Structural Studies on Bio-active Compounds. Part 3.[1] Re-examination of the Hydrolysis of the Antimalarial Drug Pyrimethamine and Related Derivatives and Crystal Structure of a Hydrolysis Product

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Hydrolysis of the diaminopyrimidine pyrimethamine and some of its 5-(3′-substituted)phenyl derivatives with 6M-hydrochloric acid, or deamination with nitrous acid, affords mixtures of isomeric aminopyrimidinones. The ratio of products is influenced by the nature of the substituent in the phenyl ring.

The mixture of aminopyrimidinones from the hydrolysis of pyrimethamine crystallised from 95% ethanol as a hydrated equimolar duplex. Crystals exhibited monoclinic symmetry, space group $P2_1/n$, with unit cell dimensions a = 24.297(6), b = 7.917(2), c = 13.312(4) Å and β = 98.09(3)°. The two isomeric molecules are linked by three hydrogen bonds in the manner of a Watson-Crick cytosine–guanine base pair.

Elucidation of the structure of folic acid in 1946[2] was the catalyst for the development of a series of 2,4-diamino-1,3-diazines which inhibit the enzyme dihydrofolate reductase (DHFR).[3] Such has been the dominating interest in this type of molecule for nearly four decades that the expression 'antifolate' has become immutably associated with diaminoazines and their inhibition of DHFR. However, Jones[4] has drawn attention to other enzymes in the folate network which are potential targets for rational drug design, particularly of antitumour agents. Intriguingly, there is evidence also that the antitumour activity of small molecule lipophilic diaminopyrimidines cannot be explained by DHFR inhibition alone.[5,6]

In this paper[7] we describe results of our successful efforts to deaminate the amino groups in a simple 2,4-diaminopyrimidine—the antimalarial drug pyrimethamine (**1a**)—in the expectation that this process would destroy its DHFR-inhibitory activity.[8] We also report a crystal structure determination of a hydrolysis product which has an unusual Watson-Crick duplex arrangement.

Hydrolysis of Diaminopyrimidines in 6M-*hydrochloric Acid.*—It has been claimed that hydrolysis of (**1a**) in 6M-hydrochloric acid affords the 2-aminopyrimidin-4(3*H*)-one (**2a**) exclusively.[9] In the present work, analysis of the mixture of hydrochloride salts formed from the hydrolysis of pyrimethamine in boiling 6M-hydrochloric acid (18 h) by ^{1}H n.m.r. (Figure 1) and h.p.l.c. confirms that the product ratio 4-one (**2a**):2-one (**3a**) is 1:2. The starting material is consumed completely and no other products were detected. The component hydrochloride salts were separated by fractional crystallisation from 2M-hydrochloric acid and their structures have been confirmed by *X*-ray crystallography.[10] Basification of the salts liberated aminopyrimidinones, one of which was identical with an authentic sample of (**3a**) prepared by treating the methoxyacrylonitrile (**5**)‡ with urea in sodium ethoxide solution. The free bases (above) and many other pyrimidines described in this paper crystallised from ethanol but retained variable amounts of solvent of crystallisation which was held, tenaciously, even at elevated temperatures (80 °C) *in vacuo*. Elemental microanalysis gave unreliable results on most of these compounds and they were characterised by ^{1}H n.m.r. (Table 1) and mass spectroscopy.

^{1}H N.m.r. spectroscopy also proved to be useful for the analysis of mixtures of the isomeric aminopyrimidinones (**2**) and (**3**). In particular, the methylene group at C(6) of the free bases (in [$^2H_6$]-DMSO) in the 2-aminopyrimidin-4(3*H*)-one series (**2a**—**d**) appeared as a quartet centred at δ 2.24 (±0.04) whereas the comparable signal in the 4-aminopyrimidin-2(1*H*)-one series (**3a**—**d**) was upfield at δ 2.14 (±0.05).

Nitration of pyrimethamine (**1a**) in a nitric–sulphuric acid mixture at 45 °C gave the 3′-nitro derivative (**1b**) which crystallised from dimethylformamide as a solvate. The unsolvated nitropyrimethamine was hydrolysed in 6M-hydrochloric acid to a mixture of (**2b**) and (**3b**) in the ratio 1:1.2 (analysed by ^{1}H n.m.r. spectroscopy). Separate nitrations of the free bases of aminopyrimidinones (**2a**) and (**3a**) gave authentic samples of the corresponding nitroaminopyrimidinones (**2b**) and (**3b**).

Introduction of the 3′-nitro group activated the 4′-chloro substituent of pyrimethamine towards nucleophiles. The piperidinopyrimidine (**1c**) formed from (**1b**) and boiling piperidine, crystallised as a solvate from dimethylformamide and afforded a cream dihydrochloride salt in cold ethanolic 10M-hydrochloric acid. The dihydrochloride dissociated in water to form red prisms of a monohydrochloride monohydrate; the structure of the latter has been confirmed by *X*-ray crystallography.[10] No evidence for the production of benzimidazoles by intervention of an '*ortho*-nitro interaction'[11,12] was adduced in the 6M-hydrochloric acid hydrolysis of (**1c**): the only products were the isomeric pyrimidinones (**2c**) and (**3c**) which were formed in a ratio of 1:0.4 (^{1}H n.m.r.). The isomers were separated by exploiting the differential solubilities of the respective free bases. Nitropyrimidin-4-one (**2c**) was soluble in cold acetone whereas the isomeric nitropyrimidin-2-one (**3c**) was insoluble. The structures of the bases were corroborated by independent syntheses from piperidine and the chloronitropyrimidinones (**2b**) and (**3b**), respectively.

Reduction of nitropyrimethamine (**1b**) with stannous chloride–hydrochloric acid or hydrazine–Raney nickel afforded the corresponding amine (**1d**) which when hydrolysed with 6M-hydrochloric acid yielded a mixture of the 4-one (**2d**) and 2-one (**3d**) in a ratio 1:0.8 (^{1}H n.m.r.) although the individual components of the mixture were not separated.

Trattner and colleagues[9] compared the hydrolysis of

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‡ *Geometrical configuration unknown, but depicted in the Z-geometry for ease of visualisation of the reaction.*

