

**SYNTHESIS AND EVALUATION OF SOME
NITROGEN HETEROCYCLES AS
ANTIMALARIALS**

A Thesis
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To my Mother & Father

and

my brother Santi

for their encouragement to study in Australia

CERTIFICATE OF ORIGINALITY

The work described in this thesis was carried out by the candidate at The Australian National University. Where the work of others was employed or quoted, appropriate references are given.

Umyit Iravinyu,

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ABSTRACT

A large number of heterocyclic compounds has been prepared and tested for antimalarial activity.

Di-Mannich bases derived from 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)-phenol have been prepared from 5-trifluoromethylpyridin-3-amine through the new 4-chloro (and 4-hydroxy)-7-trifluoromethyl-1,5-naphthyridine followed by nucleophilic displacement of the chloro substituent with 2,6-di-Mannich bases of 4-aminophenol. Di-Mannich bases derived from 4-(7-trifluoromethylquinazolin-4-ylamino)phenol and 4-[7-chloro (and 7-trifluoromethyl)quinolin-4-ylamino]-3-methylphenol were prepared similarly.

Series of mono-Mannich bases such as 4'-chloro-3-(substituted amino)methyl-5-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]biphenyl-2-ols ("Tebuquine" analogues) and analogous compounds which incorporated the 7-trifluoromethyl (quinolin-4-yl and quinazolin-4-yl) substituents, and mono-Mannich bases derived from 4-[7-bromo (and 7-trifluoromethyl)-1,5-naphthylidin-4-ylamino]-5,6,7,8-tetrahydronaphth-1-ols have also been described.

The mefloquine analogue, α -(piperidin-2-yl)- α -(7-trifluoromethylquinolin-4-yl)-methanol has been prepared, and syntheses attempted for the α -[7-trifluoromethyl (and 7-bromo)-1,5-naphthyridin-4-yl] analogues.

Physical properties such as ^1H n.m.r. and ^{13}C n.m.r. (including two dimensional n.m.r. studies); some u.v. and i.r. spectra; and ionization constants have been determined and discussed.

All of these compounds were evaluated for antimalarial activity in a preliminary *in vitro* screen against the chloroquine-sensitive strain of the human malaria *Plasmodium falciparum* (the FCQ-27 isolate) using the incorporation of [^3H]-hypoxanthine as a measure of parasite growth. Some of the more active compounds were also tested using a morphological technique in an *in vitro* test against both chloroquine-sensitive and

chloroquine-resistant (K-1) isolates, respectively, of *P. falciparum*; and the suitability of the visual microtest technique as a preliminary screen was also investigated.

Selected compounds were also examined for activity *in vivo* against *P. vinckei vinckei* in mice.

The most active compounds in the *in vitro* tests against the FCQ-27 isolate of *P. falciparum*, were the di-Mannich bases derived from 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol and 4-(7-chloroquinolin-4-ylamino)-3-methylphenol. In comparative tests against the FCQ-27 and K-1 isolates, no significant differences were observed for each compound examined.

The *in vivo* tests against *P. vinckei vinckei* in mice revealed that some of the di-Mannich bases derived from 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol and the "Tebuquine" analogues which incorporated the 7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-yl substituent were effective when administered in a single dose of 200 mg/kg.

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CHAPTER I

CHAPTER I Introduction

I-1 Malaria: Historic background

The word "malaria" comes from the Italian *mal'aria*, meaning bad air, because it was thought to result from bad air issuing from swamps and marshy ground. It was also known as *Ague*, *Jungle Fever*, *Marsh Fever* or *Periodic Fever*. In 1880, the causative agent of malaria was discovered by Alphonse Laveran¹ when he observed the presence of minute parasites in the corpuscles of blood of soldiers in Algeria. Golgi² in 1889 produced clear evidence of the existence of multiple species of malaria parasites by defining the characteristic periodicity of the fever in relation to the rupture of schizonts in the blood.

The curative property of the bark of the Cinchona tree (now known to contain quinine) was exploited for the treatment of malaria at least 350 years ago, long before the discovery of the causative agent. Quinine is still used to-day as a life-saving medicant in severe cases of *Plasmodium falciparum*. An old Chinese herbal treatment, using Qinghao (a febrifugine) known now to contain Qinghaosu or Artemisinin,^{3,4} although toxic, was also used for the treatment of malaria in that part of the world.

In the early part of the 20th century considerable effort, particularly by German workers, was directed at the synthesis of compounds for evaluation as antimalarials to replace or supplement quinine. Pamaquine was the first of many successful synthetic antimalarials such as chloroquine, amodiaquine and more recently mefloquine.

The widespread use of chloroquine for the treatment of malaria in Latin America, South-east Asia, the South-west Pacific and Africa has led to the widespread development of chloroquine resistance. This was recognised in the early 1960s, and it is caused by spontaneous mutation of the malaria parasites.^{5a} Quinine retains a high level of efficacy for the control of *P. falciparum* and the radical cure of chloroquine-resistant infections. The development of drug resistance by malaria parasites has led to the use of drug combinations, such as pyrimethamine with sulphadoxine (and later sulphalene) for radical cures of such infections.⁶⁻⁸

Concern about drug resistance in the last three decades has stimulated research directed towards the development of malaria vaccines.

Malaria presently occurs principally in the tropical areas of Africa, Asia (and the Pacific) and Central and South America. The distribution as at 1988, provided by the World Health Organisation in 1990,⁹ is indicated in Figure I-1.

According to WHO,¹⁰ about 100 million people are clinically ill with malaria at any given time and of the four species which affect humans (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*); *P. falciparum* is responsible for the most severe infections and the highest mortality rates.

I-2 The Malaria Parasites

I-2.1 Introduction

In 1898, Sir Ronald Ross¹¹ was able to demonstrate the genus *Anopheles* as the insect in which development of the malaria parasite takes place. All malaria parasites are members of the subphylum *Sporozoa*, the family *Plasmodiidae*, and the genus *Plasmodium*. For convenience, the genus is split into a number of subgenera; there are three in mammals (*Plasmodium*, *Laverania* and *Vinckeia*), four in avians (*Haemamoeba*, *Giovannolaia*, *Novyella* and *Huffia*), and two in reptiles (*Carinamoeba* and *Sauramoeba*). There are at least one hundred species of malaria parasites, of which only four species occur in humans i.e. *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The first species belongs to the subgenus *Laverania*, and the latter three species belong to the subgenus *Plasmodium*.

P. falciparum and *P. vivax* are the most important and widespread infections in man. *P. malariae* is also widespread, but more patchy in distribution than *P. falciparum* or *P. vivax*. *P. malariae* is most common in parts of Central and North Africa, and it also occurs in India, Malaysia, Indonesia, Papua New Guinea and parts of South America. *P. ovale* primarily occurs in tropical Africa, and also in South-east Asia and Papua New Guinea.

Another subgenus that occurs in mammalian parasites is *Vinckeia*, which includes many species such as *P. berghei* and *P. vinckei*; most of these occur in lower mammals, such as rats, mice and bats.

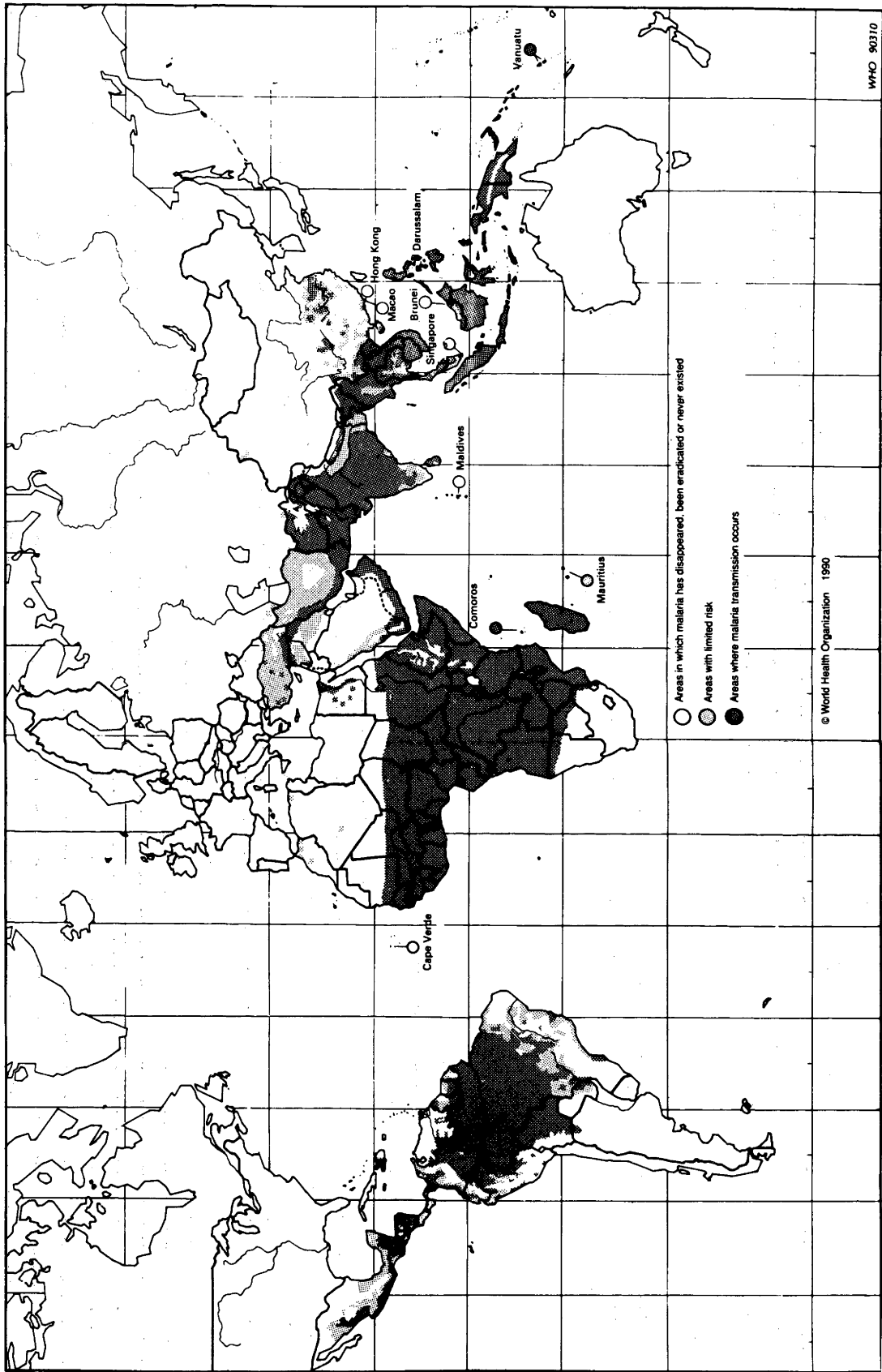


Figure I-1 Epidemiological assessment of the status of malaria, 1988

In nature, malaria parasites have two hosts, a mosquito and a vertebrate, and malaria parasites are transmitted by female anopheline mosquitoes.

I-2.2 General life cycle of human malaria parasites

A concept of the life cycles of the *Plasmodium* species is not only essential knowledge in chemotherapy, but also in epidemiology, pathogenesis and immunology. Different groups (mammalian, avian and reptilian) of malaria parasites, of course, have different life cycles.

The life cycle of *Plasmodium* consists of three different phases or cycles of development (Figure I-2).^{12a} Two types of host are involved, mosquitoes and vertebrates (i.e. man). The sexual phase occurs in the mosquitoes, and two asexual cycles occur in the vertebrate, one in blood cells and the other in liver tissues. Both exo- and intra-erythrocytic stages are developed in the vertebrate host. The male and female gametocytes are also produced in the erythrocytes, and malaria pigment (haemazoin) is formed during the intra-erythrocytic stages in man.

I-3 Investigations of Malaria Vaccines: Future Prospects

The spread of drug-resistant *P. falciparum* in many areas, and the occurrence of severe epidemic outbreaks of this disease, lend particular urgency to recent attempts to develop a malaria vaccine.¹³

In recent years, since the development of the first *in vitro* cultures by Trager and Jensen¹⁴ in 1976, the contribution made by various techniques for purification and characterisation of antigens (such as chromatography, affinity chromatography, electrofocalisation, HPLC, autoradiography, etc), and lastly a better knowledge of laboratory animals suitable for use in the study of human malaria,^{15,16} have helped to make some promising progress in vaccine investigation.

Theoretically, against the *Plasmodium* species, four types of vaccines are possible: those affecting sporozoites, exoerythrocytic forms, asexual blood forms, and gametocytes.

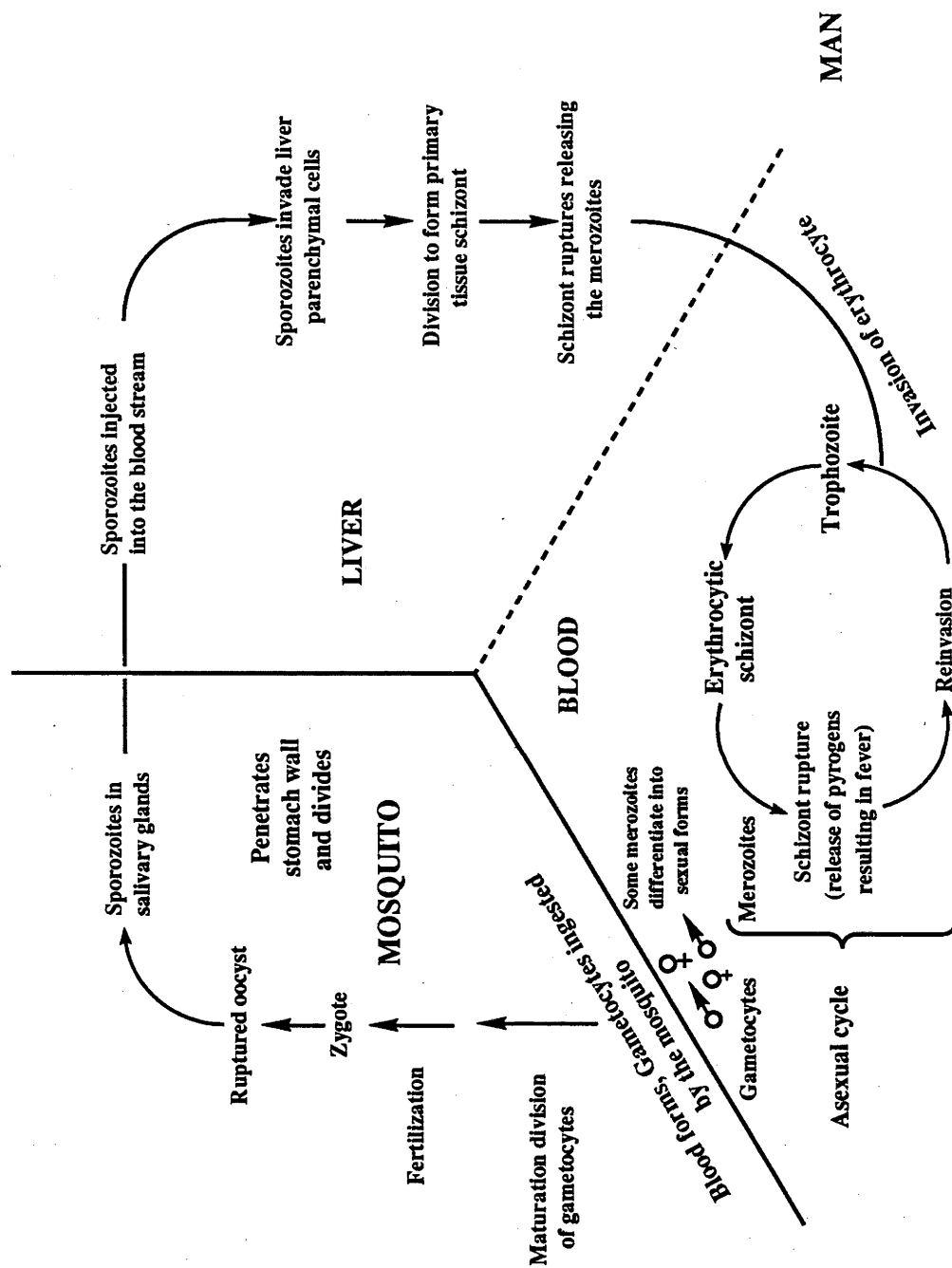


Figure I-2 Schematic diagram of the life cycle of the malaria parasite^{12a}

In practice, three types of vaccines have been investigated, an anti-sporozoite vaccine to abort the infection at its source,¹⁷ an anti-merozoite or anti-blood stage vaccine to prevent the clinical disease,¹⁸ and an anti-gamete vaccine which would block transmission.¹⁹

Efforts to develop a malaria vaccines to 1983, have been reviewed.²⁰ In 1984, successful cloning of the genes coding respectively for surface antigens of *P. knowlesi*²¹ and *P. falciparum*,²² and of the genes expressing *P. falciparum* schizont and merozoite-specific polypeptides²³ were reported.

Despite much research effort directed to the development of malaria vaccines, no successful vaccine has yet been developed. Two articles published in 1990^{10,24} discuss this situation at some length.

I-4 Mode of Antimalarial Drug Action

I-4.1 Definition of "chemotherapy"

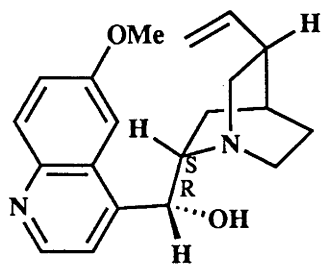
There is a number of definitions of the term "chemotherapy". Dr Frank Hawking,²⁵ defined chemotherapy as: "The search for chemical compounds which will destroy infective parasites or an organism without destroying their animal host, and employment of such compounds to corresponding diseases".

I-4.2 Development of antimalarial drugs

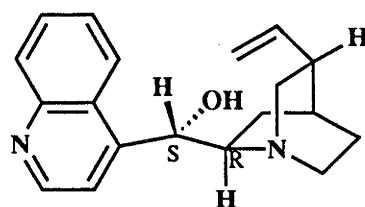
The bark of the Cinchona tree was reputed to have introduced into Europe (from South America) about 1630-1640. It was shown by the French chemists, Pelletier and Caventou^{26,27} in 1820, to contain the alkaloids quinine (**I.1a**) and cinchonine (**I.1b**). The interesting history of quinine has been reviewed by many authors including McHale.²⁸

In 1891, Guttman and Ehrlich,²⁹ were the first to attempt to treat patients suffering from malaria with a synthetic dyestuff, methylene blue (**I.2**).

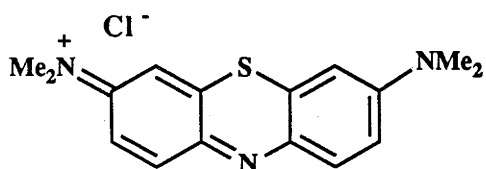
Stimulated by the lack of quinine during World War I, German workers, in 1925 produced the first synthetic antimalarial compound, pamaquine (**I.3**).³⁰ This was closely



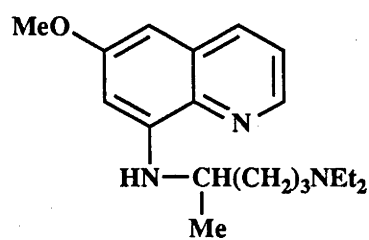
I.1a



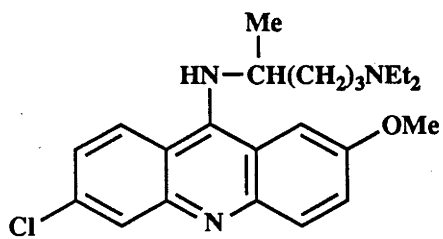
I.1b



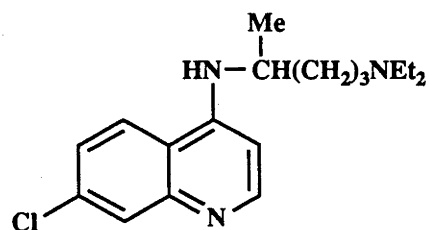
I.2



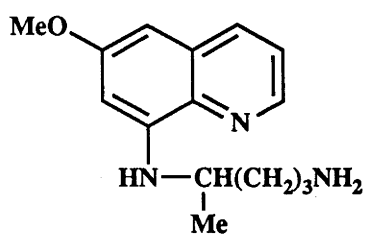
I.3



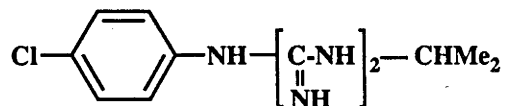
I.4



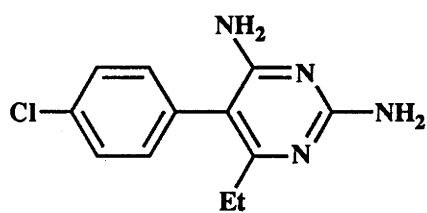
I.5



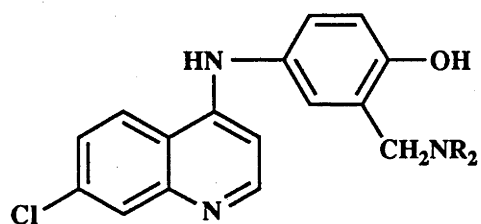
I.6



I.7



I.8

I.9 a; NR₂=NEt₂b; NR₂=pyrrolidin-1-yl

followed, in 1930, by mepacrine (quinacrine or Atebrin) (I.4) which was the first synthetic blood schizontocidal drug.³¹

Chloroquine (I.5), first known as Resochin, was prepared in 1934 by Andersag *et al.*³² and was released as a commercial antimalarial about 1946. It is generally considered as one of the most fascinating, useful and versatile antimalarial drugs.

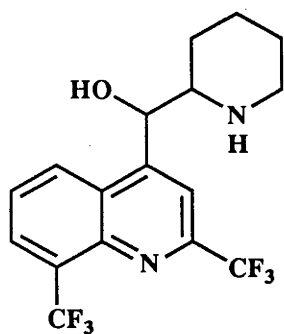
During World War II, antimalarial drug research was actively promoted in Germany, United States, United Kingdom and in Australia. This programme ultimately yielded the 4-aminoquinolines, such as chloroquine (as mentioned above) as blood schizontocidal drugs, and the improved 8-aminoquinolines, such as primaquine (I.6) as tissue schizontocidals. British scientists also developed dihydrofolate reductase-inhibiting compounds, such as proguanil (i.e. Paludrine, I.7)³³ and pyrimethamine (Daraprim, I.8),³⁴ which became widely used.

Amodiaquine (I.9a), a Mannich base-type antimalarial and related compounds (I.9b), were also developed³⁵⁻³⁷ in which the 7-chloroquinoline nucleus was attached through the amino group to a mono-Mannich base derivative of *p*-aminophenol.

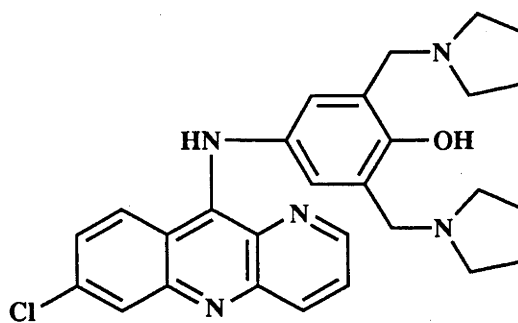
Schmidt *et al.*³⁸ highlighted the advantage of amodiaquine (I.9a) and amopyroquine (I.9b) against chloroquine-resistant strains of *P. falciparum* in *Aotus* monkeys.

During the 1950s and 1960s, superior antimalarial drugs, such as chloroquine, were employed on a massive scale for suppressive treatment, and this led to the development of drug resistance in the major human parasite, *P. falciparum*. Events in South-east Asia caused the US Army Research and Development Command, based on the Walter Reed Army Institute of Research (WRAIR), since 1964, to carry out a massive antimalarial research development programme. This involved the primary screening of over 300,000 candidate compounds and basic studies of the biology and immunology of malaria.

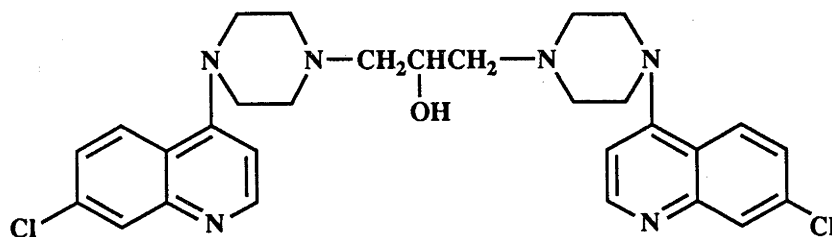
From this programme, a new antimalarial drug, i.e. mefloquine (I.10), a quinoline methanol, was developed. It was subjected to clinical trials in areas where multiple-resistant *P. falciparum* is a major problem such as in South-east Asia³⁹ and South America.⁴⁰ This drug has a long half-life in man, and it is now commercially available.



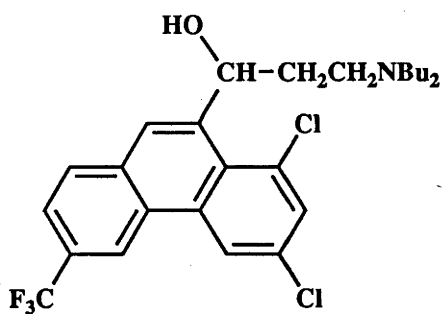
I.10



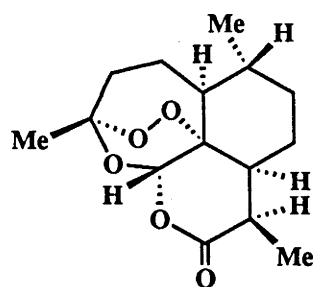
I.12



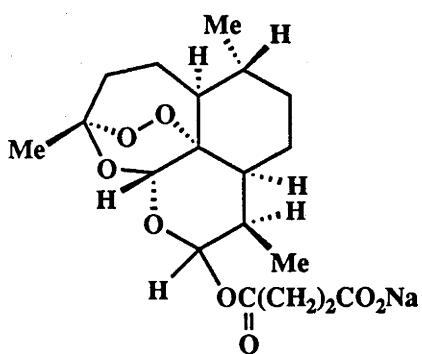
I.11



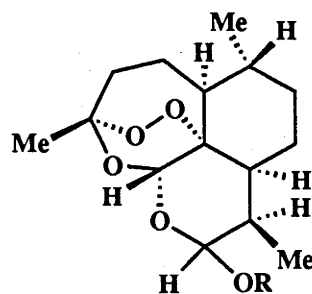
I.13



I.14



I.15

I.16 a; R=Me
b; R=H

More recently, new compounds such as hydroxypiperaquine (**I.11**), pyronaridine (**I.12**) and halofantrene (**I.13**) have been subjected to clinical trials.⁴¹⁻⁴⁴ Halofantrene is now in use in the Francophone regions of West Africa.

Qinghao, a constituent of the plant *Artemisia annua*, has been used by the Chinese as an antimalarial since 168BC.^{5b} In 1972 the chemical structure (**I.14**) of the active component Qinghaosu or artemisinin was determined⁴ as an endoperoxide of a sesquiterpenoid lactone; quite different from any other human antimalarial agents. This compound is a highly potent blood schizontocide, but it has poor solubility. Semi-synthetic derivatives have been prepared and evaluated. Two of these, artesunate (**I.15**) and artemether (**I.16a**) are more soluble and active, and artesunate is currently being developed for further clinical trials in the treatment of cerebral malaria^{3,45} (one of the more severe complications seen in patients ill with *P. falciparum* malaria).

I-4.3 Terminology and site of drug action

Expressed simply, the specific actions of antimalarials can be summarised as in Figure I-3.

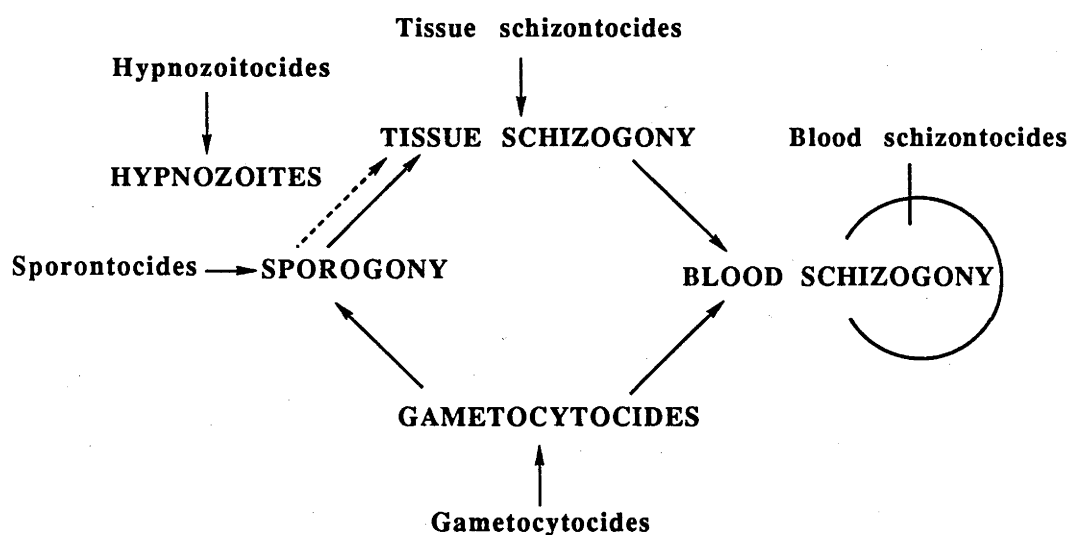


Figure I-3 Diagram of specific actions of antimalarials

Terminology and site of drug action are explained further as following:

Blood schizontocide (erythrocytic schizontocide) - refers to a drug that destroys asexual parasites in the blood.

Tissues schizontocide (exoerythrocytic schizontocide) - refers to a drug which destroys asexual parasites in the tissues. It was commonly divided into 'primary' and 'secondary' tissue schizontocides:

- a. *Primary tissue schizontocide* - denotes a drug which acts on pre-erythrocytic (primary exoerythrocytic) forms.
- b. *Secondary tissue schizontocide* - denotes a drug which acts on secondary exoerythrocytic forms.

Gametocytocide - refers to a drug which destroys the sexual forms of the parasite in the blood.

Sporontocide - refers to a drug which acts on the sporogonic forms in the mosquito after having fed on a treated host.

The activity of the major antimalarial drugs against different forms of *P. vivax* and *P. falciparum* are summarised in Table I-1 .

I-5 Mechanism of Antimalarial Drug Action

The compounds listed in Table I-1 possess different mechanisms of action. Some of these are discussed below under the headings folate pathway antagonists, blood schizontocides, antibiotics, Qinghaosu, naphthoquinone derivatives and tissue schizontocides.

I-5.1 Folate pathway antagonists

(a) Sulphonamides (I.17) and sulphones (I.18)

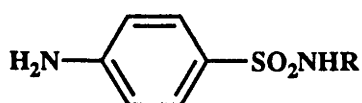
These drugs act on all multiplying stages in the life-cycle of the malaria parasites. In animals, they act on both tissue and blood schizogony, and thus effect only the asexual blood cycle. In the insect vector they act on sporogony.

Table I-1 Activity of the major antimalarial drugs against different forms of *P. falciparum* and *P. vivax*^{12a}

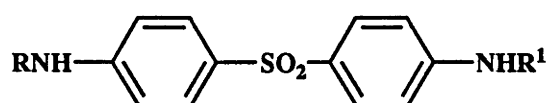
Drug	Blood schizontocidal activity		Tissue schizontocidal activity	Gametocidal activity	
	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. vivax</i>
Chloroquine	+	+	-	-	-
Mefloquine	+	+	-	-	-
Quinine	+	+	-	-	±
Primaquine	-	±	+	+	+
Pyrimethamine	+	+	-	-	-
Sulfonamides and sulfones	+	±	-	-	-
Tetracyclines	±	?	?	?	?

+ active, - inactive, ± slightly active, ? effect unknown

Sulphonamides are not used alone, but usually used in combination with dihydrofolate reductase inhibitors, such as pyrimethamine (I.8) with which they are synergistic, and effect the production of tetrahydrofolic acid which is necessary for the synthesis of malarial DNA.



I.17



I.18

p-Aminobenzoic acid (PABA) is an essential growth factor for both *P. berghei*⁴⁶ and *P. falciparum*.⁴⁷ The effectiveness of sulphonamides as antimalarials is due to their competition with PABA (antagonist of PABA) for binding sites to the enzyme, dihydropteroate synthase.⁴⁸ This effect is indicated in Figure I-4.

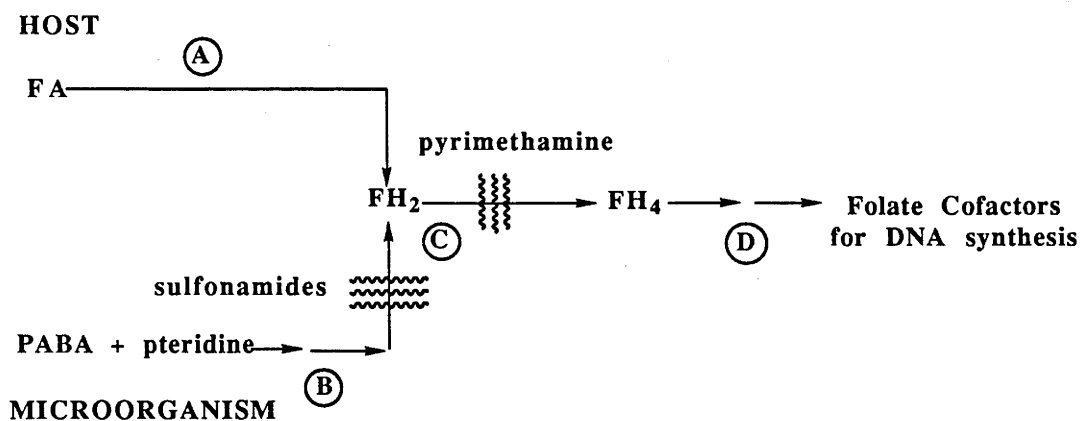


Figure I-4 Folate cofactor biosynthesis pathway⁴⁸

FA, folic acid; FH₂, dihydrofolate; FH₄, tetrahydrofolate.

Wavey lines indicate loci of action of inhibitors.

- (A) Folate reductase;
- (B) PABA + pteridine condensing system;
- (C) Dihydrofolate reductase;
- (D) Systems for addition of 1-carbon units to FH₂

(b) Dihydrofolate reductase inhibitors⁴⁹

Dihydrofolate reductase (DHF) is a key enzyme in folate metabolism as indicated in Figure I-4. Both parasite and host convert dihydrofolate to tetrahydrofolate using dihydrofolate reductase. 2,4-Diaminopyrimidines, such as pyrimethamine and the triazines, such as cycloguanil, are considered as DHF inhibitors. These drugs bind more tightly to plasmodial DHF than to that of host tissue.⁵⁰ Some relevant data are given in references.⁵¹⁻⁵³

I-5.2 Blood schizontocides: chloroquine and related compounds

The mechanism(s) of action of chloroquine have been reviewed recently by Howell⁵⁴ and by Wernsdorfer and Trigg.^{55a} Two proposals are discussed below.

(a) *Proposed mechanism of action: pigment clumping*

Blood schizontocides such as chloroquine (I.5), amodiaquine (I.9a), quinine (I.1a) and mefloquine (I.10) exhibit effects on the erythrocytes of malaria parasites. These compounds have been divided into two groups, on the basis of chemical structure and the effects on the morphology of malaria parasites.^{12b} The first group, exemplified by chloroquine, causes swelling and fusion of the adjacent digestive vacuoles,⁵⁶ followed by encirclement of the membrane to give an antophagic vacuole^{57,58} (so called chloroquine induced pigment clumping, CIPC⁵⁹) which causes the aggregation of hemozoin pigment. Peters,⁶⁰ and Warhurst and Thomas⁵⁹ have proposed that the clumping disrupts the production of amino acids required by the parasite.

O'Brien and Hahn⁶¹ have proposed that the quinoline ring of chloroquine intercalates between base pairs of deoxyribonucleic acid (DNA), and Marquez *et al.*⁶² suggests that the conformation of the quinoline and phenol rings of amodiaquine may be important with respect to its interaction with DNA.

The second group exemplified by quinine and mefloquine, do not cause pigment clumping⁵⁹ but morphological change can be observed, such as swelling and vesiculation of outer parasite membranes, swelling of the digestive vacuoles and decrease of their pigment density which is then followed by cytoplasmic degeneration and vacuolisation.

Davidson *et al.*^{63,64} could find no evidence for intercalation of mefloquine (and its analogues) with DNA but they did find that mefloquine binds weakly to DNA phosphate groups. Mefloquine is an active competitive inhibitor⁵⁹ of chloroquine-induced pigment clumping (CIPC), with an affinity for the "clumping site" of 100 times that of quinine.

(b) *Chloroquine-ferriprotoporphylin IX (FP)-complex formation*

Fitch and his coworkers⁶⁵ have proposed that within *P. berghei*, chloroquine complexes with ferriprotoporphylin IX (FP)⁶⁶ (a degradation product of haemoglobin within the parasites). This ultimately leads to damage to the parasite membrane as shown in Figure I-5.

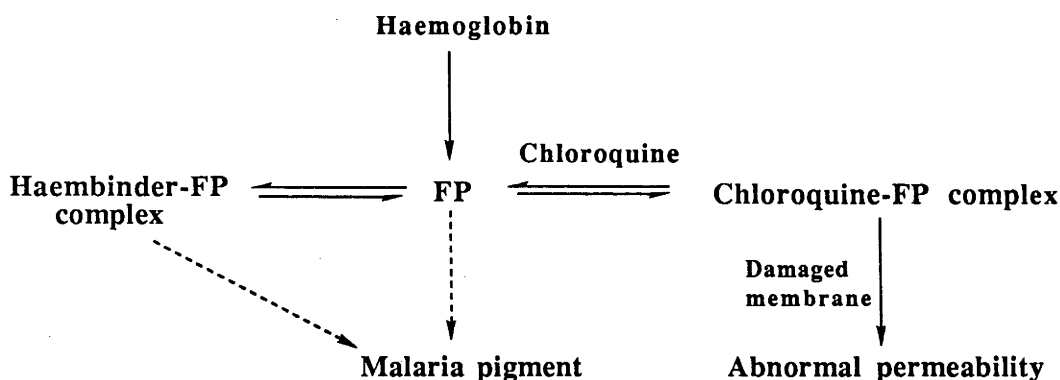


Figure I-5 Diagrammatic representation of a hypothesis to explain the mode of action of chloroquine as an antimalarial drug

I-5.3 Antibiotics

The first reports of an antimalarial effect by antibiotics appeared in 1949, when chlortetracycline was found effective against avian and human malaria parasites.^{67,68} Coatney and Greenberg⁶⁹ in 1952 further reported that only nine of 31 antibiotics tested showed any antimalarial activity. The antibiotics, chlortetracyclines, oxytetracycline and chloramphenicol, have blood schizontocidal, as well as causal prophylactic activity, and are of potential value for malaria chemotherapy. However they are relatively slow acting compared to chloroquine. Other antibiotics such as clindamycin, lincomycin, minocycline, doxycycline and erythromycin also have been shown to have blood schizontocidal effects. Minocycline and doxycycline have useful activity against the liver stages,^{55b} and erythromycin⁷⁰ has a synergistic effect with chloroquine against chloroquine-resistant *P. berghei*.

The mode of action of antibiotics in malaria has not yet been established. Their primary mode of action in bacteria is by inhibition of ribosomal protein synthesis.⁷¹

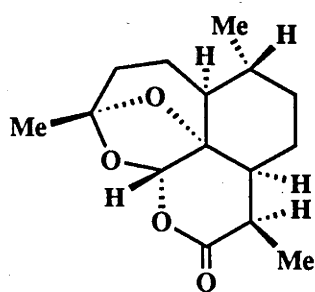
I-5.4 Qinghaosu and its derivatives

Qinghaosu (I.14) (artemisinin, qing hau sau, QHS), meaning active principle of qing hao, is a novel antimalarial drug, and it was isolated in 1971 from the leafy portions of the traditional Chinese herbal remedy, Qinghao (*Artemisia annua*). QHS is a sesquiterpene lactone that bears a peroxide group and; unlike most other antimalarials, lacks a nitrogen-containing heterocyclic ring system. It shows marked activity against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. In 1987, Luo and Shen reviewed the chemistry, pharmacology and clinical application of Qinghaosu and its derivatives.⁷²

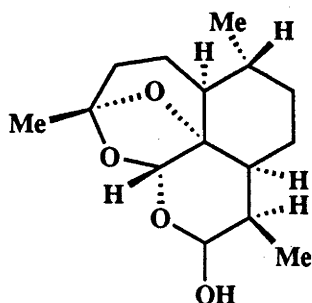
Derivatives of QHS, such as artemether (I.16a); dihydroqinghaosu (I.16b), and the water-soluble sodium artesunate (I.15), appear to be more potent than QHS itself.

The actions of this novel drug are different from other antimalarials in that it has no detectable prophylactic effects on the preerythrocytic stages,⁷³ no competition with CIPC inhibition^{73a} as well as no interference with the folic acid metabolism of the parasite.^{73,74} Gu and co-workers⁷⁵ have suggested that the prevention of protein synthesis may be the primary target of the QHS family of drugs, rather than the inhibition of nucleic acid synthesis. These workers⁷⁵ showed that the incorporation of tritiated isoleucine (as essential exogenous amine) into protein by *P. falciparum* was inhibited by QHS.

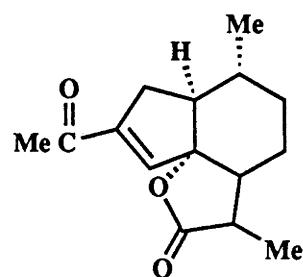
Another possible mechanism may involve the peroxide part of the molecule (see compound I.14). It has been shown³ that QHS (I.14) gives the three metabolites (I.19, I.20, and I.21) (which lack the peroxide moiety), and these are inactive against *P. berghei* in mice.



I.19



I.20

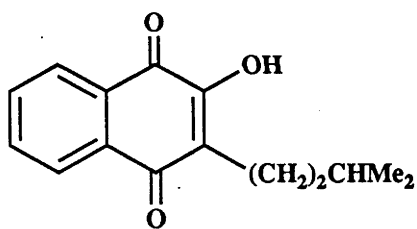


I.21

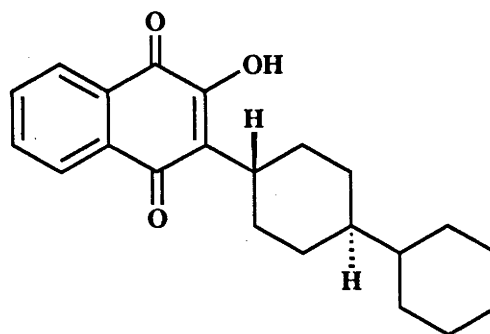
I-5.5 Naphthoquinones

In the early 1940s, the hydroxynaphthoquinones,⁷⁶ such as hydrolapachol (**I.22**), originally prepared by Hooker⁷⁷ in 1936, were found to have antimalarial properties.⁷⁸ Structure-activity relationships among the naphthoquinones against *P. lophurae* in ducks have been summarised by Fieser *et al.*⁷⁸ As the alkyl chain of hydrolapachol was lengthened, the activity of the resulting compounds increased to a maximum at nine carbon atoms and thereafter decreased. Other groups such as alicyclic or aryl groups appeared to shift the peak activity. Compound **I.23** was the most potent compound examined in this work, and the trans-isomer (depicted) was 13 times more potent than its cis-isomer.

Naphthoquinones show activity against both primary tissue and blood forms, and could have a role to play as causal prophylactic drugs. The mode of action of these drugs is unknown, but inhibition of the plasmodial electron transfer process has been proposed.^{79,80}



I.22



I.23

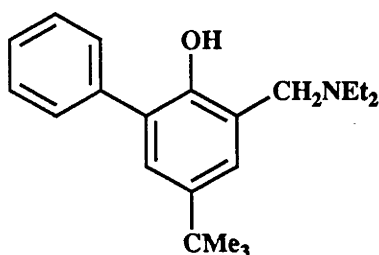
I-5.6 8-Aminoquinolines as tissue schizontocides

The 8-aminoquinoline, pamaquine (**I.3**)^{30,81} was the first effective synthetic antimalarial. Primaquine (**I.6**),⁸² in fact is clinically effective against the persistent tissue stages of *P. vivax* or *P. ovale*. This drug acts on all stages of the malaria life-cycle, but is generally only used as a tissue schizontocide and a gametocytocide, because of its significant toxicity in man and its limited blood schizontocidal activity.

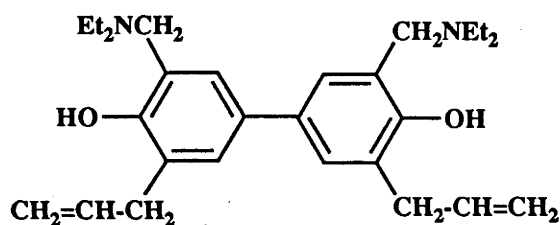
The molecular mechanism of the mode of action of primaquine is unknown. Nevertheless, this drug has been shown to cause mitochondrial swelling in both the exoerythrocytic⁸³ and erythrocytic stages⁸⁴ of malaria parasites. New tissue schizontocidal antimalarial drugs have been reviewed by Davidson *et al.*⁸⁵

I-6 Mannich Bases as Potential Antimalarial Agents

Burckhalter and his colleagues⁸⁶ in 1946 reported the synthesis of a series of Mannich base compounds, of which compound **I.24**, 5-*t*-butyl-3-diethylaminomethyl-biphenyl-2-ol (SN 7,744) and compound **I.25** (bialamicol, SN 6,771) showed antimalarial activity. This work was later extended to heterocycles as detailed below.



I.24



I.25

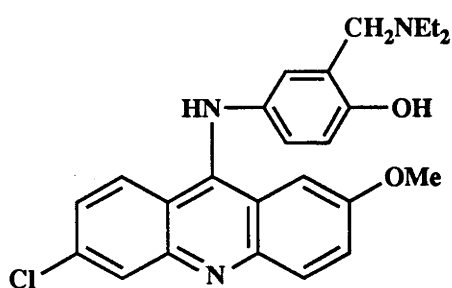
I-6.1 Di-Mannich (and mono-Mannich) base derivatives of 4-(quinolin-4-ylamino)phenols

In 1948, Burckhalter and coworkers³⁵ reported the synthesis of amodiaquine (SN 10,751; trade name, camoquin)³⁶ (**I.9a**), which joined the 7-chloroquinoline portion present in chloroquine and quinine, through a nitrogen atom at position 4 to a Mannich base of a phenol. This compound exhibited high antimalarial activity as did its analogue (SN 8,617) (**I.26**)³⁵ [which proved to be as active as quinacrine (SN 390) (**I.4**)].

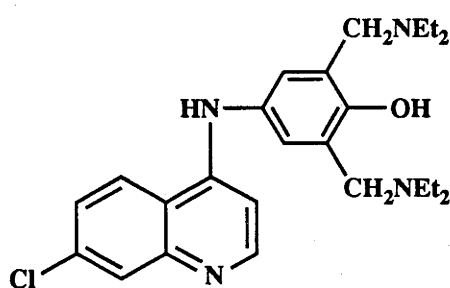
Singh and coworkers^{87,88} and Elslager and coworkers⁸⁹ (and others) have studied modifications to the side chain and to the quinoline ring system respectively in analogous compounds.

The di-Mannich compound, cycloquine (Haloquine)⁹⁰ (I.27) was synthesised and widely used as an antimalarial in the USSR in 1965.

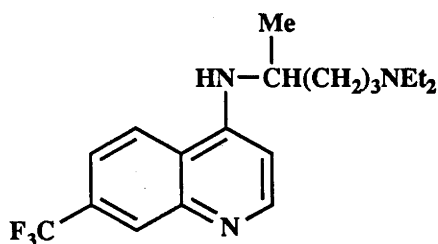
It was also shown⁹¹ that when the 7-chloro substituent of chloroquine was replaced by a trifluoromethyl group, the resulting compound (I.28) retained potent antimalarial activity. Various 7-trifluoromethylquinolinemethanols such as compound I.29 have been prepared⁹² and evaluated for activity against *P. berghei* in mice. The test data indicated that the 7-trifluoromethyl compounds such as I.29 were more potent than the chloro analogue; the curative dose of compound I.29 was 40 mg/kg.



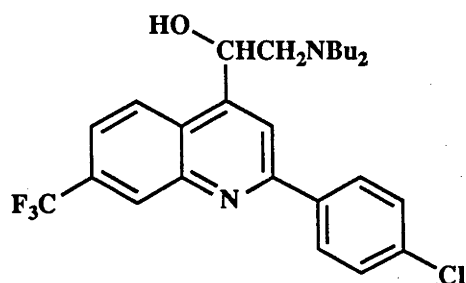
I.26



I.27



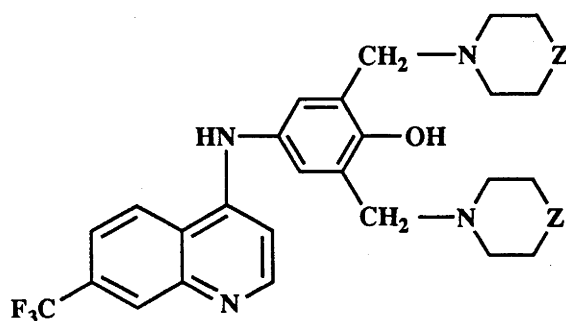
I.28



I.29

In the mid 1980s, two independent groups of workers, Shen *et al.*⁹³ and Barlin *et al.*^{94,95} reported the synthesis of Mannich base derivatives of 4-(7-trifluoromethylquinolin-4-ylamino)phenol and studies of their antimalarial activity. Shen *et al.*⁹³ found that the compound I.30 and I.31 suppressed *P. berghei* infections in mice at a dosage of 2.5 mg/kg, whereas Barlin *et al.*⁹⁴ found that compound I.30 (and others) were highly effective against *P. vinckei vinckei* in mice.

Hu *et al.*⁹⁶ and Scott *et al.*^{97,98} have shown independently that the above compounds are also effective against a chloroquine-resistant ANKA strain (EC123) of *P. berghei* in mice.⁹⁶ Scott *et al.*^{97,98} have studied the effect of their compounds on a chloroquine-sensitive (FCQ-27) and a chloroquine-resistant (K-1) strain of *P. falciparum* *in vitro*, and many were found to be highly effective. For example, compound **I.30** gave IC₅₀ values of 0.7 and 1.1 nmol l⁻¹ against the FCQ-27 and K-1 strains, respectively.



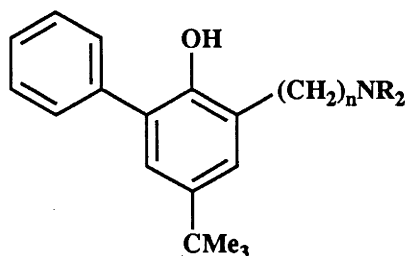
I.30 ; Z=CH₂
I.31 ; Z=O

I-6.2 Mono-Mannich base derivatives of 5-(quinolin-4-ylamino)biphenyl-2-ols

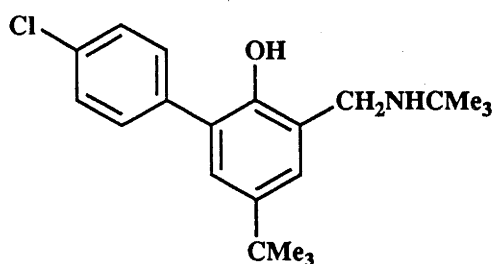
In 1969, prompted by the antimalarial activity of Mannich base derivatives of 5-*t*-butylbiphenyl-2-ols⁸⁶ such as **I.24** (SN 7,744) and **I.32** (n=1, R=Me) (SN 7,282) and amodiaquine (**I.9a**),³⁵ Duncan and Henry⁹⁹ further investigated the synthesis and biological activity of biphenyl-2-ols (**I.32**). Compound **I.32** (n=1, R=Me) was the most active member of this series, and the activity decreased as "n" was increased. The compound **I.32** (n=2, R=Me) possessed significant antimalarial activity, but it was less potent and more toxic than **I.32** (n=1, R=Me). The demethyl analogue **I.32**, (n=2, R=H) was completely inactive.

Compounds WR-194,965 (**I.33**) and WR-204,165 (**I.34**), synthesised subsequently, had similar levels of efficacy to compound **I.32** (n=1, R=Et) in owl

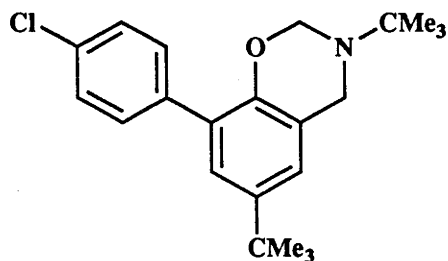
monkeys infected with the multidrug-resistant "Vietnam Smith" strain of *P. falciparum*,¹⁰⁰ and they did not show acute toxicity at 640 mg/kg.



I.32



I.33



I.34

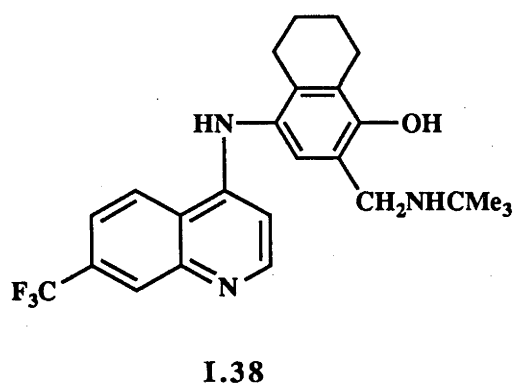
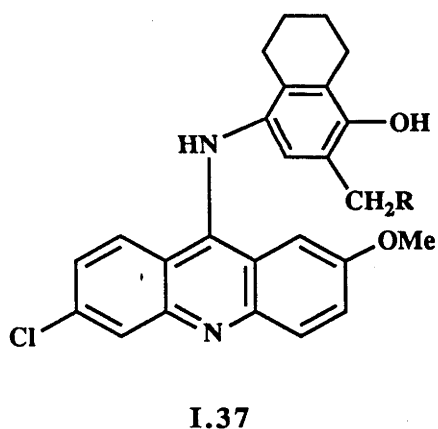
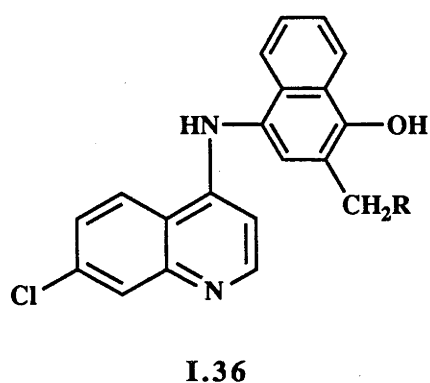
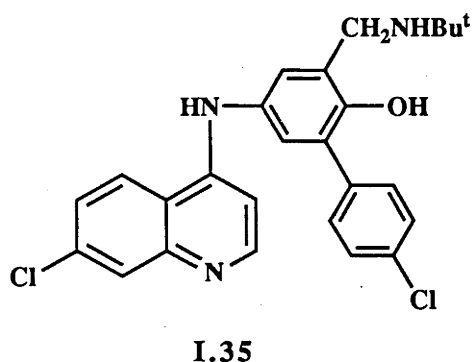
Recently, Werbel *et al.*¹⁰¹ described the synthesis of 3-(*t*-butylaminomethyl)-4-chloro-5-(7-chloroquinolin-4-ylamino)biphenyl-2-ol ["Tebuquine" (I.35)], a series of analogues, and some *N*-oxides. The antimalarial activity and quantitative structure-activity relationships of this series were also reported.

I-6.3 Mono-Mannich base derivatives of 4-(quinolin-4-ylamino)-5,6,7,8-tetrahydronaphth-1-ols

Some 1,4-naphthoquinones show considerable antimalarial activity^{78,102,103} and their mode of action appears different from the 4-aminoquinoline-type antimalarials. In 1972, Nabih *et al.*¹⁰⁴ prepared compounds such as compound I.36 and I.37 in the hope that these compounds might exhibit interesting antimalarial activity.

Compound I.36, ($R=NEt_2$) was subsequently shown to have both curative and prophylactic effects against *P. berghei* in mice; but in further tests¹⁰⁵ it was less potent than a series of biphenyl-2-ols.

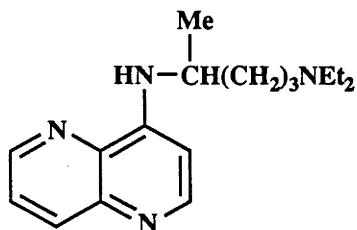
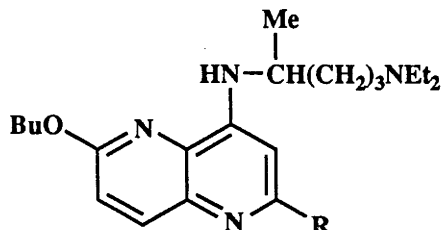
Twelve years later, Shen *et al.*⁹³ prepared 2-(*t*-butylaminomethyl)-4-(7-trifluoromethylquinolin-4-ylamino)-5,6,7,8-tetrahydronaphth-1-ol hydrochloride (I.38), and showed that it suppressed *P. berghei* in infected mice at a dosage of 2.5 mg/kg.



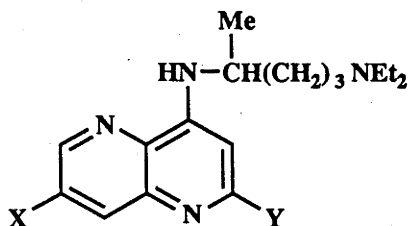
I-6.4 Mannich base derivatives of 4-(1,5-naphthyridin-4-ylamino)phenols

The antimalarial activity shown by the 4-aminoquinolines such as chloroquine stimulated interest in aza-analogues, including the 1,5-naphthyridines. 4-Amino-1,5-naphthyridine may be regarded as such an analogue of either 4- or 8-aminoquinoline.

Adams *et al.*¹⁰⁶ prepared compound **I.39** and Goldberg *et al.*¹⁰⁷ prepared compound **I.40** (R=H): the former was active against *P. gallinaceum* in chicks and against *P. berghei* in mice¹⁰⁷ but compound **I.40** (R=Me) was inactive.¹⁰⁷

**I.39****I.40**

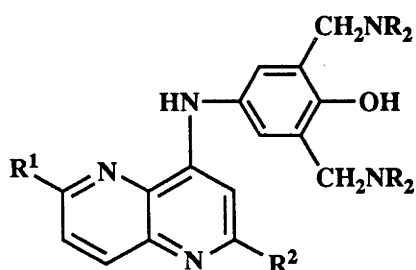
In 1970 McCaustland and Cheng¹⁰⁸ synthesised several 1,5-naphthyridine congeners (**I.41** and **I.42**) of chloroquine and found that the compound **I.41b** possessed very good antimalarial activity against *P. berghei* in mice, also it was much less toxic than the existing 4- and 8-aminoquinoline drugs. No acute toxicity was noted at a dose of 640 mg/kg, whereas chloroquine was 100% lethal at a dose of 320 mg/kg. In contrast, compounds **I.41a** and **I.41c** were inactive when tested in the same model.



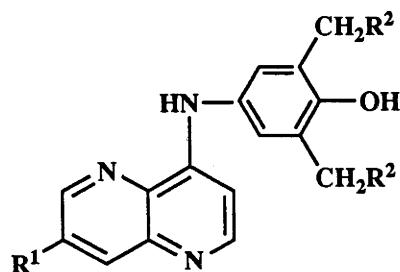
I.41 a; X=Cl, Y=OMe
 b; X=Cl, Y=H
 c; X=H, Y=OMe

More recently, several unsubstituted and 2,6-disubstituted 1,5-naphthyridines carrying di-Mannich basic chains of *p*-aminophenol (e.g. **I.42a**) were synthesised by Chen and his colleagues,¹⁰⁹ and these proved to be effective antimalarials.

Di-Mannich bases of 4-[7-chloro-(and 7-bromo)-1,5-naphthyridin-4-ylamino]-phenols (**I.43**) were prepared by Barlin and Tan^{95,110,111} in 1986. These compounds were shown to exhibit activity in *in vivo* tests against *P. vinckei vinckei* in mice. Subsequent tests by Scott *et al.*^{97,98} against *P. falciparum* *in vitro* revealed significant activity against both the chloroquine-sensitive (FCQ-27) and chloroquine-resistant (K-1) strains. The chloro compound (**I.43a**) and the bromo compound (**I.43b**) showed only a small difference in activity.



- I.42** a; $R^1=R^2=H$, $NR_2=NC_4H_8$
 b; $R^1=OMe$, $R^2=Me$, $NR_2=NEt_2$
 c; $R^1=OMe$, $R^2=Me$, $NR_2=NC_5H_{10}$

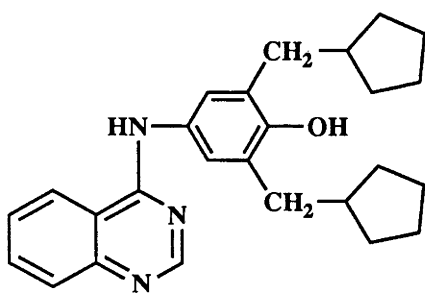


- I.43** a; $R^1=Cl$
 b; $R^1=Br$

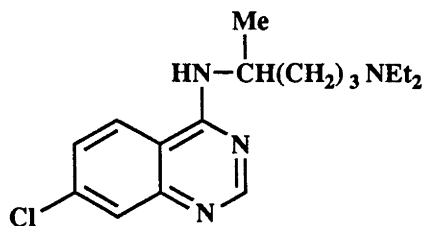
I-7 Quinazoline Derivatives as Potential Antimalarials

Prior to the present work no Mannich base derivatives of 4-(quinazolin-4-ylamino)phenol had been tested for antimalarial activity. However the di-Mannich base, changrolin (**I.44**)^{112,113} has been described as an effective antiarrhythmic agent, and some articles^{114,115} claim a relationship between antimalarial and antiarrhythmic activity.

Magidson and Golovchinskaya,¹¹⁶ Price *et al.*¹¹⁷ and Chapman *et al.*¹¹⁸ have prepared a variety of quinazolines for evaluation as antimalarials. The compound **I.45**, the quinazoline analogue of chloroquine, was found to have suppressive activity against the blood forms of *P. gallinaceum* in chicks at a dosage of 20 mg/kg¹¹⁸ and was equivalent or slightly superior to quinine.¹¹⁷

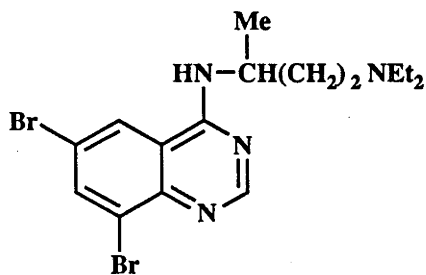


I.44

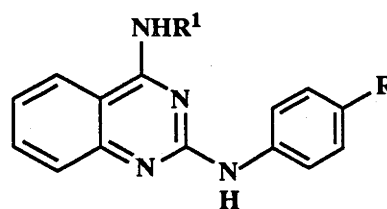


I.45

Antimalarial activity has been demonstrated¹¹⁹ in compound **I.46**, and the compound **I.47** showed marked activity¹²⁰ against asexual blood forms of *P. gallinaceum* in chicks; but no activity against the tissue stages.

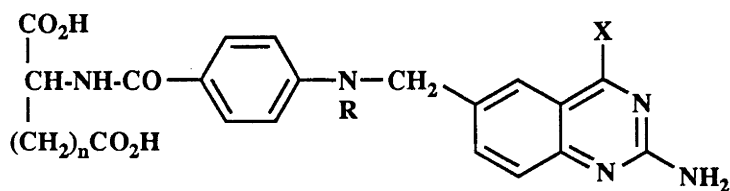


I.46

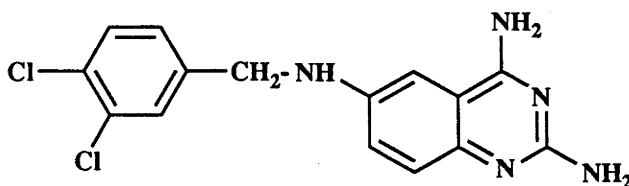


I.47 R=Cl, R¹=(CH₂)₂NEt₂

In 1970, Davoll and Johnson¹²¹ prepared compound **I.48** (n=2, R=Me, X=OH), a folate antagonist, which was shown to have good oral activity against *P. berghei* injections in mice.¹²² Much effort has since been directed to the study of folate antagonists including the 6-substituted 2,4-aminoquinazolines (the so-called non-classical folate antagonists). In early work, compound **I.49** showed strong antimalarial activity against *P. berghei* in mice (4 to 11 times as potent as quinine)¹¹⁹ and many compounds of this type have been examined.¹²³⁻¹³⁰



I.48: $n=1, 2$; $R=H, Me$; $X=OH, NH_2$



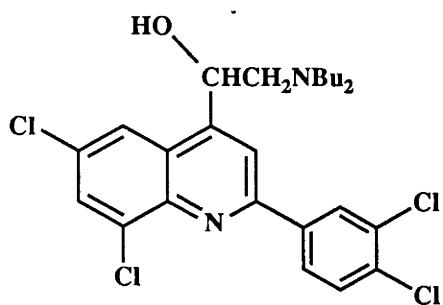
I.49

I-8 Mefloquine and its Analogues as Potential Antimalarials^{131,132}

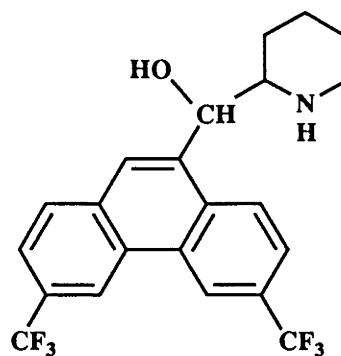
The preparation and antimalarial activity of mefloquine (WR 142,490) (I.10) and its 2,6- and 2,7-bistrifluoromethyl isomers was first reported in 1971 by Ohnmacht *et al.*¹³³ Like quinine (I.1a) and the active antimalarial (I.50; WR 30090),¹³⁴ mefloquine (I.10) is a quinolinemethanol; the active antimalarials (I.51; WR 122,455)¹³⁵ and halofantrine (I.13; WR 171,669)¹³⁶ are also methanol derivatives. Mefloquine was 100% curative at 40 mg/kg against *P. berghei* in mice, twice as effective as the 2,7-isomer and 4 times as effective as the 2,6-isomer.¹³³ In a test involving increased survival time of mice infected with *P. berghei*, mefloquine was at least 8 times better than chloroquine and 64 times better than quinine.¹³⁷ Further details of testing in animals are reported in references 131 and 132.

Mefloquine is now available for clinical use. It acts as a potent blood schizontocide and is effective against the multi-drug resistant "Smith" strain of *P. falciparum*.¹³⁸

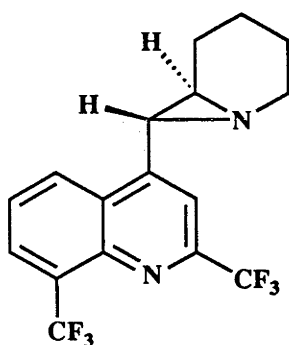
Compound I.52, an analogue of mefloquine containing a trans-aziridine ring, was devoid of antimalarial activity in the standard *P. berghei* mouse model.¹³⁹ Other structural analogues of mefloquine which also showed potent antimalarial activity are WR 184,806 (I.53)¹⁴⁰ and WR 226,253 (I.54).¹⁴¹



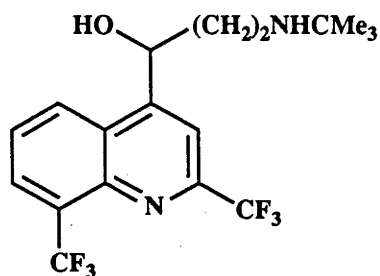
I.50



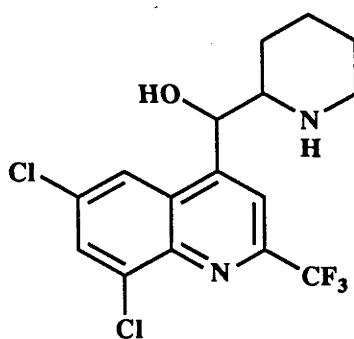
I.51



I.52



I.53



I.54

I-9 Scope of the Present Work

Chemical syntheses of Mannich base derivatives of heterocyclic amino phenols, the synthesis of a mefloquine analogue, and preliminary testing of these compounds for antimalarial activity have been undertaken in this work. The aim was to prepare novel compounds which may be more effective antimalarials than the classical compounds (such

as chloroquine and amodiaquine), particularly against the chloroquine-resistant strain of *P. falciparum*.

In the present work new di-Mannich base derivatives of 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol, 4-(7-trifluoromethylquinazolin-4-ylamino)phenol, 4-[7-chloro (and 7-trifluoromethyl)quinolin-4-ylamino]-3-methylphenols, 4-(7-bromo-1,5-naphthyridin-4-ylamino)-3-methylphenol; mono-Mannich base derivatives of 4-chloro-5-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]biphenyl-2-ols and 4-chloro-5-[7-trifluoromethylquinolin (and quinazolin)-4-ylamino]biphenyl-2-ols ("Tebuquine" analogues) and 4-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]-5,6,7,8-tetrahydronaphthols; and the 7-trifluoromethylquinolin-4-yl analogue of mefloquine have been prepared. The physical properties (such as ^1H n.m.r., ^{13}C n.m.r., ultraviolet, infrared and mass spectra, and ionization constants) of these compounds are also reported and discussed.

These compounds have been tested for antimalarial activity against *P. falciparum* *in vitro* and *in vivo* tests against *P. vinckei vinckei* in mice, and the results compared with those for analogous compounds.

CHAPTER II

CHAPTER II 4-Chloro-(and 4-Hydroxy)-7-(trifluoromethyl-1,5-naphthyridine, Di-Mannich Bases of 4-(7-Trifluoromethyl-1,5-naphthyridin-4-yl-amino)phenol and *N*⁴-(4-Diethylamino-1-methylbutyl)-7-trifluoromethyl-1,5-naphthyridin-4-amine

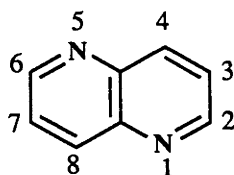
The work reported in this Chapter relates to the preparation of di-Mannich bases of 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol for testing for antimalarial activity. This work was undertaken because the high activity of di-Mannich bases of 4-(7-trifluoromethylquinolin-4-ylamino)phenol^{93,94,98} and the curative properties of di-Mannich bases of 4-[7-chloro (and 7-bromo)-1,5-naphthyridin-4-ylamino]phenol^{97,98,111} against malarial plasmodia have been described previously. In addition, the 7-halogeno-1,5-naphthyridin-4-amines^{97,108} have been found to have lower toxicity than the corresponding quinolines (See Chapter I-6.1 and I-6.2). Thus it was decided that the di-Mannich bases derived from 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol should be examined.

In this Chapter, the literature relating to the preparation and to the chemical and physical properties of simple 1,5-naphthyridines is surveyed; the methods employed for the present synthetic work are discussed; and the physical properties (such as ¹H n.m.r., i.r., u.v. and pK_a values) of these compounds are then reported and discussed. Results of testing for antimalarial activity by *in vitro* tests against *P. falciparum* and by *in vivo* tests against *P. vinckei vinckei* in mice will be described in Chapter VIII.

II-1 Simple 1,5-Naphthyridines

1,5-Naphthyridine (II.1) is a heterocyclic system consisting of two fused six-membered aromatic rings, each ring containing one nitrogen atom. Alternatively, it can be considered as 5-azaquinoline or a pyridopyridine; it has ten delocalised π -electrons. The doubly bound ring nitrogen atoms withdraw electrons from other parts of the ring system and render it electron-deficient (such ring systems are said to be π -electron

deficient): accordingly they are highly susceptible to nucleophilic attack, particularly at the 2- and 4-positions, and correspondingly deactivated to electrophilic attack.



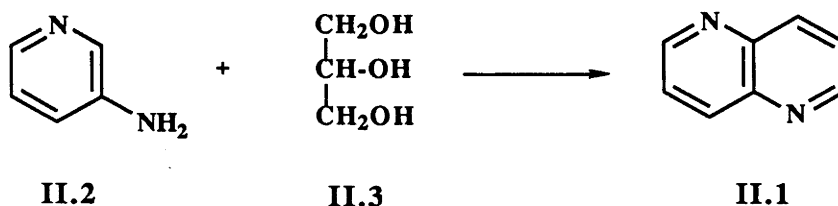
II.1

II-1.1 Syntheses of simple 1,5-naphthyridines

Two principal methods are generally employed in 1,5-naphthyridine syntheses, namely, the Skraup reaction and the ethoxymethylenemalonic ester (EMME) reaction. In addition to these methods, there are some modifications which are mentioned below:

(a) *The Skraup reaction*

Unsubstituted 1,5-naphthyridine can be synthesised easily by heating pyridin-3-amine (II.2) with glycerol (II.3) in the presence of concentrated sulphuric acid and of arsenic pentoxide¹⁴² as a condensing agent.



Several modifications^{143,144a} of this reaction have been described. Likewise, substituted 1,5-naphthyridines can be prepared by using substituted pyridin-3-amines with different condensing agents, such as a methylacrolein and acetaldehyde. Some literature preparations of 1,5-naphthyridines are summarised in Table II-1, in which reagents, products and references are listed.

Table II-1 Some literature preparations of 1,5-naphthyridines

Substituted 3-aminopyridine	Condensing agent	Substituted 1,5-naphthyridine	Reference
-	Glycerol	-	145
-	Crotonaldehyde	2-Methyl-	145
-	Methyl vinyl ketone	4-Methyl-	145
-	2-Ethylacrolein	3-Ethyl-	145
4-Hydroxy	Glycerol	4-Hydroxy-	146
6-Hydroxy	Acetaldehyde + acetone	6-Hydroxy-2,4-dimethyl-	147
6-Chloro	Acetaldehyde	6-Chloro-2-methyl-	148

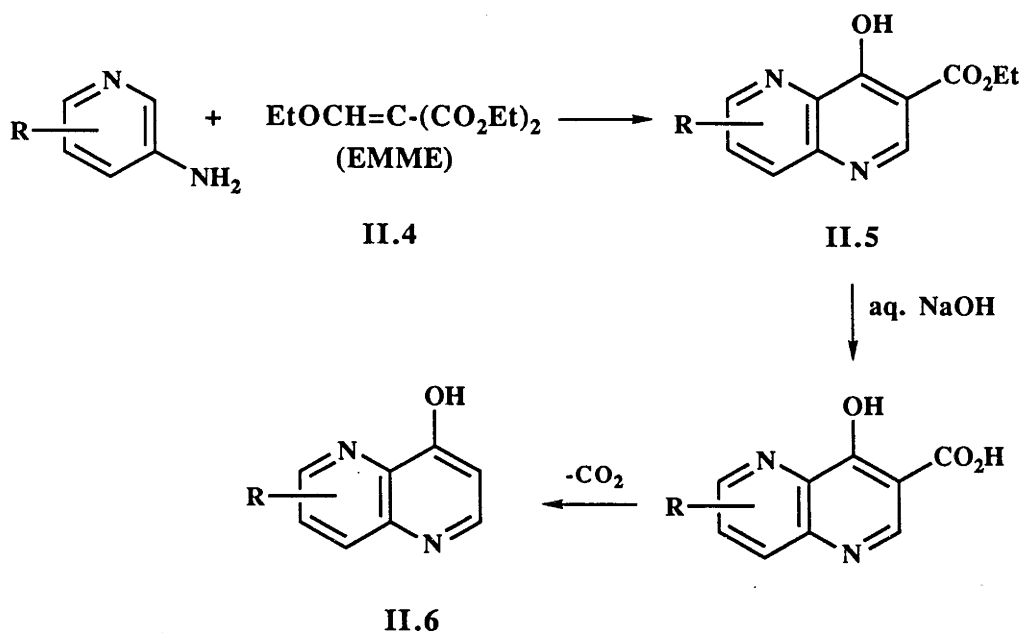
Rapoport and Batcho¹⁴⁵ reported that the cyclisation of pyridin-3-amine with anhydrous glycerol gave 1,5-naphthyridine, by cyclisation at the 2-position. No 1,7-naphthyridine (which could have been formed by cyclisation to the 4-position) could be detected in the reaction products.

When the 2-position in pyridin-3-amine was blocked by another group differing results were obtained. For example, 3-aminopyridin-2-ol gave 1,7-naphthyridin-8-ol¹⁴⁹ (formed by cyclisation to the 4-position) but 2-halogenopyridin-3-amine gave 1,5-naphthyridine^{144a} (presumably involving replacement of the 2-halogeno substituent).

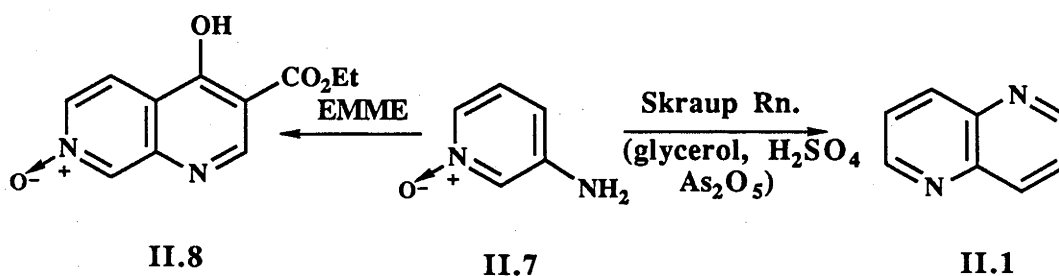
(b) *The ethoxymethylenemalonate ester (EMME) reaction*

The synthesis of 4-hydroxyquinoline-3-carboxylic esters from aniline and ethoxymethylenemalononic ester developed by Price and Roberts¹⁵⁰ following the earlier work of Gould and Jacobs¹⁵¹ has been extended to the preparation of 1,5-naphthyridines.^{106,142,150}

In this modification pyridin-3-amine (or substituted pyridin-3-amines) were heated with ethyl ethoxymethylene malonate (EMME) (II.4) in refluxing "Dowtherm A" (an eutectic mixture of diphenyl ether and biphenyl) to afford ethyl 4-hydroxy-1,5-naphthyridine-3-carboxylate (II.5). This compound (II.5) was hydrolysed and decarboxylated to give the 1,5-naphthyridin-4-ol (II.6) in good yields.^{106-108,152}



The EMME reaction applied to pyridin-3-amine 1-oxide (II.7) gave the 1,7-naphthyridine *N*⁷-oxide (II.8);¹⁵³ but interestingly, compound II.7 under the condition of the Skraup reaction gave 1,5-naphthyridine (II.1).¹⁵³ [Deoxygenation of pyridine *N*-oxides in the presence of acid and oxidising agents has been reported.^{154,155} Therefore under the condition of the Skraup reaction compound II.7 was probably first deoxygenated to form pyridin-3-amine¹⁵³ which then underwent the Skraup reaction to give compound II.1].

