Structural Studies on Bio-active Compounds. Part 25[1] Synthesis and Properties of Potential Metabolites of the Diaminopyrimidine Antifolate 2,4-Diamino-5-(3-azido-4-chlorophenyl)-6-ethylpyrimidine (MZPES)

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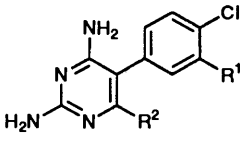
A series of potential metabolites of the antitumour diaminopyrimidine *m*-azidopyrimethamine (**2**, MZP) has been prepared. Selective reduction of the nitro substituent of the nitropyrimidine 3-oxide **8**, prepared by performic acid oxidation and diazotisation–azidation of the resulting amine *N*-oxide **10**, afforded MZP 3-oxide **11**. Direct oxidation of compound **2** with performic acid gave compound **11** and the isomeric 1-oxide **12**, without azide decomposition. Sodium tungstate-catalysed oxidation of 2,4-diamino-5-(3-amino-4-chlorophenyl)-6-ethylpyrimidine (**3**, MAP) furnished the corresponding nitropyrimidine **6** and its 3-oxide **8**. Protection of the hydroxyl substituent of 6-(1-hydroxyethyl)-pyrimethamine **13** as the heptafluorotolyl ether enabled successful aromatic nitration, but hydrogenolysis to afford **3** occurred upon reduction with hydrazine–Raney nickel.

Although the nitropyrimidine 3-oxide **8** retained inhibitory activity, other putative MZP metabolites were all markedly less active than the parent azide **2** as inhibitors of rat liver dihydrofolate reductase, consistent with the short biological half-life required for the antifolate *in vivo*.

Lipophilic or non-classical antifolates currently attract considerable interest as alternatives to methotrexate (MTX) for the chemotherapy of neoplastic disease.[2] However, the potential clinical efficacy of several lipophilic antifolates in the treatment of MTX-resistant malignancies has been hampered by toxicity thought to emanate, at least in part, from the protracted biological half-life ($t_{1/2}$) of these highly lipid-soluble compounds. Thus, metoprine **1**, the prototype lipophilic diaminopyrimidine antifolate (octanol–water, log P = 2.82), exhibits a biological half-life of approximately 216 h in man.[3]



1	R^1 = Cl,	R^2 = Me
2	R^1 = N_3,	R^2 = Et
3	R^1 = NH_2,	R^2 = Et
4	R^1 = Cl,	R^2 = Et
5	R^1 = H,	R^2 = Et
6	R^1 = NO_2,	R^2 = Et
7	R^1 = NHAc,	R^2 = Et

2,4-Diamino-5-(3-azido-4-chlorophenyl)-6-ethylpyrimidine (**2**; '*m*-azidopyrimethamine'; MZP) was selected as the most promising of a series of azido-substituted diaminopyrimidine antifolates designed to circumvent the toxicity problems observed with metoprine.[4] The liphilic azido substituent was introduced to facilitate inhibition of the target enzyme dihydrofolate reductase (DHFR), but also to undergo metabolic or chemical reduction *in vivo* to the corresponding polar arylamine **3** ('*m*-aminopyrimethamine'; MAP), a 50-fold weaker inhibitor of the enzyme. The subsequent rapid elimination of compound **3** was expected to reduce the half-life, and hence the toxicity, of the parent drug. MZP exhibited a good spectrum of antitumour activity against a panel of murine tumour cell lines *in vivo*,[5] and the biological half-life of the water-soluble ethanesulfonate salt ('*m*-azidopyrimethamine ethanesulfonate'; MZPES) was determined as approximately 4 h in mice, the predicted metabolite **3** being detectable in the plasma of treated animals.[6]

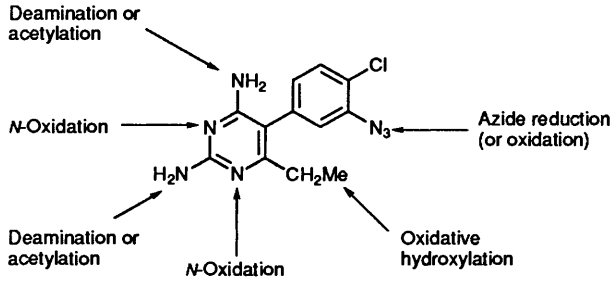
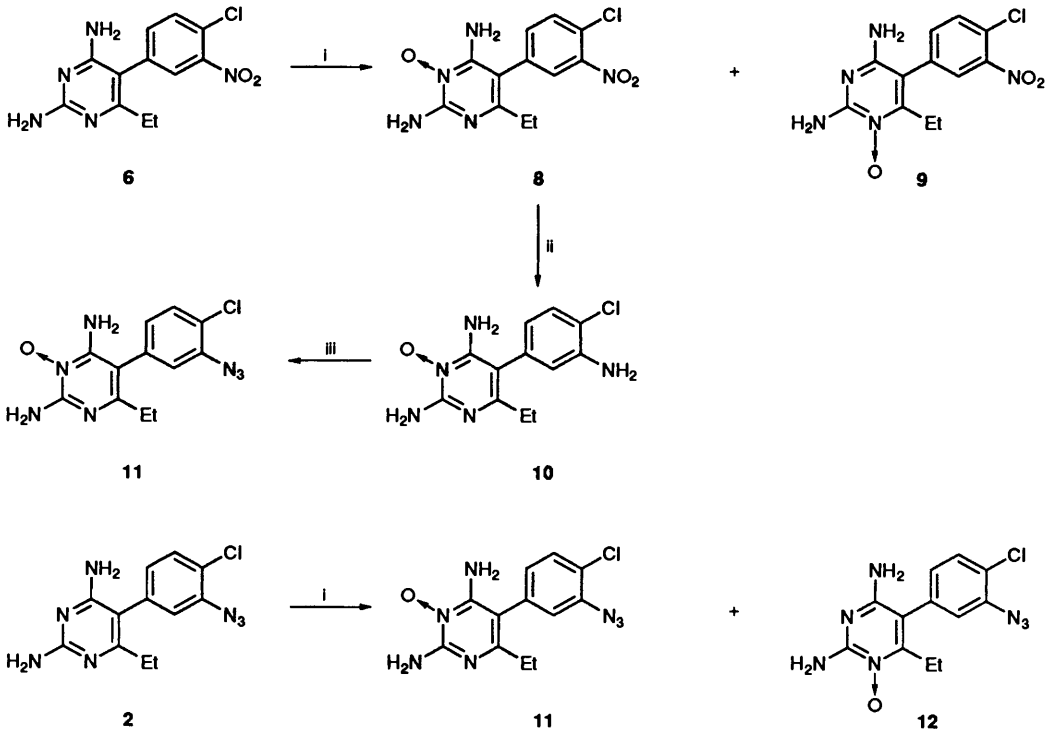


