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SYNTHESIS OF A 2,4-DIAMINODIHYDROHOMOPTERIDINE, 6-ACETYL-2,4-DIAMINO-7,8-DIHYDRO-9*H*-PYRIMIDO[4,5-b][1,4]DIAZEPINE, USING A FURAZANO[3,4-d]PYRIMIDINE PRECURSOR

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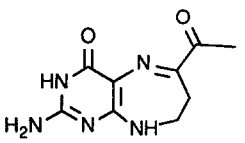
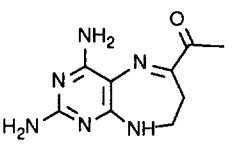
(*Received in UK* 20 *March* 1991)

Abstract *5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine (3) reacts readily with a variety of amines and hydroxylamines under mild conditions, with the introduction of an N-substituent at position 7. Using this reaction of (3) with 1-amino-4,4-diethoxy-3-pentanol (16), a synthesis of 6-acetyl-2,4-diamino-7,8-dihydro-9*H*-pyrimido[4,5-b][1,4]diazepine, (2), was achieved. Compound (2) is an amino analogue of a naturally occurring dihydrohomopterin (1).*

The pyrimidodiazepine (1), which can also be regarded as a dihydrohomopterin, occurs naturally in *Drosophila melanogaster*, where it is a biosynthetic precursor of the eye pigment drosopterin.[1] We have recently described its chemical synthesis,[2] and evidence is now emerging that it can exert an important biological effect on human cells.[3] Because of the well known biological activity of the 4-amino analogs of folates, we also developed[4] a synthesis of compound (2), which is the 4-amino analog of the naturally occurring compound (1), and in the present paper we wish to describe an alternative synthesis of (2) based on a furazanopyrimidine precursor (3).



(1)



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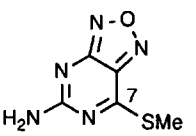
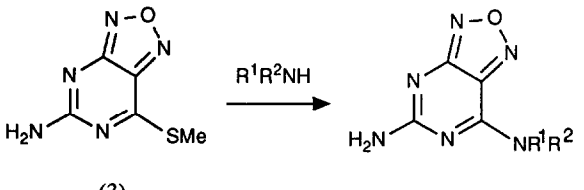


(3)

Some time ago Taylor and coworkers observed that a nitrogen substituent at position 7 of a furazano[3,4-d]-pyrimidine ring was particularly susceptible to substitution by both nitrogen and carbon nucleophiles, and they exploited this observation in a new synthesis of purines and pteridines.[5] Oxygen nucleophiles could not

be introduced readily by this method, however, and we therefore developed use of the 7-methylthio group as an especially good leaving group for this purpose.[6] In the present work, we found that the methylthio group in 5-amino-7-(methylthio)furazano[3,4-d]pyrimidine (3) is also readily attacked under mild conditions by amino or substituted amino groups, leading to a series of new 7-substituted derivatives (see scheme 1). Thus, primary and secondary aliphatic amines, as well as hydroxylamines, were found to react with (3) at room temperature, and the more weakly nucleophilic aniline reacts at 45°C. Even the highly hindered *tert*-butylamine reacts slowly, although in this case it was found advantageous to add mercuric bromide to the reaction mixture, which markedly accelerates the rate of reaction. The structures of the resulting new 7-substituted furazanopyrimidines (4)-(9) rest on their spectroscopic properties and elemental analyses.



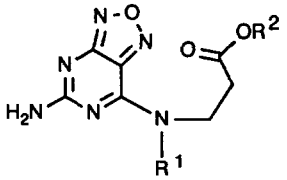
(3) (4) - (9)

Scheme 1

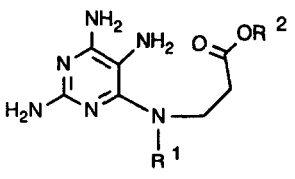
	R^1	R^2
(4)	H	$-CH(CH_3)_2$
(5)	H	$-CH_2CH_2OH$
(6)	H	$-C(CH_3)_3$
(7)	H	Ph
(8)	H	OH
(9)	CH_3	OH