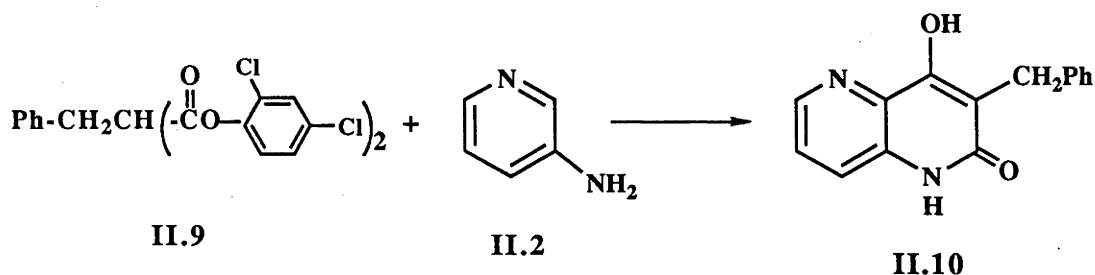


This EMME reaction was also used in the present work for the synthesis of 7-trifluoromethyl-1,5-naphthyridin-4-ol.

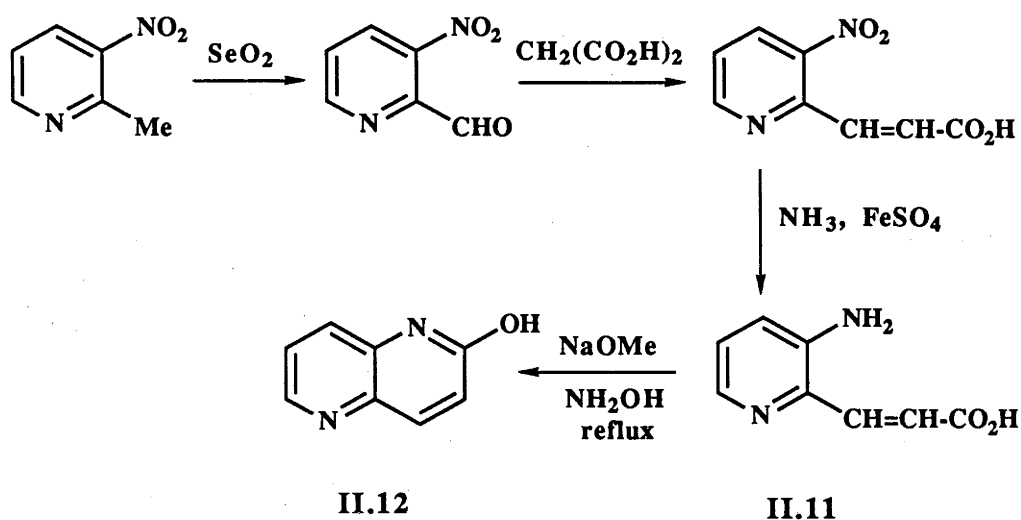
(c) *Miscellaneous preparations*

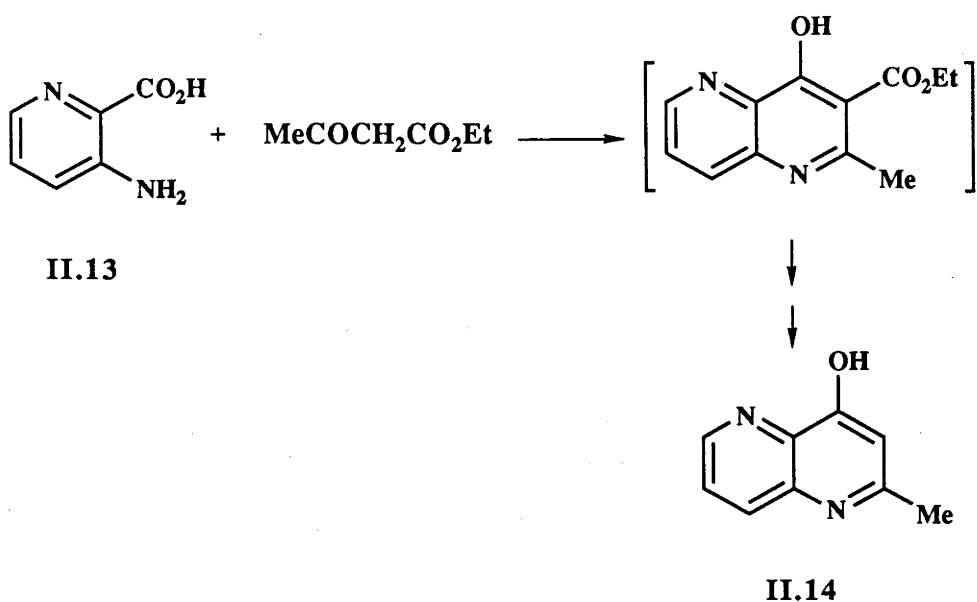
The following preparations of 1,5-naphthyridines have also been described.

Ziegler and Noelken¹⁵⁶ prepared the 1,5-naphthyridine (II.10) by the condensation of compounds II.9 and II.2.

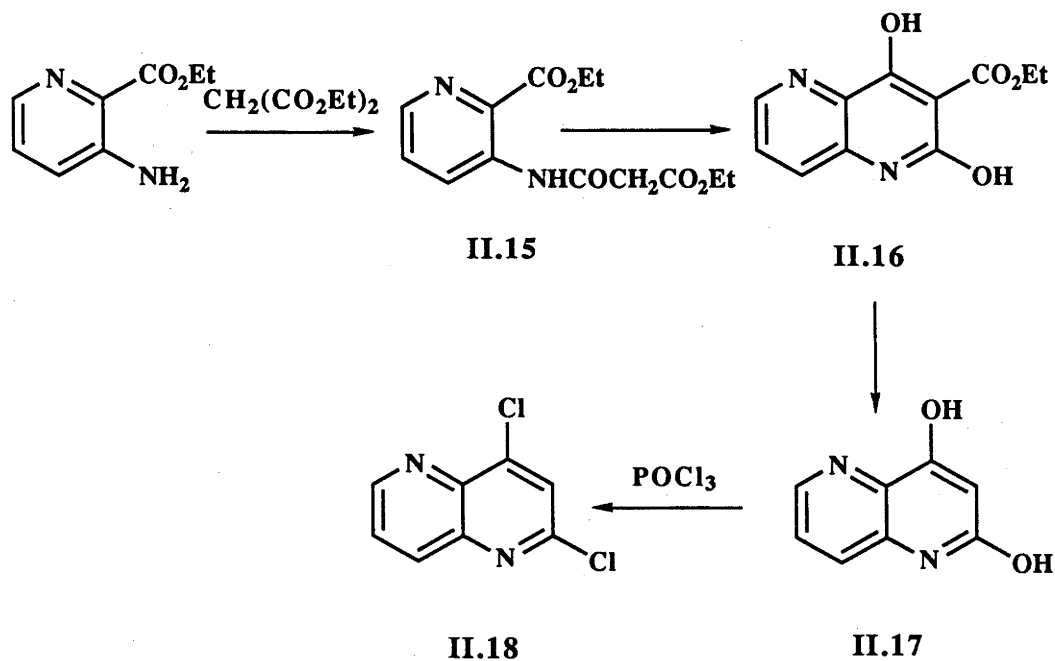


Baumgarten *et al.*¹⁵⁷ converted the 2,3-disubstituted pyridines, compounds II.11 and II.13, into 1,5-naphthyridin-2-ol (II.12) and its 2-methyl derivative (II.14) respectively,¹⁵⁷ as in the reaction sequences illustrated below.



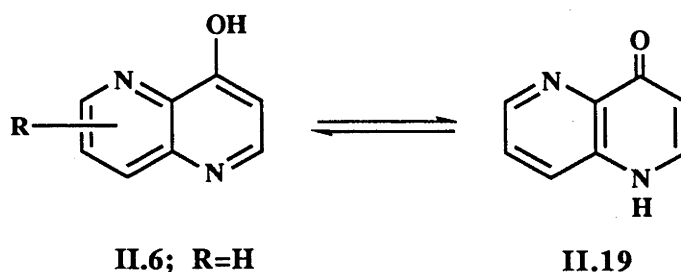


Oakes and Rydon¹⁵⁸ have reported the preparation of 1,5-naphthyridin-2,4-diol (II.17), the 2,4-dichloro derivative (II.18), and (2-amino-4-chloro- and 4-chloro-2-hydroxy)-1,5-naphthyridines. Dieckmann cyclisation of the substituted malonamic ester (II.15) gave the ester II.16 which after hydrolysis and decarboxylation afforded 1,5-naphthyridin-2,4-diol (II.17).



II.1.2 Physical properties of simple 1,5-naphthyridines

Physical properties of 1,5-naphthyridine [^1H n.m.r., i.r., u.v. and mass spectra; and ionization constant (pK_a)] have been tabulated by Paudler and Kress.^{144b} The keto-enol tautomerism of 1,5-naphthyridin-4-ol (**II.6**, $\text{R}=\text{H}$) has been studied by i.r. spectroscopy both in solution and the solid state by Bailey *et al.*¹⁵⁹ They found that in polar solvents, the keto form (**II.19**) predominates, whereas the enol form (**II.6**) is predominant in non-polar solvents. The infrared absorption bands such as the $\text{C}=\text{O}$ and N-H stretching regions have been reported for several other hydroxy-naphthyridines.^{160,161}

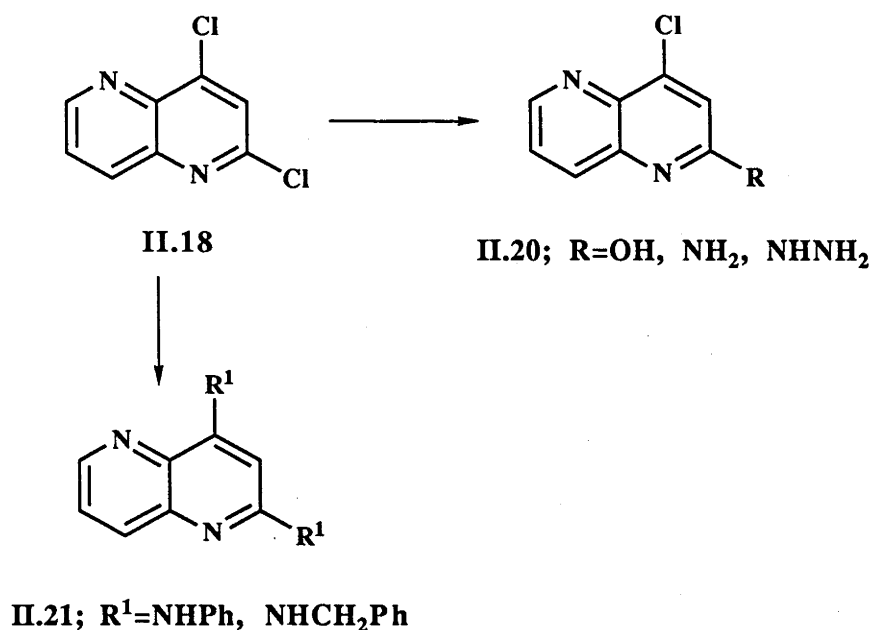


II-1.3 Reactivity of halogeno substituents in 1,5-naphthyridines

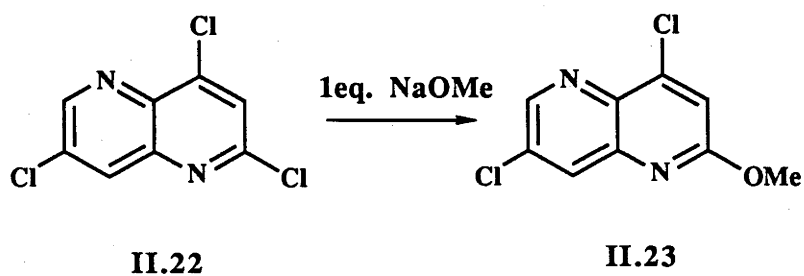
In unsubstituted 1,5-naphthyridine (**II.1**) there are only three different ring-positions (2-, 3- and 4-), which are activated to nucleophilic substitution both by resonance and by induction. The reactions involving displacement of a halogeno substituent from substituted naphthyridines have been reviewed.¹⁶²

Oakes and Rydon,¹⁵⁸ by application of an approximate quantum mechanical calculation,¹⁶³⁻¹⁶⁵ were able to calculate that the 2-chloro substituent in 2,4-dichloro-1,5-naphthyridine is more reactive towards nucleophiles than the 4-chloro substituent. Accordingly preferential displacement, by water, ammonia or hydrazine, occurs at the 2-position to give 4-chloro-2-substituted-1,5-naphthyridines (**II.20**).¹⁵⁸ No differential reactivity of the 2- and 4-chloro substituents in compound **II.18** was observed when it was allowed to react with aniline or benzylamine and the 2,4-disubstituted compound

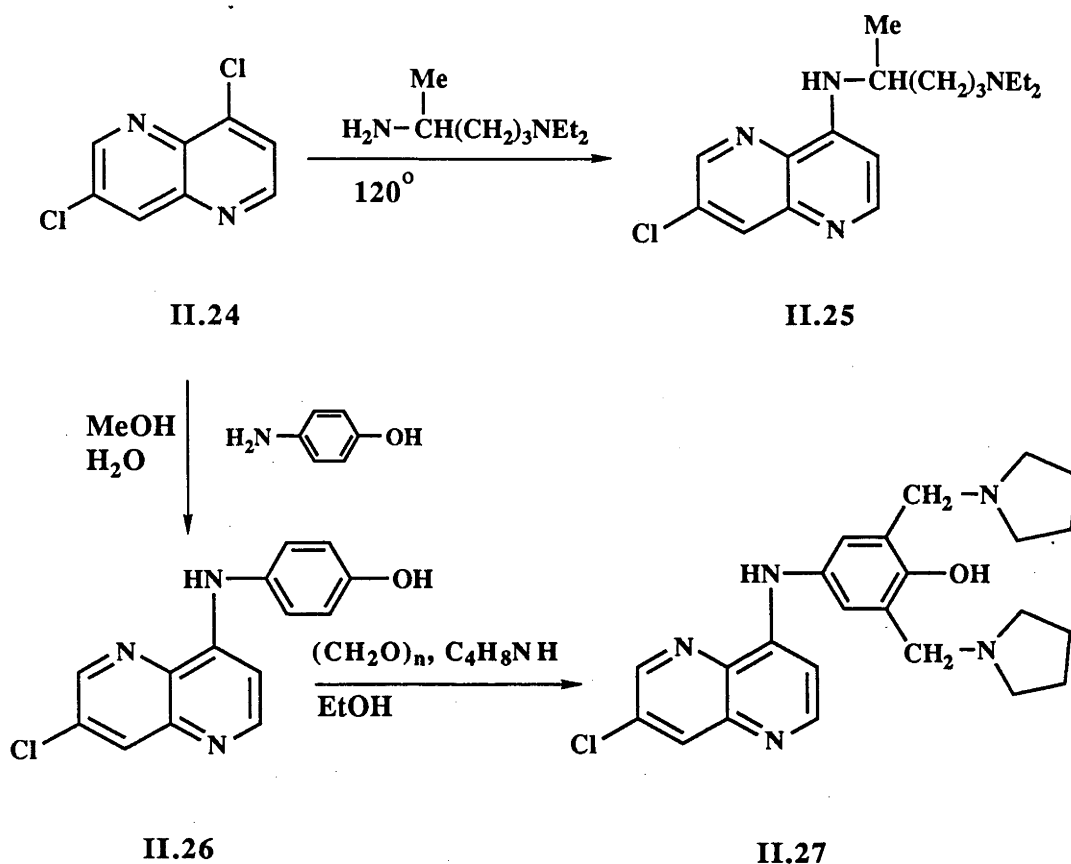
(II.21, $R^1 = \text{NHPh}$) was formed, even with only one equivalent of aniline in dioxane solution.¹⁵⁸



McCautland and Cheng¹⁰⁸ studied the nucleophilic substitution of 2,4,7-trichloro-1,5-naphthyridine (II.22) with one equivalent of sodium methoxide in methanol, using ^1H n.m.r. They found that the major product was 4,7-dichloro-2-methoxy-1,5-naphthyridine (II.23) rather than its 4-methoxy isomer.

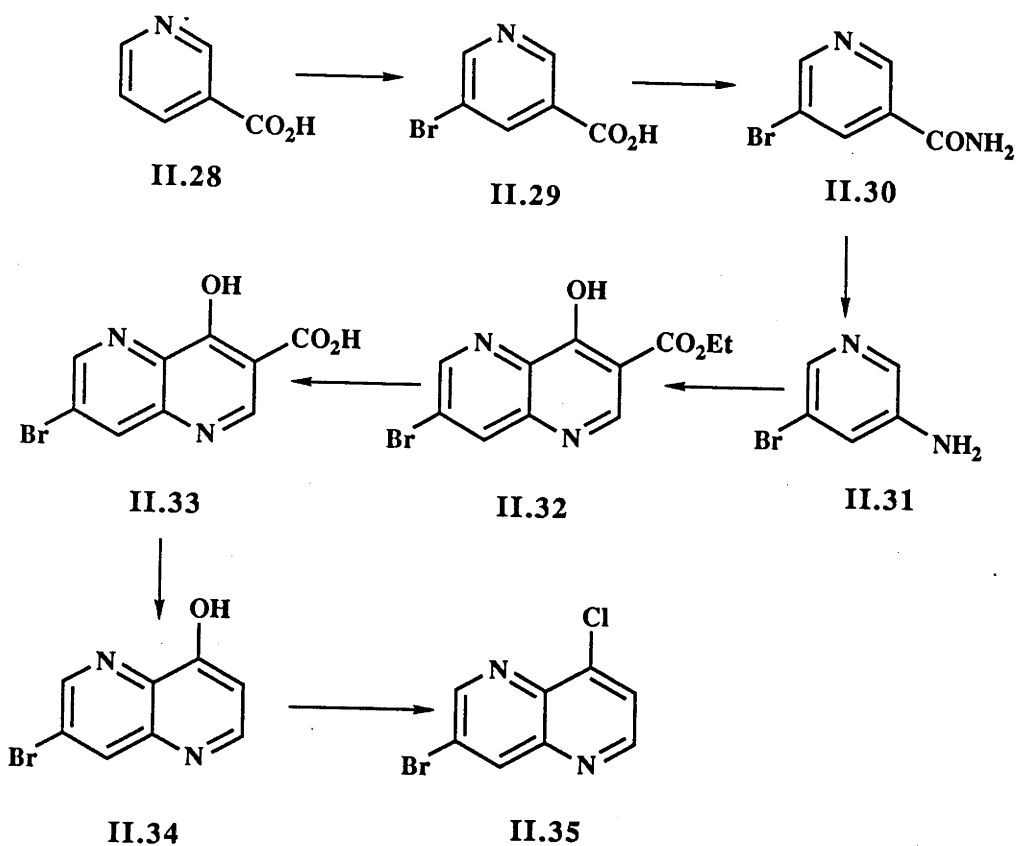


As expected compounds (II.25)¹⁰⁸ and (II.26)⁹⁵ were produced by nucleophilic displacements involving 4,7-dichloro-1,5-naphthyridine (II.24) (both compounds II.25 and II.27 showed good antimalarial activity).

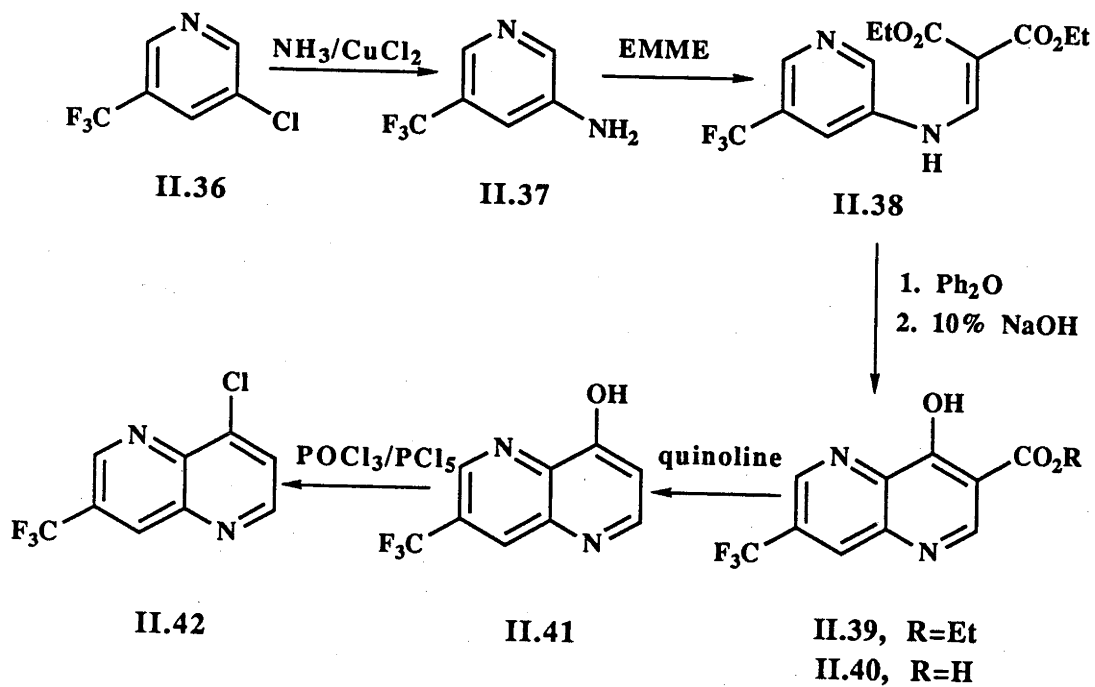


II-2 Syntheses of 7-Trifluoromethyl-1,5-naphthyridin-4-ol and 4-Chloro-7-trifluoromethyl-1,5-naphthyridine

3-Bromo-8-chloro-1,5-naphthyridine¹¹⁰ (**II.35**; Scheme II-1) has been prepared previously from the known ethyl 7-bromo-4-hydroxy-1,5-naphthyridine-3-carboxylate¹⁶⁶ (**II.32**). This compound (**II.32**) was prepared from nicotinic acid (**II.28**) through 5-bromonicotinic acid (**II.29**),^{167,168} its amide (**II.30**)¹⁶⁸ and 5-bromopyridin-3-amine (**II.31**).^{168,169} The compound **II.31** was condensed with EMME and ring-closed in boiling diphenyl ether (instead of "Dowtherm A" in reference 166) to give compound **II.32**. This ester (**II.32**) was hydrolysed in aqueous sodium hydroxide to give the corresponding acid (**II.33**) which was decarboxylated in refluxing quinoline to afford the 7-bromo-1,5-naphthyridin-4-ol (**II.34**). Chlorination of this hydroxy compound (**II.34**) by refluxing with phosphoryl chloride for 10 h, gave 3-bromo-8-chloro-1,5-naphthyridine (**II.35**) in good yields (Scheme II-1).



Scheme II-1



Scheme II-2

In the present work 7-trifluoromethyl-1,5-naphthyridin-4-ol (**II.41**) and 4-chloro-7-trifluoromethyl-1,5-naphthyridine (**II.42**) were prepared by a similar method. These two compounds were prepared from the known 3-chloro-5-trifluoromethylpyridine (**II.36**) (commercially available) by an initial treatment with 28% aqueous ammonia in the presence of cuprous chloride at 170° in a high pressure vessel for 48 h, and gave good yields of 5-trifluoromethylpyridin-3-amine (**II.37**).¹⁷⁰ This compound (**II.37**) was subsequently allowed to react with EMME (*i.e.* the EMME reaction) at 100° for 2 h and gave diethyl 5-trifluoromethylpyridin-3-ylaminomethylenemalonate (**II.38**), which was then heated in refluxing diphenyl ether to yield ethyl 4-hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylate (**II.39**). This ethyl ester (**II.39**) was hydrolysed in 10% aqueous sodium hydroxide to give the corresponding acid (**II.40**) which was decarboxylated in boiling quinoline to give 7-trifluoromethyl-1,5-naphthyridin-4-ol (**II.41**). Chlorination¹⁷¹ of compound **II.41** with a mixture of phosphorus pentachloride and phosphoryl chloride at 110° for 3 h, readily gave 4-chloro-7-trifluoromethyl-1,5-naphthyridine (**II.42**) in excellent yields, as shown in Scheme II-2.

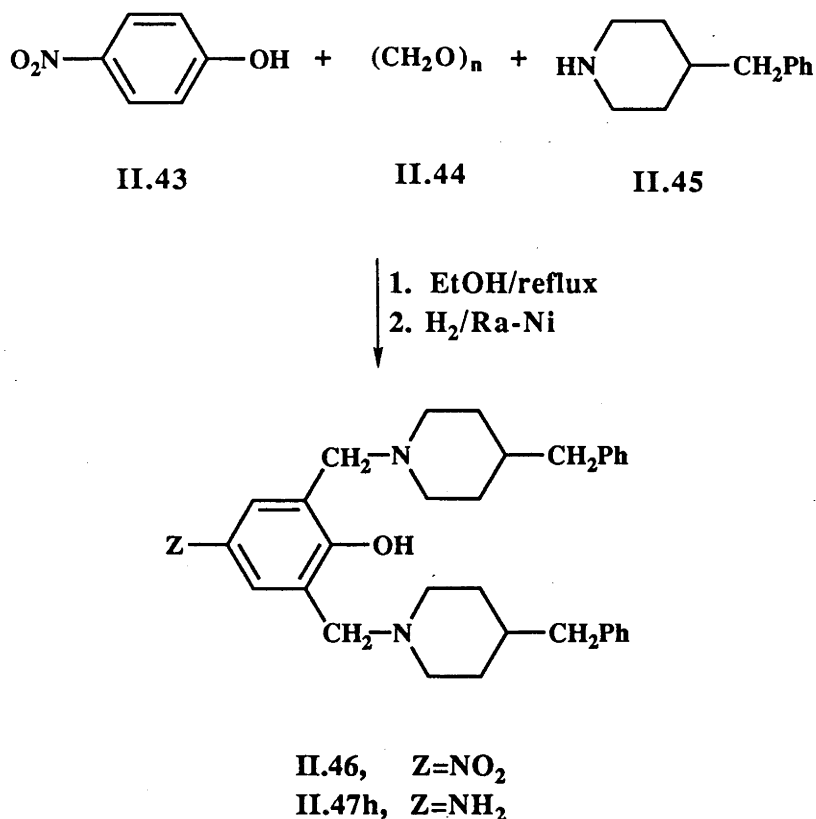
II-3 Synthesis of New Di-Mannich Bases of 4-Nitrophenol

Barlin and Ireland¹⁷² have described the preparation of the di-Mannich bases of 4-aminophenol from the inexpensive 4-nitrophenol with paraformaldehyde and appropriate amines, through the readily purified di-Mannich bases of 4-nitrophenol (for further detail of Mannich reaction see Appendix 2).

In the present work, the new 2,6-bis(4-benzylpiperidin-1-ylmethyl)-4-nitrophenol (**II.46**) and the corresponding 4-amino compound (**II.47h**) were prepared by a similar procedure, as shown in Scheme II-3.

4-Nitrophenol (**II.43**) with a considerable excess of 4-benzylpiperidine (**II.45**) and paraformaldehyde (**II.44**) in a small volume of ethanol at reflux for 16 h gave compound **II.46**. This nitro compound (**II.46**) was readily purified by recrystallisation and was catalytically reduced with hydrogen gas in the presence of Raney nickel to afford

the corresponding amine (**II.47h**) which was sufficiently pure for condensation directly with 4-chloro-7-trifluoromethyl-1,5-naphthyridine (**II.42**).

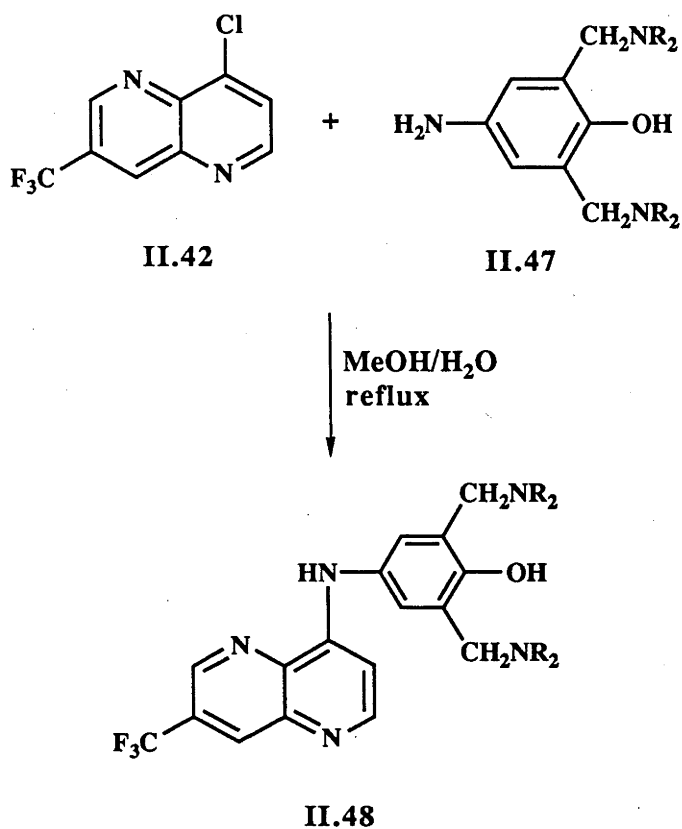


Scheme II-3

II-4 Syntheses of Di-Mannich Bases of 4-(7-Trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol

The preparation of compounds such as the di-Mannich bases of 4-(7-trifluoromethylquinolin-4-ylamino)phenol by the condensation of di-Mannich bases of 4-amino-phenol with 4-chloro-7-trifluoromethylquinoline has been described¹⁷² recently. In the present work, the di-Mannich bases of 4-(7-trifluoromethyl-1,5-naphthyridin-4-yl-amino)phenol (**II.48**) were also prepared in a similar way by condensation of appropriate di-Mannich bases (**II.47**) with 4-chloro-7-trifluoromethyl-1,5-naphthyridine

(II.42) in refluxing aqueous methanol solution (approximately at pH 2.5-3.0) containing a few drops of concentrated hydrochloric acid for 3.5 h to 8 h (Scheme II-4).



II.47, II.48

	NR ₂		NR ₂
a	NEt ₂	e	3-methylpiperidin-1-yl
b	NPr ₂	f	3,5-dimethylpiperidin-1-yl
c	pyrrolidin-1-yl	g	4-methylpiperidin-1-yl
d	piperidin-1-yl	h	4-benzylpiperidin-1-yl

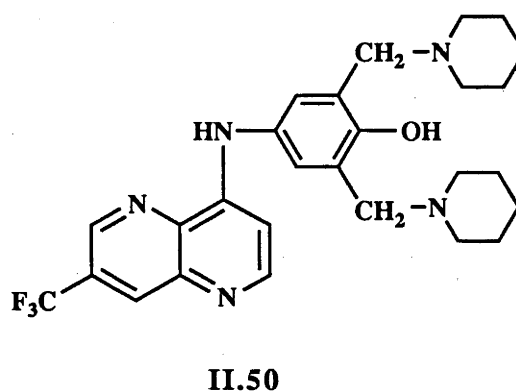
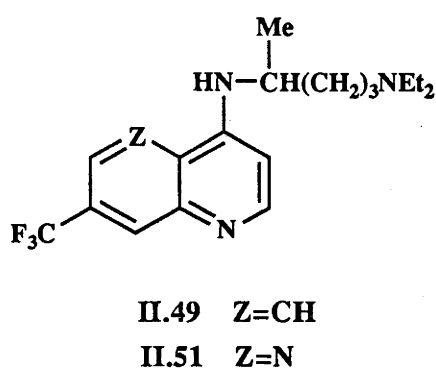
Scheme II-4

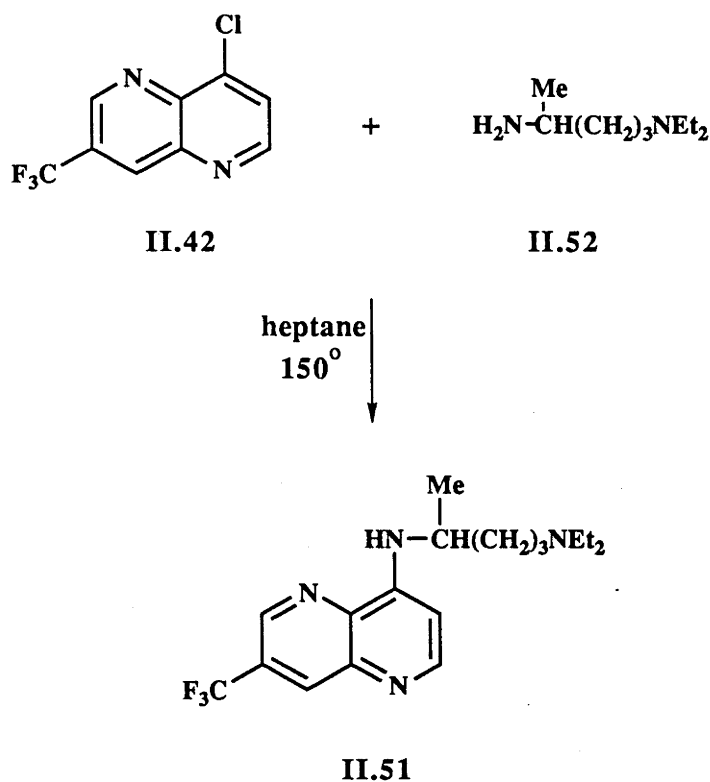
II-5 Synthesis of *N*⁴-(4-Diethylamino-1-methylbutyl)-7-trifluoromethyl-1,5-naphthyridin-4-amine

Literature methods for the preparation of the *N*⁴-substitutedquinolin-4-amines¹⁷¹ and naphthyridin-4-amines^{106-108,110} have been described. Some of these compounds have shown antimalarial activity, for example 7-chloro-*N*⁴-(4-diethylamino-1-methylbutyl)quinoline (chloroquine) and 7-chloro-*N*⁴-(4-diethylamino-1-methylbutyl)-1,5-naphthyridin-4-amine.^{108,109}

Replacement of the 7-halogeno substituent in the quinoline nucleus of known antimalarials by a trifluoromethyl group have given compounds with potent activities, e.g. compound **II.49**⁹¹ and **II.50**,⁹⁵ the novel drug, mefloquine, also contains trifluoromethyl groups. Accordingly it was decided to investigate *N*⁴-(4-diethylamino-1-methylbutyl)-7-trifluoromethyl-1,5-naphthyridin-4-amine (**II.51**).

The compound **II.51** was prepared similarly to the 7-bromo analogue.^{98,110} Compound **II.42** was heated with *N*¹,*N*¹-diethylpentane-1,4-diamine (**II.52**) in heptane at 150° in a Teflon-lined reaction vessel for 20 h and gave *N*⁴-(4-diethylamino-1-methylbutyl)-7-trifluoromethyl-1,5-naphthyridin-4-amine (**II.51**) as shown in Scheme II-5.





Scheme II-5

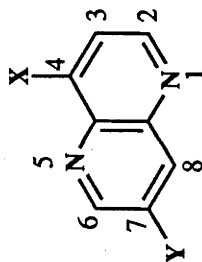
II-6 Physical Properties

II-6.1 Nuclear magnetic resonance spectra

The ^1H n.m.r. spectrum of unsubstituted 1,5-naphthyridine (II.1) has been published,^{144b,173} as an $\text{A}_2\text{B}_2\text{X}_2$ spin system.¹⁷⁴ As both rings in unsubstituted 1,5-naphthyridine are symmetrical, the protons at position 2, 3 and 4; and at 6, 7 and 8 are equivalent. In deuteriochloroform, the chemical shifts of these protons were found to be at δ 8.97, 7.58 and 8.40 for H2 (H6), H3 (H7) and H4 (H8), respectively, with coupling constants of $J_{2,3}$ 4.1 Hz, $J_{2,4}$ 1.8 Hz, $J_{3,4}$ 8.0 Hz and $J_{4,8}$ 0.6 Hz.^{144b,174}

In contrast, the 4-substituted-7-trifluoromethyl-1,5-naphthyridines, prepared in the present work, showed relatively simple ^1H n.m.r. spectra (Table II-2, II-3 and Experimental section).

Table II-2 ^1H n.m.r. spectral data^{a, b} for 7-trifluoromethyl (and 7-bromo)-1,5-naphthyridin-4-ols and 4-chloro-7-trifluoromethyl (and 7-bromo)-1,5-naphthyridines



Compound	X	Y	Solvent	H2	H3	H4	H6	H7	H8	J _{2,3}	J _{3,4}	J _{4,8}	J _{6,8}
II.1	H	H	CDCl ₃	8.97	7.58	8.40	8.97	7.58	8.40	4.1	8.0	0.6	1.8
II.41	CF ₃	OH ^c	NaOD/D ₂ O	8.39	6.75	-	8.87	-	8.45	6	-	-	2
II.42	CF ₃	Cl	CDCl ₃	8.97	7.89	-	9.27	-	8.73	5	-	-	2
II.34 ^d	Br	OH ^c	NaOD/D ₂ O	8.29	6.64	-	8.65	-	8.33	6	-	-	2
II.35 ^d	Br	Cl	CDCl ₃	8.85	7.77	-	9.07	-	8.62	5	-	-	2

^a Chemical shifts are reported as δ values downfield from tetramethylsilane (TMS) as internal standard in CDCl₃ and sodium 3-trimethylsilylpropanesulphonate as internal standard in NaOD/D₂O.

^b Coupling constants (J values) are reported in Hz.

^c As anions.

^d Published data from reference 110.

Examination of Table II-2 revealed that the ring protons in compound **II.41** (as an anion) appeared upfield of those in compound **II.42** (as neutral molecule); the effects were greatest at the 3- and 2-positions. This is due to a combination of the electron-donating qualities of the anion of compound **II.41**, and the electron-withdrawing qualities of the chloro substituent in compound **II.42**. A similar pattern was observed in the bromo analogues (**II.34** and **II.35**) with the protons of compound **II.34** upfield of those in **II.35**.

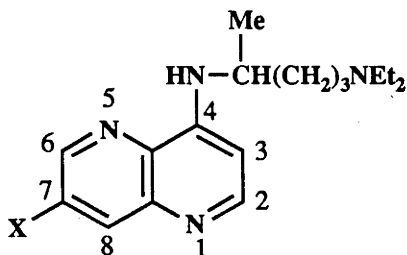
In contrast, the chemical shifts of 7-bromo (**II.34**) and 7-trifluoromethyl (**II.41**) derivatives of 1,5-naphthyridin-4-ol (as anions) showed only small differences; as did similar derivatives (**II.35** and **II.42**) of 4-chloro-1,5-naphthyridine (as neutral molecules). These differences of 0.1-0.2 ppm implied that the bromo and trifluoromethyl groups had similar electron-withdrawing (inductive) effects, which were slightly greater in the trifluoromethyl derivatives.

Inspection of the ^1H n.m.r. spectra in the Experimental section for the compounds **II.48a-II.48h** revealed that, of the protons attached to the naphthyridine ring, H6 was the most deshielded due to the adjacent ring nitrogen atom and trifluoromethyl group; and the most shielded proton was H3. The ring protons occurred for H2 at δ 8.58-8.60, for H3 at 6.95-7.06, for H6 at 8.92-8.94 and for H8 at 8.51-8.53; and the coupling constant applicable to each position was consistent, e.g. $J_{2,3}$ was 5.5 Hz and $J_{6,8}$ was 2 Hz.

The methylene group in all Mannich side chains appeared as a singlet; and its position was dependent on the amine present in the Mannich base. For those compounds containing the dialkylamino substituent it occurred at δ 3.72-3.73, for the pyrrolidinyl substituent at δ 3.80 and for the various piperidinyl derivatives at δ 3.63-3.70.

The ^1H n.m.r. spectra of the 7-trifluoromethyl-1,5-naphthyridine (**II.51**) and the 7-bromo-1,5-naphthyridine (**II.53**) are listed in Table II-3. As expected (and in agreement with the data in Table II-2) there were only slight differences in the spectra of these two compounds. For the aromatic protons this difference was 0.09-0.17 ppm and for the protons of the side chain it was 0.01-0.1 ppm.

Table II-3 ^1H n.m.r. spectral data (δ)^a for *N*⁴-(4-diethylamino-1-methyl-butyl)-7-substituted-1,5-naphthyridin-4-amine



Compound	X	H2	H3	H6	H8	1'-Me	CHMe	(CH ₂) ₂	CH ₂ N	NCH ₂ Me	NCH ₂ Me
II.51	CF ₃	8.60	6.61	8.82	8.47	1.36	3.72	1.66	2.41	2.53	1.01
II.53	Br ^b	8.48	6.52	8.65	8.36	1.33	3.62	1.60	2.50	2.52	1.01

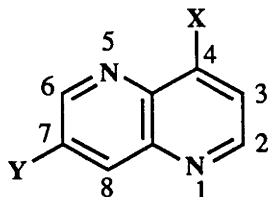
^a Chemical shifts are reported as δ values downfield from tetramethyl silane (TMS) as internal standard in deuteriochloroform as solvent.

^b Data from reference 110.

The ^{13}C n.m.r. spectrum of 4-chloro-7-trifluoromethyl-1,5-naphthyridine (II.42) (with 1,5-naphthyridine^{173,175} for comparison) and 2,6-bis(diethylaminomethyl)-4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol (II.48a) are recorded in Table II-4 and Figure II-1, respectively.

The assignments of the ^{13}C chemical shifts were carried out by running a decoupled ^{13}C n.m.r. spectrum, 2-D heteronuclear one-bond correlated (HETCOR) spectrum and 2-D heteronuclear multiple-bond correlated (HMBC) spectrum [for example see Appendix 1 (Figure A1 and A2)]. The HETCOR spectrum was used to determine the C-H connectivities present in the molecule; and the HMBC spectrum, to determine the C-H long-range coupling (especially for determining the quaternary carbons which do not appear in the HETCOR spectrum).

Table II-4 ^{13}C n.m.r. spectra (ppm) in CDCl_3 of 4-chloro-7-trifluoromethyl-1,5-naphthyridine (II.42) and 1,5-naphthyridine^{173,175} (for comparison)

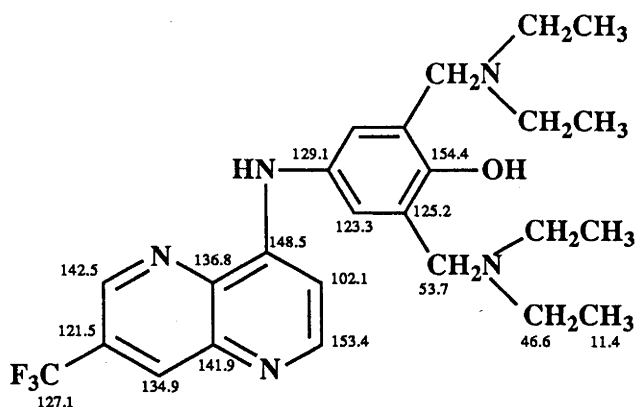


X	Y	C2	C3	C4	C6	C7	C8	C9	C10	CF ₃
H	H	151.0	124.1	137.2	151.0	124.1	137.2	144.0	144.0	-
Cl	CF ₃	152.2	126.2	143.2	147.2	124.6	135.9	144.5	142.5	127.8

As both rings in unsubstituted 1,5-naphthyridine ($\text{X}=\text{Y}=\text{H}$) are equivalent, four peaks only were observed for C2(C6), C3 (C7), C4 (C8) and C10 (C9) as shown in Table II-4. In the spectrum of 4-chloro-7-trifluoromethyl-1,5-naphthyridine ($\text{X}=\text{Cl}$, $\text{Y}=\text{CF}_3$), the most deshielded carbon was C2; and the least deshielded was C7.

The assignments of the ^{13}C chemical shifts for compound II.48a (Figure II-1) revealed that the most deshielded carbon was C1 in the phenolic ring (at 154.4 ppm), followed by C2' (at 153.4 ppm) in the naphthyridine ring, and the most shielded carbon was the methyl group (at 11.4 ppm). In the naphthyridine ring, obviously C6' (at 142.5 ppm) was more shielded than C2' (at 153.4 ppm) due to the adjacent trifluoromethyl group at position-7.

In the proton decoupled ^{13}C -spectrum, the carbon atom of the trifluoromethyl group appeared as a quartet with a coupling constant *ca* 30 Hz, due to C-F coupling.



II.48a

Figure II-1 The chemical shifts (ppm) of the carbon atoms in 2,6-bis(diethylaminomethyl)-4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol (II.48a)

II-6.2 Ionization constants; ultraviolet spectra and infrared spectra

The basic ionization constant (pK_a) of 7-trifluoromethyl-1,5-naphthyridin-4-ol (II.41) was determined by spectrophotometric methods¹⁷⁶ to be 1.05 ± 0.03 . Ionization constants have been published for 1,5-naphthyridine (pK_a 2.84)¹⁷⁷ and 1,5-naphthyridin-4-ol (basic pK_a 2.85, acidic pK_a 10.01).¹⁷⁸ Thus compound II.41 is a weaker base by 1.8 units compared to its 7-unsubstituted analogue; and this is due to the electron-withdrawing properties of the trifluoromethyl group which reduces basic strength. Protonation of compound II.41 probably also involves N-5. Ionization constants of the more complex (and less soluble) Mannich bases were not examined.

The ultraviolet spectrum of compound II.41 (basic pK_a 1.05) was measured at pH 5.27 (It was unchanged in the range pH 4.6-6.2). This spectrum is probably the neutral species of the keto form because it has been shown that α - and γ -hydroxy derivatives of nitrogen heterocycles as neutral species in aqueous solution exist predominantly in this form.¹⁷⁹ The spectrum consisted of two main absorption bands at 248 nm ($\log \epsilon$ 4.27) and 336 nm ($\log \epsilon$ 3.85). These absorption maxima are at longer wavelengths than in 1,5-naphthyridin-4-ol [$\lambda_{\max}$ 240 nm ($\log \epsilon$ 4.43), 322 nm ($\log \epsilon$

4.02) at pH 6.0]¹⁸⁰ and the bathochromic shift (red shift) is due to the 7-trifluoromethyl substituent (which withdraws electrons from the ring system).

The ultraviolet spectrum of compound **II.48a** was measured in aqueous solution at pH 8.15 (it was constant in the range pH 7.6-8.42) and consisted of three main absorption bands at λ_{max} 261 nm (log ϵ 4.35), 320 nm (log ϵ 3.72) and 370 nm (log ϵ 3.84).

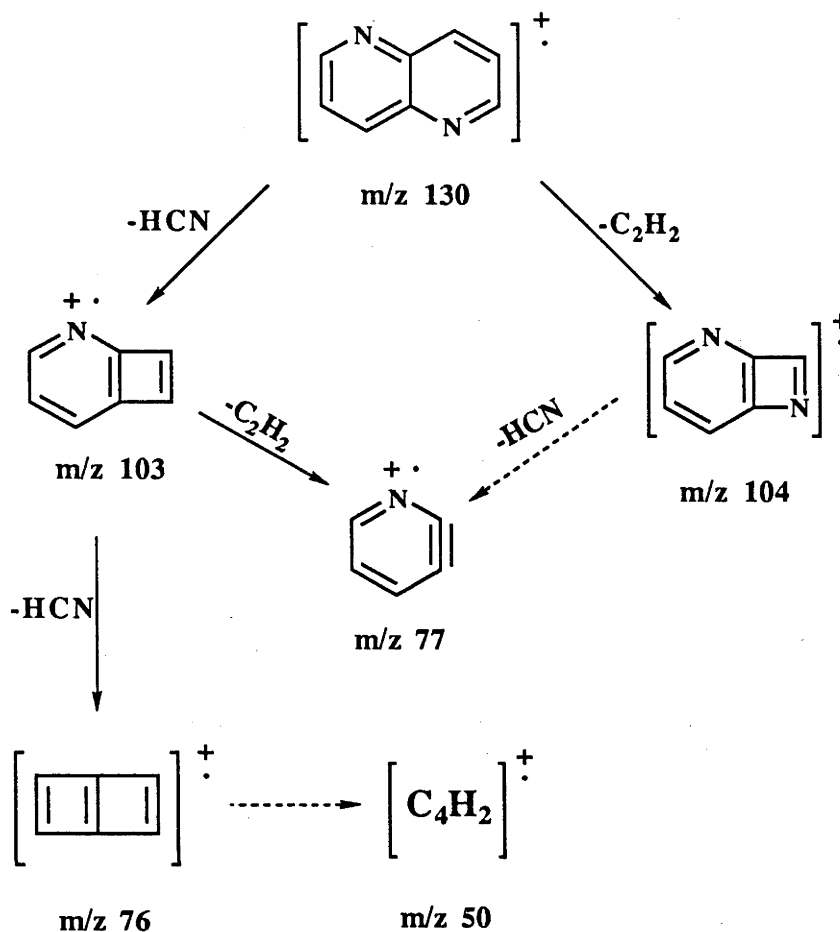
The infrared spectrum of the 1,5-naphthyridin-4-ol has been examined in the solid state and in chloroform solution.¹⁶⁰ It displayed N-H and O-H stretching at 3202 (w) and 3108 (m) cm^{-1} , and double bond stretching at 1624 (s) and 1586 (m) cm^{-1} indicative of both keto and enol tautomeric forms. The spectrum of 7-trifluoromethyl-1,5-naphthyridin-4-ol (**II.41**), measured in the present work in KBr disc, shows N-H stretching at 3470 (w) cm^{-1} , and double-bond stretching at 1624 (s), 1614 (s) (indicative of the keto form) and 1517 (s) cm^{-1} ; and C-F stretching at 1136 cm^{-1} .

The infrared spectrum of 2,6-bis(4-methylpiperidin-4-ylmethyl)-4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol (**II.48g**) displayed strong O-H and N-H stretching absorptions at 3460 and 3400 cm^{-1} , and was devoid of absorption in the carbonyl region.

II-6.3 Mass spectra

Mass spectra of the unsubstituted 1,5-naphthyridine and its methyl derivatives have been reported.¹⁸¹ In unsubstituted 1,5-naphthyridine, the major cleavage involves loss of HCN and C_2H_2 , and the fragmentation pattern is depicted in Scheme II-6.

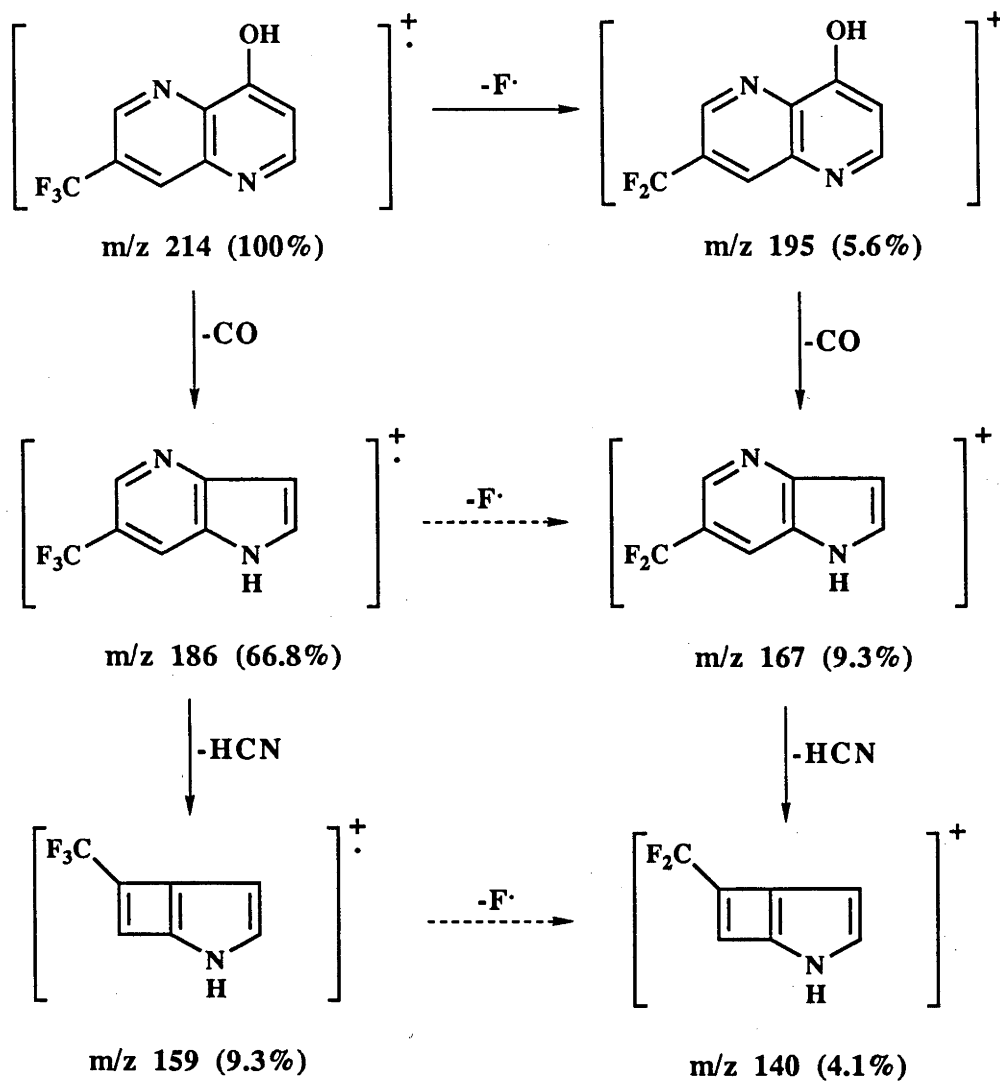
In this work, the mass spectra of the 4-substituted-7-trifluoromethyl-1,5-naphthyridines [(**II.41**), (**II.42**) and (**II.48d**)] were examined. The spectrum of compound **II.41** shows an intense peak for the molecular ion (m/z 214) and another strong peak at m/z 186 ($\text{M}^+ - \text{CO}$) and peaks at m/z 195, 167, 159 and 140. The fragmentation pattern is depicted in Scheme II-7, and involves the loss of CO, HCN and F.



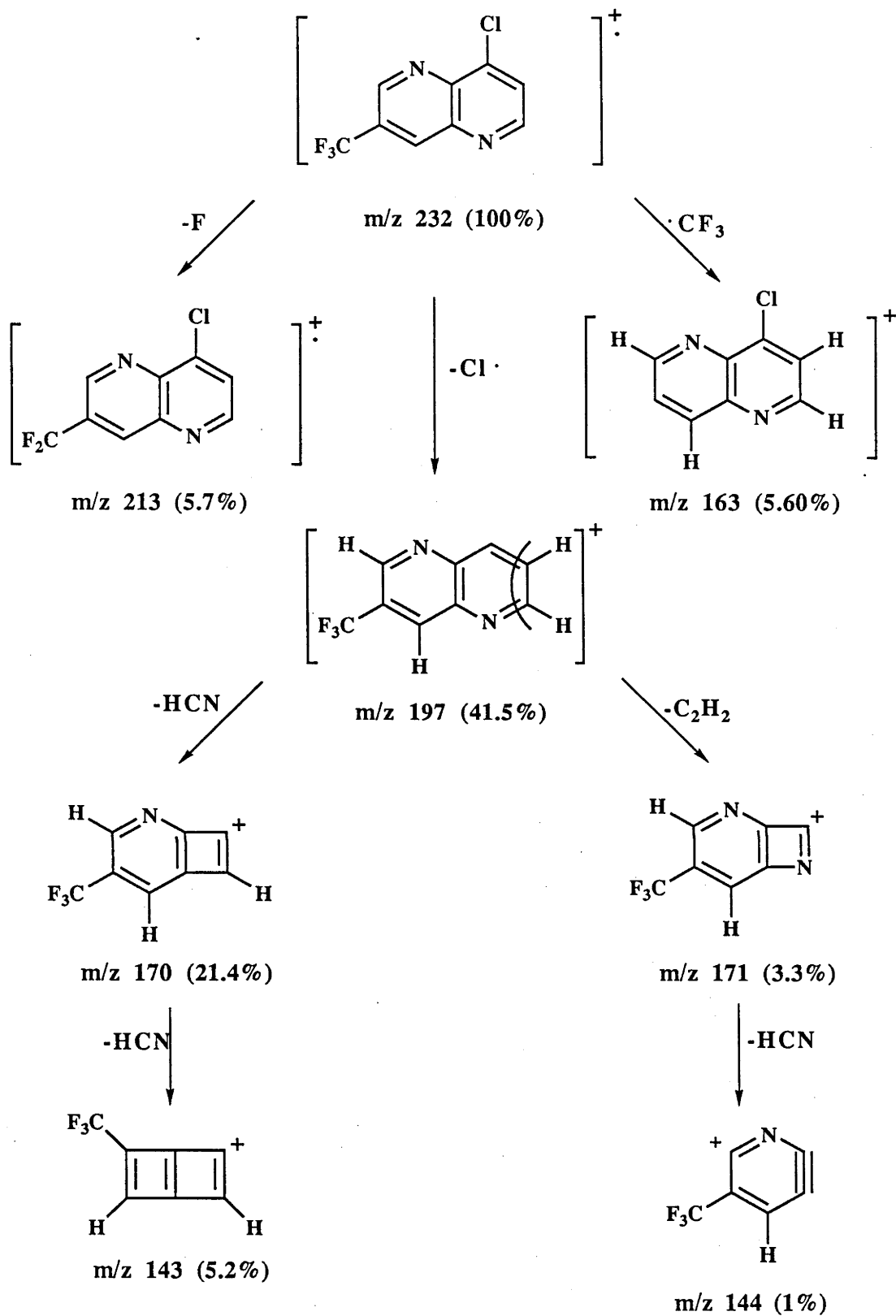
Scheme II-6 Fragmentation of 1,5-naphthyridine

The mass spectrum of the chloro compound (II.42) shows the two molecular ion peaks at m/z 232 and 234 with relative intensities of the lines in the ratio 3:1. This is characteristic of ions from chlorine-containing compounds and is engendered by the ^{35}Cl and ^{37}Cl isotopes.¹⁸² The fragmentations observed involve the loss of Cl, 2HCN and F, and it is shown in Scheme II-8.

The fragmentation of the di-Mannich base (II.48d; piperidinyl derivative; M^+ 499) differed from the above two compounds; the most intense peak is due to the loss of $C_5H_{10}N$ (piperidinyl group) (m/z 84) to give the fragment of $M-C_5H_{10}N$ (m/z 414).



Scheme II-7 The major fragmentation pattern of 7-trifluoromethyl-1,5-naphthylidin-4-ol (II.41)



Scheme II-8 The major fragmentation pattern of 4-chloro-7-trifluoromethyl-1,5-naphthylidin-4-ol (II.42)

II-7 Experimental

II-7.1 General Topics

- (a) Melting points (m.ps.) were taken in Pyrex capillaries with a Gallenkamp melting point apparatus and were uncorrected.
- (b) Infrared (i.r) spectra were recorded on a Unicam SP 1000 infrared spectrophotometer as potassium bromide discs. The following abbreviations were adopted: br (broad); s (sharp); m (medium); mw (medium weak); w (weak). Assignment of the absorptions were attempted if appropriate. The 1602 cm^{-1} and 1495 cm^{-1} regions of the i.r spectra were calibrated by the absorption of polystyrene film.
- (c) Ultraviolet (u.v.) spectra were recorded on a CARY 219 UV-visible spectrophotometer between 200-500 nm, in aqueous solution at 18-20° and at the specific pH. The wavelengths of absorption maxima (λ_{max}) and the extinction coefficients (ϵ) were checked on a Perkin-Elmer Lambda I uv-visible spectrophotometer. The buffers were of constant ionic strength, $I=0.02$.¹⁸³
- (d) Ionization constants were determined using the spectrophotometric method of Albert and Serjeant.¹⁷⁶
- (e) ^1H Nuclear magnetic resonance spectra (^1H n.m.r.) were recorded either at 90 MHz and 30° on a Jeol FX90Q or at 200 or 300 MHz on a Varian XL200E or VXR300 fourier-transform spectrometer. Data were presented in the following order: chemical shift (δ) relative to tetramethylsilane in organic solvent or sodium 3-trimethylsilylpropane sulfonate in deuterated sodium hydroxide/deuterium oxide; multiplicity; coupling constant (J) in Hz; and assignment (where possible). Samples were run in CDCl_3 unless specified otherwise. The following abbreviations were adopted: s (singlet); d (doublet); t (triplet); br s (broad singlet); m (multiplet); dd (doublet of doublets). Exchangable protons were identified by their disappearance upon addition of deuterium oxide.
- (f) ^{13}C Nuclear magnetic resonance techniques were performed at 300 MHz on a Varian VXR300 instrument at 25°. Samples were run in CDCl_3 . Data were presented in the following order: chemical shift (ppm) relative to deuterated solvent peak and assignments where appropriate.

- (g) Two dimensional (2-D) nuclear magnetic resonance spectra such as the heteronuclear one-bond correlated (HETCOR) spectra, the multiple-bond correlated (HMBC) spectra and the proton-proton correlation spectroscopy (COSY) spectra run by Ms P. Simmonds, University Nuclear Magnetic Resonance Centre, the Australian National University, using a Varian VXR 300 spectrometer.
- (h) Low resolution mass spectra (MS) were recorded on an Incos data system attached to a VG-Micromass 7070F double focusing mass spectrometer using electron impact (EI) at 70 eV or chemical ionization (CI) with ammonia at the Research School of Chemistry under the supervision of Dr J.K. MacLeod. Data are presented in the following order: m/z value; relative intensity as a percentage of the base peak. The mass spectra are EI unless specified otherwise.
- (i) Microanalyses were performed by The Australian National University Analytical Service Unit, Canberra. All solids were dried for at least 4 hours under vacuum (0.2 mmHg) and at 60-100° prior to analysis, unless otherwise specified.
- (j) Analytical thin layer chromatography (t.l.c.) was performed on glass plates and aluminium plates with Merck Kieselgel 60 F₂₅₄ or Merck aluminium oxide 60 F₂₅₄ neutral (type E) of 0.25 mm and 0.2 mm thickness, respectively. Preparative thin layer chromatography (PTLC) was performed on glass plates precoated with Merck aluminium oxide 60 F₂₅₄ (type E) of 2 mm thickness, or with Merck silica gel 60F₂₅₄ of 2 mm thickness. Columns for chromatography were packed using Merck aluminium oxide 90 active neutral (0.063-0.200 mm, 70-230 mesh ASTM) or aluminium oxide for chromatography (M&B Laboratory Reagent, MX6390).
- (j) Reaction temperatures refer to external oil bath temperatures unless stated otherwise.
- (l) Where full experimental details are not recorded, percentage yields are given in brackets.
- (m) Dry solvents were obtained by standard procedures according to D.D. Perrin, W.L.F. Armarego, and D.R. Perrin, Purification of Laboratory Chemistry (2nd Ed., Pergamon Press, 1980).

II-7.2 Synthetic work

5-Trifluoromethylpyridin-3-amine¹⁷⁰ (II.37)

A mixture of 3-chloro-5-trifluoromethylpyridine (10.0 g), cuprous chloride (Prepared by passing sulphur dioxide into a solution of 70.0 g cupric sulphate pentahydrate and 18.3 g sodium chloride in 250 ml water), and aqueous ammonia (190 ml; 28-30%) was heated with stirring in a bomb at 170° for 48 h. After cooling, the reaction mixture was extracted with methylene chloride, the extract dried (Na₂SO₄), and the solvent evaporated to leave an oil (7.72 g). It was purified by chromatography in methylene chloride over a column of alumina (18 cm) to give the title compound as a white solid (7.49g), m.p. 40-41.5° (Found, for a sample dried in air at 20° for 24 h: C, 44.3; H, 2.9; N, 17.2. Calc. for C₆H₅F₃N₂: C, 44.4; H, 3.1; N, 17.3%). ¹H n.m.r. δ 3.82, br, NH₂; 7.13 br s, H₄; 8.21, s, H 2; 8.24, s, H 6.

Diethyl (5'-trifluoromethylpyridin-3'-ylaminomethylene)malonate (II.38)

A mixture of 5-trifluoromethylpyridin-3-amine (2.976 g) and diethyl ethoxy methylenemalonate (3.888 g) was heated at 100° for 2 h. On cooling it gave a waxy solid which was dissolved in methanol (36 ml), heated to 50° and diluted with an equal volume of water. On scratching and chilling it gave *diethyl (5'-trifluoromethylpyridin-3'-ylaminomethylene)malonate* (6.00 g), m.p. 65-67° (Found, for a sample dried at 20° *in vacuo* for 2 days: C, 50.3, H, 4.5; N, 8.3. C₁₄H₁₅F₃N₂O₄ requires C, 50.6; H, 4.6, N, 8.4%). ¹H n.m.r. δ 1.35, t, J 7 Hz, 1.39, t, J 7 Hz, MeCH₂; 4.28, q, J 7 Hz, 4.33, q, J 7 Hz, MeCH₂; 7.66, br s, H₄'; 8.39, s, 8.53, s, NCH=; 8.65, br s, H₂',6'.

Ethyl 4-hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylate (II.39)

Diethyl (5'-trifluoromethylpyridin-3'-ylaminomethylene)malonate (4.8 g) was added cautiously and in small portions to refluxing diphenyl ether (65 ml) and when the addition was complete it was refluxed for 2-3 h. After cooling, the mixture was diluted with hexane (260 ml), and the pale solid (2.1 g; m.p. >300°) was filtered off and washed with acetone to give the *title compound* (Found: C, 50.3; H, 3.1; N, 10.0.

$C_{12}H_9F_3N_2O_3$ requires C, 50.4; H, 3.2; N, 9.8%). 1H n.m.r. ($CD_3SOCD_3 + NaOD/D_2O$) δ 1.27, t, J 7 Hz, $MeCH_2$; 4.19, q, J 7 Hz, $MeCH_2$; 8.25, br s, H8; 8.72, br s, H6; 8.74, s, H2.

4-Hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylic acid (II.40)

Ethyl 4-hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylate (7.69 g) was refluxed with 10% aqueous sodium hydroxide (89.0 ml) at 120° ; all the solid had dissolved after 30 min. The refluxing was continued for a further 1 h and on cooling a solid separated. This mixture was diluted with water (295 ml), heated to boiling to dissolve the solid, filtered with charcoal, and the filtrate adjusted with hydrochloric acid to pH 2-3 and chilled. A light grey precipitate separated and it was filtered off to give *4-hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylic acid* (6.14 g), m.p. $>300^\circ$ (Found: C, 46.7; H, 2.0; N, 10.9. $C_{10}H_5F_3N_2O_3$ requires C, 46.6; H, 2.0; N, 10.9%). 1H n.m.r. ($D_2O + NaOD$) δ 8.44, br s, H8; 8.64, s, H2; 8.87, br s, H6.

7-Trifluoromethyl-1,5-naphthyridin-4-ol (II.41)

4-Hydroxy-7-trifluoromethyl-1,5-naphthyridine-3-carboxylic acid (6.14 g) was added in small portions to boiling quinoline (230 ml) and then the mixture was refluxed for 1 h. After cooling, the mixture was diluted with three volumes of acetone to give a precipitate. The grey product (4.7 g) was filtered off, washed with acetone and recrystallised from water or methoxyethanol to give *7-trifluoromethyl-1,5-naphthyridin-4-ol*, m.p. $>300^\circ$ (Found: C, 50.7; H, 2.4; N, 12.8. $C_9H_5F_3N_2O$ requires C, 50.5; H, 2.4; N, 13.1%). 1H n.m.r. ($D_2O + NaOD$) δ 6.75, d, $J_{2,3}$ 6 Hz, H3; 8.39, d, $J_{2,3}$ 6 Hz, H2; 8.45, br s, H8; 8.87, d, $J_{6,8}$ 2 Hz, H6. I.r. ν_{max} 3470 (w) (NH st), 2800 (br s), 2080 (m), 1640 (s) + 1517 (s) (C=O st, C=C st), 1624 (s), 1614 (s) (ar skeletal st), 1570 (m) (ar skeletal st), 1437 (m), 1376 (m), 1136 (m), 940 (mw), 841 (m) cm^{-1} . U.v. λ_{max} (pH 5.27) 248 nm (log ϵ 4.27), 336 nm (log ϵ 3.85). MS m/z 214 (M^+) (100%), 195 (M^+-F) (5.6%), 186 (M^+-CO) (66.8%), 167 [$(M^+-F)-CO$] (9.3%), 159 [$(M^+-CO)-HCN$] (9.3%), 140 (4.1%).

4-Chloro-7-trifluoromethyl-1,5-naphthyridine (II.42)

7-Trifluoromethyl-1,5-naphthyridin-4-ol (0.122 g), phosphorus pentachloride (0.255 g) and phosphoryl chloride (2.5 ml) were refluxed at 110° for 3 h, and excess phosphoryl chloride was distilled under reduced pressure. The residue was treated with ice and then saturated sodium carbonate solution until it was basic; the mixture was extracted with chloroform, the extract dried (Na₂SO₄), and the solvent evaporated. The product was subjected to column chromatography (alumina; benzene) and recrystallised from heptane to give white crystals of *4-chloro-7-trifluoromethyl-1,5-naphthyridine* (0.119 g), m.p. 147-149° (Found, for a sample dried at 20° and 0.2 mmHg for 24 h: C, 46.5; H, 1.7; N, 11.9. C₉H₄ClF₃N₂ requires C, 46.4; H, 1.7; N, 12.0%). ¹H n.m.r. δ 7.89, d, J 5 Hz, H3; 8.73, br s, H8; 8.97, d, J 5 Hz, H2; 9.27, d, J 2 Hz, H6. MS m/z 232 (M⁺) (100%), 234 (M⁺⁺²) (31%), 213 (M^{+-F}) (5.7%), 197 (M^{+-Cl}) (41.5%), 171 (3.3%), 170 [(M^{+-Cl})-HCN] (21.5%), 163 (M^{+-CF₃}) (5.6%), 144 (1%), 143 (5.2%).

2,6-Bis(diethylaminomethyl)-4-(7'-trifluoromethyl-1',5'-naphthyridin-4'-ylamino)phenol (II.48a)

A mixture of 4-chloro-7-trifluoromethyl-1,5-naphthyridine (0.066 g), 4-amino-2,6-bis(diethylaminomethyl)phenol¹⁷² (0.080 g), methanol (1.3 ml), water (0.4 ml) and concentrated hydrochloric acid (4 drops) was refluxed at 100° for 4 h. The methanol was evaporated under reduced pressure and the residue diluted with a little water and adjusted with N ammonium hydroxide to pH 9-10. The product was extracted into chloroform, and subjected to PTLC (alumina; 7% methanol in methylene chloride; then alumina; 1% methanol in chloroform) to give, as a yellow oil, *2,6-bis(diethylaminomethyl)-4-(7'-trifluoromethyl-1',5'-naphthyridin-4'-ylamino)phenol* (0.126 g) (Found: C, 63.2; H, 6.9; N, 14.4. C₂₅H₃₂F₃N₅O requires C, 63.1; H, 6.8; N, 14.7%). ¹H n.m.r. δ 1.12, t, J 7 Hz, MeCH₂; 2.65, q, J 7 Hz, MeCH₂; 3.73, s, Ar CH₂N; 6.99, d, J 5.5 Hz, H3'; 7.15, s, H3,5; 8.36, br s, NH?; 8.53, br s, H8'; 8.60, d, J 5.5 Hz, H2'; 8.93, d, J 2 Hz, H6'. U.v. λ_{max} (pH 8.15) 261 nm (log ε 4.35), 320 nm (log ε 3.72), 370 nm (log ε 3.84). The ¹³C n.m.r. data are presented in Figure II-1.

2,6-Bis(dipropylaminomethyl)-4-(7'-trifluoromethyl-1',5'-naphthyridin-4'-ylamino)phenol (II.48b)

In a similar manner to that described above (but refluxed for 5 h) 4-chloro-7-trifluoromethyl-1,5-naphthyridine (0.061 g) and 4-amino-2,6-bis(dipropylaminomethyl)phenol¹⁷² (0.088 g) gave a crude product which was purified by PTLC (alumina; chloroform) to give the *title compound* as a yellow oil (0.104 g) (Found: C, 65.5; H, 7.9, N, 13.2. $C_{29}H_{40}F_3N_5O$ requires C, 65.5; H, 7.6; N, 13.2%). 1H n.m.r. δ 0.90, t, J 7 Hz, $MeCH_2$; 1.56, m, $MeCH_2CH_2N$; 2.50, m, $MeCH_2CH_2N$; 3.72, s, $ArCH_2N$; 7.00, d, J 5.5 Hz, $H_{3'}$; 7.16, s, $H_{3,5}$; 8.33, br s, $NH?$; 8.53, br s, $H_{8'}$; 8.58, d, J 5.5 Hz, $H_{2'}$; 8.93, d, J 2 Hz, $H_{6'}$.

2,6-Bis(pyrrolidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)phenol (II.48c)

4-Chloro-7-trifluoromethyl-1,5-naphthyridine (0.125 g) and 4-amino-2,6-bis-(pyrrolidin-1'-ylmethyl)phenol¹⁷² (0.148 g), treated similarly but refluxed for 8 h, and extracted with ether, gave a crude product which was purified by PTLC (alumina; 1% methanol/chloroform) to give the *title compound* as a yellow oil (0.156 g) (Found, for a sample dried at 20°/0.1 mmHg for 1 h: C, 63.5; H, 6.3; N, 14.5. $C_{25}H_{28}F_3N_5O \cdot 0.2 H_2O$ requires C, 63.2; H, 6.0; N, 14.7%). 1H n.m.r. δ 1.86, complex, $H_{3,4}$; 2.67, complex, $H_{2,5}$; 3.80, s, $ArCH_2N$; 6.99, d, J 5.5 Hz, $H_{3''}$; 7.13, s, $H_{3,5}$; 8.29, br, NH ; 8.53, br s, $H_{8''}$; 8.60, d, J 5.5 Hz, $H_{2''}$; 8.94, d, J 2 Hz, $H_{6''}$.

2,6-Bis(piperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)phenol (II.48d)

4-Chloro-7-trifluoromethyl-1,5-naphthyridine (0.050 g) and 4-amino-2,6-bis-(piperidin-1'-ylmethyl)phenol¹⁷² were similarly refluxed for 3.5 h, and the product purified by PTLC (alumina; 1% methanol in methylene chloride), to give the *title compound* as a yellow oil (0.059 g) (Found, for a sample dried at 20°/0.2 mmHg for 4 h: C, 64.3; H, 6.9; N, 13.7. $C_{27}H_{32}F_3N_5O \cdot 0.3 H_2O$ requires C, 64.2; H, 6.5; N, 13.9%). 1H n.m.r. δ 1.58, complex, $H_{3',4',5'}$; 2.58, complex, $H_{2',6'}$; 3.69, s,

ArCH₂N; 7.04, d, J 5.5 Hz, H3"; 7.17, s, H3,5; 8.31, br s, NH?; 8.52, br s, H8"; 8.60, d, J 5.5 Hz, H2"; 8.92, d, J 2 Hz, H6". MS m/z 499 (M⁺) (26.8%), 414 (M⁺-C₅H₁₀N) (97%), 84 (C₅H₁₀N) 100%).

2,6-Bis(3'-methylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)phenol (II.48e)

This compound was prepared from 4-chloro-7-trifluoromethyl-1,5-naphthyridine (0.078 g) and 4-amino-2,6-bis(3'-methylpiperidin-1'-ylmethyl)phenol¹⁷² (0.111 g) as above but refluxed for 5 h. It was purified by PTLC (alumina; chloroform) to give the *title compound* as a yellow oil (0.130 g) (Found: C, 66.0; H, 7.2; N, 12.9. C₂₉H₃₆F₃N₅O requires C, 66.0; H, 6.9; N, 13.3%). ¹H n.m.r. δ 0.88, d, J 5.5 Hz, Me; 1.72, complex, H3',4',5'; 2.91, complex, H2',6'; 3.70, s, ArCH₂N; 7.06, d, J 5.5 Hz, H3"; 7.17, s, H3,5; 8.34, br s, NH(?); 8.53, br s, H8"; 8.60, d, J 5.5 Hz, H2"; 8.93, d, J 2 Hz, H6".

2,6-Bis(3',5'-dimethylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)phenol (II.48f)

This compound was prepared in a similar manner from 4-chloro-7-trifluoromethyl-1,5-naphthyridine (0.034 g) and 2,6-bis(3',5'-dimethylpiperidin-1'-ylmethyl)phenol¹⁷² (0.054 g). The product was purified by PTLC (alumina; 4% methanol in chloroform) to give the *title compound* (0.092 g) (Found, for a sample dried at 20°/0.2 mmHg for 20 h: C, 66.7; H, 7.4; N, 12.4. C₃₁H₄₀F₃N₅O 0.2 H₂O requires C, 66.6; H, 7.3; N, 12.5%). ¹H n.m.r. δ 0.85, d, J 5.5 Hz, Me; 1.70, complex, H3',4',5'; 2.93, m, H2',6'; 3.65, s, ArCH₂N; 7.00, d, J 5.5 Hz, H3"; 7.11, s, H3,5; 8.31, br s, NH(?); 8.53, br s, H8"; 8.59, d, J 5.5 Hz, H2"; 8.93, d, J 2 Hz, H6".

2,6-Bis(4'-methylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)phenol (II.48g)

4-Chloro-7-trifluoromethyl-1,5-naphthyridine (0.101 g) and 4-amino-2,6-bis(4'-methylpiperidin-1'-ylmethyl)phenol¹⁷² (0.144 g) reacted as above, and the product was purified by PTLC (alumina; 1% methanol in chloroform) to give the *title compound*

(0.134 g) (Found: C, 65.8; H, 6.9; N, 13.2. $C_{29}H_{36}F_3N_5O$ requires C, 66.0; H, 6.9; N, 13.3%). 1H n.m.r. δ 0.94, d, J 4 Hz, Me; 1.55, complex, 2.12, m, 2.98, m, $H_{2',3',4',5',6'}$; 3.65, s, $ArCH_2N$; 6.97, d, J 5.5 Hz, $H_{3'}$; 7.11, s, $H_{3,5}$; 8.32, br s, $NH?$, 8.53, br s, $H_{8''}$; 8.60, d, J 5.5 Hz, $H_{2''}$; 8.93, d, J 2 Hz, $H_{6''}$. I.r. ν_{max} 3460 (w) (OH st), 3400 (w) (NH st), 2970 (w), 2940 (m) (CH_2 st), 1595 (m) (ar. st), 1580 (m), 1475 (s), 1372 (s), 1162 (s), 1136 (s).

