Specific Inhibitors in Vitamin Biosynthesis. Part 9.[1] Reactions of 7,7-Dialkyl-7,8-dihydropteridines of Use in the Synthesis of Potential Inhibitors of Tetrahydrofolate Biosynthesis

Saiba S. Al-Hassan, Robert Cameron, Sydney H. Nicholson, David H. Robinson, Colin J. Suckling,* and Hamish C. S. Wood*
Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL

Reactions of 7,7-dialkyl-7,8-dihydropteridines which are of potential use in modifying substituents on the pyrazine ring to yield compounds with inhibitory activity against 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase and dihydrofolate reductase are described. These enzymes lie along the pathway leading to the coenzyme tetrahydrofolate. 6-Methyl substituents showed typical reactivity of alkyl groups α- to a pyrazine nitrogen atom and underwent exchange of protium for deuterium under acidic and basic conditions: however, they failed to undergo clean bromination or aldol condensation. Autoxidation of alkyl groups at this position provided ready access to pteridines substituted with carbonyl groups at C-6. 6-Formyl derivatives underwent Wittig-type reactions to yield 6-aralkylidene compounds that are potential inhibitors of dihydrofolate reductase. Alkylation of the anion of 2,4-diamino-7,8-dihydro-6,7,7-trimethylpteridine occurred at N-8 in low yield. The reduction of the blocked dihydropteridine system was readily accomplished using catalytic hydrogenation in a manner analogous to that used for normal pteridines.

In the previous two papers in this series[1,2] we described ring syntheses of compounds that are inhibitors of 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase, the enzyme that activates the pteridine fragment to coupling with 4-aminobenzoic acid during the biosynthesis of tetrahydrofolate. Although several extremely potent inhibitors were obtained, the available syntheses were not flexible, making an extensive study of structure–activity relationships difficult. We therefore wished to see whether it would be possible to introduce substituents into a simple, readily available, blocked dihydropterin so that the characteristics required for potent enzyme inhibition could be explored using a divergent synthetic approach. In particular, the introduction of polar substituents at carbon attached to C-6 and the presence of hydrophobic substituents in the region of C-7 and N-8 of the pteridine have been shown to be important.[1,2] In this paper we outline the reactivity of 6,7,7-trialkyl-7,8-dihydropteridines in this context together with the properties of some 6-formyl derivatives.

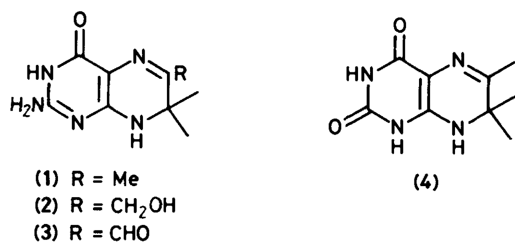
Reactions of C-6 *Alkyl Substituents*.—(a) *Bromination*. An early synthesis of folic acid[3] used bromination of 2-amino-6-methylpteridin-4(3*H*)-one to activate the 6-position prior to reaction with 4-aminobenzoic acid. In our series, a similar reaction could lead to a large number of potential enzyme inhibitors. Accordingly, several attempts were made to brominate 2-amino-7,8-dihydro-6,7,7-trimethylpteridin-4(3*H*)-one (**1**) (Table). Allylic brominating agents such as *N*-bromosuccinimide (NBS) under irradiation led to recovery of starting pteridine but reaction with bromine up to 100 °C led to a multiplicity of products, presumably *N*-brominated derivatives together with the required 6-bromination product. For our purposes, the extensive use of protecting groups to overcome side reactions was undesirable and accordingly a more thorough study of bromination was not attempted.

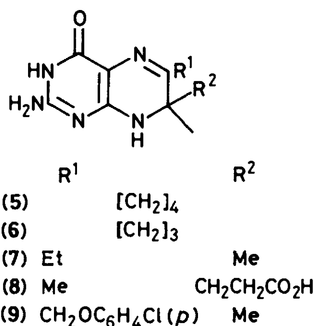
(b) *Deuterium exchange*. It was hoped that the activity of the 6-methyl group might be revealed by its ability to undergo exchange of deuterium for protium, a reaction characteristic of such substituted heterocyclic systems. Complete exchange was observed, in 6 h at 100 °C, of the 6-methyl protons exclusively in both the pteridine (**1**) and its 2-oxo analogue (**4**). The reaction occurred under either acidic or basic conditions. No other carbon-bound proton underwent exchange as would be expected. The implication of this observation is that nucleophilic reactivity can be generated at C-6 and that condensation reactions with aldehydes might be possible. Despite literature precedent,[4] the pteridine (**1**) failed to add to a very electrophilic aldehyde, chloral. Condensation with 4-nitrobenzaldehyde did occur but the reaction was not clean and was not investigated in detail.