The same reaction with methyl 3-aminopropanoate, as well as with ethyl N-methyl-3-aminopropanoate, afforded compounds (10) and (11). The ^1H nmr spectrum of (11) is of interest in that at room temperature some of the side chain signals appear to be duplicated. For example, the N-methyl group gives rise to two singlets of approximately equal intensity, 0.34 p.p.m. apart, which collapse to a singlet at higher temperatures, and separate again on cooling. This is most probably due to restricted rotation around the bond joining the exocyclic nitrogen atom to the ring, due to resonance interaction between the nitrogen and the ring. Both compounds (10) and (11) underwent palladium catalysed hydrogenolysis of the furazan ring to the the corresponding 4,5-diaminopyrimidines, (12) and (13) respectively. These were too unstable to be easily isolated, but when the N-methyl compound (13) was refluxed in methanol it cyclised to (14), thus thus opening up a new route to the preparation of pyrimido[4,5-b][1,4]diazepines. Surprizingly, all efforts to cyclise the non-N-methylated compound (12) failed, although the formation of (12) was demonstrated by allowing it to condense with benzil, when the 6,7-diphenylpteridine (15) was isolated and characterised.

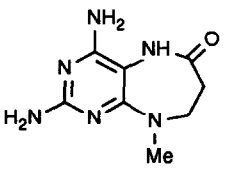
Based on these preliminary experiments, the desired target molecule (2) was synthesised successfully as follows. The methylthiofurazanopyrimidine (3) was allowed to react with the β-amino alcohol[2] (16) by stirring in ethyl acetate solution at room temperature, when the α-hydroxyacetal (17) was obtained in 70% yield. Compound (17) decomposed under a wide variety of oxidising conditions, or else did not react at all if the conditions were too mild. However the ketoacetal (18) could be obtained in 80% yield by treatment of (17) with a dimethyl sulphoxide/acetic anhydride system over two days, or in 67% yield by treatment with



(10) R^1 = H R^2 = Me
(11) R^1 = Me R^2 = Et



(12) R^1 = H R^2 = Me
(13) R^1 = Me R^2 = Et



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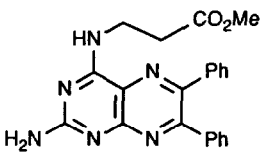
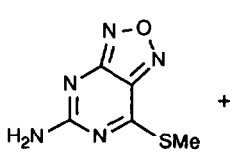


(15)

pyridine/chromium trioxide for four days. Treatment of (18) at 60°C with methanolic sulphuric acid at pH 2-3 led to hydrolysis of the protecting acetal group, with the formation of diketone (19). This was too unstable to be isolated or properly characterised, but it could be converted *in situ* to the 2,4,5-triaminopyrimidine (20) by hydrogenolysis of the furazan ring with hydrogen and a palladised charcoal catalyst. The triaminopyrimidine (20) was also too unstable to be isolated, but it could be cyclised to the pyrimidodiazepine target compound (2) by warming it to 50°C in solution at pH 3. Both heat and acid were found to be necessary in order to effect this cyclisation. No pyrimidodiazepine (2) could be detected, either when a neutral solution of (20) was warmed to 50°C, or when a solution of (20) at pH 3 was maintained at room temperature for several hours.



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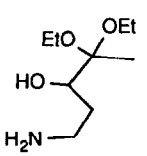

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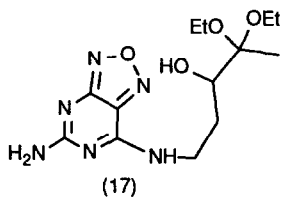

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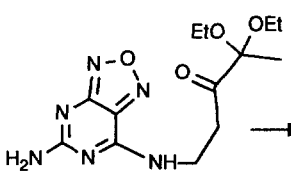

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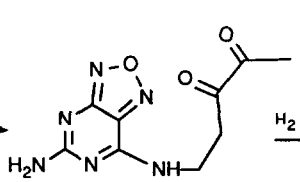

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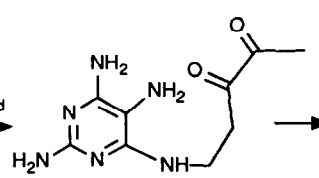
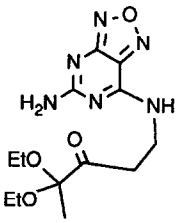


(20)