2,6-Bis(4'-benzylpiperidin-1'-ylmethyl)-4-nitrophenol (II.46)

Paraformaldehyde (0.86 g) and 4-benzylpiperidine (4.98 g) in ethanol (5.0 ml) were warmed until homogeneous. Then 4-nitrophenol (1.0 g) was added and the mixture was refluxed at 90-95° for 16 h. After cooling, the mixture was diluted with hexane and a little chloroform, and the yellow solid (3.74 g) was filtered off. It was recrystallised from a mixture of dichloromethane and light petroleum (b.p. 60-80°) to give yellow crystals of 2,6-bis(4'-benzylpiperidin-1'-ylmethyl)-4-nitrophenol (2.416 g), m.p. 110-111.5° (Found: C, 72.9; H, 8.1; N, 8.0. $C_{32}H_{39}N_3O_3 \cdot 0.8 H_2O$ requires C, 72.8; H, 7.8; N, 8.0%). 1H n.m.r. δ 1.12-2.21, complex, 2.95, m, $H_{2',3',4',5',6'}$; 2.53, br s, CH_2Ph ; 3.66, s, $ArCH_2N$; 7.22, complex, Ph; 8.00, s, $H_{3,5}$.

2,6-Bis(4'-benzylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)phenol (II.48h)

2,6-Bis(4'-benzylpiperidin-1'-ylmethyl)-4-nitrophenol (0.402 g) was dissolved in ethanol (10.0 ml) and saturated ethanolic ammonia (30.0 ml) and shaken with hydrogen over Raney nickel until hydrogen uptake ceased and the yellow colour was discharged. The catalyst was filtered off on Celite and the filtrate evaporated on the rotary evaporator to give the amine as a purple liquid (0.116 g). 1H n.m.r. δ 1.16 - 2.37, complex, 2.95, m, $H_{2',3',4',5',6'}$; 2.52, br s, CH_2Ph ; 3.53, s, $ArCH_2N$; 6.46, s, $H_{3,5}$; 7.21, complex, Ph.

A mixture of 4-chloro-7-trifluoromethyl-1,5-naphthyridine (0.056 g) and 4-amino-2,6-bis(4'-benzylpiperidin-1'-ylmethyl)phenol (0.116 g) in aqueous methanol containing hydrochloric acid was refluxed for 8 h. The crude product was subjected to PTLC (alumina; methylene chloride, then 30% ethyl acetate in hexane) to give the *title*

compound (0.106 g) (Found: C, 71.5; H, 6.8; N, 10.0. $C_{41}H_{44}F_3N_5O$ requires C, 71.4; H, 6.7; N, 10.1%). 1H n.m.r. δ 1.20 - 2.20, complex, 2.95, m, $H_{2',3',4',5',6'}$; 2.54, br s, CH_2Ph ; 3.63, s, $ArCH_2N$; 6.95, d, J 5.5 Hz, $H_{3''}$; 7.09, s, $H_{3,5}$; 7.22, complex, $CH_2C_6H_5$; 8.29, br s, $NH?$; 8.51, br s, $H_{8''}$; 8.58, d, J 5.5 Hz, $H_{2''}$; 8.92, br s, $H_{6''}$.

***N*⁴-(4'-Diethylamino-1-methylbutyl)-7-trifluoromethyl-1,5-naphthyridin-4-amine (II.51)**

4-Chloro-7-trifluoromethyl-1,5-naphthyridine (0.05 g), *N*¹,*N*¹-diethylamino-pentane-1,4-diamine (0.39 g) and heptane (1.0 ml) were heated in a Teflon-lined reaction vessel at 150° for 20 h. The solvents were then removed on a rotary evaporator, and the residue subjected to PTLC (alumina; 30% ethyl acetate in hexane) to give *N*⁴-(4'-diethylamino-1'-methylbutyl)-7-trifluoromethyl-1,5-naphthyridin-4-amine (0.68 g) as a yellow oil (0.068 g) (Found, for a sample dried at 55°/0.2 mmHg for 4 h: C, 60.5; H, 7.5; N, 16.1%. $C_{18}H_{25}F_3N_4$ requires C, 61.0; H, 7.1; N, 16.1%. 1H n.m.r. δ 1.01, t, J 7 Hz, $MeCH_2$; 1.36, d, J 6.5 Hz, $MeCH$; 1.66, complex, $CH_2CH_2CH_2N$; 2.41, complex, CH_2N ; 2.53, q, J 7 Hz, CH_2Me ; 3.72, complex, $CHMe$; 6.61, d, J 5.5 Hz, H_3 ; 6.70, br s, $NH?$; 8.47, br s, H_8 ; 8.60, d, J 5.5 Hz, H_2 ; 8.85, d, J 2 Hz, H_6 .

II-7.3 Determination of the basic ionization constant of 7-trifluoromethyl-1,5-naphthyridin-4-ol (II.41)

Substance: 7-Trifluoromethyl-1,5-naphthyridin-4-ol

$\text{C}_9\text{H}_5\text{F}_3\text{N}_2\text{O}$; MW 214.146

Concentration: 1.0×10^{-4} M. Recrystallised compound, m.p. $>300^\circ$, was dried at $20^\circ/0.2$ mmHg overnight and 0.0107 g was dissolved in boiling water (200 ml), then cooled and made up to 500.0 ml with water. This stock solution (3.0 ml) was diluted with appropriate buffers (1.0 ml).

Analytical wavelength: 332 nm; cell, 1 cm

Buffer: Hydrochloric acid and sulphuric acid buffers (I=0.1)

Species: Neutral and cationic species

Temperature: 20°

Result:

H_0 value:	1.4	1.2	0.98	0.8	0.6
pK_a calc.:	1.01	1.03	1.06	1.06	1.02
$\text{pK}_a =$	1.04 ± 0.03				

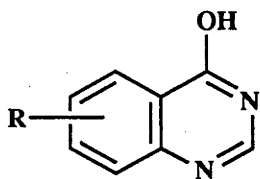
CHAPTER III

CHAPTER III Di-Mannich Bases of 4-(7-Trifluoromethylquinazolin-4-yl-amino)phenol and *N*⁴-(4-Diethylamino-1-methylbutyl)-7-trifluoromethyl-quinazolin-4-amine

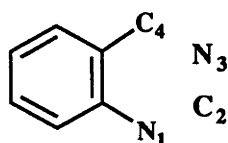
III-1 Introduction

In this chapter, the syntheses of di-Mannich bases of 4-(7-trifluoromethyl-quinazolin-4-ylamino)phenol, and *N*⁴-(4-diethylamino-1-methylbutyl)-7-trifluoromethyl-quinazolin-4-ylamine are reported. The required intermediates for these two derivatives are di-Mannich bases of 4-nitrophenol, which have been mentioned in Chapter II; and 4-chloro (and 4-hydroxy)-7-trifluoromethylquinazoline, which will be discussed below.

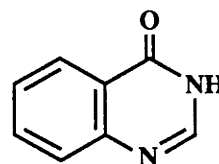
There is a number of methods for the preparation of quinazolin-4-ol and its derivatives (III.1); these have been reviewed by Armarego.¹⁸⁴ Most of these syntheses involved an *o*-disubstituted benzene (III.2) which was then condensed with the C2 N3 segment to give quinazolines in various ways.¹⁸⁴⁻¹⁸⁶ Quinazolines with substituents in the pyrimidine ring or benzene ring or in both rings could thus be prepared.



III.1



III.2



III.3

The most common synthesis, and that most relevant to the present work, was that known as Niementowski's synthesis.

III-2 Literature Preparations of Quinazolin-4-ol and its Derivatives *via* Niementowski's Synthesis

The original method of Niementowski's synthesis involved the reaction of anthranilic acid with formamide at 120-130° for 3 h to give quinazolin-4-ol.¹⁸⁷

Homologous amides such as acetamide were also used to give 2-substituted quinazolin-4-ols.¹⁸⁷

This type of the reaction also proceeds with substituted anthranilic acids containing substituents such as halogeno,^{188,189} alkyl¹⁹⁰ and trifluoromethyl^{189,191} groups. Further details¹⁸⁴ and the mechanism^{192,193} of Niementowski's synthesis have been reported.

Quinazolin-4-ol as the neutral molecule in aqueous solution has been shown^{194,195} to exist mainly in the *o*-quinonoid form (**III.3**).

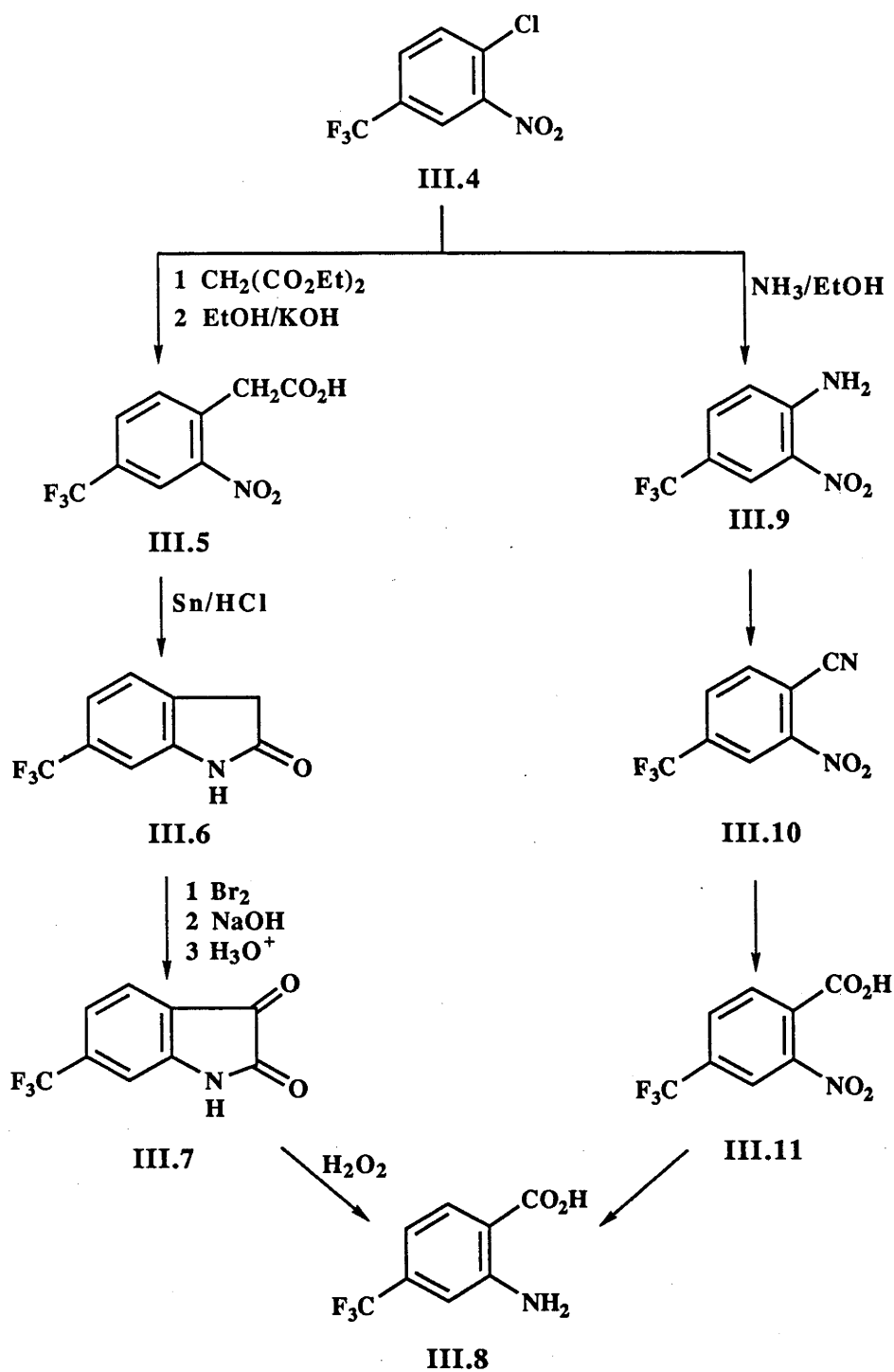
III-3 Synthesis of 2-Amino-4-trifluoromethylbenzoic Acid

The necessary intermediate for the preparation of the 4-chloro (and 4-hydroxy)-7-trifluoromethylquinazolines described in this chapter was 2-amino-4-trifluoromethylbenzoic acid (**III.8**). Two methods have been described for the synthesis of compound **III.8**. The first pathway has been described by Simet¹⁹⁶ (Simet's pathway) from 1-chloro-2-nitro-4-trifluoromethylbenzene (**III.4**) through (2-nitro-4-trifluoromethylphenyl)-acetic acid (**III.5**), 6-trifluoromethyloxindole (**III.6**) and 6-trifluoromethylisatin (**III.7**). The other pathway has been described by Hauptschein *et al.*¹⁹⁷ (Hauptschein's pathway) from 2-nitro-4-trifluoromethylaniline (**III.9**) through 2-nitro-4-trifluoromethylbenzonitrile (**III.10**) and 2-nitro-4-trifluoromethylbenzoic acid (**III.11**) (shown in Scheme III-1).

In the present work, the procedure described by Simet was found to be the preferred route, mainly because of difficulties with the diazotisation in the alternative procedure. Details of these methods are discussed below.

The acid compound (**III.5**) was prepared by alkylation of 1-chloro-2-nitro-4-trifluoromethylbenzene (**III.4**) with ethyl malonate, in refluxing ethanol for 2.5 h; followed by hydrolysis and decarboxylation with 10% aqueous potassium hydroxide solution at reflux for 1.5 h. This compound **III.5** was reduced by tin in boiling 9 N hydrochloric acid solution to give the corresponding oxindole (**III.6**). The compound **III.6** reacted with bromine in refluxing carbon tetrachloride for 15 minutes, followed by hydrolysis with 5% sodium hydroxide to afford 6-trifluoromethylisatin (**III.7**). Oxidation of the isatin (**III.7**)

with 1.5% hydrogen peroxide solution in 2% sodium hydroxide at 80° afforded 2-amino-4-trifluoromethylbenzoic acid (III.8).

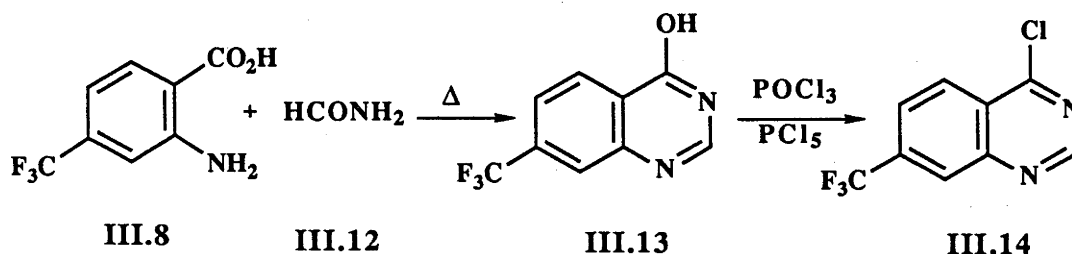


Scheme III-1

The second (less preferred) pathway required the diazotisation of 2-nitro-4-trifluoromethylaniline (**III.9**) [prepared as described by Pettit and Tatlow¹⁹⁸ by the reaction of 1-chloro-2-nitro-4-trifluoromethylbenzene (**III.4**) with saturated ethanolic ammonia in a sealed vessel at 120° for 5 h] to compound **III.10**. This diazotisation was carried out in a mixture of glacial acetic acid and sulphuric acid as described for the isomer, 4-nitro-3-trifluoromethylaniline;¹⁹⁹ the alternative method in aqueous sulphuric acid described by Hauptschein *et al.*¹⁹⁷ was found to be less satisfactory. Thus a solution of compound **III.9** in glacial acetic acid was diazotised by addition to a mixture of sodium nitrite in concentrated sulphuric acid at 10-20°, and then added to a solution of potassium nickel cyanide as described by Hauptschein *et al.*¹⁹⁷ Compound **III.10** was isolated from the reaction mixture by steam distillation. The nitrile (**III.10**) was hydrolysed in 55% sulphuric acid at 165° for 1 h as described by Hauptschein *et al.* to the 2-nitro-4-trifluoromethylbenzoic acid (**III.11**) which was reduced by iron powder in aqueous ammonium chloride at 50° for 1 h to 2-amino-4-trifluoromethylbenzoic acid (**III.8**).

III-4 Syntheses of 4-Chloro (and 4-Hydroxy)-7-trifluoromethylquinazoline

The above compounds were prepared as outlined by Armarego²⁰⁰ and Armarego and Smith¹⁹¹ from the reaction of 2-amino-4-trifluoromethylbenzoic acid with formamide followed by chlorination (as in Scheme III-2).



Scheme III-2

2-Amino-4-trifluoromethylbenzoic acid (**III.8**) was allowed to react with formamide (**III.12**) at 125° for 45 minutes and then at 171° for 2 h, and gave 4-hydroxy-7-trifluoromethylquinazoline (**III.13**).^{191,200} This hydroxy compound (**III.13**) with a mixture of phosphorus pentachloride and phosphoryl chloride, at reflux for 3 h,^{191,200} afforded 4-chloro-7-trifluoromethylquinazoline (**III.14**).

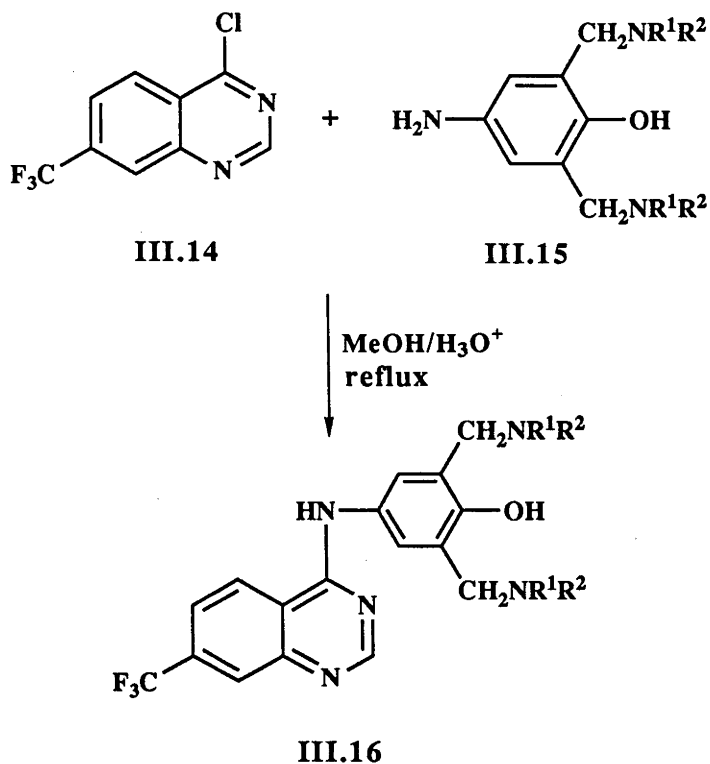
III-5 Syntheses of di-Mannich Bases of 4-(7-Trifluoromethylquinazolin-4-ylamino)phenol

The starting materials required for the syntheses of the di-Mannich bases (**III.16**) were the appropriate di-Mannich bases of *p*-aminophenol (**III.15**) [prepared previously by Mannich reactions on *p*-nitrophenol, followed by catalytic reduction with hydrogen over Raney nickel as described by Barlin and Ireland;¹⁷² other compounds were prepared in a similar manner] and 4-chloro-7-trifluoromethylquinazoline (**III.14**) described in Chapter III-4. A mixture of the compounds **III.14** and **III.15** (in 1:1 molar ratio) in aqueous methanol adjusted to pH *ca* 4.0-4.5 was refluxed for 5-10 h, and gave high yields of the desired di-Mannich bases (**III.16**) of 4-(7-trifluoromethylquinazolin-4-ylamino)phenol (as shown in Scheme III-3).

III-6 Synthesis of *N*⁴-(4-Diethylamino-1-methylbutyl)-7-trifluoromethylquinazolin-4-amine

*N*⁴-(4-Diethylamino-1-methylbutyl)-7-trifluoromethylquinazolin-4-amine (**III.18**), the 3-aza-7-trifluoromethyl analogue of chloroquine, was synthesised from 4-chloro-7-trifluoromethylquinazoline (**III.14**) and *N*¹,*N*¹-diethylpentane-1,4-diamine (**III.17**) in heptane in a Teflon lined screw-top reaction vessel at 140°, for 20 h.

Each of the compounds **III.16** and **III.18** was purified by preparative thin layer chromatography (PTLC). The results of testing of these compounds for antimalarial activity are given in Chapter VIII.

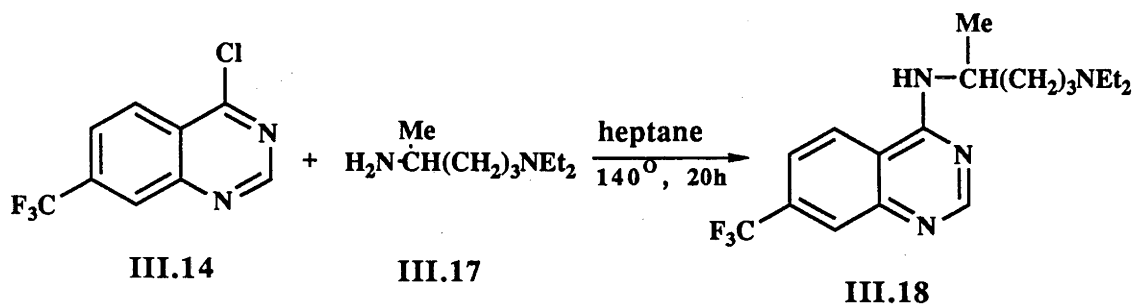


III.15, II.16

	NR ¹ R ²
a	NMe ₂
b	NEt ₂
c	NPr ₂
d	N(C ₅ H ₁₁) ₂
e	NBuMe
f	pyrrolidin-1-yl

	NR ¹ R ²
g	piperidin-1-yl
h	3-methylpiperidin-1-yl
i	3,5-dimethylpiperidin-1-yl
j	4-methylpiperidin-1-yl
k	4-benzylpiperidin-1-yl

Scheme III-3

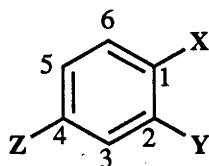


III-7 Physical Properties

III-7.1 ^1H Nuclear magnetic resonance spectra

The ^1H n.m.r. spectra of 1,2-disubstituted-4-trifluoromethyl (and 4-chloro) benzenes (III.8 - III.11 and III.19 - III.21) are listed in Table III-1. All spectra are reported in CDCl_3 solution, except for compound III.8 in CD_3SOCD_3 and III.19 in a mixture of CD_3SOCD_3 and CDCl_3 .

Table III-1 The chemical shifts for 1,2-disubstituted-(4-trifluoromethyl and 4-chloro)benzene



Compound	X	Y	Z	Solvent	H3	H5	H6
III.8	COOH	NH ₂	CF ₃	CD ₃ SOCD ₃	7.11(br s)	6.76(dd)	7.87(d)
III.9	NH ₂	NO ₂	CF ₃	CDCl ₃	8.43(s)	7.56(dd)	6.91(d)
III.10	CN	NO ₂	CF ₃	CDCl ₃	8.62(s)	8.14(s)	8.14(s)
III.11	COOH	NO ₂	CF ₃	CDCl ₃	8.20(s)	8.02(s)	8.02(s)
III.19 ^a	COOH	NH ₂	Cl	CD ₃ SOCD ₃ /CDCl ₃	6.85(d)	6.5(dd)	7.8(d)
III.20	NH ₂	NO ₂	Cl	CDCl ₃	8.12(d)	7.32(dd)	6.78(d)
III.21	COOH	NO ₂	Cl	CDCl ₃	7.83(d)	7.67(dd)	7.92(d)

^a C.J. Pouchert, *The Aldrich Library of NMR Spectra*, Vol.2 (Aldrich Chemical Company, Inc., 1983), p. 214B.

An examination of the spectra of compounds III.8 to III.11 revealed that the protons in the cyano compound (III.10) were downfield of those of the corresponding acid (III.11); and, as expected the signals in the corresponding amino compound (III.9) were upfield of those in compound III.10 with the greatest effect on H6. Although a direct comparison of the spectrum of compound III.8 (in CD_3SOCD_3) with compound

III.11 (in CDCl_3) - was not possible the significant differences in spectra of the amino and corresponding nitro compounds were apparent.

Comparison of the spectra of the trifluoromethyl compounds (**III.9**) and (**III.11**) with the corresponding chloro compounds (**III.20**) and (**III.21**) (all in CDCl_3) confirmed the greater electron-withdrawing power of the trifluoromethyl group with the proton signals in compounds **III.9** and **III.11** being downfield of those in the chloro compounds (**III.20**) and (**III.21**). The signals of compound **III.8** (in CD_3SOCD_3) were also downfield of its chloro analogue (**III.19**, in $\text{CD}_3\text{SOCD}_3/\text{CDCl}_3$).

A comparison of the ^1H n.m.r. spectrum of 4-chloro-7-trifluoromethylquinazoline (**III.14**) with that of the 4-hydroxy analogue (**III.13**) given in the Experimental section revealed that all protons of compound **III.14** are downfield of those in compound **III.13**; with the effect being most pronounced at H2. This is due to the strong inductive electron-withdrawal of the chloro substituent (relative to the hydroxy group).

An examination of the ^1H n.m.r. spectra of the di-Mannich bases of 4-(7-trifluoromethylquinazolin-4-ylamino)phenol given in the Experimental section revealed that the Mannich methylene protons occurred at δ 3.55-3.74, the phenolic protons at δ 7.35-7.43, and the protons of the quinazoline ring as follows: H2, δ 8.72-8.76; H5, 7.99-8.16; H6, 7.64-7.71; and H8, 8.11-8.18.

III-7.2 Ionization constants

The basic ionization constant of 7-trifluoromethylquinazolin-4-ol (**III.13**) was determined spectroscopically as pK_a 1.43, and accordingly it is a weaker base than quinazolin-4-ol (basic pK_a 2.06¹⁷⁹). This reflects the strong electron-withdrawing properties of the trifluoromethyl group which usually exceeds that of the chloro substituent. Unfortunately, the basic pK_a value of 7-chloroquinazolin-4-ol is unknown. However the superior electron-withdrawing effect of the trifluoromethyl group (compared to the chloro substituent) is revealed by a comparison of the pK_a values of the following compounds: quinoline, 4.9;²⁰¹ 7-chloroquinoline, 3.85;²⁰² 7-trifluoromethylquinoline, 3.16;²⁰³

quinazolin-8-ol, 5.02;²⁰⁴ 7-chloroquinazolin-8-ol, 4.0;²⁰⁵ and 7-trifluoromethylquinazolin-8-ol, 2.4.²⁰⁶

Ionization constants (which probably overlap) for the more complex di-Mannich bases compounds (**III.16a** - **III.16k**) were not examined.

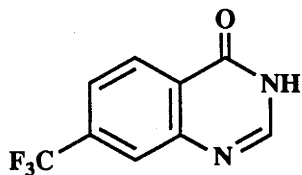
III-7.3 Ultraviolet, infrared and mass spectra

The ultraviolet spectrum of the neutral molecule of 7-trifluoromethylquinazolin-4-ol (pK_a 1.43) was measured in aqueous solution at pH 6.7. Its spectrum consisted of four absorption bands (λ_{max}) as follows: 226 nm ($\log \epsilon$ 4.35), 264 nm ($\log \epsilon$ 3.85), 308 nm ($\log \epsilon$ 3.69) and 320 nm ($\log \epsilon$ 3.60).

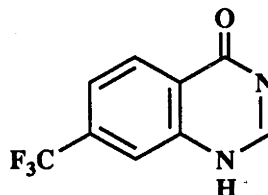
This spectrum closely resembles the spectrum of the neutral molecule of quinazolin-4-ol:^{195,207} at 224 nm ($\log \epsilon$ 4.36), 265 nm ($\log \epsilon$ 3.81), 300 nm ($\log \epsilon$ 3.60) and 331 nm ($\log \epsilon$ 3.54).

The ultraviolet spectrum of the di-Mannich base (**III.16b**) was measured at pH 6.7 (it was unchanged in the range pH 6.07-6.7) and displayed two main absorption bands at 206 nm ($\log \epsilon$ 4.73) and 316 nm ($\log \epsilon$ 3.98) with a inflexion point at 246-248 nm ($\log \epsilon$ 4.12).

The infrared spectrum of 7-trifluoromethylquinazolin-4-ol (**III.13**) (in KBr) displayed NH stretching absorption at 2900 cm^{-1} and CO stretching at 1720 and 1620 cm^{-1} ; and it is similar to the spectrum of quinazolin-4-ol.²⁰⁸ Thus like quinazolin-4-ol, compound **III.13** probably exists mainly as a mixture of the tautomeric forms [(**III.22**) and (**III.23**)].



III.22



III.23

The mass spectrum of 2,6-bis(diethylaminomethyl)-4-(7-trifluoromethylquinazolin-4-ylamino)phenol (**III.16**, $R^1=R^2=Et$), a representative of the di-Mannich compounds, showed a weak intensity molecular ion peak at m/z 475. The major cleavages appeared to be at the C-N bond of one or both Mannich bases to give (stable) fragments appearing as peaks at m/z 403 and 331, respectively. Other cleavages were also observed such as the loss of ethyl groups to give an ion peak at m/z 446, and the cleavage of the C4'-N bond to give the 7-trifluoromethylquinazoline fragment (m/z 197) and then the likely loss of hydrogen cyanide to give to peak at m/z 170.

III-8 Experimental

III-8.1 Synthetic work

2-Nitro-4-trifluoromethylphenylacetic acid (III.5)

This compound was prepared from 1-chloro-2-nitro-4-trifluoromethylbenzene with ethyl malonate as described by Simet.¹⁹⁶ It had m.p. 143-145° (lit.¹⁹⁶ 145-146.5°). ¹H n.m.r. δ 4.15, s, CH₂; 6.35, br, COOH; 7.54, d, J_{5,6} 8 Hz, H₆; 7.89, br d, J_{5,6} 8 Hz, H₅; 8.42, br s, H₃. I.r. $\nu_{\max}$ 3140 (br) (OH st), 1730 (s) (C=O st), 1570 (m), 1520 (mw), 1370 (mw), 1340 (s) (NO st), 1190 (s), 1100 (s), 875 (m), 780 (mw), 710 (w) cm⁻¹.

6-Trifluoromethyloxindole (III.6)

This compound was prepared from 2-nitro-4-trifluoromethylphenyl acetic acid with tin in boiling 9 N hydrochloric acid as described by Simet.¹⁹⁶ It had m.p. 186-188° (lit.¹⁹⁶ 186-188°). ¹H n.m.r. δ 3.63, s, CH₂; 7.18, s, H₇; 7.33, q, J_{5,6} 8 Hz, H_{5,6}; 8.70, br, NH. I.r. $\nu_{\max}$ 3160 (br) (NH st), 1710 (s) (C=O st), 1640 (w), 1470 (m), 1329 (m), 1309 (m), 1265 (m), 1170 (s), 1120 (s), 1055 (m) cm⁻¹.

6-Trifluoromethylisatin (III.7)

This compound was prepared from 6-trifluoromethyloxindole with bromine in refluxing carbon tetrachloride as described by Simet.¹⁹⁶ It had m.p. 193-195° (lit.¹⁹⁶ 192-194.5°) (Found: C, 50.4; H, 1.8; F, 26.5; N, 6.4. Calc. for C₉H₄F₃NO₂: C, 50.2; H, 1.9; F, 26.5; N, 6.5%). ¹H n.m.r. (CD₃SOCD₃) δ 4.5, br, NH; 7.12, s, H₇; 7.41, d, J_{4,5} 8 Hz, H₅(4); 7.71, d, J_{4,5} 8 Hz, H₄(5). I.r. $\nu_{\max}$ 3140 (br) (NH st), 1780 (s) and 1765 (s) and 1730 (s) (C=O st) 1650 (m) (C=O st?), 1468 (m), 1330 (s), 1190 (s), 1145 (s), 1065 (m) cm⁻¹.

2-Nitro-4-trifluoromethylaniline (III.9)

This compound was prepared from 1-chloro-2-nitro-4-trifluoromethylbenzene with ethanolic ammonia at 120° as described by Pettit and Tatlow.¹⁹⁸ It had m.p. 105-107° (lit.¹⁹⁸ 106-107°). ¹H n.m.r. δ 6.45, br s, NH₂; 6.91, d, J_{5,6} 8.5 Hz, H₆; 7.56, dd, J_{5,6} 8.5 Hz; J_{3,5} 2 Hz, H₅; 8.43, s, H₃. I.r. $\nu_{\max}$ 3518 (m) (NH st), 3390 (s) (NH sym st),

1667 (s) (NO₂ st?), 1590 (m) (ar skeleton st), 1340 (s) (NO₂ st), 1290 (m), 1130 (s), 916 (m), 840 (m), 700 (m), 630, (m) cm⁻¹.

2-Nitro-4-trifluoromethylbenzonitrile (III.10)

A solution of 2-nitro-4-trifluoromethylaniline (7.0 g) in glacial acetic acid (54.0 ml) was gradually poured into a stirred solution of sodium nitrite (2.58 g) in concentrated sulphuric acid (18.0 ml) maintained at 10-20°. The excess nitrous acid was decomposed with urea, and then the diazonium solution was then treated with potassium nickel cyanide as described¹⁹⁷ and the product isolated by steam distillation to give the *title compound* (2.30 g) as a low melting solid (Found: C, 44.9; H, 1.4; N, 12.7. Calc. for C₈H₃F₃N₂O₂: C, 44.4; H, 1.4, N, 12.9%). ¹H n.m.r. δ 8.14, s, H_{5,6}; 8.62, s, H₃.

2-Nitro-4-trifluoromethylbenzoic Acid (III.11)

This compound was prepared as described by Hauptschein *et al.*¹⁹⁷ and recrystallised from a mixture of methylene chloride and benzene. It had m.p. 132-134° (lit.¹⁹⁷ 140-140.5°) (Found, for a sample dried at 20°/0.2 mmHg for 2 h: C, 40.8; H, 1.4; N, 5.9. Calc. for C₈H₄F₃NO₄: C, 40.8; H, 1.7, N, 6.0%). ¹H n.m.r. δ 8.02, s, H_{5,6}; 8.20, s, H₃; 8.81, br s, COOH. I.r. ν_{max} 3300-2300 (br) (OH st), 3140 (w) (CH st), 1732 (s) (C=O st), 1570 (ms) (NO₂ st?), 1370 (m), 1340 (s) (NO₂ st), 1190 (s), 1100 (s), 875 (m), 780 (m), 710 (w) cm⁻¹.

2-Amino-4-trifluoromethylbenzoic acid (III.8)

This compound was best prepared according to Simet¹⁹⁶ and recrystallised from aqueous methanol. It had m.p. 174-176° (lit.¹⁹⁶ 175-177°) (Found, for a sample dried at 20°/0.2 mmHg for 2 h: C, 46.7 H, 2.9; F, 28.1; N, 6.8. Calc. for C₈H₆F₃NO₂: C, 46.8; H, 2.9; F, 27.8; N, 6.8%). ¹H n.m.r. (CD₃SOCD₃) δ 6.76, dd, J_{5,6} 8.5 Hz, J_{3,5} 1.5 Hz, H₅; 7.11, br s, H₃; 7.87, d, J_{5,6} 8.5 Hz, H₆. I.r. ν_{max} 3550 (m) (NH aysm st), 3430 (m) (NH sym st) 3100 (br) (OH st), 1706 (s) (C=O st), 1610 (m), 1350 (m), 1250 (s), 1190 (s), 1100 (m), 940 (m), 790 (m) cm⁻¹.

It was also prepared from 2-nitro-4-trifluoromethylbenzoic acid as described by Hauptschein *et al.*¹⁹⁷ but in our hands this method was less satisfactory.

7-Trifluoromethylquinazolin-4-ol (III.13)

2-Amino-4-trifluoromethylbenzoic acid (1.705 g) was mixed with formamide (1.4 ml) and heated at 125° for 45 min, and then at 171° for 2.5 h. After cooling, the mixture was diluted with water and the product filtered and washed. It was recrystallised from ethanol with charcoal filtration to give 7-trifluoromethylquinazolin-4-ol (1.24 g), m.p. 225-227° (lit.¹⁹¹ 227°) (Found: C, 50.8; H, 2.3; F, 27.0; N, 13.1. Calc. for C₉H₅F₃N₂O: C, 50.5; H, 2.3; F, 26.6; N, 13.1%). ¹H n.m.r. (CD₃SOCD₃) δ 7.83, br d, J_{5,6} 8 Hz, H₆; 7.98, br s, H₈; 8.24, s, H₂; 8.33, br d, J_{5,6} 8 Hz, H₅. I.r. ν_{max} 2900 br (w) (NH st), 1720 (s) (C=O st), 1620 (w), 1455 (mw), 1330 (m), 1265 (m), 1200 (m), 1140 (m), 890 (mw), 800 (w) cm⁻¹. U.v. λ_{max} (pH 6.7) 226 nm (log ε 4.35), 264 nm (log ε 3.85), 308 nm (log ε 3.69), 320 nm (log ε 3.60).

4-Chloro-7-trifluoromethylquinazoline (III.14)

This compound was prepared from 7-trifluoromethylquinazolin-4-ol by a literature procedure^{191,200} but the mixture of 7-trifluoromethylquinazolin-4-ol (2.35 g), phosphorus pentachloride (4.86 g) and phosphoryl chloride (15.0 ml) was refluxed for 3 h after all the solid had dissolved. After chromatography in benzene over alumina (15 cm) and recrystallisation from light petroleum (b.p. 60-80°) the 4-chloro-7-trifluoromethylquinazoline (1.56 g) had m.p. 62-64° (lit.¹⁹¹ 62°). ¹H n.m.r. δ 7.91, dd, J_{5,6} 8.5 Hz, J_{6,8} 1.5 Hz, H₆; 8.36, br s, H₈; 8.41, d, J_{5,6} 8.5 Hz, H₅; 9.14, s, H₂. I.r. ν_{max} 3080 (w) (CH st), 1580 (m), 1330 (s), 1235 (m), 1150 (s), 1075 (m), 845 (m), 690 (m) cm⁻¹.

2,6-Bis(dipentylaminomethyl)-4-nitrophenol

Dipentylamine (2.10 g) was added to a chilled mixture of paraformaldehyde (0.402 g) in ethanol (1.0 ml), *p*-nitrophenol (0.460 g) added and the mixture refluxed at 90-95° for 18 h. The solvent was then evaporated under vacuum and the crude product chromatographed in dichloromethane over a column of alumina to give as an oil, 2,6-bis(dipentylaminomethyl)-4-nitrophenol (0.905 g) (Found: C, 70.4; H, 10.6. C₂₈H₅₁N₃O₃ requires C, 70.4; H, 10.8. ¹H n.m.r. δ 0.88, t, J 7 Hz, Me; 1.26, complex, NCH₂CH₂CH₂CH₂; 2.50, t, NCH₂; 3.71, s, ArCH₂N; 8.05, s, H_{3,5}).

2,6-Bis(*N*-butyl-*N*-methylaminomethyl)-4-nitrophenol

N-Butylmethylamine (5.060 g), paraformaldehyde (1.740 g), ethanol (4.0 ml) and *p*-nitrophenol (2.0 g) were allowed to react in a manner similar to that described above. The product was chromatographed in chloroform over a column of alumina and gave as an oil 2,6-bis(*N*-butyl-*N*-methylaminomethyl)-4-nitrophenol (4.592 g) (Found: C, 64.3; H, 9.3. $C_{18}H_{31}N_3O_3$ requires C, 64.1; H, 9.3%). 1H n.m.r. δ 0.91, complex, CH_2Me ; 1.46, complex, $NCH_2CH_2CH_2$; 2.28, s, NMe ; 2.50, t, J 7 Hz, NCH_2CH_2 ; 3.66, s, $ArCH_2N$; 8.02, s, $H_{3,5}$.

2,6-Bis(4'-benzylpiperidin-1'-ylmethyl)-4-nitrophenol

4-Benzylpiperidine (4.980 g) paraformaldehyde (0.860 g), ethanol (5.0 ml) and *p*-nitrophenol (1.0 g) were allowed to react as above. After cooling, the mixture was diluted with hexane and the yellow solid was filtered off, and recrystallised from a mixture of dichloromethane and light petroleum (b.p. 60-80°) to give the *title compound* (2.416 g), m.p. 110-111.5° (Found: C, 72.9; H, 8.1; N, 8.0. $C_{32}H_{39}N_3O_3 \cdot 0.8H_2O$ requires C, 72.8; H, 7.8; N, 8.0%). 1H n.m.r. δ 1.12-2.21, complex, 2.95, m, $H_{2',3',4',5',6'}$; 2.53, br s, CH_2Ph ; 3.66, s, $ArCH_2N$; 7.22, complex, Ph ; 8.00, s, $H_{3,5}$.

2,6-Bis(dipentylaminomethyl)-4-(7'-trifluoromethylquinazolin-4'-ylamino)-phenol (III.16d)

2,6-Bis(dipentylaminomethyl)-4-nitrophenol (0.400 g) was dissolved in a mixture of ethanol (15.0 ml) and saturated ethanolic ammonia (15.0 ml), Raney nickel added and the mixture shaken with hydrogen until uptake ceased. The catalyst was filtered off on Celite and the product subjected to PTLC (alumina; chloroform) to give the amine (0.370 g) which was used directly in the next reaction. It had 1H n.m.r. δ 0.87, br t, Me ; 1.28, complex, $NCH_2CH_2CH_2CH_2$; 2.46, t, J 7 Hz, NCH_2CH_2 ; 3.58, s, $ArCH_2N$; 6.50, s, $H_{3,5}$.

Treatment of this product (0.131 g) and 4-chloro-7-trifluoromethylquinazoline (0.068 g) as described above (but refluxed for 8 h) gave a crude product which was purified by PTLC (alumina; ethyl acetate/hexane, 3:7) to give the *title compound* (0.053 g) as an oil which solidified on standing (Found: C, 68.9; H, 8.7. $C_{37}H_{56}F_3N_5O$ requires C,

69.0; H, 8.8%). ^1H n.m.r. δ 0.89, complex, Me; 1.27, complex, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$; 2.54, complex, NCH_2CH_2 ; 3.72, s, ArCH_2N ; 7.43, s, H_{3,5}; 7.59, br, OH, NH; 7.71, dd, J_{5',6'} 8.5 Hz, J_{6',8'} 1.5 Hz, H_{6'}; 8.01, d, J_{5',6'} 8.5 Hz, H_{5'}; 8.18, br s, H_{8'}; 8.76, s, H_{2'}.

2,6-Bis(N-butyl-N-methylaminomethyl)-4-(7'-trifluoromethylquinazolin-4'-ylamino)phenol (III.16e)

2,6-Bis(*N*-butyl-*N*-methylaminomethyl)-4-nitrophenol (0.800 g) was reduced with hydrogen over Raney nickel as for the dipentylamino analogue above. After column chromatography (alumina, chloroform) it gave as an oil 4-amino-2,6-bis(*N*-butyl-*N*-methylaminomethyl)phenol (0.552 g) which was used directly in the reaction below. It had ^1H n.m.r. δ 1.68, br t, CH_2Me ; 2.22, complex, $\text{NCH}_2(\text{CH}_2)_2$; 3.01, s, NMe; 3.20, t, J 7 Hz, NCH_2CH_2 ; 4.30, s, ArCH_2N ; 7.22, s, H_{3,5}.

The above crude phenol (0.149 g) and 4-chloro-7-trifluoromethylquinazoline (0.113 g) [under similar reaction conditions to those described above but refluxed for 10 h and the crude product purified by PTLC (alumina; ethyl acetate/hexane, 1:3, developed twice] gave the *title compound* (0.102 g) (Found: C, 64.3; H, 7.5. $\text{C}_{27}\text{H}_{36}\text{F}_3\text{N}_5\text{O}$ requires C, 64.4, H, 7.2%). ^1H n.m.r. δ 0.93, complex, CH_2Me ; 1.50, complex, $\text{NCH}_2\text{CH}_2\text{CH}_2$; 2.29, s, NMe; 2.51, complex, NCH_2CH_2 ; 3.62, s, ArCH_2N ; 7.42, s, H_{3,5}; 7.57, br, OH, NH; 7.70, dd, J_{5',6'} 8.5 Hz, J_{6',8'} 1.5 Hz, H_{6'}; 7.99, d, J_{5',6'} 8.5 Hz, H_{5'}; 8.16, s, H_{8'}; 8.75, s, H_{2'}.

2,6-Bis(pyrrolidin-1'-ylmethyl)-4-(7"-trifluoromethylquinazolin-4"-yl-amino)phenol (III.16f)

A similar reaction of 4-amino-2,6-bis(pyrrolidin-1'-ylmethyl)phenol¹⁷² (0.139 g) and 4-chloro-7-trifluoromethylquinazoline (0.118 g), but refluxed for 6 h, gave a crude product which was purified by PTLC (alumina; chloroform) to give the *title compound* as a yellow oil (0.083 g) (Found: C, 63.7; H, 6.2, F, 11.9; N, 14.5. $\text{C}_{25}\text{H}_{28}\text{F}_3\text{N}_5\text{O}$ requires C, 63.7; H, 6.0; F, 12.1; N, 14.8%). ^1H n.m.r. δ 1.79, complex, H_{3',4'}; 2.60, complex, H_{2',5'}; 3.74, s, ArCH_2N ; 7.37, s, H_{3,5}; 7.64, br d, J_{5'',6''} 9 Hz, H_{6''}; 8.10, br d, J_{5'',6''} 9

Hz, H5"; 8.14, br s, H8"; 8.36, br, OH, NH; 8.72, s, H2". I.r. $\nu_{\max}$ 3320 (br) (OH st and NH st), 2970 (m) (CH₂ st), 1580 (s), 1480 (s), 1320 (s), 1130 (s) cm⁻¹.

2,6-Bis(dimethylaminomethyl)-4-(7'-trifluoromethylquinazolin-4'-yl-amino)phenol (III.16a)

A mixture of 4-amino-2,6-bis(dimethylaminomethyl)phenol¹⁷² (0.214 g), 4-chloro-7-trifluoromethylquinazoline (0.223 g), methanol (1.5 ml), water (0.4 ml) and concentrated hydrochloric acid (2 drops) was refluxed at 90° for 5 h. The solvent was evaporated under reduced pressure, the residue diluted with water and neutralised with dilute ammonium hydroxide. The product was extracted into chloroform, the extract dried (Na₂SO₄) and the solvent evaporated. The crude product was purified by PTLC (alumina; 0.5% methanol in chloroform) to give *2,6-bis(dimethylaminomethyl)-4-(7'-trifluoromethylquinazolin-4'-yl-amino)phenol* (0.262 g) as an oil (Found, for a sample dried at 120°/0.2 mmHg for 5 h: C, 60.4; H, 6.1; N, 16.8. C₂₁H₂₄F₃N₅O requires C, 60.1; H, 5.8; N, 16.7%). ¹H n.m.r. δ 2.28, s, Me; 3.55, s, CH₂N; 7.36, s, H3,5; 7.64, br d, J_{5',6'} 8.5 Hz, H6'; 8.11, br s, H8'; 8.16, br d, J_{5',6'} 8.5 Hz, H5'; 8.73, s, H2'. I.r. $\nu_{\max}$ 3700-2400 (br) (OH st and NH st), 1580 (s), 1485 (s), 1465 (s), 1320 (s), 1145 (s) cm⁻¹.

2,6-Bis(diethylaminomethyl)-4-(7'-trifluoromethylquinazolin-4'-ylamino)-phenol (III.16b)

4-Amino-2,6-bis(diethylaminomethyl)phenol¹⁷² (0.117 g) and 4-chloro-7-trifluoromethylquinazoline (0.097 g) were allowed to react as for the analogue above. The crude product was purified by PTLC (alumina; 7% methanol in methylene chloride) and the product (0.154 g) was crystallised from a mixture of methylene chloride and light petroleum (b.p. 60-80°) to give the *title compound* m.p. 75-85°. (Found, for a sample dried at 20°/0.2 mmHg for 24 h: C, 62.3; H, 7.0, N, 14.4. C₂₅H₃₂F₃N₅O.0.3H₂O requires C, 62.4; H, 6.8; N, 14.5%). ¹H n.m.r. δ 1.07, t, J 7 Hz, Me; 2.61, q, J 7 Hz, CH₂Me; 3.69, s, ArCH₂N; 7.40, s, H3,5; 7.65, br d, J_{5',6'} 8.5 Hz, H6'; 8.09, br d, J_{5',6'} 8.5 Hz, H5'; 8.13, br s, H8'; 8.73, s, H2'. U.v. $\lambda_{\max}$ (pH 6.7) 206 nm (log ϵ 4.73), 246-248

nm (log ϵ 4.12), 316 nm (log ϵ 3.98). MS m/z 475 (M^+), 446 (2.4%), 403 (1.4%), 373 (9.3%), 331 (11.0%), 197 (3.9%), 170 (2.8%).

2,6-Bis(dipropylaminomethyl)-4-(7'-trifluoromethylquinazolin-4'-ylamino)-phenol (III.16c)

4-Amino-2,6-bis(dipropylaminomethyl)phenol¹⁷² (0.123 g) and 4-chloro-7-trifluoromethylquinazoline (0.086 g) were allowed to react as above. The crude product was purified by PTLC (alumina; chloroform, developed twice and then alumina; 1% methanol in chloroform) to give the *title compound* (0.156 g) (Found: C, 64.3; H, 7.8; N, 12.8. $C_{29}H_{40}F_3N_5O \cdot 0.5 H_2O$ requires C, 64.4; H, 7.6; N, 12.9%). 1H n.m.r. δ 0.87, t, J 7 Hz, Me; 1.49, complex, CH_2Me ; 2.48, complex, NCH_2CH_2 ; 3.69, s, $ArCH_2N$; 7.42, s, H_{3,5}; 7.66, br d, J_{5',6'} 8.5 Hz, H_{6'}; 7.84, br s, NH; 8.07, br d, J_{5',6'} 8.5 Hz, H_{5'}; 8.14, br s, H_{8'}, OH; 8.74, s, H_{2'}. I.r. ν_{max} 3500 (br) (H_2O), 2970 (w) (CH aliph st); 1580 (m), 1480 (s), 1130 (s) cm^{-1} .

2,6-Bis(piperidin-1'-ylmethyl)-4-(7''-trifluoromethylquinazolin-4''-ylamino)phenol (III.16g)

The product from 4-amino-2,6-bis(piperidin-1'-ylmethyl)phenol¹⁷² (0.240 g) and 4-chloro-7-trifluoromethylquinazoline (0.184 g) when refluxed as above (but for 5 h) and purified by PTLC (alumina; chloroform and alumina; 0.5% methanol in chloroform) gave as an oil the *title compound* (0.197 g) (Found: C, 62.9; H, 6.8; N, 13.3. $C_{27}H_{32}F_3N_5O \cdot H_2O$ requires C, 62.6; H, 6.6; N, 13.5%). 1H n.m.r. δ 1.51, complex, H_{3',4',5'}; 2.49, complex, H_{2',6'}; 3.60, s, $ArCH_2N$; 7.37, s, H_{3,5}; 7.67, br d, J_{5'',6''} 8.5 Hz, H_{6''}; 7.79, br s, OH, NH; 8.03, br d, J_{5'',6''} 8.5 Hz, H_{5''}; 8.15, br s, H_{8''}; 8.74, s, H_{2''}. I.r. ν_{max} 3360 (br) (OH st and NH st), 2940 (m) (CH_2 st), 1580 (s), 1480 (s), 1320 (s), 1130 (s), cm^{-1} .

2,6-Bis(3'-methylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethylquinazolin-4''-ylamino)phenol (III.16h)

This compound was prepared similarly from 4-amino-2,6-bis(3'-methylpiperidin-1'-ylmethyl)phenol¹⁷² (0.227 g) and 4-chloro-7-trifluoromethylquinazoline (0.159 g) and

the crude yellow solid was subjected to PTLC (alumina; 0.3% methanol in chloroform) to give the *title compound* (0.318 g) (Found, for a sample dried at 120°/0.2 mmHg for 5 h: C, 64.7; H, 7.0; N, 12.8. $C_{29}H_{36}F_3N_5O \cdot 0.65 H_2O$ requires C, 64.6; H, 7.0; N, 13.0%) 1H n.m.r. δ 0.86, d, J 5.5 Hz, Me; 1.65, complex, H3',4',5'; 2.85, complex, H2',6'; 3.62, s, ArCH₂N; 7.38, s, H3,5; 7.67, br d, J_{5'',6''} 9 Hz, H6''; 7.67, br, OH, NH; 8.02, br d, J_{5'',6''} 9 Hz, H5''; 8.15, br s, H8''; 8.75, s, H2''. I.r. ν_{max} 3400 (br) (H₂O), 2940 (w) (CH aliph st), 1580 (s), 1480 (s), 1320 (m), 1130 (m) cm⁻¹.

4,6-Bis(3',5'-dimethylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethylquinazolin-4''-ylamino)phenol (III.16i)

The 3',5'-dimethyl analogue was prepared from 4-amino-2,6-bis(3',5'-dimethylpiperidin-1'-ylmethyl)phenol¹⁷² (0.290 g) and 4-chloro-7-trifluoromethylquinazoline (0.188 g). The product was subjected to PTLC (alumina; 20% ethyl acetate in hexane) to give the *title compound* (0.223 g) (Found: C, 66.8; H, 7.6; N, 12.5. $C_{31}H_{40}F_3N_5O$ requires C, 67.0; H, 7.3; N, 12.6%). 1H n.m.r. δ 0.85, complex, Me; 1.65, complex, H3',4',5'; 2.90, complex, H2',6'; 3.60, s, ArCH₂N; 7.36, s, H3,5; 7.65, br d, J_{5'',6''} 8.5 Hz, H6''; 8.09, br d, J_{5'',6''} 8.5 Hz, H5''; 8.14, br s, H8''; 8.72, s, H2''. I.r. ν_{max} 2960 (w) (CH aliph st), 1580 (m), 1475 (m), 1320 (m), 1130 (m) cm⁻¹.

2,6-Bis(4'-methylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethylquinazolin-4''-ylamino)phenol (III.16j)

The 4-methyl isomer was prepared similarly from 4-amino-2,6-bis(4'-methylpiperidin-1'-ylmethyl)phenol¹⁷² (0.309 g) and 4-chloro-7-trifluoromethylquinoline (0.217 g), it precipitated as a yellow solid, and purified by PTLC (alumina; 25% ethyl acetate in hexane) to give the *title compound* (0.182 g) (Found: C, 65.9; H, 7.1; F, 10.4; N, 13.0. $C_{29}H_{36}F_3N_5O$ requires C, 66.0; H, 6.9; F, 10.8; N, 13.3%). A sample of this product was dissolved in dilute hydrochloric acid and reprecipitated by addition of ammonium hydroxide to pH 9. The solid was filtered off, washed with water and dried in the air. It melted at *ca* 130° (Found: C, 64.0; H, 7.4; N, 12.7. $C_{29}H_{36}F_3N_5O \cdot H_2O$ requires C, 63.8; H, 7.0; N, 12.8%). 1H n.m.r. δ 0.91, br s, Me; 1.47, complex, 2.04, br t, J 11 Hz,

2.93, br d, J 11 Hz, H2',3',4',5',6'; 3.59, s, ArCH₂N; 7.35, s, H3,5; 7.64, br d, J_{5'',6''} 8.5 Hz, H6''; 8.08, br d, J_{5'',6''} 8.5 Hz, H5''; 8.04, br s, OH, NH; 8.13, br s, H8''; 8.72, s, H2''. I.r. $\nu_{\max}$ 2980 (w) and 2950 (w) (CH aliph st), 1590 (m), 1485 (m), 1330 (m), 1140 (m) cm⁻¹.

4,6-Bis(4'-benzylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethylquinazolin-4''-ylamino)phenol (III.16k)

2,6-Bis(4'-benzylpiperidin-1'-ylmethyl)-4-nitrophenol (0.260 g) in ethanolic ammonia was reduced as described above to give the corresponding amino compound (0.127 g). ¹H n.m.r. δ 1.16-2.37, complex, 2.95, m, H2',3',4',5',6'; 2.52 br s, CH₂Ph; 3.53, s, ArCH₂N; 6.46, s, H3,5; 7.21, complex, Ph.

The above crude phenol (0.127 g) and 4-chloro-7-trifluoromethylquinazoline (0.061 g) were allowed to react under conditions similar to those described above and the crude product was purified by PTLC (alumina, dichloromethane) to give the *title compound* (0.085 g), m.p. 98-102° (Found: C, 72.2; H, 6.8, N, 10.1. C₄₁H₄₄F₃N₅O. 0.2H₂O requires C, 72.1; H, 6.6; N, 10.2%). ¹H n.m.r. δ 1.12-2.23, complex, 2.98, m, H2',3',4',5',6'; 2.54, br s, CH₂Ph; 3.64, s, ArCH₂N; 7.22, complex CH₂Ph; 7.40, s, H3,5; 7.68, br d, J_{5'',6''} 8.5 Hz, H6''; 8.00, br d, J_{5'',6''} 8.5 Hz, H5''; 8.16, br s, H8''; 8.74, s, H2''.

N⁴-(4'-Diethylamino-1'-methylbutyl)-7-trifluoromethylquinazolin-4-amine (III.18)

A mixture of 4-chloro-7-trifluoromethylquinazoline (0.070 g), N¹,N¹-diethylpentane-1,4-diamine (0.6 ml; 0.490 g) and heptane (1.4 ml) was heated in a teflon lined screw-top reaction vessel at 140° for 20 h. The solvent was evaporated and the product subjected to PTLC (alumina; 30% ethyl acetate in hexane, developed twice) to give the *title compound* (0.082 g), m.p. 89-91° (Found: C, 60.8; H, 7.1; N, 15.6. C₁₈H₂₅F₃N₄ requires C, 61.0; H, 7.1; N, 15.8%). ¹H n.m.r. δ 1.03, t, J 7 Hz, MeCH₂; 1.34, d, J 6.5 Hz, MeCH; 1.68, complex, CH₂CH₂CH₂N; 2.47, complex, CH₂CH₂CH₂N; 2.55, q, J 7

compound (0.082 g), m.p. 89-91° (Found: C, 60.8.; H, 7.1; N, 15.6. C₁₈H₂₅F₃N₄ requires C, 61.0; H, 7.1; N, 15.8%). ¹H n.m.r. δ 1.03, t, J 7 Hz, MeCH₂; 1.34, d, J 6.5 Hz, MeCH; 1.68, complex, CH₂CH₂CH₂N; 2.47, complex, CH₂CH₂CH₂N; 2.55, q, J 7 Hz, MeCH₂; 4.50, complex, MeCH; 6.75, br d, NH; 7.59, dd, J_{5,6} 9 Hz, J_{6,8} 1.5 Hz; H₆; 7.93, br d, J_{5,6} 9 Hz, H₅; 8.09, br s, H₈; 8.69, s, H₂.

III-8.2 Determination of the basic ionization constant of 7-trifluoromethylquinazolin-4-ol (III.13)

Substance: 7-Trifluoromethylquinazolin-4-ol

C₉H₅F₃N₂O; MW 214.146

Concentration: 1.0×10^{-4} M. Recrystallised compound, m.p. 227-228°, was dried at 20° and 0.2 mmHg overnight and 0.0107 g was dissolved in warm ethanol (5.0 ml) then made up to 500.0 ml with water. This stock solution (3.0 ml) was diluted with relevant buffer (1.0 ml).

Analytical wavelength: 316 nm; cell, 1 cm

Buffer: Hydrochloric acid and sulphuric acid buffer (I=0.1)

Species: Neutral and cationic species

Temperature: 20°

Result:

H₀ value: 2.0 1.8 1.6 1.4 1.2 0.98 0.8 0.6

pK_a calc.: 1.51 1.48 1.42 1.39 1.41 1.36 1.46 1.4

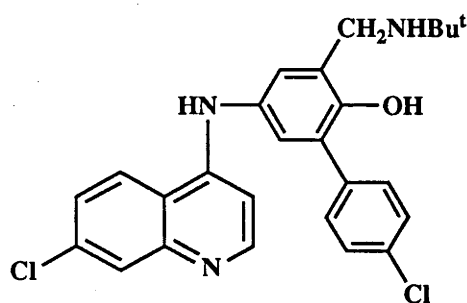
$$\text{pK}_a = 1.43 \pm 0.08$$

CHAPTER IV

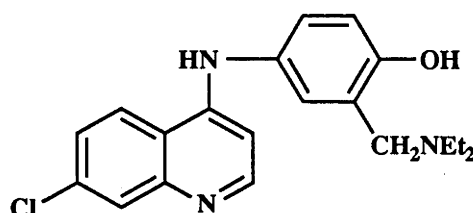
CHAPTER IV 4'-Chloro-3-(substituted amino)methyl-5-[7-substituted quinolin (quinazolin and 1,5-naphthyridin)-4-ylamino]biphenyl-2-ols

IV-1 Introduction

Werbel *et al.*,¹⁰¹ in 1986, described the syntheses, antimalarial activity (against *P. berghei* infections in mice) and semi-quantitative structure-activity relationships of 3-(*t*-butylaminomethyl)-4'-chloro-5-(7-chloroquinolin-4-ylamino)biphenyl-2-ol, "Tebuquine" (IV.1), a series of analogues, and their *N*-oxides.



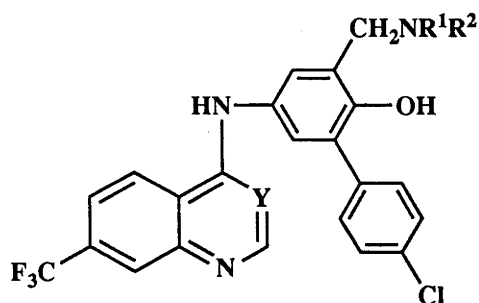
IV.1



IV.2

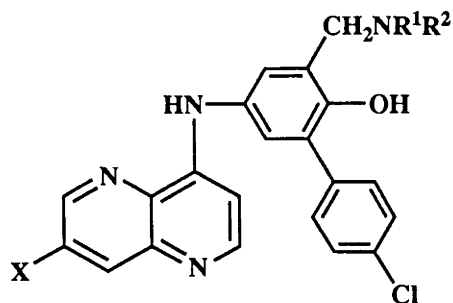
The high antimalarial activities shown by "Tebuquine" and the antimalarial amodiaquine⁸⁶ (IV.2) both of which contain mono-Mannich base side chains, and the antimalarial activity of the di-Mannich bases recently reported by Barlin and coworkers,^{95,97,98} prompted the present work to prepare analogues of "Tebuquine", such as compounds IV.3, IV.4, IV.5 and IV.6, which incorporated different heterocyclic nuclei.

The syntheses and physical properties of these compounds are discussed in this chapter, and the results of testing for antimalarial activity in *in vitro* tests against *P. falciparum* and *in vivo* tests against *P. vinckei vinckei* in mice are presented in Chapter VIII.



IV.3 Y=CH

IV.4 Y=N



IV.5 X=Br

IV.6 X=CF₃

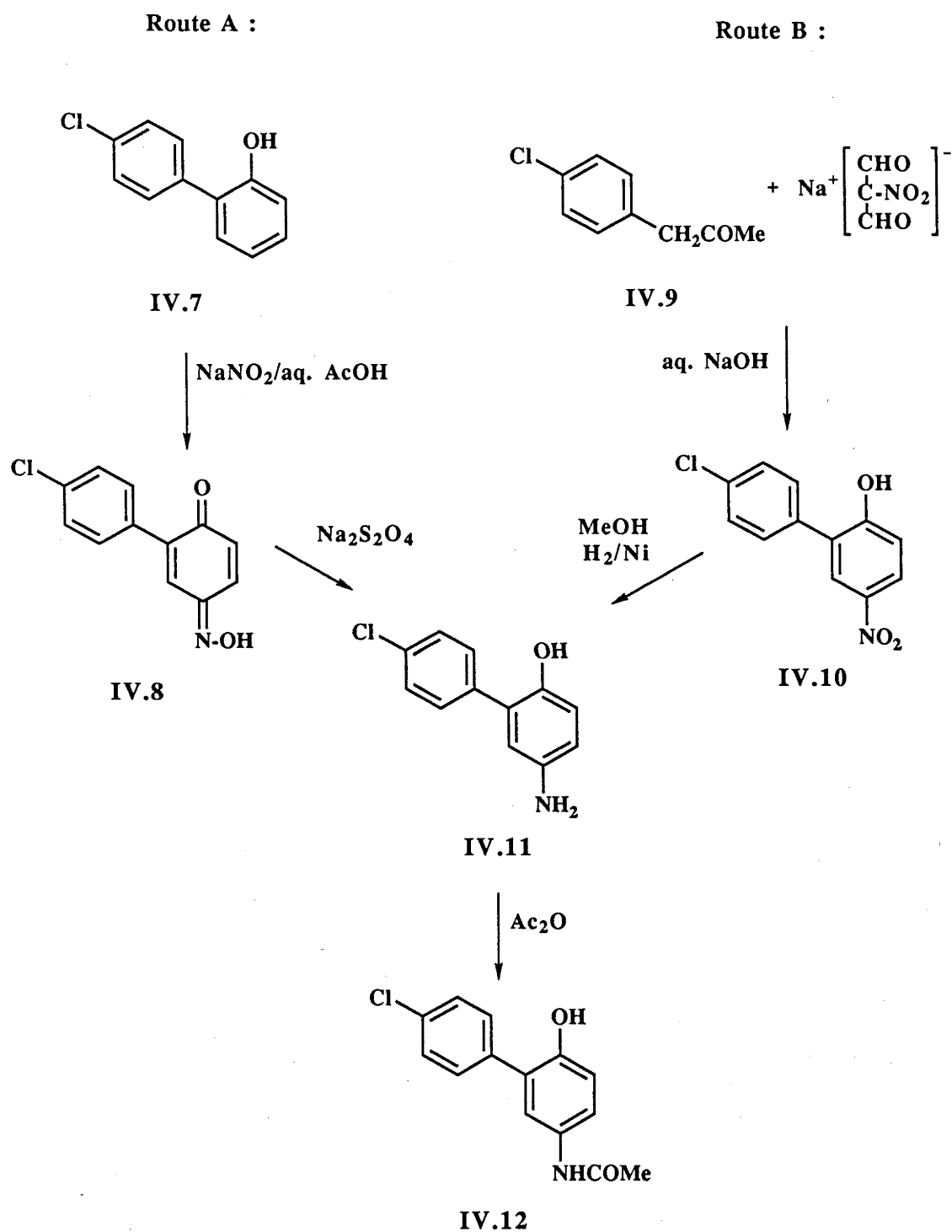
IV-2 Literature Preparations of 4'-Chloro-5-nitrobiphenyl-2-ol, *N*-(4'-Chloro-4-hydroxybiphenyl-3-yl)acetamides and "Tebuquine".

Werbel *et al.*¹⁰¹ in their synthesis of "Tebuquine" and its analogues, used as intermediates the *N*-(4-hydroxybiphenyl-3-yl)acetamides such as *N*-(4'-chloro-4-hydroxybiphenyl-3-yl)acetamide (IV.12), and its preparation was described by two routes (Scheme IV-1).

Route A, (Scheme IV-1) involved the nitrosation²⁰⁹ of 4'-chlorobiphenyl-2-ol (IV.7) (prepared by an Ullmann reaction between 1-chloro-4-iodobenzene and 1-iodo-2-methoxybenzene to give 4'-chloro-2-methoxybiphenyl,^{101,210} followed by hydrolysis^{101,211} of the methoxy group) with sodium nitrite in aqueous acetic acid to give 2-(4-chlorophenyl)-2,5-cyclohexadiene-1,4-dione 4-oxime (IV.8). Reduction of compound IV.8 with sodium dithionite gave the amino compound IV.11 which was acetylated with acetic anhydride to afford the acetamide (IV.12).

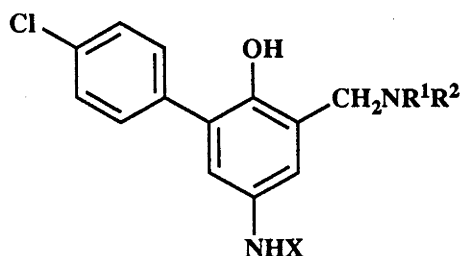
The alternative, route B (Scheme IV-1), was superior to route A,¹⁰¹ in terms of yield, availability of starting materials, and wide applicability to the syntheses of multisubstituted aromatic ring systems from aliphatic components. It was first reported by Hill and Hale in 1905.²¹² Thus condensation of 1-(4-chlorophenyl)-2-propanone (IV.9) with sodium nitromalonaldehyde hydrate in the presence of sodium hydroxide gave the nitrobiphenyl (IV.10). Catalytic hydrogenation of the nitro compound (IV.10) gave the

corresponding amino compound (IV.11) which with acetic anhydride, afforded the acetamide (IV.12).



Scheme IV-1

The *N*-(4'-chloro-6-hydroxybiphenyl-3-yl)acetamide (IV.12) was then subjected to the Mannich reaction with *t*-butylamine and formaldehyde to give the mono-Mannich base (IV.13; $\text{NR}^1\text{R}^2=\text{NHCMe}_3$). Hydrolysis of this compound gave the free amine (IV.14; $\text{NR}^1\text{R}^2=\text{NHCMe}_3$) which was then condensed with 4,7-dichloroquinoline to afford "Tebuquine" (IV.1).



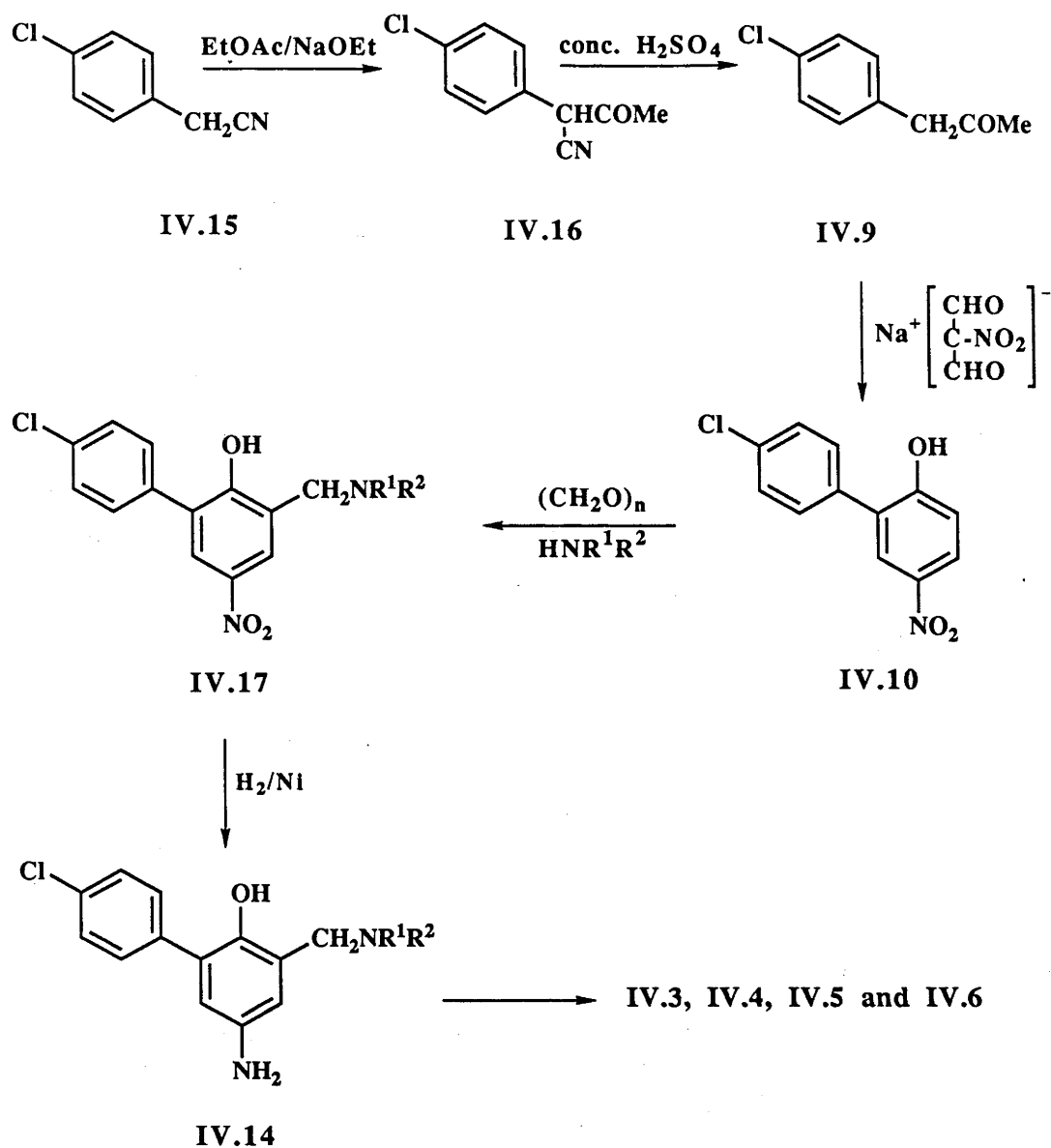
IV.13 $\text{X}=\text{COMe}$

IV.14 $\text{X}=\text{H}$

IV-3 Syntheses of 4'-Chloro-3-(substituted amino)methyl-5-[7-substituted quinolin (quinazolin and 1,5-naphthyridin)-4-ylamino]biphenyl-2-ols

In the present work, the 5-amino-4'-chloro-3-(substituted aminomethyl)biphenyl-2-ols (IV.14) required for the synthesis of "Tebuquine" analogues were prepared from 4-chlorobenzyl cyanide (IV.15) through α -acetyl- α -(4-chlorophenyl)acetonitrile (IV.16)²¹³ and 1-(4-chlorophenyl)propan-2-one (IV.9) (Scheme IV-2) and 4'-chloro-5-nitrobiphenyl-2-ol (IV.10) by literature procedures.¹⁰¹ The last compound (IV.10) was then subjected to the Mannich reaction to give compounds IV.17 which were subsequently reduced to compounds IV.14. This differs from the procedure used by Werbel and coworkers,¹⁰¹ (and described above) in which the Mannich reactions were conducted on the acetamido compounds (IV.12).

The amino compounds (IV.14) were then condensed with the relevant 4-chloro heterocycles to give the "Tebuquine" analogues.



IV.3, IV.4, IV.5, IV.6, IV.14 and IV.17

	NR ¹ R ²		NR ¹ R ²
a	NEt ₂	e	3-methylpiperidin-1-yl
b	NHBu ^t	f	4-methylpiperidin-1-yl
c	pyrrolidin-1-yl	g	4-benzylpiperidin-1-yl
d	piperidin-1-yl	h	4-benzylpiperazin-1-yl

Scheme IV-2

Thus compound **IV.15** with ethyl acetate in the presence of sodium ethoxide in ethanol at reflux for 3 h,²¹⁴ gave compound **IV.16**, which in concentrated sulphuric acid²¹⁴ was hydrolysed and decarboxylated to compound **IV.9**.

The ketone compound **IV.9** with sodium α -nitromalonaldehyde hydrate²¹⁵ in a mixture of ethanol and N sodium hydroxide at room temperature for 24 h, gave 4'-chloro-5-nitrobiphenyl-2-ol (**IV.10**). Under the conditions of the Mannich reaction,¹⁷² the nitro compound (**IV.10**) underwent reaction with paraformaldehyde and amines (such as diethylamine, *t*-butylamine, pyrrolidine, piperidine, 3- and 4-methylpiperidine, 4-benzylpiperidine and 4-benzylpiperazine) in refluxing ethanol for 15-20 h to afford good yields of 4'-chloro-5-nitro-3-(substituted amino)methylbiphenyl-2-ols (**IV.17**).

The nitro compounds (**IV.17**) dissolved easily in ethanolic ammonia, and were reduced therein with hydrogen in the presence of Raney nickel to give the corresponding amines (**IV.14**) by the general procedures described by Barlin and Ireland.¹⁷² These amines (**IV.14**) were condensed subsequently with 4-chloro-7-trifluoromethylquinoline, 4-chloro-7-trifluoromethylquinazoline or 7-bromo (or 7-trifluoromethyl)-4-chloro-1,5-naphthyridine in refluxing aqueous methanol (containing a little hydrochloric acid) for 5-8 h, to give the mono-Mannich base derivatives (**IV.3**, **IV.4**, **IV.5** and **IV.6**) of 4'-chloro-5-[7-trifluoromethylquinolin (and quinazolin)-4-ylamino]biphenyl-2-ols and 4'-chloro-5-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]biphenyl-2-ols, respectively.

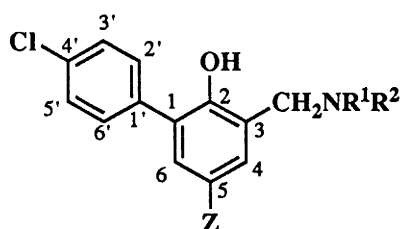
IV-4 Physical Properties

IV-4.1 ¹H Nuclear magnetic resonance spectra

The ¹H n.m.r. spectra of nitro compounds (**IV.17a** and **b**) and amino compounds (**IV.14a** and **b**) are given in Table IV-1. Inspection of the data revealed that the protons (H4 and H6) of the phenolic ring of the nitro compounds (**IV.17a** and **b**) appeared at lower field by *ca* 0.5 ppm relative to those of the corresponding amino compounds (**IV.14a** and **b**), due to the strong electron-withdrawing power of the nitro group compared to the electron-donating properties of the amino group. Similarly, the methylene protons of the Mannich side chain in these nitro compounds (**IV.17a** and **b**) were at lower

field by 0.22 ppm; the reduced effect reflected the greater spacial separation of the interacting groups.

Table IV-1 Chemical shifts (δ)^a of some mono-Mannich base derivatives of 4'-chloro-5-nitro (and 5-amino)biphenyl-2-ols



Compound	Z	NR ¹ R ²	Alkyl Group	CH ₂ N	H2',3',5',6'	H4	H6
IV.17a	NO ₂	NEt ₂	1.16, Me; 2.73, MeCH ₂	3.94	7.40, 7.58	7.92	8.18
IV.17b	NO ₂	NHBu ^t	1.27, Me	4.09	7.42, 7.57	7.98	8.16
IV.14a	NH ₂	NEt ₂	1.09, 1.17, Me; 2.62, MeCH ₂	3.72	7.34, 7.54	6.40	6.60
IV.14b	NH ₂	NHBu ^t	1.19, Me	3.87	7.34, 7.52	6.42	6.59

^a Reported as parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard in deuteriochloroform (CDCl₃) solution.