Table. Conditions for attempted bromination and oxidation of compound (1)

Reagent	Temp. (°C)	Time	Product(s)
Br_2–HOAc–H_2SO_4	reflux	5 min	multiple
Br_2–HOAc	100	1 h	multiple
Br_2–H_2O	20	3 h	multiple
Br_2–aq. NaOAc	20	2 h	multiple
NBS–CCl_4; hν	reflux	10 h	no reaction
2M-$KMnO_4$–NaOH	20	—	multiple
30% H_2O_2–Cl_3CCO_2H	20	40 h	multiple

(c) *Autoxidation*. The failure of the above reactions to afford synthetically useful procedures suggests that the reactivity of 6-methyl groups in this blocked dihydropteridine series is unsuitable for elaboration into a series of enzyme inhibitors, perhaps because of the interference of unprotected acidic and basic groups in this heterocyclic system. The somewhat atypical character of this series of compounds was extended by the discovery of a surprisingly ready autoxidation of 6-alkyl substituents to the corresponding carbonyl derivatives. It is well known that unblocked dihydropteridines are readily autoxidised to the fully conjugated heterocyclic compounds,[5,6] but in contrast, the 7,7-dialkyl-7,8-dihydropteridines are stable with respect to this reaction. The pteridine (**1**) is also inert to alkaline permanganate and to hydrogen peroxide in the presence of trichloroacetic acid. However, in the presence of acetic acid, it was found that 2-amino-6,7,7-trialkyl-7,8-dihydropteridin-4(3*H*)-ones are oxidised in good yield to the corresponding 6-carbonyl derivatives simply by warming in air (Figure 1). It was already known that 6-hydroxymethyl and 6-alkylaminomethyl pteridines readily undergo autoxidation to the corresponding 6-formyl compound[7] and indeed the same reaction





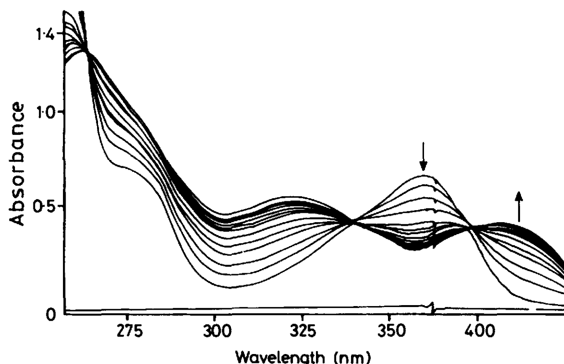


Figure 1. U.v.–visible spectrum of reaction mixtures during oxidation of compound (5)

occurred with the 6-hydroxymethyl-blocked dihydropteridine (2).[8] In this case, the reaction was particularly effective if a mixture of sulphur dioxide and oxygen was passed through a heated solution of the substrate: autoxidation of the 6-methylpteridine (1) and its more substituted analogues (5)—(8) was thus surprising in several respects and merited further investigation. Unlike reactions with unblocked dihydropteridines, this autoxidation reaction did not take place with concomitant hydroxylation of phenylalanine.[9] Free-radical scavengers such as quinol did not prevent reaction nor was light essential for it to occur. It therefore appeared that a free-radical mechanism was not operating. The conditions required for complete oxidation differed in time and temperature for the compounds studied. The cycloalkyl compounds (5) and (6) were readily oxidised at room temperature whereas 6-methyl-substituted compounds (1) and (8) required 24 h at 80 °C for complete conversion into the carbonyl compounds. The 6-ethylpteridine (7) showed behaviour intermediate between the two, and the 6-chlorophenoxymethylpteridine (9) oxidised readily at 80 °C to the aldehyde (3) and *p*-chlorophenol. The preference for oxidation at a secondary site with respect to a primary one is consistent with the occurrence of either radical or carbenium ion intermediates and, in view of the observations mentioned above, a carbenium ion intermediate seems preferable. Such an intermediate would require the abstraction of hydride from the substrate by a suitable acceptor. It is interesting that the cycloalkyl compounds (5) and (6) underwent more rapid oxidation even than the 6-ethyl analogue; this fact can be rationalised by considering that breakage of a C–H bond in the cycloalkyl compounds is stereoelectronically favoured with respect to the same reaction in the ethyl analogue in which the conformation of the α-C–H bonds is not restricted.

In order to shed some light on the nature of the possible hydride-abstracting species, we examined the kinetics of autoxidation of the cyclohexyl compound (5). At concentrations of 10^{-4}M or less, the reaction did not occur at 66.5 °C. On raising the concentration to 10^{-3}M, the reaction became second order in the pteridine and at concentrations greater than 10^{-3}M it was cleanly first order (Figure 2). These results suggest that the

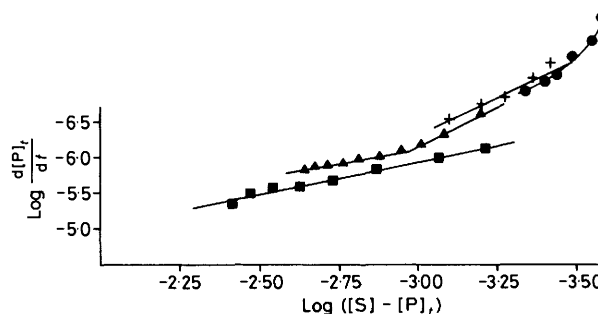
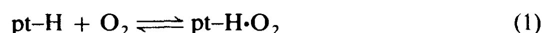
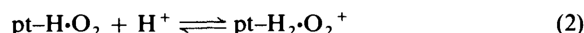


Figure 2. Kinetics of oxidation of compound (5). [(5)] = 4.36 × 10^{-3}M (■), 2.44 × 10^{-3}M (▲), 8.29 × 10^{-4}M (+), 4.80 × 10^{-4}M (●)

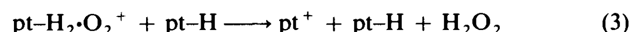
hydride-abstracting species is a derivative of the pteridine and can be accommodated by reactions (1)—(4) in which reaction 2 is rate limiting at low pteridine concentration, and reaction 1 rate limiting at high pteridine concentration (pt–H = pteridine).