As mentioned above, treatment of the ketoacetal (18) with methanolic sulphuric acid at pH 3 afforded the unstable α-diketone (19) by hydrolysis of the acetal grouping. In contrast, when a solution of (18) in dioxane was brought to pH 3 by the addition of dilute sulphuric acid, and heated at 60°C for three hours, a stable new compound was obtained. Although the structure of this new product cannot be regarded as firmly established, the evidence available suggests that it contains a novel tetracyclic ring system, as in structure (23) or its isomer (24). These could be formed by an intramolecular cyclisation of initially formed diketone (19), to give the tricyclic intermediate (21), followed by a second cyclisation to give the tetracyclic structure (22). Addition of a molecule of water to one of the carbon-nitrogen double bonds in (22) would then lead to either (23) or (24). The new product exhibits an upfield (1.46 p.p.m.) methyl singlet in its pmr spectrum, together with four deuterium exchangeable singlets, and it shows no carbonyl absorption in its infrared spectrum, and these data are incompatible with both structures (21) and (22). The ultraviolet spectrum of the new product shows maxima at 233 and 312 nm, which are at shorter wavelengths than are normally observed for the furazano[3,4-d]pyrimidine ring system, as in compounds (4) - (9), and (17) - (18), and this is accommodated by structures (23) and (24), which have less highly conjugated double bond systems than do the furazanopyrimidine compounds. Finally, elemental analysis corresponds to a molecular formula of $C_9H_{12}N_6O_4$, as required by (23) or (24).



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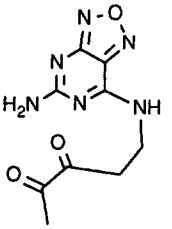

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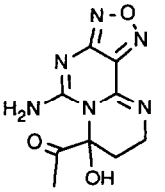

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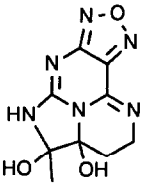

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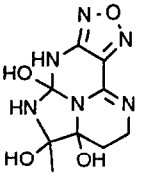

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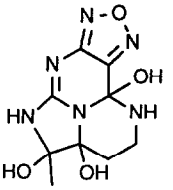

(24)

ACKNOWLEDGEMENT

We are grateful to EOLAS for financial support of this work.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer 298 spectrophotometer, and ultraviolet spectra on a Pye Unicam PU8800 spectrophotometer. Nuclear magnetic resonance spectra were run either on a Bruker WP80 or a Jeol GX 270 instrument using tetramethylsilane as internal standard. Electron-ionization mass spectra were recorded using a VG analytical 70-E mass spectrometer, with samples introduced by direct probe (ionizing energy 70 ev, ion source temperature 200°C). TLC was carried out on DC-Alufolien Kieselgel 60 F254 0.2 mm plates and on DC-Alufolien cellulose F 0.1 mm plates. Kieselgel 60 (400-230 mesh) was used for silica flash chromatography with freshly distilled solvents. Column chromatography on cellulose used cellulose CF11 (Whatman) or cellulose 33830 (Riedel-de Haën). All oxygen sensitive reactions were performed under an atmosphere of oxygen free nitrogen. Microanalyses were carried out in the microanalytical laboratories of University College Dublin. Some samples required the addition of tungstic oxide to ensure complete combustion, and these analyses were carried out in the microanalytical laboratories of University College, London. Dimethyl sulphoxide was dried by standing over calcium hydride and 4Å molecular sieve. pH values were measured using narrow range indicator papers.

*5-Amino-7-(isopropylamino)furazano[3,4-*d*]pyrimidine (4)*

5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine[6] (3) (500 mg, 2.74 mmol) was stirred at room temperature with isopropylamine (4 ml) for 5 min, during which time the yellow solution rapidly turned green. Evaporation of the excess isopropylamine and chromatography of the residue on a column of silica gel, eluting with ethyl acetate-hexane (6:4), gave white crystals of (4) (410 mg, 59%), m.p. 157-8 °C (from ethanol) (Found: C, 43.51; H, 5.43; N, 42.93. $C_7H_{10}N_6O$ requires C, 43.28; H, 5.19; N, 43.29%); ν_{max} (Nujol) 3 500, 3 350, 1 660, 1 600, 1 540, 870, and 790 cm^{-1}; λ_{max} (EtOH) 280 and 340 nm (log ϵ 4.04 and 3.82); δ_H (80 MHz; CD_3OD) 1.34 (6H, d, J=7Hz, 2 x CH_3), and 3.31 (1H, m, $CH(CH_3)_2$); m/z 194 (M^+, 52%), 164 (M^+- NO, 100), 122 (68), 95 (82), and 70 (39).

