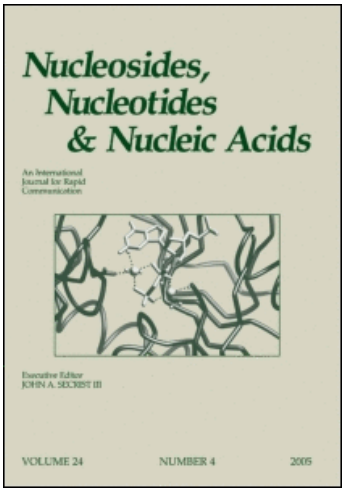

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Nucleosides. LXII. Synthesis of 6-Methyl-8-(2-deoxy-β-D-ribofuranosyl)-isoxanthopterin and Derivatives

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To cite this Article Melguizo, M. , Gottlieb, M. , Charubala, R. and Pfleiderer, W.(1998) 'Nucleosides. LXII. Synthesis of 6-Methyl-8-(2-deoxy-β-D-ribofuranosyl)-isoxanthopterin and Derivatives', Nucleosides, Nucleotides and Nucleic Acids, 17: 1, 175 — 186
To link to this Article: DOI: 10.1080/07328319808005168
URL: http://dx.doi.org/10.1080/07328319808005168

NUCLEOSIDES & NUCLEOTIDES, 17(1-3), 175-186 (1998)

NUCLEOSIDES LXII[1] SYNTHESIS OF 6-METHYL-8-(2-DEOXY-ß-D-RIBOFURANOSYL)-ISOXANTHOPTERIN AND DERIVATIVES

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ABSTRACT: The syntheses of 6-methyl-8-(2-deoxy-ß-D-ribofuranosyl)isoxanthopterin (**21**) and its protected 3'-*O*-phosphoramidite **23** were achieved from 6-methyl-2-methylthio-8-(2-deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranosyl)-3H,8H-pteridine-4,7-dione (**8**) in several steps. The new building block for oligonucleotide syntheses is highly fluorescent and can be considered as a substitute for 2'-deoxyguanosine.

INTRODUCTION. Pteridine-N-1 and -N-8 nucleosides show a close structural relationship to the common, naturally occurring pyrimidine and purine nucleosides and can therefore be regarded as interesting substitutes for various purposes. There are, however, substantial differences what the chemistry of the nucleo-bases is concerned and this is also reflected in the physical properties of this type of molecules. A striking feature of the pteridines is seen in their strong fluorescence which can be applied for various labeling experiments in biochemistry and molecular biology. Only recently the fluorescence of the pteridine nucleus has been considered as an alternative possibility to label oligonucleotides at specific sites of the chain in order to study interactions during hybridizations, intermolecular loop formations and stacking effects.[2,3,4] Furthermore, fluorescence modified oligonucleotides may play a special role in various sequencing techniques.

In the past our interest has mainly been focussed on the synthesis of pteridine ß-D-ribonucleosides[5-12] which can be obtained in a stereospecific manner by direct glycosylation reactions of the nucleo-bases applying preferentially the Hilbert-Johnson-Birkofer method.

In memoriam of Prof. Tsujiaki Hata and in admiration of his valuable and important contributions to nucleic acid chemistry

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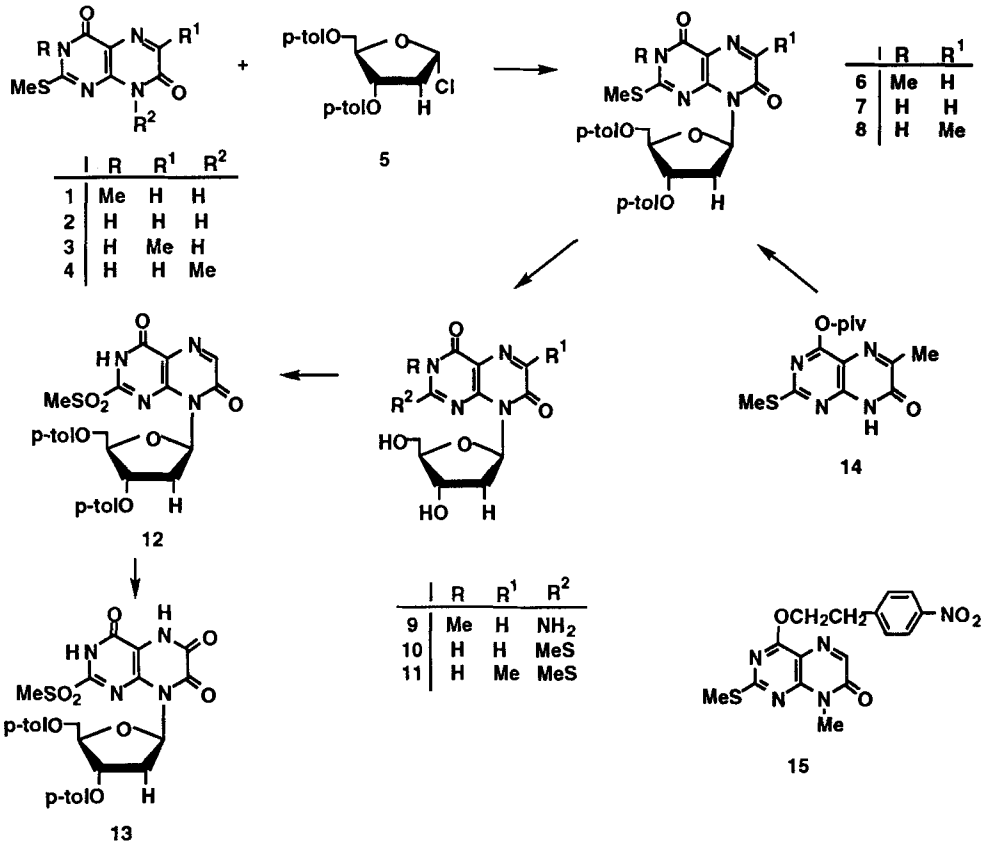
The synthesis of the corresponding 2'-deoxy-ß-D-ribofuranosides , however, is much more complicated due to the formation of α,ß-anomeric mixtures[13] which are commonly difficult to be separated into the pure components. The very high quantum yields of isoxanthopterin derivatives[3] called our attention to synthesize 8-(2-deoxy-ß-D-ribofuranosyl)-isoxanthopterin (**24**) and its properly protected 3'-*O*-phosphoramidite as an appropriate monomeric building block for machine-aided oligonucleotide synthesis on solid support materials.[14]

