Bull Soc Chim Fr (1996) 133, 491-500

Synthesis of new deazatetrahydropterins as potential NO synthase modulators

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(Received 30 January 1996; accepted 3 April 1996)

Summary — As part of our research on NO synthase modulators, a series of deazatetrahydropterins were synthesized and their effects were assessed. In this paper, we describe the synthesis of new substituted (especially at the 4a position) 2-amino-4-hydroxy-3,4,4a,5,6,7-hexahydropyrido[2,3-*d*]pyrimidines. These compounds showed no interesting pharmacological activity.

deazatetrahydropterin / nitric oxide / NOS modulator / NOS inhibitor

Résumé — Synthèse de nouveaux déazatétrahydroptérines modulateurs de la NO synthase. *Dans le cadre de nos recherches sur les modulateurs de la NO synthase, nous avons synthétisé une nouvelle série de déazatétrahydroptérines et déterminé les effets pharmacologiques des différents composés. Dans cet article, nous décrivons la synthèse de 2-amino-4-hydroxy-3,4,4a,5,6,7-hexahydropyrido[2,3-*d*]pyrimidines substituées (plus particulièrement à la position 4a); ces composés ne présentent pas d'activité pharmacologique intéressante.*

déazatétrahydroptérine / oxyde nitrique / modulateur de la NO synthase / inhibiteur de la NO synthase

Introduction

Nitric oxide (NO) is an important intercellular messenger which plays a significant role in different physiological processes. It is generated by at least two classes of NO synthase enzymes: the constitutive enzyme (cNOS) that generates small amounts of NO and the inducible enzyme (iNOS) that generates large amounts of NO over a long period of time. Although increased NO has generally beneficial effects, an overproduction of iNOS (and hence NO) may be dangerous; for example, in response to sepsis, an overproduction of NO may cause septic shock. Consequently it would be helpful to be able to block or modulate NO production as a therapeutic aim.

Tetrahydrobiopterin (BH_4) **1** is one of the cofactors used by NOS for the catalytic oxidation of L-arginine to L-citrulline and NO; its 4a-peroxy-H4 biopterin derivative **2** is a proposed structure of the hydroxylating species which plays a role in a possible mechanism suggested by Stuehr [1] and depicted in figure 1. As 6(*R*,*S*)-methyl-5-deazatetrahydropterin **3j** has been found to inhibit iNOS of murine macrophage [2], we decided to synthesize new 5-deazatetrahydropterin derivatives bearing a substituent at the 4a position, in order to evaluate their effects on different tests and to compare them to the results obtained with **3j**. We chose to prepare compounds bearing a methyl, ethyl, isopropyl, benzyl or phenyl group at the 4a position, since these have different values of parameters π, σ and Es [3]. Secondly, we decided to synthesize compounds with a methyl on the carbon 6 and either a methyl or benzyl group at the 4a position; thirdly, to complete this study, we also attempted to prepare compounds bearing methyl groups on carbon number 5 or carbons 5 and 6. However, it was impossible to carry out the preparation of compounds bearing a methyl on the carbon number 5 and a methyl or benzyl group at the 4a position, since the synthesis failed at the last step.

Chemistry

Among the various options for obtaining 5-deazatetrahydropterin, our experience in the field of 2-piperidinones [4] prompted us to choose a synthetic pathway using them as intermediates. Target compounds were prepared as depicted in scheme 1, according to a procedure worked out by Pyatin and Glushkov [5–7] with guanidine and a lactim ether of the corresponding 2-piperidinone at the last step.

The first reaction consisted of the preparation of substituted nitrile esters **6**; apart from **6h**, which was ob-

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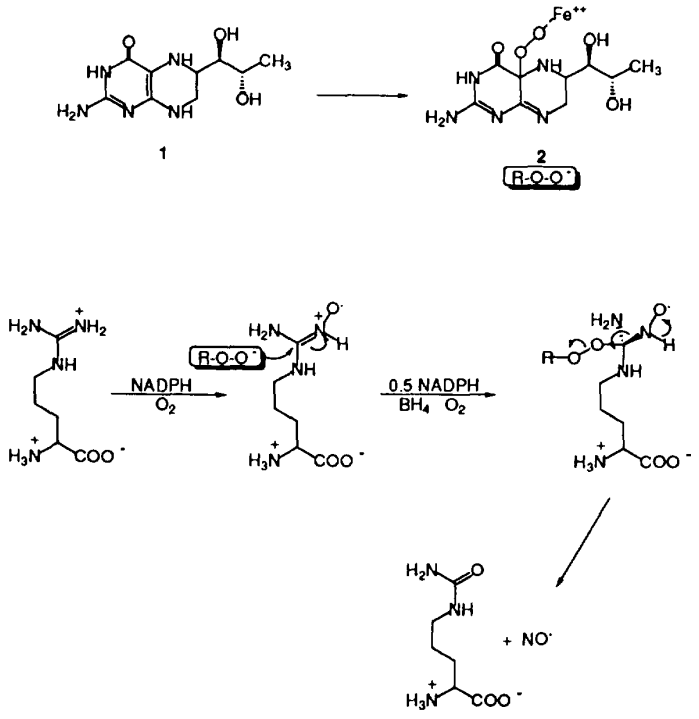