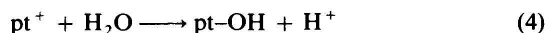
$$\text{pt-H} + O_2 \rightleftharpoons \text{pt-H}\cdot O_2 \qquad (1)$$



$$\text{pt-H}\cdot O_2 + H^+ \rightleftharpoons \text{pt-}H_2\cdot O_2^{\,+} \qquad (2)$$

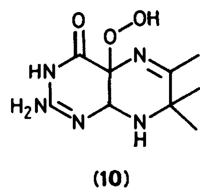


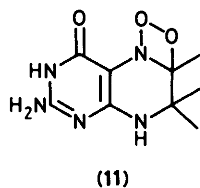
$$\text{pt-}H_2\cdot O_2^{\,+} + \text{pt-H} \longrightarrow \text{pt}^+ + \text{pt-H} + H_2O_2 \qquad (3)$$

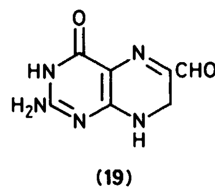


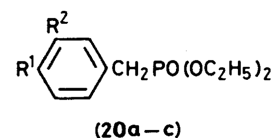
$$\text{pt}^+ + H_2O \longrightarrow \text{pt-OH} + H^+ \qquad (4)$$

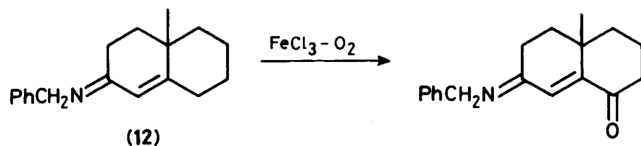
The structure of the oxygen adduct which is postulated to carry out the hydride abstraction (step 3) is not known but consideration of the reactivity of the dihydropteridine and recent studies on the mechanism of action of pteridine-dependent hydroxylases[10] suggests that the structure (10) is possible although other structures such as (11) cannot be ruled out. Analogies for such a clean oxidation are scarce but a similar reaction was observed for the amine (12) in the presence of iron(III) chloride.[11] Just as the bromination and condensation behaviour of this series of compounds seemed idiosyncratic, so the success of this oxidation reaction seems to depend critically upon the substitution pattern of the blocked dihydropteridine. Thus the diamino analogue (13), the thiadiazolopteridine[1] (14), the 2-oxo analogue (4), the pyrimido-oxazine (15), and the quinoxaline (16) all oxidised only partly under the standard conditions and mixtures of products were obtained. It is probable, however, that a detailed investigation of the reaction rate as a function of pH of reaction medium and pK_a of substrate would yield conditions applicable to the oxidation of these compounds. In addition to these limitations, we also found that if a labile substituent offered an alternative oxidation

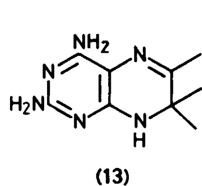


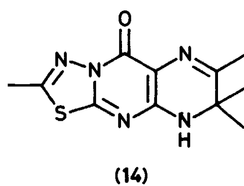


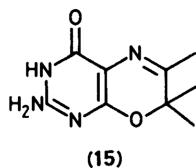


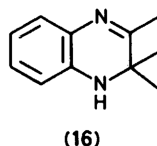


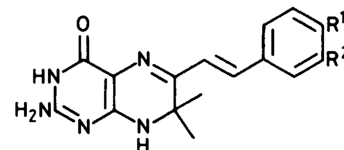




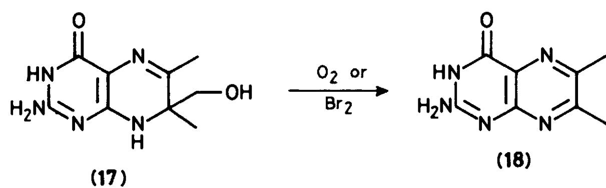




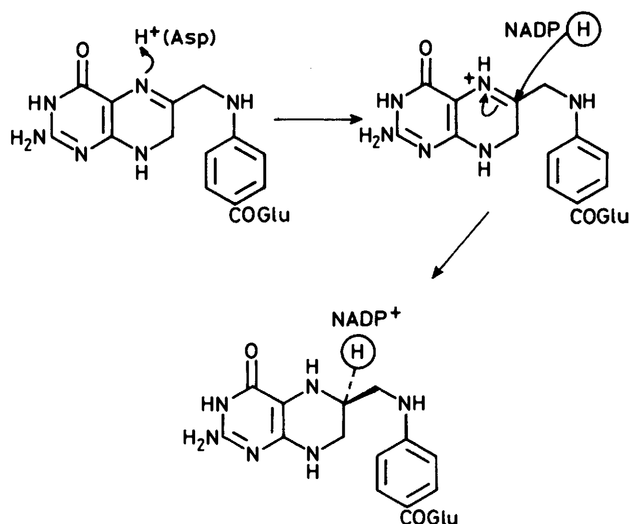




pathway leading to the fully conjugated pteridine, then autoxidation of the 6-substituent did not occur. Thus attempted autoxidation or bromination of the 7-hydroxymethyl-7-methylpteridine (**17**)[1] led to loss of the hydroxymethyl group and formation of the 6,7-dimethylpteridine (**18**).



