Synthesis of a Naturally Occurring Dihydrohomopteridine Insect Metabolite, 6-Acetyl-2-amino-7,8-dihydro-9*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one[1]

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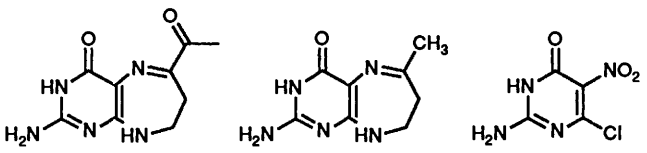
The naturally occurring dihydrohomopterin derivative 6-acetyl-2-amino-7,8-dihydro-9*H*-pyrimido-[4,5-*b*][1,4]diazepin-4(3*H*)-one (**1**) has been synthesised from 1-amino-4,4-diethoxypentan-3-ol (**10**) and 2-amino-6-chloro-5-nitropyrimidin-4(3*H*)-one (**3**). A novel tricyclic intermediate, 2,2a-dihydroxy-2-methyl-6-nitro-1,2,2a,3,4,5-hexahydro-1,5,8,8b-tetra-aza-acenaphthylen-7-one was isolated.

The diazepine ring system remains rare in nature, with the commonest known sources being bacteria and fungi.[2] To our knowledge, no diazepines have been found to occur naturally in mammals, and only two diazepines have been discovered in the animal kingdom, both in *Drosophila melanogaster*. The first of these is the eye pigment drosopterin, which has a complex pentacyclic structure.[3] The second is the pyrimidodiazepine (**1**)



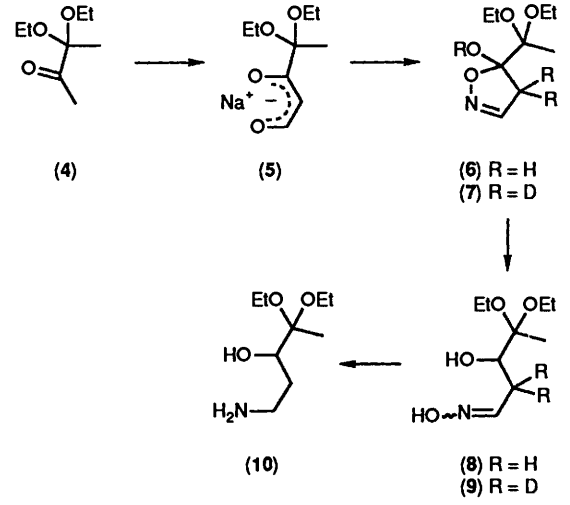
(1) (2) (3)

which was first isolated in 1977 from *Drosophila* by Wilson and Jacobson,[4] was further investigated by Wiederrecht, Paton and Brown,[5] and was finally characterised completely by Jacobson, Pfleiderer and co-workers[6] in 1982. Interest in this pyrimidodiazepine (**1**) first arose during a study on the biosynthesis of drosopterin,[7] and it has excited much attention because of the evidence[8,9] that it arises biosynthetically from 6-pyruvoyltetrahydropterin. This is a key intermediate in the biosynthesis of tetrahydrobiopterin,[9] and the latter is the focus of an intense research effort at the moment because of its connection with nerve transmitter diseases such as Parkinson's disease.[10] These relationships raise the possibility that compound (**1**) might affect some of the pteridine pathways in the metabolism of animals, and some preliminary results have already been obtained suggesting that this may indeed be the case.[11] An unambiguous chemical synthesis of the compound has become desirable, therefore, and such a synthesis forms the subject of the present paper.

The first synthesis of a pyrimido[4,5-*b*][1,4]diazepine was achieved in 1965 by Nyberg,[12] and further examples have since been reported by other workers. Senda *et al.* developed a photochemical synthesis using 6-azido-1,3-dimethyluracil,[13] but most other syntheses have been based on the condensation between a 4,5-diaminopyrimidine and a 1,3-dicarbonyl compound.[14] This method suffers from poorly defined regiospecificity, however, as in the Isay pteridine synthesis.[15] Furthermore, practically all the pyrimidodiazepines synthesised by these methods carry only oxygen substituents on the pyrimidine ring, or else have no substituents at all. The only synthetic pyrimidodiazepine containing the pterin 2-amino-4-oxo- substitution pattern, as in (**1**) was reported recently by Ayling, Bailey and co-workers,[16] who prepared 2-amino-6-methyl-7,8-dihydro-9*H*-pyrimido[4,5-*b*][1,4]diazepin-4-(3*H*)-one (**2**) using a Boon and Leigh type method. We now describe the first and unambiguous synthesis of the naturally occurring compound. (**1**).

Results and Discussion

In this synthesis, the pyrimidine moiety of (**1**) was derived from the known 2-amino-6-chloro-5-nitropyrimidin-4(3*H*)-one (**3**), while four of the diazepine ring atoms of (**1**) together with the side chain acetyl group were derived from the β-amino alcohol (**10**). This latter incorporates a protected acetyl group, and was prepared *via* an isoxazoline intermediate as outlined in Scheme 1. The mono diethyl acetal (**4**) of biacetyl was formylated with



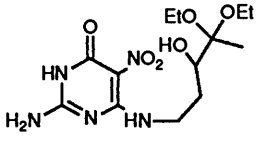
(4) (5) (6) R = H (7) R = D (10) (8) R = H (9) R = D

Scheme 1.

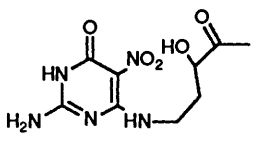
ethyl formate and sodium hydride to give the sodium enolate salt (**5**) of 4,4-diethoxy-3-oxopentanal. This salt was a somewhat unstable cream coloured solid, which could not be obtained analytically pure, but it was characterised by its ^{1}H NMR spectrum. When (**5**) was stirred at room temperature in a buffered solution at pH 7 with hydroxylamine hydrochloride, the aldehyde carbon atom reacted preferentially, and the resulting mono-oxime cyclised spontaneously to give the isoxazoline (**6**) in 83% yield. When (**6**) was reduced with sodium borohydride, an isomeric pair of (*E*)- and (*Z*)-oximes (**8**) was obtained. The mixture could be separated by flash chromatography to afford one isomer as a crystalline solid and the other as an oil, but while the former was stable, the oil reverted once more to a mixture of the two isomers with time at room temperature. Preparation of the deuteriated analogues (**7**) and (**9**) confirmed the NMR assignments. Reduction of the mixture

of oximes (**8**) with lithium aluminium hydride afforded the desired 1-amino-4,4-diethoxypentan-3-ol (**10**) in 80% yield. The same product was obtained on direct reduction of the isoxazoline (**6**) with lithium aluminium hydride, but overall yields were much lower. 5-Hydroxyisoxazolines are well known in the literature, and may exist in equilibrium with the corresponding open chain β-keto-oximes.[17] Surprisingly, however,[18] it was not found possible to dehydrate (**6**) to the corresponding aromatic isoxazole.