Fig 1

tained from **4f**, and the organozinc compound derived from propionitrile [8], they were synthesized by reaction of ethyl malonate or substituted ethyl malonate 4 and acrylonitrile or substituted acrylonitrile **5** [9–11]. In the second step, hydrogenation of nitrile esters [12, 13] gave corresponding amino esters which cyclized spontaneously to yield 2-piperidinone **7** or **8**; this cyclization sometimes had to be completed by slight heating. Preparation of piperidinone **7b** and **7c** was undertaken because of the poor yield obtained in the preparation of sterically-hindered nitrile ester; they were used as intermediates to obtain 3-disubstituted 2-piperidinones **8g**, **8h** and **8i**. Determination of the configuration of piperidinones with two or three asymmetric carbons was not important for the further steps; however, it was noticed that **7c**, **8h** and **8i** were pure diastereomers after recrystallization and that **7b**, **8f** and **8g** consisted of mixtures of two diastereomers. Although **8j** contained three asymmetric carbones, it also corresponded to a mixture of two diastereomers (see the *Experimental section*). Despite the possibility of synthesizing 5-deazatetrahydropterin from substituted 2-piperidinones **8**, a better yield was obtained from lactim ether derivatives **9** after treatment by triethyloxonium fluoroborate [14–16]. There was no change in the configuration compared to that of **8**. 5-Deazatetrahydropterins **3** were synthesized in satisfactory yields by reaction of the different compounds **9** with guanidine and sodium ethylate in dichloromethane. However, it was impossible in several trials to obtain 5-deazatetrahydropterin derivatives R_1 and R_2 substituted from **9h** and **9i**. Hydrochloride and trifluoroacetate salts of **3** were generally prepared, either to facilitate their purification by recrystallization or to improve their solubility. The reference compound **3j** was prepared according to the procedure described by Moad et al [17].

Pharmacological results

Three pharmacological tests were used to assess the activities of compounds **3**.

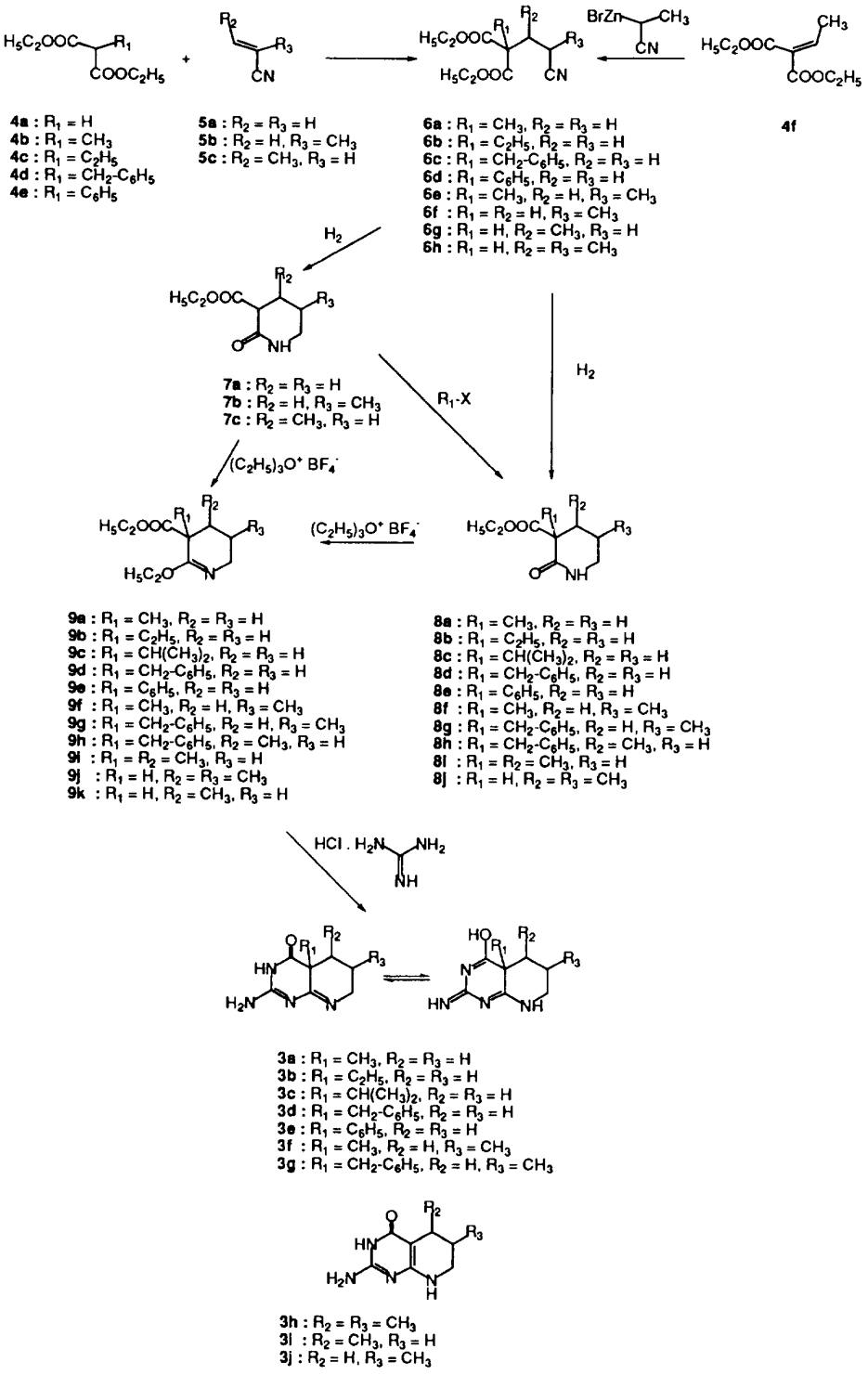
Mouse macrophages were used for two tests: screenings were performed with lipopolysaccharide/interferon-γ (LPS/IFN-γ) macrophages stimulated over a period of 4 h. Compounds were either administered after expression of iNOS for a period of 2 h or during expression of iNOS with a drug incubation of 4 h in the presence of LPS/IFN-γ. Compared to the reference substance (**3j**, aminoguanidine or L-nitro arginine) known to inhibit iNOS, our compounds did not exhibit any significant effect.

Rat primary astroglial cells in culture stimulated by LPS were also used to test our substances. Compounds were administered together with LPS during the induction period; they were either inactive or had very little effect.

Considering these disappointing results and the emergence of another more attractive series, the study of this family was discontinued.