As expected the signals due to the protons H2',3',5',6' (and the alkyl groups in the Mannich side chain) did not show large differences between the nitro and amino compounds, and the H2',3',5', and 6' protons revealed an AA'BB' pattern.

Inspection of the ¹H n.m.r. spectra of the "Tebuquine" analogues (IV.6) reported in the Experimental section revealed that, like the di-Mannich base derivatives of 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol described in Chapter II, the proton at position 6 of the naphthyridine ring was the most deshielded (δ 8.93-8.95) whereas the most shielded proton was that at position 3 (δ 6.98-7.01). The signal due to H8 (δ 8.54-8.58) was also slightly upfield of that due to H2 (δ 8.57-8.61). The methylene protons of the Mannich side chains appeared in the range δ 3.77-4.05.

The ^1H n.m.r. spectra of the compounds **IV.3**, **IV.4** and **IV.5** are also reported in the Experimental section.

IV-4.2 Ultraviolet, infrared and mass spectra

The ultraviolet spectrum of α -acetyl- α -(4-chlorophenyl)acetonitrile (**IV.16**) was measured at pH 3.42 (The spectrum did not change in the range pH 3.0-3.5). At this pH, the compound was assumed to be the neutral molecule, and the spectrum consisted of two absorption maxima at 221 nm ($\log \epsilon$ 4.18) and 266 nm ($\log \epsilon$ 4.39).

The infrared spectrum of compound **IV.16** displayed a strong absorption at 2133 cm^{-1} due to $\text{C}\equiv\text{N}$ stretching and a medium absorption at 1632 cm^{-1} , probably due to $\text{C}=\text{C}$ stretching rather than $\text{C}=\text{O}$ stretching (which is normally a much stronger absorption band). Absorption peaks at 3130 (br) and 700 (br) cm^{-1} , were probably due to the O-H stretching and O-H out of plane stretching, which may imply that compound **IV.16** exists as a mixture of keto and enol forms.

The nitro compound **IV.17f** (**IV.17**, $\text{NR}^1\text{R}^2=4\text{-methylpiperidin-1-yl}$), a representative of the Mannich nitro derivatives, displayed infrared absorptions due to O-H stretching at 3465 cm^{-1} and two absorption peaks due to NO_2 stretching at 1495 and 1338 cm^{-1} . Likewise the quinazoline and naphthyridine; compounds **IV.4f** (**IV.4**; $\text{NR}^1\text{R}^2 = 4\text{-methylpiperidin-1-yl}$) and **IV.5f** (**IV.5**; $\text{NR}^1\text{R}^2 = 4\text{-methylpiperidin-1-yl}$) showed absorptions due to O-H stretching at 3450 and 3470 cm^{-1} , respectively.

The mass spectra of compounds **IV.6a** and **IV.6f** (**IV.6**, $\text{NR}^1\text{R}^2=\text{NEt}_2$ and 4-methylpiperidin-1-yl; respectively) were examined. Each spectrum showed two molecular ion peaks associated with an M+1 peak, the molecular ion peaks appeared at m/z 500 (and 502) and 526 (and 528), for compounds **IV.6a** and **IV.6f**, respectively; with relative intensities of the lines in the ratio 3:1. As mentioned in Chapter II-6.3, this is characteristic of ions from chlorine containing compounds. Similar fragmentation of the Mannich N to C (benzylic) bond also occurred in these two compounds giving the same fragmentation peaks at m/z 427 for both compounds **IV.6a** and **IV.6f**.

IV-5 Experimental

α -Acetyl- α -(4-chlorophenyl)acetonitrile (IV.16)

This compound was prepared²¹³ from 4-chlorobenzyl cyanide. It had m.p. 120-122° (from toluene) (lit.²¹³ 124-125°) (Found, for a sample dried at 20°/0.2 mmHg for 20 h: C, 62.4; H, 4.4; Cl, 18.1; N, 7.2. Calc. for $C_{10}H_8ClNO$: C, 62.2, H, 4.2; Cl, 18.3; N 7.2%). 1H n.m.r. δ 2.30, s, Me; 4.65, s, CH; 7.31, d, 7.43, d, J 9 Hz, H2',3',5',6'. I.r. ν_{max} 3130 (br) (OH st ?), 2133 (s) ($C\equiv N$ st), 1632 (s) ($C=C$, $C=O$, st), 1499 (s), 1356 (s) (OH st), 1091 (m), 1011 (w), 918 (w), 831 (s), 700 (br) (OH out of plane st). U.v. (pH 3.42) λ_{max} 221 nm (log ϵ 4.18), 266 nm (log ϵ 4.39).

4'-Chloro-5-nitrobiphenyl-2-ol (IV.10)

This compound was prepared from α -acetyl- α -(4-chlorophenyl)acetonitrile through 1-(4-chlorophenyl)propan-2-one.¹⁰¹ It was purified by column chromatography (silica; benzene/methylene chloride) and by distillation: b.p. 78-80°/0.3 mmHg (lit.²¹⁶ 110-112°/6 mmHg) (Found, for the distillate: C, 64.4; H, 5.5; Cl, 20.4. Calc. for C_9H_9ClO : C, 64.1; H, 5.4; Cl, 21.0%). 1H n.m.r. δ 2.18, s, Me; 3.68, s, CH_2 ; 7.12, d, 7.31, d, J 9 Hz, H2',3',5',6'.

This last named compound and sodium α -nitromalonaldehyde hydrate,²¹⁵ by a method similar to that described in detail for 5-nitro-3'-trifluoromethylbiphenyl-2-ol by Werbel *et al.*,¹⁰¹ gave the title compound, m.p. 163-165° (lit.¹⁰¹ 161-163°) (Found: C, 57.6; H, 3.0; N, 5.4. Calc. for $C_{12}H_8ClNO_3$: C, 57.7; H, 3.2; N, 5.6%). 1H n.m.r. δ 1.56, s, OH; 7.08, d, J 9 Hz, H3; 7.42, d, 7.55, d, J 9 Hz, H2',3',5',6'; 8.17, d, J 3 Hz, H6; 8.19, d, J 9 Hz, H4.

4'-Chloro-3-(diethylaminomethyl)-5-nitrobiphenyl-2-ol (IV.17a)

A mixture of paraformaldehyde (0.054 g), diethylamine (0.19 ml) and ethanol (2.0 ml) was heated until it formed a clear solution. This mixture was chilled and 4'-chloro-5-nitrobiphenyl-2-ol (0.150 g) was added and the mixture refluxed at 95° for 16 h. After cooling, the yellow solid (0.261 g) was filtered off. The filtrate was subjected to PTLC (alumina; 26% ethyl acetate in hexane) to give a further 0.026 g of product. It was

recrystallised from ethanol to give the yellow crystals of the *title compound*, m.p. 149-150° (Found: C, 60.7; H, 6.0; Cl, 10.4; N, 8.2. $C_{17}H_{19}ClN_2O_3$ requires C, 61.0; H, 5.7; Cl, 10.6; N, 8.4%). 1H n.m.r. δ 1.16, t, J 7.0 Hz, Me; 2.73, q, J 7 Hz, $MeCH_2$; 3.94, s, CH_2N ; 7.40, d, 7.58, d, J 9 Hz, $H_{2',3',5',6'}$; 7.92, d, J 3 Hz, H_4 ; 8.18, d, J 3 Hz, H_6 .

3-(*t*-Butylaminomethyl)-4'-chloro-5-nitrobiphenyl-2-ol (IV.17b)

Paraformaldehyde (0.144 g), *t*-butylamine (0.51 ml), ethanol (3.0 ml) and 4'-chloro-5-nitrobiphenyl-2-ol (0.40 g) were refluxed for 15 h and the precipitate (0.421 g) was filtered off. It was purified by PTLC (alumina; 40% ethyl acetate in hexane) and recrystallised from ethanol to give the *title compound*, m.p. 211-213°. The filtrate gave a further quantity (0.070 g) after PTLC (Found: C, 61.1; H, 5.9; N, 8.4. $C_{17}H_{19}ClN_2O_3$ requires C, 61.0; H, 5.7; N, 8.4%). 1H n.m.r. δ 1.27, s, Me; 3.06, br s, NH; 4.09, s, CH_2 ; 7.42, d, 7.57, d, J 9 Hz, $H_{2',3',5',6'}$; 7.98, d, J 3 Hz, H_4 ; 8.16, d, J 3 Hz, H_6 .

4'-Chloro-5-nitro-3-(pyrrolidin-1"-ylmethyl)biphenyl-2-ol (IV.17c)

In a similar manner from paraformaldehyde (0.144 g), pyrrolidine (0.39 ml), ethanol (4.0 ml) and 4'-chloro-5-nitrobiphenyl-2-ol (0.40 g) there was obtained after PTLC (alumina; 40% ethyl acetate in hexane) and recrystallisation from chloroform/methanol, yellow needles of the *title compound* (0.494 g), m.p. 155-156° (Found: C, 59.7; H, 5.3; N, 8.0. $C_{17}H_{17}ClN_2O_3 \cdot 0.5 H_2O$ requires C, 59.7; H, 5.3; N, 8.2%). 1H n.m.r. δ 1.91, complex, $H_{3'',4''}$; 2.78, complex, $H_{2'',5''}$; 4.01, s, CH_2 ; 5.24, br s, OH; 7.40, d, 7.58, d, J 9 Hz, $H_{2',3',5',6'}$; 7.94, d, J 3 Hz, H_4 ; 8.19, J 3 Hz, H_6 .

4'-Chloro-5-nitro-3-(piperidin-1"-ylmethyl)biphenyl-2-ol (IV.17d)

This compound (0.478 g) was prepared from paraformaldehyde (0.086 g), piperidine (0.28 ml), ethanol (4.0 ml) and 4'-chloro-5-nitrobiphenyl-2-ol (0.40 g) by refluxing for 20 h. The crystalline solid (0.478 g) was purified by recrystallisation from ethanol and had m.p. 199-200° (Found: C, 62.0; H, 5.8; N, 8.1. $C_{18}H_{19}ClN_2O_3$ requires C, 62.3; H, 5.5; N, 8.1%). 1H n.m.r. δ 1.65, complex, 2.65, complex, $H_{2'',3'',4'',5'',6''}$; 3.84, s, $ArCH_2N$; 7.41, d, 7.57, d, J 9 Hz, $H_{2',3',5',6'}$; 7.91, d, J 3 Hz, H_4 ; 8.17, d, J 3 Hz, H_6 .

4'-Chloro-3-(3''-methylpiperidin-1''-ylmethyl)-5-nitrobiphenyl-2-ol (IV.17e)

This compound was prepared similarly from paraformaldehyde (0.082 g), 3-methylpiperidine (0.32 ml), ethanol (2.3 ml) and 4'-chloro-5-nitrobiphenyl-2-ol (0.40 g). The reaction mixture was evaporated and the viscous liquid purified by PTLC (alumina; 30% ethyl acetate in hexane) to give the *title compound* (0.570 g) as a thick oil (Found: C, 63.6; H, 6.0; N, 7.5. $C_{19}H_{21}ClN_2O_3$ requires C, 63.2; H, 5.9; N, 7.8%). 1H n.m.r. δ 0.91, d, J 6 Hz, Me; 1.72, complex, 3.00, complex, $H_{2'',3'',4'',5'',6''}$; 3.84, s, $ArCH_2N$; 7.41, d, 7.59, d, J 9 Hz, $H_{2',3',5',6'}$; 7.92, d, J 3 Hz, H_4 ; 8.18, d, J 3 Hz, H_6 .

4'-Chloro-3-(4''-methylpiperidin-1''-ylmethyl)-5-nitrobiphenyl-2-ol (IV.17f)

Paraformaldehyde (0.086 g), 4-methylpiperidine (0.34 ml), ethanol (4.0 ml) and 4'-chloro-5-nitrobiphenyl-2-ol (0.40 g) refluxed for 20 h gave yellow crystals (0.504 g) which were recrystallised from ethanol/water to give the *title compound*, m.p. 135-137° (Found: C, 63.0; H, 6.1; N, 7.5. $C_{19}H_{21}ClN_2O_3$ requires C, 63.2; H, 5.9; N, 7.8%). 1H n.m.r. δ 0.95, d, J 5 Hz, Me; 1.5, complex, 2.25, complex, 3.01, complex, $H_{2'',3'',4'',5'',6''}$; 3.84, s, $ArCH_2N$; 7.40, d, 7.58, d, J 9 Hz, $H_{2',3',5',6'}$; 7.92, d, J 3 Hz, H_4 ; 8.16, d, J 3 Hz, H_6 . I.r. ν_{max} 3465 (br w) (OH st), 2970 (w) (CH aliphatic st), 1597 (m) (aromatic st), 1495 (s) (NO_2 st), 1460 (aromatic st), 1338 (s) (NO_2 st), 1310 (br m), 838 (m), 750 (m) cm^{-1} .

3-(4''-Benzylpiperidin-1''-ylmethyl)-4'-chloro-5-nitrobiphenyl-2-ol (IV.17g)

Paraformaldehyde (0.062 g), 4-benzylpiperidine (0.37 ml), ethanol (2.0 ml) and 4'-chloro-5-nitrobiphenyl-2-ol (0.177 g) refluxed for 16 h and chilled gave a crystalline solid. This solid was filtered off, washed with light petroleum (b.p. 60-80°) and recrystallised from a mixture of methylene chloride and light petroleum to give the *title compound* (0.257 g), m.p. 132-134° (Found: C, 68.7; H, 5.9; N, 6.4. $C_{25}H_{25}ClN_2O_3$ requires C, 68.7; H, 5.8; N, 6.4%). 1H n.m.r. δ 1.66, complex, 2.25, complex, 3.07, complex, $H_{2'',3'',4'',5'',6''}$; 2.54, d, J 6.5 Hz, CH_2Ph ; 3.86, s, $ArCH_2N$; 7.23, m, Ph; 7.41, d, 7.57, d, J 9 Hz, $H_{2',3',5',6'}$; 7.92, d, J 3 Hz, H_4 ; 8.18, d, J 3 Hz, H_6 .

3-(4''-Benzylpiperazin-1''-ylmethyl)-4'-chloro-5-nitrobiphenyl-2-ol

(IV.17h)

Paraformaldehyde (0.097 g), *N*-benzylpiperazine (0.56 ml), ethanol (3.5 ml) and 4'-chloro-5-nitrobiphenyl-2-ol (0.30 g) were refluxed as above and gave, after concentration and dilution with chloroform, a yellow solid (0.458 g) which was filtered off and washed with ethanol. It was recrystallised from ethanol to give light yellow needles of the *title compound*, m.p. 135-136° (Found: C, 65.3; H, 5.9; N, 9.6. $C_{24}H_{24}ClN_3O_3 \cdot 0.2 H_2O$ requires C, 65.3; H, 5.6; N, 9.5%). 1H n.m.r. δ 2.67, br, $H_{2'',3'',5'',6''}$; 3.57, s, CH_2Ph ; 3.87, s, $ArCH_2N$; 7.41, d, 7.55, d, J 9 Hz, $H_{2',3',5',6'}$; 7.31, s, Ph 7.94, d, J 3 Hz, H_4 ; 8.18, d, J 3 Hz, H_6 .

4'-Chloro-3-(diethylaminomethyl)-5-(7''-trifluoromethylquinolin-4''-yl-amino)biphenyl-2-ol (IV.3a)

4'-Chloro-3-(diethylaminomethyl)-5-nitrobiphenyl-2-ol (0.122 g) was dissolved in ethanolic ammonia (5.0 ml) and ethanol (5.0 ml) and shaken with hydrogen over Raney nickel until the yellow colour was discharged and hydrogen uptake ceased. The catalyst was filtered on Celite and the filtrate evaporated to give the crude amino compound (0.085 g). 1H n.m.r. δ 1.09, t, 1.17, t, J 7 Hz, Me; 2.62, q, $2 \times MeCH_2$; 3.72, s, $ArCH_2N$; 5.29, br s, NH_2 ; 6.40, d, J 3 Hz, H_4 ; 6.60, d, J 3 Hz, H_6 ; 7.34, d, 7.54, d, J 9 Hz, $H_{2',3',5',6'}$.

This amine (0.10 g) and 4-chloro-7-trifluoromethylquinoline (0.076 g) in methanol (1.7 ml) and water (0.7 ml) with two drops of concentrated hydrochloric acid were refluxed for 8 h. The solvent was evaporated and the residue dissolved in water and adjusted by addition of ammonium hydroxide to pH 10, to give a yellow precipitate. This was extracted into chloroform, the extract dried (Na_2SO_4) and the solvent evaporated to give the crude product (0.162 g). It was purified by PTLC (alumina; 30% ethyl acetate in hexane), and recrystallised from acetonitrile to give yellow needles of the *title compound*, m.p. 213-215° (Found: C, 64.6; H, 5.4; N, 8.3. $C_{27}H_{25}ClF_3N_3O$ requires C, 64.9; H, 5.0; N, 8.4%). 1H n.m.r. δ 1.16, t, J 7 Hz, Me; 2.70, q, J 7 Hz, $MeCH_2$; 3.86, s, $ArCH_2N$; 6.79, d, J 5.5 Hz, $H_{3''}$; 6.98, d, J 2.5 Hz, 7.20, d, J 2.5 Hz, $H_{4,6}$; 7.38, d, 7.59, d, J 9

Hz, H2',3',5',6'; 7.59, d, J 9 Hz, H6"; 8.08, d, J 9 Hz, H5"; 8.33, br s, H8"; 8.55, d, J 5.5 Hz, H2".

3-(*t*-Butylaminomethyl)-4'-chloro-5-(7"-trifluoromethylquinolin-4"-yl-amino)biphenyl-2-ol (IV.3b)

3-(*t*-Butylaminomethyl)-4'-chloro-5-nitrobiphenyl-2-ol (0.230 g) in ethanolic ammonia (30.0 ml) and ethanol (10.0 ml) was reduced with hydrogen over Raney nickel as above to give the amine (0.190 g). ¹H n.m.r. δ 1.19, s, Me; 3.87, s, ArCH₂N; 4.60, br s, NH₂; 6.42, d, J 3 Hz, H4; 6.59, d, J 3 Hz, H6; 7.34, d, 7.52, d, J 9 Hz, H2',3',5',6'.

4-Chloro-7-trifluoromethylquinoline (0.071 g) and the above amine (0.094 g) were allowed to react as described in the previous preparation. The crude product was purified by PTLC (alumina; 40% ethyl acetate in hexane, developed twice) to give the *title compound* (0.147 g), m.p. 92-98° (Found: C, 64.8; H, 4.8; N, 8.1. C₂₇H₂₅ClF₃N₃O requires C, 64.8; H, 5.0; N, 8.4%). ¹H n.m.r. δ 1.27, s, Me; 4.04, s, ArCH₂N; 6.78, d, J 5.5 Hz, H3"; 7.03, d, 7.18, d, J 3 Hz, H4,6; 7.38, d, 7.57, d, J 9 Hz, H2',3',5',6'; 7.66; d, J 9 Hz, H6"; 8.10, d, J 9 Hz, H5"; 8.33, br s, H8"; 8.53, d, J 5.5 Hz, H2".

4'-Chloro-3-(pyrrolidin-1"-ylmethyl)-5-(7'''-trifluoromethylquinolin-4'''-ylamino)biphenyl-2-ol (IV.3c)

4'-Chloro-5-nitro-3-(pyrrolidin-1"-ylmethyl)biphenyl-2-ol (0.250 g) in ethanolic ammonia (12.5 ml) and ethanol (12.5 ml) was reduced with hydrogen over Raney nickel to give the corresponding amine (0.187 g).

The above amine (0.101 g) and 4-chloro-7-trifluoromethylquinoline (0.077 g) were allowed to react as described earlier. The yellow precipitate (0.151 g) obtained after adjustment to pH 9 was subjected to PTLC (alumina; 30% ethyl acetate in hexane) and recrystallised from heptane to give the *title compound*, m.p. 210-214° (Found: C, 65.2; H, 4.8; N, 8.3. C₂₇H₂₃ClF₃N₃O requires C, 65.1; H, 4.7; N, 8.4%). ¹H n.m.r. δ 1.87, complex, H3",4"; 2.71, complex, H2",5"; 3.92, s, ArCH₂N; 6.79, d, J 5.5 Hz, H3"; 6.98, d, J 3 Hz, 7.19, d, J 3 Hz, H4,6; 7.38, d, 7.58, d, J 9 Hz, H2',3',5',6'; 7.66, d, J 8.5 Hz, H6"; 8.07, J 8.5 Hz, H5"; 8.32, br s, H8"; 8.56, d, J 5.5 Hz, H2".

4'-Chloro-3-(piperidin-1''-ylmethyl)-5-(7'''-trifluoromethylquinolin-4'''-ylamino)biphenyl-2-ol (IV.3d)

4'-Chloro-5-nitro-3-(piperidin-1''-ylmethyl)biphenyl-2-ol (0.2 g) in ethanolic ammonia was reduced with hydrogen over Raney nickel as described above to give the corresponding amine (0.107 g).

This amine (0.076 g) and 4-chloro-7-trifluoromethylquinoline (0.056 g) as above gave a crude product which was purified by PTLC (alumina; 30% ethyl acetate in hexane) to give the *title compound* (0.112 g), m.p. 203-205° (Found: C, 65.9; H, 5.0; N, 8.0%. $C_{28}H_{25}ClF_3N_3O$ requires C, 65.7; H, 4.9; N, 8.2%). 1H n.m.r. δ 1.62, br, 2.60, br, $H_2'',3'',4'',5'',6''$; 3.75, s, $ArCH_2N$; 6.77, d, J 5.5 Hz, H_3''' ; 6.98, d, J 3 Hz, 7.19, d, J 3 Hz, $H_{4,6}$; 7.38, d, 7.58, d, J 9 Hz, $H_{2',3',5',6'}$; 7.67, d, J 5.5 Hz, H_6''' ; 8.17, d, J 5.5 Hz, H_5''' ; 8.33, br s, H_8''' ; 8.53, d, J 5.5 Hz, H_2''' .

4'-Chloro-3-(3''-methylpiperidin-1''-ylmethyl)-5-(7'''-trifluoromethylquinolin-4'''-ylamino)biphenyl-2-ol (IV.3e)

4'-Chloro-3-(3''-methylpiperidin-1''-ylmethyl)-5-nitrobiphenyl-2-ol (0.2 g) was reduced as described above to give the amine (0.16 g).

This amine (0.080 g) and 4-chloro-7-trifluoromethylquinoline (0.056 g) as for the piperidino analogue gave the *title compound* (0.135 g), m.p. 197-200° (Found: C, 66.4; H, 5.5; N, 7.9. $C_{29}H_{27}ClF_3N_3O$ requires C, 66.2; H, 5.2; N, 8.0%). 1H n.m.r. δ 0.90, d, J 5 Hz, Me; 1.71, complex, 2.90, complex, $H_2'',3'',4'',5'',6''$; 3.75, s, $ArCH_2N$; 6.79, d, J 5.5 Hz, H_3''' ; 6.96, d, J 3 Hz, 7.19, d, J 3 Hz, $H_{4,6}$; 7.38, d, 7.58, d, J 9 Hz, $H_{2',3',5',6'}$; 7.77, d, J 9 Hz, H_6''' ; 8.06, d, J 9 Hz, H_5''' ; 8.32, br s, H_8''' ; 8.56, d, J 5.5 Hz, H_2''' .

4'-Chloro-3-(4''-methylpiperidin-1''-ylmethyl)-5-(7'''-trifluoromethylquinolin-4'''-ylamino)biphenyl-2-ol (IV.3f)

4'-Chloro-3-(4''-methylpiperidin-1''-ylmethyl)-5-nitrobiphenyl-2-ol (0.04 g) was reduced as above to give the corresponding amine (0.038 g). 1H n.m.r. δ 0.90, d, J 3 Hz,

Me; 1.56, complex, 2.07, complex, 2.96, complex, H^{2''},3'',4'',5'',6''; 3.34, br s, ArCH₂N; 5.80, br, NH₂; 7.38, complex, H_{4,6,2',3',5',6'}.

This amine (0.11 g) and 4-chloro-7-trifluoromethylquinoline (0.077 g) as above gave the *title compound* (0.100 g), m.p. 175-178° (Found: C, 66.4; H, 5.5; N, 7.8. C₂₉H₂₇ClF₃N₃O requires C, 66.2; H, 5.2; N, 8.0%). ¹H n.m.r. δ 0.94, d, J 5 Hz, Me; 1.53, complex, 2.17, complex, 3.03, complex, H^{2''},3'',4'',5'',6''; 3.76, s, CH₂N; 6.78, d, J 5.5 Hz, H^{3'''}; 6.95, d, J 3 Hz, 7.18, d, J 3 Hz, H_{4,6}; 7.38, d, 7.58, d, J 9 Hz, H^{2',3',5',6'}; 7.67, d, J 9 Hz, H^{6'''}; 8.08, d, J 9 Hz, H^{5'''}; 8.32, br, H^{8'''}; 8.56, d, J 5.5 Hz, H^{2'''}.

3-(4''-Benzylpiperidin-1''-ylmethyl)-4'-chloro-5-(7'''-trifluoromethyl-quinolin-4'''-ylamino)biphenyl-2-ol (IV.3g)

3-(4''-Benzylpiperidin-1''-ylmethyl)-4'-chloro-5-nitrobiphenyl-2-ol (0.2 g) was reduced in a manner similar to that described above to give the corresponding amine. ¹H n.m.r. δ 1.58, complex, 2.04, complex, 2.98, complex, H^{2''},3'',4'',5'',6''; 2.50, d, J 6 Hz, CH₂Ph; 3.62, s, CH₂N; 4.42, br, NH₂; 6.37, d, J 3 Hz, H₄; 6.59, d, J 3 Hz, H₆; 7.15, complex, Ph; 7.35, d, 7.53, d, J 9 Hz, H^{2',3',5',6'}.

This amine (0.116 g) and 4-chloro-7-trifluoromethylquinoline (0.066 g) gave, as above, and after PTLC (alumina; methylene chloride), the *title compound* (0.161 g), m.p. 112-115° (Found: C, 69.8; H, 5.3; N, 6.9. C₃₅H₃₁ClF₃N₃O requires C, 69.8; H, 5.2; N, 7.0%). ¹H n.m.r. δ 1.65, complex, 2.11, complex, 3.05, complex, H^{2''},3'',4'',5'',6''; 2.53, d, J 6 Hz; CH₂Ph; 3.74, s, CH₂N; 6.77, d, J 5.5 Hz, H^{3'''}; 6.96, d, J 3 Hz, 7.18, d, J 3 Hz, H_{4,6}; 7.23, m, Ph; 7.38, d, 7.58, d, J 9 Hz, H^{2',3',5',6'}; 7.66, d, J 9 Hz, H^{6'''}; 8.10, d, J 9 Hz, H^{5'''}; 8.32, br s, H^{8'''}; 8.52, d, J 5.5 Hz, H^{2'''}.

3-(4''-Benzylpiperazin-1''-ylmethyl)-4'-chloro-5-(7'''-trifluoromethyl-quinolin-4'''-ylamino)biphenyl-2-ol (IV.3h)

3-(4''-Benzylpiperazin-1''-ylmethyl)-4'-chloro-5-nitrobiphenyl-2-ol (0.281 g) was reduced as described above to the corresponding amine (0.252 g). ¹H n.m.r. δ 2.56, br,

H²",3",5",6"; 3.51, s, CH₂Ph; 3.66, s, ArCH₂N; 4.59, br s, NH₂; 6.40, d, J 3 Hz, H₄; 6.60, d, J 3 Hz, H₆; 7.29, s, Ph, 7.35, d, 7.54, d, J 9 Hz, H²',3',5',6'.

This amine (0.140 g) and 4-chloro-7-trifluoromethylquinoline (0.080 g), as above, and the product purified by PTLC (alumina, methylene chloride) and recrystallised from a mixture of methylene chloride and hexane, gave pale orange crystals of the *title compound* (0.124 g), m.p. 188-189° (Found: C, 67.5; H, 4.9; N, 9.1. C₃₄H₃₀ClF₃N₄O requires C, 67.7; H, 5.0; N, 9.3%). ¹H n.m.r. δ 2.62, br, H²",3",5",6"; 3.54, s, CH₂Ph; 3.78, s, ArCH₂N; 6.76, d, J 5.5 Hz, H³"; 7.00, d, J 3 Hz, 7.21, d, J 3 Hz, H_{4,6}; 7.30, s, Ph, 7.38, d, 7.55, d, J 9 Hz, H²',3',5',6'; 7.64, d, J 8.5 Hz, H⁶"; 8.22, d, J 8.5 Hz, H⁵"; 8.33, br s, H⁸"; 8.47, d, J 5.5 Hz, H²".

3-(*t*-Butylaminomethyl)-4'-chloro-5-(7"-trifluoromethylquinazolin-4"-yl-amino)biphenyl-2-ol (IV.4b)

Crude 5-amino-3-(*t*-butylaminomethyl)-4'-chlorobiphenyl-2-ol (0.102 g; prepared by reduction of the corresponding nitro compound) and 4-chloro-7-trifluoromethylquinazoline (0.078 g) in a mixture of methanol (1.8 ml), water (0.8 ml) and concentrated hydrochloric acid (2 drops) were refluxed at 100° for 8 h. The crude product (0.153 g) was subjected to PTLC (alumina; 35% ethyl acetate in hexane) and then reprecipitated from dilute acetic acid solution by addition of 0.9 N ammonium hydroxide to give the *title compound*, m.p.180° (dec) (Found: C, 59.6; H, 4.9; N, 10.5. C₂₆H₂₄ClF₃N₄O.1.3 H₂O requires C, 59.6; H, 5.1; N, 10.7%). ¹H n.m.r. δ 1.30, s, Me; 4.01, s, CH₂N; 7.46, complex, H_{4,6,2',3',5',6'}; 7.66, d, J 9 Hz, H⁶"; 8.11, s, H⁸"; 8.14, d, J 9 Hz, H⁵"; 8.68, s, H²".

In a similar manner the following compounds were prepared from 4-chloro-7-trifluoromethylquinazoline and 3-substituted-5-amino-4'-chlorobiphenyl-2-ols.

4'-Chloro-3-diethylaminomethyl-5-(7"-trifluoromethylquinazolin-4"-yl-amino)biphenyl-2-ol (IV.4a)

The *title compound* (73%) had m.p. ca 110° [after PTLC (alumina; 34% ethyl acetate in hexane)] (Found: C, 62.4; H, 5.0; N, 11.2. C₂₆H₂₄ClF₃N₄O requires C, 62.3;

H, 4.8; N, 11.2%). ^1H n.m.r. δ 1.14, t, J 7 Hz, Me; 2.70, q, J 7 Hz, MeCH_2 ; 3.87, s, CH_2N ; 7.42, s, H_{4,6}; 7.36, d, 7.58, d, J 9 Hz, H_{2',3',5',6'}; 7.57, d, J 9 Hz, H_{6''}; 8.00, d, J 9 Hz, H_{5''}; 8.17, br s, H_{8''}; 8.76, s, H_{2''}.

4'-Chloro-3-(pyrrolidin-1''-ylmethyl)-5-(7'''-trifluoromethylquinazolin-4'''-ylamino)biphenyl-2-ol (IV.4c)

This compound (72%) had m.p. 115-120° [after PTLC (alumina; methylene chloride)] (Found: C, 62.8; H, 4.5; N, 10.9. $\text{C}_{20}\text{H}_{22}\text{ClF}_3\text{N}_4\text{O}$ requires C, 62.6; H, 4.4; N, 11.2%). ^1H n.m.r. δ 1.86, complex, 2.72, complex, H_{2'',3'',4'',5''}; 3.93, s, CH_2N ; 5.53, br, NH; 7.42, s, H_{4,6}; 7.37, d, 7.57, d, J 9 Hz, H_{2',3',5',6'}; 7.69, d, J 9 Hz, H_{6'''}; 8.02, d, J 9 Hz, H_{5'''}; 8.17, br s, H_{8'''}; 8.76, s, H_{2'''}.

4'-Chloro-3-(piperidin-1''-ylmethyl)-5-(7'''-trifluoromethylquinazolin-4'''-ylamino)biphenyl-2-ol (IV.4d)

The title compound (81%) had m.p. ca 135° with sublimation [after PTLC (alumina; 30% ethyl acetate in hexane; then alumina; methylene chloride)] (Found: C, 63.0; H, 4.6; N, 10.6. $\text{C}_{27}\text{H}_{24}\text{ClF}_3\text{N}_4\text{O}$ requires C, 63.3; H, 4.7; N, 10.9%). ^1H n.m.r. δ 1.59, complex, 2.60, complex, H_{2'',3'',4'',5'',6''}; 3.78, s, CH_2N ; 4.42, br s, NH; 7.43, s, H_{4,6}; 7.38, d, 7.57, d, J 9 Hz, H_{2',3',5',6'}; 7.70, d, J 9 Hz, H_{6'''}; 8.03, d, J 9 Hz, H_{5'''}; 8.17, br s, H_{8'''}; 8.72, s, H_{2'''}.

4'-Chloro-3-(3''-methylpiperidin-1''-ylmethyl)-5-(7'''-trifluoromethylquinazolin-4'''-ylamino)biphenyl-2-ol (IV.4e)

The title compound (69%) had m.p. 118-122° [after PTLC (alumina; 30% ethyl acetate in hexane)] (Found: C, 63.8; H, 5.2; N, 10.3. $\text{C}_{28}\text{H}_{26}\text{ClF}_3\text{N}_4\text{O}$ requires C, 63.8; H, 5.0; N, 10.6%). ^1H n.m.r. δ 0.87, d, J 5.5 Hz, Me; 1.72, complex, 3.02, complex, H_{2'',3'',4'',5'',6''}; 3.75, s, CH_2N ; 7.43, s, H_{4,6}; 7.38, d, 7.59, d, J 9 Hz, H_{2',3',5',6'}; 7.68, d, J 9 Hz, H_{6'''}; 8.01, d, J 9 Hz, H_{5'''}; 8.19, br s, H_{8'''}; 8.77, s, H_{2'''}.

4'-Chloro-3-(4''-methylpiperidin-1''-ylmethyl)-5-(7'''-trifluoromethyl-quinazolin-4'''-ylamino)biphenyl-2-ol (IV.4f)

This compound (56%) had m.p. 119-123° [after PTLC (alumina; 36% ethyl acetate in hexane)] (Found: C, 63.8; H, 5.0; N, 10.3. $C_{28}H_{26}ClF_3N_4O$ requires C, 63.8; H, 5.0; N, 10.6%). 1H n.m.r. δ 0.93, d, J 3.5 Hz, Me; 1.51, complex, 2.20, complex, 3.06, complex, $H_{2'',3'',4'',5'',6''}$; 3.80, s, CH_2N ; 7.42, s, $H_{4,6}$; 7.39, d, 7.58, d, J 9 Hz, $H_{2',3',5',6'}$; 7.68, d, J 9 Hz, $H_{6''}$; 8.01, d, J 9 Hz, $H_{5''}$; 8.19, br s, $H_{8''}$; 8.77, s, $H_{2''}$. I.r. ν_{max} 3450 (br) (OH st), 2945 (w) (aliphatic CH st), 1584 (s), 1472 (m), 1184 (ms), 1140 (s) (CF_3 st), 1084 (m) cm^{-1} .

3-(4''-Benzylpiperidin-1''-ylmethyl)-4'-chloro-5-(7'''-trifluoromethyl-quinazolin-4'''-ylamino)biphenyl-2-ol (IV.4g)

The title compound (78%) after PTLC (alumina; methylene chloride then alumina; 80% methylene chloride in hexane, developed twice) had m.p. ca 140° (after filtration from dilute hydrochloric acid) (Found: C, 67.9; H, 5.3; N, 9.0. $C_{34}H_{30}ClF_3N_4O$ requires C, 67.7; H, 5.0; N, 9.2%). 1H n.m.r. δ 1.62, complex, 2.14, complex, 3.07, complex, $H_{2'',3'',4'',5'',6''}$; 2.52, d, J 5.5 Hz, CH_2Ph ; 3.78, s, CH_2N ; 7.41, s, $H_{4,6}$; 7.39, d, 7.58, d, J 9 Hz, $H_{2',3',5',6'}$; 7.69, d, J 9 Hz, $H_{6''}$; 8.01, d, J 9 Hz, $H_{5''}$; 8.16, s, $H_{8''}$; 8.75, s, $H_{2''}$.

3-(4''-Benzylpiperazin-1''-ylmethyl)-4'-chloro-5-(7'''-trifluoromethyl-quinazolin-4'''-ylamino)biphenyl-2-ol (IV.4h)

This compound was obtained in 64% yield [after PTLC (alumina; 40% ethyl acetate in hexane)]. It was dissolved in dilute hydrochloric acid and reprecipitated with aqueous ammonium hydroxide to give a yellow solid, m.p. 144-150° (Found: C, 63.2; H, 4.7; N, 10.9. $C_{33}H_{29}ClF_3N_5O \cdot 1.4 H_2O$ requires C, 63.0; H, 5.1; N, 11.1%). 1H n.m.r. δ 2.71, complex, $H_{2'',3'',5'',6''}$; 3.63, s, CH_2Ph ; 3.83, s, CH_2N ; 7.44, complex, $H_{4,6,2'',3'',5'',6''}$; 7.73, d, J 9 Hz, $H_{6''}$; 8.06, d, J 9 Hz, $H_{5''}$; 8.19, br s, $H_{8''}$; 8.78, s, $H_{2''}$.

3-(4''-Benzylpiperidin-1''-ylmethyl)-5-(7'''-bromo-1''',5'''-naphthyridin-4'''-yl)amino-4'-chlorobiphenyl-2-ol (IV.5g)

A mixture of 3-bromo-8-chloro-1,5-naphthyridine¹¹⁰ (0.059 g) and crude 5-amino-3-(4''-benzylpiperidin-1''-ylmethyl)-4'-chlorobiphenyl-2-ol [0.100 g, prepared by reduction, as above, of 3-(4''-benzylpiperidin-1''-ylmethyl)-4'-chloro-5-nitrobiphenyl-2-ol] in a mixture of methanol (1.8 ml), water (0.6 ml) and concentrated hydrochloric acid (2 drops) was refluxed at 100° for 8 h. The product was isolated as above and purified by PTLC (alumina, methylene chloride) and recrystallised from a mixture of methylene chloride and hexane with concentration to give as a pale yellow solid the *title compound* (0.10 g), m.p. 169-170.5° (Found: C, 64.7; H, 4.8; N, 9.0. C₃₃H₃₀BrClN₄O requires C, 64.6; H, 4.9; N, 9.1%). ¹H n.m.r. δ 1.63, complex, 2.09, complex, 3.04, complex, H2'',3'',4'',5'',6''; 2.49, d, J 5.5 Hz, CH₂Ph; 3.73, s, CH₂N; 6.90, d, J 5.5 Hz, H3'''; 6.96, d, 7.17, d, J 3 Hz, H4,6; 7.38, d, 7.58, d, J 9 Hz, H2',3',5',6'; 8.21, br, NH; 8.41, d, J 2 Hz, H8'''; 8.48, d, J 5.5 Hz, H2''; 8.72, d, J 2 Hz, H6''.

In a similar manner the following compounds were prepared from the corresponding 3-bromo-8-chloro-1,5-naphthyridine¹¹⁰ and 3-substituted 5-amino-4'-chlorobiphenyl-2-ols.

5-(7''-Bromo-1'',5''-naphthyridin-4''-ylamino)-4'-chloro-3-diethylamino-methylbiphenyl-2-ol (IV.5a)

The *title compound* was obtained in 96% yield after PTLC (alumina; 20% ethyl acetate in hexane). Recrystallisation from a mixture of ether and light petroleum (b.p. 60-80°) gave a pale yellow solid, m.p. 171-173° (Found: C, 59.0; H, 4.8; N, 11.1. C₂₅H₂₄BrClN₄O requires C, 58.7; H, 4.7; N, 11.0%). ¹H n.m.r. δ 1.14, t, J 7 Hz, Me; 2.68, q, J 7 Hz, MeCH₂; 3.85, s, CH₂N; 6.92, d, J 5.5 Hz, H3''; 7.00, d, 7.24, d, J 3 Hz, H4,6; 7.38, d, 7.59, d, J 9 Hz, H2',3',5',6'; 8.24, br, NH; 8.44, d, J 2 Hz, H8''; 8.50, d, J 5.5 Hz, H2''; 8.75, d, J 2 Hz, H6''.

5-(7''-Bromo-1'',5''-naphthyridin-4''-ylamino)-3-(t-butylaminomethyl)-4'-chlorobiphenyl-2-ol (IV.5b)

The *title compound* (66%) had m.p. ca 97° [after PTLC (alumina; methylene chloride)] (Found: C, 58.7; H, 4.8; N, 10.8. $C_{25}H_{24}BrClN_4O$ requires C, 58.7; H, 4.7; N, 10.9%). 1H n.m.r. δ 1.23, s, Me; 3.98, s, CH_2N ; 6.87, d, J 5.5 Hz, H3''; 6.96, d, J 2 Hz, 7.18, d, J 2 Hz, H4,6; 7.35, d, 7.56, d, J 9 Hz, H2',3',5',6'; 8.20, br, NH; 8.38, d, J 2 Hz, H8''; 8.47, d, J 5.5 Hz, H2''; 8.70, d, J 2 Hz, H6''.

5-(7''-Bromo-1'',5''-naphthyridin-4''-ylamino)-4'-chloro-3-(pyrrolidin-1'''-ylmethyl)biphenyl-2-ol (IV.5c)

This *compound* (68%) had m.p. 198-201° [from PTLC (alumina; 20% ethyl acetate in hexane)] (Found: C, 58.5; H, 4.3; N, 10.7. $C_{25}H_{22}BrClN_4O$ requires C, 58.9; H, 4.3; N, 11.0%). 1H n.m.r. δ 1.90, complex, H3''',4'''; 2.76, complex, H2''',5'''; 3.96, s, CH_2N ; 6.95, d, J 5.5 Hz, H3''; 7.05, d, 7.27, d, J 3 Hz, H4,6; 7.40, d, 7.60, d, J 9 Hz, H2',3',5',6'; 8.30, br, NH; 8.49, br s, H8''; 8.53, d, J 5.5 Hz, H2''.

5-(7''-Bromo-1'',5''-naphthyridin-4''-ylamino)-4'-chloro-3-(piperidin-1'''-ylmethyl)biphenyl-2-ol (IV.5d)

This *title compound* (91%) had m.p. 173-175° [after PTLC (alumina; 30% ethyl acetate in hexane, developed twice)] (Found: C, 59.5; H, 4.7; N, 10.4. $C_{26}H_{24}BrClN_4O$ requires C, 59.6; H, 4.6; N, 10.7%). 1H n.m.r. δ 1.59, complex, 2.60, complex, H2''',3''',4''',5''',6'''; 3.75, s, CH_2N ; 6.92, d, J 5.5 Hz, H3''; 6.99, d, 7.24, d, J 3 Hz, H4,6; 7.37, d, 7.59, d, J 9 Hz, H2',3',5',6'; 8.24, br, NH; 8.47, s, H8''; 8.48, d, J 5.5 Hz, H2''; 8.75, d, J 2 Hz, H6''.

5-(7''-Bromo-1'',5''-naphthyridin-4''-ylamino)-4'-chloro-3-(3'''-methylpiperidin-1'''-ylmethyl)biphenyl-2-ol (IV.5e)

The *title compound* (78%) was obtained after PTLC (alumina; 30% ethyl acetate in hexane; then alumina; methylene chloride). It had m.p. 86-88° (Found: C, 60.3; H, 4.7; N, 10.3. $C_{27}H_{26}BrClN_4O$ requires C, 60.3; H, 4.9; N, 10.4%). 1H n.m.r. δ 0.90, d, J 5.5

Hz, Me; 1.69, complex, 2.92, complex, H^{2'''},3^{'''},4^{'''},5^{'''},6^{'''}; 3.75, s, CH₂N; 6.93, d, J 5.5 Hz, H^{3''}; 7.00, d, 7.24, d, J 3 Hz, H^{4,6}; 7.39, d, 7.59, d, J 9 Hz, H^{2',3',5',6'}; 8.24, br s, NH; 8.44, d, J 2 Hz, H^{8''}; 8.51, d, J 5.5 Hz, H^{2''}; 8.74, d, J 2 Hz, H^{6''}.

5-(7''-Bromo-1'',5''-naphthyridin-4''-ylamino)-4'-chloro-3-(4'''-methylpiperidin-1'''-ylmethyl)biphenyl-2-ol (IV.5f)

The title compound (95%) had m.p. 207-209° [after PTLC (alumina; 33% ethyl acetate in hexane)] (Found: C, 60.5; H, 5.0; N, 10.3. C₂₇H₂₆BrClN₄O requires C, 60.3; H, 4.9; N, 10.4%). ¹H n.m.r. δ 0.93, d, J 4.5 Hz, Me; 1.25, complex, 3.04, complex, H^{2'''},3^{'''},4^{'''},5^{'''},6^{'''}; 3.77, s, CH₂N; 6.92, d, J 5.5 Hz, H^{3''}; 7.00, d, 7.26, d, J 3 Hz, H^{4,6}; 7.39, d, 7.58, d, J 9 Hz, H^{2',3',5',6'}; 8.33, br, NH; 8.48, br s, H^{8''}; 8.51, d, J 5.5 Hz, H^{2''}; 8.76, br s, H^{6''}. I.r. ν_{max} 3470 (br) (OH st), 3390 (w) (NH st), 2964 (w) (aliphatic CH st), 1598 (m), 1570 (s), 1530 (m), 1502 (m), 1470 (s), 1370 (ms), 842 (m) cm⁻¹.

3-(4''-Benzylpiperazin-1''-ylmethyl)-5-(7'''-bromo-1''',5'''-naphthyridin-1'''-ylamino)-4'-chlorobiphenyl-2-ol (IV.5h)

This compound (81%) had m.p. ca 107-110° [after PTLC (alumina; methylene chloride; and alumina; 25% ethyl acetate in hexane, developed twice)] (Found: C, 62.5; H, 4.8; N, 11.2. C₃₂H₂₉BrClN₅O requires C, 62.5; H, 4.8; N, 11.4%). ¹H n.m.r. δ 2.66, complex, H^{2''},3^{''},5^{''},6^{''}; 3.56, s, PhCH₂N; 3.80, s, ArCH₂N; 6.92, d, J 5.5 Hz, H^{3'''}; 7.02, d, 7.26, d, J 3 Hz, H^{4,6}; 7.39, d, 7.58, d, J 9 Hz, H^{2',3',5',6'}; 8.26, br, NH; 8.46, d, J 2 Hz, H^{8'''}; 8.50, d, J 5.5 Hz, H^{2'''}; 8.75, d, J 2 Hz, H^{6'''}.

4'-Chloro-3-diethylaminomethyl-5-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)biphenyl-2-ol (IV.6a)

4'-Chloro-3-diethylaminomethyl-5-nitrobiphenyl-2-ol (0.122 g) was dissolved in half-saturated ethanolic ammonia (10.0 ml), and shaken with hydrogen over Raney nickel at room temperature until uptake ceased. The catalyst was filtered off on Celite, and the filtrate evaporated to give crude 5-amino-4'-chloro-3-diethylaminomethylbiphenyl-2-ol (0.085 g).

This amine (0.085 g) and 4-chloro-7-trifluoromethyl-1,5-naphthyridine (0.061 g) in a mixture of methanol (1.5 ml), water (0.5 ml) and concentrated hydrochloric acid (3 drops) at pH 2.5 was refluxed at 95-100° for 5 h. The solvent was evaporated, the residue diluted with water (5.0 ml) and adjusted with 0.9 N sodium hydroxide to give a yellow precipitate. The product was extracted into chloroform, the extract washed with water, dried (Na₂SO₄), and the solvent evaporated to give a yellow oil. It was purified by PTLC (alumina; 20% ethyl acetate in hexane) to give the *title compound* (0.118 g), m.p. 140-142° (Found: C, 62.6; H, 5.0; N, 11.1. C₂₆H₂₄ClF₃N₄O requires C, 62.3; H, 4.8; N, 11.3%). ¹H n.m.r. δ 1.16, t, J 7 Hz, Me; 2.73, q, J 7 Hz, MeCH₂; 3.88, s, CH₂N; 7.01, d, J 5.5 Hz, H^{3''}; 7.04, 7.26, br s, H^{4,6}; 7.38, d, 7.59, d, J 9 Hz, H^{2',3',5',6'}; 8.36, br s, NH; 8.58, br s, H^{8''}; 8.61, d, J 5.5 Hz, H^{2''}; 8.95, d, J 2 Hz, H^{6''}. MS m/z 500 (9.4%), 502 (3.4%), 501 (2.6%), 503 (0.9%), 427 (17.7%).

In a similar manner the following compounds were prepared from 4-chloro-7-trifluoromethyl-1,5-naphthyridine and the corresponding 3-substituted-5-amino-4'-chlorobiphenyl-2-ols.

3-(*t*-Butylaminomethyl)-4'-chloro-5-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)biphenyl-2-ol (IV.6b)

The *title compound* (68%) after PTLC (alumina; 30% ethyl acetate/hexane) and reprecipitation from aqueous acid by neutralisation with ammonium hydroxide gave a yellow solid, had m.p. 189-192° (Found: C, 61.6; H, 4.6; N, 10.8. C₂₆H₂₄ClF₃N₄O. 0.25 H₂O requires C, 61.8; H, 4.9; N, 11.1%). ¹H n.m.r. δ 1.25, s, Me; 4.05, s, CH₂N; 7.14, d, J 5.5 Hz, H^{3''}; 7.18, br s, 7.24, d, J 2 Hz, H^{4,6}; 7.40, d, 7.59, d, J 9 Hz, H^{2',3',5',6'}; 8.34, br s, NH; 8.54, d, J 2 Hz, H^{8''}; 8.64, d, J 5.5 Hz, H^{2''}; 8.95, d, J 2 Hz, H^{6''}.

4'-Chloro-3-(pyrrolidin-1''-ylmethyl)-5-(7'''-trifluoromethyl-1''',5'''-naphthyridin-4'''-ylamino)biphenyl-2-ol (IV.6c)

The *title compound* (78%) [after PTLC (alumina; 20% ethyl acetate in hexane)] had m.p. 125-127° (Found: C, 62.5; H, 4.3; N, 11.2. C₂₆H₂₂ClF₃N₄O requires C, 62.6; H,

4.4; N, 11.2%). ^1H n.m.r. δ 1.89, complex, 2.73, complex, $\text{H}^{2'',3'',4'',5''}$; 3.93, s, CH_2N ; 7.01, d, J 5.5 Hz, $\text{H}^{3''}$; 7.04, br s, 7.27, br s, $\text{H}^{4,6}$; 7.38, d, 7.59, d, J 9 Hz, $\text{H}^{2',3',5',6'}$; 8.32, br s, NH; 8.54, br s, $\text{H}^{8''}$; 8.61, d, J 5.5 Hz, $\text{H}^{2''}$; 8.93, br s, $\text{H}^{6''}$.

***4'-Chloro-3-(piperidin-1''-ylmethyl)-5-(7'''-trifluoromethyl-1''',5'''-naphthyridin-4'''-ylamino)biphenyl-2-ol* (IV.6d)**

This compound (77%) had m.p. 165-166° [after PTLC (alumina; 25% ethyl acetate in hexane)] (Found: C, 62.3; H, 4.8; N, 10.6. $\text{C}_{27}\text{H}_{24}\text{ClF}_3\text{N}_4\text{O}$ requires C, 63.3; H, 4.7; N, 10.9%). ^1H n.m.r. δ 1.64, complex, 2.63, complex, $\text{H}^{2'',3'',4'',5'',6''}$; 3.77, s, CH_2N ; 7.01, d, J 5.5 Hz, $\text{H}^{3''}$; 7.00, br s, 7.27, br s, $\text{H}^{4,6}$; 7.40, d, 7.60, d, J 9 Hz, $\text{H}^{2',3',5',6'}$; 8.32, br s, NH; 8.55, br s, $\text{H}^{8''}$; 8.59, d, J 5.5 Hz, $\text{H}^{2''}$; 8.94, br s, $\text{H}^{6''}$.

***4'-Chloro-3-(3''-methylpiperidin-1''-ylmethyl)-5-(7'''-trifluoromethyl-1''',5'''-naphthyridin-4'''-ylamino)biphenyl-2-ol* (IV.6e)**

It was obtained after PTLC (alumina; 25% ethyl acetate in hexane) as a yellow oil (87%) (Found: C, 63.9; H, 5.1; N, 10.3. $\text{C}_{28}\text{H}_{26}\text{ClF}_3\text{N}_4\text{O}$ requires C, 63.8; H, 5.0; N, 10.6%). ^1H n.m.r. δ 0.90, d, J 5.5 Hz, Me; 1.73, complex, 2.35, complex, 2.98, complex, $\text{H}^{2'',3'',4'',5'',6''}$; 3.79, s, CH_2N ; 7.01, J 5.5 Hz, $\text{H}^{3''}$; 7.04, br s, 7.26, br s, $\text{H}^{4,6}$; 7.38, d, 7.58, d, J 9 Hz, $\text{H}^{2',3',5',6'}$; 8.36, br s, NH; 8.57, br s, $\text{H}^{8''}$; 8.60, d, J 5.5 Hz, $\text{H}^{2''}$; 8.93, br s, $\text{H}^{6''}$.

***4'-Chloro-3-(4''-methylpiperidin-1''-ylmethyl)-5-(7'''-trifluoromethyl-1''',5'''-naphthyridin-4'''-ylamino)biphenyl-2-ol* (IV.6f)**

The title compound (84%) after PTLC (alumina; 20% ethyl acetate in hexane), had m.p. 164-167° (Found: C, 64.0; H, 5.2; N, 10.5. $\text{C}_{28}\text{H}_{26}\text{ClF}_3\text{N}_4\text{O}$ requires C, 63.8; H, 5.0; N, 10.6%). ^1H n.m.r. δ 0.94, d, J 4.5 Hz, Me; 1.54, complex, 2.19, complex, 3.05, complex, $\text{H}^{2'',3'',4'',5'',6''}$; 3.78, s, CH_2N ; 6.99, d, J 5.5 Hz, $\text{H}^{3''}$; 7.03, br s, 7.24, br s, $\text{H}^{4,6}$; 7.38, d, 7.58, d, J 9 Hz, $\text{H}^{2',3',5',6'}$; 8.32, br s, NH; 8.55, br s, $\text{H}^{8''}$; 8.60,

d, J 5.5 Hz, H2'''; 8.93, br s, H6'''. MS m/z 526 (66.8%), 528 (26.4%), 527 (21.8%), 529 (7.1%), 427 (63.5%).

3-(4''-Benzylpiperidin-1''-ylmethyl)-4'-chloro-5-(7'''-trifluoromethyl-1''',5'''-naphthyridin-4'''-ylamino)biphenyl-2-ol (IV.6g)

This compound (82%) after reprecipitation and PTLC (alumina; methylene chloride), had m.p. ca 95° (Found: C, 68.0; H, 5.3; N, 9.2. C₃₄H₃₀ClF₃N₄O requires C, 67.7; H, 5.0; N, 9.3%). ¹H n.m.r. δ 1.64, complex, 2.13, complex, 3.05, complex, H2'',3'',4'',5'',6''; 2.53, d, J 5.5 Hz, CH₂Ph; 3.76, s, CH₂N; 6.98, d, J 3 Hz, H3'''; 7.02, br s, 7.16, br s, H4,6; 7.39, d, 7.59, d, J 9 Hz, H2',3',5',6'; 8.32, br s, NH; 8.54, br s, H8'''; 8.57, d, J 5.5 Hz, H2'''; 8.93, br s, H6'''.

3-(4''-Benzylpiperazin-1''-ylmethyl)-4'-chloro-5-(7'''-trifluoromethyl-1''',5'''-naphthyridin-4'''-ylamino)biphenyl-2-ol (IV.6h)

The title compound (87%) after PTLC (alumina; 30% ethyl acetate in hexane) crystallised as a yellow solid from a mixture of methylene chloride and hexane and had m.p. 181-183° (Found: C, 63.8; H, 4.8; N, 11.0. C₃₃H₂₉ClF₃N₅O.H₂O requires, C, 63.7; H, 5.0; N, 11.3%). ¹H n.m.r. δ 2.67, complex, H2'',3'',5'',6''; 3.60, s, PhCH₂N; 3.82, s, ArCH₂N; 7.00, d, J 7 Hz, H3'''; 7.04, br s, 7.28, br s, H4,6; 7.39, d, 7.58, d, J 9 Hz, H2',3',5',6'; 8.32, br s, NH; 8.54, br s, H8'''; 8.60, d, J 5.5 Hz, H2'''; 8.94, d, J 2 Hz, H6'''.

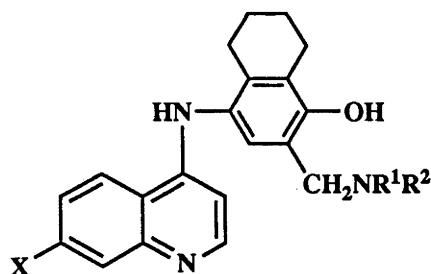
CHAPTER V

CHAPTER V Mono-Mannich Bases of 4-[7-Bromo (and 7-Trifluoromethyl)-1,5-naphthyridin-4-ylamino]-5,6,7,8-tetrahydronaphth-1-ols

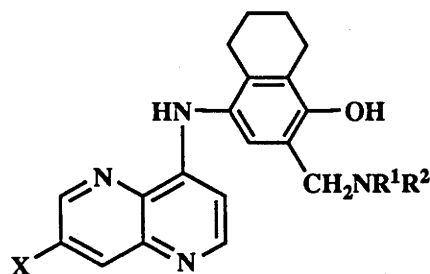
V-1 Introduction

Nabih *et al.*¹⁰⁴ were the first to synthesise mono-Mannich bases of 4-(7-chloroquinolin-4-ylamino and 6-chloro-2-methoxy-9-acridin-4-ylamino)-5,6,7,8-tetrahydronaphth-1-ols for evaluation for antimalarial activity.²¹⁷

Recent publications by Kesten *et al.*¹⁰⁵ and Shen *et al.*⁹³ have reported the synthesis and antimalarial activity against *P. berghei* in mice of Mannich base derivatives of 4-[7-chloro (and 7-trifluoromethyl)quinolin-4-ylamino]-5,6,7,8-tetrahydronaphth-1-ols (V.1; X=Cl and CF₃, respectively). Kesten *et al.*¹⁰⁵ found that the compound V.1 (X=Cl, NR¹R²=NEt₂) exhibited antimalarial activity (as measured by increased mean survival time) when administered in a single dose of 10 mg/kg and that it effected partial cures at 40 mg/kg.



V.1 X=Cl, CF₃



V.2 X=Br
V.3 X=CF₃

In an attempt to discover other compounds which may have good antimalarial activity, mono-Mannich bases of 4-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]-5,6,7,8-tetrahydronaphth-1-ols (V.2 and V.3) have been synthesised in the present work for testing for antimalarial activity. The synthesis of the starting material, *N*-(4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (V.10) is mentioned first in this

Chapter, and then the Mannich base derivatives (V.2 and V.3) are described. Their physical properties are also discussed briefly. The results of testing for antimalarial activity against *P. falciparum* *in vitro*, and the results of *in vivo* tests of two selected compounds against *P. vinckei vinckei* are discussed in Chapter VIII.

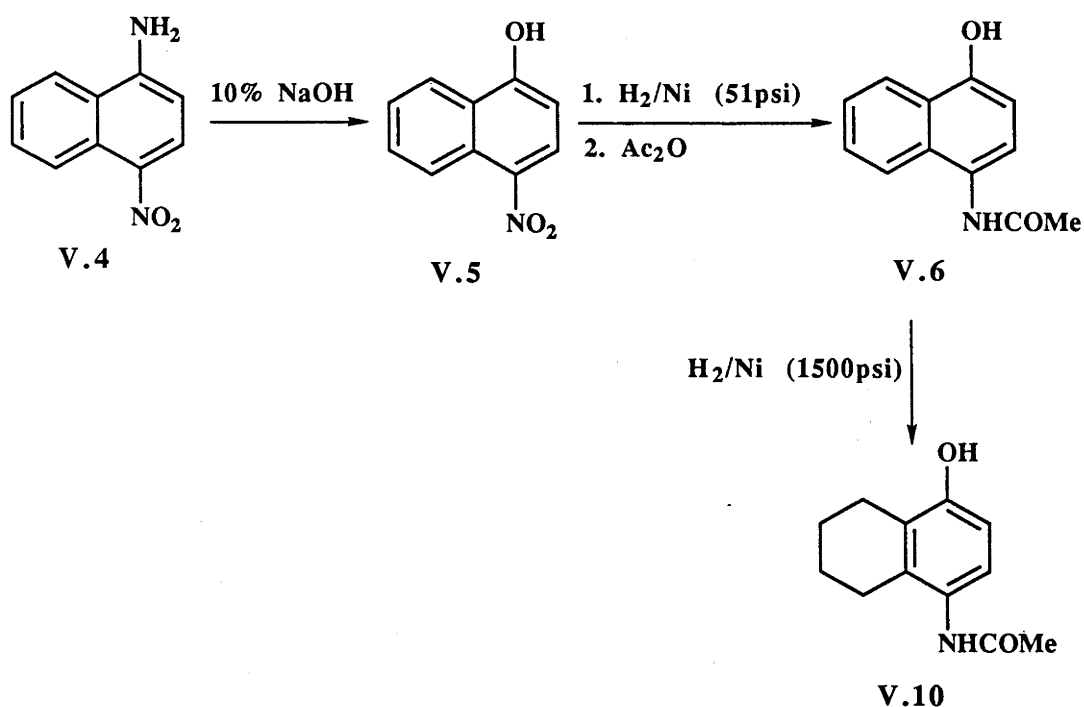
V-2 Syntheses of *N*-(4-Hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide

N-(4-Hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (V.10) was the necessary precursor for the present work. Two syntheses of compound V.10 have been reported. The first (Method A; Scheme V-1) was described by Kesten *et al.*¹⁰⁵ and commenced from the commercially available 4-nitronaphthyl-1-amine (V.4). It involved the hydrolysis of compound V.4 in refluxing 10% sodium hydroxide to afford 4-nitronaphth-1-ol (V.5) in good yields. Catalytic hydrogenation of compound V.5 over Raney nickel at 51 psi and subsequent acetylation with acetic anhydride provided *N*-(4-hydroxynaphth-1-yl)acetamide (V.6) in excellent yields. A further hydrogenation under high pressure (1500 psi) in the presence of Raney nickel gave *N*-(4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (V.10) in high yields.

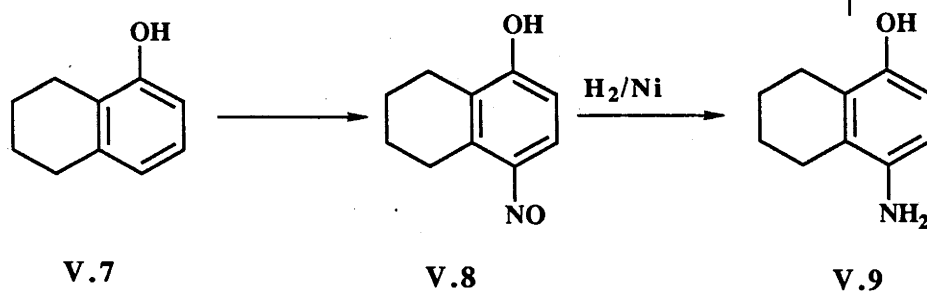
The second approach, Method B (Scheme V-1), involved the nitrosation²¹⁸⁻²²⁰ of the commercially available 5,6,7,8-tetrahydronaphth-1-ol (V.7) with sodium nitrite in sodium hydroxide solution in an ice-bath for 15 minutes then in 10% sulphuric acid at 0° for 1.5 h to afford 4-nitroso-5,6,7,8-tetrahydronaphth-1-ol (V.8). The reduction of compound V.8 with stannous chloride and hydrochloric acid²¹⁸ or with aqueous ammonium sulphide²²¹ has been described to give the amino analogue (V.9) which was then *N*-acetylated with a mixture of acetic anhydride and acetic acid (1:5) at 40° for 5 h to the compound V.10.

In the present work, Method B was chosen (in preference to Method A) to avoid the high pressure hydrogenation of a naphthalene to a tetrahydronaphthalene. It was also found preferable to reduce compound V.8 (dissolved in ethanolic ammonia) with hydrogen over Raney nickel.

Method A



Method B

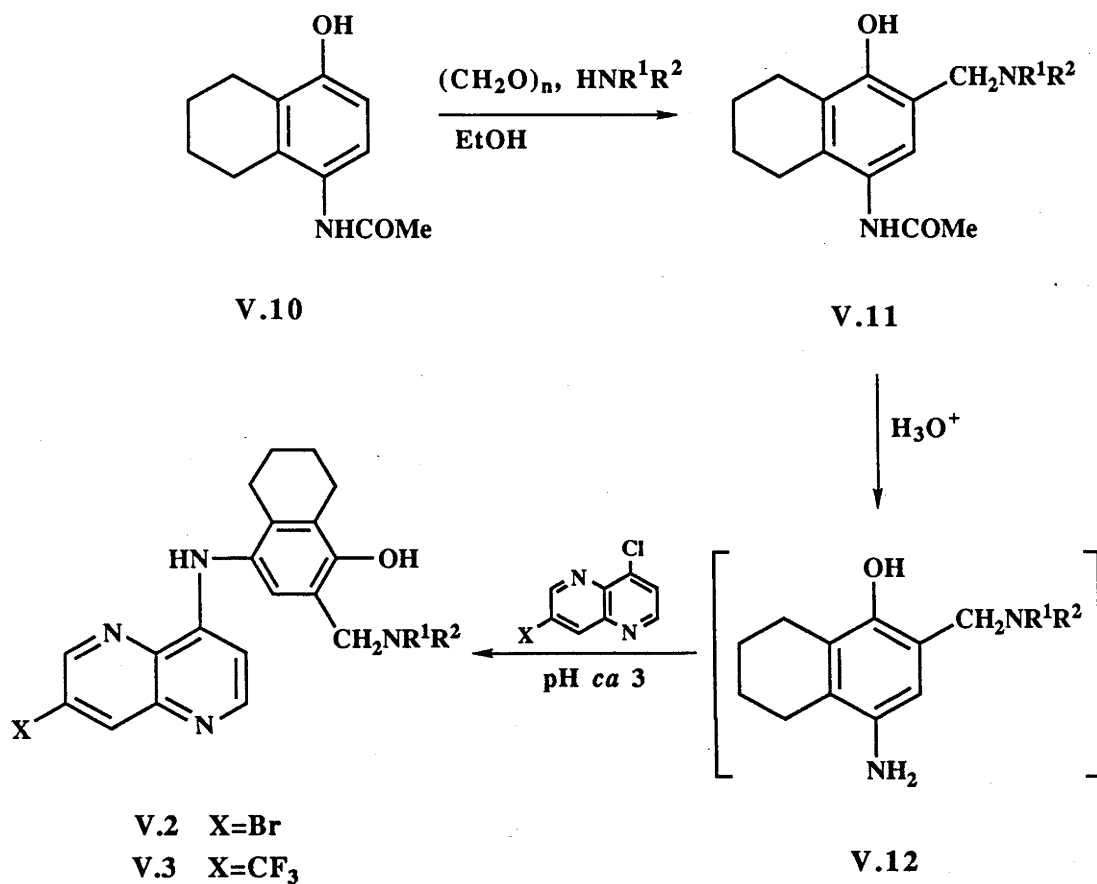


Scheme V-1

V-3 Syntheses of Mono-Mannich Bases of 4-[7-Bromo (and 7-Trifluoromethyl)-1,5-naphthyridin-4-ylamino]-5,6,7,8-tetrahydronaphth-1-ols

Compound **V.10** underwent the Mannich reaction (as has been reported previously¹⁰⁴ for the amines, diethylamine or piperidine and formaldehyde) to give mono-Mannich compounds (**V.11**). In this work, further amines; namely, dipropylamine, *t*-butylamine, pyrrolidine, 3-methylpiperidine, 4-methylpiperidine, 4-benzylpiperidine and

4-benzylpiperazine, and paraformaldehyde reacted with *N*-(4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (V.10) in refluxing ethanol for 12 h and gave the mono-Mannich compounds (V.11) (Scheme V-2).



V.2, V.3, and V.11

	NR ¹ R ²
a	NEt ₂
b	NPr ₂
c	NHBu ^t
d	pyrrolidin-1-yl

	NR ¹ R ²
e	piperidin-1-yl
f	3-methylpiperidin-1-yl
g	4-methylpiperidin-1-yl
h	4-benzylpiperidin-1-yl
i	4-benzylpiperazin-1-yl

Scheme V-2

These compounds **V.11** were readily *N*-deacetylated on hydrolysis with concentrated hydrochloric acid in ethanol at 95° for 5-10 h to give the amino analogues (**V.12**). The reaction solution was then adjusted pH *ca* 3 with sodium hydroxide solution, and used directly for condensation with 7-bromo (and 7-trifluoromethyl)-4-chloro-1,5-naphthyridine at 100° for 8-10 h, to give the mono-Mannich bases of 4-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]-5,6,7,8-tetrahydronaphth-1-ols (**V.2** and **V.3**, respectively).

V-4 Physical Properties

The ^1H n.m.r. spectra of compounds **V.2**, **V.3** and **V.11** are recorded in the Experimental section. The 2-D COSY spectrum and the ^1H n.m.r. spectrum of compound **V.3g** were also measured on a XL-300 spectrometer. The latter spectrum is shown in Figure V-1. The assignment of the methylene protons in this compound (**V.3g**) was confirmed by the 2-D COSY spectrum (Figure V-2).

The ^{13}C n.m.r. spectrum of compound **V.3g** has also been determined and the results are shown in Figure V-3. The assignments of the ^{13}C n.m.r. chemical shifts were carried out by running a decoupled ^{13}C n.m.r. spectrum, and 2-D HETCOR and HMBC spectra.

The assignments made using the HETCOR spectrum for aromatic carbons (C2",3",6",8",3); methyl, methylene and methine carbons; and the other carbons, such as quaternary carbons (not connected with any protons) were assigned from the HMBC spectrum. For example carbon C-1' was assigned on the basis of the cross peak between the resonance at δ 154.7 and the hydrogen atom peaks assigned to H3 and 2-CH₂N. These cross peaks are due to three-bond coupling.

Comparison of the ^{13}C chemical shifts of the 1,5-naphthyridine nucleus in compound **V.3g** (Figure V-3) with the 1,5-naphthyridine nucleus in the di-Mannich base **II.48a** (see Figure II-1) revealed that the signals for C7" and C4" in compound **V.3g** (δ 125.2 and 149.4) showed the biggest downfield shift from those in compound **II.48a** (δ 121.5 and 148.5); whereas at other positions the differences were slight (0.0-0.2 ppm).