Fig. 1 Prospective sites for the biotransformation of *m*-azidopyrimethamine **2**

The metabolism of diaminopyrimidine antifolates has been reported to occur at a number of sites. Thus, metoprine **1** is metabolised principally to the 1-oxide and its glucuronide conjugate, while the major urinary metabolities of the 6-ethyl analogues etoprine **4** and pyrimethamine **5**, are the 3-oxides hydroxylated at the α-position of the 6-ethyl substituent.[3] Implicit in the design of compound **2** was the expectation that incorporation of an azido substituent would introduce a metabolic 'handle' into the molecule, thereby facilitating the biotransformation to one polar, inactive and rapidly excretable metabolite **3**. Clearly, the arylamine **3** may represent only one of a multitude of possible biotransformation products of compound **2**, as illustrated in Fig. 1, and it was therefore necessary to undertake a detailed study of the metabolism of this compound. In this paper, we detail the synthesis and the DHFR-inhibitory activity of several putative metabolites useful for studies concerning the biological fate of compound **2**.

Results and Discussion

Syntheses of compound **3** and 2,4-diamino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidine (**6**; '*m*-nitropyrimethamine'), putative products of the respective reductive and oxidative metabolism of compound **2**, have been described previously.[7] Methods for the preparation of the 3'-(acetylamino)pyrimidine derivative **7** and a series of mono-, di- and tri-acetylated



Scheme 1 *Reagents and conditions:* i, HCO_3H–HCO_2H, 25 °C; ii, $SnCl_2$, EtOH; iii, $NaNO_2$ aq. HCl, 5 °C; NaN_3

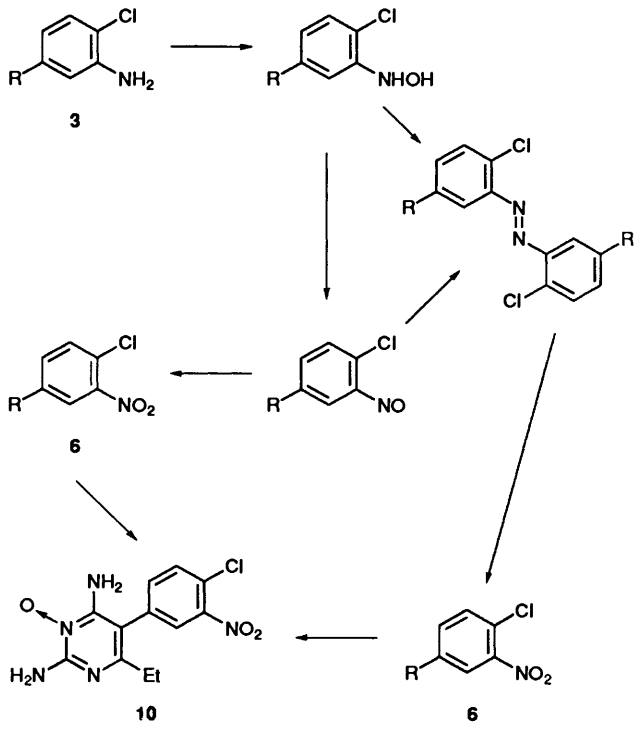
derivatives of compounds **3**, **5** and **6** have also been documented.[4,8]

N-*Oxidation of* 2,4-*Diaminopyrimidines.*—Two strategies were adopted for the synthesis of the required diaminopyrimidine *N*-oxides; the first utilised our previously described synthetic route[4] to form the *m*-azidopyrimethamine *via* the reduction and the azidation of compound **6**, following the *N*-oxidation of the pyrimidine ring. Initial attempts to synthesise the desired pyrimidine *N*-oxide derivatives using *m*-chloroperbenzoic acid as the oxidant, reportedly an efficient method for the preparation of diaminopyrimidine *N*-oxides,[9] were hampered by the poor solubility of the pyrimidines in suitable solvents. The use of peracetic acid, as reported by Jovanovich,[10] was also without effect. Thus, treatment of compound **6** with peracetic acid in acetic acid at 70 °C for 12 h, or under reflux, gave only starting materials. The successful oxidation of compound **6** was eventually achieved in performic acid–formic acid at 25 °C, affording a moderate yield of the nitropyrimethamine 3-oxide **8**. However, chromatographic separation of the reaction mixture furnished only a small quantity of the isomeric pyrimidine 1-oxide **9**, and elevated temperatures or protracted reaction times were without effect on the relative proportion of each oxide. Selective reduction of the nitro substituent of the diaminopyrimidine 3-oxide **8** was achieved utilising tin(II) chloride in refluxing ethanol to yield the corresponding *m*-aminopyrimethamine 3-oxide **10**; concomitant reduction of the *N*-oxide function was not observed. However, all attempts to isolate the isomeric aminopyrimethamine 1-oxide from compound **9** under identical reaction conditions proved unsuccessful, and insufficient quantities of compound **9** could be prepared to pursue alternative methods. Subsequent diazotisation and azidation of compound **10** furnished the target azidopyrimidine 3-oxide **11** in good yield (Scheme 1).