Experimental section

Melting points were determined on a Büchi apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 1720 X infrared Fourier transform spectrometer. ^{1}H NMR



Scheme 1

spectra were recorded at 60 MHz on a Varian A60 spectrometer and at 300 MHz on a Bruker AM 300 spectrometer. TMS was used as internal reference standard. Chemical shifts are expressed in ppm values. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, q = quartet, sep = septet, m = multiplet or massif, dd = double doublet, ddd = triple doublet, dddd = quadruple doublet, dm = double massif, dt = double triplet, dq = double quartet, ddt = double double triplet, ddq = double double quartet. Microanalyses were performed by Société française Hoechst or Service central d'analyse du CNRS and results obtained were within ±0.4% of the theoretical values. In chromatographic purifications, silica gel of type Kieselgel 60, 230–400 mesh ASTM was used.

Synthesis of substituted 4,4-bis(ethoxycarbonyl)butanenitriles **6**

According to the procedure described by Ansell [9] and Bruson [10] using substituted malonate **4b**, **4c**, **4d** and **4e** with acrylonitrile **5a**, we prepared **6a** (bp 110 °C/0.04 mmHg; lit [9]: 110/0.04), **6b** (mp 47 °C; lit [10]: 47 °C), **6c** (mp 47 °C; lit [10]: 47 °C) and **6d** (mp 37 °C; lit [9]: 37 °C).

Using **4b** and methacrylonitrile **5b**, **6e** was prepared according to the same procedure; after distillation, the fraction between 110 and 125 °C(0.1 mmHg) containing impure **6e** was chromatographed (ethyl acetate/heptane 1:4) to give pure oily **6e** in poor yield (<5%).

IR: 2 985, 2 240, 1 730 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 4.13 (q, 4H, J = 7.1 Hz, 2×CH_2 (ethyl)), 2.69 (ddq, 1H, J = 10, 3.4 and 3×7.1 Hz, Ha), 2.17 (dd, 1H, J = 14.5 and 10 Hz, Hb), 1.99 (dd, 1H, J = 14.5 and 3.4 Hz, Hb), 1.43 (s, 3H, CH_3g), 1.30 (d, 3H, J = 7.1 Hz, CH_3a), 1.19 (t, 6H, J = 7.1 Hz, 2 × CH_3 (ethyl)).

According to the procedure described by Colonge [11], compounds **6f** (bp 160 °C/15 mmHg; lit [11]: bp 150 °C/12 mmHg) and **6g** (bp 155–160 °C/15 mmHg; lit [11]: bp 154 °C/11 mmHg) were prepared from **4a** and methacrylonitrile **5b** and **4a** and crotonitrile **5c** respectively.

The procedure described by Goasdoué [8] using ethyl ethylidenemalonate **4f**, α-bromopropionitrile and Zn/Ag in THF gave **6h** (bp 92 °C/0.02 mmHg; lit [8]: 92 °C/0.02 mmHg).

Synthesis of substituted 2-piperidinones **7**

Except **7a**, which was purchased from Aldrich, substituted 2-piperidinones **7** were prepared according to the following procedure: a solution of nitrile ester **6** (0.15 mol) in acetic acid (140 mL) was hydrogenated at 40 bar and room temperature for 20 h with Ni Raney. The mixture was filtered on celite and evaporation gave a green oil that was refluxed for 2.5 h in ethanol. The solvent was evaporated and the oily residue chromatographed on silica (ethyl acetate then ethyl acetate/methanol 9:1 then 4:1). The ^1H NMR spectra are described in table I.

• *Ethyl 5-methyl-2-oxopiperidine-3-carboxylate* **7b**

Compound **6f** (4.56 g, 0.02 mol) gave 3 g (81%) of a solid. It was recrystallized in ethyl acetate to give 2.4 g of **7b** (66% *cis* and 33% *trans*); mp 101 °C.

IR: 2 963, 1 734, 1 657 cm^{-1}.

• *Ethyl 4-methyl-2-oxopiperidine-3-carboxylate* **7c**

Compound **6g** (34.3 g, 0.15 mol) gave 24 g (86%) of a solid. It was recrystallized in ethyl acetate to give 15.5 g (55.7%) of **7c** (*trans*); mp 101 °C.

IR: 2 690, 1 738, 1 662 cm^{-1}.

Synthesis of substituted 2-piperidinones **8**

Compounds **8a** (mp 86–88 °C; lit [18]: 86–88 °C), **8b** (mp 46 °C; lit [19]: 46–49 °C), **8d** (mp 52 °C; lit [13]: 51–52 °C), **8e** (mp 142 °C; lit [20]: 142–143 °C) **8f** and **8j** were prepared according to the modified method published by Govindachari [12]; hydrogenation was carried out at 60 bar and 50 °C, then the crude compound was recrystallized or chromatographed (ethyl acetate) before recrystallization. The ^1H NMR spectra are described in table I.

• *Ethyl 3,5-dimethyl-2-oxopiperidine-3-carboxylate* **8f**

Compound **6e** (19.2 g) gave 6.7 g (42%) of crystallized **8f** consisting of a mixture 1:1 of two diastereomers.

IR: 2 952, 1 723, 1 669, 1 254 cm^{-1}.

• *Ethyl 4,5-dimethyl-2-oxopiperidine-3-carboxylate* **8j**

Compound **6h** (2.1 g) gave 1.65 g (95%) of oily **8j** consisting of a mixture 1:1 of two diastereomers (A: $COOC_2H_5$ and both methyl groups are equatorial; B: $COOC_2H_5$ is equatorial and both methyl groups are axial).

IR: 2 975, 1 731, 1 651, 1 254 cm^{-1}.

8c, **8g**, **8h** and **8i** were prepared according to the modified procedure published by Rodriguez [13]. The ^1H NMR spectra are described in table I.