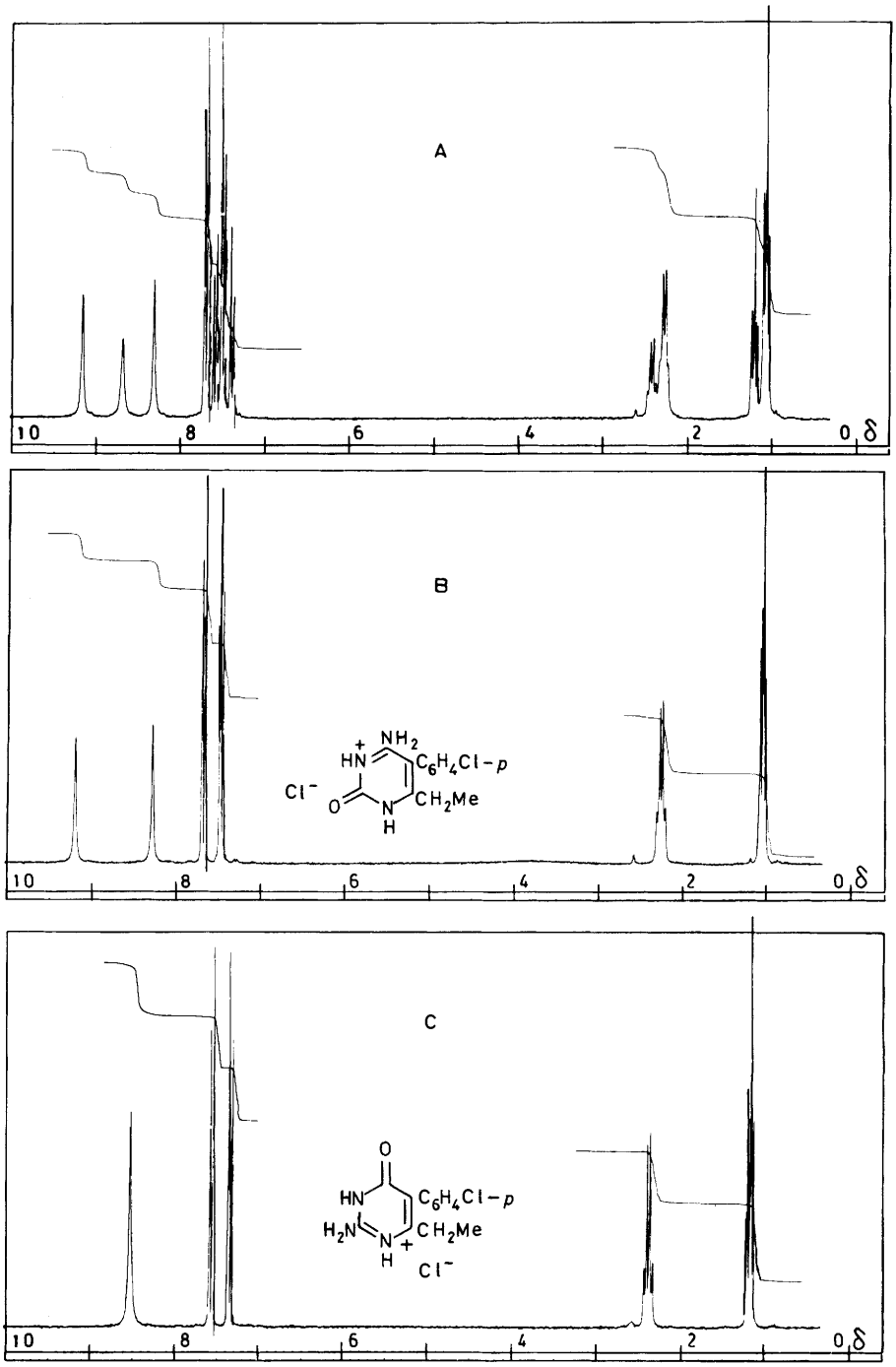
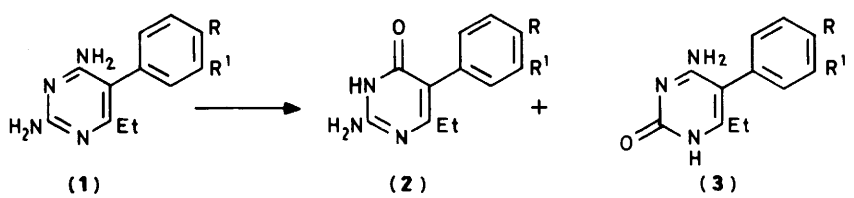


Figure 1. 220 MHz ^{1}H N.m.r. spectra of A: mixture of hydrochloride salts formed from the 6M-hydrochloric acid hydrolysis of pyrimethamine (**1a**); B: authentic 4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2(1*H*)-one hydrochloride; C: authentic 2-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-4(3*H*)-one hydrochloride. All spectra were recorded in [$^2H_6$]DMSO.



a; R = Cl, R′ = H
b; R = Cl, R′ = NO_2
c; R = NC_5H_{10}, R′ = NO_2
d; R = Cl, R′ = NH_2

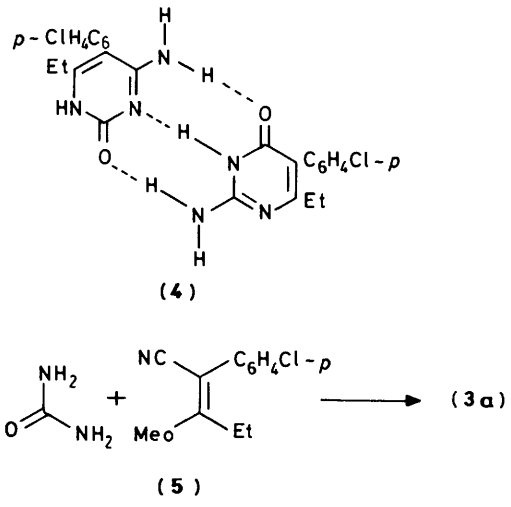
Table 1. ^{1}H N.m.r. spectra[a] (δ values) of diaminopyrimidines and aminopyrimidinones

Compound	Solvent	Me[c]	CH_2[a]	2′-H	3′-H	5′-H	6′-H	Other[b]
(**1a**)	A	0.96	2.12	7.25 d and 7.53 d: AA′BB′				5.62 (2 H, NH) 5.91 (2 H, NH)
(**1a**) (hydrochloride)	A	1.00	2.27	7.38 d and 7.63 d: AA′BB′				6.95 (1 H, NH) 7.81 (2 H, NH) 8.22 (1 H, NH) 13.20 (1 H, NH^+)
(**1b**) (DMF solvate)	A	1.00	2.17	7.94 d	—	7.86 d	7.59 dd	2.77 (3 H, s, NMe) 2.93 (3 H, s, NMe) 6.01 (2 H, NH) 6.10 (2 H, NH) 8.02 (1 H, s, *H*CONMe$_2$)
(**1c**)	A	1.00	2.15	7.59 br s	—	7.37 m		1.62 (6 H, br s, $[CH_2]_3$) 3.04 (4 H, br s, $[CH_2]_2$) 5.67 (2 H, NH) 5.87 (2 H, NH)
(**1c**) (3 × DMF solvate)	A	0.98	2.14	7.58 br s	—	7.36 m		1.60 (6 H, br s, $[CH_2]_3$) 2.74 (9 H, s, 3 × NMe) 2.90 (9 H, s, 3 × NMe) 3.02 (4 H, br s, $[CH_2]_2$) 5.69 (2 H, NH) 5.88 (2 H, NH) 7.98 (3 H, s, 3 × *H*CONMe$_2$)
(**1c**) (hydrochloride hydrate)	A	1.12	2.29	7.76 d	—	7.41 d	7.49 dd	1.62 (6 H, br s, $[CH_2]_3$) 3.07 (4 H, br s, $[CH_2]_2$) 7.1 (2 H, NH) 8.2 (2 H, NH)
(**1c**) (dihydrochloride)	A	1.14	2.31	7.79 d	—	7.40 m		1.64 (6 H, br s, $[CH_2]_3$) 3.13 (4 H, br s, $[CH_2]_2$) 5 35 (2 H, NH) 7.13 (1 H, NH) 8.23 (1 H, NH)
(**1d**)[c]	A	1.00	2.18	6.65 d	—	7.25 d	6.38 dd	5.35 (2 H, NH) 5.69 (2 H, NH) 5.90 (2 H, NH)
(**1d**) (hydrochloride)	A	1.12	2.30	6.68 d	—	7.28 d	6.40 dd	5.50 (2 H, NH) 7.7 (1 H, NH)
(**2a**)	A	1.04	2.23	7.27 d and 7.47 d: AA′BB′				6.72 (2 H, NH) 10.95 (1 H, NH)
(**2a**) (hydrochloride)	A	1.16	2.39	7.35 d and 7.57 d: AA′BB′				8.52 (2 H, NH) 12.9 (2 H, NH)
(**2b**)	A	1.07	2.28	7.95 d	—	7.80 d	7.60 dd	6.79 (3 H, NH)
(**2b**)[d]	B	1.10	2.43	8.47 d	—	8.24 d	8.07 dd	*e*
(**2c**)	A	1.03	2.23	7.56 d	—	7.38 d	7.24 d	1.63 (6 H, br s, $[CH_2]_3$) 3.0 (4 H, br s, $[CH_2]_2$) 6.58 (2 H, NH) 10.60 (2 H, NH)
(**2d**)[f]	A	1.03	2.22	6.69 d	—	7.18 d	6.39 dd	*g*
(**3a**)	A	0.99	2.10	7.31 d and 7.55 d: AA′BB′				5.9 (3 H, NH)
(**3a**) (hydrochloride)	A	1.04	2.26	7.46 d and 7.67 d: AA′BB′				3.5—5.0 (1 H, NH) 8.29 (1 H, NH) 9.20 (1 H, NH) 12.39 (1 H, NH^+)
(**3b**)	A	1.00	2.13	7.98 d	—	7.85 d	7.61 dd	6.2 (1 H, NH) 7.15 (2 H, NH)

Table 1 (*continued*)