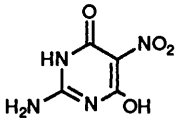
Condensation of the amino alcohol (**10**) with chloronitropyrimidinone (**3**) in ethanol solution in the presence of solid sodium hydrogen carbonate afforded the α-hydroxy acetal (**11**) in 80% yield. The acetal group in this and related compounds was found to be very labile, and when (**11**) was refluxed in water for 30 min the corresponding α-hydroxy ketone (**12**) was obtained in 90% yield. If the condensation between (**10**) and (**3**) was carried out in ethanol in the absence of base, the α-hydroxy ketone (**12**) was obtained directly. When potassium acetate was used as the base for the condensation the yield of (**11**) was much reduced (30%), and the hydrolysed pyrimidinone derivative (**13**) was obtained as a major by-product (43%).



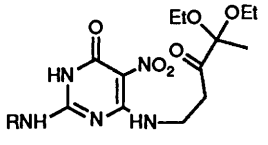
(**11**)



(**12**)



(**13**)

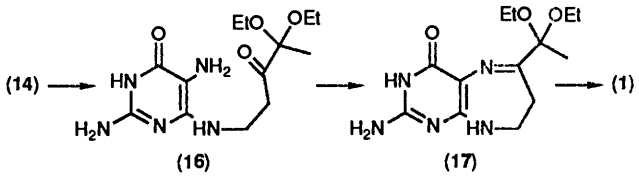


(**14**) R = H
(**15**) R = Ac

The secondary alcohol group in the α-hydroxy acetal (**11**) was successfully oxidised to the α-keto acetal (**14**) in 75% yield with the chromium trioxide–pyridine complex of Sarett.[19] Only very low yields were obtained using a pyridine chlorochromate reagent.[20] Oxidation was also achieved by stirring the alcohol (**11**) in dimethyl sulphoxide and acetic anhydride[21,22] for 43 h at room temperature, but concomitant acetylation of the amino group occurred under these conditions and the product obtained was the 2-acetamido α-keto acetal (**15**). The oxidation could be speeded up using higher temperatures, but yields were lower. The desired α-keto acetal (**14**) could be obtained in 96% yield from (**15**) by dilute alkaline hydrolysis of the 2-acetamido group in the latter. Attempted oxidation of (**11**) with dimethyl sulphoxide and phosphorus pentoxide gave only the hydroxy ketone (**12**), also in 96% yield, while the dimethyl sulphoxide and trifluoroacetic anhydride system[21] gave only very poor yields of (**14**) from (**11**). A constraint in the desired oxidation of (**11**) was that the acetal grouping preferably had to remain intact, since its loss would lead to an α-diketone, and thus introduce an ambiguity during the subsequent cyclisation to the diazepine ring.

The structures assigned to all these new compounds follow from their method of preparation, elemental analyses, and spectroscopic properties. ^{1}H NMR spectra showed that, as expected,[23] the methylene protons of the ethoxy groups in each of the acetal compounds are magnetically non-equivalent. As well, however, the two acetal methyl groups in the α-hydroxy acetals (**6**), (**8**), (**10**), and (**11**) are also non-equivalent, appearing as a pair of overlapping three-proton triplets, although the corresponding acetal methyl groups in the α-keto acetals (**14**) and (**15**) appeared as a single six-proton triplet. In compounds (**11**), (**12**), (**14**), and (**15**), the side-chain secondary NH proton appeared consistently as a triplet. This established the location of the *N*-acetyl group at position 2 in compound (**15**).

Conversion of the α-keto acetal (**14**) into the naturally occurring pyrimidodiazepine (**1**) necessitated reduction of the nitro group, cyclisation to the diazepine, and cleavage of the acetal moiety (Scheme 2). Reduction of (**14**) was achieved

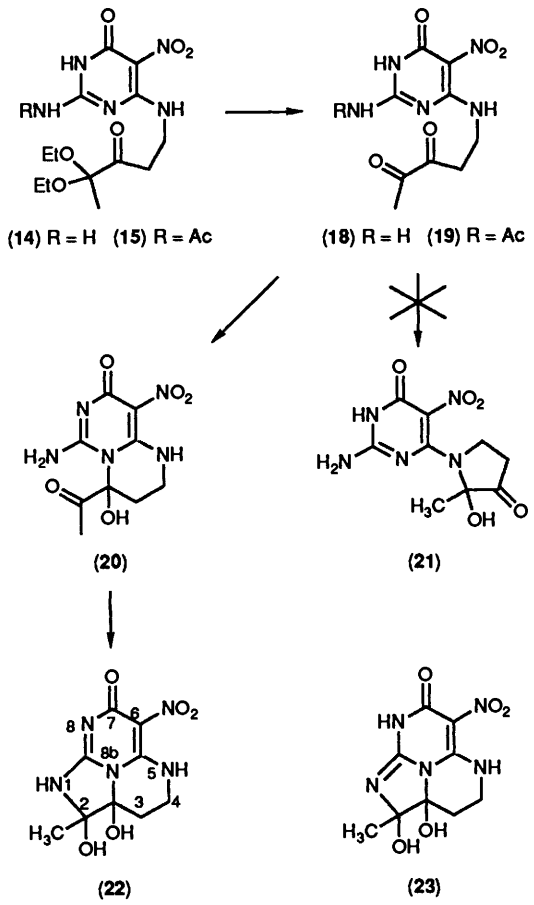


(**16**) (**17**)

Scheme 2.

successfully using sodium dithionite in aqueous *N*,*N*-dimethylformamide containing sodium hydroxide[16,24] under an atmosphere of nitrogen. Very little reduction occurred either with sodium dithionite in water or in ethanol, or by hydrogenation over a palladium catalyst in a variety of solvents including water, ethanol, and *N*,*N*-dimethylformamide. These results are consistent with observations made earlier by Nair and coworkers,[25] and have been explained by a possible interaction between the 5-nitro group and the carbonyl group on the side chain. The initial 5-aminopyrimidine reduction product (**16**) was unstable in air and was not isolated, although its formation could be monitored by TLC. It was converted into (**1**) by treatment with a solution of barium chloride, the pH being maintained at 3 for 20 min at room temperature, and then neutralisation to pH 7. In one experiment when a solution of (**16**) was kept at pH 3 for only a few minutes, and then neutralised to pH 7, a small amount of material was obtained which from its NMR spectrum appeared to be the cyclised acetal compound (**17**). This was converted into (**1**) on further treatment with acid. The sequence of reactions thus appears to be as shown in Scheme 2. While 50% yields of (**1**) from (**14**) could be achieved, very careful control of the reaction conditions was necessary, since the acid treatment required to hydrolyse the acetal also tended to decompose the product (**1**). This sensitivity to acid has also been noted for the naturally occurring material.[6,26] For example, Jacobson found that a solution of the latter in aqueous 3% ammonium chloride decomposed completely within a few hours, and that decomposition was twice as fast at pH 4.9 as at 7.5. The 6-methyl analogue (**2**) is also unstable, possibly due to ring strain leading to hydrolytic ring opening of the imine bond, with subsequent autoxidation of the resulting 5-aminopyrimidine.[16] The three steps shown in Scheme 2 could also be carried out in a one-pot reaction using iron in dilute methanolic hydrochloric acid as the reducing agent, with the acidic medium serving also to catalyse cyclisation and to hydrolyse the acetal.