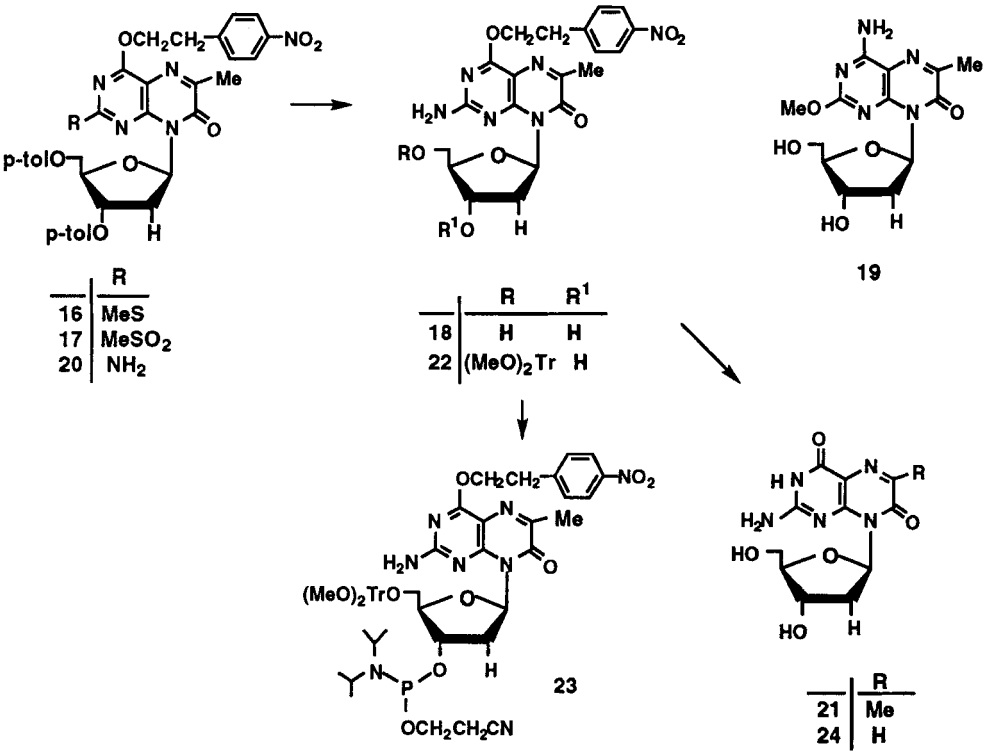
SYNTHESIS AND RESULTS. Isoxanthopterin,[15] one of the natural wing-pigments of butterflies, is a very insoluble material, which so far resisted all efforts of direct glycosylation with 2-deoxy-1-chloro-3,5-di-*O*-*p*-toluoyl-D-ribofuranose (**5**) and afforded only complex mixtures from which no pure component could be isolated. However, a model reaction between 3-methyl-2-methylthio-3H,8H-pteridine-4,7-dione (**1**) led in acetonitrile in presence of DBU as a base in a highly stereospecific manner to the corresponding 8-(2-deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranoside) **6** which reacted under mild conditions with NH_3/MeOH under nucleophilic displacement of the methylthio group and simultaneous cleavage of the sugar protecting groups to 3-methyl-8-(2-deoxy-ß-D-ribofuranosyl)isoxanthopterin (**9**) in good yield.[2] An analogous reaction between 2-methylthio-3H,8H-pteridine-4,7-dione (**2**) and **5** led to a mixture of compounds from which the desired 8-(2-deoxy-ß-D-ribofuranoside) **7** could easily be isolated chromatographically in 46% yield since almost no α-anomer had been formed under the applied reaction conditions. The interconversion of **7** into the isoxanthopterin series, however, was not possible since ammonia treatment caused only deacylation at the sugar moiety to the corresponding pteridine-2'-deoxyriboside **10** whereas displacement of the methylthio group was suppressed due to anion formation and electronic repulsion in the anticipated substitution reaction. Activation of the methylthio group by peracid oxidation to the methylsulfonyl derivative **12** was also no alternative approach since analogously to the ribo series[12] simultaneous hydroxylation at the 6-position took place affording 2-methylsulfonyl-8-(2-deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranosyl)-3H,5H,8H-pteridine-4,6,7-trione (**13**).