Compound	Solvent	Me[c]	CH_2[a]	2′-H	3′-H	5′-H	6′-H	Other[b]
(**3b**)[d]	B	1.03	2.33	8.60 d	—	8.36 d	8.15 dd	*e*
(**3c**)	A	0.95	2.11	7.65 d	—	7.35 d	7.42 dd	1.61 (6 H, br s, $[CH_2]_3$) 3.03 (4 H, br s, $[CH_2]_2$) 10.6 (3 H, NH)
(**3c**) (3 × DMF solvate)	A	1.00	2.13	7.66 d	—	7.35 d	7.43 dd	1.62 (6 H, br s, $[CH_2]_3$) 2.76 (9 H, s, 3 × NMe) 2.92 (9 H, s, 3 × NMe) 3.05 (4 H, br s, $[CH_2]_2$) 8.02 (3 H, s, 3 × *H*CONMe$_2$) 10.6 (3 H, NH)
(**3d**)[f]	A	1.03	2.19	6.73 d	—	7.35 d	6.47 dd	*g*

Solvents; A, [$^2H_6$]DMSO; B, DCl–D_2O.
[a] Spectra were recorded on a 220 MHz Perkin-Elmer R34 spectrometer. [b] All NH absorptions appeared as broad singlets, exchangeable with D_2O. [c] Anhydrous base. [d] Chemical shifts abstracted from the spectrum of the mixture formed from the 6M-hydrochloric acid hydrolysis of 2,4-diamino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidine. [e] All NH protons exchanged. [f] Chemical shifts abstracted from the spectrum of the mixture formed from the 6M-hydrochloric acid hydrolysis of 2,4-diamino-5-(3-amino-4-chlorophenyl)-6-ethylpyrimidine. [g] NH protons unassigned.
t = triplet, q = quartet, d = doublet, dd = double doublet, m = multiplet, br s = broad singlet.



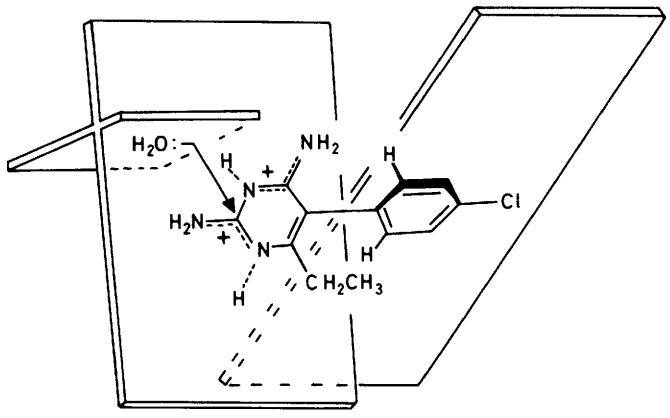


Figure 2. Hydrolysis of N(1), N(3) diprotonated pyrimethamine (**1a**) at the C(2) position.

pyrimethamine with that of several planar fused-ring diaminopyrimidines (2,6-diaminopurine, 2,4-diamino-7-butyl-6-propylpyrido[2,3-*d*]pyrimidine, 2,4-diaminoquinazoline and 5,7-diamino-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidine). In all these latter cases the 4-amino group* in the pyrimidine ring was claimed to be more vulnerable to acid hydrolysis than the 2-amino group. This selectivity was rationalised on the grounds that the tetrahedral intermediates formed by attack of water at the 4-amino groups of the protonated fused diaminopyrimidines are stabilised by '*para*-quinoid' mesomeric forms whereas attack at the 2-amino positions acquires less-favoured '*ortho*-quinoid' stabilisation. This is clearly not so in the case of pyrimethamine.

The initial protonation of pyrimethamine occurs at N(1)[13] with a pK_a of 7.34;[14] in the 6M-hydrochloric acid medium the pyrimethamine is presumably in the N(1), N(3) diprotonated form. Preferential nucleophilic attack at C(2) may be determined, in part, by the shielding effect of the hydrophobic chlorophenyl group which would repel orthogonal approach of water at C(4) (Figure 2).

* Position numbered as in a monocyclic pyrimidine for convenient comparison with pyrimethamine.

The 3′-nitro group in nitropyrimethamine (**1b**) weakly influences the isomer ratio of aminopyrimidinones formed on acidic hydrolysis (Table 2) but this effect is not as marked as that elicited by an additional protonated basic substituent (**1c**) and (**1d**) where the 4-one isomers (**2c**) and (**2d**) now become the major products at the expense of the 2-one products (**3c**) and (**3d**). An *X*-ray crystallographic analysis of the monohydrochloride (monohydrate) salt of (**1c**)[10] confirms that the pyrimidinyl and aryl rings achieve greater coplanarity (angle τ = 57°) than that found in the monocation of pyrimethamine (67°).[13] This could allow for easier attack at C(4) in this case, although this explanation should be regarded as tentative.

Deamination of Diaminopyrimidines with Nitrous Acid.— Interaction of pyrimethamine (**1a**) in hot 1M-sulphuric acid with nitrous acid under standard conditions employed by Trattner *et al*[9] did not yield exclusively the 2-one (**3a**) as previously claimed, but a mixture of 4-one (**2a**) and 2-one (**3a**). Because of the presence of substantial amounts of unchanged pyrimethamine (*ca.* 50%) analysis of the mixture by ^1H n.m.r. was not possible: h.p.l.c. analysis gave a ratio (**2a**):(**3a**) of 1:1.5. Crystallisation of the mixture from 95% ethanol gave an initial crop of crystals of the hydrate of duplex (**4**). Deamination of the

Table 2. Products formed on deamination of 2,4-diaminopyrimidines.

Starting material	Reaction conditions	Products (Yield)
(**1a**)	A	(**2a**) (33%)[a]:(**3a**) (67%)
	B	(**2a**) (40%)[b]:(**3a**) (60%)[a,b]
(**1b**)	A	(**2b**) (45%):(**3b**) (55%)
(**1c**)	A	(**2c**) (70%):(**3c**) (30%)
(**1d**)[c]	A	(**2d**) (55%):(**3d**) (45%)

Reaction conditions: A – Substrate (0.01 mol equiv.) boiled in 6M-hydrochloric acid (25 ml) for 18 h. Mixture basified with aqueous ammonia–ice; products collected and analysed by ^{1}H n.m.r. (see Table 1). B – See deamination of 2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine (**1a**) as in reference 9.

[a] Trattner *et al.*[9] record exclusive formation of this isomer under stated conditions. [b] Excluding unchanged starting material in yield calculation. Mixture analysed by h.p.l.c. [c] Anhydrous base.

nitro (**1b**) and piperidine derivative (**1c**) under the same conditions was incomplete. T.l.c. examination of the mixtures confirmed the presence of isomeric aminopyrimidinones and in both cases the respective 2-ones (**3b**) and (**3c**) predominated. Repeated crystallisation of the mixture produced from the nitrous acid deamination of nitropyrimethamine (**1b**) afforded a meagre yield of pure aminopyrimidinone (**3b**). The aminopyrimethamine (**1d**) was not subjected to nitrous acid deamination because of competing diazotisation of the arylamine substituent.

Base-Pairing in Isomeric Aminopyrimidinones.—That the recrystallised product from the hydrolysis or deamination of pyrimethamine (**1a**) might be other than a simple mixture was indicated by h.p.l.c. analysis, which showed that the ratio of isomers (**2a**):(**3a**) was precisely 1:1. Confirmation of its duplex nature (see later) was obtained by its near quantitative preparation by co-crystallisation of an equimolar mixture of the two isomers from ethanol.

In the mixture of neutral molecules there are alternatives to duplex formation: by co-crystallisation of the 1*H* and 3*H* tautomers of either isomer on its own it is still possible to form three hydrogen bonds between molecules, as is observed[15] in crystalline isocytosine (**7**). In the hydrochloride salts the absence of duplex formation was expected since protonation at N(3) in the 4-aminopyrimidin-2(1*H*)-one isomer (**3a**) would inhibit H-bonding in the Watson-Crick sense.

Attempts to effect cross-crystallisations were also unsuccessful: thus the 4-one (**2c**) failed to co-crystallise with the 2-one (**3a**) in 95% ethanol. Our efforts to exploit the 2-one (**3a**) as a Watson-Crick H-bonding crystallisation aid for intransigently uncrystallisable folate compounds was not realised because, disappointingly, it failed to co-crystallise with either folic acid or the novel quinazoline thymidylate synthetase inhibitor CB 3717 (**6**).[4] To our knowledge this approach to the crystallisation of folates had not been attempted previously.