*5-Amino-7-(2-hydroxyethylamino)furazano[3,4-*d*]pyrimidine (5)*

5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine[6] (3) (310 mg, 1.69 mmol) and 2-aminoethanol (4 ml) were stirred together at room temperature for 5 min, by which time the reaction mixture had become greenish black in colour. Ethanol was added and the black precipitate was filtered off. Removal of ethanol under reduced pressure, followed by addition of water to the residue, caused crystals of (5) to precipitate (240 mg, 69%), m.p. 203-204°C (Found: C, 36.68; H, 4.25; N, 42.63. $C_6H_8N_6O_2$ requires C, 36.72; H, 4.11; N, 42.85%); ν_{max}(Nujol) 3 330, 3 100, 1 670, 1610, 1550, 1130, 1000, 860, and 790 cm^{-1}; λ_{max} (EtOH) 286 and 349 nm (log ϵ 3.88 and 3.72); δ_H (270 MHz; CF_3COOD) 2.84 (2H, t, J=5Hz, CH_2OH), 3.31 (2H, t, J=5Hz, CH_2NH).

*5-Amino-7-(*tert*-butylamino)furazano[3,4-*d*]pyridimine (6)*

5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine[6] (3) (990 mg, 5.41 mmol) and mercury II bromide (1.95 g, 5.41 mmol) were refluxed for 5 h with *tert*-butylamine (10 ml), and the mixture was then stirred overnight at room temperature. The mercury salt was removed by filtration and the filtrate was evaporated. Chromatography of the residue on silica gel with ethyl acetate-hexane (4:6) as eluant gave cream coloured crystals of (6) (690 mg, 47%) m.p. 150-152°C (from ether-hexane) (Found: C, 45.97; H, 5.83; N, 39.66. $C_8H_{12}N_6O$ requires C, 46.13; H, 5.81; N, 40.37%); ν_{max}(Nujol) 3 530, 3 420, 3 150, 1 600, 1 220, 990, 870 and 800 cm^{-1}; λ_{max}(EtOH) 276 and 341 nm (log ϵ 4.02 and 3.75); δ_H (80 MHz; $CDCl_3$) 1.58 (9H, s, 3 x CH_3), 5.51 (2h, br s, NH_2), and 6.08 (1H, br s, N*H*); m/z 208 (M^+, 70%), 178 (M^+- NO, 52), 122 (100), and 95 (27).

*5-Amino-7-anilinofurazano[3,4-*d*]pyrimidine (7)*

5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine[6] (3) (750 mg, 4.1 mmol) and aniline (6 ml) were stirred at 45°C for 30 min, by which time the mixture had become black in colour. Ethyl acetate was added and the black precipitate was removed by filtration. The ethyl acetate solution was taken to dryness, affording a yellow solid which was chromatographed on silica gel using ethyl acetate-hexane (4:6) as eluant. Recrystallisation from absolute ethanol gave yellow needles of (7) (350 mg, 30%), m.p. 220°C (Found: C, 52.10; H, 3.79; N, 36.23. $C_{10}H_8N_6O$ requires C, 52.61; H, 3.54; N, 36.84%); ν_{max}(Nujol) 3 500, 3 480 , 3 350, 1 670, 1 610, 1 580, 1 490, 870, 790, and 760 cm^{-1}; λ_{max}(EtOH) 224 and 362 nm (log ϵ 3.96 and 3.68); δ_H (80 MHz; CD_3OD) 7.1 - 8.05 (5H, m, C_6H_5); m/z 228 (M^+, 36%), 198 (M^+- NO, 53), 129 (50), and 77 (100).