The low yield of the nitropyrimethamine 1-oxide **9** obtained *via* the oxidation of compound **6** led us to investigate a second approach, namely the possibility of preparing the requisite amino- and azido-pyrimidine *N*-oxides by the direct oxidation of the amine **3** or the azide **2**, respectively. Unfortunately, all attempts to oxidise compound **3** with performic acid were to no avail, and only unchanged starting materials were recovered from the reaction mixture. The use of sodium tungstate to catalyse *N*-oxidation by hydrogen peroxide has been reported previously[11] and this method was investigated for the oxidation of compound **3**. Treatment of a methanol solution of compound **3** with sodium tungstate and hydrogen peroxide at room temperature, gave an intensely yellow solution with the consumption of starting material (TLC) to afford two products. Surprisingly, chromatographic separation of these two components did not yield the expected isomeric aminopyrimethamine *N*-oxides, but instead furnished the nitropyrimethamine **6** and the corresponding nitropyrimidine 3-oxide **8** in approximately equal proportions. These compounds were characterised as identical (^{1}H NMR, UV, mass spectrum, TLC) with authentic samples of both compounds previously described.

The sodium tungstate catalysed oxidation of compound **3** by hydrogen peroxide to give a mixture of the nitropyrimidine **6** and the corresponding 3-oxide **8**, is thought to occur *via* the formation of the tungstate peroxo anion (WO_8^{2-}), which subsequently serves as oxidant. Oxidation of the arylamine must then proceed through the intermediacy of a hydroxylamino and thence a nitroso species, which suffers further oxidation to form the nitropyrimethamine **6**. An alternative mechanism has also been proposed[11] whereby the nitroso and hydroxylamine intermediates condense to form an azoxy derivative, with subsequent oxidative cleavage affording two molecules of compound **6**, as illustrated in Scheme 2. Presumably, *N*-oxidation of compound **6** then gives the pyrimidine 3-oxide **8** although the alternative route, where *N*-oxidation of the pyrimidine precedes the oxidation of the arylamine, is also possible.

The direct oxidation of the azidopyrimethamine **2** with performic acid proved more successful and afforded, after chromatography on silica, the desired azidopyrimidine 3-oxide **11** and the corresponding 1-oxide **12** in a ratio of approximately 3:1, together with the unchanged starting compound **2** and



Scheme 2 Putative mechanism for the sodium tungstate-catalysed oxidation of **3** (R = 2,4-diamino-6-ethylpyrimidin-5-yl). *Reagents and conditions:* i, $Na_2WO_42H_2O$, 30% H_2O_2, MeOH, 25 °C.

traces of an intensely red component. Compound **11** proved identical (^{1}H NMR, UV, mass spectrum, TLC) with the azidopyrimidine *N*-oxide synthesised *via* the alternative route discussed above. The decomposition of the aromatic azides in peracids with formation of azo and azoxy compounds has been documented,[12] and may account for the traces of coloured material observed, although insufficient of this contaminant was isolated to permit characterisation. It is of interest to note that the *m*-azidopyrimethamine 3-oxide **11** proved considerably more soluble than the isomeric 1-oxide **12** in ethanol, although separation of these by fractional crystallisation of the reaction mixture was precluded by the presence of the unreacted compound **2**.

The *N*-oxidation of diaminopyrimidines has not been fully investigated. Ochai[13] has reported that while pyrazines may form the mono- or the di-oxide products, pyridazines and pyrimidines invariably afford only the mono-*N*-oxides, and these observations are borne out in the present study. Thus, the mass spectra of the diaminopyrimidine *N*-oxides exhibited the expected molecular ions (M^+), and characteristic fragments ($M^+ - 16$) corresponding to loss of oxygen. For the azidopyrmidine *N*-oxides **11** and **12**, loss of oxygen was observed to precede the M − 28 fragment characteristic of nitrogen elimination. Definitive information regarding the extent and orientation of the *N*-oxidation was provided by analysis of the ^{1}H NMR spectra, and supported by the reported spectral data for metoprine **1** and related diaminopyrmidines.[9] Thus, pyrimidine ring substituents juxtaposed to the *N*-oxide were subject to a deshielding influence, this effect being particularly pronounced for the methylene protons of the 6-ethyl substituent of the diaminopyrimidine 1-oxides **9** and **12** (δ 2.46 and 2.52). In contrast, the chemical shift of the 6-ethyl methylene protons of the 3-oxides differed little from that of the parent diaminopyrimidine **6** (δ 2.15). The overall deshielding effect of an *N*-oxide on the N-H protons of both the 2- and 4-amino substituents, was also especially evident for the 2-amino group of a pyrimidine 1-oxide where a large downfield shift was observed. Oxidation of diaminopyrimidines purportedly elicits a bathochromic shift in the UV spectrum, particularly following oxidation at N-1. However, in the present study only a small shift to longer absorption wavelength was observed for the diaminopyrimidine 3-oxides (**8**, **10** and **11**) and, surprisingly, the 1-oxides **9** and **12** exhibited an hypsochromic shift in both cases. The spectral properties of the diaminopyrimidine *N*-oxides are shown in comparison with the parent pyrimidines in Table 2.

The predominant formation of the diaminopyrimidine 3-oxides in these studies is probably attributable to several interactive factors. Jovanovic,[10] in considering the effect of substituents upon the position of oxidation of disubstituted pyrimidines, concluded that simple alkyl groups offer little steric hindrance and that ring nitrogens *ortho* or *para* to electron-donating substituents suffer preferential oxidation. The biological oxidation of the diaminopyrimidines may, however, be subject to steric factors since metoprine **1**, a 6-methylpyrimidine, suffers oxidation at N-1, whereas the 6-ethyl homologue etoprine **4** and the closely related diaminopyrimidine pyrimethamine **5** are metabolised predominantly at N-3.[3] Although any extrapolation to more complex tetra-substituted pyrimidines must be regarded with caution, the presence of two amino groups *ortho* to N-3 as compared with the *ortho*/*para* disposition relative to N-1 should favour oxidation at the latter ring nitrogen, electron-donation being most pronounced with amino substituents *para* to pyrimidine ring nitrogens.[14] This is consistent with our previous observation that the N-1 position of several diaminopyrimidine antifolates, including **2** and **6**, is the most basic centre,[15] and protonation at this site under the acidic reaction conditions employed will presumably favour oxidation at N-3.