Crystal Structure of the Hydrated Duplex (4).

Crystal Data.—$C_{12}H_{12}N_3OCl{\cdot}C_{12}H_{12}N_3OCl{\cdot}H_2O$, M = 517.4. Monoclinic, a = 24.297(6), b = 7.917(2), c = 13.312(4) Å, β = 98.09(3)°, V = 2 535.3 Å^3 (by least-squares refinement on diffractometer angles for 25 automatically centred reflections, λ = 0.710 69 Å), space group $P2_1/n$ (alt. $P2_1/c$, No. 14), D_m = 1.34 g cm^{-3}, Z = 4, D_x = 1.355 g cm^{-3}. Colourless tabular or platey crystals from ethanol. Crystal dimensions 0.28 × 0.16 × 0.04 mm, μ(Mo-K_α) = 2.44 cm^{-1}.

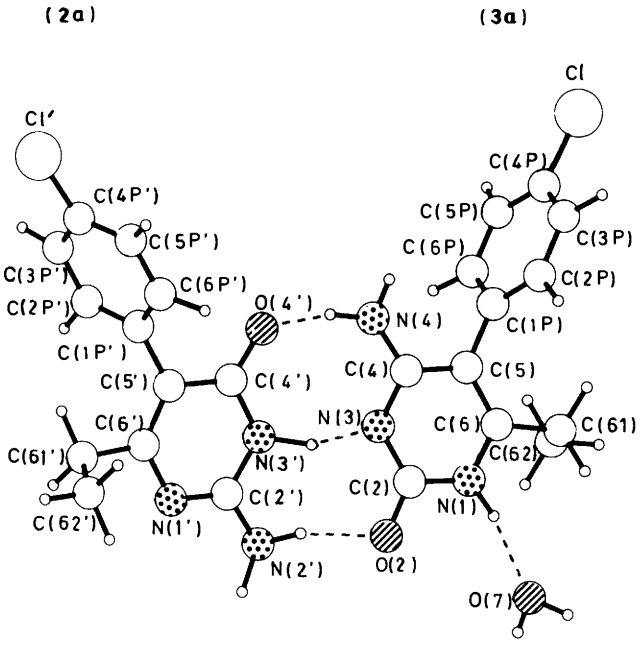


Figure 3. PLUTO[17] drawing of the hydrated base-pair projected onto its least-squares plane.

Data Collection and Processing.—CAD4 diffractometer, $\omega/2\theta$ mode with scan width = 0.88 + 0.35 tan θ, scan speed 0.16 to 3.4° min^{-1}, graphite-monochromated Mo-K_α radiation; 2 669 reflections measured ($2.0 \leqslant \theta \leqslant 20.5°$, $\pm h$, $-k$, $-l$), 2 529 unique (merging R = 0.033, no absorption correction made), 1 486 considered observed with $F < 1.5\sigma(F)$. Linear and isotropic crystal decay, *ca.* 10%, corrected during processing.

Structure Analysis and Refinement.—Direct methods revealed all non-hydrogen atoms, which were input to least-squares refinement with carbon scattering factors. Isotropic temperature factor values and bond distances clearly indicated N and O atoms. Hydrogen positions found in difference electron density syntheses for heterocyclic and amino H atoms, calculated for ethyl and phenyl. Final refinement by full-matrix least-squares with non-hydrogen atoms anisotropic, H atom positions fixed, common U_{iso} refined for methyl (= 0.146 Å^2) and for all other (= 0.100 Å^2) H atoms. The weighting scheme $w = 1/\sigma^2 + 0.006\,718\,F_o^2$) gave satisfactory agreement analysis. Final R and R_w values are 0.072 and 0.083 for observed data; standard deviations for non-hydrogen bond distances, *ca.* 0.01 Å; for bond angles, 0.7—0.9°. All computer programs and scattering factor data were used from the SHELX system.[16]

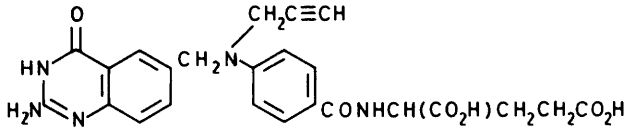
Results and Discussion.—Figure 3 is a PLUTO[17] drawing of the two isomers, 2-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-4(3*H*)-one (**2a**) and 4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2(1*H*)-one (**3a**), and the water molecule which together constitute the asymmetric unit. The numbering scheme adopted for the crystallographic work is also shown. Final atomic co-ordinates are presented in Table 3 except for hydrogen atom positions, which have been treated as part of a supplementary

Table 3. Final fractional co-ordinates ($\times 10^4$) and equivalent isotropic temperature factors ($\times 10^3$) for isomers (**3a**) (unprimed) and (**2a**) (primed)

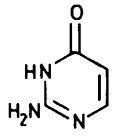
Atom	*x*	*y*	*z*	U
N(1)	4 810(3)	4 441(9)	−1 805(5)	48(3)
C(2)	4 660(4)	3 728(12)	−930(8)	51(4)
O(2)	4 906(3)	2 417(8)	−592(5)	60(3)
N(3)	4 242(3)	4 422(9)	−500(5)	51(3)
C(4)	3 990(4)	5 809(12)	−930(7)	49(4)
N(4)	3 588(4)	6 430(10)	−461(6)	72(3)
C(5)	4 124(4)	6 549(10)	−1 844(7)	48(4)
C(6)	4 548(4)	5 805(11)	−2 253(6)	47(3)
C(61)	4 742(4)	6 395(12)	−3 224(7)	61(4)
C(62)	4 454(5)	5 456(18)	−4 111(9)	105(6)
C(1P)	3 819(4)	8 059(11)	−2 271(6)	44(3)
C(2P)	4 048(4)	9 654(13)	−2 131(8)	67(4)
C(3P)	3 757(4)	11 054(12)	−2 544(8)	68(4)
C(4P)	3 251(4)	10 892(11)	−3 104(7)	48(3)
C(5P)	3 009(4)	9 331(13)	−3 238(7)	59(4)
C(6P)	3 296(4)	7 922(12)	−2 820(8)	56(4)
Cl	2 917(1)	12 686(3)	−3 628(2)	85(1)
O(7)	5 678(3)	2 924(7)	−2 623(5)	70(3)
N(1′)	4 036(3)	477(8)	2 370(6)	51(3)
C(2′)	4 167(4)	1 261(11)	1 569(8)	48(4)
N(2′)	4 493(3)	565(8)	968(6)	54(3)
N(3′)	4 000(3)	2 880(9)	1 329(5)	46(3)
C(4′)	3 629(4)	3 725(12)	1 847(7)	50(4)
O(4′)	3 464(3)	5 171(8)	1 538(5)	61(3)
C(5′)	3 466(3)	2 849(11)	2 693(6)	40(3)
C(6′)	3 687(4)	1 294(11)	2 924(7)	55(4)
C(61′)	3 587(5)	326(12)	3 860(9)	76(5)
C(62′)	4 101(6)	270(19)	4 659(9)	117(6)
C(1P′)	3 079(3)	3 718(11)	3 298(6)	42(3)
C(2P′)	2 544(4)	3 126(11)	3 325(7)	53(4)
C(3P′)	2 198(4)	3 894(12)	3 911(7)	55(4)
C(4P′)	2 385(4)	5 280(11)	4 505(7)	48(3)
C(5P′)	2 901(4)	5 940(11)	4 460(7)	52(4)
C(6P′)	3 247(4)	5 162(12)	3 871(7)	54(4)
Cl′	1 976(1)	6 125(3)	5 351(2)	69(1)

Table 4. Bond distances (Å) with estimated standard deviations in parentheses

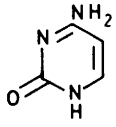
	Unprimed	Primed
N(1)–C(2)	1.39(1)	1.31(1)
C(2)–O(2)	1.25(1)	
[C(2′)–N(2′)]		1.32(1)
C(2)–N(3)	1.35(1)	1.37(1)
N(3)–C(4)	1.35(1)	1.38(1)
C(4)–N(4)	1.33(1)	
[C(4′)–O(4′)]		1.26(1)
C(4)–C(5)	1.43(1)	1.43(1)
C(5)–C(6)	1.37(1)	1.36(1)
C(5)–C(1P)	1.48(1)	1.49(1)
C(6)–N(1)	1.35(1)	1.36(1)
C(6)–C(61)	1.51(1)	1.51(1)
C(61)–C(62)	1.49(2)	1.52(2)
C(1P)–C(2P)	1.38(1)	1.39(1)
C(1P)–C(6P)	1.38(1)	1.40(1)
C(2P)–C(3P)	1.39(1)	1.37(1)
C(3P)–C(4P)	1.35(1)	1.39(1)
C(4P)–C(5P)	1.37(1)	1.37(1)
C(4P)–Cl	1.73(1)	1.74(1)
C(5P)–C(6P)	1.39(1)	1.37(1)



(**6**)



(**7**)



(**8**)

publication [Sup. No. 56285 (5 pp.)].* Tables 4, 5, 6, and 7 contain bond distances, bond angles, least-squares planes, and a list of hydrogen bond contacts.