5-Amino-7-(hydroxyamino)furazano[3,4-d]pyrimidine (8)

Hydroxylamine hydrochloride (1.20 g, 17.5 mmol) was dissolved in hot ethanol (30 ml). To the solution was added a solution of potassium hydroxide (980 mg, 17.5 mmol) in absolute ethanol and the precipitated potassium chloride was removed by filtration. An ethanolic solution of 5-amino-7-(methylthio)furazano-[3,4-d]pyrimidine[6] (3) (350 mg, 1.9 mmol) was added to the filtrate and the mixture then refluxed for 1 h. After cooling, the precipitated product was filtered off and washed with water. Recrystallisation from a mixture of 0.1M HCl and 3.5% aqueous ammonia solution gave white crystals of (8) (180 mg, 56%), m.p. >300°C (decomp.); (Found: C, 28.70; H, 2.61; N, 49.85. $C_4H_4N_6O_2$ requires C, 28.56; H, 2.40; N, 50.00%); ν_{max}(Nujol) 3 380, 3 280, 1 660, 1 590, 1 230, 1 040 and 850 cm^{-1}; λ_{max}(0.1M HCl) 239 and 301 nm (log ϵ 3.65 and 3.77); m/z 168 (M^+, 85%), 151 (30), 138 (81), 122 (33), and 68 (52).

*5-Amino-7-(*N*-hydroxy-*N*-methylamino)furazano[3,4-d]pyrimidine (9)*

5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine[6] (3) (2.00 g, 10.9 mmol) was stirred with a methanolic solution of N-methylhydroxylamine (0.56 g, 12.1 mmol) overnight. The yellow precipitate was filtered off and recrystallised from dimethylformamide-water to give (9) (1.64 g, 83%), m.p. 235°C;

ν_{max}(Nujol) 3 280, 3 100, 1 645, 1 050 and 850 cm^{-1}; λ_{max}(EtOH) 304 and 370 nm (log ϵ 3.46 and 3.71); δ_H (80 MHz; CD_3SOCD_3) 3.96 (3H, s, CH_3); m/z 182 (M^+, 45%), 152 (M^+- NO, 100), 136 (9), 125 (4), 109 (4), 100 (15), 93 (4), 83 (39), and 67 (59).

Methyl N-*(5-amino-7-furazano[3,4-d]pyrimidinyl)-3-aminopropanoate (10).*

5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine[6] (3) (816 mg, 4.4 mmol) was added to methyl 3-aminopropanoate (4.0 ml) and the mixture stirred vigorously at room temperature. All solid material had dissolved after 3 min and a precipitate appeared after 4 min. Stirring was continued for 12 h and the reaction mixture was then poured on to hexane (100 ml). The solid product was collected and washed with hexane. Recrystallisation from ethanol afforded yellow plates of (10) (988 mg, 93%) m.p. 178-79°C (decomp.) Found: C, 40.03; H, 4.40; N, 35.09. $C_8H_{10}N_6O_3$ requires: C, 40.32; H, 4.23; N, 35.29%); ν_{max}(Nujol) 3 450, 3 360, 3 250, 1 735, 1 615, 1 500, 1 390, 1 100, 880 and 800 cm^{-1}; λ_{max}(EtOH) 203, 284 and 342 nm (log ϵ 4.20, 3.94, and 3.65); δ_H (80 MHz; CD_3SOCD_3) 2.76 (2H, t, J=7Hz, CH_2), 3.63 (3H, s, OCH_3), 3.76 (2H ,t, J=7Hz, CH_2), 7.15 (2H, br s, NH_2), and 9.22 (1H, br s, N*H*).

Ethyl N-*(5-amino-7-furazano[3,4-d]pyrimidinyl)*-N-*methyl-3-aminopropanoate (11).*

