INVESTIGATIONS ON NEW ANTITUMOUR AGENTS

by

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SUMMARY

Two series of compounds have been synthesised based on two non myelosuppressive antineoplastic agents, N-methylformamide (NMF) and 2-amino-5-bromo-6-phenylpyrimidine-4(3H)-one (ABPP).

Presented in part B of the thesis is the synthesis, chemistry, NMR spectroscopy, and structure-antitumour activity relationship of a series of compounds based on NMF, potential metabolites of NMF, and potential prodrugs of NMF. These agents were evaluated for antitumour activity on a number of murine models including the M5076 ovarian sarcoma and TLX5 lyphoma as well as on human xenografts implanted into nude mice such as in the MX-1 tumour model. Modification of the strucure of NMF by replacement of (i) the N-methyl group by CD_3, $CHCH_2CH_2$, CH_2CF_3, CH_2OH, CH_2OEt, or CH_2NMe_2 or (ii) the hydrogen attached to nitrogen by CH_2OAc or CH_2OPh or (iii) the formyl group by $NO_2CH=CH$, $(CN)_2C=CH$, $CNCH=CH$, $CHO(Me)C=CH$ did not afford any analogue which was as active as NMF. However, NN-dimethyl-2-nitroethenamine ($NO_2CH=CHNMe_2$) showed marginal activity against the M5076 ovarian sarcoma and TLX5 lymphoma. NMR spectroscopy showed that these formamides exhibited rotational isomerism about the amide bond and that the predominant rotamer was that expected by comparison with literature findings. However, N-(hydroxymethyl)formamides and ethers and esters of N-(hydroxymethyl)formamides had a higher percentage of the E rotamer than the corresponding N-alkylformamide. The rotameric populations of some N-(alkyl)nitroethenamines were compared to those of the corresponding N-alkylformamides.

In part C of the thesis is presented the chemistry and a structure-antitumour activity study of a series of compounds based on the biological reponse modifier ABPP. The compounds were evaluated mainly using the M5076 ovarian sarcoma and B16 melanoma murine tumour models. Some analogues with increased water solubility showed activity against the B16 melanaoma. In a combination study against the M5076 ovarain sarcoma NMF and ABPP did not enhance the reduction in the total WBC produced by cyclophosphamide.

Key Words

N-Methylformamide (NMF)
2-Amino-5-bromo-6-phenylpyrimidin-4(3H)-one (ABPP)
Antitumour
Biological Response Modifier
Interferon Inducer

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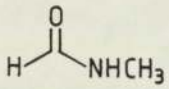
LIST OF ABBREVIATIONS

ABMP	2-Amino-5-bromo-6-methylpyrimidin-4(3H)-one
ABPP	2-Amino-5-bromo-6-phenylpyrimidin-4(3H)-one
BRM	Biological response modifier
CA	Chemical abstracts
CY	Cyclophosphamide
DME	1,2-Dimethoxyethane
DMF	Dimethylformamide
F	Formamide
glc	Gas liquid chromatography
GSH	Glutathione
HMF	N-Hydroxymethylformamide
HMMF	N-Hydroxymethyl-N-methylformamide
IFN	Interferon
im	Intramuscularly
ip	Intraperitoneally
i.r.	Infra-red
IST	Increase in survival time
iv	Intavenously
NK	Natural killer cell
NMF	N-Methylformamide
NMR	Nuclear magnetic resonance
sc	Subcutaneously
u.v.	ultra-violet

1. AIMS AND LAYOUT OF THE THESIS

The aim of the work described in this thesis was to synthesise, investigate the chemistry of, and investigate the structure anti-tumour activity relationship of analogues of two non myelosuppressive antineoplastic agents with a view to finding agents with enhanced therapeutic efficacy. These two agents are N-methylformamide (NMF, 1) and 2-amino-5-bromo-6-phenyl-pyrimidine-4(3H)-one (ABPP, 2).



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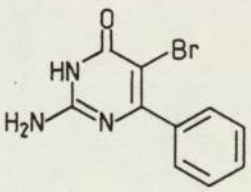

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Presented in part B of this thesis is an investigation into the chemistry, NMR spectroscopy, and structure antitumour activity requirements of a series of compounds based on (i) NMF, (ii) potential metabolites of NMF, and (iii) potential prodrugs of NMF. Part C of this thesis is concerned with the chemistry and structure antitumour-activity study of a series of compounds based on ABPP. Part D contains the experimental sections together with a number of appendices of antitumour and haematological toxicology data for some compounds described in parts B and C. Also given in part D are the bibliographic details of the thesis.

PART B

FORMAMIDE ANTITUMOUR AGENTS

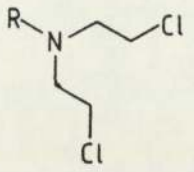
2. INTRODUCTION

2.1. INTRODUCTION

In their textbook on 'The Anticancer Drugs' Pratt and Ruddon[1] indicate that cancer in all its forms is the second biggest cause of death (ca 20%) in westernised societies. The first sucessful systemic use of an anticancer agent, nitrogen mustard (3, R= Me) was reported by Gilman and Philips[2] in 1946, although the clinical evaluation had taken place during the second world war. This trial marked the begining of modern cancer chemotherapy and although many new antineoplastic agents have been developed the prognosis for patients with solid tumours has remained poor. The small percentage of cancers for which chemotherapy contributes to a life span approaching normal life expectancy belong to the class which have a high proportion of rapidly dividing cells.

2.2. REQUIREMENT OF THE N-METHYL GROUP IN ANTICANCER DRUGS

The inclusion of certain chemical moieties in some antitumour agents has been noted as a requirement for activity[3]. One of the best examples of such a chemical grouping is the 2-chloroethylamino function which appears in NN-bis-(2-chloroethyl)amines (3)[3], cyclophosphamide (CY, 4), nitrosoureas (5)[3], aryltriazenes (6)[3], and imidazotetrazinones (7)[4]. However, the N-methyl group has also emerged as a chemical function which included into some molecules leads to antitumour agents[3]. Among this group are aryltriazenes (8)[5], nitrosoureas (9)[3], imidazotetrazinones (10)[3], procarbazine (11)[6,7], hexamethylmelamine (12)[3,8], and NMF[9]. Comparison of the N-(2-chloroethyl) and N-methyl containing groups of antitumour agents shows



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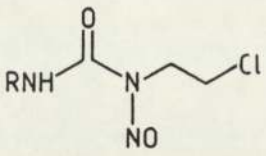

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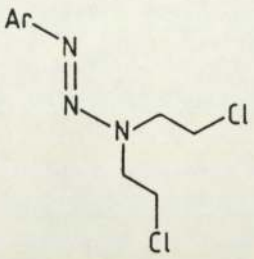

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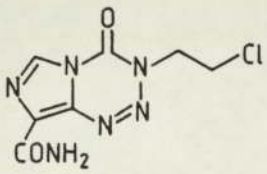

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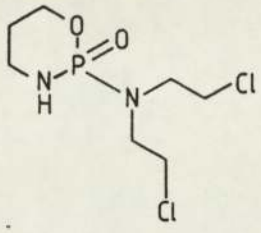

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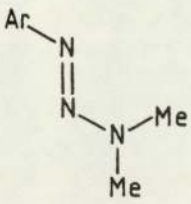

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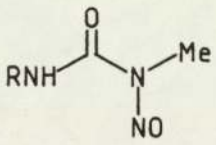

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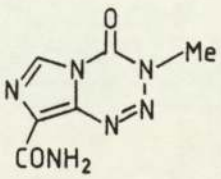

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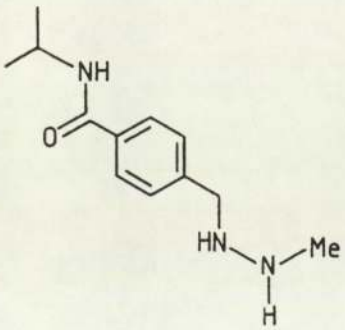

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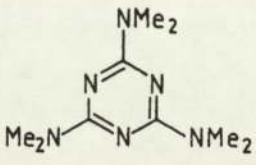


(12)

considerable overlap, with the N-(2-chloroethyl) compound usually the more active. Replacement of N-methyl by N-ethyl or higher alkyl group leads to a loss of activity. It is unclear why in these groups of medicinal agents only the N-(2-chloroethyl) and N-methyl analogues are active.

2.3. METABOLISM OF N-METHYL CONTAINING ANTITUMOUR AGENTS

The oxidative metabolism of N-methyl containing xenobiotics to stable progenitors of formaldehyde was discussed by Gescher *et al*[10]. Direct oxidation of the methyl group or oxidation on nitrogen and rearrangement would give the N-hydroxymethyl compound (carbinolamine or N-methylol, 15), scheme 1. These N-hydroxymethylamines were considered to be unstable and breakdown chemically to the nor-methylamine and formaldehyde[11]. However, the attachment of electron withdrawing groups onto nitrogen leads to more stable N-hydroxymethyl compounds[10-17]. Thus 3,3-dimethylaryltriazenes[10,12,17], hexamethylmelamine[16], N-methylbenzamide (24)[13-15], NN-dimethylbenzamide (21)[13-15] and N-(4-chlorophenyl)-N'N'-dimethylurea (28)[13] have been metabolised in mice to stable N-hydroxymethyl compounds, scheme 2, route A. Ross *et al*[14,15] have shown additionally that N-hydroxymethylbenzamide (25) is metabolised to N-formylbenzamide (27) in mice both *in vivo* and *in vitro* (mouse liver preparations and isolated hepatocytes), scheme 2, route C. The chemical breakdown of these N-hydroxymethyl compounds is given in scheme 2, route B.

A series of unsymetrically 3,3-substituted aryltriazenes were tested for antitumour activity *in vivo* in mice[5]. 3-N-Methyl-3-N-alkyltriazines which possessed an α-hydrogen in the N-alkyl group were active whereas those not possessing an α-hydrogen were inactive.

Scheme 1

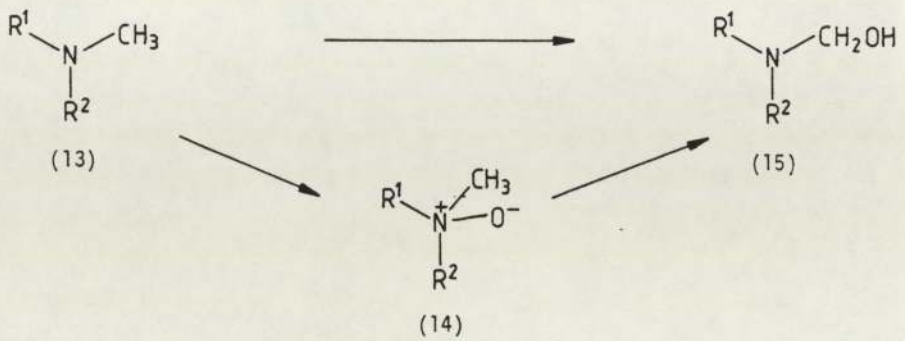
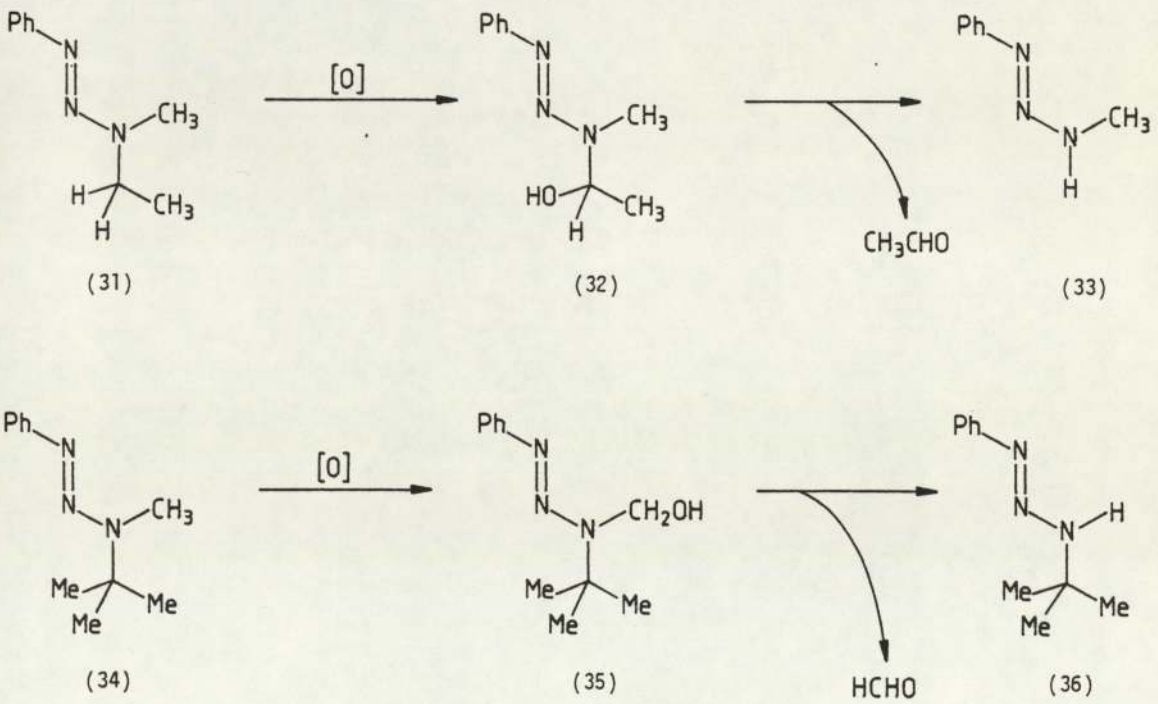


Table 1, The antitumour activity of some 3-(N-methyl)phenyltriazenes[a]

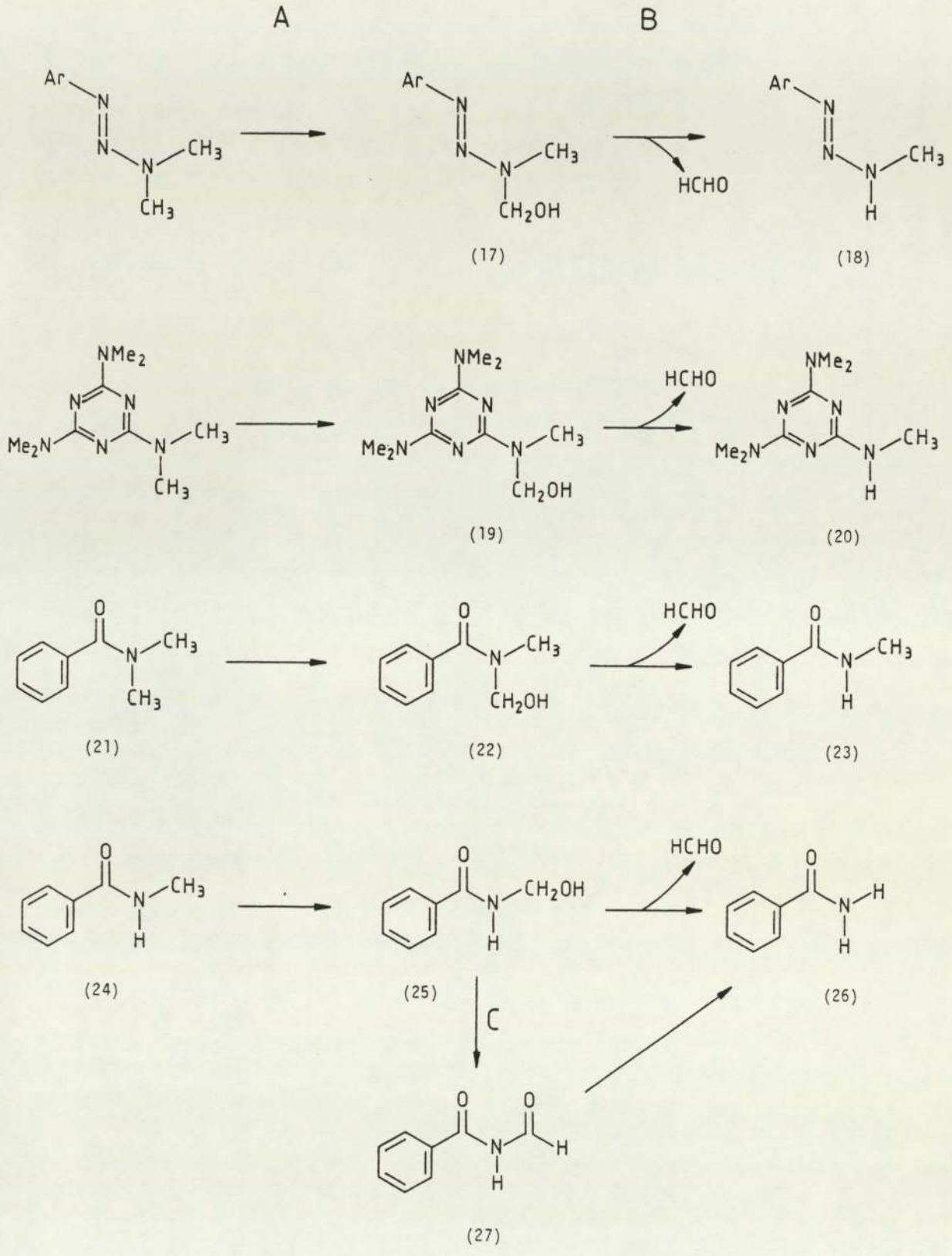
Triazene Cpd No	PhN=N-NRMe R	Antitumour Activity
31	Et	+
34	Bu^t	-

a From Audette et al ref 5

Scheme 3,



Scheme 2

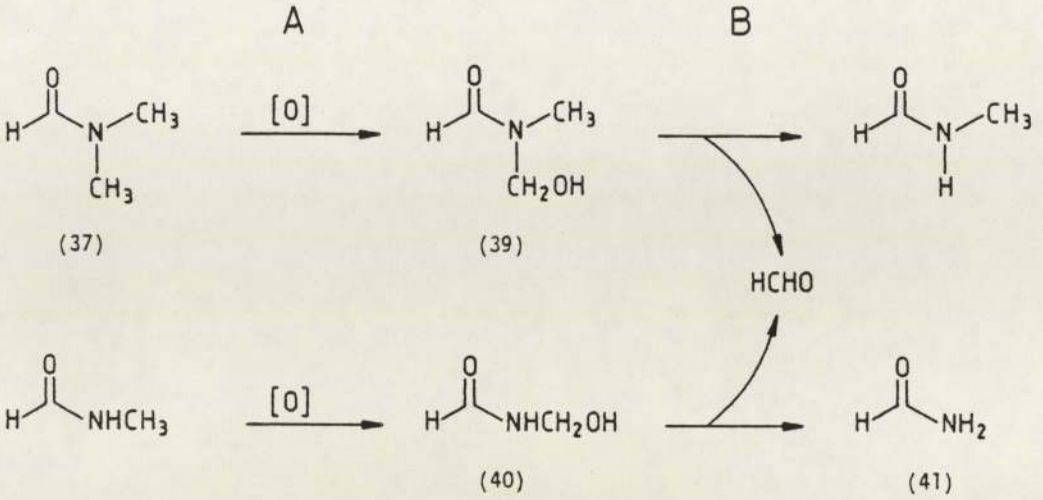

A
B
Ar
N
N
CH3
N
CH3
CH2OH
HCHO
H
(17)
(18)
NMe2
Me2N
(19)
(20)
O
(21)
(22)
(23)
(24)
(25)
(26)
C
(27)
Cl
(28)
(29)
(30)

Thus, 3-N-ethyl-3-N-(methyl)phenyltriazene (31) was active whereas 3-N-^{t}butyl-N-(methyl)phenyltriazene (34) was inactive, table 1. The explanation was that 3-N-alkyl groups bearing α-hydrogens are metabolised on this alkyl group in preference to the N-methyl group, thus generating 3-N-(methyl)phenyltriazene (33) which is the active species, scheme 3. On the other hand, in the case of N-alkyl groups not possessing an α-hydrogen metabolism occurs on the N-methyl which does not generate an active antitumour species.

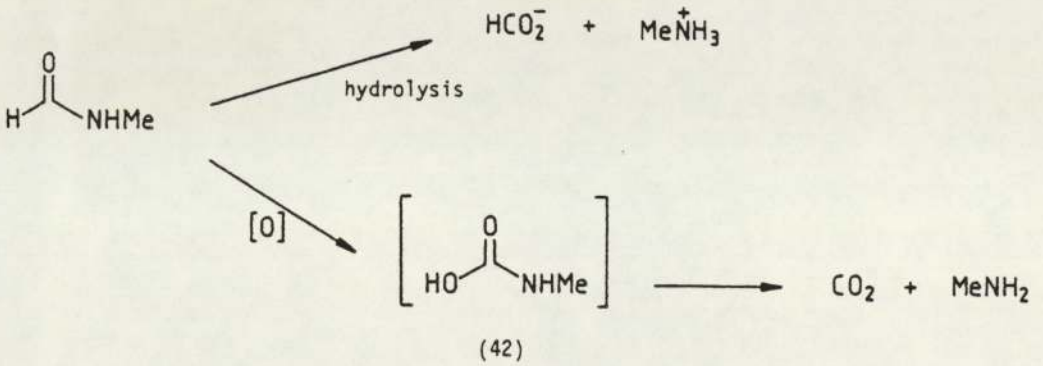
2.4. METABOLISM OF FORMAMIDES

Metabolic oxidation of the N-methyl groups of NMF and dimethylformamide (DMF, 37) would lead to the N-(hydroxymethyl)amides (carbinolamides) N-hydroxymethylformamide (HMF, 40) and N-hydroxymethyl-N-methylformamide (HMMF, 39) repectively, scheme 4, route A. Studies in rats[18,19] and humans[21] on DMF, a widely used industrial solvent, have shown NMF and formamide (F, 41) to be metabolites and inferred that NMF was responsible for its hepatotoxicity. Glc analysis has shown that both F and NMF are plasma and urinary metabolites of DMF[22] and that F is a plasma and urinary metablolite of NMF in mice[23]. However, it was noted[21-23] that glc does not distinguish between N-(hydroxymethyl)formamides and their nor N-hydroxymethyl compound as the carbinolamides under the conditions of the glc analysis break down to formaldehyde and the amide, scheme 4, route B. Brindley *et al*[22] have shown that the urine of mice treated with DMF required alkaline hydrolysis to liberate formaldehyde, as assayed colormetrically by the Nash reagent, whereas alkalinised urine of control urine did not give a positive test result for formaldehyde. As HMMF releases formaldehyde only in alkali it was concluded that the species identified as NMF in the plasma and urine

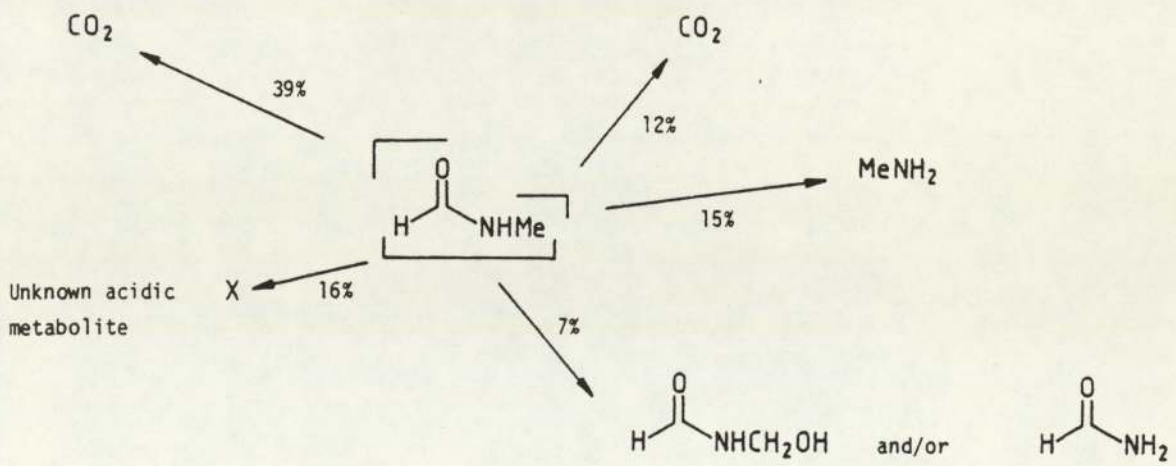
Scheme 4



Scheme 5



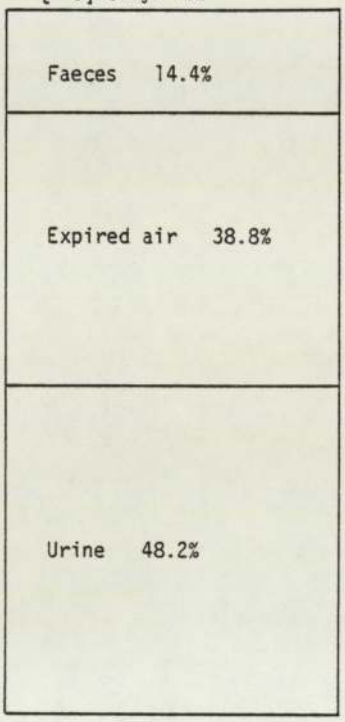
Scheme 6



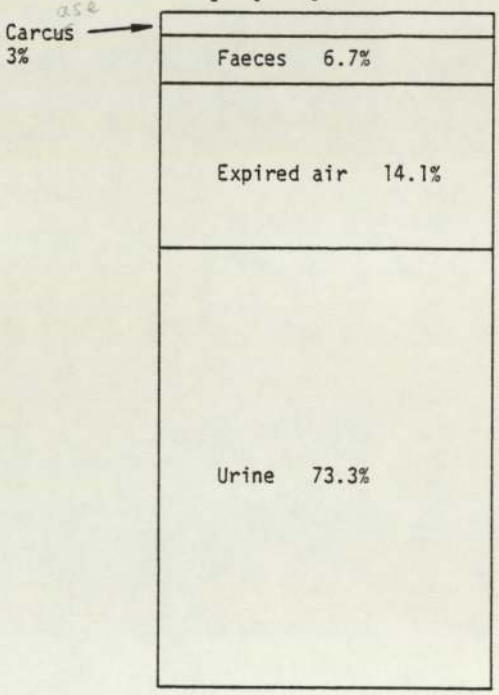
Stacked Pie Charts 1-4

The excretion of radioactivity upto 72 hr after the administration of 400mg/Kg ^{14}C labelled NMF to male CBA/CA mice

1 $[^{14}C]$Formyl-NMF

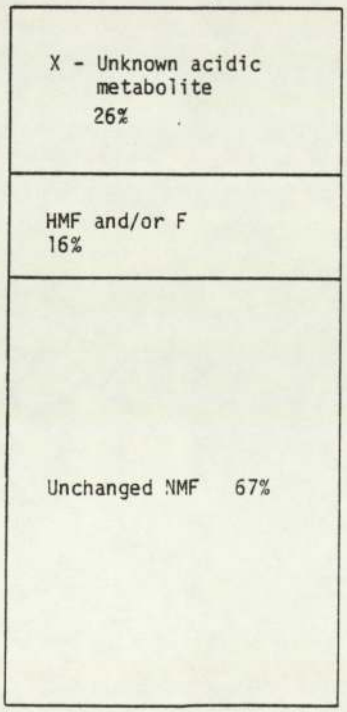


2 $[^{14}C]$Methyl-NMF

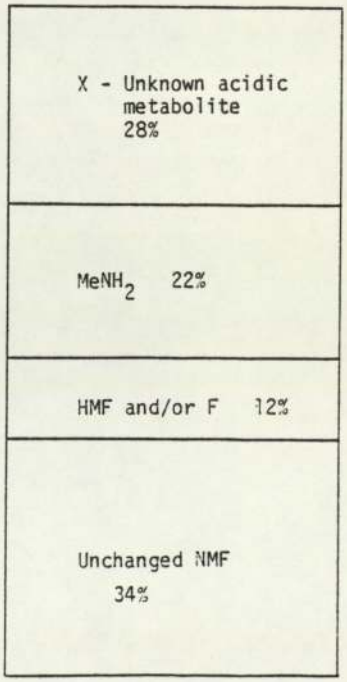


The distribution of metabolites in a 24 hr urine collection from male CBA/CA mice after the administration of 400mg/Kg ^{14}C labelled NMF

3 $[^{14}C]$Formyl-NMF



4 $[^{14}C]$Methyl-NMF



of mice treated with DMF may well have been HMMF. Analogous ivestigations[23] inferred that HMF was a metabolite of NMF. Using NMR, Kestell *et al*[24], have recently unequivocally shown that HMMF rather than NMF is the major urinary metabolite of mice treated with DMF.

The metabolism of NMF in mice has been demonstrated *in vivo*[9,23,25] but could not be shown *in vitro*[9,25]. Brindley *et al*[23] using [^{14}C]methyl-NMF showed that the plasma concentration of radioactivity versus time was superimposable on the curve for NMF (measured by glc) versus time for the initial 24 hours. Radioactivity was still detectable in the plasma 8 days after NMF administration. Of the total radioactivity injected 74% was recovered in the urine after 24 hours (26% of this as unchanged NMF) whereas 3% of the radioactivity was exhaled in 7 hours as $^{14}CO_2$ at a constant rate of 0.007% of the dose per minute. Less than 2% of the dose was eliminated as F (or HMF). In a recent study Kestell *et al*[26] have investigated *in vivo* murine metabolism of NMF using both [^{14}C]methyl-NMF and [^{14}C]formyl-NMF. The results are summarised in scheme 6 and stacked pie charts 1-4. The results have revealed that methylamine, although an endogenous amine, is a major metabolite of NMF as well as an unknown acidic compound. The methylamine could conceivably arise from hydrolysis of NMF or from metabolism possibly via N-methylcarbamic acid (42), which would spontaneously decompose to methylamine and CO_2, scheme 5. 39% of the [^{14}C]formyl-NMF dose is exhaled and labelled formate could not be detected. Differences in the metabolism of NMF in various strains of mice have been reported by Pearson *et al*[27,30] and Gescher *et al*[28,29] who have also shown that the radioactivity of either [^{14}C]formyl-NMF or [^{14}C]methyl-NMF is covalently bound into hepatic protein.

2.5. HEPATIC AND HAEMATOPOEITIC TOXICITY OF NMF

In a study involving five patients in 1956 Myers *et al*[31] showed NMF to be hepatotoxic in man as indicated by serum liver function tests. In mice an effective antitumour dose (400mg/Kg) caused a 60% depletion of non protein thiol after 1 hour[9]. This depletion was not however brought about by F or N-ethylformamide (NEF, 44) and may indicate the production of an electrophilic species from NMF. The depletion of glutathione (GSH, 43) *in vitro* as well as *in vivo* has been shown by Whitby *et al*[32] who noted that the depletion *in vitro* was only observable after 1 hour as was lipid peroxidation. NMF was more toxic in acute rather than chronic regimens. Glutathione has been depleted *in vitro* by high concentrations (>100mM) of DMF[33], although the effect could be negated by pre-treatment with L-cysteine. Liver necrosis and elevated sorbitol dehydrogenase (SDH) were observed in mice[32] after the administration of NMF (400 mg/Kg). Similar signs of toxicity have been seen in dogs[34] for NMF and in rats for DMF[35]. The ability[36] of murine mitochondria to sequester Ca^{2+} was also affected by NMF but not by F, DMF, or HMF.

Langdon *et al*[37] have investigated the effect of NMF on the blood forming system as well as on the plasma levels of SDH, L-alanine aminotransferase (ALT), and L-aspartate aminotransferase (AST) in mice. An LD_{10} dose of NMF (800 mg/Kg, BALB/c) did not produce any change in peripheral white blood cell count and an LD_{50} dose (2300 mg/Kg) produced only a mild granulocytosis. No enhanced toxicity was observed towards the blood forming units over that produced by cyclophosphamide (CY) alone or when NMF and CY were used in combination with each other against the M5076 ovarian sarcoma murine tumour model[38]. Since the antitumour response was more than additive Langdon

et al proposed NMF as a candidate for combination chemotherapy with myelosuppresive drugs.

2.6. CLINICAL EVALUATIONS OF NMF

NMF has been evaluated for antineoplastic activity in phase I clinical trials both in the 1950's[31] and more recently[39-41] and is now entering phase II clinical evaluation in Europe and the USA. Interest in NMF lapsed after the trial in the 1950's, which reported no activity but observed hepatotoxicity, and was only regained in the early 1980's.

3. DISCUSSION

3.1. STRUCTURE ANTITUMOUR-ACTIVITY RELATIONSHIPS OF THE FORMAMIDES

3.1.1. Introduction

Clarke et al[42] following the use of some aliphatic amides as solvents for the parenteral administration of organic chemicals in the sarcoma 180 murine tumour model reported the activity of NMF on that model. This was the first report of the antitumour activity of NMF which was more active than F while DMF was not active. The activity took the form of a delay in growth rather than an abolition of the sc tumour and the delay in growth could be observed if NMF was administered 24 or 96 hours after implantation of the tumour. Activity was found when NMF was given ip or orally. Some structure-activity investigations were performed by Furst et al[43] who used the Erlich ascites tumour model and administered the amides in food-stuffs, usually at a concentration of 0.1%. The N-formyl derivatives of some primary and secondary aliphatic and aromatic amines as well as some acetamides and HMF were investigated. The only amide which was active was NMF. The later studies by Gescher et al[9] and Langdon et al[44] employed the murine tumour models of the sarcoma 180, TLX5 lyphoma and the slower growing solid M5076 ovarian sarcoma: these authors evaluated the results from the analysis of dose-response curves. The chemosensitivity of the M5076 murine sarcoma is given in table 2. NMF was the only amide to show activity, confirming the, at best, marginal activity of F, NEF, DMF, and HMF. The antitumour activity of NMF is more pronounced in chronic as opposed to acute regimens against the M5076 murine ovarian sarcoma model[45]. In addition Langdon et al[44] showed, table 3, that replacing the formyl

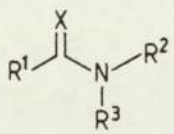
Table 2, Chemosensitivity of the M5076 murine sarcoma model[a]

Compound	Activity[d]
Cyclophoshamide	++
cis-Platinum	++
CCNU	++
Methotrexate	-
Adriamycin	-
Procarbazine	++
Hexamethylmelamine	+
N-Methylformamide	++

a Langdon et al ref 44

d ++ Therapeutic index > 2
+ Therapeutic index > 1

Table 3. Activity of analogues of NMF on the M5076 murine sarcoma model[a,b]



Cpd No	X	R^1	R^2	R^3	LD_{10}[c]	LD_{50}[c]	Optimal Dose[c]	T:C[d] (%)
41	O	H	H	H	200	270	200	37
1	O	H	Me	H	220	300	200	0
44	O	H	Et	H	320	420	300	78
37	O	H	Me	Me	1130	1280	100	40
45	O	Me	Me	H	880	1240	800	100
46	O	Me	Me	Me	900	1420	800	78
47	O	CF_3	Me	H	800		800	95
48	O	NHMe	Me	H	1730	2260	1600	66
49	O	NMe_2	Me	Me	440	640	400	67
50	S	H	Me	Me	185	260	200	97
51	S	NHMe	Me	H	450	720	400	91
52	S	NMe_2	Me	Me	430	565	400	79
53	NH	NMe_2	Me	Me	107	142	100	100

a Langdon et al ref 44,
b Compounds ip on days 1-17 [QO1DX17(1)]
c mg/Kg/dose
d % tumour growth for optimal dose assessed on day 24

proton of formamides with amino or substituted amino, or replacement of the carbonyl oxygen with sulphur still lead to a loss of activity, although N-methylthioformamide was not tested.

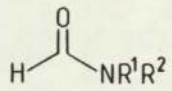
The effect of NMF on nucleic acid synthesis was investigated[42,46-51] in the 1950's but the results were inconclusive. More recently the ability of NMF to induce differentiation has been demonstrated[30,52]. However, the concentration of drug required (>100 mM) is far greater then the peak[53] plasma concentration achieved *in vivo* (ca 7 mM) and is close to a lethal dose. DMF (inactive in murine experimental tumour models) has also been shown to induce differentiation of tumour cells[33,54] *in vitro* and Langdon *et al*[55] have shown that the ability of a number of related 'small' molecules to induce differentiation of tumour cells *in vitro* is related to their molecular weight.

3.1.2. Rationale and antitumour results

A series of analogues of NMF were tested for antitumour activity using mainly the M5076 ovarian sarcoma and TLX5 murine lymphoma tumour models. The activity of these agents is given in table 4 and appendix 1. The chemosensitivity of the TLX5 lymphoma is given in table 7.

In vitro antitumour studies[9] on simple formamides showed that NMF was no more toxic to murine tumour cell lines than any other aliphatic amide tested which contrasts sharply with *in vivo* results. This *in vitro* toxicity was assumed to be non specific and implied that *in vivo* metabolism of NMF generated the 'active' species. However, advantageous pharmacokinetic characteristics can not be ruled out as responsible for the *in vivo* antitumour responses of NMF.

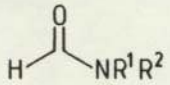
Table 4, Activity of NMF and analogues against the M5076 sarcoma and the TLX5 lymphoma murine tumour models.



Cpd No	X	R^1	R^2	TLX5[a] Dose[c] Range	Optimal Dose[c]	Wt Dif (g)[d]	Day of Death[i] C	T	T:C[e] (%)	M5076[b] Dose[c] Range	Optimal Dose[c]	Wt Dif (g)[d]	Tumour Vol[f] C	T	T:C[e] (%)
41	0	H	H	1200-50	800	g	9.1	11.2	**123**	400-100	100	g	2.06	0.76	**37**
1	0	H	Me	800-50	800	-4.3	9.2	17.6	**191**	400-6.25	200	-2.9	2.72	0.0	**0**
55	0	H	CD_3	800-50	400	-4.8	13.2	18.8	**142**[h]						
44	0	H	Et	800-100	800		9.2	12.6	**137**	600-100	300		2.49	1.95	**78**
56	0	H	$CHCH_2CH_2$							800-25	400	-1.8	4.2	3.0	**71**
40	0	H	CH_2OH	1600-100	1600	-1.2	9.6	10.2	**106**	2500-600	1500		2.06	0.77	**37**
57	0	H	CH_2OEt	6400-50	1600	-0.4	12.0	13.0	**108**						
58	0	H	CH_2NHCHO	800-50	400	-2.4	11.6	11.2	**97**						
59	0	H	CH_2NMe_2	800-50	100	+0.5	11.8	11.8	**100**						
60	0	H	CH_2CF_3	800-50	400	-3.6	13.2	15.8	**120**						
61	0	H	CH_2CH_2Cl	900-28	450	-2.7	11.6	12.6	**108**						
62	0	Me	OH	1600-100	400	-2.0	10.3	12.2	**118**						
37	0	Me	Me	3200-50	1600	-1.1	12.2	13.8	**113**	1500-600	1000	-0.3	2.49	0.99	**40**
39	0	Me	CH_2OH	1600-25	800	-1.1	10.0	10.4	**104**	1200-100	400	-1.5[g]	3.20	2.43	**76**
63	0	Me	CH_2OAc	2000-50	800	-0.8	11.6	12.0	**103**	400-100	100	-1.6	2.89	2.33	**80**
64	0	Me	CH_2OCOPh	800-50	400	+0.3	11.0	10.6	**96**						

cont

cont



Cpd No	X	R^1	R^2	TLX5[a] Dose[c] Range	Optimal Dose[c]	Wt Dif (g)[d]	Day of Death[i] C	T	T:C[e] (%)	M5076[c]b Dose[c] Range	Optimal Dose[c]	Wt Dif (g)[d]	Tumour Vol[f] C	T	T:C[e] (%)
65	$N(CHO)CH_2N(CHO)CH_2N(CHO)CH_2$			800-50	>800	+0.4	12.4	11.4	**92**						
66	$CMeCHO$	H	Me	800-50	>800	-2.6	11.7	12.4	**106**						
67	CHCN	Me	Me	800-50	50	0.0	11.7	12.0	**103**						
68	$C(CN)_2$	Me	Me	800-50	50	-0.2	12.4	11.6	**94**						
69	$CHNO_2$	H	H	800-50	100	0.0	11.7	12.0	**103**						
70	$CHNO_2$	H	Me	400-25	200	-1.4	11.6	11.0	**95**						
71	$CHNO_2$	Me	Me	400-25	100	-4.0	11.1	15.4	**139**	200-25	50	-1.6	1.4	0.5	**36**
72	$CHNO_2$	$-CH_2CH_2OCH_2CH_2-$		3200-50	800	+0.2	12.4	11.8	**95**						

a Compounds administered ip on days 3-7 Q01DX05(3)

b Compunds administered ip on days 1-17 Q01DX17(1)

c mg/Kg/dose, see appendix 1

d Weight difference (treated minus control) on last day of treatment

e Treated compared to control (%)

f On day 24, mean tumour volume in cm^{-3}

g One death due to toxicity

h Positive control, NMF 400 mg/Kg/dose, T:C(%) 139

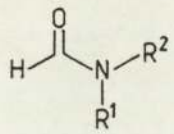
i Mean day of death

Table 5, Chemosensitivity of the mammary MX-1 sub-renal capsule xenograft tumour model[a]

Compound	Activity (T/C, %)
Cyclophosphamide	-37
Methotrexate	93
Adriamycin	59
cis Platinum	-17
BCNU	17
DMF	-13

a A Goldin et al, ref 58. Fragments of the MX-1 brest xenograft were implanted under the renal capsule of recipient mice on day 0 and the tumour length (a) and width (b) were measured in situ. On day 11 the tumour was again measured and activity was based on the the change in average tumour diameter of treated animals compared with control animals. Thus T/C(%) = DT/DC where DT is the mean tumour diameter (a+b)/2 of the treated group on day 11 less the mean tumour diameter on day 0, and DC is the change in mean tumour diameter for the control group over the same period.

Table 6, Activity of NMF and analogues on the mammary MX-1 sub-renal capsule xenograft tumour model in Nu/Nu BALB/c athymic mice



Cpd No	R^1	R^2	Dose Range[a] mg/Kg/dose	Optimum[a] Dose mg/Kg/dose	For optimum dose Cont body change	Wt Dif (T-C)	T:C (%)
1	H	Me	100-800	400	+1.4	+0.2	-74
40	H	CH_2OH	300-2000	2000	+1.8	-1.0	5
63	Me	CH_2OAc	300-2400	>2400	-1.2	+0.6	-8

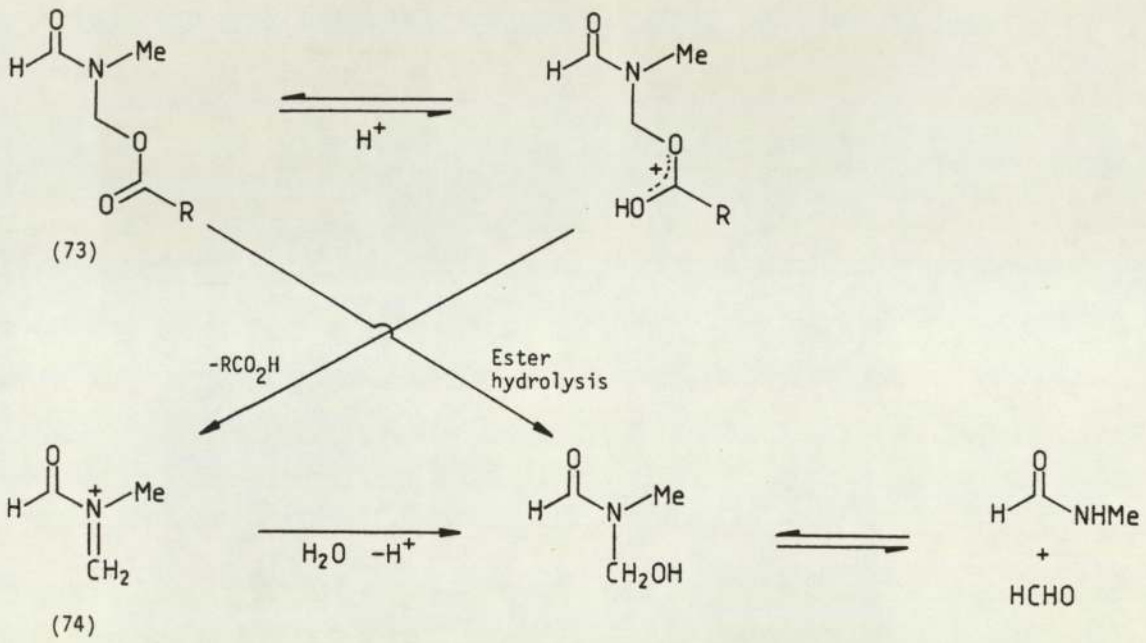
a All compounds given ip on days 1-10, [Q01DX10(1)]

Table 7, The chemosensitivity of the TLX5 murine lymphoma[a]

Compound	T:C (%)
Triazene[b]	167
BCNU	297
Methotrexate	155
Cytosine Arabinoside	139

a From R C S Audette et al, ref 5
b 5-(3,3-Dimethyl-1-triazeno)-4-carbethoxy-2-methylimidazole

Scheme 7



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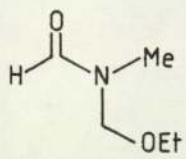
(74)



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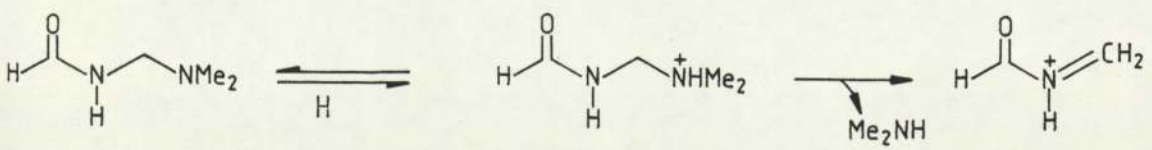
Scheme 8



(59)

The metabolism of NMF and DMF has been demonstrated *in vivo*[9,22,23,56] but could not be shown *in vitro*[9,56]

HMF showed only marginal activity against murine tumour models, but did inhibit the growth of the human xenogaft mammary tumour MX-1 implanted in the sub renal capsule in nude mice, table 6. NMF however, was more active on xenograft tumour models. The chemosensitivity of the MX-1 xenograft model is given in table 5. *In vitro* HMF in comparison to NMF was more cytotoxic to TLX5 cells and inhibited the incorporation of radiolabelled thymidine, uridine, formate, and leucine[57]. This enhanced inhibitory effect of HMF could however, be abolished by the preincubation of these cells with semicarbazide. These inhibitory effects were therefore assigned to the equilibrium amount of formaldehyde present in HMF (about 1% in normal saline at pH 7.4).

HMMF did not show any significant antitumour activity although it is a potential pro-drug of NMF; the formation of N-hydroxymethylamides is a revesible reaction and breakdown *in vivo* would liberate NMF and formaldehyde. However, as mentioned above Brindley *et al*[22] and Kestell *et al*[24] have shown that HMMF is stable in urine and probably stable in plasma.

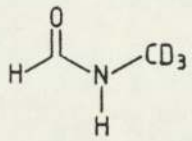
To increase the stability of these amides bearing a σ bonded oxygen in the α carbon of the N-alkyl group ethers (ethyl) of HMF and esters (acetyl and benzoyl) were tested for antitumour activity. None showed activity against the TLX5 lymphoma but N-acetoxymethyl-N-methylformamide (73, R=Me) showed activity against the MX-1 subrenal xenograft model, table 6. This activity was not as high as that for NMF although a potential a pro-drug of HMMF and NMF, scheme 7. The N-formyl-N-methylmethyleneiminium ion (74) is proposed by S Ross *et*

al[59] as an intermediate in the acid catalysed $A_{AL}1$ reactions of the analogous formate ester N-formyloxymethyl-N-methylformamide (73, R=H), scheme 7, route A. S Ross et al reported that (73, R=H) was stable to ethanol but produced N-ethoxymethyl-N-methylformamide (75) immediately on the addition of 1 drop of concentrated hydrochloric acid. Under basic conditions (sodium ethoxide) ester exchange results in the formation of ethylformate and HMMF.