Difference electron density maps clearly show that hydrogen atoms are bound to ring nitrogen atom N(3′) in isomer (**2a**) and on ring atom N(1) in isomer (**3a**). This conclusion is further strengthened by the intra-annular bond angles at N atoms, which are $>120°$ only for C(6)–N(1)–C(2) and C(4′)–N(3′)–C(2′). Thus isomer (**2a**) resembles one of the tautomeric forms of isocytosine (**7**) present in the crystalline state[15] while isomer (**3a**) is analogous to cytosine (**8**). A comparison of (**3a**) and (**8**)[18] reveals no difference exceeding 0.02 Å between the length of analogous bonds except for C(5)–C(6) which is lengthened presumably by repulsion of bulky substituents in (**3a**); isomer (**2a**) and (**7**) differ even less. Bond angles within the pyrimidine rings of (**3a**) and (**8**) agree to within 2°; so do corresponding angles in (**2a**) and (**7**).

As is the case with similar molecules,[13] the phenyl and pyrimidinyl rings are prevented by steric hindrance from attaining coplanarity. In (**3a**) where the phenyl ring abuts amino and ethyl groups, the angle between least-squares planes through the two rings is 78°. In (**2a**) where the adjoining substituents are the less sterically demanding carbonyl oxygen atom as well as the ethyl group, the corresponding angle decreases to 67°. Deviations from the ring planes (Table 6) indicate slight puckering of the pyrimidine ring in (**3a**) and somewhat more puckering in (**2a**); in both cases the most profound effect is on the 6-ethyl substituent.

One molecule of (**2a**) joins with one molecule of (**3a**) to form a triply hydrogen-bonded base pair. The hydrogen bonds are of the same nature as those in a guanine:cytosine base pair. They are almost identical with those in the pyrimidine:pyrimidine base pair formed by the two tautomeric forms of isocytosine[15] (Table 7). The least-squares planes of the two pyrimidine ring partners intersect at a 14° angle. Isomer (**2a**) is a 3*H* tautomer like ring B of isocytosine and plays a similar role in hydrogen bonding. The pyrimidine ring of (**3a**) is reflected about N(3)···C(6) with respect to ring A of isocytosine, thereby achieving similar positions for the carbonyl and amino groups in both base pairs. Once the triply hydrogen-bonded base-pair is formed, further hydrogen bonding is still possible by proton donation from H(1), H(4B), and H(2A′), and proton acceptance by N(1′), O(2), and O(4′). Different base pairs are linked by N(2′)H(2A′)···O(2) bonding. A water molecule associates with H(1) and orients its protons toward N(1′) and in the general direction of O(4′). Only H(4B) is left unused in the intricately hydrogen-bonded structure. Two pyrimidine rings of

* For details of the Supplementary publications scheme, see Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1985, Issue 1.

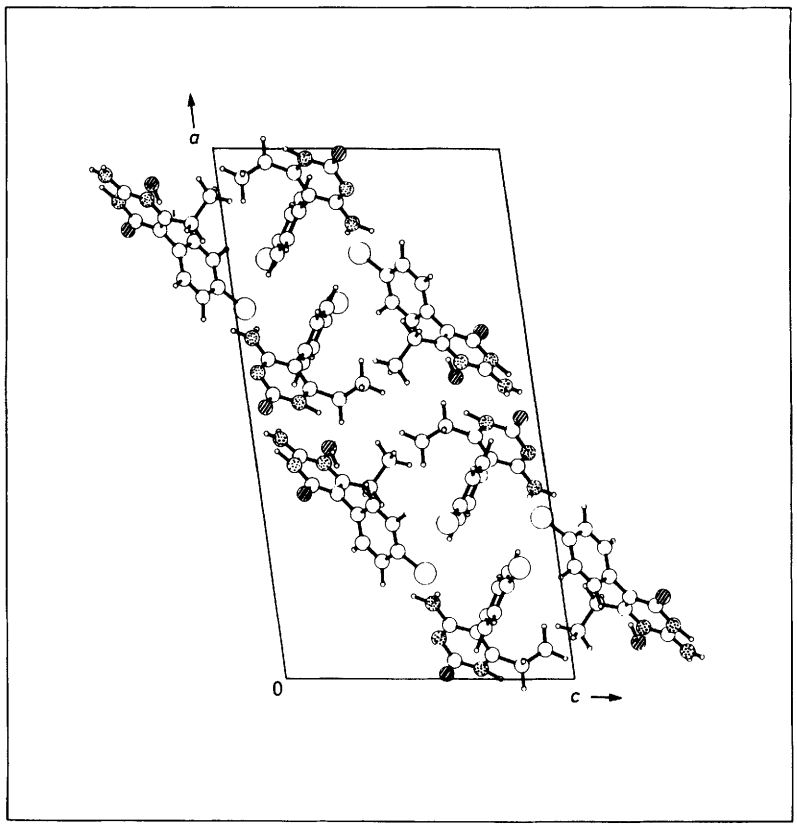


Figure 4. PLUTO[17] drawing of the crystal structure projected onto the *a*—*c* plane. Nitrogen atoms are stippled and oxygen atoms are hatched.

(**3a**) are brought into close proximity by the symmetry operation $1 - x$, $1 - y$, $-z$. The ring planes are parallel and separated by 3.31 Å, but only the C(2)–N(3) bonds come close to overlapping. Atom C(2) is expected to be electron-deficient. The crystal packing (Figure 4) creates domains of saturated hydrocarbon chains (near the centre), chlorophenyl moieties (upper left and lower right) and hydrogen-bonded pyrimidines.

Experimental

Ethanol refers to 95% ethanol and ether to diethyl ether. All melting points are uncorrected. U.v. spectra were recorded on a Pye Unicam SP8000 Ultraviolet Recording Spectrometer and i.r. spectra on a Unicam SP200 Infrared Spectrometer as potassium bromide discs.

The t.l.c. systems employed Kieselgel $60F_{254}$ (0.25 mm) as the adsorbent and either toluene–acetone (10:3), toluene–acetone–methanol (1:3:3) or toluene–acetone–ethanol (7:3:3) as the developing solvent.

H.p.l.c. separations were conducted on a C_{18} ODS column with a 5 mm internal diameter (radial pack compression system). The solvent system was methanol–phosphate buffer pH 4.5 (60:40) with a flow rate of 2 ml min^{-1} and u.v. detection at 280 nm.

2,4-*Diamino*-5-(4-*chlorophenyl*)-6-*ethylpyrimidine* (*Pyrimethamine*) (**1a**).—Supplies of pyrimethamine were obtained from The Wellcome Foundation Ltd., Dartford, Kent. The hydrochloride salt was prepared from the base by crystallisation from 2M-hydrochloric acid, m.p. > 320 °C; $\nu_{max.}$ 3 350br and 3 150 (NH); $\lambda_{max.}$ (2M-hydrochloric acid) 223 and 277 nm.

2,4-*Diamino*-5-(4-*chloro*-3-*nitrophenyl*)-6-*ethylpyrimidine* (**1b**).—Pyrimethamine (**1a**) (5.0 g) was added (10 min) to a stirred mixture of nitric acid (*d* 1.42; 15 ml) and sulphuric acid (15 ml). The mixture was heated at 50 °C (1 h) and then kept at 25 °C (18 h). The syrupy golden solution was poured into concentrated aqueous ammonia–ice and the cream solid collected. The product crystallised from aqueous ethanol as yellow rosettes, m.p. 203—205 °C[19] [Found: M^+, 293 (295). Calc. for $C_{12}N_{12}ClN_5O_2$: *M*, 293 (295)]; $\nu_{max.}$ 3 450, 3 290, and 3 100br (NH), and 1 530 and 1 340 cm^{-1} (NO_2); $\lambda_{max.}$(EtOH) 218 and 287 nm.

