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**EXPLORING THE POTENTIAL OF XANTHENE DERIVATIVES AS
INHIBITORS OF TRYPANOTHIONE REDUCTASE AND
RESISTANCE REVERSAL AGENTS**

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MASTER OF SCIENCE

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Dr Kelly Chibale**

"If we knew what it is we were doing, it would not be called research, would it?"

Albert Einstein (1879 – 1955)

DECLARATION

I declare that "**Exploring the potential of xanthene derivatives as inhibitors of trypanothione reductase and resistance reversal agents**" is my own work and that all sources I have used or quoted have been indicated and acknowledged by means of complete reference.

Mark Ronald Visser

Signed by candidate

ACKNOWLEDGEMENTS

The completion of this project has been both challenging and rewarding. It would not have been possible without the financial, emotional and academic support I have received from people who were close to me over this period. Thus, I would like to thank the following people for their contribution to the success of this project.

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ABSTRACT

Since molecules containing the xanthene moiety were not previously described as trypanothione reductase (TryR) inhibitors and chloroquine (CQ) resistance reversal agents, two new classes of tricyclic compounds, namely 9,9-dimethyl-4,5-disubstituted and 9-monosubstituted xanthene derivatives were designed and synthesised. Within these classes, amide, amine, isothiurea, sulphonamide and urea analogues were synthesised and evaluated for inhibitory activity against TryR, *in vitro* activity against the causative agents of trypanosomiasis and leishmaniasis, and as potential CQ resistance reversal agents in a resistant strain of *Plasmodium falciparum*.

9,9-Dimethyl-4,5-disubstituted xanthenes synthesised were generally found to be weak inhibitors of TryR. From this, the best inhibitors were polyamine *N*-[3-(5-{3-[3-(Dimethyl-amino)-propylamino]-propyl}-9,9-dimethyl-9*H*-xanthen-4-yl)-propyl]-*N,N'*-dimethyl-propane-1,3-diamine (63 % inhibition at 100 μ M) and α,β -unsaturated amide *N*-(3-Dimethylamino-propyl)-3-{5-[2-(3-dimethylamino-propylcarbamoyl)-vinyl]-9,9-dimethyl-9*H*-xanthen-4-yl}-acrylamide (IC_{50} = 35.7 μ M). High *in vitro* anti-parasitic activity was observed for some derivatives with 1-[3-Dimethyl-amino)-propyl]-3-[3-{3-[3-(dimethyl-amino)-propylthioureido]-propyl}-9,9-dimethyl-9*H*-xanthen-4-yl)-propyl]-thiourea showing high anti-parasitic activity against *T. brucei* (ED_{50} = 0.098 μ M). A general lack of correlation between inhibitory potency against TryR and *in vitro* anti-parasitic activity was observed; suggesting inhibition of TryR is not totally responsible for the observed *in vitro* antiparasitic activities.

Of the different compounds tested, the 9-monosubstituted xanthene derivatives showed the most promise as potential CQ resistance reversal agents. The corresponding sulphonamide, amide and urea derivatives were less efficient than their parent amine [*N,N*-Dimethyl-*N'*-(9*H*-xanthen-9-ylmethyl)-propane-1,3-diamine]. The 9,9-dimethyl-4,5-disubstituted xanthene aryl amine *N,N*-Bis-(4-diethylamino-1-methyl-butyl)-9,9-dimethyl-9*H*-xanthene-4,5-diamine showed *in vitro* anti-malarial activity against a resistant *P. falciparum* strain (K1) comparable to that of CQ but also showed an improvement in CQ accumulation and a reversal effect greater than verapamil (VPL). The 9,9-dimethyl-4,5-disubstituted xanthene sulphonamide *N*-(3-Dimethylamino-propyl)-*N*-[3-(5-{3-[(3-dimethylamino-propyl)-4-methyl-benzenesulphonyl-amino]-propyl}-9,9-dimethyl-9*H*-xanthen-4-yl)-propyl]-4-methyl-benzenesulphonamide had an IC_{50} for antimalarial activity against K1 far superior to that of CQ but also showed a CQ resistance reversal effect at 0.5 μ M.

ABBREVIATIONS

4-AQ	4-aminoquinoline
ABC	ATP binding cassette
AD	Anno domini
Anal.	analytical
Ar	aryl
aq	aqueous
ATP	adenosine triphosphate
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
br	broad (in NMR)
Bu	butyl
<i>t</i> Bu	<i>tert</i> -butyl
Bz	benzoyl
°C	degrees Celsius
Ca ²⁺	calcium ion
Calcd.	calculated
CDCl ₃	deutero chloroform
CH ₂ Cl ₂	dichloromethane
CHCl ₃	chloroform
CNS	central nervous system
CO ₂ ⁻	carboxylate ion
CQ	chloroquine
CQR	chloroquine resistant
CQS	chloroquine sensitive
D	<i>dextro</i> isomer
d	doublet (in NMR)
DCC	<i>N,N</i> -dicyclohexylcarbodiimide
dd	doublet of doublets (in NMR)
DIAD	diisopropyl azodicarboxylate

DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
δ	chemical shift in parts per million downfield from tetramethylsilane
dt	doublet of triplets (in NMR)
ED ₅₀	effective dose required to inhibit 50% of parasite growth
EDC.HCl	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
EI	electron impact (in mass spectrometry)
Et	ethyl
Et ₃ N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
F	phenylalanine
FAB	fast atom bombardment
FAD	flavin adenine dinucleotide
g	gram (s)
Glu	glutamic acid
GR	glutathione reductase
GSH	glutathione
GSSG	glutathione disulphide
h	hour (s)
HAT	human African trypanosomiasis
hGR	human glutathione reductase
His	histidine
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
Hz	hertz
IC ₅₀	inhibitory concentration to inhibit 50% of enzyme
IFN- γ	interferon gamma
<i>i</i> Pr ₂ NEt	diisopropylethylamine
IR	infrared

J	coupling constant (in NMR)
K_a	affinity constant
$KHSO_4$	potassium hydrogen sulphate
K_i	inhibitory constant
L	litre (s), leucine, <i>levo</i> isomer
<i>L.</i>	<i>Leishmania</i>
Leu	leucine
LAH	lithium aluminium hydride
μ	micro (10^{-6})
M	methionine, moles per litre (mol.dm^{-3})
m	multiplet (in NMR), meter(s), milli
<i>m</i>	<i>meta</i>
Me	methyl
MDR	multidrug resistant
Me_3Al	trimethylaluminium
MeOH	methanol
Met	methionine
min	minute(s)
MgSO_4	magnesium sulphate
MHz	megahertz
mL	millilitre
mol	mole(s)
mp	melting point
MP	macroporous
m/z	mass to charge ratio (in mass spectra)
Na_2SO_4	sodium sulphate
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced NADP
NaHCO_3	sodium bicarbonate
NaOtBu	sodium <i>tert</i> -butoxide
<i>n</i> -BuLi	<i>normal</i> butyllithium
NH_3	ammonia

NMR	nuclear magnetic resonance
Nu	nucleophile
<i>o</i>	<i>ortho</i>
<i>p</i>	<i>para</i>
P	proline
<i>P.</i>	<i>Plasmodium</i>
Pgh1	P-glycoprotein homologue 1
Ph	phenyl
pH	- log [H ⁺]
Phe	phenylalanine
PhMe	toluene
Pr	propyl
<i>i</i> Pr	isopropyl
Pro	proline
PS	polymer-supported
q	quartet (in NMR)
R _f	retention factor (in chromatography)
RBC	red blood cell
RNA	ribonucleic acid
rt	room temperature
s	singlet (in NMR), second(s)
Sb	Antimony
Ser	serine
SiO ₂	silicon oxide (silica gel)
t	triplet (in NMR)
<i>T.</i>	<i>Trypanosoma</i>
td	triplet of doublets (in NMR)
<i>tert</i>	tertiary
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	<i>N,N,N',N'</i> -tetramethyl-1,2-ethylenediamine
Trp	tryptophan

TryR	trypanothione reductase
Try[S] ₂	trypanothione disulphide
Try[SH] ₂	trypanothione
Ts	<i>p</i> -toluenesulphonyl, tosyl
Tyr	tyrosine
V	valine
VPL	verapamil
W	tryptophan
WHO	World Health Organisation
Z	benzyloxycarbonyl

University of Cape Town

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CHAPTER 1

TRYPANOETHIONE REDUCTASE AND ITS INHIBITION

1.1 INTRODUCTION

Present chemotherapy for trypanosomiasis and leishmaniasis is inadequate, toxic or both. African trypanosomiasis (caused by *Trypanosoma brucei* spp.) can be treated effectively if detected early but complications arise in the later stages where parasites have invaded the CNS. This stage necessitates the use of drugs that have to cross the blood-brain barrier and toxic arsenicals are still widely used for this purpose. Little advance has been made in the chemotherapy of American trypanosomiasis (caused by *Trypanosoma cruzi*). Current drugs in use have no effect on the chronic stage of the disease and continuing lack of confidence in their effectiveness and safety presents a bleak future for people affected by this disease. Leishmaniasis (caused by *Leishmania* spp.), is currently treated by expensive and moderately toxic antimonial drugs and in unresponsive cases, the use of highly toxic drugs are required. There is a clear and urgent need for new treatments for these parasitic diseases that are safe, effective and inexpensive enough to permit widespread use.

A metabolic difference between trypanosomatids and their mammalian host has been described. It was shown that the parasites utilise a different process to control their intracellular reducing environment. Trypanosomatids utilise trypanothione for this task rather than glutathione used by the host. Essential to this metabolism is the enzyme trypanothione reductase (TryR), a homologue of the host glutathione reductase (GR). This biochemical difference between parasites and mammalian cells suggests trypanothione metabolism as a possible target for anti-parasite drugs and it might be possible to combat all three diseases with a single agent. Effective inhibition of TryR would compromise the parasites ability to defend itself against reactive oxygen molecules like hydrogen peroxide and hydroxyl radicals; agents known to destroy DNA and cellular membranes.

Over the past decade a number of molecules, structurally different to the substrate, have been described as inhibitors of TryR. Of particular importance is the requirement for a hydrophobic moiety, an alkylamino chain and positively charged terminal tertiary nitrogen, especially for the tricyclic inhibitors.

1.2 Oxidative stress

Oxidative stress refers to a cellular environment where the mechanisms responsible for the defence of the cell against reactive oxygen species are challenged. These reactive oxygen species include superoxide anions (O_2^-), pernitrite ($ONOO^-$), hydroxyl radicals ($HO^\bullet$), water radicals ($H_2O^\bullet$) and hydrogen peroxide (H_2O_2). The body protects itself against bacteria, parasites and tumour cells by producing superoxide anions which are converted to highly toxic hydroxyls. These reactive oxygen species are cytotoxic due to their known ability to modify nucleic acids, thiol-containing proteins and membrane lipids. Thus, oxidative stress provides an important strategy in developing new anti-parasitic agents.

1.3 The glutathione redox cycle

During normal aerobic respiration, reactive oxygen species like those mentioned above are produced. The body's main defence system against these molecules is the glutathione redox cycle (figure 1.1). One of the most important enzymes in this cycle is the flavoenzyme GR which catalyses the reduction of glutathione disulfide 1 (GSSG) to two molecules of glutathione 2 (GSH) (scheme 1.1). This reaction is NADPH dependent.

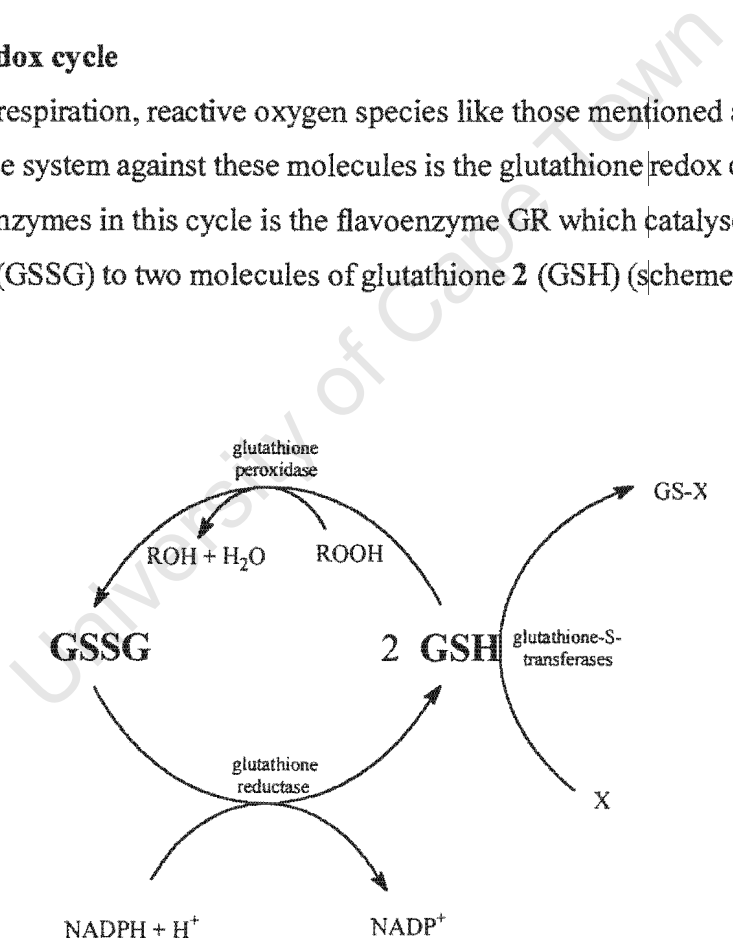
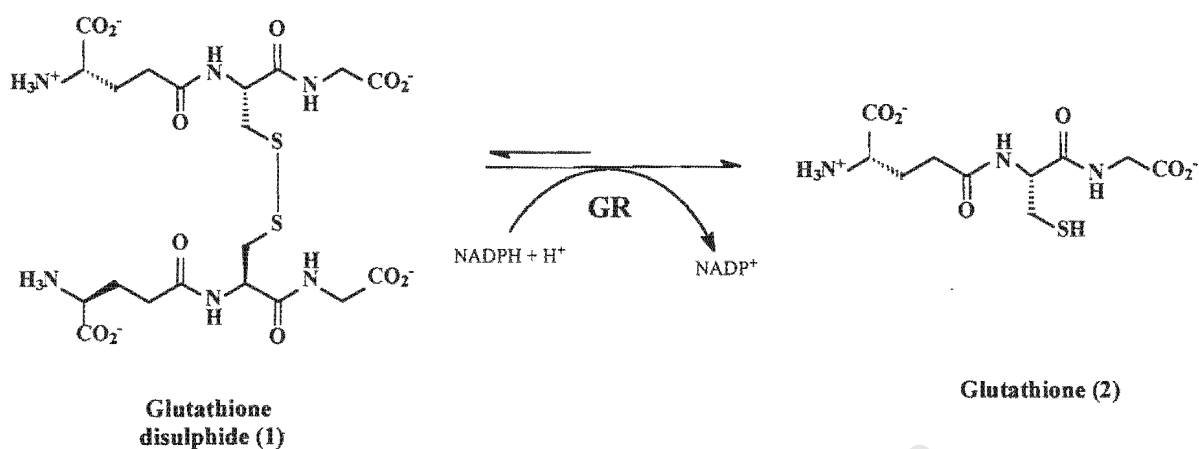


Figure 1.1. Simplified representation of the human glutathione redox cycle



Scheme 1.1. Representative diagram of the reduction of glutathione disulphide to glutathione catalysed by the enzyme GR.

The concentration of GSH is maintained at 20-fold greater than that of GSSG. Glutathione is used to remove hydrogen peroxide and organic peroxides (ROOH in figure 1.1). This process involves the enzyme glutathione peroxidase. GSH also binds various cytotoxic substances (X in figure 1.1) mediated by glutathione-S-transferases.

1.4 Trypanothione

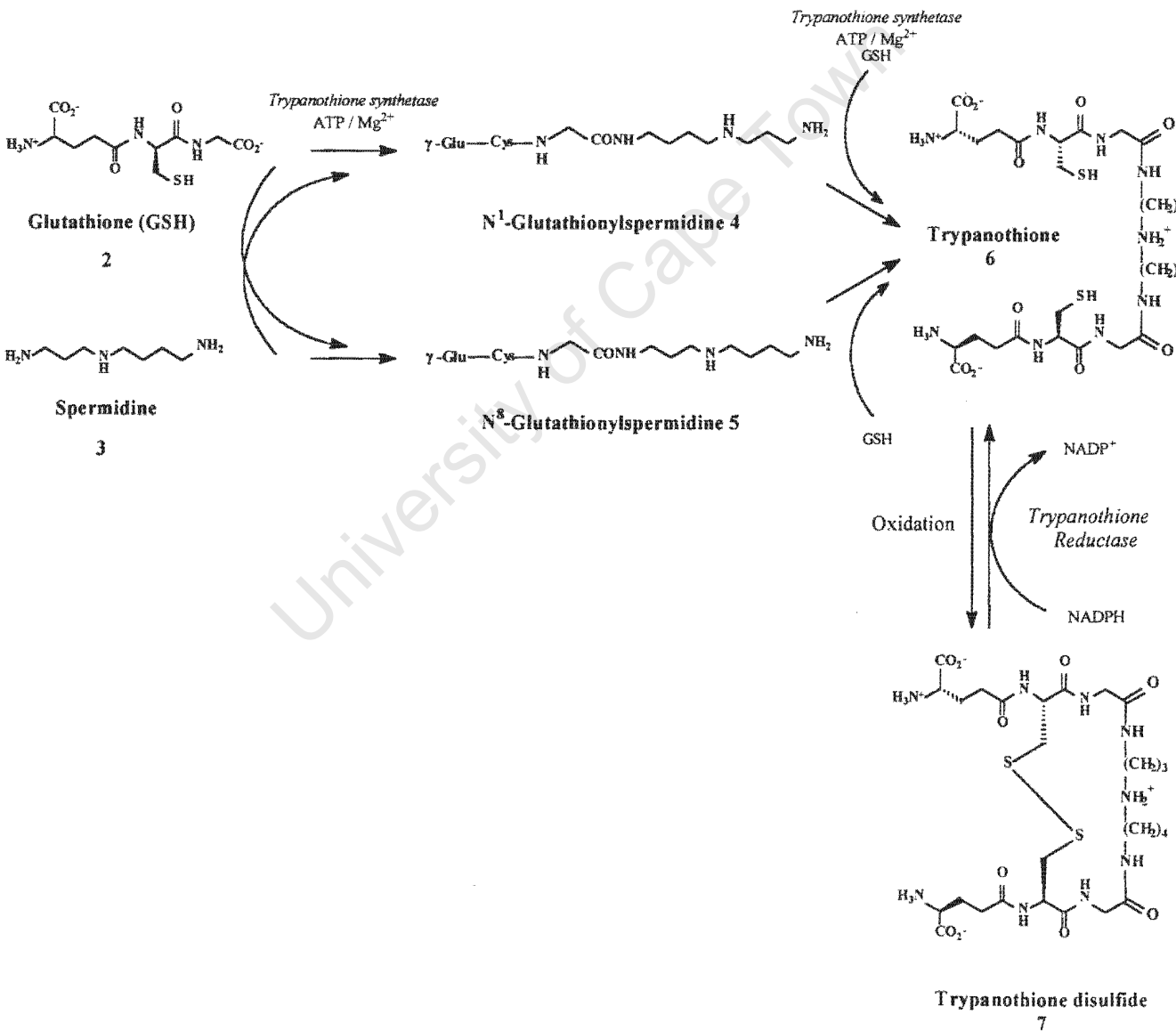
Trypanosomes and leishmanias have no GR and hence no glutathione redox cycle. Important thiols in these parasites are glutathionylspermidine (Gsp; 4 and 5) and trypanothione (Try[SH]₂) 6 (scheme 1.2).¹ Spermidine 3 is combined with glutathione 2 by trypanothione synthetase, which is an ATP dependent enzyme. The carboxyl of the cysteinyl moiety (the carboxyl closest to the -SH group) in glutathione forms a peptide bond with either the N¹- or N⁸- amine of spermidine to form N¹- or N⁸- glutathionylspermidine (4 or 5). When either of these is combined with another glutathione molecule, again by trypanothione synthetase, 6 is formed. Note that, the peptide bond is once again formed at the cysteinyl carboxyl of glutathione.

Trypanosomatids lack enzymes like catalase and glutathione peroxidase, which in other organisms detoxifies H₂O₂ and organic peroxides. Earlier belief was that these substances are removed by trypanothione in an uncatalyzed reaction to produce trypanothione disulphide (Try[S]₂) 7 (equation 1).



Recently, it was discovered that the parasite detoxifies hydroperoxides *via* a unique cascade of oxidoreductases mediated by trypanothione. The flux of reducing equivalents goes from trypanothione to tryparedoxin and, finally, via tryparedoxin peroxidase to H₂O₂. However, the parasite system is far less efficient than the mammalian glutathione peroxidases.²

During oxidative stress or in the presence of peroxides, trypanothione (Try[SH]₂) **6** is oxidised to **7**. For survival, the parasite has to re-generate Try[SH]₂ from Try[S]₂. This is done by the NADPH-dependent TryR. Thus, TryR maintains the parasites' reducing intracellular environment by keeping trypanothione in the dithiol state.



Scheme 1.2 Metabolism of trypanothione in trypanosomes and leishmanias.

Trypanothione also has other functions in the parasite, which include ascorbate homeostasis (regulation),³ thiol disulphide exchanges, conjugation of metals and drugs (sequestration/export),⁴ synthesis of deoxyribonucleotides⁵ and reduction of hydroperoxides.⁶

1.5 Trypanothione reductase is essential

Since *Trypanosoma* and *Leishmania* are known to be sensitive to oxidative stress,^{7,8} inhibition of TryR in the presence of the host analogue, glutathione reductase, is a plausible strategy to combat diseases caused by these parasites.

Several experiments have been performed to demonstrate the importance of TryR during oxidative stress. Over expression of the *tyr* gene led to the finding that mutant parasites were equally susceptible to damage by hydrogen peroxide as their normal counterparts.⁷ This suggested that the ability to re-generate trypanothione from trypanothione disulphide was not the rate-limiting step in the metabolism of hydrogen peroxide. In gene knockout experiments with *L. donovani* in which the *tyr* gene was disabled, trypanothione levels were comparable to normal cells but showed a decreased ability to survive when exposed to human macrophages.⁹ This experiment led to the conclusion that one of TryR's physiological roles in *L. donovani* is to combat oxidative stress inside human macrophages.⁹ By producing a mutant *T. brucei* strain that contained a single inducible *tyr* gene, H₂O₂ hypersensitivity was observed when the gene was "switched off".¹⁰ This group also demonstrated that trypanosomes lacking TryR lost their ability to infect a host.¹¹ Taken together, it can be deduced that the absolute concentrations of Try[S]₂ and Try[SH]₂ is not important but rather the ability to re-generate Try[SH]₂ during oxidative stress, i.e. TryR is important during oxidative stress.^{2,10,11}

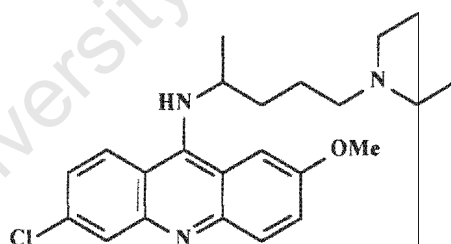
1.6 Structural and binding features of TryR

TryR and GR are dimeric proteins with a monomer mass of 52 kDa. Each subunit folds into 4 domains; the FAD-binding, the NADPH-binding, the central and interface domains. The active site is formed by residues of the FAD, NADPH and central domain of one monomer and interface domain of the other. The redox-active disulphide, formed between Cys53 and Cys58, and the active base (His461') are involved in the catalysis and is located at the bottom of the active site.¹²

Despite a 41 % sequence homology between *T. congolense* TryR and human GR, the two enzymes display mutual exclusivity with respect to their disulphide substrates.^{13,14} Differing substrate specificities

of hGR and TryR have been explained on the basis of the structure of the active site of native TryR. There are close similarities in the active sites of the two enzymes. Almost all active site residues distinguishing TryR from GR are located in the region binding the spermidine moiety of $[\text{TS}]_2$. There are two factors that confer substrate specificity. Firstly, the TryR active site is larger to accommodate the larger substrate¹⁵ and secondly, the overall charge of active site residues in TryR interacting with $\text{Try}[\text{S}]_2$ is negative whilst those in GR is positive. This is explained by the fact that $\text{Try}[\text{S}]_2$ is positively charged at NH_2^+ (scheme 1.2) of the spermidine bridge whilst in GSSG it is negatively charged (scheme 1.1).¹⁶

In 1996, the first crystal structure of mepacrine-trypanothione reductase complex was described.¹⁷ Mepacrine **8** is a competitive inhibitor of TryR¹⁸ and in its complex with TryR is bound at the substrate-binding site with the tricyclic moiety close to Trp 21 and Met 113 (forming the hydrophobic cleft, figure 1.2), with the ring nitrogen pairing with Met 113, the chlorine atom with Trp 21 and the methoxy group with Ser 109. The alkylamino chain is fixed with Glu18 *via* a water-mediated hydrogen bond.¹⁷ More recently, the three-dimensional structure of TryR in complex with its substrate trypanothione has been characterised.¹² A hydrogen bond interaction between an active site histidine and a carbonyl of the substrate was explained as the basis for the substrate specificity observed between TryR and GR.