In order to avoid oxidation in 6-position a new series starting from 6-methyl-2-methylthio-3H,8H-pteridine-4,7-dione (**3**) was chosen and led in the first step of the reaction sequence on glycosylation with **5** in 31% yield to **8**. An improvement of the relatively low yield could be achieved if **3** was first acylated with pivaloyl chloride to form 6-methyl-2-methylthio-4-pivaloyloxy-8H-pteridin-7-one (**14**) and followed by treatment with 5 in acetonitrile and in presence of DBU as a base. Subsequent short treatment with NH_3/MeOH to cleave the pivaloyl blocking group and chromatographical work-up afforded the ß-anomer **8** in 67% yield. In the next step the amide function in **8** was



protected at O^4 by the 2-(4-nitrophenyl)ethyl group introduced by a Mitsunobu reaction[16] yielding 93% of **16**. This protection reaction was based upon a model study in which 8-methyl-2-methylthio-3H,8H-pteridine-4,7-dione (**4**) was treated with 2-(4-nitrophenyl-ethanol, triphenylphosphine and diisopropyl azodicarboxylate in dioxane at room temperature to give 8-methyl-2-methylthio-4-[2-(4-nitrophenyl)ethoxy]-8H-pteridin-7-one (**15**) in 88% yield.

Oxidation of **16** with m-chloroperbenzoic acid proceeded well in the expected manner to give the 2-methylsulfonyl derivative **17** in 86% yield. The nucleophilic displacement of the methylsulfonyl group and simultaneous cleavage of the sugar protecting groups, however, caused problems since the use of MeOH/NH_3 led to a mixture of 8-(2-deoxy-ß-D-ribofuranosyl)-6-methyl-O^4-[2-(4-nitrophenyl)ethyl]isoxanthopterin (**18**) and 4-amino-8-(2-deoxy-ß-D-ribofuranosyl)-2-methoxy-6-methyl-8H-pteridin-7-one (**19**) in almost equal amounts. Treatment of **17** with gaseous NH_3 in CH_2Cl_2 worked well and only displacement of the methylsulfonyl group took place in almost quantitative yield to **20** without harming the other blocking groups. Selective removal of the p-toluoyl groups from



the sugar moiety was achieved by sodium thiophenolate in CH_2Cl_2/MeOH to form **18** in 81% yield and the final elimination of the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc)-protecting group was done with DBU in pyridine to give 8-(2-deoxy-ß-D-ribofuranosyl)-6-methyl-isoxanthopterin (**21**) in 71% yield. Finally compound **18** was dimethoxytritylated at the 5'-OH group to **22** and subsequently phosphitylated with bis-diisopropylamino-ß-cyanoethoxyphosphine under tetrazole catalysis to give 8-(2-deoxy-5-*O*-dimethoxytrityl-ß-D-ribofuranosyl)-6-methyl-O^4-[2-(4-nitrophenyl)ethyl]isoxanthopterin-3'-*O*-(ß-cyanoethyl, N-diisopropyl)-phosphoramidite (**23**) in good yield and which has been proven as an interesting fluorescent building block in modified oligonucleotide synthesis.[3]

Physical Properties. The newly synthesized compounds have been characterized by elemental analysis, UV- and NMR spectra. The structural assignments of the glycosidic linkages in the new nucleosides has been derived from the ^{1}H-NMR spectra showing widely separated signals for H-C(2'and 2") characteristic for the ß-configuration.[13] The structure of **19** was assigned on the basis of its UV spectrum which shows a striking resemblance to the model substance 4-amino-2-methoxy-8H-pteridin-7-one.[19]

EXPERIMENTAL

General. TLC: Precoated silica gel thin-layer sheets *F1500 LS 254* from *Schleicher & Schuell.* Flash chromatography (FC): silica gel (*Baker*, 30-60 mm); 0.2-0.3 bar. Column chromatography (CC): silica gel 60, *Merck 60* (0.063-0.2 mesh). Mp: *Gallenkamp* or *Büchi*, Model *Dr. Tottoli* melting point apparatus; no corrections. UV/VIS: *Perkin-Elmer*, Lambda 15; λ_{max} in nm (log ε); [ ] shoulder. ^{1}H-NMR: *Bruker AC 250*: δ in ppm rel. to TMS as internal standard. ^{31}P-NMR: *Jeol GX-400*; δ in ppm rel. to H_3PO_4. The substances were dried either at 100°C in the oven or at room temperature in a vacuum desiccator.

6-Ethoxycarbonylmethyl-2-methylthio-3H,8H-pteridine-4,7-dione. A mixture of 5,6-diamino-2-methylthio-3H-pyrimidin-4-one (17.2 g, 0.1 mol) and sodium diethyl oxalylacetate (22.6 g) was heated in AcOH (200 ml) with stirring in an oilbath to 80 °C. After cooling the precipitate was collected, washed with H_2O and then the crude product dissolved in H_2O/MeOH (1:1, 800 ml) by heating and addition of a saturated solution of sodium bicarbonate (170 ml). The solution was treated with charcoal, filtered and then the filtrate added to hot AcOH (200 ml). After cooling overnight the precipitate was filtered by suction, washed with cold H_2O, some MeOH and ether to give after drying at 100 °C a yellowish crystal powder (18.9 g, 64%) of mp >210 °C (decomp.). UV (MeOH): 217 (4.36), [244 (4.04)], 296 (3.91), 340 (4.21), [420 (2.95)]. ^{1}H-NMR (DMSO-d_6): 12.97 (s, 2H, H-N), 4.08 (q, 2H, *CH*$_2$-Me), 3.69 (s, 2H, CH_2), 2.55 (s, 3H, S-Me), 1.15 (t, 3H, C-Me). Anal. calc. for $C_{11}H_{12}N_4O_4S$ (296.3): C 44.59, H 4.08, N 18.91. Found: C 44.49, H 4.03, N 18.88.