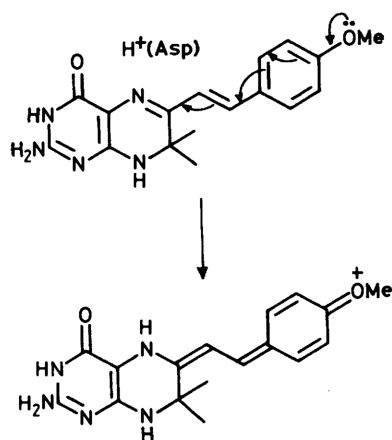
(d) *Reactivity of 6-formyl derivatives.* One of the most studied enzymes in chemotherapy is dihydrofolate reductase, and with a series of blocked dihydropteridines in hand it was of interest to see whether it would be possible to obtain derivatives that would inhibit this enzyme. We chose to investigate the synthesis of a potential suicide inhibitor of dihydrofolate reductase. The target compound was designed based upon the mechanism of action of the enzyme suggested by *X*-ray crystallography.[12] In this mechanism (Scheme 1) it is believed that Asp-26 of the *Lactobacillus casei* enzyme delivers a proton to N-5 of dihydrofolate, the most basic site, thereby activating it to reduction by hydride from NADPH. Our strategy was to use this protonation to encourage the formation of an electrophilic side chain in co-operation with an electron-donating aromatic ring (Scheme 2); this intermediate might then be captured by a nucleophile at the active site. In order to synthesize such pteridines, it was necessary to carry out condensation reactions at a C-6 formyl substituent. We attempted such reactions with



Scheme 1.

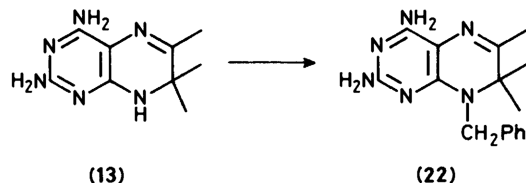
2-amino-6-formyl-7,8-dihydropteridin-4(3*H*)-one (**19**) but, owing to the insolubility of this compound, no success was found. However, the 6-formyl-blocked dihydropteridine (**3**), prepared as described above from the corresponding alcohol, reacted readily in dimethyl sulphoxide (DMSO) solution at 70 °C with the anions of benzylic phosphonates generated with sodium hydride. The products showed an additional long-wavelength absorption in their electronic spectra ($\lambda_{max.}$ 450, 328, and 263 nm in HCl; and 419 and 218 nm in aqueous NaOH; *cf.* 355 and 256 nm in HCl; and 330 and 280 nm in aqueous NaOH for a typical 6,7,7-trialkyldihydropteridine).

Modification at the Ring System.—(a) *Alkylation.* In view of our findings[1,2] that hydrophobic substituents in the region of C-7 greatly enhanced the inhibitory potency of blocked dihydropteridines with respect to the kinase enzyme, a direct method for the insertion of such substituents in this region avoiding lengthy synthesis was required. The amino-oxo-blocked dihydropteridines are unattractively profuse with potentially alkylatable functional groups. Alkylation of an anion would certainly lead to attack on the pyrimidine ring at N-3 and the oxo group. However, if the 2,4-diamino analogue

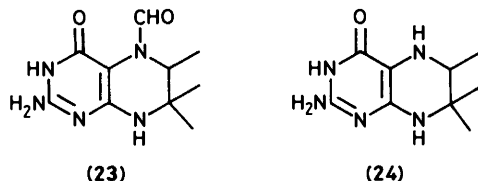


Scheme 2.

was used, alkylation of an anion formed at N-8 would be expected to occur; the 4-amino group could then be hydrolysed to reform the oxo substituent.[13] A further advantage of this strategy was that it was possible that the diaminopteridines prepared might also be inhibitors of bacterial dihydrofolate reductases. We found that the anion formed by treatment of the blocked diaminopteridine (**13**) could be alkylated by benzyl chloride in low yield at the expected site to give the pteridine (**22**). The reaction has not been further investigated pending results on the inhibitory activity of the products.



(b) *Hydrogenation.* The final reaction which we studied was catalytic hydrogenation and, in this sense, the blocked dihydropteridines showed themselves as close relatives of the normal dihydro compounds. Hydrogenation occurred over either platinum or palladium catalysts at atmospheric pressure in acidic solution. In 98% formic acid, the product formed from (**1**) after uptake of 1 mol equiv. of hydrogen was formylated after a time to give the 5-formamide (**23**). In dilute hydrochloric acid the blocked tetrahydropteridine (**24**) was obtained as the



hydrochloride. This compound, as expected, was oxidised in air to the starting dihydropteridine.

From the results described above, it is clear that the properties of this series of blocked dihydropteridines are subtly idiosyncratic and that a general synthesis of enzyme inhibitors based upon this class of heterocycle has yet to be devised. The properties of several of the 6-carbonyl derivatives as inhibitors of the kinase enzyme have been described in previous papers,[1,2] and the properties of the dihydrofolate reductase inhibitors synthesized as described above will be reported elsewhere.