• *Ethyl 3-isopropyl-2-oxopiperidine-3-carboxylate* **8c**

To a solution of sodium ethoxide (from sodium 0.58 g, 25 mmol) in absolute ethanol (40 mL), **7a** (4.3 g, 25 mmol) was added at room temperature. After stirring for 5 min, isopropyl bromide (4.7 mL, 50 mmol) and NaI (100 mg) were added and the mixture was heated at 65 °C for 7 h. After cooling, water (300 mL) was added and the mixture was extracted with ethyl acetate, the combined organic extracts were washed with water, dried over Na_2SO_4 and concentrated to afford an oil (2.8 g) containing a mixture of **7a** and **8c**. Chromatography (ethyl acetate/heptane 3:1 then ethyl acetate) gave 1.95 g (37%) of crystallized **8c** (mp 82–83 °C).

IR: 2 968, 1 718, 1 678, 1 636, 1 243 cm^{-1}.

• *Ethyl 3-benzyl-5-methyl-2-oxopiperidine-3-carboxylate* **8g**

Using the same procedure as for **8c**, this was prepared from **7b** (4.6 g, 25 mmol) and an equimolecular amount (4.32 g, 25.2 mmol) of benzyl bromide. The mixture was heated at 65 °C for 2 h without NaI; after treatment it afforded an oil (6.8 g) containing a mixture of two diastereomers of **8g** (66:33) which was used directly in the next step.

IR: 2 959, 1 739, 1 671, 1 239 cm^{-1}.

• *Ethyl 3-benzyl-4-methyl-2-oxopiperidine-3-carboxylate* **8h**

Using the same procedure as for **8g**, 5 g (27.15 mmol) of **7c** afforded an oil (7.3 g) which crystallized after chromatography; recrystallization in ethyl acetate gave 5.4 g (73%) of a white solid corresponding to one diastereomer of **8h**; mp 137–138 °C.

IR: 2 967, 1 733, 1 674, 1 631, 1 194 cm^{-1}.

• *Ethyl 3,4-dimethyl-2-oxopiperidine-3-carboxylate* **8i**

Using the same procedure as for **8g**, this was prepared from **7c** (10 g, 54.3 mmol) and two equimolecular amounts of methyl iodide. The mixture was heated at 42 °C for 7 h and afforded a crude solid compound (8.5 g) that was recrystallized in ethyl acetate to give 6.45 g (60%) of **8i** corresponding to one diastereomer; mp 94–95 °C.

IR: 2 937, 1 734, 1 660, 1 197 cm^{-1}.

Synthesis of substituted ethyl 2-ethoxy-3,4,5,6-tetrahydropyridine-3-carboxylates **9**

To a solution of triethyloxonium fluoborate (8 g, 42 mmol) in dry dichloromethane (25 mL) [16], a solution of piperidinone (36.5 mmol) in dichloromethane (30 mL) was added dropwise under argon. The mixture was stirred for 15 h, poured into 50 mL water and allowed to stand for 30 min. The organic layer was washed with 30 mL of a saturated solution of $NaHCO_3$ then with water and dried over Na_2SO_4. Evaporation gave an oil which was chromatographed (ethyl acetate or a mixture ethyl acetate/petroleum ether). The ^1H NMR spectra are presented in table II.

Table I. ^{1}H NMR chemical shifts (δ ppm, TMS) of **7** and **8** in $CDCl_3$ (300 MHz).

	NH	*H3*	*H4*	*H4′*	*H5*	*H5′*	*H6*	*H6′*	*CH_2*	*CH_3*	*CH*	*ArH*
7b	6.38 1H m	3.33 1H m	2.13 1H m	1.84 2/3H q 12.3 Hz 2.09 1/3H m	1.98 1H m		3.42 1H m	3.03 2/3H t 11.3 Hz 2.95 1/3H m	4.24 2H q	1.31 (c) 1.05 (d) 1.04 (e)		
7c	6.47 1H m	2.98 1H d 10.4 Hz	2.29 1H m		1.89 1H dq 13.5, 3 × 3.4 Hz	1.50 1H ddt 13.5, 6, 2 × 11 Hz	3.43 to 2H m	3.28	4.24 2H q 7.1 Hz	1.29 (c) 1.05 (f)		
8c	6.50 1H m		2.16 1H dt 2 × 3.2, 13.5 Hz	1.64 1H ddd 13.5, 13.2, 3.6 Hz	1.99 1H m	1.83 1H dm 13.5 Hz	3.28 1H m	3.28 1H m	4.20 2H m	1.28 (c) 0.96, 0.90 (g)	2.71 1H sep (a) 6.8 Hz	
8f	6.60 1H m		2.19 1/2H dm 13 Hz 1.99 1/2H t 12.8 Hz	1.76 1/2H dm 13 Hz 1.95 1/2H t 12.8 Hz	2.12 1H m		3.31 1H m	3.02 1/2H t 11.2 Ha 2.92 1/2H t 11.5 Hz	4.20 2M h	1.53, 1.47 (h) 1.284, 1.280 (i) 1.02, 0.98 (j)		
8g (1)	6.60 1H m		2.3	to 3H m	1.5		3.70 to 2H m	2.65	4.20 2H q 7.1 Hz	1.20 (c) 0.85 (k) 0.750 (l)	3.68 to 2.65 2H (b)	7.31 5H s
8h	6.34 1H m		1.94 1H ddq 12.6, 3 × 6.5, 2.3 Hz		2.04 1H ddt 12.6, 2 × 11.8 4.8 Hz	1.46 1H dm 11.8 Hz	3.17 1H dddd 11.8, 4.9, 4.8, 1.9 Hz	2.82 1H dt 2 × 11.8, 3.9 Hz	4.25 2H m	1.31 (c) 1.02 (f)	3.69, 3.09 2 × 1H d (b) 13.8 Hz	7.26 5H m
8i	6.95 1H m		1.84 1H ddq 11.1, 3 ×6.8, 2.75 Hz		2.01 1H dddd 12.7, 12.1, 11.1, 5.8 Hz	1.62 1H dm 12.7 Hz	3.35 1H m	3.29 1H dt 2 × 12.1 4.3 Hz	4.18 2H m	1.45 3H s 1.27 (c) 0.96 (g)		
8j	7.29 B 1/2H m 7.18 A 1/2H m	3.10 A 1/2H d 7.3 Hz 2.98 B 1/2H d 11 Hz	2.34 A 1/2H ddq 7.3, 3.3, 3 × 7 Hz	1.92 B 1/2H tq 2 × 11, 3 × 6.6 Hz	2.11 A 1/2H m	1.58 B 1/2H dtq 4.5, 2 × 11, 3 × 6.6 Hz	3.41 A 1/2H ddd 4.8, 12.2, 1.8 Hz 3.26 B 1/2H dt 12.2 2 × 5.5 Hz	3.04 A and B 1H m	4.20 2H	1.30, 1.29 (i) 1.00, 0.99 (j) 0.98, 0.96 (m)		