6-Methyl-2-methylthio-4,7(3H,8H)pteridinedione (3). A solution of 6-ethoxycarbonylmethyl-2-methylthio-3H,8H-pteridine-4,7-dione (19.7 g, 66.5 mmol) in 2.5 N NaOH (120 ml) was heated in an oilbath at 80 °C for 30 min, then treated with charcoal and filtered with stirring into hot AcOH (50 ml). A precipitate is formed and was collected after cooling, washed with H_2O and MeOH and dried at 100 °C to give a yellow crystal powder (14.3 g, 96%) of mp >275 °C (decomp.). UV (pH 3): 218 (4.34), [244 (4.04)], 293 (4.04), 334 (4.22). ^{1}H-NMR (DMSO-d_6): 12.0 (bs, H-N), 2.34 (s, S-Me), 2.22 (s, C-Me). Anal. calc. for $C_8H_8N_4O_2S$ (224.3): C 42.85, H 3.60, N 24.99. Found: C 42.79, H 3.59, N 25.06.

8-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranosy)-2-methylthio-3H,8H-pteridine-4,7-dione (7). To a suspension of 2-methylthio-3H,8H-pteridine-4,7-dione (**2**)[17] (0.21 g, 1 mmol) in dry acetonitrile (15 ml) was added DBU (0.3 g, 2 mmol) and then stirred at room temperature till a clear solution was obtained. It was cooled by acetone/dry ice to -45 °C, 2-deoxy-3,5-di-*O*-p-toluoyl-α-D-ribofuranosyl chloride (**5**)[18] (0.485 g, 1.25 mmol) added and the mixture stirred for 1.5 h while the bath-temperature raised to -20 °C. The reaction was quenched by addition of MeOH (50 ml), H_2O (5 ml) and AcOH (0.2 ml), stirred for 2 h,

then evaporated in vacuum, the residue taken up in CH_2Cl_2, washed with H_2O, the organic layer dried over Na_2SO_4, filtered and again evaporated. The resulting sirup was dissolved in little CH_2Cl_2, put onto a silica gel column for chromatography with a gradient CH_2Cl_2/MeOH (100:1 - 100:4) and the main fraction collected. Evaporation gave a colorless amorphous solid (0.264 g, 46%). Recrystallization from toluene gave colorless crystals of mp 142 °C. UV (MeOH): 204 (4.54), 238 (4.61), 282 (3.75), 295 (3.75), [333 (3.99)], 346 (4.06), [358 (4.00)]. ^{1}H-NMR ($CDCl_3$): 13.0 (bs, 1 H, H-N). 8.02 (s, 1 H, H-C(6)), 7.92 7.92 (d, 4 H, o-tol), 7.46 (dd, 1 H, H-C(1')), 7.19 (m, 4 H, m-tol), 5.95 (m. 1H, H-C(3')), 5.0-4.5 (m, 3 H, H-C(4'), H-C(5')), 3.37 (m, 1 H, H-C(2')), 2.64 (s, 3 H, S-Me), 2.53 (m, 1 H, H-C(2")), 2.42 (s, 3 H, Me), 2.36 (s, 3 H, Me). Anal. calc. for $C_{28}H_{26}N_4O_7S$ (562.6): C 64.21, H 5.23, N 8.56. Found: C 64.18, H 5.27, N 8.48.

8-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranosyl)-6-methyl-2-methylthio-3H,8H-pteridine-4,7-dione (8). a) A suspension of (**3**) (4.0 g, 16.8 mmol) in dry acetonitrile (240 ml) was treated with DBU (8 ml, 53.6 mmol) and stirred at room temperature till a clear solution was obtained (30 min). Then **5** (4.63 g, 11.9 mmol) was added and the mixture stirred for 10 min. It was then evaporated in vacuum to a sirupy residue which was dissolved in little CH_2Cl_2, put onto a silica gel column (8.5 x 16 cm) and chromatographed by toluene/AcOEt (1:1, 2.5 l), then (1:2, 2 l) and finally with CH_2Cl_2/MeOH (100:3, 3 l). The main fraction was collected, evaporated and recrystallized from toluene to give colorless crystals (2.12 g, 31%) of mp 196-197 °C.

b) A suspension of 6-methyl-2-methylthio-4-pivaloyloxy-8H-pteridin-7-one (**14**) (1.19 g, 3.86 mmol) in dryacetonitrile (50 ml) was treated at room temperature with DBU (0.58 ml, 3.86 mmol) and stirred till a clear solution was obtained. Then **5** (1.5 g, 3.86 mmol) was added and stirring continued for 1 h. It was evaporated under reduced pressure, the residue dissolved in CH_2Cl_2 (25 ml) and saturated methanolic ammonia added which caused the instantaneous formation of a gel. After a few min was again evaporated, coevaporated several times with CH_2Cl_2 till a solid foam was obtained. The residue was dissolved in a large amount of CH_2Cl_2, then washed with buffer pH 7 (2 x 50 ml) and H_2O and the organic layer dried over Na_2SO_4. After evaporation the residue was recrystallized from toluene to give colorless crystals (1.03 g, 46%) of mp 197 °C and isolation from the filtrate by silica gel chromatography gave another crop (0.46 g, 21%). UV (MeOH): 205 (4.60), 238 (4.66), [288 (3.88)], 298 (3.93), [330 (4.10)], 341 (4.14), [354 (4.05)]. ^{1}H-NMR ($CDCl_3$): 12.6 (bs, 1 H, H-N), 7.94 (d, 2 H, o-tol), 7.92 (d, 2 H, o-tol), 7.46 (dd, 1 H, H-C(1')), 7.23 (d, 2 H, m-tol), 7.16 (d, 2 H, m-tol), 6.00 (m, 1 H, H-C(3')), 4.9-4.5 (m, 3 H, H-C(4'), H-C(5')), 3.34 (m, 1 H, H-C(2')), 2.64 (s, 3 H, S-Me), 2.53 (m, 1 H, H-C(2")), 2.51 (s, 3 H, Me-C(6)), 2.42 (s, 3 H; Me-tol), 2.37 (s, 3 H, Me-

tol). Anal. calc. for $C_{29}H_{28}N_4O_7S$ (576.6): C 60.41, H 4.89, N 9.72. Found: C 60.26, H 4.96, N 9.68.