Mepacrine (8)

Other studies showed that TryR could process non cross-linked substrates.¹⁹ This was done by systematically varying the spermidine crosslink of $\text{Try}[\text{S}]_2$. It was found that both the α -carboxylate and α -amino groups of the γ -glutamyl moieties of the substrate were important to substrate processibility.²⁰ However, the γ -glutamyl functions could be replaced by any of several structures, many hydrophobic, but maintains the carboxylate and amino groups.²⁰

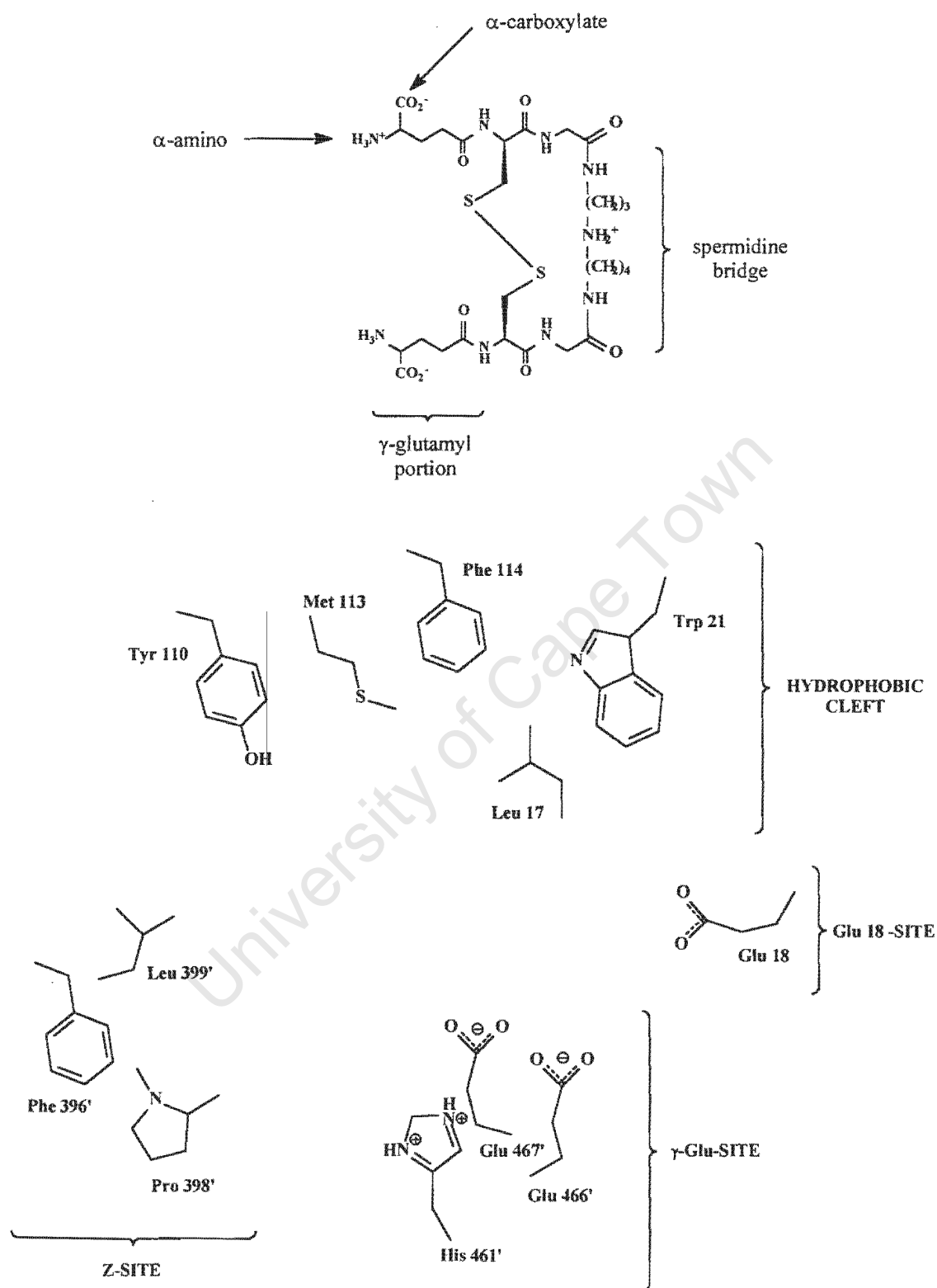
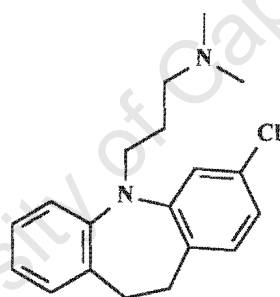


Figure 1.2. Schematic summary of regions of the active site of TryR (not to scale). Adapted from Chan *et al.*²¹

1.7 Tricyclic compounds as inhibitors of trypanothione reductase

1.7.1 Lead discovery

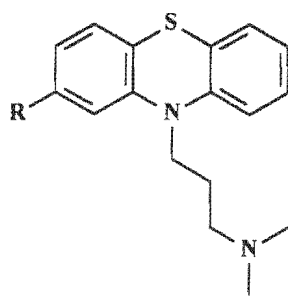
The initial design approach was to target the hydrophobic wall (figure 1.2) in the active site of TryR, since this site is responsible for the enzymes' substrate specificity. Various aromatic structures were filled into this hydrophobic pocket using a computer-modelling program (AUTODOCK²²). For solubility purposes and to position the ligand into its supposed binding site, a cationic ammonium site was introduced in the prototype inhibitors. This was envisaged to conveniently interact with a site sandwiched between two glutamic acid side chains (Glu466' and Glu467', figure 1.2) [the apostrophe next to the residue refers to amino acids on the second monomer] in the region of the active site disulphide.¹⁶ The structures that began to emerge in the design process closely resembled those of the tricyclic anti-depressants such as clomipramine **9**. These molecules were tested against TryR and GR and proved to be strong competitive inhibitors of TryR with little inhibition of hGR.²³ What follows are an overview of tricyclic compounds that have been shown to inhibit TryR.



Clomipramine (**9**)

1.7.2 Phenothiazine inhibitors

It had been shown previously that tricyclic antidepressants based on phenothiazines (figure 1.3) possessed weak antitrypanosomal and antileishmanial activities.²¹



R = H, promazine; IC_{50} = 108 μ M
R = CF₃, trifluopromazine; IC_{50} = 110 μ M
R = Cl, chlorpromazine; IC_{50} = 35.4 μ M

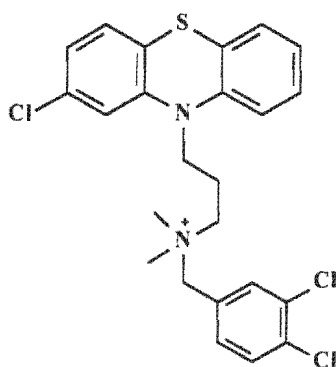
Figure 1.3. Representative phenothiazine inhibitors of *T. cruzi* TryR

A tertiary amine is important in the propylamino substituent since inhibition weakens when the terminal $N(CH_3)_2$ is replaced by $NHCH_3$ or NH_2 . Furthermore, a negative charge at the terminus, e.g. a carboxyl group is also disfavoured and derivatives with an alkyl-amino side chain were more potent than an acyl-amino chain.

There are indications that the phenothiazine nucleus can take up more than one position within the active site of TryR. This can be explained by the observation that the hydrophobic pocket in TryR is slightly bigger than the tricyclic nucleus, so the inhibitors have the freedom to bind at different angles. The effective area of the hydrophobic pocket available to inhibitors is not limited to the hydrophobic wall formed by M113, F114 and W21 used to recognise the spermidinyl sector of the TryR substrate. This region is adjacent to another hydrophobic region (F396', P398', L399', V58 and V53), the Z-site (figure 1.2), not used to recognise the natural substrate but which may bind tricyclic molecules.²¹

Phenothiazine inhibitors were also tested for *in vitro* activity against *T. brucei*, *T. cruzi* and *L. donovani*. The bloodstream trypomastigotes of *T. brucei* showed the highest *in vitro* sensitivity while the intracellular amastigote stages of *L. donovani* and *T. cruzi* had lower *in vitro* sensitivities to the phenothiazines. From the *in vitro* studies it was observed that no correlation between the ED_{50} for *in vitro* anti-parasitic activities and TryR inhibition existed and if the anti-parasitic action of these compounds arose from TryR inhibition, molecular features other than TryR binding are dominant e.g. parasitic cellular penetration or metabolism. It has been observed from other antitrypanosomal/antileishmanial activities of tricyclic compounds that no one structural feature seems to control activity.²⁴

More recent ligand design studies indicated that it should be possible to more effectively sequester the TryR active site than the first-generation tricyclics by quaternization of the tertiary amino site of chlorpromazine **9** with suitable hydrophobic groups.²⁵ Quaternary arylalkylammonium chlorpromazines were synthesised and TryR inhibition studies as well as *in vitro* anti-parasitic activity evaluations were performed. The rationale was to link the hydrophobic cleft putatively occupied by the tricyclic nucleus with the Z site (figure 1.2). These compounds were strong inhibitors of *T. cruzi* TryR with quarternised phenothiazine **10** giving the best inhibition of TryR ($IC_{50} = 0.78 \mu M$, $K_i = 0.12 \mu M$, ED_{50} against *T. brucei* = $0.546 \mu g/mL$).



10

Several of these quaternary phenothiazines completely inhibited *T. brucei* parasitic growth *in vitro* at <1 μM . Although active against *L. donovani*, none of the analogues showed major improvement in this activity relative to chlorpromazine or other nonquaternized phenothiazines. It was concluded that the binding strength of the tricyclic phenothiazine lead for TryR was improved with no loss of specificity over host GR, by enlisting a second hydrophobic pocket (the Z site) in addition to the original design target hydrophobic wall²³ and vectoring the inhibitor's interaction by means of a third electrostatic site (the γ -Glu site, figure 1.2) giving rise to the three-point inhibitor concept which may be used in future inhibitor designs.²⁵

1.7.3 Dibenzazepine inhibitors

Imipramine **11** (figure 1.4), a known anti-depressant, was found to be a linear competitive inhibitor of *T. cruzi* TryR.²⁶ Once again, any group other than a tertiary amine resulted in a weaker inhibition.

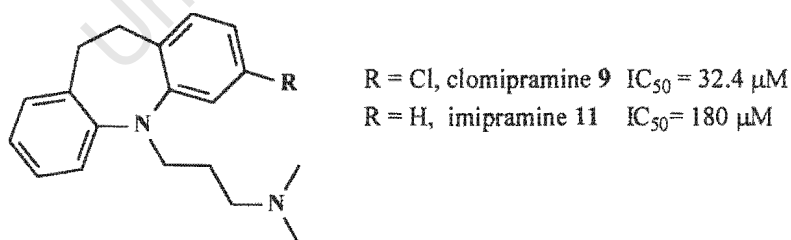


Figure 1.4. Representative benzazepines shown to be inhibitors of TryR

So far, it is evident from studies with the phenothiazines and benzazepines that the optimum chain must be flexible, five units long, aliphatic, unbranched, and present at position 1 containing a dimethylamino function as terminal group.

1.7.4 Acridine inhibitors

Acridines are known to possess anti-trypanosomal, anti-bacterial and anti-tumour properties and have been considered for the treatment of several protozoan infections.^{18,27} In a serial screening of compounds licensed for human treatment, mepacrine **8** (also known as quinacrine) was one of the most active drugs against *T.cruzi* *in vitro* and the mepacrine-TryR complex has been described as mentioned before.

A range of 9-aminoacridines related to mepacrine were synthesised and tested for inhibition of *T.cruzi* TryR.²⁸ Within this series, the importance for the alkylamino chain was reiterated but an increase in one methylene within the alkyl chain led to improved inhibition relative to mepacrine. The introduction of an additional methylene group could make direct contact between the terminal diethylamino group with the protein possible instead of through a water mediated hydrogen bond, as seen in the TryR-mepacrine complex. Acridine itself and anionic derivatives did not bind TryR.

Recently, our laboratory has also investigated the utilisation of acridines as inhibitors of TryR. The rationale was to incorporate methylene spacers between the tricyclic moiety of mepacrine and a second hydrophobic aromatic moiety that would interact with the Z-site. This led to the preliminary design of the sulphonamides **12** and ureas **13** (figure 1.5).²⁹

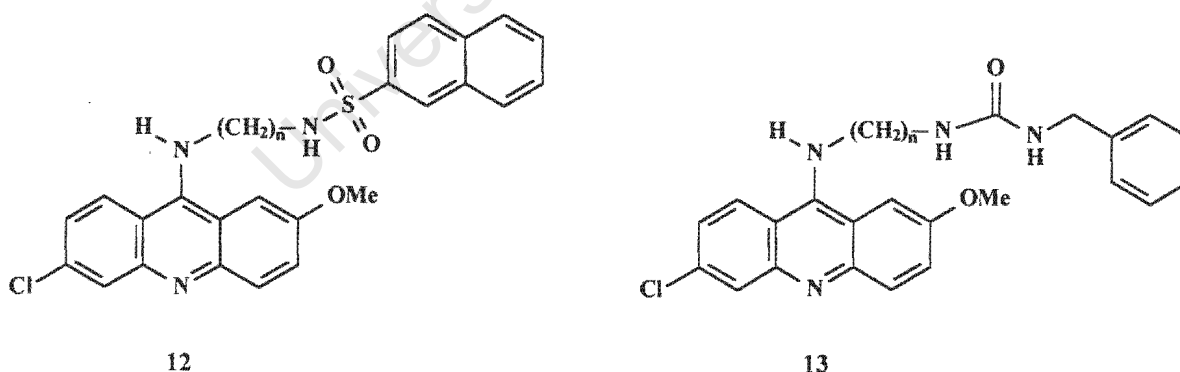


Figure 1.5. Structures of sulphonamide **12** and urea **13** acridine derivatives.

From the inhibition studies it was apparent that compounds **12** and **13** were superior to mepacrine in inhibiting TryR and within the series, the sulphonamides were superior to the ureas, but this may be due to the more hydrophobic naphthalene moiety in **12** compared to the benzyl moiety in **13**.²⁹ Despite their superior inhibitory activities against TryR, they were also inhibitors of human GR. The reason for this was ascribed to the lack of a positive charge that is normally provided by the tertiary amino group of

mepacrine. The optimal length of the methylene spacers for TryR inhibition was three and four, with the best inhibitor in the sulphonamide series ($n = 3$, $IC_{50} = 3.3 \pm 0.3 \mu M$).

Compounds **12** and **13** were also evaluated for their anti-parasitic activity against *L.donovani*, *T.cruzi* and *T.brucei*. It was observed that the inhibition data did not correlate with the *in vitro* data with the urea compounds showing significant activity against all three parasites. The compounds were also cytotoxic at the same concentrations, indicating a lack of selectivity against these parasites. The mode of action of these compounds is not clear and needs further investigation.²⁹

1.8 Conclusion

Many structurally diverse compounds have been shown to inhibit TryR, other than the tricyclic derivatives. Some of these include the arsenicals³⁰ and antimonials³¹ currently in use in the treatment of trypanosomiasis and leishmaniasis, polyamine derivatives based on spermine and spermidine³²⁻³⁴ and the 2-amino-diphenylsulphides³⁵⁻³⁹ which are structurally related to the tricyclics.

Excellent work has been done on TryR and has resulted in the development of highly selective inhibitors of this enzyme. However, there is still uncertainty regarding the mechanism of action in the cellular environment, since many potent inhibitors of TryR show no correlation between inhibitory potency and *in vitro* anti-parasitic activities. While continued work on TryR is justified, investigation into other targets may provide an alternative approach.

Other targets to combat trypanosomiasis and leishmaniasis that are beginning to emerge include the protozoal cysteine proteases, serine oligopeptidase, transporters of raw materials for nucleic acid synthesis and polyamines, glycolytic enzymes in the glycolytic pathway, phosphoribosyl transferases, tubulin and folate biosynthesis. A detailed review on recent developments in the field of drug discovery using different targets in trypanosomiasis, leishmaniasis and malaria has been published.⁴⁰

CHAPTER 2

CQ RESISTANCE IN MALARIA AND ITS REVERSAL

2.1 MALARIA

2.1.1 General considerations

Malaria results from infection with the *Plasmodium* parasite, of which 4 species are responsible for the disease. These are, *P. vivax*, *P. ovale*, *P. malariae* and *P. falciparum*; the latter species being responsible for the majority of infections. The parasite is transmitted to its human host by blood sucking female *Anopheles* mosquitoes. An infection with *P. falciparum* gives rise within weeks to periodic bouts of chills, fever and sweating, the soaring body temperature coinciding with multiplication within, and re-invasion of the red blood cells. Swelling of the spleen sets in as defensive white blood cells and parasitised red blood cells accumulate there. Lysis of infected cells leads to anaemia. Death can result from aggregation of parasitised erythrocytes in the capillaries of the viscera, causing damage to the kidneys, lungs and most critically, the brain.

The WHO estimates that 300 million to 500 million people are infected with malaria worldwide. One quarter of the world's population is at risk of malaria infection. An estimated 1.5 to 2.7 million people, primarily children, die of malaria each year, most of them in Africa. Malaria continues to be a major health threat throughout the tropical world and, while potential demand for anti-malarials are high, drug resistance is spreading faster than alternatives are being developed, the flow of new drugs in the pipeline having all but stopped. In a short time, certain parts of the world may have no effective anti-malarial drugs available at all. This unfolding crisis has made it essential to identify novel strategies for the development of antimalarial therapies. Central to the development of new drugs is the issue of drug resistance itself. Of particular significance is the recent emergence of multidrug-resistant malarial parasites.⁴¹

2.1.2 Life cycle of *Plasmodium*

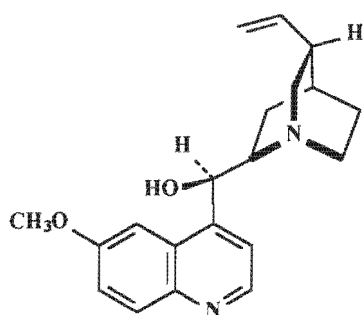
The protozoal *Plasmodium* is transmitted by the bite of the female *Anopheles* mosquito, which is the insect vector of the organism. *Sporozoites* in the salivary gland are injected into the human host. The *sporozoites* rapidly leave the circulating blood and invade hepatic (liver) parenchyma cells to begin exoerythrocytic schizogony, which occurs 6-12 days after exposure. Hepatic cells rupture, releasing

merozoites that invade circulating red blood cells (RBC's), and the erythrocytic life cycle schizogony begins.

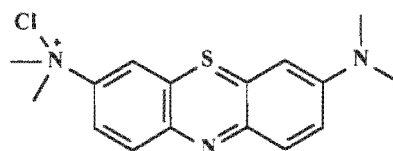
Inside the erythrocyte, the *merozoite* is nourished by the cell's contents. It metabolises the haemoglobin and grows intracellularly. The *merozoite* becomes a ring form, which grows into a late ring or *amoeboid trophozoite*, then into an early *schizont* (chromatin dividing), and finally into a *schizont* that contain *merozoites*. The *merozoites* are released from the erythrocyte and invade other cells.⁴² A female mosquito feeding on an infected host will then ingest the circulating *merozoites*, in which sexual fusion takes place, leading to the accumulation of thousands of new invasive forms in the salivary glands of the insect.

2.1.3 History of chemotherapy

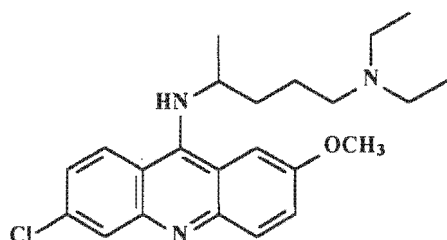
Malaria chemotherapy began in the 17th century when the wife of a Spanish viceroy in Peru was miraculously cured from a severe attack of malaria after taking a remedy made from the cinchona tree bark. In 1820 the active compound, quinine **14** (figure 2.1), was isolated from the cinchona tree bark. In 1891, it was discovered that methylene blue **15** had anti-malarial activity. During the Second World War, quinacrine **8** (discovered in 1932) was the standard anti-malarial agent in Asia and the South Pacific. Chloroquine **16** was synthesised in 1934 and replaced quinacrine as the drug of choice for the prevention and treatment of malaria. Resistance to chloroquine was first noticed in the 1960's and quinine was re-introduced. Other anti-malarial drugs used worldwide are proguanil **17**, pyrimethamine **18**, tetracyclines and sulphonamides. Newer agents are mefloquine **19**, a quinoline methanol; and halofantrine **20**, a 9-phenanthrene methanol. Artemisinin⁴³ **21**, an enantiomerically pure sesquiterpene lactone bearing an endoperoxide function, extracted from the Chinese traditional medicinal herb qinghaosu, which has been used for febrile (fever causing) illnesses in China since 341 AD was rediscovered in 1971 to have anti-malarial activity.⁴⁴



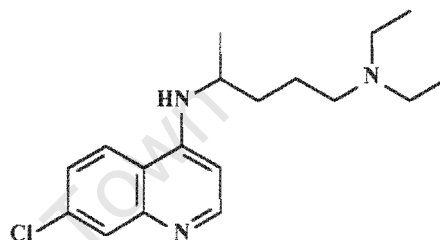
quinine (14)



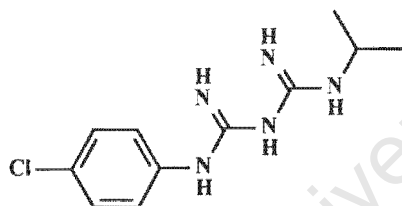
methylene blue (15)



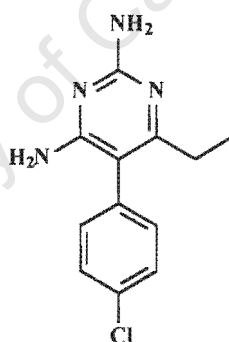
quinacrine (8)



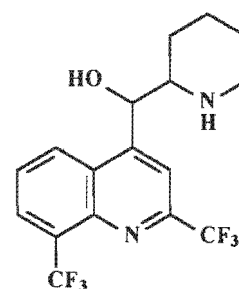
chloroquine (16)



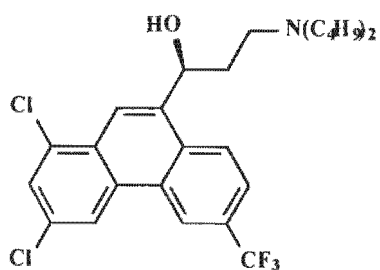
proguanil (17)



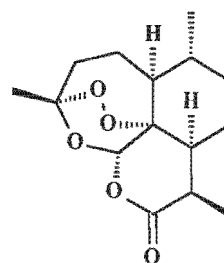
pyrimethamine (18)



mefloquine (19)



halofantrine (20)



artemisin (21)

Figure 2.1. Chemical structures of some anti-malarial agents.

2.2 CHLOROQUINE RESISTANCE

2.2.1 Introduction

"If a parasite population is exposed to drug concentrations $C_x < C_{min}$, the drug will eliminate the sensitive part of the population but leave individuals able to withstand C_x , which results in a new population that is less sensitive compared to the population prior to exposure. Since drug response is genetically determined, the new population will maintain its response pattern in the absence of further drug pressure. Upon renewed drug pressure, further selection will take place, resulting in enhanced resistance." This was the view of Wensdorfer as an explanation of the phenomenon of drug resistance in his report on the epidemiology of resistance in malaria.⁴⁵

In 1973, the WHO defined drug resistance as "the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in a dose equal to or higher than those usually recommended but within the limits of tolerance of the subject". Chloroquine resistant malaria parasites are stable phenotypes even in the absence of continued drug pressure and thus the resistant parasites may be transmitted by local mosquitoes to other people in the immediate area or may be carried by a migrant host to other places where mosquitoes may be present to establish transmission.⁴⁶

Chloroquine has been the first line of treatment of suspected or confirmed malaria since its synthesis in 1934. Its success could be contributed to its rapid onset of action, low cost, good tolerability, ease of synthesis and suitability for both prophylactic and curative purposes. Chloroquine resistance was first reported in 1959 and 1960 from 2 different areas. Since then, many more reports of chloroquine resistance have been received in all areas where the disease is endemic. This tragedy was compounded by reports of malaria parasites resistant to other quinoline containing anti-malarials, such as mefloquine, amodiaquine and quinine. There have been few conclusive reports of chloroquine resistance in species causing human malaria other than *P.falciparum*. CQ now has been lost as a first-line drug for the treatment of falciparum malaria in most endemic areas of the world.⁴⁵ There is a broad agreement amongst researchers that chloroquine resistant parasites accumulate less drug than their susceptible counterparts. Therefore, transport and accumulation of chloroquine into the parasite are not only essential for activity but are also intimately related to the resistance phenotype.⁴⁷

2.2.2 Mechanisms of chloroquine resistance

The exact mechanism of chloroquine resistance still remains unclear. To date there are a number of theories that may account for resistance, and include (1) a membrane efflux pump similar to the one

observed in multi-drug resistant (MDR) mammalian cancer cells and (2) a difference in initial uptake rates between susceptible and resistant strains of *P.falciparum*.

a) The biochemical basis of chloroquine resistance

Available data locates the accumulation of chloroquine to the acid food vacuole (lysosome) of the parasite.⁴⁸ Since chloroquine accumulation is energy dependent, saturable and inhibited by other 4-aminoquinolines (4-AQ's),⁴⁹ it was concluded that there must be a high-affinity receptor for binding 4-AQ's in malaria infected erythrocytes and it was suggested by Fitch⁴⁹ that this could be ferriprotoporphyrin IX (heme).

Chloroquine is a diprotic (two protonation sites) weak base that accumulates within the parasite as a consequence of the pH gradient that exists between the extracellular space and the lysosome. Protonation at lower pH of CQ inside the lysosome leads to the trapping of this species since protonated CQ cannot cross the membrane. This maintains the concentration gradient for inward movement of the unprotonated CQ into the lysosome. Furthermore, it was shown that CQ accumulation into *P.berghei* and *P.falciparum* is absolutely dependent on the existence of a pH gradient between the accumulating compartment and the extracellular environment.⁴⁹

It has been shown that the initial uptake rate of CQ is altered in resistant parasites and verapamil (VPL) **22** (figure 2.2), a Ca^{2+} -antagonist used in cardiovascular diseases, could increase this initial rate selectively in resistant isolates.⁵⁰ Many researchers have described the biphasic concentration-dependent accumulation of 4-AQ's which comprise a high-affinity, low capacity phase that is an energy dependent and saturable process and a low-affinity, non-saturable energy independent phase. A mathematical model based on the hypothesis that only high-affinity drug accumulation is pharmacologically relevant has been developed. This model suggests that the apparent K_a (affinity constant, measured in nmol/l) for high-affinity uptake is increased for resistant isolates, i.e. resistant cells have a slower rate of drug uptake. This apparent K_a is decreased by chemosensitizers without affecting the low-affinity uptake process. They concluded that saturation of the high-affinity "receptor" is responsible for anti-malarial activity but does not explain the mechanism of resistance or its reversal.

Both anti-malarial activity and chemosensitization are dependent on normal vacuolar acidification⁵¹ suggesting that the high-affinity site is located within the lysosome. If unpolymerized heme is the receptor and sequestration of heme into heamazoin loses its affinity for the drug, any mechanism that

can reduce the concentration of drug available to bind per unit time would have the effect of increasing the apparent affinity. This could take the form of (1) a VPL sensitive efflux pump decreasing drug uptake directly, (2) any process capable of altering vacuolar pH or (3) a membrane alteration capable of altering CQ permeability.⁴⁷

b) The molecular basis of chloroquine resistance

The mechanism of resistance in multidrug resistant (MDR) cancer has been confirmed to be drug efflux mainly (but not exclusively) *via* an ABC (ATP-binding cassette)-transporter, P-glycoprotein.⁵² It was reported that the rate of efflux of pre-accumulated CQ from resistant parasites was some forty times faster than their susceptible counterparts.⁵³ The ability of VPL **22** to reverse CQ resistance in resistant *P. falciparum* have prompted the mechanistic comparison of CQ resistance in this parasite with MDR in mammalian cancer cells,⁵⁴ since VPL is also a known chemosensitizer of mammalian MDR cancer cells.