6-(1-*Hydroxyethyl*)*pyrimidines*.—The synthetic accessibility of compounds **2**, **3** and **6** from the commercially available pyrimethamine **5**, suggested a possible route to the requisite 6-(1-hydroxyethyl)pyrimidines *via* the direct oxidation of the methylene group of the 6-ethyl substituent. Initial studies were conducted with compound **5** as a model substrate, and an authentic sample of racemic 2,4-diamino-5-(4-chlorophenyl)-6-(1-hydroxyethyl)pyrimidine **13**, the expected oxidation product of compound **5**, prepared according to the method of Rees *et al.*[16] Unfortunately, all attempts to convert compound **5** into compound **13** with potassium permanganate or activated manganese dioxide were unsuccessful, affording either unchanged compound **5** or an intractable tar, presumably as a result of oxidative degradation of the pyrimidine ring. The propensity of diaminopyrimidines to suffer oxidative cleavage on treatment with permanganate has been documented previously.[17]

Acetoxylation of benzylic methylene groups with lead tetraacetate and subsequent hydrolysis of the acetoxy derivative, has been reported to afford α-aryl alcohols,[18] and although good yields were only obtained with electron-rich aryl substituents, this method was investigated as a possible alternative route to 6-(1-hydroxyethyl)pyrimidines (Scheme 3). Thus, treatment of compound **5** with lead tetraacetate in acetic acid at 80 °C for 18 h furnished, after extensive purification, a meagre yield of a product giving the expected molecular ion (M^+) for the 6-(1-acetoxyethyl)pyrimidine **17**, and a fragmentation pattern consistent with the presence of an *O*-acetoxy substituent, notably fragments corresponding to loss of MeCO ($M^+ - 43$) and $MeCO_2$ ($M^+ - 59$). However, further characterisation of compound **17** by NMR spectroscopic methods was precluded by the insolubility of the compound and the signal broadening observed.

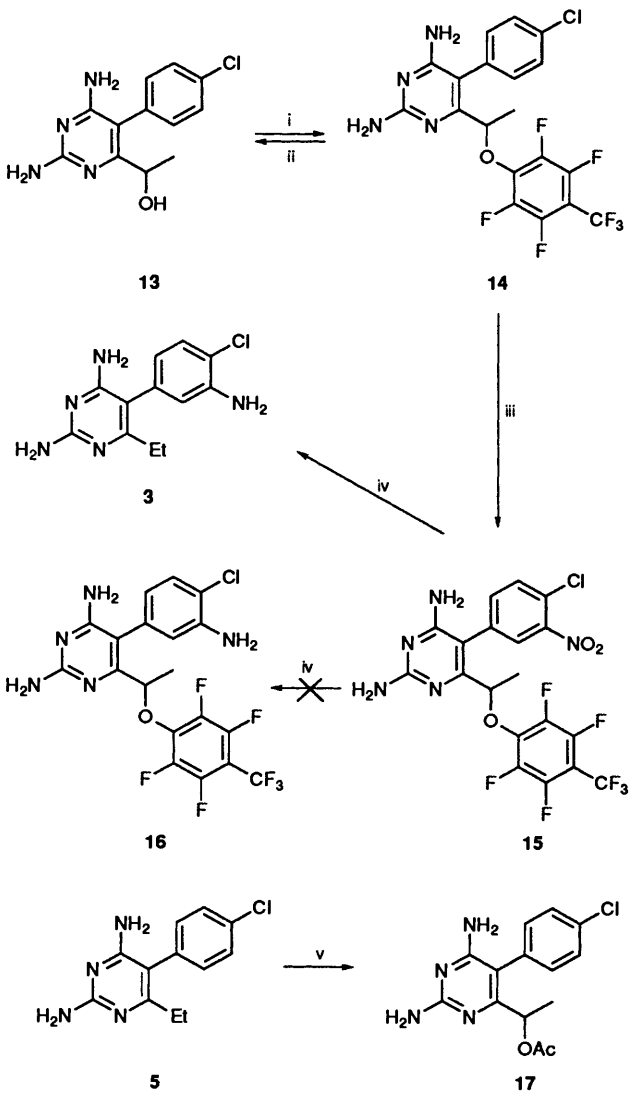
Problems encountered with the oxidation of compound **5** to the 6-(1-hydroxyethyl) derivative **13** led us to investigate the possibility of nitrating the available 6-(1-hydroxyethyl)pyrimethamine **13** directly. Although pyrimethamine **5** nitrated

exclusively at the 3-position on treatment with nitric acid–sulfuric acid at 25 °C, a similar reaction conducted with compound **13** was unsuccessful, affording an intractable mixture. A variety of alternative nitrating systems, including sodium nitrate or nitric acid in anhydrous acetic or trifluoroacetic acid, sodium nitrate in acetic anhydride,[19] and sodium nitrite in trifluoroacetic acid,[20] were also investigated utilising compound **5** as the model substrate, but either failed to react or resulted in complete degradation of the diaminopyrimidine.

The instability of compound **13** to the strongly acidic conditions necessary for nitration presumably results from protonation of the hydroxyl substituent, and thus a suitable protective group was sought, although identification of derivatives likely to be stable to such vigorous conditions proved surprisingly difficult. Jarman *et al.* have reported that heptafluoro-*p*-tolyl and pentafluoropyridyl ethers are highly stable to mineral acids and oxidants, and as such represent convenient protected forms of aliphatic alcohols and phenols.[21,22] Reaction of compound **13** with octafluorotoluene, under conditions of phase-transfer catalysis, gave the requisite heptafluorotolyl ether **14** in acceptable yield, although prolonged reaction times (24 h) proved necessary and the complete conversion of compound **13** into the product was not observed, despite addition of an excess of octafluorotoluene. The ease of removal of the protecting group by the prescribed method[21] was established by treating compound **14** with sodium methoxide in dry dimethylformamide, whereupon quantitative conversion into the parent alcohol **13** was observed. However, compound **14** was found to be poorly soluble in organic solvents, including dimethylformamide, and the reaction was conducted at 60 °C to ensure compound dissolution.