The generation of methyleneiminium ions in vivo and in vitro has been recently reviewed by Overton et al[60]. N-Alkyl amines have been shown to be metabolised α to nitrogen on the N-alkyl group to give the α hydroxy derivative which dehydrates to give the iminium ion. Gidley et al[61] correlated the antibacterial activity of some N-hydroxymethylamines to their ability to produce their corresponding methyleneiminium ion rather than to the level of free formaldehyde. Overton et al[60] argued that N-hydroxymethylamides are less likely to generate methyleneiminium ions in vitro or in vivo than are N-hydroxymethylamines because the lone pair of the amide nitrogen is less available to participate in the elimination reaction which produces the imine.

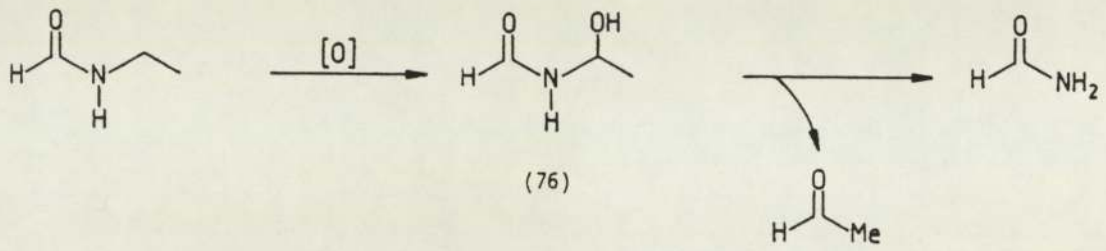
Although N-(dimethylaminomethyl)formamide (59) could potentially furnish the N-(formyl)methyleneiminium ion in vivo, scheme 8, it proved inactive against the TLX5 lymphoma.

The metabolism of NMF to HMF could be either an activation or deactivation pathway. If this route of metabolism is an activation pathway and is rate limiting then the primary isotope effect reducing the rate of production of the carbinolamide should result in a reduction in antitumour activity for N-(trideuteromethyl)formamide (55). On the other hand, if the pathway is a deactivation pathway



(55)

Scheme 9



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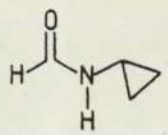

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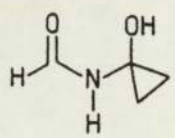

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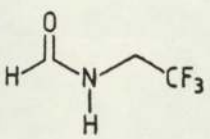

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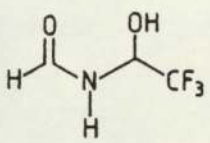

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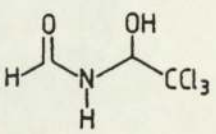

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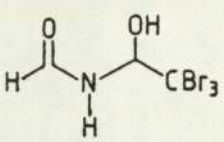

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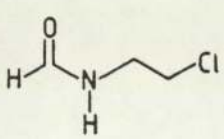

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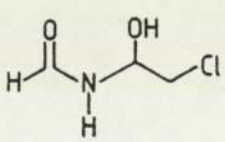

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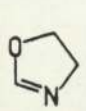

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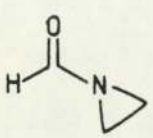
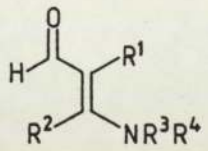


(82)

then an increase in activity should result. However, the trideuteromethyl analogue had the same antitumour activity as NMF against the TLX5 lymphoma, table 4, but was more toxic.

If the production of the N-(α-hydroxyalkyl)amide (i.e. HMF) from NMF is a requirement for activity then since N-(1-hydroxyethyl)-formamide (76) would breakdown spontaneously to F and acetaldehyde the marginal inactivity of NEF could be explained, scheme 9. However, if metabolised in the same way N-cyclopropylformamide (56) and N-(2,2,2-trifluoroethyl)formamide (60) should lead to more stable N-(α-hydroxyalkyl)formamides [i.e. N-(1-hydroxycyclopropyl)formamide (77) and N-(2,2,2-trifluoro-1-hydroxyethyl)formamide (78)]. The expected stability of (77) is a result of decreased ring strain; the prefered >CO bond angle in cyclopropanone is 120° whereas the real angle is 60° but, in (77) the prefered angle[62] is 109.5°. Attachment of electron withdrawing moieties on to the methylene group of HMF should lead to a more stable carbinolamides. (78) (Fluoralformamide) has not been reported but the analogous N-(2,2,2-trichloro-1-hydroxyethyl)-formamide (chloralformamide, 79) and N-(2,2,2-tribromo-1-hydroxy-ethyl)formamide (bromalformamide, 80) are well characterised stable compounds. Neither N-cyclopropylformamide or N-(2,2,2-trifluoro-ethyl)formamide showed activity against the TLX5 lyphoma. The carbinolamide N-(2-chloro-1-hydroxyethyl)formamide (81) which would be produced from N-(2-chloroethyl)formamide (61) by metabolic oxidation at the α carbon has not been reported in the literature.

Replacement of the methyl group of NMF by a 2-chloroethyl moiety to give N-(2-chloroethyl)formamide lead to a loss of activity, even though an analogous change in other series of antitumour agents such as the aryltriazenes[63], imidazotetrazinones[64], nitrosoureas[65], and N-



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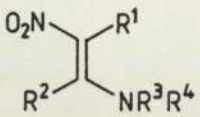

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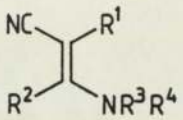

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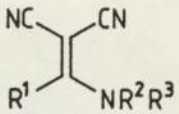

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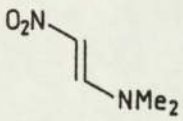

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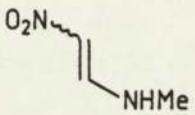


(70)

nitroso-N-nitroguanidines[66] did not result in an abolition of activity. Although N-(2-chloroethyl)formamide contains the 2-chloroethylamine moiety and therefore could potentially form an aziridine species (82), treatment of the amide with base (aqueous 50% potassium hydroxide)[67] yields 2-oxazoline (83) via intramolecular alkylation of oxygen.

In the above studies on the antitumour properties of formamides NMF is by far the most active, reflecting the importance of the methyl group. However, other formamides did show marginal activity against the TLX5 lymphoma or M5076 ovarian sarcoma models; F (M5076 63% inhibition, TLX5 23% IST), DMF (M5076 60% inhibition), NEF (TLX5 37% IST). However, all of the acetamides, ureas, and thioureas tested were inactive. This perhaps reflects a requirement for the formyl group. If the carbonyl group is considered as a π bonding system possessing a dipole then the attachment of electron withdrawing groups to an olefinic group may lead to pseudo-formyl moieties or electronic isosteres. Various 'push-pull' olefins were tested, table 4, for antitumour activity; β-aminoacroleins (84, formyl replaced by 2-formylethenyl, vinylogous amides), 2-nitroethenamines (85, formyl replaced by 2-nitroethenyl), β-aminoacrylonitriles (86, formyl replaced by 2-cyanoethenyl), 2-cyano-3-aminopropenenitriles (87, formyl replaced by 'methylenemalononitrile'). Only NN-dimethyl-2-nitroethenamine (71) showed activity (marginal) against the tumour models (TLX5 lyphoma and M5076 ovarian sarcoma), whereas the N-methyl analogue (70) proved to be inactive although bearing a closer superficial resemblance to the lead antitumour compound NMF.

In summary, analogues of NMF have been tested which are (i) potential pro-drugs of NMF, (ii) potential metabolites of NMF or DMF,

(iii) could be metabolised to stable N-(α-hydroxyalkyl)amides, (iv) potential precursors of N-(acyl)methyleneimines, (v) pseudo-amides. However, all the analogues tested were inferior to NMF.

3.2. SYNTHESIS OF ANALOGUES OF NMF

3.2.1. Simple Amides

There are several general methods for the synthesis of amides. Probably the most common involves the coupling of an amine with an acylating agent such as an acid chloride or anhydride. In the case of formamides formyl chloride and formyl anhydride are unstable at normal temperatures. The mixed anhydride formylacetic anhydride has been used but usually ethylformate is reactive enough to convert amines into formamides, scheme 10. Amides (55, 56, 61, 88) were produced essentially by the reaction of ethylformate on the appropriate amine. 2,2,2-Trifluoroethylformamide was produced by the fusion of the amine hydrochloride and sodium formate.

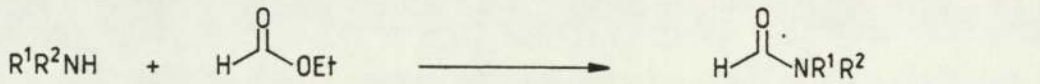
An alternative approach involves the direct alkylation of primary and secondary amides on nitrogen. However, the syntheses often involves[68] strong bases such as sodium, sodium amide, sodium hydride, or potassium tertiary butoxide. In 1981 Yamawaki *et al*[69] reported high yield N-alkylations of amides with alkylhalides under gentle conditions using potassium fluoride on alumina as catalyst, scheme 11. The use of similar conditions to ethylate NMF or F gave only the starting amide.

Differences in the infra-red spectra of NMF and its trideutero-methyl analogue (55) allowed the assignment of certain absorbance bands to vibrations of the methyl group (ν_{CH}/ν_{CD} =1.37). These assignments are given in table 8.

Table 8: Infra-red spectra (film, cm^{-1}) of NMF and its trideuteromethyl analogue (55).

V(NMF)	V(55)	V(NMF)/V(55)	Assignment
3 400	3 400	1.00	NH
3 060	3 040	1.01	CH
3 060	2 230	1.37	CH_3
2 950	2 110	1.40	CH_3
2 880	2 080	1.38	CH_3
1 660	1 660	1.00	Amide I
1 550	1 520	1.02	Amide II
1 420	1 050	1.35	CH_3
1 390	1 385	1.00	CH
1 240	1 250	0.99	Amide III
1 150	870	1.32	CH_3
960	700	1.37	CH_3

Scheme 10



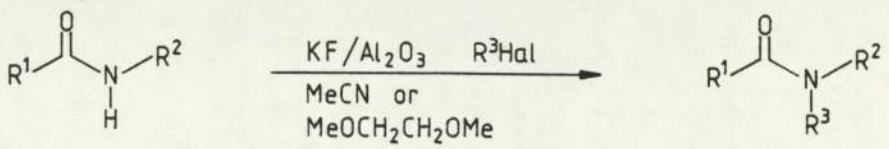
(55) R^1 = H, R^2 = CD_3

(61) R^1 = H, R^2 = CH_2CH_2Cl

(56) R^1 = H, R^2 = $CHCH_2CH_2$

(88) R^1 = Me, R^2 = Et

Scheme 11



(89) (90)

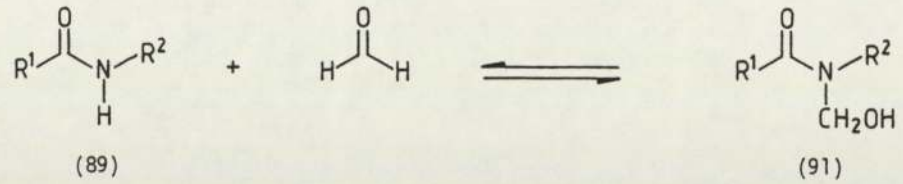
3.2.2. Synthesis of carbinolamides and related compounds

Carbinolamides (N-hydroxymethylamides, 91) are often synthesised by the reaction of the approriate amide with formaldehyde[70] scheme 12, whereas analogous carbinolamines (15) either polymerise or breakdown to the component alkylamine and formaldehyde[71]. There is considerable variation in the stability of carbinolamides. The addition of the amide to formaldehyde to produce the N-hydroxymethyl compound is a reversible reaction and Slota and LeHenaff[72] have investigated the position of equilibrium in a series of carbinolamides. This is more towards the carbinolamide for primary amides than for their N-methyl secondary amide analogues. The reaction is catalysed by both acid and base but is usually carried out in moderately basic conditions.

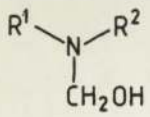
Grady and Stott[73] patented their method of synthesis of N-hydroxymethylformamide and claimed that the reaction of formamide with an anhydrous polymer of formaldehyde, preferably paraformaldehyde, in the presence of an alkali metal hydroxide as catalyst gave an almost water-white pure product in 98% yield. The analysis of HMF proved inconclusive. The micro-analysis was in agreement with the formula $C_2H_5NO_2$, but could also arise from other conceivable mixtures, eg. (a) a 1:1 mixture of formamide and formaldehyde, (b) a 1:1 mixture of N-(formamidomethyl)formamide (58) and bis NN-(hydroxymethyl)formamide (92) or, (c) a mixture of all these species in addition to HMF. The ^1H NMR spectrum of HMF [$(CD_3)_2SO$, 220 MHz] showed more resonances than could be accounted for by the N-hydroxymethylformamide structure.

Derivatization of HMF to ethers or to esters was achieved in low yield and as mixtures of products. Ethers of HMF have been reported

Scheme 12

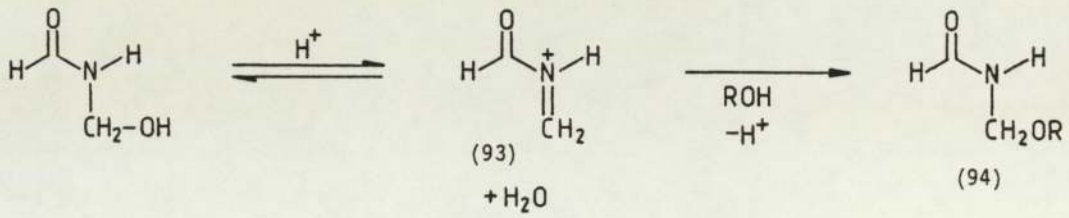


(89) (91)



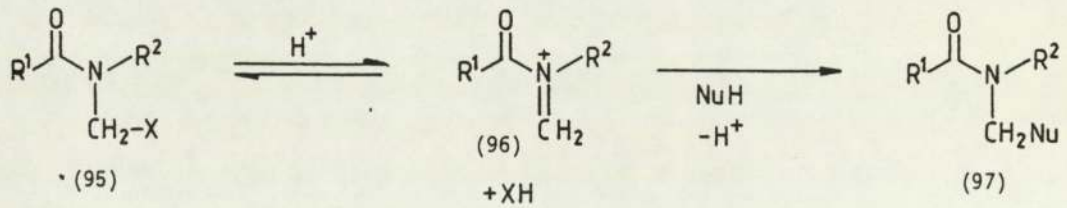
(15)

Scheme 13



(93) (94)

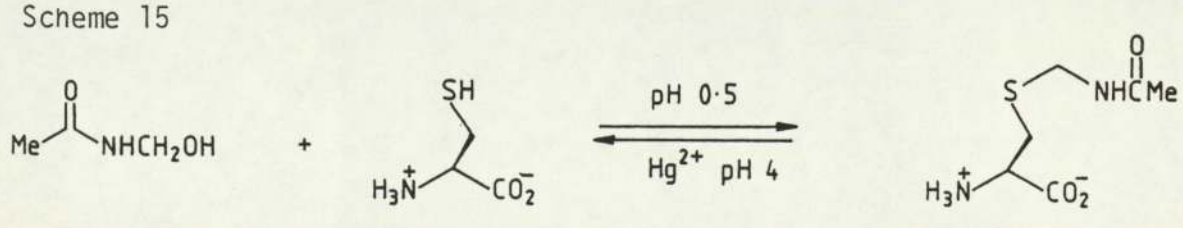
Scheme 14



(95) (96) (97)

NuH = nucleophile

Scheme 15



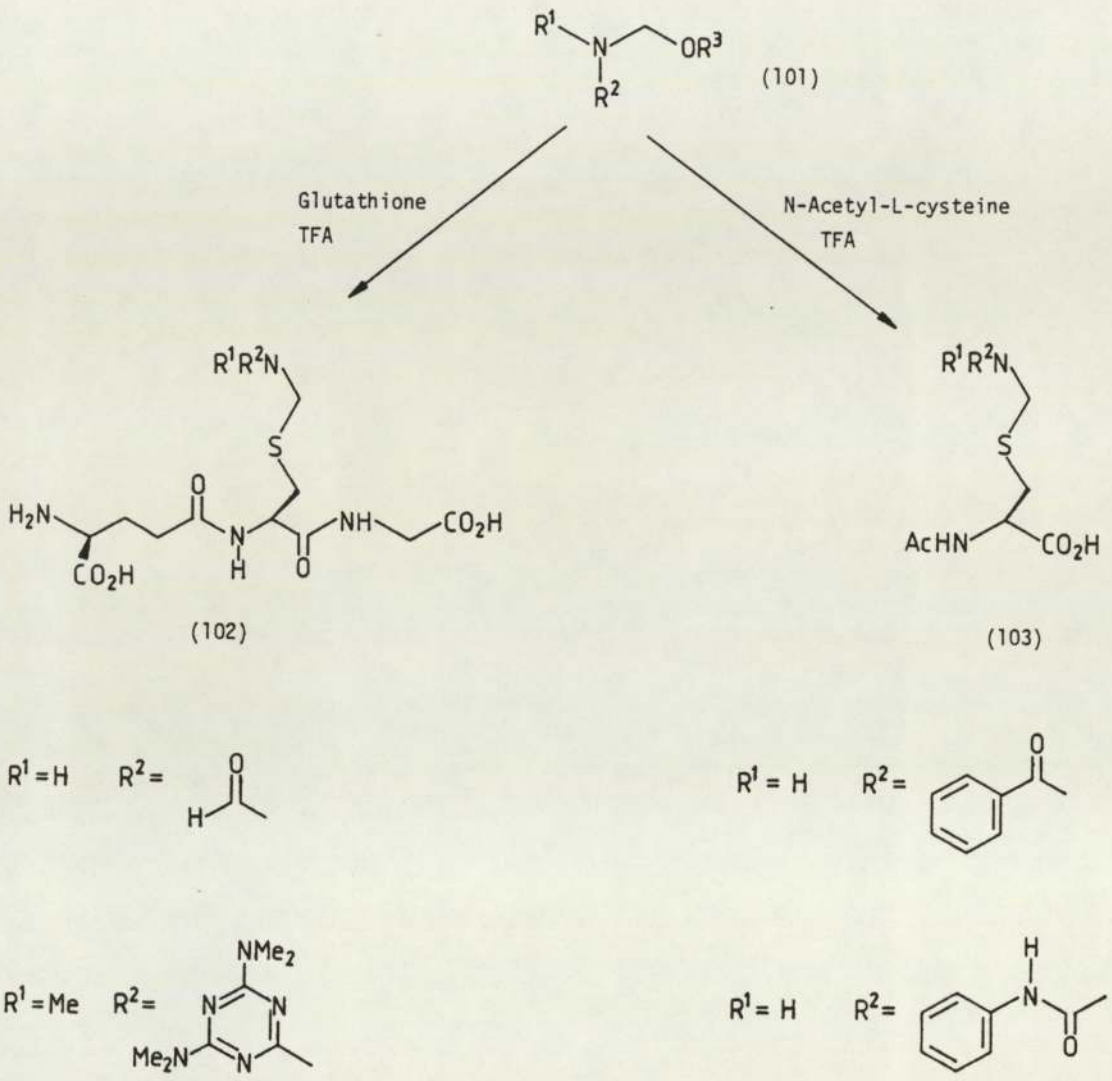
(98) (99) (100)

and ethers (94, R=Me) and (94, R=Et) were synthesised using modified methods of published procedures[74]. The synthesis, scheme 13, can be considered as the amidomethylation of the alcohol ROH via the N-(formyl)methyleneiminium ion (93).

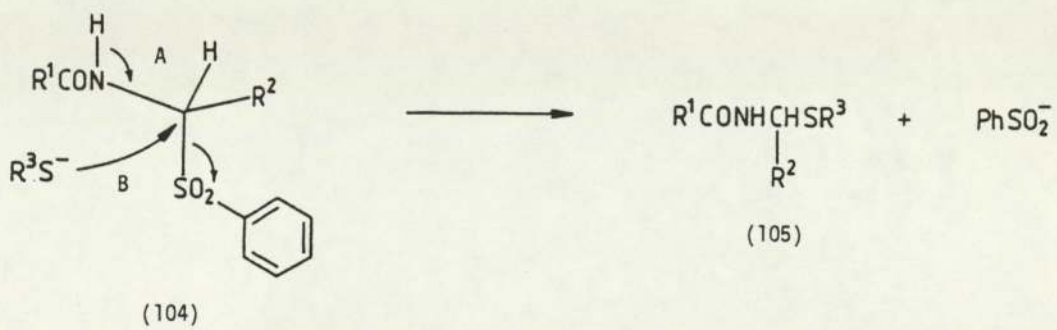
The amidomethylation reaction is a useful reaction applicable to a range of nucleophiles. The reaction is mainly carried out in strong acidic media with the N-(acyl)methyleneiminium ion being generated from a variety of precursors (95, X= OH, OAlk, O_2CR, NHCOR, NR_2, and $\overset{+}{N}R_3$) scheme 14. If the nucleophile could also act as a leaving group then the reaction is reversible. Such is the case in the formation of N-(alkoxymethyl)formamides and presumably the yields are low because of unwanted side reactions. The yields in amidomethylation reactions involving nucleophiles which irreversibly sequester the acylmethylene iminium ion are generally good whereas those with other nucleophiles are poor. Examples of irreversibly sequestering nucleophiles are those of carbon and sulphur. α-Amidoalkylation of carbon has recently been comprehensively reviewed by Zaugg[75] who gives many examples involving aromatic and olefinic substrates . The acetamidomethyl group has been used by Veber *et al*[76] as a protecting group for SH group of L-cysteine (99), scheme 15. The synthesis of a series of S-glutathione and mercapturic acid derivatives of putative metabolites of some N-methyl containing antineoplatic agents and herbicides has been achieved by Addison *et al*[77]. The methylene imminium ion generated in anhydrous trifluoroacetic acid from either the appropriate hydroxymethyl or alkoxymethyl compound was trapped with either glutathione (GSH) or N-acetyl-L-cysteine scheme 16.

Basic conditions[78,79] have also been used to produce amidomethyl sulphides and sulphones. Morton *et al*[79] proposes that the sulphur

Scheme 16



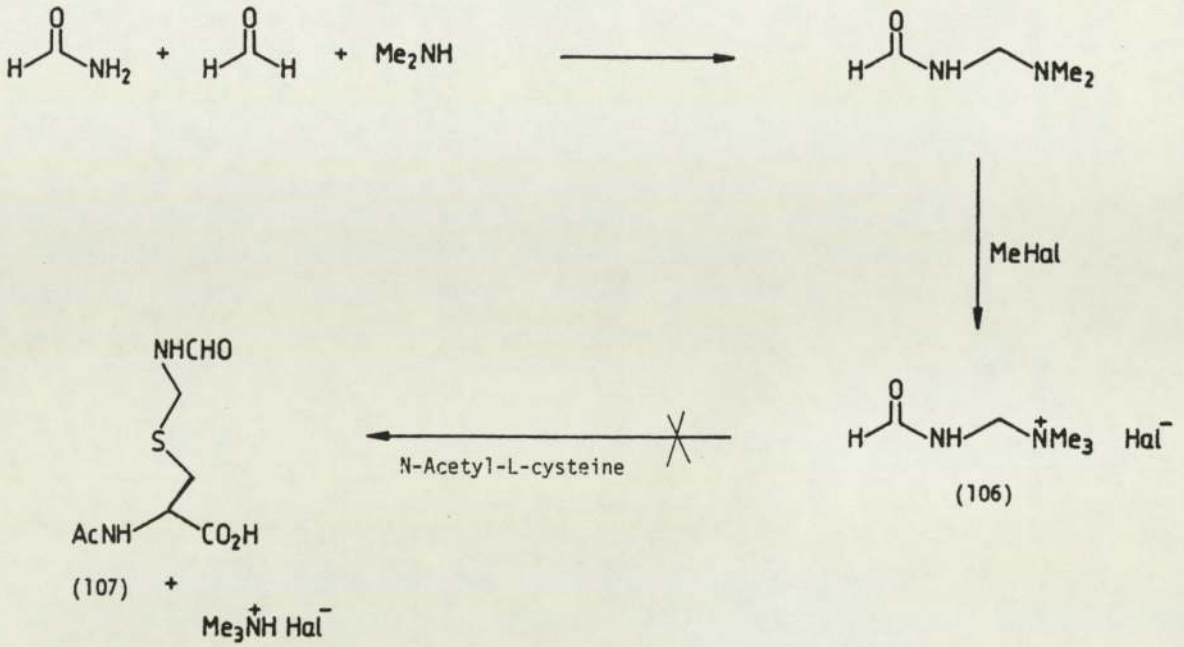
Scheme 17



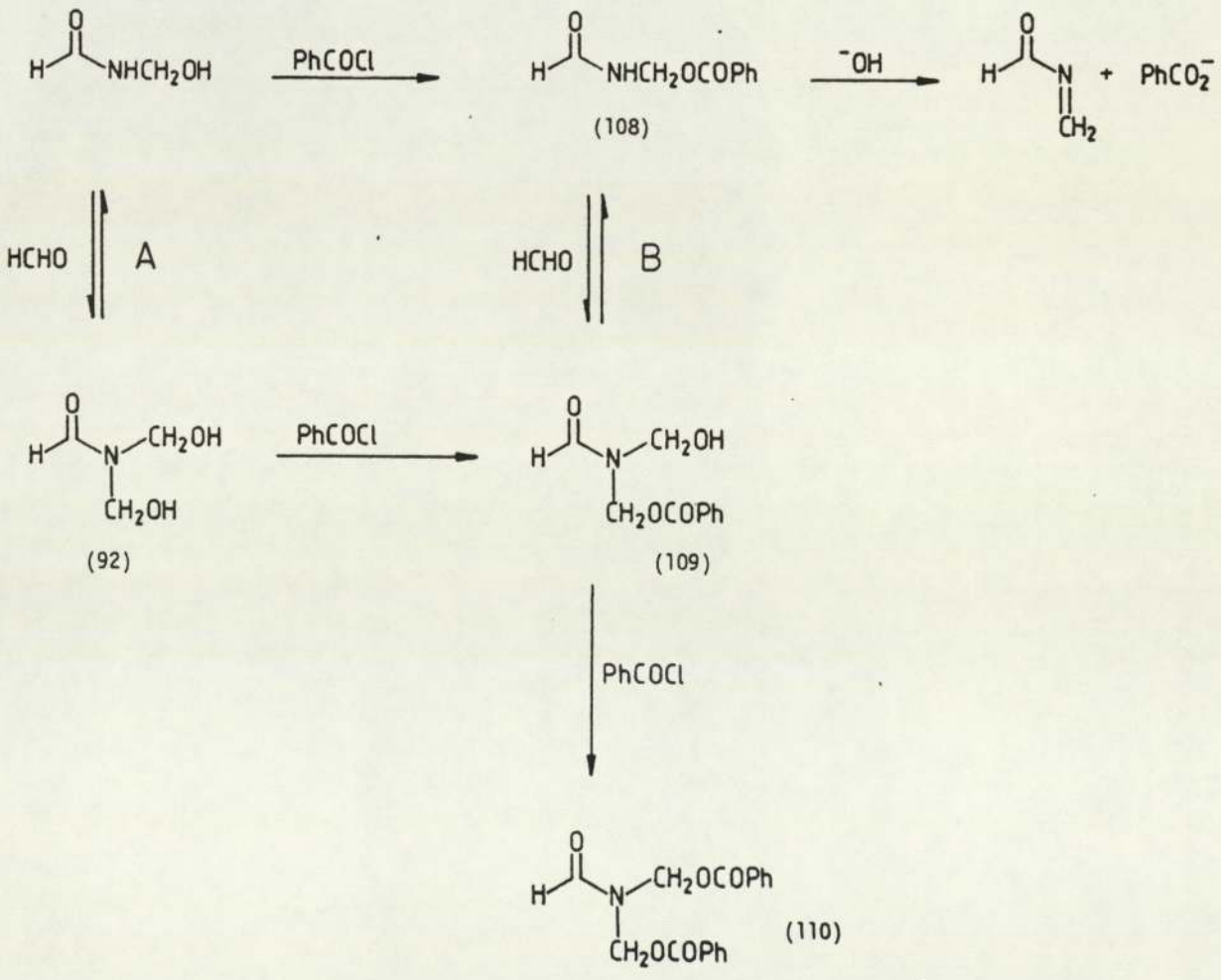
anion present under basic conditions could either react, scheme 17, with any acylimine generated, route A, or could react in a direct SN_2 sulphinate displacement, route B. Bohme and Fuchs[78] used the quaternary ammonium compound (106, Hal =Br^-) to produce sulphides and sulphones under basic conditions. The analogous iodide salt was used in an effort to produce the mercapturic acid (107) scheme 18. However, mixtures of products were produced in all cases

Treatment of HMF with acylating agents such as benzoyl chloride, 4-nitrobenzoyl chloride, or acetic anhydride invariably gave a mixture of several products. Chromatography of the brown oil obtained from the benzoylation of HMF, scheme 19, gave three major products, benzoic anhydride, NN-bis(benzoyloxymethyl)formamide (113), and N-benzoyloxymethylformamide (108). The identity of these materials was determined primarily by ^1H NMR. It is likely that there will be a small but significant amount of the bis methylol (92) present in HMF as part of the equilibrium mixture and benzoylation of this would give the bis ester (110), route A. However, although the overall isolated yield of these esters is low (approx. 1%) they were isolated in similar amounts and had similar intensities on tlc (uv quenching 254 nm). The acetyl and benzoyl esters of the analogous HMMF were prepared by acylation of HMMF in moderate yield by published methods[74,80]. The exact reason why the benzoate ester of HMF is isolated in such low yield is unclear but may be due to breakdown of the ester by base catalysed elimination of benzoic acid, scheme 19. Those amido-esters which do not possess an NH would not be suseptible to this mechanism. An alternative route to the bis ester could be the formation of the NH-mono ester followed by hydroxymethylation then further esterification, route B. It is unclear which route predominates.

Scheme 18



Scheme 19



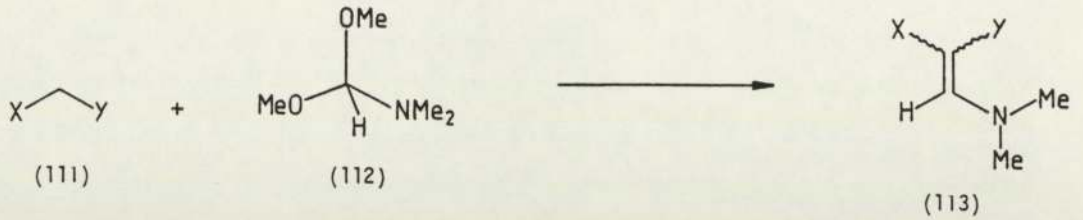
3.2.3. Vinylogous amides and related 'push-pull' olefins

Dimethylamino push-pull olefins have been synthesised by the condensation of 1-dimethylamino-1,1-dimethoxymethane (DMF-DMA, 112) with a reactive methylene or methyl compound[81], scheme 20. The reaction could be applied to other amide acetals. NN-Dimethyl-2-nitroethenamine (113, X = NO_2, Y = H) and 2-cyano-3-dimethylamino-propenenitrile (113, X = Y = CN) were synthesised by the method of Meerwein et al[81] by condensing nitromethane and malononitrile respectively with DMF-DMA. Nitroacetonitrile (115), made[82] via nitro-acetaldehyde oxime (114, methazonic acid), gave several products (tlc) when reacted with DMA-DMF at 0°C, rather than the desired but unknown 3-dimethylamino-2-nitropropenenitrile (116), scheme 21.

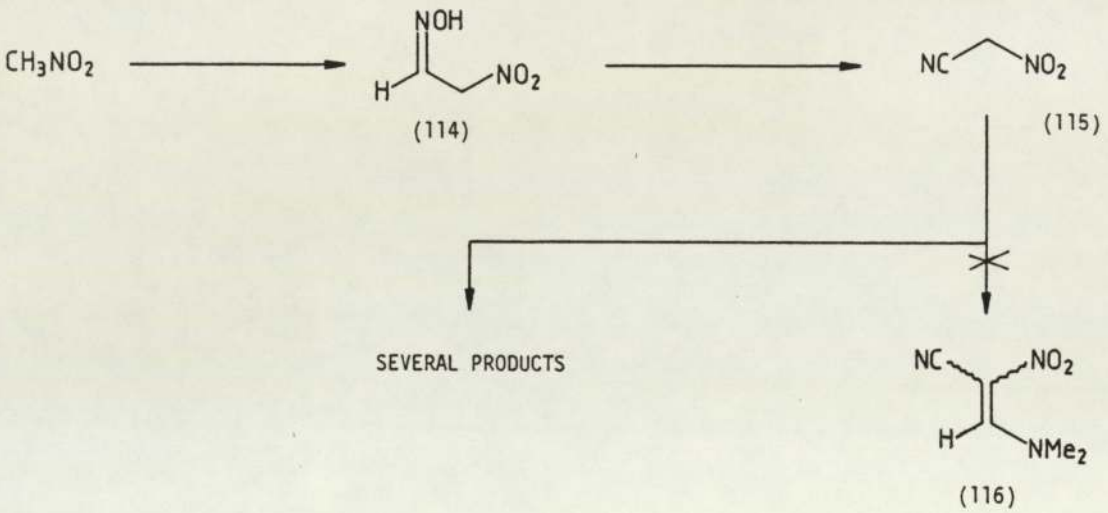
The reaction of methylamine with ethoxymethylene compounds (117) gave the corresponding N-methyl derivatives, scheme 22. The mechanism involves addition-elimination at electophilic C(1). Substituted 2-nitroethenamines (72, 121) were produced by the method of Faulques et al[83] by the reaction of triethylorthoformate, nitromethane, and secondary amine in the presence of toluene-p-sulphonic acid, scheme 23. Presumably the ethoxymethylene compound analogous to (117) is formed as an intermediate and reacts with the amine present to give the final nitroethenamine. Transamination of N-methyl-2-nitro-N-phenylethenamine (121)[84] with methylamine or ammonia gave N-methyl (70) and the unsubstituted 2-nitroethenamine (69). Again, the mechanism involves addition-elimination at C(1). Repeated recrystalliztion of N-methyl-2-nitroethenamine from D_2O gave its N-D derivative (122).

Rajappa[85], in his review of nitroethenamines, lists three other, though less important routes to nitroethenamines; (i) nitration of

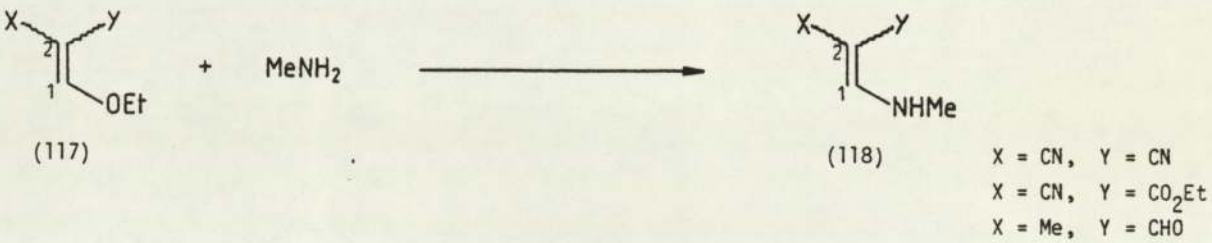
Scheme 20



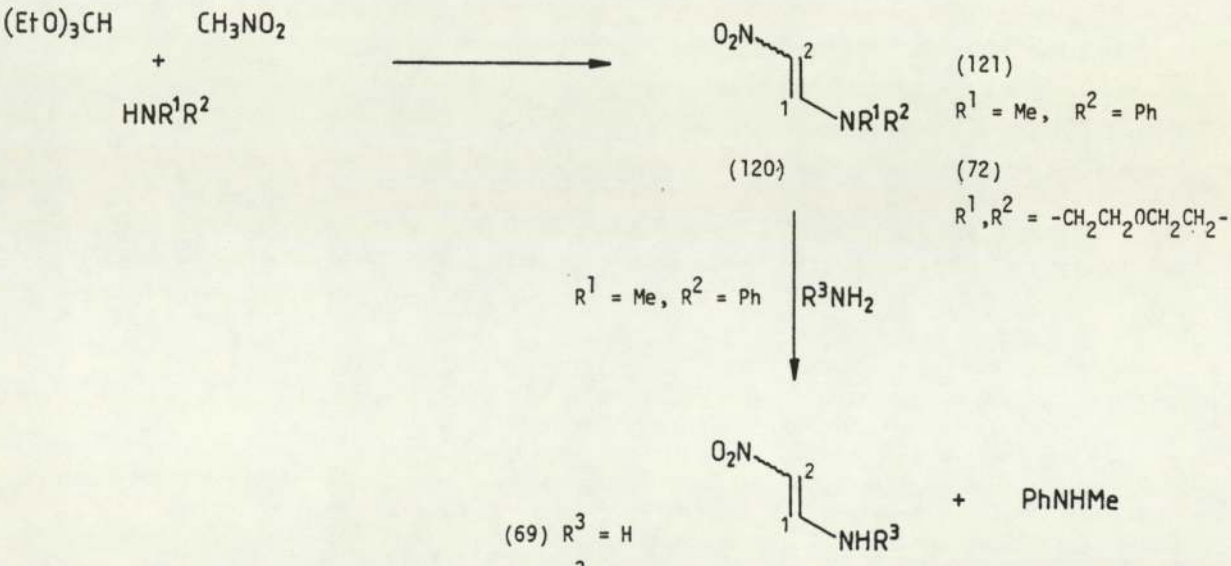
Scheme 21

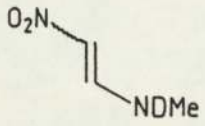


Scheme 22



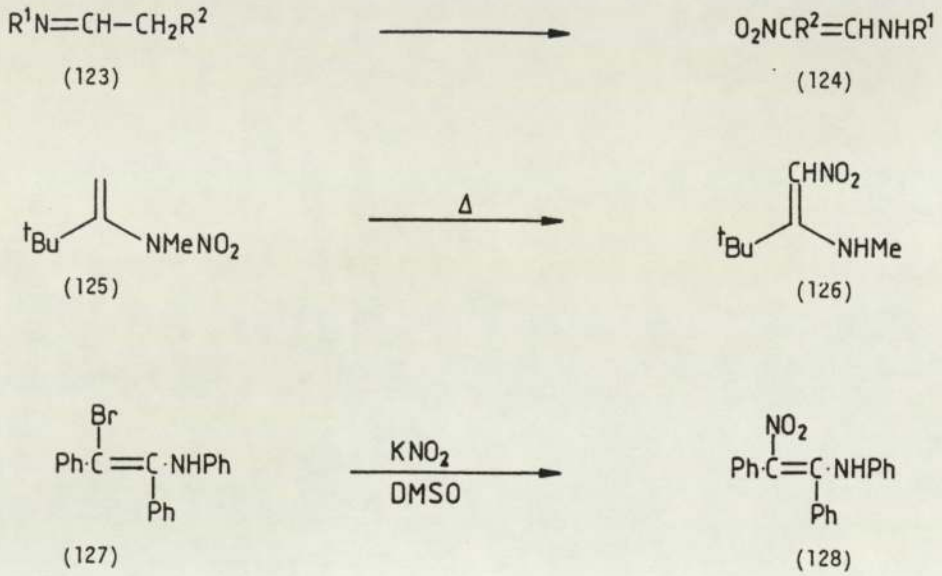
Scheme 23



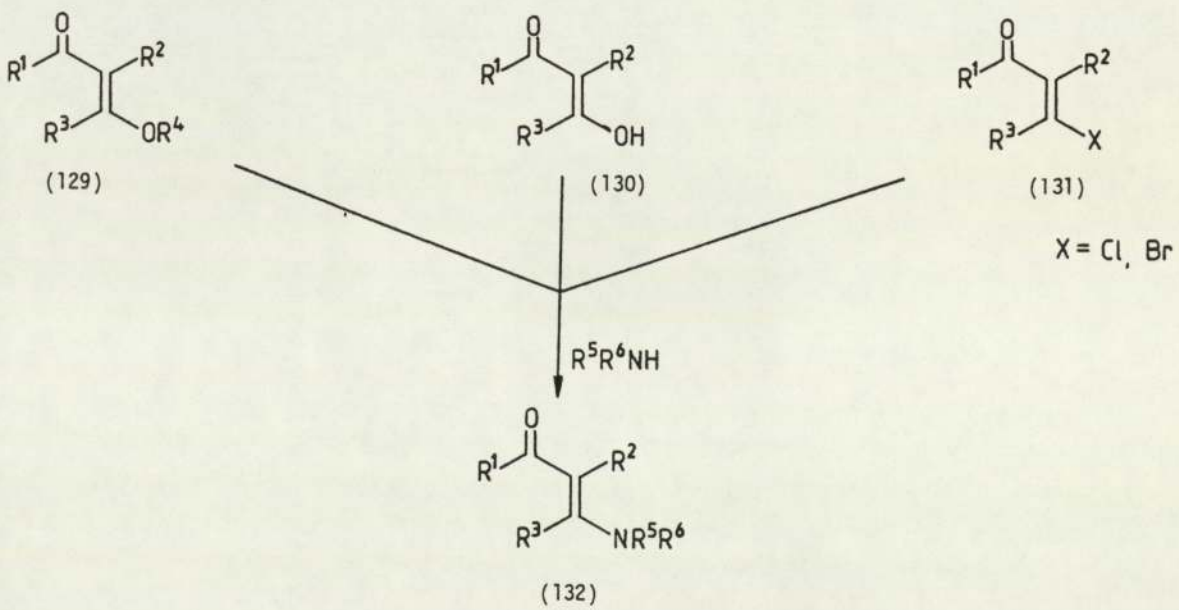


(122)

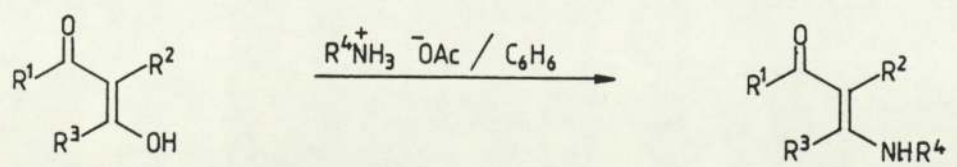
Scheme 24



Scheme 25



Scheme 26



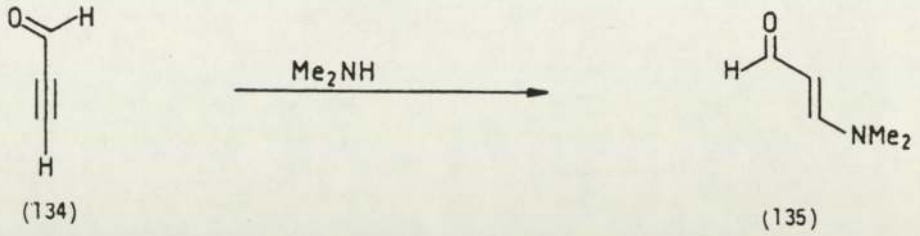
imines[86], (ii) rearrangement of N-nitroenamines[87], and (iii) from β-halogenoenamines[88], scheme 24.

In his review of enaminones (vinylogous amides) Greenhill[89] describes their preparation from 1,3-diketones (vinylogous acids, 130), or in the case of weak or low boiling point amines from 3-alkoxyacroleins (vinylogous esters, 129) or 3-haloacroleins (vinylogous acid halides, 131), scheme 25. Italian workers[90] recently reported an efficient synthesis (from 1,3-diketones) of unsubstituted or N-ethyl enaminones using the amine acetate as the source of the otherwise troublesome low boiling point amine, scheme 26.

Malhorta and Whiting[91] used Michael addition into propynal (propargaldehyde, 134) with dimethylamine to produce 3-dimethylamino-propenal (3-dimethylaminoacrolein, 135) in 68% yield, scheme 27. 3-(N-Monoalkyl)acroleins have been reported by Russian workers from the reaction of primary amines and propynal[92] or 3-chloropropenal (131, $R^1=R^2=R^3=H$, X=Cl)[93]. However yields were low due to unwanted side reactions.

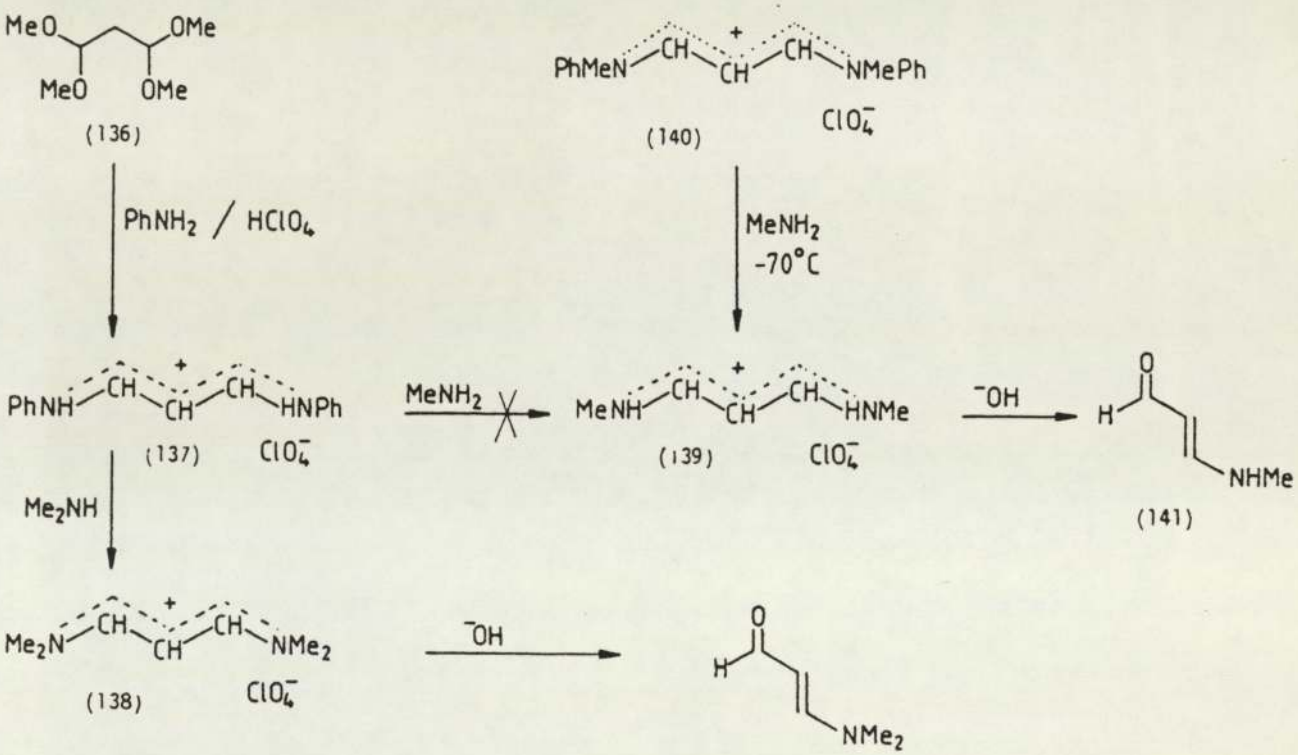
Kikugawa et al[94] in their study of the condensation products between 1,1,3,3-tetramethoxypropane [malondialdeyde bis(dimethyl-acetal), 136] and aliphatic amines found that dimethylamine gave the expected enaminone (135) but methylamine gave a mixture of products[95]. The N-methyl enaminone, 3-methylaminopropenal (vinylogous NMF, 141) was produced in only 2.4% yield after chromatography and is the only literature reference to this compound. In an attempt to synthesise this compound by a more productive route an analogous approach to McNab's[96] synthesis of 3-dimethylaminopropenal via the methine dye perchlorate (138) was investigated, scheme 28. Transamination of (137) with methylamine at 0°C did not afford the

Scheme 27



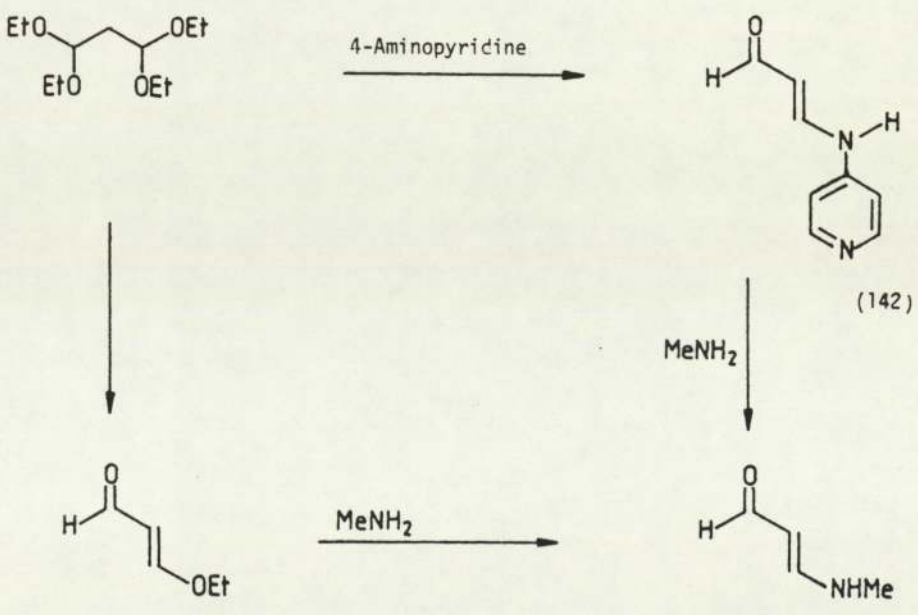
(134) (135)

Scheme 28



(136) (140) (137) (139) (141) (138)

Scheme 29



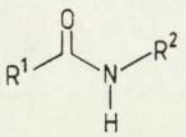
(142) (142)

desired perchlorate salt (139). However, Grimm and Dahne[97] described the transamination of the N-methylanilino methine dye (140) with methylamine at -78°C to give (139). Other potential routes to vinylogous NMF are given in scheme 29 involving 3-ethoxyacrolein (142)[98] or 3-(4-pyridyl)acrolein (143)[99].