The mammalian MDR phenotype results from the amplification of *mdr* genes resulting in increased expression of P-glycoprotein. The search for a similarity in *P. falciparum* resulted in the finding of 2 *mdr* homologues, *pfmdr1* and *pfmdr2*. The latter codes for a 110 kDa protein but there is little evidence to suggest a link with CQ resistance. However, there is a considerable amount of data implicating *pfmdr1* involvement in the mechanism of resistance. This gene codes for a 162 kDa protein, named P-glycoprotein homologue 1 (Pgh1) and has a 54 % amino acid sequence homology to the *mdr1* gene product.⁵⁵ It was observed that Pgh1 is expressed throughout the erythrocytic cycle and is located predominantly on the vacuolar membrane of *trophozoites*.⁵⁶ However, no correlation between over-expression and drug resistance was observed with equivalent amounts of Pgh1 in CQ resistant and susceptible parasites.⁵⁶

2.2.3 In vitro chemosensitization of resistant cells

a) Chemosensitization of mammalian MDR cells

Ideal chemosensitizers have been described as compounds that are without any intrinsic or inherent cytotoxicity, which can completely reverse resistance of cells to the cytotoxic action of drugs.⁵⁷ VPL is the classical chemosensitizer of MDR cells and provided the first evidence for a mechanistic similarity between resistant cancer cells and chloroquine resistant *P. falciparum*. Since the introduction of VPL, many diverse and structurally different compounds were found to modulate MDR cells *in vitro*. In the presence of appropriate concentrations of chemosensitizers, drug cytotoxicity is greatly potentiated in resistant cancer cells and in many cases the IC₅₀ approaches that of susceptible cells.⁵⁷ VPL had the

ability to modulate a variety of MDR cell lines.⁵⁸ Sensitive cells from which MDR cell lines were selected displayed no significant change in drug sensitivity in the presence of VPL.⁵⁸

Verapamil is a calcium-channel blocker⁵⁹ but its mode of action in MDR cells is independent of calcium ion transport.⁶⁰ Moreover, the D and L isomer is equipotent in modulating MDR cells⁶⁰ whilst only the L-isomer interacts with the calcium-channel.⁵⁹ The chemosensitization effect of VPL is associated with an increase in accumulation of drug and this process is thought to be the mechanistic basis of chemosensitization.⁶¹ VPL was shown to completely reverse resistance in moderately resistant cells but only a partial reversing effect was observed in highly resistant cells.⁶¹ The potential clinical utility for resistance reversal *in vivo* is limited by its cardiotoxicity at resistance reversing concentrations⁶² but provides a good model for future investigations.

b) Chemosensitization of CQR *Plasmodium falciparum*

There are several phenotypic similarities of the reversal of drug resistance by VPL in malaria parasites and mammalian cells. Similar concentrations of VPL (1 – 10 μM) are required. Chemosensitization of CQ-resistant parasites by VPL are also not stereospecific with both D and L isomers being equally efficient⁶³ and the modulation activity of VPL is distinct from any calcium-channel blocking activity.⁶⁴ In addition, VPL does not increase free calcium concentration in the parasite.⁶⁵ Thus it can be concluded that VPL reverses resistance in *P.falciparum* by a mechanism unrelated to the movement of free calcium. VPL was also shown to increase the steady-state accumulation of CQ and this accumulation was restricted to CQ resistant isolates.⁵³

Verapamil often increases accumulation of drugs in MDR cells to levels equivalent to sensitive cells but CQ accumulation in CQR *trophozoites* in the presence of verapamil is always substantially lower than sensitive parasites.^{53,66} This points to a mechanism of modulation of malaria parasites that is subtly different from that of mammalian MDR cells. It is likely that VPL acts specifically to increase the binding of CQ to a high-affinity low capacity receptor inside the parasite that is responsible for the activity of CQ.⁶⁷

c) Chloroquine resistance reversal agents

Many compounds have been identified to reverse CQ resistance *in vitro*. Interestingly, most compounds are tricyclic molecules that are already being used for human treatment of various conditions. Also, most

of these compounds contain a protonatable nitrogen atom (figure 2.2) whereas many chemosensitizers of MDR are uncharged.⁶⁷ Compounds include Ca^{2+} antagonists, methoxyverapamil, diltiazem **23**, amlodipine⁶⁸ **24**; tricyclic anti-depressants like chlorpromazine (figure 1.3) and analogues⁶⁹, desipramine **25**, protriptyline **28**, imipramine **11** (figure 1.4), doxepin⁶⁶ **26**; tricyclic anti-histaminics like cyproheptadine⁷⁰ **27**, chlorpheniramine⁷¹ **29**; as well as selective serotonin re-uptake inhibitors like fluoxetine⁷² **30**. It is not known whether all these compounds have a CQ accumulation effect but it has been shown for desipramine⁶⁶ and diltiazem.⁵³ There have been cases where a molecule could chemosensitize CQ resistant parasites without affecting CQ accumulation.⁷³

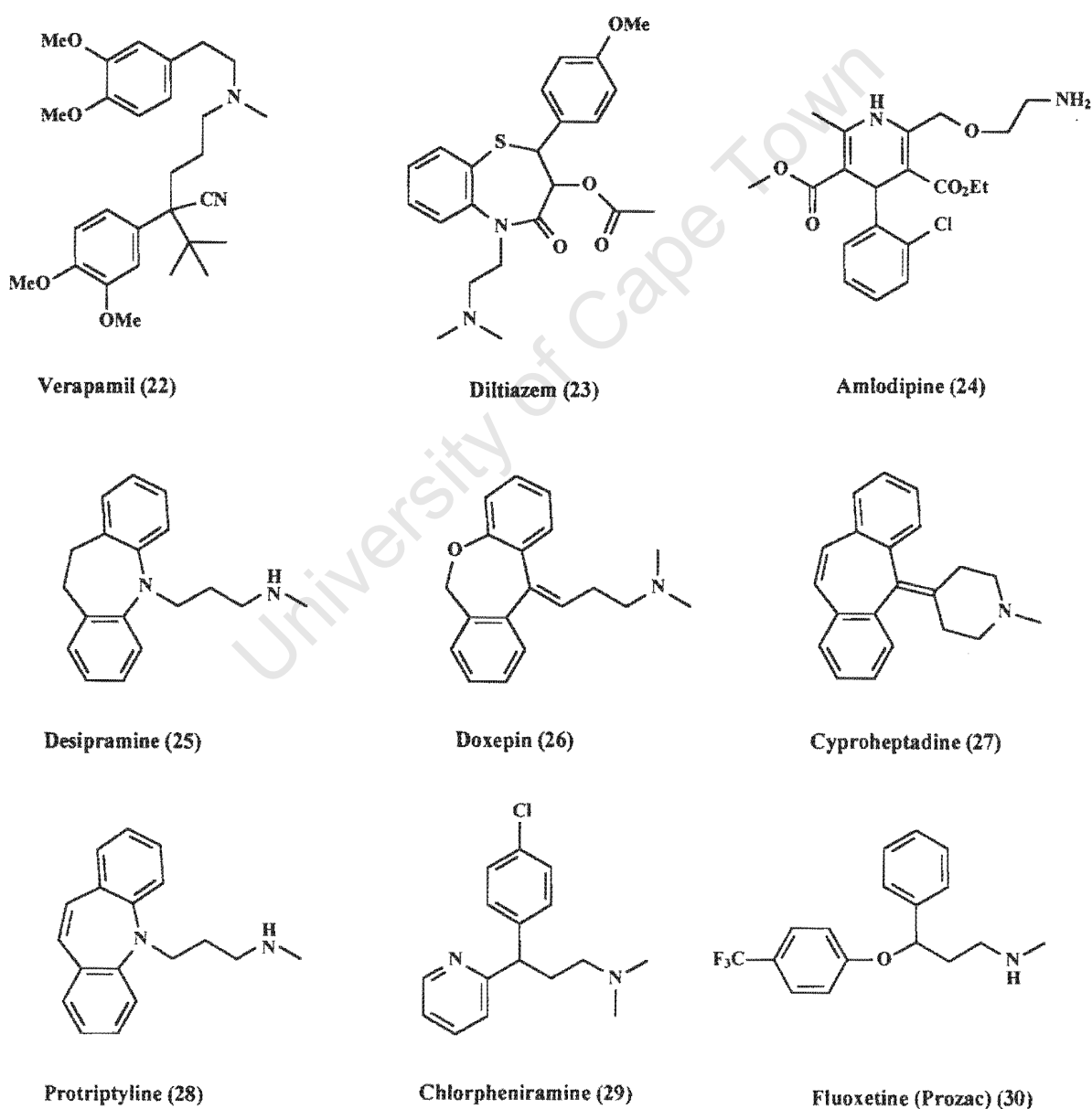


Figure 2.2. Chemical structures of some compounds that reverse CQ resistance *in vitro*.

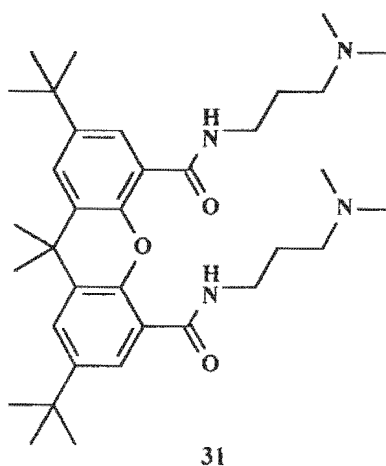
CHAPTER 3

9,9-DIMETHYL-4,5-DISUBSTITUTED XANTHENE DERIVATIVES

3.1 RATIONALE

As earlier indicated, tricyclic compounds exemplified by acridines, benzazepines, phenothiazines and pyridoquinolines have provided drug leads in the area of chemotherapy of trypanosomiasis and leishmaniasis.^{1,21,23} A literature search revealed that tricyclics based on the 9,9-dimethylxanthene moiety have not been previously investigated as potential TryR inhibitors and CQ resistance reversal agents. Thus, it was decided to conduct an investigation into the potential of the subject compounds exemplified by **32 – 39** (figure 3.1).

For TryR, the rationale behind the choice of the subject compounds was as follows: First, being an aromatic hydrophobic tricyclic moiety, the 9,9-dimethylxanthene moiety bears resemblance to the aromatic hydrophobic tricyclic moieties found in other tricyclic compounds already reported as competitive inhibitors of TryR, where the tricyclic moiety binds in the hydrophobic pocket involved in the recognition of the spermidine moiety of trypanothione disulfide, the substrate for TryR.¹⁷ Second, the chemically reactive 2,7 and 4,5 positions of the xanthene moiety provide potential multiple sites for introducing chemical diversity.^{74,75} This would ultimately aid analogue synthesis and exploration of structure-activity relationships within this class of compounds. Third, a terminal amino group (exemplified by the dimethylamino group) was incorporated into these compounds to provide a positive charge, which has been shown to favour TryR over glutathione reductase (GR), the closest related host enzyme.⁷⁶ Intermediary functional groups (amides, amines and thioureas) were incorporated to improve solubility properties.



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The 2,7-*tert*-butyl amide derivative **31** had earlier been synthesised and shown to possess excellent anti-parasitic activity against *L. donovani*, *T. cruzi* and *T. brucei*.⁷⁷ This is despite being a weak inhibitor of TryR. Thus, further investigation into this new class of inhibitors was justified and provided the impetus for the current research project on TryR inhibitors.

In view of the tricyclic nature of some known CQ resistance reversal agents alluded to earlier, we also wished to explore the potential of selected derivatives in this regard.

University of Cape Town

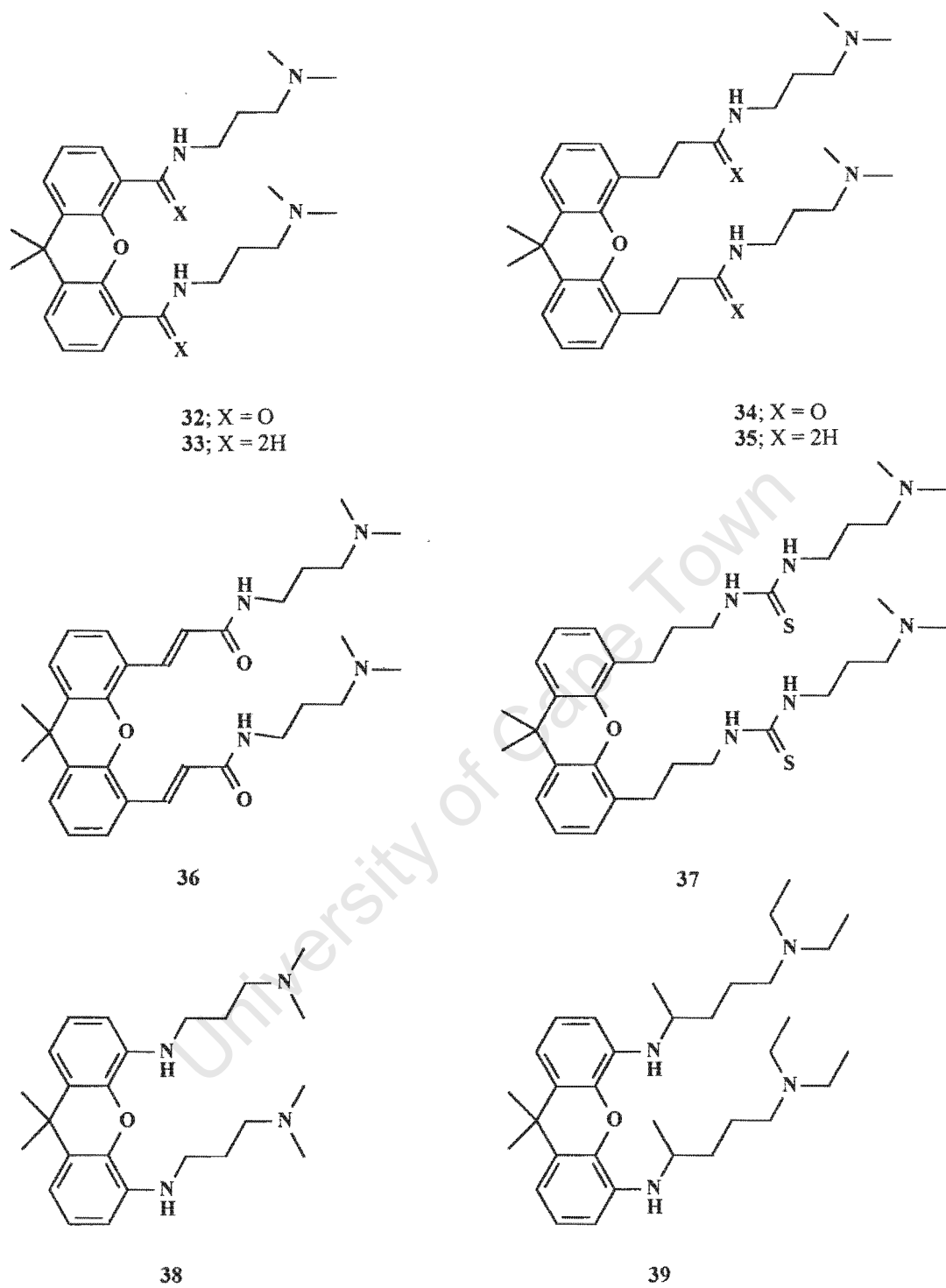
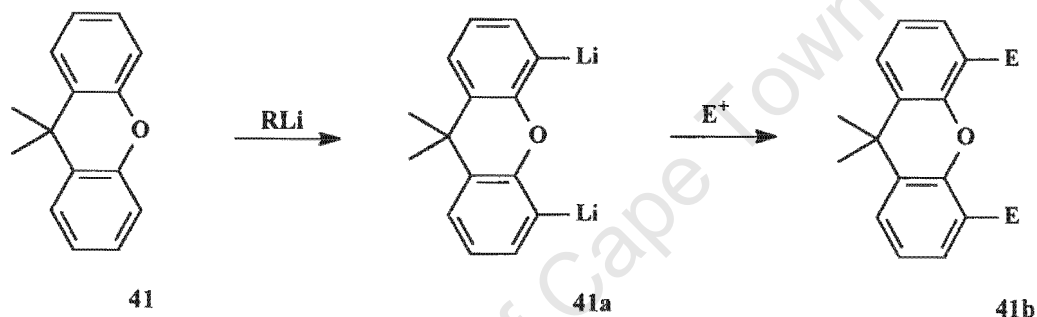


Figure 3.1. Structures of 9,9-dimethylxanthene compounds targeted for synthesis.

3.2 CHEMICAL SYNTHESIS

3.2.1 Synthesis of amide 32 and amine 33

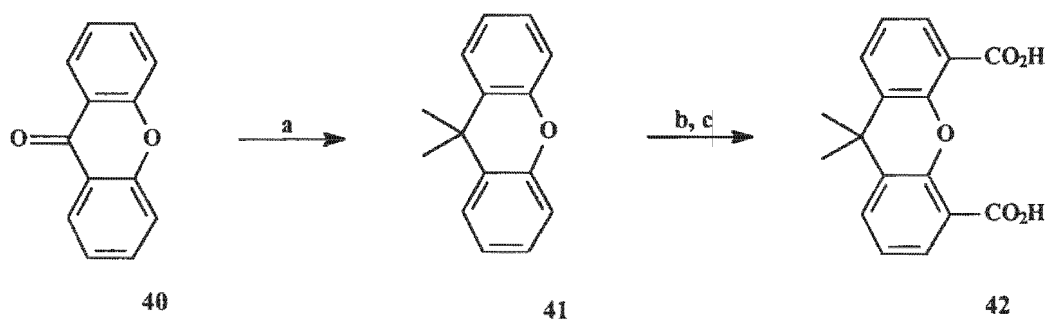
Amides can be synthesised from the corresponding acid by activation of the carboxylic functionality and coupling with the desired amine. Thus, the acid **42** (scheme 3.2) was synthesised through a directed *ortho*-metalation (DoM) procedure. The DoM reaction comprises the deprotonation by a strong base of a site *ortho* to a heteroatom containing a directed metalation group (DMG). In our case the heteroatom is the oxygen atom of xanthene **41**. The strong base is normally an alkyllithium reagent (RLi), leading to an *ortho*-lithiated species **41a**. This species, upon treatment with electrophilic reagents (E⁺) will lead to the 4,5-disubstituted xanthene **41b** (scheme 3.1).⁷⁸



Scheme 3.1. The directed *ortho* metalation reaction.

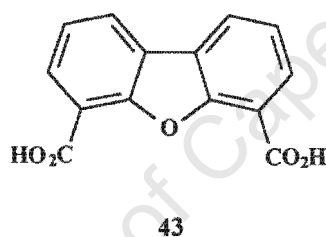
In hydrocarbon solvents, alkyllithiums tend to aggregate; i.e. *n*-butyllithium (*n*-BuLi) in hexane occurs as a hexamer. Addition of basic solvents such as THF or Et₂O causes dissociation by an acid-base reaction, e.g. THF coordination to (*n*-BuLi)₆ leads to solvated (*n*-BuLi)₄ and addition of Et₃N leads to acceleration in dissociation to the dimer. Bidentate ligands such as *N,N*-tetramethylethylenediamine (TMEDA) effectively break down alkyllithium aggregates forming monomers and dimers in solution and thereby significantly increase their basicity.⁷⁸

Thus, 9,9-dimethylxanthene **41**, prepared *via* exhaustive methylation of xanthone **40**,⁷⁹ was subjected to *ortho*-lithiation using *n*-butyllithium in THF followed by quenching with solid CO₂ and acidification (scheme 3.2).

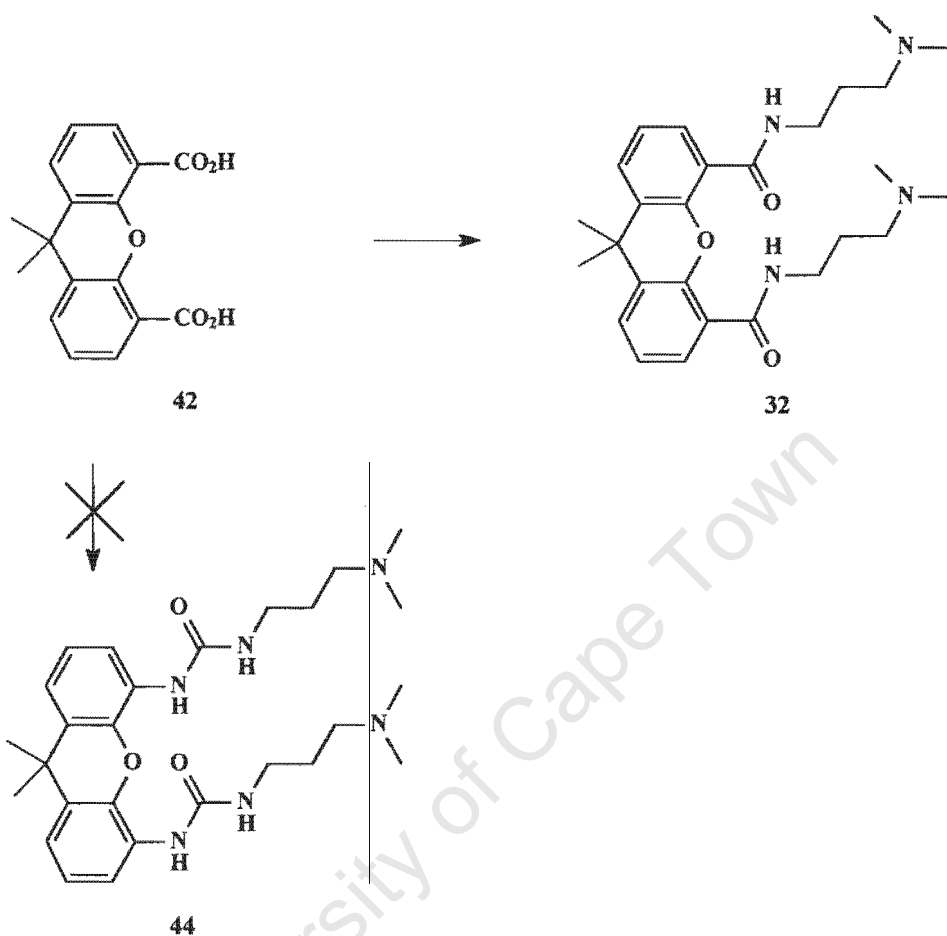


Scheme 3.2. Reagents and conditions: (a) 2.5 equiv. of Me_3Al , PhMe , $0\text{ }^\circ\text{C}$, 3h, then $25\text{ }^\circ\text{C}$, 16h, 98 %; (b) 3.0 equiv. of $n\text{-BuLi}$, THF , $-40\text{ }^\circ\text{C}$, 1h, then (c) excess dry ice (CO_2), H_2O , pH 1, 60 %.

Compound **42** was difficult to purify due to its lack of solubility in most organic solvents used. Others have also noted similar difficulties in the synthesis of related 4,6-dibenzofurandicarboxylic acid **43**.⁸⁰

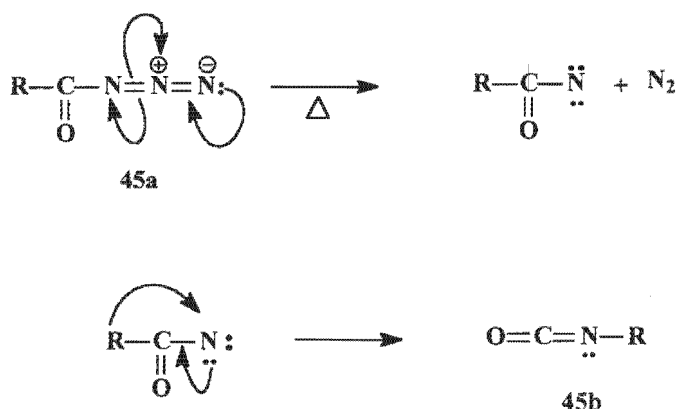


Initially, we wished to synthesise the urea derivative **44** (scheme 3.3) by treatment of acid **42** with diphenyl phosphoryl azide (DPPA), 3-dimethylamino-1-propylamine in the presence of triethylamine as base and heating to $85\text{ }^\circ\text{C}$ for 18 hours. Instead, only the amide analogue **32** was isolated. Evidence in support of the amide came from the ^1H NMR spectrum where the amide proton resonated at 8.5 ppm, whereas the urea NH was expected to resonate at 6 ppm. In addition, the structure was confirmed by HRMS.



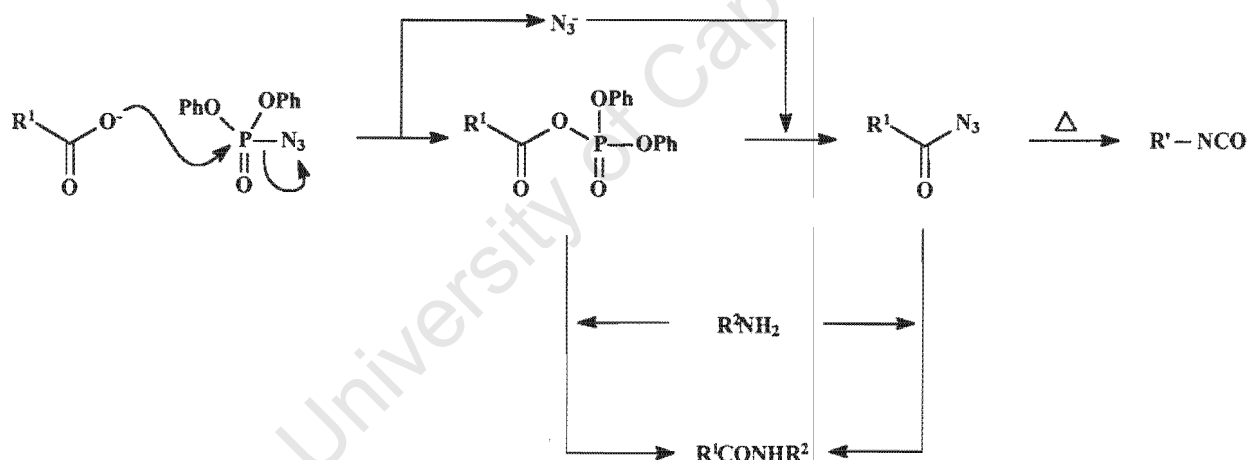
Scheme 3.3. Reagents and conditions: 2.5 equiv. of $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, 2.0 equiv. of Et_3N , 2.0 equiv. of $\text{H}_2\text{N}(\text{CH}_2)_3\text{NMe}_2$, PhMe , 85°C , 18h, 65 %.

Usually when a carboxylic acid is treated with DPPA in the presence of triethylamine, an acyl azide (scheme 3.4, **45a**) is produced. Upon heating, the acyl azide undergoes a Curtius rearrangement (scheme 3.4)⁸¹ to form the isocyanate **45b** *in situ*. Formation of the nitrene and subsequent rearrangement happens simultaneously upon heating of the acyl azide. The isocyanate is then trapped by the amine to produce the urea.



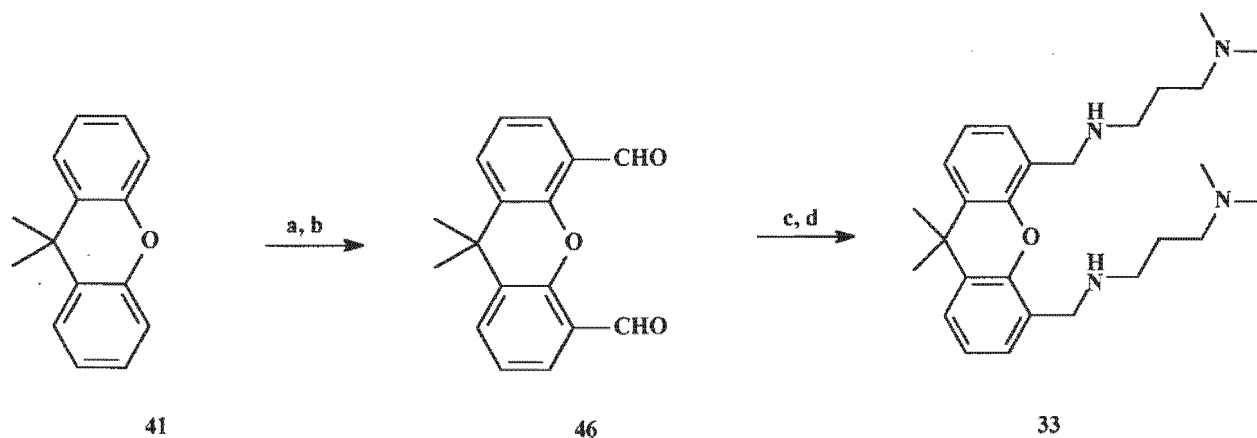
Scheme 3.4. Schematic representation of the Curtius rearrangement

In our case, presumably in the presence of the amine, a nucleophile substitution reaction involving the diphenyl phosphoryl anhydride or the acyl azide occurred in competition with the Curtius rearrangement (scheme 3.5). As it turned out, the former reaction was favoured under the reaction conditions.



Scheme 3.5. Illustration of possible products using diphenyl phosphoryl azide as activating agent

The target amine **33** was synthesised through the bis-aldehyde intermediate **46**, prepared by quenching the 4,5-bis anion formed from the *ortho*-lithiation and trapping with DMF, *via* reductive amination in ethanol by reaction of 3-dimethylamino-1-propylamine with bis-aldehyde **46** (scheme 3.6). Filtration gave the amine in quantitative yield. This procedure provided an efficient synthesis of the amine since it is a one-pot procedure and does not require harsh conditions, like that required if the amine was synthesised by reducing the amide **32** with borane.