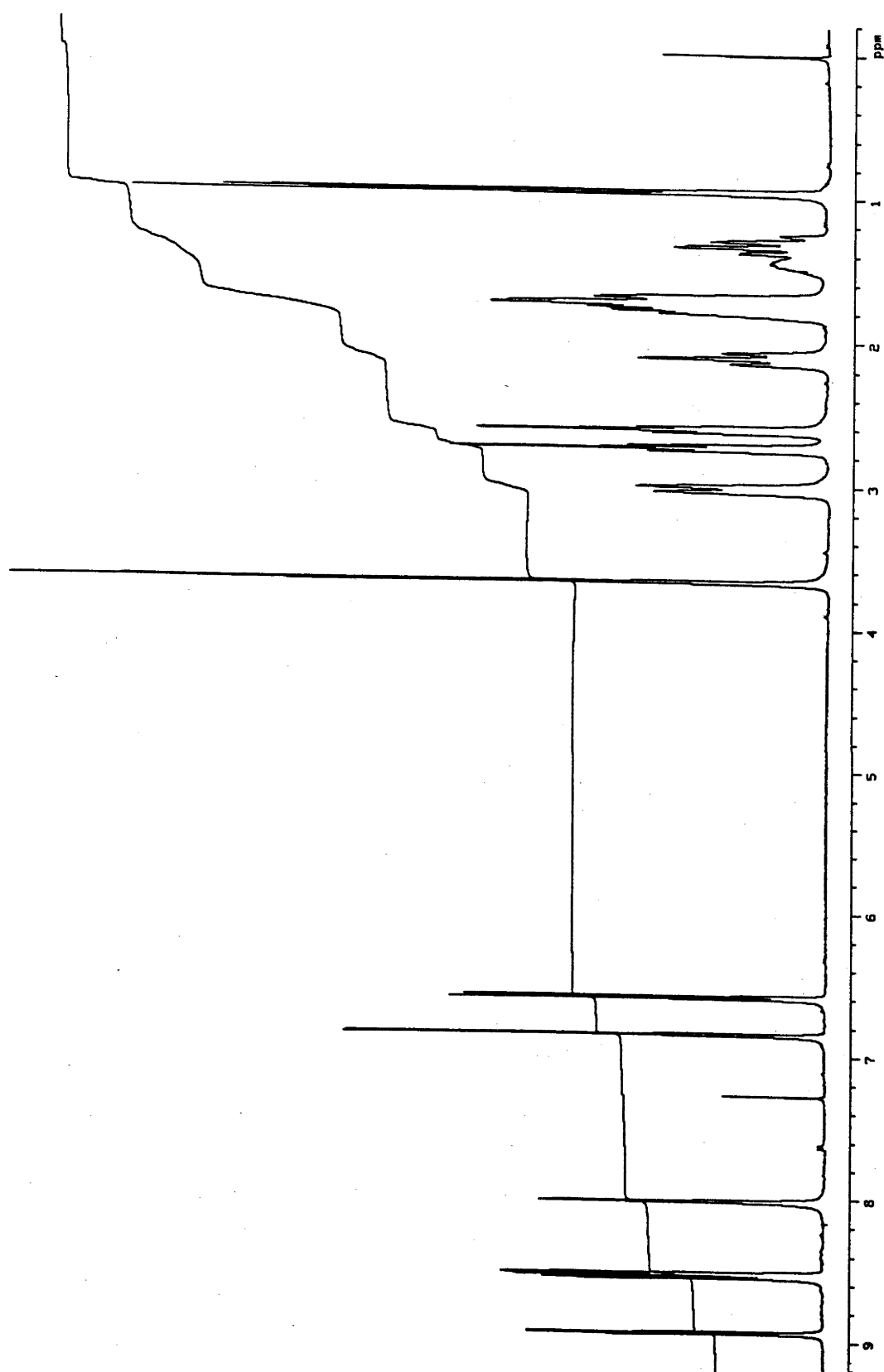


Figure V-1 ^1H n.m.r. spectrum of 2-(4'-methylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3g) in CDCl_3

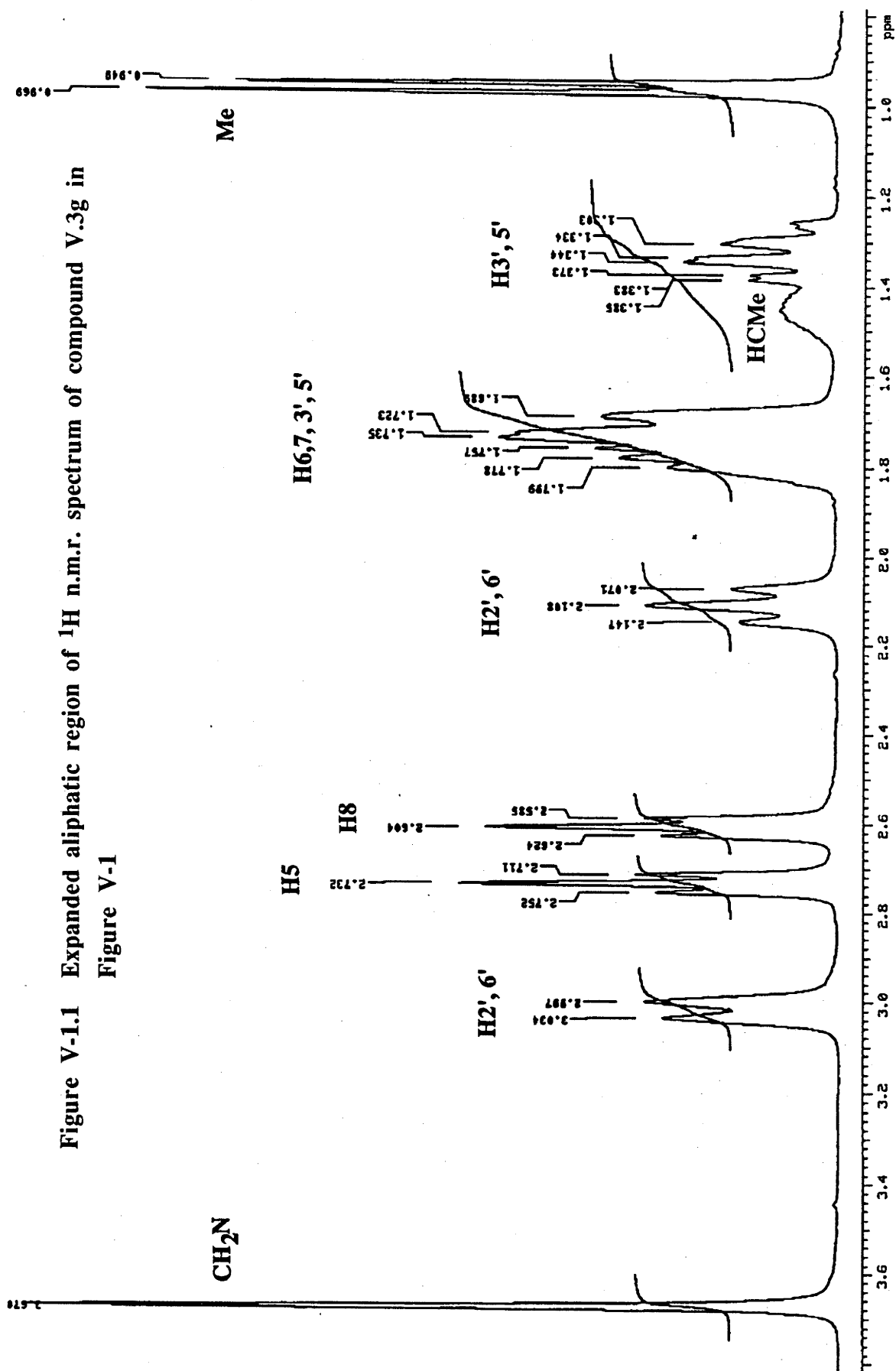


Figure V-1.2 Expanded aromatic region of ^1H n.m.r. spectrum of compound V.3g in Figure V-1

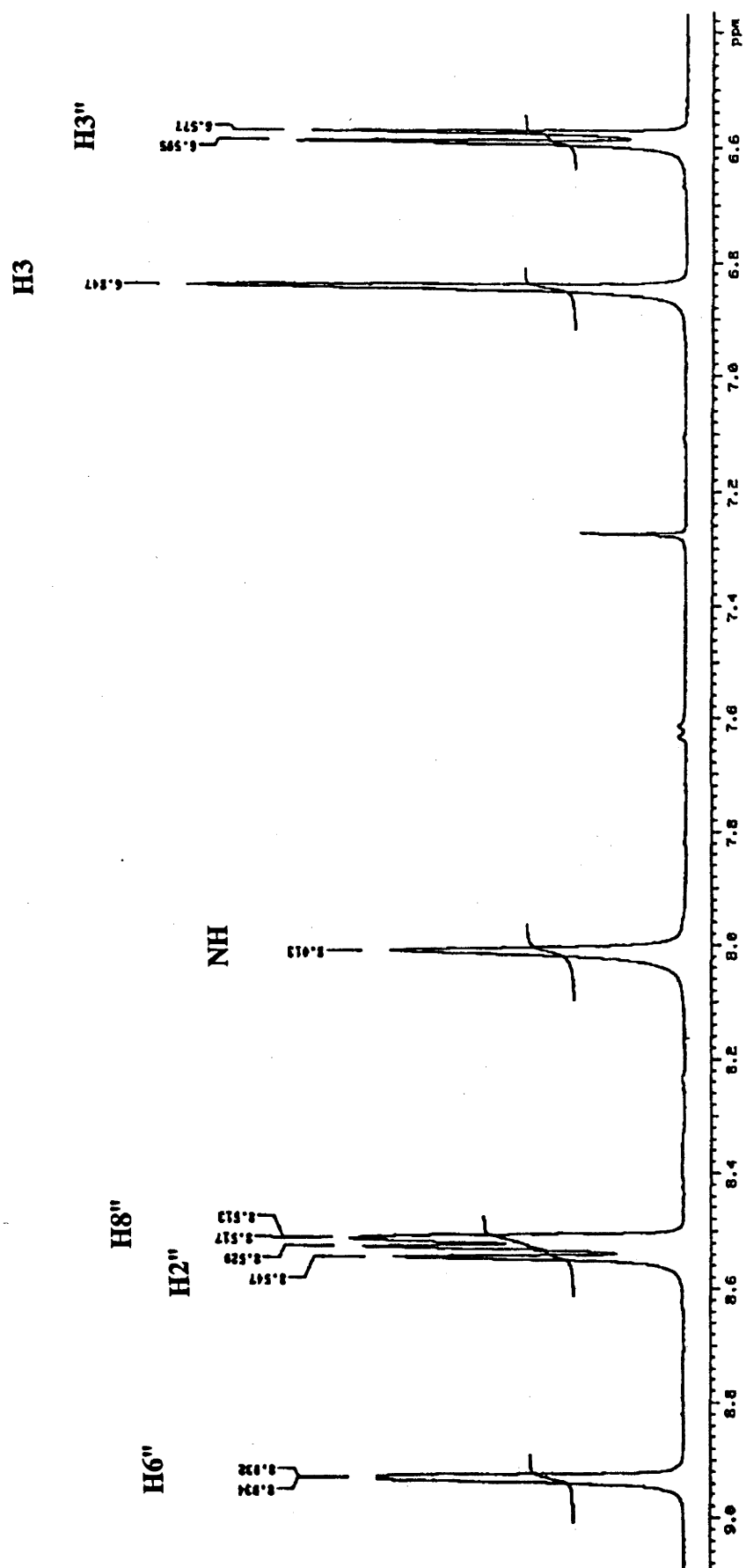


Figure V-2 2-D COSY spectrum of 2-(4'-methylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3g) in CDCl_3

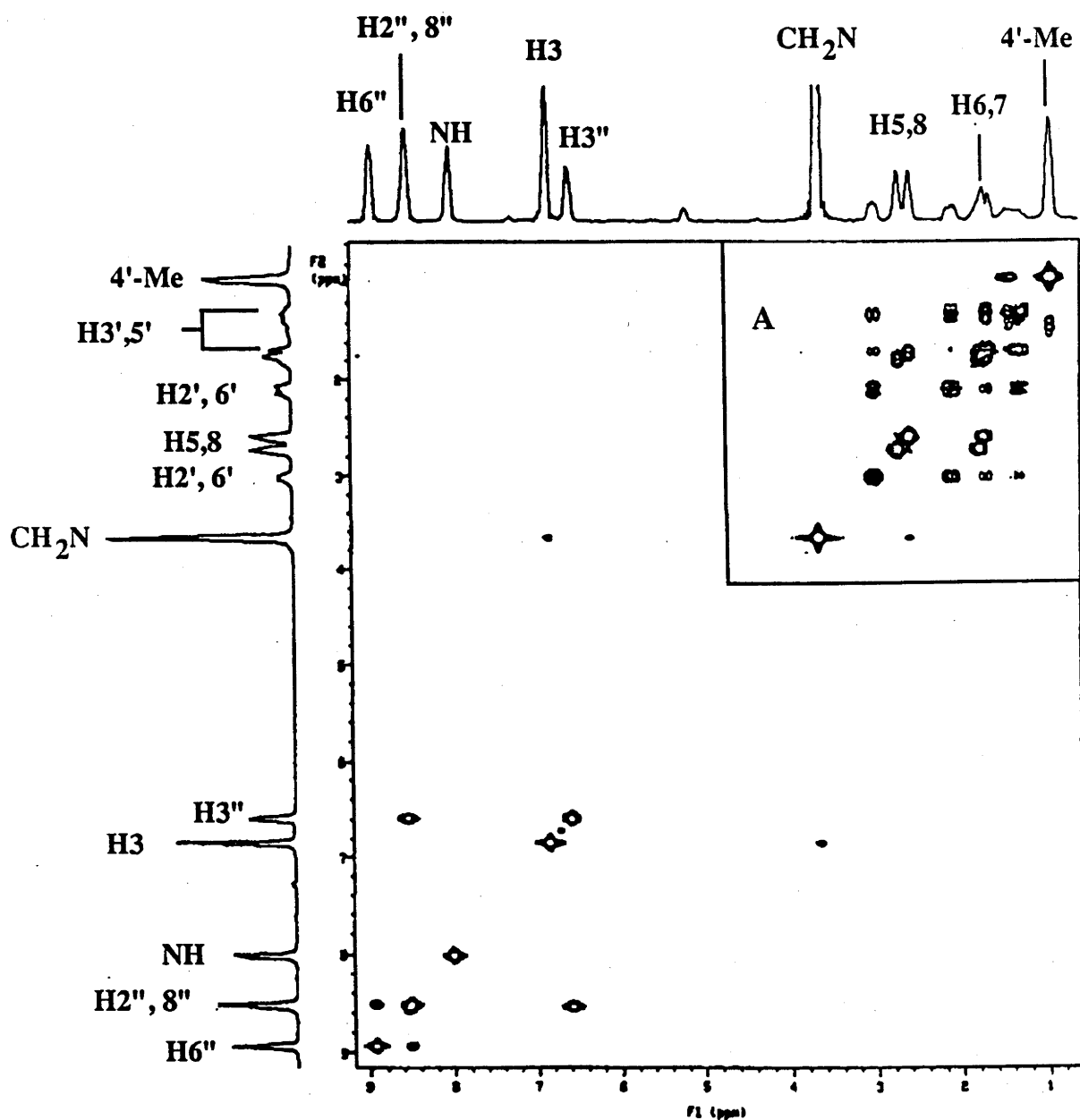
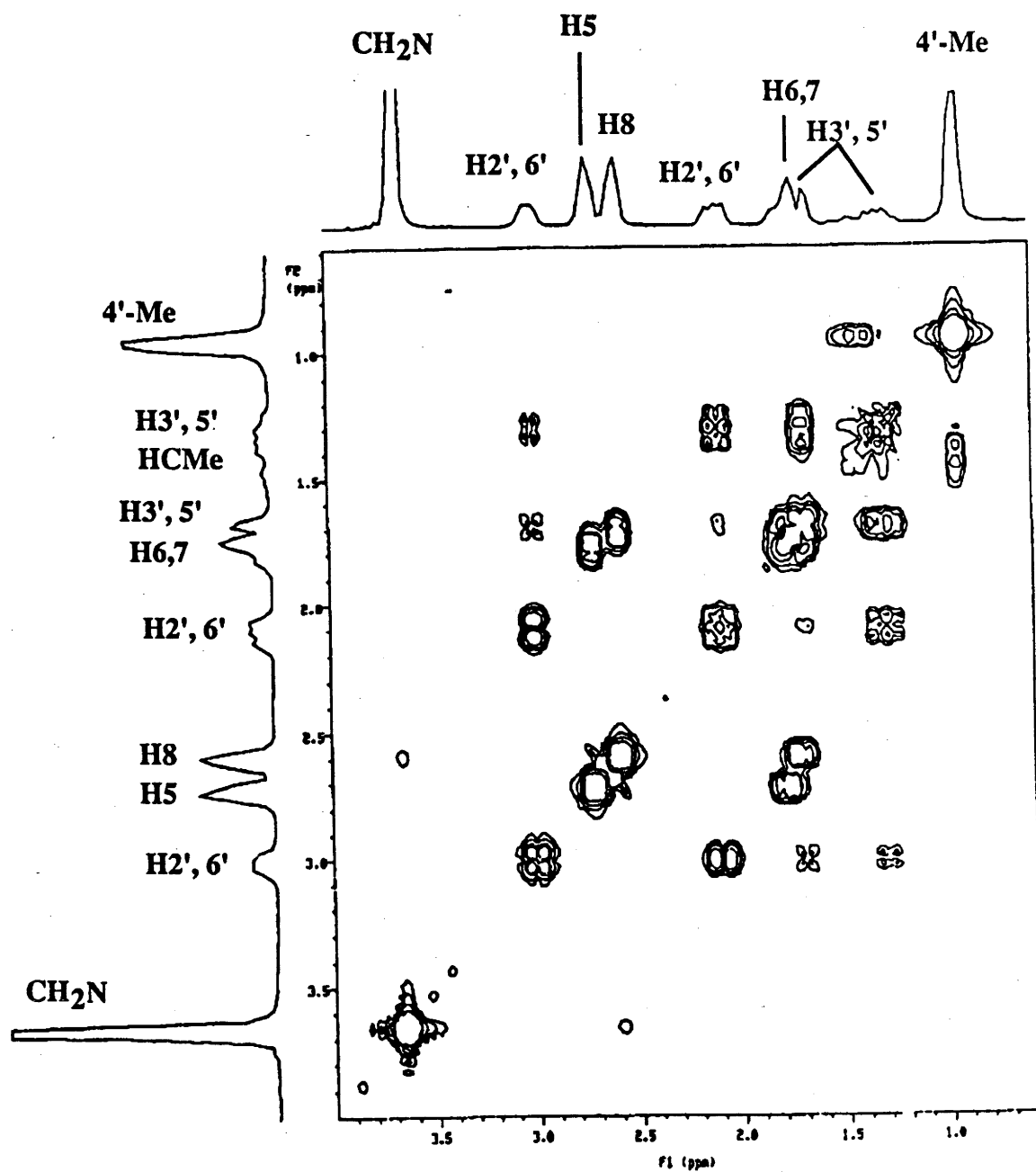


Figure V-2.1 Expansion of Figure V-2 at region A



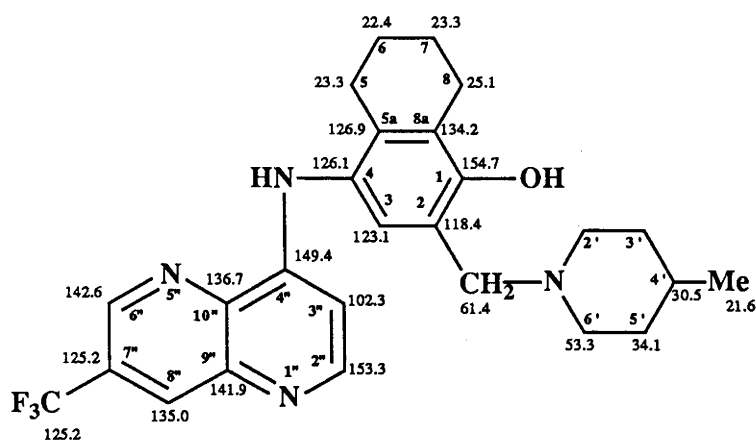


Figure V-3 The chemical shifts (ppm) of the carbon atoms in 2-(4-methylpiperidin-1-ylmethyl)-4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3g)

V-5 Experimental

N-(4-Hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (V.10)

This compound was prepared from 5,6,7,8-tetrahydronaphth-1-ol by nitrosation²¹⁸⁻²²⁰ to give 4-nitroso-5,6,7,8-tetrahydronaphth-1-ol [m.p. 157-159° (from aqueous ethanol) (lit.²¹⁸ 161-163° (dec.) (Found: C, 67.6; H, 6.3; N, 7.8. Calc. for C₁₀H₁₁NO₂: C, 67.8; H, 6.3; N, 7.9%). ¹H n.m.r. δ 1.71, complex, 2.51, complex, H_{5,6,7,8}; 6.41, d, J_{2,3} 10 Hz, H₂; 7.72, d, J_{2,3} 10 Hz, H₃] which was then reduced as follows.

4-Nitroso-5,6,7,8-tetrahydronaphth-1-ol (2.0 g) was dissolved in half-saturated ethanolic ammonia (200 ml) and shaken with hydrogen gas over Raney nickel until uptake ceased. The mixture was filtered through Celite and the filtrate evaporated to give 4-amino-5,6,7,8-tetrahydronaphth-1-ol (1.843 g), m.p. 145-146° (lit.²²¹ 144-146°). ¹H n.m.r. δ 1.81, complex, 2.50, complex, H_{5,6,7,8}; 6.43, d, 6.54, d, J_{2,3} 9 Hz, H_{2,3}.

This amino compound (1.950 g), acetic acid (59.0 ml) and acetic anhydride (12.0 ml) were heated at 40° for 5 h. The mixture was then evaporated to dryness under reduced pressure and the residue recrystallised from a mixture of acetone and light petroleum (b.p. 60-80°) to give the *N*-(4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (1.752 g), m.p. 192-194° (lit.²²¹ 188-189°) (Found, for a sample dried at 120°/0.2 mmHg for 2 h: C, 69.9; H, 7.6; N, 6.8. Calc. for C₁₂H₁₅NO₂: C, 70.2; H, 7.4; N, 6.8%). ¹H n.m.r. (CD₃OD) δ 1.72, complex, 2.58, complex, H_{5,6,7,8}; 2.08, s, Me; 6.55, d, 6.83, d, J 9 Hz, H_{2,3}.

N-(3-Diethylaminomethyl-4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)-acetamide (V.11a)

This compound was prepared from *N*-(4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)-acetamide, paraformaldehyde and diethylamine as described by Nabih *et al.*¹⁰⁴ It had m.p. 154-155° (from aqueous ethanol and aqueous acetone) (lit.¹⁰⁵ 156-157°). ¹H n.m.r. δ 1.11, t, J 7 Hz, MeCH₂; 1.80, complex, 2.59, complex, H_{5,6,7,8}; 2.15, s, MeCO; 2.63, q, J 7 Hz, MeCH₂; 3.72, s, CH₂N; 6.63, br s, 6.75, br s, NH, OH; 7.10, s, H₃.

***N*-(3-Dipropylaminomethyl-4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)-acetamide (V.11b)**

A mixture of *N*-(4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (0.603 g), paraformaldehyde (0.167 g), dipropylamine (0.76 ml) and ethanol (7.2 ml) was refluxed at *ca* 95° for 12 h. The solvent was evaporated under reduced pressure and the yellow oily residue was washed with water and then extracted into chloroform. The product was subjected to column chromatography in methylene chloride over alumina and then PTLC (alumina; chloroform) to give the *title compound* (0.648 g), m.p. 80-83° (Found C: 72.0; H, 9.8; N, 8.8. C₁₉H₃₀N₂O₂ requires C, 72.0; H, 9.5; N, 8.8%). ¹H n.m.r. δ 0.89, t, J 7 Hz, MeCH₂; 1.56, complex, MeCH₂CH₂; 1.76, complex, 2.56 complex, H_{5,6,7,8}; 2.14, s, MeCO; 2.48, t, J 7 Hz, MeCH₂CH₂; 3.70, s, CH₂N; 6.62, br s, 6.77; br s, NH, OH; 7.08, H₃.

***N*-(3-*t*-Butylaminomethyl-4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)-acetamide (V.11c)**

A mixture of paraformaldehyde (0.223 g) and *t*-butylamine (0.77 ml) in ethanol (6.0 ml) was refluxed to give a clear solution, chilled, *N*-(4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (0.504 g) added and the refluxing continued for 12 h. The solvent was evaporated and the residue washed with water to give a brown solid (0.643 g). It was recrystallised from aqueous acetone with charcoal filtration to give white crystals of the *title compound*, m.p. 190-191° (Found, for a sample dried at 20°/0.2 mmHg for 24 h: C, 70.3; H, 9.3; N, 9.3. C₁₇H₂₆N₂O₂ requires C, 70.3; H, 9.0; N, 9.6%). ¹H n.m.r. δ 1.19, s, Me₃C; 1.73, complex, 2.56, complex, H_{5,6,7,8}; 2.16, s, MeCO; 4.06, s, CH₂N; 6.66, br, 6.74, br, 2xNH; 7.18, br s, H₃.

***N*-[4-Hydroxy-3-(pyrrolidin-1'-ylmethyl)-5,6,7,8-tetrahydronaphth-1-yl]-acetamide (V.11d)**

This compound (73%) as white needles had m.p. 191-193° (from aqueous acetone) (Found: C, 70.4; H, 8.5 N, 9.7. C₁₇H₂₄N₂O₂ requires, C, 70.8; H, 8.4; N, 9.7%).

^1H n.m.r. δ 1.81, complex, 2.66, complex, $\text{H2}',3',4',5'$; 1.81, complex, 2.66, complex, $\text{H5},6,7,8$; 2.16, s, MeCO ; 3.78, s, CH_2N ; 6.64, br s, NH ; 7.11, s, H3 .

***N*-[4-Hydroxy-3-(piperidin-1'-ylmethyl)-5,6,7,8-tetrahydronaphth-1-yl]-acetamide (V.11e)**

This compound was prepared in an analogous manner by the method of Nabih *et al.*¹⁰⁴ It had m.p. 164-166° (lit.¹⁰⁵ 164-166°). ^1H n.m.r. δ 1.70, complex, 2.58, complex, $\text{H2}',3',4',5',6'$ and $\text{H5},6,7,8$; 2.16, s, Me ; 3.62, s, CH_2 ; 6.62, br s, 6.73, br s, NH , OH ; 7.09, s, H3 . I.r. ν_{max} 3285 (br) (NH st), 2950 (s) (CH aliph st), 1668 + 1648 (s) (C=O st), 1556 (s) (ar skeleton st), 1470 (s), 1232 (m), 879 (m), 790 (m) cm^{-1} .

The following compounds were prepared by similar procedures to those described or referred to above.

***N*-[4-Hydroxy-3-(3'-methylpiperidin-1'-ylmethyl)-5,6,7,8-tetrahydronaphth-1-yl]acetamide (V.11f)**

The title compound (72%) had m.p. 122-125° (after chromatography in methylene chloride over alumina, and triturated with cyclohexane) (Found: C, 72.8; H, 9.3; N, 8.5. $\text{C}_{19}\text{H}_{28}\text{N}_2\text{O}_2 \cdot 0.2\text{C}_6\text{H}_{12}$: C, 72.8; H, 9.2; N, 8.4%). ^1H n.m.r. δ 0.86, d, J 5.5 Hz, MeC ; 1.74, complex, 2.59, complex, 2.85, complex, $\text{H2}',3',4',5',6'$ and $\text{H5},6,7,8$; 2.12, s, MeCO ; 3.57, s, CH_2N ; 6.98, s, H3 ; 7.08, br s, NH .

***N*-[4-Hydroxy-3-(4'-methylpiperidin-1'-ylmethyl)-5,6,7,8-tetrahydronaphth-1-yl]acetamide (V.11g)**

The title compound was obtained by extraction with chloroform and it was purified by chromatography in chloroform over alumina. After triturated with light petroleum (b.p. 60-80°) it was obtained as white crystals (79%), m.p. 143-145° (Found: C, 72.0; H, 9.0; N, 8.8. $\text{C}_{19}\text{H}_{28}\text{N}_2\text{O}_2$ requires C, 72.1; H, 8.9; N, 8.8%). ^1H n.m.r. δ 0.93, d, MeC ; 1.37, complex, 1.76, complex, 2.61, complex, 2.98, complex, $\text{H2}',3',4',5',6'$ and $\text{H5},6,7,8$; 2.15, s, MeCO ; 3.64, s, CH_2N ; 6.62, br s, 6.72, br s, NH , OH ; 7.09, s, H3 .

***N*-[3-(4'-Benzylpiperidin-1'-ylmethyl)-4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl]acetamide (V.11h)**

This *compound* (73%) had m.p. 193-194° (after crystallisation from methylene chloride/acetone) (Found: C, 76.0; H, 8.9; N, 6.8. $C_{25}H_{32}N_2O_2$ requires C, 75.9; H, 8.9; N, 7.0%). 1H n.m.r. δ 1.76, complex, 2.58, complex, 2.95, complex, H2',3',4',5',6' and H5,6,7,8; 2.55, d, CH_2Ph ; 3.63, s, CH_2N ; 6.62, br s, 6.72, br s, NH, OH; 7.09, s, H3; 7.20, complex, Ph.

***N*-[3-(4'-Benzylpiperazin-1'-ylmethyl)-4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl]acetamide (V.11i)**

The *title compound* (70%) had m.p. 221-222° (from aqueous acetone) (Found: C, 73.4; H, 8.2; N, 10.3. $C_{24}H_{31}N_3O_2$ requires C, 73.3; H, 7.9; N, 10.7%). 1H n.m.r. δ 1.76, complex, 2.58, complex, H2',3',4',5',6' and H5,6,7,8; 2.16, s, MeCO; 3.54, s, CH_2Ph ; 3.66, s, CH_2N ; 6.67, br, NHCO; 7.08, s, H3; 7.31, s, Ph.

***4*-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-diethylaminomethyl-5,6,7,8-tetrahydronaphth-1-ol (V.2a)**

N-[3-Diethylaminomethyl-4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl]acetamide (0.063 g), ethanol (2.0 ml) and concentrated hydrochloric acid (0.28 ml) were refluxed for 10 h. The mixture was cooled, adjusted to pH 4.5 with aqueous sodium hydroxide, 3-bromo-8-chloro-1,5-naphthyridine (0.042 g) and a little ethanol added and the mixture refluxed for 10 h. It was then evaporated to dryness, the residue diluted with water, and some undissolved solid removed by filtration. The filtrate (pH 3) was adjusted with aqueous ammonia to pH 8.5-9 to give a yellow precipitate (0.045 g). It was recrystallised from aqueous ethanol to give the *title compound*, m.p. 167-169° (Found: C, 60.5; H, 6.0; N, 12.0. $C_{23}H_{27}BrN_4O$ requires C, 60.7; H, 6.0; N, 12.3%). 1H n.m.r. δ 1.15, t, J 7 Hz, Me; 1.75, complex, 2.67, complex, H5,6,7,8; 2.67, q, J 7 Hz, $MeCH_2$; 3.76, s, CH_2N ; 6.53, d, J_{2',3'} 5.5 Hz, H3'; 6.85, s, H3; 7.96, br s, NH; 8.43, d, J_{2',3'} 5.5 Hz, H2'; 8.48, d, J_{6',8'} 2 Hz, H8'; 8.76, d, J_{6',8'} 2 Hz, H6'.

4-(7'-Bromo-1';5'-naphthyridin-4'-ylamino)-2-(*t*-butylaminomethyl)-5,6,7,8-tetrahydronaphth-1-ol (V.2c)

A mixture of *N*-(3-*t*-butylaminomethyl-4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (0.097 g), concentrated hydrochloric acid (0.43 ml) and ethanol (3.2 ml) was refluxed for 5 h; cooled, adjusted to pH 3, 3-bromo-8-chloro-1,5-naphthyridine (0.092 g) added and then refluxed for a further 8 h. It was diluted with aqueous ethanol and adjusted to pH 9 to give a precipitate (0.14 g). This was purified by PTLC (alumina; methylene chloride) and reprecipitated from methylene chloride/light petroleum (b.p. 60-80°)/hexane to give the yellow *title compound* (0.066 g), m.p. 181-183° (Found: C, 60.7; H, 6.2; N, 12.2. $C_{23}H_{27}BrN_4O$ requires C, 60.7; H, 6.0; N, 12.3%). 1H n.m.r. δ 1.23, s, Me_3C ; 1.75, complex, 2.67, complex, $H_{5,6,7,8}$; 3.93, s, CH_2N ; 6.60, d, J 5.5 Hz, $H_{3'}$; 6.92, s, H_3 ; 7.92, br s, NH; 8.45, d, J 2 Hz, $H_{8'}$; 8.45, d, J 5.5 Hz, $H_{2'}$; 8.75, d, J 2 Hz, $H_{6'}$.

4-(7'-Bromo-1';5'-naphthyridin-4'-ylamino)-2-(pyrrolidin-1''-ylmethyl)-5,6,7,8-tetrahydronaphth-1-ol (V.2d)

N-[2-Hydroxy-3-(pyrrolidin-1''-ylmethyl)-5,6,7,8-tetrahydronaphth-1-yl]acetamide (0.044 g), ethanol (1.5 ml), and concentrated hydrochloric acid (0.2 ml; 37%) were refluxed for 5 h. The mixture was then adjusted to pH 4.7 with dilute sodium hydroxide. 3-Bromo-8-chloro-1,5-naphthyridine (0.037 g) and a little ethanol were added and the mixture refluxed for 5 h, and then evaporated under reduced pressure. The residue was dissolved in water, the solution filtered, and the filtrate adjusted to pH 8.5 with aqueous ammonia. The yellow precipitate was collected, washed with water, and recrystallised from aqueous ethanol to give the *title compound* (0.013 g), m.p. 167-169° (Found, for a sample dried at 110°/0.2 mmHg for 5 h: C, 61.3; H, 5.9; N, 12.2. $C_{23}H_{25}BrN_4O$ requires C, 60.9; H, 5.6; N, 12.4%). 1H n.m.r. δ 1.76, complex, 2.67, complex, $H_{2'',3'',4'',5''}$ and $H_{5,6,7,8}$; 3.86, s, NCH_2 ; 6.53, d, $J_{2',3'}$ 5.5 Hz, $H_{3'}$; 6.88, s, H_3 ; 7.97, br s, NH; 8.44, d, $J_{2',3'}$ 5.5 Hz, $H_{2'}$; 8.48, d, $J_{6',8'}$ 2.5 Hz, $H_{8'}$; 8.75, d, $J_{6',8'}$ 2.5 Hz, $H_{6'}$.

In a similar manner the following compounds were prepared.

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-(piperidin-1''-ylmethyl)-5,6,7,8-tetrahydronaphth-1-ol (V.2e)

The *title compound* (75%) had m.p. 180-182° (from acetone) (Found, for a sample dried at 110°/0.2 mmHg for 5 h: C, 61.9; H, 6.1; N, 12.0. $C_{24}H_{27}BrN_4O$ requires C, 61.7; H, 5.8; N, 12.0%). 1H n.m.r. δ 1.68, complex, 2.65, complex, $H_{2'',3'',4'',5'',6''}$ and $H_{5,6,7,8}$; 3.69, s, CH_2N ; 6.52, d, $J_{2',3'}$ 5.5 Hz, $H_{3'}$; 6.85, s, H_3 ; 8.02, br s, NH; 8.43, d, $J_{2',3'}$ 5.5 Hz, $H_{2'}$; 8.53, d, $J_{6',8'}$ 2 Hz, $H_{8'}$; 8.77, d, $J_{6',8'}$ 2 Hz, $H_{6'}$.

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-(3''-methylpiperidin-1''-ylmethyl)-5,6,7,8-tetrahydronaphth-1-ol (V.2f)

The *title compound* (70%) had m.p. 210-212° [after PTLC (alumina; methylene chloride) and recrystallisation from methylene chloride/hexane] (Found: C, 60.3; H, 6.3; N, 10.9. $C_{25}H_{29}BrN_4O \cdot H_2O$ requires C, 60.1; H, 6.3; N, 11.2%). 1H n.m.r. δ 0.91, d, MeC; 1.77, complex, 2.63, complex, 2.89, complex, $H_{2'',3'',4'',5'',6''}$ and $H_{5,6,7,8}$; 3.65, s, CH_2N ; 6.52, d, J 5.5 Hz, $H_{3'}$; 6.84, s, H_3 ; 7.89, br s, NH; 8.43, d, J 2 Hz, $H_{8'}$; 8.45, d, J 5.5 Hz, $H_{2'}$; 8.74, d, J 2 Hz, $H_{6'}$.

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-(4''-methylpiperidin-1''-ylmethyl)-5,6,7,8-tetrahydronaphth-1-ol (V.2g)

The *title compound* (47%) had m.p. 201-203° [after PTLC (alumina; 25% ethyl acetate in methylene chloride, then methylene chloride) and trituration with hexane] (Found: C, 62.1; H, 6.2; N, 11.5. $C_{25}H_{29}BrN_4O$ requires C, 62.4; H, 6.1; N, 11.6%). 1H n.m.r. δ 0.97, d, MeC; 1.40, complex, 1.77, complex, 2.13, complex, 2.66, complex, 3.03, complex, $H_{2'',3'',4'',5'',6''}$ and $H_{5,6,7,8}$; 3.67, s, CH_2N ; 6.51, d, J 5.5 Hz, $H_{3'}$; 6.84, s, H_3 ; 6.90, br s, NH; 8.43, d, J 2 Hz, $H_{8'}$; 8.45, d, J 5.5 Hz, $H_{2'}$; 8.75, d, J 2 Hz, $H_{6'}$.

2-(4'-Benzylpiperidin-1'-ylmethyl)-4-(7''-bromo-1'',5''-naphthyridin-4''-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.2h)

This *compound* (51%) had m.p. ca 120° (with prior softening) [after PTLC (alumina; methylene chloride)] (Found: C, 66.9; H, 6.2; N, 9.8. $C_{31}H_{33}BrN_4O$ requires C, 66.8; H, 6.0; N, 10.0%). 1H n.m.r. δ 1.76, complex, 2.09, complex, 2.63, complex,

3.05, complex, H2',3',4',5',6' and H5,6,7,8; 2.58, d, J 5 Hz, CH₂Ph; 3.67, s, CH₂N; 6.50, d, J 5.5 Hz, H3"; 6.84, s, H3; 7.22, complex, Ph; 7.92, br s, NH; 8.43, d, J 5.5 Hz, H2"; 8.45, d, J 2 Hz, H8"; 8.75, d, J 2 Hz, H6".

2-(4'-Benzylpiperazin-1'-ylmethyl)-4-(7''-bromo-1'',5''-naphthyridin-4''-yl-amino)-5,6,7,8-tetrahydronaphth-1-ol (V.2i)

The *title compound* (65%) had m.p. 141-143° [after PTLC (alumina; 30% ethyl acetate in hexane) and recrystallisation from methylene chloride/hexane)] (Found: C, 64.5; H, 6.0; N, 12.5. C₃₀H₃₂BrN₅O requires C, 64.5; H, 5.8; N, 12.4%). ¹H n.m.r. δ 1.76, complex, 2.60, complex, H2',3',5',6' and H5,6,7,8; 3.54, s, CH₂Ph; 3.68, s, CH₂N; 6.49, d, J 5.5 Hz, H3"; 6.85, s, H3; 7.30, complex, Ph; 7.88, br s, NH; 8.43, d, J 2 Hz, H8"; 8.43, d, J 5.5 Hz, H2"; 8.73, d, J 2 Hz, H6".

2-(4'-Methylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3g)

N-[2-Hydroxy-3-(4'-methylpiperidin-1'-ylmethyl)-5,6,7,8-tetrahydronaphth-1-yl] acetamide (0.1 g) with concentrated hydrochloric acid (0.4 ml) in ethanol (3.2 ml) was refluxed for 6 h, and evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of methanol (1.8 ml) and water (0.8 ml) and 2.5 N sodium hydroxide added until a test sample had pH 2. 4-Chloro-7-trifluoromethyl-1,5-naphthyridine (0.074 g) was added and the mixture was refluxed for 10 h. After cooling a little white solid was removed and the filtrate adjusted to pH *ca* 9 and the yellow product extracted into chloroform to give an oil. This was subjected to PTLC (alumina; 25% ethyl acetate in hexane) and the product (0.092 g) triturated with hexane to give the *title compound*, m.p. 192-194° (Found: C, 66.7; H, 6.5; N, 11.9. C₂₆H₂₉F₃N₄O requires C, 66.4; H, 6.2; N, 11.9%). ¹H n.m.r. δ 0.97, d, MeC; 1.34, complex, 1.77, complex, 2.13, complex, 2.68, complex, 3.02, complex, H2',3',4',5',6' and H5,6,7,8; 3.67, s, CH₂N; 6.59, d, J 5.5 Hz, H3"; 6.85, s, H3; 7.98, br s, NH; 8.52, s, H8"; 8.55, d, J 5.5 Hz, H2"; 8.93, J 2 Hz, H6".

In a similar manner to the preparations described above were prepared the following compounds.

2-Diethylamino-4-(7'-trifluoromethyl-1',5'-naphthyridin-4'-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3a)

N-(3-Diethylaminomethyl-4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide (0.080 g), ethanol (2.4 ml) and concentrated hydrochloric acid (0.32 ml) were refluxed for 10 h, and then evaporated under reduced pressure. The residue was dissolved in a mixture of methanol (1.5 ml) and water (0.7 ml), adjusted with 2.5 N sodium hydroxide to pH 2, 4-chloro-7-trifluoromethyl-1,5-naphthyridine (0.064 g) added and the mixture refluxed for 8 h. After cooling a white solid (0.015 g) was filtered and rejected and the filtrate adjusted to pH 8.5 to give a yellow solid (0.079 g). It was subjected to PTLC (alumina; 30% ethyl acetate in hexane) to give the *title compound*, m.p. 147-148° (Found: C, 64.7; H, 6.3; N, 12.4. C₂₄H₂₇F₃N₄O requires C, 64.8; H, 6.1; N, 12.6%). ¹H n.m.r. δ 1.16, t, J 7 Hz, MeCH₂; 1.76, complex, 2.65, complex, H_{5,6,7,8}; 2.69, q, J 7 Hz, MeCH₂; 3.78, s, CH₂N; 6.60, d, J 5.5 Hz, H_{3'}; 6.87, s, H₃; 8.01, br s, NH; 8.51, s, H_{8'}; 8.54, d, J 5.5 Hz, H_{2'}; 8.95, d, J 2 Hz, H_{6'}.

2-Dipropylaminomethyl-4-(7'-trifluoromethyl-1',5'-naphthyridin-4'-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3b)

This *compound* (46%) had m.p. 115-117° [after PTLC (alumina; 30% ethyl acetate in hexane)] (Found: C, 66.3; H, 7.0; N, 11.6. C₂₆H₃₁F₃N₄O requires C, 66.1; H, 6.6; N, 11.9%). ¹H n.m.r. δ 0.93, t, J 7 Hz, Me; 1.65, complex, MeCH₂CH₂; 1.75, complex, 2.55, complex, H_{5,6,7,8}; 2.53, t, J 7 Hz, MeCH₂CH₂; 3.75, s, CH₂N; 6.60, d, J 5.5 Hz, H_{3'}; 6.86, s, H₃; 8.0, s, NH; 8.52, s, H_{8'}; 8.55, d, J 5.5 Hz, H_{2'}; 8.94, d, J 2 Hz, H_{6'}. MS m/z 472 (1.3%) (M⁺), 371 (6.8%).

2-(*t*-Butylaminomethyl)-4-(7'-trifluoromethyl-1',5'-naphthyridin-4'-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3c)

The *title compound* (53%) had m.p. 178-180° [after PTLC (alumina; 30% ethyl acetate in cyclohexane, and crystallisation from methylene chloride/hexane)] (Found: C,

64.9; H, 6.2; N, 12.5. $C_{24}H_{27}F_3N_4O$ requires C, 64.9; H, 6.1; N, 12.6%). 1H n.m.r. δ 1.22, s, Me_3C ; 1.75, complex, 2.67, complex, $H_{5,6,7,8}$; 3.93, s, CH_2N ; 6.64, d, J 5.5 Hz, $H_{3'}$; 6.91, s, H_3 ; 7.97, br s, NH; 8.52, s, $H_{8'}$; 8.55, d, J 5.5 Hz, $H_{2'}$; 8.85, d, J 2 Hz, $H_{6'}$. MS m/z 444 (8.9%) (M^+), 371 (48.7%).

2-(Pyrrolidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-yl-amino)-5,6,7,8-tetrahydronaphth-1-ol (V.3d)

This compound (56%) had m.p. 154-157° [after PTLC (alumina; 30% ethyl acetate in cyclohexane)] (Found, for a sample dried at 20°/0.2 mmHg for 3 days: C, 65.1; H, 6.0; N, 12.4. $C_{24}H_{25}F_3N_4O$ requires C, 65.1; H, 5.7; N, 12.7%). 1H n.m.r. δ 1.81, complex, 2.66, complex, $H_{2',3',4',5'}$ and $H_{5,6,7,8}$; 3.83, s, CH_2N ; 6.59, d, J 5.5 Hz, $H_{3''}$; 6.87, s, H_3 ; 7.99, br s, NH; 8.53, s, $H_{8''}$; 8.55, d, J 5.5 Hz, $H_{2''}$; 8.94, d, J 2 Hz, $H_{6''}$.

2-(Piperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-yl-amino)-5,6,7,8-tetrahydronaphth-1-ol (V.3e)

The title compound (50%) had m.p. 169-171° [after PTLC (alumina; 20% ethyl acetate in hexane)] (Found: C, 65.1; H, 6.3; N, 11.8. $C_{25}H_{27}F_3N_4O \cdot 0.3H_2O$ requires C, 65.0; H, 6.0; N, 12.1%). 1H n.m.r. δ 1.67, complex, 2.60, complex, $H_{2',3',4',5',6'}$ and $H_{5,6,7,8}$; 3.66, s, CH_2N ; 6.58, d, J 5.5 Hz, $H_{3''}$; 6.84, s, H_3 ; 8.00, br s, NH; 8.50, s, $H_{8''}$; 8.53, d, J 5.5 Hz, $H_{2''}$; 8.93, d, J 2 Hz, $H_{6''}$. I.r. ν_{max} 3320 (br) (NH st), 2960+2945 (m) (CH_2 aliph. st), 1600 (m), 1576 (m), 1540 (s), 1375 (ms), 1164 (s), 1140 (s) (CF_3 st), 744 (w), 690 (w) cm^{-1} .

2-(3'-Methylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3f)

This compound (15%) had m.p. 154-156° [after PTLC (alumina; 26% ethyl acetate in hexane then alumina; methylene chloride)] (Found: C, 65.1; H, 6.3; N, 11.5. $C_{26}H_{29}F_3N_4O$ requires C, 65.1; H, 6.3; N, 11.7%). 1H n.m.r. δ 0.92, d, J 5 Hz, MeC ; 1.77, complex, 2.68, complex, 2.90, complex, $H_{2',3',4',5',6'}$ and $H_{5,6,7,8}$; 3.67, s, CH_2N ; 6.60, d, J 5.5 Hz, $H_{3''}$; 6.86, s, H_3 ; 7.99, br s, NH; 8.52, br s, $H_{8''}$; 8.56, d, J 5.5 Hz, $H_{2''}$; 8.95, d, J 2 Hz, $H_{6''}$.

2-(4'-Benzylpiperidin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3h)

The *title compound* (61%) had m.p. 166-169° [after PTLC (alumina; 26% ethyl acetate in hexane)] (Found: C, 70.2; H, 6.4; N, 9.9. $C_{32}H_{33}F_3N_4O$ requires C, 70.3; H, 6.1; N, 10.2%). 1H n.m.r. δ 1.76, complex, 2.67, complex, 3.06, complex, H2',3',4', 5',6' and H5,6,7,8; 2.59, d, CH_2Ph ; 3.69, s, CH_2N ; 6.57, d, J 5.5 Hz, H3''; 6.85, s, H3; 7.22, complex, Ph; 8.00, br s, NH; 8.53, d, J 5.5 Hz, H2''; 8.56, s, H8''; 8.94, d, J 2 Hz, H6''.

2-(4'-Benzylpiperazin-1'-ylmethyl)-4-(7''-trifluoromethyl-1'',5''-naphthyridin-4''-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (V.3i)

The *title compound* (61%) had m.p. 193-195° [after PTLC (alumina; chloroform) and trituration with hexane] (Found: C, 67.6; H, 5.9; N, 12.4. $C_{31}H_{32}F_3N_5O \cdot 0.3H_2O$ requires C, 67.6; H, 5.9; N, 12.4%). 1H n.m.r. δ 1.75, complex, 2.60, complex, H2',3',5',6' and H5,6,7,8; 3.56, s, CH_2Ph ; 3.70, s, $ArCH_2N$; 6.57, d, J 5.5 Hz, H3''; 6.86, s, H3; 7.25, complex, Ph; 7.97, br s, NH; 8.50, br s, H8''; 8.53, d, J 5.5 Hz, H2''; 8.92, br s, H6''.

CHAPTER VI

Chapter VI Di-Mannich Bases of 4-[7-Chloro (and 7-Trifluoromethyl)-quinolin-4-ylamino]-3-methylphenols and 4-(7-Bromo-1,5-naphthyridin-4-ylamino)-3-methylphenols

VI-1 Introduction

In yet unpublished work, Barlin and coworkers have observed that the 3- and 5-methyl derivatives of 4,6-bis(pyrrolidin-1-ylmethyl)-2-(7-trifluoromethylquinolin-4-ylamino)phenol exhibit higher solubility and antimalarial activity against *P. falciparum* *in vitro* than their demethyl analogues.

Accordingly, it was decided to examine the 3-methyl derivatives of some 4-aminophenols, such as the di-Mannich bases derived from 4-(7-trifluoromethylquinolin-4-ylamino)phenol and 4-(7-bromo-1,5-naphthyridin-4-ylamino)phenol (parent compounds found previously to possess high antimalarial activity⁹⁸) together with di-Mannich bases derived from 4-(7-chloroquinolin-4-ylamino)-3-methylphenol [cf. Amodiaquine (I.9a)].

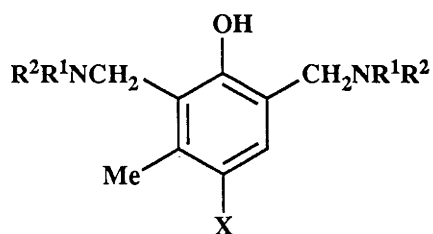
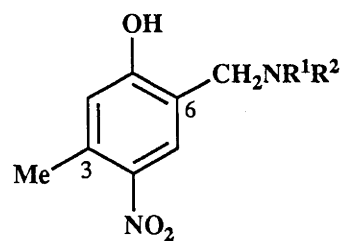
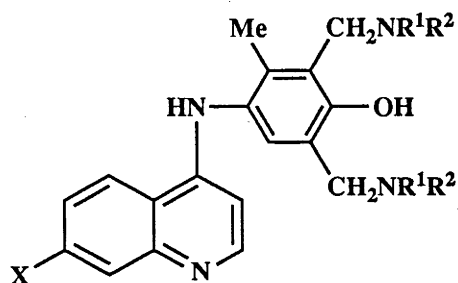
In the first part of this chapter, the syntheses of di-Mannich bases of 3-methyl-4-nitrophenol and di-Mannich bases of some 4-[7-substituted quinolin (and 1,5-naphthyridin)-4-ylamino]-3-methylphenols are reported. Physical properties (such as the nuclear magnetic resonances and mass spectra) are then discussed. Test results for antimalarial activity against *P. falciparum* *in vitro*, using both the hypoxanthine uptake method²²² and a visual method²²³ are discussed in Chapter VIII.

VI-2 Syntheses of Di-Mannich Bases of 3-Methyl-4-nitrophenol

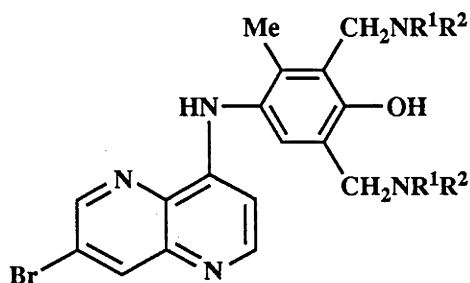
The nitro compounds required in this work, were prepared by standard procedures described by Barlin and coworkers to make di-Mannich base derivatives of 4-aminophenol¹⁷² and 2-aminophenol.²²⁴

3-Methyl-4-nitrophenol with four to ten equivalents of paraformaldehyde and the appropriate amines, dimethylamine, diethylamine, *t*-butylamine, pyrrolidine and piperidine

in refluxing ethanol for an extended period (*ca* 65 h) gave di-Mannich base (VI.1) of 3-methyl-4-nitrophenol. These di-Mannich compounds were readily purified by column and

VI.1, X=NO₂VI.3, X=NH₂VI.2, NR¹R²=pyrrolidin-1-yl

VI.4, X=Cl

VI.5, X=CF₃

VI.6

VI.1, VI.3, VI.4, VI.5 and VI.6

	NR ¹ R ²
a	NMe ₂
b	NEt ₂
c	NHBu ^t
d	pyrrolidin-1-yl
e	piperidin-1-yl

then preparative thin layer chromatography (PTLC) over alumina. Interestingly, when a lower proportion of paraformaldehyde and pyrrolidineP (*ca* 3 equivalents) was used and the

reflux was conducted for 19 h only, more complex mixtures including mono-Mannich products such as compound **VI.2** were obtained. Obviously under these conditions which favour mono-Mannich base formation, steric hindrance by the 3-methyl group in 3-methyl-4-nitrophenol hinders substitution at position 2 and the mono-Mannich side chain is inserted at position 6.

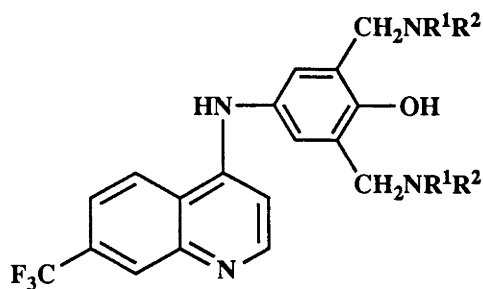
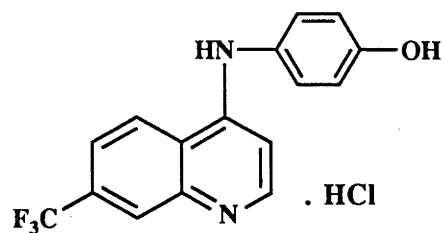
The separation of mixtures of mono- and di-Mannich bases such as compounds **VI.2** and **VI.1** could not be achieved readily on alumina because they possessed similar R_f values. However separation was readily effected by PTLC over silica gel.

VI-3 Syntheses of Di-Mannich Bases of 4-[7-Chloro (and 7-Trifluoromethyl)quinolin-4-ylamino]-3-methylphenol and 4-(7-Bromo-1,5-naphthyridin-4-ylamino)-3-methylphenol

The nitro compounds (**VI.1**) were subjected to catalytic reduction with hydrogen over Raney nickel in ethanolic ammonia at atmospheric pressure and room temperature to afford the corresponding amines (**VI.3**). These were then condensed with the appropriate 4-halogenoheterocycles, namely 4,7-dichloroquinoline, 4-chloro-7-trifluoromethylquinoline and 3-bromo-8-chloro-1,5-naphthyridine,¹¹⁰ in aqueous methanol containing a few drops of concentrated hydrochloric acid (the mixture had pH *ca* 4.7) at reflux for 14-19 h. The product was precipitated by addition of dilute ammonium hydroxide and purified by PTLC. In this way di-Mannich bases of 4-[7-chloro (and 7-trifluoromethyl)quinolin-4-ylamino]-3-methylphenol (**VI.4** and **VI.5**) and 4-(7-bromo-1,5-naphthyridin-4-ylamino)-3-methylphenol (**VI.6**) were prepared.

Di-Mannich bases of the demethyl analogues, 2,6-bis(dipentylaminomethyl)-4-(7-trifluoromethylquinolin-4-ylamino)phenol (**VI.7d**) and 2,6-bis(*N*-butyl-*N*-methylaninomethyl)-4-(7-trifluoromethylquinolin-4-ylamino)phenol (**VI.7e**) were also synthesised for comparative studies with quinazolines.

The testing of these compounds for antimalarial activity is reported in Chapter VIII.

VI.7d, $\text{NR}^1\text{R}^2 = \text{NC}_5\text{H}_{12}$ VI.7e, $\text{NR}^1\text{R}^2 = \text{NBuMe}$ 

VI.8

VI-4 Physical Properties

VI-4.1 ^1H Nuclear magnetic resonance spectra

The structure of the mono-Mannich compound (VI.2) was established from its ^1H n.m.r. It showed singlets for H2 and H5 at δ 6.67 and 7.84, respectively, that due to H5 adjacent to the nitro group was downfield; and the methylene protons at C-6 appeared at δ 3.87.

The corresponding di-Mannich compound VI.1d gave signals for methylene protons at C-2 and C-6 at δ 3.73 and 3.88, respectively. The methylene protons in 4-nitro-2,6-bis(pyrrolidin-1-ylmethyl)phenol have been shown to occur at δ 3.80.¹⁷²

The ^1H n.m.r. spectra of compounds VI.4, VI.5 and VI.6 are given in the Experimental section. The assignments in the spectrum of compound VI.5e were checked by running 2-D COSY²²⁵ (see Appendix 1, Figure A3), in which coupling between the 3-methyl protons and the methylene protons at C-2 was observed.

VI-4.2 ^{13}C Nuclear magnetic resonance spectra

The ^{13}C n.m.r. spectral data for 2,6-bis(piperidin-1-ylmethyl)-4-(7-trifluoromethyl-quinolin-4-ylamino)-3-methylphenol (VI.5e) and quinoline (for comparison) are shown in Figures VI-1 and VI-2, respectively.

The assignments of the ^{13}C chemical shifts were carried out by running a decoupled ^{13}C n.m.r. spectrum, and 2-D HETCOR and HMBC spectra. The chemical shifts of carbon

atoms connecting with one or more protons were assigned from the HETCOR spectrum, other carbons such as quaternary carbons (not-connected with any protons) were assigned from the HMBC spectrum.

Comparison of the ^{13}C chemical shifts of the quinoline nucleus in compound **VI.5e** with those of quinoline itself revealed that C4" in **VI.5e** was downfield of that in quinoline by 13.9 ppm, but C3" was upfield by 19.5 ppm; presumably due to the 4-(substituted amino) group. All carbon atoms in the benzene ring of quinoline were downfield of the corresponding atoms in compound **VI.5e** by 1.3-8.5 ppm. The carbon of the trifluoromethyl group (with the strong electron-withdrawing fluoro substituents) appeared as a quartet at 130.8 ppm, J 32 Hz. [compared with the carbon of the 3-methyl (phenolic) group at 13.7 ppm].

In contrast with the ^1H n.m.r. spectrum of compound **VI.5e** the signal in the ^{13}C spectrum due to the carbon of the 2- CH_2 group (attached to the phenol) was downfield of that due to the 6- CH_2 group.

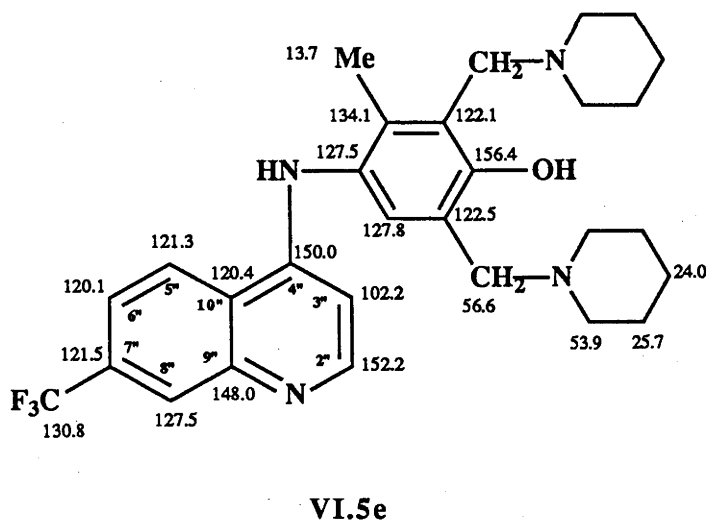


Figure VI-1 The chemical shifts (ppm) of the carbon atoms in 3-methyl-2,6-bis(piperidin-1-ylmethyl)-4-(7-trifluoromethylquinolin-4-ylamino)phenol (**VI.5e**)

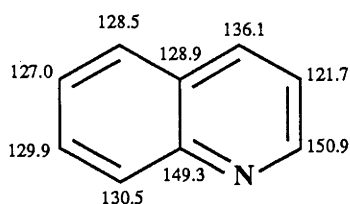


Figure VI-2 The chemical shifts (ppm) of the carbon atoms in quinoline

VI-4.3 Ionization Constants

The basic ionization constant (pK_a) of 4-(7-trifluoromethylquinolin-4-ylamino)-phenol^a (VI.8) (determined spectroscopically by methods described in Chapter II-6.2) was found to be 7.60 ± 0.05 .

Ionization constants have been published for 4-aminoquinoline (pK_a 9.17),^{201,226} 4-amino-7-chloroquinoline (pK_a 8.23)²²⁷ and 4-aminophenol (pK_a 5.5 and 10.3).²²⁸ The pK_a of compound VI.8 which is a weaker base by 0.63 units compared to 4-amino-7-chloroquinoline, obviously reflects the effects of the greater electron-withdrawing power of the trifluoromethyl group compared to the chloro substituent and the electronic effect of the *N*-(4-hydroxyphenyl) substituent in compound VI.8.

VI-4.4 Ultraviolet and mass spectra

Some ultraviolet spectra of compounds VI.5a, VI.6a and VI.8 are recorded in the Experimental section. At pH 8.8, compound VI.8 (pK_a 7.6) existed as a neutral molecule, and had a long-wavelength absorption maximum at 342 nm ($\log \epsilon$ 3.98); which in the cation, at pH 4.47, was at 348 nm ($\log \epsilon$ 4.14). This bathochromic shift on protonation at the ring nitrogen atom is consistent with similar effects in 2-, 3- and 4-aminopyridines.²²⁹

^a Kindly prepared by S.J. Ireland

The ultraviolet spectrum of compound **VI.6a** at pH 8.6 (it was constant in the range pH 8.5 to 9.5) was probably that of the mono-cation involving protonation of a Mannich side chain (the pK_a values of dimethylamine and triethylamine at 20° are 10.9 and 9.9, respectively).²³⁰

The mass spectra of compound **VI.2** showed high intensity peaks due to the molecular ion (at m/z 236) and also M^++1 . The major fragmentations observed with this compound involved the loss of pyrrolidinyl group and then loss of the nitro group to give fragments of m/z 166 and 120.

In compounds **VI.5** (a, b and e) and **VI.6** (d and e), the major fragmentations involved the cleavage at the C-N benzylic bonds (loss of dimethylamine and diethylamine in compounds **VI.5a** and **VI.5b** gave m/z 387 and 415, respectively), and the loss of the CHO fragment (such as compound **VI.5b**; m/z 386).

The bromo compounds (**VI.6d** and **VI.6e**) showed two molecular ion peaks in the ratio 1:1 due to the two major isotopes of bromine.

VI-5 Experimental

VI-5.1 Synthetic work

3-Methyl-4-nitro-2,6-bis(piperidin-1'-ylmethyl)phenol (VI.1e)

A mixture of paraformaldehyde (1.569 g), piperidine (5.16 ml) and ethanol (10.0 ml) was heated until all dissolved. After cooling, 3-methyl-4-nitrophenol (2.01 g) was added and the mixture refluxed at 90-95° for 65 h. The solvent was evaporated under reduced pressure and the residue diluted with water and extracted with methylene chloride. The extract was washed with 10% aqueous sodium chloride, dried (Na₂SO₄) and evaporated to leave an orange liquid. The product was purified by chromatography in methylene chloride over a column of alumina (25cm x 4cm) to give the oily *title compound* (4.4 g) which was purified further for analyses by PTLC (alumina; methylene chloride, developed twice) (Found C: 65.5; H, 8.5; N, 11.8. C₁₉H₂₉N₃O₃ requires C, 65.7; H, 8.4; N, 12.1%). ¹H n.m.r. δ 1.52, complex, 2.45, complex, H_{2'},3',4',5',6'; 2.45, s, Me; 3.56, s, 2-CH₂; 3.65, s, 6-CH₂; 7.73, s, H₅.

In a similar manner were prepared the following compounds.

2,6-Bis(dimethylaminomethyl)-3-methyl-4-nitrophenol (VI.1a) (75%) [after chromatography over a column of alumina (20cm x 6cm) in methylene chloride and elution with methylene chloride-methanol, and PTLC (alumina; 3% methanol in chloroform)] (Found C: 58.1; H, 8.1; N, 15.5. C₁₃H₂₁N₃O₃ requires C, 58.4; H, 7.9; N, 15.7%). ¹H n.m.r. δ 2.33, s, MeN; 2.48, s, MeC; 3.55, s, 2-CH₂; 3.66, s, 6-CH₂; 7.73, s, H₅.

2,6-Bis(diethylaminomethyl)-3-methyl-4-nitrophenol (VI.1b) (90%) [after chromatography over a column of alumina in methylene chloride and then 2% methanol in methylene chloride followed by PTLC (alumina; chloroform)] (Found C: 63.4; H, 9.2; N, 13.3. C₁₇H₂₉N₃O₃ requires C, 63.1; H, 9.0; N, 13.0%). ¹H n.m.r. δ 1.06, t, 1.08, t, J 7 Hz, MeCH₂; 2.46, s, 3-Me; 2.60, q, J 7 Hz, MeCH₂; 3.64, s, 2-CH₂; 3.77, s, 6-CH₂; 7.80, s, H₅. MS m/z 323 (0.3%) (M⁺), 221 (24.4%).

2,6-Bis(*t*-butylaminomethyl)-3-methyl-4-nitrophenol (VI.1c) (70%) [after the crude product was extracted (with hexane) and chromatographed in chloroform over a column of alumina, and PTLC (alumina; chloroform, developed twice)] (Found C: 60.2; H, 7.0; N, 13.7. $C_{25}H_{34}BrN_5O$ requires C, 60.0; H, 6.9; N, 14.0%). 1H n.m.r. δ 1.20, s, Me_3C ; 2.37, s, 3-Me; 3.67, s, 2- CH_2 ; 4.05, s, 6- CH_2 ; 7.86, s, H5.

3-Methyl-4-nitro-2,6-bis(pyrrolidin-1'-ylmethyl)phenol (VI.1d) (86%) [after column chromatography over alumina in methylene chloride with addition of methanol and PTLC (alumina; 1% methanol in chloroform)] (Found C: 63.7; H, 8.1; N, 13.3. $C_{17}H_{25}N_3O_3$ requires C, 63.9; H, 7.9; N, 13.2%). 1H n.m.r. δ 1.84, complex, 2.65, complex, H2',3',4',5'; 2.47, s, Me; 3.73, s, 2- CH_2 ; 3.88, s, 6- CH_2 ; 7.78, s, H5.

3-Methyl-4-nitro-6-(pyrrolidin-1'-ylmethyl)phenol (VI.2)

This compound was obtained (14%), together with the di-Mannich compound, when a mixture of pyrrolidine (1.721 g), paraformaldehyde (0.782 g) and 3-methyl-4-nitrophenol (1.2 g) in ethanol (10.0 ml) was refluxed for 19 h. The crude product was obtained by extraction with chloroform and methylene chloride. It was subjected to PTLC (alumina; 1% methanol in chloroform) and then PTLC (silica; 6% methanol in chloroform) and the product at higher R_f was recrystallised from cyclohexane to give the *title compound*, m.p. 110-113° (Found C: 61.8; H, 6.9; N, 11.3. $C_{12}H_{16}N_2O_3 \cdot 0.1 C_6H_{12}$ requires C, 61.9; H, 7.1; N, 11.4%). 1H n.m.r. δ 1.89, complex, 2.67, complex, H2',3',4',5'; 2.58, s, 3-Me; 3.87, s, 6- CH_2 ; 6.67, s, H2; 7.84, H5. MS m/z 237 ($M+1$) (2.2%), 236 (M^+) (12.2%), 166 (4.5%), 120 (3.7%), 84 (cyclohexane) (32.4%).

4-(7'-Chloroquinolin-4'-ylamino)-3-methyl-2,6-bis(piperidin-1''-ylmethyl)phenol (VI.4e)

3-Methyl-4-nitro-2,6-bis(piperidin-1-ylmethyl)phenol (0.6 g) in saturated ethanolic ammonia (30.0 ml), ethanol (20.0 ml) and Raney-nickel were shaken with hydrogen until uptake ceased and the yellow colour had discharged. The catalyst was removed on Celite and the solvent evaporated to give the crude amine (0.556 g). 1H n.m.r. δ 1.14, complex,

2.40, complex, 2.46, complex, H_{2'},3',4',5',6'; 2.10, s, Me; 3.50, s, 2-CH₂; 3.58, s, 6-CH₂; 5.69, br s, NH, OH; 6.47, s, H₅.

4,7-Dichloroquinoline (0.121 g) and the above amine (0.194 g) with methanol (2.2 ml), water (0.6 ml) and concentrated hydrochloric acid (3 drops) (pH mixture 4.7) were refluxed at 90-95° for 14.5 h. The mixture was diluted with water, made alkaline with ammonium hydroxide and the precipitate collected. It was subjected to PTLC (alumina; chloroform, developed twice) and the product (0.221 g) reprecipitated from dilute hydrochloric acid with ammonium hydroxide to give as a white solid the *title compound*, m.p. 181-184° (Found C: 66.7; H, 7.4; N, 10.9. C₂₈H₃₅ClN₄O.1.4H₂O requires C, 66.7; H, 7.6; N, 11.1%). ¹H n.m.r. δ 1.55, complex, 2.53, complex, H_{2''},3'',4'',5'',6''; 2.12, s, 3-Me; 3.60, s, 2-CH₂; 3.68, s, 6-CH₂; 6.13, d, J 5.5 Hz, H_{3'}; 6.50, br s, NH; 7.08, s, H₅; 7.41, d, 7.43, d, J_{5',6'} 9 Hz, J_{6',8'} 2 Hz, H_{6'}; 7.85, d, J 9 Hz, H₅; 8.00, d, J 2 Hz, H_{8'}; 8.44, d, J_{2',3'} 5 Hz, H_{2'}.

In a similar manner the following compounds were prepared.

3-Methyl-2,6-bis(piperidin-1'-ylmethyl)-4-(7''-trifluoromethylquinolin-4''-ylamino)phenol (VI.5e) (73%) as yellow crystals m.p. 186-188° [after PTLC (alumina; 1% methanol in methylene chloride, developed twice) and recrystallisation from a mixture of ethanol and light petroleum (b.p. 60-80°)] (Found: C, 68.1; H, 7.1; N, 10.7. C₂₉H₃₅F₃N₄O requires C, 68.0; H, 6.9; N, 10.9%). ¹H n.m.r. δ 1.54, complex, 2.50, complex, H_{2'},3',4',5',6'; 2.12, s, Me; 3.57, s, 2-CH₂; 3.68, s, 6-CH₂; 6.23, d, J 5.5 Hz, H_{3''}; 6.58, br s, NH; 7.06, s, H₅; 7.63, d, 7.65, d, J_{5'',6''} 9 Hz, J_{6'',8''} 2 Hz, H_{6''}; 8.04, d, J 9 Hz, H₅; 8.31, br s, H_{8''}; 8.52, d, J 5.5 Hz, H_{2''}. MS m/z 512 (3.0%) (M⁺), 427 (7.1%). ¹³C n.m.r. (CDCl₃) 13.7, 24.0, 25.7, 53.9, 56.6, 102.2, 120.1, 120.4, 121.3, 122.1, 122.5, 125.8, 127.5, 127.8, 130.8, 134.1, 148.0, 152.2, 156.4 ppm.

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-3-methyl-2,6-bis(piperidin-1''-ylmethyl)phenol (VI.6e) (71%) as white crystals, m.p. 184-186° [after PTLC (alumina; 0.5% methanol in chloroform) and recrystallisation from a mixture of ethanol and light petroleum (b.p. 60-80°)] (Found, C, 61.7; H, 6.6; N, 12.9. C₂₇H₃₄BrN₅O requires

C, 61.8; H 6.5; N, 13.3%). ^1H n.m.r. δ 1.55, complex, 2.51, complex, $\text{H}2'',3'',4'',5'',6''$; 2.17, s, Me; 3.57, s, 2- CH_2 ; 3.68, s, 6- CH_2 ; 6.39, d, J 5 Hz, $\text{H}3'$; 7.10, s, $\text{H}5$; 7.96, br s, NH; 8.41, d, J 2 Hz, $\text{H}8'$; 8.44, d, $\text{J}_{2',3'}$ 5 Hz, $\text{H}2'$; 8.75, d, J 2 Hz, $\text{H}6'$. MS m/z 523 (2.3%), 525 (2.3%) (M^+) 438 (4.8%), 440 (5.8%), 354 (13.3%).

4-(7'-Chloroquinolin-1'-ylamino)-2,6-bis(dimethylaminomethyl)-3-methylphenol (VI.4a)

2,6-Bis(dimethylaminomethyl)-3-methyl-4-nitrophenol (0.712 g) was reduced as described above to give the amine (0.656 g). ^1H n.m.r. δ 2.14, s, 3-Me; 2.28, s, MeN; 3.44, s, 2- CH_2 ; 3.55, s, 6- CH_2 ; 6.43, s, $\text{H}5$.

The above amine (0.299 g) and 4,7-dichloroquinoline (0.191 g) gave, after PTLC (alumina; 3% methanol in methylene chloride), the *title compound* (61%). It was recrystallisation twice from a mixture of cyclohexane and ethanol and then from methylene chloride and light petroleum (b.p. 60-80°) to give yellow needles, m.p. 177-179° (Found, C, 66.1; H, 6.8; N, 13.5. $\text{C}_{22}\text{H}_{27}\text{ClN}_4\text{O}$ requires C, 66.2; H, 6.8; N, 14.0). ^1H n.m.r. δ 2.17, s, 3-Me; 2.29, s, 2.33, s, MeN; 3.51, s, 2- CH_2 ; 3.65, s, 6- CH_2 ; 6.12, d, J 5.5 Hz, $\text{H}3'$; 6.56, br s, NH; 6.97, s, $\text{H}5$; 7.39, d, 7.42, d, $\text{J}_{5',6'}$ 9 Hz, $\text{J}_{6',8'}$ 2 Hz, $\text{H}6'$; 7.86, d, J 9 Hz, $\text{H}5'$; 7.99, d, J 2 Hz, $\text{H}8'$; 8.42, d, J 5.5 Hz, $\text{H}2'$.

2,6-Bis(dimethylaminomethyl)-3-methyl-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (VI.5a) (63%), as yellow needles, m.p. 183-186° [after PTLC (alumina; 2.3% methanol in methylene chloride) and recrystallisation from a mixture of ethanol and light petroleum (b.p. 60-80°)] (Found C: 64.1; H, 6.5; N, 13.0. $\text{C}_{23}\text{H}_{27}\text{F}_3\text{N}_4\text{O}$ requires C, 63.9; H, 6.3; N, 13.0%). ^1H n.m.r. δ 2.15, s, 3-Me; 2.30, s, 2.34, s, MeN; 3.52, s, 2- CH_2 ; 3.66, s, 6- CH_2 ; 6.22, d, J 5.5 Hz, $\text{H}3'$; 6.65, br s, NH; 6.99, s, $\text{H}5$; 7.62, d, 7.64, d, $\text{J}_{5',6'}$ 9 Hz, $\text{J}_{6',8'}$ 2 Hz, $\text{H}6'$; 8.06, d, J 9 Hz, $\text{H}5'$; 8.30, br s, $\text{H}8'$; 8.51, d, J 5.5 Hz, $\text{H}2'$. I.r. ν_{max} 3480 (br) (OH st), 3200 (m) (NH st), 2975 + 2954 (m) (aliph. CH st), 1598 (s), 1582 (s), 1535 (s), 1477 (s), 1390 (s), 1388 (s), 1156 (s), 1131 (s) (CF_3 st), 1079 (m), 828 (m), 743 (mw), 690 (ms) cm^{-1} . U.v. λ_{max} (pH

8.34) 212 nm (log ϵ 4.77), 244 nm (log ϵ 4.12) (as inflexion), 330 nm (log ϵ 4.16). MS m/z 432 (2.0%) (M^+), 387 (11.3%).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis(dimethylamino-methyl)-3-methylphenol (VI.6a) (48%) [after PTLC (alumina; 3% methanol in methylene chloride, developed twice)]. It was recrystallised from a mixture of cyclohexane and ethanol to give a white solid, m.p. 117-119° (Found C: 56.5; H, 5.7; N, 15.5. $C_{21}H_{26}BrN_5O$ requires C, 56.8; H, 5.9; N, 15.8%). 1H n.m.r. δ 2.19, s, 3-Me; 2.31, s, 2.35, s, MeN; 3.54, s, 2-CH₂; 3.66, s, 6-CH₂; 6.37, d, J 5.5 Hz, H3'; 7.06, s, H5; 7.95, br s, NH; 8.42, d, J 2 Hz, H8'; 8.44, d, J 5.5 Hz, H2'; 8.75, d, J 2 Hz, H6'. I.r. ν_{max} 3460 (br) (OH st), 3230 (br) (NH st), 2964 (mw), 1602 (m) (ar. CH skeleton st), 1575 (s), 1528 (m), 1476 (multiplet), 1369 (s), 1476 (s) cm^{-1} . U.v. λ_{max} (pH 8.6) 263 nm (log ϵ 4.44), 314-316 nm (log ϵ 3.89), 354 nm (log ϵ 3.93).

4-(7'-Chloroquinolin-4'-ylamino)-2,6-bis(diethylaminomethyl)-3-methylphenol (VI.4b)

2,6-Bis(diethylaminomethyl)-3-methyl-4-nitrophenol (90.8 g) was reduced as above to give the corresponding amine (0.772 g). 1H n.m.r. δ 1.05, t, J 7 Hz, MeCH₂; 2.10, s, 3-Me; 2.55, q, 2.57, q, J 7 Hz, MeCH₂; 3.58, s, 2-CH₂; 3.68, s, 6-CH₂; 5.47, br s, NH, OH; 6.56, s, H5.

This amine (0.297 g) and 4,7-dichloroquinoline (0.202 g) as above gave, after PTLC (alumina; 3% methanol in methylene chloride) and recrystallisation from a mixture of ethanol and light petroleum (b.p. 60-80°), the *title compound* (52%), m.p. 137-139° (Found, C, 68.8; H, 7.8; N, 12.1. $C_{26}H_{35}ClN_4$ requires C, 68.6; H, 7.8; N, 12.3%). 1H n.m.r. δ 1.06, t, 1.11, t, J 7 Hz, MeCH₂; 2.14, s, 3-Me; 2.59, q, 2.63, q, J 7 Hz, MeCH₂; 3.64, s, 2-CH₂; 3.78, s, 6-CH₂; 6.14, d, J 5.5 Hz, H3'; 6.58, br s, NH; 7.08, s, H5; 7.40, d, 7.42, d, J_{5',6'} 9 Hz, J_{6',8'} 2 Hz, H6'; 7.87, d, J 9 Hz, H5'; 7.99, d, J 2 Hz, H8'; 8.42, d, J 5.5 Hz, H2'. MS m/z , 454 (M^+), 425 (M^+ -Et).

2,6-Bis(diethylaminomethyl)-3-methyl-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (VI.5b) (69%) as white crystals, m.p. 155-157° [after PTLC

(alumina; 2.5% methanol in methylene chloride, developed twice) and recrystallisation from a mixture of cyclohexane and methylene chloride] (Found, C, 66.6; H, 7.4; N, 11.3. $C_{27}H_{35}F_3N_4O$ requires C, 66.4; H, 7.2; N, 11.5%). 1H n.m.r. δ 1.10, t, 1.14, t, J 7 Hz, $MeCH_2$; 2.15, s, 3-Me; 2.65, q, J 7 Hz, $MeCH_2$; 3.70, s, 2- CH_2 ; 3.81, s, 6- CH_2 ; 6.24, d, J 5.5 Hz, $H3'$; 6.54, br s, NH; 7.15, s, $H5$; 7.65, br d, J 9 Hz, $H6'$; 8.05, d, J 9 Hz, $H5'$; 8.31, s, $H8'$; 8.53, d, J 5 Hz, $H2'$. MS m/z , 488 (0.5%) (M^+), 459 (2.4%) ($M^+ - Et$), 415 (3.5%), 386 (6.0%), 344 (17.6%).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis(diethylaminomethyl)-3-methylphenol (VI.6b) (80%) [after PTLC (alumina; 3% methanol in methylene chloride, developed twice) and reprecipitation from aqueous acid with ammonium hydroxide] (Found, C, 59.6; H, 6.6; N, 13.7. $C_{25}H_{34}BrN_5O$ requires C, 60.0; H, 6.8; N, 14.0%). 1H n.m.r. δ 1.08, t, 1.12, t, J 7 Hz, $MeCH_2$; 2.19, s, 3-Me; 2.62, q, 2.64, q, J 7 Hz, $MeCH_2$; 3.68, s, 2- CH_2 ; 3.79, s, 6- CH_2 ; 6.40, d, J 5.5 Hz, $H3'$; 7.17, s, $H5$; 7.97, br s, NH; 8.42, d, J 2 Hz, $H8'$; 8.44, d, J 5 Hz, $H2'$; 8.75, d, J 2 Hz, $H6'$.

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis(*t*-butylaminomethyl)-3-methylphenol (VI.6c)

2,6-Bis(*t*-butylaminomethyl)-3-methyl-4-nitrophenol (0.602 g) was reduced as above to give the corresponding amine (0.523 g). 1H n.m.r. δ 1.20, s, 1.23, s, Me_3C ; 2.14, s, 3-Me; 2.95, br, NH; 3.75, s, 2- CH_2 ; 3.83, s, 6- CH_2 ; 6.42, s, $H3$.

This amine (0.152 g) and 3-bromo-8-chloro-1,5-naphthyridine (0.149 g) as above gave after PTLC (alumina; 3% methanol in chloroform) the *title compound* (0.054 g). It was recrystallised from light petroleum (b.p. 60-80°) and had m.p. 149-151° (Found: C, 60.2; H, 7.0; N, 13.7. $C_{25}H_{34}BrN_5O$ requires C, 59.9; H, 6.9; N, 14.0%). 1H n.m.r. δ 1.22, s, 1.25, s, Me_3C ; 2.20, s, 3-Me; 3.84, s, 2- CH_2 ; 3.91, s, 6- CH_2 ; 6.39, d, J 5.5 Hz, $H3'$; 7.01, s, $H5$; 7.90, br s, NH; 8.42, d, J 2 Hz, $H8'$; 8.44, d, J 5.5 Hz, $H2'$; 8.75, d, J 2 Hz, $H6'$.

4-(7'-Chloroquinolin-4'-ylamino)-3-methyl-2,6-bis(pyrrolidin-1''-yl-methyl)phenol (VI.4d)

3-Methyl-4-nitro-2,6-bis(pyrrolidin-1'-ylmethyl)phenol (0.701 g) was reduced as above to the corresponding amine (0.578 g). ^1H n.m.r. δ 1.75, complex, 2.58, complex, $\text{H}2',3',4',5'$; 2.13, s, Me; 3.61, s, 2- CH_2 ; 3.73, s, 6- CH_2 ; 6.41, br s, $\text{H}5$.

This amine (0.175 g) and 4,7-dichloroquinoline (0.123 g) as above gave after precipitation, PTLC (alumina; 3% methanol in methylene chloride) and recrystallisation from a mixture of cyclohexane and ethanol, the *title compound* (0.144 g), m.p. 174-176° (Found: C, 67.2; H, 6.5; N, 11.5. $\text{C}_{27}\text{H}_{31}\text{F}_3\text{N}_4\text{O}$ requires C, 66.9; H, 6.5; N, 11.6%). ^1H n.m.r. δ 1.79, 1.83, complex, 2.59, 2.65, complex $\text{H}2'',3'',4'',5''$; 2.12, s, Me; 3.69, s, 2- CH_2 ; 3.85, s, 2- CH_2 ; 6.12, d, J 5.5 Hz, $\text{H}3'$; 6.56, br s, NH; 7.02, s, $\text{H}5$; 7.39, d, 7.41, d, $\text{J}_{5',6'} 9$ Hz, $\text{J}_{6',8'} 2$ Hz, $\text{H}6'$; 7.85, d, 9 Hz, $\text{H}5''$; 7.99, d, J 2 Hz, $\text{H}8'$; 8.41, d, J 5.5 Hz, $\text{H}2'$.

3-Methyl-2,6-bis(pyrrolidin-1'-ylmethyl)-4-(7''-trifluoromethylquinolin-4''-ylamino)phenol (VI.5d) (49%), as a white solid, m.p. 178-180° [after PTLC (alumina; 3% methanol in methylene chloride, developed twice) and recrystallisation from a mixture of cyclohexane and a little ethanol] (Found, C, 67.2; H, 6.5; N, 11.5. $\text{C}_{27}\text{H}_{31}\text{F}_3\text{N}_4\text{O}$ requires C, 66.9; H, 6.5; N, 11.6%). ^1H n.m.r. δ 1.81, 1.85, complex, 2.62, 2.68, complex, $\text{H}2',3',4',5'$; 2.15, s, Me; 3.72, s, 2- CH_2 ; 3.88, s, 6- CH_2 ; 6.24, d, J 5.5 Hz, $\text{H}3''$; 6.59, br s, NH; 7.06, s, $\text{H}3$; 7.60, d, 7.69, d, $\text{J}_{5'',6''} 9$ Hz, $\text{J}_{6'',8''} 2$ Hz, $\text{H}6''$; 8.04, d, J 9 Hz, $\text{H}5''$; 8.31, br s, $\text{H}8''$; 8.52, d, J 5.5 Hz, $\text{H}2''$.