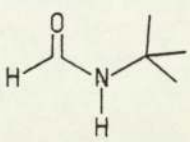
3.3. SOLID STATE AND SOLUTION CONFORMATIONS OF FORMAMIDES AND RELATED 'PUSH-PULL' OLEFINS

3.3.1. Formamides

Rotational isomerism about the amide bond of N-alkylamides has been extensively studied by ^{1}H, ^{13}C NMR[100,101] and recently by natural abundance ^{15}N NMR[102]. This barrier to rotation in the C-N bond is a consequence of the delocalization of the lone pair of nitrogen into the π bonding system of the carbonyl group. The two methyl groups of DMF give rise in NMR spectra to separate signals and it has been shown[103] that the methyl group <u>syn</u> to oxygen resonates at higher field. However, in most instances the methylenes of N-ethyl and the methines of N-isopropyl amides which are <u>syn</u> to oxygen resonate at lower field[100].



(144)



(145)

In monosubstituted amides (144) generally the Z rotamer is found to predominate[100]. This is still the case in formamides (144, R^1=H), even though steric considerations would have predicted the E rotamer to dominate. Interestingly, the percentage of the E rotamer of N-^{t}butylformamide (145) increases from 18% in dilute benzene solution

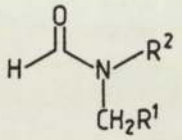
to 63% in sulphuric acid solution[104]. Protonation on O would increase steric repulsion, thus favouring the E rotamer.

Table 9 gives, for some formamides in various solvents, the ^{1}H NMR chemical shifts, rotameric ratio, and where observable the value of the coupling constant to the formyl proton. Assignment of signals to rotamers is based on the higher field resonance of N-methyl groups syn to the carbonyl oxygen[100,103] and to the larger trans coupling of the formyl proton. In secondary formamides the trans $^{3}J_{C(1)H,NH}$ is about 11 Hz whereas the corresponding value for the cis coupling is 0-2 Hz and is usually not observed in the formamides studied. In HMMF an additional trans coupling [$^{4}J_{(HCNCH)(CH_2)}$ = 2.8 Hz] is observable in the Z rotamer where the formyl and methylene groups are anti-periplanar. Less emphasis was placed on the diagnostic value of the position of the N-CH_2 chemical shift since the difference between the E and Z rotamers is smaller than for N-CH_3 resonances and may be the reverse of that expected[100]. The chemical shift, for example, of the methylenes of (46, R^1=OAc, R^2= Me) in $(CD_3)_2SO$ fall at 5.28 and 5.34 ppm for the Z and the E rotamers respectively but in $CDCl_3$ the positions are reversed with the E rotamer resonating at higher field with values of 5.33 and 5.27 ppm. In agreement with the conclusion of LaPlanche and Rogers[105] the E rotamer is favoured in tertiary formamides with the bulkier group anti to the carbonyl oxygen.

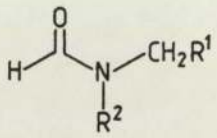
The ^{13}C NMR spectral data for N-methoxymethylformamide (147) and other amides is given in table 10. Assignments of signals to E and Z rotamers was confirmed by comparision of the ^{13}C-H coupling constants (table 11) in the undecoupled ^{13}C NMR spectrum in $CDCl_3$ of (147), figure 1, with those reported for the corresponding conformations of NMF[101]. The formyl carbon of the Z rotamer can be assigned by its

Table 9, giving rotamer populations and ^1H chemical shift data (and where observable coupling to the formyl proton) for some substituted formamides in various solvents.

(146)



E



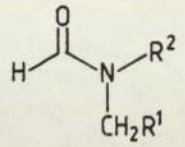
Z

Cpd No	R^1	R^2	Solvent	%E	%Z	Rot	Chemical Shift (δ) CHO	CH_2	R^1	R^2
41	a	a	$(CD_3)_2SO$	-	-	-	8.13(13 and 1Hz)			
1	H	H	$CDCl_3$	9	91	E	7.95	2.93		NS
						Z	8.20	2.83		7.3
55	b	b	$CDCl_3$	10	90	E	8.03			
						Z	8.27			
60	CF_3	H	$(CD_3)_2SO$	10	90	E	8.11(11.5Hz)	NS	-	NS
						Z	8.23	3.96	-	8.75
61	CH_2Cl	H	$CDCl_3$	10	90	E	8.1(10Hz)	NS	NS	NS
						Z	8.2(1Hz)	3.6	3.6	7.6
106	$\overset{+}{N}Me_3$	H	$(CD_3)_2SO$	25	75	E	8.57	5.0	NS	NS
						Z	8.45	4.75	3.1	9.4
56	c	H	$CDCl_3$	40	60	E	8.29(12Hz)	2.65[d]	0.75	6.0
						Z	8.18	2.65	0.75	6.0
147	OMe	H	$CDCl_3$	40	60	E	8.20(11.5Hz)	4.61	3.32	5.85
						Z	8.32	4.72	3.36	5.85
148	OCOPh	H	$CDCl_3$	33	67	E	8.48(11Hz)	5.52	e	6.94
						Z	8.35	5.58	e	7.07
37	H	Me	$CDCl_3$	-	-	(E)	8.0	2.97		2.85
			D_2O	-	-	(E)	7.93	3.01		2.85
149	Me	Me	$CDCl_3$	60	40	E	8.07	3.3	1.17	2.83
						Z	8.0	3.37	1.17	2.93
39	OH	Me	$CDCl_3$	80	20	E	8.1	4.77	5.2	2.93
						Z	8.0	4.77	5.2	3.03
			D_2O	83	17	E	8.16	4.84	-	2.91
						Z	8.03	4.82	-	3.05
63	OAc	Me	$CDCl_3$	85	15	E	8.27	5.27	2.07	2.85
						Z	8.07	5.33	2.07	3.05
			$(CD_3)_2SO$	85	15	E	8.29	5.34	2.00	2.77
						Z	8.12	5.28	2.00	2.97

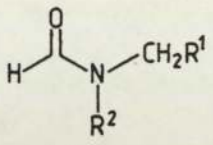
cont

cont

(146)



E



Z

Cpd No	R^1	R^2	Solvent	%E	%Z	Rot	Chemical Shift (δ) CHO	CH_2	R^1	R^2
64	OCOPh	Me	$CDCl_3$	83	17	E	8.07	5.5	e	3.0
						Z	8.23	5.6	e	3.1
			$(CD_3)_2SO$	85	15	E	8.50	5.72	e	2.93
						Z	8.28	5.64	e	3.14
150	OCOPh	CH_2OCOPh	$CDCl_3$	-	-	-	8.63	5.75 and 5.8		

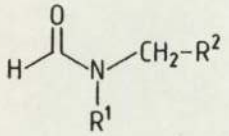
a - Formamide, b - N-(Trideuteromethyl)formamide
c - Cyclopropyl, d - CH
e - Aromatic, NS - Not seen or not observable.

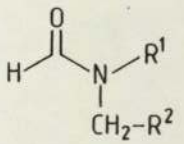
Table 11, ^{13}C - ^{1}H Coupling constants (Hz) in N-(methoxymethyl)-formamide (147) in $CDCl_3$

	148Z	148E
$^1J(CH)(C=O)$	195.2	191.5
$^2J(CNH)(CO)$	4.3	a
$^3J(CNCH)(CO)$	4.3	6.65
$^1J(CH)(CH_2)$	157.3	155.7
$^3J(COCH)(CH_2)$	5.4	5.4
$^3J(CNCH)(CH_2)$	5.4	a
$^1J(CH)(CH_3)$	142.1	141.9
$^3J(COCH)(CH_3)$	5.6	5.4

a not resolved

Table 10, ^{13}C Chemical shift data for N-(methoxymethyl)formamide (147) and other formamides





Cpd No	R^1	R^2	Solvent	%Z	%E	Z Rotamer C=O	Z Rotamer CH_2	Z Rotamer R^1	Z Rotamer R^2	E Rotamer C=O	E Rotamer CH_2	E Rotamer R^1	E Rotamer R^2
148	H	OMe	$CDCl_3$	56	44	161.74	69.72		56.02	165.28	74.55		54.8
			D_2O	62	38	167.92	72.22		57.99	171.29	77.15		57.2
40	H	OH	D_2O	43	57	167.28	67.25			168.2	73.1		
39	Me	OH	D_2O	13	87	167.26	69.45	35.9		168.0	76.0	31.1	
63	Me	OAc	D_2O	17	83	167.9	70.7	36.4	22.5 174.6	168.3	76.6	31.6	22.6 174.7

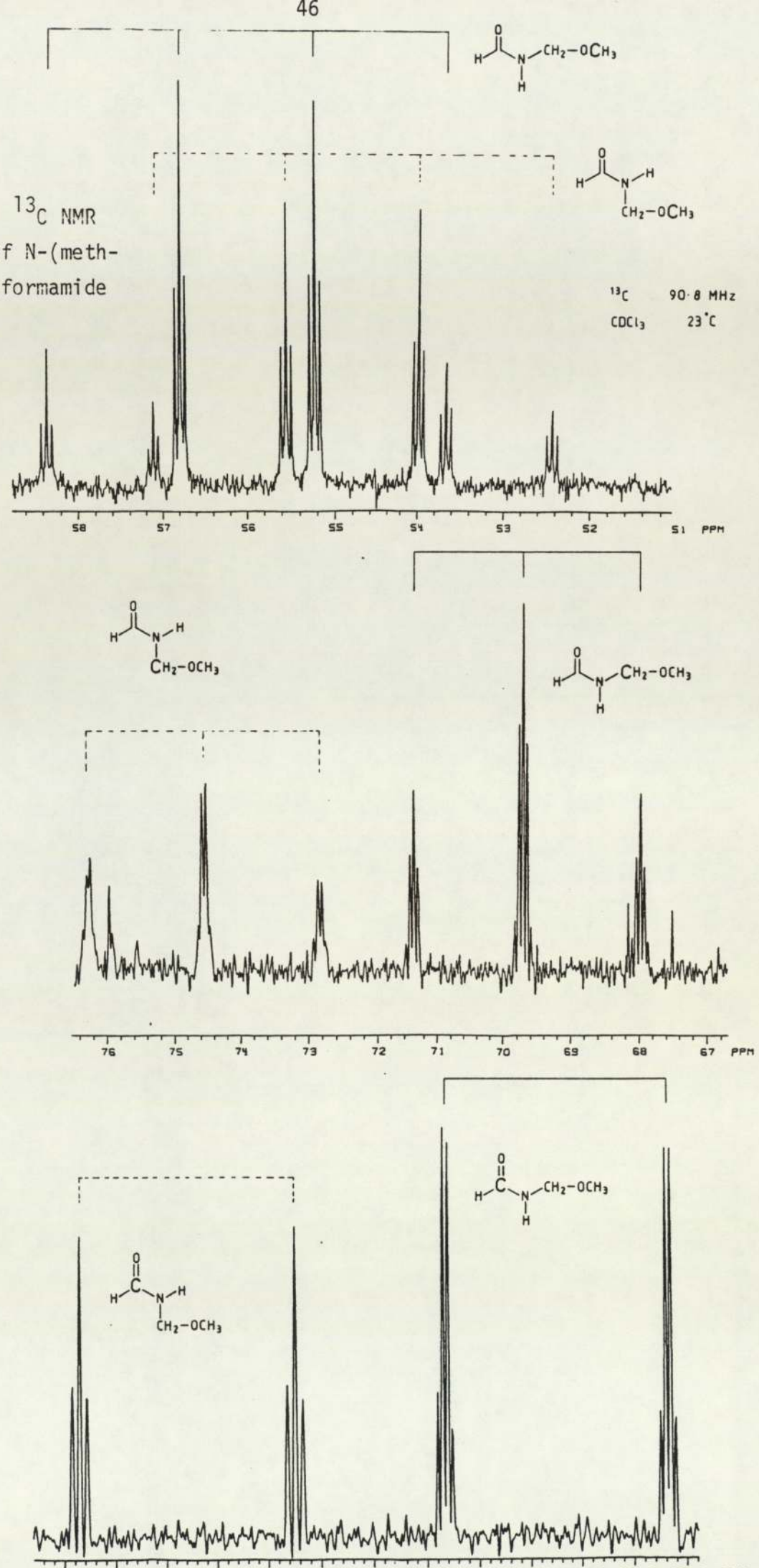


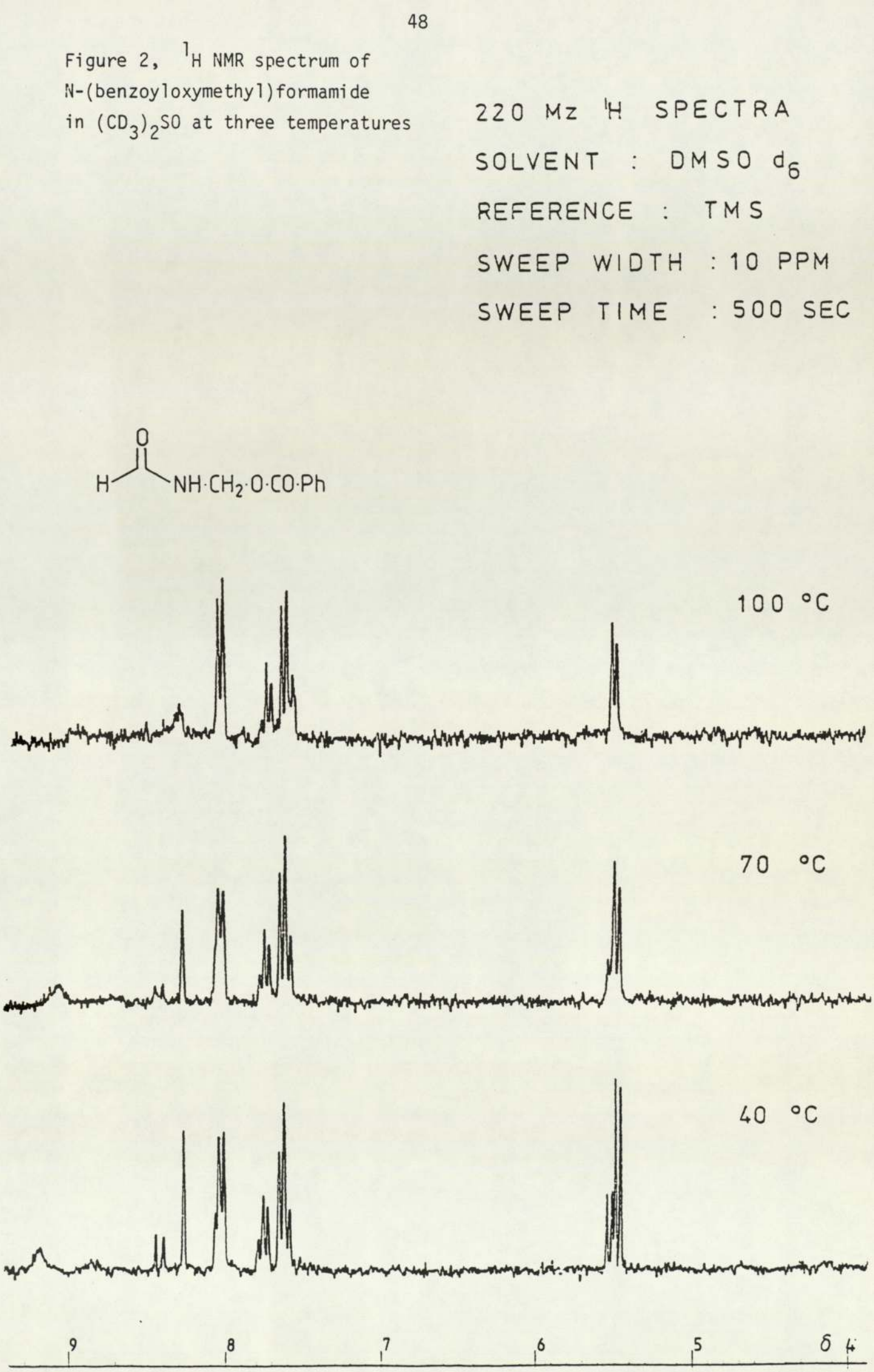
Figure 1, ^{13}C NMR spectrum of N-(methoxymethyl)formamide (147)

larger $^{1}J_{(CH)}$ value (195.2 compared with 191.5 Hz in the E rotamer) and appears as a doublet of quartets because of equivalence of $^{2}J_{(CNH)}$ and $^{3}J_{(CNCH)}$ (4.3 Hz). A doublet of triplets is observed for the correponding E rotamer with $^{2}J_{(CNH)}$ not resolved. The higher $^{3}J_{(CNCH)}$ value of 6.65 Hz for (147E), compared with 3.1 Hz is an effect[106] of the electronegative methoxy substituent on the coupled system. The methylene of the E rotamer appears as a triplet of quartets whereas the Z rotamer appears as a triplet of quintets. The additional multiplicity is probably due to coupling to the formyl H which is suitably antiperiplanar.

The ^{1}H and ^{13}C NMR spectra of HMF show more resonances than expected and is probably due to the presence of impurities. Given in table 10 are the resonances tentatively assigned to $OHCNHCH_2OH$ by comparison with other formamides.

The secondary formamides listed in tables 9 and 10 show a preference for the Z rotamer in agreement with literature conclusions[100,104,107]. However, the effect of introducing the α oxygen containing substituent into the N-alkyl group of N-methylformamide on the rotamer population is significantly greater than the change brought about by simply increasing the size of the alkyl group. The replacement of the methyl group in NMF by the n-propyl group results in a slight shift towards the E rotamer (8 to 14%), whereas the substitution by a methoxymethyl group results in a more significant shift to about 40% of the E rotamer. The interaction between the methoxymethyl group and the carbonyl oxygen can not simply be explained by the size of the methoxymethyl group, and may involve an electronic repulsion not present in simple N-alkylformamides.

Figure 2, ^{1}H NMR spectrum of N-(benzoyloxymethyl)formamide in $(CD_3)_2SO$ at three temperatures



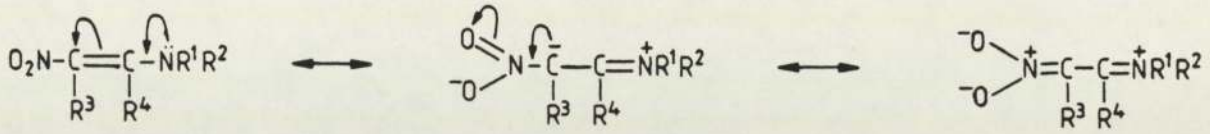
Given in figure 2 is the ^1H NMR spectrum of N-(benzoyloxymethyl)formamide (148) in $(CD_3)_2SO$ at 40, 70, and 100°C. As expected coalescence of signals of the E and Z rotamers is seen as the temperature is raised.

In summary, the general preference of the Z rotamer in secondary formamides and a preference for the bulkier substituent to be anti to the carbonyl oxygen in tertiary formamides has been confirmed. However, those possessing an oxygen atom in the α position of one of the N-sustituents (tertiary formamides) or in the N-substituent (secondary formamides) have a greater percentage of the E rotamer than can be acconted for by steric considerations alone.

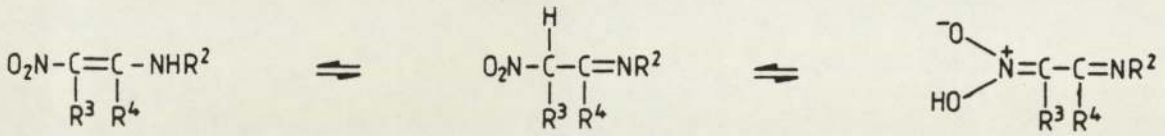
3.3.2. Related 'push-pull' olefins

2-Nitroethenamines can exist in three canonical forms (151a-c) and if (151, R^1=H) then three tautomers are possible (152a-c). In his recent review Rajappa[85] reports that most of this class of compound exist as the enamine tautomer (152a) with only two examples reported[87] of the 2-nitroimine (152b). Analogous to amides delocalization of the lone pair of electrons of the amine nitrogen into the π bonding system of the nitroethylene moiety would lead to a contribution from canonical form (151c) and to a restriction of rotation about the C(1)-N bond.

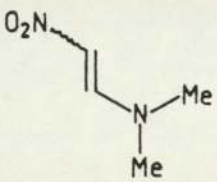
Table 12 gives the ^1H NMR data for nitroethenamines (69-71, 122) in various solvents. In both $CDCl_3$ and $(CD_3)_2SO$ NN-dimethyl-2-nitroethenamine (71) shows the presence, as reported[108,109], of only one geometrical isomer, and inequivalence of the methyl groups. The value of the C(1)H-C(2)H coupling constant (11 Hz) is of little diagnostic value in determining the stereochemistry about that bond, being



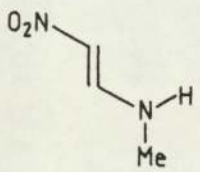
(151) a b c



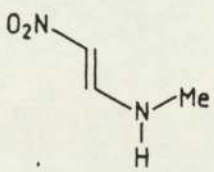
(152) a b c



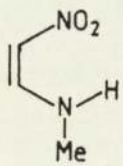
(71)



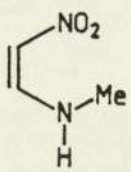
(70) E/E



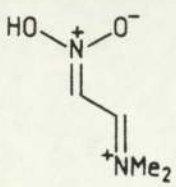
E/Z



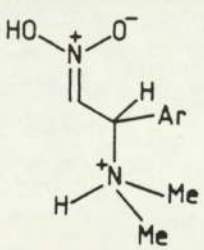
Z/E



Z/Z

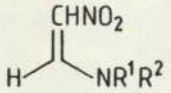


(156)



(157)

Table 12. ^{1}H NMR data for nitroethenamines 69-71 and 122



69 $R^1 = R^2 = H$

70 $R^1 = H$, $R^2 = Me$

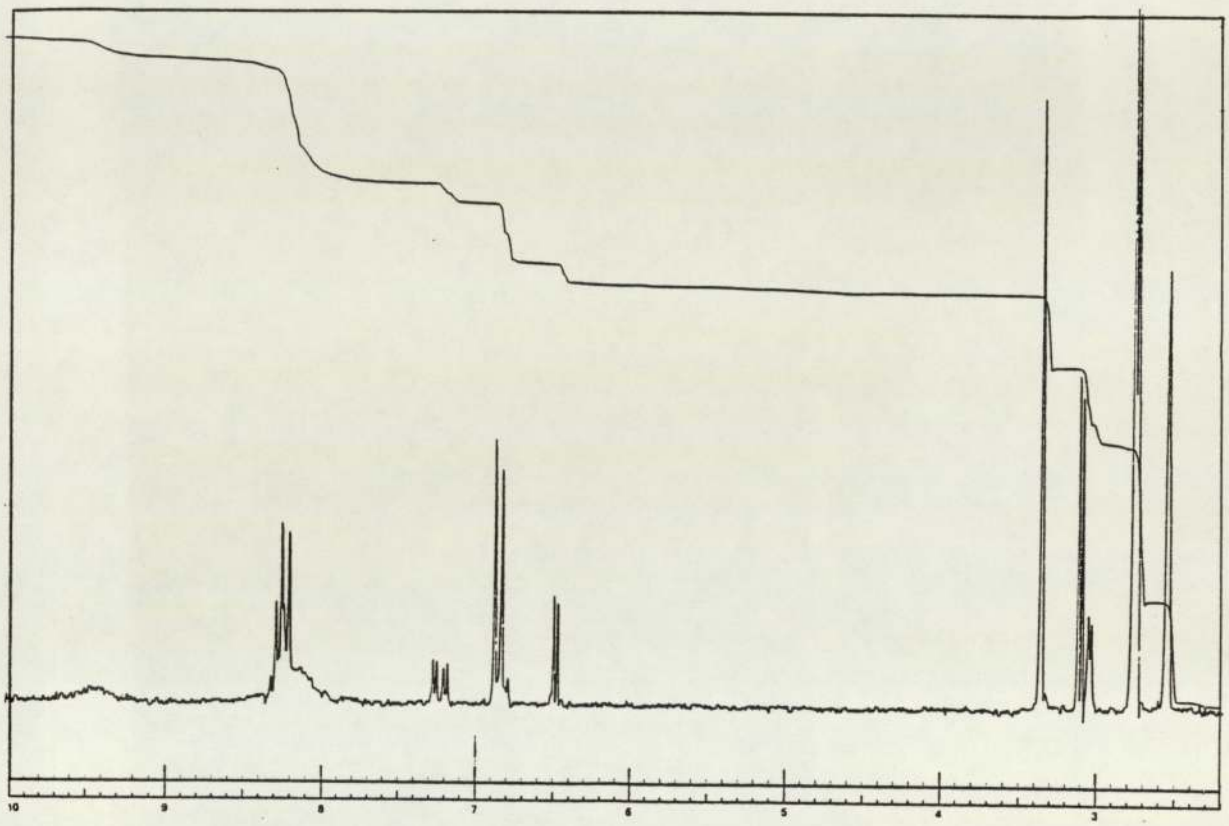
71 $R^1 = R^2 = Me$

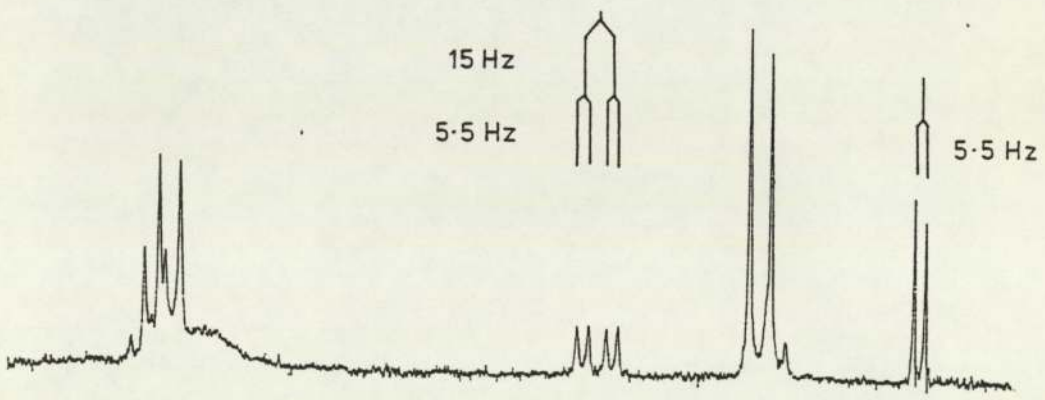
122 $R^1 = D$, $R^2 = Me$

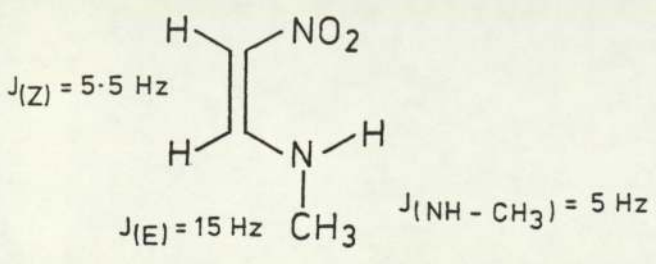
Cpd No	Solvent	Stereochemistry C(1)-C(2)	C(1)-N	% of Rot	Chemical shift (δ) C(1)H	C(2)H	NH	CH_3
71	$CDCl_3$	E		100	8.20 (d, J 11 Hz)	6.65 (d, J 11 Hz)		2.90br, 3.25br
	$(CD_3)_2SO$	E		100	8.30 (d, J 11 Hz)	6.80 (d, J 11 Hz)		2.85 (s), 3.20 (s)
70	$CDCl_3$	Z	E	100	6.79 (dd, J 14, 5.5 Hz)	6.5 (d, J 5.5 Hz)	9.1br	3.21 (d, J 5 Hz)
	$(CD_3)_2SO$	Z	E	23.5	7.22 (dd, J 15, 6 Hz)	6.48 (d, J 6 Hz)	9.4br	3.09 (d, J 6 Hz)
		E	E	7.7	8.29 (d, J 10.5 Hz)	6.80 (d, J 10.5 Hz)	8.15br	3.03 (d, J 4.5 Hz)
		E	Z	68.8	8.24 (dd, J 7.5, 10.5 Hz)	6.82 (d, J 10.5 Hz)	8.15br	2.74 (d, J 4.5 Hz)
	D_2O	Z		63	7.55	6.81 (d, J 6 Hz)		3.28 (s)
		E		37	8.51 (d, J 10.5 Hz)	7.08 (d, J 10.5 Hz)		2.96 (s)
	CF_3CO_2H	Adduct (70a)		47.5	5.06 (m)	5.27 (dd, J 5.5, 16 Hz) 5.48 (dd, J, 7, 16 Hz)		3.10 (t, J 5.5 Hz)
		Species (70b)		52.5	7.90 (d, J 15 Hz)	8.2 (m)	8.2br	3.49 (d, J 5.5 Hz)
69	$(CD_3)_2SO$	Z			7.20 (m)	6.45 (d, J 6 Hz)	8.1br	
		E			6.80br (d, J 11 Hz)	8.25 (d, J 11 Hz)	8.1br	
122	$(CD_3)_2SO$	Z	E		7.20 (m)	6.46 (d, J 6 Hz)		3.08 (s)
		E	E		8.24 (d, J 10 Hz)	6.79 (d, J 10 Hz)		3.02 (s)
		E	Z		8.22 (d, J 11 Hz)	6.83 (d, J 11 Hz)		2.73 (s)

^1H NMR spectrum and scale expansion of N-methyl-2-nitroethenamine in $(CD_3)_2SO$ (220 MHz 34°C)

Figure 3







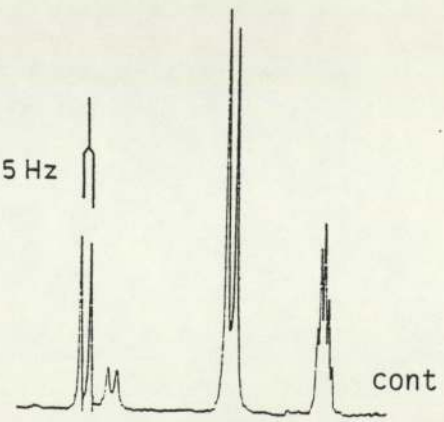
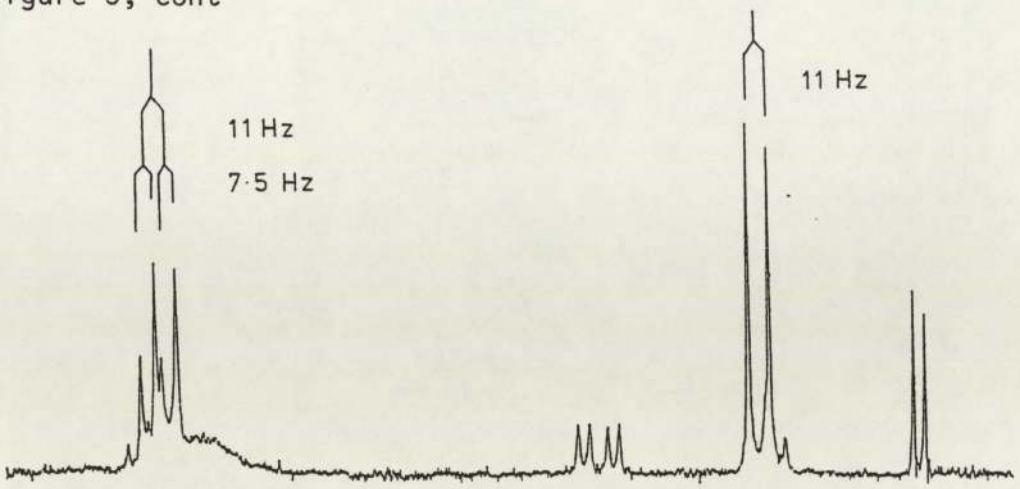
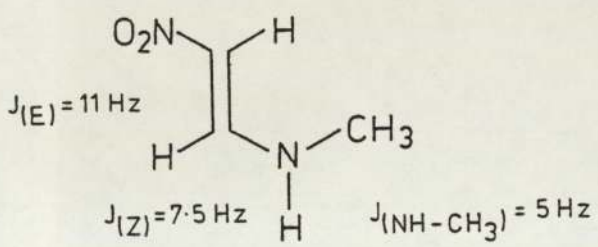
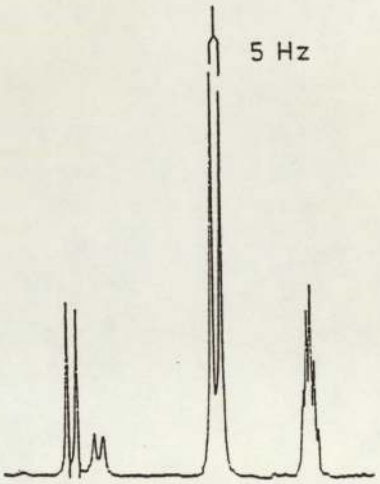
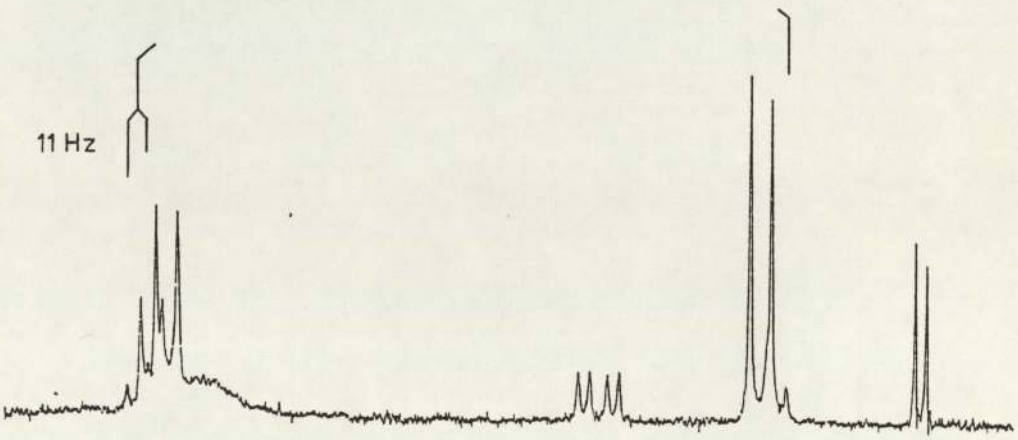


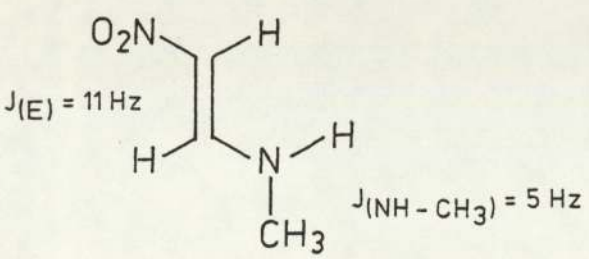
Figure 3, cont











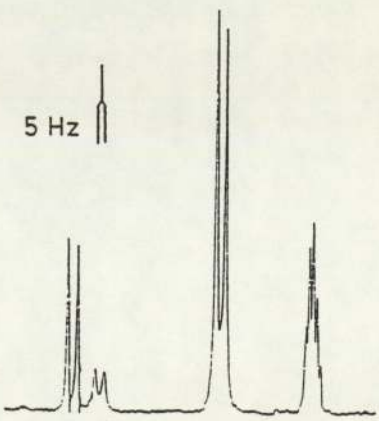


Figure 4 ^1H NMR spectrum (and scale expansion) of N-methyl-2-nitroethenamine (70) in trifluoroacetic acid

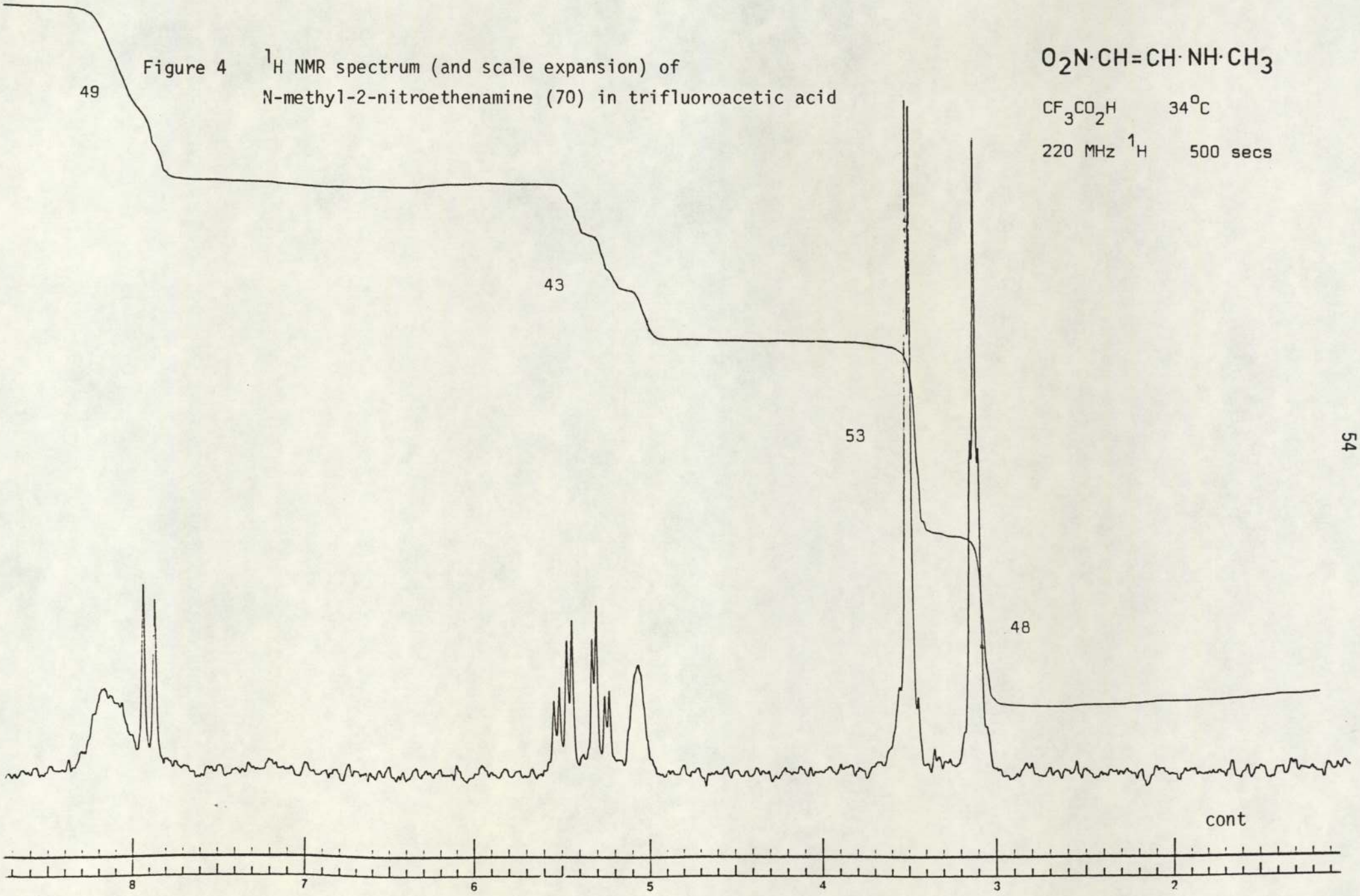
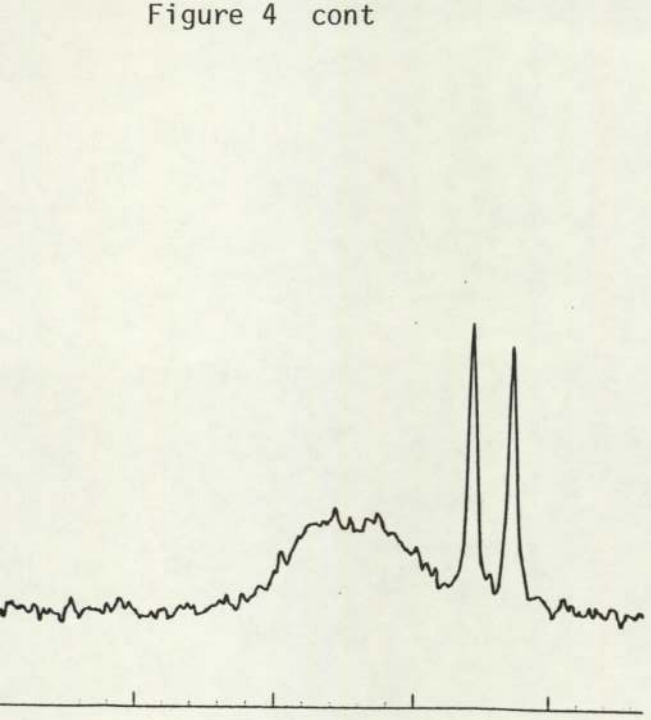
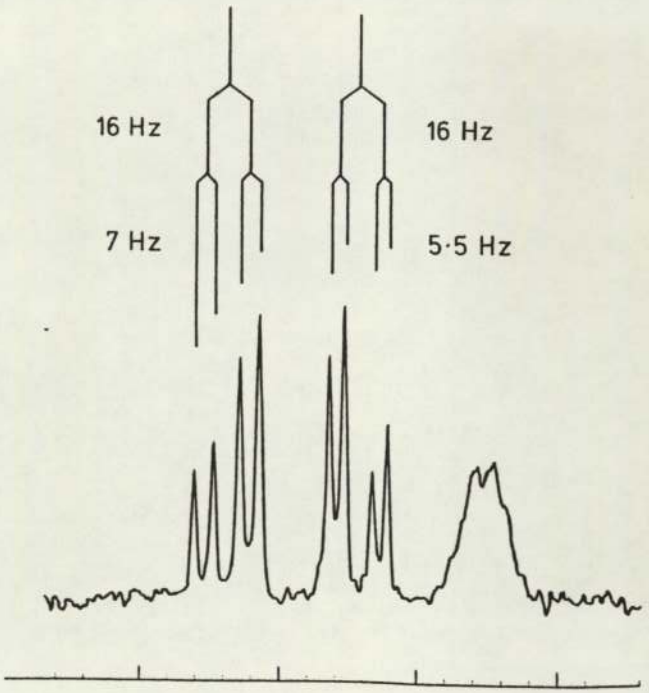
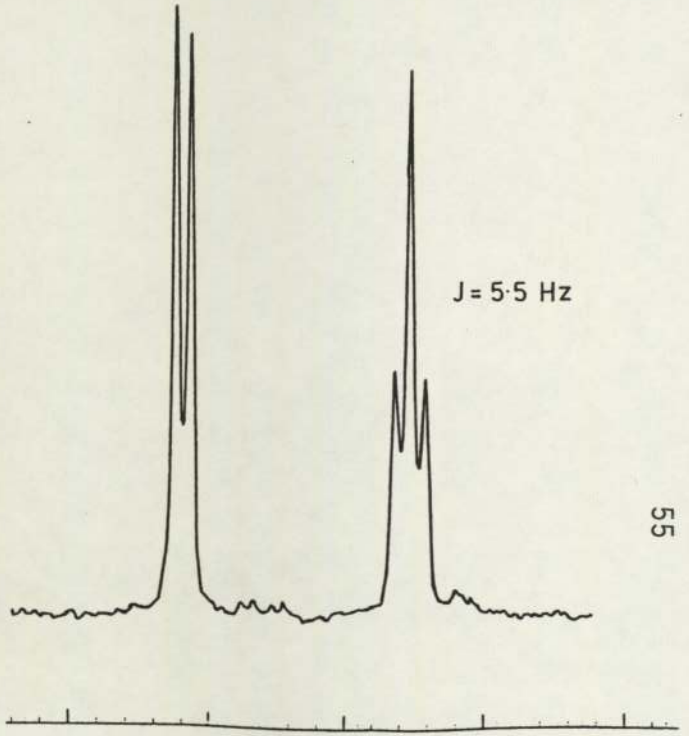
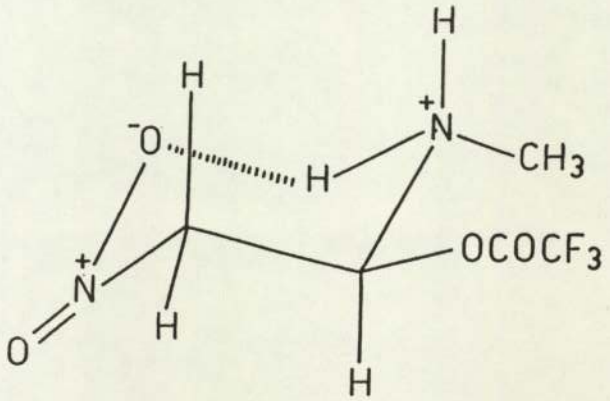


Figure 4 cont





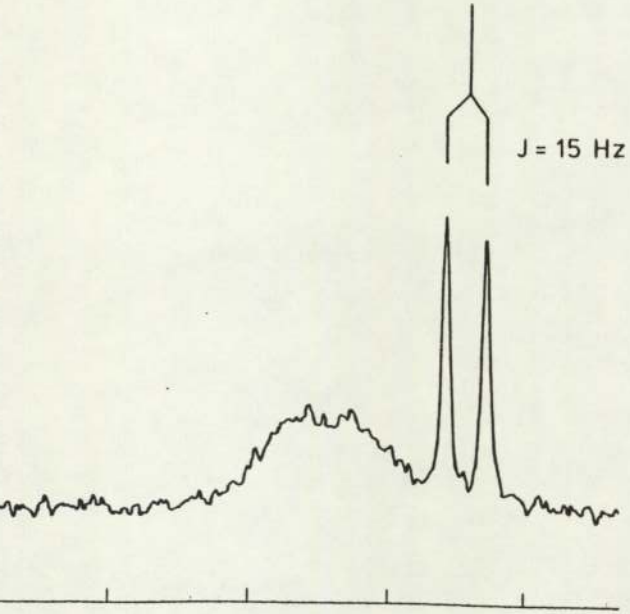


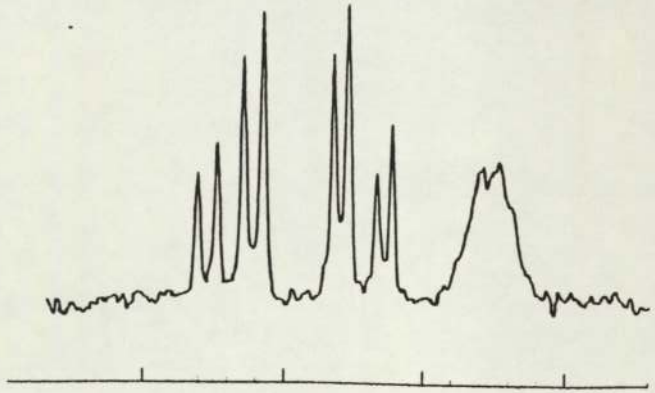


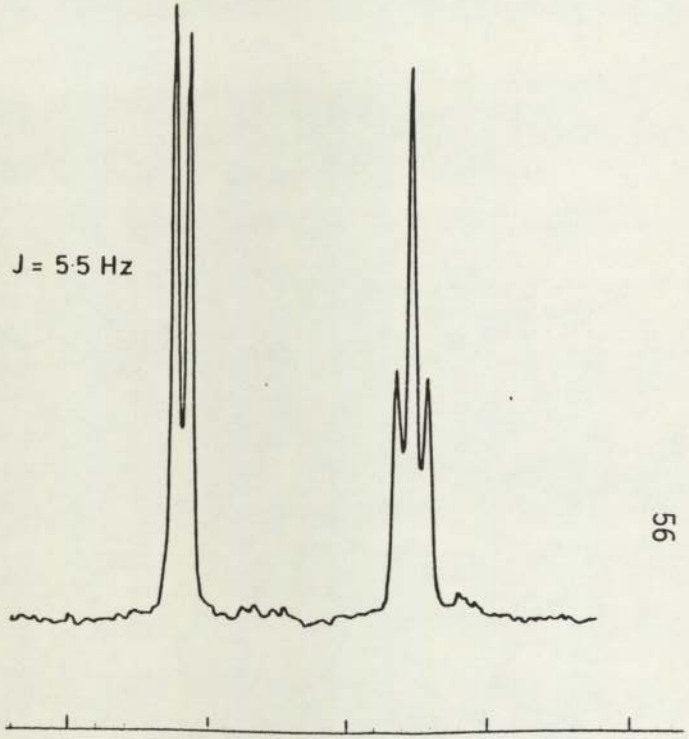
$CF_3CO_2^-$

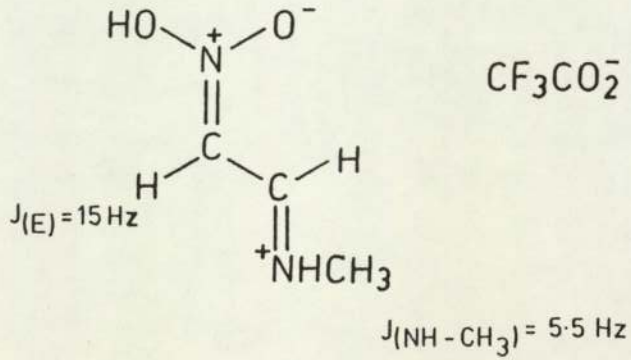
cont

Figure 4 cont









$CF_3CO_2^-$

$J_{(NH-CH_3)} = 5{\cdot}5$ Hz

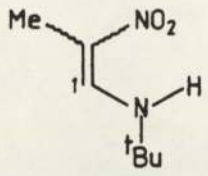
neither clearly Z or E as noted by Buchi[108]. The methyl signals in $CDCl_3$ are broadened when compared to those in $(CD_3)_2SO$ implying that the coalescence temperature is higher in DMSO than in chloroform (Mannschreck[110] gives a coalescence temperature of 52°C in $CDBr_3$). The origins of this effect are unclear but may arise from the greater viscosity of DMSO, or from such factors as the dielectric constant or dipole moment of the solvent.