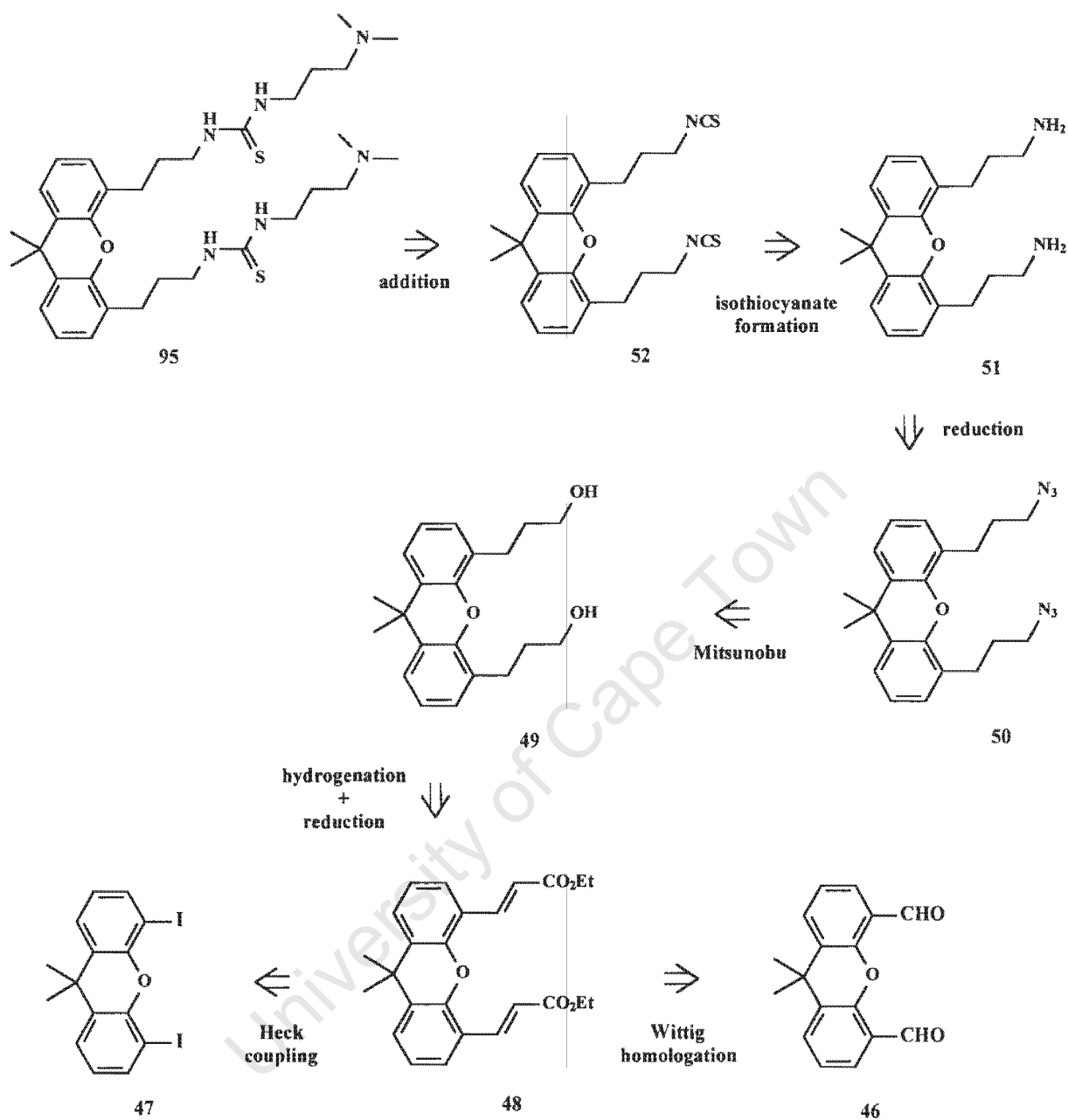


Scheme 3.6. Reagents and conditions: (a) 3.0 equiv. of *n*-BuLi, 3.0 equiv. of TMEDA, Et₂O, reflux, 18h; then (b) 3.0 equiv. of DMF, Et₂O, 25 °C, 24h, 72 %; (c) 1.8 equiv. of H₂N(CH₂)₃NMe₂, MeOH, 25 °C, 15h; (d) 4.0 equiv. of Amberlite IRA-400 borohydride resin, 25 °C, 18h, 100%.

3.2.2 Synthesis of isothioureia 37

a) Retrosynthetic analysis

To synthesise 9,9-dimethylxanthene derivatives with increased spacer length between the xanthene moiety and the reactive groups in the chain, a two-carbon spacer needs to be introduced. As outlined in the retrosynthetic scheme (scheme 3.7), the key intermediate α,β -unsaturated ester **48** could be synthesised by performing a palladium catalysed Heck coupling on the di-iodide **47** using ethyl acrylate or *via* Wittig homologation. The former procedure requires a palladium catalyst and a phosphine ligand. These reagents are considered expensive in light of the relatively inexpensive Wittig reaction reagents and since the starting bis-aldehyde **46** was already available, **48** was synthesised using the Wittig approach.

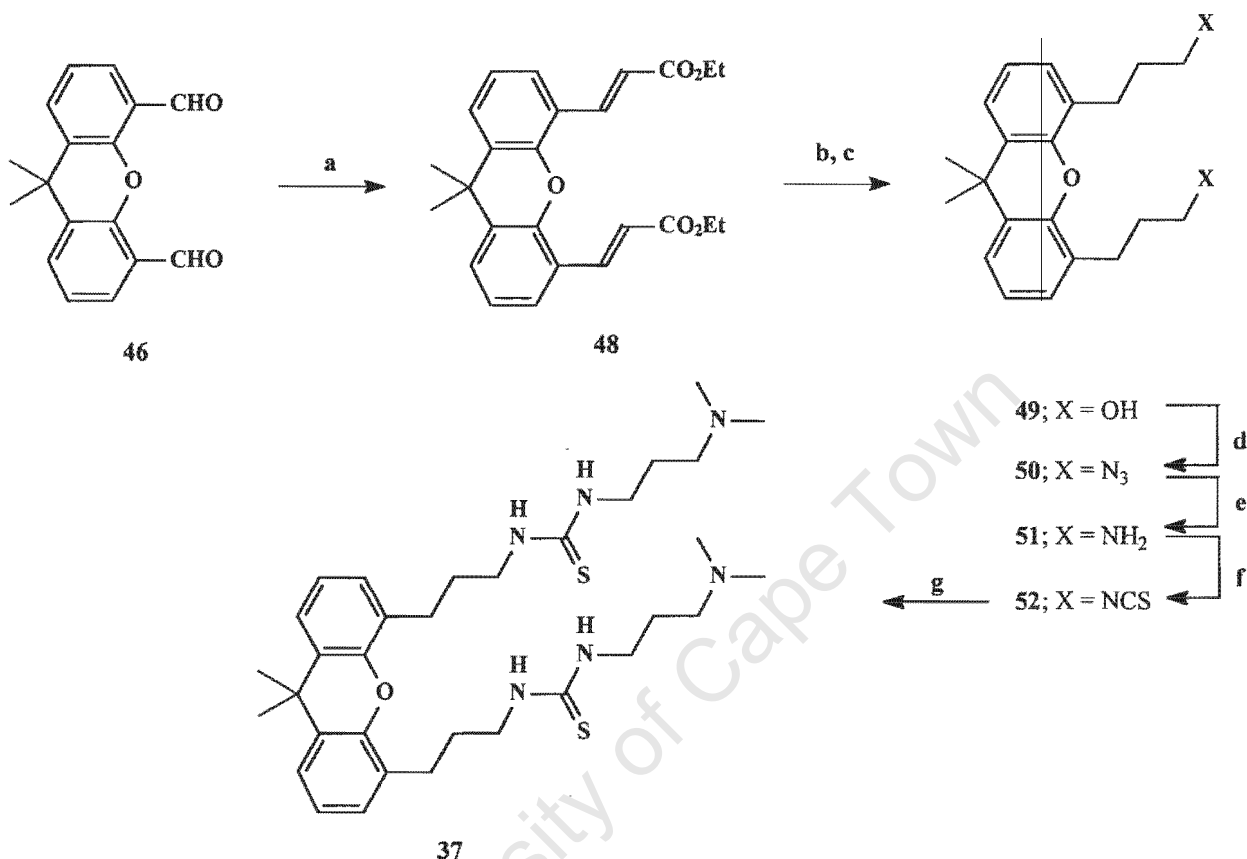


Scheme 3.7. Retrosynthetic scheme for isothioureia **95**.

b) Synthesis

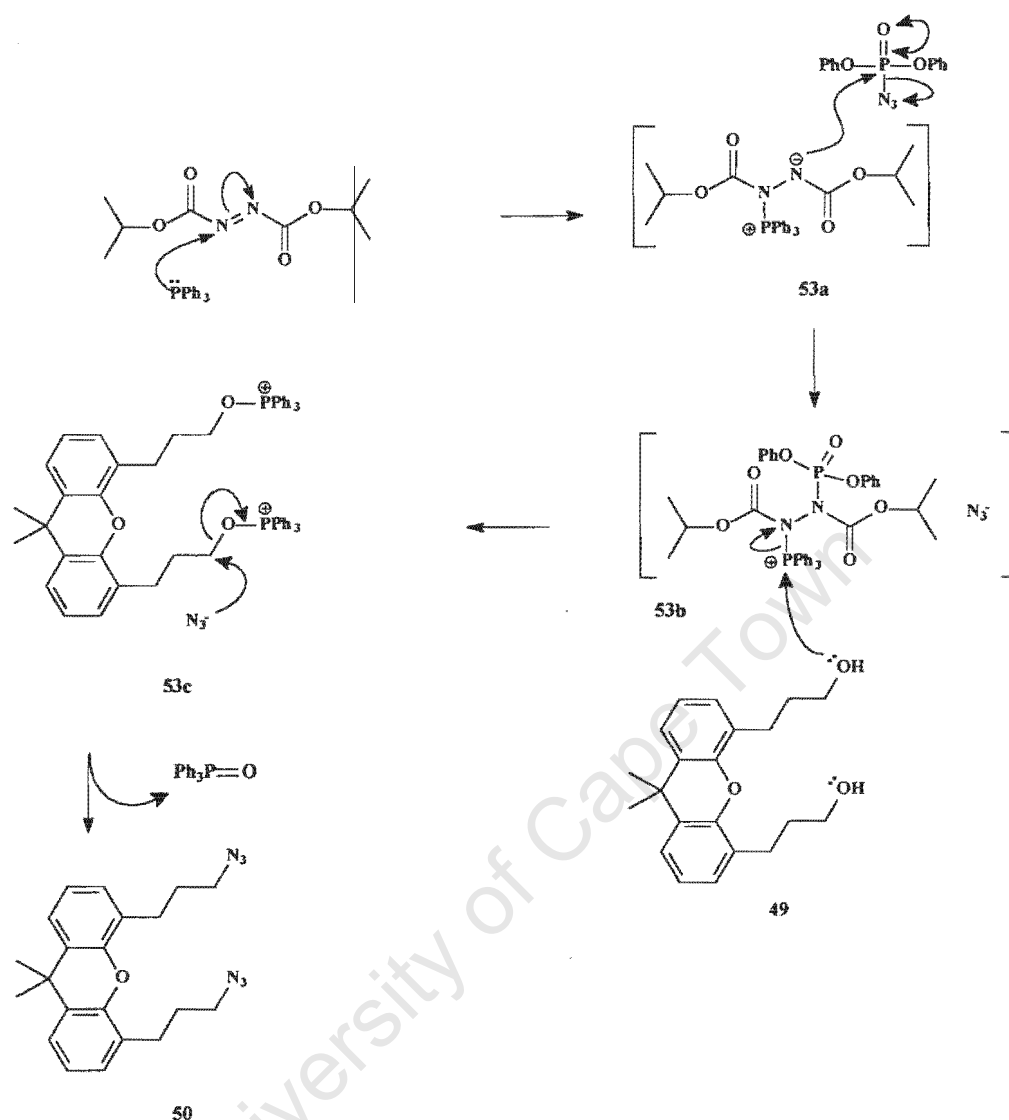
Bis-aldehyde **46** and ethyl (triphenylphosphoranylidene) acetate ($\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$) were refluxed in dry dichloromethane for 3 hours and subsequent chromatography gave the α,β -unsaturated ester **48** (scheme 3.8) in good yield. ^1H NMR revealed two doublets at 8.3 and 6.5 ppm with coupling constants (J) of 16 Hz, confirming the presence of the new olefinic bonds, which had an *E*-geometry. The ester was

converted to the saturated bis-alcohol **49** by reducing the olefinic bonds *via* catalytic hydrogenation (Pd-C) followed by reduction using lithium aluminium hydride (scheme 3.8).



Scheme 3.8. Reagents and conditions: (a) 3.0 equiv. of $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$, CH_2Cl_2 , reflux, 3h, 82%; (b) H_2 , Pd-C, EtOAc, 25 °C, 24h, 100%; (c) 2.0 equiv. of LiAlH_4 , Et_2O , 0 °C, 0.5 h, 97%; (d) 2.0 equiv. of Ph_3P , 2.0 equiv. of DIAD, 2.0 equiv. of $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, THF, -15 to 25 °C, 4h, 57%; (e) H_2 , Pd-C, EtOAc, 25 °C, 18h, 91%; (f) 14.0 equiv. of CS_2 , 2.0 equiv. of DCC, THF, 25 °C, 18h, 20%; (g) 2.6 equiv. of $\text{H}_2\text{N}(\text{CH}_2)_3\text{NMe}_2$, CH_2Cl_2 , 25 °C, 18h, 100%.

Alcohol **49** was converted to azide **50**, using standard Mitsunobu⁸² reaction conditions (scheme 3.8). This reaction is believed to proceed through: (a) addition of triphenylphosphine to diisopropyl azodicarboxylate (DIAD) (scheme 3.9) giving a quaternary phosphonium salt **53a**, (b) reaction of the phosphonium salt with diphenyl phosphoryl azide to release the azido ion (N_3^-) **53b**, (c) formation of the alkoxyphosphonium salt **53c** and (d) displacement of **53c** by the azido ion to give the azide **49** and triphenylphosphine oxide ($\text{Ph}_3\text{P}=\text{O}$, scheme 3.9).

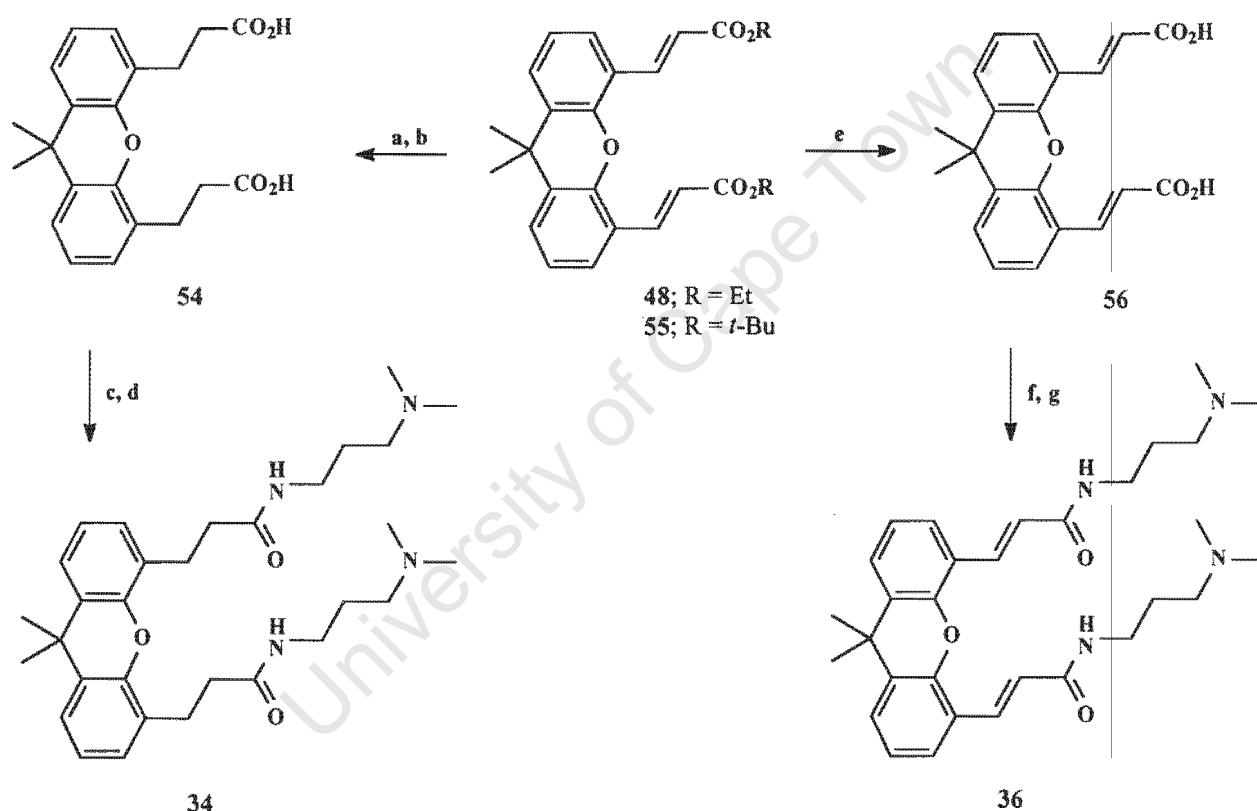


Scheme 3.9. Proposed mechanism for modified Mitsunobu reaction.

Reduction of the azide *via* catalytic hydrogenation gave the amine **51** in excellent yield, which upon treatment with DCC and carbon disulfide gave the isothiocyanate **52** albeit in poor yield. The poor yield may be explained on the basis that isothiocyanates are very reactive and thus reacted with the starting amine or decomposed upon purification. Addition of 3-dimethyl-1-propylamine to the isothiocyanate gave thiourea **37** in quantitative yield (scheme 3.8). The IR spectrum of thiourea **37** revealed a band at 1547 cm^{-1} , consistent with a thiourea $\text{C}=\text{S}$ stretch. HRMS further confirmed that the thiourea had indeed been synthesised.

3.2.3 Synthesis of amide derivatives 34 and 36

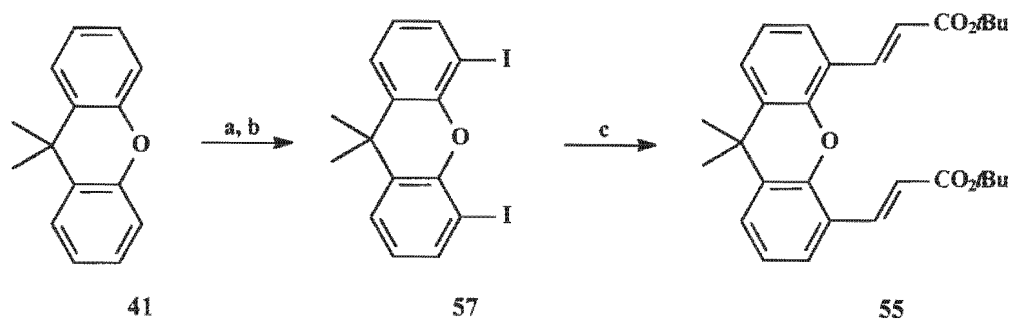
Bis-carboxylic acid **54** was synthesised in quantitative yield from the α,β -unsaturated ethyl ester **48**, described earlier, in a two-step process involving catalytic hydrogenation (Pd-C) followed by hydrolysis using lithium hydroxide monohydrate (scheme 3.10). The saturated acid was subjected to activation with oxalyl chloride to give the corresponding acid chloride. 3-Dimethylamino-1-propylamine was then added slowly to a cooled solution of the acid chloride in CH_2Cl_2 . After stirring for 12 hours at room temperature and subsequent purification by column chromatography amide **34** was obtained in moderate yield (scheme 3.10).



Scheme 3.10. Reagents and conditions: (a) H_2 , Pd-C, EtOAc, 25 °C, 24 h; then (b) 3.0 equiv. of $\text{LiOH}\cdot\text{H}_2\text{O}$, THF:H₂O (3:1), 25 °C, 18 h, 100 %; (c) 3.0 equiv. of $(\text{COCl})_2$, DMF (cat.), CH_2Cl_2 , reflux 3h, then (d) 2.0equiv of $\text{H}_2\text{N}(\text{CH}_2)_3\text{NMe}_2$, 2.0 equiv Et_3N , CH_2Cl_2 , 25 °C, 12h, 65%; (e) TFA, CH_2Cl_2 , 0 °C, 20 min, rt, 2h, 99 %; (f) 6.0 eq. of $(\text{COCl})_2$, CH_2Cl_2 , reflux, 3h; then (g) 5.0 eq. of $\text{H}_2\text{N}(\text{CH}_2)_3\text{NMe}_2$, CH_2Cl_2 , 0 °C $\rightarrow$ rt, 1h, 75 %.

Using *tert*-butyl acrylate and palladium acetate, the unsaturated ester **55** was synthesised in excellent yield from the bis-iodide **57** using Heck chemistry, which in turn was obtained from 9,9-dimethylxanthene **41** (scheme 3.11). Synthesis of the bis-iodide had been previously described in the

literature.⁸³ The ester was hydrolysed to the corresponding unsaturated free acid **56** using trifluoroacetic acid (TFA).

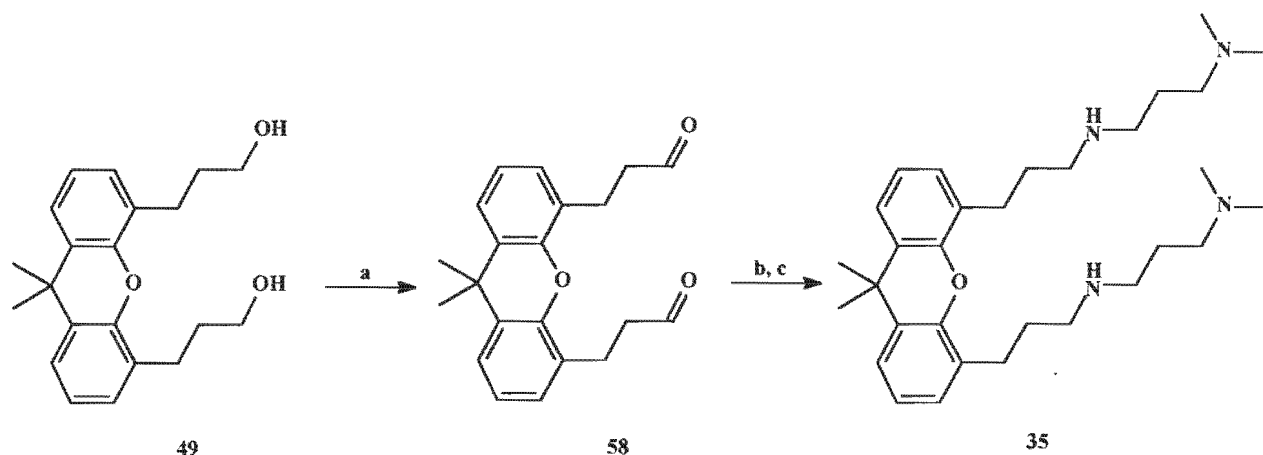


Scheme 3.11. Reagents and conditions: (a) 2.5 eq. of TMEDA, 3.0 eq. of *n*-BuLi, Et₂O, -78 °C → rt, 21h; (b) 2.6 eq. of CH₂I₂ at 0 °C, Et₂O, -78 °C → rt, 12h, 67 %; (c) 10 mol % Pd(OAc)₂, 20 mol % PPh₃, 11.0 eq. of *tert*-butyl acrylate, 2.0 eq. of Et₃N, DMF, 75 °C, 12h, 94 %;

Acid **56** was activated by conversion to the acid chloride followed by trapping with 3-dimethylamino-1-propylamine to give the unsaturated amide **36** (scheme 3.10). At this stage, a number of structurally diverse amines can be used to produce a library if so desired. For the purpose of this project, only this simple amide was synthesised so that it could be evaluated for potential as a biologically active molecule within the context of inhibitors of TryR and CQ resistance reversal agents.

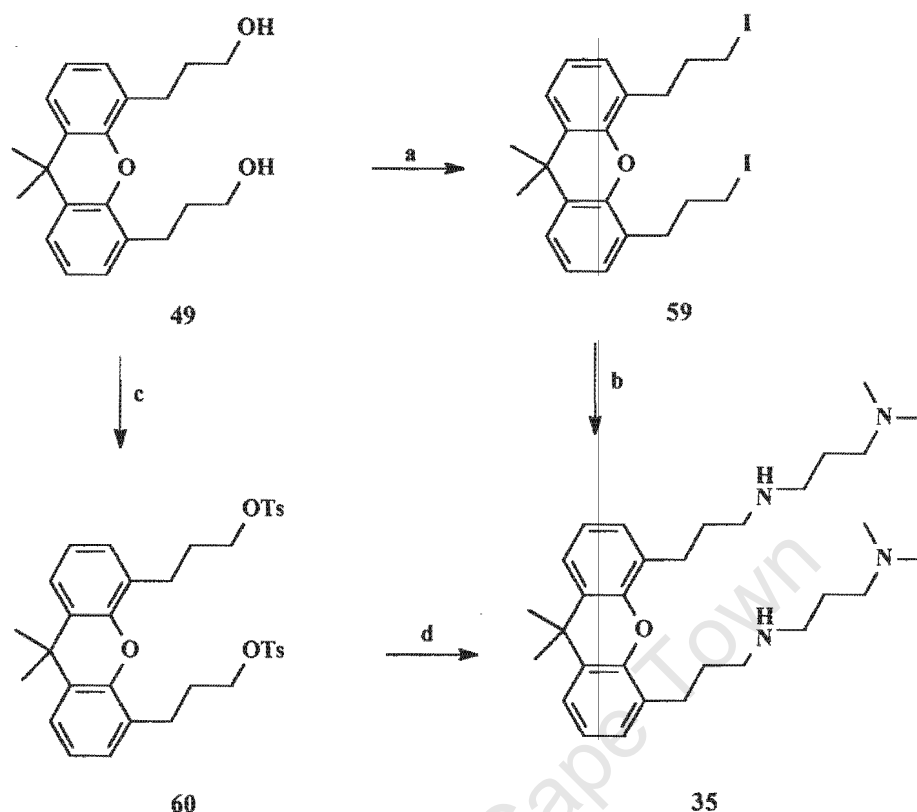
3.2.4 Synthesis of amine **35**

Since the alcohol **49** had been synthesised previously, two routes to amine **35** were envisaged: (i) reductive amination of bis-aldehyde **58** (scheme 3.12) or (ii) conversion of the hydroxy group in **49** to a good leaving group followed by nucleophilic substitution. Thus, oxidation of bis-alcohol **49** using sulphur trioxide pyridine complex in DMSO/ Et₃N gave **58** in moderate yield after purification by column chromatography. Reductive amination resulted in a poor yield of **35** (scheme 3.12). This may presumably be due to the instability of the bis-aldehyde.



Scheme 3.12. Reagents and conditions: (a) 5.2 equiv. of SO_3 , pyridine, 31.5 equiv. of DMSO, 16.0 equiv. of Et_3N , THF, $0\text{ }^\circ\text{C}$, 15 min, $25\text{ }^\circ\text{C}$, 30 min, 58 %; (b) 2.0 equiv. of $\text{H}_2\text{N}(\text{CH}_2)_3\text{NMe}_2$, MeOH, 18h, $25\text{ }^\circ\text{C}$; then (c) 4.0 equiv. of Amberlite IRA-400 borohydride resin, $25\text{ }^\circ\text{C}$, 24h, 21 %.

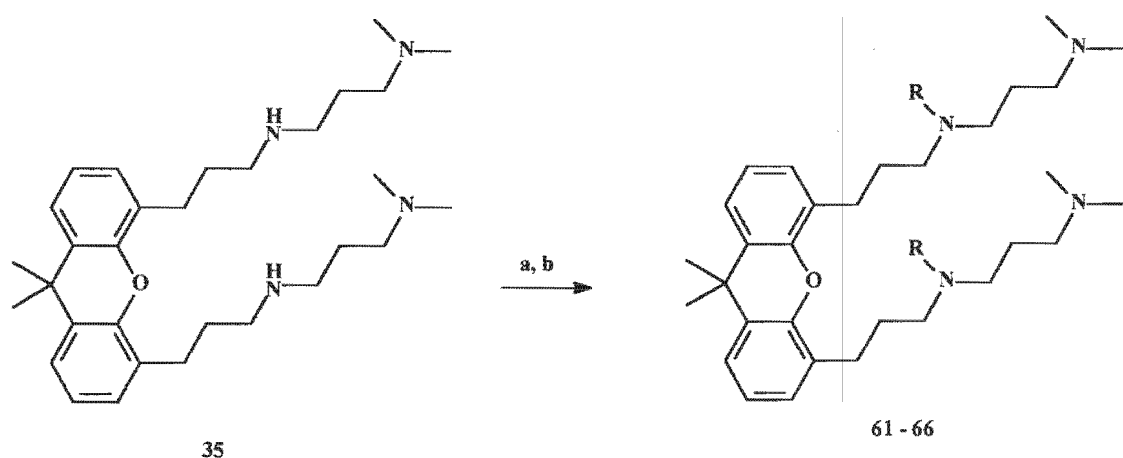
The second route to polyamine **35** involving displacement of a leaving group, either an iodide or sulphonate, from **59** and **60**, respectively, gave poor to moderate yields of **35**. A poor yield was obtained in the case of **60** while a moderate yield was obtained in the case of **59** (scheme 3.13). The poor yield in the former may have been due to the fewer number of equivalents of 3-dimethyl-1-propylamine that may have resulted in the product **35** reacting further with **60**. This over-alkylation is a common side reaction in related reactions, e.g. those involving opening of epoxides with primary amines. To prevent the aforementioned side reaction, a large excess of the primary amine is normally used. At this juncture, our main interest was in accessing **35** for biological evaluation without due regard to chemical yield optimisation.