(1) Recorded at 60 MHz, (a): CH (isopropyl), (b): CH (benzyl), (c): 3H, t, 7.1 Hz, (d): 2H, d, 6.45 Hz, (e): 1H, d, 6.45 Hz, (f): 3H, d, 6.6 Hz, (g): 3H, d, 6.8 Hz, (h): 3/2H, s, (i): 3/2H, t, 7.1 Hz, (j): 3/2H, d, 6.3 Hz, (k): 2H, d, 6 Hz, (l): 1H, d, 6 Hz, (m): 1.5H, d, 7 Hz, A: diastereomer A, B: diastereomer B.

Table II. ^{1}H NMR chemical shifts (δ ppm, TMS) of **9** in $CDCl_3$ (300 MHz).

	H3	*H4*	*H4′*	*H5*	*H5′*	*H6*	*H6′*	CH_2	CH_3	*CH*	*ArH*
9c		1.59 1H ddd 13.5 12.7, 3.6 Hz	1.97 1H dddd 13.5, 2.7, 2.5, 2.1 Hz	1.80–1.66 1H m	1.80–1.66 1H m	3.25 1H ddd 15.6, 11.2, 4.45 Hz	3.6 1H dddd 15.6, 4.5, 2.4, 2.1 Hz	4.14 (1) 2H m 4.01 (2) 2H q 7 Hz	1.23 (1) (c) 1.21 (2) (c) 0.91, 0.86 2 × (d)	2.52 (a) 1H sep 6.9 Hz	
9f		1.85–1.65 1H m	2.04 1H m	1.85–1.65 1H m		3.02 B 0.4H dd 15.9, 10.3 Hz 2.92 A 0.6H dd 15.8, 10.9 Hz	3.65–3.50 1H m	4.25–3.92 4H m (1) and (2)	1.42 B 1.2H s 1.39 A 1.8H s 1.28 1.16 6H m (1) and (2) 0.95 B 1.2H d 5.55 Hz 0.89 A 1.8H d 6.55 Hz		
9g		1.63 A 0.6H t 13.2 Hz 1.38 B 0.4H t 13.7 Hz	1.88–1.78 1H m	1.88–1.78 B 0.4H m 0.83 A 0.6H m		2.86 A 0.6H dd, 15.6, 10.7 Hz 2.39 B 0.4H dd 15.6, 10.6 Hz	3.39 B 0.4H ddd 15.6, 4.4 2.2 Hz 3.34 A 0.6H ddd 15.6, 4.4, 2.2 Hz	4.29–3.96 4H m (1) and (2)	1.32–1.21 6H m (1) and (2) 0.77 B 1.2H d 6.4 Hz 0.69 A 1.8H d 6.4 Hz	3.46 B (b) (h) 3.36 A (b) (i) 3.09 A (b) (i) 2.93 B (b) (h)	7.37–7.16 5H m
9h		1.85 1H ddq 12.7, 3.3, 3 × 6.8 Hz		1.67 1H m	1.67 1H m	2.77 1H ddd 15.7, 11.8, 3.9 Hz	3.47 1H ddd 15.7,4.4, 1.6 Hz	4.30–4.05 4H m (1) and (2)	1.31 (c) 1.28 (c) 0.96 (d)	3.49, 3.07 (b) 2 × 1H d 14.1 Hz	7.27–7.17 3H m 7.10–7.07 2H m
9i		1.72 1H m		1.72 1H m	1.48 1H ddd 10, 4.3, 2 Hz	3.38 1H ddd 15.7, 11.3, 4.3 Hz	3.68 1H m	4.18 (1) 2H m 4.02 (2) 2H q 7 Hz	1.40 3H s 1.25 (c) 1.20 (c) 0.94 (e)		
9j	2.89 A 0.5H d 6.2 Hz 2.82 B 0.5H ddd 11, 2.6, 1.1 Hz	1.75 B 0.5H tq 2 × 11, 3 × 7 Hz	2.18 A 0.5H ddq 6.2, 3.6, 3 × 7 Hz	1.37–1.23 B 0.5H m	1.89 A 0.5H m	3.24 A 0.5H ddd 16.3, 6.6, 1.1 Hz 3.06 B 0.5H ddd 16.3, 11, 2.45 Hz	3.56 A 0.5H ddd 16.3, 4.95, 1.1 Hz 3.54 B 0.5H ddd 16.3, 4.8, 1.1 Hz	4.19 2H m 4.08 2H m	1.27 B, 1.26 A 1.21 A, 1.20 B (f) 0.97 B, 0.95 B 0.93 A, 0.85 A (g)		

(1): Ester, (2): ether, (a): CH (isopropyl), (b): CH (benzyl), (c): 3H, t, 7 Hz, (d): 3H, d, 6.9 Hz, (e): 3H, d, 6.4 Hz, (f): 3/2H, t, 7.1 Hz, (g): 3/2H, d, 7 Hz, (h): 0.4H, d, 13.7 Hz, (i): 0.6H, d, 13.6 Hz, A: diastereomer A, B: diastereomer B.

• *Ethyl 2-ethoxy-3-methyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9a**

Compound **8a** (6.76 g) gave 5.56 g of chromatographed **9a** (71.5%).

IR: 2 981, 2 942, 1 739, 1 681, 1 277 cm^{-1}.

• *Ethyl 2-ethoxy-3-ethyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9b**

Compound **8b** (5 g) gave 2.8 g of chromatographed **9b** (53%).