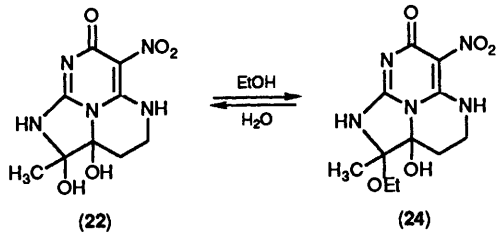
The product (**1**) prepared as described above was purified by chromatography on cellulose columns, and the pure material analysed correctly for the expected molecular formula. Its IR, UV, NMR, and mass spectra corresponded with those reported in the literature[5,6,26] for the naturally occurring compound, and final confirmation of identity was obtained by direct TLC and HPLC comparison between the synthetic material and a sample isolated from *Drosophila*, kindly provided by Dr. Jacobson. As noticed previously,[6,26] the behaviour of (**1**) on TLC is very characteristic. When performed on a cellulose plate using 3% aqueous ammonium chloride it appears under UV light as a dark absorbing spot. If the freshly developed plate is immersed in liquid nitrogen, however, and is then viewed immediately



Scheme 3.

under UV light (366 nm) the spot appears with a green yellow fluorescence.

Hydrolysis of the α-keto acetal (**14**) in dilute methanolic hydrochloric acid led to diketone (**18**) as a pale yellow solid which decomposed within minutes on exposure to air. Hydrolysis of (**14**) at 60 °C in aqeuous dioxane at pH 1–2, however, gave a new compound, the chemical and spectroscopic properties of which are consistent with the novel tricyclic structure (**22**). This could be formed from the diketone (**18**) by cyclisation *via* the intermediate (**20**) as shown in Scheme 3. The IR spectrum of the new compound showed no carbonyl band above 1 690 cm^{-1}, no lowfield carbonyl carbon signal in the ^{13}C NMR spectrum, and four one-proton deuterium exchangeable signals in the ^{1}H NMR spectrum. While consistent with structure (**22**), these data are inconsistent with isomeric structures (**20**) and (**21**). As well, the proton NMR spectrum revealed a methyl singlet resonance at δ 1.53 and a methylene multiplet resonance at δ 2.05, and these signals are too highfield respectively for a methyl ketone as in (**20**) or for either of the methylene groups in the five-membered ring of (**21**). Elemental analysis, together with an accurate fast atom bombardment mass spectrum lent further support to structure (**22**) for the new product. This structure was favoured over isomeric structure (**23**) because of its greater degree of conjugation. The spectral data do not admit of a clear distinction between the two, however, nor do they allow stereochemical assignment of the two hydroxy groups. Chemical evidence supporting the idea that the 2-amino group of (**14**) was involved in the formation of the new compound (**22**) came from the observation that compound (**15**), which is the acetylated derivative of (**14**), failed to give any cyclised product when subjected to the same conditions of acid hydrolysis in dioxane as had been used for (**14**). The only product obtained was the unstable diketone (**19**). When (**22**) was heated with ethanol another new compound (**24**) was obtained, which on boiling with water was reconverted quantitatively into (**22**) again. The new compound had spectral properties very similar to those of its precursor (**22**), but it did contain a new ethyl group, and only three deuterium-exchangeable protons. These results, together with elemental analysis and an accurate mass spectrum, suggest that the new product was formed by replacement of a hydroxy group in (**22**) by an ethoxy group. Structure (**24**) is consistent with these observations, as would be the isomeric structure with the positions of the ethoxy and hydroxy groups interchanged.



Compound (**22**) could be converted into the target compound (**1**) by reduction with either iron or sodium dithionite. The iron reducing system gave the higher yield (57%) and an easier work-up procedure, and is now the method of choice for the preparation of (**1**). The reaction shows, moreover, that no profound or irreversible structural change occurs in the formation of (**22**), again consistent with the proposed structure. Use of dithionite for the reduction of (**22**) gave a lower (44%) yield of (**1**) and led to the formation of large amounts of inorganic salts which were difficult to separate. The pyrimidodiazepine (**1**) prepared as described here is proving to have interesting biological properties which are currently being investigated. For example, preliminary results obtained by Jacobson and Ziegler[11] suggest that (**1**) may replace tetrahydrobiopterin in the modulation of interleukin-2-directed DNA synthesis of T-cells, and that the level of tetrahydrobiopterin in these cells may increase when they are cultured in the presence of (**1**).

Experimental

IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer, and UV spectra on a Pye Unicam PU8800 spectrometer. NMR spectra were run on JEOL JNM-GX 270, Bruker WP-80, or JEOL PMX-60 instruments. Electron ionisation mass spectra were recorded using a VG analytical 70-E mass spectrometer. Melting points were determined in open capillaries and are uncorrected. TLC was carried out on DC-Alufolien Kieselgel 60 F254 0.2 mm plates and on DC-Alufolien cellulose F 0.1 mm plates. Standard methods of microanalysis failed to give satisfactory results with some samples, due to incomplete combustion. Addition of tungstic oxide to these samples, however, overcame this problem.

4,4-*Diethoxy*-3-*oxopentanal Sodium Salt* (**5**).—Sodium hydride (63%; 1.35 g, 30 mmol) was washed with hexane under nitrogen to remove the mineral oil. Ethyl formate (8 g, 0.11 mol), which had been dried over potassium carbonate and phosphorus pentoxide, and sodium-dried ether (50 ml), were then added. The flask was immersed in cold water and 3,3-diethoxybutan-2-one[27] (4.6 g, 29 mmol) was added dropwise to the mixture. More ether was added when necessary to keep the mixture mobile. The mixture was stirred overnight after which the title compound (**5**) was collected by filtration at the pump as

a cream-coloured solid, and washed with dry ether (4.82 g, 79%), m.p. > 300 °C (decomp.); ν_{max}(Nujol) 1 640, 1 520, 1 140, and 1 070 cm^{-1}; δ_H (80 MHz; D_2O) 1.19 (6 H, t, *J* 7.3 Hz, 2 × OCH_2CH_3), 1.42 (3 H, s, CH_3), 3.51 (2 H, q, *J* 6.9 Hz, OCH_2CH_3), 3.53 (2 H, q, *J* 6.9 Hz, OCH_2CH_3), 5.56 (2 H, d, *J* 10.3 Hz, CH_2CHO, exch. D_2O), and 9.10 (1 H, t, *J* 5 Hz, CH_2CHO).