Scheme 3.13. Reagent and conditions: (a) 3.0 equiv. of I_2 , 3.0 equiv. of Ph_3P , 3.0 equiv. of imidazole, $Et_2O:CH_3CN$ (3:1), 25 °C, 5h, 72%; (b) 16.0 equiv of $H_2N(CH_2)_3NMe_2$, THF, reflux, 1h, 68%; (c) 2.2 equiv. of *p*-TsCl, 3.0 equiv. of Et_3N , CH_2Cl_2 , 0 °C, 20 min, then 25 °C, 40 min, 53 %; (d) 2.2 equiv. of $H_2N(CH_2)_3NMe_2$, PhMe, reflux, 3h, 16 %.

3.2.5 Exploring the potential of compound library generation *via* parallel synthesis on a bifunctional scaffold

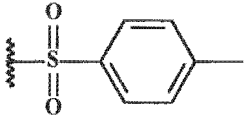
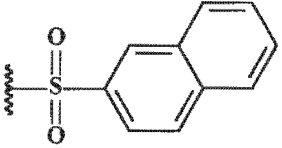
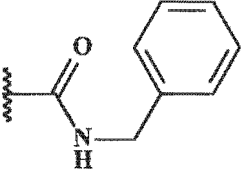
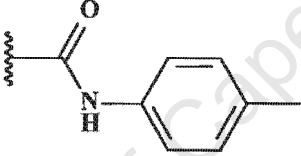
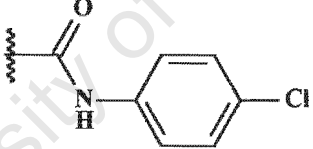
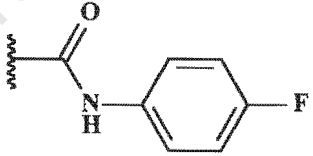
The idea of using a core molecule possessing multiple functional groups to synthesise libraries was initially introduced by Rebek.⁷⁵ It was envisaged that by attaching building blocks on a multifunctional scaffold at random, every possible combination could theoretically be generated. For example, a library of 55 compounds can be produced theoretically by using a bi-functional scaffold and 10 building blocks. Principally, condensing a variety of multifunctional core molecules with a variety of building blocks, nearly limitless, well-defined libraries of small organic molecules with biological significance can be generated. Thus, polyamine **35** presented us with an attractive core molecule to explore the synthesis of libraries containing molecules with diverse structures.



Scheme 3.14. Reagents and conditions: (a) 2.1 eq. of ArSO_2Cl or ArNCO , 2.2 eq. of PS-Morpholine for ArSO_2Cl , CH_2Cl_2 , rt, 5-24h; (b) 4.0 eq. of PS-tris(2-aminoethyl) amine, CH_2Cl_2 , rt, 12 h.

A parallel solution phase synthesis approach was applied. Polyamine **35** was divided into six vials and dissolved in a small amount of CH_2Cl_2 . To these solutions were added 2.1 equivalents of either aryl sulphonyl chloride or aryl isocyanate. For the sulphonyl chloride reactions, 2.2 equivalents of polymer-supported morpholine were added to remove HCl formed during the reaction (scheme 3.14). The vials were shaken at room temperature. After 5 hours, the isocyanate reactions were complete, whilst the sulphonyl chloride reactions were slow with a total reaction time of 24 hours required for completion. This was expected since isocyanates are more reactive than sulphonyl chlorides. The sulphonamide solutions were filtered to remove the PS-morpholine. Excess sulphonyl chloride and isocyanate was removed from the mixture by addition of 4 equivalents of polymer-supported tris (2-aminoethyl) amine. To ensure complete removal, the suspension was shaken for a further 12 hours. Filtration gave the sulphonamides and ureas as "one-spot" products on TLC in yields ranging from 50 – 83 % (table 1). ^1H NMR analyses revealed minor impurities and thus, the compounds were purified on silica gel in order to obtain pure molecules for biological evaluation.

Table 1. Table showing substituents (R) from sulphonamides and ureas and their synthetic yields.

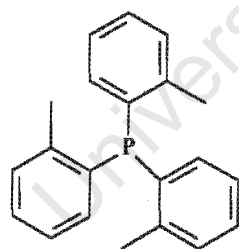
Compound no.	R	% Yield
61		50 %
62		60 %
63		56 %
64		55 %
65		83 %
66		62 %

3.2.6 Synthesis of aryl amines 38 and 39 via palladium-catalysed amination

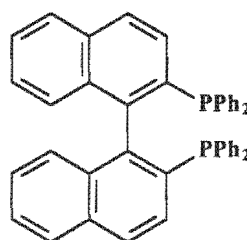
a) Introduction to palladium catalysed amination

Compounds containing an *N*-aryl moiety occur in biologically important molecules and thus effective methodologies to synthesise these types of compounds are of great importance. Although there exist a number of traditional methods for aryl C-N bond construction, problems such as limited generality, harsh conditions, the need to employ stoichiometric quantities of valuable/expensive reagents, numerous synthetic steps or regiochemical ambiguities presents a limitation to the synthesis.⁸⁴

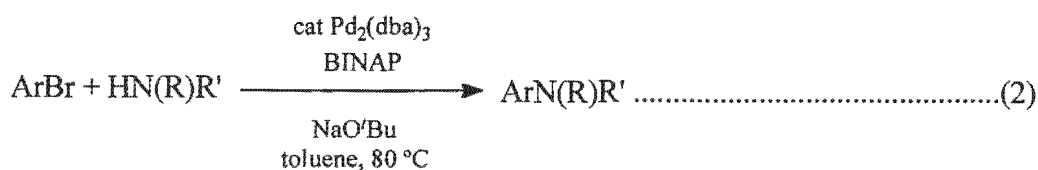
Since 1983, with Migita's *N,N*-diethylaminotributyltin⁸⁵ procedure, efforts to improve this methodology have been underway. Hartwig and Buchwald have been actively involved in the transition metal-catalyzed approach, which involves the simple cross coupling of an amine with an aryl halide. An important finding was that a phosphine ligand such as $P(o\text{-tolyl})_3$ **67**, results in reasonable yields of the aniline products. However, with this ligand poor results were obtained in the cross coupling of primary amines with aryl bromides. Investigation into the ligand BINAP [2,2'-bis(diphenylphosphino)-1,1'-binaphthyl] **68** have lead to the discovery that the combination of tris(dibenzylideneacetone) dipalladium(0) $[Pd_2(dba)_3]$ and BINAP in the presence of NaO'Bu constituted a superior catalyst system for the cross coupling of amines with aryl bromides (equation 2)⁸⁶ which could be extended to aryl chlorides, aryl iodides and aryl sulphonates.⁸⁴



$P(o\text{-tolyl})_3$ (**67**)



BINAP (**68**)



The mechanism for the catalytic cycle is shown in figure 3.3. Firstly, the BINAP combines with $Pd_2(dba)_3$ to produce the $Pd(0)$ species $[(BINAP)Pd]$ which is thought to be the active catalyst. The

catalytic cycle involves the oxidative addition of the aryl halide (1), coordination (2) and deprotonation (3) of the amine followed by reductive elimination (4) of the N-aryl product (figure 3.3).⁸⁶

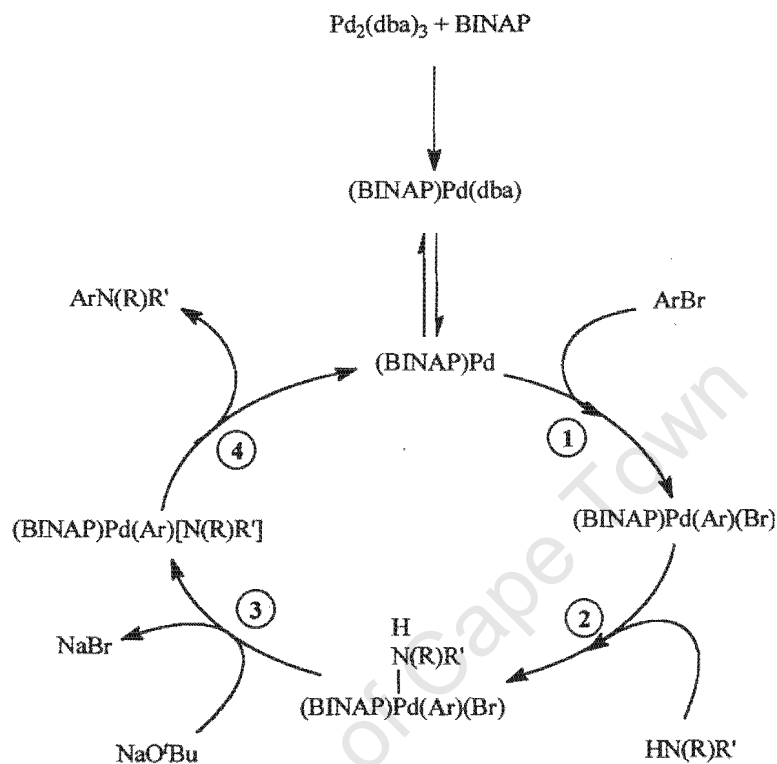
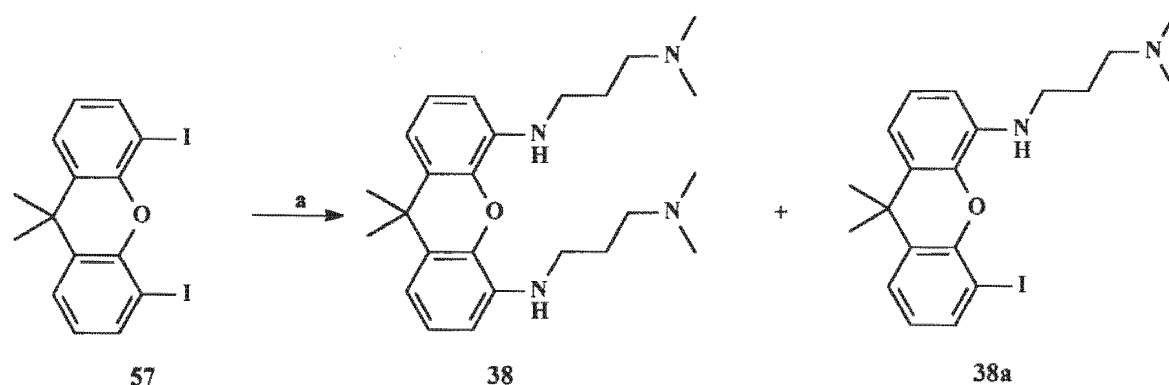


Figure 3.3 Proposed catalytic cycle⁸⁶

b) Synthesis of aryl amine 38

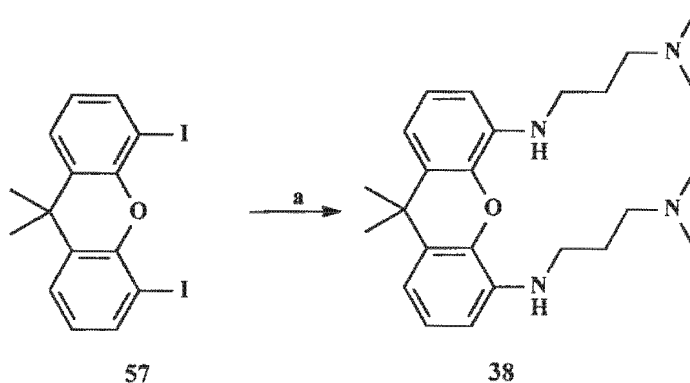
4,5-Diiodo-9,9-dimethylxanthene **57** described earlier provided a starting point in the synthesis of aryl amines based on 9,9-dimethylxanthene. The representative procedure described by Buchwald⁸⁶ was applied on this system using 3-dimethylamino-1-propylamine. In the procedure described by Buchwald, it is proposed that 0.5 % Pd at 80 °C for 2 hours was sufficient for complete arylation. However, when these conditions were applied to the di-iodide **57**, a 96 % conversion of the starting material with a 1:1 ratio of bis-amine **38** to mono-amine **38a** products was obtained (scheme 3.15). Even after an extended reaction time of 48 hours, complete conversion could not be achieved.



Scheme 3.15. Reagents and conditions: (a) 0.5 mol % $\text{Pd}_2(\text{dba})_3$, 1.5 mol % BINAP, 2.8 eq. of NaOtBu , 2.1 eq. of $\text{H}_2\text{N}(\text{CH}_2)_3\text{NMe}_2$, PhMe , 80°C , 48h.

The major side product in the arylation of primary amines is diaryl(alkyl)amines resulting from over-arylation of the primary amine substrate. In a paper by Buchwald *et al.*, where he examined the scope and limitations of this catalytic system, a variety of ligands were examined and it was found that all ligands were inferior to BINAP, i.e. this chelating ligand gave the best monoaryl/diaryl product ratio ($\sim 10\text{-}30/1$).⁸⁷ It was found that a higher catalyst loading (2 mol % Pd) improved this ratio (39/1) under dilute reaction conditions (9 ml toluene/mmol halide). At more concentrated conditions (2 ml toluene/mmol halide) and 0.5 % Pd, the ratio was diminished ($\sim 11.5/1$). However when an excess of the amine (4.0 eq.) is employed at a higher concentration (2 ml toluene/mmol halide) the ratio of monoaryl/diaryl product was greatly improved to 80/1.⁸⁷

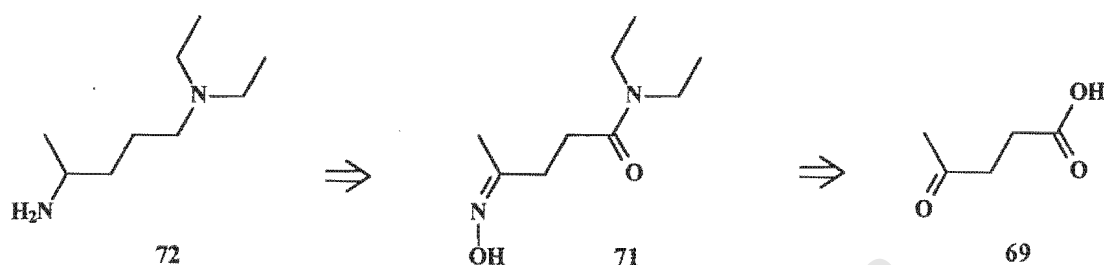
The reaction conditions in scheme 3.15 were adjusted to a slight excess of the amine (2.5 eq.), increased catalyst loading (2 mol % Pd), and a higher temperature (100°C). The concentration of the reaction was kept at 2 ml toluene/mmol halide. Under these conditions, the reaction went to completion within 18 hours giving the bis-amine product exclusively in 89 % yield (scheme 3.16).



Scheme 3.16. Reagents and conditions: (a) 2.0 mol % $\text{Pd}_2(\text{dba})_3$, 6.0 mol % BINAP, 2.8 eq. of NaOtBu , 2.5 eq. of $\text{H}_2\text{N}(\text{CH}_2)_3\text{NMe}_2$, PhMe , 100°C , 18h, 89 %.

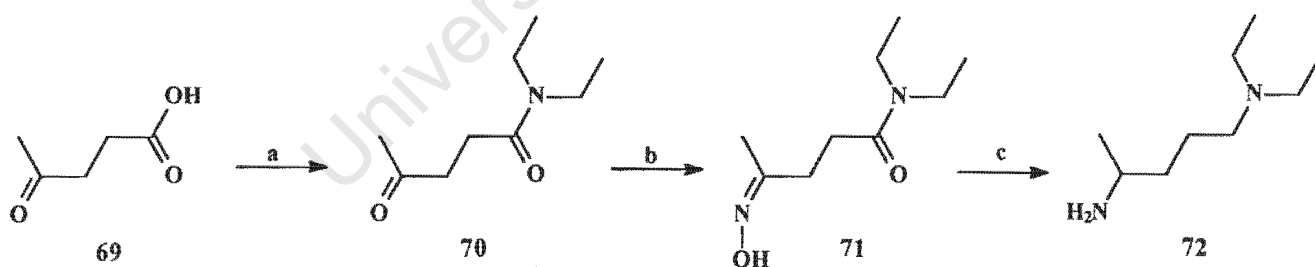
c) Synthesis of aryl amine 39.

Now that a general procedure for amination of the bis-iodide **57** was established, it was proposed that this methodology could be extended to a variety of different amines. The first amine substituent was the side chain of chloroquine. A proposed retrosynthetic approach for the amine is outlined in scheme 3.17.



Scheme 3.17. Retrosynthetic scheme for the synthesis of amine **72**.

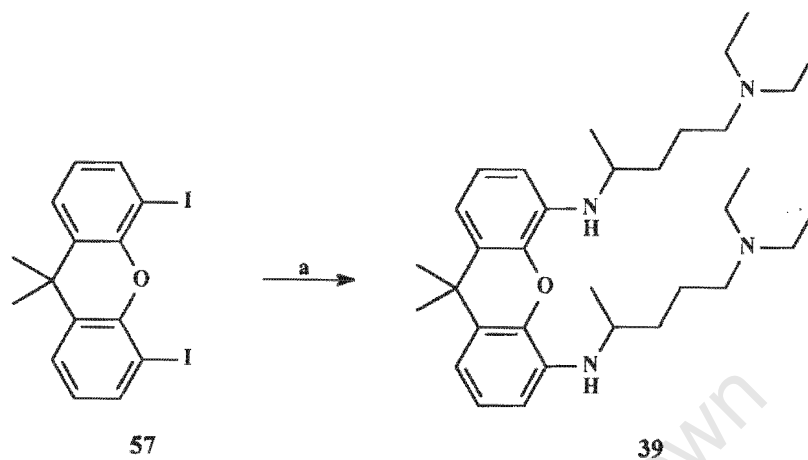
The synthesis was achieved through the "oxime-amide" intermediate **71** (scheme 3.17), which is obtained from commercially available levulinic acid **69** through a two-step procedure. In an attempt to synthesise the amide **70** (scheme 3.18), *en route* to **71** through the acid chloride, using either oxalyl chloride or thionyl chloride, the product seemed to undergo decomposition. Condensation of the acid **69** with diethylamine was achieved using the coupling agent 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC.HCl) to give the amide **70** in moderate yield (scheme 3.18).



Scheme 3.18. Reagents and conditions: (a) 1.1 eq. of EDC.HCl, 1.1 eq. of Et₂NH, CH₂Cl₂, 0 °C, 1h, rt, 18h, 60 %; (b) 1.6 eq. of NH₂OH.HCl, 3.3 eq. of NaOH, EtOH, reflux, 2h, 92 %; (c) 3.0 eq. of LiAlH₄, THF, 0 °C → reflux, 6h, 67 %.

The ketone functionality of **70** was transformed to oxime **71** by refluxing in ethanol with hydroxylamine hydrochloride and sodium hydroxide in excellent yield (scheme 3.18). In the final step, both the oxime and amide functionality of **71** were simultaneously reduced by lithium aluminium hydride to give the amine **72** in moderate yield.

The same conditions used in the synthesis of **38** were applied to the synthesis of aryl amine **39** using **72** as the primary amine. The reaction was complete within 6 hours and gave the aryl amine **39** in 60% yield (scheme 3.19).



Scheme 3.19. Reagents and conditions: (a) 2.0 mol % $\text{Pd}_2(\text{dba})_3$, 6.0 mol % BINAP, 2.8 eq. of NaOtBu, 2.5 eq. of **72**, PhMe, 100 °C, 6h, 60 %.

CHAPTER 4

9-MONOSUBSTITUTED XANTHENE DERIVATIVES

4.1 INTRODUCTION

9-Monosubstituted xanthene derivatives **73** – **77** (figure 4.1) were initially targeted for preliminary investigation as modulators of CQ resistance. These included amine, amide, sulphonamide and urea compounds. It was decided that before embarking on the parallel synthesis of new xanthene derivatives for structure-activity studies, representative compounds be synthesised for preliminary biological evaluation.

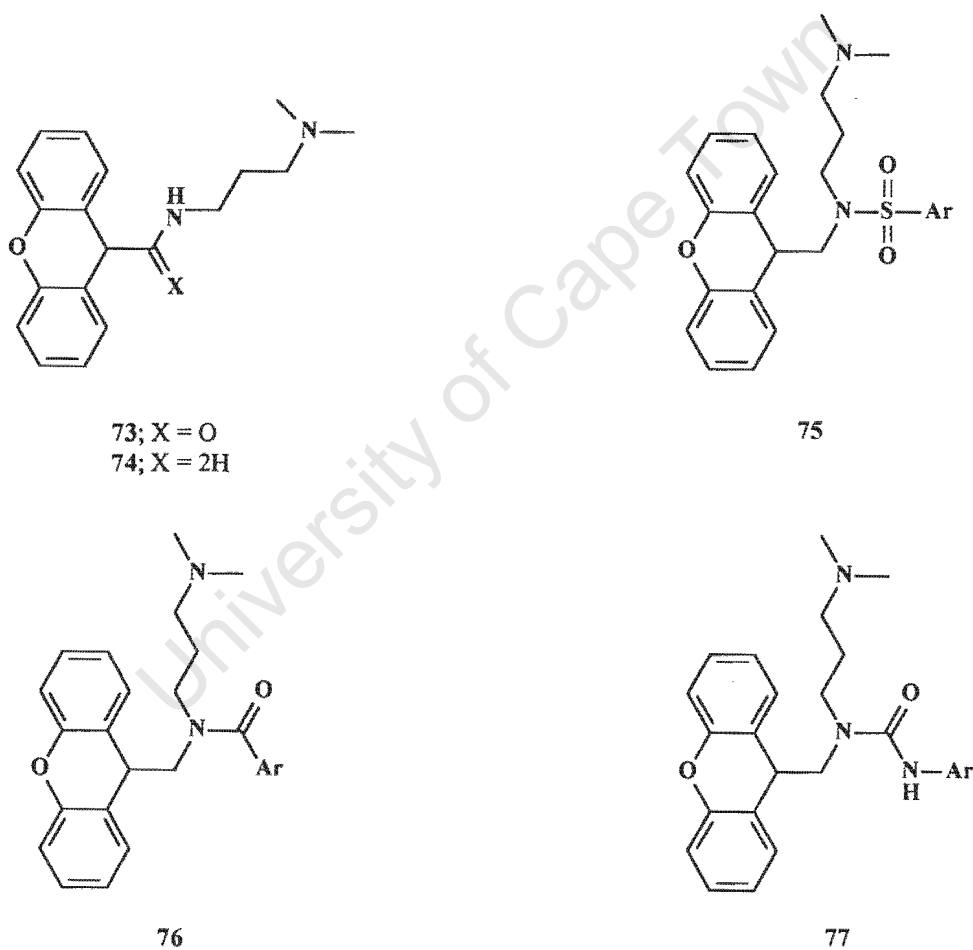
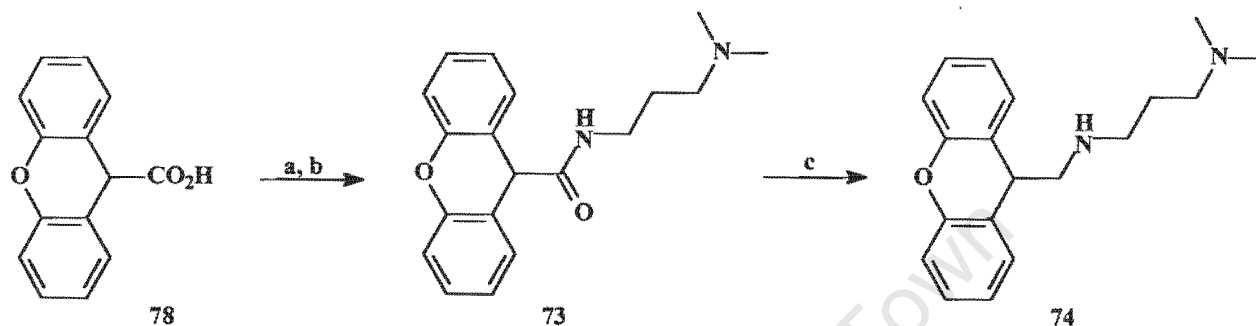


Figure 4.1 Tricyclic target compounds for anti-malarial modulators

4.2 CHEMICAL SYNTHESIS

4.2.1 Synthesis of amine 74

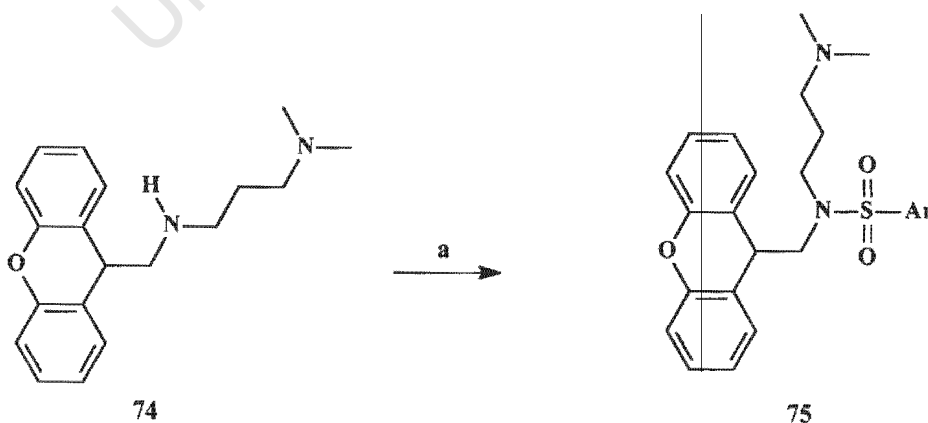
Commercially available xanthene-9-carboxylic acid **78** was treated with oxalyl chloride to form the acyl chloride with subsequent addition of 3-dimethyl-1-propylamine giving the amide **73** in excellent yield (scheme 4.1). Reduction of the amide with borane gave the amine in moderate yield



Scheme 4.1. Reagents and conditions: (a) 3.0 eq. of (COCl)₂, cat. DMF, CH₂Cl₂, reflux, 3h; then (b) 1.2 eq. of H₂N(CH₂)₃N(CH₃)₂, CH₂Cl₂, 0 °C → rt, 1h, 98 %; (c) 2.5 eq. BH₃.THF, THF, reflux, 18h, 66 %.

4.2.2 Synthesis of sulphonamides, amides and ureas

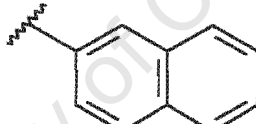
Our initial goal was to study the effect of replacing the secondary amino group in **74** with sulphonamide, amide and urea moieties. Before embarking on the parallel synthesis of these derivatives, we needed to ascertain that these types of compounds are viable as CQ resistance modulators. With no particular preference, three different sulphonamides were synthesised using amine **74** (scheme 4.2).



Scheme 4.2. Reagents and conditions: (a) 1.2 eq. of ArSO₂Cl, 1.5 eq. of PS-Morpholine, CH₂Cl₂, rt, 18h, 59 – 78 %.

The amine **74** was dispensed into three vials and dissolved in a minimal amount of CH_2Cl_2 . The sulphonyl chlorides (1.2 equivalents) and polymer-supported morpholine was added and the suspension shaken for 18 hours at room temperature. The solutions were separated from the polymer by filtration and washing with small amounts of CH_2Cl_2 . Excess sulphonyl chlorides were removed by column chromatography to give the sulphonamides in moderate yields (table 2). In a typical parallel synthesis protocol, excess sulphonyl chlorides would have been scavenged using polymer supported tris (2-aminoethyl) amine.

Table 2. Table showing R groups for compounds **75** and their chemical yields.