IR: 2 976, 2 941, 1 737, 1 678, 1 241 cm^{-1}.

• *Ethyl 2-ethoxy-3-isopropyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9c**

Compound **8c** (65.5 g) gave 5.4 g of chromatographed **9c** (86.5%).

IR: 2 974, 2 942, 1 740, 1 727, 1 677, 1 264 cm^{-1}.

• *Ethyl 3-benzyl-2-ethoxy-3,4,5,6-tetrahydropyridine-3-carboxylate* **9d**

Compound **8d** (19 g) gave 18.3 g of chromatographed **9d** (87%).

IR: 2 978, 2 938, 1 734, 1 681, 1 238 cm^{-1}.

• *Ethyl 2-ethoxy-3-phenyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9e**

Compound **8e** (6.8 g) gave 6.6 g of chromatographed **9e** (86.5%).

IR: 2 978, 2 938, 1 745, 1 727, 1 682, 1 268 cm^{-1}.

• *Ethyl 2-ethoxy-3,5-dimethyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9f**

Compound **8f** (6 g) gave 5.45 g of chromatographed **9f** (79.5%) corresponding to a mixture of two diastereomers A/B (3:2).

IR: 2 979, 2 940, 1 739, 1 682, 1 245 cm^{-1}.

• *Ethyl 3-benzyl-2-ethoxy-5-methyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9g**

Compound **8g** (6.8 g) gave 5.7 g of chromatographed **9g** (76%) corresponding to a mixture of two diastereomers A/B (3:2).

IR: 2 978, 2 931, 1 737, 1 682, 1 235 cm^{-1}.

• *Ethyl 3-benzyl-2-ethoxy-4-methyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9h**

Compound **8h** (4.95 g) gave 5.7 g of chromatographed **9h** (86%) corresponding to a pure diastereomer.

IR: 2 976, 2 938, 1 737, 1 682, 1 223 cm^{-1}.

• *Ethyl 2-ethoxy-3,4-dimethyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9i**

Compound **8i** (5.8 g) gave 5.93 g of chromatographed **9i** (89.5%) corresponding to a pure diastereomer.

IR: 2 978, 2 938, 1 739, 1 682 cm^{-1}.

• *Ethyl 2-ethoxy-4,5-dimethyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9j**

Compound **8j** (1.64 g) gave 1.05 g of chromatographed **9j** (56%) corresponding to a mixture of two diastereomers 1:1 (A: $COOC_2H_5$ and both methyl groups are equatorial; B: $COOC_2H_5$ is equatorial and both methyl groups are axial).

IR: 2 977, 2 932, 1 739, 1 682, 1 279, 1 157 cm^{-1}.

• *Ethyl 2-ethoxy-4-methyl-3,4,5,6-tetrahydropyridine-3-carboxylate* **9k**

Compound **7c** (6.85 g) gave 5.5 g (70%) of chromatographed **9k** which was used directly in the next step.

IR: 2 933, 1 738, 1 685 cm^{-1}.

Synthesis of substituted 2-amino-4-hydroxy-3,4,4a,5,6,7-hexahydropyrido[2,3-d]pyrimidine and 2-amino-4-hydroxy-5,6,7-tetrahydro pyrido [2,3-d]pyrimidine **3**

To a solution of sodium ethanolate (from 1.5 g Na, 65.2 mmol) in dry ethanol (65 mL), guanidine hydrochloride (2.5 g, 26.2 mmol) and substituted 2-ethoxy-3-ethoxycarbonyl-3,4,5,6-tetrahydropyridine **9** (26.2 mmol) were added successively. The mixture was boiled for 24 h, the alcohol was evaporated off and the residue triturated with ether (150 mL). After removal of the etheral layer, water (10 mL) was added to the residue and the solution neutralized with 1N HCl to pH 7.5 and cooled. The solid was filtered off and recrystallized; evaporation of mother liquors to a few milliliters generally gave a second crop of the substance.

The compound was generally purified after conversion into the trifluoroacetate or hydrochloride salt according to the following procedures:

Trifluoroacetate salt: Crude **3** (40 mmol) was dissolved in 3 mL trifluoroacetic acid; the solution was evaporated to dryness and the oily residue triturated with ether to give a white solid which was filtered and recrystallized.

Hydrochloride salt: Crude **3** (1.5 mmol) was dissolved by slight heating in a solution of hydrogen chloride (1.7 N) in methanol (20 mL). Solvent was evaporated and the residue was triturated with ether to give a solid; after filtration, it was recrystallized. ^1H NMR and ^{13}C NMR spectra are presented in tables III and IV.

• *2-Amino-4a-methyl-4a,5,6,7-tetrahydropyrido [2,3-d]pyrimidin-4(3H)-one trifluoroacetate* **3a**

Compound **9a** (5.9 g) gave 3.4 g of crystallized 5-deazapterin that was converted into the trifluoroacetate salt; recrystallization in ethanol gave 3.8 g (47%) of **3a**; mp = 202–203 °C.

IR: 3 363, 1 690, 1 595, 1 206 cm^{-1}.

Anal calc for $C_8H_{12}N_4O{\cdot}CF_3COOH$: C, 40.82; H, 4.45; N, 19.04. Found: C, 41.0; H, 4.6; N, 19.0.

• *2-Amino-4a-ethyl-4a,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4(3H)-one hydrochloride* **3b**

Compound **9b** (5.9 g) gave 2.6 g (51.2%) of 5-deazapterin; mp (water) = 255–257 °C.

Anal calc for $C_9H_{14}N_4O$: C, 55.65; H, 7.27; N, 28.84. Found: C, 55.2; H, 7.5; N, 29.1.

Conversion into the hydrochloride salt and recrystallization in methanol gave 2.7 g (45%) of **3b**; mp = 271 °C.

IR: 3 136, 1 724, 1 672, 1 618, 1 562, 1 509, 1 460, 1 321, 1 255 cm^{-1}.