5-(1,1-*Diethoxyethyl*)-5-*hydroxy*-4,5-*dihydroisoxazole* (**6**).—Hydroxylamine hydrochloride (2.65 g, 38 mmol) and the sodium enolate (**5**) (4.0 g, 19 mmol) were dissolved in a 0.4 M solution of dipotassium hydrogen phosphate (200 ml) and the mixture stirred at room temperature for 3 h. The mixture was extracted into ethyl acetate and the extract was dried ($MgSO_4$) and evaporated. Recrystallisation of the residue from hexane gave white needles of the *title compound* (**6**) (3.2 g, 83%), m.p. 78 °C (Found: C, 53.25; H, 8.35; N, 6.95. $C_9H_{17}NO_4$ requires C, 53.20; H, 8.43; N, 6.89%); ν_{max}(Nujol) 3 470, 1 600, 1 200, and 1 060 cm^{-1}; δ_H (80 MHz; $CDCl_3$) 1.18 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.22 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.37 (3 H, s, CH_3), 3.03 (2 H, dq, *J* 19 and 1.7 Hz, CH_2CH=N), 3.62 (4 H, m, 2 × OCH_2CH_3), 4.19 (1 H, br s, OH), and 7.19 (1 H, t, *J* 1.7 Hz, CH=N).

4,4-*Diethoxy*-3-*hydroxypentanal Oxime* (**8**).—The dihydroisoxazole (**6**) (1 g, 5 mmol) was dissolved in aqueous ethanol (70% v/v, 50 ml). A solution of sodium borohydride (0.58 g, 15 mmol) in ethanol was added dropwise, and the mixture was stirred overnight at room temperature. The ethanol was removed under reduced pressure and the aqueous layer was extracted with methylene dichloride. The extract was dried (Na_2CO_3) and evaporated to afford a mixture of the two isomeric *oximes* of 4,4-diethoxy-3-hydroxypentanal (**8**) (0.75 g, 73%). Chromatography of the mixture on a column of silica gel, eluting with ethyl acetate–hexane (1:1), gave one isomer as a white crystalline solid, m.p. 89 °C (Found: C, 52.4; H, 9.3; N, 6.85. $C_9H_{19}NO_4$ requires C, 52.66; H, 9.33; N, 6.82%); ν_{max}(Nujol) 3 460 and 1 650 cm^{-1}; δ_H (80 MHz; $CDCl_3$) 1.17 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.19 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.28 (3 H, s, CH_3), 2.46 (2 H, m, CH_2CH=NOH), 3.53 (4 H, m, 2 × OCH_2CH_3), 3.84 (1 H, m, CHOH), 6.93 (1 H, t, *J* 5 Hz, CH=N), and 8.17 (1 H, br s, NOH). The other isomer was obtained as an oil which was unstable and rearranged with time to a mixture of the two isomers, with an NMR spectrum identical with that of the initially isolated mixture before chromatography. This isomer had ν_{max} 3 380 and 1 650 cm^{-1}; δ_H (80 MHz; $CDCl_3$) 1.16 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.19 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.28 (3 H, s, CH_3), 2.37 (2 H, m, CH_2CH=N), 2.73 (1 H, br s, OH), 3.54 (4 H, m, 2 × OCH_2CH_3), 3.9 (1 H, m, CHOH), 7.54 (1 H, t, *J* 5 Hz, CH=N), and 8.32 (1 H, br s, NOH).

1-*Amino*-4,4-*diethoxypentan*-3-*ol* (**10**).—A mixture of the isomeric oximes (**8**) (4.0 g, 19.5 mmol) was dissolved in dry ether (70 ml) and the solution added dropwise to lithium aluminium hydride (2.0 g, 52.6 mmol), also in dry ether (250 ml). The mixture was refluxed for 2.5 h and was then stirred overnight at room temperature, after which excess lithium aluminium hydride was destroyed by addition of ethyl acetate, followed by water. After filtration, the ether layer was dried and evaporated to afford the *title compound* (**10**), as a white solid (3.1 g, 83%), m.p. 88 °C (Found: C, 56.25; H, 10.65; N, 7.6. $C_9H_{21}NO_3$ requires C,56.52; H, 11.07; N, 7.32%); ν_{max}(Nujol) 3 330, 3 100, 1 600, and 1 140 cm^{-1}; δ_H (80 MHz; $CDCl_3$) 1.15 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.19 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.26 (3 H, s, CH_3), 1.64 (2 H, m, CH_2CHOH), 2.16 (1 H, s, OH), 2.96 (2 H, m, CH_2NH_2), 3.50 (4 H, m, 2 × OCH_2CH_3), and 3.84 (1 H, m, CHOH); *m/z* 176 (2%, $M^+ - CH_3$), 146 (14, $M^+ - OCH_2CH_3$), 117 (100), 100 (39), 89 (95), 61 (100), and 43 (58).

2-*Amino*-6-(4,4-*diethoxy*-3-*hydroxypentylamino*)-5-*nitropyrimidin*-4(3H)-*one* (**11**).—(*i*) A stirred suspension of 2-amino-6-chloro-5-nitropyrimidin-4(3*H*)-one (**3**)[24] (0.84 g, 4.4 mmol) and sodium hydrogen carbonate (1.0 g) in ethanol (250 ml) was brought to reflux. 1-Amino-4,4-diethoxypentan-3-ol (**10**) (0.837 g, 4.4 mmol) and sodium hydrogen carbonate (1.2 g) in ethanol (30 ml) were added to the pyrimidinone suspension. The vigorously stirred reaction mixture was heated under reflux for 1.45 h, the progress of the reaction being monitored by silica gel TLC (9:1 methanol–dichloromethane). After 1.5 h all the starting material had been consumed. The hot reaction mixture was filtered to give sodium hydrogen carbonate and sodium chloride (1.8 g). The filtrate was taken to dryness under reduced pressure at room temperature to give a brown–yellow residue, which was triturated with acetonitrile to give a pale yellow solid product (**11**) (1.22 g, 80%), m.p. 188 °C (from acetonitrile) (Found C, 45.35; H, 6.7; N, 20.25. $C_{13}H_{23}N_5O_6$ requires C, 45.21; H, 6.71; N, 20.27%); ν_{max}(Nujol) 3 560, 3 460, 3 280, 3 180, 1 685 (lactam C=O), 1 645, 1 585, 1 500, 1 140, 865, and 790 cm^{-1}; λ_{max}(0.05M NaOH) 345 nm (ε 14 668) and 214 nm (ε 14 465); δ_H (80 MHz; CD_3SOCD_3) 1.06 (3 H, t, *J* 7.1 Hz, OCH_2CH_3), 1.09 (3 H, t, *J* 7.1 Hz, OCH_2CH_3), 1.14 (3 H, s, CH_3), 1.74 (2 H, m, CH_2CHOH), 3.45 (7 H, m, 2 × OCH_2CH_3, CH_2NH, and CHOH), 4.61 (1 H, d, *J* 4.9 Hz, CHO*H*, exchangeable with D_2O), 7.15 (2 H, br s, NH_2, exchangeable with D_2O), 9.68 (1 H, t, *J* 5.3 Hz, CH_2NH, exchangeable with D_2O), and 10.61 (1 H br s, HNCO, exchangeable with D_2O).