Compound no.	R	% Yield
75a		78 %
75b		59 %
75c		59 %

These sulphonamides proved useful as chloroquine resistance reversal agents (see biological evaluation). To evaluate the effect of changing the sulphonamide functionality, amides and ureas were included. It was decided to synthesise these derivatives using a "catch and release" strategy as a means of purifying the products without resorting to scavenging protocols. This approach presents a new and exciting addition to purification protocols for solution phase library generation. Here, focus is placed on the product and not the impurity, i.e. the product is removed from solution instead of the impurities. This is useful since it does not require one to know the chemical nature of the impurities, only that of the product. For our purposes, macroporous *p*-toluene sulphonic acid (MP-TsOH) was utilised. This reagent protonates the amine components of the product, thus sequestering the product onto the now negatively charged sulphonate groups (figure 4.2). After filtration and washing with a solvent to remove excess reagents, the product is released into solution by addition of a solution of ammonia in methanol.

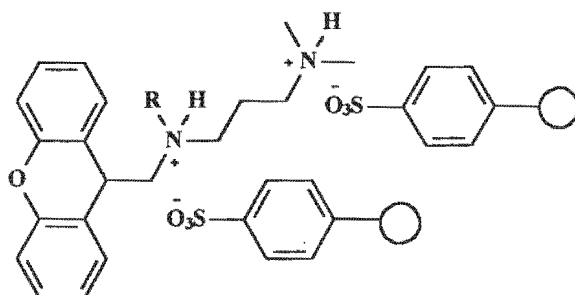
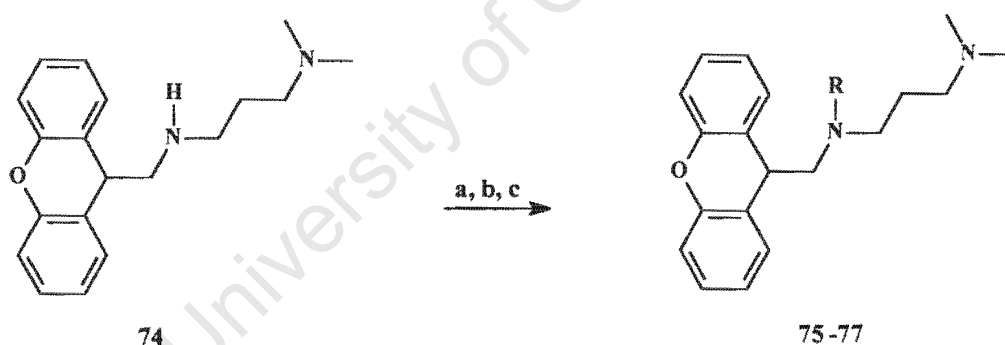


Figure 4.2. Illustration of the "catch" process of the amine product using MP-TsOH.

One drawback of this strategy is that it requires complete conversion of starting amines to products, since both product and starting material could be sequestered by the MP-TsOH.

This time the building blocks were restricted to benzene and its *p*-chloro derivatives. For illustrative purposes sulphonyl chlorides were included. As before, the amine was dispensed into 6 vials, diluted in a small amount of CH_2Cl_2 , the building blocks and PS-morpholine added, except for isocyanates (scheme 4.3).

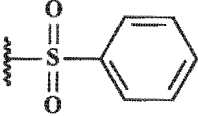
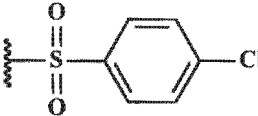
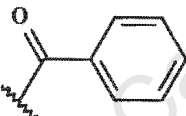
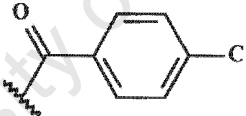
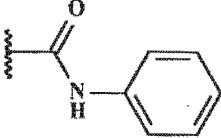
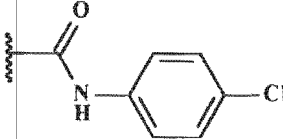


Scheme 4.3. Reagents and conditions: (a) 3.0 eq. of building block, 1.0 eq. of PS-Morpholine, CH_2Cl_2 , rt, 2h; (b) 3.0 eq. of MP-TsOH, CH_2Cl_2 , rt, 4h; (c) 2M NH_3/MeOH , rt, 15h.

Previously, only 1.2 equivalents of the building block were used and required 18 hours for complete reaction. Using 3 equivalents, the reaction was complete in 1 hour and MP-TsOH added to the solution. After 4 hours of shaking at room temperature, the product was removed from the solution, as judged by TLC, and only the excess building blocks remained in solution. The solution was drained from the polymer and washed with CH_2Cl_2 to remove all traces of impurities. Ammonia (2 M in methanol) was then added to the polymer bound product in order to release it in solution. Most of the product was released after 10 minutes but some still remained on the beads. The polymer was allowed to shake in this ammonia-methanol solution over night to ensure maximum release of the product. The solution is

once again drained from the polymer to give the pure (as judged by TLC and NMR) products. Yields ranged from 60 to 96 % (table 3).

Table 3. Table showing R groups for compounds 75 - 77 and their chemical yields¹.

Compound no.	R	% Yield
75a		73 %
75d		73 %
76a		96 %
76b		83 %
77a		84 %
77b		63 %

¹ Purity was judged by TLC and NMR

CHAPTER 5

BIOLOGICAL RESULTS AND DISCUSSION

5.1 INHIBITORS OF TRYPANOTHIONE REDUCTASE

5.1.1 Inhibition and antiparasitic activities of derivatives 31, 32, 33, 34, 35 and 37

The data in table 4 shows the activity of these derivatives against TryR, *L. donovani* amastigotes, *T. cruzi* amastigotes as well as *T. brucei* bloodstream form trypomastigotes. The data for standard control drugs is included for comparison purposes. As far as the inhibition of TryR is concerned compounds 31, 32 and 33 bearing either one (33) or no methylene spacer (31 and 32) between the tricyclic moiety and the secondary nitrogen atom generally show weaker inhibition of TryR compared to derivatives with a two or three carbon methylene spacer (34, 35 and 37).

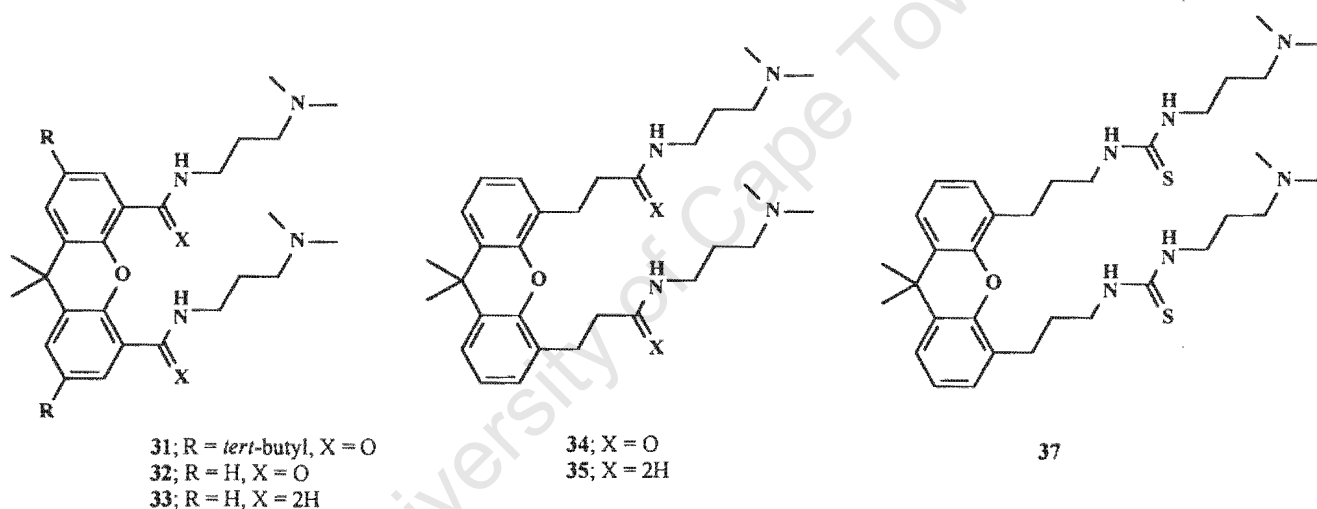


Figure 5.1. Structures of 9,9-dimethylxanthene derivatives discussed in this section.

For *in vitro* antiparasitic activity, the rapidly dividing extracellular *T. brucei* bloodstream-form trypomastigotes showed the highest *in vitro* sensitivity to these derivatives with ED₅₀ values below 5 µg/ml in all cases while the intracellular slow dividing amastigote stages of *L. donovani* and *T. cruzi* were the least sensitive. Compounds 31, 35 and 37 showed the highest *in vitro* potency against *T. brucei*. However, the most notable compound is 31, which showed high activity against all three parasites with ED₅₀ values either comparable (*T. brucei*) or superior (*L. donovani* and *T. cruzi*) to the standard control drugs.

Table 4: Inhibition of TryR and *in vitro* antiprotozoal activity of control drugs and xanthene derivatives¹

Compound	% Inhibition of TryR	ED ₅₀ (µg/ml)			Cytotoxicity MIC, µg/ml
		<i>L. donovani</i>	<i>T. cruzi</i>	<i>T. brucei</i>	
Pentostam^a	—	12.5 ^b	—	—	
	—	—	8.5	—	
Benznidazole	—	—	—	0.01	
Pentamidine					
31	28.4	0.32	0.28	0.011	>30
32	40	>30	>30	4.5	>300
33	13.8	>30	>30	3.32	>300
34	58	17.03	>30	4.3	>300
35	63	>30	>30	0.07	>30
37	52	>30	>30	0.06	>30

^aPentostam is sodium stibogluconate and the ED₅₀ value is expressed as µg Sb^V/ml

¹Biological Assays were performed as described before^{77,88,89}

Although compound **34** showed comparable activity against TryR to **35** and **37** it does not possess comparable high *in vitro* activity against *T. brucei*. Coupled with the lack of activity of **35** and **37** against *L. donovani* and *T. cruzi* but high activity against *T. brucei*, this suggests that TryR inhibition is not totally responsible for the observed *in vitro* activities of these compounds. Other factors such as cell penetration and metabolism etc. may be playing a crucial role. It is noteworthy that for the intracellular *L. donovani* and *T. cruzi* amastigotes, the drug needs to pass through the macrophage to reach the amastigote. Hence achieving selective toxicity is a greater challenge in *L. donovani* and *T. cruzi* than in *T. brucei*. For compound **31**, which showed high broad activity against all three parasites but even weaker inhibitory activity against TryR, it is apparent, that TryR is not the target. Nevertheless it is encouraging that the more potent compounds **31**, **35** and **37** are not overtly toxic to mammalian macrophages at or below a concentration of 30 µg/ml (estimated microscopically).

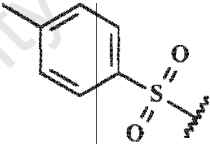
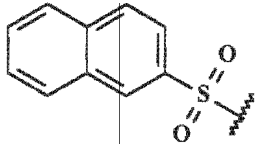
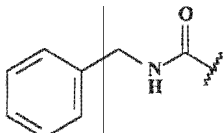
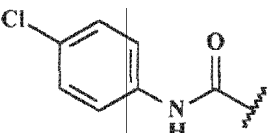
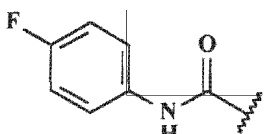
In conclusion, within this series of compounds: (i) there is no clear correlation between potency as inhibitors of TryR and the *in vitro* antiparasitic activities, and (ii) there is no apparent single structural feature controlling *in vitro* antiparasitic activities. These observations are consistent with conclusions of previous findings.^{21,24,90} In the case of compound **31**, which show pronounced activity against all three

parasites, TryR is clearly not the target. The mechanism of action of the promising derivatives **31**, **35** and **37** needs investigation if they are to serve as useful leads for rational drug design.

5.1.2 Inhibition of TryR by sulphonamide and urea derivatives of polyamine 35

By derivatizing the polyamine **35**, it is observed that the percentage inhibition is decreased compared to the parent amine (table 5). There is no clear advantage of sulphonamides over ureas in this case.

Table 5. Percentage inhibition of TryR by polyamine derivatives

Compound no.	R	% Inhibition at 100 μ M ^a
35	H	63.0
61		13.9
62		26.8
63		21.5
65		11.8
66		11.1

^a TryR assays were performed as described before^{77,88}

Three-dimensional structures of amine **35**, sulphonamide **62** and urea **63** were constructed using SymApps V 6.0 molecular modelling software from Bio-Rad laboratories (figure 5.2). SymApps has a built-in molecular mechanics program which utilises a modified MM2 force field minimization module to convert 2-D structures to 3-D models. It is unclear whether the program performed an energy minimization calculation on the models. Although the exact interaction with the active site of TryR of these molecules is not known, looking at the three-dimensional pictures (figure 5.2), it is observed that the terminal amino group of these derivatives are located differently in space compared to the parent amine. It is possible that the interaction of this group is displaced from the putative binding site exemplified by the glutamic acid residues (Glu 467' and Glu 466') in the active site of TryR.

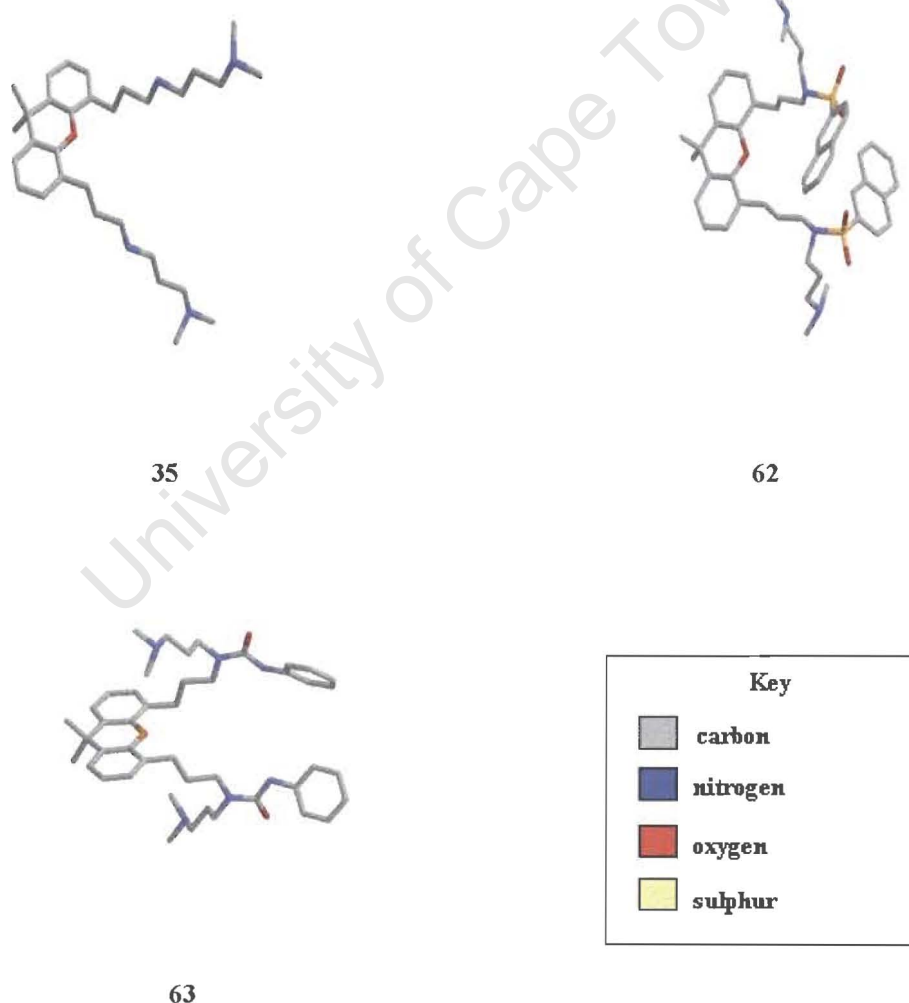


Figure 5.2. Three-dimensional representations of polyamine **35**, sulphonamide **62** and urea **63**.

Further investigation into these molecules is needed to test this theory. As modifications to these molecules, a chemical structure optimisation strategy is proposed that would (i) vary the spacer length between the xanthene and reactive secondary amino group, (ii) increase the spacer length between the amine and the terminal amino group, (iii) increase the spacer length between the sulphonamide or urea and the hydrophobic moiety or (iv) introduce bulkier hydrophobic groups. Varying the spacer length between the xanthene and reactive amine and that between sulphonamides or ureas and the hydrophobic group might determine the optimal length for the hydrophobic group to interact with the Z-site. Increasing the spacer length between the amine and terminal amino group might improve interaction with glutamic acid residues, perhaps even with E18 (as in mepacrine). Introduction of a more bulky moiety might improve the interaction with the Z-site.

5.1.3 Inhibition of TryR by derivatives 38, 39 and 36

Aryl amines 38 and 39 and α,β -unsaturated amide 36 (figure 5.3) represent a new class of 9,9-dimethylxanthene derivatives and thus we were interested to see their inhibitory effects on TryR. The IC_{50} values are presented in table 6.

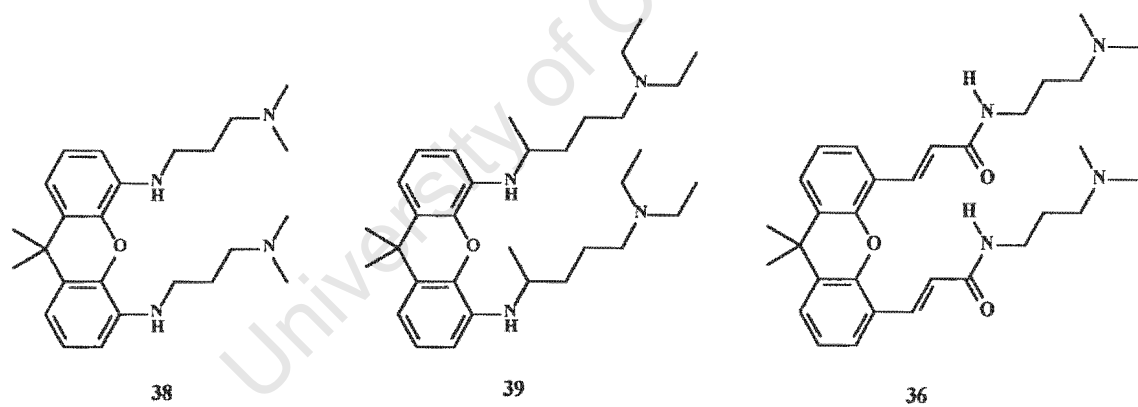


Figure 5.3. Chemical structures of aryl amines and the α,β -unsaturated amide.

Table 6. Inhibition of TryR by derivatives 36, 38 and 39 [†] .	
Compound no.	IC_{50} (μ M)
38	108 $\pm$ 1
39	105 $\pm$ 8
36	35.7 $\pm$ 1.5

[†] TryR inhibition assays were performed as described before²⁹

The aryl amines showed comparable activity to each other but the amide was superior in inhibiting the enzyme. The IC_{50} values were comparable to the phenothiazine derivatives like promazine ($IC_{50} = 108 \mu M$) and chlorpromazine ($IC_{50} = 35.4 \mu M$)²¹ and the dibenzazepine derivative clomipramine ($IC_{50} = 32.4 \mu M$).²⁶ Derivative **36** was also a better inhibitor than its saturated analogue (**34**), i.e. at $100 \mu M$ the enzyme was only 58% inhibited (table 4). Further investigation into these classes of compounds would be worthwhile.

5.2 CHLOROQUINE RESISTANCE REVERSAL IN A RESISTANT *P.falciparum* STRAIN

5.2.1 The effect of xanthene derivatives on chloroquine accumulation

Figure 5.4 shows the fold increase in tritiated (^3H) chloroquine accumulation in both sensitive (D10) and resistant (K1) strains of *P.falciparum* in the presence of $5 \mu M$ of the xanthene compounds (summarised in figure 5.3). The values are the average of six readings. In the sensitive strain, there was no improvement of CQ accumulation when co-administered with the various derivatives.

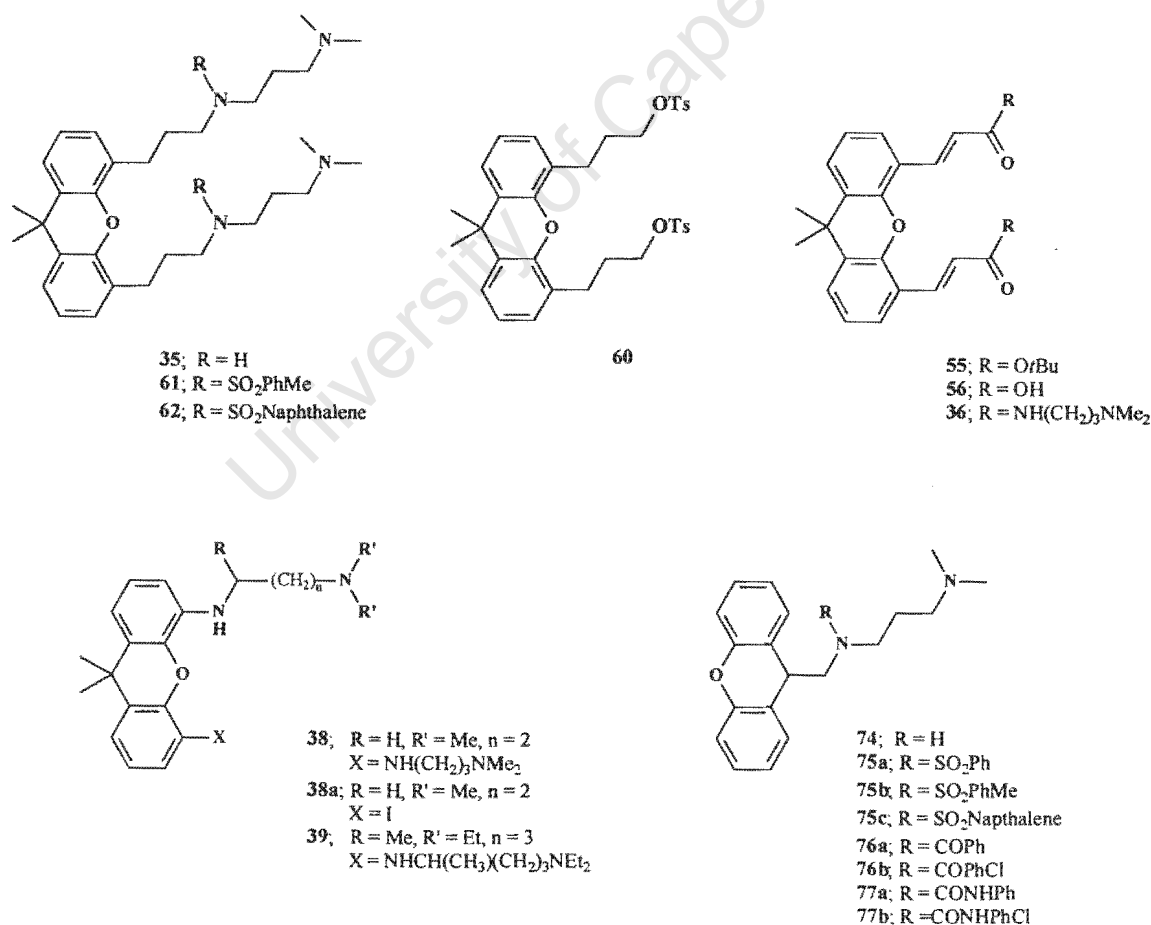


Figure 5.3. Summary of structures of derivatives used in chloroquine resistance reversal studies.

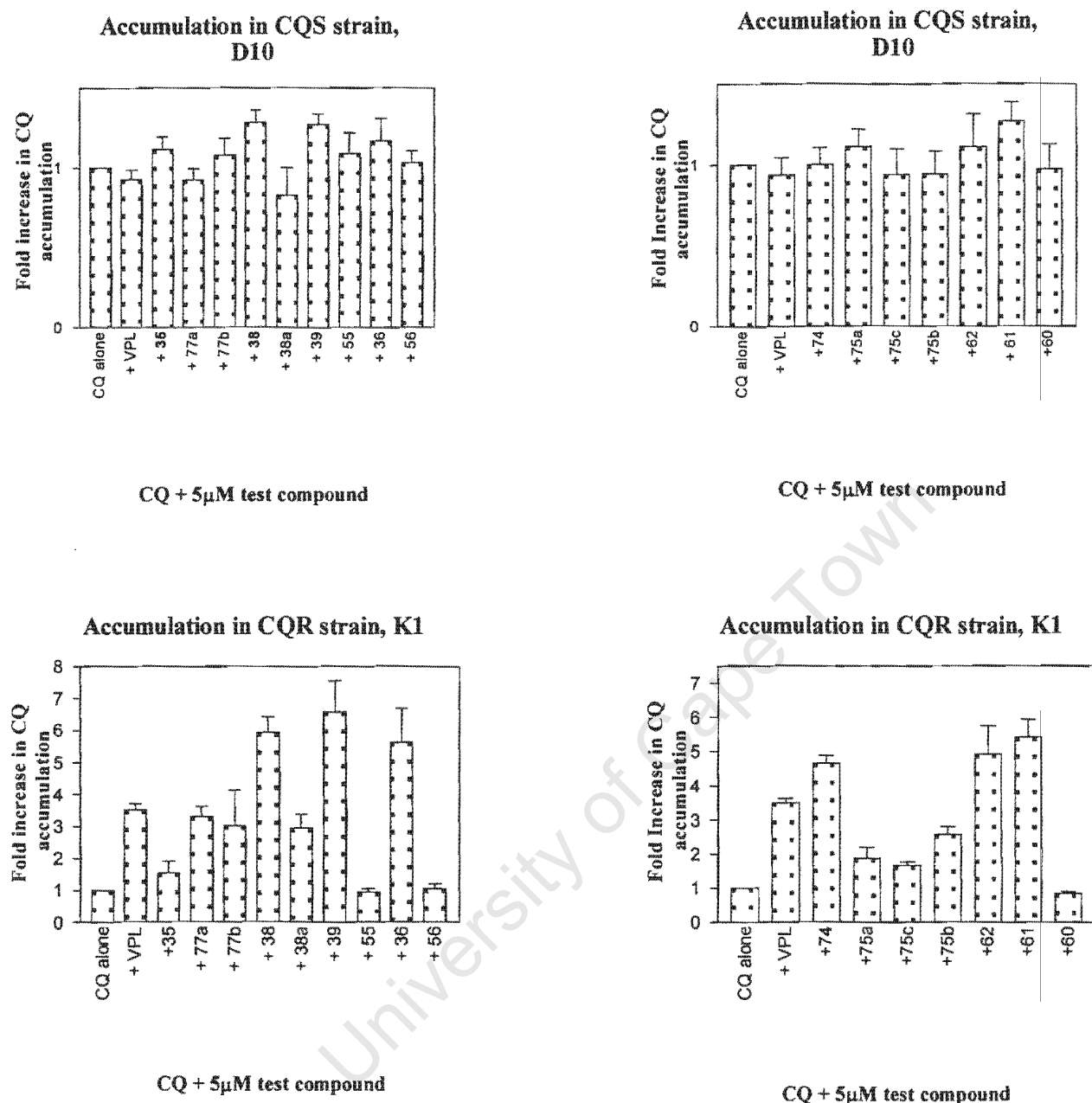


Figure 5.4. Accumulation of tritiated CQ in CQS (D10) and CQR (K1) *P. falciparum* in the presence of 5 μ M xanthene derivatives expressed as fold increase in CQ accumulation.

For the resistant strain (K1), compounds showed a variation in CQ accumulation. The well-known CQ resistance reverser verapamil, showed a 3.5 fold increase in accumulation. Compounds **60**, **55** and **56** showed no significant accumulation above that of CQ alone in the CQR strain. These compounds contain no terminal or other amino groups, confirming the trend observed by other compounds that the presence of this functionality is essential.^{67,72} Derivatives **35**, **38a** and **75a - c** showed an increase in accumulation in K1 but were not as good as VPL at enhancing CQ accumulation. Compounds **76a**, **76b**,

77a and **77b** showed accumulation that was comparable to VPL. Derivatives **36**, **38**, **39**, **61**, **62** and **74** showed an increase in accumulation above that of VPL with **36**, **38** and **39** showing ~5-6 fold increase (approximately 1.7 times better than VPL) whilst the others showed ~5 fold increase (~ 1.4 times better than VPL).