Anal calc for $C_9H_{14}N_4O{\cdot}HCl\ 0.55H_2O$: C, 44.92; H, 6.73; N, 23.28; Cl, 14.73. Found: C, 44.8; H, 6.4; N, 23.3; Cl, 14.7.

• *2-Amino-4a-isopropyl-4a,5,6,7-tetrahydropyrido-[2,3-d]pyrimidin-4(3H)-one hydrochloride* **3c**

Compound **9c** (4.25 g) gave 1.15 g (31%) of crude 5-deazapterin (mp = 253 °C) that was converted into the hydrochloride. Recrystallization in water gave 0.75 g (17.5%) of **3c**; mp = 268 °C.

IR: 3 139, 1 742, 1 661, 1 580, 1 510, 1 464, 1 406, 1 245 cm^{-1}.

Anal calc for $C_{10}H_{16}N_4O{\cdot}HCl\ 0.1H2O$: C, 48.72; H, 7.02; N, 22.72; O, 7.13. Found: C, 48.6; H, 7.0; N, 22.7; O, 6.8.

Table III. ^{1}H NMR chemical shifts (δ ppm, TMS) of **3** (300 MHz).

	NH(8)	*NH(2)*	*OH*	*H7*	*H7′*	*H5*	*H5′*	*H6*	*H6′*	*CH*	*CH_3*	*ArH*
3a (1)	10.19 1H s	8.8 1H s	8.54 1H s	3.44 1H dm 13.4 Hz	3.29 1H m	1.97	to 4H	m	1.84		1.49 3H s	
3b (1)	10.17 1H s	8.83 1H s	8.04 1H s	3.44 1H dm 14 Hz	3.31 1H m	2.05		to 4H m	+	1.73 2H (a)	0.83 3H t 7.4 Hz	
3c (2)				3.89 1H m	3.89 1H m	2.56 1H m	2.19 1H m	2.42 to 2H	2.31 m	2.65 1H m (b) 6.8 Hz	1.35, 1.28 3H d 6.8 Hz	
3d (3)				3.65 1H ddm 15.05, 5 Hz	3.48 1H m	2.01 1H m	2.01 1H m	2.24 1H m	2.24 1H m	3.36, 3.12 1H d (c) 13.3 Hz		7.31 3H m 7.08 2H m
3e (4)				3.75 1H m	3.75 1H m	2.02 1H m	1.71 1H m	2.84 1H m	2.30 1H m			7.51 3H s 7.34 2H s
3f (3)				3.62 1H dd 14.2, 5.75 Hz	3.01 1H dd 14.2, 11.3 Hz	2.07 1H dd 13.5, 2.3 Hz	1.68 1H dd 13.5, 12.8 Hz	2.34 1H m			1.64 3H s 1.04 3H d 6.4 Hz	
3g (3)				3.74 1H dd 14.25, 6.1 Hz	3.08 1H dd 14.25, 11 Hz	2.22 1H dd 13.3, 2.7 Hz	1.72 1H t 13.3 Hz	2.57 1H m		3.39, 3.18 1H d (c) 13.2 Hz	1.09 3H d 6.45 Hz	7.36 3H m 7.11 2H m
3h (3)				3.39 A 1/2H dd 12.7, 3.6 Hz 3.11 B 1/2H dd 12.4, 4.7 Hz	2.99 A, B 1H m	2.69 B 1/2H dq 4.5, 6.9 Hz 2.46 A 1/2H dq 1.8, 6.9 Hz		1.87 A 1/2H m	1.79 B 1/2H m		1.01 A 0.95 B 0.86 B 0.82 A 4 × 3/2H d 6.9 Hz	
3i (3)				3.35 1H m	3.35 1H m	2.84 1H m		1.72 1H m	1.72 1H m		1.04 3H d 6.9 Hz	

(1) CD_3SOCD_3 + CF_3COOH, (2): D_2O, 58 °C, (3): D_2O, (4): CF_3COOH + $CDCl_3$, (a): CH_2 (ethyl), (b): CH (isopropyl), (c): CH (benzyl), A: diastereomer A, B: diastereomer B.

Table IV. ^{13}C NMR chemical shifts (δ ppm, TMS) of **3** (300 MHz).

Carbon	*8a*	*4*	*2*	*4a*	*7*	*5*	*6*	*C*	*CH_3*	*Ar*
3a (5)	175.74	172.56	156.97	41.07	41.02	24.78	16.27		27.08	
3b (1)	175.50	171.02	156.99	45.20	40.81	21.59	16.05	31.98 CH_2(Et)	7.80	
3c (2)	178.90	174.84	160.20	52.96	44.37	24.83	19.79	40.57 CH(iPr)	19.72 19.66	
3d (3)	176.23	174.49	156.83	48.29	46.77	26.58	25.11	41.39 CH_2(Bz)		133.63 130.62 128.93 128.82
3e (4)	172.53	169.48	156.17	52.42	43.30	27.29	15.66			134.45 130.68 130.49 125.89
3f (3)	176.78	175.18	157.26	42.32	48.88	33.21	22.89		28.20 18.29	
3g (3)	174.50	173.30	156.83	48.97	48.86	32.76	22.96	47.18 CH_2(Bz)	18.34	133.67 130.71 128.93 128.83
3h (3)				91.60	43.77 42.73	31.54 29.82	32.37 31.78		22.29 18.98 15.85 14.50	
3i (3)	150.75	161.68	150.68	90.06	36.65	23.08	26.31		19.66	

(1) CD_3SOCD_3 + CF_3COOH, (2): D_2O, 58 °C, (3): D_2O, (4): CF_3COOH + $CDCl_3$, (5): CD_3SOCD_3.

• *2-Amino-4a-benzyl-4a,5,6,7-tetrahydropyrido-[2,3-*d*]pyrimidin-4(3*H*)-one trifluoroacetate* **3d**

Compound **9d** (10 g) gave 8.3 g (93%) of crude 5-deazapterin (mp = 243–244 °C).