(*ii*) A solution of potassium acetate (2.37 g, 24 mmol) and 1-amino-4,4-diethoxypentan-3-ol (**10**) (4.6 g, 24 mmol) in ethanol (10 ml) was added to a refluxing suspension of the pyrimidinone (**3**)[24] (4.6 g, 24 mmol) in ethanol (400 ml). The progress of the reaction was monitored by silica gel TLC (9:1, methanol–dichloromethane). After the mixture had been stirred for 1 h at reflux temperature all the starting materials had been consumed, and it was then filtered whilst hot to give a pale yellow solid (2.5 g). The solid was washed successively with water, ethanol, and ether, and then recrystallised from methanol to give 2-amino-6-hydroxy-5-nitropyrimidin-4(3*H*)-one (**13**) (1.78 g, 43%), m.p. 251 °C (decomp.) (lit.[28] 251 °C decomp.), δ_H (80 MHz; CD_3SOCD_3) 7.53 (2 H, br s, NH_2) and 4.09 (2 H, br s, 2 × OH). The filtrate was evaporated to dryness under reduced pressure to give a brown oil (8.15 g). This was dissolved in ethyl acetate (250 ml) and the solution washed with aqueous sodium hydrogen carbonate (1M; 100 ml) followed by water (200 ml). With time, a pale yellow solid precipitated out of the solution, and this was collected, washed with water (20 ml) and ethanol (20 ml), and dried, to give the title compound (**11**) (2.5 g, 30%); identical with that prepared as described above.

2-*Amino*-6-(3-*hydroxy*-4-*oxopentylamino*)-5-*nitropyrimidin*-4(3H)-*one* (**12**).—(*i*) To a boiling suspension of the pyrimidinone (**3**) (2.3 g, 12 mmol) in ethanol (200 ml) was added the pentanol (**10**) (2.3 g, 12 mmol) in ethanol (15 ml). The mixture was heated under reflux for 1 h. On cooling, a pale yellow solid (1.6 g) separated, and this was collected and washed with ether (20 ml). The mother liquor was evaporated to half its volume and set aside when a second crop (0.9 g) was obtained; this was combined with the first crop to give a total yield of (2.5 g). Recrystallisation from water gave a white semi-solid which on trituration with ethanol, filtration, and drying, afforded the *title compound* (**12**) (2.4 g, 73%) as a white powder, m.p. 242 °C (Found: C, 40.05; H, 5.05; N, 25.9. $C_9H_{13}N_5O_5$ requires C, 39.85; H, 4.83; N, 25.82%); ν_{max}(Nujol) 3 360, 3 250, 3 110, 1 705, 1 680, and 795 cm^{-1}; λ_{max}(0.05M NaOH) 345 nm (ε 14 870) and 215 nm (14 465); δ_H (80 MHz; CD_3SOCD_3) 1.86 (2 H, m, CH_2CHOH), 2.13 (3 H, s, $COCH_3$), 3.57 (2 H, q, *J* 6.4 Hz, $NHCH_2$), 3.97 (1 H, m, CHOH), 5.49 (1 H, d, *J* 5.4 Hz, CHO*H*, exchangeable with D_2O), 7.15 (2 H, br s, NH_2, exchangeable

with D_2O), 9.64 (1 H, t, *J* 5.7 Hz, N*H*CH_2, exchangeable with D_2O), and 10.55 (1 H, br s, NHCO, exchangeable with D_2O); δ_C (270 MHz; CD_3SOCD_3) 211.28 (s, C=O), 159.33 (s), 156.29 (s), 154.27 (s), 110.74 (s), 74.99 (d, CHOH). 37.98 (t, CH_2NH), 32.28 (t, *C*H_2CHOH), and 25.58 (q, *C*H_3CO).

(*iii*) A suspension of the pyrimidinone (**11**) (100 mg, 0.28 mmol) in water (15 ml) was stirred at reflux temperature until all the material had dissolved (30 min); on cooling, a white solid separated from solution. This was filtered off, washed with ethanol (10 ml) and ether (5 ml), and dried to give (**12**) as a white solid (70 mg, 90%); identical with the same product prepared as described above in method (i).

2-*Amino*-6-(4,4-*diethoxy*-3-*oxopentylamino*)-5-*nitropyrimidin*-4(3H)-*one* (**14**).—(i) Chromium trioxide (130 mg) was added portion wise with stirring to dry pyridine (4 ml) at 0 °C. The resulting solution was allowed to warm to room temperature and was then mixed with a solution of the pyrimidinone (**11**) in dry pyridine (4 ml). The reaction flask was stoppered, and the contents were mixed thoroughly and stirred at room temperature for 48 h. The mixture was evaporated to dryness, and the resulting dark brown residue dissolved in water (60 ml) and extracted several times with warm ethyl acetate (400 ml total). The ethyl acetate extracts were combined, dried ($MgSO_4$), and evaporated to give a white solid (85 mg). Column chromatography of this on silica gel, eluting with chloroform, gave the *title compound* (**14**) as a white solid (75 mg, 75%), m.p. 210 °C (Found: C, 45.2; H, 6.25; N, 20.6. $C_{13}H_{21}N_5O_6$ requires C, 45.47; H, 6.16; N, 20.39%); ν_{max}(Nujol) 3 400, 3 260, 1 730 (CO), 1 685 (CONH), and 785 cm^{-1}; λ_{max}(MeOH) 329 (ε 14 825), 236 (17 450), and 213 nm (24 995); δ_H (80 MHz; $CDCl_3$) 1.25 (6 H, t, *J* 7.3 Hz, 2 × OCH_2CH_3), 1.40 [3 H, s, $C(OEt)_2CH_3$)], 3.00 (2 H, t, *J* 6.1 Hz, CH_2CO), 3.48 (2 H, q, *J* 7.3 Hz, OCH_2CH_3), 3.50 (2 H, q, *J* 7.3 Hz, OCH_2CH_3), 3.80 (2 H, m, $NHCH_2$), 8.5 (2 H, br s, NH_2), 9.73 (1 H, t, N*H*CH_2), and 10.71 (1 H, br s, NHCO).