Nitration of the heptafluorotolyl ether **14** proceeded in good yield and without detriment to the protecting group, the product **15** giving a fragment in the mass spectrum corresponding to the loss of the heptafluorotolyloxy fragment ($M^+ - C_7F_7O$). That nitration had occurred *ortho* to the chloro substituent was established from the ^1H NMR spectrum and also by the treatment of a sample of compound **15** with hot benzylmethylamine, whereupon a deep red colouration developed immediately. This was consistent with amine substitution of the now activated chloro substituent and analogous to our previous observations for the reaction of nitropyrimethamine with various amines.[7,23] To avoid the likelihood of nucleophilic displacement of the chloro substituent of compound **15** upon removal of the protecting group with sodium methoxide in DMF, it was decided to proceed with the reduction of the 3-nitro substituent prior to deprotection. However, the reaction of compound **15** with Raney nickel–hydrazine did not give the expected amine **16**, but instead afforded a product which proved identical with *m*-aminopyrimethamine **3**. Thus, in an apparently hitherto unprecedented reaction, reduction of the 3-nitro substituent of compound **15** was accompanied by cleavage of the heptafluorotolyl ether to give compound **3** as the only product, and in near quantitative yield (Scheme 3). Sadly, insufficient quantities of compound **15** were available to enable the further investigation of possible alternative methods for the reduction of the nitro group, but studies to establish the generality of perfluorotolyl ether hydrogenolysis are underway.

Interestingly, the 300 MHz ^1H NMR spectrum of the heptafluorotolypyrimidine **14** did not show the conventional AA'BB' *para*-disubstitution pattern observed for other 2,4-diamino-5-(4-chlorophenyl)-6-alkylpyrimidines, but gave four doublets centred at δ 6.91, 7.19, 7.33 and 7.45. We have previously observed atropisomerism arising from the restricted rotation about the aryl–pyrimidine C–C bond of analogous diaminopyrimidines bearing substituents in the hindered *ortho*-position of the aryl ring.[4] The bulky heptafluorotolyl substituent of compound **14**, located at the chiral 6-position of the

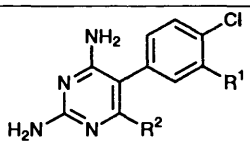


Scheme 3 *Reagents and conditions:* i, C_7F_8, Bu_4NHSO_4, CH_2Cl_2, aq. NaOH; ii, NaOMe, dimethylformamide, 60 °C; iii, HNO_3, H_2SO_4; iv, Raney nickel, N_2H_4, 60 °C; v, $Pb(OAc)_4$, AcOH, 80 °C

diaminopyrimidine, accentuates restricted rotation such that signals emanating from the diastereotopic 2' and 6' protons of the phenyl ring are clearly observable. In contrast, the less hindered 6-(1-hydroxyethyl)pyrimidine **13**, did not show this resonance pattern, although some signal broadening was observable for the aromatic protons.

That atropisomerism was responsible for the NMR spectrum exhibited by compound **14**, was confirmed by a dynamic ^1H NMR experiment in [$^2H_6$]DMSO (Fig. 2).[24] Thus, the pattern observed at 297 K was seen to broaden with increasing temperature, coalescence occurring at approximately 337 K. Although some decomposition of this compound was observed in the NMR spectrum at the maximum temperature utilised (377 K), presumably due to loss of the heptafluorotolyl substituent, the original signal pattern was observed when the spectrum was re-determined at 297 K (not shown). Unfortunately, insufficient data were available to enable the free energy of rotation to be calculated. Restricted rotation in compound **15**, where a 3'-nitro substituent is introduced onto the 5-phenyl ring, gives rise to four atropisomers and hence two diastereoisomeric pairs. Indeed, this was evident in the ^1H NMR of compound **15** where the methine proton of the 6-[1-(heptafluorotolyloxy)]ethyl group appeared as a pair of doublets. The successful resolution of enantiomeric rotational

Table 1 Activity of 2,4-diaminopyrimidines against rat liver dihydrofolate reductase[a]



Compound	Solvent	R_1	R^2	*N*-Oxide	I_{50}(μmol dm^{-3})[b]
1	B	Cl	Me	—	0.10
2[c]	A	N_3	Et	—	1.30
3	B	NH_2	Et	—	62.0
6	A	NO_2	Et	—	0.08
7	B	NHAc	Et	—	>25.0[d]
8	B	NO_2	Et	3	2.50
10	B	NH_2	Et	3	>25.0
11	B	N_3	Et	3	>25.0
12	B	N_3	Et	1	>25.0

Solvents: A, water; B, 0.1 mol dm^{-3} hydrochloric acid. [a] Partially purified rat liver DHFR (E.C.1.5.1.3) was prepared by the method of Bertino and Fischer[27] and assayed spectrophotometrically by a previously published method.[28] [b] Defined as the final concentration of inhibitor in the assay system necessary to reduce the enzymatic reaction rate to 50% of the uninhibited rate. I_{50} values were determined by conducting inhibitory assays in duplicate at four different concentrations estimated to reduce DHFR activity by 20, 40, 60 and 80% of control values. [c] Ethanesulfonic acid salts. [d] Compounds giving I_{50} values of greater than 25 μmol dm^{-3} are considered essentially inactive as DHFR inhibitors.