In the case of N-methyl-2-nitroethenamine four rotamers are possible [70, E/E, E/Z, Z/E, Z/Z; the orientation about C(1)-C(2) is given first]. Krowczynski and Kozerski[84] report a 60:40 ratio Z:E of isomers for (70) in $CDCl_3$ solution and that for related compounds signals attributable to Z isomers are only observed in dilute $CDCl_3$ solution or in $(CD_3)_2SO$. However, it is unclear whether reference is being made to geometrical isomerism about the C(1)-C(2) or C(1)-N bonds, and no data is given for DMSO solutions. In contrast, a single rotamer was obtained in $CDCl_3$ solution, table 12. In this single rotamer the magnitudes of the C(1)H-C(2)H coupling constant (5.5 Hz) and of $J_{C(1)H-NH}$ (14 Hz) are consistant with Z and E configurations respectively (ie 70Z/E). This arrangement is stabilized by an intramolecular hydrogen bond between the NH and nitro group; which is confirmed by the immutability of the chloroform i.r. spectrum upon dilution. In $(CD_3)_2SO$ three of the four rotamers are observed, figure 3, with three-quarters of the total population adopting an E configuration about C(1)-C(2) reflecting the facility of hydrogen bonding to solvent. However, of these C(1)-C(2) E species, the majority adopt the Z arrangement about the C(1)-N bond (ie 70E/Z). Configurations are derived from coupling constants; $^3J_{C(1)H-C(2)H}$ cis and trans of 5.5 and 11 Hz and $^3J_{C(1)H-NH}$ cis and trans of 7.5 and 15 Hz respectively. By analogy, the uncertainty of the stereochemistry

of the NN-dimethyl analogue is resolved, the 11 Hz coupling is trans $^{3}J_{C(1)H\text{-}C(2)H}$ (ie 71E). Similarly, the N-Me signals of (71) in $(CD_3)_2SO$ can be assigned; the signal at δ2.85 is for the methyl syn to C(2) while the lower field signal at δ 3.20 is for the methyl anti to C(2). The value of $^{3}J_{C(1)H\text{-}NH}$ (15 Hz) is consistant with that reported by Fetell and Feuer[86] for N-t-butyl-2-nitroprop-1-ene (153) $^{3}J_{C(1)H\text{-}NH}$ (14 Hz). Of those molecules of (70) that are E about C(1)-C(2) the E to Z ratio about C(1)-N is 1:8.9, a proportion which is intrigingly similar to the 1:9 ratio E:Z for rotamers of NMF.

In trifluoroacetic acid, (70) forms two distinct species (70a) and (70b) in a ratio of 47.5 : 52.5. The spectrum of (70a), figure 4, contains an ABX system with J_{gem} of the prochiral methylene at C(2) being 16 Hz. The prochirality arises from the adjacent asymetric centre at C(1). The N-Me resonates as a triplet coupled now to two protons on N(1). The adduct (70a) arises from addition of trifluoroacetic acid across the formal bouble bond C(1)-C(2) and subsequent protonation of the amine nitrogen N(1). The other species (70b) appears to be the protonated nitronic acid tautomer having E stereochemistry about C(1)-C(2), $^{3}J_{C(1)H\text{-}C(2)H}$ is 15 Hz. Buchi and Mak[108] proposed the intermediacy (156) and (157) in the 2-nitroethenylation of indoles and other aromatics by NN-dimethyl-2-nitroethenamine in trifluoroacetic acid.

The reaction of nitroenamines with electrophiles has been discussed by Rajappa[85,109] who compares their reactions with those of other push-pull olefins such as 1,1-diamino-2-nitroethenes (158), β-aminoacroleins (159) and enamines (160). The degree of enaminic character at (C)2 was correlated with (i) the ^{1}H and ^{13}C chemical shifts[109] at C(2), and (ii) the rank order of reactivity with



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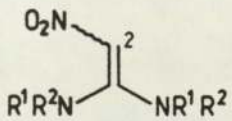

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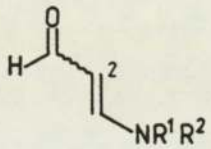

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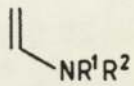
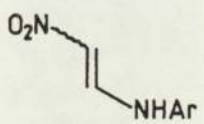


(162)

Scheme 31



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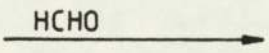
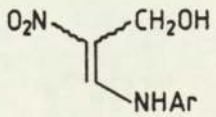
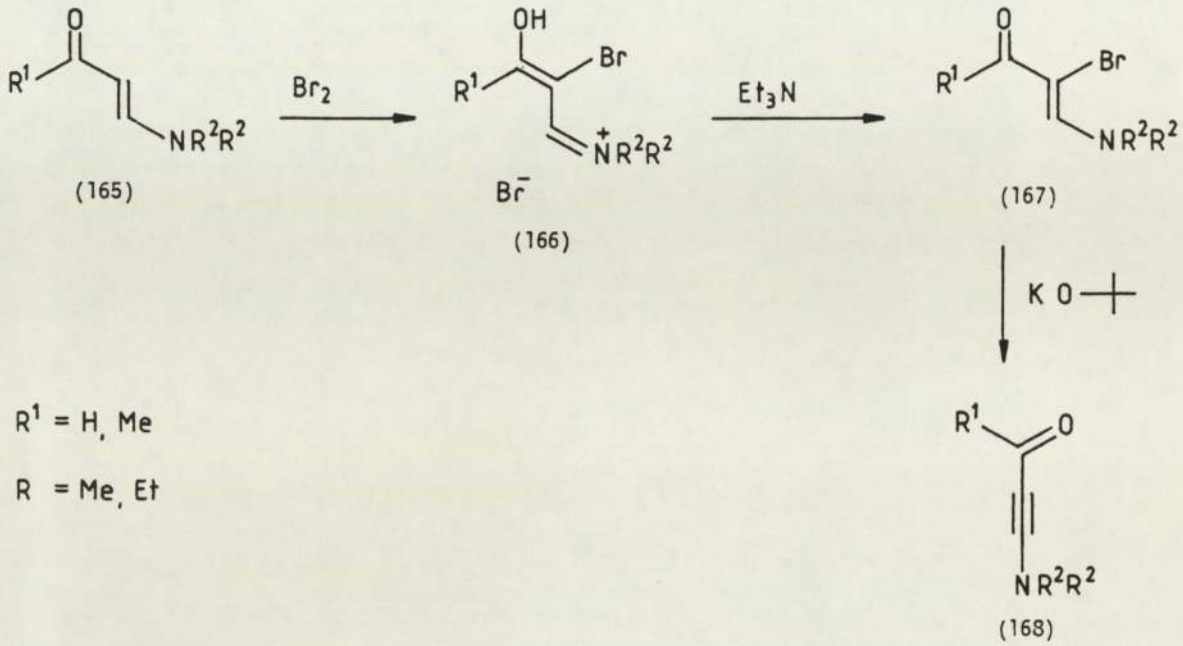




(164)

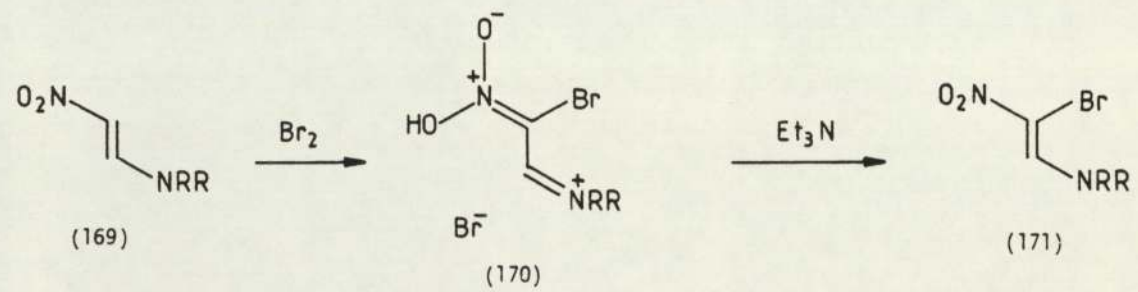
Scheme 32



R^1 = H, Me

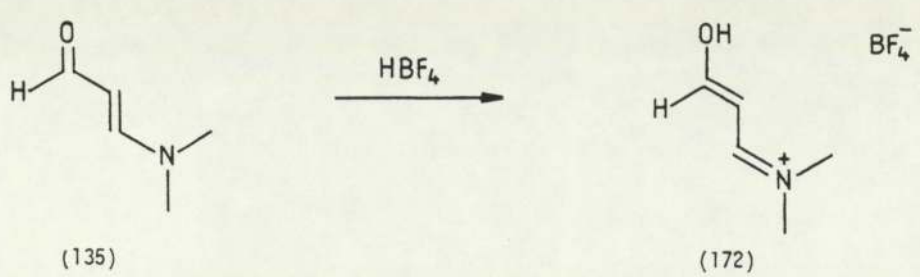
R = Me, Et

Scheme 33



R = Me, Et

Scheme 34



electrophiles at C(2)[85]; 2-nitroethenamines < 1,1-diamino-2-nitroethenes < β-aminoacroleins < enamines. Treatment of N-(4-chlorophenyl)-2-nitroethenamine (163, Ar= 4-chlorophenyl) with formaldehyde gives the 2-hydroxymethyl derivative[111] (164). Burgi et al[112] and Lienhard et al[113] have brominated 3-dialkylaminoacroleins[112,113] and NN-dialkyl-2-nitroethanamines[112], scheme 32, to produce the bromo derivatives, which in the acrolein series were treated with strong base to eliminate HBr and produce the 'push-pull' acetylene (168), scheme 32. In contrast, Fetell and Feuer[86] found that bromination, in the presence of pyridine, of the nitroenamine N-t-butyl-2-nitroprop-1-ene (153), which possesses an NH and no hydrogen on C(2), leads to cleavage. Protonation of 3-dimethylaminoacrolein with HBF_4 affords the O protonated species (174) with E/E stereochemistry[112], scheme 34.

Crystallographic determination[114] of the structure of (70) revealed an E stereochemistry around C(1)-C(2) [as for the NN-dimethyl analogue (71) and having inter (N(3)-H(3)···O(7) bond distance of 2.937Å)] rather than intramolecular hydrogen bonding, figure 5. Simple steric considerations would predict E stereochemistry about the C(1)-N(3) bond, but it is found to be Z in the crystal. The amine nitrogen N(3) is trigonal and that the molecule is virtually planar with a N(3)-C(1)-C(2)-N(5) torsion angle of 177.4°. The amide bond length of NMF (calculated)[115] and the 'pro-amide' [C(1)-N(3)] bond lengths of NN-dimethyl[116] (71) and N-methyl (70) derivatives of 2-nitroethenamine are given in table 13. There is more double bond character in the formal single pro-amide bond of the nitroethenamines than in the amide bond of NMF.

Figure 5, The structure of N-methyl-2-nitroethenamine (70) in the crystal

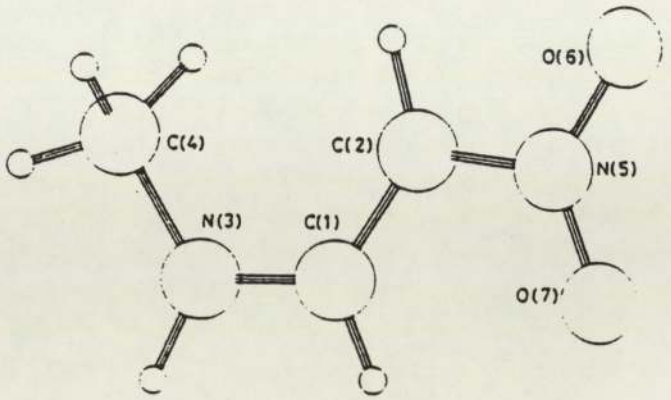
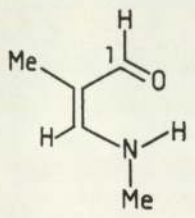


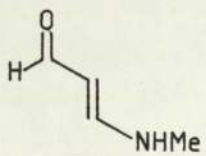
Table 13, Amide or 'pro-amide bond' lengths for NMF and N-methyl-2-nitroethenamine

Amide or pro-amide bond	Bond length Å
OHC-NHMe	1.405[a]
$O_2NCH{=}HC{-}NHMe$	1.303

a calculated ref 115

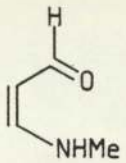


(66 Z/Z/E)

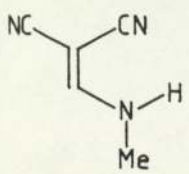


E/E/-

(141)



Z/Z/-



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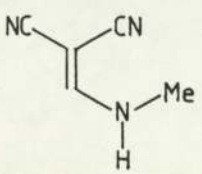

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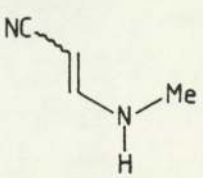


(174)

In $CDCl_3$ only a single rotamer of 2-methyl-3-methylaminopropenal (66) could be detected. The stereochemistry about C(3)-N can be designated as E ($^3J_{C(3)H-NH}$ of 15 Hz) but, the orientation about the olefinic linkage can not be deduced; although it would be tempting to propose the intramolecularly hydrogen bonded form (66 Z/Z/E). Stereochemistry for 3-aminopropenals is given in the order of C(1)-C(2), C(2)-C(3) and then C(3)-N. In $(CD_3)_2SO$ a more complex spectrum is obtained with the C(3)H and NH signals not resolved from each other (60 MHz). 3-N-Methylaminopropenal (141) which possesses three bonds which have a restriction of rotation could potentially have eight rotamers in solution. Kikugawa et al[95] does not report rotamers for (141) about the C(3)-N bond (pro-amide) but by analysis of coupling constants shows that (141 E/E/-) is exclusively formed in $(CD_3)_2SO$ but in $CDCl_3$ a 2:1 ratio (141 E/E/-) : (141 Z/Z/-) is found. The E:Z ratio of rotamers about the C(1)-N bond of 2-cyano-3-methylaminopropenenitrile (173) in $(CD_3)_2SO$ is 5:1. This preference for the E rotamer perhaps reflects a significant interaction between the N-substituent syn to the olefinic linkage with the cyano group syn to the amino group. Interestingly, although Wakamatsu[118] does not report any rotational isomerism about the C(3)-N bond of 3-N-methylaminopropenenitrile (174) in $CDCl_3$ the value of the C(3)H-NH coupling constant (7.2 Hz) would infer a Z orientation

In summary, in two parameters (populations of rotamers about the C(1)-N bond and the corresponding bond order) it has been possible to compare NMF with a 'push-pull' olefin analogue (N-methyl-2-nitroethenamine, 70). The ratio of non-intramolecularly hydrogen bonded rotamers of (70) are similar to that found in NMF, but the pro-amide [C(1)-N] bond of (70) has more double bond character than the amide bond of NMF.

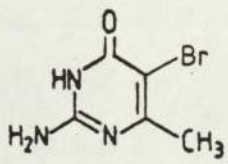
PART C

ANTITUMOUR 6-ARYL-5-HALOISOCYTOSINES

4. INTRODUCTION

4.1. INTERFERON INDUCING AND ANTIVIRAL PROPERTIES OF 6-ARYL-5-HALO-ISOCYTOSINES

There are many agents[125-128] which have been shown to induce interferon (IFN) either *in vitro* and or *in vivo* and include both naturally occuring and synthetic agents. Examples of naturally occuring inducers include viruses, bacteria, bacterial extracts, and antigens while synthetic inducers include polynucleotides such as poly I: poly C, poly carboxylates such as pyran copolymer and low molecular weight compounds such as tilorone, the purine complex isoprinosine, the tetrapeptide tuftsin, and isocytosines such as ABPP.



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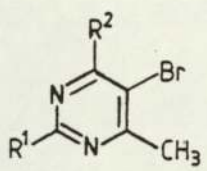

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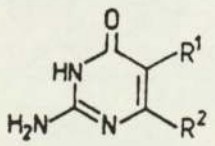
The stimulation of murine IFN by the isocytosine 2-amino-5-bromo-6-methylpyrimidine-4(3H)-one (180, ABMP) was desribed[129] in 1976 together with its antiviral activity. Mice were protected against intranasal encephalomyocardis virus challange when ABMP was administered repeatedly prior to viral inoculation. 6-Alkyl analogues of ABMP were tested for antiviral and IFN inducing ability although only at one dose level. However, at this one dose level (50 mg/Kg) the antiviral activity of these pyrimidines decreased as the size of the 6-alkyl group increased and iodine could replace bromine at the 5 position with retention of activity. Removal from ABMP of the amino group (181, R^1= H, R^2= OH) or replacement with methylamino (181, R^1=

MeNH, R^2= OH) resulted in loss of activity as did replacement of the oxygen atom of the 4-oxo function with methyl (181, R^1= NH_2, R^2= Me) or amino (181, R^1= R^2= NH_2).

In a comparitive study of the antiviral activity of ABMP with tilorone and poly I: poly C it was shown[130] that in mice the IFN response is progresively lost on repeated daily administration of ABMP. This hyporeactivity is a phenomenum which is seen with other IFN inducers[125,131] but mice hyporeactive to ABMP still responded to tilorone or poly I: poly C or vice versa. Animals became responsive after 4 to 6 days without treatment with ABMP. However, hyporeactivity was also noted as a consequence of viral infection. Restoration of the IFN response in animals hyporeative to ABMP was brought about by administration of prostaglandins[132]. In mice this hyporeactivity was also eliminated by pretreatment with low levels of IFN[133].



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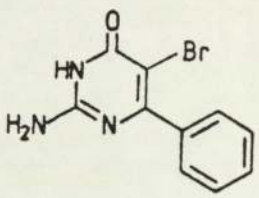

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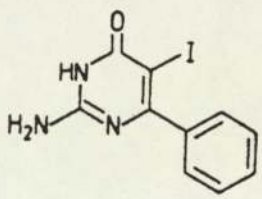


(183)

The low solubility of ABMP resulted in nephrotoxicity due to crystal formation in the kidney in a variety of animal species[134] and so greatly reduced the clinical potential of ABMP. However, some 6-aryl analogues (182, R^1= Hal, R^2= Aryl) of ABMP were not nephrotoxic but were still antiviral agents and/or inducers of IFN[134-144]. Thus the 6-phenyl anaologue of ABMP (2, ABPP) was active as an IFN inducer and antiviral agent at lower doses than ABMP and did not produce renal toxicity in experimental systems. In IFN inducing and antiviral

structure activity studies[135,139,145] the antiviral activity of these 6-aryl-5-haloisocytosines could not be corelated with the induction of IFN in mice. Antiviral effectiveness was assessed on the ability of these agents to protect against a normally lethal dose of a variety of viruses when administered to mice prior to innoculation with the viruses and was expressed as the minimum dose required to afford protection to 50% of the animals. These aminopyrimidinones were generally effective when administered ip but some were also active when given sc or orally 6-Aryl-5-chloroisocytosines were less effective antiviral agents than their 5-bromo or 5-iodo analogues. Substitution of the 6-phenyl ring of (182, R^1= Cl, Br, or I, R^2= Ph) with F, Cl, Br, CF_3, MeO, Me, or NO_2 retained activity however, di-substitution of the phenyl ring led to less active compounds. The 6-phenyl group could be repaced by other aromatic moieties such as 1-naphthyl, 2 or 3-pyridyl, and 2-furyl although activity was lost with 2-naphthyl, 1-furyl, 4-pyridyl, or 2-quinolinyl. However, owing to the low solubility of these agents it is difficult to assess whether the absence of activity with some of these compounds is due to intrinsic inactivity or to poor bioavailibility.

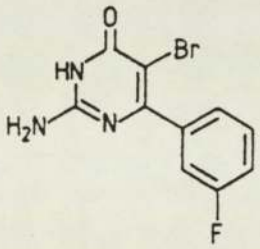
The ability of some of these agents to induce IFN in vivo and in vitro in a variety of animal species[129,146-149] and in vitro in human tonsilar tissue[134,150] has been demonstrated. However, Stringfellow[149-151] in reviewing the antiviral and IFN inducing properties of these pyrimidinones noted the lack of corelation between IFN induction and antiviral activity. Thus for example, 2-amino-5-iodo-6-phenylpyrimidin-4(3H)-one (183, AIPP) was as effective as ABPP as an antiviral agent but induced only low levels of IFN. Other biological activities of these pyrimidines have been investigated and include the induction of murine natural killer (NK) cells[137,152-155],

enhancement of macrophage cytotoxicity[136], increased T killer cell cytotoxicity[138], antibody formation[136,156], effects on thymus and spleen lymphocytes[157], and membrane fluidity in murine thymus and spleen cells[158]. ABPP is therefore included in that group of agents known as 'biological response modifiers' (BRMs).

4.2. ANTINEOPLASTIC PROPERTIES OF 6-ARYL-5-HALOISOCYTOSINES

ABPP was first synthesised by Brown and Stevens[159] in the early 70's and was evaluated for antitumour activity together with some analogues. Stringfellow[149,150] reported the activity of ABPP, AIPP and ABMP against the B16 melanoma and noted that a more favourable resopnse was obtained as the initial tumour burden was lowered and that these isocytosines reduced the number of pulmonary metastases. The effect on metastatic rate was further investigated by Milas *et al*[153] who found that the reduction in the rate of metastasis did not correlate with tumour immunogenicity or with peak serum IFN levels induced by these agents. The most effective reduction was seen when the pyrimidines were given prior to the iv injection of tumour cells. However, whole body irradiation prior to tumour induction abolished the reduction in the metastic rate brought about by these molecules. ABPP was slightly better than AIPP both of which were superior to ABMP. The induction of NK cells by ABPP, AIPP, and 2-amino-5-bromo-6-(3-fluorophenyl)pyrimidin-4(3H)-one (184, ABmFPP) has been shown by Lotzova *et al*[154] in peritoneal exudates of mice. Enhanced NK cytotoxicity was observed after 6 hours but peaked at 2-4 days post isocytosine injection and was still evident after 12 days. The spleen, bone marrow, and peripheral blood also showed increased NK activity. However, the capability to induce NK cells did not correlate with IFN

induction (characterised as IFN α) as AIPP induced negligible levels of IFN but was as effective as ABPP in inducing NK cells. In combination therapy[160] ABPP, ABmFPP, and 2-amino-5-bromo-6-(2-fluorophenyl)pyrimidin-4(3H)-one (185, ABoFPP) with cyclophosphamide (CY)



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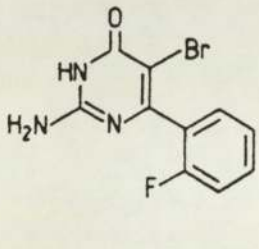


(185)

were found to have additive antitumour effects against the B16 melanoma and synergism against the P388 leukaemia. The authors claimed that the synergistic activity of ABoFPP and ABmFPP was superior to that of ABPP. However, the antitumour reponse appears to be dependant on both the dose of CY and the isocytosine and only a narrow dose range was presented for ABPP. The most efficacious reponse was obtained when CY was given on day 1 (day 0 = inoculation with tumour cells) and the pyrimidinone on day 2 then every 4 days for a total of 7 injections. This together with more pronounced synergism when higher doses of CY were used led the authors to conclude that the effects of these pyrimidines were more pronounced as the initial tumour burden was reduced by CY. The antitumour effectiveness of ABPP against a murine neuroblastoma was shown by Oku[133] to be enhanced by pretreatment with IFN. Recently Li et al[155] showed that the antitumour activity of ABmFPP against the B16 melanoma and the P388 leukaemia tumour models was almost abolished by X-irradiation 5 days prior to inoculation with tumour cells. The activity against the B16 melanoma was also reduced by administration of anti-asialo monosialoganglioside antibody. The induction of NK

cells and macrophages in peritoneal exudates by ABmFPP was confirmed but when ABmFPP and CY were used in combination the augmentation of both effector cells was delayed but cytotoxicity was more pronounced. Since the antitumour activity of ABmFPP and the augmentation of NK and macrophages in peritoneal exudates were diminished by X-irradiation and/or in combination with anti-asialo monosialoganglioside antibody it was concluded that the antitumour activity of ABmFPP was at least in part mediated through NK cell and macrophage activity.

ABPP was recently evaluated for antineoplastic activity in two phase I trials[161,162] In 16 patients[161] with solid tumours ABPP was given orally as two, four, six, or eight 1g doses at three hourly intervals and the course repeated weekly upto three weeks. Dose limiting toxicity (8g) was mild orthostatic hypotension. A partial response was seen in a patient with a lymphoma who was given the lowest dose of ABPP. The authors noted that the peak plasma level of ABPP was very variable between patients and that an improved formulation was required to enhance the bioavailability. In the other trial[162] involving 59 patients ABPP was given at doses upto $5g/M^{-2}$ orally with minimal toxicity (naussea and vomitting in 18% of patients). One patient with malignent melanoma did show evidence of tumour regression. Generally IFN induction was not consistently observed and modification of patients defence parameters were not observed. The poor bioavailability of ABPP was noted.

4.3 SUMMARY

Isocytosines based on ABPP have been shown to be IFN inducers, antiviral agents, antineoplastic agents, and BRMs. Attention was

first drawn to these molecules because of their ability to induce IFN in experimental animals but there appears to be no corelation between IFN induction and antiviral activity and antitumour activity. Their antineoplastic activity may at least in part be mediated via NK cells. The structural requirements for antiviral activity have been more thoroughly investigated than those for antitumour activity. The clinical potential of ABPP as an antineoplastic agent is currently being investigated but is hampered by the poor bioavailability of the drug.

5. DISCUSSION

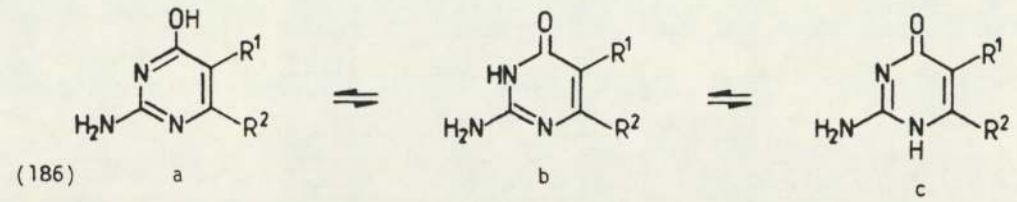
5.1. SYNTHESIS AND REACTIONS OF 2-AMINO-6-SUBSTITUTED PYRIMIDIN-4(3H)-ONES (6-SUBSTITUTED ISOCYTOSINES)

2,4-, and 6-Hydroxypyrimidines exist as their oxo tautomers[163]. 2-Amino-4-hydroxypyrimidines (isocytosines) exist as the pyrimidin-4(3H)-one (186b) or the pyrimidin-4(1H)-one (186c) tautomer and have been named as 4(3H)-pyrimidinones.

The most used synthetic route[163-165] to 6-alkyl or 6-aryl-isocytosines involves disconection of the pyrimidine ring accross the 1,6 and 3,4 bonds which gives guanidine and β-ketoesters as precursors. 6-Methylisocytosine (189) and 6-arylisocytosines (188, R=Ar) were prepared by the reaction of guanidinium carbonate and the appropriate β-ketoester in refluxing ethanol, scheme 35. The reaction time was greater for ethyl 3-phenyl-3-oxopropanoates (ethyl benzoyl-acetates) than for ethyl 3-oxobutanoate (ethyl acetoacetate) which is a reflection of the greater enolic component[166] of the aromatic β-ketoesters and of steric hindrance.

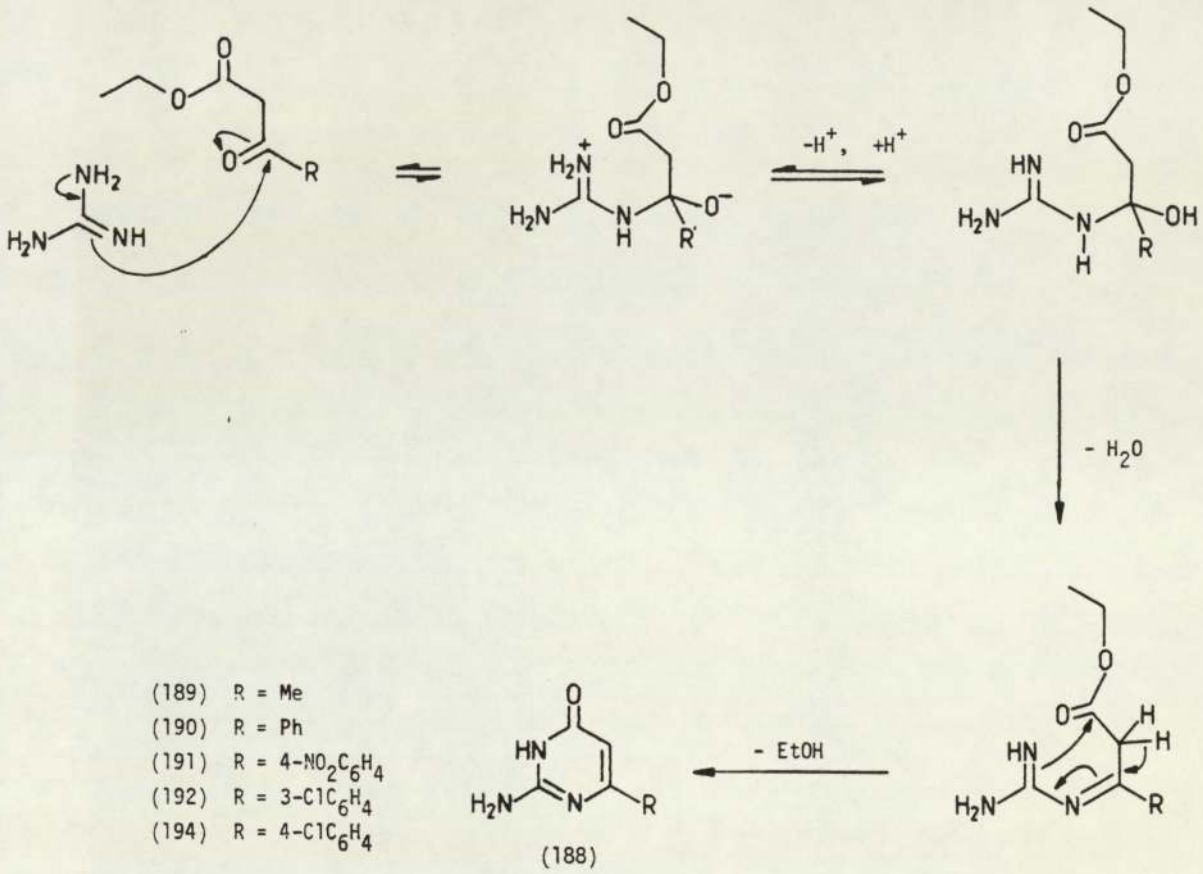
Non-commercially available β-ketoesters were prepared by acylation of the dilithium salt of ethyl hydrogen malonate as reported by Wierenga and Skulnick[167], scheme 36. The β-ketoesters produced by this method contained high boiling hydrocarbons but were used for further reactions without purification.

Electrophilic substitution of the pyrimidine ring has only been reported for the 5-position[163-165] which easily monitored by the disappearence of the 5-H signal at about δ6.3 from the NMR spectrum. Bromination of 6-methylisocytosine afforded the 5-bromo derivative



(186) a b c

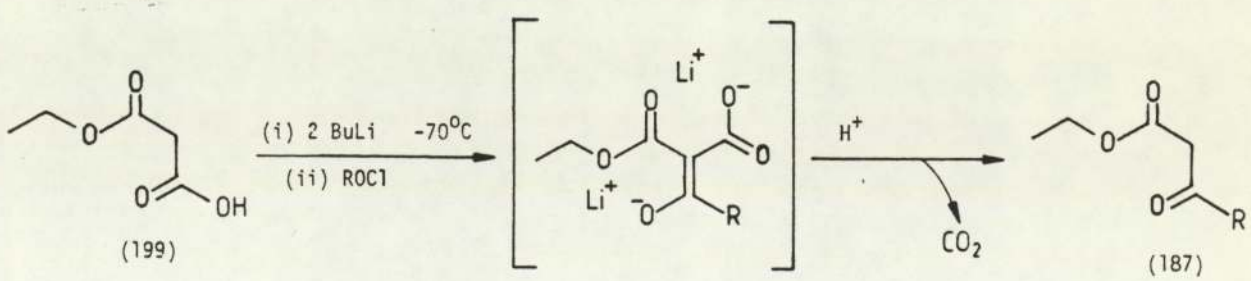
Scheme 35



(189) R = Me
(190) R = Ph
(191) R = 4-$NO_2C_6H_4$
(192) R = 3-ClC_6H_4
(194) R = 4-ClC_6H_4

(188)

Scheme 36

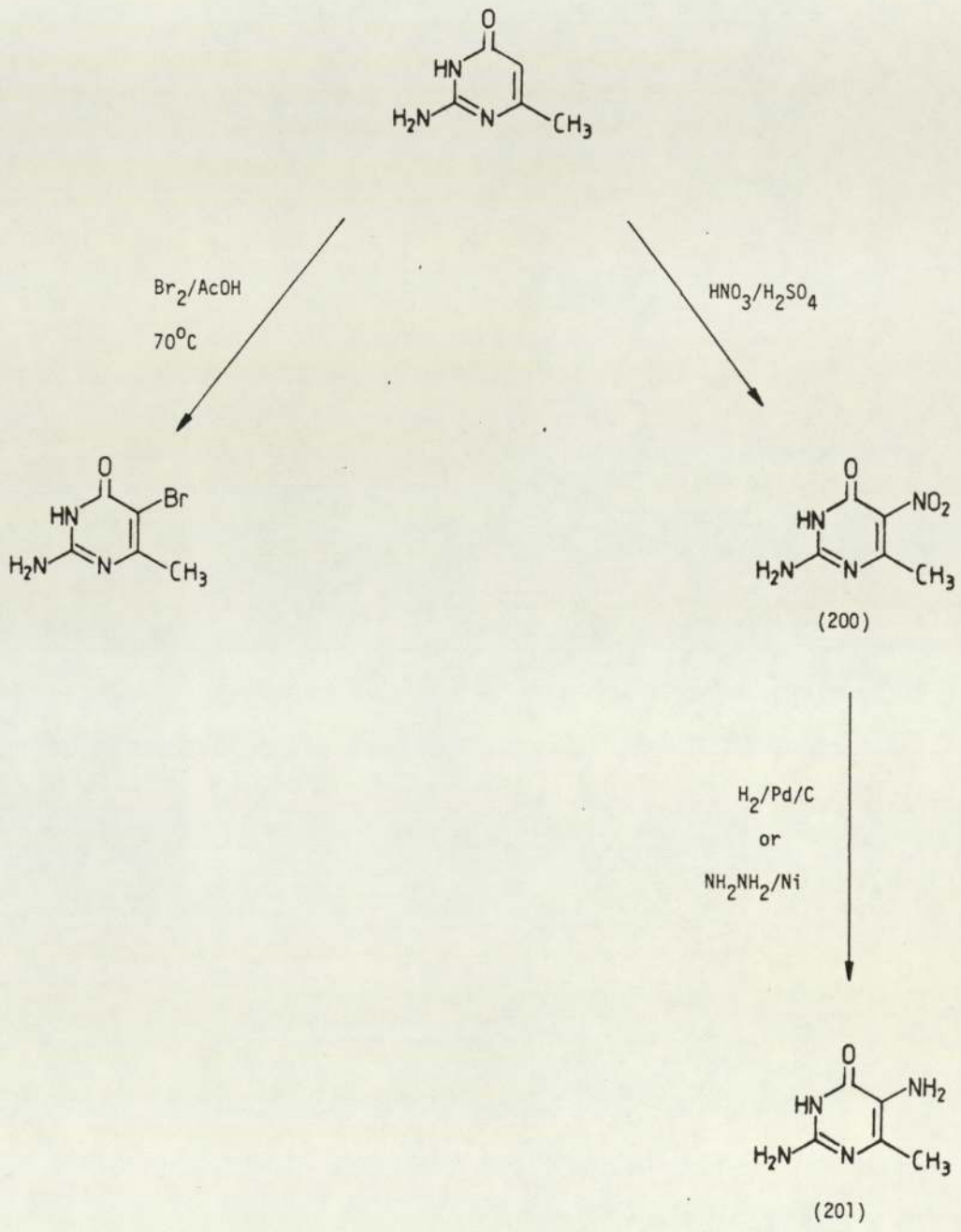


(199)

(194) R = Me, (195) R = Ph
(196) R = 4-$NO_2C_6H_4$
(197) R = 3-ClC_6H_4 (198) R = 4-ClC_6H_4

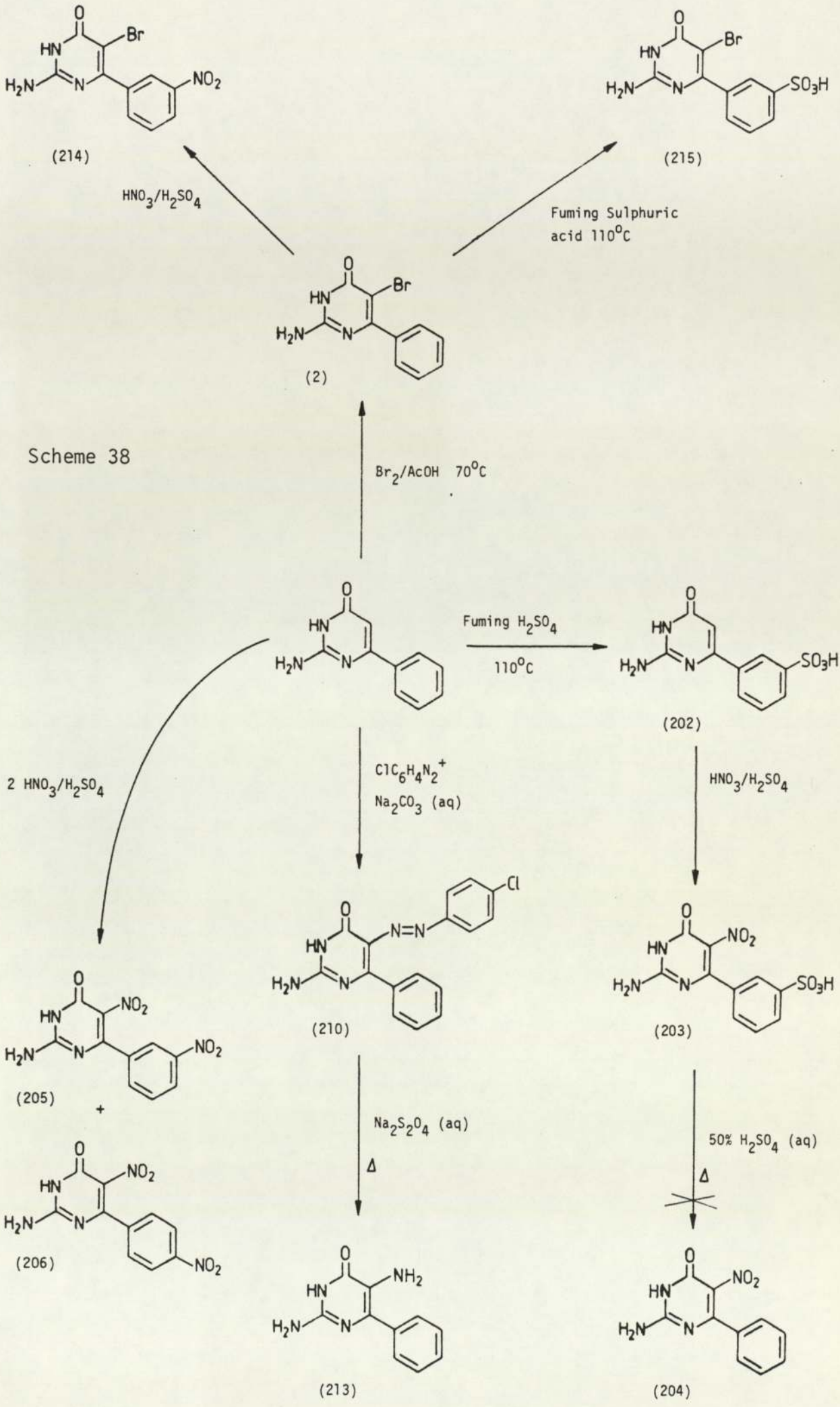
(187)

Scheme 37

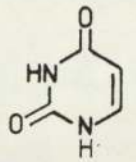


and nitration using 70% nitric acid in concentrated sulphuric acid (nitrating mixture) gave the 5-nitro analogue which was reduced with hydrogen as previously reported[168] or with hydrazine and Raney nickel, scheme 37, to the 5-aminopyrimidine (201).

