than the starting material **2** and has different physicochemical properties, i.e., reversed optical rotation. The appearance of the blue fluorescence on the TLC-plate after several hours of exposure to air requires the release of the methyl group. The UV-spectrum (see Table 1) is similar to that of N(5)-methyl-tetrahydropterin (7) and also pH-dependent (amphoteric character), with p*K*_a determination possibilities. Chirale HPLC control analysis as for the diastereoisomers 6 α /6 β -separation of tetrahydro-L-biopterin has so far not been done.

The spectral data of the new compound are shown in Figures 1 and 2 (¹H-NMR), and in Table 1 and Figure 3 (UV), followed by mass spectrometry data.

¹H-NMR

(0.1 N DCl/D₂O) (Fig. 1): 4.05-3.99 d_q, J=5, H-C (7 eq); 3.98-3.92 m, H-C (6) and H-C (2' =); 3.84-3.78 d_q, J=5, H-C (7 ax); 3.43 broad s, H-C (1'); 3.22 s, CH₃-N (5); 1.31 d, J=2, CH₃-C (2'). After irradiation at 3.43 (double resonance) (Fig. 2), the peak 3.43 disappears and the m changes in the new signals at 3.98-3.96 d, J=2, H-C (2') and 3.92 t, H-C (6). For the assignment of the ¹H-NMR peaks of **3** we use the results we have already obtained with other tetrahydropterin derivatives (10-12).

GC/MS Analysis of 6 β -N(5)-methyl-tetrahydro-L-biopterin·2 HCl

The sample was derivatized by a mixture of

Table 1. UV spectrum of 6 β -N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl.

Solution	pH	λ_1 nm (ϵ_1 =molar extinction)	λ_2 nm (ϵ_2 =molar extinction)	Ion state
1 N HCl	0	212 (10315)	262 (15025)	Di-cation
0.1 N HCl	1	220 (16500)	264 (12700)	
1/15 M KH ₂ PO ₄	3.5*	222 (17815)	264 (11500)	(mono-) cation
H ₂ O	4	222 (17770)	264 (10055)	
1/15 M KH ₂ PO ₄ / Na ₂ HPO ₄ (Socrensen)	8.2	220 (17262)	286 (11129)	zwitterion (neutral molecule)
0.1 N NaOH	13	222 (12540)	278 (9035)	anion (stability?)

*pH adjusted with 1 N HCl. The largest pH-dependent bathochromic shift (see λ_2) occurs between cation ion state and neutral molecule. The max. molecular extinction (MW: 328.20) is in the same pH range (see ϵ_1) in accordance with Fig. 3.

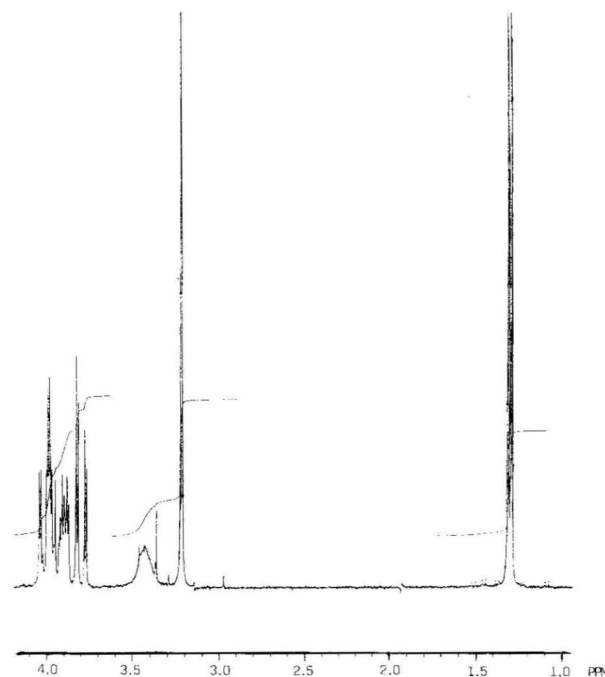


Figure 1. ¹H-NMR spectrum of 6 β -N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl in 0.1 DCl/D₂O.

BSTFA/acetonitrile (1:3, v/v) for 60 min at 100°C to the TMS ethers, as described (13). GC: Carlo Erba, 10 m fused silica SE-54 column, 0.8 bar He; injector 320°C, temperature program 80°C/min to 200°C within 12 min, then increased to 250°C at a rate

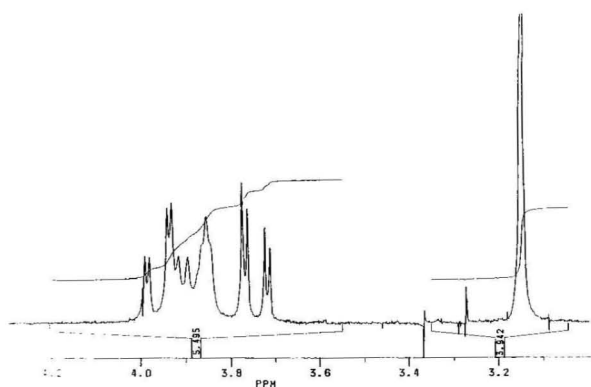


Figure 2. $^1\text{H-NMR}$ spectrum of **3**, irradiated at H-C (1'), 3.43 ppm.