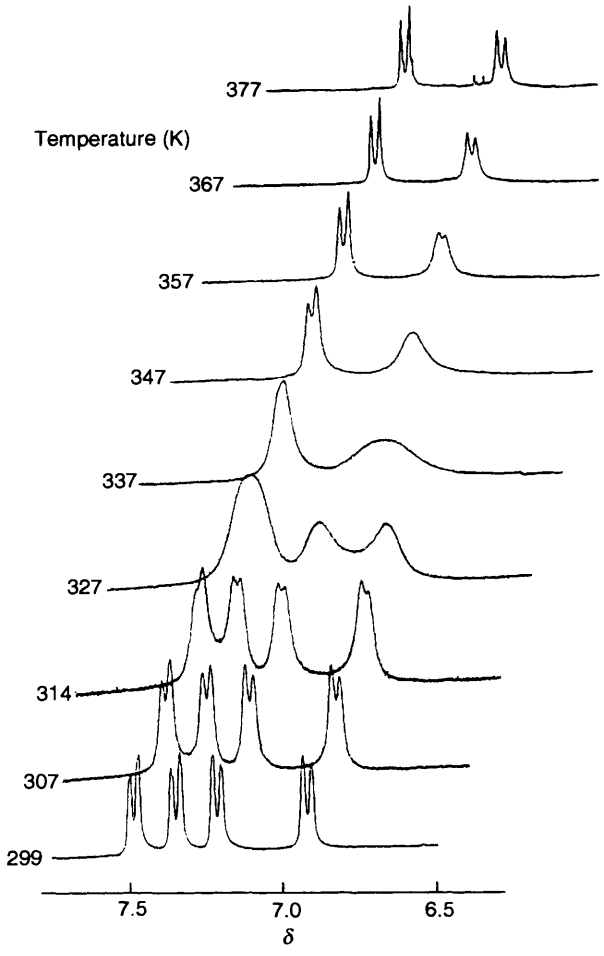


Fig. 2 300 MHz Dynamic ^{1}H NMR spectrum of compound **14**

isomers of 6-alkyl-2,4-diamino-5-phenylpyrimidines has yet to be achieved, and is desirable in light of our previous observations implying that rotameric diaminopyrimidines exhibit differing binding affinities for DHFR.[25,26] Our results suggest that the introduction of a heptafluorotolyl group may facilitate the separation of atropisomeric diaminopyrimidines, and future studies will address this possibility.

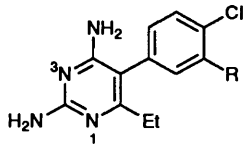
Biological Properties of Potential Metabolites of 2,4-*Diamino*-5-(4-*azido*-3-*chlorophenyl*)-6-*ethylpyrimidine* **2**.—The putative diaminopyrimidine metabolites were screened for inhibitory activity against rat liver DHFR and I_{50} values are recorded in Table 1. The two azidopyrimidine *N*-oxides **11** and **12** were at least an order of magnitude less active than the parent compound **2**, with values in excess of 25 μmol dm^{-3}. Thus, *N*-oxidation of *m*-azidopyrimethamine **2** *in vivo* would be expected to greatly reduce or abolish DHFR-inhibitory activity. The *N*-acetylated derivative **7** also exhibited very weak activity compared with compound **2**, implying that metabolic *N*-acetylation of compound **3**, the amine metabolite of compound **2**, would not restore DHFR-inhibitory activity. Interestingly, the nitropyrimidine 3-oxide **8** still retained significant activity, albeit some twenty-fold less than the parent *m*-nitropyrimethamine **6**. Metabolic reduction of the azido substituent of compound **2** *in vivo*, was predicted to dramatically lower DHFR-inhibitory activity, and facilitate elimination of the polar amine metabolite **3**. The results of this study suggest that should metabolism of compound **2** arise by processes other than simple azide reduction, it is likely that the products formed will also be devoid of significant activity against the enzyme.

Experimental

Ethanol refers to 95% ethanol; light petroleum refers to the fraction b.p. 60–80 °C. All m.p.s were measured on an electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Unicam SP200 Infrared Spectrometer as potassium bromide discs. Mass spectra were recorded on a V.G. Micromass 12 instrument at 70 eV; source temperature 250–300 °C. UV spectra were recorded on a Pye Unicam SP8000 recording spectrophotometer. ^{1}H NMR Spectra were recorded either on a Bruker WH300 (300 MHz) or 2 WH400 spectrometer (400 MHz) using tetramethylsilane as the internal standard. All spectra were recorded in [$^2H_6$]DMSO as solvent and NH resonances appeared as broad singlets which were exchangeable with D_2O. The TLC systems employed Kieselgel $60F_{254}$ (0.25 mm) as the adsorbent and either chloroform–methanol (4:1) or toluene–ethanol–acetone (7:5:3) as the developing solvent.

N-Oxidation of 2,4-*Diamino*-5-(4-*chloro*-3-*nitrophenyl*)-6-*ethylpyrimidine* **6**.—Hydrogen peroxide solution (30% v/v; 2.8

Table 2 Spectral properties of the diaminopyrimidine *N*-oxides



Compd	*N*-Oxide	R	^{1}H NMR (δ)[a] 2-NH_2[c]	4-NH_2[c]	6-CH_2	Other absorptions	UV[b] (λ_{max}/nm)
6	—	NO_2	6.01	6.10	2.15	1.00 (3 H, s, Me) 7.59 (1 H, dd, 6-H) 7.86 (1 H, d, 5-H) 7.94 (1 H, d, 2-H)	283
8	3	NO_2	7.02	7.22	2.17	0.99 (3 H, s, Me) 7.59 (1 H, dd, 6-H) 7.84 (1 H, d, 5-H) 7.98 (1 H, d, 2-H)	290
9	1	NO_2	7.92	7.08	2.46	1.05 (3 H, s, Me) 7.65 (1 H, dd, 6-H) 8.05 (1 H, d, 5-H) 7.90 (1 H, d, 2-H)	250
3	—	NH_2	5.69	5.90	2.18	1.00 (3 H, s, Me) 6.38 (1 H, dd, 6-H) 6.65 (1 H, d, 2-H) 7.25 (1 H, d, 5-H)	284
10	3	NH_2	6.65	7.14	2.19	1.00 (3 H. s. Me) 6.39 (1 H, dd, 6-H) 6.64 (1 H, d, 2-H) 7.26 (1 H, d, 5-H)	293
2	—	N_3	5.84	5.96	2.18	1.00 (3 H, s, Me) 7.00 (1 H, dd, 6-H) 7.19 (1 H, d, 2-H) 7.55 (1 H, d, 5-H)	284
11	3	N_3	6.92	7.25	2.22	1.00 (3 H, s, Me) 7.11 (1 H, dd, 6-H) 7.34 (1 H, d, 2-H) 7.63 (1 H, d, 5-H)	290
12	1	N_3	7.88	7.01	2.52	1.06 (3 H, s, Me) 7.11 (1 H, dd, 6-H) 7.48 (1 H, d, 2-H) 7.69 (1 H, d, 5-H)	246