2-*Acetamido*-6-(4,4-*diethoxy*-3-*oxopentylamino*)-5-*nitropyrimidin*-4(3H)-*one* (**15**).—To a solution of the pyrimidinone (**11**) (100 mg, 0.289 mmol); in dry dimethyl sulphoxide (2 ml), was added freshly distilled acetic anhydride (320 mg, 3.1 mmol). The reaction mixture was stirred at room temperature for 43 h and then diluted with ethanol (1 ml) and stirred for a further 5 min. The resulting solution was cooled in an ice bath, diluted with water (3 ml), and made basic (pH 8.5) with concentrated ammonium hydroxide. The temperature was maintained at 0–5 °C and stirring was continued until a precipitate formed. The precipitate was filtered off, washed with ice-water (20 ml), and dried, to give a white solid (95 mg). Recrystallisation from ethanol afforded the *title compound* (**15**) (86 mg, 78%) as white crystals, m.p. 189 °C (Found: C, 46.35; H, 5.75; N, 18.0. $C_{15}H_{23}N_5O_7$ requires C, 46.75; H, 6.0; N, 18.2%); ν_{max}(Nujol) 3 320, 3 200, 3 140, 1 725 (CO), 1 680 (CONH), 1 635 (CONH), and 840 cm^{-1}; λ_{max}(EtOH) 336 (ε 12 980), 286 (6 870), and 221 nm (25 120); δ_H (80 MHz, CD_3SOCD_3) 1.12 (6 H, t, *J* 6.8 Hz, 2 × OCH_2CH_3), 1.30 [3 H, s, $C(OEt)_2CH_3$], 2.19 (3 H, s, CH_3CONH), 3.00 (2 H, t, *J* 6.8 Hz, CH_2CO), 3.38 (2 H, q, *J* 6.8 Hz, CH_3CH_2O), 3.43 (2 H, q, *J* 6.8 Hz, CH_3CH_2O), 3.79 (2 H, m, CH_2NH), 9.66 (1 H, t, *J* 5.1 Hz, N*H*CH_2, exchangeable with D_2O), and 11.54 (2 H, br s, CH_3CONH and lactam-NH, exchangeable with D_2O).

2-*Amino*-6-(4,4-*diethoxy*-3-*oxopentylamino*)-5-*nitropyrimidin*-4(3*H*)-*one* (**14**).—The pyrimidinone (**15**) (644 mg, 1.6 mmol) was stirred with aqueous sodium hydroxide (30 ml, 0.125 M) at room temperature for 4 h. Dilute acetic acid was added dropwise to the reaction mixture until a white precipitate was formed, and this was extracted with ethyl acetate (200 ml). The extract was washed with water (20 ml), dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a white solid (0.5 g), which on recrystallisation from methanol gave pure title compound (**14**) (0.43 g, 96%), identical with that prepared by oxidation of (**11**).

6-*Acetyl*-2-*amino*-7,8-*dihydro*-9H-*pyrimido*[4,5-*b*][1,4]*diazepin*-4(3H)-*one* (**1**), *from* (**14**).—(i) A deaerated solution of the pyrimidinone (**14**) (343 mg, 1 mmol); in *N*,*N*-dimethylformamide (10 ml), water (9.5 ml), and aqueous sodium hydroxide (0.125M; 5 ml) was heated to 70 °C, under nitrogen. Sodium dithionite (3.9 g) was added portionwise with stirring over 2.5 h, the reaction mixture being maintained at 70 °C. After a further 2 h, deaerated aqueous sodium hydroxide (0.125 M; 5 ml) and water (5 ml) were added to keep the pH in the range 7–8. The progress of the reaction was monitored by silica gel TLC (1:1, methanol–chloroform). When all the starting material had been consumed (*ca.* 3.5 h), the mixture was cooled under nitrogen to room temperature. Aqueous barium chloride (1 M; 20 ml) was added and the precipitated barium salts were filtered off under a nitrogen atmosphere and washed with deaerated distilled water (20 ml). The washings were added to the filtrate, and the resulting solution had a pH of 3. After a period of 20 min from the addition of the aqueous barium chloride, the solution was brought to pH 7 with deaerated aqueous sodium hydroxide (0.125 M) and then evaporated at room temperature under reduced pressure to give a greenish yellow solid. This was dissolved in distilled water and chromatographed at 4 °C on a column of cellulose (4.5 × 40 cm) which had been pre-washed with distilled water adjusted to pH 7 with a few drops of ammonia solution. The column was eluted with aqueous ammonium chloride (0.25%). The fractions (30 ml) were monitored by TLC on cellulose plates (aqueous ammonium chloride 3%). The 6-acetyltetrahydrohomopterin (**1**) appeared as a dark spot under UV light (365 nm) at room temperature, but showed a green-yellow fluorescence if the TLC sheet was cooled in liquid nitrogen before viewing under the UV light. The fractions containing pure 6-acetyltetrahydrohomopterin (**1**) were combined, cooled in ice, and concentrated by evaporation under reduced pressure, the temperature of the flask being kept below 5 °C during evaporation. Fine green-yellow needle crystals were precipitated. These were filtered off, washed with ice–water (1 ml), and dried, to give 25 mg of a first crop of product. The filtrate was then chromatographed on a second column. This column was prepared as before but was eluted with methanol–distilled water (1:3) under the same conditions. The fractions containing 6-acetyltetrahydrohomopterin (**1**) were combined and the methanol was evaporated. The resulting aqueous solution was then lyophilised to dryness to give a second crop of (**1**) (95 mg) as a green-yellow powder. The total yield of (**1**) was 120 mg (50%); m.p. 165 °C (decomp.) (Found: C, 44.95; H, 5.9; N, 29.25. $C_9H_{11}N_5O_2 \cdot H_2O$ requires C, 45.18; H, 5.48, N, 29.28%); ν_{max}(Nujol) 3 390, 3 320, 1 675, 1 658, and 725 cm^{-1}; λ_{max}(10 mM phosphate buffer) 263 (ε 16, 595), and 383 nm (ε 12 155); δ_H (80 MHz; CD_3SOCD_3) 2.33 (3 H, s, CH_3CO). 2.78 (2 H, m, $NHCH_2CH_2$), 3.28 (2 H, m, $NHCH_2$), 6.48 (2 H, br s, NH_2), 7.50 (1 H, br s, N*H*CH_2), and 10.35 (1 H, br s, NHCO); *m*/*z* 221 (M^+, 100%), 206 (2), 193 (2), 178 (56), 161 (27), 152 (21), 136 (24), 126 (1), 119 (3), 108 (5), 94 (3), 81 (3), 69 (5), 54 (4), and 43 (25).

In a repeat experiment following this procedure, the solution was neutralised to pH 7 with aqueous sodium hydroxide (0.125 M) immediately after the precipitated barium salts had been filtered off to give a very small amount of (**17**) as a pale yellow solid; δ_H (80 MHz; CD_3SOCD_3) 1.23 (6 H, t, *J* 6.8 Hz, 2 × OCH_2CH_3), 1.73 [3 H, s, $C(OEt)_2CH_3$], 3.21 (2 H, m, $NHCH_2CH_2$), 4.00 (6 H, m, $NHCH_2$, 2 × OCH_2CH_3), 6.89 (2 H, br s, NH_2), 8.25 (1 H, br s, N*H*CH_2), and 11.11 (1 H, br s, NHCO).