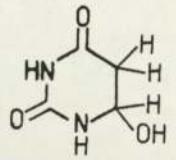
Electrophilic substitution of 6-phenylisocytosine could occur either in the pyrimidine or phenyl rings or both. Bromination with bromine in glacial acetic acid gave 2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone (ABPP, 2) whereas sulphonation with fuming sulphuric acid at 110°C gave the phenyl sulphonic acid (202), scheme 38, which was characterised as its sodium salt. IUPAC nomenclature gives a high priority to the sulphonic acid moiety and (202) should be named 3-(2-amino-4(3H)-oxopyrimidin-6-yl)benzenesulphonic acid. The difference in the site of electrophilic substitution may arise from deactivation of the (protonated) pyrimidine ring in strongly acidic media. Nitration of 6-phenylisocytosine with two equivalents of nitrating mixture gave the 5-nitro-6-(3-nitrophenyl) derivative (205) and the 5-nitro-6-(4-nitrophenyl) derivative (206) in a proportion of 70:30 repectively and were characterised by NMR. A previous report[159] indicated that <u>para</u> nitration of the phenyl ring occured. Nitration with one equivalent of nitric acid gave a mixture of the starting material and the dinitrated products. The exact reason for this is unclear. However, if the first product of nitration reduced the pKa of the pyrimidine ring then subsequent nitration of the unprotonated nitropyrimidine may occur at a rate faster than nitration of the unreacted starting material. The pKa's of ABPP and conjugate acid are about 8.6 and 3 repectively[169]. 6-Phenylisocytosine or 6-methylisocytosine could not be nitrated using 70% nitric acid, potassium nitrate in concentrated sulphuric acid, dilute aqueous nitrc acid, 98% nitric acid in glacial acetic acid, or 70% nitric acid in acetic


(214)
(215)
HNO3/H2SO4
Fuming Sulphuric acid 110°C
(2)
Br2/AcOH 70°C
Fuming H2SO4
110°C
(202)
2 HNO3/H2SO4
ClC6H4N2+
Na2CO3 (aq)
HNO3/H2SO4
(210)
(203)
(205)
+
(206)
Na2S2O4 (aq)
Δ
50% H2SO4 (aq)
Δ
(213)
(204)

Scheme 38

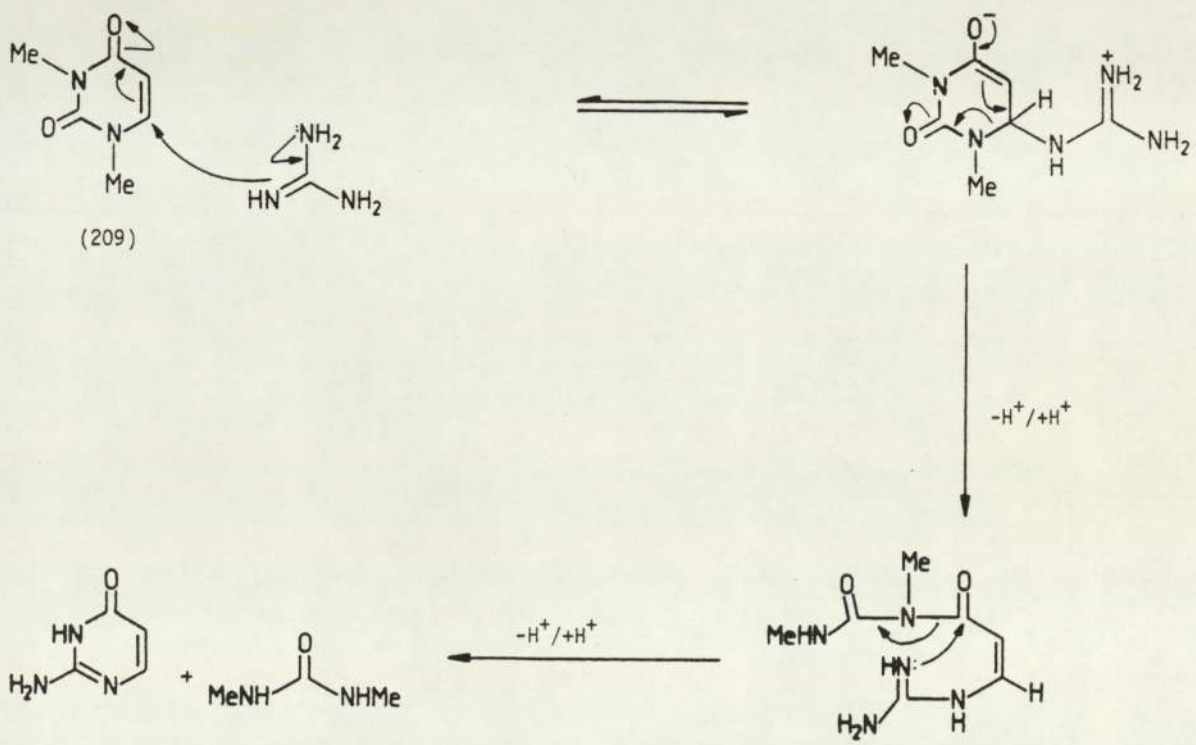


(207)



(208)

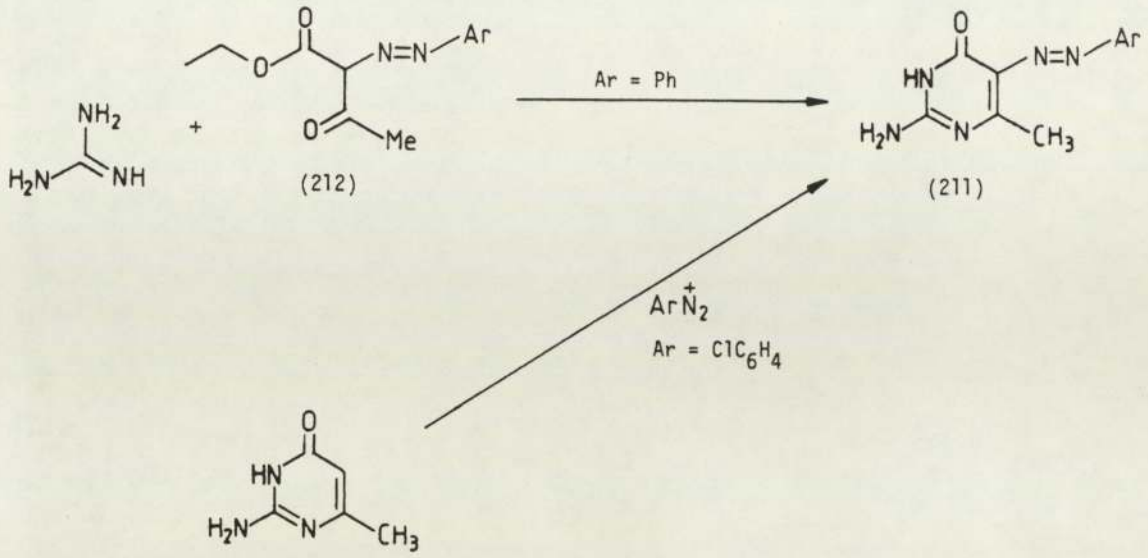
Scheme 39



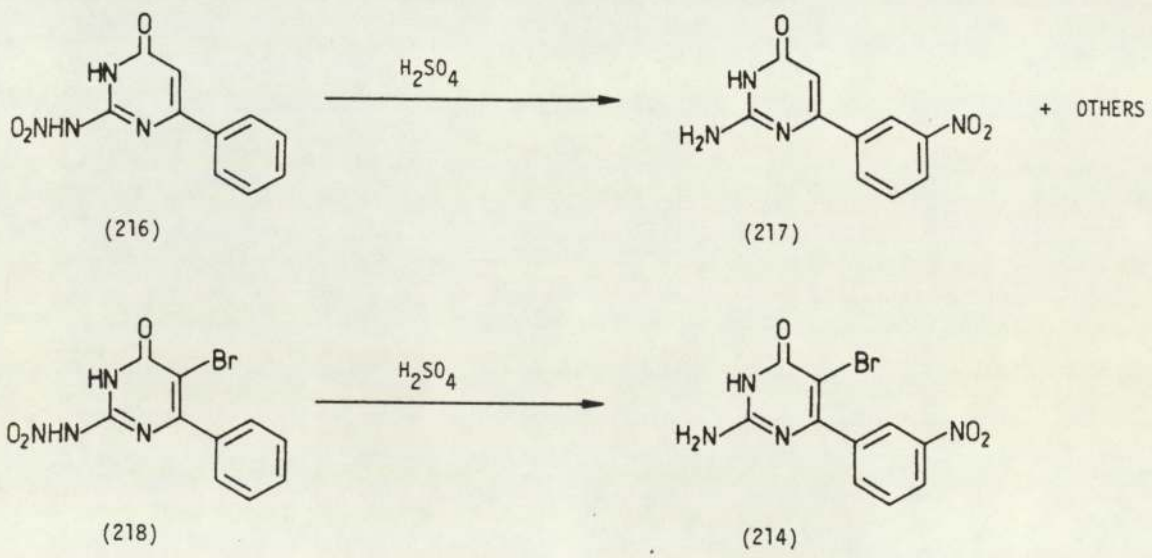
anhydride and glacial acetic acid. Although nitration of the 5-position of the sulphonic acid (202) was readily achieved, scheme 38, attempted desulphonation of the nitrosulphonic acid (203) (reluxing 50% sulphuric acid) resulted in decomposition to several products. This may be a result of addition across the 5-6 bond of the pyrimidine ring which would be expected to be more susceptible to nucleophiles than the un-nitrated precursor. The 6-position of the pyrimidine ring will be more electron difficient because of the presence of the nitro group. Uracil (207) is known to form a covalent hydrate (208) on u.v. irradiation of an aqueous solution[163] and Hirota et al[170] propose nucleophilic addition across the 5-6 position of NN'-dimethyluracil (209) as a step in the mechanism for the conversion of this uracil into isocytosine, scheme 39.

Coupling of the 5-position of the pyrimidine ring of 6-phenyl-isocytosine was achieved in aqueous sodium carbonate by treatment with diazotized 4-chloroaniline, scheme 38. Aqueous sodium acetate or sodium bicarbonate were not suitable media for coupling. 5-Arylazo-6-methylisocytosines (211) have been synthesed from guanidine and ethyl 2-arylazo-3-oxobutanoates (212)[171], scheme 40, or from coupling of 6-methylisocytosine with aryl diazonium ions[172]. Reduction of azo dyes is often achieved[164,165,173] using sodium dithionite, stannous chloride and acid, and by hydrogenation although hydrazo compounds can be the product of hydrogenation[173]. Reduction of 5-(4-chloro-phenylazo)-6-phenylisocytosine (210) with stannous chloride in hydrochloric acid gave a product which could not be separated from the tin complexes also present in the reaction mixture. Reduction with zinc in acetic acid produced a mixture of several products. However, the use of sodium dithionite as the reducing agent afforded a low yield (15%) of 5-amino-6-phenylisocytosine (213), scheme 38.

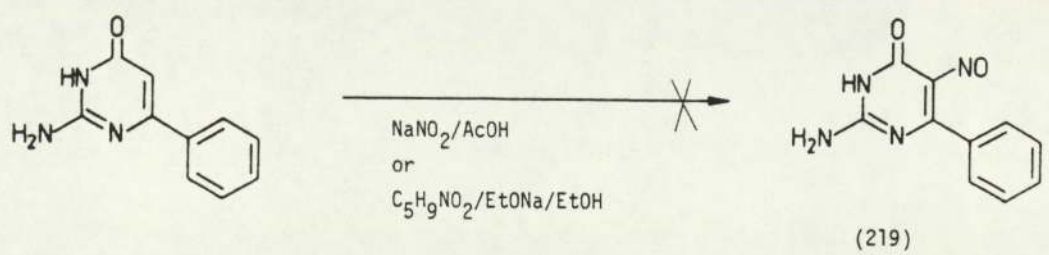
Scheme 40



Scheme 41



Scheme 42



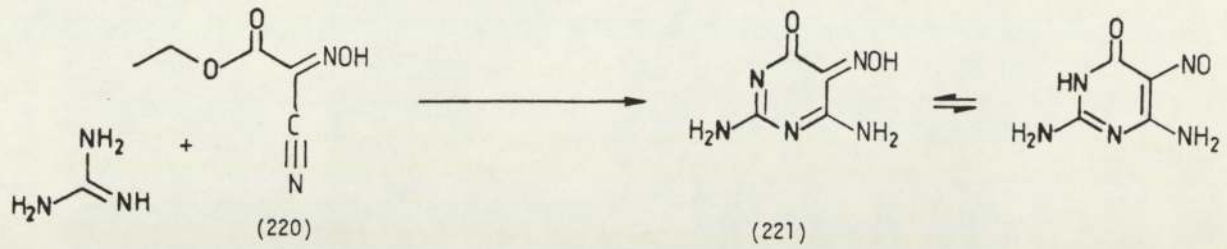
Rearrangement of the nitramine 2-nitramino-6-phenylpyrimidin-4(3H)-one (216) in concentrated sulphuric acid, scheme 41, gave mixture of products (tlc). However, the major component was 2-amino-6-(3-nitrophenyl)pyrimidin-4(3H)-one (217) which was identified after purification by repeated crystallisation by ^{1}H NMR. Rearrangement of this nitramine was reported[159] to give the 5-nitropyrimidinone (204). The position of nitration may be affected by the pH of the reaction medium with nitration of the pyrimidine ring being less favoured in more acidic conditions. Rearrangement in the presence of anisole did not give nitroanisole. Rearrangement of the 5-bromonitramine (218) gave the 6-(3-nitrophenyl) isomer (214) which had an ir spectrum identical to the product produced by nitration of ABPP with nitrating mixture.

Sodium nitrite in glacial acetic acid failed to nitrosate 6-phenylisocytosine, scheme 42. However, direct nitrosation of the pyrimidine ring in the 5-position requires the presence of two electron releasing groups (amino or hydroxy) in the 4 and 6 positions[163]. Resorcinols and certain heterocycles, such as indoles, bearing an acidic NH have been reported[174] to be nitrosated (in the 3 position for indoles) by amyl nitrite and sodium ethoxide in ethanol. However, under these conditions 6-phenylisocytosine failed to nitrosate.

An alternative approach to 5-aminoisocytosines is a primary synthesis of the pyrimidine ring with inclusion of either the nitroso, nitro, or acetamido groups in the synthetic precursors.

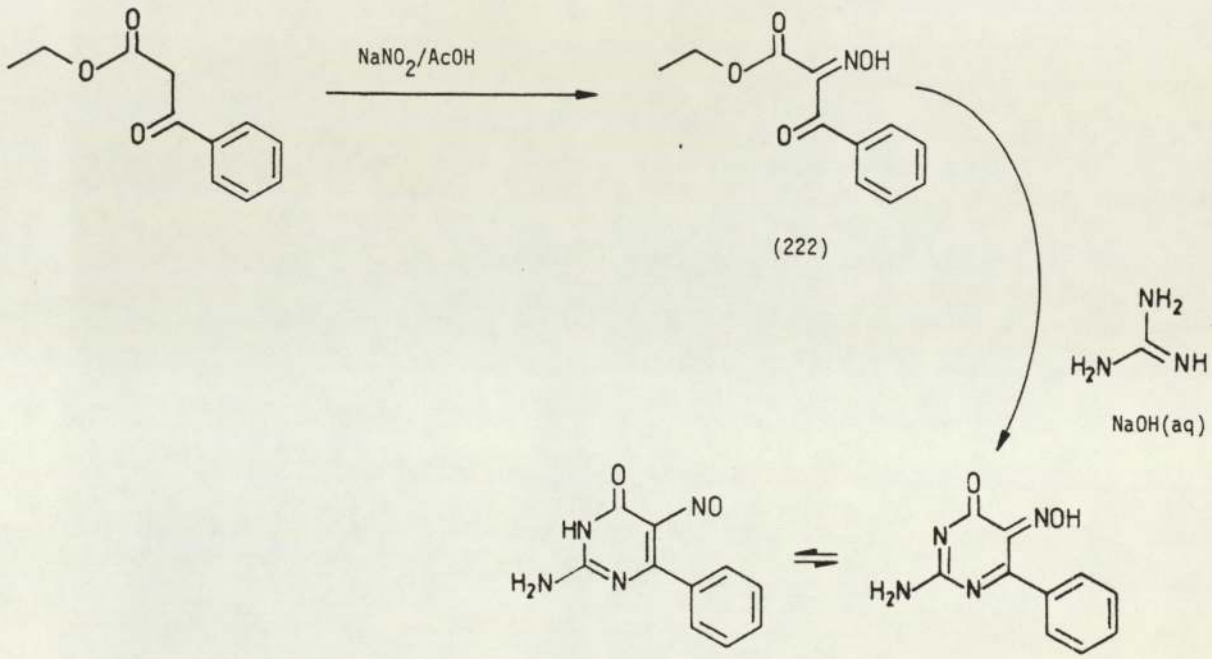
Landauer and Rydon[175] used ethyl 2-cyano-2-hydroxyiminoacetate (220) and guanidine to prepare 2,4-diamino-6-hydroxy-5-hydroxyiminopyrimidine (221), which is a tautomer of the 5-nitroso-

Scheme 43



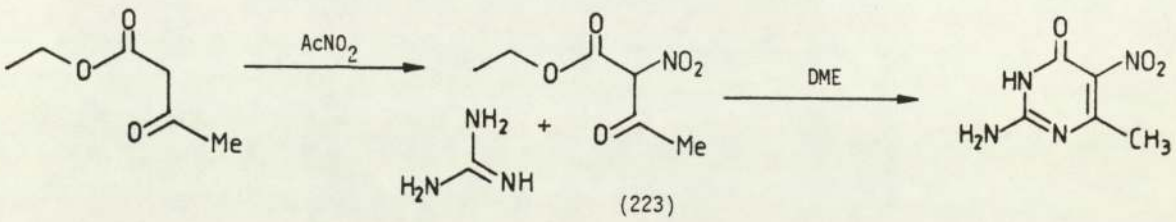
(220) (221)

Scheme 44

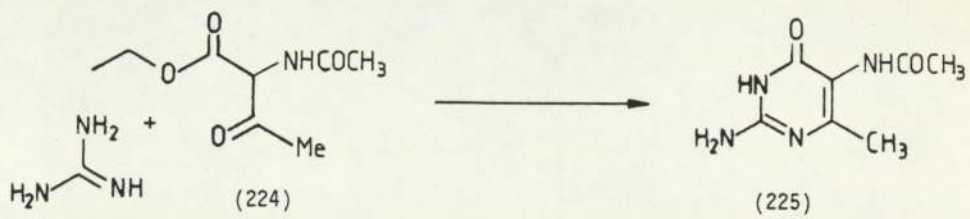


(222)

Scheme 45



(223)



(224) (225)

pyrimidine, scheme 43. The reaction of guanidine hydrochloride and ethyl 2-hydroxyimino-3-oxo-3-phenylpropanoate (222) in aqueous sodium hydroxide gave a green solution which on acidification gave a colourless high melting (>330°C) solid. The NMR spectrum of this product was in agreement with the structure of 5-hydroxyimino-6-phenylisocytosine (219), scheme 44, but mass spectral analysis gave the highest m/z of 183 rather than the required 216. Reduction with sodium dithionite gave mixtures of products, while oxidation with hydrogen peroxide in trifluoroacetic acid did not give any products recognisable as the 5-nitro analogue. Taylor and McKillop[176] have used hydrogen peroxide in trifluoroacetic acid to oxidise 5-nitroso-pyrimidines to 5-nitropyrimidines.

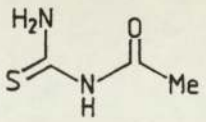
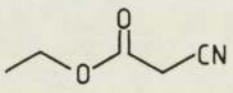
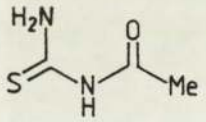
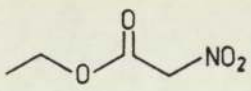
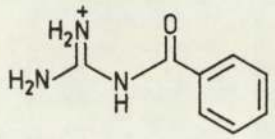
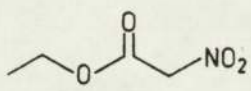
Ethyl 2-nitro-3-oxobutanoate (ethyl nitroacetoacetate, 223) did not cyclise with guanidine carbonate in 1,2-dimethoxyethane to give 6-methyl-5-nitroisocytosine, scheme 45. However, ethyl 2-acetamido-3-oxobutanoate (224) has been cyclised with guanidine to give 5-acetamido-6-methylisocytosine (225)[177].

Summerised in table 14 are the conditions used in an attempt to synthesise pyrimidines by the condensation of an N-C-N-C and a C-C fragment arising from a disconection of the 3,4 and 5,6 bonds. However, ring closure was not accomplished by any of the conditions.

6-Phenylisocytosine or ABPP failed to react with sodium azide in glacial acetic acid.

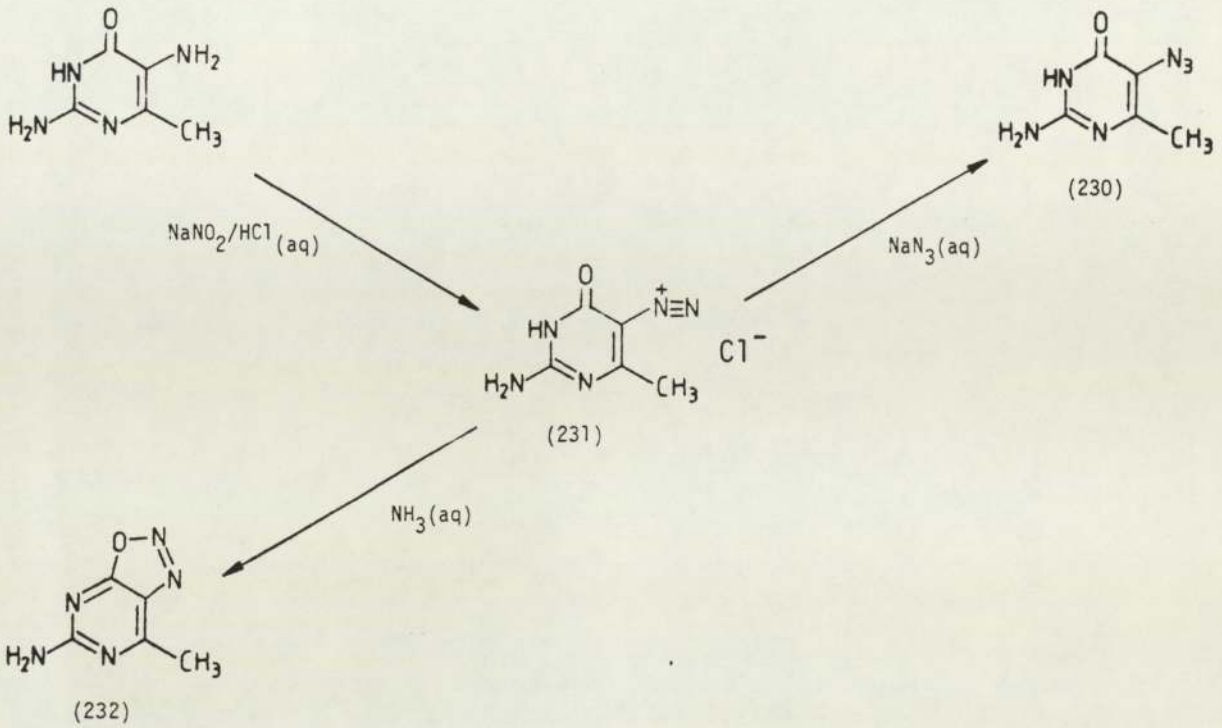
The conversion of 5-amino-6-methylisocytosine to the 5-azido-pyrimidine (230) was achieved by treatment of of the diazotized amine with sodium azide in aqueous mineral acid, scheme 46. Cyclization of the diazonium ion (231) to the pyrimido-[5,4-d]-1,2,3-oxadiazole

Table 14. Summary of conditions used in attempted synthesis of pyrimidinones by a disconection of the 3,4 and 5,6 bonds.

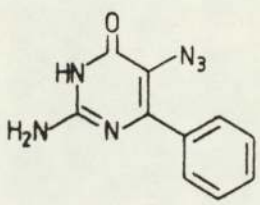
Fragment N-C-N-C	Fragment C-C	Reaction Conditions	Product[a]
 (226)	 (227)	EtOH/20°C	sm
		EtOH/reflux	sm
		EtOH/EtONa/ 20°C	water soluble products
		EtOH/KOH/ reflux	water soluble products
	 (228)	DME/pyridine/ 20°C	sm
		DME/pyridine/ reflux	sm sm and others
 (229)		EtOH/EtONa/ 20°C	water soluble products
		EtOH/EtONa/ reflux	water soluble products
		EtOH/NaOAc/ reflux	mixture of products

a sm = starting material

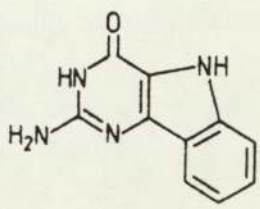
Scheme 46



(230) (231) (232)

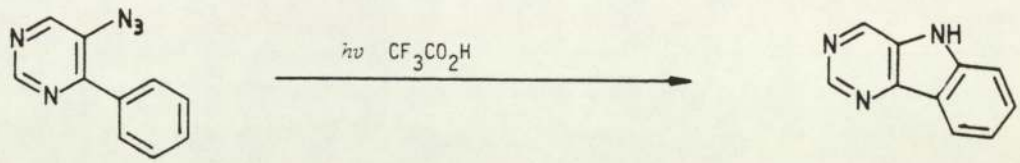


(233)

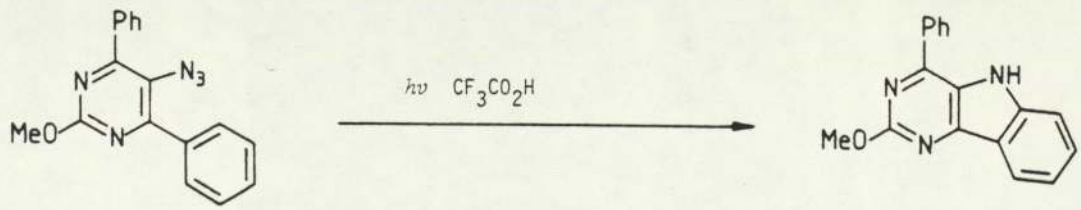


(234)

Scheme 47



(235) (236)



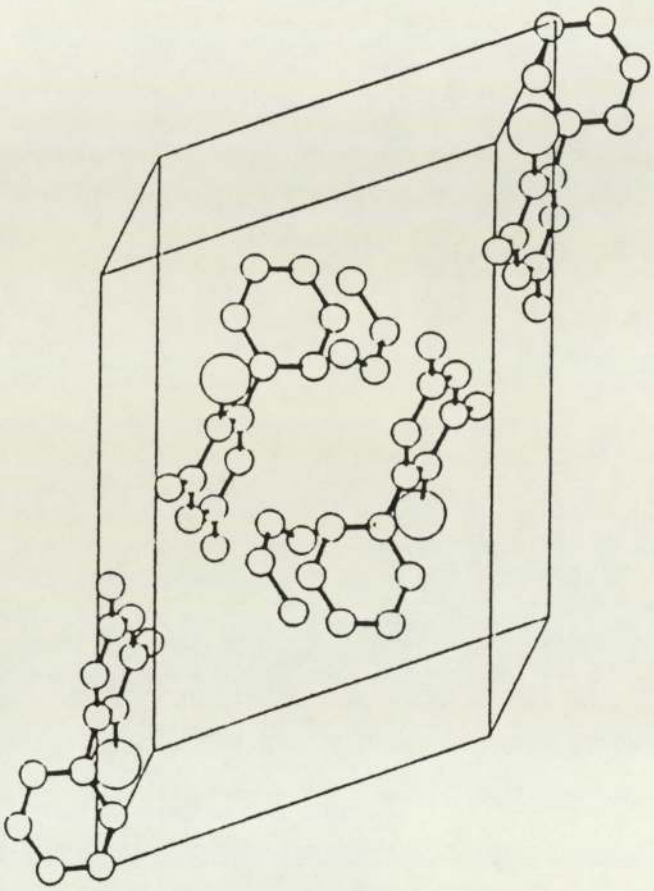
(237) (238)

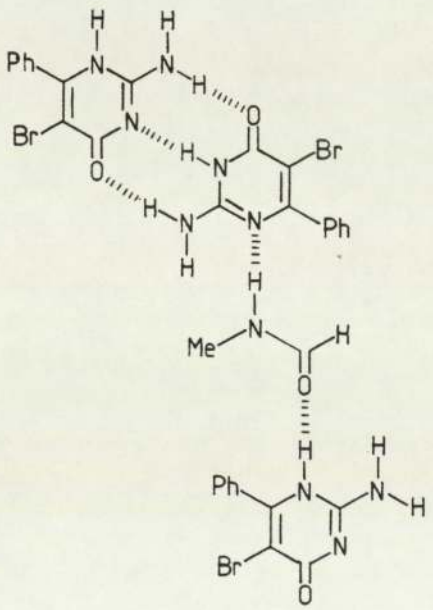
(232) has been reported by Rose[178] but only occured on treatment of the diazonium species with aqueous ammonia while the coupling of diazonium ions with azide ion is reported to be complete within[179] one minute at 0°C. The azide showed the characteristic band in the i.r. spectrum at 2 120 cm^{-1} and melted explosively at 108°C. Treatment of the azide with HBr in glacial acetic acid gave the 5-aminopyrimidine. On boiling in ethanol the azide decomposed with effervescence to give a mixture of compounds (tlc) one of which comigrated with the 5-aminopyrimidine and was presumably formed by hydrogen abstraction from the solvent by the triplet nitrene. The product formed from the treatment of diazotised 5-amino-6-phenylisocytosine with sodium azide also decomposed to several products (tlc) when heated in boiling ethanol. The product which comigrated with the 5-amino compound represented only a minor product. Intramolecular insertion into the 6-phenyl ring to form 2-amino-3,5-dihydro-4-oxopyrimido-[5,4-b]-indole (234) would be an attractive candidate for a decomposition product. Russian workers[180,181] have formed this ring system by the photolysis of 5-azidopyrimidines, scheme 47.

Thiourea cyclised with ethyl benzoylacetate to give 6-phenyl-pyrimidin-4(3H)-one-2(1H)-thione (239), scheme 48.

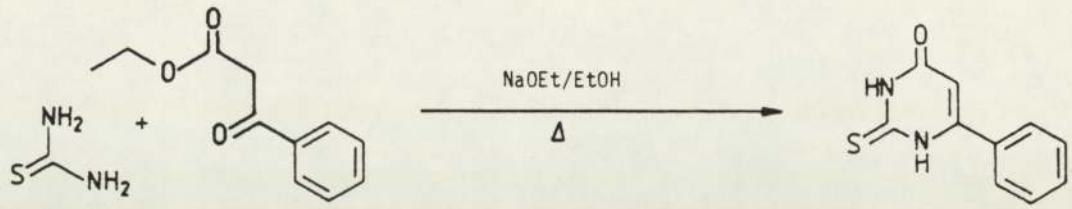
ABPP on recrystallisation from NMF formed a hemisolvate. X-ray crystallographic studies[182] showed the unit cell to be comprised of two ABPP molecules, one as the 4(3H) tautomer the other as the 4(1H) tautomer, and one molecule of NMF, figure 6. The intermolecular hydrogen bonding of the 3H tautomer to the 1H tautomer is consistant with that found in isocytosine itself[183] but additionally possesses a hydrogen bond between the carbonyl oxygen of NMF and the N(1)H of the 1H tautomer and between the NH of NMF and N(1) of the 3H tautomer.

Figure 6, The structure of ABPP hemi NMF solvate in the crystal



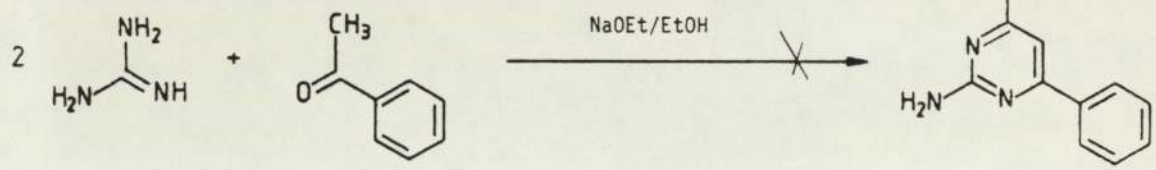


Scheme 48



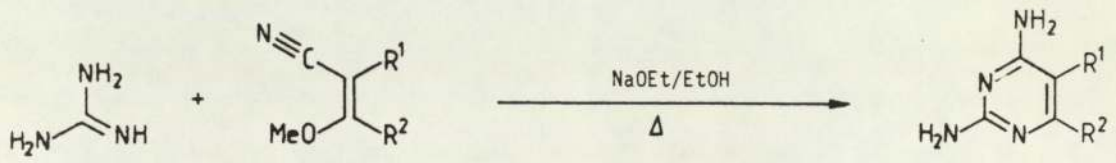
(239)

Scheme 49



(240)

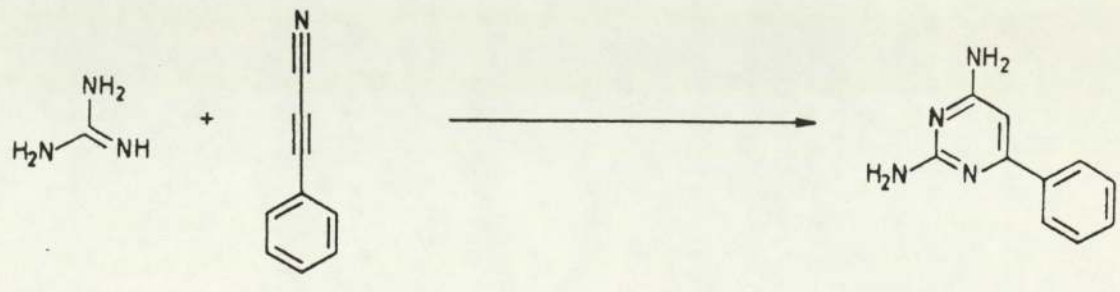
Scheme 50



(241)

(242)

Scheme 51



(243)

X-Ray crystallographic studies have been reported for ABPP in an abstract[184] though no details were given.

Wendelin et al[185] reported a one stage route to 2,4-diamino-6-phenylpyrimidine (240) although in low yield by the reaction of guanidine with acetophenone, scheme 49. However, on repeating this reaction the diaminopyrimidine could not be isolated. 2,4-Diaminopyrimidines are often synthesised by the cyclisation of guanidine with the enol ether of β-acylacetonitriles (241), scheme 50. However, a recent synthesis[186] of 2,4-diamino-6-phenylpyrimidine involved the addition and subsequent cyclisation of guanidine to 3-phenylpropynenitrile (243), scheme 51.

In summary a series of isocytosines has been synthesised. Electrophilic substitution of 6-aryl-5-unsubstitutedisocytosines has been achieved at the 5-position of the pyrimidine ring and/or in the 6-phenyl ring. However, the position of substitution depends on the reaction conditions. A sucessful synthesis of 5-nitro-6-phenylisocytosine was not achieved.

5.2. ANTITUMOUR ACTIVITY OF ABPP AND RELATED ISOCYTOSINES

The antitumour activity of ABPP and a limited number of analogues is summarised in tables 15 and 16, and shows that ABPP is highly active against the B16 melanoma and is active against the Colon 38 and the M5076 ovarian sarcoma murine tumour models. Replacement of the bromine atom at the 5 postion by amino or 4-chlorophenylazo leads to a loss of activity against the B16 melanoma as does the replacement of the 2-amino group by nitramino. Interestingly, the sodium sulphonates (202) and (203) showed activity

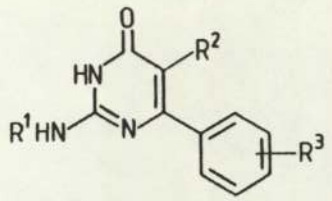


Table 15, The activity of ABPP and analogues against the M5076 ovarian sarcoma and B16 melanoma murine tumour models

Cpd No	R^1	R^2	R^3	M5076[a] Dose[c] Range	M5076[a] Optimal Dose	M5076[a] T:C[d] (%)	B16[b] Dose[c] Range	B16[b] Optimal Dose	B16[b] T:C[e] (%)
2[f]	H	Br	H	2000-16	250	**38**	400-50	100	**189**
185[g]	H	Br	2-F	4000-125	2000	**26**			
184[g]	H	Br	3-F	2000-125	500	**28**			
215	H	Br	3-$NaSO_3$	2000-125	500	86			
202	H	H	3-$NaSO_3$				106-13	13	**130**
203	H	NO_2	3-$NaSO_3$				103-13	103	**131**
213	H	NH_2	H				90-11	45	107
210	H	4-$ClC_6H_4N{=}N$	H				75-9	19	90
216	NO_2	H	H				88-11	44	105

a Compounds administered ip in a schedule of Q04DX04(1)

b Compounds administered ip in a schedule of Q01DX09(1)

c mg/Kg/dose, see appendix 1

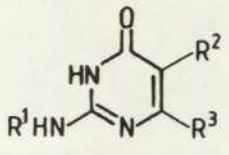
d Tumour inhibition on day 24 for optimal dose, Treated compared to control (%); values in bold indicate active compounds

e Median survival for optimal dose, Treated compared to control (%); values in bold indicate active compounds

f ABPP active against the Colon 38 tumour inhibition model [T:C of 36% at 1600 mg/Kg/dose on a schedule of Q07DX02(2)] but not active against Lewis Lung carcinoma, P388 lymphocytic leukaemia, CDF_1 mammary tumour, MX-1 breast xenograft, CX-1 colon xenograft, or LX-1 lung xenograft tumour models

g Gift from W Wierenga, Upjohn, Kalamazoo, USA

Table 16, Inactivity of ABPP and analogues[a] against the L1210 murine lymphoid leukaemia using various schedules



Cpd No	R^1	R^2	R^3	Schedule[b] QO1DXO9(1) Optimum Dose[c]	T:C (%)	QO4DXO3(1) Optimum Dose[c]	T:C (%)	QO1DXO2(2) Optimum Dose[c]	T:C (%)	QO1DXO1(1) Optimum Dose[c]	T:C (%)
2	H	Br	Ph	100	103	100	104				
245	H	Br	4-$N_3C_6H_4$			400	100				
246	H	NO_2	4-$NO_2C_6H_4$	3.13	96					12.5	94
247	H	NH_2	4-$NH_2C_6H_4$			400	93			100	96
248	H	2-$NO_2C_6H_4CONH$	Me							400	97
249	H	2-$NH_2C_6H_4CONH$	Me							100	94
250	H	4-$NH_2C_6H_4CONH$	Me							400	Tox
251	H	4-$N_3C_6H_4CONH$	Me	25	93	25	97			25	95
252	H	d	Me							100	92
216	NO_2	H	Ph			100	100				
218	NO_2	Br	Ph					100	103		

a Compounds for these antitumour tests synthesised by T B Brown

b See appendix 1 for notation

c mg/Kg/dose given ip

d 4-oxobenzotriazin-3-yl

even though they do not possess a bromine atom at the 5 position of the pyrimidine ring. The inclusion of a chemical grouping which is fully ionised at physiological pH often results in a poor pharmakokinetic profile and the inactivity of the bromo sodium sulphonate (215) the M5076 ovarian sarcoma may at least in part be due to disadvantageous distribution *in vivo*. The introduction of a fluorine atom in the 2 or 3 position of the 6-phenyl ring of ABPP (i.e. ABoFPP and ABmFPP) allowed antineoplastic activity to be retained aginst the M5076 ovarian sarcoma. The insolubility of the pyrimidinones necessitated their administration (ip) as a suspension in 10% tween 80 in saline. However, unabsorbed ABoFPP was found after examination of the peritoneal cavity of mice bearing the M5076 sarcoma sacrificed on day 24 after treated with ABoFPP [4000 mg/Kg, Q01DX04(1)] administered as a suspension.

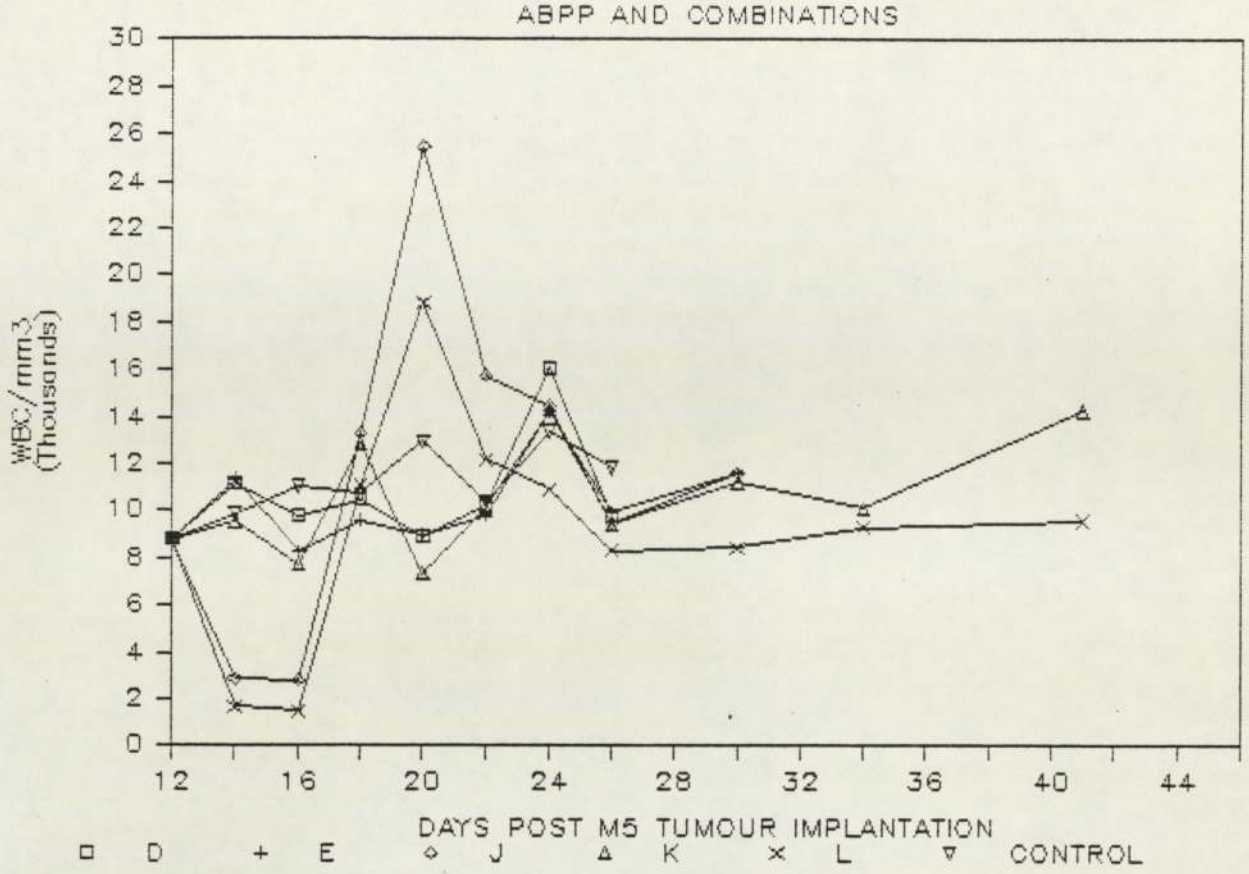
Drugs possessing non overlapping toxicities are often employed in combination chemotherapies in the treatment of cancer to obtain enhanced therapeutic responses over the single agents. Myelosupression is the dose limiting toxicity of some anticancer agents e.g. CY[187]. The use of CY in combination with agents that are not toxic to the haematopoietic system may be of potential value.

Using the M5076 ovarian sarcoma model the antitumour affects and toxicity to the WBC of CY, NMF, and ABPP (alone or in combination) were investigated. Given in table 17 are the doses and schedules of the compounds used in this study. Tumour weight was measured every four days from day 12 while total WBC and differential lymphocyte and granulocyte counts were measured on every other day from day 12, the results of which are given in appendix 2. Graphs 1-4 show the total WBC from day 12, while graphs 5-8 gives tumour weight from day 12.