Experimental

^{1}H N.m.r. spectra were recorded on Perkin-Elmer R10, R14, or R32 spectrometers. Chemical shifts are reported on the δ scale relative to $(CH_3)_4Si$ as internal standard. Spectra marked [a] were obtained at 90 MHz, [b] at 100 MHz, and [c] at 60 MHz. BAW refers to n-butanol–acetic acid–water (5:2:3).

Attempted Condensation of 2-*Amino*-7,8-*dihydro*-6,7,7-*trimethylpteridin*-4(3H)-*one* (**1**) *with Aldehydes.*—(a) 2-Amino-7,8-dihydro-6,7,7-trimethylpteridin-4(3*H*)-one (**1**) (0.3 g) was dissolved in hot 2M-sodium hydroxide (steam-bath) and 4-nitrobenzaldehyde (0.23 g) was added portionwise. After the addition of 4-nitrobenzaldehyde was complete, the solution was cooled, and the dark-red solid which formed was collected by centrifugation. Paper chromatography showed that it was not pure. Attempts to purify the solid failed because of its high solubility in water.

(b) 2-Amino-7,8-dihydro-6,7,7-trimethylpteridin-4(3*H*)-one (**1**) (0.5 g, 0.3 mol equiv.), dry pyridine (20 ml), and chloral (0.48 g, 0.4 mol equiv.) were heated on the steam-bath for 2 h. The pyridine was removed as completely as possible by evaporation under reduced pressure. A black tar-like mass remained which was thoroughly mixed with 2M-hydrochloric acid (50 ml). The solution was treated with charcoal, filtered, and the filtrate was made alkaline with dil. aqueous ammonium hydroxide and evaporated to dryness under reduced pressure. The residue was extracted with hot benzene and the benzene extract was evaporated to dryness. No solid product was obtained.

Oxidation of 2-*Amino*-6-(4-*chlorophenoxymethyl*)-7,8-*dihydro*-7,7-*dimethylpteridin*-4(3H)-*one* (**9**).—The title pteridine (**9**)* (200 mg) was stirred in BAW (10 ml) for 24 h at 80 °C. After the solvent had been removed under reduced pressure, a solution of the residue in 50% aqueous methanol was applied to a column of CG-50 ion-exchange resin (15 cm × 2.5 cm) (H^+ form). The column was eluted with water and that fraction of the eluate containing the product was evaporated to dryness to give the 6-formylpteridine (**3**) (76 mg, 57%), identical (i.r., u.v., paper chromatography) with an authentic sample.

Repetition of the above procedure in an atmosphere of nitrogen and using degassed BAW gave an 80% recovery of starting material.

The Kinetics of the Oxidation of 2-*Amino*-6,7,8,9,9a,10-*hexahydro*-9a-*methylbenzo*[g]*pteridin*-4(3H)-*one* (**5**).—*General method.* The pteridine (**5**)[2] (4—200 mg) was dissolved in stirred BAW (100 ml) at 66.5 °C under nitrogen. The reaction vessel was surrounded with aluminium foil in order to restrict the entry of light. When the pteridinone (**5**) had completely dissolved, air was admitted to the system. Samples were removed periodically and appropriately diluted with water in order to obtain the u.v. spectrum. The concentration of starting material (**5**) was determined at time $t = 0$, at 364 nm (ε 7 400). The concentration of the ketone was measured at various times, t, at 430 nm (ε 7 746) in order to avoid complications arising from the tail of the pteridinone (**5**) absorption. A plot of the product concentration at time t, $[P]_t$, against time was obtained. If [S] = initial concentration of starting pteridine (**5**) then a plot of log $(d[P]_t)/dt$ against log $([S] - [P]_t)$ gave straight lines from which values of the rate constant k and the reaction order n were obtained.

* Sample provided by Dr. A. Stuart and prepared by the methods described in Part 7 (ref. 2).

6-(1,1-*Dimethylacetonylamino*)-5-*nitrouracil Semicarbazone*.—6-Chloro-5-nitrouracil (2.0 g, 1 mol equiv.) was added to a solution of 3-amino-3-methylbutan-2-one semicarbazone hydrochloride (2.03 g, 1 mol equiv.) and triethylamine (2.2 g, 2 mol equiv.) in ethanol (25 ml). The resulting solution was refluxed for 16 h and then cooled. The pyrimidine semicarbazone was collected and purified by dissolution in aqueous ammonium hydroxide to yield the *ammonium salt of the pyrimidine* (2.55 g, 77%) as pale yellow crystals, m.p. 255—257 °C (Found: C, 36.2; H, 5.8; N, 34.2. $C_{10}H_{14}N_7O_5 \cdot NH_4$ requires C, 36.4; H, 5.5; N, 33.9%).

The above salt semicarbazone was dissolved in the minimum quantity of water and the solution was carefully brought to pH 6 with dil. hydrochloric acid, when the *title pyrimidine semicarbazone* separated as a white solid, m.p. 262—264 °C (Found: C, 38.5; H, 5.1; N, 31.0. $C_{10}H_{15}N_7O_5$ requires C, 38.3; H, 4.8; N, 31.3%).