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-3-methyl-2,6-bis(pyrrolidin-1''-ylmethyl)-phenol (VI.6d) (62%), m.p. 182-184° [after PTLC (alumina; 2.5% methanol in methylene chloride, developed twice) and recrystallisation from a mixture of methylene chloride and hexane] (Found, C, 60.7; H, 6.2; N, 14.0. $\text{C}_{25}\text{H}_{30}\text{BrN}_5\text{O}$ requires C, 60.5; H, 6.1; N, 14.1%). ^1H n.m.r. δ 1.86, complex, $\text{H}3'',4''$; 2.20, s, Me; 2.70, complex, $\text{H}2'',5''$; 3.76, s, 2- CH_2 ; 3.90, s, 6- CH_2 ; 6.40, d, J 5.5 Hz, $\text{H}3'$; 7.14, s,

H5; 7.95, br s, NH; 8.42, br s, H8' 8.45, d, J 5.5 Hz, H2'; 8.75, d, J 2 Hz, H6'. MS m/z 495 (1.4%), 497 (1.4%) (M^+), 426 (2.5%), 355 (7.3%).

2,6-Bis(dipentylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)-phenol (VI.7d)

4-Amino-2,6-bis(dipentylaminomethyl)phenol (0.371 g; see above), 4-chloro-7-trifluoromethylquinoline (0.151 g), methanol (3.0 ml), water (1.0 ml) and concentrated hydrochloric acid (0.1 ml) were refluxed at 90° for 18 h. The methanol was evaporated under reduced pressure, the residue diluted with water and adjusted with ammonium hydroxide to pH 7 and the mixture extracted with chloroform. The extract was dried (Na_2SO_4) and evaporated to give an oil which was subjected to PTLC (alumina; dichloromethane) to give *2,6-bis(dipentylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol* (0.243 g) (Found: C, 70.2; H, 9.1; F, 8.7; N, 8.5. $\text{C}_{38}\text{H}_{57}\text{F}_3\text{N}_4\text{O} \cdot 0.5 \text{H}_2\text{O}$ requires C, 70.0; H, 9.0; F, 8.7; N, 8.6%). ^1H n.m.r. δ 0.87, t, J 7 Hz, Me; 1.26, complex, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$; 2.5, t, J 7 Hz, NCH_2CH_2 ; 3.69, s, ArCH_2N ; 6.74, d, $J_{2',3'}$ 5.5 Hz, H3'; 7.07, s, H3,5; 7.63, br d, $J_{5',6'}$ 9 Hz, H6'; 8.04, d, $J_{5',6'}$ 9 Hz, H5'; 8.31, s, H8'; 8.54, d, $J_{2',3'}$ 5.5 Hz, H2'.

2,6-Bis(N-butyl-N-methylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (VI.7e)

4-Amino-2,6-bis(*N*-butyl-*N*-methylaminomethyl)phenol (0.551 g; see above) and 4-chloro-7-trifluoromethylquinoline (0.413 g) as for the analogue above gave a crude product which was purified by PTLC (alumina; ethyl acetate/hexane, 1:5) to yield the *title compound* (0.352 g). Found: C, 60.4; H, 6.1; N, 16.8. $\text{C}_{21}\text{H}_{24}\text{F}_3\text{N}_5\text{O}$ requires C, 60.1; H, 5.8; N, 16.7%). ^1H n.m.r. δ 0.90, t, J 7 Hz, MeCH_2 ; 1.45, complex, $\text{NCH}_2(\text{CH}_2)_2$; 2.26, s, NMe; 2.46, t, J 7 Hz, NCH_2CH_2 ; 3.61, s, NCH_2Ar ; 6.74, d, $J_{2',3'}$ 5 Hz, H3'; 7.04, s, H3,5; 7.30, br s, NH; 7.58, br d, $J_{5',6'}$ 9 Hz, H6'; 8.12, br d, $J_{5',6'}$ 9 Hz, H5'; 8.28, br s, H8'; 8.54, d, $J_{2,3}$ 5 Hz, H2'. I.r. ν_{max} 2960 (w), 1580 (m), 1480 (s), 1125 (m) cm^{-1} .

VI-5.2 Determination of the basic ionization constant and ultraviolet spectra of 4-(7-trifluoromethylquinolin-4-ylamino)phenol (VI.8)

Substance: 4-(7-Trifluoromethylquinolin-4-ylamino)phenol hydrochloride,^a

$C_{16}H_{11}F_3N_2O \cdot HCl$; MW 340.729.

Concentration: $1:1 \times 10^{-4}$ M. Recrystallised compound (m.p.315-317°) was dried at 20°/0.2 mmHg overnight and a sample (0.0094 g) was dissolved in ethanol (2.5 ml) added to hot water (200 ml), then cooled and made up to 250.0 ml with water. This stock solution (2.0 ml) was diluted with appropriate buffers (2.0 ml).

Analytical wavelength: 350 nm; cell, 1 cm

Buffer: Potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate buffers (I = 0.1)

Species: Neutral and cationic species

Temperature: 20°

Result:

pH measured: 7.69 7.62 7.51 7.42 7.32 7.13 6.91

pK_a calc.: 7.55 7.59 7.59 7.63 7.55 7.65 7.64

$pK_a = 7.60 \pm 0.05$

The ultraviolet spectra were recorded as follows:

u.v. λ_{max} (pH 8.8) 342 nm (log ϵ 3.98)

(pH 4.47) 348 nm (log ϵ 4.14)

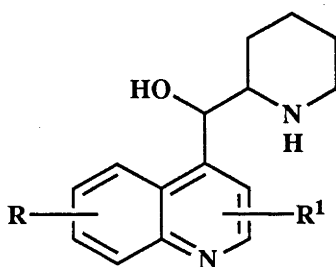
^a Kindly prepared by S.J. Ireland

CHAPTER VII

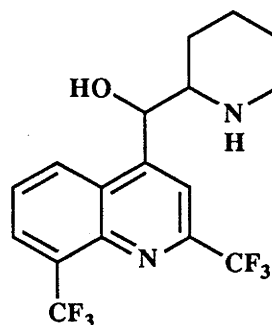
CHAPTER VII α -(Piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)-methanol, its 1'-Oxide and Related Compounds

VII-1 Introduction

A series of quinolinylmethanols (VII.1), which are structurally related to quinine (I.1a), have been shown to possess high antimalarial activity; and mefloquine [α -(2,8-bistrifluoromethylquinolin-4-yl)- α -(piperidin-2-yl)methanol] (VII.2) is now widely used as an antimalarial.



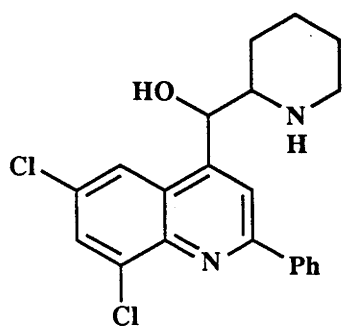
VII.1



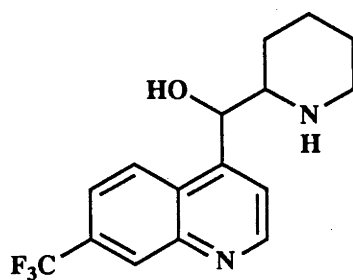
VII.2

Prior to the discovery of mefloquine, a number of α -(piperidin-2-yl)- α -(quinolin-4-yl)methanols²³¹⁻²³³ was shown to possess high antimalarial activity. This activity was associated with a substituent in the 2-position of the quinoline nucleus, particularly the phenyl substituent, which prevented oxidation at that position (the cinchona alkaloids and related compounds were rapidly biotransformed in man to the inactive carbostyryl derivatives²³⁴). Thus α -(6,8-dichloro-2-phenylquinolin-4-yl)- α -(piperidin-2-yl)methanol²³⁵ (SN 10,275) (VII.3) was 8 times more active than quinine against *P. cathemerium* in ducks;¹⁴¹ but this compound was also reported to cause photosensitisation in mice²³⁶ and man.²³⁷ Further evaluation of the phototoxicity of quinolinylmethanol derivatives (VII.1) has been reported by Rothe and Jacobus.²³⁶ Surprisingly, quinine (a well-known quinolinylmethanol lacking a 2-substituent) does not cause any phototoxicity.

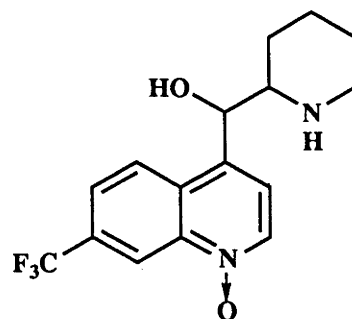
A consideration of the above data and of the high antimalarial activity of a series of di-Mannich bases of 4-(7-trifluoromethylquinolin-4-ylamino)phenol^{97,98} and related compounds reported previously from this laboratory prompted the present work to synthesise the α -(piperidin-2-yl)- α -(2-unsubstituted-7-trifluoromethylquinolin-4-yl)-methanol (VII.4) and related compounds, such as compounds VII.5, VII.6 and VII.7.



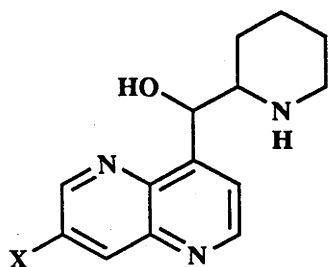
VII.3



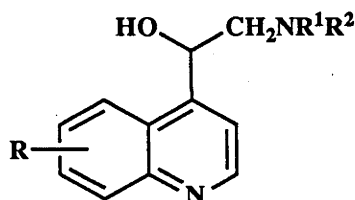
VII.4



VII.5



VII.6 X=Br

VII.7 X=CF₃

VII.8

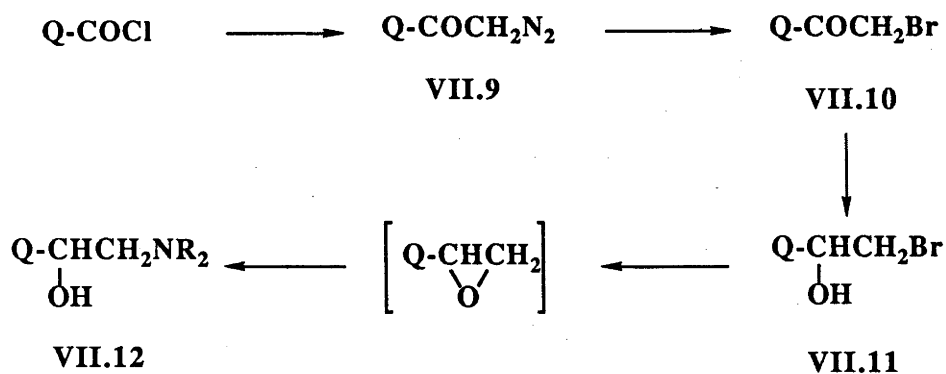
VII-2 Some Literature Preparations Relating to the Synthesis of α -(Quinolin-4-yl)methanols

Literature syntheses relating to two types of α -(substituted quinolin-4-yl)methanols are discussed in this Chapter: α -alkyl (and α -dialkyl)aminomethyl- α -(quinolin-4-yl)-methanols (VII.8) in Chapter VII-2.1; α -(piperidin-2-yl)- α -(quinolin-4-yl)methanols (VII.1 except mefloquine) in Chapter VII-2.2; and mefloquine in Chapter VII-4.

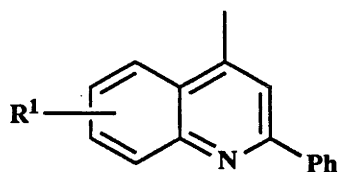
VII-2.1 Preparations of α -alkyl (and α -dialkyl)aminomethyl- α -(2-phenylquinolin-4-yl)methanols

The general methods used to prepare α -alkyl (and α -dialkyl)aminomethyl- α -(2-phenylquinolin-4-yl)methanols have commenced from the relevant quinoline-4-carboxylic acid or ester and have involved two synthetic methods. The first method which was more generally used (Scheme VII-1) involved the reaction of the acid chloride to give the diazomethyl ketone (VII.9).²³¹ Hydrobromination of compound VII.9 gave the α -bromo methyl ketone (VII.10),²³⁸ and then an aluminium isopropoxide reduction afforded the bromohydrin (VII.11).²³⁹ Epoxidation and subsequent condensation of compound VII.11 with appropriate amines afforded the amino alcohols (VII.12).²⁴⁰

The other method, which has been used in a few cases, involved the Claisen condensation between the quinolin-4-carboxylic ester and ethyl acetate to give the β -keto ester (VII.13),²⁴¹⁻²⁴³ followed either by simultaneous bromination and hydrolysis²⁴¹ to the bromomethyl ketone (VII.10) or by hydrolysis and then decarboxylation to the methyl

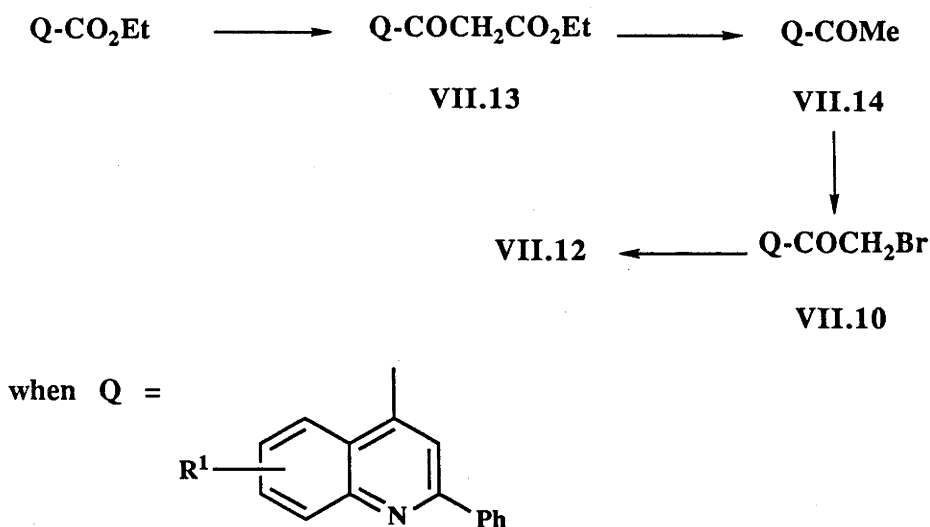


when Q =



Scheme VII-1

ketone (VII.14) with a subsequent bromination to compound VII.10. Amination then afforded the desired compound VII.12 (Scheme VII-2).

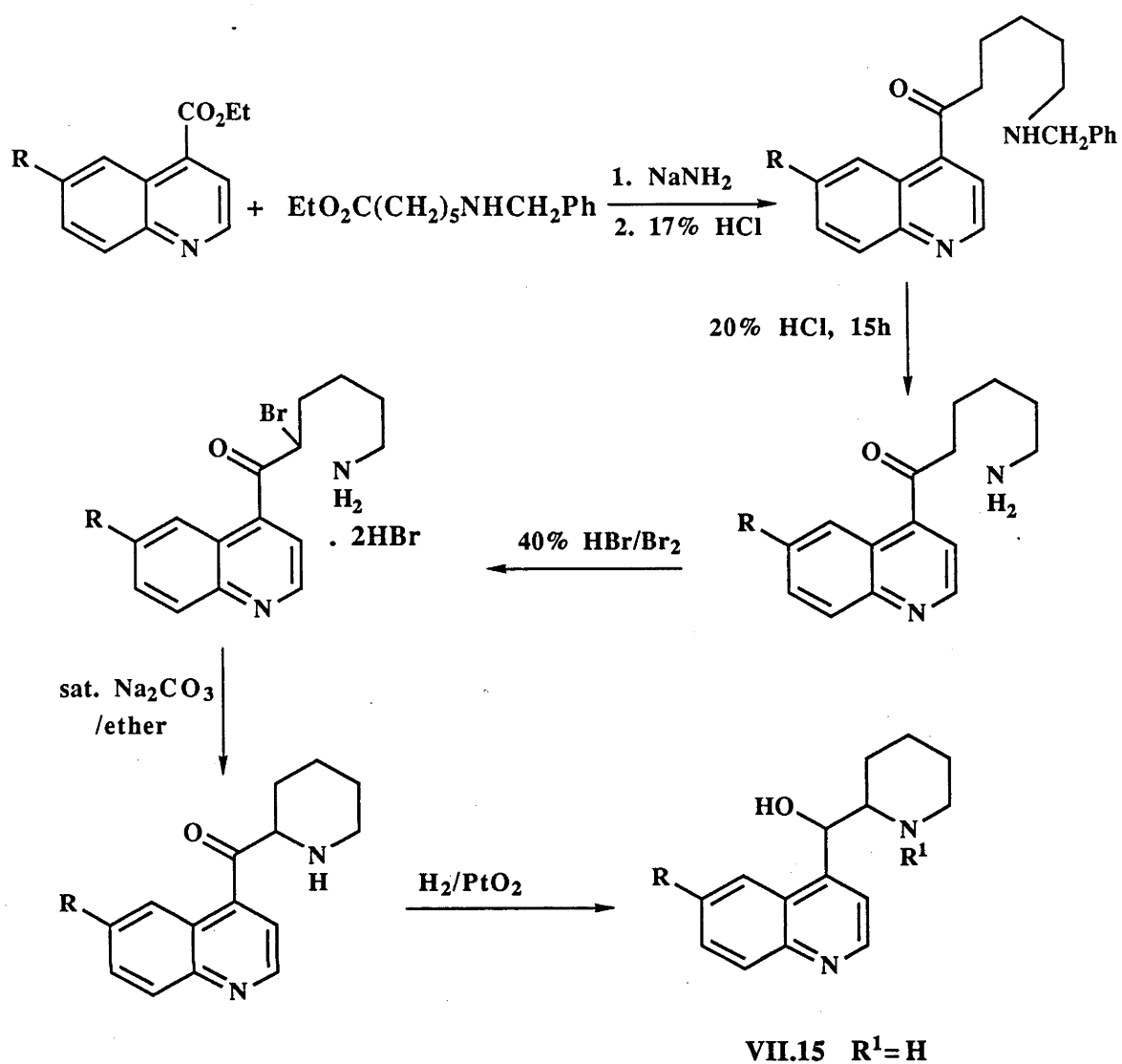


Scheme VII-2

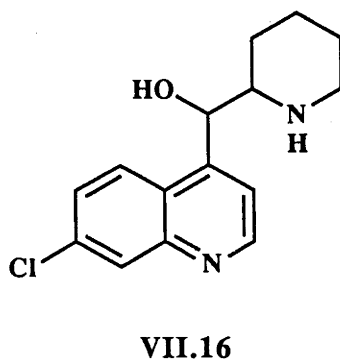
VII-2.2 Preparations of α -(piperidin-2-yl)- α -(2-substituted quinolin-4-yl)methanols

The α -(piperidin-2-yl)- α -(quinolin-4-yl)methanols [(VII.15), R=R¹=H; R=OMe, R¹=H; R=H or OMe, R¹=alkyl, allyl or crotyl] were first synthesised and evaluated for antimalarial activity by Ainley and King in 1938.²³¹ They found that only compound VII.15 (R=OMe, R¹=H) and its diastereoisomer were active against malaria parasites. Their synthetic procedures are summarised in Scheme VIII-3.

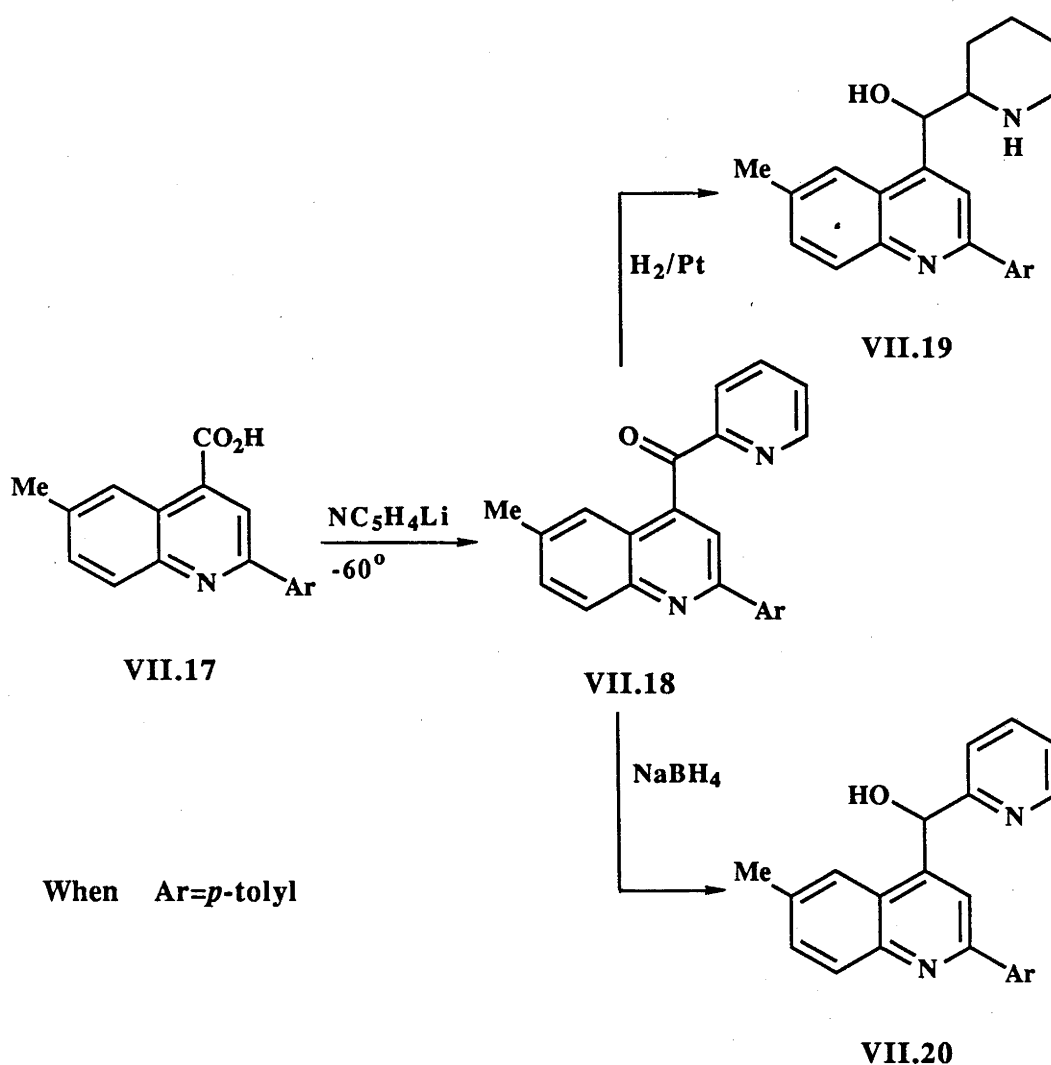
α -(7-Chloroquinolin-4-yl)- α -(piperidin-2-yl)methanol (VIII.16) was synthesised (in a similar manner to that described above) by Senear *et al.*²⁴⁴ from ethyl 7-chloroquinoline-4-carboxylate using the improved²⁴⁵ procedure of Ainley and King.²³¹ The 5-chloro isomer of compound VII.16, however, could not be obtained by this method, presumably due to the steric effect of the 5-chloro substituent.



Scheme VII-3

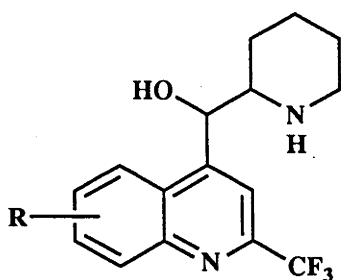
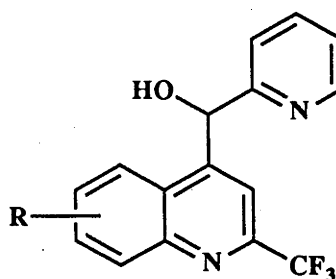


In 1967, Boykin *et al.*²³³ reported a new and more convenient two-step synthesis from the corresponding quinoline-4-carboxylic acids to give the α -(2-arylquinolin-4-yl)- α -(piperidin-2-yl)methanols (VII.19). The first step involved conversion of 6-methyl-2-(*p*-tolyl)quinoline-4-carboxylic acid (VII.17) by 2-pyridinyl lithium into the pyridin-2-yl ketone (VII.18). The second step was a selective reduction of compound VII.18: catalytic hydrogenation reduced both the carbonyl and pyridinyl groups and gave compound VII.19, whereas sodium borohydride reduced only the carbonyl group to afford the α -(pyridin-2-yl)methanols (VII.20) (Scheme VII-4).



Scheme VII-4

Pinder and Burger,¹⁴¹ have reported the syntheses (using the method described by Boykin *et al.*²³³) and antimalarial activity of a series of α -(piperidin-2-yl and pyridin-2-yl)- α -(2-trifluoromethylquinolin-4-yl)methanols (**VII.21** and **VII.22**). They found that compound **VII.21** showed moderate antimalarial activity but induced photosensitisation, the unsubstituted compound (**VII.21**, R=H) was inactive; and the α -(pyridin-2-yl) methanols (**VII.22**) were inactive and did not cause photosensitisation.

**VII.21****VII.22**

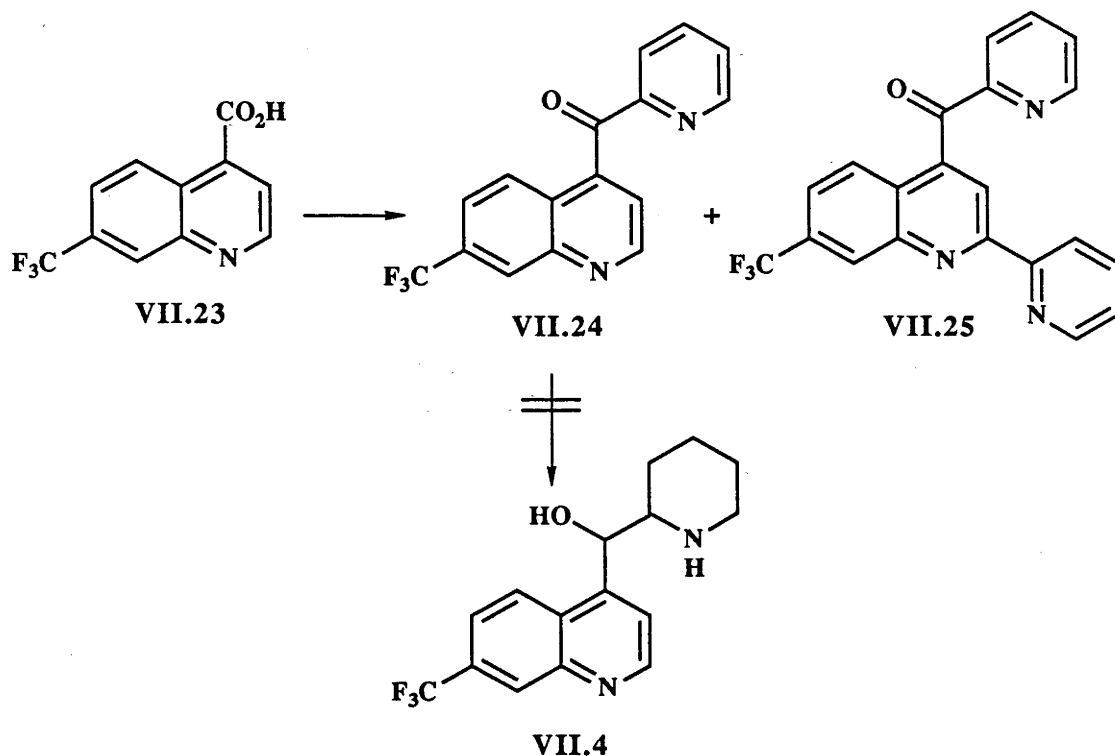
Patel and coworkers²⁴⁶ attempted the synthesis of compound **VII.4** but were unsuccessful. In this 7-trifluoromethylquinoline-4-carboxylic acid (**VII.23**) with 2-pyridinyl lithium gave low yields of the pyridin-2-yl ketone (**VII.24**), and compound **VII.25**; but the ester derivative of compound **VII.23** with 2-pyridinyl lithium in ether gave compound **VII.24** in good yields. However the selective hydrogenation of compound **VII.24** to compound **VII.4** was unsuccessful (Scheme VIII-5).

The syntheses of α -(2,8-bistrifluoromethylquinolin-4-yl)- α -(piperidin-2-yl)-methanol (mefloquine) (**VII.2**) by Ohnmacht,¹³³ using a procedure similar to that described above, is mentioned in Chapter VII-4.

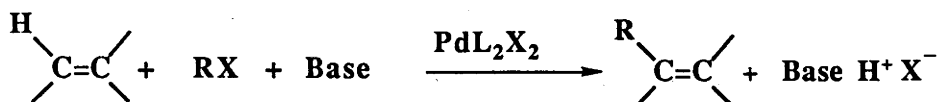
VIII-3 Palladium-catalysed Vinylation of Organic Halides

The palladium-catalysed vinylation of organic halides provides a very convenient method for carbon-carbon bond formation at unsubstituted vinylic positions, and the reaction can be carried out in one step. The general reaction²⁴⁷ is shown in Scheme VII-6.

The base needed for this type of reaction may be a secondary²⁴⁸ or tertiary amine,^{249,250} or sodium bicarbonate,^{251,252} and a variety of organophosphine palladium complexes have been used as catalysts in these vinylic hydrogen substitution reactions.²⁴⁷



Scheme VII-5



R = Aryl, heteroaryl, benzyl or vinyl

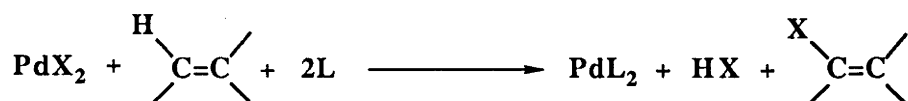
X = Bromide, iodide, or (rarely) chloride

L = Ligand

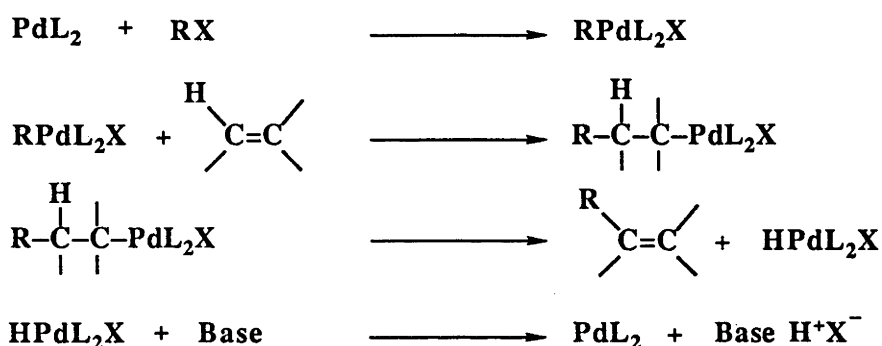
Scheme VII-6

The detailed mechanism of the reaction has not been established. However, Heck²⁴⁷ has proposed a mechanism (Scheme VII-7) which is based on the large amount of information available from related studies with other organopalladium reactions.

Catalyst formation :



Catalytic cycle :



Scheme VII-7

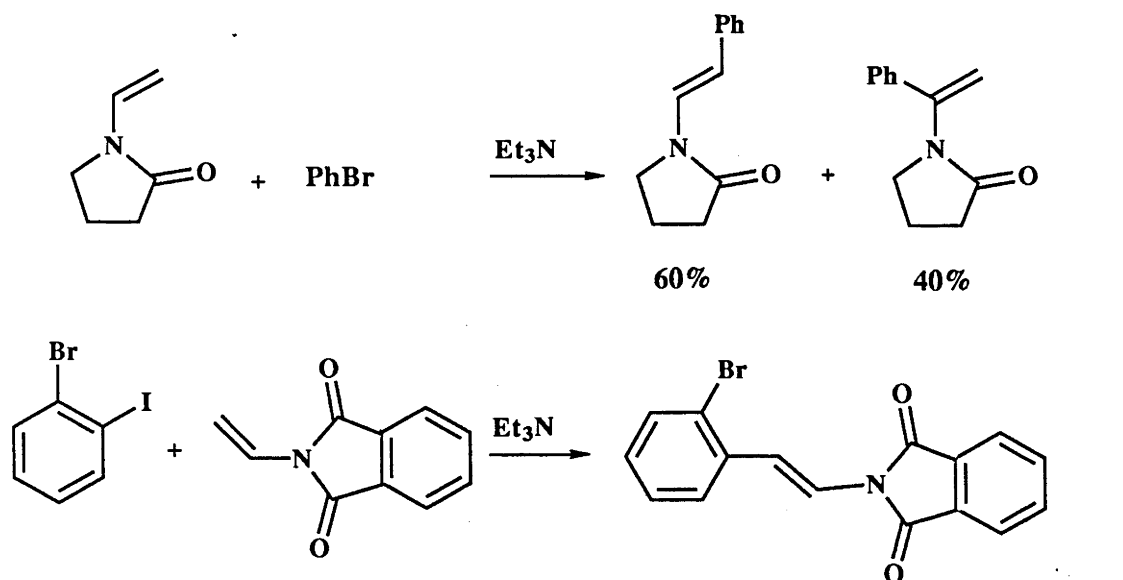
Ziegler and Heck²⁵³ have reported reactions of *N*-vinylamides such as *N*-vinylpyrrolidin-2-one and *N*-vinylphthalimide with bromobenzene (Scheme VII-8) and Frank *et al.*²⁵⁴ have described the synthesis of nornicotine (VII.26) in four steps from 3-bromopyridine and *N*-(but-3-enyl)phthalimide (Scheme VII-9).

Palladium-catalysed vinylations of organic halides have been reviewed by Heck.²⁴⁷

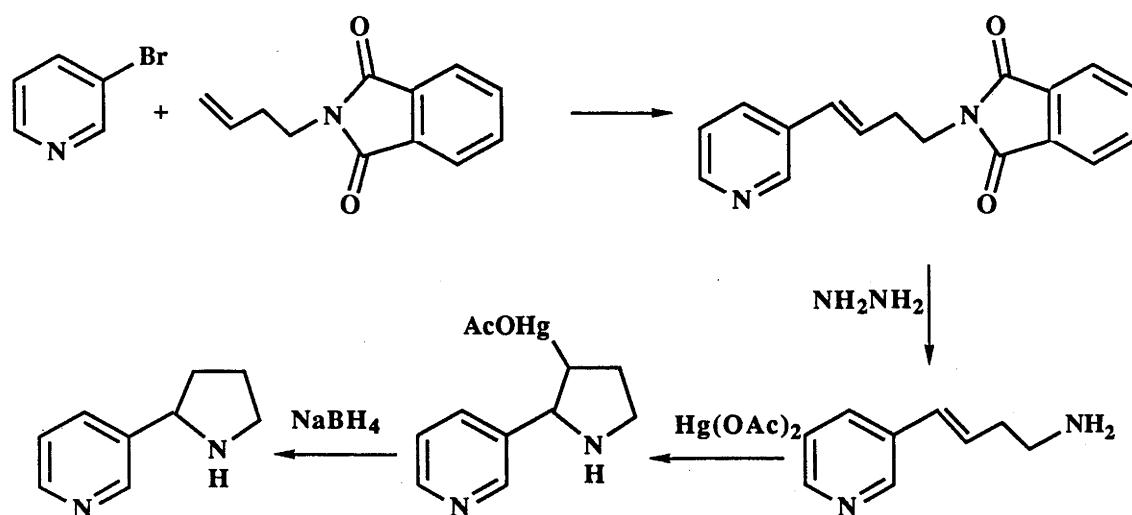
VII-4 Literature Preparations of α -(2,8-Bistrifluoromethylquinolin-4-yl)- α -(piperidin-2-yl)methanol (Mefloquine)

Mefloquine, registered under the name Lariam (F. Hoffmann-La Roche), is a highly active blood schizontocide against multidrug-resistant *P. falciparum*.¹³³ Three syntheses of this compound have been described.

The first synthesis was reported in 1971 by Ohnmacht *et al.*¹³³ and involved the conversion of 2,8-bistrifluoromethylquinolin-4-one (VII.27) (obtained by PPA condensation of 2-trifluoromethylaniline and ethyl 4,4,4-trifluoroacetoacetate using adaptations of previously described procedures^{141,255}) by phosphoryl bromide into the 4-



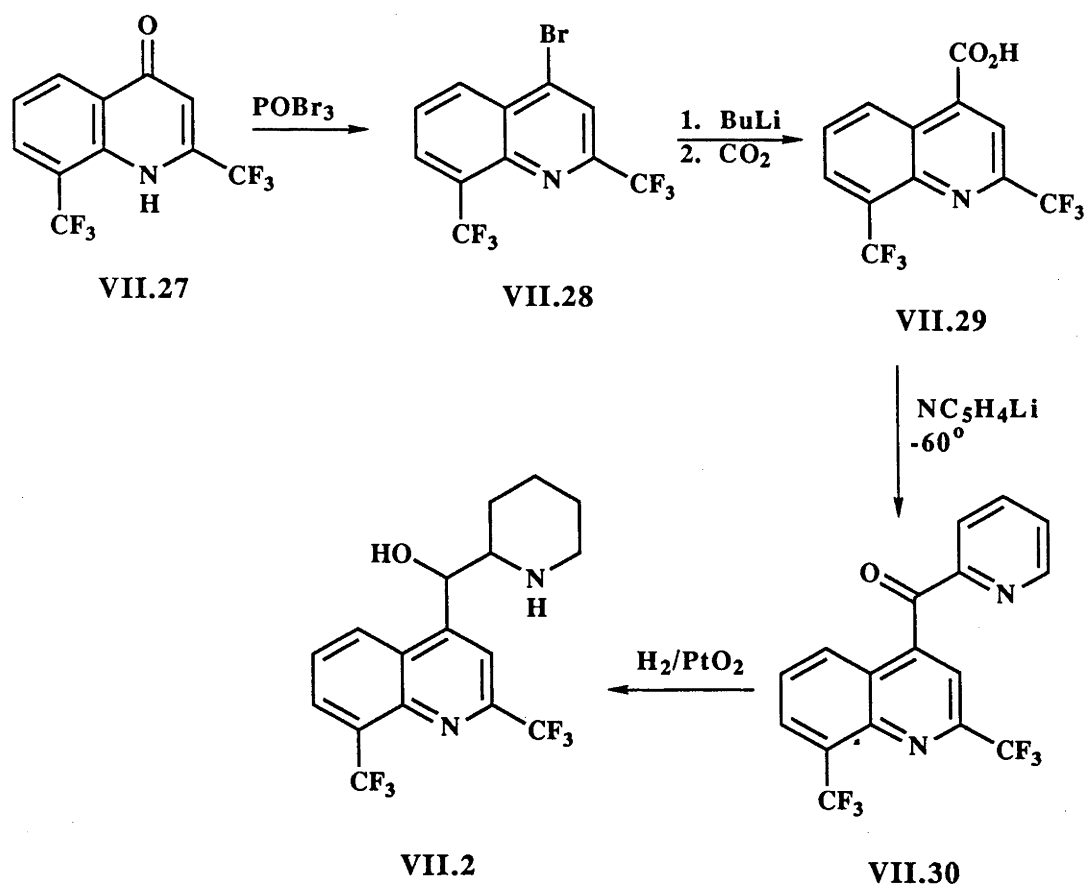
Scheme VII-8



VII.26

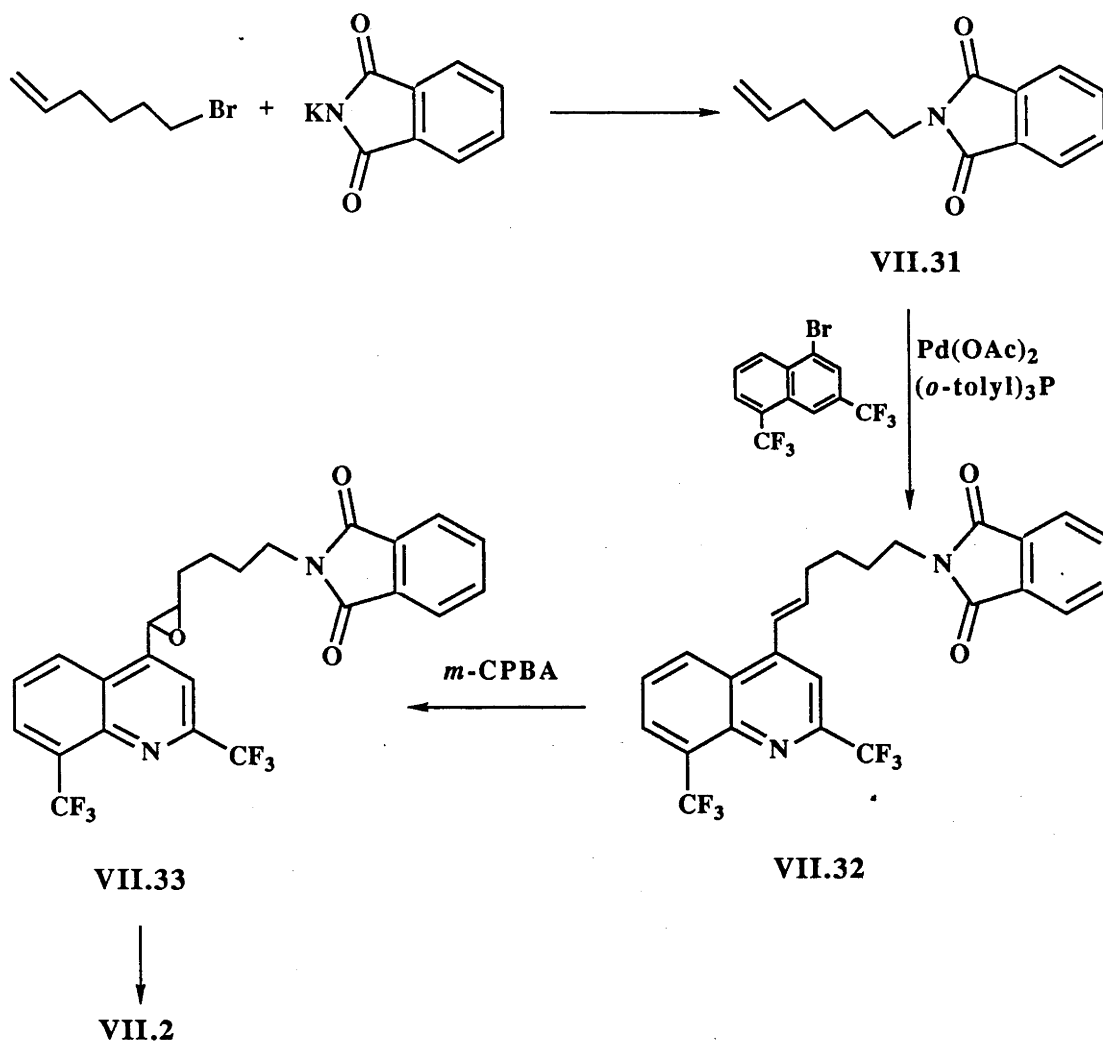
Scheme VII-9

bromoquinoline (VII.28) and its conversion in the presence of butyllithium and carbon dioxide to the quinoline-4-carboxylic acid (VII.29). Addition of 2-pyridinyl lithium to compound VII.29 afforded the pyridinyl ketone (VII.30). Reduction of VII.30 with hydrogen over platinum oxide gave good yields of compound VII.2 (Scheme VII-10).



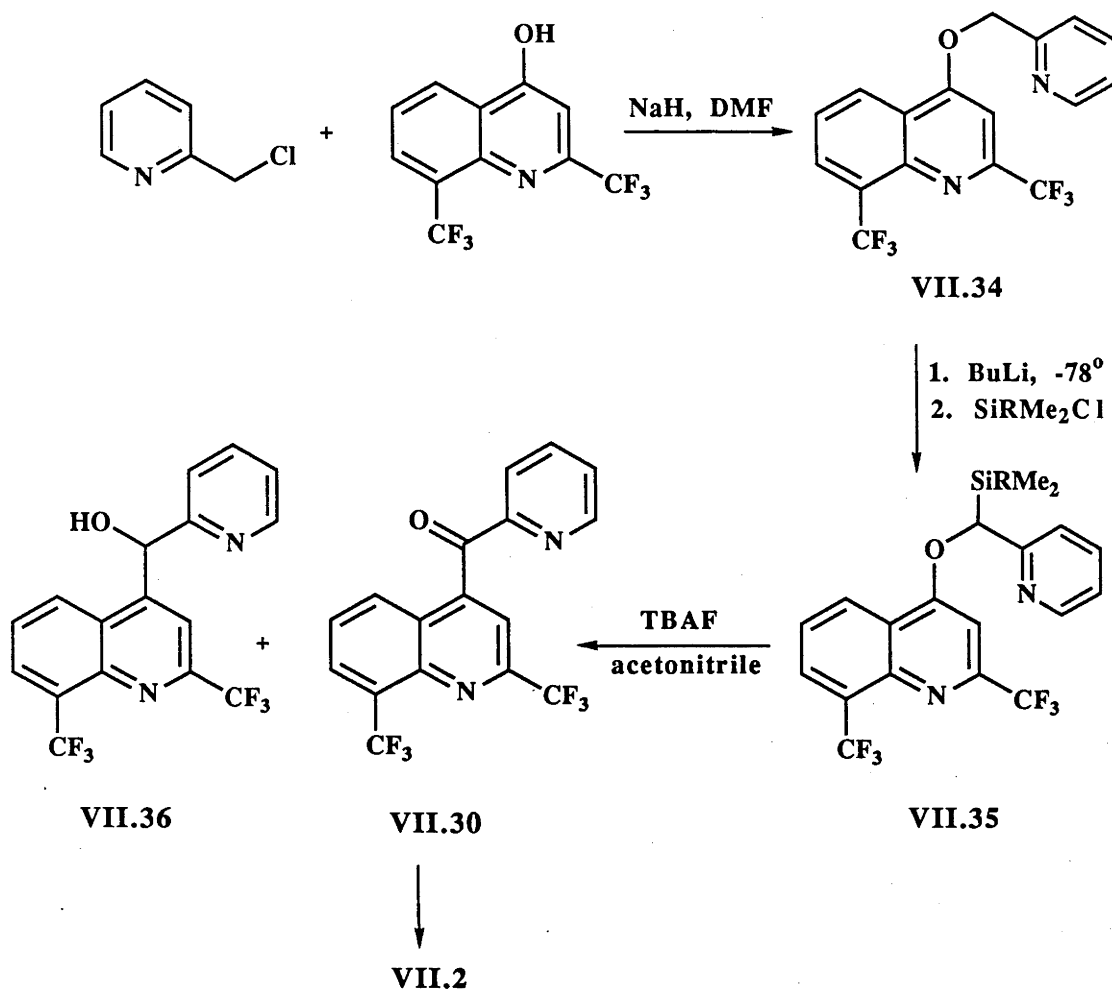
Scheme VII-10

The second synthesis²⁵⁶ (Scheme VII-11) involved the reaction of 6-bromohex-1-ene with potassium phthalimide in *N,N*-dimethylacetamide to give the *N*-(hex-5-en-1-yl) compound (**VII.31**), which was subjected to a palladium-catalysed vinylation reaction. Compound **VII.31** was condensed with 4-bromo-2,8-bis(trifluoromethyl)quinoline in the presence of tri-*o*-tolylphosphine and palladium acetate to give compound **VII.32**. Epoxidation of **VII.32** with *m*-chloroperoxybenzoic acid afforded **VII.33** and then cyclisation by treatment with hydrazine hydrate gave mefloquine (**VII.2**).



Scheme VII-11

A third synthesis of mefloquine has been reported²⁵⁷ and involved the application of the Wittig rearrangement of aryl ethers to arylcarbinols.^{258,259} The condensation of 2-chloromethylpyridine and 4-hydroxy-2,8-bistrifluoromethylquinoline¹³³ gave the ether (VII.34) in 90% yield. This ether (VII.34) was treated with butyllithium at -78° in THF and then subjected to silylation affording (after flash chromatography) compound VII.35 (Scheme VII-12). Treatment of compound VII.35 with a catalytic amounts of tetrabutylammonium fluoride (TBAF) (0.2-0.5 equivalents) in acetonitrile at room temperature generated green solutions and gave a mixture of the expected carbinol (VII.36) and the ketone (VII.30). Catalytic hydrogenation of compound VII.30 over platinum oxide gave the desired compound (VII.2).



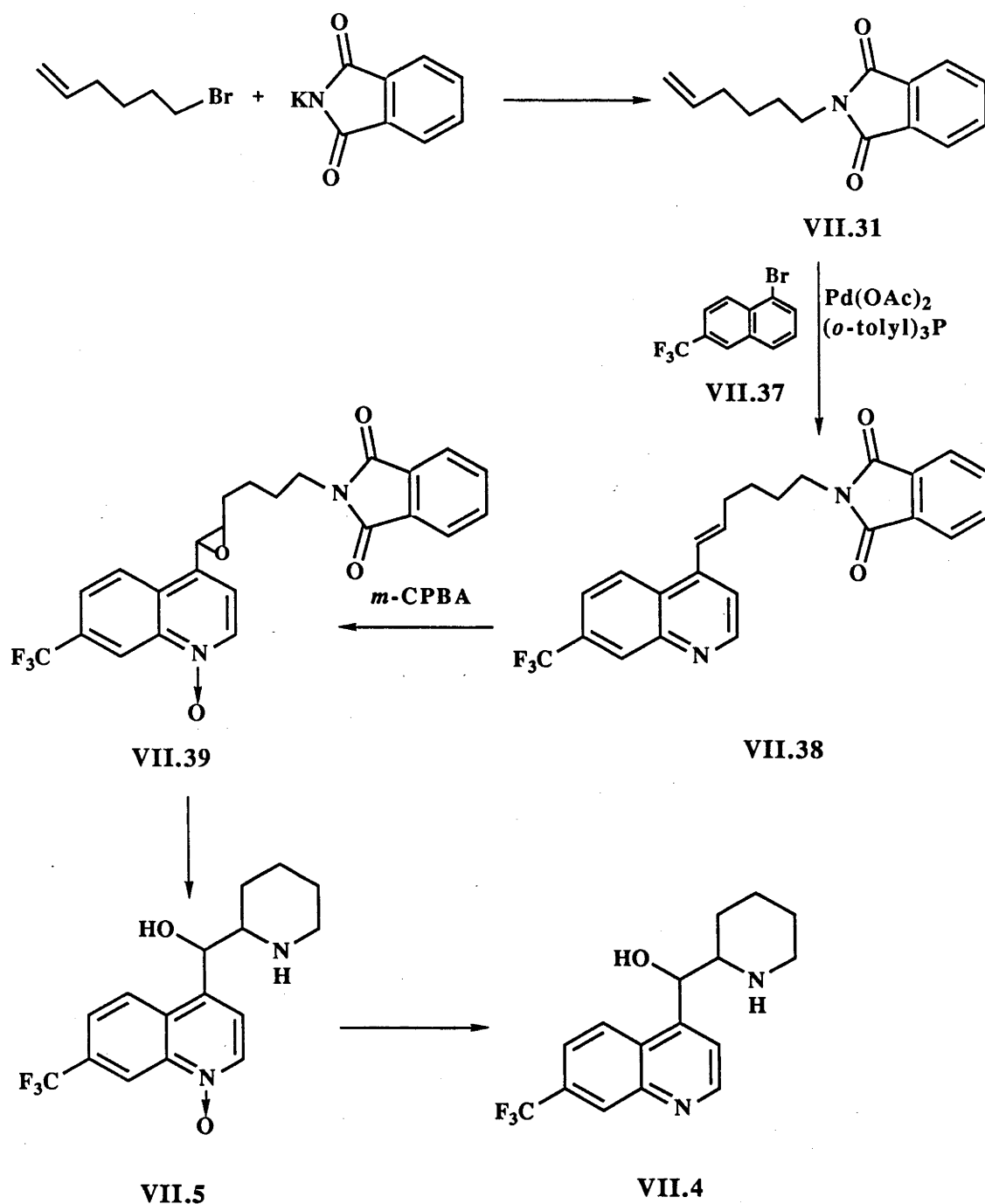
Scheme VII-12

Carrol and Blackwell²⁶⁰ tested various optically active isomers of mefloquine for antimalarial activity but found no significant differences between such isomers.

VII-5 Syntheses of α -(Piperidin-2-yl)- α -(7-trifluoromethylquinolin-4-yl)-methanol and Related Compounds

VII-5.1 α -(Piperidin-2-yl)- α -(7-trifluoromethylquinolin-4-yl)methanol

The synthesis of the above compound employed in this work is illustrated in Scheme VII-13 and the details are discussed in sections (a) and (b) below.



Scheme VII-13

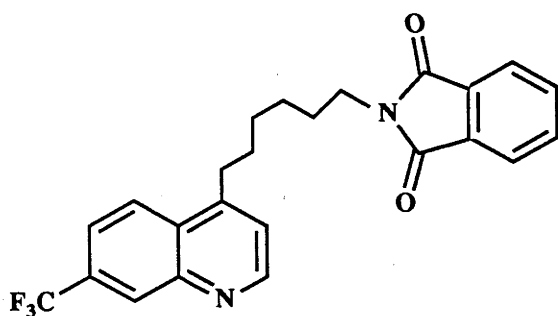
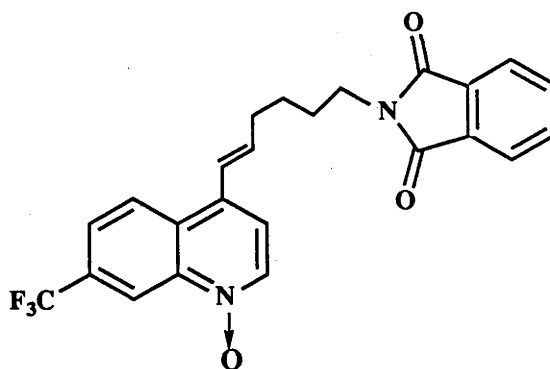
(a) *Synthesis of α -(piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)-methanol 1'-oxide (VII.5)*

6-Bromohex-1-ene with potassium phthalimide²⁶¹ in dry *N,N*-dimethylacetamide at room temperature as described by Adam,²⁵⁶ gave the vinyl compound (VII.31). This compound (VII.31) with 4-bromo-7-trifluoromethylquinoline (VII.37) [prepared by

bromination of the 4-hydroxy analogue with phosphoryl bromide at 140° for 4 h]¹⁴¹ and a mixture of palladium acetate, tri-*o*-tolylphosphine, sodium iodide and tributylamine in DMF at 100° for 17 h (a modification of literature procedures^{249,252,256} described above) gave compound **VII.38**. Surprisingly, when this reaction was carried out at 160° for 8 h the saturated compound (**VII.40**) was obtained. This palladium-catalysed reaction did not proceed when 4-chloro-7-trifluoromethylquinoline was used in place of its bromo analogue (**VII.37**).

When compound **VII.38** was treated with excess *m*-chloroperoxybenzoic acid (*ca* 3 equivalents) in refluxing chloroform, it gave compound **VII.39**; but with 1.5-2.0 equivalents of the peroxyacid at room temperature for 3 h, it gave the 1'-oxide (**VII.41**). This differed from the oxidation of compound **VII.32**²⁵⁶ in which the 2-trifluoromethyl group apparently hindered *N*-oxidation.

Hydrolysis and cyclisation of compound **VII.39** with hydrazine hydrate in methanol at reflux gave the α -(piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)methanol 1'-oxide (**VII.5**).

**VII.40****VII.41**

(b) Attempted synthesis of α -(piperidin-2-yl)- α -(7-trifluoromethylquinolin-4-yl)methanol (VII.4**)**

In the limited time available, attempts were made to remove the *N*-oxide group from compound **VII.5** by two methods. Compound **VII.5** with phosphorus trichloride²⁶² in chloroform at reflux for 4 h gave a product differing in physical properties from **VII.5**. It

had different R_f values on tlc (alumina; 5% methanol-chloroform), different ^1H n.m.r. spectrum and mass spectrum, and its analysis approached the calculated values for compound **VII.4**. Another attempt to remove the *N*-oxide group by hydrogenation²⁶³ of compound **VII.5** in saturated methanolic hydrogen chloride gave mixtures of products.

VII-5.2 Attempted syntheses of α -[(7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-yl)- α -(piperidin-2-yl)methanols

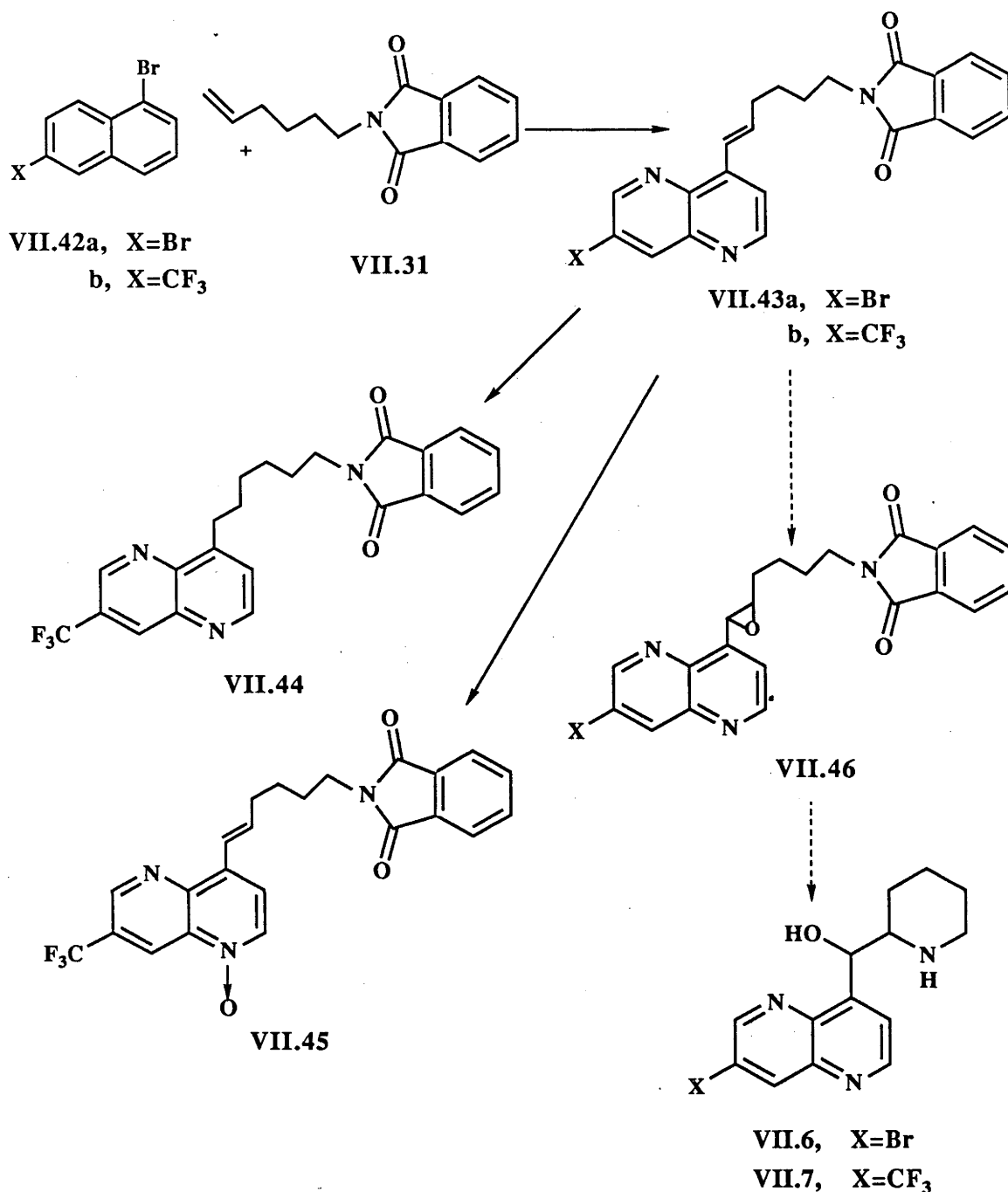
In the limited time available, and with the difficulties encountered in the syntheses described in Chapter VII-5.1 and common to this Section (VII-5.2), it was not possible to complete the syntheses of the above named compounds. The synthetic approaches employed and envisaged are shown in Scheme VII-14; some of these are described in detail below.

(a) Syntheses of *N*-[6-(7-substituted-1,5-naphthyridin-4-yl)hex-5-en-1-yl]phthalimides

Palladium-catalysed vinylation of compound **VII.31** with 4-bromo-7-trifluoromethyl-1,5-naphthyridine (**VII.42b**) (prepared from the 4-hydroxy compound with phosphoryl bromide at 140° for 4 h) in the presence of a mixture of palladium acetate, tri-*o*-tolylphosphine and tributylamine in hexamethylphosphoramide (HMPA) at 100° for 12 h gave compound **VII.43b**.

However when the reaction was carried out at 150° for 15 h the hexylphthalimide derivative (**VII.44**) was obtained as the major product. Mixtures of compounds **VII.43b** and **VII.44** proved difficult to separate by PTLC.

4,7-Dibromo-1,5-naphthyridine (**VII.42a**) (prepared from the 4-hydroxy compound with phosphoryl bromide) with compound **VII.31**, palladium acetate, tri-*o*-tolylphosphine, tributylamine and sodium iodide in dimethylformamide (DMF) at 100° for 21.5 h afforded compound **VII.43a**.



Scheme VII-14

(b) *Synthesis of N-[6-(1-oxido-7-trifluoromethyl-1,5-naphthyridin-4-yl)-hex-5-en-1-yl]phthalimide (VII.45)*

Compound VII.43b was treated with *m*-chloroperoxybenzoic acid (1.5 equivalents) in chloroform at 65° for 2 h to give compound VII.45 by oxidation at the ring nitrogen atom. The required intermediate epoxide (VII.46, X=CF₃) was not obtained.

This difference in behavior of compound **VII.43b** relative to compound **VII.32** (Scheme VII-11) is obviously related to the presence of the 2-trifluoromethyl group in the latter compound.

VII-6 Physical Properties

VII-6.1 ^1H Nuclear magnetic resonance spectra

The ^1H n.m.r. spectrum of *N*-(hex-5-en-1-yl)phthalimide (**VII.31**) is shown in Figure VII-1, A. The assignments of *N*-allylic side chain protons were confirmed by means of proton-proton decoupling (Figures VII-1 and VII-1.1; B-C and D, respectively). Spectrum B shows that irradiation of the protons at δ 2.1 (H4), the complex pattern of the peaks at δ 5.53-6.08 (H5) was changed to a doublet of doublets, and the signal at δ 1.50 was also changed. Spectrum C revealed that irradiation at δ 5.53-6.08 (H5) causes the peaks at δ 2.06 (H4) and 4.97 (H6) to change; and spectrum D shows that irradiation of the triplet at δ 3.7 (H1) causes a change in the complex peak at δ 1.50 (H2,3).

The ^1H n.m.r. spectra of the *N*-(hex-5-en-1-yl)phthalimides (**VII.38** and **VII.43**) (see the Experimental section) revealed that they existed as the *trans*-isomers with coupling constants of 16 Hz; and H6 was at lower field than H5. *Cis*-olefinic coupling constants have been reported to occur in the range 7-11 Hz and the *trans*-olefinic coupling constant in the range 12-18 Hz.²⁶⁴

Whereas the hex-5-en-1-ylphthalimides (**VII.38** and **VII.43**) exhibited olefinic protons in the region δ 6.0-7.20 (olefinic protons are reported to occur in the range δ 4.5-6.5),²⁶⁴ as expected, the hexylphthalimides (**VII.40** and **VII.44**) did not show any signals in this region. These spectral differences were of assistance in the identification of the epoxides and *N*-oxides in subsequent reactions.

The ^1H n.m.r. spectra of compound **VII.4** and its 1'-oxide (**VII.5**) revealed that, as expected from data in the pyridine series,²⁶⁵ the signal for H2' at δ 8.37 in the 1'-oxide (**VII.5**) was upfield of that in **VII.4** (δ 8.99) whereas the signal of H8' in the 1'-oxide (**VII.5**) at δ 8.98 was significantly downfield of that in compound **VII.4** at δ 8.42.

Figure VII-1 ^1H n.m.r. spectra of *N*-(hex-5-en-1-yl)phthalimide (VII.31); VII.31 in CDCl_3 (A), irradiated at 2.1 ppm (B), and irradiated at 5.75 ppm (C)

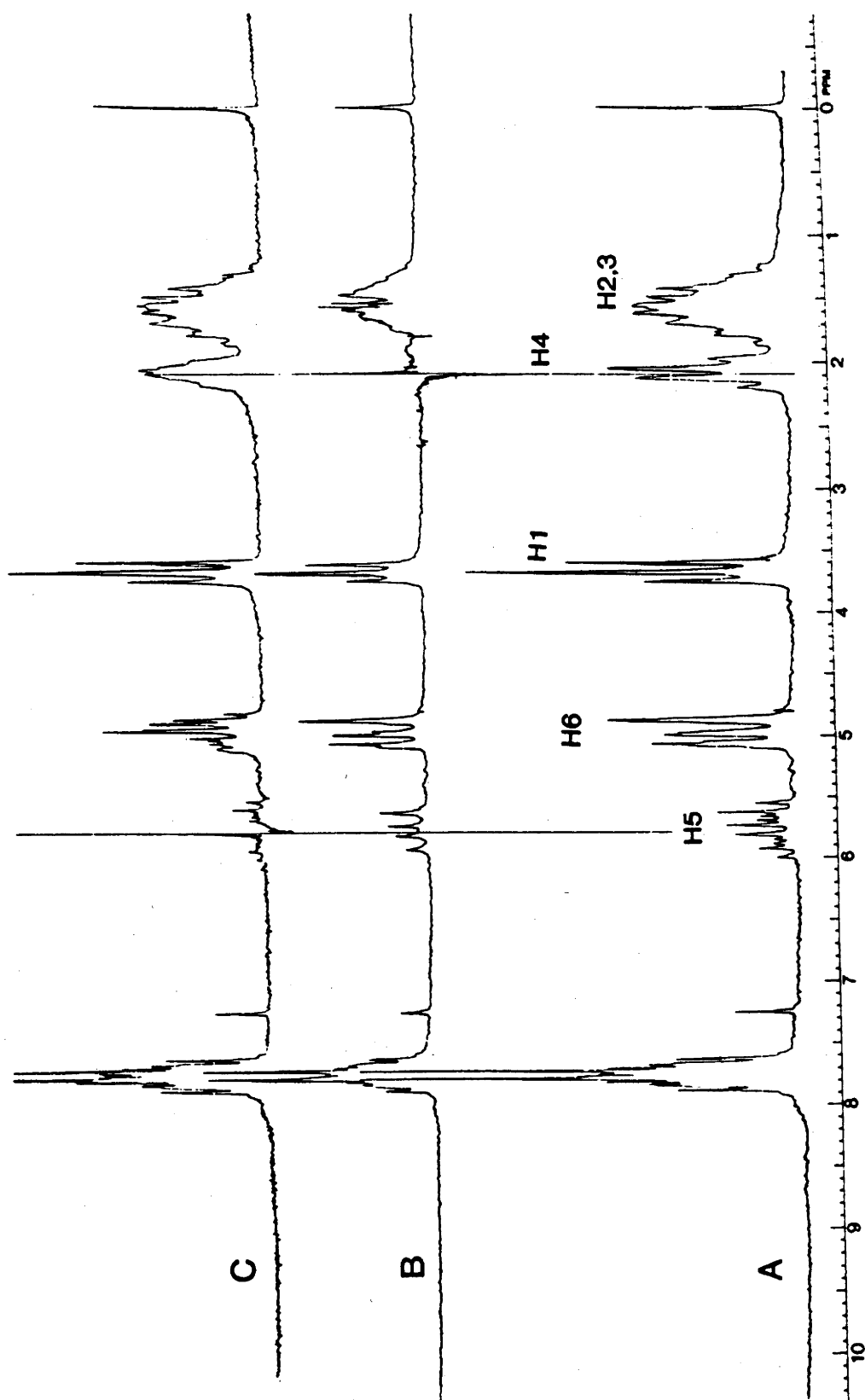
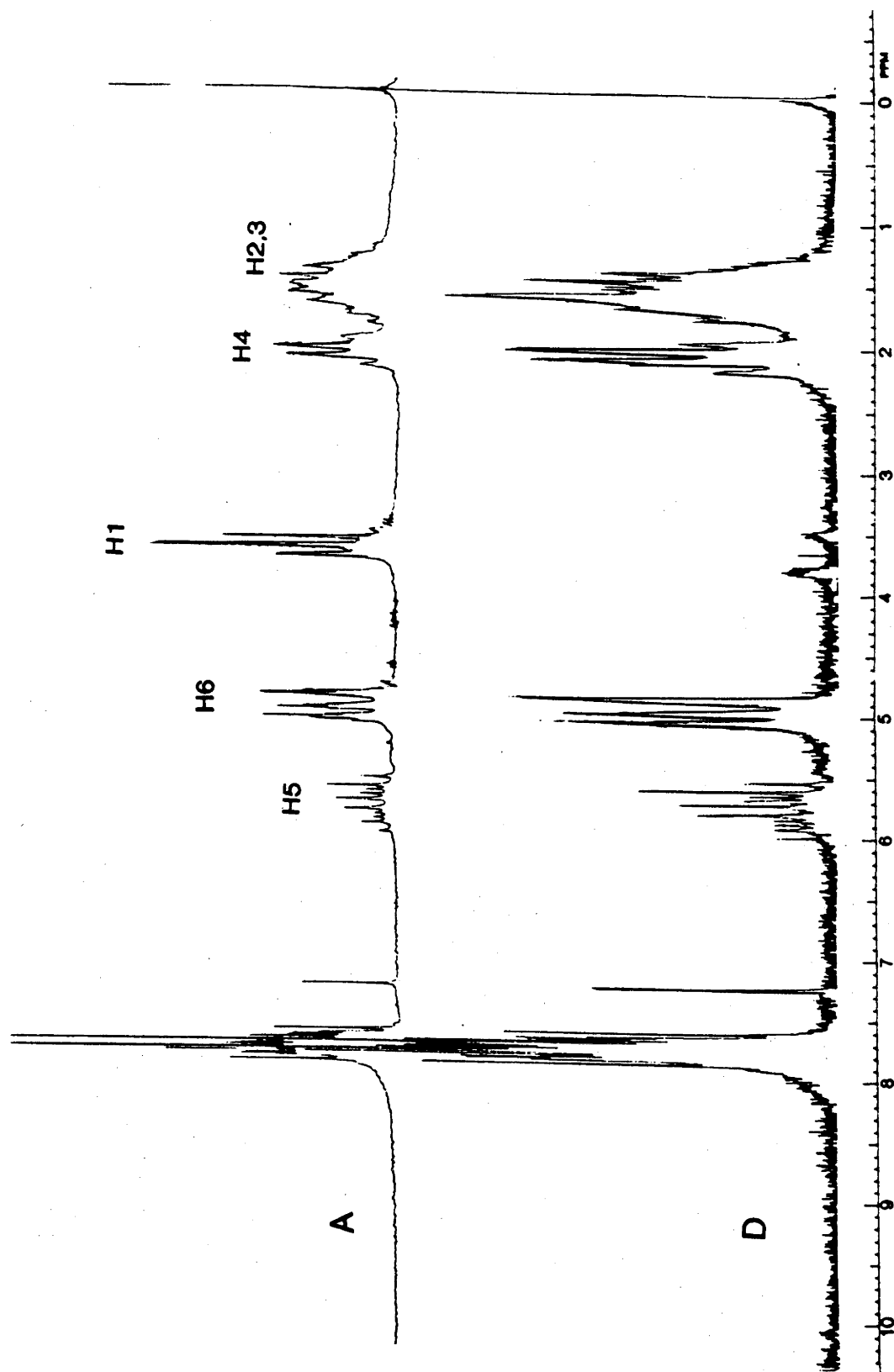


Figure VII-1.1 ^1H n.m.r. spectra of *N*-(hex-5-en-1-yl)phthalimide (VII.31); VII.31 in CDCl_3 (A), irradiated at 3.65 ppm (D)



VII-6.2 ^{13}C Nuclear magnetic resonance spectra

The assignment of the ^{13}C n.m.r. spectral data for α -(piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)methanol (VII.4) and its 1'-oxide (VII.5) are shown in Figures VII-2 and VII-3, respectively.

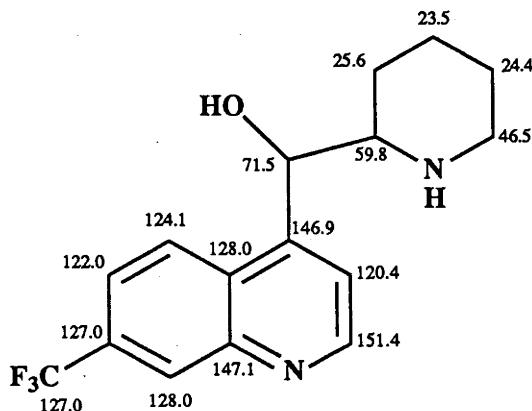


Figure VII-2 The chemical shifts (ppm) of the carbon atoms in α -(piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-ylamino)methanol (VII.4)

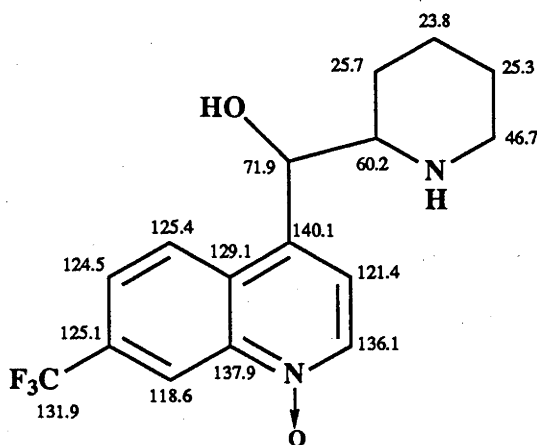


Figure VII-3 The chemical shifts (ppm) of the carbon atoms in α -(piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-ylamino)methanol 1'-oxide (VII.5)

The assignments of the ^{13}C chemical shifts for these two compounds were carried out by running decoupled ^{13}C n.m.r. spectra and 2-D HECTOR spectra. The ^{13}C chemical

shifts of carbon atoms with attached hydrogen atoms (C2',3',5',6',8'; C2,3,4,5,6; and C- α) were assigned from the HETCOR spectrum; the carbon of the CF₃ group of VII.5 appeared as a quartet (carbon-fluorine coupling); and the remaining carbon atoms were assigned similarly to compound VI.5e.

VII-6.3 Mass spectra

The mass spectra of *N*-[6-(7'-trifluoromethylquinolin-4'-yl)hex-5-en-1-yl]-phthalimide (VII.38) and the 7-bromo-1,5-naphthyridine analogue (VII.43a) were recorded (see Experimental section). The spectra showed the molecular ion peaks at *m/z* 424 and 435 (associated with a peak at *m/z* 437; ratio 1:1), respectively. Major fragmentations of these two compounds involved C-C bond cleavages between C1 and C4 to give the fragments at *m/z* M-160, M-174, M-188 and M-202. The loss of fluorine (mass 19) and bromine substituents (mass 79) were also observed for compounds VII.38 and VII.43a to give fragments at *m/z* 405 and 356.

Surprisingly, the pattern of fragmentations of the oxiran *N*-oxide (VII.39) are different from those mentioned above; the stable fragments were *m/z* 160 (100%) and 211 (87%), which are probably due to the loss of *N*-methylenephthalimide (C₉H₆NO₂) and 4-methyl-7-trifluoromethylquinoline (C₁₁H₈F₃N) fragments. The spectrum of compound VII.39 showed the molecular ion peak at *m/z* 456, and the loss of oxygen (cleavage of the N-O bond)¹⁸² and carbon dioxide (from the phthalimide group)^{266,267} at *m/z* 440 (12%) and 412 (2%), respectively (alternatively it may involve loss of oxygen followed by loss of carbon monoxide from the phthalimido group).

The molecular ion peaks for α -(piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)methanol (VII.4) and its *N*-oxide (VII.5) were not observed in their electron impact mass spectra, but the chemical ionization spectra [CI(NH₃)] showed peaks due to the addition of a proton (MH⁺ peak) at *m/z* 311 and 327, respectively. The simultaneous loss of oxygen and water from compound VII.4 gave peaks at *m/z* 295 (88%) and 293 (48%), and from compound VII.5 gave peaks at *m/z* 311 (50%) and 309 (13%).

VII-7 Experimental

4-Bromo-7-trifluoromethylquinoline (VII.37)

7-Trifluoromethylquinolin-4-ol (4.0 g) and phosphoryl bromide (8.0 g) were heated at 140° for 4 h, cooled, and the residue was treated with ice and then with saturated sodium bicarbonate solution until it was alkaline. The grey precipitate was filtered off, washed with water, air dried and then recrystallised from isopropyl alcohol-water to give colourless needles of *4-bromo-7-trifluoromethylquinoline* (4.7 g), m.p. 72-73° (Found, for a sample dried at 20°/0.2 mmHg for 3 h: C, 43.6; H, 1.6; Br, 28.5; N, 4.8. $C_{10}H_5BrF_3N$ requires C, 43.5; H, 1.8; Br, 28.9; N, 5.1%). 1H n.m.r. δ 7.82, dd, $J_{5,6}$ 9 Hz; $J_{6,8}$ 2 Hz, H6; 7.82, d, $J_{2,3}$ 4.5 Hz, H3; 8.34, d, $J_{5,6}$ 9 Hz, H5; 8.43, br s, H8; 8.77; d, $J_{2,3}$ 4.5 Hz, H2.

4,7-Dibromo-1,5-naphthyridine (VII.42a)

7-Bromo-1,5-naphthyridine-4-ol (5.0 g) and phosphoryl bromide (20.0 g) were allowed to react as above. After neutralisation with 0.9 N ammonium hydroxide, the resulting brown solid was filtered off and recrystallised from heptane; to give colourless needles of the *title compound* (4.48 g), m.p. 190-192° (Found: C, 33.6; H, 1.2; N, 9.6. $C_8H_4Br_2N_2$ requires C, 33.4; H, 1.4; N, 9.7%). 1H n.m.r. δ 7.99, d, J 4.5 Hz; H3; 8.61, d, J 2 Hz, H8; 8.74, d, J 4.5 Hz, H2; 9.07, d, J 2 Hz, H6.

4-Bromo-7-trifluoromethyl-1,5-naphthyridine (VII.42b)

7-Trifluoromethyl-1,5-naphthyridin-4-ol (1.0 g) and phosphoryl bromide (2.0 g) were allowed to react as above. The crude product was purified by column chromatography (alumina; benzene) and the product (0.916 g) was recrystallised from heptane to give colourless needles of *4-bromo-7-trifluoromethyl-1,5-naphthyridine*, m.p. 139-141° (Found, for a sample dried at 20°/0.2 mmHg for 19 h: C, 39.0; H, 1.4; Br, 28.7; N, 10.1. $C_9H_4BrF_3N_2$ requires C, 39.0; H, 1.5; Br, 28.8; N, 10.1%). 1H n.m.r. δ 8.11, d, J 4.5 Hz, H3; 8.73, br s, H8; 8.86, d, J 4.5 Hz, H2; 9.27, d, J 2 Hz, H6.