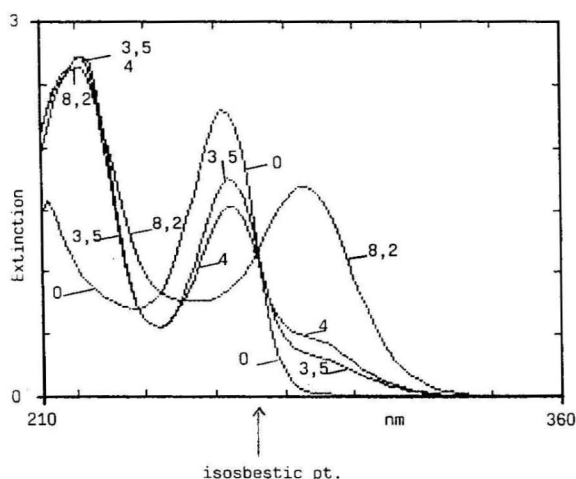


Figure 3. UV spectrum of 6 β -N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin 2 HCl. Curve number represents pH of solution (C=50 mg 3/L).

of 4°C/min. *MS*: VG-7070F, EI^+ with 40 eV, ion source temperature 220°C, emission current 50 μA , scan range m/e 100-750 at a rate of 1.5 sec. *Result*: The title compound showed a weak signal for the molecular ion with four trimethylsilyl groups (m/e 543, 0.5% relative intensity), as common for pteridines; the spectrum showed peaks for silyl derivatives with fewer (three) TMS groups (m/e 471); the base peak (m/e 324) is formed by loss of the entire side chain at C-6 and two remaining TMS groups.

TLC

Silica gel K60F₂₅₄ plates (Merck); solvent: isopropanol- H_3BO_3 (0.5 M, in H_2O)- H_2O (4:1:0.5). **3**: R_F 0.59 (fluorescence appeared only after 24 h exposure to air); **4**: R_F 0.34.

HVE

Electrophoresis on paper (Schleicher-Schüll No. 2043B) with buffer, pH 1.9: acetic acid-formic acid-water (120:26:850 v/v). **3**: R_x 203; **4**: R_x 248 in relation to L-biopterin: R=100.

Experimental Part

Apparatus

The optical rotation was measured with a Perkin Elmer Polarimeter Type 241; the $^1\text{H-NMR}$ spectrum was recorded on a Bruker 300 MHz NMR spectrometer, and the UV spectrum was registered with a Diode Array HP8451A spectrophotometer.

Chemicals

Formaldehyde, 36% in H_2O , stabilized with 10% methanol, platinum IV-oxide (Adams catalyst), methanol puriss. and diethylether abs. (Fluka, CH-9470 Buchs, Switzerland), 6- β -5,6,7,8-tetrahydro-L-biopterin-2 HCl (Schircks Laboratories, CH-8645 Jona, Switzerland).

6 β -N(5)-Methyl-5,6,7,8-tetrahydro-L-biopterin \cdot 2 HCl (**3**): In a hydrogenation apparatus (round bottom 3-neck flask, 250 ml) with magnetic stirrer, nitrogen flow device, and H_2 -balloon (connected with a hydrogen bomb for temporary reloading), acetic acid (3 ml, 2 N) and methanol (40 ml) were added to the PtO_2 -catalyst (90 mg maximum). After N_2 flow the catalyst was prehydrogenated for approx. 20 min under H_2 -balloon pressure, stirring, and cooling (ice water bath). 6 β -5,6,7,8-Tetrahydro-L-biopterin \cdot 2 HCl (315 mg, 1.0 mmol) was added (solid) under N_2 -flow in the reaction bulb. A mixture of acetic acid (3 ml, 2 N) and methanol (20 ml), followed by formaldehyde 36% (0.48 ml, 6.2 mmol) was added and the hydrogenation (i.e. the reductive methylation) was started under stirring and H_2 -balloon pressure for 2 h at 15°C. After N_2 -flow a second portion of formaldehyde 36% (0.3 ml, 3.8 mmol) was added and again hydrogenated for 2 h (ca. 15°C) under stirring. The catalyst was filtered off, protected under N_2 -flow over a glass filter D4, covered with Celite 535 (argon protection of the filter to avoid self ignition of catalyst), and rinsed with methanol 90% (15 ml). The filtrate was evaporated (Rotavapor) to ca. 10 ml, then HCl (100 μl , 6 N) and ethanol abs. (20 ml) were added and the filtrate was reconcentrated to 5 ml. Ethanol (abs., 30 ml) was added again and evapora-

ted to a slurry (ca. 5 ml). On addition of ether (abs., 30 ml) under ice bath cooling, the white dihydrochloride was left to crystallize overnight at 4°C. The product was collected (D4 glass filter), washed with a few ml of a mixture of ethanol-ether (abs., 1:1), followed by ether and dried in a vacuum desiccator over P₂O₅ and NaOH pellets separately at room temperature: 0.31 g dihydrochloride (**3**), yield ca. 95 %.

For recrystallization the dihydrochloride (0.3 g) was dissolved in warm methanol (20 ml) under addition of HCl (50 μ l, 6 N) and water (600 μ l). After addition of carbon (10 mg) the solution was filtered (glass filter D4, covered with Celite 535), and the filtrate was chilled. HCl (800 μ l, 6 N) and, gradually, diethylether (25 ml) were added under crystallization of the product. After 2 h in the ice bath the crystals were filtered, washed with up to 10 ml cold ether/methanol (2:1), then washed with ether and dried in a vacuum desiccator; yield: 0.25 g 6 β -N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl.

Analysis: calc. for C₁₀H₁₇N₅O₃·2 HCl (328.20): C 36.60, H 5.84, Cl 21.60, N 21.34%; found C 36.40, H 5.68, Cl 21.32, N 21.08%.

Specific optical rotation: $[\alpha]_{D(589\text{ nm})}^{21}$ +44.8° (C=1.056; 0.1 N HCl). (In comparison 6 β -tetrahydro-L-biopterin: $[\alpha]_{D}^{21}$ -65.2° (C=1.135; 0.1 N HCl).

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manuscript.

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