8-(2-Deoxy-ß-D-ribofuranosyl)-2-methylthio-8H-pteridin-7-one (10). In a mixture of EtOH (10 ml), dioxane (10 ml) and conc. NH_3 (10 ml) **7** (0.225 g, 0.4 mmol) was stirred for 24 h at 50 °C in a closed flask. The clear solution was evaporated, the residue treated with ether by stirring for 3 h, filtered by suction, then the residue stirred with AcOEt (50 ml) for 12 h, collected by suction and dried in a vacuum desiccator to give a yellowish powder (0.1 g, 80%). UV (MeOH): 348 (4.08), 295 (3.71), [265 (4.00)], 227 (4.70). ^{1}H-NMR (D_6-DMSO): 11.12 (bs, 1 H, H-N), 7.84 (s, 1 H, H-C(6)), 7.13 (m, 1 H, H-C(1')), 5.14 (d, 1 H, HO-C(3')), 4.58 (bs, 1 H, H-C(5')), 4.40 (m, 1 H, H-C(3')), 3.80 (m, 1 H, H-C(4')), 3.71 (m, 2 H, H-C(5',5")), 2.80 (m, 1 H, H-C(2')), 2.43 (s, 3 H, S-Me), 2.04 (m, 1 H, H-C(2")). Anal. calc. for $C_{12}H_{14}N_4O_5S$ x H_2O (344.3): C 41.86, H 4.68, N 16.27, S 9.29. Found: C 42.11, H 4.76, N 15.79, S 8.78.

8-(2-Deoxy-ß-D-ribofuranosyl)-6-methyl-2-methylthio-8H-pteridin-7-one (11). A suspension of **8** (0.108 g, 0.18 mmol) in conc. NH_3 (5 ml) and dioxane (3 ml) was stirred at 50 °C for 8 h till a clear solution was obtained. It was evaporated to dryness, the residue stirred with ether (50 ml) for 2 h, filtered and the solid stirred with AcOEt for 20 h. After drying in a vacuum desiccator, a yellowish powder (45 mg, 73%) was obtained. UV (MeOH): [356 (4.06)], 341 (4.15), [299 (3.90)], [266 (3.79)], 217 (4.36). ^{1}H-NMR (DMSO-d_6): 11.23 (bs, 1 H, H-N), 7.29 (pt, 1 H, H-C(1')), 5.15 (d, 1 H, HO-C(3')), 4.66 (bs, 1 H, HO-C(5')), 4.40 (m, 1 H, H-C(3')), 3.85 (m, 1 H, H-C(4')), 3.70 (m, 2 H, H-C(5',5")), 2.85 (m, 1 H, H-C(2')), 2.46 (s, 3 H, S-Me), 2.25 (s, 3 H, Me-C(6)), 2.15 (m, 1 H, H-C(2")). Anal. calc. for $C_{13}H_{16}N_4O_5S$ x H_2O (358.3): C 43.57, H 5.06, N 15.63. Found: C 44.10, H 4.88, N 15.88.

8-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranosyl)-2-methylsulfonyl-3H,5H,8H-pteridine-4,6,7-trione (13). A solution of **7** (0.1 g, 0.178 mmol) in dry $CHCl_3$ (5 ml) was treated with m-chloroperbenzoic acid (Fluka 70%; 0.17 g, 0.7 mmol) by stirring at room temperature for 24 h. During this time the solution became turbid. It was evaporated to dryness, the residue treated with ether (30 ml) by stirring for 15 min, filtered by suction, washed again with ether and dried under vacuum at 40 °C to give a colorless powder (76 mg, 70%). UV (MeOH): 202 (4.78), 237 (4.59), 305 (4.04). ^{1}H-NMR ($CDCl_3$): 11.94 (s, 1 H, H-N), 7.87 (d, 2 H, o-tol), 7.80 (d, 2 H, o-tol), 7.32 (d, 2 H, m-tol), 7.22 (d, 2 H, m-tol), 7.13 (dd, 1 H, H-C(1')), 5.89 (m, 1 H, H-C(3')), 4.63 (m, 1 H, H-C(4')), 4.55 (m, 2 H, H-C(5')), 3.37 (s, 3 H, SO_2-Me), 3.09 (m, 1 H, H-C(2')), 2.54 (m, 1 H, H-C(2")), 2.38 (s, 3 H, Me-tol), 2.33 (s, 3 H, Me-tol). Anal. calc. for $C_{28}H_{26}N_4O_{10}S$ x H_2O (619.5): C 53.50, H 4.49, N 8.91. Found: C 53.38, H 4.45, N 9.07.