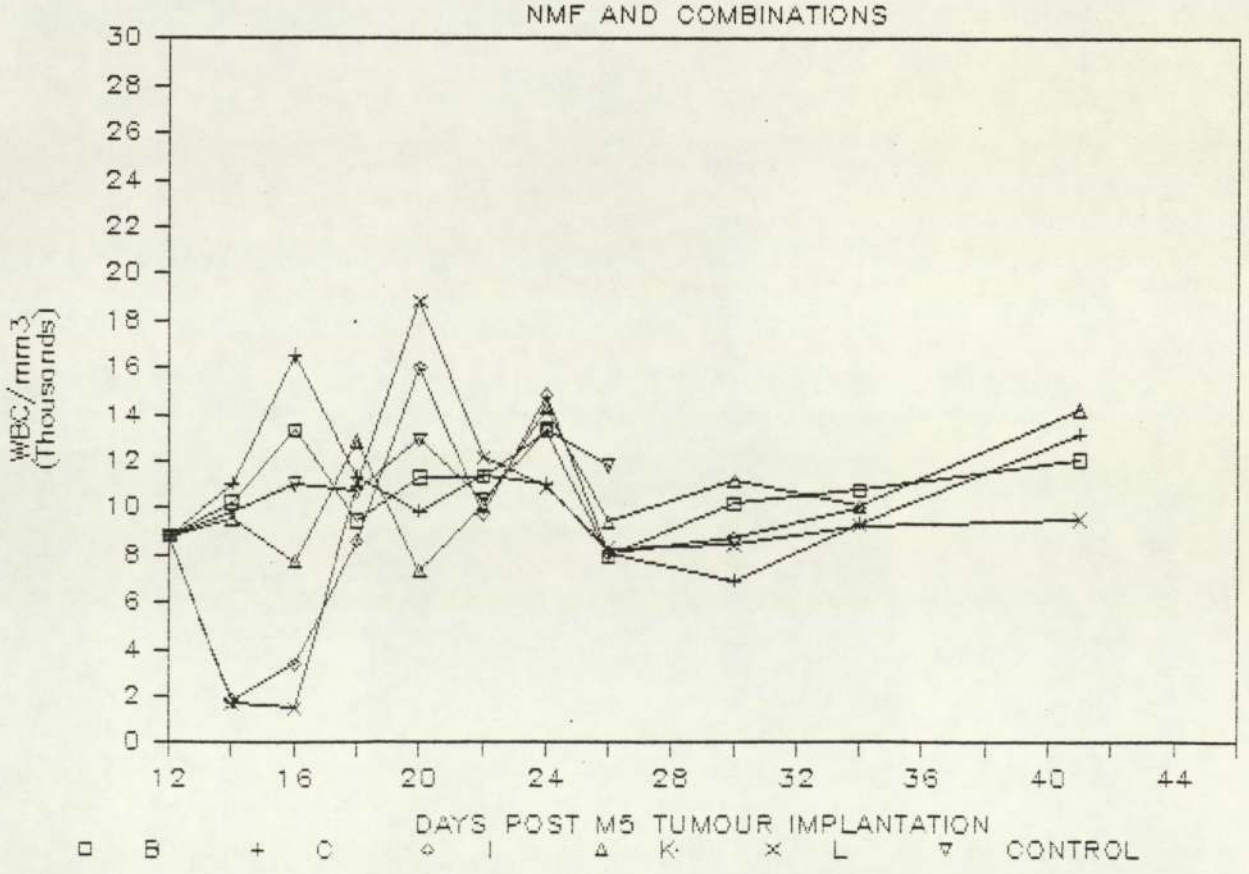
Table 17, Regimens used in the antitumour evaluation of CY, NMF, and ABPP in combination against the M5076 ovarian sarcoma in BDF_1 mice

Group	Dose mg/Kg/injection			Days of treatment		
	CY	NMF	ABPP	CY	NMF	ABPP
B		200			15-24	
C		400			15-24	
D			125			12, 16, 20, 24
E			250			12, 16, 20, 24
F	160			12		
G	320			12		
H	160			12, 15		
I	160	200		12	15-24	
J	160		125	12		12, 16, 20, 24
K		200	125		15-24	12, 16, 20, 24
L	160	200	125	12	15-24	12, 16, 20, 24
M		Control				

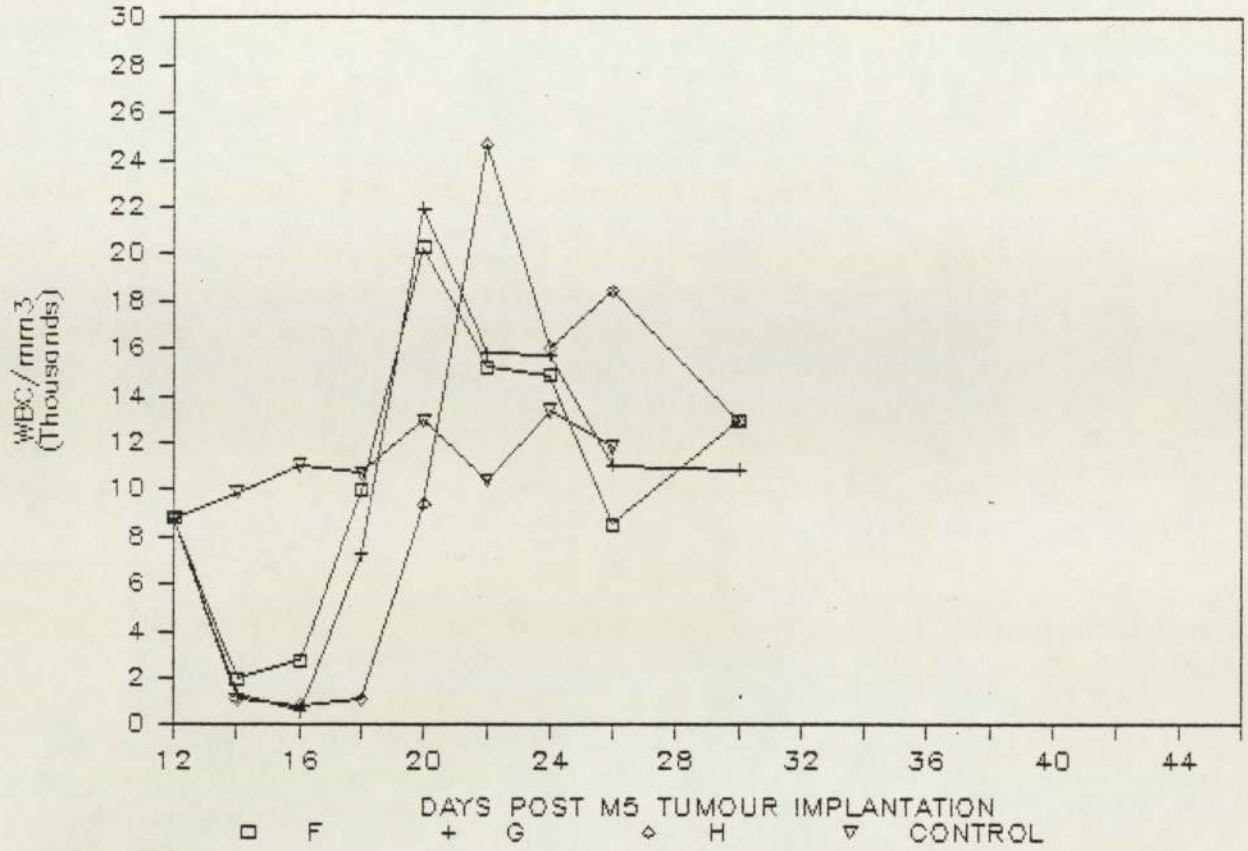
1. WBC FOR MICE TREATED WITH ABPP AND COMBINATIONS



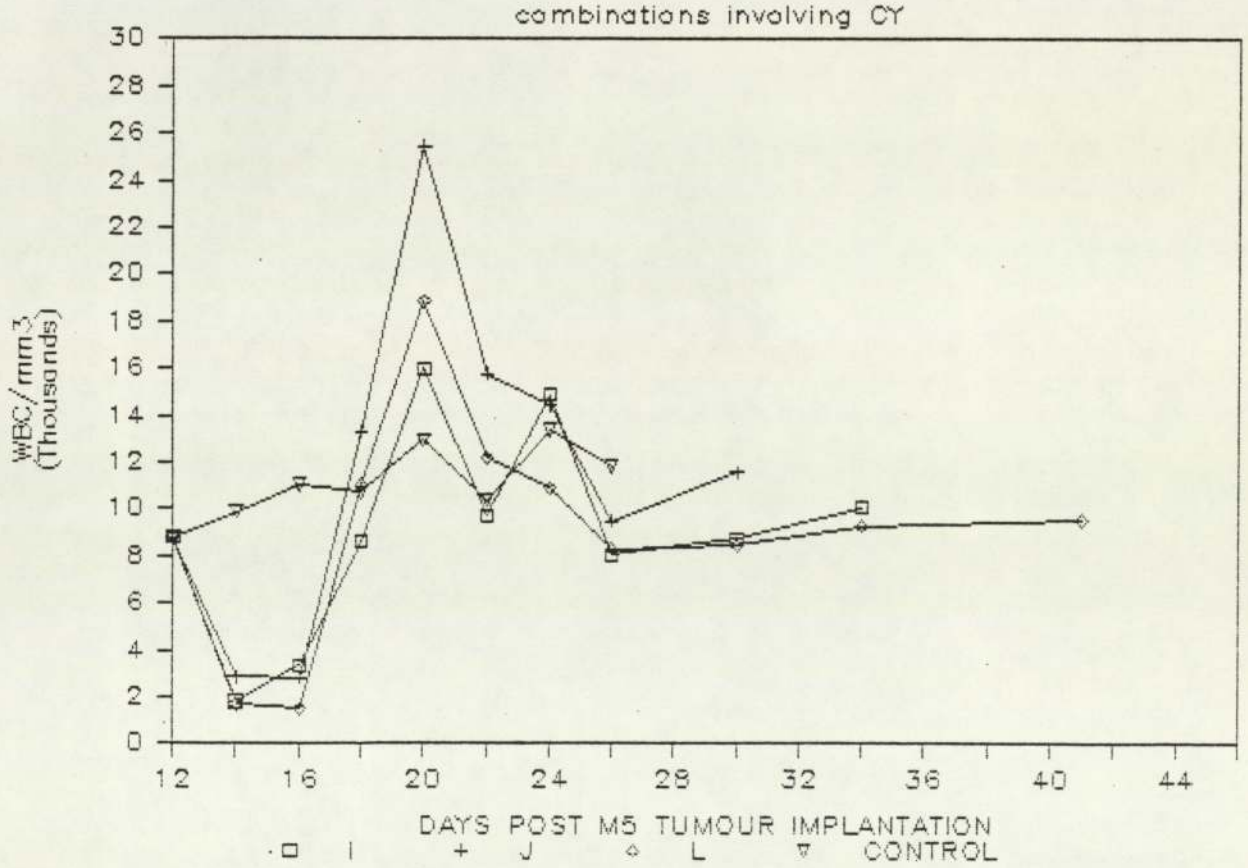
2. WBC FOR MICE TREATED WITH NMF AND COMBINATIONS



3. WBC count for mice treated with CY



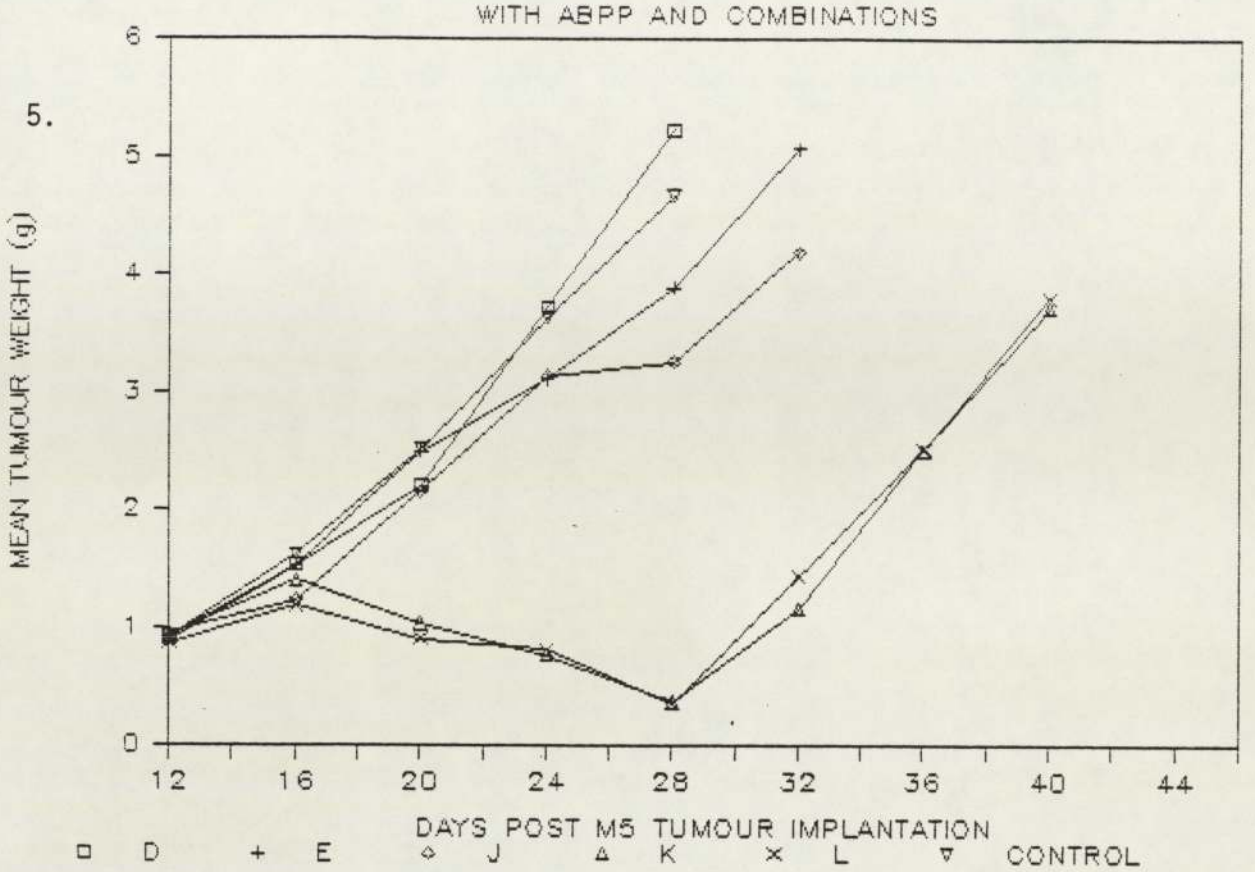
4. WBC count for mice in combinations involving CY



5.

MEAN TUMOUR WEIGHTS FOR MICE TREATED

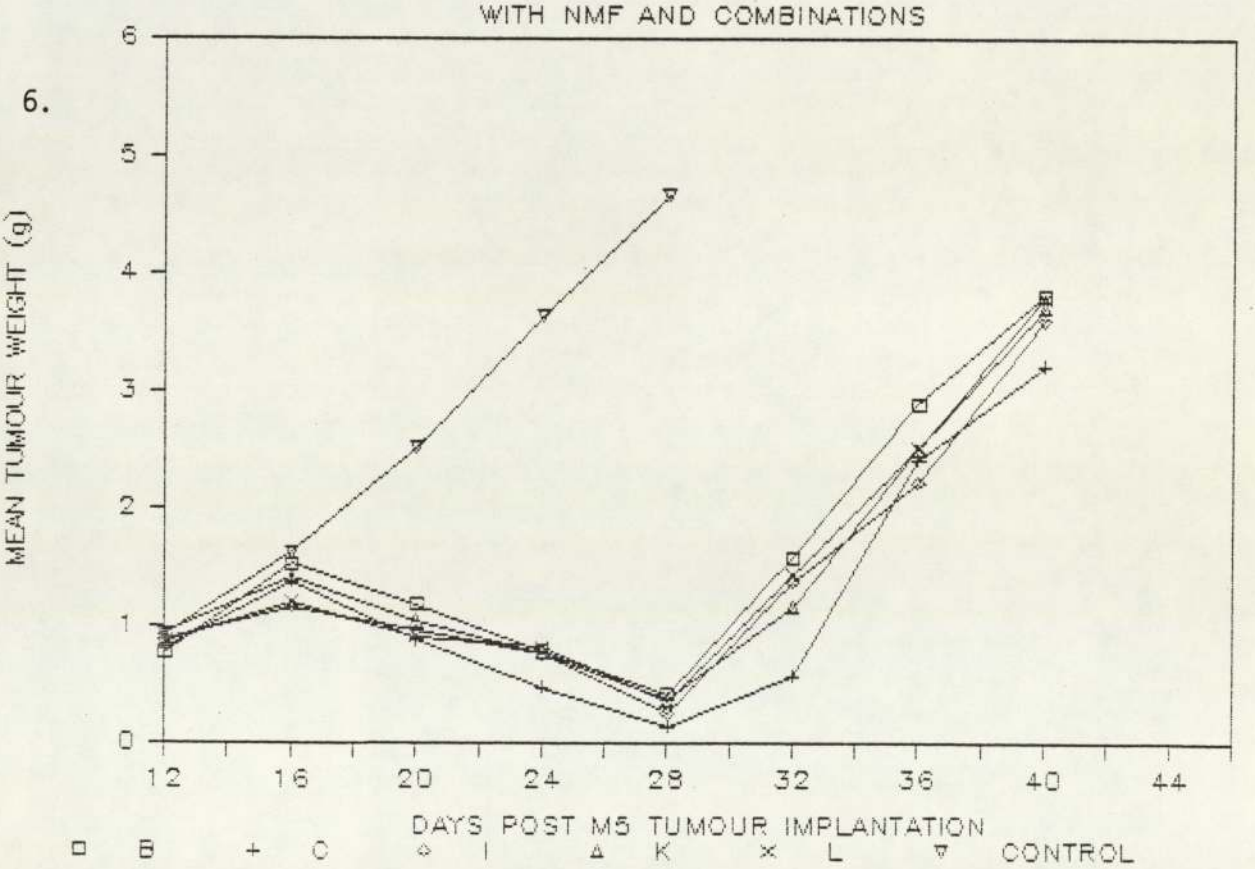
WITH ABPP AND COMBINATIONS



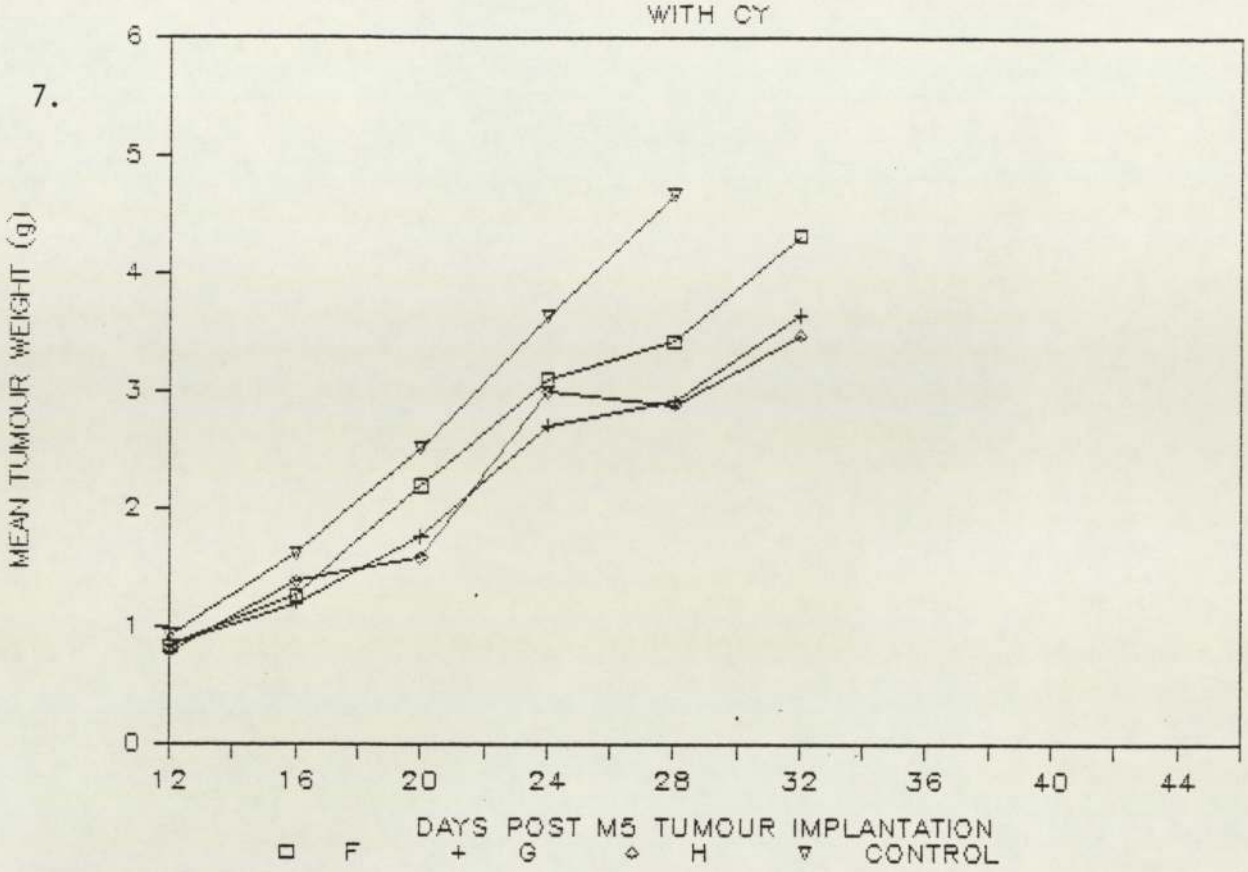
6.

MEAN TUMOUR WEIGHTS FOR MICE TREATED

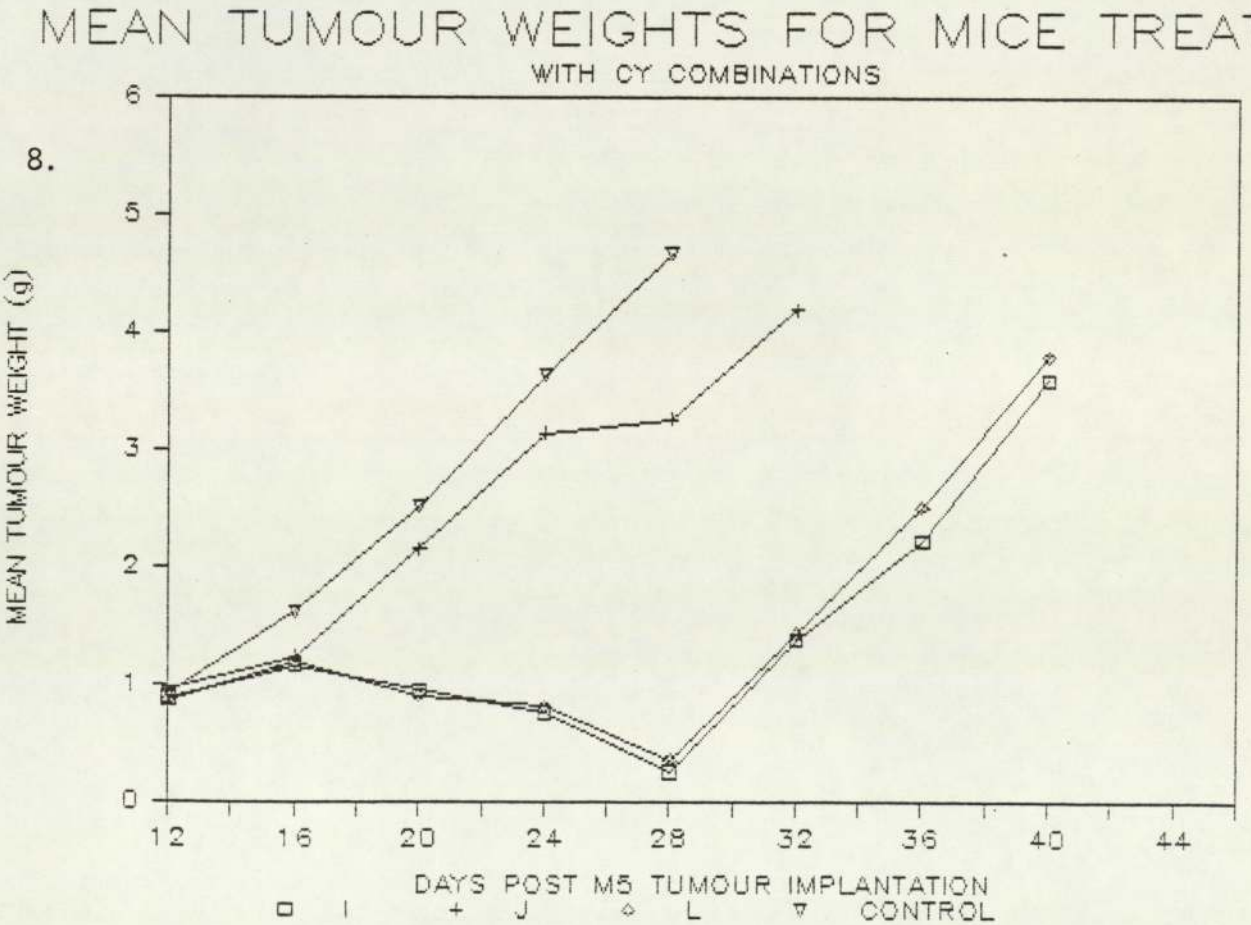
WITH NMF AND COMBINATIONS



7.


MEAN TUMOUR WEIGHTS FOR MICE TREATED
WITH CY
MEAN TUMOUR WEIGHT (g)
0
1
2
3
4
5
6
12
16
20
24
28
32
36
40
44
DAYS POST M5 TUMOUR IMPLANTATION
F
G
H
CONTROL

8.

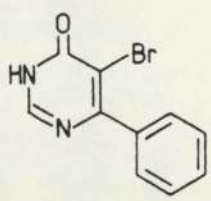

MEAN TUMOUR WEIGHTS FOR MICE TREATED
WITH CY COMBINATIONS
MEAN TUMOUR WEIGHT (g)
0
1
2
3
4
5
6
12
16
20
24
28
32
36
40
44
DAYS POST M5 TUMOUR IMPLANTATION
I
J
L
CONTROL

Against this tumour line CY or ABPP did not enhance the antitumour action of NMF. Three way analysis of variance (groups B, D, F, I, J, K, L, M) of total WBC on day 16 shows that CY produces a highly significant reduction in the total WBC. The addition of either NMF or ABPP or both into the regimens of mice treated with CY does not enhance the myelosuppression. On day 18 only the group which received CY on days 12 and 15 (i.e. group H) had a total WBC highly significantly lower than control (all groups, one way analysis of variance and Tukey's test). The reduction in the total WBC caused by CY is in agreement with that found by DeWys *et al*[188]. NMF, at two dose levels, does not affect the total WBC, lymphocyte or grannulocyte count and does not enhance the toxicity of CY to these cells which confirms the work of Langdon *et al*[37,38]. ABPP or ABPP with NMF does not cause a reduction in the total WBC and does not enhance the myelosuppression of CY either in the pressence or absence of NMF.

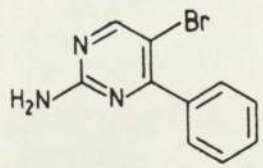
5.3. RELATED POTENTIALLY INTERESTING COMPOUNDS

The requirement of the 2-amino-4-oxo function of these isocytosines if hydrogen bonding is important for their antitumour activity could be tested by removal or exchange of the amino or oxo moiety. Thus the biological activity of reported 5-bromo-6-phenylpyrimidin-4(3H)-one (253)[189], 2-amino-5-bromo-6-phenylpyrimidine (254)[190], and the unreported 5-bromo-6-phenylcytosine (255), and 2-amino-5-bromo-6-phenylpyrimidine-4(3H)-thione (256) may be of interest.

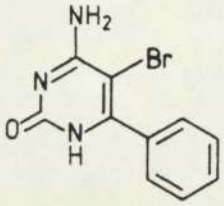
Nucleophilic substitution of the chlorine atom of 2-amino-5-bromo-6-(4-chloro-3-nitrophenyl)pyrimidin-4(3H)-one (257) by amines, scheme 52, would afford in the case of diamines molecules possessing



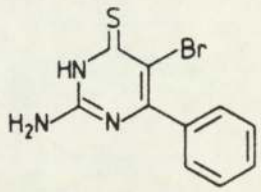
(253)



(254)

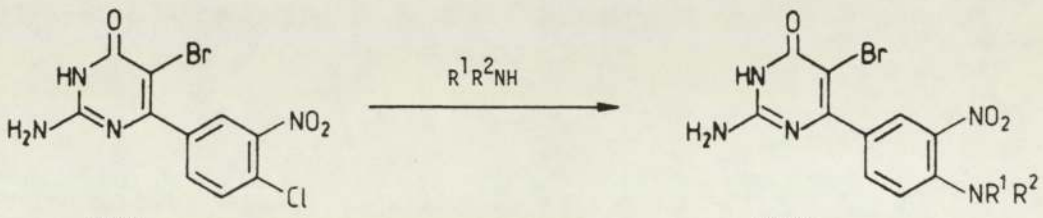


(255)



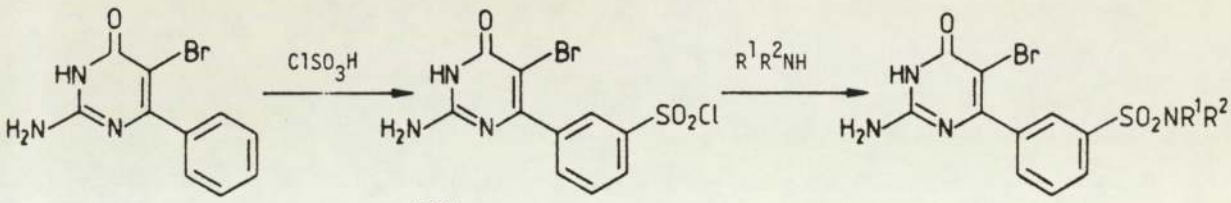
(256)

Scheme 52



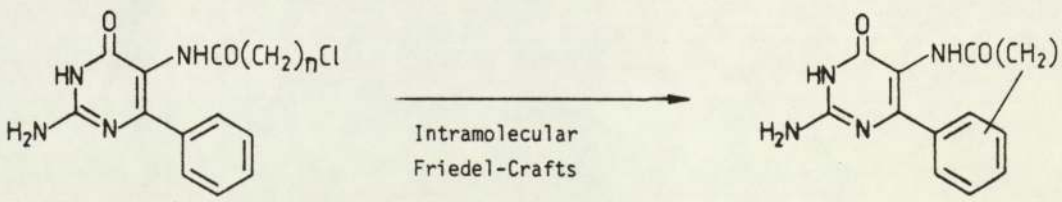
(257) (258)

Scheme 53

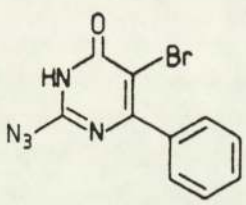


(259) (260)

Scheme 54

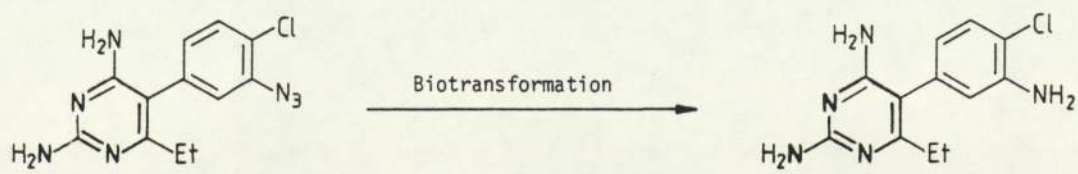


(261) (262)



(263)

Scheme 55



(264) (265)

a charged centre at physiological pH. A charged centre in this region of the molecule may have a profound effect on biological activity. Molecules with similar basic side chains could be reached via the sulphonyl chloride (259) by the reaction with diamines, scheme 53.

If the angle between the pyrimidine and phenyl rings is important then the angle between these two rings could be constrained by constructing a third ring between the phenyl ring and the 5 position of the pyrimidine ring, scheme 54.

A potential prodrug of ABPP is 2-azido-5-bromo-6-phenyl-pyrimidin-4(3H)-one (263) which if the azido group is biotransformed to an amino group would give ABPP. The biotransformation of the arylazide 'meta-azidopyrimethamine' (MZP, 264) to the amino-pyrimethamine (265) has been reported[191], scheme 55.

5.4. SUMMARY

A number of analogues of ABPP have been synthesised and evaluated for antitumour activity against the B16 melanoma and M5076 ovarian sarcoma murine tumour models. ABPP and NMF in combination with CY did not enhance the myelosuppression of CY in mice bearing the M5076 ovarian sarcoma. Some analogues of ABPP with increased water solubility showed activity against the B16 melanoma.

PART D

6. EXPERIMENTAL

6.1. DETAILS OF INSTRUMENTATION

All melting points are uncorrected.

Infra-red spectra were recorded on either a Perkin-Elmer 1310 or Pye-Unicam SP200 spectrophotometer

NMR spectra were recorded on Varian EM 360A (60 MHz, ^{1}H), Brucker WP 80 (80 MHz, ^{1}H), Jeol FX 90 (90 MHz, ^{1}H), Perkin Elmer R34 (220 MHz, ^{1}H), or Nicolet 360 NB (360 MHz, ^{1}H; 90.8 MHz, ^{13}C) spectrophotometers and were referenced to TMS. Unless otherwise stated spectra are ^{1}H at 60 MHz.

Electron impact mass spectra were recorded from a single focusing Micro Mass 12B spectrophotometer

6.2. SYNTHESIS OF FORMAMIDES AND RELATED COMPOUNDS

6.2.1. Aliphatic amides

6.2.1.1 N-Ethyl-N-methylformamide (88)

Anhydrous potassium carbonate (10g) and ethyl formate (10ml) were stirred together in absolute ethanol (15ml) at -40°C. N-Methylethylamine (0.7g), previously cooled to -40°C, was added and the mixture allowed to warm to room temperature then stirred overnight at room temperature. After the addition of ether (50ml) the mixture was filtered and concentrated under reduced pressure to give an oil which was Kugelrohr distilled, to give N-ethyl-N-methyl-formamide as a colourless liquid (0.31g, 30%); $b.p._2$ 35-40°C (lit.,[105] $b.p._{44}$ 82°C); ν_{max} (film): 2 970, 2 930, and 2 870 (CH), 1 660 cm^{-1} (CO); $\delta(CDCl_3)$, E rotamer first: 1.17 (1.7H, t, J 7Hz, $CH_2\underline{CH_3}$), 2.83 (1.7H, s, NMe), 3.3 (1.12H, q, J 7Hz, NCH_2), 8.07 (0.56H, s, CHO); Z rotamer 1.1 (1.3H, t, J 7Hz, $CH_2\underline{CH_3}$), 2.93 (1.3H, s, NMe), 3.37 (0.88H, q, J 7Hz, NCH_2), 8.0 (0.44H, s, CHO), m/z 87 ($\underline{M}^+$).

6.2.1.2. N-Cyclopropylformamide (56)

To a stirred mixture of ethyl formate (50ml), anhydrous sodium carbonate (30g), and absolute ethanol (50ml) at -5°C was added cyclopropylamine (11.4g). The mixture was allowed to warm to room temperature, then stirred overnight at room temperature. After filtration the filtrate was concentrated under reduced pressure, then distilled to give N-cyclopropylformamide as a water white product (10g, 59%);

b.p.$_3$ 86-88°C. ν_{max} (film): 3 260 (NH), 3 020 and 2 880 (CH), 1 670 and 1 535 cm^{-1} (CO); δ(220 MHz; $CDCl_3$): 0.75 (4H, m, CH_2CH_2), 2.65 (1H, m, $\underline{CH}CH_2$), 5.5-6.5 (1H, br s, NH), 8.18 (0.6H, s, CHO(Z)), 8.29 (0.4H, d, J 12Hz, CHO(E)); m/z 85 ($\underline{M}^+$)

6.2.1.3. N-(2,2,2-trifluoroethyl)formamide (60)

Sodium formate (0.68g) and recently recrystalised (isopropanol) 2,2,2-trifluoroethylamine hydrochloride (1.35g) were gentley fused together and the liquid product refluxed for 20 mins, then distilled. This product was Kugelrohr distilled to give the amide as a water white liquid (1.05g, 83%); b.p.$_3$ 70-80°C; ν_{max} (film): 3 310 (NH), 3 060, 2 970, and 2 900 (CH), 1 680 and 1 540 cm^{-1} (CO); δ[220 MHz; $(CD_3)_2SO$] Z isomer first, 3.96 (1.8H, dq, J 6.5 and 10Hz, CH_2CF_3), 8.23 (0.9H, s, CHO), 8.75 (0.9H, br, NH), E isomer where observable, 8.11 (0.1H, d, J 11.5Hz, CHO); m/z 127 ($\underline{M}^+$).

6.2.1.4. N-(2-chloroethyl)formamide (61)

Sodium metal (2.3g) was disolved in stirring absolute ethanol (100ml). The mixture was cooled to -5°C and ethylformate (25ml) and anhydrous sodium carbonate (10g) were added followed by 2-chloroethylamine hydrochloride (8.15g) over 30 mins and the mixture allowed to stir overnight at room temperature. After filtration the combined filtrate and absolute ethanol washings were concentrated under reduced pressure then distilled. One main fraction was collected (5.5g, 51%); b.p.$_2$ 98-100°C (lit.,[67] b.p.$_2$ 70-90°C); (Found: C, 30.7; H ,6.0; N, 12.0. Calc for $C_3H_6ClNO.\frac{1}{2}H_2O$: C, 30.9; H, 6.0; N, 12.0%). ν_{max} (film): 3 290 (NH), 3 040, 2 960, and 2 880 (CH), 1 670 and 1 520 cm^{-1} (CO); $\delta(CDCl_3)$ Z rotamer 3.53-3.7 (4H, m, CH_2CH_2), 7.0-

7.93 (1H, br, NH), 8.2 (0.9H, d, J 1Hz, CHO), E rotamer where observable 8.1 (0.1H, d, J 10Hz, CHO); m/z 109 (M^+), 107 (M^+).

6.2.1.5 N-Trideuteromethylformamide (55)

Sodium metal (0.66g) was dissolved in absolute ethanol (30ml) and the solution cooled to -5°C. Trideuteromethylamine hydrochloride (2g) was added, followed by ethylformate (25ml) and anhydrous sodium carbonate (10g). The mixture was stirred at -5°C for 1 h. then overnight at room temperature. After filtration the filtrate and ethylformate washings were concentrated under reduced pressure to 10ml, then Kugelrohr distilled to give N-trideuteromethylformamide as a colourless liquid (1.35g, 77%); b.p.$_3$ 65-70°C; ν_{max} (film): 3 500 to 3 200 (NH), 3 040 (CH), 2 230, 2 110, and 2 080 (CD), 1 660 and 1 520 (NH), 1 385 (CH), 1 250, 1 050, 870, and 700 cm^{-1} (CD); $\delta(CDCl_3)$: 7.3 (1H, br, NH), 8.03 (0.1H, s, CHO(E)), 8.27 (0.9H, s, CHO(Z)); m/z 62 (M^+).

6.2.1.6. Attempted alkylation of formamide and N-methylformamide.

Direct alkylation of formamide and N-methylformamide using the method of Yamawaki[69] with ethyliodide in either acetonitrile or 1,2-dimethoxyethane with potassium fluoride on alumina as catalyst produced only the starting amide (i.r. and NMR).

6.2.2. Amidals and hemiamidals

6.2.2.1. N-Hydroxymethylformamide (40)

The method of Grady and Stott[73] was used. To stirred formamide (47g) at 95°C was added over 1 hour paraformaldehyde (32.2g). 10%

sodium hydroxide solution (0.4ml) was added initially and after 0.5 h. The mixture was stirred at 95°C for a further hour then filtered hot after the addition of filter aid (0.5g) to give a clear water white viscous liquid (72g); (Found: C, 31.7; H, 6.8; N, 18.2. Calc for $C_2H_5NO_2$: C, 32.0; H, 6.8; N, 18.6%). ν_{max} (film): 3 400 to 3 100 (NH and OH), 2 900 (CH), 1 670 and 1 520 cm^{-1} (CO).

Distillation of this product resulted in decomposition.

6.2.2.2. N-Methoxymethylformamide (147)

Aqueous 40% potassium hydroxide (2ml) was added to a stirred mixtue of formamide (45g) and paraformaldehyde (33g). After 0.5hr, methanol (200ml) and concentrated sulphuric acid (3ml) were added to the clear mixture, which was stirred for a further 6hrs. After filtration to remove a white precipitate, dry ether (25ml) was added to the the filtrate, which was allowed to stand over sodium bicarbonate overnight. After filtration, the mixture was concentrated under reduced pressure and the residue dissolved in water (50ml) and extracted with dichloromethane (5x100ml). The combined extracts were washed with water, dried and evaporated under reduced pressure to give an oil (12g), which was distilled at 3 torr. Three fractions were collected: (i) 1.9g, 80-90°C, (ii) 1.7g, 90-98°C, (iii) 2.4g, 98-110°C (lit.,[119] $b.p._{0.1}$ 55-58°C). Chromatography on silica gel of combined fractions (i) and (ii) with chloroform as eluent yielded N-methoxymethylformamide as a clourless liquid (0.7g). TLC (silica gel, chloroform-methanol 19:1) single spot R_F 0.5, developed with iron(III) chloride after conversion to the hydroxamic acid. Gas chromatographic analysis of the product (1%w/v solution in acetone) injected on to a glass column (1.5mx4mm i.d) packed with 10% w/w PEGA

on chromosorb W AWDMCS (100-200 mesh) maintained at 180°C in a Pye Unicam 204 gas chromatograph with the injector and detector maintained at 200 and 250°C repectively, gave a single peak with a retension time of 4.3 mins (carrier gas , N_2 at a flow rate of 25 $ml.min^{-1}$) using flame ionization detection (H_2, 45$ml.min^{-1}$; air, 325 $ml.min^{-1}$); $\delta(CDCl_3$, 360 MHz) Z rotamer 3.36 (1.8H, s, OMe), 4.72 (1.2H, d, J 7.2Hz, NCH_2), 5.7-6.0(0.6H, sbr, NH), 8.32 (0.6H, s, CHO), E rotamer 3.32 (1.2H, s, CHO), 4.61 (0.8H, d, J 7.2Hz, NCH_2), 5.7-6.0 (0.4H, sbr, NH), 8.20 (0.4H, d, J 11.5Hz, CHO), m/z 89 (M^+).

6.2.2.3. N-Ethoxymethylformamide (57)

Aqueous 40% potassium hydroxide (2ml) was added to a stirred mixture of formamide (45g) and paraformaldehyde (33g). After 0.5 h, absolute ethanol (200ml) and concentrated sulphuric acid (3ml) were added to the clear mixture, which was stirred for a further 6h. After filtration to remove a white precipitate, dry ether (25ml) was added to the filtrate, which was allowed to stand over sodium bicarbonate overnight. After filtration the mixture was concentrated under reduced pressure, the residue dissolved in water (50ml) and extracted with dichloromethane (5x100ml). The combined extracts were washed with water, dried and evaporated under reduced pressure, then distilled to give N-ethoxymethylformamide as a colourless liquid (22g, 21%), $b.p._1$ 108-20°C (lit.,[119] $b.p._{0.01-0.1}$ 71-82°C); ν_{max} (film) 3 450br (NH), 2 975, 2 925, and 2 875 (CH), 1 670 cm^{-1} (NH); $\delta(CDCl_3)$ 1.16 (3H, t, J 7Hz, Me), 3.53 (2H, m, CH_2CH_3), 4.7 (2H, m, NCH_2), 7.13 (1H, br, NH), 8.27 (1H, m, CHO); m/z 103 (M^+).

6.2.2.4. N-Benzoyloxymethylformamide (148)

To stirred N-hydroxymethylformamide (10g) and triethylamine (20ml) in THF (100ml) at 0°C was added dropwise benzoylchloride (17ml). The mixture was stirred for a further 1hr at 0°C, then filtered and concentrated under reduced pressure. The residue was dissolved in chloroform, washed with aqueous sodium bicarbonate and water, dried, and concentrated under reduced pressure to give a brown oil. Chromatography on silica gel with chloroform as eluent gave (i) N-benzoylmethylformamide (0.24g) and NN-bis(benzoyloxymethyl)-formamide (110) (0.1g) as oils. N-benzoyloxymethylformamide m.p. 86-8°C (MeOH-water), (Found C, 60.5; H, 5.3; N, 7.95. $C_9H_9NO_2$ requires C, 60.3; H, 5.05; N, 7.8%); ν_{max} (KBr) 3 250 (NH), 3 150 (CH), 1 710 and 1 690 cm^{-1} (CO); δ (220 MHz; $CDCl_3$) Z isomer 5.58 (1.33H, d, J 8.5Hz, CH_2), 7.07 (0.67H, br, NH), 7.52 (1.33H, m, 3 and 5-H), 7.66 (0.67H, m, 4-H), 8.11 (1.33H, m, 2 and 6-H), 8.35 (0.67H, s, CHO), E isomer 5.52 (0.67H, d, J 8.5Hz, CH_2), 6.94 (0.33H, br, NH), 7.52 (0.67H, m, 3 and 5-H), 7.66 (0.33H, m, 4-H), 8.11 (0.67H, m, 2 and 6-H), 8.48 (0.33H, d, J 11Hz, CHO); m/z (chemical ionisation, isobutane) 180 (MH^+). N N-Bis (benzoyloxymethyl)formamide ν_{max} (film) 1 720 and 1 710 infl cm^{-1} (CO); $\delta(CDCl_3)$ 5.75 and 5.8 (4H, 2xs, CH_2), 7.3 (6H, m, 3, 4, and 5-H), 7.75 (4H, m, 2 and 6-H), 8.63 (1H, s, CHO).

6.2.2.5. N-Hydroxymethyl-N-methylformamide (39)

The method of Grady and Stott[73] was used. To stirred N-methyl-formamide (59g) at 95°C was added over 1 h paraformaldehyde (30.5g). 10% sodium hydroxide solution (0.4ml) was added initially and after 0.5hr. The mixture was stirred at 95°C for a further hour then

filtered hot after the addition of filter aid (0.5g) to give a clear water white viscous liquid (72.5g); ν_{max} (film) 3 400br (OH), 2 900 (CH), 1 660 cm^{-1} (CO).

This product contains about 5% NMF (NMR) and decomposed on distillation.

6.2.2.6. N-Benzoyloxymethyl-N-methylformamide (64)

N-Hydroxymethyl-N-methylformamide (9g) and benzoylchloride (11.5ml) were stirred together in 5% aqueous sodium hydroxide (120ml) at 0°C for 0.5h. The lower oily layer was seperated and washed with aqueous sodium bicarbonate, then distilled. The middle fraction gave N-benzoyloxymethyl-N-methylformamide as a colourless oil (5.2g, 27%), b.p.$_3$ 154°C (lit.,[120] b.p.$_{0.02}$ 115°C); ν_{max} (film) 2 950 (CH), 1 720 and 1 690 cm^{-1} (CO); δ [220 MHz, $(CD_3)_2SO$], E isomer 2.94 (2.55H, s, NMe), 5.72 (1.7H, s, NCH_2), 7.66 (2.55H, m, 3, 4, and 5-H), 8.08 (1.7H, m, 2 and 6-H), 8.50 (0.85, s, CHO), Z isomer 3.14 (0.45H, s, NMe), 5.64 (0.3H, s, NCH_2), 7.66 (0.45H, m, 3, 4, and 5-H), 8.08 (0.3H, m, 2 and 6-H), 8.28 (0.15H, s, CHO); m/z 193 (M^+).

6.2.2.7. N-Acetoxymethyl-N-methylformamide (63)

N-Hydroxymethyl-N-methylformamide (22.25g), acetic anhydride (37.5ml), and pyridine (25ml) were stirred together at 0°C for 0.5 h, then overnight at room temperature. Excess reagents were removed under reduced pressure and the residue distilled. The final fraction (28g, 86%) was collected, b.p.$_2$ 80°C (lit.,[74] b.p.$_{0.3}$ 66°C), but contained about 5% NMF. This impure fraction (12g) was dissolved in dichloromethane-ether (3:1, 250ml) and washed with formamide (50ml) and water (50ml), dried and the solvents removed under reduced

pressure to give N-acetoxymethyl-N-methylformamide free from NMF as an off white oil (4.7g); ν_{max} (film) 2 950 (CH), 1 740 and 1 690 cm^{-1} (CO), δ[$(CD_3)_2SO$, 220 MHz] Z rotamer 2.00 (045H, s, OAc), 2.97 (0.45H, s, NMe), 5.28 (0.3H, s, NCH_2), 8.12 (0.15H, s, CHO), E rotamer 2.00 (2.55H, s, OAc), 2.77 (2.55H, s, NMe), 5.34 (1.7H, s, NCH_2), 8.29 (0.85H, s, CHO).

6.2.2.8. N-Hydroxymethylacetamide (175)

The method of Milkowski *et al*[121] was used. Acetamide (5.9g), potassium carbonate (0.4g), and 37% formaldehyde solution (8.2ml) were swirled together on a hot water-bath for 3 min then stirred overnight at room temperature. Solid CO_2 (1g) was added and the mixture concentrated under reduced pressure at room temperature. Anhydrous sodium sulphate (7.5g) was added to the residue and after 6 h acetone (50ml) was added. The mixture was filtered, dried with further anhydrous sodium sulphate and the solvents removed under reduced pressure to give N-hydroxymethylacetamide as a colourless oil (8.4g); ν_{max} (film) 3 500 (NH and OH), 2 960 (CH), 1 660 cm^{-1} (CO); δ[$(CD_3)_2SO$] 1.85 (3H, s, Me), 4.57 (2H, s, CH_2), 5.6(2H, sbr, NH and OH).

6.2.2.9. N-(Dimethylaminomethyl)formamide (59)

Aqueous 37% formaldehyde (112G) was dripped into a stirred solution of formamide (45g) and aqueous 40% dimethylamine (70g) at 0°C then stirred overnight at room temperature. After concentration under reduced pressure the mixture was distilled to give N-(dimethylaminomethyl)formamide as a water white oil (68.3g, 67%), b.p.$_3$ 100°C (lit.,[78] b.p.$_{0.05}$ 62-5°C); ν_{max} (film) 3 300br (NH), 2 970, 2 920,

2 860, and 2 770 (CH), 1 650 cm^{-1}(CO); δ($CDCl_3$) 2.25 (6H, m, NMe), 4.0 (2H, m, CH_2), 7.1 (1H, sbr, NH), 8.2 (1H, m, CHO); m/z 102 (M^+)

6.2.2.10. N-(Formamidomethyl)trimethylammonium iodide (106)

Iodomethane (10ml) was added portionwise to stirred N-(dimethylaminomethyl)formamide (5.1g) in ether at 0°C, then stirred for a further 2 h at 0°C. Evaporation to dryness gave a white solid (11.6g). N-(Formamidomethyl)trimethylammonium iodide was crystallised from acetonitrile-ether (5.9g, 58%), m.p. 135-9°C (lit.,[122] 151-6°C EtOH); ν_{max} (KBr) 3 300 (NH), 3 050 (CH), 1 695 cm^{-1} (CO); δ[$(CD_3)_2SO$] Z isomer 3.1 (7.2H, s, NMe), 4.75 (1.6H, d, J 7Hz, CH_2), 8.45 (0.8H, s, CHO), 9.4 (0.8H, t, J 7Hz, NH), E isomer where observable 5.0 (0.4H, d, J 7Hz, CH_2), 8.57 (0.2H, m, CHO).

6.2.2.11. Attempted synthesis of N-acetyl-S-(formamidomethyl)-L-cysteine (107)

The following methods gave mixtures of products (tlc).

(a) N-(formamidomethyl)trimethylammonium iodide (1 equiv) N-acetyl-L-cysteine (1 equiv), and either sodium hydroxide (2 equiv) or pyridine (2 equiv) in hot or cold water, methanol, or acetonitrile.

(b) N-Acetyl-L-cysteine (1 equiv) and either N-(formamidomethyl)trimethylammonium iodide (1 equiv) or N-hydroxymethylformamide (1 equiv) in acetonitrile at room temperature.

(c) Formamide (1 equiv), formaldehyde (1 equiv), and N-acetyl-L-cysteine (1 equiv) in water.

6.2.2.12. 1,3,5,-Triformylhexahydro-1,3,5-triazine (65)

Acetic anhydride (21.4g) was added over 20 mins to stirred 98% formic acid (9.7g) at -5°C, then heated at 50°C for 15 mins and recooled to -5°C. Hexamethylenetetramine (6.7g) was added so that the temperature was maintained at 10°C (about 1.5 h). After allowing to warm to room temperature over 2 h, water (100ml) was added and the mixture neutralized with solid potassium carbonate. After evaporation to dryness under reduced pressure the residue was extracted with chloroform which was dried, then evaporated under reduced pressure to give a hygroscopic solid (2.4g). The hexahydro-triazine was crystallised from absolute ethanol, m.p. 170.5-171.5 (lit.,[123] 171-2°C); (Found: C, 42.4; H, 5.5. Calc for $C_6H_9N_3O_3$: C, 42.1; H, 5.3%); ν_{max} (KBr) 3 030, 2 940, and 2 890 (CH), 1 710 and 1 675 cm^{-1}; δ ($CDCl_3$) 5.2 (6H, s, CH_2), 8.25 (3H, s, CHO), δ [$(CD_3)_2SO$] 5.2 (6H, s, CH_2), 8.23 (3H, m, CHO), δ (CF_3CO_2H) 5.5 (6H, m, CH_2), 8.46 (3H, m, CHO); m/z 171 (M^+)

6.2.2.13. N-Acetyl-L-cystine methyl ester (176)

N-Acetyl-L-cysteine and concentrated sulphuric acid (1.5ml) were refluxed together in methanol for 4 h. After standing overnight at room temperature the mixture was concentrated under reduced pressure. The residue was dissolved in ethylacetate, washed with aqueous sodium bicarbonate, water, and dried to give a white solid. The cystine ester was crystallised from chloroform-hexane as white crystals (0.42g), m.p. 127-8°C (lit.,[124] 129-30°C), ν_{max} (KBr) 3 320 (NH), 3 050, 2 990, 2 890, and 2 830 (CH), 1 750, 1 735, 1 650, and 1 520 cm^{-1} (CO); δ($CDCl_3$) 2.07 (6H, s, COMe), 3.2 (4H, d, J 6Hz, CH_2), 3.8 (6H, s, OMe), 4.9 (2H, dt, J 8 and 6Hz, CH), 6.9 (2H, d, J 8Hz, NH); m/z 352.