From these data, it is apparent that the 9-monosubstituted xanthene derivatives are not superior to VPL at increasing CQ accumulation. But it is also apparent that the ureas (**77a-b**) and amides (**76a-b**) were superior to the sulphonamides (**75a-c**) within this series, at increasing CQ accumulation. Also, the parent amine **74** was far superior compared to its sulphonamide, amide and urea derivatives (1.3 fold better than VPL). Therefore, it can be concluded that the 9,9-dimethylxanthene derivatives were more efficient at increasing CQ accumulation. Interestingly, and to the contrary, the parent amine **35** within the 9,9-dimethylxanthene series was inferior to its sulphonamide derivatives at increasing CQ accumulation. The aryl amines **38** and **39** and α,β -unsaturated amide **36** show promise for increasing CQ accumulation in CQR *P.falciparum* strains.

It has been observed that compounds could reverse CQ resistance *in vitro* without affecting CQ accumulation and *vice versa*.^{67,73} Thus accumulation data alone cannot be used solely as a measure of the compounds' ability to reverse CQ resistance in *P.falciparum*.

5.2.2 The intrinsic anti-malarial activity of xanthene derivatives

As earlier mentioned, an ideal resistance-reversing agent or chemosensitizer is one that does not have inherent anti-malarial activity but restores sensitivity of the resistant cells to the cytotoxic drug. A typical chemosensitizer has no effect on the sensitive strain. To effectively determine the potential of these derivatives to modulate CQ resistance, the assay must be performed at a concentration that is not cytotoxic to the parasites. Thus, determining the intrinsic antimalarial activity of these compounds would give an idea of the concentration to be used in the reversal assays. This was done for both the sensitive (D10) and resistant (K1) strains of *Plasmodium falciparum* (table 7).

Most compounds were relatively anti-malarial to both sensitive and resistant strains but were not as good as chloroquine with the exception of derivatives **61** and **62**. Compounds **36**, **38** and **39** were comparable to chloroquine in the resistant strain but derivatives **61** and **62** were far superior to chloroquine in the same strain.

Table 7. A comparison of the intrinsic anti-malarial activity of xanthene derivatives in a CQS (D10) and CQR (K1) strain of *P.falciparum*.

Compound	Mean IC ₅₀ ± std deviation in CQS strain (µM)	Mean IC ₅₀ ± std deviation in CQR strain (µM)
Chloroquine	0.032 ± 0.002	0.239 ± 0.028
35	1.853 ± 0.027	1.844 ± 0.390
61	0.049 ± 0.008	0.059 ± 0.024
62	0.097 ± 0.025	0.169 ± 0.027
60	2.134 ± 0.117	3.798 ± 0.582
38	2.444 ± 0.111	0.209 ± 0.084
38a	5.086 ± 0.206	1.022 ± 0.389
39	2.868 ± 0.114	0.229 ± 0.130
55	25.52 ± 0.863	21.25 ± 2.497
56	37.24 ± 2.878	38.48 ± 3.179
36	1.748 ± 0.115	0.392 ± 0.078
74	3.120 ± 0.124	1.950 ± 0.624
75a	2.807 ± 0.231	3.757 ± 0.499
75b	2.213 ± 0.502	2.319 ± 0.323
75c	1.130 ± 0.279	1.175 ± 0.293
76a	n.d ^a	13.76 ± 1.468
76b	n.d ^a	4.25 ± 0.575
77a	18.21 ± 2.030	2.972 ± 1.054
77b	26.61 ± 0.962	9.641 ± 1.027

^a not determined

5.2.3 Resistance reversal effects of derivatives CQR *P.falciparum*

Because the intrinsic antimalarial activities covered a big range, a concentration of 500 nM (0.5 µM) was chosen for the reversal assays. Although at this concentration, some compounds are cytotoxic to the parasites, it would enable the comparison of the effects of all the compounds. To determine whether the compounds could reverse CQ resistance, the test compound was added to the parasites at a concentration of 500 nM at differing concentrations of CQ (table 8). The response modification index (R.M.I), calculated by dividing the IC₅₀ for the compounds combined with CQ with that of CQ alone, was determined for each compound. This gives a fraction, which represents an indication of the activity of the compound relative to CQ.

$$\text{R.M.I} = \frac{\text{IC}_{50} \text{ of (compound + CQ)}}{\text{IC}_{50} \text{ of CQ alone}}$$

An R.M.I < 1 indicates a resistance reversing effect, an R.M.I around 1 shows no activity and an R.M.I > 1 indicates an antagonistic effect, i.e. the compound is antagonising the action of CQ.

There was no change in R.M.I for the CQS strain D10 (data not shown) indicating that there was no effect of these compounds on CQ action. This is expected since chloroquine is working at its maximum capacity in the sensitive strain and any potentiating effect by the derivatives would not be observed.

Table 8. Table of the IC_{50} values in the CQR strain (K1) at 500 nM test compound and Response Modification Indices (R.M.I.'s) of xanthene derivatives in combination with CQ.

Combination	Mean $\text{IC}_{50} \pm \text{std deviation (nM)}$	R.M.I
CQ alone	239.3 $\pm$ 27.71	1.000
CQ + VPL	75.72 $\pm$ 4.83	0.316
CQ + 35	302.4 $\pm$ 5.82	1.264
CQ + 61	29.68 $\pm$ 1.81	0.124
CQ + 62	19.94 $\pm$ 7.00	0.083
CQ + 60	226.5 $\pm$ 40.02	0.947
CQ + 38	60.78 $\pm$ 20.97	0.254
CQ + 38a	181.1 $\pm$ 19.83	0.753
CQ + 39	29.99 $\pm$ 7.20	0.125
CQ + 56	299.1 $\pm$ 29.11	1.250
CQ + 55	281.3 $\pm$ 40.75	1.176
CQ + 36	234.1 $\pm$ 37.98	0.978
CQ + 74	46.68 $\pm$ 15.07	0.195
CQ + 75a	186.2 $\pm$ 35.14	0.778
CQ + 75b	153.6 $\pm$ 20.65	0.642
CQ + 75c	162.4 $\pm$ 1.83	0.678
CQ + 76a	109.37 $\pm$ 20.64	0.457
CQ + 76b	90.99 $\pm$ 3.46	0.380
CQ + 77a	127.6 $\pm$ 6.72	0.533
CQ + 77b	141.2 $\pm$ 13.56	0.590

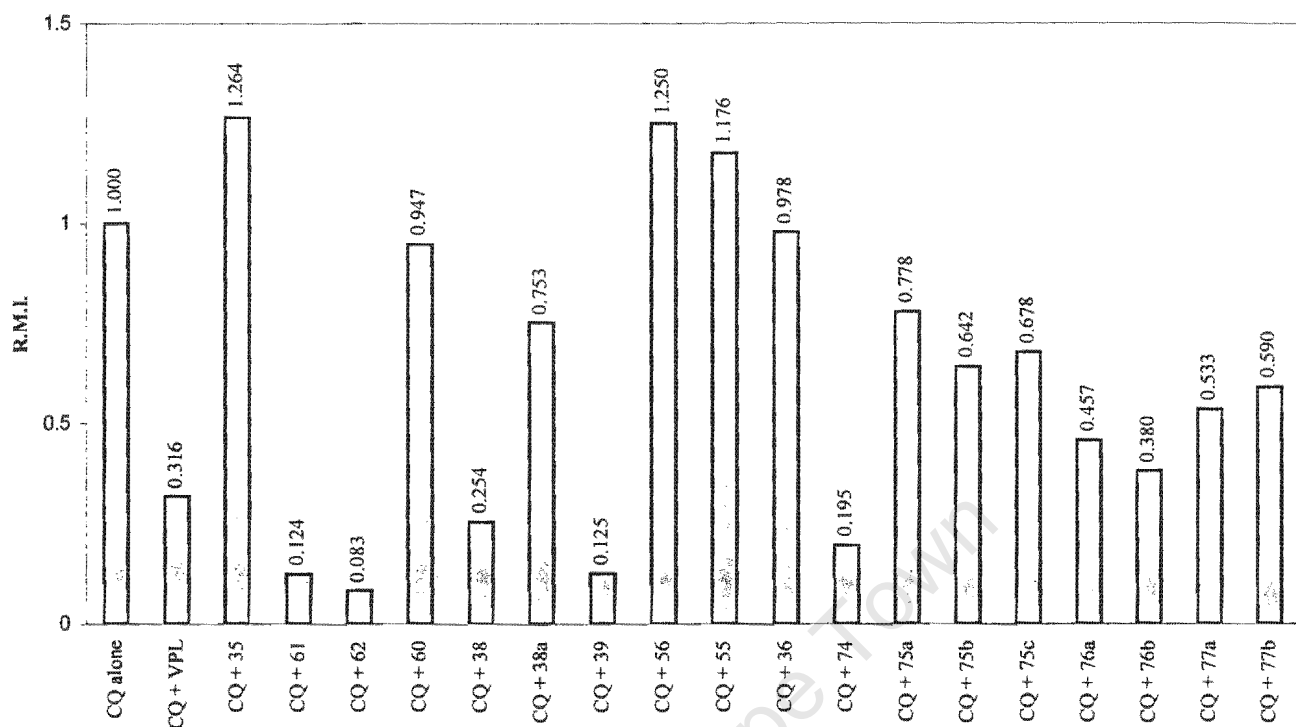


Figure 5.5. Chart showing R.M.I. values for combinations of CQ with various xanthene derivatives.

It is clear from the R.M.I values (table 8 and figure 5.5) that derivatives **35**, **36**, **60**, **38a**, **55**, **56** and **75a** shows no reversal effect at 500 nM with compounds **35**, **55** and **56** showing a slight antagonistic effect. It is noteworthy that the α,β -unsaturated amide **36** had no reversal effect since this derivative showed a 5.6 fold increase in CQ accumulation in CQR parasites. Also, compounds **35** and **75a** that showed a slight increase in CQ accumulation did not have a reversal effect.

Most of the derivatives that had mild reversal activity but not as good as VPL were the 9-monosubstituted xanthene derivatives (**75b-c**, **76a-b**, **77a-b**) as well as the mono aryl amine derivative **38a**. Coincidentally, these compounds did not show CQ accumulation greater than that of VPL either. Compounds showing activity greater than VPL were **38**, **39**, **61**, **62** and **74**. The best activity was observed with sulphonamide **62** followed by **61** and aryl amine **39** (table 8). These compounds also showed CQ accumulation greater than that of VPL.

5.2.4 Summary of results

Most of the 9-monosubstituted xanthene derivatives showed properties that resemble a typical resistance reverser. These were derivatives **75b-c**, **76a-b**, **77a-b** and the mono aryl amine **38a**. Although these

compounds were relatively anti-malarial, they did show a reversal effect on the CQR strain (K1) of *P.falciparum* at 0.5 μ M. All of the mentioned compounds have IC₅₀'s above this concentration, i.e. these compounds showed a reversal effect at a sub-lethal concentration, which is a requisite for resistance reversal agents. The effects observed were not as good as verapamil and this is also reflected by their CQ accumulation profile.

The parent 9-monosubstituted xanthene amine **74** showed the most promise. At 0.5 μ M, the compound is not acting as an anti-malarial but showed a reversal effect superior to that of verapamil. It also showed CQ accumulation above that of VPL.

The aryl amines **38** and **39** showed anti-malarial activity comparable to CQ in K1. Although these derivatives showed reversal activity superior to VPL, the compounds are lethal to resistant parasites at a concentration of 0.5 μ M. These compounds have potential as reversal agents since they showed the best CQ accumulation effect (about 2 fold better than VPL). Thus, if the compounds are assayed at a concentration sub-lethal to parasites, they might still show an effect that is comparable to VPL.

Sulphonamide derivatives **61** and **62** were more promising as anti-malarials than being resistant reversal agents, since their IC₅₀'s were far superior to CQ in K1 but also showed excellent reversal effects at 0.5 μ M. It should be noted that although the aryl amines and aforementioned sulphonamides possess high anti-malarial activity, it is not necessarily a disadvantage since they could still be utilised as dual-purpose drugs in resistant *P.falciparum* as long as they are not cytotoxic to mammalian cells.

The aforementioned compounds showed correlation between CQ accumulation and resistant reversal effects, except the α,β -unsaturated amide **36**. This derivative had anti-malarial activity comparable to CQ and increased CQ accumulation in resistant parasites 5.6 fold above that of CQ alone. However, it is not surprising to observe that this derivative had no effect on resistance reversal. This supports earlier observations that increase in CQ accumulation is not solely responsible for resistance reversal. This result is consistent with results obtained with anticancer anthracyclines and *vinca* alkaloids which only raise CQ accumulation but do not act as chemosensitizers.⁵³

CHAPTER 6

EXPERIMENTAL

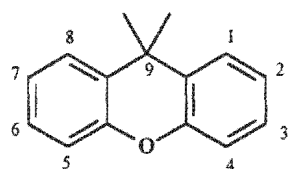
6.1 GENERAL

Reactions were monitored by thin layer chromatography on aluminium-backed silica gel 60 F₂₅₄ plates (Merck) and visualised with a combination of ultraviolet light (254 nm) and either anisaldehyde spray [freshly prepared from a 2.5 % solution of *p*-methoxybenzaldehyde (20 cm³) and 18 M sulphuric acid (1 cm³)] or ceric ammonium sulphate in 8 M sulphuric acid, followed by heating at 200 °C. Column chromatography was carried out using Merck Kieselgel 60: 70-230 mesh. Where TLC solutions refer to aqueous ammonia [NH₃(aq)], a 25 % solution of ammonia in water was used.

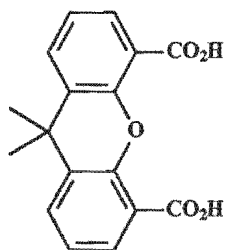
Melting points were determined on a Reichert-Jung hot stage apparatus and are uncorrected. ¹H NMR spectra were recorded on either a Varian VXR-200 at 200 MHz, Varian Mercury 300 MHz or a Varian Unity spectrometer at 400 MHz and are recorded in parts per million (ppm) as measured from tetramethylsilane. ¹³C NMR spectra were recorded on the same instruments operating at 50, 75 and 100 MHz respectively. Coupling constants *J* are measured in Hertz. Infrared (IR) spectra were recorded on a Satellite FTIR Spectrometer. Mass spectra were recorded on a VG micromass 16F spectrometer and accurate mass determinations were performed on a Kratos Limited MS9/50 spectrometer. Microanalysis for C, H, N and S were performed using a Fison's Instruments Elemental Analyser EA1108.

Tetrahydrofuran, benzene, and diethyl ether were dried over sodium wire prior to use using benzophenone as an indicator. Triethylamine were dried over and distilled from calcium hydride and stored over potassium hydroxide pellets. Dichloromethane were dried over phosphorous pentoxide and distilled. Solvents not mentioned above which were used in reactions were anhydrous unless otherwise stated.

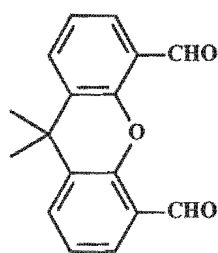
6.2 PROCEDURES FOR 9,9-DIMETHYLBXANTHENE ANALOGUES



9,9-Dimethyl-9H-xanthene (41). – This compound was synthesized as described in the literature.⁹¹ 9-Xanthone (5.0 g, 25.51 mmol) was suspended in toluene (30 cm³) and cooled to 0°C in an ice-water bath. Trimethylaluminium solution (32 cm³ of a 2.0 M solution in toluene, 64.0 mmol) was added over 30 minutes. The yellow solution was allowed to warm to room temperature over 3 hours and stirred for a further 16 hours. The resulting dark red solution was poured into a manually stirred solution of 25 cm³ concentrated hydrochloric acid and 400 cm³ of ice. The organic phase was separated, dried over MgSO₄, filtered and concentrated, which afforded 5.24 g (98%) **41** as yellow oil after drying *in vacuo*. The material was used without further purification: *R*_f 0.63 (30 % EtOAc – Hexane); ¹H NMR (CDCl₃, 400 MHz) δ 7.45 (2H, dd, *J* = 1.4 and 7.5 Hz, aromatic), 7.25 – 7.21 (2H, m, aromatic), 7.13 – 7.08 (4H, m, aromatic), 1.67 (6H, s, 9-(CH₃)₂).

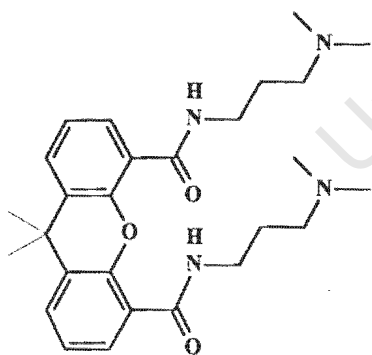


9,9-Dimethyl-9H-xanthene-4,5-dicarboxylic acid (42). – To a stirred solution of 9,9-Dimethylxanthene (0.53 g, 2.52 mmol) in 10 ml THF at -40°C was added *n*-Butyllithium (4.50 cm³ of a 1.6 mol.dm⁻³ solution in hexane, 7.56 mmol).⁹² The solution is stirred at -40°C for 1 hour and then allowed to warm to room temperature and stirred for a further 4 hours. The dark solution is poured onto excess dry ice and CO₂ allowed to evaporate. Water (100 cm³) is added to the resulting yellow residue and the pH of the solution adjusted to 1, using concentrated hydrochloric acid. A yellow precipitate is formed which is filtered by suction to give 1.03 g of acid-acid: mp > 400°C; *R*_f 0.24 (10% MeOH - CH₂Cl₂); ¹H NMR (400 MHz; CD₃OD) δ 8.0 (2H, dd, *J* = 2.0 and 8.0 Hz, 1-H and 8-H), 7.83 (2H, dd, *J* = 2.0 and 8.0 Hz, 3-H and 6-H), 7.28 (2H, t, *J* = 8.0 Hz, 2-H and 7-H), 1.66 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz; CD₃OD) δ 168.0, 150.3, 132.8, 132.4, 125.2, 119.1, 35.2, 32.6; IR (CHCl₃ film) ν/cm⁻¹ 3504 (OH), 1697 (C=O); [HRMS (FAB) Found: *M*⁺ 298.0839. C₁₇H₁₄O₅ requires *M*, 298.0841]. This carboxylic acid could not be purified by column chromatography or crystallisation due to its low solubility in organic solvents. Therefore, it was used in the following transformation without further purification.



9,9-Dimethyl-9H-xanthene-4,5-dicarbaldehyde (46). - To a solution of 9,9-Dimethylxanthene (2.38 g, 14.22 mmol) and *N,N,N',N'*-Tetramethylethylenediamine (TMEDA) (5.2 cm³, 35.23 mmol) in 100 cm³ dry diethyl ether was added *n*-Butyllithium (20.0 cm³ of a 1.6 mol. dm⁻³ solution in hexanes, 35.23 mmol).⁹³ The solution was refluxed for 18 hours and cooled in an ice-water bath. *N,N'*-

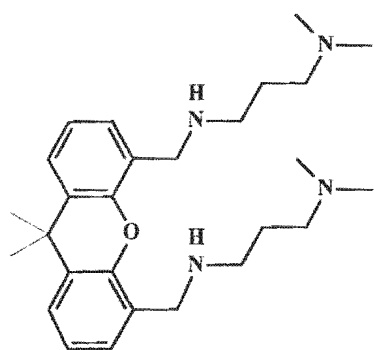
Dimethylformamide (2.62 cm³, 35.23 mmol) was added drop wise over 5 minutes. The mixture was allowed to warm to room temperature and stirred for 24 hours. The resulting dark solution was poured into 150 cm³ of ice-cold 1 M hydrochloric acid, upon which a yellow brown precipitate formed. The phases were separated and the acidic water phase extracted with dichloromethane (5 x 50 cm³). The combined organic fractions were washed with 1 M hydrochloric acid (200 cm³), water (200 cm³) and brine (200 cm³), dried over MgSO₄, filtered and evaporated under reduced pressure. The compound was chromatographed (SiO₂, 30% EtOAc-Hexane) to give 2.72 g (72 %) of dicarbaldehyde **46** as yellow crystals: mp 181-184 °C; *R_f* 0.39 (30% EtOAc - Hexane); ¹H NMR (400 MHz; CDCl₃) δ 10.86(2H, s, 4-CHO), 7.80 (2H, dd, *J* = 2.0 and 8.0 Hz, 1-H and 8-H), 7.70 (2H, dd, *J* = 2.0 and 8.0 Hz, 3-H and 6-H), 7.25 (2H, t, *J* = 7.0 Hz, 2-H and 7-H), 1.69 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz; CDCl₃) δ 188.5, 151.1, 132.3, 130.9, 127.6, 124.1, 123.8, 33.8, 32.2; IR (CHCl₃ film) ν /cm⁻¹ 2871, 2756 (H of aldehyde), 1685 (C=O); [HRMS (EI) Found: *M*⁺ 266.095. C₁₇H₁₄O₃ requires *M*, 266.092); Anal. (Calculated for C₁₇H₁₄O₃: C, 76.7; H, 5.3 Found: C, 76.7; H, 5.5].



9,9-Dimethyl-9H-xanthene-4,5-dicarboxylic acid bis [(3-dimethylaminopropyl) amide] (32). - To a stirred solution of acid **42** (0.29 g, 0.96 mmol) in dry toluene (20 cm³) was added triethylamine (0.27 cm³, 1.93 mmol), diphenylphosphoryl azide (0.52 cm³, 2.41 mmol) and 3-Dimethylamino-1-propylamine (0.24 cm³, 1.93 mmol). The solution was heated to 85°C and stirred for 18 hours. After cooling to room temperature, toluene was removed by evaporation. The resulting

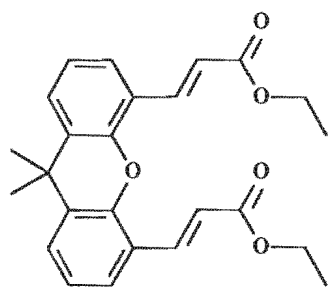
brown residue was re-dissolved in CH₂Cl₂ and washed with 2% Na₂CO₃ (2 x 50 cm³). The organic phase was dried over K₂CO₃, filtered and concentrated. The compound was chromatographed (SiO₂, 5 % NH₃(aq) - MeOH) to give 0.29 g (65 %) of amide **32** as a brown oil: *R_f* 0.14 (5 % NH₃(aq) - MeOH); ¹H NMR (400 MHz; CDCl₃) δ 8.56 (2H, t, *NH*), 7.73 (2H, dd, *J* = 1.8 and 7.8 Hz, 1-H and 8-H), 7.52 (2H, dd, *J* = 1.8 and 7.8 Hz, 3-H and 6-H), 7.16 (2H, t, *J* = 7.6 Hz, 2-H and 7-H), 3.58 (4H, q, *J* = 6.7 Hz, -CO-N-CH₂-C), 2.49 (4H, t, *J* = 6.9 Hz, -C-CH₂-N(CH₃)₂), 2.27 (12H, s, -N(CH₃)₂), 1.86 (4H,

quintet, $J = 6.7$ Hz, $-C-CH_2-C-N(CH_3)_2$, 1.64 (6H, s, $9-(CH_3)_2$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 165.6, 148.0, 130.5, 128.6, 128.4, 123.3, 123.1, 58.2, 45.2, 39.4, 34.1, 32.4, 26.4; IR ($CHCl_3$ film) ν/cm^{-1} 3358 (NH), 1640 (C=O); [HRMS (EI) Found M^+ 466.2948. $C_{27}H_{38}O_3N_4$ requires M , 466.2942].



***N*-{5-[(3-Dimethylamino)-propylamino-methyl]-9,9-dimethyl-9*H*-xanthen-4-yl-methyl}-*N*',*N*'-dimethyl-propane-1,3-diamine (33).** – 3-Dimethylamino-1-propylamine (1.88 cm³, 15.04 mmol) was added to a solution of the aldehyde **46** (2.0 g, 7.52 mmol) in methanol. The reaction mixture was stirred at room temperature for 15 hours. The stirrer bar was removed and polymer supported borohydride (Aldrich, 2.5 mmol BH_4^-/g loading, 12.0 g, 30.08 mmol) added. The suspension

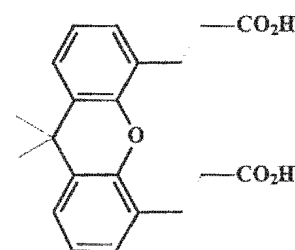
was shaken at room temperature for 18 hours. The polymer beads were removed by filtration and washed with several aliquots of methanol. The filtrate was concentrated under reduced pressure and the resulting residue chromatographed (SiO_2 , 5 % $NH_3(aq)$ - MeOH) to give 2.96 g (100 %) of amine **33** as a yellow oil: R_f 0.40 (5 % $NH_3(aq)$ - MeOH); ^1H NMR (400 MHz; $CDCl_3$) δ 7.33 (2H, dd, $J = 1.6$ and 7.9 Hz, 1-H and 8-H), 7.20 (2H, dd, $J = 1.6$ and 7.4 Hz, 3-H and 6-H), 7.04 (2H, t, $J = 7.7$ Hz, 2-H and 7-H), 3.97 (4H, s, Ar- CH_2-N), 2.72 (4H, t, $J = 6.9$ Hz, $-NH-CH_2-C$), 2.31 (4H, t, $J = 7.1$ Hz, $-C-CH_2-N(CH_3)_2$), 2.19 (12H, s, $-N(CH_3)_2$), 1.70 (4H, quintet, $J = 7.3$ Hz, $-C-CH_2-CH_2-N(CH_3)_2$), 1.62 (6H, s, $9-(CH_3)_2$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 148.0, 130.0, 127.5, 127.0, 124.9, 122.8, 58.0, 48.9, 47.9, 45.5, 34.0, 32.3, 28.1; IR ($CHCl_3$ film) ν/cm^{-1} 3393 (NH); [HRMS (EI) Found M^+ 438.3355. $C_{27}H_{42}N_3O$ requires M , 438.3358].



3-[5-(2-Ethoxycarbonyl-vinyl)-9,9-dimethyl-9*H*-xanthen-4-yl]-acrylic acid ethyl ester (48). – To a stirred solution of the aldehyde **46** (1.90 g, 7.14 mmol) dissolved in dry CH_2Cl_2 (50 cm³) was added ethyl (triphenylphosphoranylidene) acetate (7.45 g, 21.42 mmol) and the solution refluxed for 3 hours. After cooling, the solvent is removed by evaporation and the resulting residue chromatographed (SiO_2 , 30 % EtOAc – Hexane) to

give 2.37 g (82 %) of ester **48** as an oil: R_f 0.46 (30% EtOAc - Hexane), ^1H NMR (400 MHz; $CDCl_3$) δ 8.33 (2H, d, $J = 16$ Hz, Ar- $CH=C-CO$), 7.50 (2H, dd, $J = 1.5$ and 7.5 Hz, 1-H and 8-H), 7.45 (2H, dd, $J = 1.6$ and 8.0 Hz, 3-H and 6-H), 7.12 (2H, t, $J = 7.8$ Hz, 2-H and 7-H), 6.51 (2H, d, $J = 16$ Hz, Ar- $C=CH-CO$), 4.34 (4H, q, $J = 7.1$ Hz, $-CO-O-CH_2-C$), 1.63 (6H, s, $9-(CH_3)_2$), 1.37 (6H, t, $-CO-O-C-CH_3$).

); ^{13}C NMR (100 MHz; CDCl_3) δ 166.7, 148.5, 138.1, 130.9, 127.6, 125.5, 123.4, 123.1, 120.1, 60.5, 34.4, 31.8, 14.3; IR (CHCl_3 film) ν/cm^{-1} 3030 (olefinic CH), 1654 (C=O), 1618 (C=C); [HRMS (EI) Found M^+ 406.1782. $\text{C}_{25}\text{H}_{26}\text{O}_5$ requires 406.1780].