6-Methyl-2-methylthio-4-pivaloyloxy-8H-pteridin-7-one (14). To a suspension of **3** (2.0 g, 8.9 mmol) in dry pyridine (25 ml) pivaloyl chloride (1.2 g, 9.8 mmol) was added dropwise with stirring at room temperature. After 2 h the mixture was evaporated, the residue dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$ solution and H_2O, dried over Na_2SO_4, evaporated and the residue rightaway purified by silica gel flash chromatography in toluene/AcOEt (5:1, 1 l) and (1:1, 0.5 l). The main fraction gave on evaporation a colorless solid (1.88 g, 68%). A sample was recrystallized from CH_2Cl_2/ toluene to give crystals of mp 178 - 180 °C. UV (MeOH): 216 (4.32), 284 (3.87), 329 (4.24), [343 (4.12)]. ^{1}H-NMR (DMSO-d_6): 2.56 (s, 3H, S-Me), 2.34 (s, 3 H, Me-C(6)). Anal. calc. for $C_{13}H_{16}N_4O_3S$ (308.4): C 50.63, H 5.23, N 18.17. Found: C 50.84, H 5.26, N 17.71.

8-Methyl-2-methylthio-4-[2-(4-nitrophenyl)ethoxy]-8H-pteridin-7-one (15). To a suspension of 8-methyl-2-methylthio-3H,8H-pteridine-4,7-dione (**4**)[12] (0.224 g, 1 mmol) in dioxane (20 ml) was added first 2-(4-nitrophenyl)ethanol (0.25 g, 1.5 mmol), then triphenylphosphine (0.4 g, 1.5 mmol) and finally diisopropyl azodicarboxylate (0.3 g, 1.5 mmol) and stirred at room temperature for 24 h. The reaction mixture was diluted with MeOH (100 ml) and the resulting precipitate of **15** collected. The filtrate was partially evaporated, cooled in the ice-box for 2 h and the new precipitate filtered off to give after washing with MeOH colorless crystals (0.183 g, 88%) of mp 163-164 °C. UV (MeOH): 203 (4.32), 222 (4.51), 250 (4.26), [326 (4.20)], 337 (4.25), [350 (4.15)]. ^{1}H-NMR (DMSO-d_6): 8.18 (d, 2 H, o- to NO_2), 8.13 (s, 1 H, H-C(6)), 7.48 (d, 2 H, m to NO_2), 4.82 (t, 2 H, O-CH_2), 4.82 (t, 2 H, CH_2), 2.61 (s, 3 H, S-Me), Anal. calc. for $C_{16}H_{15}N_5O_4S$ (373.4): C 51.47, H 4.05, N 18.76. Found: C 51.52, H 3.96, N 18.74.

8-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranosyl)-6-methyl-2-methylthio-4-[2-(4-nitrophenyl)ethoxy]-8H-pteridin-7-one (16). A solution of dioxane (75 ml) containing **8** (2.19 g, 3.8 mmol), 2-(4-nitrophenyl)ethanol (0.96 g, 5.7 mmol) and triphenylphosphine (1.53 g, 5.7 mmol) was treated with diisopropyl azodicarboxylate (1.56 g, 5.7 mmol) at room temperature with stirring for 2.5 h. The mixture was evaporated and separated by flash chromatography on a silica gel column (5.3 x 15 cm) with toluene (0.3 l), toluene/ AcOEt (8:1, 0.25 l) and (6:1, 0.6 l). The main fraction was collected and evaporated to give a glass (2.57 g, 93%). Recrystallization from CH_2Cl_2/AcOEt gave colorless crystals (2.34 g, 85%) of mp 122-125 °C. UV (MeOH): 205 (4.66), 218 (4.58), 240 (4.64), [272 (4.21)], 329 (4.20), [345 (4.08)]. ^{1}H-NMR ($CDCl_3$): 8.18 (d, 2 H, o- to NO_2), 7.94 (m, 4 H, o-tol), 7.47 (d, 2 H, m to NO_2), 7.45 (dd, 1 H, H-C(1')), 7.24 (d, 2 H, m-tol), 7.15 (s 3 H, m-tol), 6.04 (m, 1 H, H-C(3')), 4.9-4.5 (m, 3 H, H-C(4'), H-C(5')), 4.82 (t, 2 H, O-CH_2), 3.32 (m, 3 H, H-C(2'), CH_2), 2.59 (s, 3 H, S-Me), 2.55 (m, 1 H, H-C(2")), 2.53 (s, 3 H,

Me-C(6)), 2.42 (s, 3 H, Me-tol), 2.37 (s, 3 H, Me-tol). Anal. calc. for $C_{37}H_{35}N_5O_9S$ (725.8): C 61.23, H 4.86, N 9.65. Found: C 61.18, H 4.95, N 9.67.

8-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranosyl)-6-methyl-2-methylsulfonyl-4-[2-(4-nitrophenyl)ethoxy]-8H-pteridin-7-one (17). A solution of **16** (2.27 g, 3.1 mmol) in abs. CH_2Cl_2 (100 ml) was treated with m-chloroperbenzoic acid (80-90% purity, 1.4 g) for 24 h with stirring at room temperature. The solution was concentrated to about 10 - 15 ml under reduced pressure whereby m-chlorobenzoic acid crystallized out. It was removed by suction, the solid washed with little CH_2Cl_2 , the filtrates united and put onto a silica gel column (5.3 x 14 cm) for flash chromatography with toluene/AcOEt (2.5 : 1). Evaporation of the solvent resulted in crystallization to give colorless crystals (2.04 g, 86%) of mp 193 °C. UV (MeOH): 244 (4.64), 272 (4.25), 293 (4.18), 305 (4.17), [316 (4.08)]. ^{1}H-NMR ($CDCl_3$): 8.19 (d, 2 H, o to NO_2), 7.93 (d, 2 H, o-tol), 7.91 (d, 2 H, o-tol), 7.51 (d, 2 H, m to NO_2), 7.36 (dd, 1 H, H-C(1')), 6.04 (m, 1 H, H-C(3')), 4.92 (t, 2 H, O-CH_2), 4.8 - 4.5 (m, 3 H, H-C(4')), 3.35 (t, 2 H, CH_2), 3.35 (s, 3 H, Me-SO_2), 3.32 (m, 1 H, H-C(2')), 2.62 (s, 3 H, Me-C(6)), 2.58 (m, 1 H, H-C(2")), 2.42 (s, 3 H, Me-tol), 2.37 (s, 3 H, Me-tol). Anal. calc. for $C_{37}H_{35}N_5O_{11}S$ (757.8): C 58.65, H 4.66, N 9.24. Found: C 58.77, H 4.69, N 9.30.