5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine[6] (3) (1.009 g, 5.5 mmol) was stirred with ethyl N-methyl-3-aminopropanoate[7] (4 ml) at room temperature for 12 h and the reaction mixture then poured on to carbon tetrachloride (200 ml). The solid was collected and washed with carbon tetrachloride. Recrystallisation from ethanol afforded (11) (995 mg, 69%) m.p. 175-78°C (decomp.) (Found: C, 44.85; H, 5.46; N, 31.79). $C_{10}H_{14}N_6O_3$ requires: C, 45.09; H, 5.30; N, 31.57%); ν_{max}(Nujol) 3 460, 3 115, 1 745, 1 600, 1 495, 1 375, 1 360 and 1 180 cm^{-1}; λ_{max}(EtOH) 207, 298 and 350 nm (log ϵ 4.32, 3.88 and 3.75); δ_H (60 MHz; CD_3SOCD_3) 1.15 and 1.18 (3H, 2 x t, J=7Hz, CH_2CH_3), 2.78 (2H, t, J=7Hz, CH_2CO), 3.28 and 3.62 (3H, 2 x s, NCH_3), 4.00 and 4.33 (2H, 2 x t, J=7Hz, NCH_2), 4.05 (2H, q, J=7Hz, CH_2CH_3), and 7.00 (2H, br s, NH_2).

2,4-Diamino-7,8-dihydro-9-methyl-6(5H)-pyrimido[4,5-b][1,4]diazepinone (14)

Ethyl N-(5-amino-7-furazano[3,4-d]pyrimidinyl)-N-methyl-3-aminopropanoate (11) (213 mg, 0.8 mmol) was hydrogenated in oxygen-free methanol (100 ml) at atmospheric pressure over a 10% palladised charcoal catalyst (104 mg) until hydrogen uptake ceased (c. 3h). The catalyst was removed by filtration and the filtrate refluxed under nitrogen for 8 h. Evaporation of the solvent and several recrystallisations from methanol afforded (11) (52mg, 31%) m.p. 269-70°C (decomp.) (Found: C, 46.11; H, 5.91, N, 40.09. $C_8H_{12}N_6O$ requires C, 46.13; H, 5.81; N, 40.37%); ν_{max} (Nujol) 3 470, 3 420, 3 320, 3 180, 1 668, 1 620, and 1 435 cm^{-1}; λ_{max}(EtOH) 230 and 290 nm (log ϵ 4.39 and 4.12); δ_H (80 MHz, CD_3SOCD_3) 2.42 (2H, t, J=6Hz, CH_2), 2.80 (3H, s, CH_3), 3.54 (2H, t, J=6Hz, CH_2), 5.53 (2H, br s, NH_2), 5.73 (2H, br s, NH_2), and 8.19 (1H, br s, N*H*).

Methyl N-*(2-amino-6,7-diphenyl-4-pteridinyl)-3-aminopropionate (15)*

A solution of methyl N-(5-amino-7-furazano[3,4-d]pyrimidinyl)-3-aminopropanoate (10) (400 mg, 1.7 mmol) in oxygen-free methanol (150 ml) was hydrogenated at atmospheric pressurure over a 10% palladised charcoal catalyst until hydrogen uptake ceased (c. 2h). The catalyst was filtered off and benzil (500 mg, 2.4 mmol) added to the filtrate, which was then refluxed for 5 h. The methanol was evaporated under reduced pressure and the residue extracted with warm hexane. Evaporation of the extract and chromatography of the resulting solid on a silica gel column, eluting with ethanol-dichloromethane (1:9) afforded (15) (380 mg, 56%) m.p. 178°C (Found: C, 65.61; H, 5.10; N, 20.83. $C_{22}H_{20}N_6O_2$ requires C, 65.97; H, 5.04; N, 21.00%); ν_{max}(Nujol) 3 480, 3 330, 1 735, 1 630, 1 595, 1 446, 1 353, 1 200, 1 179, and 900 cm^{-1}; δ_H (80 MHz; $CDCl_3$) 2.75 (2H, t, J=6Hz, CH_2), 3.70 (3H, s, CH_3), 3.95 (2H, t, J=6Hz, CH_2), 5.40 (2H, br s, NH_2), 7.32 (10H, m, 2 x C_6H_5); λ_{max}(EtOH) 198, 222, 280 and 394 nm (log ϵ 4.65, 4.46, 4.41 and 4.10).