6-(1,1-*Dimethylacetonylamino*)-5-*nitrouracil*.—The above pyrimidine semicarbazone (1.0 g) was hydrolysed in hot 2M-hydrochloric acid (steam-bath) for 1 h. The solution was cooled and neutralised to pH 7 with dil. aqueous ammonium hydroxide. The pyrimidine (0.71 g, 86%) was collected and crystallised from aqueous ethanol. Purification was also achieved by dissolution of the pyrimidine in dil. aqueous ammonium hydroxide and reprecipitation with dil. hydrochloric acid to give the *title pyrimidine* as white crystals, m.p. 280 °C (decomp.) (Found: C, 42.1; H, 4.8; N, 22.0. $C_9H_{12}N_4O_5$ requires C, 42.2; H, 4.7; N, 21.9%).

7,8-*Dihydro*-6,7,7-*trimethyl-lumazine* (**4**).—6-(1,1-Dimethylacetonylamino)-5-nitropyrimidine (1.0 g) was dissolved in the minimum amount of hot 0.1M-sodium hydroxide (steam-bath), and sodium dithionite was added portionwise until a colourless solution was obtained. The solution was cooled and brought to pH 7 with 2M-acetic acid. Refrigeration of the solution gave a white solid. The solid was collected and purified by dissolution in the minimum of 2M-ammonium hydroxide and reprecipitation of the product with glacial acetic acid. The *dihydrolumazine* (**4**) (0.66 g, 81%) was obtained as crystals, m.p. 300 °C (decomp.) (Found: C, 51.7; H, 5.7; N, 26.7. $C_9H_{12}N_4O_2$ requires C, 51.9; H, 5.8; N, 26.9%); δ_H(NaOD in D_2O)[c] 2.05 (3 H, s, CH_3C=N) and 1.32 (6 H, s, 2 × CH_3).

2,4-*Diamino*-6-(1,1-*dimethylacetonylamino*)-5-*nitropyrimidine Semicarbazone*.—2,4-Diamino-6-chloro-5-nitropyrimidine (1.5 g, 1 mol equiv.), 3-amino-3-methylbutan-2-one semicarbazone hydrochloride (1.54 g, 1 mol equiv.), and triethylamine (1.8 g, 2 mol equiv.) were refluxed in ethanol (25 ml) for 16 h and the solution was then cooled. The product was collected and recrystallised from ethanol to give the *title diaminopyrimidine semicarbazone* (2.2 g, 88%), m.p. 232—234 °C (Found: C, 38.3; H, 5.3; N, 40.2. $C_{10}H_{17}N_9O_3$ requires C, 38.6; H, 5.5; N, 40.5%).

2,4-*Diamino*-6-(1,1-*dimethylacetonylamino*)-5-*nitropyrimidine*.—The above pyrimidine semicarbazone (0.5 g) was hydrolysed in dil. hydrochloric acid (steam-bath) for 30 min. The solution was cooled and neutralised with dil. ammonium hydroxide to give the pyrimidine. Recrystallisation from aqueous ethanol gave the *title diaminonitropyrimidine* (0.38 g, 95%), m.p. 225—226 °C (Found: C, 42.8; H, 5.8; N, 32.9. $C_9H_{14}N_6O_3$ requires C, 42.5; H, 5.5; N, 33.1%).

2,4-*Diamino*-7,8-*dihydro*-6,7,7-*trimethylpteridine* (**13**).—2,4-Diamino-6-(1,1-dimethylacetonylamino)-5-nitropyrimidine (0.8 g) was dissolved in the minimum of hot water, and sodium dithionite was added portionwise until a colourless solution was obtained. The solution was then cooled and the dihydropteridine was collected by filtration. Recrystallisation from water gave the 2,4-*diaminodihydropteridine* (**13**) (0.06 g, 95%) as needles, m.p. 187 °C (decomp.) (Found: C, 52.8; H, 6.85; N, 40.5. $C_9H_{14}N_6$ requires C, 52.4; H, 6.8; N, 40.8%); $\delta_H(CF_3CO_2H)$[c] 2.71 (3 H, s, CH_3C=N) and 1.82 (6 H, s, 2 × CH_3).

2-Amino-6-hydroxypyrimidin-4(3*H*)-one and its 5-nitroso and 5-amino derivatives were prepared by published procedures.[14] 3-Bromo-3-methylbutan-2-one was prepared by the method of Jones.[15]

2-*Amino*-6,7,7-*trimethyl*-7H-*pyrimido*[4,5-b][1,4]*oxazin*-4(3H)-*one* (**15**).—A solution of the above diaminopyrimidine (2.06 g) in water (50 ml) was mixed with a solution of the above bromo ketone (3.3 g) in ethanol (50 ml). The resulting mixture was heated under reflux for 2 h whilst a solution of sodium hydrogen carbonate (1.68 g) in water (25 ml) was added dropwise. The mixture was then stored at 0 °C overnight to give the *pyrimido-oxazine* (**15**) (1.6 g, 76%) as pale yellow crystals, m.p. >260 °C (decomp.) (Found: C, 51.6; H, 6.0; N, 26.7. $C_9H_{12}N_4O_2$ requires C, 51.9; H, 5.8; N, 26.9%); $\lambda_{max.}$ (pH 1) 217, 267, and 337 (ε 6 007, 7 380, and 8 112); (pH 13) 225, 267, and 312 nm (ε 4 759, 8 680, and 8 680 dm^3 mol^{-1} cm^{-1}); $\delta_H(CF_3CO_2H)$[c] 2.77 (3 H, s, CH_3C=N) and 1.9 (6 H, s, 2 × CH_3).