[a] Solvent [$^2H_6$]DMSO, 1% TMS. [b] Solvent 95% ethanol. [c] Broad singlet exchangeable on deuteration and assigned in accordance with literature[9]

g) was added dropwise over a period of 1 h to a stirred solution of compound **6** (1.0 g) in formic acid (90%; 20 cm^3), and the mixture stirred for 72 h at 25 °C. Following the addition of water (50 cm^3), the pale yellow solution was basified with conc. aq. ammonia solution and the resultant cream solid collected by filtration. Recrystallisation from ethanol gave orange needles of 2,4-diamino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidine 3-oxide **8** (0.57 g, 54%), m.p. 250–252 °C (Found: C, 46.8; H, 3.9; N, 22.2. $C_{12}H_{12}ClN_5O_3$ requires C, 46.5; H, 3.9; N, 22.6%).

In addition, the crude reaction product was subjected to chromatography on silica gel, with chloroform–methanol (4:1) as the eluent, to give the pyrimidine 3-oxide **8** and a meagre yield of the isomeric 2,4-diamino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidine 1-oxide **9** (3%), m.p. 264–266 °C (Found: C, 46.8; H, 3.7; N, 22.5%).

2,4-*Diamino*-5-(3-*amino*-4-*chlorophenyl*)-6-*ethylpyrimidine* 3-*Oxide* **10**.—Tin(II) chloride dihydrate (3.3 g) was added to a solution of the nitropyrimethamine 3-oxide **8** (1.1 g) in ethanol (40 cm^3) and the mixture was refluxed for 1 h, concentrated to a quarter of the original volume and, after cooling, the solution was basified to pH 12 with 10 mol dm^{-3} aq. sodium hydroxide solution. The cream precipitate was collected by filtration, washed with water and recrystallised from ethanol to give, after the removal of a persistent colloidal material by filtration through a pad of Kieselguhr, the aminopyrimethamine 3-oxide **10** in moderate yield, m.p. 288–290 °C (Found: C, 51.6; H, 5.3; N, 24.8. $C_{12}H_{14}ClN_5O$ requires C, 51.5; H, 5.0; N, 25.0%).

2,4-*Diamino*-5-(3-*azido*-4-*chlorophenyl*)-6-*ethylpyrimidine* 3-*Oxide* **11**.—A solution of the amine 3-oxide **10** (2.0 g) in 5 mol dm^{-3} hydrochloric acid (50 cm^3) was diazotised at 0 °C by the addition of sodium nitrite (0.54 g) in water (2 cm^3), over a period of 20 min with constant stirring. Sodium azide (1.4 g) was added in portions over 15 min and the mixture was stirred for a further 1 h at 10 °C, then diluted with water (50 cm^3) and basified to pH 9 with conc. aq. ammonia solution. The cream product which deposited was collected by filtration and recrystallised from aqueous ethanol to furnish photosensitive microprisms of the azidopyrimidine 3-oxide **11** (1.5 g, 68.6%), m.p. 189–190 °C (decomp.) (Found: 47.7; H, 4.1; N, 32.2. $C_{12}H_{12}ClN_7O$ requires C, 47.1; H, 3.9; N, 32.1%).

Oxidation of 2,4-*Diamino*-5-(3-*azido*-4-*chlorophenyl*)-6-*ethylpyrimidine* **2**.—A solution of the *m*-azidopyrimethamine **2** (2.0 g) in formic acid (90%; 40 cm^3) was oxidised by the addition of hydrogen peroxide (30% v/v; 6 g) in portions over 30 min. The yellow solution was protected from the light and stirred for a further 12 h at 25 °C. After dilution with water (50 cm^3), the mixture was basified with conc. aq. ammonia solution and the

resultant precipitate was collected by filtration. Chromatography on silica gel with chloroform–methanol (9:1) as the eluent afforded the azidopyrimidine 3-oxide **11** (0.51 g, 24.2%) and the isomeric azidopyrimidine 1-oxide **12** (0.23 g, 10.9%). Recrystallisation from aqueous ethanol gave analytical samples of each compound. For **12**, m.p. 210–212 °C (decomp.) (Found: C, 47.4; H, 4.2; N, 32.3. $C_{12}H_{12}ClN_7O$ requires C, 47.1; H, 3.9; N, 32.1%).

Oxidation of 2,4-*Diamino*-5-(3-*amino*-4-*chlorophenyl*)-6-*ethylpyrimidine* **3**.—A solution of the aminopyrimethamine **3** (1.0 g) in methanol (100 cm^3) was stirred at room temp. and hydrogen peroxide solution (30% v/v; 50 g) was added cautiously over 30 min. Sodium tungstate dihydrate (0.5 g) was added and the mixture was stirred for a further 12 h, then diluted with water (100 cm^3) and extracted with chloroform (3 × 50 cm^3). The chloroform layer was then dried (Na_2SO_4) and the solvent evaporated under reduced pressure to afford an intensely yellow crystalline solid. Chromatography on silica gel with chloroform–methanol (9:1) as the eluent gave 2,4-diamino-5-(4-chloro-3-nitrophenyl)-6-ethylpyrimidine **6** (0.27 g, 24%) and the corresponding pyrimidine 3-oxide **8** (0.24 g, 20%) as the only products.