5-Amino-7-(4,4-diethoxy-3-hydroxypentylamino)furazano[3,4-d]pyrimidine (17)

5-Amino-7-(methylthio)furazano[3,4-d]pyrimidine (3) (1.27 g, 6.94 mmol) was stirred with a solution of 1-amino-4,4-diethoxy-3-pentanol (16) (1.50 g, 7.85 mmol) in ethyl acetate (25 ml) overnight. The precipitated product (17) was collected at the pump, and a second crop was obtained on addition of hexane to the filtrate (total yield 1.60 g, 71%), m.p. 148°C (from ethyl acetate-hexane) (Found: C, 47.41; H, 6.56; N, 25.63. $C_{13}H_{22}N_6O_4$ requires: C, 47.83; H, 6.80; N, 25.76%); ν_{max}(Nujol) 3 430, 3 120, 1 600, 1 100, 870, and 790 cm^{-1}; λ_{max}(EtOH) 288 and 343 nm (log ϵ 3.86 and 3.80); δ_H (80 MHz; $CDCl_3$) 1.17 (3H, t, J=7Hz, OCH_2CH_3), 1.19 (3H, t, J=7Hz, OCH_2CH_3), 1.23 (3H, s, CH_3), 1.89 (2H, m, CH_2CHOH), 3.53 (6H, m, 2 x OCH_2CH_3 and CH_2NH), 3.95 (1 H, m, CHOH), 5.50 (2H, br s, NH_2) and 7.55 (1H, brs, NH); m/z 326 (M^+, 6%), 281 (48), 251 (58), 235 (66), 209 (39), 117 (82), and 89 (33).

5-Amino-7-(4,4-diethoxy-3-oxopentylamino)furazano[3,4-d]pyrimidine (18)

(i) Chromium trioxide (300 mg) was added portionwise with stirring to dry pyridine (15 ml) at 0°C. The resulting solution was allowed to warm up to room temperature and was then mixed with a solution of 5-amino-7-(4,4-diethoxy-3-hydroxypentylamino)furazano[3,4-d]pyrimidine (17) (300 mg, 0.92 mmol) in dry pyridine (10 ml). After 4 days at room temperature reaction was complete. The mixture was evaporated under reduced pressure to give a dark brown residue, which was dissolved in distilled water (150 ml) and then extracted with boiling ethyl acetate (1 l). The ethyl acetate extract was dried over magnesium sulphate and then evaporated, to give a pale yellow solid (250 mg). Silica gel flash column chromatography using ethyl acetate:hexane (1:1) as eluant gave a white solid (18) (200 mg, 67%) m.p 164°C (Found: C, 48.20; H, 6.27; N, 25.90. $C_{13}H_{20}N_6O_4$ requires C, 48.13; H, 6.22; N, 25.92%); ν_{max}(Nujol) 3 460, 3 360, 3 240, 1 725, 1 655, 1 600, 1 530, 1 410, 1 235, 1 150, 1 090, 1 080, 1 040, 1 010, 990, 970, 930, 870, 790, and 760 cm^{-1}; λ_{max}(MeOH) 340 and 288 nm (log ϵ 3.74 and 3.85); δ_H (80 MHz; $CDCl_3$) 1.20 (6H, t, J=7Hz, 2 x OCH_2CH_3), 1.40 (3H, s, CH_3), 3.05 (2H, t, J=6Hz, CH_2CO), 3.47 (2H, q, J=7Hz, OCH_2CH_3), 3.49 (2H, q, J=7Hz, OCH_2CH_3), 3.88 (2H, t, J=6Hz, NCH_2), 5.58 (2 H, br s, NH_2), and 7.04 (1H, br s, NH-CH_2).

(ii) Freshly distilled acetic anhydride (320 mg, 3.13 mmol) was added to a solution of 5-amino-7-

(4,4-diethoxy-3-hydroxypentylamino)furazano[3,4-d]pyrimidine (17) (100 mg, 0.306 mmol) in dry dimethyl sulphoxide (2.0 ml). The reaction mixture was stirred at room temperature for 48 h and was then diluted with ethanol (1 ml) and stirred for a further 5 min at room temperature. The resulting solution was cooled in an ice bath, diluted with water (3.5 ml) and brought to pH 8 with concentrated ammonia solution, keeping the reaction vessel in the ice bath. A precipitate formed and was filtered off, washed with ice-water (15 ml), and dried at room temperature to give a pale yellow solid (85 mg). Recrystallisation from ethanol gave a white crystals of (18) (80 mg, 80%), m.p. 164°C, identical to the material prepared by method (i) described above.