Oxidation of 2-*Amino*-6,7,7-*trimethyl*-7H-*pyrimido*[4,5-b]-[1,4]-*oxazin*-4(3H)-*one* (**15**).—The pyrimido-oxazine (**15**) (200 mg) was dissolved in BAW (20 ml) at 80 °C and the solution was heated and stirred in this solvent for 24 h, allowing free access of air to the reaction mixture. The solvent was removed under reduced pressure and the residue was dissolved in water. This solution was applied to a column of CG-50 ion-exchange resin (20 cm × 2.5 cm) (H^+-form) which was eluted with water. The relevant portion of the eluate was evaporated under reduced pressure to give starting material (32 mg, 16%) as a pale yellow solid. No further homogenous material could be eluted from the ion-exchange column even when eluted with 1M-aqueous formic acid.

1,2-Dihydro-2,2,3-trimethylquinoxaline (**16**) was prepared as previously described.[16] Diethyl 4-methoxybenzylphosphonate (**20a**)[17] and diethyl 4-chlorobenzylphosphonate (**20b**)[18] were prepared by heating the appropriate benzyl chloride with triethyl phosphite at 160 °C.

Diethyl 3,4-*Methylenedioxybenzylphosphonate* (**20c**).—A solution of 3,4-methylenedioxybenzyl alcohol (10 g, 6.6 × 10^{-1} mol) in thionyl chloride (25 ml) was refluxed for 2 h. The excess of thionyl chloride was removed by evaporation, and triethyl phosphite (16.6 g, 1 × 10^{-1} mol) was added to the residue. The resulting solution was refluxed for 3 h, the solution was cooled, and excess of triethyl phosphite was removed by distillation. The residue was distilled to give the *benzylphosphonate* (**20c**) as a liquid (11.3 g, 63%), b.p. 170 °C/3 Torr (Found: C, 52.9; H, 6.54%; M^+, 272.0827. $C_{12}H_{17}O_5P$ requires C, 52.7; H, 6.60%; *M*, 272.0814); $\nu_{max.}$(neat) 2 980, 2 900, 1 600, 1 490, 1 240, and 1 030 cm^{-1}; $\delta_H(CDCl_3)$[a] 6.9 (3 H, m, ArH), 5.9 (2 H, s, OCH_2O), 4.0 (4 H, m, 2 × CH_2), 3.1 (2 H, d, ArCH_2), and 1.3 (6 H, t, 2 × CH_3).

2-*Amino*-6-(4-*chlorostyryl*)-7,8-*dihydro*-7,7-*dimethylpteridin*-4(3H)-*one* (**21b**).—Sodium hydride (50% dispersion in oil) (0.04 g, 8.4 × 10^{-4} mol) was added to a constantly stirred solution of diethyl 4-chlorobenzylphosphonate (0.217 g, 8.4 × 10^{-4} mol) in dry DMSO (1 ml). A solution of 2-amino-6-formyl-7,8-dihydro-7,7-dimethylpteridin-4(3*H*)-one (**3**) (0.1 g, 4.2 × 10^{-4} mol) in warm DMSO (t < 35 °C; 1 ml) was added

dropwise to the stirred mixture and the resulting yellow solution was heated at 50 °C for 2 h during which time a deep red colour formed.

The solution was cooled and adjusted to pH 8 by the dropwise addition of glacial acetic acid. The solution was then added dropwise to constantly stirred ice–water (20 ml) and the mixture was stirred for 30 min. The yellow precipitate was collected by filtration, washed successively with water (20 ml), light petroleum (b.p. 60—80 °C; 20 ml), and ether (20 ml), and and dried to give the crude pteridine (**21b**) as a bright yellow powder (0.1 g, 66.6%), m.p. 260 °C (decomp.). A sample was dissolved in the minimum of hot ethanol, the solution was filtered, and the filtrate was evaporated to half volume and cooled. The resultant precipitate was collected by filtration and dried to give the *pteridine* (**21b**) as a yellow powder (Found: C, 58.1; H, 4.8; Cl, 10.9; N, 21.6. $C_{16}H_{16}ClN_5O$ requires C, 58.3; H, 4.9; Cl, 10.8; N, 21.2%); $\lambda_{max.}$ (pH 1) 450, 328, and 263 (ε 14 433, 10 309, and 11 340); (pH 13) 419 and 281 nm (ε 13 917 and 15 463 $dm^3 mol^{-1} cm^{-1}$); $\delta_H[(CD_3)_2SO]^a$ 7.4—6.0 (6 H, m, ArH and CH=CH) and 1.3 (6 H, s, 2 × CH_3).