A pure sample of the pyrimidine 3-oxide **8** was also obtained following recrystallisation of the crude product from ethanol.

Attempted Oxidation of 2,4-*Diamino*-5-(4-*chlorophenyl*)-6-*ethylpyrimidine* **5**.—To a stirred solution of pyrimethamine **5** (1.6 g) in glacial acetic acid (90 cm^3) was added acetic anhydride (0.5 cm^3) and lead tetraacetate (3.25 g). The mixture was stirred at 80 °C for 18 h, when a starch–iodide test proved negative, then poured onto ice-aqueous ammonia and the resultant dark solid precipitate was collected. Repeated recrystallisation from ethyl acetate afforded an amorphous brown powder (0.08 g, 4%), m.p. 189–194 °C (Found: M^+, 308.1192[306]. $C_{14}H_{15}ClN_4O_2$ requires *M*, 308.1195[306]); ν_{max}/cm^{-1} 1550, 1610, 1700, 3150 and 3250.

2,4-*Diamino*-5-(4-*chlorophenyl*)-6-{[1-(2,3,5,6-*tetrafluoro*-4-*trifluoromethyl*)*phenoxy*]*ethyl*}*pyrimidine* **14**.—A mixture of 2,4-diamino-5-(4-chlorophenyl)-6-(1-hydroxyethyl)pyrimidine **13** (0.5 g), octafluorotoluene (0.5 g), tetrabutylammonium hydrogen sulfate (0.5 g), dichloromethane (30 cm^3) and aq. sodium hydroxide (2 mol dm^{-3}; 30 cm^3) was stirred at 25 °C for 24 h, with the further addition of octafluorotoluene (0.25 g) after 12 h. The organic phase was then separated, the aqueous layer extracted with dichloromethane (2 × 25 cm^3) and the combined organic layers washed with water (2 × 25 cm^3), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to furnish a cream solid. Chromatography on silica gel with ethyl acetate–light petroleum (4:1) as the eluent gave the pyrimethamine derivative **14** (0.31 g, 34%), m.p. 190–192 °C (Found: C, 47.4; H, 2.7; N, 11.9. $C_{19}H_{12}ClF_7N_4O$ requires C, 47.47; H, 2.52; N, 11.65%); δ_H 1.52 (3 H, d, Me), 5.18 (1 H, q, CH), 5.85 (1 H, br s, NH), 6.10 (3 H, br s, 3 × NH), 6.91, 7.19, 7.33 and 7.45 (4 H, 4 × d, Ph); *m/z* (FAB, 3-nitrobenzyl alcohol) 483 ([M + H], 34%), 481 ([M + H], 250 (10.5), 249 (16.7), 248 (31.7) and 247 (34.5).

Deprotection of the Perfluorotolyl Derivative **14** *of Pyrimethamine*.—A solution of the heptafluorotolylpyrimidine **14** (0.1 g) in dry dimethylformamide (5 cm^3) containing sodium methoxide (0.2 g), was stirred at 60 °C for 2 days. After dilution with water (20 cm^3), the mixture was extracted with ethyl acetate (2 × 20 cm^3) and the solvent was evaporated to give the 6-(1-hydroxyethyl)pyrimethamine **13** in quantitative yield.

2,4-*Diamino*-5-(4-*chloro*-3-*nitrophenyl*)-6-{[1-(2,3,5,6-*tetrafluoro*-4-*trifluoromethyl*)*phenoxy*]*ethyl*}*pyrimidine* **15**.—The heptafluorotolylpyrimidine **14** (0.5 g) was added over a period of 2 h to a solution of nitric acid (*d* 1.42; 0.21 g) in conc. sulfuric acid at 0 °C. The mixture was then stirred for a further 2 h at 25 °C and then poured onto ice–water. The solution was neutralised by the cautious addition of sodium hydrogen carbonate and then extracted with ethyl acetate (2 × 50 cm^3). After washing with water (50 cm^3), the ethyl acetate layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure. Chromatography on silica gel with chloroform–methanol (9:1) as the eluent gave the nitropyrimethamine derivative **15** (as a mixture of atropisomers) as pale yellow needles (0.90 g, 83%), m.p. 208–210 °C (Found: C, 43.1; H, 2.2; N, 13.3. $C_{19}H_{11}ClF_7N_5O_3$ requires C, 43.42; H, 2.11; N, 13.34%); δ_H 1.54 (3 H*, m, Me), 5.15–5.25 (1 H*, dd, CH), 6.11 (2 H, br s, NH_2), 6.22 (2 H, br s, NH_2) and 7.31–7.90 (3 H*, m, Ph); *m/z* (FAB, 3-nitrobenzyl alcohol) 528 ([M + H], 39%), 526 ([M + H], 100) and 293 (19.5).

Attempted Preparation of 2,4-*Diamino*-5-(3-*amino*-4-*chlorophenyl*)-6-{[2-(2,3,5,6-*tetrafluoro*-4-*trifluoromethyl*)*phenoxy*]*ethyl*}*pyrimidine* **16**.—To a mixture of the nitropyrimidine **15** (0.8 g) and Raney nickel (*ca.* 1 g) in ethanol (30 cm^3) at 60–65 °C, was added a solution of hydrazine hydrate (20 cm^3) in ethanol (20 cm^3) over 30 min. When the effervescence had subsided, the mixture was filtered through Celite and the solvent was evaporated off to give a cream solid. Chromatography on silica gel with chloroform–methanol (8:2) as the eluent furnished colourless microcrystals (0.31 g), which proved identical with an authentic sample of 2,4-diamino-5-(3-amino-4-chlorophenyl)-6-ethylpyrimidine (*m*-aminopyrimethamine; **3**) (lit.,[7] m.p. 215–217 °C).

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* Indicates the total contribution from each diastereoisomer.

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