6.2.3. 'Push-Pull' Olefins

6.2.3.1. 1,5-Diaza-1,5-diphenyl-(1H)-pentadienium perchlorate (137)

To stirred malonaldehyde bis (dimethylacetal) (16.4g) and aniline (18.6g) in ethanol (5ml) at 0°C was added over 20 mins 70% perchloric acid (17ml). The precipitated perchlorate was collected, washed with cold methanol then crystallised from ethanol (26.7g), m.p. 222-4 (lit.,[96] 220-1°C); ν_{max} (nujol) 3 250 (NH), 1 640 cm^{-1} (C=N); δ [$(CD_3)_2SO$] 6.2 (1H, t, J 12Hz, CHCHCH), 7.45 (10H, m, aromatic), 8.76 (2H, t, J 12Hz, NCH), 13.1 (2H, d, J 14Hz, NH).

6.2.3.2. NN-Dimethyl-2-nitroethenamine (71)

Nitromethane (6.1g) and dimethylformamide dimethylacetal (12.9g) were heated together at 70°C for 1 h. Concentration under reduced pressure gave a brown product from which NN-dimethyl-2-nitroethenamine was crystallised (toluene) as orange crystals (8.5g, 75%), m.p.104-5°C (lit.,[81] 104°C); ν_{max} (KBr) 3 100, 3 030, 2 920, and 2 820 (CH), 1 630 cm^{-1} (C=N); m/z 116 (M^+).

6.2.3.3. N-methyl-2-nitro-N-phenylethenamine (121)

Nitromethane (30.5g), N-methylaniline (10.7g), triethylorthoformate (29.6g), and toluene sulphonic acid monohydrate (0.5g) were refluxed together for 7 h. Concentration under reduced pressure gave a brown liquid which was passed down a neutral alumina column (200g) with dichloromethane as eluent. Evaporation of the eluent under reduced pressure and crystallisation from toluene-cyclohexane gave the nitro-olefin as yellow plates (10.3g), m.p. 93-4°C (lit.,[83] 94°C), ν_{max} (KBr) 3 120 and 3 050 (CH), 1 630 cm^{-1} (C=C); δ ($CDCl_3$)

3.35 (3H, s, Me), 6.9 (1H, d, J 11 Hz, 2-H), 7.3 (5H, m, Ar), 8.55 (1H, d, J 11Hz, 1-H); m/z 178 (M^+)

6.2.3.4. NN-Diethyl-2-nitroethenamine (177)

Diethylamine (15ml) was added to a stirred suspension of N-methyl-2-nitro-N-phenylethenamine (1.78g) in ether (20ml) and the mixture stirred for 48 h at room temperature. The solvent and excess reagents were removed under reduced pressure to give an orange oil. Chromatography on silica gel with ethylacetate as eluent afforded NN-diethyl-2-nitroethenamine as an orange liquid (0.63g, 44%), (lit.,[83] m.p. 36°C); ν_{max} (flim) 3 150, 2 960, 2 920, and 2 870 (CH), 1 620 cm^{-1} (C=C); δ ($CDCl_3$) 1.27 (6H, tbr, Me), 3.33 (4H, qbr, CH_2), 6.7 (1H, d, J 11Hz, 2-H), 8.2 (1H, d, J 11Hz, 1-H); m/z 144 (M^+)

6.2.3.5. N-Methyl-2-nitroethenamine (70)

Methylamine gas was bubbled through a stirred suspension of N-methyl-2-nitro-N-phenylethenamine (5.3g) in ether (30ml) at 0°C and the mixture stirred overnight at room temperature. After evaporation of the solvent under reduced pressure the residue was tritruated and washed with dry ether (2x30ml). The nitro-olefin was cystallized from ethylacetate as yellow crystals (2.6g, 86%), m.p. 121-3°C (lit.,[84] 114-6°C), (Found: C, 35.0; H, 5.8; N, 27.3. Calc for $C_3H_6N_2O_2$ C, 35.3; H, 5.9; N, 27.4%); ν_{max} (KBr) 3 250 (NH), 3 100 and 3 040 (CH), 1 615 cm^{-1} (C=N), ν_{max} (Nujol) 3 250 (NH), 1 615 cm^{-1} (C=N), ν_{max} (0.5%, 1mm, $CHCl_3$) 3 300 cm^{-1} (NH), ν_{max} (0.1%, 5mm, $CHCl_3$) 3 300 cm^{-1} (NH), m/z 102 (M^+).

6.2.3.6. N-Deutero-N-methyl-2-nitroethenamine (122)

N-Methyl-2-nitroethenamine (0.15g) was dissolved in warm deuterium oxide (99%+; Aldrich 0.75ml) followed by evaporation of the solvent under reduced pressure. the process was rpeated and the residue was recrystallised from deuterium oxide to give the N-deutero compound as brown needles (0.1g, 65%), m.p. 119-20°C, ν_{max} (KBr) 3 100 and 3 050 (CH), 2 370 (ND), 1 620 cm^{-1} (C=N), ν_{max} (Nujol) 2 370 (ND), 1 610 cm^{-1} (C=N).

6.2.3.7. 2-Nitroethenamine (69)

Ammonia gas was bubbled through a solution of N-methyl-2-nitro-N-phenylethenamine (2.4g) in chloroform at 0°C and allowed to stand in the fridge for 48 h. After evaporation of the solvent under reduced pressure the residue was tritruated and washed with dry ether (2x10ml) Crystallisation from ethylacetate-chloroform afforded 2-nitroethenamine as yellow needles (0.75g, 63%), m.p. 102-4°C (lit.,[84] 101°C), ν_{max} (KBr) 3 370 (NH), 3 150 (CH), and 1 625 cm^{-1} (C=N); m/z 88 (M^+)

6.2.3.8. N-(2-nitroethenyl)morpholine (72)

Triethylorthoformate (29.6g), morpholine (8.7g), nitromethane (30.5g), and 4-toluenesulphonic acid monohydrate (0.5g) were refluxed together for 1 h. After cooling and removal of excess reagents under reduced pressure the residue was passed down a silica gel column (70g) with dichloromethane as eluent. Removal of the eluent and crystallisation from absolute ethanol afforded N-(2-nitroethenyl)-morpholine as yellow needles (10.4g, 73%), m.p. 142.5°C (lit.,[83] 140-1°C); ν_{max} (KBr) 3 125, 3 000, 2 975, 2 950,and 2 875 (CH), 1 625

cm^{-1} (C=C); ($CDCl_3$) 3.3 (4H, m, NCH_2), 3.77 (4H, m, OCH_2), 6.65 (1H, d, J 11Hz, 2-H), 8.3 (1H, d, J 11Hz, 1-H), δ[$(CD_3)_2SO$] 3.63 (8H, m, CH_2), 7.0 (1H, d, J 11Hz, 2-H), 8.3 (1H, d, J 11Hz, 1-H).

6.2.3.9. 2-Methyl-3-methylaminopropenal (66)

33% Methylamine in ethanol (12.5ml) was dripped into stirred 3-ethoxy-2-methylpropenal (11.4g) at 0°C then the mixture stirred at room temperature overnight. Evaporation of the solvent under reduced pressure gave a yellow oil which was distilled. The major fraction ($b.p._2$ 114-8°C, 8.1g) was redistilled to give 2-methyl-3-methylaminopropenal as a yellow oil ($b.p._1$ 111-4°C) which solidified on standing (5.7g, 57%), m.p. 55°C, (Found: C, 60.7; H, 9.5; N, 14.2. C_5H_9NO requires C, 60.6; H, 9.15; N, 14.1%); ν_{max} (film) 3 260br (NH), 2 930, 2 790, and 2 720 (CH), 1 660 and 1 580 cm^{-1} (CO); δ(80 MHz, $CDCl_3$) 1.65 (3H, s, CMe), 3.07 (3H, d, J 3Hz, NMe), 6.54 (1H, br, NH), 6.86 (1H, d, J 14Hz, NCH), 8.84 (1H, s, CHO), m/z 99 (M^+).

6.2.3.10. 2-Cyano-3-dimethylaminopropenenitrile (68)

Dimethylformamide dimethylacetal (4.76g) and malononitrile (2.64g) were stirred together at room temperature for 5 h. then concentrated under reduced pressure to give a yellow solid. Crystallisation from chloroform-toluene gave an impure product. Chromatography on silca gel with ethylacetate as eluent afforded the aminonitrile as a white solid (1g, 21%), m.p. 80.5-2°C (lit.,[81] 82-3°C); ν_{max} (KBr) 3 025, 2 980, and 2 940 (CH), 2 220 and 2 210 inf (CN), 1 650 cm^{-1} (C=C); δ($CDCl_3$) 3.23 and 3.37 (6H, 2xs, NMe), 7.1 (1H, s, CH); m/z 121 (M^+)

6.2.3.11. 2-Cyano-3-methylaminopropenenitrile (173)

Methylamine gas was bubbled through a suspension of 2-cyano-3-ethoxypropenenitrile (1.22g) in ether (50ml) at 0°C and the mixture stirred at room temperature overnight. Evaporation of the solvent under reduced pressure and crystallisation from absolute ethanol afforded the amine as orange microcrystals (0.77g, 72%), m.p. 191-4°C (lit.,[117] 191-2°C), ν_{max} (KBr) 3 250 (NH), 3 020 and 2 960 (CH), 2 220 inf and 2 200 (CN), 1 640 cm^{-1} (C=C); $\delta[(CD_3)_2SO]$ E rotamer 2.97 (2.5H, d, J 4Hz, NMe), 7.83 (0.83H, d, J 15Hz, CH), 8.8 (0.83H, br, NH), Z rotamer where observable 3.07 (0.5H, NMe), 7.53 (0.17H, d, 9Hz, CH); m/z 107 (M^+).

6.2.3.12. 2-Nitroacetaldehyde oxime (114)

Nitromethane (40g) was dripped into stirred sodium hydroxide (40g) in water (80ml) at 40-5°C so that the temperature was maintained (about 2 h.). After cooling to 0°C concentrated hydrochloric acid (85ml) was added dropwise maintaining the temperature at 0-5°C (about 1 h.). The white precipitate formed was filtered then dried between filter paper to give a now pale orange solid which was dissolved in ether (1200ml) and dried overnight over calcium chloride. After filtering through diatomaceous earth the solvent was evaporated under reduced pressure to give 2-nitroacetaldehyde oxime[82] as yellow crystals (6.0g, 18%); $\delta(CDCl_3)$ 5.37 (2H, d, J 5Hz, CH_2), 7.15 (1H, t, J 5Hz, CH), 11.5 (1H, m, OH).

6.2.3.13 Nitroacetonitrile (115)

Freshly distilled thionyl chloride (4.3ml) was dripped into a refluxing solution of 2-nitroacetaldehyde oxime (6g) in dry ether

(40ml) over 5 min and the mixture refluxed for a further 1 h. Filtration and evaporation of excess reagent and solvent gave a yellow oil which was dissolved in ether, washed with water and dried with calcium chloride to give nitroacetonitrile as a brown oil (2.9g, 58%), ν_{max} (film) 3 100 and 3 050 (CH), 1 560 and 1 350 $cm^{-1}(NO_2)$; δ [$CDCl_3$-$(CD_3)_2SO$ 3:1] 5.85 (s, CH_2); m/z 120 (M^+).

6.2.3.14. Attempted synthesis of 3-dimethylamino-2-nitropropene-nitrile (116)

The reaction of dimethylformamide dimethyl acetal with nitroacetonitrile at 0°C gave a mixture of several products (tlc).

6.3. 2-AMINOPYRIMIDIN-4(3H)-ONES (ISOCYTOSINES) AND RELATED COMPOUNDS

6.3.1. β-Ketoesters and related compounds

6.3.1.1. Ethyl hydrogen malonate (199)

The method of Breslow et al[192] was used. Potassium hydroxide (14g) in absolute ethanol (160ml) was dripped into diethyl malonate (40g) in absolute ethanol (160ml) over 1 h and the mixture stirred overnight. The mixture was boiled and filtered hot to remove any di-potassium salt. On cooling the filtrate deposited a crystalline product which was collected and washed with ether which, together with subsequent crops gave ethyl potassium malonate as white crystals (34.6g, 81%), m.p. 197°C

Concentrated hydrochloric acid (10ml) was added dropwise over 5 min to stirred ethyl potassium malonate (15g) in water (15ml) at 0°C. The mixture was extracted with ether (3x50ml), dried, concentrated under reduced pressure then dried at 50°C under reduced pressure (4 torr) for 4 h to give ethyl hydrogen malonate as a colourless oil (9.8g, 84%), ν_{max} (film) 2 950 (CH) and 1 720 cm^{-1} br (CO); δ ($CDCl_3$) 1.3 (3H, t, J 7Hz, Me), 3.33 (2H, s, CH_2CO), 4.25 (2H, q, J 7 Hz, CH_2O), 11.5 (1H, s, CO_2H).

6.3.1.2. Ethyl 3-(4-chlorophenyl)-3-oxopropanoate (198)

The method of Wierenga and Skulnick was used[167]. Butyl lithium in hexane (1.55M, 96ml) was added in portions to stirred ethyl hydrogen malonate (9.8g) in dry THF (distilled from CaH_2), with 2,2'-bipyridyl as indicator, at -70°C under N_2. The temperature was allowed to rise to -5°C which produced a pink heterogeneous mixture . After recooling the mixture again to -70°C, 4-chlorobenzoyl chloride (7.64g) was added and the mixture stirred for a further hour. After being allowed to warm to room temperature the mixture was poured into ether (150ml) and washed with 1M hydrochloric acid (200ml), saturated aqueous sodium bicarbonate (2x50ml), water (50ml), then dried, and concentrated under reduced pressure to give ethyl 3-(4-chlorphenyl)-3-oxopropanoate mixed with high boiling hydrocarbons as an orange oil (12.6g); ν_{max} (film) 1 740 and 1 690 cm^{-1} (CO); δ ($CDCl_3$) excluding methyl and hydrocarbon signals, 3.96 (1.8H, s, CH_2), 4.25 (2H, m, OCH_2), 5.63 (0.1H, s, =CH), 7.5 (2H, d, J 9 Hz, 3 and 5-H), 7.93 (2H, d, J 9 Hz, 2 and 6-H), 12.7 (0.1H, brs, OH); m/z 228 (M^+), 226 (M^+).

6.3.1.3. Ethyl 3-(3-chlorophenyl)-3-oxopropanoate (197)

Ethyl 3-(3-chlorophenyl)-3-oxopropanoate was prepared by the same method as ethyl 3-(4-chlorophenyl)-3-oxopropanote (6.3.1.2) from ethyl hydrogen malonate (2.24g), butyl lithium in hexane (1.55M, 23ml), and 3-chlorobenzoyl chloride (1.75g). Ethyl 3-(3-chlorophenyl)-3-oxopropanoate was produced as an orange oil (containing high boiling) hydrocarbons (2.78g); ν_{max} (film) 1 740 and 1 690 cm^{-1} (CO); $\delta(CDCl_3)$ excluding methyl and hydrocarbon signals, 3.97 (1.1H, s, CH_2), 4.25 (2H, m, OCH_2) 5.67 (0.45H, s, =CH), 7.2-8.1 (4H, m, Ar-H); m/z 228 (M^+), 226 (M^+).

6.3.1.4. Ethyl 2-hydroxyimino-3-oxo-3-phenylpropanoate (222)

Sodium nitrite (3.8g) in water (5ml) was added over 10 mins to a stirred solution of ethyl 3-oxo-3-phenylpropanoate (9.6g) in glacial acetic acid (10ml). After stirring for a further 30 mins the precipitate was collected, washed with water then dried at 60°C (8.62g) and was not purified further. ν_{max} (KBr) 3 350 (OH), 1 720 and 1 670 cm^{-1} (CO); $\delta[(CD_3)_2SO]$ 1.2 (3H, t, J 7Hz, Me), 4.3 (2H, q, J 7Hz, CH_2), 7.4-8.1 (5H, m, Ar-H), 11.2-14.0 (1H, sbr, OH).

3.3.1.5. Ethyl 2-nitro-3-oxobutanoate (223)

The method of Sifniades was used[193]. Nitric acid (70%, d 1.42, 0.63ml) was added slowly to a stirred mixture of acetic anhydride (5.7ml) and concentrated sulphuric acid (2 drops) at 10°C. Ethyl 3-oxobutanoate (1.3g) was dripped into the mixture at -10°C over 5 mins and the mixture stirred for a further 1 h at -10°C then allowed to warm to room temperature. Dry sodium carbonate was added until the colour changed from yellow to orange-yellow. The mixture was

concentrated under reduced pressure at 40°C to give ethyl 2-nitro-3-oxobutanoate as a yellow oil (0.91g) contaminated with acetic anhydride (1:8 molar ratio). $\delta(CDCl_3)$ 1.33 (3H, t, 7Hz, $CH_2\underline{CH_3}$), 2.3 (0.5H, s, OH), 2.27 (0.125 molar ratio Ac_2O), 2.43 (3H, s, COMe), 4.4 (2H, q, J 7Hz, CH_2), 5.85 (0.5H, s, $CHNO_2$).

6.3.2. 6-Methylisocytosines

6.3.2.1. 2-Amino-6-methylpyrimidin-4(3H)-one (189)

Ethyl 3-oxopropanoate (72g), guanidinium carbonate (52g), and ethanol (200ml) were boiled under reflux for 1 h, then cooled and poured into iced water acidified with acetic acid. The white product was collected by filtration, washed with water and oven dried at 80°C overnight to give 2-amino-6-methylpyrimidin-4(3H)-one (26.8g, 56%), m.p. >300°C (lit[170]., 290-2°C dec); ν_{max} (KBr) 3 450 (NH), 1 660 cm^{-1} (CO), $\delta(CF_3CO_2H)$ 2.4 (3H, s, Me), 6.07 (1H, s, 5-H), 7.9 (2H, sbr, NH); m/z 126, 125 ($\underline{M}^+$).

6.3.2.2. 2-Amino-5-bromo-6-methylpyrimidin-4(3H)-one (180)

Bromine (1.76g), in glacial acetic acid (5ml), was added dropwise over 15 mins to stirred 2-amino-6-methylpyrimidin-4(3H)-one (1.25g) in glacial acetic acid (10ml) at 70°C. On cooling, the solution deposited the bromopyrimidinone which was washed with ethanol then recrystallised from absolute ethanol (1.06g, 52%), m.p. 252-3°C (lit[170] 250°C dec), ν_{max} 3 380 (CH), 1 690 and 1 635 cm^{-1} (CO); $\delta[(CD_3)_2SO]$ 2.33 (3H, s, Me), 8.5 (3H, mbr, NH); m/z 205 ($\underline{M}^+$), 203 ($\underline{M}^+$).

6.3.2.3. 2-Amino-6-methyl-5-nitropyrimidin-4(3H)-one (200)

2-Amino-6-methylpyrimidin-4(3H)-one (25g) was added in portions over 3 h to a stirred mixture of nitric acid (70%, d 1.42, 14ml) and concentrated sulphuric acid (50ml) at -5°C. After stirring overnight at room temperature, the mixture was poured onto ice and neutralised with aqueous ammonia. After acidification with glacial acetic acid the crude product was collected by filtration and washed with water. This crude product was dissolved in hot 1.25M aqueous sodium hydroxide then cooled. The sodium salt produced was dissolved in hot water and the nitropyrimidinone was reprecipitated with glacial acetic acid. Washing with water and drying at 80°C gave 2-amino-6-methyl-5-nitropyrimidin-4(3H)-one (9.94g), m.p. 309°C dec; ν_{max} (KBr) 3 550 (NH), 1 700 and 1 660 (CO), 1 550 and 1 320 cm^{-1} (NO_2) (lit.,[168] 1 550 and 1 325 cm^{-1} (NO_2)); $\delta[(CD_3)_2SO]$ 2.3 (3H, s, Me), 3.8 (1H, sbr, NH), 7.5 (2H, sbr, NH); m/z 170 (M^+).

6.3.2.4. 2,5-Diamino-6-methylpyrimidin-4(3H)-one (201)

2-Amino-6-methyl-5-nitropyrimidin-4(3H)-one (1.25g) was treated with hydrogen in the presence of 10% pallidised charcoal (270mg) at 2 atm for 4 h. After filtration the filtrate was evaporated under reduced pressure, washed with methanol, dissolved in water, and neutralised with sodium bicarbonate to precipitate the diaminopyrimidine. Recrystallisation from water gave 2,5-diamino-6-methylpyrimidin-4(3H)-one (1.35g, 96%), m.p. 285-7°C (lit[168] 270-5°C); ν_{max} (KBr) 3 300 (NH), 1 670 and 1 640 cm^{-1} (CO); $\delta(CF_3CO_2H)$ 2.5 (s, Me); m/z 140 (M^+).

Reduction of the 5-nitropyrimidine with hydrazine hydrate and Raney nickel gave a product identical to that produced by hydrogenation.

6.3.2.5. 2-Amino-5-azido-6-methylpyrimidin-4(3H)-one (230)

Sodium nitrite was added in portions over 10 mins to a stirred solution of 2,5-diamino-6-methylpyrimidin-4(3H)-one in 2M aqueous hydrochloric acid (25ml) at -5°C. Sodium azide (1.0g) was added in portions over 30 mins then allowed to stir for a further 2 h. The suspension was neutralised with sodium acetate then filtered and washed with water. The azide was dried under reduced pressure at room temperature to give a cream solid (0.6g), m.p. 108°C expl; ν_{max} (Kbr) 3 420 (NH), 2 120 (N_3), 1 685 and 1 650 cm^{-1} (CO).

Attemped crystallisation (EtOH) of this product resulted in decomposition of the azide with effervescence. Decomposition also occured on storage.

The azide was decomposed by 30% HBr in glacial acetic acid at 10°C, mainly to the corresponding 5-amino compound (tlc, silica, ammoniacal chloroform-methanol 4:1).

6.3.3. 6-Phenylisocytosines and related compounds

6.3.3.1 2-Amino-6-phenylpyrimidin-4(3H)-one (190)

Ethyl 3-oxo-3-phenylpropanoate (134.4g), guanidinium carbonate (63g), and ethanol (250ml) were boiled under reflux with stirring for 7 days. After cooling the mixture was poured on to aqueous acetic acid to give a white precipitate which was washed with ethanol and water then dried at 80°C (82.5g, 63%). 2-Amino-6-phenylpyrimidin-4(3H)-one was recrystallised from glacial acetic acid, m.p. 306-8°C (lit.,[194] 304°C), ν_{max} (KBr) 3 400 (NH) and 1 660 cm^{-1} (CO);

$\delta(CF_3CO_2H)$ 6.5 (1H, s, 5-H), 7.7 (5H, s, 6-Ph), 8.1 (2H, sbr, NH); m/z 187 (M^+).

6.3.3.2. 3-(2-Amino-4(3H)-oxopyrimidin-6-yl)benzenesulphonic acid (202)

2-Amino-6-phenylpyrimidin-4(3H)-one (3.74g) was added over 15 mins to stirred fuming sulphuric acid (10ml) at room temperature, then stirred for 1.5 h at 110°C. The solution produced was cooled and poured onto ice. The precipitate was washed with water and dried at 80°C to give 3-(2-amino-4(3H)-oxopyrimidin-6-yl)benzenesulphonic acid (3.98g, 75%), m.p. >330°C, ν_{max} 1 690 (CO) and 1 175 cm^{-1} (SO). The sulphonic acid (1.3g) was suspended in 5M aqueous sodium hydroxide (1ml) and the sodium salt was recrystallised from water-ethanol, m.p. >330°C; δ(220 MHz, D_2O-NaOD) 6.29 (1H, s, 5-H), 7.72 (1H, t, J 9Hz, 5'-H), 7.97 (1H, d, J 9Hz, 6'-H), 8.05 (1H, d, J 9Hz, 4'H), 8.25 (1H, s, 2'-H).

6.3.3.3. 3-(2-amino-5-nitro-4(3H)-oxopyrimidin-6-yl)benzenesulphonic acid (203)

3-(2-Amino-4(3H)-oxopyrimidin-5-yl)benzenesulphonic acid (2.67g) was added to a stirred mixture of nitric acid (70%, d 1.42, 0.75ml) and concentrated sulphuric acid (5ml) at room temperature, then stirred at 55°C for 2.5 h. The mixture was cooled, poured onto ice and the yellow precipitate was washed with with water and dried at 80°C to give the nitrosulphonic acid (2.61g, 84%). The sulphonic acid (1.5g) was suspended in 5M aqueous sodium hydroxide (1ml) and the sodium sulphonate was crystallised from water-ethenol as yellow crystals, m.p. >330 °C, ν_{max} (KBr) 1 680 (CO), 1 550 and 1 340 (NO_2),

1 170 cm^{-1} (SO); δ (C_5H_5N-D_2O) 7.45 (1H, m, 5'-H), 7.8 (1H, m, 6'-H), 8.65 (1H, m, 4'-H), 8.8 (1H, m, 2'-H).

6.3.3.4. Attempted desulphonation of 3-(2-amino-5-nitro-4(3H)-oxo-pyrimidin-6-yl)benzenesulphonic acid

3-(2-Amino-5-nitro-4(3H)-oxopyrimidin-6-yl)benzenesulphonic acid (0.94g) and 50% aqueous sulphuric acid were boiled under reflux for 24 h. The solution was cooled, diluted with ice, neutralised with ammonia, then acidified with glacial acetic acid which gave a yellow precipitate. This precipitate was collected by filtration, washed with water and oven dried at 60°C (0.15g), tlc (silica, BuOH-AcOH-H_2O 12-5-3) several spots; m/z 439, 425, 411, 397, 383, 293, 279, 275, 223.

6.3.3.5. 2-Amino-5-bromo-6-phenylpyrimidin-4(3H)-one (2)

Bromine (70.4g, 22.7ml) in glacial acetic acid (50ml) was dripped into 2-amino-6-phenylpyrimidin-4(3H)-one (78.8g) in glacial acetic acid at 70°C over 1 h with stirring. The mixture was cooled and the precipitate was collected by filtration, washed with ethanol, aqueous sobium bicarbonate and water then oven dried at 80°C (89.6g, 82%). 2-Amino-5-bromo-6-phenylpyrimidin-4(3H)-one was recrystallised from 2-ethoxyethanol-water and dried in vacuo at 95°C, m.p. 285-9°C (lit.,[159] 246-8°C), (Found: C, 45.2; H, 3.3; N, 16.1. Calc for $C_{10}H_8BrN_3O$: C, 45.1; H, 3.0; N, 15.8%), ν_{max} (KBr) 3 300 (NH) and 1 680 cm^{-1} (CO); δ[$(CD_3)_2SO$] 6.83 (2H, sbr, NH) 7.5 (5H, m, Ar-H), 11.4 (1H, sbr, NH); m/z 267 (M^+), 265 (M^+).

Recrystallisation from NMF (at 120°C) afforded the hemi solvate (73%), m.p. 292-4°C with effervescence at 185°C, (Found: C, 44.7; H,

3.5; N, 16.6. $C_{11}H_{10.5}BrN_{3.5}O_{1.5}$ requires C, 44.7; H, 3.6; N, 16.6%), ν_{max} (KBr) 3 380 (NH) and 1 650 br cm^{-1} (CO); δ [$(CD_3)_2SO$] 2.6 (0.5x3H, d, J 5Hz, Me), 6.7 (2H, sbr, ABPP NH), 7.4 (5H, m, ArH), 8.0 (0.5x1H, s, CHO)

6.3.3.6. 3-(2-Amino-5-bromo-4(3H)-oxopyrimidin-6-yl)benzenesulphonic acid (215)

2-Amino-5-bromo-6-phenylpyrimidin-4(3H)-one (5.3g) was added to stirred fuming sulphuric acid (10ml) and the mixture stirred at 110°C for 30 mins. After cooling, the mixture was poured onto ice and the precipitate washed with water and the bromosulphonic acid was dried at 80°C (6.57g, 95%). The sulphonic acid (1.7g) was suspended in 5M aqueous sodium hydroxide (1ml) and the sodium sulphonate was crystallised from water-ethanol, m.p. 322-5°C, (Found: C, 31.1; H, 2.1; N, 11.0. $C_{10}H_7BrN_3O_4SNa.H_2O$ requires C, 31.1; H, 2.3; N, 10.9%), ν_{max} (KBr) 3 400 and 3 300 (NH), 1 680 cm^{-1} (CO); δ(220 MHz, $(CD_3)_2SO$) 6.90 (2H, s, NH), 7.46 (1H, t, J 9Hz, 5'-H), 7.64 (1H, d, J 9 Hz, 6'-H), 7.73 (1H, d, 9 Hz, 4'-H), 7.92 (1H, s, 2'-H).

6.3.3.7. 2-Amino-5-bromo-6-(3-nitrophenyl)pyrimidin-4(3H)-one (214)

2-Amino-5-bromo-6-phenylpyrimidin-4(3H)-one (5.32g) was added in portions over 1 h to a stirred mixture of nitric acid (70%, d 1.42, 1.3ml) and concentrated sulphuric acid (15ml) at 0°C and then stirred at room temperature for 1 h. The yellow solution was poured onto ice and neutralised with ammonia then acidified with glacial acetic acid. The precipitate was washed with water and oven dried at 80°C (5.9g, 95%). Recrystallisation from 2-ethoxyethanol-water afforded 2-amino-5-bromo-6-(3-nitrophenyl)pyrimidin-4(3H)-one as yellow crystalls

(4.6g, 74%), m.p. 294-5°C; ν_{max} (KBr) 3 400 and 3 200 (NH), 1 690 and 1 650 (CO), 1 540 and 1 360 cm^{-1} (NO_2); δ[220 MHz, $(CD_3)_2SO$] 6.95 (2H, sbr, NH), 7,84 (1H, t, J 8 Hz, 5'-H), 8.16 (1H, d, J 8 Hz, 6'-H), 8.39 (1H, d, 8 Hz, 4'-H), 8.48 (1H, s, 2'-H); m/z 312 (M^+), 310 (M^+).

6.3.3.8. 2-Amino-6-(4-nitrophenyl)pyrimidin-4(3H)-one (191)

Ethyl 3-(4-nitrophenyl)-3-oxopropanoate (11.9g), guanidinium carbonate (5g) and ethanol (25ml) were boiled under reflux with stirring for 24 h. After cooling the mixture was poured into iced aqueous acetic acid and the precipitate was washed with aqueous acetic acid and water then dried at 80°C (9.3g, 80%). Recrystallisation from 2-ethoxyethanol-DMF afforded 2-amino-6-(4-nitrophenyl)pyrimidin-4(3H)-one as yellow needles (5.5g), m.p. >310°C (lit.,[195] 334°C dec), ν_{max} (KBr) 3 500 and 3 400 (NH), 1 670 (CO), 1 530 and 1 350 cm^{-1} (NO_2); δ [$(CD_3)_2SO$] 6.6 (1H, s, 5-H), 8.0 (2H, d, J 8Hz, 2' and 6'-H), 8.3 (2H, sbr, NH), 8.57 (2H, d, J 8Hz, 3' and 5'-H)

6.3.3.9. 2-Amino-5-bromo-6-(4-nitrophenyl)pyrimidin-4(3H)-one (268)

Bromine (1.8g) in glacial acetic acid (5ml) was dripped into stirred 2-amino-6-(4-nitrophenyl)pyrimidin-4(3H)-one (2.32g) in glacial acetic acid (10ml) at 70°C over 20 mins. After sirring at 70°C for a further 30 mins the mixture was cooled and the precipitate washed with ethanol, aqueous sodium bicarbonate, and water then oven dried at 80°C (2.79g, 90%). 2-amino-5-bromo-6-(4-nitrophenyl)-pyrimidin-4(3H)-one was recrystallised from DMF-water (2.18g, 70%), m.p. 301-3°C; ν_{max} (KBr) 3 400 (NH), 1 660 and 1 640 cm^{-1} (CO);

$\delta(CF_3CO_2H)$ 7.9 (2H, d, J 9Hz, 2' and 6'-H), 8.2 (1H, s, NH), 8.5 (2H, d, J 9Hz, 3' and 5'-H); m/z 312 (M^+), 310 (M^+).

6.3.3.10. 2-Amino-6-(4-chlorophenyl)pyrimidin-4(3H)-one (193)

Ethyl 3-(4-chlorophenyl)-3-oxopropanoate (9.0g), guanidinium carbonate (3.6g), and ethanol (50ml) were boiled under reflux with stirring for 36 h. After cooling, the mixture was poured into aqueous acetic acid and the precipitate was washed with water and ethanol then dried at 70°C to give 2-amino-6-(4-chlorophenyl)pyrimidin-4(3H)-one (4.91g, 56%), m.p. >300°C (lit.,[195] 344-7°C), ν_{max} (KBr) 3 400 (NH) and 1 670 cm^{-1} (CO); $\delta(CF_3CO_2H)$ 6.47 (1H, s, 5-H), 7.63 (4H, s, 6-ArH), 8.2 (2H, sbr, NH); m/z 223 (M^+), 221 (M^+).

6.3.3.11. 2-Amino-5-bromo-6-(4-chlorophenyl)pyrimidin-4(3H)-one (269)

Bromine (3.2g, 1.05ml) in glacial acetic acid (20ml) was dripped into stirred 2-amino-6-(4-chlorophenyl)pyrimidin-4(3H)-one (4.4g) in glacial acetic acid (25ml) at 70°C and the mixture stirred for a further 1 h. After cooling the mixture was poured into aqueous acetic acid and the precipitate was washed with ethanol, water, aqueous sodium bicarbonate, and water then dried at 70°C (5.05g, 84%). Recrystallisation from 2-ethoxyethanol-water yielded 2-amino-5-bromo-6-(4-chlorophenyl)pyrimidin-4(3H)-one as white crystals (4.22g), m.p. 284-6°C dec; ν_{max} (KBr) 3 400 (NH) and 1 670 cm^{-1} (CO); $\delta(CF_3CO_2H)$ 7.6 (4H, m, 6-ArH), 8.1 (2H, sbr, NH); m/z 303 (M^+), 301 (M^+), 299 (M^+).

6.3.3.12. 2-Amino-5-bromo-6-(4-chloro-3-nitrophenyl)pyrimidin-4(3H)-one (257)

To concentrated nitric acid (70%, d 1.42, 0.22ml) and concentrated sulphuric acid (3ml) at 0-5°C was added 2-amino-5-bromo-6-(4-chlorophenyl)pyrimidin-4(3H)-one (0.9g) in portions over 1 hour. After stirring at room temperature for 1 h the mixture was poured onto ice and neutralised with sodium bicarbonate. The precipitate was washed with water then oven dried at 70°C to give a yellow powder (1.03g, 99%). 2-Amino-5-bromo-6-(4-chloro-3-nitrophenyl)pyrimidin-4(3H)-one was recrystallised from 2-ethoxyethanol-water (0.69g, 65%), m.p. 303°C, ν_{max} (KBr) 3 460 and 3 360 (NH), 1 660 cm^{-1} (CO); $\delta[(CD_3)_2SO]$ 7.05 (2H, s, NH), 7.9 (2H, m, 5' and 6'-H), 8.3 (1H, m, 2'-H); m/z 348 (M^+), 346 (M^+), 344 (M^+).

6.3.3.13. 2-Amino-6-(3-chlorophenyl)pyrimidin-4(3H)-one (192)

Ethyl 3-(3-chlorophenyl)-3-oxopropanoate (2.26g), guanidinium carbonate (1.0g), and ethanol (25ml) were boiled under reflux for 24 h. After cooling the mixture was poured into aqueous acetic acid and the precipitate was washed with water then dried at 70°C to give a pale brown solid (1.93g, 83%). Recrystallisation from DMF-water gave 2-amino-6-(3-chlorophenyl)pyrimidin-4(3H)-one as white crystals, m.p. 324-7°C, ν_{max} (KBr) 3 400 (NH) and 1 660 cm^{-1} (CO); $\delta[(CD_3)_2SO]$ 6.3 (1H, s, 5-H), 6.75 (2H, s, NH), 7.5-8.3 (4H, m, 6-ArH); m/z 223 (M^+), 221 (M^+).

6.3.3.14. 2-Amino-5-(4-chlorophenylazo)-6-phenylpyrimidin-4(3H)-one (210)

To stirred 4-chloroaniline (2.55g) in 1M hydrochloric acid (100ml) at 0°C was addded sodium nitrite (1.45g) in water (10ml) in poetions. 2-Amino-6-phenylpyrimidin-4(3H)-one (3.74g) was added and the mixture was basified with sodium carbonate. After 1 h, the mixture was filtered and the orange retentate washed with water then dried at 70°C (6.50g, 99%). Recrystallisation from glacial acetic acid gave 2-amino-5-(4-chlorophenylazo)-6-phenylpyrimidin-4(3H)-one (2.95g, 46%) as orange crystals, m.p. 279-81°C, ν_{max} (KBr) 3 400 (NH) and 1 650 cm^{-1} (CO); $\delta(CF_3CO_2H)$ 7.4-8.2 (m, Ar-H); m/z 327 ($\underline{M}^+$), 325 ($\underline{M}^+$).

6.3.3.15. 2,5-Diamino-6-phenylpyrimidin-4(3H)-one (213)

To a stirred boiling suspension of 2-amino-5-(4-chlorophenylazo)-6-phenylpyrimidin-4(3H)-one (1.62g) in water (50ml) was added in portions sodium dithionite until decolourisation (to light orange colour) occured (ca 4g). The suspension was cooled, filtered and the retentate was washed with acetone (3x5ml) and water then dried at 70°C. The crude amine was dissolved in 2M hydrochloric acid (25ml) and filtered to remove unreduced azo dye. The filtrate was neutralised with sodium bicarbonate and the reprecipitated 2,5-diamino-6-phenylpyrimidin-4(3H)-one was washed with water and acetone (0.15g, 15%), m.p. 269-72°C; ν_{max} (KBr) 3 400 (NH) and 1 690 cm^{-1} (CO); $\delta(CF_3CO_2H)$ 7.0-9.0 (2H, br, NH), 7.4-7.9 (5H, m, Ar-H); m/z 202 ($\underline{M}^+$).

6.3.3.16. Attempted azidation of 2,5-diamino-6-phenylpyrimidin-4(3H)-one

To a stirred suspension of crude 2,5-diamino-6-phenylpyrimidin-4(3H)-one (1.0g) in 2M hydrochloric acid (50ml) at 0°C was added in portions sodium nitrite (0.62g) followed by sodium azide (0.6g) over 30 mins (effervescence). After stirring for a further 2 h the mixture was neutralised with sodium acetate. The precipitate was collected by filtration and washed with water then air dried. Attempted recrystallisation from ethanol resulted in decomposition (tlc).

6.3.3.17. Reaction of guanidine with ethyl 2-hydroxyimino-3-oxo-3-phenylpropanoate

To stirred guanidine hydrochloride (4g) in 2M sodium hydroxide solution (100ml) was added over 20 mins ethyl 2-hydroxyimino-3-oxo-3-phenylpropanoate (4.42g). The green solution was stirred for a further 30 min then acidified slowly with conc hydrochloric acid (colour changed from green to turquoise to colourless). The white precipitate produced was washed with water and dried at 70°C (3.07g) then recrystallised from water (2.5g), m.p. >330°C, ν_{max} (KBr) 3 300, 3 100, 2 900, 1 720, 1 670, 1 640, 1 600 cm^{-1}; $\delta[(CD_3)_2SO]$ 4.7-6.8 (2H, br), 7.3-8.1 (5H, m), $\delta[(CD_3)_2SO\text{-}D_2O]$ 4.3 (3H, s, HOD), 7.4-8.2 (5H, m); m/z 183, 167, 144,122, 116, 105, 83, 78, 77; tlc (silica, BuOH-AcOH-H_2O 12-5-3) single spot R_F 0.55

Attempted reduction of this product with sodium dithionite, hydrazine hydrate and Raney nickel, or sodium in ethanol produced mixtures of products (tlc).

6.3.3.18. 6-Phenylpyrimidin-4(3H)-one-2(1H)-thione (239)

Sodium (9.7g) was dissolved in absolute ethanol (150ml) then thiourea (21.28g) and ethyl 3-oxo-3-phenylpropanoate (40.32g) were added and the mixture was boiled under reflux for 12 h. After concentration under reduced pressure the residue was dissolved in water (400ml) then filtered. The filtrate was acidified with conc hydrochloric acid and the precipitate washed with water and dried at 60°C (31.8g, 73%). Recrystallisation from glacial acetic acid yielded 6-phenylpyrimidin-4(3H)-one-2(1H)-thione as needles (22.37g, 52%), m.p. 265°C (lit.,[196] 259°C), ν_{max} (KBr) 3 400 (NH) and 1 670 cm^{-1} (CO); $\delta[(CD_3)_2SO]$ 6.1 (1H, s, 5-H), 7.6 (5H, m, 6-ArH), 12.5 (2H, s, NH).

6.3.3.19. Benzoylguanidine hydrochloride (229)

Guanidine hydrochloride (9.5g) and benzoyl chloride (14.05g) were heated together (with the evolution of hydrogen chloride) at 180-5°C, 190-5°C, and 210-20°C each for 25 mins. Absolute ethanol (40ml) was added cautiously yet quickly to the mixture with stirring. The product was crystallised from absolute ethanol (9.27g, 47%), m.p. 208-9°C (lit.,[197] 210-12°C), ν_{max} (KBr) 3 250 (NH) and 1 700 cm^{-1} (CO); $\delta[(CD_3)_2SO]$ 7.77 (3H, m, 3, 4 and 5-H), 8.35 (2H, m, 2 and 6-H), 8.9 (4H, sbr, NH), 12.3 (1H, s, NH).

6.3.4. 2-Nitraminopyrimidinones

6.3.4.1. 2-Nitramino-6-phenylpyrimidin4(3H)-one (216)

Nitroguanidine (80%, 14.3g), ethyl 3-oxo-3-phenylpropanoate (21.2g), potassium hydroxide (4.0g) and 2-methoxyethanol (100ml) were boiled under reflux for 24 h. Water was added to the cooled mixture and the unreacted nitroguanidine filtered off and washed with alkaline 2-methoxyethanol. The combined filtrate was acidified with 2M hydrochloric acid and the precipitate was washed with water. Recrystallisation from ethanol-water yielded 2-nitramino-6-phenyl-pyrimidin-4(3H)-one (4.3g), m.p. 210-12°C (lit.,[198] 206°C); ν_{max} (KBr) 3 420 (NH), 1 660 (CO), and 1 530 cm^{-1} (NO_2); δ[$(CD_3)_2SO$] 6.37 (1H, s, 5-H), 7.63 (5H, m, 6-ArH), 8.2-12.0 (2H, br, NH).

6.3.4.2. Rearrangement of 2-nitramino-6-phenylpyrimidin-4(3H)-one

2-Nitramino-6-phenylpyrimidin-4(3H)-one (5.8g) was added slowly to concentrated sulphuric acid (15ml) at 30°C. The mixture was stirred for a further 4 h then poured onto ice and neutralised with sodium bicarbonate. The precipitate was washed with water then oven dried at 60°C (5.2g, m.p. 250-75°C). This product was recrystallised from DMF-water (2.5g, m.p. 276-80°C) which gave a mixture of three compounds; tlc (AcOH-$CHCl_3$ 1:10) R_F minor 0.24, minor 0.29, major 0.48. Recrystallisation from DMF-water and ethanol-water yielded almost pure 2-amino-6-(3-nitrophenyl)pyrimidin-4(3H)-one; R_F 0.48; δ[220 MHz, $(CD_3)_2SO$] 6.25 (1H, s, 5-H), 6.82 (2H, s, NH), 7.79 (1H, t, J 8Hz, 5'-H), 8.36 (1H, d, J 8 Hz, 6'-H), 8.45 (1H, d, J 8Hz, 4'-H), 8.88 (1H, s, 2'-H).

6.3.4.3. 5-Bromo-2-nitramino-6-phenylpyrimidin-4(3H)-one (218)

Bromine (8g, 2.6ml) in glacial acetic acid (25ml) was dripped into stirred 2-nitramino-6-phenylpyrimidin-4(3H)-one (10.4g) in glacial acetic acid (50ml) at 70°C over 30 mins. After stirring at room temperature for a further 6 h the precipitate was washed with ethanol, water then dried (9.87g, 71%). Crystallisation from ethanol-water yielded 5-bromo-2-nitramino-6-phenylpyrimidin-4(3H)-one (8.6g, 61%), m.p. 195°C (lit.,[159] 192-3°C), ν_{max} (KBr) 3 400 (NH), 1 680 (CO), and 1 540 cm^{-1} (NO_2); $\delta[(CD_3)_2SO]$ 7.6 (m, Ar-H).

6.3.4.4. Rearrangement of 5-bromo-2-nitramino-6-phenylpyrimidin-4(3H)-one

5-Bromo-2-nitramino-6-phenylpyrimidin-4(3H)-one (0.62g) was slowly added to stirred concentrated sulphuric acid (1.5ml) at 30°C then stirred for a further 4 h. The mixture was poured onto ice then neutralised with sodium bicarbonate and the precipitate washed with water and dried at 80°C (0.55g). The ir spectrum was identical to 2-amino-5-bromo-6-(3-nitrophenyl)pyrimidin-4(3H)-one.