Prepared by a similar method were (*a*) *2-amino-7,8-dihydro-6-(4-methoxystyryl)-7,7-dimethylpteridin-4(3H)-one* (**21a**), m.p. 265 °C (decomp.) (Found: M^+, 325.1542. $C_{17}H_{19}N_5O_2$ requires *M*, 325.1539); $\lambda_{max.}$ (pH 1) 454, 350, and 263 (ε 10 266, 5 660, and 7 466); (pH 13) 412 and 285 nm (ε 8 333 and 7 000 $dm^3 mol^{-1} cm^{-1}$); $\delta_H[(CD_3)_2SO]^a$ 7.4—6.2 (6 H, m, ArH and CH=CH), 3.7 (3 H, s, OCH_3), and 1.4 (6 H, s, 2 × CH_3); and (*b*) *2-amino-7,8-dihydro-7,7-dimethyl-6-(3,4-methylenedioxystyryl)-pteridin-4(3H)-one* (**21c**), m.p. 265 °C (decomp.) (Found: M^+, 339.1326. $C_{17}H_{17}N_5O_3$ requires *M*, 339.1331); $\lambda_{max.}$ (pH 1) 455 and 265 (ε 6 666 and 5 697); (pH 13) 415 and 285 nm (ε 5 815 and 4 242 $dm^3 mol^{-1} cm^{-1}$).

2,4-Diamino-8-benzyl-7,8-dihydro-6,7,7-trimethylpteridine (**22**).—A 2.5M-solution of butyl-lithium in hexane (0.4 ml, 1 mmol) was added dropwise to a stirred solution of 2,4-diamino-7,8-dihydro-6,7,7-trimethylpteridine (**13**) (200 mg, 0.97 mmol; dried at 80 °C *in vacuo*) in dry DMSO (5 ml). The mixture was stirred at room temperature for 2 min, a solution of benzyl chloride (150 mg, 1.2 mmol) in DMSO (1.0 ml) was added slowly, and the mixture was kept at room temperature for a further 15 min. The solution was transferred to a 50 ml centrifuge tube and cooled to 2 °C. Water (40 ml) was added to the tube while the contents were stirred, and the mixture stored at 0 °C for 1 h. The heavy precipitate was collected by centrifugation, the supernatant liquid was decanted, the residue was dissolved in absolute ethanol (2 ml), and the solution was evaporated to dryness. The resulting semi-solid gum was dissolved in ethanol (1 ml) and applied to a column of silica gel (1.5 × 15 cm). The column was washed with chloroform and the product was eluted with 15% ethanol in chloroform. The fractions containing the product were pooled and evaporated to give a gum; the residue was stored overnight in ethanolic solution (2 ml) and the resulting crystals (37 mg, 13%) of the *pteridine* (**22**) were collected. The pale yellow crystals had m.p. 185 °C (Found: C, 64.3; H, 6.9; N, 28.6. $C_{16}H_{20}N_6$ requires C, 64.8; H, 6.8; N, 28.4%); $\lambda_{max.}$(EtOH) 328 and 294 nm (ε 9 190 and 6 000 $dm^3 mol^{-1} cm^{-1}$); $\delta_H[(CD_3)_2SO]^b$ 7.10 (5 H, s, ArH), 5.63 (2 H, s, NH_2) 5.58 (2 H, s, NH_2), 4.80 (2 H, s, CH_2Ph), 2.01 (3 H, s, CH_3), and 1.18 (6 H, s, 2 × CH_3).

2-Amino-5-formyl-5,6,7,8-tetrahydro-6,7,7-trimethylpteridin-4(3H)-one (**23**).—2-Amino-7,8-dihydro-6,7,7-trimethylpteridin-4(3*H*)-one (**1**) (0.2 g) was dissolved in 98—100% formic acid (20 ml), and the mixture was hydrogenated over platinum oxide (20 mg). After uptake of the theoretical amount of hydrogen, the flask was filled with nitrogen and acetic anhydride (5 ml) was added. After the mixture had been kept for 24 h at room temperature, the catalyst was filtered off and the filtrate was concentrated under reduced pressure to afford a syrup. This was diluted with water and the solution was adjusted to pH 7. Refrigeration of the solution gave the 5-*formyltetrahydropteridine* (**23**) (0.17 g, 74%) as crystals, m.p. >300 °C (Found: C, 50.4; H, 6.05; N, 29.2. $C_{10}H_{15}N_5O_2$ requires C, 50.6; H, 6.3; N, 29.5%); $\delta_H(CF_3CO_2H)^c$ 9.4—9.15 (1 H, br s, CHO), 5.0—4.8 (1 H, m, CH), 1.55—1.4 (3 H, d, CH_3), 1.34 (3 H, s, CH_3), and 1.22 (3 H, s, CH_3).

2-Amino-5,6,7,8-tetrahydro-6,7,7-trimethylpteridin-4(3H)-one Hydrochloride (**24**)·HCl.—2-Amino-7,8-dihydro-6,7,7-trimethylpteridin-4(3*H*)-one (**1**) (0.2 g) was dissolved in 5M-hydrochloric acid (20 ml) and the solution was hydrogenated over palladium–charcoal (40 mg). After the theoretical amount of hydrogen was taken up, the solution was filtered, and the filtrate was concentrated under reduced pressure at room temperature until crystallisation occurred. The crystals were filtered off very quickly because the tetrahydropteridine hydrochloride decomposed in solution. The tetrahydropteridine hydrochloride (**24**)·HCl (0.18 g, 78%) was collected as needles. The u.v. spectrum showed that oxidation of the tetrahydropteridine took place in solution to give the dihydropteridine. Instability of the solid hydrochloride prevented ultimate analysis.

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