N-(Hex-5-en-1-yl)phthalimide²⁵⁶ (VII.31)

Potassium phthalimide²⁶¹ (1.34 g) and 6-bromohex-1-ene (0.9 ml) in dry *N,N*-dimethylacetamide (9.0 ml; dried over BaO and then distilled) were stirred at room

temperature for 20 h. The mixture was extracted with ether and the crude product (1.56 g) was subjected to column chromatography [alumina; 5% ethyl acetate in light petroleum (b.p. 60-80°)] and gave the title compound (1.320 g), m.p. 27-28° (Found, for a sample dried at 20°/0.2 mmHg for 18 h: C, 73.1; H, 6.9; N, 5.8. Calc. for $C_{14}H_{15}NO_2$: C, 73.3; H, 6.6; N, 6.1%). 1H n.m.r. δ 1.50, complex, H_{2,3}; 2.06, complex, H₄; 3.70, t, J 6 Hz, H₁; 4.95, d, J_{6,6} 9 Hz; 4.99, d, J_{5,6} 16 Hz, H₆; 5.53-6.08, complex, H₅; 7.78, complex, ArH.

***N*-[6-(7'-Trifluoromethylquinolin-4'-yl)hex-5-en-1-yl]phthalimide (VII.38)**

4-Bromo-7-trifluoromethylquinoline (2.3 g), *N*-(hex-5-en-1-yl)phthalimide (2.1 g), palladium acetate (0.020 g), tri-*o*-tolylphosphine (0.053 g), sodium iodide (0.030 g) and tributylamine (2.0 ml) in dimethylformamide (3.0 ml) were heated at 100° under nitrogen for 17 h. The residue was diluted with water, extracted with ethyl acetate, and the extract washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, water, and then dried (Na_2SO_4). The solvent was evaporated and the product subjected to column chromatography (alumina; 10-15% ethyl acetate in hexane) and recrystallised from ethanol to give white crystals of the *title compound* (0.719 g), m.p. 103.5-105° (Found: C, 67.8; H, 4.7; N, 6.5. $C_{24}H_{19}F_3N_2O_2$ requires C, 67.9; H, 4.5; N, 6.6%). 1H n.m.r. δ 1.70, complex, H_{2,3}; 2.44, complex, H₄; 3.76; t, J 7 Hz, H₁; 6.47, dt, J_{5,6} 16 Hz, J_{4,5} 7 Hz, H₅; 7.08, d, J_{5,6} 16 Hz, H₆; 7.51, d, J_{2',3'} 5 Hz, H_{3'}; 7.75, complex, phthalimido H and H_{6'}; 8.23, d, J_{5',6'} 9 Hz, H_{5'}; 8.39, br s, H_{8'}; 8.91, d, J_{2',3'} 5 Hz, H_{2'}. MS m/z 424 (M^+) (60%), 405 (7%), 277 (12%), 264 (31%), 250 (100%), 236 (29%), 211 (77%), 160 (81%), 147 (2%).

***N*-{4-[3'-(1''-Oxido-7''-trifluoromethylquinolin-4''-yl)oxiran-2'-yl]but-1-yl}phthalimide (VII.39)**

To *N*-[6-(7-trifluoromethylquinolin-4-yl)hex-5-en-1-yl]phthalimide (0.261 g) in chloroform (3.0 ml), *m*-chloroperoxybenzoic acid (*m*-CPBA) (80%; 0.422 g) was added; the mixture was refluxed at 70° for 5 h. A white precipitate (0.075 g) was filtered off, and the filtrate was washed with 10% sodium sulfite solution, saturated sodium bicarbonate,

10% sodium chloride, and the chloroform evaporated to give the crude product. This product was subjected to PTLC (alumina; 2.5% methanol-chloroform) and recrystallised from methanol to afford white crystals of the *title compound* (0.135 g), m.p. 146-147.5° (Found: C, 63.1; H, 4.2; N, 5.9. $C_{24}H_{19}F_3N_2O_4$ requires C, 63.2; H, 4.2; N, 6.1%). 1H n.m.r. δ 1.82, complex, H_{2,3,4}; 2.97, dt, J_{2',3'} 2 Hz, J_{2',4} 5 Hz, H_{2'}; 3.77, t, J 6 Hz, H₁; 4.18, d, J_{2',3'} 2 Hz, H_{3'}; 7.35, d, J_{2'',3''} 6 Hz, H_{3''}; 7.80, complex, phthalimido H and H_{6''}; 8.23, d, J_{5'',6''} 9 Hz, H_{5''}; 8.53, d, J_{2'',3''} 6 Hz, H_{2''}; 9.13, br s, H_{8''}. MS m/z 457 (M⁺+1) (2%), 456 (M⁺) (7%), 440 (12%), 412 (2%), 211 (87%), 160 (100%).

α -(Piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)methanol 1'-oxide
(VII.5)

N-{4-[3'-(1''-Oxido-7''-trifluoromethylquinolin-4''-yl)oxiran-2'-yl]but-1-yl}phthalimide (0.147 g) and hydrazine hydrate (0.10 ml) in methanol (1.5 ml) were refluxed at 90-95° for 6 h. The solvent was evaporated and the residue warmed with acetone and the white solid was filtered off. The filtrate was evaporated and the residue subjected to PTLC (alumina; 5% methanol-chloroform) and recrystallised from ethanol to afford white crystals of the *title compound* (0.045 g), m.p. 136-138° (Found: C, 58.8; H, 5.2; N, 8.2. $C_{16}H_{17}F_3N_2O_2$ requires C, 58.9; H, 5.2; N, 8.6%). 1H n.m.r. δ 1.27, 1.52, 1.82, complex, H_{3,4,5}; 2.66, 3.04, complex, H_{2,6}; 5.23, d, J 4.5 Hz, CHOH; 7.56, d, J_{2',3'} 6 Hz, H_{3'}; 7.68, dd, J_{5',6'} 9 Hz, J_{6',8'} 2 Hz, H_{6'}; 8.12, d, J_{5',6'} 9 Hz, H_{5'}; 8.37, d, J_{2',3'} 6 Hz, H_{2'}; 8.98, br s, H_{8'}. MS m/z 327 (MH⁺) (33%), 311 (50%), 309 (13%), 242 (17%), 226 (100%).

α -(Piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)methanol (VII.4)

α -(Piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)methanol 1'-oxide (0.025 g) and phosphorus trifluoride (0.1 ml) in chloroform (1.0 ml) were refluxed for 4 h and the solvent was evaporated. The residue was treated with ice and then 0.9 N ammonium hydroxide solution until it was neutral; the mixture was extracted with chloroform, the extract was washed with 10% sodium chloride solution, dried (Na₂SO₄), and the solvent evaporated. The product was subjected to PTLC (alumina; 8% methanol-chloroform) and

recrystallised from ethanol-cyclohexane to afford the off-white crystals of the *title compound* (0.009 g), m.p. 151-154° (Found: C, 61.3; H, 5.7; N, 8.5. $C_{16}H_{17}F_3N_2O$ requires C, 61.9; H, 5.5; N, 9.0%). 1H n.m.r. δ 1.15, 1.53, 1.77, complex, H_{3,4,5}; 2.70, t, 3.08, complex, H_{2,6}; 3.59, br s, NH; 5.41, d, J 4 Hz, CH-OH; 7.66, d, J 9 Hz, H_{6'}; 7.77, d, J 5 Hz, H_{3'}; 8.11, d, J 9 Hz, H_{5'}; 8.42, s, H_{8'}; 8.99, d, J 5 Hz, H_{2'}. MS m/z 311 (MH^+) (53%), 295 (89%), 293 (48%), 288 (96%), 212 (100%).

***N*-[6-(7'-Trifluoromethyl-1',5'-naphthyridin-4'-yl)hex-5-en-1-yl]phthalimide (VII.43b)**

4-Bromo-7-trifluoromethyl-1,5-naphthyridine (0.174 g), *N*-(hex-5-en-1-yl)phthalimide (0.144 g), tri-*o*-tolylphosphine (0.050 g), palladium acetate (0.008 g) and tributylamine (0.15 ml) in hexamethylphosphoramide (HMPA) (1.0 ml) were heated in a Teflon-lined reaction vessel at 100° for 12 h. The mixture was then diluted with ethyl acetate and washed twice with water, dried (Na_2SO_4) and the solvent evaporated. The product (0.393 g) was subjected to PTLC (alumina; 14% ethyl acetate-hexane) and recrystallised from ethanol to give the white crystals of the *title compound* (0.047 g), m.p. 137-138° (Found: C, 64.7; H, 4.1; N, 9.6. $C_{23}H_{18}F_3N_3O_2$ requires C, 64.9; H, 4.3; N, 9.9%). 1H n.m.r. δ 1.82, complex, H_{2,3}; 2.49, q, H₄; 3.75, t, J 6 Hz, H₁; 6.80, dt, J 16 Hz, H₅; 7.58, d, J 16 Hz, H₆; 7.78, complex, phthalimido H and H_{3'}; 8.68, br s, H_{8'}; 8.97, d, J 5 Hz, H_{2'}; 9.14, d J 2 Hz, H_{6'}.

***N*-[6-(1'-Oxido-7'-trifluoromethyl-1',5'-naphthyridin-4'-yl)hex-5-en-1-yl]-phthalimide (VII.45)**

N-[6-(7'-Trifluoromethyl-1',5'-naphthyridin-4'-yl)hex-5-en-1-yl]phthalimide (0.092 g) and *m*-chloroperoxybenzoic acid (80%; 0.050 g) in chloroform (1.5 ml) were heated at 65° for 2 h. The mixture was washed with 10% sodium sulfite solution, saturated sodium bicarbonate and 10% sodium chloride solution. The extract was dried (Na_2SO_4), and the solvent evaporated. The crude product was subjected to PTLC [alumina; 64% chloroform-petroleum ether (b.p. 60-80°)] and recrystallised from methylene chloride-ethanol to give the *title compound* (0.055 g), m.p. 145-148° (Found: C, 62.5; H, 4.4; N,

9.4. $C_{23}H_{18}F_3N_3O_3$ requires C, 62.6; H, 4.1; N, 9.5%). 1H n.m.r. δ 1.75, complex, H2,3; 2.47, complex, H4; 3.76, t, J 6 Hz, H1; 6.64, dt, J 16 Hz, H5; 7.47, d, J 16 Hz, H6; 7.79, complex, phthalimido H and H3'; 8.54, d, J 7 Hz, H2'; 9.20, br s, H6'; 9.32, br s, H8'.

***N*-[6-(7'-Bromo-1',5'-naphthyridin-4'-yl)hex-5-en-1-yl]phthalimide (VII.43a)**

4,7-Dibromo-1,5-naphthyridine (0.187 g), *N*-(hex-5-en-1-yl)phthalimide (0.179 g), palladium acetate (0.009 g), tri-*o*-tolylphosphine (0.050 g), sodium iodide (0.021 g) and tributylamine (0.3 ml) in *N,N*-dimethylformamide (1.5 ml) were heated at 100° under nitrogen for 21.5 h. The mixture was diluted with ethyl acetate, then washed with water, dried (Na_2SO_4) and evaporated. The crude product was purified by PTLC (alumina; 20% ethyl acetate-hexane) and recrystallised from hot ethanol to give the white crystals of the *title compound* (0.045 g), m.p. 119-121° (Found: C, 60.6; H, 3.9; Br, 18.3; N, 9.5. $C_{22}H_{18}BrN_3O_2$ requires C, 60.6; H, 4.2; Br, 18.3; N, 9.6%). 1H n.m.r. δ 1.75, complex, H2,3; 2.44, complex, H4; 3.75, t, J 6 Hz, H1; 6.78, dt, J 16 Hz, H5; 7.60, d, J 16 Hz, H6; 7.78, complex, phthalimido H and H3'; 8.60, d, J 2 Hz, H8'; 8.86, d, J 5 Hz, H2'; 8.96, d, J 2 Hz, H6'. MS m/z 437, 435 (M^+) (73, 75%), 356 (18%), 290 (13%), 288 (15%), 277 (15%), 275 (19%), 263 (33%), 261 (36%), 249 (92%), 247 (100%), 235 (56%), 233 (55%), 160 (46%).

***N*-[6-(7'-Trifluoromethylquinolin-4'-yl)hexyl]phthalimide (VII.40)**

4-Bromo-7-trifluoromethylquinoline (0.199 g), *N*-(hex-5-en-1-yl)phthalimide (0.163 g), tri-*o*-tolylphosphine (0.040 g), palladium acetate (0.007 g) and tributylamine (0.17 ml) in HMPA (1.0 ml) were heated in Teflon-lined vessel at 160° for 8 h. The mixture was diluted with ethyl acetate, then washed with water, 10% sodium chloride solution, dried (Na_2SO_4) and the solvent evaporated. The crude product was subjected to PTLC (alumina; 10% ethyl acetate-hexane) and recrystallised from ethanol to give white crystals of *N*-[6-(7'-trifluoromethylquinolin-4'-yl)hexyl]phthalimide, m.p. 92-93° (Found, for a sample dried at 20°/0.2 mmHg for 19 h: C, 67.5; H, 5.0; N, 6.4. $C_{24}H_{21}F_3N_2O_2$

requires C, 67.6; H, 5.0; N, 6.6%). ^1H n.m.r. δ 1.50, complex, H_{3,4}; 1.71, complex, H_{2,5}; 3.10, t, J 9 Hz, H₆; 3.70, t, J 9 Hz, H₁; 7.35, d, J 4 Hz, H_{3'}; 7.78, complex, phthalimido H and H_{6'}; 8.16, d, J 9 Hz, H_{5'}; 8.43, br s, H_{8'}; 8.90, br d, J 4 Hz, H_{2'}.

***N*-[6-(7'-Trifluoromethyl-1',5'-naphthyridin-4'-yl)hexyl]phthalimide
(VII.44)**

4-Bromo-7-trifluoromethyl-1,5-naphthyridine (0.174 g), *N*-(hex-5-en-1-yl)phthalimide (0.144 g), tri-*o*-tolylphosphine (0.053 g), palladium acetate (0.011 g) and tributylamine (0.15 ml) in HMPA (1.0 ml) were allowed to react as for the preparation of VII.40 above, but heated at 150° for 15 h. The product was purified by PTLC (alumina; 10% ethyl acetate) and recrystallisation from ethanol to afford a white crystal of the *title compound*, m.p. 112-114° (Found: C, 64.6; H, 4.7; N, 9.8. $\text{C}_{23}\text{H}_{20}\text{F}_3\text{N}_3\text{O}_2$ requires C, 64.6; H, 4.7; N, 9.8%). ^1H n.m.r. δ 1.49, 1.78, complex, H_{2,3,4,5}; 3.31, t, J 9 Hz, H₆; 3.69, t, J 9 Hz, H₁; 7.58, d, J 5 Hz, H_{3'}; 7.77, complex, phthalimido H; 8.66, br s, H_{8'}; 8.96, d, J 5 Hz, H_{2'}; 9.15, d, J 2 Hz, H_{6'}.

CHAPTER VIII

CHAPTER VIII Biological Evaluation of Antimalarial Activity

VIII-1 Introduction to Techniques for Antimalarial Testing

Adequate techniques for the efficient evaluation of candidate compounds in malaria chemotherapy are essential; moreover the choice of laboratory methods or screening procedures can be critical. History has revealed many discrepancies between promising laboratory results and clinical utility. Several major problems may arise in the evaluation of antimalarial drugs. These include the variations in drug susceptibility among life cycle stages of the parasites and the diversity of objectives. The latter involves treatment of the acute attack, radical cure, suppression, prophylaxis, and interrupting transmission.

Various experimental models are now available to evaluate the activity of established and new potential antimalarial compounds. These test procedures include the use of avian, rodent and simian malarias; also the development of techniques for the continuous *in vitro* culture of the human malaria *Plasmodium falciparum*. The latter have provided unprecedented opportunities to advance the search for new antimalarial drugs and for the examination of their mechanism of action.

In the work described in this thesis, test compounds were evaluated first in an *in vitro* test against *P. falciparum* (in which a semi-automated microdilution technique was employed), and then some of the more active compounds were screened (after appropriate toxicity tests) for *in vivo* activity against *P. vinckei vinckei* in mice.

The applicability of a morphological technique and a visual test microtechnique to determine *in vitro* activity against *P. falciparum* has also been examined.

VII-1.1 *In vivo* evaluation of antimalarial activity

Early methods using avian malarias (e.g. *P. gallinaceum*) in chicks have now been superseded by the use of rodent malarias²⁶⁸ such as *P. berghei* and *P. yoelii* in mice. A standard test for blood schizontocidal activity against drug-sensitive or drug-

resistant isolates of *P. berghei* or *P. yoelii* was described by Peters.²⁶⁹ *P. berghei* is an excellent model for the evaluation of blood schizontocides and useful to forecast the potential drug activity against chloroquine-resistant *P. falciparum*.²⁷⁰

The rodent malaria models are often used as a primary and secondary screen before monkey malaria screens. Blood schizontocidal screens in rodent malaria are usually performed in single-dose regimens (these include the Rane test²⁷¹ and Fink-Kretschmar test²⁷²) or multiple-dose regimens (these include the early test procedures, four-day test, drug-diet methods and the six-day test as summarised by Ager²⁷³).

The discovery that the South American owl monkey (*Aotus trivirgatus*) was susceptible to *P. falciparum* and *P. vivax*, permitted new studies by Schmidt on the activity of many antimalarial compounds against various drug-resistant lines of these two parasites *in vivo*.²⁷⁴ Another South American animal, the squirrel monkey (*Saimiri sciureus*), was also shown to be susceptible to *P. falciparum*.²⁷⁵ This small animal, which is relatively easy to colonise, may replace *Aotus* as a host for antimalarial drug research on human malaria parasites.

VIII-1.2 *In vitro* evaluation of potential antimalarials

Short-term culture systems of *P. berghei* in an *in vitro* test for blood schizontocidal compounds were described by Richards and Williams in 1973.²⁷⁶ This method was based on the inhibition by the antimalarial of [³H]-leucine uptake by the parasite during a 24 h incubation. Later, Canfield *et al.*²⁷⁷ developed an *in vitro* test system involving *P. knowlesi* for screening potential antimalarial drugs. This technique was later adapted for use with *P. falciparum* and reported by Richards and Williams.²⁷⁸

The long-term (continuous) cultivation of *P. falciparum* was developed in 1976 by Trager and Jensen¹⁴ (by use of the "candle jar" technique) and by Haynes *et al.*²⁷⁹; it provided the basis for several improved procedures *in vitro* for testing the response of the human parasite, *P. falciparum* to drugs. Another *in vitro* technique, the automated culture system, developed for massive screening of potential antimalarials has been described by Desjardins *et al.*²²² This technique is an automated, computerised system,

in which the incorporation of [^3H]-hypoxanthine into the parasites nucleic acid is determined to provide a measure of parasite growth. More details are described in Chapter VIII-2.1.

For field-work, three important *in vitro* techniques have been used for determining the response of the human parasite, *P. falciparum*, to chloroquine. The original "macrotest" technique was described by Rieckmann *et al.*²⁸⁰ This test required a large quantity of parasitised blood (10-15 ml), and the criterion of drug response in this macrotest was morphological (i.e. the failure of the asexual stages to develop to schizogony) when cultured (in air) for 24 h at 38-40°. A further application of the "candle jar" technique was the "microtest" also described by Rieckmann *et al.*²⁸¹ This microtest required only 50 µl of blood which can be easily collected from a finger-prick; this simplified the *in vitro* drug-susceptibility test, particularly in young children. The third technique, reported by Rieckmann,²²³ involves a visual test and a modification of the microtest (using capillary-blood specimens). The criterion for this test involves the determination by observation of the presence or absence of dark pigmented precipitates in the wells containing various concentrations of the drug.

VIII-2 Determination of Antimalarial Activity

VIII-2.1 *In vitro* screen against *P. falciparum*

In this work, three different *in vitro* techniques have been employed for the determination of antimalarial activity against *P. falciparum*. These techniques were the [^3H]-hypoxanthine uptake method modified from the semi-automated microdilution technique described by Desjardines *et al.*²²² and others,^{98,282} the modified morphological microtest described by Rieckmann *et al.*,²⁸¹ and the visual observation of pigment precipitation.²²³

Measurements of *in vitro* antimalarial activity against the chloroquine-sensitive FCQ-27 isolate of *P. falciparum* involving uptake of [^3H]-hypoxanthine were carried out in the laboratories of Dr G.A. Butcher, Department of Zoology, Australian National

University (ANU), Canberra; and at the Army Malaria Research Unit (AMRU) (Prof. K.H. Rieckmann), Milpo, Ingleburn, NSW.

Studies involving other techniques with the FCQ-27 isolate as well as all measurements (*in vitro*) involving the chloroquine-resistant K-1 strain were made at AMRU, Ingleburn.

Parasites

The parasites used in this work were from the following sources:

FCQ-27 isolate: The FCQ-27 line of *P. falciparum* (origin: Papua New Guinea, first isolated at the Walter and Eliza Hall Institute, Melbourne)²⁸³ was routinely cultured by Dr G.A. Butcher, ANU,²⁸² and also in continuous culture at the AMRU, under the supervision of Professor K.H. Rieckmann.¹⁴

K-1 isolate: The K-1 line (origin: Kanchanaburi, Thailand, isolated in Bangkok)²⁸⁴ was maintained in routine culture at AMRU.¹⁴

(a) Incorporation of [³H]-hypoxanthine

The procedures used in this test at the ANU and AMRU were similar, as described low.

Test procedure

The compounds under the test were dissolved in ethanol and subsequently diluted in RPMI 1640 medium to the required highest drug concentration. Dilution of this solution with RPMI 1640 gave the range of drug concentrations required for the assay. Chloroquine diphosphate (as a standard) was dissolved in water. Microculture plates (Falcon, 96 well flat bottom) containing 50 µl of RPMI 1640 with or without inhibitor (test compound) and 50 µl of 2% haematocrit of unsynchronised (at Zoology, ANU) and synchronised (at AMRU, Ingleburn) parasitised erythrocytes (Group O or A) (3-5% parasitaemia) were incubated in a gas mixture (5%CO₂, 5%O₂ and 90%N₂) at 37.5°. At

18-24 h, 20 μ l of [3 H]-hypoxanthine (10 μ Ci/ml; prepared by dilution of the product of 2.8 Ci/mmol supplied by Amersham, UK) in RPMI 1640 was added to all wells and the culture continued for a further 18-24 h. The drug concentrations in the test wells ranged from 200-1.6 nM, and triplicate or quadruplicate tests with each compound were carried out.

The contents of the wells were harvested on to filters (Chemtec, Australia) with a semi-automated cell harvester (Skatron harvester), washed through with distilled water for about 15 seconds, and suctioned for a further 15 seconds. After harvesting, the filters were dried at 37° for 2 h, placed in polyethylene scintillation vials (Packard) with phase combining system (PCS) scintillation fluid (Amersham) (2.5 ml) and the radioactivity was measured on a LKB 1217 or Packard Rack Beta liquid scintillation spectrometer. This gave a measure of the incorporation of [3 H]-hypoxanthine into the nuclei acid of the parasites.

Analyses of the resulting data required application of non-linear curve fitting techniques. The data for each active compound were fitted to a generalised logistic-logarithmic function between various concentrations of inhibitor and percentage of inhibition of parasite growth.

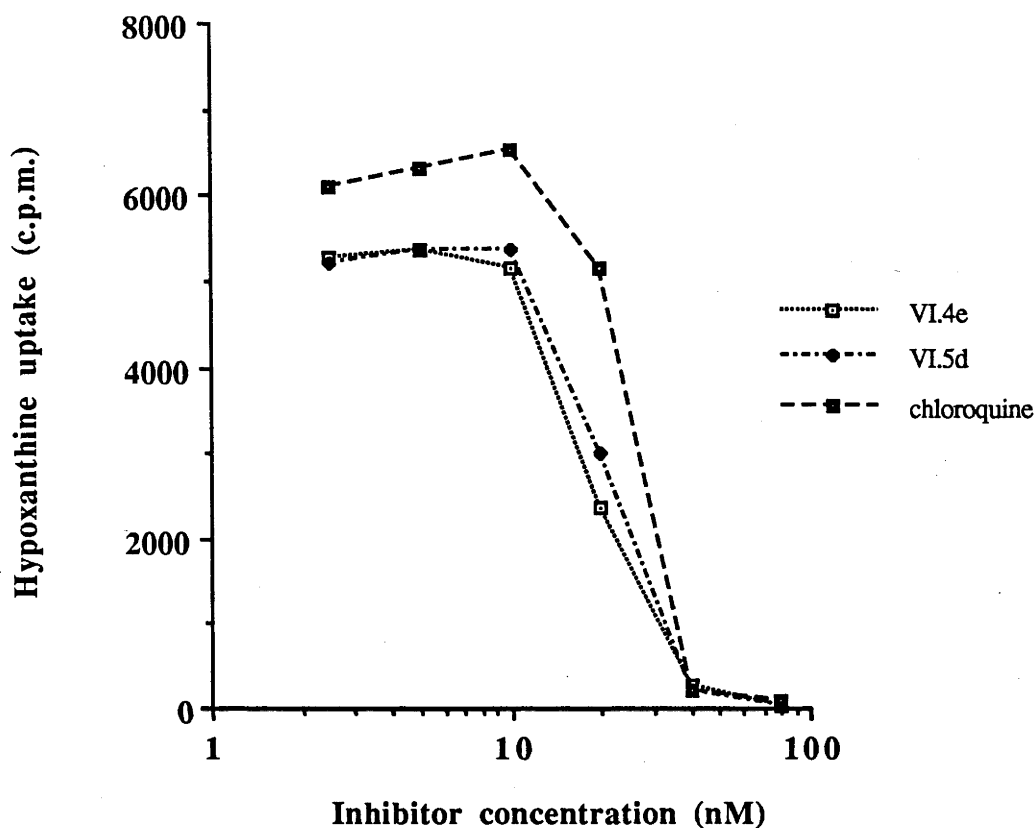
The inhibition (%) of parasite growth was simply calculated from the following expression:

$$100 - \left[\frac{\text{c.p.m. in test wells}}{\text{c.p.m. in control wells}} \right] \times 100$$

and from these results the inhibitory concentration required to cause 50% inhibition of parasite growth (IC₅₀) was calculated. In some cases IC₉₀ values were also determined. These results are presented in Tables VIII-1 to VIII-7.

In an analysis of the data, the [3 H]-hypoxanthine uptake (measured in counts per minute, c.p.m.) was plotted against the concentration of inhibitor for the test compounds VI.4e, VI.5d and chloroquine. The results are shown as sigmoidal curves in Figure VII-1, and reveal a similar response to that obtained by Desjardines *et al.*²²² in tests with chloroquine against "African-Uganda I" and "Vietnam Smith" strains of *P. falciparum* (chloroquine-sensitive and chloroquine-resistant strains, respectively).

Figure VIII-1 [^3H]-Hypoxanthine uptake in cultures of the FCQ-27 isolate of *P. falciparum* in the presence of various concentrations of di-Mannich bases (VI.4c and VI.5d) and chloroquine



(b) *Modified morphological microtest*

Tests using this technique were carried out at AMRU, Ingleburn, as described by Rieckmann.²⁸¹ Some selected compounds were tested against both the FCQ-27 and the K-1 isolates of *P. falciparum*. For this method cultures were prepared as described below, and from these thick blood films were prepared (with Romanowsky strain²⁸⁵) and examined under an oil immersion microscope. The number of parasite infected red cells (as schizonts) per 200 erythrocytes was then determined by application of the following guide lines.

The form of parasites were classified²⁸⁶ as follows: those parasites which did not yet contain any haemazoin were classified as ring forms; those parasites which contained haemazoin (dark-brown dot) with one piece of nuclear chromatin (pink dot) were classified as trophozoites; and those parasites containing haemazoin and at least two pieces of nuclear chromatin were classified as schizonts. In this work, to avoid problems due to multi-infection, schizonts were considered as containing at least three chromatin dots.

The IC₅₀ value was the concentration of the inhibitor required to reduce the number of erythrocytes containing schizonts (as revealed on the control slide) to half that value on the slide from the culture containing the inhibitor under test.

Test procedure

The compounds under test were dissolved in methanol and sequently diluted with RPMI 1640 medium. Microculture plates (as mentioned above) were prepared with each well containing 20 µl of inhibitor solution and 80 µl of innoculum [i.e. 100 µl, which contained 2% heamatocrit and 0.5% synchronised culture (at young ring stage)]. The concentration of inhibitor in these tests against the FCQ-27 and the K-1 strains of *P. falciparum* ranged from 100-1.6 nM; duplicate tests for each compound were also examined. Control wells consisted of 20 µl of RPMI 1640 medium in place of inhibitor solution; otherwise the procedure was the same. The cultures were incubated at 37.5° for 24 h, then thick film slides were prepared with Romanowsky stain²⁸⁵ and the slides were examined under the microscope using the criteria outlined above. Thus it was possible to determine the concentration of compound (inhibitor) required to produce 50% inhibition (IC₅₀) of growth of the parasite. The results from the use of this morphological technique are presented in Table VIII-8.

(c) Visual observation of pigment precipitation

A visual technique,²²³ modified by Prof. K.H. Rieckmann at AMRU, was also used in this work to determine by a simple procedure the minimum concentration at which the test compound was an effective inhibitor of the malaria parasite.

Test procedure

Culture cells were prepared essentially as described in Section VIII-2.1a above but with the following modifications.

Each well of the microculture plate contained 50 μ l of inhibitor solution and 50 μ l of inoculum (4% haematocrit, 0.5% synchronised culture at ring stage) which was then incubated at 37.5° for 24 h. The concentration of inhibitor in the culture tests were in the range 200-12.5 nM, with duplicate tests for each compound.

After incubation, 20-25 μ l of a mixture (1:1 of N sodium hydroxide and N sodium chloride) was added to each well and the plate was allowed to stand for 3-5 minutes. The plates were then shaken for 5 minutes (at this time control wells gave a greenish-yellow hue with a turbid coagulum which presumably resulted from denaturation of serum and cell proteins). It was allowed to stand for 5-30 minutes and then tapped gently. The presence of a precipitate was then clearly visible under illumination. The dark-brown precipitates were present only in wells in which parasites had developed from ring stages to schizonts (the more mature pigment-containing stages). The results for this visual test are shown in Table VIII-9 against both the FCQ-27 and the K-1 isolates.

VIII-2.2 *In vivo* screen against *P. vinckei vinckei*

In this work, *P. vinckei vinckei* was selected as the malaria parasite for use in the mouse. This parasite was isolated from Shaba, Zaire and adapted to mice in 1952. Since then it has been used successfully in numerous malaria studies.^{282,287}

Animals:

Male CBA mice (6-8 weeks old) were bred and maintained in the John Curtin School of Medical Research Animal Breeding Establishment (under specific pathogen-free conditions) and fed normal laboratory diet pellets and tap water *ad libitum*. Average body weight was 20 g.

(a) *Toxicity test in mice*

Selected compounds from each series described in Chapter II-V were tested for acute toxicity in three mice by injection intraperitoneally (ip.), each with a single dose in peanut oil at a dosage of 200 mg/kg of body weight. The results are shown in Table VIII-10.

(b) *Preliminary in vivo tests in mice*

P. vinckei vinckei (originally from Dr D. Walliker, Institute of Animal Genetics, Edinburgh) was stored frozen in liquid nitrogen and had been passaged several times before experimental use in CBA mice.

Infection of mice:

All infections were initiated by intraperitoneal injection with 10^5 or 10^6 parasitised erythrocytes, containing *P. vinckei vinckei* (obtained from an infected donor mouse and diluted in sterile saline).^{*} After 5 days (and daily thereafter) each mouse was examined for suitable parasitaemia levels (generally 10-20%). The percentage parasitaemia was monitored by thin blood smears taken from each mouse's tail vein; slides were fixed with methanol and stained with Diff-Quik stain; then examined under an oil immersion microscope. Counts of parasite-infected red blood cells were determined as the average of two or more counts in microscopic fields with approximately 200 cells per field in which few erythrocytes overlapped.

Drug treatment

Each test compound at a dosage at 200 mg/kg of body weight in 0.4 ml of peanut oil was given intraperitoneally to three mice with infection levels of preferably 10-20% and whose individual parasitaemias had just previously been determined. Thereafter, thin

^{*} Kindly supplied by Dr P.K. Halladay

blood smears were taken from each mouse at the time intervals indicated in Tables VIII-11 and VIII-12, and the parasitaemia assessed as above.

As controls, some mice were treated with peanut oil and other mice were treated with chloroquine (as diphosphate; at a dosage 40 mg/kg of body weight in normal saline).

VIII-3 Results of Antimalarial Testing

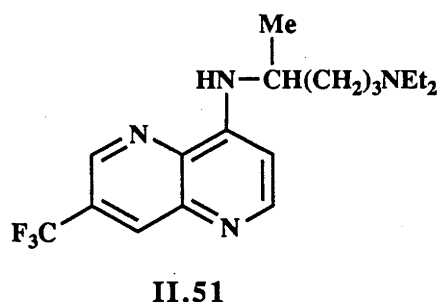
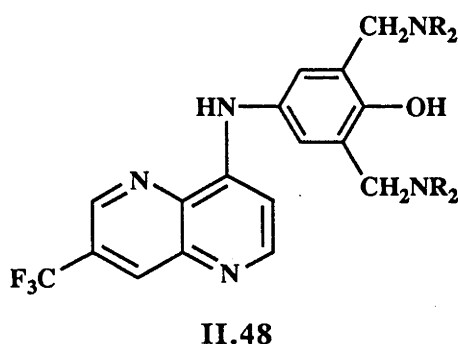
The results of preliminary testing *in vitro* against the FCQ-27 (chloroquine-sensitive) isolate of *P. falciparum* using the method of incorporation of [³H]-hypoxanthine (as described in Chapter VIII-2.1a; (at the Department of Zoology, ANU) for the compounds described in Chapters II, III, IV and V are shown in Tables VIII-1 to VIII-5.

Comparative testing results against both the FCQ-27 and the K-1 isolates (at AMRU) using the [³H]-hypoxanthine method for compounds described in Chapter VI and VII are reported in Table VII-6 and VIII-7.

Results obtained from comparative testing against the FCQ-27 and K1 isolates using morphological techniques (as described in Chapter VIII-2.1b) (at AMRU) for selected compounds prepared in Chapter II-V are presented in Table VIII-8; and those obtained using the visual techniques (see Chapter VIII-2.1c) for compounds described in Chapter VI and VII are given in Table VIII-9.

Table VIII-10 and Tables VIII-11 to VII-12 give the results for toxicity studies, and those of *in vivo* testing against *P. vinckei vinckei* in mice, respectively, for some of the more active compounds prepared in Chapter II-V.

Table VIII-1 *In vitro* antimalarial activity of some di-Mannich bases (II.48) of 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol and its chloroquine analogue (II.51) against the FCQ-27 isolate of *P. falciparum*. Literature values⁹⁸ for the corresponding 7-bromo analogues (I.43b) are also given for comparison.



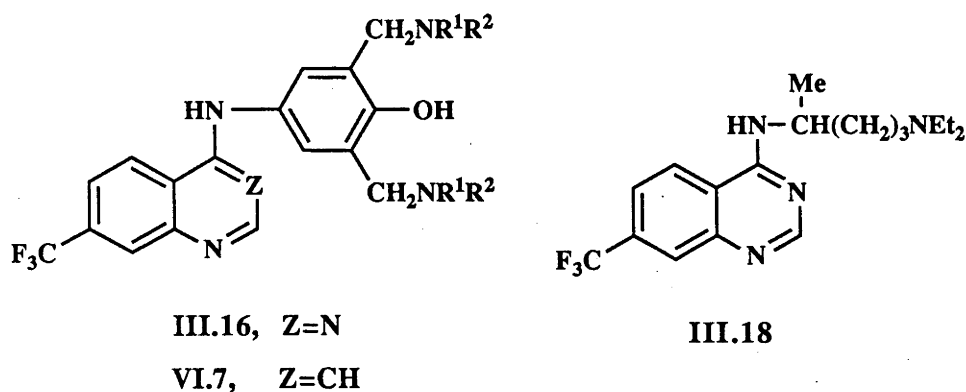
Inhibitor	IC ₅₀ ^A of II.48	CH-factor ^B	IC ₅₀ of corresponding I.43b
II.48; NR ₂ =			
a NEt ₂	12	0.9	5.4
b NPr ₂	17	0.6	3.8
c pyrrolidin-1-yl	6	1.8	3.6
d piperidin-1-yl	7.5	1.5	34.8
e 3-methylpiperidin-1-yl	6	1.8	-
f 3,5-dimethylpiperidin-1-yl	5	2.2	1.9
g 4-methylpiperidin-1-yl	8	1.4	>64
h 4-benzylpiperidin-1-yl	12	0.9	-
II.51	>200	-	-
Chloroquine ^C	11	1	13.0

^A The IC₅₀ values (the concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l⁻¹.

^B The CH-factor (Chloroquine factor) is the comparative activity of the inhibitor under test compared to chloroquine; and is the ratio of the IC₅₀ value for the inhibitor over that for chloroquine.

^C As diphosphate salt.

Table VIII-2 *In vitro* antimalarial activity of some di-Mannich bases (III.16 and VI.7) of 4-(7-trifluoromethylquinazolin-4-ylamino)phenol and 4-(7-trifluoromethylquinolin-4-ylamino)phenol against the FCQ-27 isolate of *P. falciparum*.



Inhibitor	Compounds III.16		Corresponding compounds VI.7	
	IC ₅₀ ^A	CH-factor ^B	IC ₅₀ ^A	CH-factor ^B
III.16; NR¹R²=				
a NMe ₂	>200	<0.08	-	-
b NEt ₂	150	0.1	1.3 ^C	11.0
c NPr ₂	50	0.3	1.7 ^C	8.8
d N(C ₅ H ₁₁) ₂	90	0.2	175	0.09
e NBuMe	30	0.5	10	1.5
f pyrrolidin-1-yl	30	0.5	0.9 ^C	16.7
g piperidin-1-yl	70	0.2	0.7 ^C	21.4
h 3-methylpiperidin-1-yl	12	1.3	1.6 ^D	9.4
i 4-methylpiperidin-1-yl	40	0.4	1.5	10
j 3,5-dimethylpiperidin-1-yl	20	0.8	1.4 ^D	10.7
k 4-benzylpiperidin-1-yl	40	0.4	-	-
III.18	>200	<0.08	-	-
Chloroquine ^E	15	1		

^A The IC₅₀ values (the concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l⁻¹.

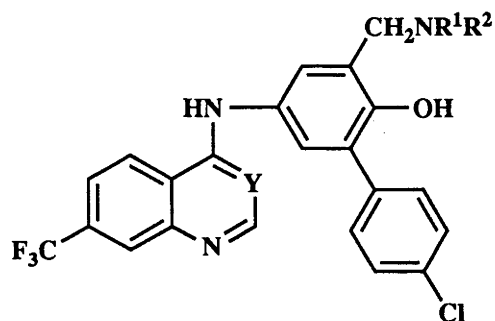
^B The CH-factor (Chloroquine factor) is the comparative activity of the inhibitor under test compared to chloroquine; and is the ratio of the IC₅₀ value for the inhibitor over that for chloroquine.

^C Data from reference 98.

^D H.V. Scott, G.B. Barlin, and S.J. Ireland, unpublished data.

^E As diphosphate salt.

Table VIII-3 *In vitro* antimalarial activity of 4'-chloro-3-(substituted amino)methyl-5-(7-trifluoromethylquinolin-4-ylamino)biphenyl-2-ols (IV.3) and 5-(7-trifluoromethylquinazolin-4-ylamino)biphenyl-2-ol analogues (IV.4) against the FCQ-27 isolate of *P. falciparum*.



IV.3; Y=CH

IV.4; Y=N

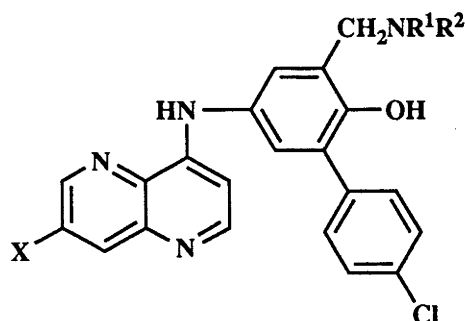
Substituent	Compounds IV.3		Compounds IV.4	
	IC ₅₀ ^A	CH-factor ^B	IC ₅₀ ^A	CH-factor ^B
NR ¹ R ² =				
a NEt ₂	20	0.6	80	0.2
b NHCMe ₃	20	0.6	20	0.6
c pyrrolidin-1-yl	8	1.5	30	0.4
d piperidin-1-yl	24	0.5	120	0.1
e 3-methylpiperidin-1-yl	22	0.5	75	0.2
f 4-methylpiperidin-1-yl	45	0.3	140	0.1
g 4-benzylpiperidin-1-yl	160	0.1	>200	<0.06
h 4-benzylpiperazin-1-yl	80	0.2	>200	<0.06
Chloroquine ^C	12	1.0		

^A The IC₅₀ values (the concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l⁻¹.

^B The CH-factor (Chloroquine factor) is the comparative activity of the inhibitor under test compared to chloroquine; and is the ratio of the IC₅₀ value for the inhibitor over that for chloroquine.

^C As diphosphate salt.

Table VIII-4 *In vitro* antimalarial activity of 4'-chloro-3-(substituted amino)methyl-5-(7-bromo-1,5-naphthylridin-4-ylamino)biphenyl-2-ols (IV.5) and 5-(7-trifluoromethyl-1,5-naphthylridin-4-ylamino)biphenyl-2-ol analogues (IV.6) against the FCQ-27 isolate of *P. falciparum*.



IV.5; X=Br

IV.6; X=CF₃

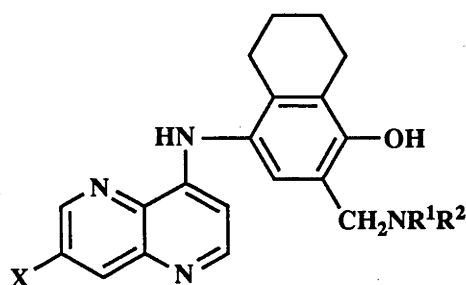
Substituent	Compounds IV.5		Compounds IV.6	
	IC ₅₀ ^A	CH-factor ^B	IC ₅₀ ^A	CH-factor ^B
NR ¹ R ² =				
a NEt ₂	60	0.20	170	0.07
b NHCMe ₃	30	0.40	50	0.24
c pyrrolidin-1-yl	70	0.17	70	0.17
d piperidin-1-yl	80	0.15	90	0.13
e 3-methylpiperidin-1-yl	90	0.13	150	0.08
f 4-methylpiperidin-1-yl	160	0.08	40	0.3
g 4-benzylpiperidin-1-yl	>200	<0.06	>200	<0.06
h 4-benzylpiperazin-1-yl	145	0.08	>200	<0.06
Chloroquine ^C	12	1.0		

^A The IC₅₀ values (the concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l⁻¹.

^B The CH-factor (Chloroquine factor) is the comparative activity of the inhibitor under test compared to chloroquine; and is the ratio of the IC₅₀ value for the inhibitor over that for chloroquine.

^C As diphosphate salt.

Table VIII-5 *In vitro* antimalarial activity of a series of mono-Mannich base derivatives of 4-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]-5,6,7,8-tetrahydronaphth-ols (V.2 and V.3, respectively) against the FCQ-27 isolate of *P. falciparum*.



V.2, X=Br

V.3, X=CF₃

Substituent	Compounds V.2		Compounds V.3	
	IC ₅₀ ^A	CH-factor ^B	IC ₅₀ ^A	CH-factor ^B
NR ¹ R ² =				
a NEt ₂	42	0.3	C	-
b NPr ₂	-	-	C	-
c NHCM ₃	7	2.0	18	0.78
d pyrrolidin-1-yl	10	1.4	60	0.23
e piperidin-1-yl	40	0.35	200	0.07
f 3-methylpiperidin-1-yl	45	0.31	C	-
g 4-methylpiperidin-1-yl	47	0.3	C	-
h 4-benzylpiperidin-1-yl	50	0.28	C	-
i 4-benzylpiperazin-1-yl	120	0.12	C	-
Chloroquine ^D	14	1.0		

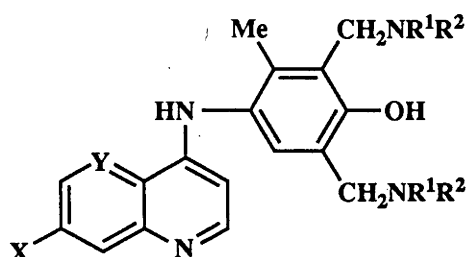
^A The IC₅₀ values (the concentration of the inhibitor required to reduce parasite growth by 50%) are expressed as nmol l⁻¹.

^B The CH-factor (Chloroquine factor) is the comparative activity of the inhibitor under test compared to chloroquine; and is the ratio of the IC₅₀ value for the inhibitor over that for chloroquine.

^C No activity at 200 nM.

^D As diphosphate salt.

Table VIII-6 *In vitro* antimalarial activity of a series of di-Mannich base derivatives (VI.4 and VI.5, and VI.6) of 4-[7-chloro (and 7-trifluoromethyl)quinolin-4-ylamino]-3-methylphenols and 4-(7-bromo-1,5-naphthyridin-4-ylamino)-3-methylphenol against the FCQ-27 and K-1 isolates of *P. falciparum*.



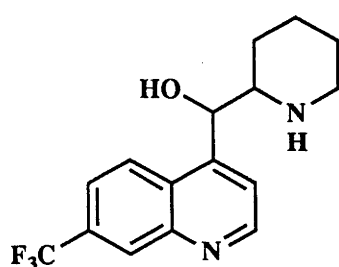
VI.4; X=Cl, Y=CH
 VI.5; X=CF₃, Y=CH
 VI.6; X=Br, Y=N

Inhibitor	FCQ-27		K-1		Resistance factor ^A
	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀	
VI.4; NR ¹ R ² =					
a NMe ₂	19	28	55	82	2.9
b NEt ₂	23	37	27	38	1.2
c NHCMe ₃	36	67	32	67	0.9
d pyrrolidin-1-yl	28	39	16	26	0.6
e piperidin-1-yl	22	36	12	19	0.5
VI.5; NR ¹ R ² =					
a NMe ₂	23	31	46	76	2.0
b NEt ₂	29	39	32	47	1.1
d pyrrolidin-1-yl	23	37	9	32	0.4
e piperidin-1-yl	24	37	12	25	0.5
VI.6; NR ¹ R ² =					
a NMe ₂	46	73	53	78	1.2
b NEt ₂	41	58	45	71	1.1
c NHCMe ₃	6	28	47	77	7.8
d pyrrolidin-1-yl	23	44	32	82	1.4
e piperidin-1-yl	30	69	28	88	1.0
Chloroquine ^B	23	31	221	304	10

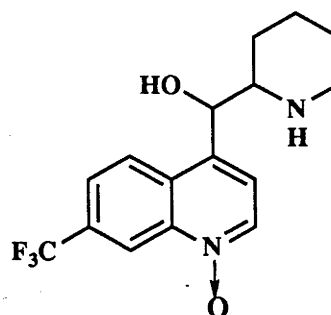
^A Resistance factor is the IC₅₀ value against the K-1 isolate divided by that for the FCQ-27 isolate.

^B As diphosphate salt.

Table VIII-7 *In vitro* antimalarial activity of α -(piperidin-2-yl)- α -(7-trifluoromethylquinolin-4-yl)methanol (VII.4), the *N*-oxide derivative (VII.5), mefloquine and chloroquine against the FCQ-27 and K-1 isolates of *P. falciparum*.



VII.4



VII.5

Inhibitor	FCQ-27 isolate	K-1 isolate
	IC ₅₀ ^A	IC ₅₀ ^A
VII.4	no activity at 80 nM	99
VII.5	no activity at 80 nM	172
Mefloquine	10.1 ^B	4.9 ^B
Chloroquine	23	221

^A The IC₅₀ values are expressed as nmol l⁻¹.

^B Data from H.V. Scott, W.L. Tan, and G.B. Barlin, *Ann. Trop. Med. Parasitol.*, 1987, **81**, 85.

Table VIII-8 *In vitro* antimalarial activity of some selected compounds against the FCQ-27 and K-1 isolates of *P. falciparum* determined by using the morphological method. Previous results determined for the FCQ-27 isolate by the use of the [³H]-hypoxanthine method are recorded for comparison.

Results (IC₅₀ values) are expressed in nmol l⁻¹.

Inhibitor	IC ₅₀ values Morphological method		Resistance- factor ^A	IC ₅₀ values [³ H]-hypoxanthine method
	FCQ-27	K-1		FCQ-27
II.48a	50	22	0.4	13
II.48b	-	19	-	17
II.48e	15	17	1.1	6
II.48g	29	-	-	9
II.48h	44	52	1.2	21
III.16h	30	38	1.3	12
III.16j	36	29	0.8	25
IV.3b	-	40	-	18
IV.5a	-	69	-	60
IV.5b	75	54	0.7	30
IV.5c	71	-	-	70
IV.6b	-	69	-	43
IV.6f	-	74	-	73
V.2c	31	-	-	10
V.2d	-	48	-	12
VI.7e	22	11	0.5	10
Chloroquine ^B	17	385	22.6	15

^A Resistance factor is the IC₅₀ value against the K-1 isolate divided by that for the FCQ-27 isolate.

^B As diphosphate salt.

Table VIII-9 Estimation of IC_{50} values from visual inspection of pigment precipitation in tests with the FCQ-27 and K-1 isolates of *P. falciparum* against di-Mannich bases (VI.4-6) of *N*-substituted 4-amino-3-methylphenols and the α -(piperidin-2-yl)methanols (VII.4 and VII.5).

0 signifies no precipitate, i.e. complete inhibition of parasite growth;

$\pm$ signifies some precipitate formation;

+ signifies precipitate formation, as in uninhibited parasite cultures.

The region between 0 and + indicates therefore the approximate IC_{50} value.

Inhibitor	Isolate	Concentrations of inhibitor (nmol l ⁻¹)				
		200	100	50	25	12.5
Chloroquine ^A	FCQ-27	0	0	0	$\pm$	+
	K-1	+	+	+	+	+
VI.4d	FCQ-27	0	0	0	+	+
	K-1	0	0	0	+	+
VI.4e	FCQ-27	0	0	0	0	+
	K-1	0	0	0	0	+
VI.5a	FCQ-27	0	0	0	$\pm$	+
	K-1	0	0	$\pm$	+	+
VI.6a	FCQ-27	0	0	0	+	+
	K-1	0	0	$\pm$	+	+
VII.4	FCQ-27	+	+	+	+	+
	K-1	+	+	+	+	+
VII.5	FCQ-27	+	+	+	+	+
	K-1	+	+	+	+	+
Control (no inhibitor)	FCQ-27	+	+	+	+	+
	K-1	+	+	+	+	+

^A As diphosphate salt.

Table VIII-10 Toxicity testing in mice.^A Times given are those after i.p. injection of the chemical (200 mg/kg) under test in peanut oil (0.4 ml). Time : h, hours; d, days. For formulae of compounds see Tables VIII-11 and VIII-12.

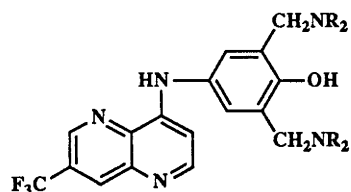
Compound	No. of mice in test	No. of mice living at the following times after intraperitoneal injection				
		0.5 h	1 h	24 h	7 d	60 d
II.48a	3	3	3	3	3	3
II.48c	3	3	3	3	3	3
II.48d	3	3	3	3	3	3
II.48g	3	3	3	3	3	3
II.48h	3	3	3	3	1	B
II.51	3	3	2	2	2	2
IV.3b	3	3	3	3	3	3
IV.3c	3	3	3	3	3	3
IV.4b	3	3	3	3	3	3
IV.5b	3	3	3	3	3	3
IV.6b	3	3	3	3	3	3
V.2c	3	3	3	3	3	3
V.3d	3	3	3	3	3	3
Control (peanut oil)	3	3	3	3	3	3
Amodiaquine ^C	6	1	1	1	1	
Chloroquine ^C (100 mg/kg)	2	0	0	0	0	

^A For details of test procedure see section VIII-2.2a.

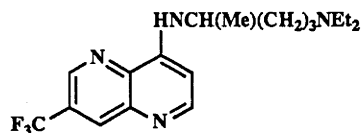
^B Two mice died on day 6.

^C Results from reference 97. Amodiaquine hydrochloride and Chloroquine diphosphate in normal saline.

Table VIII-11 Preliminary antimalarial screening results for compounds II.48 and II.51 against *P. vinckei vinckei* in mice.^A Times are those after injection of the chemical under test. Time : h, hours; d, days; 0h denotes pretreatment.



II.48 a; NR₂=NEt₂
c; NR₂=pyrrolidin-1-yl
d; NR₂=piperidin-1-yl
g; NR₂=4-methylpiperidin-1-yl
h; NR₂=4-benzylpiperidin-1-yl



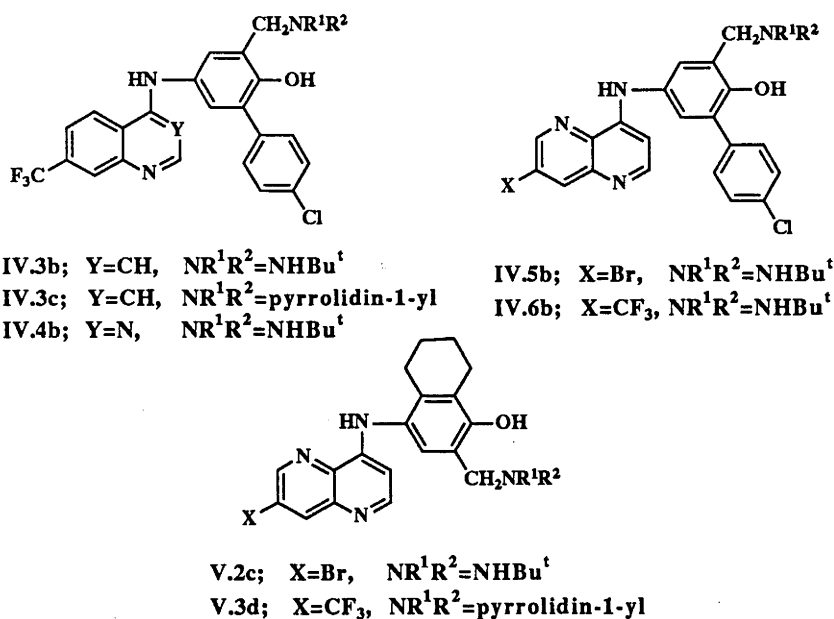
II.51

Compound (IC ₅₀ nM)	Solvent; ^B Dosage (mg/kg)	Mouse													
		no	0h	17h	24h	48h	3d	4d	9d	10d	11d	12d	14d	15d	60d
II.48a (12 nM)	PO; 200	1	<1	<1	<1	<1	1	5	1	3	2	4	<1	<1	C
		2	8	4	1	<1	<1	1	2	5	9	29	75	D	C
		3	1	<1	<1	<1	<1	1	2	4	6	2	<1	<1	C
II.48c (6 nM)	PO; 200	1	<1	<1	<1	<1	<1	3	1	2	7	<1	<1	<1	C
		2	7	4	<1	<1	<1	2	1	3	3	<1	<1	<1	C
		3	6	<1	<1	<1	<1	1	1	1	2	<1	<1	<1	C
II.48d (7.5 nM)	PO; 200	1	2	<1	<1	<1	<1	1	6	31	60	75	D		
		2	1	<1	<1	<1	<1	2	3	8	20	35	77	D	
		3	3	<1	<1	<1	<1	1	3	9	35	42	D		
II.48g (8 nM)	PO; 200	1	1	<1	<1	<1	<1	2	3	3	2	4	31	39 ^E	
		2	3	<1	<1	<1	<1	2	3	3	2	2	18	33 ^E	
		3	1	<1	<1	<1	<1	1	1	1	5	2	<1	<1	C
II.48h (12 nM)	PO; 200	1	51	60	83	55	D								
		2	48	71	66	59	D								
		3	40	71	76	64	D								
II.51 (>200 nM)	PO; 200	1	46	D											
		2	1	1	1	7	24	50	F						
		3	32	32	25	24	17	30		23		43	42		C
Control	PO	1	2	5	9	43 ^G									
		2	1	4	8	16 ^G									
		3	6	14	19	48 ^G									
Chloroquine ^H (13 nM)	NS; 40	1	<1	<1	<1	<1	<1	2	2	4	1	<1	<1	<1	C
		2	1	1	<1	<1	<1	2	39	60	62	D			
		3	6	1	<1	<1	<1	4	58 ^G						

^A For details of test procedure see section VIII-2.2b. ^B PO, Peanut oil; NS, Normal saline. ^C Mouse alive at day 60.

^D Mouse dead. ^E Mouse dead at day 17. ^F Mouse dead at day 7. ^G Mouse was killed. ^H Diphosphate salt.

Table VIII-12 Preliminary antimalarial screening results for compounds IV.3 to IV.6 and V.2 to V.3 against *P. vinckei vinckei* in mice.^A Times are those after injection of the chemical under test. Time : h, hours; d, days; 0h denotes pretreatment.



Compound (IC ₅₀ nM)	Solvent, ^B Dosage (mg/kg)	Mouse no	0h	17h	24h	48h	3d	4d	9d	10d	11d	12d	14d	18d	60d
IV.3b (20 nM)	PO; 200	1	7	23	35	45	54 ^C								
		2	11	15	30	50	71 ^C								
		3	11	34	33	38	48 ^C								
IV.3c (8 nM)	PO; 200	1	10	16	32	50	67 ^C								
		2	17	32	49	75	51 ^C								
		3	9	13	23	34	43 ^C								
IV.4b (20 nM)	PO; 200	1	20	16	15	29	62	52 ^C							
		2	19	8	16	16	26	54 ^C							
		3	11	25	42	73	72 ^C								
IV.5b (30 nM)	PO; 200	1	<1	<1	<1	<1	<1	3	1	2	7	<1	<1	<1	D
		2	7	4	<1	<1	<1	2	1	3	3	<1	<1	<1	D
		3	6	<1	<1	<1	<1	1	1	1	2	<1	<1	<1	D
IV.6b (50 nM)	PO; 200	1	13	39	44	54	28	9	3	7	10	3	1	<1	D
		2	<1	<1	<1	<1	<1	1	5	2	5	4	<1	<1	D
		3	<1	<1	<1	<1	<1	3	3	4	6	4	<1	E	
V.2c (7 nM)	PO; 200	1	12	20	9	2	1	1	1	3	4	6	30	F	
		2	8	10	6	1	1	1	2	3	6	2	17	F	
		3	7	7	1	<1	<1	1	2	3	6	2	<1	30 ^G	
V.3d (60 nM)	PO; 200	1	35	43	E										
		2	61	E											
		3	48	E											
Control	PO	1	53	78	81	E									
Chloroquine ^H (13 nM)	NS; 40	1	<1	<1	<1	<1	<1	2	2	4	1	<1	<1	<1	D
		2	1	1	<1	<1	<1	2	39	60	62	E			
		3	6	1	<1	<1	<1	4	58 ^C						

^A For details of test procedure see section VIII-2.2b. ^B PO, Peanut oil; NS, Normal saline. ^C Mouse was killed.

^D Mouse alive at day 60. ^E Mouse died. ^F Mouse dead at day 17. ^G Mouse dead at day 20. ^H Diphasphate salt.

VIII-4 Discussion of Results

Some results for the inhibition of parasite growth (as measured by the inhibition of [^3H]-hypoxanthine uptake) plotted against concentration of inhibitor for some di-Mannich bases of N-substituted 4-amino-3-methylphenols (such as compounds **VI.4d**, **VI.5d** and **VI.6d**, selected as representative of the compounds tested) are shown in Figures VIII-2 and VIII-3 for tests against the K-1 and FCQ-27 isolates, respectively. Each datum point was the mean for the sample tested in quadruplicate in a single experiment. The approximate IC_{50} value can be obtained from the sigmoidal curve by interpolation after logarithmic transformation of both concentration and percentage inhibition of parasite growth (at 50% inhibition).

Examination of Figures VIII-2 and VIII-3 indicates that with a common di-Mannich side chain, the response varies with different heterocyclic nuclei. In each case the best response was observed with compounds containing the 7-chloroquinolin-4-yl group (e.g. **VI.4d**).

VIII-4.1 *In vitro* test results against *P. falciparum*

(a) Results from incorporation of [^3H]-hypoxanthine

The results given in Table VIII-1 for the testing of the series of di-Mannich bases (**II.48**) derived from 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol against the FCQ-27 isolate of *P. falciparum* revealed IC_{50} values in the range 5-17 nM (chloroquine, 11 nM). The most active compound was **II.48f** (IC_{50} 5 nM) which was *ca* 2.2 times more active than chloroquine. The least active compound (**II.48b**; IC_{50} 17 nM) was 0.6 times as active as chloroquine.

A comparison of the activity of these compounds (**II.48**) with values published⁹⁸ for the corresponding bromo analogues (**I.43b**) revealed that the 7-bromo analogues were generally more active by 1.7-4.5-fold, except for compounds **II.48d** and **II.48g** which were more active than their bromo analogues.

Figure VIII-2 Plots of the % inhibition of parasite growth in cultures of the K-1 isolate of *P. falciparum* containing various concentrations of three different inhibitors (see Table VII-6 for relevant formulae).

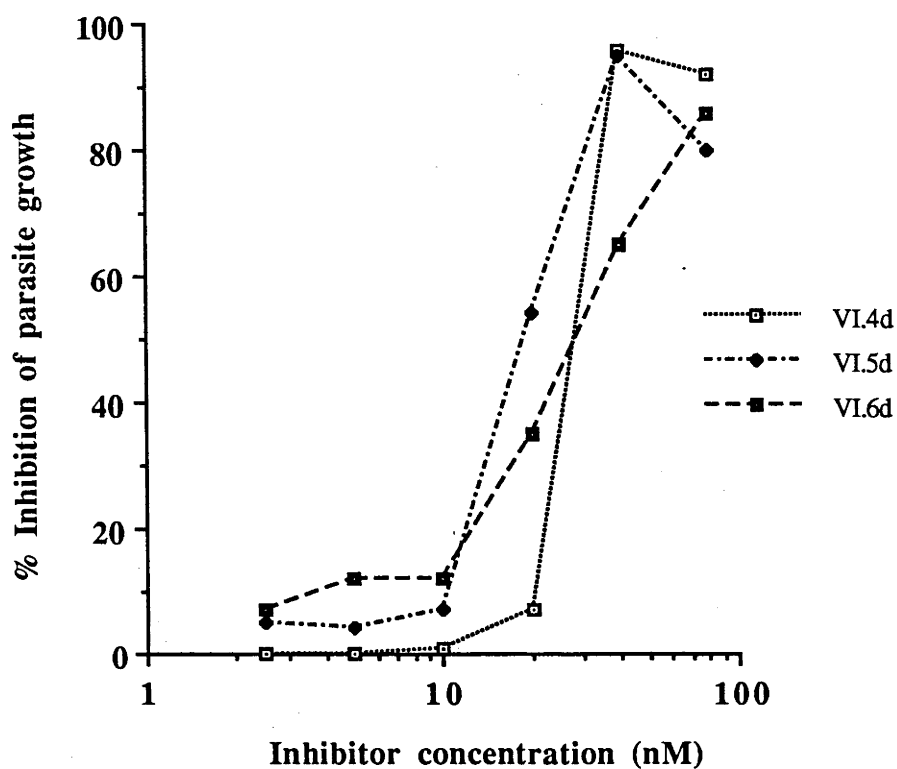
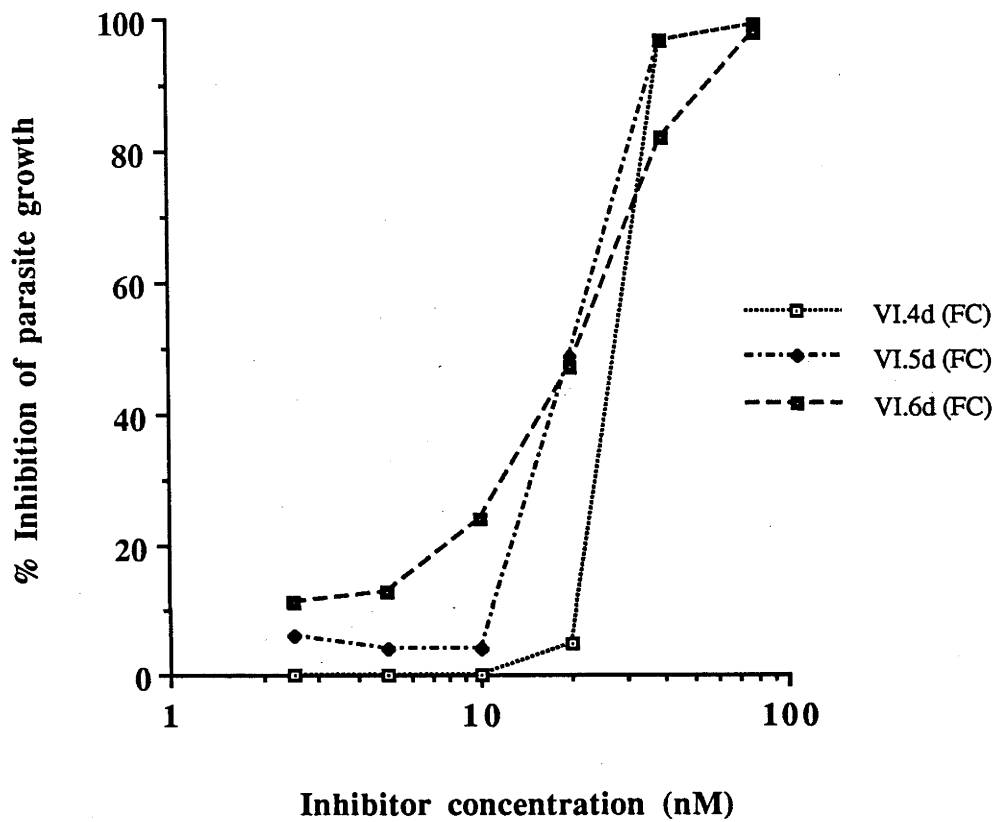


Figure VIII-3 Plots of the % inhibition of parasite growth in cultures of the FCQ-27 isolate of *P. falciparum* containing various concentrations of three different inhibitors (see Table VII-6 for relevant formulae).



The IC_{50} values for the di-Mannich bases (**III.16**) of 4-(7-trifluoromethylquinazolin-4-ylamino)phenol and the 7-trifluoromethylquinoline analogues (**VI.7**) are given in Table VIII-2. These reveal IC_{50} values in the range 12-200 nM and 0.7-10 nM for compounds **III.16** and **VI.7**, respectively, except for compound **VI.7d** which gave an IC_{50} value of 175 nM.

Six of di-Mannich derivatives of 4-(7-trifluoromethylquinazolin-4-ylamino)phenol (**III.16**) gave IC_{50} values of less than 50 nM, and the two most active compounds, those from 3-methylpiperidine (**III.16h**) and 3,5-dimethylpiperidine (**III.16j**), gave IC_{50} values of 12 and 20 nM, respectively. The di-Mannich base from diethylamine (**III.16b**) was appreciably less active, with an IC_{50} value of 150 nM.

Examination of the new data in Table VIII-2 for the di-Mannich bases (**VI.7**) derived from 4-(7-trifluoromethylquinolin-4-ylamino)phenol revealed that compound **VI.7e** with the butylmethylamino group had an IC_{50} value of 10 nM and it was less active than the diethylamino analogue;⁹⁸ this trend continued in the corresponding dipentylamino compound (**VI.7d**) which had an IC_{50} value of 175 nM. The 3- and 4-methylpiperidin-1-yl compounds (**VI.7h** and **VI.7i**) had activity comparable with the demethyl analogue (**VI.7g**).

Comparison of the quinazolines (**III.16**) with the quinolines (**VI.7**) listed in Table VIII-2 revealed that, with the exception of the dipentylamino compound (**III.16d**), the quinazolines were less active than the corresponding quinolines. This lower activity of the quinazolines varied from *ca* 110-fold for the diethylamino compound (**III.16b**) (in which it was most marked) to *ca* 3-fold for compound **III.16e**.

Thus the presence of N-3 in the quinazolines (**III.16**) reduces the antimalarial activity relative to the corresponding quinoline (**VI.7**). Likewise comparison of the trifluoromethylquinolines (**VI.7**) in Table VIII-2 with the 7-trifluoromethyl-1,5-naphthyridines (**II.48**) in Table VIII-1 reveals that the naphthyridines (with N-5) are from 3.6 to 10.7 times less active than the corresponding quinolines, except for compounds **VI.7d** and **VI.7e**.

The generally lower antimalarial of the compounds **II.48** and **III.16** relative to the corresponding quinolines (**VI.7**) may be due to many factors including a lower basicity of N-1 in compounds **II.48** and **III.16** relative to **VI.7**.

The results for the "Tebuquine" analogues, compounds **IV.3** and **4**, and **IV.5** and **6** are reported in Table VIII-3 and Table VIII-4, respectively. The most active compound of these series was compound **IV.3c** (pyrrolidin-1-yl derivative), which had an IC₅₀ value of 8 nM.

Inspection also revealed that, in each of the four series of compounds, the Mannich base containing a *t*-butylamino group was the most active (except for compound **IV.3c** and **IV.6f**), followed closely by those containing the pyrrolidin-1-yl group. Generally the least active compounds were those containing the benzylpiperidin-1-yl or benzylpiperazin-1-yl groups but this may be related to poor solubility under the test conditions.

A comparison of the activities of the quinolines (**IV.3**) with those of the quinazolines (**IV.4**) in Table VIII-3 revealed that the activities of the quinolines were generally higher (as also applied to the data in Table VIII-2). Perusal of the data in Table VIII-4 revealed (despite some inconsistencies) relatively small differences between the 7-bromo and 7-trifluoromethyl-1,5-naphthyridine (**IV.5**) and (**IV.6**), respectively.

Inspection of the Mannich bases (**IV.3**) derived from 4-chloro-5-(7-trifluoromethylquinolin-4-yl)biphenyl reported in Table VIII-3 with the di-Mannich bases (**VI.7**) derived from 4-(7-trifluoromethylquinolin-4-ylamino)phenol listed in Table VIII-2 revealed that the di-Mannich bases (**VI.7**) are from 8.9 to 34.3 times more active than the corresponding "Tebuquine" analogues (**IV.3**).

The quinazolines (**IV.4**) (Table VIII-3), however did not show consistent or marked differences in activity compared with the corresponding di-Mannich bases (**III.16**) (Table VIII-2).

The results reported in Table VIII-4 for the compounds **IV.5** when compared to those reported^{97,98} for the di-Mannich bases of 4-(7-bromo-1,5-naphthyridin-4-ylamino)phenol (**I.43b**, see also Table VIII-1) revealed that the di-Mannich bases

(I.43b) were from 2.3 to 19.4 times the more active. For example, the IC₅₀ value recorded for the di-Mannich compound (I.43b, NR₂ = pyrrolidinyl) was 3.6 nM whereas that for the "Tebuquine" analogue (IV.5c) was 70 nM.

Data for the 7-trifluoromethyl-1,5-naphthyridines (IV.6) (Table VIII-4) and II.48 (Table VIII-1) revealed a similar pattern; the di-Mannich compounds (II.48) exhibited superior activity (5 to >40 times).

The results for a series of mono-Mannich base derivatives of 4-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]-5,6,7,8-tetrahydronaphthols (V.2 and V.3), recorded in Table VIII-5, indicated that in each series the most active compound was that containing the *t*-butylamino group (V.2c, IC₅₀ 7 nM and V.3c, IC₅₀ 18 nm). Compounds containing the pyrrolidinyl group were the next most active.

The bromo compounds (V.2) were consistently more active than the corresponding trifluoromethyl compounds (V.3). This observation mirrors the conclusions reported above for di-Mannich base derivatives of 4-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]phenol.

Results of testing the di-Mannich base derivatives of the *N*-substituted 4-amino-3-methylphenols (VI.4 to VI.6) for antimalarial activity *in vitro* (see Chapter VI) against the chloroquine-sensitive (FCQ-27) and chloroquine-resistant (K-1) isolates of *P. falciparum* are presented in Table VIII-6.

Examination of these results revealed modest differences in activity only between the various Mannich bases with differing amines; for individual compounds there were no significant differences in activity between the FCQ-27 and K-1 isolates except for compound VI.6c. The resistance factors (obtained by dividing for individual inhibitors the IC₅₀ against the K-1 isolate by that for the FCQ-27 isolate) for compounds VI.4 were in the range 0.5-2.9; for compounds VI.5, 0.5-2.0; and for compounds VI.6 (except VI.6c), 1.0-1.4 whereas that for chloroquine was *ca* 10.

Comparison of the results given in Table VIII-6 with those reported by Scott *et al.*^{97,98} for the relevant 3-demethyl analogues, revealed that the 3-methyl derivatives (such as VI.5 and VI.6) had lower activity than the analogues lacking the methyl group.

For example the IC₅₀ values for the FCQ-27 isolate for the compounds **VI.5** were from 5 to 34 times less active than their demethyl analogue^{97,98} (the largest difference of 34 times related to Mannich bases derived from piperidine) whereas for the compounds (**VI.6**), it varied from 6 to 16 times except for compound **VI.6e** (piperidin-1-yl) which was slightly more active than its demethyl analogues.⁹⁷

Whereas McCuastland and Cheng¹⁰⁸ reported that the chloroquine analogue [(**I.41b**), containing the 7-chloro-1,5-naphthyridine nucleus] possessed very good antimalarial activity against *P. berghei* in mice, and Chapman *et al.*¹¹⁸ reported that the chloroquine analogue [(**I.45**), containing the 7-chloroquinazoline nucleus] revealed suppressive activity against *P. gallinaceum* in chicks at a dosage of 20 mg/kg, the chloroquine analogues (**II.51**) and (**III.18**) (containing the 7-trifluoromethyl-1,5-naphthyridine and quinazoline nuclei, respectively) prepared in the present work revealed no activity at 200 nM in tests against *P. falciparum* *in vitro*.

The results of testing the mefloquine analogue, α -(piperidin-2-yl)- α -(7-trifluoromethylquinolin-4-yl)methanol (**VII.4**) and its *N*-oxide (**VII.5**), against the FCQ-27 and K-1 isolates of *P. falciparum* are reported in Table VIII-7. In tests against the K-1 isolate, compounds **VII.4** and **VII.5** gave IC₅₀ values of 99 and 172 nM, respectively. Against the FCQ-27 isolate both compounds were inactive at 80 nM. Compared to mefloquine (IC₅₀ values of 10.1 and 4.9 nM against the FCQ-27 and K-1 isolates, respectively), compound **VII.4** was much less active.

Whereas di-Mannich bases derived from 4-(7-trifluoromethylquinolin-4-ylamino) phenol have been shown to possess high antimalarial activity,^{97,98} the compound **VII.4** was much less active and may indicate a different mechanism of action.

(b) Results from morphological evaluations

Some of the more active compounds reported in Tables VIII-1 to 6 were selected for testing against the FCQ-27 and K-1 isolates using the morphological technique

(Chapter VIII-2.1b), and the results are reported in Table VIII-8. For comparison, the IC_{50} values obtained from the incorporation of [3H]-hypoxanthine are also given.

Inspection of these results revealed differences in IC_{50} values which are probably due to differences in the experimental procedures (and in laboratory techniques) and to the different line of the FCQ-27 parasite (at ANU and AMRU). The results obtained from incorporation of [3H]-hypoxanthine consistently gave lower IC_{50} values.

However, the results of testing selected compounds against both isolates in Table VIII-8 showed that compounds **II.48a**, **III.16j**, **VI.7b** and **IV.5b** are slightly more active against the K-1 than against the FCQ-27 strain by a resistance factor of 0.4-0.8; moreover compounds **II.48e**, **II.48h** and **III.16h** are slightly less active against the K-1 than against the FCQ-27 strain by a resistance factor of 1.1-1.3. In contrast, the resistance factor for chloroquine against these two strains was 22.6.

These small differences in activity towards different strains, when compared to the large difference in the chloroquine sensitivity, indicate that there is no significant cross-resistance with these selected compounds.

(c) Results from visual observation of pigment precipitation

The results from visual observation of pigment precipitation for selected compounds described in Chapters VI and VII against both the FCQ-27 and K-1 isolates are given in Table VIII-9 (for comparison IC_{50} values obtained for these compounds by the use of [3H]-hypoxanthine are given in Tables VIII-6 and VIII-7).

As the concentrations of inhibitor were increased, chloroquine (as standard) showed activity around 25 nM against the FCQ-27 isolate and no activity at 200 nM against the K-1 isolate (the IC_{50} values in Table VIII-6 are 23 and 221 nM, respectively). Compound **VI.4d** (IC_{50} values 28 and 16 nM against the FCQ-27 and K-1 isolates, respectively, from Table VIII-6) first exhibited activity in the range 25-50 nM against both isolates; compound **VI.4e** (IC_{50} values of 22 and 12 nM) showed activity in the range 12.5-25 nM against both isolates; compound **VI.5a** (IC_{50} values of 23 and 46 nM) showed activity at approximately 25 and 50 nM against the FCQ-27 and K-1 isolates,

respectively; and compound VI.6a (IC₅₀ values of 46 and 53 nM) exhibited activity in the range 25-50 nM and approximately 50 nM against the FCQ-27 and K-1 isolates, respectively.

In the visual test, the Mefloquine analogue (VII.4) and its *N*-oxide (VII.5) (see Table VIII-9) revealed no activity at 200 nM against both strains whereas the results obtained with [³H]-hypoxanthine and recorded in Table VIII-7 revealed no activity by either compound at 80 nM against the FCQ-27 isolate but IC₅₀ values of 99 and 172 nM for compounds VII.4 and VII.5, respectively against the K-1 isolate.

Thus it appears that this visual technique may be used as a preliminary guide prior to primary screening for antimalarial activity of Mannich base type compounds such as compounds VI.4-VI.6. However its application to mefloquine type compounds has yet to be established. Then more accurate determinations of IC₅₀ values could be made by use of the morphological method or by incorporation of [³H]-hypoxanthine.

VIII-4.2 *In vivo* test results in mice

(a) *Toxicity tests in mice*

The preliminary toxicity tests of thirteen selected compounds (preparations described in Chapter II to V) are reported in Table VIII-10. It is clear that most of selected compounds (11 of 13 compounds) did not produce any acute toxicity at a dosage of 100 mg/kg. Thus these eleven compounds are considerably less toxic than amodiaquine [reference 97 reported that when amodiaquine hydrochloride (200 mg/kg) was given ip. to 6 mice, one only survived to day 7] and chloroquine [neither of two mice injected ip. with chloroquine diphosphate (200 mg/kg) survived to 0.5 h].⁹⁷

Compound II.51 (a chloroquine analogue) was less toxic than chloroquine because two of three mice survived to day 60. However compound II.48h appeared to be the most toxic of those tested because two of three mice treated at 200 mg/kg were dead at day 6.