7. APPENDICES

APPENDIX 1
(Tables of antitumour screening data for formamides and isocytosine antitumour agents)

Key to tables#

1. Compound numbers
   Parenthasised - this thesis
   CCRG - Cancer Chemotherapy Research Group, Pharmaceutical Sciences Institute, University of Aston
   NSC - National Cancer Institute

2. Test-Syst: Test System - Six character code
   (* indicates work done at NCI)

Characters 1 and 2	Host Group	Code
	CBA/LAC	3L
	CBA/CA	3C
	Balb/C	4A
	BDF_1	02
	$CD_2F_1(CDF_1)$	06

Characters 3 and 4	Tumour	Code
	TLX5S lymphoma	T5
	M5076 sarcoma	M5
	P388 leukaemia	PS
	B16 melanocarcinoma	B1

Character 5	Parameter	Code
	Mean Tumour Weight	1
	Mean Surval Time	2
	Mean Tumour Volume	A
	Median Survival Time	3

Character 6	Site	Code
	Intraperitoneal	1
	Subcutaneous	2
	Intramuscular	6

3. Tis:

Tissue	Code
Ascitic Fluid	1
Homogenate	2
Fragment	6

4. Lvl:

Level of Innoculation	Code
Dilution 1:10	G
2×10^5 number of cells	Z
Log of cell number	1-9
1 mM Fragments	X

5. Veh:

Vehicle	Code
Saline	2
10% DMSO Arachis	$
10% Tween 80 in streile sailine	T
Distilled water / Tween 80	+

6. Rt:	Route of administration of compound	Code
	Intraperitoneal	1
	Subcutaneous	2
	Oral	3
7. Sex:	male	M
	female	F

8. Schedule : Eight character code

characters 1- 4 - 'Q' 'two digit number' 'D' which gives the interval (in days) between doses

characters 5, 6, and 7 - 'X' 'two digit number' which gives the total number of doses

character 8 - parenthasised number which gives the day of the first dose

9. Date : Date of test day, month, year

10. Dose : per injection in mg/Kg

11. Day of Death : Digits before '/' refer to the day, digits after the '/' refer to the number of animals

12. Surv Tox : Indicates the number of animals which survived drug toxicity.

13. Wt Dif : For control - refers to difference in weight (g) on last day of treatment compared to day 0

For noncontrol - refers to difference in weight (g) on last day of treatment compared to day 0

Wt Dif : T-C weight difference (g) (treated minus control) on last day of treatment. Figure for control refers to weight difference (g) on last day of treatment compared to day 0

14. Evaluation Result - Result of the parameter measured in the test-system :
mean tumour weight (g)
mean survival time (days)
median survival time (days)
mean tumour volume (cm^{-3})
(M5076 evaluated on day 24, TLX5 evaluated on day of death)

T:C - The reult of the treated group compared to the control group (%)

Adapted from refs 199 and 200

Cpd. Formamide

(41), CCRG 80016

Test-syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX04(3)	151280

Dose	Day of Death	Surv Tox	Wt Dif T-C	Evaluation Result	T:C
Cont	09/8,10/1	9/10	+1.5	9.1	100
800	09/1,11/1,12/3	5/5	-2.8	11.2	123
400	10/1,11/3,13/1	5/5	-1.3	11.2	123
200	10/4,11/1	5/5		10.2	112
100	09/4,11/1	5/5		9.4	103
50	09/3,10/1,11/1	5/5		9.6	106

Cpd. Formamide

(41), CCRG 80016

Test-syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX07(1)	200381

Dose	Day of Death	Surv Tox	Wt Dif T-C	Evaluation Result	T:C
Cont	10/7,11/3	10/10		10.3	100
1200	05/1,07/4	0/5	-1.9	6.6	63
800	07/1,09/1,10/3	4/5	-2.6	9.3	90
400	11/3,12/2	5/5	0.0	11.4	111
200	11/4,12/1	5/5	+1.4	11.2	109
100	10/2,11/3	5/5	+1.0	10.6	103

Cpd. Formamide

(41), CCRG 80016

Test-syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif T-C	Evaluation Result	T:C
Cont		27/27		2.06	100
400		0/10			
200		9/10	+0.5	0.76	37
100		10/10	+0.9	1.12	54

Cpd. N-Methylformamide

(1), CCRG 80011, NSC 003051

Test-syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3LT522	1	5	2	1	F	Q01DX04(3)	260980

Dose	Day of Death	Surv Tox	Wt Dif T-C	Evaluation Result	T:C
Cont	09/3,10/1	4/5		9.2	100
800	16/1,18/4	5/5	-4.3	17.6	191
400	14/1,17/4	5/5		16.4	178
200	11/1,12/2,13/1, 14/1	5/5		12.4	135
100	10/1,11/3,12/1	5/5		11.0	119

Cpd. N-Methylformamide

(1), CCRG 80011, NSC 003051

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	071083

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/9,12/1	10/10	+1.0	11.1	100
800	18/1,19/1,23/3	5/5	-3.1	21.2	191
400	16/3,17/2	5/5	-2.2	16.4	148
200	14/5	5/5	+0.4	14.0	126
100	12/1,13/3,14/1	5/5	+0.9	13.0	117
50	11/1,12/2,13/2	5/5	+1.0	12.2	110

Cpd. N-Methylformamide

(1), CCRG 80011, NSC 003051

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	101080

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		18/18	+2.6	2.72	100
400		1/8	-5.9	0.0	0
300		4/8	-2.3	0.0	0
200		8/8	-0.3	0.0	0
100		8/8	+0.7	0.27	10
50		8/8	+1.8	0.88	32

Cpd. N-Methylformamide

(1), CCRG 80011, NSC 003051

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		18/18	+2.7	2.95	100
25		10/10	+2.0	2.09	71
12.5		10/10	+1.6	2.36	80
6.25		10/10	+1.7	2.66	90

Cpd. N-Ethylformamide

(44), CCRG 80012

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3LT522	1	5	2	1	F	Q01DX04(3)	260980

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	09/4,10/1	5/5		9.2	100
800	11/1,12/2,14/2	5/5		12.6	137
400	11/3,12/2	5/5		11.4	124
200	10/2,11/3	5/5		10.6	115
100	09/3,10/1,12/1	5/5		9.8	107

Cpd. N-Ethylformamide

(44), CCRG 80012

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif T-C	Evaluation Result	T:C
Cont		10/10		2.49	100
600		0/10			
300		10/10		1.95	78

Cpd. N-Ethylformamide

(41), CCRG 80012

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		15/15	+0.5	3.05	100
200		10/10	+0.3	2.59	85
100		10/10	+0.2	2.83	93

Cpd. N-(Trideuteromethyl)formamide

(55), CCRG 82030

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	250583

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	12/1,13/2,14/2	5/5	+1.8	13.2	100
800	05/3,07/1,18/1	1/5	-4.0	8.0	61
400	17/1,18/1,19/2 21/1	5/5	-3.0	18.8	142
200	13/1,14/1,15/2 17/1	5/5	+1.4	14.8	112
100	12/1,13/1,14/1 16/2	5/5	+1.4	14.2	107
50	12/1,13/2,14/1 16/1	5/5	+1.9	13.6	103

Cpd. N-Methylformamide

(1), CCRG 80011, NSC 003051

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	250583

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	12/1,13/2,14/2	5/5	+1.8	13.2	100
800	05/1,18/1,19/2 21/1	5/5	-2.1	16.4	124
400	17/1,18/2,19/1 20/1	5/5	-0.1	18.4	139
200	15/1,16/3,17/1	5/5	+1.0	16.0	121
100	13/1,14/2,15/1 17/1	5/5	+1.6	14.6	111
50	12/1,13/2,14/2	5/5	+1.0	13.0	98

Cpd. N-Hydroxymethylformamide

(40), CCRG 80007, NSC 348403

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	5	2	1	F	Q01DX04(3)	141180

Dose	Day of Death	Surv Tox	Wt Dif T-C	Evaluation Result	T:C
Cont	09/2,10/3	5/5		9.6	100
1600	09/1,10/2,11/2	5/5	-1.2	10.2	106
800	09/3,11/2	5/5	-1.0	9.8	102
400	09/1,10/3,11/1	5/5		10.0	104
200	09/3,10/2	5/5		9.4	98
100	09/3,10/1,11/1	5/5		9.6	100

Cpd. N-Hydroxymethylformamide

(40), CCRG 80007, NSC 348403

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q0.5DX07(1)	160181

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	09/5	5/5	+2.6	9.0	100
1600	07/1,08/1,10/2, 11/1	4/5	-4.0	9.2	102
800	09/1,10/3,11/1	5/5	-0.4	10.0	111
400	09/2,10/2.11/1	5/5		9.8	109
200	08/1,09/2,10/2	5/5		9.2	102
100	08/3,09/2	5/5		8.4	93

Cpd. N-Hydroxymethylformamide

(40), CCRG 80007, NSC 348403

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		19/19	+1.9	3.20	100
2500		0/5			
2000		2/5	-5.1	0.54	17

Cpd. N-Hydroxymethylformamide

(40), CCRG 80007, NSC 348403

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		27/27	+1.6	2.06	100
1500		6/6		0.77	37
1000		10/10	+0.4	0.96	47
600		10/10	+0.5	1.39	67

Cpd. N-Hydroxymethylformamide

(40), CCRG 80007, NSC 348403

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
06PS31*	1	6	9	1	F	Q01DX05(1)	271081

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	09/1,10/10, 11/12,12/2	25/25	+1.7	10.7	100
4000	03/1,06/2,07/1, 08/1	1/5	-3.4	6.5	
2000	08/1,13/2,14/2	5/5	-2.1	13.5	126
1000	11/2,12/3	5/5	-0.4	11.8	110
500	11/5	5/5	+0.8	11.1	103
250	08/1,10/1,11/3	5/5	+0.6	10.8	100

Cpd. N-Ethoxymethylformamide

(57), CCRG 81044, NSC 348404

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	210881

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/3,12/6,13/2	12/12	+0.8	11.8	100
800	11/2,12/1,13/1, 14/1	5/5	+0.4	12.2	103
400	11/1,12/2,13/1, 14/1	5/5	+1.6	12.4	105
200	11/3,12/2	5/5	+1.7	11.4	97
100	10/1,11/2,12/2	5/5	+1.7	11.2	93
50	12/5	5/5	+2.0	12.0	102

Cpd. N-Ethoxymethylformamide

(57), CCRG 81044, NSC 348404

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	250981

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/2,12/1,13/2	5/5	-0.2	12.0	100
1600	12/1,13/3,14/1	5/5	-0.6	13.0	108
1200	11/1,12/1,13/3	5/5	0.0	12.4	103

Cpd. N-Ethoxymethylformamide

(57), CCRG 81044, NSC 348404

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/1,12/2,13/2	5/5	+1.2	12.2	100
6400	04/5	0/5		4.0	33
3200	05/5	0/5		5.0	41
2000	05/2,06/1,09/1, 12/1	1/5	-0.3	7.5	61

Cpd. N-Ethoxymethylformamide

(57), CCRG 81044, NSC 348404

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
06PS31*	1	6	9	1	F	Q01DX05(1)	271081

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	09/1,10/10, 11/12,12/2	25/25	+1.7	10.7	100
1000	11/2,12/2,13/1	5/5	-1.6	12.0	112
500	11/3,12/2	5/5	-0.6	11.5	107
250	10/2,11/3	5/5	-0.4	10.8	100
125	10/1,11/2,12/2	5/5	-+1.2	11.5	107

Cpd. N-(2-Chloroethyl)formamide

(61), CCRG 82031

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	100982

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/4,12/4,13/1	9/9	+1.2	11.6	100
450	11/2,12/1,13/1, 16/1	5/5 5/5	-1.5	12.6	109
225	10/1,12/1,13/3	5/5	-0.2	12.2	105
112	12/2,13/2,13/1	5/5	+0.5	12.8	110
56	12/4,13/1	5/5	+1.3	12.2	105
28	12/3,13/1,14/1	5/5	+0.4	12.6	109

Cpd. N-(2,2,2-Trifluoroethyl)formamide

(60), CCRG 83001

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	110283

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	12/1,13/2,14/2	5/5	+1.8	13.2	100
800	06/3,07/2	0/5		6.4	48
400	14/2,17/3	5/5	-1.8	15.8	120
200	12/2,13/1,13/2	5/5	+0.2	13.0	98
100	13/2,14/1,17/2	5/5	+0.9	14.8	112
50	12/1,13/1,14/2,	5/5	+2.5	13.8	104

Cpd. N-(Dimethylaminomethyl)formamide

(59)

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1		$	1	F	Q01DX05(3)	210881

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/4,12/6,13/2	12/12	+0.8	11.8	100
800	04/2,05/1,06/1, 08/1	0/5	-0.1	5.4	46
400	05/1,06/2,07/2	0/5	-2.5	6.2	53
200	07/1,08/1,12/2, 13/1	3/5	+0.8	10.4	88
100	11/1,12/4	5/5	+1.3	11.8	100
50	11/1,12/3,13/1	5/5	+1.8	12.0	102
900	04/1,05/4	0/5		4.8	36

Cpd. N-Hydroxy-N-methylformamide

(62), CCRG 81015

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1		2	1	F	Q01DX05(3)	200381

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	10/7,11/3	10/10	+1.8	10.3	100
1600	03/3,04/1,05/1	0/5		3.6	35
800	08/1,09/2,10/1, 12/1	4/5	-3.7	9.6	93
400	11/1,12/2,13/2	5/5	-0.2	12.2	118
200	10/3,11/2	5/5	+1.9	10.2	101
100	11/3,12/1	4/4	+1.0	11.3	109

Cpd. N-(Formamidomethyl)formamide

(58), CCRG 84023

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/3,12/1,13/1	5/5	+0.5	11.6	100
800	07/1,12/1,13/3	4/5	-4.2	11.6	100
400	09/1,11/1,12/3	4/5	-1.9	11.2	97
200	11/2,12/3	5/5	-0.6	11.6	100
100	11/5	5/5	-0.3	11.0	95
50	10/1,11/4	5/5	+0.2	10.8	93

Cpd. Dimethylformamide

(37), CCRG 80008

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	5	2	1	F	Q01DX05(3)	141180

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	09/2,10/3	5/5	+1.7	9.6	100
1600	09/1,10/3,11/1	5/5	+0.2	10.0	104
800	09/1,10/2,11/2	5/5	+0.6	10.2	106
400	09/3,10/1,11/1	5/5		9.6	100
200	10/4,11/1	5/5		10.2	106
100	09/1,10/4	5/5		9.8	102
50	09/2,10/2,11/1	5/5		9.8	102

Cpd. Dimethylformamide

(37), CCRG 80008

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Coht	11/3,12/2,13/5	10/10	+0.7	12.2	100
3200	05/3,08/2,10/1	1/6	-2.6	6.8	56
1600	13/2,14/2,15/1	5/5	-0.4	13.8	113

Cpd. Dimethylformamide

(37), CCRG 80008

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
O2M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		27/27	+0.5	2.49	100
1000		10/10	+0.2	0.99	40
800		10/10	+0.1	1.38	55
600		10/10	+0.6	1.54	62

Cpd. Dimethylformamide

(37), CCRG 80008

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
O2M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		27/27		2.06	100
1500		0/10			
1200		7/10		0.68	33

Cpd. N-Hydroxymethyl-N-methylformamide

(39), CCRG 80004

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	5	2	1	F	Q01DX05(3)	311080

Dose	Day of Death	Surv Tox	Wt Dif (7-3)	Evaluation Result	T:C
Cont	09/2,10/6,11/2	10/10	+0.5	10.0	100
1600	07/1,08/3,09/1	4/5	-2.3	8.0	80
800	10/4,11/1	5/5	-0.6	10.4	104
400	09/2,11/2,13/1	5/5		10.6	106
200	09/1,10/2,11/1, 12/1	5/5		10.4	104
100	09/1,10/4	5/5		9.8	98
50	10/5	5/5		10.0	100
25	09/1,10/3,11/1	5/5		10.0	100

Cpd. N-Hydroxymethyl-N-methylformamide

(39), CCRG 80004

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
O2M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		19/19	+1.9	3.20	100
1200		0/5			
800		0/5			
400		4/5	+0.4	2.43	76
200		5/5	+1.9	2.55	80
100		5/5	+1.4	2.70	84

Cpd. N-Acetoxymethyl-N-methylformamide

(63), CCRG 82032, NSC 348405

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	100982

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/4,12/4,13/1	9/9	+1.2	11.6	100
800	11/1,12/3,13/1	5/5	+0.4	12.0	103
400	12/2,13/1,14/1, 18/1	5/5	+1.7	13.8	119
200	12/3,14/1,15/1	5/5	+1.2	13.0	112
100	11/2,12/1,13/1, 15/1	5/5	+1.8	12.4	107
50	12/2,13/2,14/2	5/5	+1.5	13.2	114

Cpd. N-Acetoxymethyl-N-methylformamide

(63), CCRG 82032, NSC 348405

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	2	1	F	Q01DX05(3)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	12/2,13/3,14/5	10/10	+0.6	13.3	100
2000	05/1,06/2,07/1, 15/1	1/5	-3.9	7.8	59
1000	04/1,11/1,12/1, 14/2	4/5	-0.7	11.0	83

Cpd. N-Acetoxymethyl-N-methylformamide

(63), CCRG 82032, NSC 348405

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	130183

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		10/10	+6.5	2.89	100
400	06/2,07/2,08/1	0/5			
200	11/1,14/3,24/1	1/5	+2.4	1.52	52
100		5/5	+4.9	2.33	80

Cpd. N-Acetoxymethyl-N-methylformamide

(63), CCRG 82032, NSC 348405

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
06PS31*	1	6	9	1	F	Q01DX05(1)	271081

Dose	Day of Death	Surv Tox	Wt Dif T-C	Evaluation Result	T:C
Cont	09/1,10/10, 11/12,12/2	25/25	+1.7	10.7	100
1000	04/2,05/2,06/1	0/5	-3.5		
500	07/1,10/2,11/2	5/5	-2.5	10.5	98
250	09/2,10/3	5/5	-1.3	9.8	91
125	10/4,11/1	5/5	-0.7	10.3	96

Cpd. N-Benzoyloxymethyl-N-methylformamide

(64), CCRG 81022, NSC 348406

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1			1	F	Q01DX05(3)	050681

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/5	5/5	+0.8	11.0	100
800	04/2,05/2,11/1	1/5	+1.1	5.8	53
400	10/2,11/3	5/5	+0.9	10.6	96
200	10/4,11/1	5/5	+1.0	10.2	93
100	10/1,11/2,12/1, 13/1	5/5	+0.7	11.4	104
50	10/5	5/5	+2.9	10.0	90

Cpd. N-Benzoyloxymethyl-N-methylformamide

(64), CCRG 81022, NSC 348406

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1		$	1	F	Q01DX5(3)	210881

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/3,12/7,13/2	12/12	+0.8	11.8	100
400	09/1,12/3,13/1	5/5	-0.1	11.6	98
200	11/1,12/4	5/5	+1.9	11.8	100
100	11/2,12/2,14/1	5/5	+1.7	12.0	102
50	11/2,12/3	5/5	+2.8	11.6	98

Cpd. N-Benzoyloxymethyl-N-methylformamide

(64), CCRG 81022, NSC 348406

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
06PS31*	1	6	+	1	F	Q01DX05(1)	271081

Dose	Day of Death	Surv Tox	Wt Dif T-C	Evaluation Result	T:C
Cont	09/1,10/10, 11/2,12/2	25/25	+1.7	10.7	100
250	10/1,11/3,12/1	5/5	-2.9	11.2	102
125	09/1,10/4	5/5	-1.5	10.0	93
62.5	10/4,12/1	5/5	-0.9	10.3	96
31.25	10/1,11/3,12/1	5/5	-0.5	11.2	104

Cpd. 1,3,5-Tris(formyl)hexahydro-1,3,5-triazine

(65), CCRG 84011

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	180584

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/1,12/2,13/1, 14/1	5/5	-0.8	12.4	100
800	11/3,12/2	5/5	-0.4	11.4	92
400	11/1,12/2,13/1, 14/1	5/5	-0.4	12.4	100
200	12/3,13/2	5/5	-0.4	12.4	100
100	11/2,12/2,13/1	5/5	+0.4	11.8	95
50	11/1,12/2,13/1, 16/1	5/5	+0.2	13.0	105

Cpd. 2-Nitroethenamine

(69), CCRG 84004

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	130184

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/5,12/3,13/2	10/10	-1.4	11.7	100
800	03/5	0/5		3.0	26
400	03/5	0/5		3.0	26
200	06/1,09/1,12/2	3/4	-2.2	9.7	83
100	11/1,12/3,13/1	5/5	-1.4	12.0	103
50	11/3,12/1,13/1	5/5	-2.1	11.6	99

Cpd. N-Methyl-2-nitroethenamine

(70), CCRG 83035

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/3,12/1,13/1	5/5	+1.2	11.6	100
400	03/2,04/1,11/2	2/5	-0.8	6.4	55
200	11/5	5/5	-0.2	11.0	95
100	11/4,12/1	5/5	+1.2	11.2	96
50	11/1,11/4	5/5	+0.4	10.8	93
25	11/4,12/1	5/5	+0.2	11.2	96

Cpd. N N-Dimethyl-2-nitroethenamine

(71), CCRG 83030

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	100783

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/9,12/1	10/10	+1.0	11.1	100
400	05/5	0/5		5.0	45
200	05/5	0/5		5.0	45
100	14/2,16/2,17/1	5/5	-3.0	15.4	139
50	12/1,13/3,14/1	5/5	-0.4	13.0	117
25	10/1,12/3,13/1	5/5	+0.4	11.8	106

Cpd. N N-Dimethyl-2-nitroethenamine

(71), CCRG 83030

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	$	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		10/10	+2.2	1.4	100
200	04/1,05/4	0/5			
100	07/1,08/2,09/2	0/5			
50		5/5	+0.6	0.5	36
25		5/5	+3.8	1.3	86

Cpd. N-(2-Nitroethenyl)morpholine

(72), CCRG 84010

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	180584

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/1,12/2,13/1, 14/1	5/5	-0.8	12.4	100
800	11/2,12/2,13/1	5/5	-0.6	11.8	95
400	12/3,13/1,14/1	5/5	-0.2	12.6	102
200	11/1,12/3,13/1	5/5	0.0	12.0	97
100	11/2,12/2,13/1	5/5	+0.6	11.8	95
50	11/1,12/4	5/5	+0.4	11.8	95

Cpd. N-(2-Nitroethenyl)morpholine

(72), CCRG 84010

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	121084

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/2,12/4,13/4	10/10	+0.2	12.2	100
3200	03/2,05/3	0/5		4.2	34
1600	06/4,07/1	0/5	-1.2	6.2	51
800	10/1,11/2,12/1, 13/1	5/5	-1.0	11.4	93

Cpd. 2-Methyl-3-methylaminopropenal

(66), CCRG 84005

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	130184

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/5,12/3,13/2	10/10	-1.4	11.7	100
800	10/1,13/4	5/5	-4.0	12.4	106
400	12/3,13/2	5/5	-1.0	12.4	106
200	12/2,13/3	5/5	-2.8	12.6	108
100	11/1,12/4	5/5	+0.6	11.8	101
50	11/4,12/1	5/5	+0.6	11.2	96

Cpd. 3-(Dimethylamino)propenenitrile

(67), CCRG 84003

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	130184

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/5,12/3,13/2	10/10	-1.4	11.7	100
800	03/5	0/5		3.0	26
400	03/5	0/5		3.0	26
200	03/3,04/2	0/5		3.4	29
100	03/2,11/1,12/2	3/5	-1.7	8.2	70
50	11/2,12/1,13/2	5/5	-1.4	12.0	103

Cpd. 2-Cyano-3-(dimethylamino)propenenitrile

(68), CCRG 84009

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
3CT522	1	Z	$	1	F	Q01DX05(3)	180584

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/1,12/2,13/1, 14/1	5/5	-0.8	12.4	100
800	03/5	0/5		3.0	24
400	03/5	0/5		3.0	24
200	03/3,04/2	0/5		3.4	28
100	06/1,11/3,12/1	4/5	-0.6	10.2	82
50	11/2,12/3	5/5	-1.0	11.6	94

Cpd. N-Cyclopropylformamide

(56), CCRG 83006

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	2	1	F	Q01DX17(1)	

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		10/10	+3.3	1.9	100
200		5/5	+0.8	1.7	89
100		5/5	+2.0	1.7	89
50		5/5	+1.0	1.6	83
25		5/5	+1.8	1.7	89

Cpd. 2-Amino-5-bromo-6-phenylpyrimidine-4(3H)-one

(2), CCRG 83007, NSC 149027

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	T	1	F	Q04DX04(1)	011283

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		14/14	+3.7	4.7	100
2000	01/1,02/2,09/1, 20/1	1/5	+4.0	1.8	38
1000	02/2,09/1	2/5	+1.8	1.6	34
500		5/5	+3.2	1.5	32
250		5/5	+1.9	1.8	38
125		5/5	+1.8	1.4	30

Cpd. 2-Amino-5-bromo-6-phenylpyrimidine-4(3H)-one

(2), CCRG 83007, NSC 149027

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	T	1	F	Q01DX01(1)	200384

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		5/5	+3.2	4.2	100
500		5/5	+1.4	2.1	50
125		5/5	+2.6	2.5	60
62		5/5	+2.6	2.8	67
31		5/5	+3.4	3.2	76
16		5/5	+4.0	4.4	105

Cpd. 2-Amino-5-bromo-6-phenylpyrimidine-4(3H)-one

(2), CCRG 83007

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	T	1	F	Q04DX04(1)	120483

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		10/10	+3.3	1.9	100
400		5/5	+3.6	1.5	77
200		5/5	+2.6	0.9	49
100		5/5	+4.8	1.3	69
50		5/5	+3.2	1.6	80

Cpd. 2-Amino-5-bromo-6-(2-fluorophenyl)pyrimidine-4(3H)-one

(185), CCRG 84006

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	T	1	F	Q04DX04(1)	011283

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		14/14	+3.7	4.7	100
2000		5/5	+3.7	1.2	26
1000		5/5	+2.9	2.8	60
500		5/5	+3.5	2.8	60
250		5/5	+2.4	3.5	74
125		5/5	+3.1	5.1	109

Cpd. 2-Amino-5-bromo-6-(2-fluorophenyl)pyrimidine-4(3H)-one

(185), CCRG 84006

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	T	1	F	Q01DX01(1)	200384

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		5/5	+3.2	4.2	100
4000	14/1	4/5	+3.1	2.7	64

Cpd. 2-Amino-5-bromo-6-(3-fluorophenyl)pyrimidine-4(3H)-one

(184), CCRG 84007

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	T	1	F	Q01DX04(1)	011283

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		14/14	+3.7	4.7	100
2000	03/3,05/1,10/1	0/5			
1000	01/1,03/1	3/5	+2.5	0.7	15
500		5/5	+1.8	1.3	28
250		5/5	+2.5	2.0	43
125		5/5	+3.1	3.1	66

Cpd. 2-Amino-5-bromo-6-phenylpyrimidin-4(3H)-one

(2), CCRG 83007, NSC 149027

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
03B131*	2	G		1	M	Q01DX09(1)	170484

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	06/1,15/2,16/1, 17/7,18/7,19/5, 20/6,21/3,22/3 24/1,30/1,31/1, 34/1/35/1	40/40		19.0	100
400	22/1,23/1,27/1, 28/1,29/2,30/1, 41/1,44/1,48/1	10/10		29.3	154
200	25/1,26/1,29/1, 30/2,33/1,37/1,			33.0	173
100	29/1,30/1,32/1, 33/1,35/1,36/1, 37/1,50/1,60/1			36.0	189
50	22/1,27/1,28/2 29/1,30/2,34/1			29.8	156

Cpd. 2-Amino-5-(4-chlorophenylazo)-6-phenyl-pyrimidin-4(3H)-one
(210), NSC 36324

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
03B131*	2	G	M	1	M	Q01DX09(1)	040483

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/1,15/3,16/7, 17/6,18/6,19/1, 20/3,22/4,26/1 28	35/35	+2.4	17.7	100
75	13/5,15/3,16/2	10/10	+1.7	14.7	
37.5	12/1,14/1,15/5, 16/3	10/10	+1.3	15.2	
18.75	14/3,15/2,16/1, 17/3,18/1	10/10	+1.4	16.0	90
9.38	13/1,14/1,15/3, 16/1,17/1,18/1, 20/1,27/1	10/10	+1.9	16.0	90

Cpd. Sodium 3-[2-amino-4(3H)-oxopyrimidin-6-yl]-benzenesulphonate
(202), NSC 372960

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
03B131*	2	G		1	M	Q01DX09(1)	170484

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	06/1,15/2,16/1, 17/7,18/7,19/5, 20/6,21/3,22/3, 24/1,30/1,31/1, 34/1,35/1	40/40		19.0	100
106	06/1,16/1,18/1, 19/1,20/1,21/1, 22/1,23/1,25/1, 29/1	10/10		21.0	110
53	16/1,17/1,18/1, 19/2,20/2,21/2, 22/1	10/10		19.8	104
26.5	16/2,17/1,18/3, 19/1,25/1,26/1, 38/1	10/10		18.3	96
13.3	17/1,18/1,21/2, 23/1,25/2,26/1 27/1,28/1	10/10		24.8	130

Cpd. Sodium 3-[2-amino-5-nitro-4(3H)-oxopyrimidin-6-yl]benzenesulphonate
(203), NSC 372962

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
03B131	2	G		1	M	Q01DX09(1)	170484

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	06/1,15/2,16/1, 17/7,18/7/19/5, 20/6,21/3,22/3, 24/1,30/1,31/1, 34/1,35/1	40/40		19.0	100
103	17/1,20/4,24/1, 27/1,30/1,31/1, 40/1	10/10		25.0	131
51.5	16/2,17/1,18/1, 20/1,21/2,22/1, 23/1,27/1	10/10		20.8	109
25.8	14/1,16/1,17/2, 18/2,19/1,20/2, 21/1	10/10		18.3	98
12.9	15/1,17/2,18/1, 19/4,20/1,21/1				

Cpd. 2,5-Diamino-6-phenylpyrimidin-4(3H)-one

(213), NSC 363321

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
03B131*	2	G	M	1	M	Q01DX09(1)	040483

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	11/1,15/3,16/7, 17/6,18/6,19/1, 20/3,21/2,22/4, 26/1,28/1	35/35	+2.4	17.7	100
90	03/1,16/1,17/1, 18/2,19/1,20/1, 21/1,22/1,38/1	9/10	+1.3	19.0	107
45	15/1,16/1,17/1, 18/2,19/1,20/2, 21/2	10/10	+2.1	19.0	107
22.5	08/1,15/1,16/2, 17/1,18/2,21/1, 24/1,27/1	9/10	+2.3	17.8	100
11.3	15/2,16/3,17/2, 19/1,21/2	10/10	+2.1	16.8	94

Cpd. 2-Nitramino-6-phenylpyrimidin-4(3H)-one

(216), NSC 149029

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
03B131	2	G		1	M	Q01DX09(1)	170484

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont	06/1,15/2,16/1, 17/7,18/7,19/5, 20/6,21/3,22/3, 24/1,30/1,31/1, 34/1,35/1	40/40		19.0	100
88	15/2,16/3,17/2, 18/2,20/1	10/10		16.8	88
44	15/1,17/3,19/1, 20/1,21/1,22/1, 23/1,24/1	10/10		20.0	105
22	06/1,16/1,17/1, 18/2,19/1,21/1, 22/1,26/1,28/1	9/10		19.0	100
11	06/1,15/1,16/1, 17/3,18/3,41/1	9/10		17.3	91

Cpd. Sodium 3-(2-amino-5-bromo-4(3H)-oxopyrimidin-6-yl)benzenesulphonate (215), CCRG 84008

Test-Syst	Tis	Lvl	Veh	Rt	Sex	Schedule	Date
02M5A6	2	6	T	1	F	Q04DX04(1)	011283

Dose	Day of Death	Surv Tox	Wt Dif	Evaluation Result	T:C
Cont		5/5	+3.2	4.2	100
2000	02/5	0/5			
1000	02/3,04/1	1/5	+3.8	3.9	93
500		5/5	+2.4	3.6	86
250		5/5	+3.0	3.9	93
125		5/5	+4.2	4.2	100

APPENDIX 2
(Antitumour and haematopoietic data for
NMF, ABPP, and CY in combination therapy
aginst the M5076 sarcoma in BDF_1 mice)

(A) MATERIALS AND METHODS.

Antitumour assay

Asuspension of 10^6 M5076 sarcoma cells from a routine passage (maintained as a solid sc tumour in mice) was injected (day 0) im into the hind legs of groups of 10 female BDF_1 mice (18-23g). Mean tumour volumes were dtermined in the following manner. Tumour diameters were first measured with calipers and the volumes calculated using the following formula: volume = $(l \times w^2)/2$ where l represents the longest tumour diameter and w the diameter perpendicular to this axis . The volume of the leg without tumour is 0.1 - 0.2 cm^3 when mearsured in this manner. The density of the tumour is 1. Mice with tumours of 0.5 - 1.0g were randomised into groups 12 days after implantation of the tumour. All tumours were palpable on this day (day 12).

Drugs and Schedules

Drugs were administered in the following vehicles
NMF: Physiological saline
ABPP: As a suspension in 10% Tween 80 in physiological saline
CY: DMSO : Arachis oil (1:9)

Drugs were administered in the regimens shown in table 17

Table 17

Group	Dose mg/Kg/injection			Days of treatment		
	CY	NMF	ABPP	CY	NMF	ABPP
B		200			15-24	
C		400			15-24	
D			125			12, 16, 20, 24
E			250			12, 16, 20, 24
F	160			12		
G	320			12		
H	160			12, 15		
I	160	200		12	15-24	
J	160		125	12		12, 16, 20, 24
K		200	125		15-24	12, 16, 20, 24
L	160	200	125	12	15-24	12, 16, 20, 24
M		Control				

Peripheral blood cell counts

While BDF_1 mice bearing the M5076 sarcoma were under halothane anaethesia blood samples were collected from the tip of the tail into blood cell pipettes. Blood was diluted in a 1% actic acid - saline solution, stained with gentian violet and the white blood cells counted using a Weber B.S.A. improved Neubauer haemocytometer. For differential counts, cell smears were prepared and stained with Wright's solution. All mononuclear cells are presented as lymphocytes.

(B) KEY TO TABLES

Schedule	See appendix 1 for notation
Dose	mg/Kg/injection
Day	Days post tumour implantation
L	Lymphocytes
G	Granulocytes
TW	Tumour weight (g)
AW	Animal weight (g)

SCHEDULE	DRUG	DOSE	GROUP
Q01DX10(15)	NMF	200	B

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.7747	0.2172	22.5
14	10240	2939	63.3	36.7	10.19	6415	2007	3825	1562			
16	13360	2564	77.8	22.2	4.66	10343	1827	3017	973	1.5295	0.2545	22.0
18	9460	2368	85.2	14.8	6.22	8157	2347	1303	300			
20	11320	3521	75.6	24.4	10.19	8343	1787	2977	2035	1.1930	0.2096	21.8
22	11340	4010	62.0	38.0	6.78	6989	2670	4351	1680			
24	13320	6166	72.8	27.2	17.46	10119	6190	3201	1477	0.7929	0.2266	21.9
26	7940	1091	89.0	11.0	2.12	7074	1052	866	159			
28										0.4261	0.2475	22.8
30	10220	2424	80.6	19.4	5.03	8211	1895	2009	794			
32										1.5935	0.6040	23.5
34	10800	2006	52.2	47.8	8.35	5594	1118	5206	1510			
36										2.9061	0.4446	24.6
40										3.8308	0.3317	26.1
41	12100	2007	41.8	58.2	9.52	5004	1104	7096	1851			

SCHEDULE	DRUG	DOSE	GROUP
Q01DX10(15)	NMF	400	C

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.8121	0.2101	21.8
14	11080	2947	78.0	22.0	4.00	8675	2540	2405	639			
16	16520	3531	78.2	21.8	8.04	12937	3205	3583	1407	1.3877	0.3529	21.4
18	11325	3237	80.0	20.0	1.83	9045	2529	2281	730			
20	9860	2252	85.4	14.6	3.05	8454	2140	1406	258	0.8832	0.1800	20.8
22	11380	1858	77.8	22.2	3.03	8829	1331	2551	613			
24	11060	2490	76.6	23.4	21.71	8777	3403	2283	1417	0.4807	0.2405	20.0
26	8040	1626	52.2	47.8	15.91	4258	1545	3782	1173			
28										0.1500	ERR	22.0
30	6920	1799	80.8	19.2	6.61	5628	1673	1292	409			
32										0.5806	0.1849	23.0
34	9400	2508	51.6	48.4	9.13	4880	1567	4520	1374			
36										2.4304	0.3658	23.6
40										3.2307	0.5403	24.6
41	13200	2203	38.8	61.2	6.61	5095	1071	8105	1691			

SCHEDULE	DRUG	DOSE	GROUP
Q04DX04(12)	ABPP	125	D

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.9306	0.2188	21.5
14	11140	3341	77.2	22.8	9.09	8799	3218	2341	560			
16	9800	3526	39.8	60.2	10.62	4060	2266	5740	1822	1.5435	0.2079	21.8
18	10480	2233	66.0	34.0	10.07	6834	1405	3646	1419			
20	8900	1473	51.6	48.4	6.84	4518	376	4382	1265	2.2066	0.2999	23.0
22	10240	4151	61.8	38.2	10.89	6389	2964	3851	1970			
24	16120	5749	52.0	48.0	2.45	8419	3147	7701	2643	3.7291	0.5281	24.4
26	9760	2984	55.4	44.6	7.92	5431	1821	4329	1567			
28										5.2415	1.4478	26.1
30												
32												
34												
36												
40												
41												

SCHEDULE	DRUG	DOSE	GROUP
Q04DX04(12)	ABPP	250	E

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.8975	0.2529	22.1
14	11360	2168	71.8	28.2	7.56	8102	1447	3258	1263			
16	8244	2068	24.8	75.2	4.82	2123	762	6121	1402	1.5450	0.2922	21.7
18	9580	2774	63.2	36.8	8.81	6222	2384	3358	564			
20	8980	1773	53.4	46.6	7.02	4721	726	4259	1268	2.5040	0.4392	22.8
22	9780	3706	55.0	45.0	7.62	5448	2269	4332	1661			
24	14260	2568	54.4	45.6	6.23	7783	1858	6477	1273	3.1248	0.5300	24.1
26	9925	1287	47.5	52.5	3.87	4728	796	5197	631			
28										3.8935	0.4686	25.4
30	11600	3342	54.8	45.3	6.70	6425	2146	5176	1453			
32										5.0848	1.0026	26.6
34												
36												
40												
41												

SCHEDULE	DRUG	DOSE	GROUP
Q01DX01(12)	CY	160	F

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.8459	0.1689	22.1
14	1975	831	90.8	9.2	4.09	1808	799	167	105			
16	2760	1239	88.0	12.0	7.28	2395	966	365	356	1.2780	0.1947	21.1
18	9980	2617	38.8	61.2	3.70	3875	1070	6105	1620			
20	20280	6985	27.8	72.2	6.50	5740	2429	14540	4923	2.2060	0.4546	22.6
22	15200	3324	24.0	76.0	1.41	3675	936	11525	2405			
24	14880	3702	27.8	72.2	8.41	3964	806	10916	3509	3.1067	0.7556	24.8
26	8520	3600	41.8	58.2	9.04	3743	2116	4777	1588			
28										3.4373	0.5662	26.2
30	12980	3653	31.4	68.6	10.92	4349	2696	8631	1507			
32										4.3331	0.4274	27.6
34												
36												
40												
41												

SCHEDULE	DRUG	DOSE	GROUP
Q01DX01(12)	CY	320	G

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.8424	0.1388	20.7
14	1300	818	91.0	9.0	7.07	1337	457	113	49			
16	620	614	96.4	3.6	5.37	604	615	16	23	1.2146	0.2203	19.5
18	7280	2235	38.0	62.0	3.74	2732	761	4548	1547			
20	21920	7400	17.0	83.0	4.69	3872	2025	18048	5775	1.7728	0.4974	20.1
22	15800	4116	18.8	81.2	5.02	2967	1092	12833	3524			
24	15760	4221	19.4	80.6	6.88	2991	942	12769	3771	2.7206	0.5289	22.6
26	11060	4185	30.6	69.4	9.34	3455	2097	7605	2703			
28										2.9253	0.4264	23.4
30	10860	2967	24.4	75.6	9.24	2772	1379	8088	2018			
32										3.6587	0.5996	25.4
34												
36												
40												
41												

SCHEDULE	DRUG	DOSE	GROUP
Q03DX02(12)	CY	160	H

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.7996	0.1324	21.9
14	1120	492	95.6	4.4	4.77	1068	473	52	63			
16	840	479	95.2	4.8	5.22	800	460	40	42	1.3932	0.2272	21.6
18	1120	812	94.4	5.6	8.76	1002	623	118	207			
20	9400	2095	38.8	61.3	6.70	3713	1349	5687	1021	1.5909	0.2679	20.3
22	24680	4662	28.2	71.8	4.55	7046	2143	17634	3026			
24	16020	4563	23.6	76.4	9.13	3800	1625	12220	3776	2.9991	0.4605	23.2
26	18460	2858	19.2	80.8	3.42	3564	860	14896	2281			
28										2.8961	0.2236	25.0
30	12920	4953	28.0	72.0	4.74	3660	1716	9260	3489			
32										3.4850	0.5035	26.2
34												
36												
40												
41												

SCHEDULE	DRUG	DOSE	GROUP
Q01DX10(15)	NMF	200	I
Q01DX01(12)	CY	160	

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.8891	0.2397	21.2
14	1800	919	94.4	5.6	2.19	1697	860	103	65			
16	3360	1271	77.2	22.8	7.85	2544	826	816	565	1.1729	0.3367	20.2
18	8600	3083	39.2	60.8	3.42	3336	1220	5264	1897			
20	16000	3513	28.8	71.3	8.22	4323	1203	11052	3294	0.9631	0.2909	20.8
22	9700	2553	32.6	67.4	11.87	3339	1858	6361	1326			
24	14875	5658	23.8	76.3	4.19	3596	1510	11280	4213	0.7719	0.2614	21.6
26	8050	2244	54.4	45.6	10.48	4078	1016	3972	1577			
28										0.2618	0.1809	22.6
30	8740	2680	41.2	58.8	3.63	3647	1266	5093	1446			
32										1.3891	0.5258	23.0
34	10060	1503	41.0	59.0	8.25	4069	721	5991	1428			
36										2.2296	0.4935	24.0
40										3.6093	0.7512	25.1
41												

SCHEDULE	DRUG	DOSE	GROUP
Q01DX01(12)	CY	160	J
Q04DX04(12)	ABPP	125	

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.9670	0.2489	21.2
14	2860	1713	80.4	19.6	5.37	2267	1324	593	409			
16	2800	1174	75.6	24.4	9.21	2083	822	717	410	1.2470	0.3009	20.0
18	13320	2713	42.4	57.6	7.16	5621	1344	7699	1976			
20	25460	4086	9.0	91.0	2.24	2263	518	23197	3931	2.1613	0.4500	21.5
22	15760	4961	28.0	72.0	4.53	4565	1974	11195	3049			
24	14460	5470	30.0	70.0	5.39	4316	1561	10144	4127	3.1554	0.4479	23.3
26	9480	2325	47.0	53.0	7.91	4580	1927	4900	697			
28										3.2725	0.4251	24.3
30	11580	5213	27.0	73.0	6.28	3019	1132	8561	4241			
32										4.2018	0.3371	26.8
34												
36												
40												
41												

SCHEDULE	DRUG	DOSE	GROUP
Q01DX10(15)	NMF	200	K
Q01DX04(12)	ABPP	125	

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.9456	0.1824	21.5
14	9560	2896	69.6	30.4	7.37	6622	2034	2938	1109			
16	7720	1870	35.4	64.6	5.55	2697	647	5023	1399	1.4134	0.1773	21.3
18	12900	2705	75.4	24.6	3.36	9714	2039	3186	858			
20	7320	2970	39.8	60.2	11.17	3122	2116	4198	1180	1.0489	0.2289	21.1
22	10140	3030	79.2	20.8	7.01	8067	2676	2073	733			
24	14280	2974	84.0	16.0	3.67	12067	2962	2213	376	0.7796	0.2573	21.3
26	9420	2997	85.0	15.0	2.35	7997	2538	1423	500			
28										0.3818	0.1738	22.1
30	11200	3431	75.6	24.4	9.79	8484	2780	2716	1090			
32										1.1686	0.3408	22.9
34	10120	1789	67.2	32.8	7.53	6831	1508	3289	864			
36										2.5140	0.3207	23.7
40										3.7182	0.6114	25.9
41	14267	2982	41.7	58.3	7.51	6035	2029	8232	1434			

SCHEDULE	DRUG	DOSE	GROUP
Q01DX10(15)	NMF	200	L
Q01DX01(12)	CY	160	
Q04DX04(12)	ABPP	125	

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12										0.8735	0.1942	21.5
14	1680	1661	93.2	6.8	4.15	1519	1441	161	221			
16	1460	582	88.0	12.0	8.37	1302	593	158	133	1.2030	0.3065	20.1
18	11080	1953	43.8	56.2	4.55	4869	1094	6211	1099			
20	18825	7963	16.2	83.8	6.98	2562	1073	16263	7269	0.9179	0.1766	20.6
22	12180	3761	33.8	66.2	8.53	4179	1614	8001	2483			
24	10900	5130	30.0	70.0	4.00	3229	1481	7671	3727	0.8273	0.2261	21.8
26	8320	1996	55.6	44.4	17.95	4556	1659	3764	2047			
28										0.3663	0.2200	22.8
30	8460	1786	53.8	46.2	5.81	4601	1263	3859	679			
32										1.4509	0.5636	23.3
34	9300	3218	45.2	54.8	6.14	4310	1984	4990	1329			
36										2.5237	0.5976	23.9
40										3.8054	0.9324	26.5
41	9560	1053	37.2	62.8	5.89	3565	692	5995	782			

SCHEDULE	DRUG	DOSE	GROUP
	CONTROL		M

DAY	WBC/mm3 MEAN	WBC/mm3 SD	%L MEAN	%G MEAN	%G SD	L/mm3 MEAN	L/mm3 SD	G/mm3 MEAN	G/mm3 SD	TW(g) MEAN	TW(g) SD	AW(g)
12	8800	1104	75.0	25.0	5.06	6599	902	2201	552	0.9336	0.1429	21.4
14	9880	3569	68.8	31.2	4.92	6862	2791	3018	884			
16	11020	1141	63.4	36.6	9.79	7030	1479	3990	873	1.6271	0.3050	20.8
18	10700	2298	57.6	42.4	5.22	6196	1553	4504	970			
20	12980	1634	61.4	38.6	7.83	8014	1679	4966	882	2.5243	0.4748	22.1
22	10340	4522	55.8	44.2	7.16	5565	1902	4775	2673			
24	13375	5905	54.4	45.6	10.97	6513	2103	6863	3890	3.6424	0.6441	24.0
26	11820	3509	42.0	58.0	6.96	4964	1532	6856	2250			
28										4.6785	0.7720	25.8
30												
32												
34												
36												
40												
41												

(C) STATISICAL ANALYSIS

THREE WAY ANALYSIS OF VARIANCE

ABPP : 125mg/Kg Q04DX04(12)
NMF : 200mg/Kg Q01DX10(15)
CY : 160mg/Kg Q01DX01(12)

Total WBC for day 16
Summary table

Source Grp	Drug	SSQ	Mean SQ	DF	F Ratio
D	ABPP	9.505×10^{7}	9.505×10^{7}	1	26.52
B	NMF	2.88×10^{5}	2.88×10^{5}	1	8.034×10^{-2}
F	CY	1.242×10^{9}	1.242×10^{9}	1	346.5
K	ABPPxNMF	5.056×10^{7}	5.056×10^{7}	1	14.11
J	ABPPxCY	3.125×10^{7}	3.125×10^{7}	1	8.718
I	NMFxCY	1.25×10^{6}	1.25×10^{6}	1	0.3487
L	ABPPxNMFxCY	7.688×10^{6}	7.688×10^{6}	1	2.145
	Residual	2.581×10^{8}	3.585×10^{6}	72	
	Total	1.686×10^{9}			

$F(\alpha = 0.05)_{1,60} = 4.00$ $F(\alpha = 0.01)_{1,60} = 7.08$

Tumour Weight day 24
Summary table

Source Grp	Drug	SSQ	Mean SQ	DF	F Ratio
D	ABPP	0.08738	0.08738	1	0.413
B	NMF	134.6	134.6	1	636.0
F	CY	1.24	1.24	1	5.858
K	ABPPxNMF	3.695×10^{-6}	3.695×10^{-6}	1	1.746×10^{-5}
J	ABPPxCY	0.01953	0.01953	1	0.09231
I	NMFxCY	1.894	1.894	1	8.952
L	ABPPxNMFxCY	0.04656	0.04656	1	0.22
	Residual	15.23	0.2116	72	
	Total	153.1			

$F(\alpha = 0.05)_{1,60} = 4.00$ $F(\alpha = 0.01)_{1,60} = 7.08$

TUKEY'S TEST

Total WBC for day 18 (all groups)

Summary table (one way analysis of variance)

Var	SS	DF	MS
Total	1.75306573×10^9	119	
Trts	1.10303473×10^9	11	1.00275885×10^8
Error	6.50031×10^8	108	6.01880555×10^6

Variance ratio (F) = 16.6604294

$\sqrt{MS_{error}/n} = 775.81$

$q_{k(0.05)} = 4.81$ $\qquad$ $q_{k(0.01)} = 5.60$

Critical value (mean) = $q_k \cdot \sqrt{MS_{error}/n}$

Critical value $(mean)_{0.05} = 3732$
Critical value $(mean)_{0.01} = 4345$

Table of Means and Mean Differences

Group Mean	B 9460	C 11325	D 10480	E 9580	F 9980	G 7280	H 1120	I 8600	J 13320	K 12900	L 11080	M 10700
B 9460		1865	1020	120	520	2180	8340**	860	3860*	3440	1620	1240
C 11325			845	1745	1345	4045*	1025**	2725	1995	1575	245	625
D 10480				900	500	3200	9360**	1880	2840	2420	1820	220
E 9580					400	2300	8460**	980	3740*	3320	1500	1120
F 9980						2700	8860**	1380	3340	2920	1100	720
G 7280							6160*	1320	6040**	5620**	3800*	3420
H 1120								7480**	12200**	11780**	9960**	9580**
I 8600									4720**	4300*	2480	2100
J 13320										420	2240	2620
K 12900		* significant									1820	2200
L 11080		** highly significant										380
M 10700												

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