Two grams were converted into the trifluoroacetate salt; recrystallization in ethanol gave 1.5 g (66%) of **3d**; mp = 213–214 °C.

IR: 3 070, 1 731, 1 712, 1 681, 1 204 cm^{-1}.

Anal calc for $C_{14}H_{16}N_4O{\cdot}CF_3COOH$: C, 51.89; H, 4.63. N, 15.13; F, 15.39. Found: C, 51.9; H, 4.5; N, 15.3; F, 15.4.

Two grams were converted into the hydrochloride salt; recrystallization in methanol gave 1.45 g (50%) of **3d**; mp 261–263 °C.

IR: 3 146, 1 739, 1 666, 1 620, 1 578, 1 499 cm^{-1}.

Anal calc for $C_{14}H_{16}N_4O{\cdot}HCl$: C, 57.44; H, 5.85; N, 19.14; Cl, 12.11. Found: C, 57.8; H, 6.0; N, 19.2; Cl, 12.3.

• *2-Amino-4a-phenyl-4a,5,6,7-tetrahydropyrido-[2,3-*d*]pyrimidin-4(3*H*)-one trifluoroacetate* **3e**

Compound **9e** (6.1 g) gave 5.1 g (95%) of crude 5-deazapterin (mp = 249 °C).

Two grams were converted into the trifluoroacetate salt; recrystallization in ethanol gave 2.5 g (85%) of **3e**; mp = 217–218 °C.

IR: 3 054, 1 744, 1 680, 1 600, 1 505, 1 204 cm^{-1}.

Anal calc for $C_{13}H_{14}N_4O{\cdot}CF_3COOH$ (0.15 H_2O): C, 50.18; H, 4.29; N, 15.60 Found: C, 49.9; H, 4.1; N, 15.6.

One gram was converted into the hydrochloride salt; recrystallization in water gave 0.85 g (74%) of **3e**; mp 284–285 °C.

IR: 3 129, 1 734, 1 677, 1 621, 1 584, 1 499, 1 265 cm^{-1}.

Anal calc for $C_{13}H_{14}N_4O{\cdot}HCl$: C, 56.02; H, 5.42; N, 20.10; Cl, 12.72. Found: C, 55.7; H, 5.4; N, 19.9; Cl, 12.7.

• *2-Amino-4a,6-dimethyl-4a,5,6,7-tetrahydropyrido-[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride* **3f**

Compound **9f** (4 g) gave 2.05 g (60%) of crude 5-deazapterin corresponding to a mixture of two diastereomers (*cis/trans* 1:3); conversion into the hydrochloride salt and recrystallization in ethanol gave 1.3 g of **3f** (*cis/trans* 1:9); mp = 256 °C).

IR: 3 115, 1 732, 1 667, 1 625, 1 500, 1 268 cm^{-1}.

Anal calc for $C_9H_{14}N_4O{\cdot}HCl$: C, 46.86; H, 6.55; N, 24.29; Cl, 15.37. Found: C, 46.9; H, 6.9 N, 24.4; Cl, 15.3.

• *2-Amino-4a-benzyl-6-methyl-4a,5,6,7-tetrahydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one trifluoroacetate* **3g**

Compound **9g** (5.7 g) gave 2.03 g (40%) of crude 5-deazapterin corresponding to a mixture of two diastereomers (*cis/trans* 15:85).

One portion (1.2 g) was converted into the trifluoroacetate salt and recrystallized in ethanol to give 0.86 g (50%) of **3g** corresponding to the pure *trans* isomer; mp = 218 °C).

IR: 3 064, 1 713, 1 682, 1 615, 1 206 cm^{-1}.

Anal calc for $C_{15}H_{18}N_4O{\cdot}CF_3COOH$: C, 53.12; H, 4.98; N, 14.58. Found: C, 53.4; H, 5.1; N, 14.8.

The other portion (0.8 g) was converted into the hydrochloride salt and recrystallized in methanol to give 0.5 g (55%) of **3g** corresponding to the pure *trans* isomer; mp = 258–259 °C.

IR: 3 154, 1 737, 1 686, 1 627, 1 577 cm^{-1}.

Anal calc for $C_{15}H_{18}N_4O{\cdot}HCl\ 0.5H_2O$: C, 57.05; H, 6.37; N, 17.74; Cl, 11.22. Found: C, 57.0; H, 6.4; N, 17.7; Cl, 10.9.

• *2-Amino-5,6-dimethyl-5,6,7,8-tetrahydropyrido-[2,3-*d*]pyrimidin-4(3*H*)-one* **3h**

Compound **9j** (1.55 g) gave 0.39 g (29%) of **3h** after recrystallization in water; it corresponded to a mixture of

two diastereomers 1:1 (A = *trans*: both methyl groups are axial; B = *cis*: methyl group on carbon number 5 is axial and methyl group on carbon number 6 is equatorial); mp = 266 °C.

IR: 3 422, 1 668, 1 613, 1 554 cm^{-1}.

Anal calc for $C_9H_{14}N_4O \cdot 0.25H_2O$: C, 54.40; H, 7.35; N, 28.19. Found: C, 54.4; H, 7.9; N, 28.1.

• *2-Amino-5-methyl-5,6,7,8-tetrahydropyrido-[2,3-*d*]pyrimidin-4(3*H*)-one hydrochloride* **3i**

Compound **9k** (5.48 g) gave 3.5 g (75%) of crude 5-deazapterin; mp = 274–278 °C. It was converted into 3.7 g (66%) of the hydrochloride salt (mp = 303–304 °C). Recrystallization in methanol gave 1.5 g (27%) of **3i**; mp = 297–302 °C.

IR: 3 253, 2 962, 2 698, 1 690, 1 610, 1 522 cm^{-1}.

Anal calc for $C_8H_{12}N_4O \cdot HCl\ 0.1H_2O$: C, 44.35; H, 6.05; N, 25.86; Cl, 16.36. Found: C, 44.0; H, 6.1; N, 25.5; Cl, 16.1.

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