(ii) A deaerated solution of the pyrimidinone (**14**) (50 mg, 0.146 mmol) in aqueous methanol (1:4) (10 ml) was heated at 70 °C until all the starting material dissolved. The resulting clear

solution was cooled to room temperature, when iron filings (0.45 g) were added. The mixture was acidified to pH 3 by addition of a few drops of dilute hydrochloric acid and stirred at 45–50 °C under a nitrogen atmosphere. The progress of the reaction was monitored by TLC on cellulose plates using aqueous ammonium chloride 3% as the mobile phase. The reaction mixture gradually turned green and 6-acetyltetrahydrohomopterin (**1**) was detected as a dark spot under UV light (365 nm) at room temperature, showing a green-yellow fluorescence if the TLC sheet was first cooled in liquid nitrogen. After approximately 2 h all the starting material had been consumed. The resulting dark green mixture was brought to pH 7 with aqueous sodium hydroxide (0.125 M) and the precipitated ferric hydroxide was filtered off and washed with distilled water. The washings were added to the filtrate and the combined solution evaporated on a rotary evaporator at room temperature, to give a green–brown residue. This was purified by chromatography as described above, when pure 6-acetyltetrahydrohomopterin (**1**) (10 mg, 31%) was obtained, the properties of which were identical with those of material prepared as described above.

2-*Amino*-6-(3,4-*dioxopentylamino*)-5-*nitropyrimidin*-4(3H)-*one* (**18**).—Hydrochloric acid (5 ml 1M) was added to a solution of the pyrimidinone (**14**) (260 mg; 0.758 mmol) in methanol (25 ml). After being stirred for 30 min at 80 °C, the reaction mixture was cooled to room temperature and then evaporated under reduced pressure to give a crude product (200 mg). This was washed with a mixture of methanol (10 ml) and ether (10 ml), the solvent decanted off, and the solid washed with ether and dried to give the title compound (**18**) as an unstable pale yellow solid (150 mg, 61%), ν_{max}(Nujol) 1 712 cm^{-1}; δ_H (80 MHz; CD_3SOCD_3) 2.14 (3 H, s, CH_3CO), 3.52 (2 H, t, *J* 5.3 Hz, CH_2CO), 4.0 (2 H, m, $NHCH_2CH_2$), 7.16 (2 H, br s, NH_2), 9.64 (1 H, t, *J* 5.4 Hz, $NHCH_2$), and 10.56 (1 H, br s, NH).

2,2a-*Dihydroxy*-2-*methyl*-6-*nitro*-1,2,2a,3,4,5-*hexahydro*-1,5,8,8b-*tetra-aza-acenaphthylen*-7-*one* (**22**).—A solution of the pyrimidinone (**14**) (0.21 g, 0.612 mmol), in distilled 1,4-dioxane (15 ml) was acidified to pH 1–2 by addition of a few drops of dilute sulphuric acid. After 3 h at 60 °C with stirring, most of the starting material had been consumed and a white solid had precipitated out of the solution. This was filtered off, washed with 1,4-dioxane (5 ml), ether (20 ml), and dried, to give a first crop of product (160 mg). A second crop (28 mg) was obtained by heating the mother liquor for a further 2 h at the same temperature, giving a total yield of crude product of 188 mg. Recrystallisation from water gave very small white crystals of the *title compound* (**22**) (160 mg, 91%), m.p. 215 °C (decomp.) (Found: C, 37.45; H, 4.6; N, 24.5. $C_9H_{11}N_5O_5H_2O$ requires C, 37.63; H, 4.56; N, 24.38%); ν_{max}(Nujol) 3 580, 3 440, 3 280, 1 688, 1 655, 1 140, 795, and 780 cm^{-1}; λ_{max}(MeOH) 328 (ε 15 870), and 236 nm (ε 19 115); δ_H (270 MHz; CD_3SOCD_3) 1.53 (3 H, s, CH_3), 2.05 (2 H, m, CH_2CH_2NH), 3.52 (1 H, m, $NHCH_aH_bCH_2$), 3.78 (1 H, m, $NHCH_aH_bCH_2$), 6.42 (1 H, br s, OH, exchangeable with D_2O), 7.31 (1 H, br s, OH, exchangeable with D_2O), 9.26 (1 H, br s, NH, exchangeable with D_2O), and 9.94 (1 H, br s, NH exchangeable with D_2O); δ_C (270 MHz; CD_3SOCD_3) 163.03 (s, CONH), 151.39 (s), 148.80 (s) and 110.08 (s) (pyrimidine), 89.01 (s, COH) 87.08 (s, COH), 36.49 (t, $NHCH_2$), 23.27 (t, $NHCH_2CH_2$), and 20.29 (q, CH_3) [Found: $(M + H)^+$, 270.0847 2. $C_9H_{12}N_5O_5$ requires $(M + H)^+$, 270.08384 4].

This product (**22**) (100 mg, 0.348 mmol) was suspended in ethanol (20 ml) and heated under reflux for 2.5 h, after which time silica gel TLC plates (1:4 methanol–chloroform) showed complete conversion of starting material. The ethanol was evaporated, and the solid residue (112 mg) purified by flash column chromatography using (1:4, methanol–chloroform) as eluant. A white solid product was obtained (105 mg, 96%), m.p. 180 °C (decomp.) (Found: C, 42.05; H, 5.3; N, 22.05. $C_{11}H_{15}N_5O_5H_2O$ requires C, 41.90; H, 5.43; N, 22.21%); ν_{max}(Nujol) 3 280, 3 120, 1 685, 1 610, 1 120, and 790 cm^{-1}; λ_{max}(MeOH) 327 (ε 13 985) and 236 nm (ε 18 620); δ_H (270 MHz; CD_3SOCD_3) 1.54 (3 H, t, *J* 6.96 Hz, CH_3CH_2O), 1.61 (3 H, s, CH_3C), 2.07 (1 H, m, $NHCH_2CH_aH_b$), 2.22 (1 H, m, $NHCH_2CH_aH_b$), 3.60 (2 H, q, *J* 7.0 Hz, CH_3CH_2O), 3.63 (1 H, m, $NHCH_cH_dCH_2$), 3.70 (1 H, m, $NHCH_cH_dCH_2$), 7.25 (1 H, br s, OH, exchangeable with D_2O), 9.60 (1 H, br s, NH, exchangeable with D_2O), and 9.79 (1 H, br s, NH, exchangeable with D_2O); δ_C (270 MHz; CD_3SOCD_3) 162.75 (s, CONH), 151.08 (s), 148.48 (s) and 110.0 (s) (pyrimidine), 92.20 (s, COEt), 87.11 (s, COH), 57.30 (t, OCH_2CH_3), 36.59 (t, $NHCH_2CH_2$), 23.20 (t, $NHCH_2CH_2$), 16.31 (q, CH_3COH), and 15.45 (q, CH_3CH_2O) [Found: $(M + H)^+$ 298.1161 4. $C_{11}H_{16}N_5O_5$ requires $(M + H)^+$ 298.1151 44].