(b) *In vivo test results against P. vinckei vinckei in mice*

The results for the preliminary *in vivo* studies of the thirteen compounds (tested for toxicity above) against *P. vinckei vinckei* in mice are recorded in Tables VIII-11 and VIII-12 (the IC₅₀ values for their activity against *P. falciparum* are also recorded for comparative purposes).

An examination of the results obtained from these *in vivo* experiments revealed that all compounds except **II.48h**, **IV.3b**, **IV.3c**, **IV.4b**, and **V.3d** had a significantly beneficial effect in reducing parasitaemia levels and markedly increased survival times.

The results in Table VIII-11 (for 7-trifluoromethyl-1,5-naphthyridines) revealed that the di-Mannich bases (**II.48a** and **II.48c**) of 4-(7-trifluoromethyl-1,5-naphthyridin-4-ylamino)phenol were most effective whereas compounds **II.48d** and **II.48g** (which had similar IC₅₀ values against *P. falciparum*) were somewhat less effective. Whereas compounds **II.48h** and **II.48a** had the same IC₅₀ values against *P. falciparum*, compound **II.48h** had no beneficial effect in the *in vivo* tests. Compound **II.51** (the 7-trifluoromethyl-1,5-naphthyridine analogue of chloroquine) displayed some antimalarial activity against *P. vinckei vinckei* in mice although *in vitro* tests against *P. falciparum* revealed that the IC₅₀ was greater than 200 nM.

The results of *in vivo* testing reported in Table VIII-12 reveal the highest activity in compounds **IV.5b** and **IV.6b**, both "Tebuquine" analogues derived from 7-bromo- and 7-trifluoromethyl-1,5-naphthyridines, respectively, whereas the "Tebuquine" analogues (**IV.3b** and **IV.4b**), containing the 7-trifluoromethylquinoline and 7-trifluoromethylquinazoline nuclei, did not show significant activity; nor did the 7-trifluoromethylquinoline (**IV.3c**). The 4-(7-bromo-1,5-naphthyridin-4-ylamino)-5,6,7,8-tetrahydronaphth-1-ol (**V.2c**) showed significant activity in *in vivo* tests but the 7-trifluoromethyl-1,5-naphthyridine (**V.3d**) was inactive.

A comparison of these results for activity against *P. vinckei vinckei in vivo* with the IC₅₀ values for activity against *P. falciparum in vitro* reveals no obvious correlation. The most active compounds (**IV.5b**, **V.6b** and **V.2c**) for tests *in vivo* had IC₅₀ values

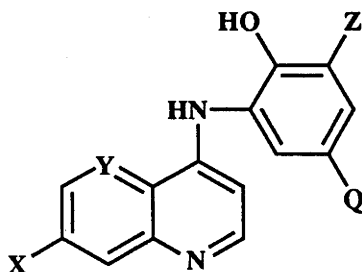
of 30, 50 and 7 nM, respectively, whereas the less active compounds (IV.4b, IV.3b, IV.3c and V.3d) had IC₅₀ values of 20, 20, 8 and 60 nM, respectively.

VIII-5 Closing Remarks

In this study only blood schizontocidal activity was examined. The development of new culturing techniques for the exoerythrocytic and gametocyte stages of the human malaria parasite in the 1980s²⁸⁸⁻²⁹¹ means that drug testing assays against these stages could soon become commonly available. If this happens it would be interesting to determine the activity of the compounds reported in this work against other stages of parasite development.

The work described in this thesis and various literature relating thereto suggests that the more active Mannich bases contain the 7-halogeno or 7-trifluoromethylquinoline or the 7-halogeno-1,5-naphthyridine nucleus. These results, together with a recent observation in this laboratory that Mannich bases of 2-aminophenol show significant activity, has focused attraction on compounds such as VIII.1.

It is hoped to develop a new class of antimalarial which may overcome or at least alleviate the existing problems of drug resistance.



VIII.1

REFERENCES

REFERENCES

- 1 A. Laveran, *Bull. Acad. Med. Paris*, 1880, **9**, 1235 through P.C.C. Garnham, *Experientia*, 1984, **40**, 1305.
- 2 C. Golgi, *Arch. Sci. Med.*, 1889, **13**, 173 through P.C.C. Garnham, *Experientia*, 1984, **40**, 1305.
- 3 D.L. Klayman, *Science*, 1985, **228**, 1049.
- 4 L.J. Bruce-Chwatt, *Br. Med. J.*, 1982, **284**, 767.
- 5 W. Peters, *Chemotherapy and Drug Resistance in Malaria*, vol. 1 (Academic Press, London, 1987), (a) p.12; (b) p. 4.
- 6 A.P. Hall, *Br. Med. J.*, 1976, **1**, 323.
- 7 A.A. Lucas, R.G. Hendricks, O.A. Okubadejo, W.H.G. Richards, R.A. Neal, and B.A.K. Kofie, *Trans. R. Soc. Trop. Med. Hyg.*, 1969, **63**, 216.
- 8 A.B.G. Laing, *Bull. W.H.O.*, 1970, **43**, 513.
- 9 W.H.O., *Weekly Epidemiological Record*, 1990, **25**, 189.
- 10 E. Marshall, *Science*, 1990, **247**, 399.
- 11 R. Ross, *Br. Med. J.*, 1897, **2**, 1786.
- 12 W.B. Pratt and R. Fekety, *The Antimicrobial Drugs* (Oxford University Press, New York, 1986), (a) p. 357; (b) pp. 362-363.
- 13 W.H. Wernsdorfer, in *Malaria*, vol. 1 (Ed. J.P. Kreier, Academic Press, New York, 1980), pp. 1-79.
- 14 W. Trager and J.B. Jensen, *Science*, 1976, **193**, 673.
- 15 Q.M. Gieman and M.J. Meagher, *Nature*, 1967, **215**, 437.
- 16 J. Gysin and T. Fandeur, *Am. J. Trop. Med. Hyg.*, 1983, **32**, 461.
- 17 A.H. Cochrane, R.S. Nussenzweig, and E.H. Nardin, in *Malaria*, vol. 3 (Ed. J.P. Kreier, Academic Press, New York, 1980), p. 163.
- 18 S.C. Cohen, *Proc. R. Soc. London, Ser. B*, 1979, **203**, 323
- 19 R. Carter and R.W. Gwadz, in *Malaria*, vol. 3 (Ed. J.P. Krier, Academic Press, New York, 1980), p. 263.
- 20 W.H.O., *Bull. W.H.O.*, 1983, **61**, 81.

- 21 V. Enea, J. Ellis, F. Zavala, D.E. Arnot, A. Asavanich, A. Masuda, I. Quakyi, and R.S. Nussenzweig, *Science*, 1984, **225**, 628.
- 22 G.N. Godson, J. Ellis, P. Svec, D.H. Schlesinger, and V. Nussenzweig, *Nature*, 1983, **305**, 29.
- 23 R.L. Coppel, A.F. Cowman, K.R. Lingelbach, G.V. Brown, R.B. Saint, D.J. Kemp, and R.F. Anders, *Nature*, 1983, **306**, 751.
- 24 J. Cherfas, *Science*, 1990, **247**, 402.
- 25 F. Hawking, in *Experimental Chemotherapy*, vol. 1 (Eds R.J. Schnitzer and F. Hawking, Academic Press, New York, 1963), p. 2.
- 26 P.J. Pelletier and J.B. Caventou, *Ann. Chim. Phys.*, 1820, **15**, 289.
- 27 P.J. Pelletier and J.B. Caventou, *Ann. Chim. Phys.*, 1820, **15**, 337.
- 28 D. McHale, *Biologist*, 1986, **33**, 45.
- 29 P. Guttman and P. Ehrlich, *Berl. Klin. Wochenschr*, 1891, **28**, 953 through P.E. Carson, in *Handbook of Experimental Pharmacology, Vol. 68/II: Antimalarial Drugs*, vol.2 (Eds W. Peters and W.H.G. Richards, Springer-Verlag, Berlin, 1984), p.83.
- 30 W. Schulemann, *Proc. R. Soc. Med.*, 1932, **25**, 897 through W.H. Wernsdorfer, in *Malaria*, vol. 1 (Ed. J.P. Kreier, Academic Press, 1980), p. 7.
- 31 H. Mauss and F. Mietzsch, *Klin. Wochschr*, 1933, **12**, 1276 through F. Hawking, in *Experimental Chemotherapy*, vol. 1 (Eds R.J. Schnitzer and F. Hawking, Academic Press, New York, 1693), p. 7.
- 32 H. Andersag, S. Breitner, and H. Jung, *Ger. Pat.*, 1939, 683692.
- 33 F.H.S. Curd, D.G. Davey, and F.L. Rose, *Ann. Trop. Med. Parasitol.*, 1945, **39**, 208.
- 34 G.H. Hitchings, I.M. Rollo, L.G. Goodwin, and G.R. Coatney, *Trans. R. Soc. Trop. Med. Hyg.*, 1952, **46**, 465.
- 35 J.H. Burkhalter, F.H. Tendick, E.M. Jones, P.A. Jones, W.F. Holcomb, and A.L. Rawlins, *J. Am. Chem. Soc.*, 1948, **70**, 1363.

- 36 E.F. Elslager, E.H. Gold, F.H. Tendick, L.M. Werbel, and D.F. Worth, *J. Heterocycl. Chem.*, 1964, **1**, 6.
- 37 W.L. Nobels, R.F. Tietz, Y.S. Koh, and J.H. Burkhalter, *J. Pharm. Sci.*, 1963, **52**, 600.
- 38 L.H. Schmidt, D. Vaughan, D. Mueller, R. Crosby, and R. Hamilton, *Antimicrob. Agents Chemother.*, 1977, **11**, 826.
- 39 T. Harinasuta, D. Bunnag, and W.H. Wernsdorfer, *Bull. W.H.O.*, 1983, **61**, 299.
- 40 J.M. de Souza, *Bull. W.H.O.*, 1983, **61**, 815.
- 41 Y. Li, Y. Hu, H. Huang, D. Zhu, W. Huang, D. Wu, and Y. Qian, *Chin. Med. J. [Engl.]*, 1981, **94**, 301.
- 42 Y. Li, Y. Qin, Y. Qu, J. Gong, and C.C. Kung, *Chin. Med. J. [Engl.]*, 1981, **94**, 303.
- 43 Y. Xu, D. Liu, Y. Wang, F. Zhou, and J. Li, *Nat. Med. J. China*, 1982, **62**, 686.
- 44 J. Rinehart, J. Arnold, and C.J. Canfield, *Am. J. Trop. Med. Hyg.*, 1976, **25**, 769.
- 45 L. Guoquiao, G. Xingbo, J. Rui, W. Zicai, J. Huaxiang, and L. Ziyang, *J. Trad. Chin. Med.*, 1982, **2**, 125 through D.L. Klayman, *Science*, 1985, **228**, 1049.
- 46 R.L. Jacobs, *Exp. Parasitol.*, 1964, **15**, 213.
- 47 W. Kretschmar, *Z. Tropenmed. Parasitol.*, 1966, **17**, 301; *ibid* 1966, **17**, 369 through H.J. Scholer, R. Leimer and R. Richle, in *Handbook of Experimental Pharmacology, Vol. 68/II: Antimalarial Drug*, vol. 2 (Eds W. Peters and W.H.G. Richards, Springer-Verlag, Berlin, 1984), p. 128.
- 48 R. Ferone and G.H. Hitchings, *J. Protozool.*, 1966, **13**, 504.
- 49 W.K. Milhous, N.F. Weatherly, J.H. Bowdre, and R.E. Desjardins, *Antimicrob. Agents Chemother.*, 1985, **27**, 525.
- 50 R. Ferone, J.J. Burchall, and G.H. Hitchings, *Mol. Pharmacol.*, 1965, **5**, 49.
- 51 I.W. Sherman, *Microbiological Reviews*, 1979, **43**, 453.
- 52 M. Aikawa and R.L. Beaudoin, *J. Cell Biol.*, 1968, **39**, 749.
- 53 W.E. Gutteridge and P.I. Trigg, *Parasitology*, 1971, **62**, 431.
- 54 R.E. Howells, *Ann. Trop. Med. Parasitol.*, 1987, **81**, 629.

- 55 W.H. Wernsdorfer and P.I. Trigg, in *Malaria Principle and Practice of Malariology*, vol. 2 (Eds W.H. Wernsdorfer and I. McGregor, Churchill Livingstone, Edinburgh, 1988), (a) p. 1574; (b) p. 1577.
- 56 D.C. Warhurst, C.A. Homewood, and V.C. Baggaley, *Ann. Trop. Med. Parasitol.*, 1974, **68**, 265.
- 57 D.C. Warhurst and D.J. Hockley, *Nature*, 1967, **214**, 935.
- 58 P.B. Macomber and H. Sprinz, *Nature*, 1967, **214**, 937.
- 59 D.C. Warhurst and S.C. Thomas, *Biochem. Pharmacol.*, 1975, **24**, 2047.
- 60 W. Peters, *Postgrad. Med. J.*, 1973, **49**, 573.
- 61 R.L. O'Brien and F.E. Hahn, *Antimicrob. Agents Chemother.*, 1965, 315.
- 62 V.E. Marquez, J.W. Cranston, R.W. Ruddon, L.B. Kier, and J.H. Burkhalter, *J. Med. Chem.*, 1972, **15**, 36.
- 63 M.W. Davidson, B.G. Griggs, D.W. Boykin, and W.D. Wilson, *Nature*, 1975, **254**, 632.
- 64 M.W. Davidson, B.G. Griggs, D.W. Boykin, and W.D. Wilson, *J. Med. Chem.*, 1977, **20**, 1117.
- 65 C.D. Fitch, *Parasitol. Today*, 1986, **2**, 330.
- 66 A.C. Chou, R. Chevli, and C.D. Fitch, *Biochemistry*, 1980, **19**, 1543.
- 67 G.R. Coatney, J. Greenberg, W.C. Cooper, and H.L. Trembley, *Proc. Soc. Exp. Biol. Med.*, 1949, **72**, 586.
- 68 W.C. Cooper, G.R. Coatney, C.A. Imboden, and G.M. Jeffery, *Proc. Soc. Exp. Biol. Med.*, 1949, **72**, 587.
- 69 G.R. Coatney and J. Greenberg, *Ann. N.Y. Acad. Sci.*, 1952, **55**, 1075.
- 70 D.C. Warhurst, B.L. Robinson, and W. Peters, *Ann. Trop. Med. Parasitol.*, 1976, **70**, 253.
- 71 E. Cundliffe and K. McQuillen, *J. Mol. Biol.*, 1967, **30**, 137.
- 72 X.D. Luo and C.C. Shen, *Med. Res. Rev.*, 1987, **7**, 28.
- 73 Qinghaosu Antimalaria Coordinating Research Group, *Chin. Med. J.*, 1979, **92**, 811.

- 73a Z. Li, *J. Trad. Chin. Med.*, 1981, **1**, 105.
- 74 China Cooperative Research Group on QHS and its derivatives as Antimalaria, *J. Trad. Chin. Med.*, 1982, **2**, 17.
- 75 H.M. Gu, D.C. Warhurst, and W. Peters, *Biochem. Pharmacol.*, 1983, **32**, 2463.
- 76 A.T. Hudson, in *Topics in Medicinal Chemistry*, Spec. Publ. No 65, the 4th SCI-RSC Medicinal Chemistry Symposium (Ed. P.R. Leeming, The Royal Society of Chemistry, London., 1988), pp. 266-283.
- 77 S.C. Hooker, *J. Am. Chem. Soc.*, 1936, **58**, 1163.
- 78 L.F. Fieser, E. Berliner, F.J. Bondhus, F.C. Chang, W.G. Dauben, M.G. Ettlinger, G. Fawaz, M. Fields, M. Fieser, C. Heidelberger, H. Heymann, A.M. Seligman, W.R. Vaughan, A.G. Wilson, E. Wilson, M.I. Wu, M.T. Leffler, K.E. Hamlin, R.J. Hathaway, E.J. Matson, E.E. Moore, M.B. Moore, R.T. Rapala, and H.E. Zaugg, *J. Am. Chem. Soc.*, 1948, **70**, 3151.
- 79 R.L. Beudoin, C.P.A. Strome, and W.G. Clutter, *Mil. Med.*, 1969, **134**, 979.
- 80 R.E. Howells, W. Peters, and J. Fullard, *Ann. Trop. Med. Parasitol.*, 1970, **64**, 203.
- 81 W. Schulemann, F. Schonhofer, and A. Wingler, *Klin. Wochenschr.*, 1932, **11**, 381.
- 82 A.P. Hall, E.B. Doberstyn, V. Mettaprakong, and P. Sonkom, *Br. Med. J.*, 1975, **2**, 15.
- 83 R.L. Beaudoin and M. Aikawa, *Science*, 1968, **160**, 1233.
- 84 W. Peters and B.L. Robinson, *Ann. Trop. Med. Parasitol.*, 1984, **78**, 561.
- 85 D.E. Davidson Jr., A.L. Ager, J.L. Brown, F.E. Chapple, R.E. Whitmire, and R.N. Rossan, *Bull. W.H.O.*, 1981, **59**, 463.
- 86 J.H. Burckhalter, F.H. Tendick, E.M. Jones, W.F. Holcomb, and A.L. Rawlins, *J. Am. Chem. Soc.*, 1946, **68**, 1894.
- 87 T. Singh, R.G. Stein, and J.H. Biel, *J. Med. Chem.*, 1969, **12**, 368.
- 88 T. Singh, R.G. Stein, and J.H. Biel, *J. Med. Chem.*, 1969, **12**, 801.

- 89 E.F. Elslager, S.C. Perricone, and D.F. Worth, *J. Heterocycl. Chem.*, 1970, **7**, 543.
- 90 S.A. Rabinovich, *Med. Parazitol.*, 1965, **34**, 91 through W. Peters, *Chemotherapy and Drug Resistance in Malaria*, vol. 2 (Academic Press, London, 1987), p. 456.
- 91 F.Y. Wiselogle, in *A Survey of Antimalarial Drugs, 1941-1945*, vol. 2 (Ed. J.W. Edwards, Ann Arbor., Michigan, 1946), p. 1236.
- 92 A.J. Saggiomo, K. Kato, and T. Kaiya, *J. Med. Chem.*, 1968, **11**, 277.
- 93 J.H. Shen, W.L. Yao, D.B. Ding, J.D. Yang, J. Wang, and F.L. Li, *Yaoxue Xuebao*, 1984, **19**, 856.
- 94 G.B. Barlin and W.L. Tan, *Aust. J. Chem.*, 1985, **38**, 1827.
- 95 G.B. Barlin and W.L. Tan, *Aust. J. Chem.*, 1986, **39**, 51.
- 96 H. Hu, X.M. Liu, B.Y. Lin, J.X. Zhang, and L.S. Huang, *Yaoxue Xuebao*, 1987, **22**, 413; *Chem. Abstr.*, 1988, **108**, 75209g.
- 97 H.V. Scott, W.L. Tan, and G.B. Barlin, *Ann. Trop. Med. Parasitol.*, 1987, **81**, 85.
- 98 H.V. Scott, W.L. Tan, and G.B. Barlin, *Ann. Trop. Med. Parasitol.*, 1988, **82**, 127.
- 99 W.G. Duncan and D.W. Henry, *J. Med. Chem.*, 1969, **12**, 711.
- 100 L.H. Schmidt and R. Crosby, *Antimicrob. Agents Chemother.*, 1978, **14**, 672.
- 101 L.M. Werbel, P.D. Cook, E.F. Elslager, J.H. Hung, J.L. Johnson, S.J. Kesten, D.J. McNamara, D.F. Ortwine, and D.F. Worth, *J. Med. Chem.*, 1986, **29**, 924.
- 102 A.T. Hudson, A.W. Randall, M. Fry, C.D. Ginger, B. Hill, V.S. Latter, N. McHardy, and R.B. William, *Parasitology*, 1985, **90**, 45.
- 103 A.T. Hudson, in *Handbook of Experimental Pharmacology, Vol. 68/II: Antimalarial Drugs*, vol. 2 (Eds W. Peters and W.H.G. Richards, Springer-Verlag, Berlin, 1984), pp. 343-361.
- 104 I. Nabih, M. Nasr, and M.A. Badawi, *J. Pharm. Sci.*, 1972, **61**, 1500.
- 105 S.J. Kesten, J. Johnson, and L.M. Werbel, *J. Med. Chem.*, 1987, **30**, 906.
- 106 J.T. Adams, C.K. Bradsher, D.S. Breslow, S.T. Amore, and C.R. Havser, *J. Am. Chem. Soc.*, 1946, **68**, 1317.

- 107 A.A. Goldberg, R.S. Theobald, and W. Williamson, *J. Chem. Soc.*, 1954, 2357.
- 108 D.J. McCaustland and C.C. Cheng, *J. Heterocycl. Chem.*, 1970, **7**, 467.
- 109 C. Chen, X. Zheng, P. Zhu, and H. Guo, *Yaoxue Xuebao*, 1982, **17**, 112; *Chem. Abstr.*, 1982, **97**, 6191n.
- 110 G.B. Barlin and W.L. Tan, *Aust. J. Chem.*, 1985, **38**, 459.
- 111 G.B. Barlin and W.L. Tan, *Aust. J. Chem.*, 1985, **38**, 905.
- 112 W. Chen, Y. Dong, and G. Ding, *Yao Hsueh Hsueh Pao*, 1979, **14**, 710; *Chem. Abstr.*, 1980, **93**, 795h.
- 113 D.M. Stout, W.L. Matier, C.B. Yang, R.D. Reynolds, and B.S. Brown, *J. Med. Chem.*, 1983, **26**, 808.
- 114 R.J. Marshall and J.A.O. Ojewole, *Toxicol. Appl. Pharmacol.*, 1978, **46**, 759.
- 115 P.H. Nelson, A.M. Strosberg, and K.G. Untch, *J. Med. Chem.*, 1980, **23**, 180.
- 116 O. Yu. Magidson and E.S. Golovchinskaya, *J. Gen. Chem. (USSR)*, 1938, **8**, 1797; *Chem. Abstr.*, 1939, **33**, 4993.
- 117 C.C. Price, N.J. Leonard, and D.Y. Curtin, *J. Am. Chem. Soc.*, 1946, **68**, 1305.
- 118 N.B. Chapman, G.M. Gibson, and F.G. Mann, *J. Chem. Soc.*, 1947, 890.
- 119 P.E. Thompson and L.M. Werbel, *Antimalarial Agents Chemistry and Pharmacology* (Academic Press, New York, 1972), pp. 324-327.
- 120 F.H.S. Curd, J.K. Landquist, and F.L. Rose, *J. Chem. Soc.*, 1947, 775.
- 121 J. Davoll and A.M. Johnson, *J. Chem. Soc.(C)*, 1970, 997.
- 122 O.B. Bird, J.W. Vaitkus, and J. Clarke, *Mol. Pharmacol.*, 1970, **6**, 573.
- 123 J. Davoll, A.M. Johnson, H.J. Davis, O.D. Bird, J. Clarke, and E.F. Elslager, *J. Med. Chem.*, 1972, **15**, 812.
- 124 E.F. Elslager, J. Clarke, L.M. Werbel, D.F. Worth, and J. Davoll, *J. Med. Chem.*, 1972, **15**, 827.
- 125 J. Davoll, J. Clarke, and E.F. Elslager, *J. Med. Chem.*, 1972, **15**, 837.
- 126 J.B. Hynes, W.T. Ashton, H.G. Merriman, and F.C. Walker, *J. Med. Chem.*, 1974, **17**, 682.

- 127 E.F. Elslager, P. Jacob, J. Johnson, L.M. Werbel, and D.F. Worth, *J. Med. Chem.*, 1978, **21**, 1059.
- 128 E.F. Elslager, M.P. Hutt, P. Jacob, J. Johnson, B. Temporelli, L.M. Werbel, and D.F. Worth, *J. Med. Chem.*, 1979, **22**, 1247.
- 129 L.M. Werbel, L. Newton, and E.F. Elslager, *J. Heterocycl. Chem.*, 1980, **17**, 497.
- 130 E.F. Elslager, P. Jacob, J. Johnson, and L.M. Werbel, *J. Heterocycl. Chem.*, 1980, **17**, 129.
- 131 T.R. Sweeney, *Med. Res. Rev.*, 1981, **1**, 281.
- 132 T.R. Sweeney, in *Handbook of Experimental Pharmacology, Vol. 68/II: Antimalarial Drugs*, vol. 2 (Eds W. Peters, and W.H.G. Richards, Springer-Verlag, Berlin, 1984), pp. 267-324.
- 133 C.J. Ohnmacht, A.R. Patel, and R.E. Lutz, *J. Med. Chem.*, 1971, **14**, 926.
- 134 R.E. Lutz, P.S. Bailey, M.F. Clark, J.F. Codington, A.J. Deinet, J.A. Freek, G.H. Harnest, N.H. Leake, T.A. Martin, Jr. Russel, J.M. Salsbury, N.H. Shearer Jr., J.D. Smith, and J.W. Wilson, *J. Am. Chem. Soc.*, 1946, **68**, 1813.
- 135 E.A. Nodiff, K. Tanabe, C. Seyfried, S. Matsuura, Y. Kondo, E.H. Chen, and M.P. Tyagi, *J. Med. Chem.*, 1971, **14**, 921.
- 136 W.T. Colwell, V. Brown, P. Christie, J. Lange, C. Reece, K. Yamamoto, and D.W. Henry, *J. Med. Chem.*, 1972, **15**, 771.
- 137 R.E. Strube, *J. Trop. Med. Hyg.*, 1975, **78**, 171.
- 138 L.H. Schmidt, R. Crosby, J. Rasco, and D. Vaughan, *Antimicrob. Agents Chemother.*, 1978, **13**, 1011.
- 139 M. Rösner, A. Brossi, and J.V. Silverton, *Heterocycles*, 1981, **15**, 925.
- 140 P. Blumber, M.S. Ao, M.P. LaMontagne, and A. Markovac, *J. Med. Chem.*, 1975, **18**, 1122.
- 141 R.M. Pinder and A. Burger, *J. Med. Chem.*, 1968, **11**, 267.
- 142 C.R. Hauser and G.A. Raynolds, *J. Org. Chem.*, 1950, **15**, 1224.
- 143 E.P. Hart, *J. Chem. Soc.*, 1954, 1879.

- 144 W.W. Paudler and T.J. Kress, *Adv. Heterocycl. Chem.*, 1970, (a) pp. 136-138; (b) pp. 123-136.
- 145 H. Rapoport and A.D. Batcho, *J. Org. Chem.*, 1963, **28**, 1753.
- 146 E.P. Hart, *J. Chem. Soc.*, 1956, 212.
- 147 K. Miyaki, *J. Pharm. Soc. Jpn.*, 1942, **62**, 257; *Chem. Abstr.*, 1951, **45**, 2950e.
- 148 T. Takahashi, T. Yatsuka, and S. Senda, *J. Pharm. Soc. Jpn.*, 1944, **64**, 9; *Chem. Abstr.*, 1952, **46**, 110g.
- 149 A. Albert and A. Hampton, *J. Chem. Soc.*, 1952, 4985.
- 150 C.C. Price and R.M. Roberts, *J. Am. Chem. Soc.*, 1946, **68**, 1204.
- 151 R.G. Gould and W.A. Jacobs, *J. Am. Chem. Soc.*, 1939, **61**, 2890.
- 152 A. Albert and W.L.F. Armarego, *J. Chem. Soc.*, 1963, 4237.
- 153 J.G. Murray and C.R. Hauser, *J. Org. Chem.*, 1954, **19**, 2008.
- 154 C.C.J. Culvenor, *Rev. Pure and Appl. Chem.*, 1953, **3**, 83.
- 155 M. Katada, *J. Pharm. Soc. Jpn.*, 1947, **67**, 56; *Chem. Abstr.*, 1951, **45**, 9537.
- 156 E. Ziegler and E. Noelken, *Monatsh. Chem.*, 1961, **92**, 1184 through W.W. Paudler and T.J. Kress, *Adv. Heterocycl. Chem.*, 1970, **11**, 137.
- 157 H.E. Baumgarten, H.C.F. Su, and R.P. Barkley, *J. Heterocycl. Chem.*, 1966, **3**, 357.
- 158 V. Oakes and H.N. Rydon, *J. Chem. Soc.*, 1958, 204.
- 159 D.N. Bailey, D.M. Hercules, and T.D. Eck, *Anal. Chem.*, 1967, **39**, 877.
- 160 S.F. Mason, *J. Chem. Soc.*, 1957, 4874.
- 161 F.J.C. Rossotti and H.S. Rossotti, *J. Chem. Soc.*, 1958, 1304.
- 162 R.G. Shepherd and J.L. Fedrick, *Adv. Heterocycl. Chem.*, 1965, **4**, 377.
- 163 H.C. Longuet-Higgins, *J. Chem. Phys.*, 1950, **18**, 283.
- 164 H.C. Longuet-Higgins, *Nature*, 1950, **166**, 139.
- 165 V. Oakes and H.N. Rydon, *J. Chem. Soc.*, 1956, 4433.
- 166 J. Heindl, H.W. Kelm, E. Dogs, A. Seeger, and C. Hermann, *Eur. J. Med. Chem.-Chim. Ther.*, 1977, **12**, 549; *Chem. Abstr.*, 1978, **88**, 152461f.
- 167 G.B. Bachman and D.D. Micucci, *J. Am. Chem. Soc.*, 1948, **70**, 2381.

- 168 E.E. Garcia, C.V. Greco, and I.M. Hunsberger, *J. Am. Chem. Soc.*, 1960, **82**, 4430.
- 169 F.E. Ziegler and G.B. Bennett, *J. Am. Chem. Soc.*, 1973, **95**, 7461.
- 170 Y. Tsujii, T. Isogai, T. Awazu, H. Jyonish, and T. Kimura, (Ishihara Sangyo Kaisha Ltd.), *Eur. Pat. Appl. EP* 228, 846; *Chem. Abstr.*, 1988, **108**, 37656t.
- 171 H.R. Snyder, H.E. Freier, P. Kovacic, and E.M. van Heyningen, *J. Am. Chem. Soc.*, 1947, **69**, 371.
- 172 G.B. Barlin and S.J. Ireland, *Aust. J. Chem.*, 1988, **41**, 1727.
- 173 M. Hirota, H. Masuda, Y. Hamada, and I. Takeuchi, *Bull. Chem. Soc. Jpn.*, 1974, **47**, 2083.
- 174 W.W. Paudler and T.J. Kress, *Chem. Ind. (London)*, 1966, 1557.
- 175 H.C. van der Plas, A. van Veldhuizen, M. Wozniak, and P. Smit, *J. Org. Chem.*, 1978, **43**, 1673.
- 176 A. Albert and E.P. Serjeant, *The Determination of Ionization Constants*, (Chapman and Hall, London, 1984), pp. 70-101.
- 177 D.D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution* (Butterworths, London, 1965), p. 274.
- 178 A. Albert and A. Hampton, *J. Chem. Soc.*, 1954, 505.
- 179 A. Albert and J.N. Phillips, *J. Chem. Soc.*, 1956, 1294.
- 180 T.D. Eck, *Anal. Chem.*, 1967, **39**, 877.
- 181 W.W. Paudler and T.J. Kress, *J. Heterocycl. Chem.*, 1967, **4**, 547.
- 182 I. Howe, D.H. Williams, and R.D. Bowen, *Mass Spectrometry Principles and Applications* (McGraw-Hill Inc., New York, 1981), pp. 117-122.
- 183 D.D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control* (Chapman and Hall, London, 1974).
- 184 W.L.F. Armarego, *Fused Pyrimidines Part 1: Quinazolines* (Interscience Publisher, New York, 1967), pp. 69-133.
- 185 W.L.F. Armarego, *Adv. Heterocycl. Chem.*, 1963, **1**, 253.
- 186 W.L.F. Armarego, *Adv. Heterocycl. Chem.*, 1979, **24**, 16.

- 187 V. Niementowski, *J. Prakt. Chem.*, 1895, **51**, 564.
- 188 C.C. Price, N.J. Leonard, and D.Y. Curtin, *J. Am. Chem. Soc.*, 1946, **68**, 1305.
- 189 B.R. Baker, R.E. Schaub, J.P. Joseph, F.J. McEvoy, and J.W. Williams, *J. Org. Chem.*, 1952, **17**, 141.
- 190 V. Oakes, H.N. Rydon, and K. Undheim, *J. Chem. Soc.*, 1962, 4678.
- 191 W.L.F. Armarego and J.I.C. Smith, *J. Chem. Soc.(B)*, 1967, 449.
- 192 M.T. Bogert and A.H. Gotthelf, *J. Am. Chem. Soc.*, 1900, **22**, 522.
- 193 J.F. Meyer and E.C. Wagner, *J. Org. Chem.*, 1943, **8**, 239.
- 194 S.F. Mason, *J. Chem. Soc.*, 1958, 674.
- 195 A. Albert and G.B. Barlin, *J. Chem. Soc.*, 1962, 3129.
- 196 L. Simet, *J. Org. Chem.*, 1963, **28**, 3580.
- 197 M. Hauptschein, E.A. Nodiff, and A.J. Saggiomo, *J. Am. Chem. Soc.*, 1954, **76**, 1051.
- 198 M.R. Petti and J.C. Tatlow, *J. Chem. Soc.*, 1954, 3852.
- 199 W.T. Caldwell and A.N. Sayin, *J. Am. Chem. Soc.*, 1951, **73**, 5125.
- 200 W.L.F. Armarego, *J. Chem. Soc.*, 1962, 561.
- 201 A. Albert, R. Goldacre, and J.N. Phillips, *J. Chem. Soc.*, 1948, 2240.
- 202 S.B. Knight, R.H. Wallick, and C. Balch, *J. Am. Chem. Soc.*, 1955, **77**, 2577.
- 203 E. Pouterman and A. Girardet, *Experientia*, 1947, **3**, 28.
- 204 R. Näsänen, P. Lumme, and A.L. Mukula, *Acta. Chem. Scand.*, 1951, **5**, 1199.
- 205 A. Albert and D. Magrath, *Biochem. J.*, 1947, **41**, 534.
- 206 R. Belcher, A. Sykes, and J.C. Tatlow, *J. Chem. Soc.*, 1955, 376.
- 207 J.M. Hearn, R.A. Morton, and J.C.E. Simpson, *J. Chem. Soc.*, 1951, 3318.
- 208 H. Culbertson, J.C. Decius, and B.E. Christensen, *J. Am. Chem. Soc.*, 1952, **74**, 4834.
- 209 W. Borsche and B.G.B. Scholten, *Chem. Ber.*, 1917, **50**, 596.
- 210 T. Migeta, N. Morikawa, and O. Simamura, *Bull. Chem. Soc. Jpn.*, 1983, **36**, 980.
- 211 J.C. Colbert, R.M. Lacy, *J. Am. Chem. Soc.*, 1946, **68**, 200.
- 212 H.B. Hill and W.J. Hale, *Am. Chem. J.*, 1905, **33**, 1.

- 213 P.B. Russell and G.H. Hitchings, *J. Am. Chem. Soc.*, 1951, **73**, 3763.
- 214 R.M. Herbst and R.H. Manske, *Org. Synth.*, 1943, **2**, 391.
- 215 P.E. Fanta, *Org. Synth.*, 1963, Coll. vol. 4, 844.
- 216 K. Kinovic, S. Vrancea, D. Grandet, J.M. Lebourg, and R. Porquet, *Chim. Ther.*, 1968, **3**, 313; *Chem. Abstr.*, 1967, **70**, 87171y.
- 217 I. Nabih, *Experientia*, 1972, **28**, 1114.
- 218 G. Schroeter, *Justus Liebigs Ann. Chem.*, 1922, **426**, 83.
- 219 M. Rowe and E. Levin, *J. Chem. Soc. (Transactions part I)*, 1927, 530.
- 220 A.G. Green and F.M. Rowe, *J. Chem. Soc. (Transactions)*, 1918, 955.
- 221 G.B. Bachman and J.W. Wetzel, *J. Org. Chem.*, 1946, **11**, 454.
- 222 R.E. Desjardins, C.J. Canfield, J.D. Haynes, and J.D. Chulay, *Antimicrob. Agents Chemother.*, 1979, **16**, 710.
- 223 K.H. Rieckmann, *Lancet*, 1982, **1**, 1333.
- 224 G.B. Barlin and J.H. Yan, *Aust. J. Chem.*, 1989, **42**, 2191.
- 225 A.E. Derome, *Modern NMR Techniques for Chemistry Research* (Pergamon Press, Oxford, 1987), pp. 183-234.
- 226 H. Hirayama and T. Kubota, *J. Pharm. Soc. Jpn.*, 1953, **73**, 140.
- 227 J. Logan Irvin and E. Moore Irvin, *J. Am. Chem. Soc.*, 1947, **69**, 1091.
- 228 R. Kuhn and A. Wassermann, *Helv. Chim. Acta*, 1928, **11**, 3.
- 229 S.F. Mason, *J. Chem. Soc.*, 1960, 219.
- 230 D.H. Everett and W.F.K. Wynne-Jones, *Proc. R. Soc. London, Ser. A*, 1941, **177**, 499.
- 231 A.D. Ainley and H. King, *Proc. R. Soc. London, Ser. B*, 1938, **125**, 60.
- 232 E.R. Buchman and D.R. Howton, *J. Am. Chem. Soc.*, 1946, **68**, 2718.
- 233 D.W. Boykin Jr., A.R. Patal, R.E. Lutz, and A. Burger, *J. Heterocycl. Chem.*, 1967, **4**, 459.
- 234 R.T. Williams, *Detoxication Mechanisms* (John Wiley and Sons, Inc. New York, 1959), p. 655.

- 235 E.R. Buchman, H. Sargent, T.C. Myers, and D.R. Howton, *J. Am. Chem. Soc.*, 1946, **68**, 2710.
- 236 W.E. Rothe and D.P. Jacobus, *J. Med. Chem.*, 1968, **11**, 366.
- 237 T.N. Pullman, L. Eichelberger, A.H. Alving, R. Jones Jr., B. Craig Jr., and C.M. Whorton, *J. Clin. Invest.*, 1948, **27**, 15.
- 238 H. King and T.S. Work, *J. Chem. Soc.*, 1940, 1307.
- 239 A.L. Wilds, *Organic Reaction II* (John Wiley and Sons, Inc., 1944), p. 193.
- 240 S. Winstein, T.L. Jacobs, R.B. Henderson, and W.H. Florsheim, *J. Org. Chem.*, 1946, **11**, 150.
- 241 H. King and T.S. Work, *J. Chem. Soc.*, 1940, 1312.
- 242 S. Winstein, T.L. Jacobs, G.B. Linden, D. Seymour, E.F. Levy, B.F. Day, J.H. Robson, R.B. Henderson, and W.H. Florsheim, *J. Am. Chem. Soc.*, 1946, **68**, 1831.
- 243 H. Gilman, R.A. Benkeser, and Leo Tolman, *J. Am. Chem. Soc.*, 1946, **68**, 1848.
- 244 A.E. Senear, H. Sargent, J.F. Mead, and J.B. Koepfli, *J. Am. Chem. Soc.*, 1946, **68**, 2695.
- 245 H. Sargent, *J. Am. Chem. Soc.*, 1946, **68**, 2688.
- 246 A.R. Patal, C.J. Ohnmacht, D.P. Clifford, A.S. Crosby, and R.E. Lutz, *J. Med. Chem.*, 1971, **14**, 198.
- 247 R.F. Heck, *Organic Reactions*, 1982, **27**, 345.
- 248 B.A. Patal and R.F. Heck, *J. Org. Chem.*, 1978, **43**, 3898.
- 249 R.F. Heck and J.P. Nolley Jr., *J. Org. Chem.*, 1972, **37**, 2320.
- 250 H.A. Dieck and R.F. Heck, *J. Am. Chem. Soc.*, 1974, **96**, 1133.
- 251 Y. Tamaru, Y. Yamada, and Z. Yoshida, *J. Org. Chem.*, 1978, **43**, 3396.
- 252 Y. Tamaru, Y. Yamada, and Z. Yoshida, *Tetrahedron*, 1979, **35**, 329.
- 253 C.B. Ziegler Jr., and R.F. Heck, *J. Org. Chem.*, 1978, **43**, 2949.
- 254 W.C. Frank, Y.C. Kim, and R.F. Heck, *J. Org. Chem.*, 1978, **43**, 2947.
- 255 A.S. Dey and M.M. Jouille, *J. Heterocycl. Chem.*, 1965, **2**, 113.

- 256 S. Adam (F. Hoffman-La Roche), *Eur. Pat. Appl.*, 1982, 103259; *Chem. Abstr.*, 1984, **101**, 38364p.
- 257 S. Adam, *Tetrahedron*, 1989, **45**, 1409.
- 258 J. March, *Advanced Organic Chemistry* (McGraw-Hill Book Co., New York, 1977), pp. 1015-1016.
- 259 D.J. Cram, *Fundamentals of Carbanion Chemistry* (Academic Press, New York, 1965), pp. 230-233.
- 260 F.I. Carroll and J.T. Blackwell, *J. Med. Chem.*, 1974, **17**, 210.
- 261 P.L. Salzberg and J.V. Supniewski, *Organic Syntheses, Coll. Vol. 1*, (Ed. A.H. Blatt, John Wiley & Sons, Inc, New York, 1984), p. 119.
- 262 C. Hansch and W. Carpenter, *J. Org. Chem.*, 1957, **22**, 936.
- 263 E.C. Taylor, A.J. Crovetti, and N.E. Boyer, *J. Am. Chem. Soc.*, 1957, **79**, 3549.
- 264 D.H. Williams and I. Fleming, *Spectroscopic Methods in Organic Chemistry* (McGraw Hill, London, 1973), p. 103.
- 265 T.J. Batterham, *NMR Spectra of Simple Heterocycles* (John Wiley & Sons, New York, 1973), p. 59.
- 266 J. Sharvit and A. Mandelbam, *Israel J. Chem.*, 1967, **5**, 33.
- 267 J.L. Cotter and R.A. Dine-Hart, *Chem. Comm.*, 1966, 809.
- 268 R. Killick-Kendrick and W. Peters, *Rodent Malaria* (Academic Press, London, 1978).
- 269 W. Peters, in *Malaria*, vol. 1 (Ed. J.P. Kreier, Academic Press, London, 1980), pp. 145-283.
- 270 W. Peters, J.H. Portus, and B.L. Robinson, *Ann. Trop. Med. Parasitol.*, 1975, **69**, 155.
- 271 T.S. Osdene, P.B. Russell, and L. Rane, *J. Med. Chem.*, 1967, **10**, 431.
- 272 E. Fink and W. Kretschmar, *Z. Tropenmed. Parasitol.*, 1970, **21**, 167 through A.L. Ager Jr., in *Handbook of Experimental Pharmacology, Vol. 68/II: Antimalarial Drugs*, vol. 1 (Eds W. Peters and W.H.G. Richards, Springer-Verlag, Berlin, 1984), p. 227.

- 273 A.L. Ager Jr., in *Handbook of Experimental Pharmacology, Vol. 68/I: Antimalarial Drugs*, vol. 1 (Eds W. Peters and W.H.G. Richards, Springer-Verlag, Berlin, 1984), p. 228.
- 274 L.H. Schmidt, *Trans. R. Soc. Trop. Med. Hyg.*, 1973, **67**, 446.
- 275 R.N. Rossan, M.D. Young, and D.C. Baerg, *Am. J. Trop. Med. Hyg.*, 1975, **24**, 168.
- 276 W.H.G. Richards and S.G. Williams, *Ann. Trop. Med. Parasitol.*, 1973, **67**, 179.
- 277 C.J. Canfield, L.B. Altstatt, and V.B. Elliot, *Am. J. Trop. Med. Hyg.*, 1970, **19**, 905.
- 278 W.H.G. Richards and S.G. Williams, *Ann. Trop. Med. Parasitol.*, 1975, **69**, 135.
- 279 J.D. Haynes, C.J. Diggs, F.A. Hines, and R.E. Desjardins, *Nature*, 1976, **263**, 767.
- 280 K.H. Rieckmann, J.V. McNamara, H. Frischer, T.A. Stockert, P.E. Carson, and R.D. Powell, *Am. J. Trop. Med. Hyg.*, 1968, **17**, 661.
- 281 K.H. Rieckmann, L.J. Sax, G.H. Campbell, and J.E. Mrema, *Lancet*, 1978, **1**, 22.
- 282 W.B. Cowden, G.A. Butcher, N.H. Hunt, I.A. Clark, and F. Yoneda, *Am. J. Trop. Med. Hyg.*, 1987, **37**, 495.
- 283 P. Chen, G. Lamont, T. Elliot, C. Kidson, G. Brown, G. Mitchell, J. Stage and M. Alpers, *South-east Asian J. Trop. Med. Public Health*, 1980, **11**, 435.
- 284 S. Thaithong and G.H. Beale, *Trans. R. Soc. Trop. Med. Hyg.*, 1981, **75**, 271.
- 285 A.A. Sandosham and V. Thomas, *Malariology* (Singapore University Press, 1982), p. 45.
- 286 R.S. Bray and P.C.C. Garnham, *Br. Med. Bull.*, 1982, **38**, 117.
- 287 W.B. Cowden and I.A. Clark, *Trans. R. Soc. Trop. Med. Hyg.*, 1987, **81**, 533.
- 288 D. Mazier, R.L. Beaudoin, S. Mellouk, P. Druilhe, B. Texier, J. Trosper, F. Miltgen, I. Landau, C. Paul, O. Brandicourt, C. Guguen-Guillouzo, and P. Langlois, *Science*, 1985, **227**, 440.
- 289 R.E. Sinden, *Parasitology Today*, 1987, **3**, 292.

- 290 R.L. Beaudoin, D. Mazier, and M.R. Hollingdale, in *Malaria Principles and Practice of Malariology* (Eds W.H. Wernsdorfer and I. McGregor, Churchill Livingstone, New York, 1988), pp. 321-330.
- 291 J.P. Vanderberg, in *Malaria Principles and Practice of Malariology* (Eds W.H. Wernsdorfer and I. McGregor, Churchill Livingstone, New York, 1988), pp. 331-348.

APPENDIX 1

Figure A1 2-D HETCOR spectrum of 2,6-bis(diethylaminomethyl)-
4-(7'-trifluoromethyl-1',5'-naphthyridin-4'-ylamino)phenol
(II.48a) in CDCl₃

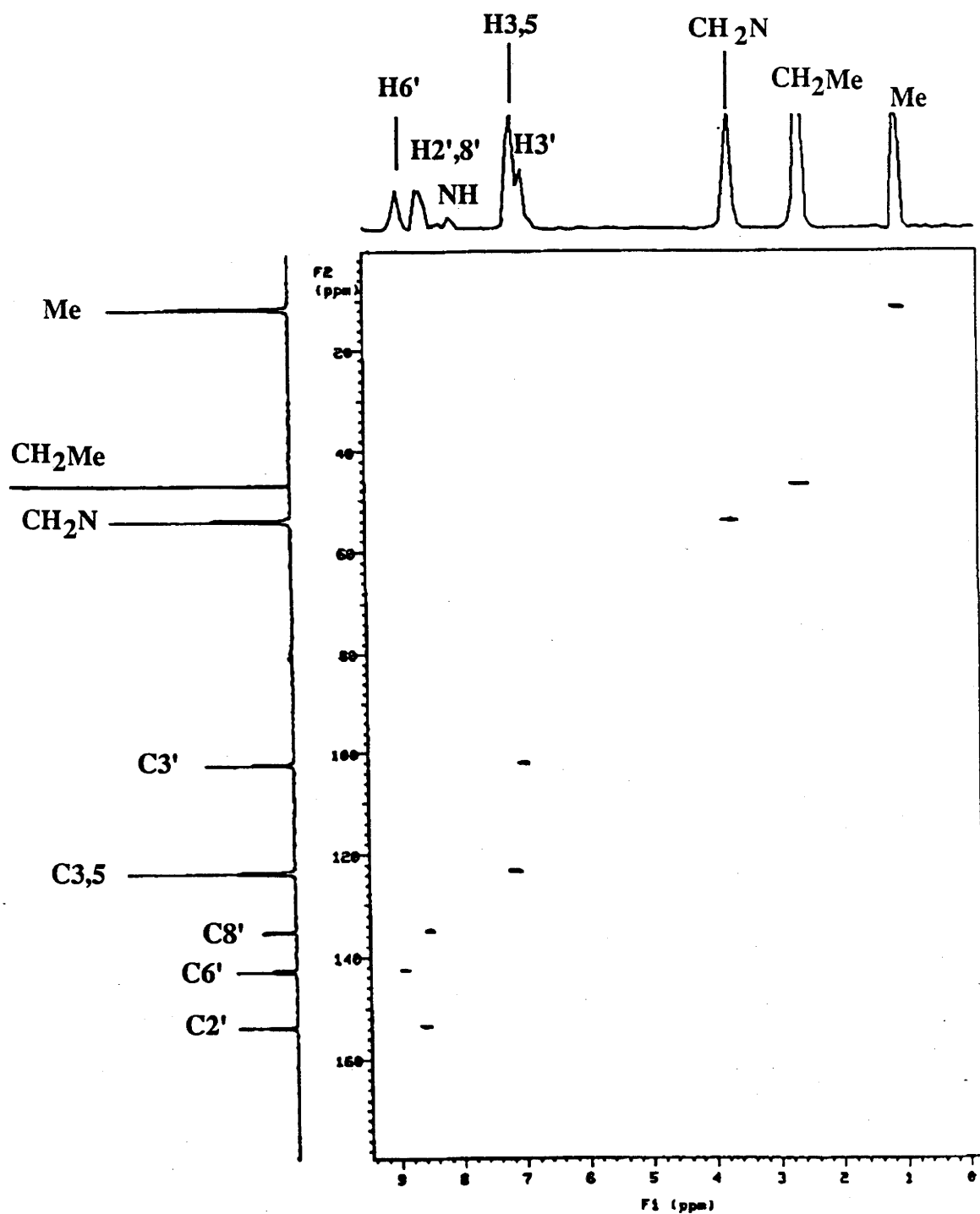


Figure A2 2-D HMBC spectrum of 2,6-bis(diethylaminomethyl)-4-(trifluoromethyl-1',5'-naphthyridin-4'-ylamino)phenol (II.48a) in CDCl_3

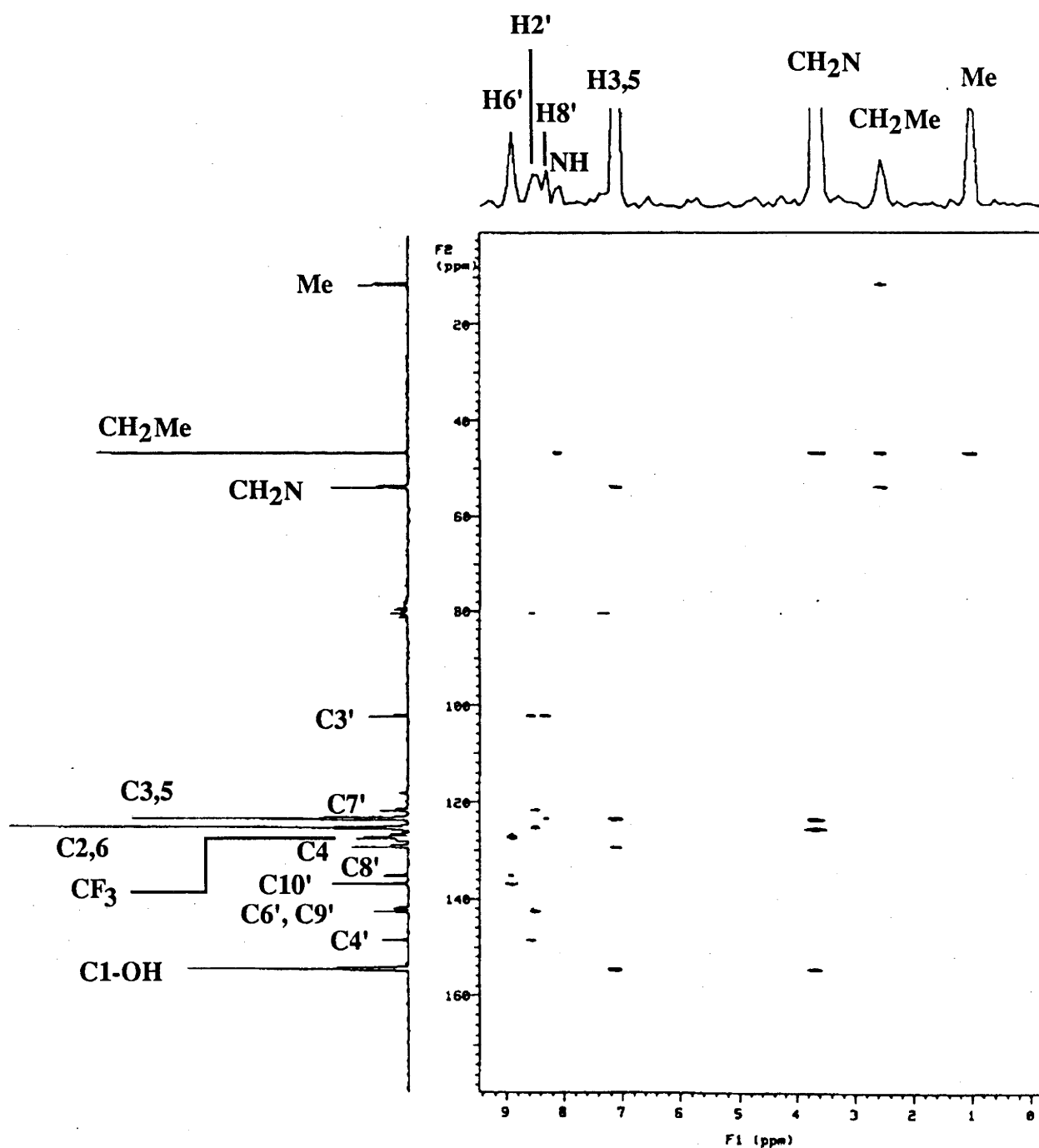


Figure A3 2-D COSY spectrum of 3-methyl-2,6-bis(piperidin-1'-ylmethyl)-4-(7''-trifluoromethylquinolin-4''-ylamino)-phenol (VI.5e)

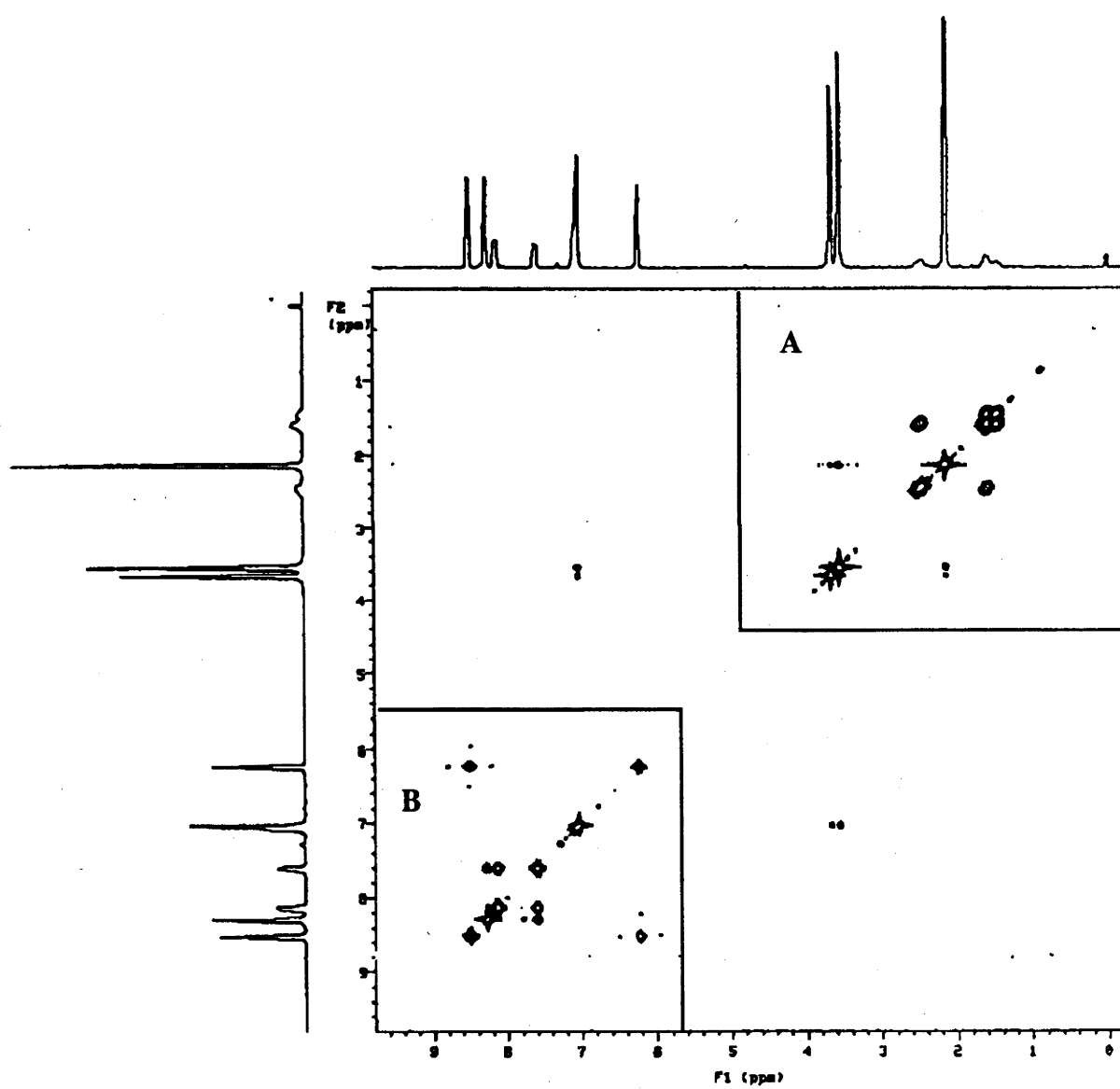


Figure A3.1 Expansion of Figure A3 at region A

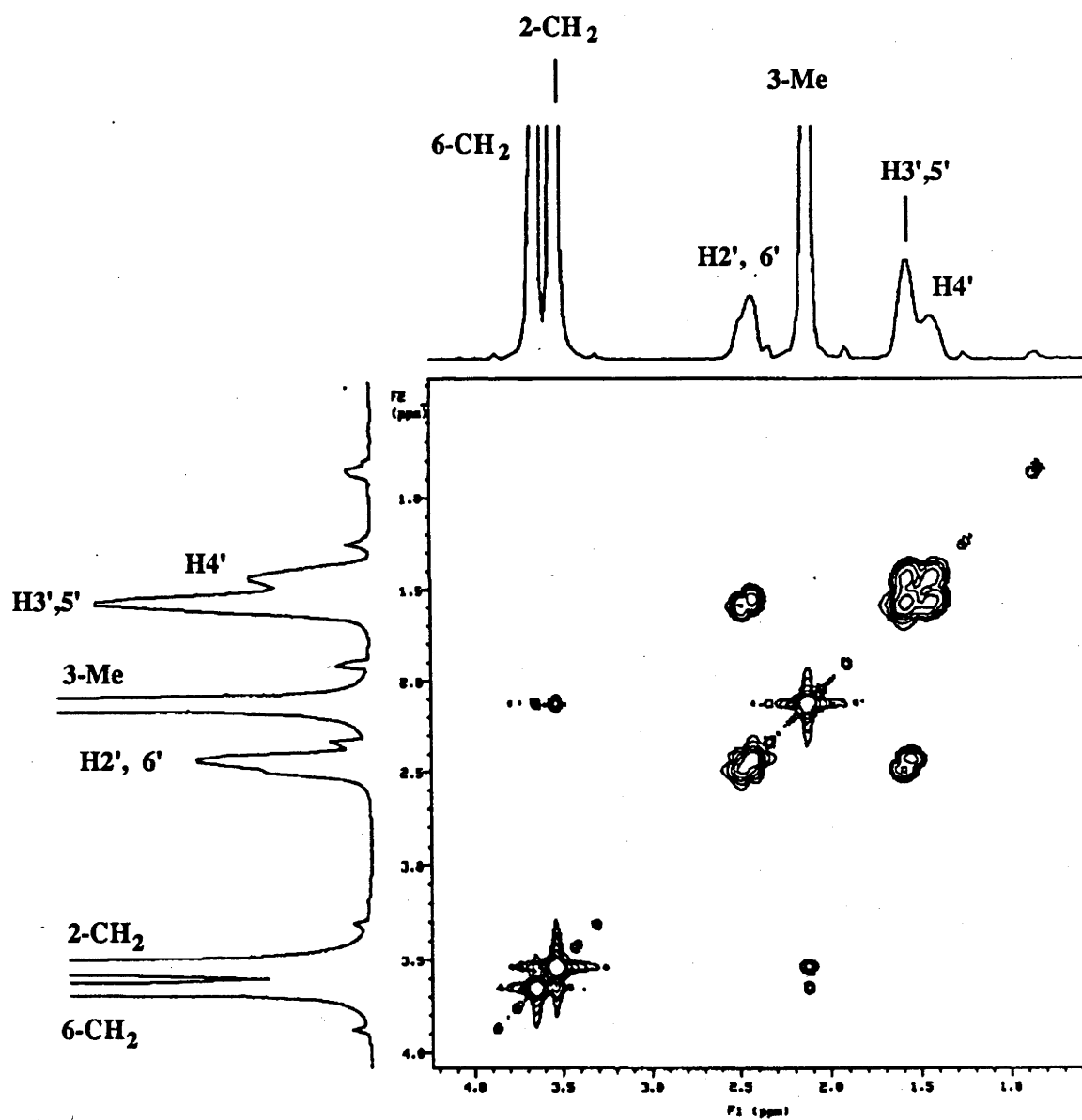
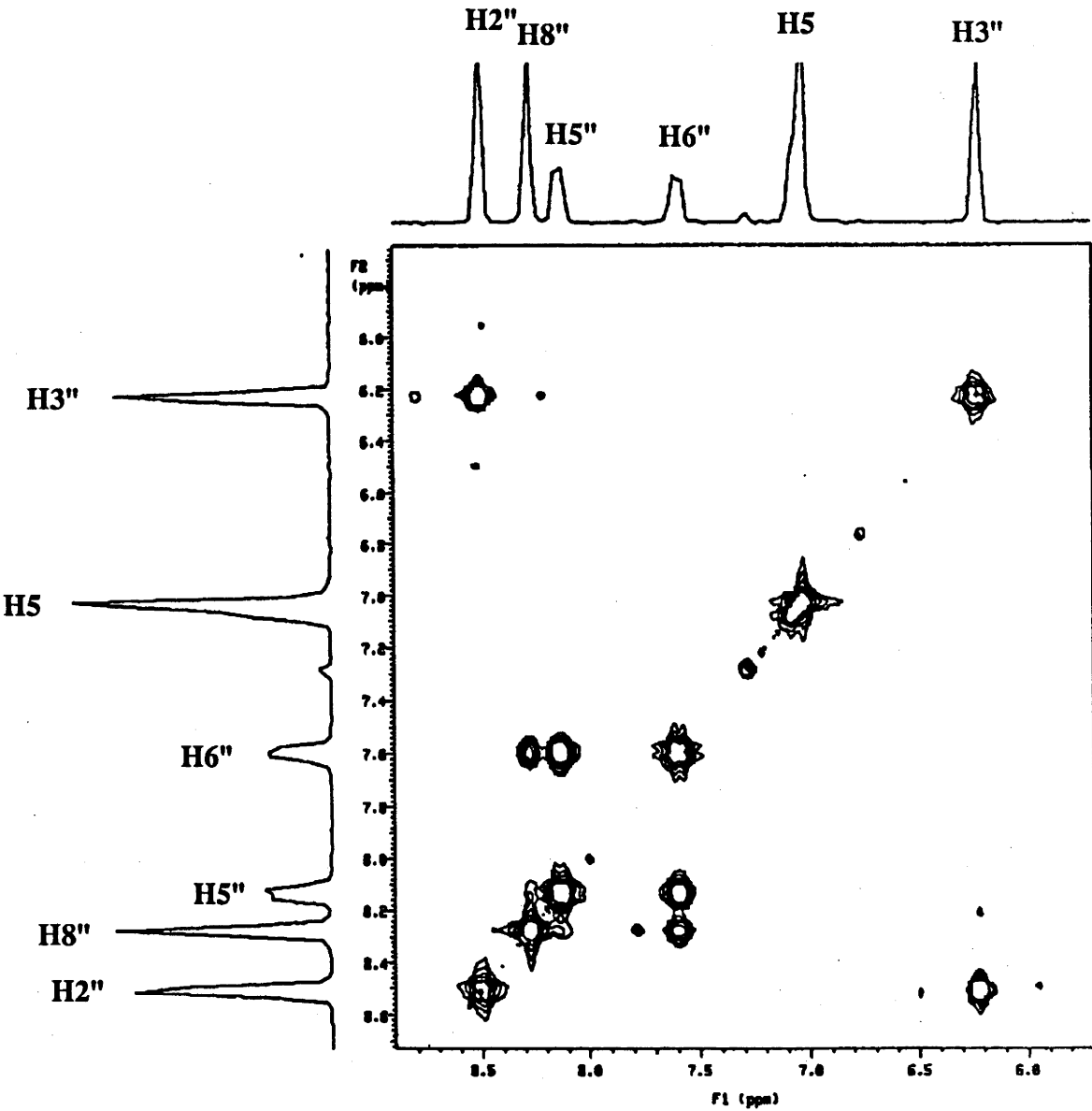


Figure A3.2 Expansion of Figure A3 at region B



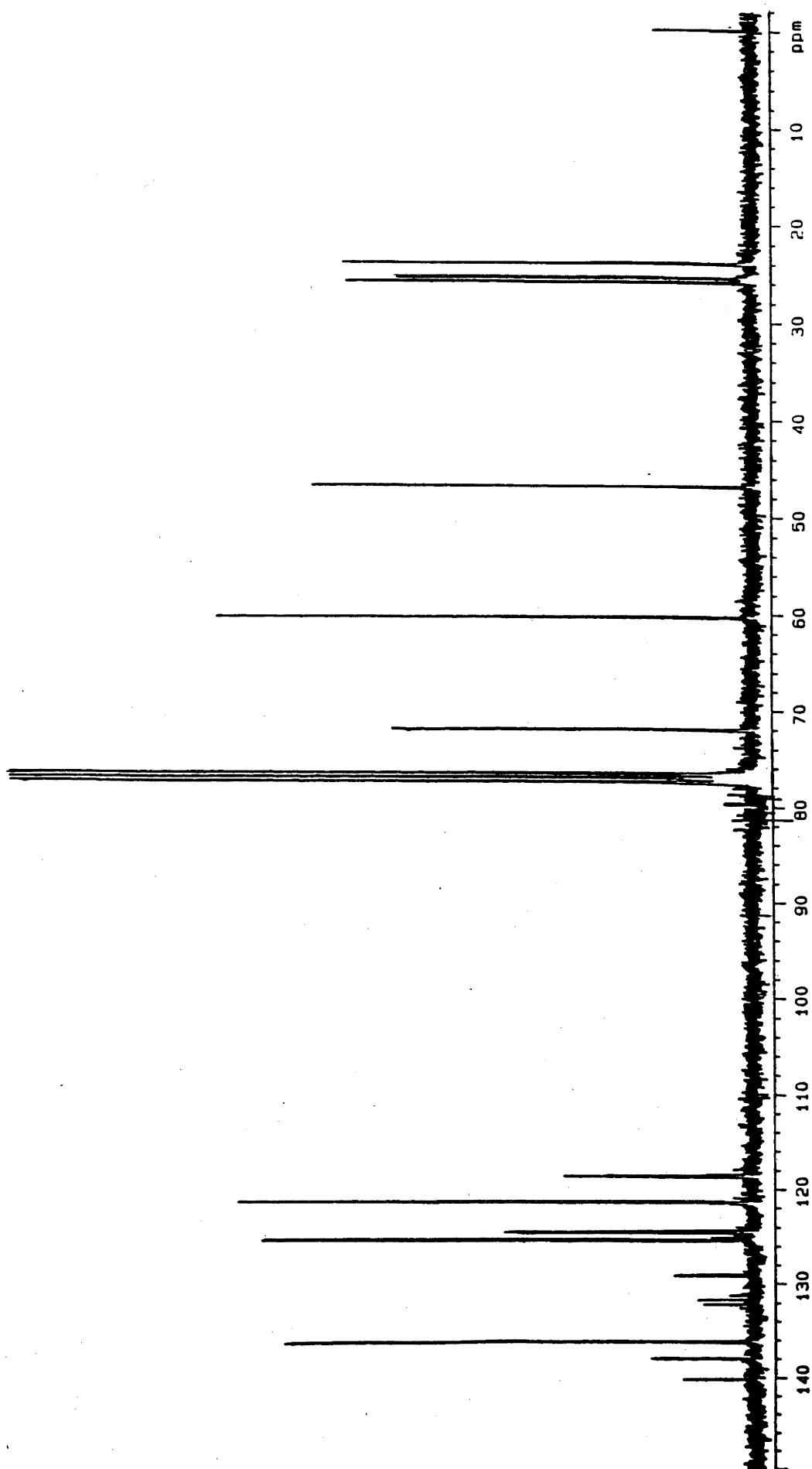


Figure A4 ^{13}C n.m.r. spectrum of α -(piperidin-2-yl)- α -(7'-trifluoromethylquinolin-4'-yl)methanol 1'-oxide (VII.5) in CDCl_3

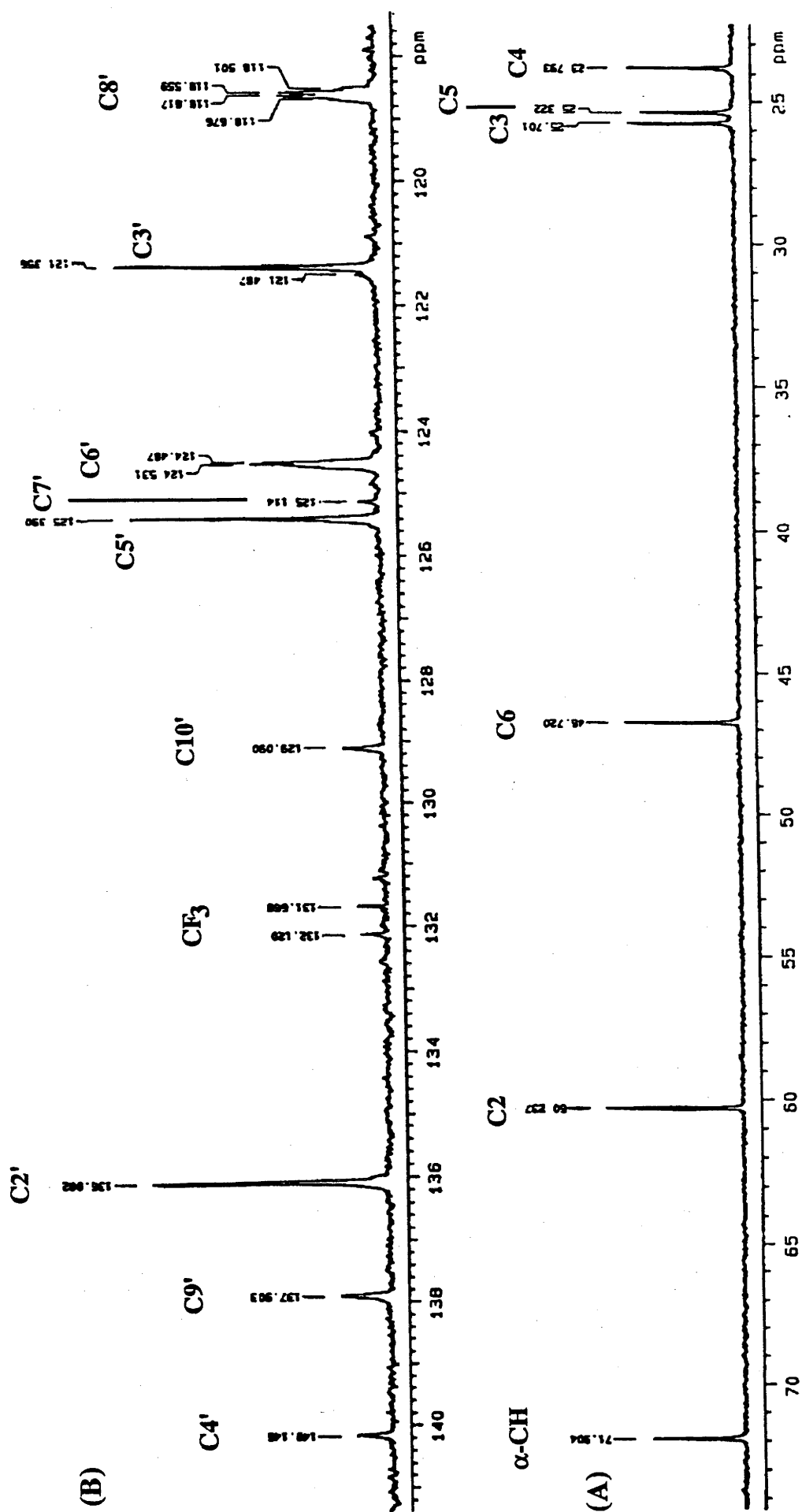


Figure A4.1 Expansion of Figure A4 at 20-80 ppm (A) and at 110-150 ppm (B)

THE MANNICH REACTION

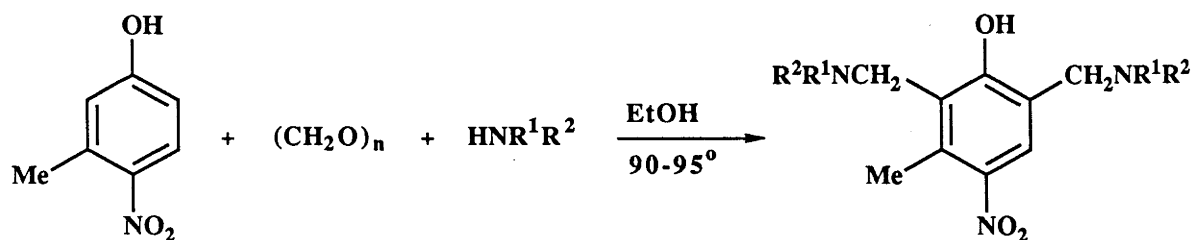
The Mannich reaction consists of the condensation of ammonia or primary or secondary amines, mostly as the hydrochloride salt with formaldehyde (or paraformaldehyde) and a compound containing one (or more) hydrogen atoms of pronounced activity such as methyl ketones or phenols. The reaction essentially involves the replacement of an active hydrogen atom by an aminomethyl or substituted aminomethyl group. The products of the condensation are known as "Mannich bases".

For example the reaction of acetophenone with formaldehyde and a secondary amine is as follows:



The earliest examples of the Mannich reaction were published by Tollens^{1,2} but Mannich^{3,4} was the first to recognise that the reaction was a general one. Numerous reports of Mannich reactions appear in the literature and many reviews of this reaction have been published.⁵⁻⁷

In the present work, di-Mannich base derivatives were prepared from 4-nitrophenol and 3-methyl-4-nitrophenol (as shown in Scheme 1) and mono-Mannich base derivatives from *N*-(4-hydroxy-5,6,7,8-tetrahydronaphth-1-yl)acetamide.



Scheme 1

3-Methyl-4-nitrophenol, as a compound containing active hydrogen atoms, reacted with excess paraformaldehyde and secondary amines (and *t*-butylamine) at reflux in ethanol to give 2,6-bis(dialkylaminomethyl)-3-methyl-4-nitrophenols (in Scheme 1).

References

1. C.M. van Marle and B. Tollens, *Ber.*, 1903, **36**, 1351.
2. H. Schäffer and B. Tollens, *Ber.*, 1906, **39**, 2181.
3. C. Mannich and W. Krösche, *Arch. Pharm.*, 1912, **250**, 647.
4. C. Mannich, *Arch. Pharm.*, 1917, **255**, 261.
5. F.F. Blicke, *Org. React.*, 1942, **1**, 303.
6. B.B. Thompson, *J. Pharm. Sci.*, 1968, **57**, 715.
7. M. Tramontini, *Synthesis*, 1973, 703.

PUBLICATIONS

PUBLICATIONS

1. Potential Antimalarials. IX
Di-Mannich Bases of 4-(7'-Trifluoromethylquinazolin-4'-ylamino)phenol and 4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol

G.B. Barlin and Chuenjit Jiravinyu
Aust. J. Chem., 1990, **43**, 311-9.
2. Potential Antimalarials. X
Di-Mannich Bases of 4-(7'-Trifluoromethyl-1',5'-naphthyridin-4'-ylamino)phenol and *N*-(4'-Diethylamino-1'-methylbutyl)-7-trifluoromethyl-1,5-naphthyridin-4-amine.

G.B. Barlin and Chuenjit Jiravinyu
Aust. J. Chem., 1990, **43**, 1175-81.
3. Potential Antimalarials. XI
4'-Chloro-3-(substituted amino)methyl-5-(7-trifluoromethylquinolin-4-ylamino)biphenyl-2-ols.

G.B. Barlin and Chuenjit Jiravinyu
Aust. J. Chem., 1990, **43**, 1301-7.
4. Potential Antimalarials. XII
4-Chloro-3-(substituted amino)methyl-5-[7-bromo (and 7-trifluoromethyl)-1,5-naphthyridin-4-ylamino]biphenyl-2-ols and 4-Chloro-3-(substituted amino)methyl-5-(7-trifluoromethylquinazolin-4-ylamino)biphenyl-2-ols.

G.B. Barlin and Chuenjit Jiravinyu
Aust. J. Chem., 1990, **43**, 1367-73.

Publication in Proof

5. Potential Antimalarials. XIII
Mono-Mannich Bases of 4-[7-Chloro (and 7-trifluoromethyl)quinolin-4-ylamino]- and 4-(7-Bromo-1,5-naphthyridin-4-ylamino)-3-methylphenol.

G.B. Barlin and Chuenjit Jiravinyu
Aust. J. Chem., 1991, **44**.

Submitted for Publication

6. Potential Antimalarials. XIV
Mono-Mannich Bases of 4-[7'-Bromo (and 7'-trifluoromethyl)-1',5'-naphthyridin-4'-ylamino]-5,6,7,8-tetrahydronaphth-1-ols.

G.B. Barlin, Chuenjit Jiravinyu and J.-H. Yan
Aust. J. Chem., Paper no 90163.