Crystallisation from aqueous dimethylformamide furnished thick yellow needles of a *dimethylformamide solvate*, m.p. 222—225 °C (sinters 175 °C) (Found: C, 49.3; H, 5.4; N, 23.1. $C_{12}H_{12}ClN_5O_2$. C_3H_7NO requires C, 49.1; H, 5.2; N, 22.9%); $\nu_{max.}$ 3 440 and 3 180 (NH), 1 666 (CO), and 1 535 and 1 360 cm^{-1} (NO_2); $\lambda_{max.}$(EtOH) 218 and 287 nm. The solvate reverted to the unsolvated form when crystallised from aqueous ethanol.

2,4-*Diamino*-6-*ethyl*-5-(3-*nitro*-4-*piperidinophenyl*)*pyrimidine* (**1c**).—A mixture of 2,4-diamino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidine (9.0 g) and piperidine (15 ml) was boiled (1 h) and poured into water. The red nitropiperidinopyrimidine (10.2 g) crystallised from a large volume of ethanol as red prisms, m.p. 248—250 °C (Found: M^+, 342. Calc. for $C_{17}H_{22}N_6O_2$: *M*, 342); $\nu_{max.}$ 3 500, 3 360, and 3 200br (NH), 2 980 and 2 860 (CH), and 1 532 and 1 350 cm^{-1} (NO_2); $\lambda_{max.}$(EtOH) 260, 290infl., and 417 nm.

The nitropiperidinopyrimidine crystallised from dimethylformamide as a trisdimethylformamide solvate, orange microneedles, m.p. 248—250 °C; $\nu_{max.}$ 3 490, 3 390 and 3 140br (NH),

2 940, 2 880 and 2 810 (CH), 1 665 (CO), and 1 550 and 1 345 cm^{-1} (NO_2).

The nitropiperidinopyrimidine furnished a *dihydrochloride salt* when crystallised from ethanol–10M-hydrochloric acid (3:1). The salt formed cream prisms, m.p. 275 °C (decomp., went orange at 140 °C) (Found: N, 20.0. $C_{17}H_{22}N_6O_2 \cdot 2HCl$ requires N, 20.24%); $\nu_{max.}$ 3 430br and 3 200br (NH), and 1 555 and 1 355 cm^{-1} (NO_2); $\lambda_{max.}$ (2M-hydrochloric acid) 217 and 272 nm. Crystallisation of the dihydrochloride salt from water afforded red needles of the *monohydrochloride hydrate*, m.p. 275 °C (decomp., went orange at 140 °C) (Found: C, 51.7; H, 6.5; N, 21.0. $C_{17}H_{22}N_6O_2 \cdot HCl \cdot H_2O$ requires C, 51.45; H, 6.3; N, 21.2%); $\nu_{max.}$ 3 500, 3 320, and 3 150br (NH), 1 520 and 1 340 cm^{-1} (NO_2); $\lambda_{max.}$(water) 209, 257, and 431 nm.

2,4-*Diamino*-5-(3-*amino*-4-*chlorophenyl*)-6-*ethylpyrimidine* (**1d**) (*with E. A. Bliss*).—The nitropyrimethamine (**1b**) (16.0 g) was added in small portions (over 15 min) to a stirred solution of tin(II) chloride dihydrate (38 g) in 10M-hydrochloric acid (160 ml) at 5—10 °C. The mixture was stirred overnight and the white stannic complex collected. A solution of the complex in hot water was basified to pH 12 with 10M-sodium hydroxide–ice. The white solid was collected and crystallised from 100% ethanol to yield amber prisms of the *anhydrous base* (9.5 g),[19] m.p. 215—217 °C; $\nu_{max.}$ 3 480, 3 320, and 3 180br (NH); $\lambda_{max.}$(EtOH) 213, 240 infl., and 291; $\lambda_{max.}$ (2M-hydrochloric acid) 216 and 273 nm [Found: C, 54.4; H, 5.1; N, 26.4%; M^+, 263 (265). $C_{12}H_{14}ClN_5$ requires C, 54.6; H, 5.3; N, 26.7%; *M*, 263 (265)].

A sample of the white solid was crystallised from 50% aqueous ethanol to afford colourless needles of the *amine hydrate*, m.p. 215—217 °C [Found: C, 50.0; H, 5.4; N, 24.9%; M^+, 263 (265). $C_{12}H_{14}ClN_5 \cdot 1.25H_2O$ requires C, 50.4; H, 5.8; N, 24.5%; M^+, 263 (265)]; $\nu_{max.}$ 3 480br and 3 200 (NH), 1 655 (H_2O).

The nitropyrimethamine (**1b**) (2.0 g) in ethanol (50 ml) was reduced with Raney nickel (2 g) and hydrazine hydrate (20 ml, added in portions over 90 min at 60—65 °C). Removal of catalyst by filtration through Kieselguhr gave a colourless solution which was evaporated to dryness. The residue was triturated with water, collected, and dried at 110 °C for 48 h. The product (1.5 g) was identical (i.r.) with a sample of the amine anhydrous base (see above).

The *hydrochloride salt* of (**1d**) was prepared by dissolving the base in hot 1M-hydrochloric acid from which pale yellow needles deposited, m.p. 310 °C (with darkening over 280 °C); $\nu_{max.}$ 3 340, 3 170 (NH), and 2 900br (protonated NH); $\lambda_{max.}$(H_2O) 209, 230 infl. and 280 nm.

4-*Amino*-5-(4-*chlorophenyl*)-6-*ethylpyrimidin*-2(1H)-*one* (**3a**).—A solution of α-propionyl-4-chlorophenylacetonitrile (18

Table 5. Bond angles (°) with standard deviations in parentheses

	Unprimed	Primed
C(6)–N(1)–C(2)	122.2(7)	117.1(7)
N(3)–C(2)–N(1)	119.5(9)	122.4(9)
O(2)–C(2)–N(1)	118.1(9)	
[N(2′)–C(2′)–N(1′)]		122.0(8)
N(3)–C(2)–O(2)	122.4(9)	
[N(3′)–C(2′)–N(2′)]		115.6(9)
C(4)–N(3)–C(2)	118.4(8)	122.0(8)
C(5)–C(4)–N(3)	123.5(8)	115.6(8)
N(4)–C(4)–N(3)	115.1(8)	
[O(4′)–C(4′)–N(3′)]		118.4(8)
C(5)–C(4)–N(4)	121.4(8)	
[C(5′)–C(4′)–O(4′)]		126.0(9)
C(6)–C(5)–C(4)	116.0(8)	118.3(8)
C(1P)–C(5)–C(4)	119.8(8)	118.0(8)
C(1P)–C(5)–C(6)	124.2(8)	123.7(8)
C(5)–C(6)–N(1)	120.3(8)	124.3(8)
C(61)–C(6)–N(1)	116.2(8)	113.0(8)
C(61)–C(6)–C(5)	123.5(8)	122.7(9)
C(62)–C(61)–C(6)	110.9(8)	112.3(9)
C(6P)–C(1P)–C(5)	121.0(8)	120.9(8)
C(2P)–C(1P)–C(5)	121.1(8)	121.5(8)
C(6P)–C(1P)–C(2P)	117.8(8)	117.6(8)
C(3P)–C(2P)–C(1P)	120.3(8)	121.2(8)
C(4P)–C(3P)–C(2P)	121.1(9)	119.8(8)
C(5P)–C(4P)–C(3P)	119.9(8)	120.3(8)
Cl–C(4P)–C(3P)	118.8(7)	120.1(7)
Cl–C(4P)–C(5P)	121.3(7)	119.5(8)
C(6P)–C(5P)–C(4P)	119.4(8)	119.5(8)
C(5P)–C(6P)–C(1P)	121.5(9)	121.4(8)