8-(2-Deoxy-ß-D-ribofuranosyl)-6-methyl-*O*^{4}-[2-(4-nitrophenyl)ethyl]-isoxanthopterin (18) and 4-Amino-8-(2-deoxy-ß-D-ribofuranosyl)-2-methoxy-6-methyl-8H-pteridin-7-one (19). a) A solution of **17** (1.76 g, 2.3 mmol) in CH_2Cl_2 (50 ml) was added to saturated methanolic ammonia (250 ml) and stirred at room temperature for 2 weeks. It was evaporated and the residue separated by silica gel flash chromatography with CH_2Cl_2 / MeOH (20:1) to give two main fractions. The first fraction gave on evaporation a colorless crystal powder (**18**) (0.3 g, 28%) of mp >250 °C (decomp.).

b) A solution of **20** (1.18 g, 1.7 mmol) in CH_2Cl_2 (30 ml) and MeOH (60 ml) was treated with sodium thiophenolate (0.45 g, 3.4 mmol) at room temperature with stirring for 16 h. Then flash silica gel (11 g) was added and evaporated under reduced pressure. The resulting solid was put on top of a flash silica gel column (5 .3 x 8.5 cm) and equilibrated with CH_2Cl_2/MeOH (100:1). Chromatography was started with the same mixture (500 ml), then 50:1 (300 ml) and finally 9:1 (500 ml). The main fraction was evaporated to give a colorless crystal powder (0.63 g, 81%) of mp >250 °C (decomp.). UV (MeOH): 209 (4.58), 233 (4.15), 278 (4.16), [285 (4.13)], 343 (4.18). ^{1}H-NMR (DMSO-d_6): 8.18 (d, 2 H, *o* to NO_2), 7.64 (d, 2 H, *m* to NO_2), 7.22 (bs, 2 H, NH_2), 7.14 (dd, 1 H, H-C(1')), 5.15 (d, 1 H, HO-C(3')), 4.70-4.55 (m, 3 H, HO-C(5'), O-CH_2), 4.42 (m, 1 H, H-C(3')), 3.71 (m, 1 H, H-C(4')), 3.65 (m, 1 H, H-C(5')), 3.51 (m, 1 H, H-C(5")), 3.26 (t, 2 H, CH_2), 2.89 (m, 1 H, H-C(2')), 2.26 (s, 3 H, Me-C(6)), 1.95 (m, 1 H, H-C(2")). Anal. calc. for $C_{20}H_{22}N_6O_7$ x 0.5 H_2O (467.4): C 51.39, H 4.96, N 17.98. Found: C 51.25, H 4.95, N 18.06.

c) The second fraction of the chromatographical separation in a) was evaporated to a small volume whereby colorless crystals (**19**) (0.26 g, 33%) of mp 169 °C were obtained. UV (MeOH): 209 (4.38), 256 (4.11), 288 (3.75), 337 (4.02). ^{1}H-NMR (DMSO-d_6): 7.74 (bs, 1 H, NH_2), 7.51 (bs, 1 H, NH_2), 7.08 (dd, 1 H, H-C(1')), 5.17 (m, 1 H, HO-C(3')), 4.64 (t, 2 H, HO-C(5')), 4.44 (m, 1H, H-C(3')), 3.85 (s, 3 H, O-Me), 3.74 (m, 1 H, H-C(4')), 3.65 (m, 1 H, H-C(5')), 3.53 (m, 1 H, H-C(5")), 2.86 (m, 1 H, H-C(2')), 2.23 (s, 3 H, Me-C(6)), 2.01 (m, 1 H, H-C(2")). Anal. calc. for $C_{13}H_{17}N_5O_5$ (323.3): C 48.29, H 5.30, N 21.66. Found: C 48.08, H 5.21, N 21.45.

8-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-ß-D-ribofuranosyl)-6-methyl-*O*^{4}-[2-(4-nitrophenyl)ethyl]-isoxanthopterin (20). A solution of **17** (1.89 g, 2.5 mmol) in CH_2Cl_2 (60 ml) was treated through a gas inlet with a slow stream of gaseous NH_3 and stirred at room temperature till all starting material has disappeared according to tlc (2-3 h). It was evaporated, the residue coevaporated twice with CH_2Cl_2 and then purified on a silica gel coulmn (5.3 x 8 cm) by flash chromatography in toluene / AcOEt (2.5 : 1). The main fraction was evaporated to a smaller volume whereby separation of colorless crystals (1.29 g, 74%) of mp 208-209 °C took place. The filtrate was evaporated to dryness and the residue treated with ether to give a chromatographically pure colorless powder (0.39 g, 22%). UV (MeOH): 207 (4.68), 238 (4.63), 275 (4.19), 343 (4.16). ^{1}H-NMR ($CDCl_3$): 8.18 (d, 2 H, o to NO_2), 7.90 (d, 2 H, o-tol), 7.88 (d, 2 H, o-tol), 7.47 (d, 2 H, m to NO_2), 7.30 (dd, 1 H, H-C(1')), 7.22 (d, 2 H, m-tol), 7.14 (d, 2H, m-tol), 6.06 (m, 1 H, H-C(3')), 5.40 (bs, 2 H, NH_2), 4.95 (m, 1 H, H-C(4')), 4.72 (t, 2 H, O-CH_2), 4.56 (m, 2 H, H-C(5')), 3.40 (m, 1 H, H-C(2')), 3.29 (t, 2 H, CH_2), 2.46 (m, 1 H, H-C(2")), 2.42 (s, 3 H, Me-tol), 2.36 (s, 3 H, Me-tol). Anal. calc. for $C_{36}H_{34}N_6O_9$ (694.7): C 62.24, H 4.93, N 12.10. Found: C 61.98, ,H 4.94, N 12.14.