*6-acetyl-2,4-diamino-7,8-dihydro-9*H*-pyrimido[4,5-b][1,4]diazepine (2).*

A solution of 5-amino-7-(4,4-diethoxy-3-oxopentylamino)furazano [3,4-d]pyrimidine (18) (140 mg, 0.432 mmol) in methanol (30 ml) was acidified to pH 3 by addition of dilute sulphuric acid, and the mixture was stirred at 60°C. The reaction which followed was monitored by TLC on silica gel plates (methanol:chloroform, 1:4) and after 6 h all the starting material had been consumed. The resulting orange-red solution was deaerated with nitrogen and stirred in an atmosphere of hydrogen with a palladised charcoal (10%) catalyst (140 mg) until no further hydrogen was absorbed (c. 2.5 h). The reaction mixture was then stirred further at 45-55°C under nitrogen, keeping the pH at 3, and monitoring the reaction on cellulose TLC plates, using a mobile phase of 3% aqueous ammonium chloride. After 3 h compound (2) appeared as a dark spot when the TLC plate was viewed under uv light (366 nm) at room temperature. The same compound showed as a yellow-orange fluorescent spot if the developed TLC plate was cooled in liquid nitrogen before viewing, and as a strong green fluorescent spot when the cellulose plate contained an indicator. The reaction mixture was heated for a further 2.5 h and was then cooled to room temperature and neutralised to pH 7.5 by deaerated aqueous sodium hydroxide (0.125 M, 20 ml). The catalyst was filtered off and the resulting green filtrate was evaporated under reduced pressure at room temperature to give a yellow-green residue. This was dissolved in (methanol:distilled water 1:3) and chromatographed on a cellulose column (4.5 x 40 cm). The column was eluted with methanol:distilled water (1:3) at a flow rate 1.5 ml/min. Fractions were monitored by TLC, and those containing 6-acetyl-2,4-diamino-7,8-dihydro-9*H*-pyrimido[4,5-b][1,4]diazepine (2) were combined and concentrated under reduced pressure. The resulting solution was cooled in an ice bath, when yellow-green needle-like crystals precipitated (20 mg). A second crop (21 mg) was obtained as yellow-green powder by lyophilisation of the filtrate. The total yield was 41 mg (43%) of pure product, identical with material previously prepared by an alternative synthesis.[4]

Acid treatment of 5-amino-7-(4,4-diethoxy-3-oxopentylamino)furazano[3,4-d]pyrimidine (18)

5-Amino-7-(4,4-diethoxy-3-oxopentylamino)furazano[3,4-d]pyrimidine (18) (138 mg, 0.425 mmol) was dissolved in 1,4-dioxane (7 ml). The resulting solution was acidified to pH 2-3 by addition of dilute sulphuric acid. The reaction was monitored by silica gel TLC plate developed in methanol:chloroform (1:4). After 3 h at 60°C all starting material had reacted. The red reaction mixture was cooled to room temperature and concentrated to 2 ml on a rotary evaporator, and the resulting solution was diluted with a mixture of methanol:chloroform (3:2) (5 ml) and chromatographed on a silica gel flash column, eluting with

methanol:chloroform (3:2). Fractions containing a pure compound were combined and evaporated to dryness. Crystallisation of the residue from water gave pale crystals (30 mg, 26%) m.p. 165°C (decomp.); (Found: C, 40.40; H, 4.40; N, 31.27. Calc for $C_9H_{12}N_6O_4$: C, 40.28; H, 4.51; N, 31.34%); ν_{max}(Nujol) 3 580, 3 360, 1 680, 1 640, 1 605, 1 500, 1 310, 1 220, 1 100, 1 090, 1 045, 1 020, 980, 940, 870, and 860 cm^{-1}; λ_{max}(EtOH) 312 and 233 nm (log ϵ 3.78 and 4.04); δ_H (80 MHz; CD_3SOCD_3) 1.46 (3 H, s, CH_3), 1.82 (2 H, m, $NHCH_2CH_2$), 3.66 (3 H, m, $NHCH_2$ and O*H*), 6.17 (1 H, br s, O*H*, exchangeable with D_2O), 6.67 (1 H, br s, O*H*, exchangeable with D_2O), 7.27 (1 H, br s, N*H*, exchangeable with D_2O) and 9.15 (1 H, br s, N*H*, exchangeable with D_2O).

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