Table 6(a). Mean planes through various groups of atoms, expressed in terms of direction cosines, *l*,*m*,*n* with respect to orthogonal axes along *a**, *b*, and *c* respectively

			l	*m*	*n*	*d*
(**3a**)	Pyrimidine ring atoms	I	0.6602	0.5920	0.4622	7.8409
	Benzene ring atoms	II	−0.4168	0.1119	0.9021	−7.0118
	Angle between the planes 78°					
(**2a**)	Primed pyrimidine ring	I′	0.7856	0.3822	0.4865	8.6139
	Primed benzene ring	II′	0.3562	−0.5915	0.7233	3.2954
	Angle between the planes 66°					

Table 6(b). Deviations ($\times 10^3$) in Å of atoms from the planes; for each plane the first six atoms define the plane

(**3a**)	I	N(1) 8, C(2) −2, N(3) −10, C(4) 15, C(5) −9, C(6) −3, O(2) −57, N(4) 20, C(61) −46, C(1P) 0.
	II	C(1P) −9, C(2P) 1, C(3P) 10, C(4P) −13, C(5P) 5, C(6P) 6, C(5) −29, Cl −46.
(**2a**)	I′	N(1′) 8, C(2′) −35, N(3′) 33, C(4′) −7, C(5′) −19, C(6′) 20, N(2′) −79, O(4′) −34, C(61′) 144, C(1P′) −23.
	II′	C(1P′) 16, C(2P′) −7, C(3P′) −13, C(4P′) 24, C(5P′) −14, C(6P′) −6, C(5′) 76, Cl′ −193.

Table 7. Hydrogen bond contact distances in the (**2a**):(**3a**) duplex and isocytosine*. In both parts of the table the molecule in the 1*H* form is given first, *viz* (**3a**) and molecule A respectively.

This work		Isocytosine	
Hydrogen bond**	X–H···Y Distance (Å)	Hydrogen bond+	X–H···Y Distance (Å)
$O(2)_I \cdots H(2B')–N(2')_I$	2.84(1)	O(8A)···H(12)–N(7B)	2.861(9)
$N(3)_I \cdots H(3')–N(3')_I$	2.86(1)	N(3A)···H(9)–N(3B)	2.908(9)
$N(4)_I–H(4A) \cdots O(4')_I$	2.90(1)	N(7A)–H(12)···O(8B)	2.904(9)
$O(2)_{II} \cdots H(2A')–N(2')_I$	2.86(1)		
$N(1)_I–H(1) \cdots O(7)_I$	2.78(1)		
$O(7)_I–H(7A) \cdots O(4')_{III}$	2.80(1)		
$O(7)_I–H(7B) \cdots N(1')_{II}$	2.79(1)		

* Ref. 15. ** I, II, III refer to the equivalent positions x, y, z; $1-x, -y, -z$; $1-x, 1-y, -z$, respectively. + Numbering scheme from ref. 15.

g) in ether (200 ml) was treated with a solution of diazomethane (8 g) in ether (500 ml) over 10 min. The mixture frothed vigorously and was stirred at 10 °C overnight. Excess of diazomethane was destroyed (acetic acid) and the ether was evaporated to give the methoxyacrylonitrile (**5**) as a yellow syrup (21 g). The methoxyacrylonitrile was heated for 5 h with urea (5.1 g) in ethanolic sodium ethoxide, prepared from sodium (4 g) and absolute ethanol (100 ml). Excess of ethanol was evaporated under reduced pressure to leave an orange syrup which solidified on cooling. Repeated crystallisation of the residue from 2M-hydrochloric acid furnished the *hydrochloride salt* of (**3a**) (1.4 g) as colourless prisms, m.p. 310 °C (decomp.) (Found: C, 50.3; H, 4.5; N, 14.6. $C_{12}H_{13}Cl_2N_3O$ requires C, 50.35; H, 4.55; N, 24.83%); $\nu_{max.}$ 3 350 and 3 160 (NH), 2 950—2 600br (bonded NH) and 1 715 cm^{-1} (CO); $\lambda_{max.}$ (2M-hydrochloric acid) 219 and 286 nm.

The *free base* of (**3a**), prepared from the hydrochloride salt and aqueous ammonia, was crystallised from ethanol as colourless leaves, m.p. 295—310 °C (decomp.) [Found: M^+, 249 (251). Calc. for $C_{12}H_{12}ClN_3O$: *M*, 249 (251)]; $\nu_{max.}$ 3 500, 3 350, and 3 170br (NH), 3 000—2 750br (bonded NH) and 1 640br cm^{-1} (CO); $\lambda_{max.}$(EtOH) 213, 222 infl., 242 infl., and 278 nm.

2-*Amino*-5-(4-*chloro*-3-*nitrophenyl*)-6-*ethylpyrimidin*-4(3H)-*one* (**2b**).—2-Amino-5-(4-chlorophenyl)-6-ethylpyrimidin-4(3*H*)-one (**1b**) (0.4 g) was nitrated with a mixture of nitric acid (*d* 1.42; 0.9 g) and sulphuric acid (10 ml) at 25 °C (18 h). The yellow solution was quenched with water and the precipitated nitrophenylpyrimidine was crystallised from ethanol–acetone to give cream crystals (0.1 g), m.p. 261—263 °C [Found: M^+, 294 (296). Calc. for $C_{12}H_{11}ClN_4O_3$: *M*, 294 (296)]; $\nu_{max.}$ 3 470, 3 350, 3 120br (NH), 3 000—2 750br (bonded NH), 1 670 (CO), and 1 535 and 1 340 cm^{-1} (NO_2); $\lambda_{max.}$(EtOH) 215, 253, and 297 nm; $\lambda_{max.}$ (2M-hydrochloric acid) 231 and 265 nm.

4-*Amino*-5-(4-*chloro*-3-*nitrophenyl*)-6-*ethylpyrimidin*-2(1H)-*one* (**3b**).—Nitration of 4-amino-5-(4-chlorphenyl)-6-ethylpyrimidin-2(1*H*)-one (**3a**) with a nitric acid–sulphuric acid mixture (as above) afforded the nitrophenylpyrimidinone (95%) which was purified by conversion into a hydrochloride salt in 10M-hydrochloric acid (recrystallisation from acetone–water) and reprecipitation as the free base with 2M-sodium hydroxide solution. The cream base crystallised as flakes from ethanol with m.p. 320 °C (decomp.) [Found: M^+, 294 (296). Calc. for $C_{12}H_{11}ClN_4O_3$: *M*, 294 (296)]; $\nu_{max.}$ 3 450, 3 100br (NH), 1 640 (CO), and 1 540 and 1 355 cm^{-1} (NO_2); $\lambda_{max.}$(EtOH) 217, 250infl., and 280infl., nm; $\lambda_{max.}$ (2M-hydrochloric acid) 218 and 284 nm.

2-*Amino*-6-*ethyl*-5-(3-*nitro*-4-*piperidinophenyl*)*pyrimidin*-4(3H)-*one* (**2c**).—2-Amino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidin-4(3*H*)-one (**2b**) (0.05 g) was boiled in piperidine (3 ml) for 30 min. The cooled mixture, was diluted with water to afford the pyrimidinone (0.055 g), m.p. 150—152 °C (decomp.) (Found: M^+, 343. Calc. for $C_{17}H_{21}N_5O_3$: *M*, 343); $\nu_{max.}$ 3 390, 3 180br (NH), 2 990 (CH), and 1 650 cm^{-1} (CO); $\lambda_{max.}$(EtOH) 256 and 296 nm; $\lambda_{max.}$ (2M-hydrochloric acid) 266 nm.

4-*Amino*-6-*ethyl*-5-(3-*nitro*-4-*piperidinophenyl*)*pyrimidin*-2(1H)-*one* (**3c**).—A sample of 4-amino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidin-2(1*H*)-one (**3b**) (0.3 g) was boiled in piperidine (10 ml) for 2 h. The cooled mixture was diluted with water (25 ml) and the orange product collected and washed with water. The nitropiperidinopyrimidinone (0.24 g) crystallised from ethanol as orange micro-prisms, m.p. 290—292 °C (Found: M^+, 343. Calc. for $C_{17}H_{21}N_5O_3$: *M*, 343); $\nu_{max.}$ 3 480 (NH), 3 070br (bonded NH), 2 960 (CH), 1 680 (CO), and 1 530 and 1 340 cm^{-1} (NO_2); $\lambda_{max.}$(EtOH) 212, 257, and 414; $\lambda_{max.}$ (2M-hydrochloric acid) 217, 250infl., and 281 nm.