8-(2-Deoxy-ß-D-ribofuranosyl)-6-methylisoxanthopterin (21). A solution of **18** (0.2 g, 0.43 mmol) in pyridine (15 ml) was treated with DBU (1.1 ml, 1.1 mmol) by stirring at room temperature for 3 h. It was evaporated under reduced pressure, the residue dissolved in H_2O (25 ml), the solution extracted with CH_2Cl_2 (3 x 25 ml), then neutralized with AcOH to pH 7, concentrated to about 5 ml and chilled in the ice-box. A colorless crystal powder (94 mg, 69%) of mp >300 °C (decomp.) separated. UV (MeOH): 214 (4.45), [229 (4.22)], 295 (3.96), 343 (4.04). $Exc._{max}$ 340 nm; $Em._{max}$ 431 nm.^{1}H-NMR (DMSO-d_6): 11.16 (bs, 1 H, H-N), 7.11 (dd, 1 H, H-C(1')), 7,10 (bs, 2 H, NH_2), 5.13 (d, 1 H, HO-C(3')), 4.66 (pt, 1 H, HO-C(5')), 4.41 (m, 1 H, H-C(3')), 3.69 (m, 1H, H-C(4')), 3.64 (m, 1 H, H-C(5')), 3.50 (m, 1 H, H-C(5")), 2.87 (m, 1 H, H-C(2')), 1.92 (m, 1 H, H-C(2")). Anal. calc. for $C_{12}H_{15}N_5O_5$ x 0.5 H_2O (318.3): C 45.28, H 5.06, N 22.00. Found: C 45.42, H 4.91, N 21.86.

8-(2-Deoxy-5-*O*-dimethoxytrityl-ß-D-ribofuranosyl)-6-methylisoxanthopterin (22). A solution of **18** (0.57 g, 1.2 mmol) in anhydrous pyridine (15 ml) was treated with dimethoxytrityl chloride (0.454, 1.34 mmol) at room temperature with stirring for 2 h. Then MeOH (5 ml) was added, stirred for 5 min, diluted with AcOEt (100 ml), washed with saturated $NaHCO_3$ solution and H_2O. The organic layer was dried over Na_2SO_4, evaporated to dryness and the residue purified by silica gel column flash chromatography withh toluene/AcOEt 1:1 . The main fraction was collected and evaporated to give a glass (0.5 g, 54%). UV (MeOH): 205 (4.85), 238 (4.50), 272 (4.23), 344 (4.05). Anal. calc. for $C_{41}H_{40}N_6O_9$ (760.8): C 64.72, H 5.30, N 11.05. Found: C 64.52, H 5.40, N 10.94.

8-(2-Deoxy-5-*O*-dimethoxytrityl-ß-D-ribofuranosyl)-6-methylisoxanthopterin-3'-*O*-(ß-cyanoethyl, N-diisopropyl)phosphoramidite (23). To a solution of **22** (1.77 g, 2.33 mmol) in anhydrous CH_2Cl_2 (60 ml) under argon atmosphere was added bis-diisopropylamino-ß-cyanoethoxyphosphine (0.84 g, 2.8 mmol) and tetrazole (81 mg, 1.16 mmol). The mixture was stirred for 3 h at room temperature, then shaken with a 5% solution of $NaHCO_3$, the organic layer dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography with toluene/AcOEt (3:1, 120 ml; then 2:1, 150 ml). The main fraction was evaporated, twice coevaporated with AcOEt to give a creme colored solid foam (1.74 g, 78%). UV (MeOH): 206 (4.91), 233 (4.51), 275 (4.21), 343 (4.16). ^{1}H-NMR ($CDCl_3$): 8.16 (d, 2 H, *o* to NO_2), 7.47-7.16 (m, 12 H, arom. H, H-C(1')), 6.74 (m, 4 H, *o* to OMe), 5.00 (bs, 2 H, NH_2), 4.80 (m, 1 H, H-C(3')), 4.70 (t, 2 H, CH_2), 4.22 (bs, 1 H, H-C(4')), 3.75 (s, 6 H, OMe), 3.70-3.35 (m, 6 H, CH_2CH_2CN, H-C(5'), 2 x $CHMe_2$), 3.28 (t, 2 H, CH_2), 3.00 (m, 1 H H-C(2")), 2.58 (t, 2 H, CH_2CH_2CN)), 2.38 (m, 4 H, Me-C(6), H-C(2")), 1.18 - 1.03 (m, 12 H, $CH(CH_3)_2$). ^{31}P-NMR ($CDCl_3$): 149.17. Anal. calc. for $C_{50}H_{57}N_8O_{10}P$ (961.0): C 62.51, H 5.98, N 11.66. Found: C 62.59, H 6.11, N 10.98.

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