2-*Acetamido*-6-(3,4-*dioxopentylamino*-(5-*nitropyrimidin*-4-(3H)-*one* (**19**).— A solution of the pyrimidinone (**15**) (100 mg, 0.259 mmol) in distilled 1,4-dioxane (15 ml), was brought to pH 1 by the addition of a few drops of dilute sulphuric acid. The reaction was monitored on silica gel TLC plates (9:1 chloroform–methanol). After being stirred at 60 °C for 6 h, the mixture was evaporated to give an oil, all the starting material having been consumed. Methanol (2 ml) was added to the residue, followed by ether until a white precipitate was formed. This solid was filtered off under nitrogen to give the unstable pyrimidinone (**19**) (60 mg, 75% crude); δ_H (80 MHz, CD_3SOCD_3) 2.20 (3 H, s, CH_3COCO), 2.26 (3 H, s, CH_3CONH), 3.09 (2 H, t, *J* 6.8 Hz, CH_2CH_2COCO), 3.74 (2 H, m, $NHCH_2CH_2$), and 9.66 (1 H, t, *J* 5.9 Hz, $NHCH_2$).

6-*Acetyl*-2-*amino*-7,8-*dihydro*-9H-*pyrimido*[4,5-b][1,4]-*diazepine*-4(3H)-*one* (**1**), *from* (**22**).—(*i*) A deaerated solution of the tetra-aza-acenaphthylenone (**22**) (50 mg, 0.185 mmol) in *N*,*N*-dimethylformamide (2.5 ml), water (2.5 ml) and aqueous sodium hydroxide (1 ml, 0.125 M) was heated to 70 °C. Sodium dithionite (0.22 g) was added portionwise with stirring over 2 h, the reaction mixture being kept at 70 °C under a nitrogen atmosphere. When all the starting material had been consumed (*ca.* 3.5 h; silica gel TLC, 1:1 methanol–chloroform), the mixture was cooled under nitrogen to room temperature. Deaerated aqueous barium chloride (20 ml, 1 M) was added and the precipitated barium salts were filtered off and washed with deaerated distilled water (10 ml). The washings were added to the filtrate. The pH of the resulting solution was 3. After a period of 20 min (critical) from the addition of the barium chloride, the solution was brought to pH 7 with aqueous sodium hydroxide (0.125 M), and then evaporated at room temperature under reduced pressure to give a greenish yellow solid (300 mg). This was dissolved in distilled water and chromatographed at 4 °C on a column of cellulose (4.5 × 40 cm) which had been pre-washed with distilled water, adjusted to pH 7 with a few drops of ammonia solution. The column was eluted with aqueous ammonium chloride (0.25%), and fractions were monitored by TLC on cellulose plates (aqueous ammonium chloride 3%). The 6-acetyltetrahydrohomopterin (**1**) appeared as a dark spot under UV light (365 nm) at room temperature, but showed a green-yellow fluorescence if the TLC sheet was cooled immediately before viewing in liquid nitrogen. The fractions containing 6-acetyltetrahydrohomopterin (**1**) were combined, cooled and lyophilised to give a yellow-green solid consisting of 6-acetyltetrahydrohomopterin (**1**) and inorganic salts. The yellow-green solid was again chromatographed on a cellulose column prepared as before but eluting with distilled water. Fractions containing 6-acetyltetrahydrohomopterin (**1**) were

combined, cooled, and lyophilised, to give a green-yellow powder of pure 6-acetyltetrahydrohomopterin (**1**) (18 mg, 44%), identical with the product obtained previously, as already described.

(*ii*)Distilled water (25 ml) and iron filings (12 g) were added to a deaerated solution of compound (**22**) (0.16 g, 0.594 mmol) in distilled *N*,*N*-dimethylformamide (25 ml). The mixture was acidified to pH 3–4 by addition of a few drops of dilute hydrochloric acid and heated at 40–45 °C with stirring under a nitrogen atmosphere. The pH was maintained at 3–4 by addition of a few drops of dilute hydrochloric acid over a period of 2 h. The progress of the reaction was monitored on cellulose TLC plates (aqueous ammonium chloride 3%). The solution gradually turned green and 6-acetyltetrahydrohomopterin (**1**) in the reaction mixture appeared on TLC as a dark spot under UV light (365 nm) at room temperature, showing a green-yellow fluorescence if the sheet was cooled in liquid nitrogen immediately prior to viewing. After 2 h at 40–45 °C all the starting material had been consumed. The dark green solution was neutralised to pH 7 with aqueous sodium hydroxide (0.125 M) and the precipitated ferric hydroxide was filtered off and washed with distilled water. The washings were added to the filtrate and the combined solution evaporated at room temperature under reduced pressure to give a brown-yellow residue. This was dissolved in distilled water and chromatographed on a cellulose column (4.5 × 40 cm) which had been pre-washed with distilled water containing a few drops of ammonia solution to bring the pH to 7. The column was run at 4 °C, eluting with aqueous ammonium chloride (0.25%). 25 Fractions of 20 ml were collected within 2 h, and the fractions which contained 6-acetyltetrahydrohomopterin (**1**) were combined and evaporated on a rotary evaporator at room temperature. During evaporation the pH of the solution was kept at 7 by addition of dilute ammonium hydroxide. The resulting green-yellow solid was applied to a second cellulose column, prepared as described above, but eluting with distilled water at 4 °C. Fractions which contained (**1**) were combined and taken to dryness on a rotary evaporator at room temperature to give a green-yellow solid. This was then applied to a third pre-washed cellulose column and eluted at 4 °C with methanol–water (1:3). Fractions containing pure 6-acetyltetrahydrohomopterin (**1**) were combined, and the methanol was removed at room temperature using a rotary evaporator. The remaining aqueous solution of (**1**) was lyophilised to give a yellow-green powder of pure 6-acetyltetrahydrohomopterin (**1**) (75 mg, 57%), identical in every respect with the samples prepared by the other methods already described.

HPLC Comparison of Synthetic 6-Acetyl-2-amino-7,8-dihydro-9H-pyrimido[4,5-b][1,4]diazepin-4(3H)-one (**1**) *with Naturally Occurring Material.*—The instrument used was a Spectra-Physics SP8000 liquid chromatograph fitted with a Spectrophysics Model 770 variable wavelength UV absorption detector, set at 263 nm. The column was a Brownlee RP-8 cartridge column (22 cm × 4.6 mm i.d., spherical particles of average 5 μm), and was operated at room temperature, with a flow rate of 1 ml min^{-1}. The mobile phase was a mixture of methanol, water, and a 10 mM ammonium phosphate buffer at pH 8, in the ratio 1:5:4. Samples were dissolved in methanol for injection. A sample of 6-acetyl-2-amino-7,8-dihydro-9*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one (**1**) which had been isolated from the eyes of *Drosophila melanogaster* was kindly donated by Dr. Jacobson, and under the above conditions this sample eluted as a single peak with a retention time of 4.8 min. Synthetic 6-acetyl-2-amino-7,8-dihydro-9*H*-pyrimido-[4,5-*b*]-[1,4]diazepine-4(3*H*)-one (**1**) prepared as described above, also eluted as a single peak with a retention time identical with that of the naturally occurring material.

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