The dimethylformamide solvate ($C_{17}H_{21}N_5O_3 \cdot 3C_3H_7NO$), m.p. 294—296 °C (decomp.) was formed when (**3c**) was recrystallised from ethanol–dimethylformamide; $\nu_{max.}$ 3 450, 3 340 (NH), 3 080br (bonded NH), 2 915 (CH), 1 673 (CO), and 1 525 and 1 340 cm^{-1} (NO_2).

Hydrolysis of 2,4-*Diamino*-5-(4-*chlorophenyl*)-6-*ethylpyrimidine* (**1a**) *in* 6M-*Hydrochloric Acid*.—The pyrimidine (**1a**) (10.0 g) was boiled in 6M-hydrochloric acid (400 ml) for 18 h and kept at 4 °C for 6 days. The product (9.4 g) was a mixture of colourless prisms and flakes. The mixture was fractionally crystallised from 2M-hydrochloric acid (300 ml) to give (after 16 h at 25 °C) colourless flakes (3.0 g) of the *hydrochloride salt* of 2-*amino*-5-(4-*chlorophenyl*)-6-*ethylpyrimidin*-4(3H)-*one* (**2a**) which after several recrystallisations from 2M-hydrochloric acid had m.p. 290—305 °C (decomp.) (Found: C, 50.1; H, 4.4; N, 14.5. $C_{12}H_{12}ClN_3O$ requires C, 50.35; H, 4.55; N, 14.7%); $\nu_{max.}$ 3 400, 3 270 and 3 170 (NH), 3 000—2 750 (bonded NH), and 1 705 and 1 685 cm^{-1} (CO); $\lambda_{max.}$ (2M-hydrochloric acid) 233 and 265 nm.

The free base of (**2a**) prepared from the hydrochloride salt and aqueous ammonia, crystallised from ethanol as colourless flakes, m.p. 285—295 °C (sinters 265 °C) [Found: M^+, 249 (251). Calc. for $C_{12}H_{12}ClN_3O$: *M*, 249 (251)]; $\nu_{max.}$ 3 450—3 300br and 3 170br (bonded NH), and 1 660 cm^{-1} (CO); $\lambda_{max.}$(EtOH) 215, 250infl., and 296 nm.

The 2M-hydrochloric acid mother liquors left after removal of the hydrochloride of (**2a**) were chilled at 4 °C overnight to yield colourless prisms (4.75 g) which were identical (m.p., i.r., u.v. and

^{1}H n.m.r.) with the authentic sample (prepared above) of the hydrochloride salt of 4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2(1*H*)-one (**3a**).

Hydrolysis of 2,4-*Diamino*-6-*ethyl*-5-(3-*nitro*-4-*piperidinophenyl*)*pyrimidine* (**1c**).—The nitropiperidinopyrimidine (**1c**) (2.2 g) was boiled in 6M-hydrochloric acid (25 ml) for 8 h to give a sherry-coloured solution. Basification with aqueous ammonia–ice gave an orange-red solid (2.0 g). T.l.c. examination of the solid showed that all the starting material had been consumed and two coloured products were formed. The product was boiled with acetone (80 ml) for 5 min and then stirred at 25 °C (30 min). The insoluble material (0.7 g) crystallised from ethanol as orange micro-prisms, m.p. 290—292 °C (decomp.) which were identical (mixed m.p., i.r., u.v. and ^{1}H n.m.r.) with the sample of 4-amino-6-ethyl-5-(3-nitro-4-piperidinophenyl)pyrimidin-2(1*H*)-one (**3c**) prepared previously.

The acetone-soluble product (1.1 g) was recovered from the evaporated solution and was identical (i.r.) with a sample of 2-amino-6-ethyl-5-(3-nitro-4-piperidinophenyl)pyrimidin-4(3*H*)-one (**2c**) prepared previously.

Products detected and analysed (without isolation) from the 6M-hydrochloric acid hydrolysis of compounds (**1b**) and (**1d**) are recorded in Table 2.

2-*Amino*-5-(4-*chlorophenyl*)-6-*ethylpyrimidin*-4(3H)-*one*: 4-*Amino*-5-(4-*chlorophenyl*)-6-*ethylpyrimidin*-2(1H)-*one Hydrate* (**4**).—(*a*) A mixture of the pyrimidin-4-one (**2a**) (0.2 g) and pyrimidin-2-one (**3a**) (0.2 g) was refluxed in ethanol (120 ml) for 2 h until all solid had dissolved. The solution was concentrated to 60 ml, filtered, and allowed to crystallise (24 h). The *duplex hydrate* (0.38 g) (colourless flakes) had m.p. 265—280 °C (decomp.) (Found: C, 55.5; H, 5.0; N, 16.1. $C_{12}H_{12}ClN_3O$: $C_{12}H_{12}ClN_3O{\cdot}H_2O$ requires C, 55.7; H, 5.0; N, 16.25%); $\nu_{max.}$ 3 450, 3 390, and 3 150 (NH), 3 000—2 700br (bonded NH), and 1 650 cm^{-1} (CO); $\lambda_{max.}$(EtOH) 211, 222infl., 245infl., and 285 nm; $\lambda_{max.}$ (2M-hydrochloric acid) 222, 233infl., and 281 nm. (*b*) The solution formed when 2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine (**1a**) (5.0 g) was hydrolysed in boiling 6M-hydrochloric acid (200 ml) was cooled and basified with concentrated aqueous ammonia–ice. The white solid (4.9 g) was a mixture of (**2a**) and (**3a**) (t.l.c. and ^{1}H n.m.r.). Crystallisation of a portion of the solid (1.0 g) from ethanol (250 ml) furnished colourless flakes of the same (i.r.) duplex hydrate (**4**) (0.15 g) as the initial crop of crystals.

Nitrous Acid Deamination of 2,4-*Diamino*-5-(4-*chlorophenyl*)-6-*ethylpyrimidine* (**1a**).—A solution of pyrimethamine (**1a**) (11.25 g) in boiling 0.5M-sulphuric acid (500 ml) was treated over 10 min with a solution of sodium nitrite (3.9 g) in water (100 ml). The solution was boiled for a further 5 min, cooled, and made alkaline with aqueous 2M-sodium hydroxide. The cream product was collected, washed with water, and dried (11.0 g). T.l.c. examination of the crude solid revealed the presence of 2-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-4(3*H*)-one (**2a**), 4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2(1*H*)-one (**3a**) and unchanged (**1a**). Crystallisation of the solid from 95% ethanol afforded the hydrate of the duplex (**4**) (1.1 g).

Nitrous Acid Deamination of 2,4-*Diamino*-5-(4-*chloro*-3-*nitrophenyl*)-6-*ethylpyrimidine* (**1b**).—A solution of the nitropyrimethamine (**1b**) (2.85 g) in boiling 0.5M-sulphuric acid (100 ml) was treated with sodium nitrite (0.8 g) in water (10 ml) as above. The mixture, basified with concentrated aqueous ammonia gave a cream precipitate (2.3 g). T.l.c. examination of the product showed the presence of (**2b**), (**3b**), and unchanged (**1b**). Recrystallisation (× 2) of the crude solid from ethanol gave a sample of 4-amino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidin-2(1*H*)-one (**3b**) (0.9 g) which was identical (t.l.c., ^{1}H n.m.r., i.r. and u.v.) with the authentic sample prepared by nitration of (**1b**) (see above).

Nitrous Acid Deamination of 2,4-*Diamino*-6-*ethyl*-5-(3-*nitro*-4-*piperidinophenyl*)*pyrimidine* (**1c**).—The nitropiperidinopyrimidine (**1c**) (3.42 g) was treated with sodium nitrite (0.8 g) in boiling 0.5M-sulphuric acid (100 ml) as above. The brown product (2.6 g), formed when the mixture was basified, was boiled in acetone (125 ml) for 2 h. The insoluble material (1.85 g) was a mixture of 4-amino-6-ethyl-5-(3-nitro-4-piperidinophenyl)pyrimidin-2(1*H*)-one (**3c**) and unchanged starting material (**1c**).

The acetone soluble material (0.75 g) was a mixture (t.l.c.) of 2-amino-6-ethyl-5-(3-nitro-4-piperidinophenyl)pyrimidin-4(3*H*)-one (**2c**) and unchanged starting material (**1c**).

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