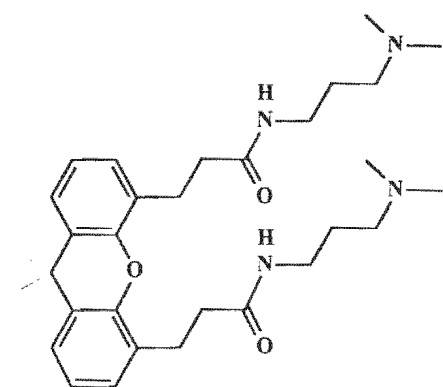


3-[5-(2-Carboxyethyl)-9,9-dimethyl-9H-xanthen-4-yl]-propionic acid (54) –

To a solution of ester **48** (0.87 g, 2.13 mmol) in ethyl acetate (20 cm^3) was added a catalytic amount (0.081 g) of Palladium (10%) on activated carbon.

The system is flushed with hydrogen and the suspension stirred at room temperature under a hydrogen atmosphere for 24 hours. The solution was

filtered over Celite and the filtrate concentrated under reduced pressure to yield a pale yellow oil (0.91 g). The oil was dissolved in a 3:1 mixture of THF/ H_2O (24 cm^3) and cooled to 0°C in an ice-water bath. Lithium hydroxide monohydrate (0.27 g, 6.53 mmol) was added and the solution stirred at room temperature for 18 hours. The resulting clear solution was poured onto 3 M hydrochloric acid (100 cm^3) and extracted with ethyl acetate (250 cm^3 in total). The ethyl acetate fractions were dried over Na_2SO_4 , filtered and concentrated to give the di-acid **54** as a pale yellow solid (0.78 g). The material was used without further purification: mp $187\text{--}190^\circ\text{C}$; R_f 0.43 (20% EtOAc - Hexane); ^1H NMR (400 MHz; acetone- d_6) δ 7.40 (2H, dd, $J = 1.6$ and 7.8 Hz, 1-H and 8-H), 7.18 (2H, dd, $J = 1.6$ and 7.6 Hz, 3-H and 6-H), 7.05 (2H, t, $J = 7.7$ Hz, 2-H and 7-H), 3.14 (4H, t, $J = 7.5$ Hz, Ar- CH_2 -C-), 2.73 (4H, t, $J = 7.5$ Hz, Ar-C- CH_2 -CO-), 1.62 (6H, s, 9-(CH_3) $_2$); ^{13}C NMR (50 MHz; acetone- d_6) 174.5, 149.5, 131.4, 129.5, 129.4, 125.8, 124.3, 35.6, 35.3, 33.2, 27.1; IR (Acetone film) ν/cm^{-1} 3590 (OH), 1757 (C=O); [HRMS (EI) Found M^+ 354.1458. $\text{C}_{21}\text{H}_{22}\text{O}_5$ requires M , 354.1467]; Anal. (Calcd. for $\text{C}_{21}\text{H}_{22}\text{O}_5$: C, 71.2; H, 6.2 Found: C, 71.2; H, 6.6].

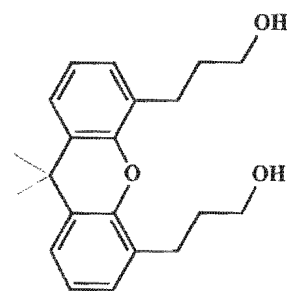


***N*-[3-(Dimethylamino)propyl]-3-(5-{2-[3-(dimethylamino)propyl carbamoyl]-ethyl}-9,9-dimethyl-9H-xanthen-4-yl)propionamide (34) –**

To a stirred suspension of acid **54** (0.5 g, 1.41 mmol) in dry CH_2Cl_2 is added oxalyl chloride (0.74 cm^3 , 8.47 mmol) and 2 drops of *N,N*-dimethylformamide and the solution refluxed for 3 hours. After cooling, the volatile substances were removed by evaporation and the resulting residue re-dissolved in dichloromethane and cooled to 0°C . 3-Dimethylamino-1-

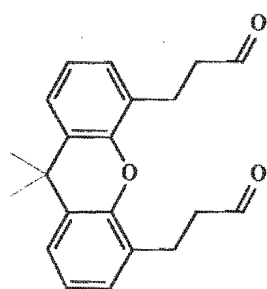
propylamine (0.39 cm^3 , 2.82 mmol) and distilled triethylamine (0.39 cm^3 , 2.82 mmol) was added and

the solution was stirred for a further 12 hours. The filtrate was concentrated and chromatographed (SiO₂, 10 % NH₃(aq) - MeOH) to give 0.48 g (65 %) of amide **34** as a brown oil: *R_f* 0.41 (10 % NH₃(aq) - MeOH); ¹H NMR (400 MHz; CDCl₃) δ 7.59 (2H, t, NH), 7.23 (2H, dd, *J* = 1.2 and 7.6 Hz, 1-H and 8-H), 7.08 (2H, dd, *J* = 1.2 and 7.2 Hz, 3-H and 6-H), 6.96 (2H, t, *J* = 7.6 Hz, 2-H and 7-H), 3.27 (4H, q, *J* = 6.4 Hz, -CO-N-CH₂-C), 3.09 (4H, q, *J* = 7.6 Hz, Ar-CH₂-C), 2.55 (4H, q, *J* = 6.4 Hz, C-CH₂-CO-N), 2.35 (4H, t, *J* = 6.8 Hz, C-CH₂-N(CH₃)₂), 2.20 (12H, s, C-N(CH₃)₂), 1.65 (4H, quintet, *J* = 6.8 Hz, -C-CH₂-C-N(CH₃)₂), 1.56 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz; CDCl₃) δ 172.7, 148.2, 129.9, 128.2, 128.1, 124.1, 122.7, 57.5, 44.9, 38.2, 37.4, 34.2, 32.3, 27.0, 26.1; IR (CHCl₃ film) ν/cm⁻¹ 3394 (NH), 1705 (C=O); [HRMS (EI) Found *M*⁺ 522.3555. C₃₁H₄₆O₃N₄ requires *M*, 522.3569].



3-[5-(3-Hydroxypropyl)-9,9-dimethyl-9H-xanthen-4-yl] propan-1-ol (49**) –**

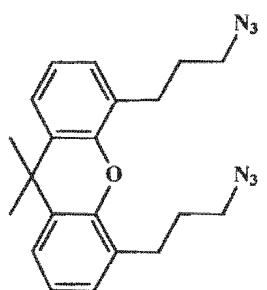
To a solution of ester **48** (1.74 g, 4.26 mmol) in ethyl acetate (20 cm³) was added a catalytic amount (0.16 g) of palladium (10%) on activated carbon. The system is flushed with hydrogen and the suspension stirred at room temperature under a hydrogen atmosphere for 24 hours. The solution was filtered over Celite and the filtrate concentrated on a rotary evaporator to yield a pale yellow oil (1.82 g). A solution of the ethyl ester (1.82 g, 4.44 mmol) in 10 cm³ dry diethyl ether was added to a suspension of lithium aluminium hydride (0.34 g, 8.88 mmol) in 10 cm³ dry diethyl ether at 0°C. The suspension was stirred at 0°C for 30 minutes and quenched by slow addition of ethyl acetate until no more fizzing occur. The solution was transferred to a separating funnel and extracted with ethyl acetate with the aid of brine. The combined organic phases were washed with 1 M hydrochloric acid and brine, dried (Na₂SO₄) and concentrated under reduced pressure to give 1.41 g (97 %) of alcohol **49** as a colourless oil which solidifies on standing: mp 59 – 61 °C; *R_f* 0.53 (10% MeOH - CH₂Cl₂); ¹H NMR (400 MHz; CDCl₃) δ 7.28 (2H, dd, *J* = 1.7 and 7.6 Hz, 1-H and 8-H), 7.08 (2H, dd, *J* = 1.7 and 7.4 Hz, 3-H and 6-H), 7.01 (2H, t, *J* = 7.4 Hz, 2-H and 7-H), 3.77 (4H, t, *J* = 6.0 Hz, -C-CH₂-OH), 2.92 (4H, t, *J* = 8.1 Hz, Ar-CH₂-C), 1.96 (4H, quintet, *J* = 8.3 Hz, C-CH₂-C), 1.62 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz; CDCl₃) 27.0, 32.0, 33.2, 34.3, 62.7, 122.6, 123.6, 127.7, 129.3, 130.1, 148.3; IR (CHCl₃ film) ν/cm⁻¹ 3622 (OH); [HRMS (FAB) Found *M*⁺ 326.1888 C₂₁H₂₆O₃ requires *M*, 326.1880]; Anal. (Calcd. for C₂₁H₂₆O₃: C, 77.3; H, 7.8 Found: C, 76.9; H, 8.0).



3-[9,9-Dimethyl-5-(3-oxopropyl)-9H-xanthen-4-yl] propionaldehyde (58) –

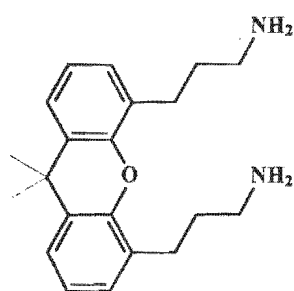
Dimethyl sulfoxide (3 cm³, 42.27 mmol) and 5 cm³ dry tetrahydrofuran was added to a solution of the alcohol **49** (0.43 g, 1.34 mmol) dissolved in dry triethylamine (3 cm³, 21.5 mmol). The solution was cooled to 0 °C and sulphur trioxide pyridine complex (1.1 g, 6.91 mmol) added. The mixture was stirred at 0°C for 15 minutes and allowed to warm to ambient temperature and stirred for a

further 30 minutes. Diluting with ether and water quenched the reaction. The ether and water layers were partitioned. The organic phase was washed with water (100 cm³) and brine (100 cm³) and subsequently dried (MgSO₄), filtered and concentrated under reduced pressure. The crude compound was chromatographed (SiO₂, 5 % MeOH-CH₂Cl₂) to give 0.25 g (58 %) of aldehyde **58** as a colourless oil: TLC R_f 0.29 (5% MeOH- CH₂Cl₂); ¹H NMR (400 MHz; CDCl₃) δ 9.87 (2H, t, *J* = 1.43 Hz, -CHO), 7.31 (2H, dd, *J* = 1.7 and 7.6 Hz, 1-H and 8-H), 7.09 (2H, dd, *J* = 1.7 and 7.4 Hz, 3-H and 6-H), 7.03 (2H, t, *J* = 7.6 Hz, 2-H and 7-H), 3.10 (4H, t, *J* = 7.4 Hz, Ar-CH₂-C), 2.58 (4H, ddd, *J* = 1.4, 6.5 and 14.6 Hz, C-CH₂-CHO), 1.62 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz; CDCl₃) 23.0, 32.3, 34.2, 43.9, 122.9, 124.4, 127.3, 127.8, 130.0, 148.3, 201.6; IR (CHCl₃) ν/cm⁻¹ 2971, 2700 (CH of aldehyde), 1711 (C=O); [HRMS (EI) Found M⁺ 322.1571. C₂₁H₂₂O₃ requires *M*, 322.1568].



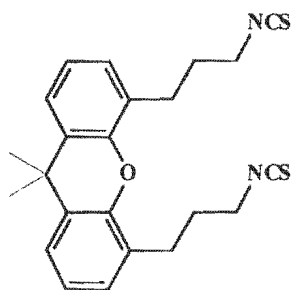
4,5-Bis (3-azidopropyl)-9,9-dimethyl-9H-xanthene (50).– A solution of the alcohol **49** (0.5 g, 1.53 mmol) and triphenylphosphine (0.82 g, 3.11 mmol) in dry tetrahydrofuran (5 cm³) was cooled to –15°C in an acetone-dry ice bath.⁹⁴ Diisopropyl azodicarboxylate (0.6 cm³, 0.63 g, 3.11 mmol) was added to this solution and stirred at –15°C for 5 minutes. Diphenyl phosphoryl azide (0.67 cm³, 0.86 g, 3.11 mmol) was added and the mixture stirred at ambient

temperature for 4 hours. The solvent was removed under reduced pressure and the resulting residue chromatographed (SiO₂, 10 % EtOAc - Hexane) to give 0.32 g (56 %) of azide **50** as a yellow oil: R_f 0.46 (10% EtOAc - Hexane); ¹H NMR (400 MHz; CDCl₃) δ 7.31 (2H, dd, *J* = 1.9 and 7.6 Hz, 1-H and 8-H), 7.07 (2H, dd, *J* = 1.9 and 7.4 Hz, 3-H and 6-H), 7.03 (2H, t, *J* = 7.6 Hz, 2-H and 7-H), 3.40 (4H, t, *J* = 6.8 Hz, -C-CH₂-N), 2.90 (4H, t, *J* = 7.4 Hz, Ar-CH₂-C), 2.01 (4H, quintet, *J* = 7.8 Hz, -C-CH₂-C), 1.63 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz; CDCl₃) δ 27.4, 29.2, 32.2, 34.2, 51.1, 122.7, 124.1, 127.9, 128.0, 130.2, 148.2; IR (CHCl₃ film) ν/cm⁻¹ 2093 (N₃); [HRMS (EI) Found M⁺ 376.2007 C₂₁H₂₄ON₆ requires *M*, 376.2010].



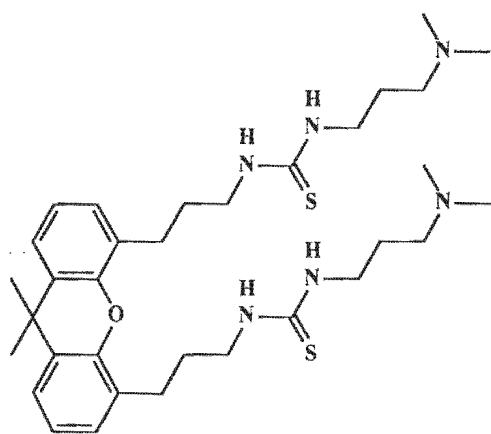
3-[5-(3-Aminopropyl)-9,9-dimethyl-9H-xanthen-4-yl]-prop-1-ylamine (51)

- 10% Palladium on carbon (0.10 g) was added to a solution of the azide **50** (0.32 g, 0.85 mmol) in a ethyl acetate-methanol (1:2) mixture. The system is flushed with hydrogen and the solution stirred at room temperature under a hydrogen atmosphere (1 atm) for 18 hours. The catalyst was removed by filtration over Celite and the filtrate concentrated under reduced pressure to give 0.25 g (91 %) of amine **51** as a pale yellow oil. The amine was used in the next step of the sequence without further purification: R_f 0.17 (10 % MeOH – CH_2Cl_2); ^1H NMR (400 MHz; CDCl_3) δ 7.27 (2H, dd, $J = 1.6$ and 7.6 Hz, 1-H and 8-H), 7.05 (2H, m, 3-H and 6-H), 7.00 (2H, t, $J = 7.6$ Hz, 2-H and 7-H), 2.83 (4H, m, Ar- CH_2 -C), 2.72 (4H, m, -C- CH_2 - NH_2), 1.88 (4H, m, -C- CH_2 -C-N), 1.61 (6H, s, 9-(CH_3)₂), 1.07 (4H, m, NH_2); ^{13}C NMR (100 MHz; CDCl_3) δ 148.3, 130.0, 129.2, 127.7, 123.7, 122.5, 42.2, 34.3, 32.1, 32.0, 22.8; IR (CHCl_3 film) ν/cm^{-1} 3410 (NH_2); [HRMS (EI) Found M^+ 324.2210. $\text{C}_{21}\text{H}_{28}\text{N}_2\text{O}$ requires M , 324.2202].



4,5-Bis (3-isothiocyanatopropyl)-9,9-dimethyl-9H-xanthene (52).

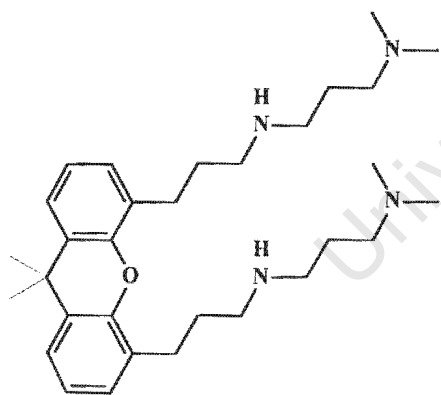
– The amine **51** (0.25 g, 0.80 mmol), dissolved in dry tetrahydrofuran (3 cm^3), was added slowly to a cooled solution of N,N' -Dicyclohexylcarbodiimide (0.33 g, 1.6 mmol) and carbon disulfide (0.67 cm^3 , 11.2 mmol) in tetrahydrofuran (5 cm^3) at -5°C .⁹⁵ The reaction mixture was stirred at room temperature for 18 hours. The solvent was removed under reduced pressure and the compound purified by column chromatography (SiO_2 , 30 % EtOAc - Hexane) to give 65 mg (20 %) of isothiocyanate **52** as a pale yellow solid: R_f 0.54 (30% EtOAc - Hexane); ^1H NMR (200 MHz; acetone- d_6) δ 7.26 (2H, dd, $J = 1.8$ and 7.5 Hz, 1-H and 8-H), 7.01 (2H, dd, $J = 1.8$ and 7.4 Hz, 3-H and 6-H), 6.92 (2H, t, $J = 7.5$ Hz, 2-H and 7-H), 3.62 (4H, t, $J = 6.5$ Hz, Ar- CH_2 -C), 2.84 (4H, t, $J = 7.3$ Hz, C- CH_2 -N), 2.01 (4H, quintet, $J = 6.5$ Hz, C- CH_2 -C); ^{13}C NMR (50 MHz, acetone- d_6) δ 195.7, 149.3, 131.5, 129.1, 125.4, 123.3, 44.1, 34.2, 32.4, 31.7, 23.5; IR (Acetone film) ν/cm^{-1} 2100 (NCS); [HRMS (EI) Found M^+ 408.1325 $\text{C}_{23}\text{H}_{24}\text{N}_2\text{OS}_2$ requires M , 408.1330].



1-[3-Dimethylaminopropyl]-3-[3-{3-[3-(dimethylamino)-propylthioureido]-propyl}-9,9-dimethyl-9H-xanthen-4-yl]-propyl]-thiourea (37) - 3-Dimethylamino-1-propylamine

(0.052 cm³, 0.41 mmol) was added to a solution of the isothiocyanate **52** (0.065 g, 0.16 mmol) in dichloromethane (5 cm³). The mixture was stirred at room temperature for 18 hours under a nitrogen atmosphere. The solvent was removed under reduced pressure and the compound chromatographed (SiO₂, 5 % NH₃(aq) - MeOH) to give 98 mg (99.9%) of the

thiourea **37** as colourless oil: *R_f* 0.39 (5% NH₃(aq) - MeOH); ¹H NMR (400 MHz; CDCl₃) δ 7.27 (2H, dd, *J* = 1.6 and 9.5 Hz, 1-H and 8-H), 7.06 (2H, dd, *J* = 1.4 and 7.2 Hz, 3-H and 6-H), 7.00 (2H, t, *J* = 7.7 Hz, 2-H and 7-H), 3.60 (8H, m, CH₂-NHCSNH-CH₂), 2.87 (4H, t, *J* = 7.2 Hz, Ar-CH₂-C), 2.42 (4H, m, C-CH₂-N), 2.20 (12H, s, N(CH₃)₂), 2.00 (4H, quintet, *J* = 7.6 Hz, Ar-C-CH₂-C), 1.71 (4H, m, C-CH₂-C-N), 1.59 (6H, s, 9-(CH₃)₂); ¹³C NMR (50 MHz; CDCl₃) δ 26.0, 27.6, 29.45, 32.1, 34.2, 44.9, 50.7, 58.8, 122.7, 123.9, 127.6, 128.6, 130.1, 148.2, 181.5; IR (CHCl₃ film) ν/cm⁻¹ 3268 (NH), 1547 (C=S); [HRMS (FAB) Found *M*⁺ 612.3629. C₃₃H₅₂ON₆S₂ requires *M*, 612.3640].



***N*-[3-(5-{3-[3-(Dimethylamino)-propylamino]-propyl}-9,9-dimethyl-9H-xanthen-4-yl)-propyl]-*N*',*N*'-dimethyl-propane-1,3-diamine (35)**

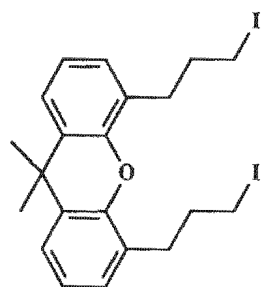
Method A: 3-Dimethylamino-1-propylamine (0.19 cm³, 1.49 mmol) was added to a solution of the aldehyde **58** (0.25 g, 0.78 mmol) dissolved in methanol and the mixture stirred at room temperature for 18 hours. The stirrer bar was removed and borohydride on Amberlite IRA-400 (Aldrich, 2.5 mmol BH₄/g

resin, 1.26 g, 3.15 mmol) was added. The suspension was shaken for 24 hours at room temperature. The resin was removed by filtration and washed with several aliquots of methanol. The compound was chromatographed (SiO₂, 10 % NH₃(aq) - MeOH) to give 0.08 g (21 %) of polyamine **35** as a pale yellow oil;

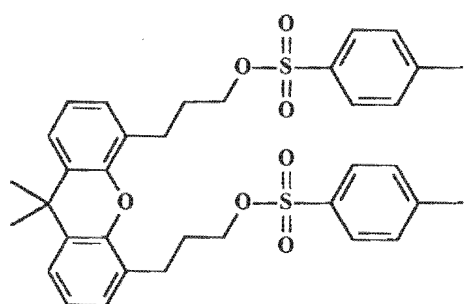
Method B: 3-Dimethylamino-1-propylamine (0.85 cm³, 6.8 mmol) was added to a solution of the sulphonic ester **60** (1.96g, 3.09 mmol) in dry toluene (20 cm³) at room temperature. The solution was

refluxed for 3 hours, cooled and toluene removed by reduced pressure. The resulting residue was chromatographed (SiO₂, 10 % NH₃(aq) - MeOH) to give 0.25 g (16 %) of the amine as a pale yellow oil;

Method C: 3-Dimethylamino-1-propylamine (1.1 cm³, 8.5 mmol) was added to a solution of iodide **59** (0.29 g, 0.53 mmol) in dry tetrahydrofuran (5 cm³). The solution was refluxed for 1 hour and the solvent removed under reduced pressure. The resulting residue was chromatographed (SiO₂, 10 % NH₃(aq) - MeOH) to give 0.18 g (68 %) of amine **35** as a pale yellow oil: *R_f* 0.27 (10 % NH₃ (aq) - MeOH); ¹H NMR (400 MHz; CDCl₃) δ 7.26 (2H, dd, *J* = 1.7 and 7.2 Hz, 1-H and 8-H), 7.05 (2H, dd, *J* = 1.7 and 7.5 Hz, 3-H and 6-H), 6.99 (2H, t, *J* = 7.6 Hz, 2-H and 7-H), 2.82 (4H, t, *J* = 7.4 Hz, Ar-CH₂-C), 2.74 (4H, t, *J* = 7.0 Hz, C-CH₂-N), 2.66 (4H, t, *J* = 7.1 Hz, C-N-CH₂-C), 2.30 (4H, t, *J* = 7.1 Hz, -C-CH₂-N(CH₃)₂), 2.20 (12H, d, *J* = 5.2 Hz, N-(CH₃)₂), 1.91 (4H, quintet, *J* = 7.4 Hz, Ar-C-CH₂-C), 1.66 (4H, quintet, *J* = 7.1 Hz, -N-C-CH₂-C), 1.60 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz; CDCl₃) δ 27.8, 28.1, 30.2, 32.1, 34.2, 45.4, 48.4, 49.8, 58.0, 122.4, 123.6, 127.7, 129.1, 130.0, 148.3; IR (CHCl₃ film) ν /cm⁻¹ 3400 ((NH)); [HRMS (EI) Found *M*⁺ 494.3970 C₃₁H₅₀N₄O requires *M*, 494.3982].

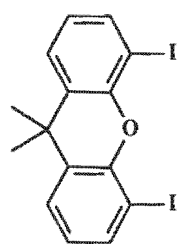


4,5-Bis (3-iodopropyl)-9,9-dimethyl-9H-xanthene (59) - Iodine (5.73 g, 22.55 mmol) was added to a stirred solution of the alcohol **49** (2.45 g, 7.52 mmol), imidazole (1.50 g, 22.55 mmol) and triphenylphosphine (5.87 g, 22.55 mmol) dissolved in a 1:3 mixture of ether/acetonitrile under nitrogen. The mixture was stirred at room temperature for 5 hours. The yellow suspension was filtered through Celite and the filtrate concentrated. Subsequent column chromatography (SiO₂, 5% MeOH-CH₂Cl₂) gave 2.97 g (72 %) of the iodide as a dark red oil: *R_f* 0.90 (10 % MeOH-CH₂Cl₂); ¹H NMR (200 MHz; CDCl₃) δ 7.31 (2H, dd, *J* = 1.9 and 7.5 Hz, 1-H and 8-H), 7.11 (2H, dd, *J* = 1.9 and 7.5 Hz, 3-H and 6-H), 7.02 (2H, t, *J* = 7.5 Hz, 2-H and 7-H), 3.29 (4H, t, *J* = 7.0 Hz, C-CH₂-I), 2.93 (4H, t, *J* = 7.0 Hz, Ar-CH₂-C), 2.24 (4H, quintet, *J* = 7.5 Hz, C-CH₂-C), 1.62 (6H, s, 9-(CH₃)₂); ¹³C NMR (50 MHz; CDCl₃) δ 148.2, 130.1, 127.9, 127.5, 124.1, 122.7, 34.2, 33.5, 32.3, 31.2, 6.69; IR (CHCl₃ film) ν /cm⁻¹ 479 (C-I); [HRMS (FAB) Found *M*⁺ 545.9928. C₂₁H₂₄OI₂ requires *M*, 545.9916].



Toluenesulfonic acid 3-[5-(3-toluenesulfonyloxypropyl)-9,9-dimethyl-9H-xanthen-4-yl]-propyl ester (60) – Triethylamine (2.44 cm³, 17.49 mmol) was added to a solution of the alcohol **49** (1.90 g, 5.83 mmol) in dichloromethane (10 cm³). The solution was cooled to 0 °C in an ice-water bath. *p*-Toluenesulphonyl chloride (2.4 g, 12.82 mmol) dissolved in dichloromethane (5

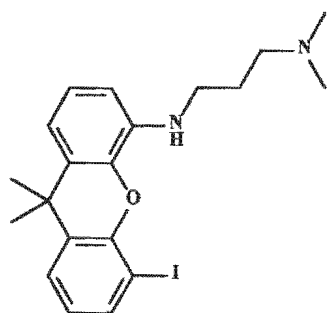
cm³) was added slowly to this cooled solution and stirred for 20 minutes at 0 °C. The reaction mixture is allowed to warm to room temperature and stirred for a further 40 minutes. The solution is transferred to a separating funnel and washed with 2M HCl (2 x 20 cm³) and saturated sodium bicarbonate (20 cm³). The organic phase is dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was chromatographed (SiO₂, 20 % EtOAc-Hexane) to give 1.96 g (53 %) of the ester as a colourless oil: *R*_f 0.3 (30 % EtOAc – Hexane); ¹H NMR (400 MHz, CDCl₃) δ 7.78 – 7.82 (4H, m, Ar-H on toluene rings), 7.33 (4H, d, *J* = 8.0 Hz, Ar-H on toluene rings), 7.27 (2H, dd, *J* = 2.0 and 7.2 Hz, 1-H and 8-H), 6.97 (2H, t, *J* = 7.2 Hz, 2-H and 7-H), 6.94 (2H, dd, *J* = 2.0 and 7.2 Hz, 3-H and 6-H), 4.09 (4H, t, *J* = 6.2 Hz, -CH₂-O-SO₂Tol), 2.80 (4H, t, *J* = 7.1 Hz, Ar-CH₂-C), 2.44 (6H, s, CH₃ on toluene rings), 2.02 (4H, m, -C-CH₂-C-), 1.58 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ 148.1, 144.7, 133.2, 130.1, 129.8, 128.0, 127.9, 127.5, 124.2, 122.8, 69.9, 34.2, 32.0, 29.0, 26.2, 21.8; IR (CHCl₃ film) ν/cm⁻¹ 1442 (S=O), 1357, 1201 (SO₂-O-); [HRMS (EI) Found *M*⁺ 634.2051. C₃₅H₃₈O₇S₂ requires *M*, 634.2059].



4,5-Diiodo-9,9-dimethylxanthene (57) – 9,9 dimethylxanthene (9.0 g, 42.86 mmol) was dissolved in dry diethyl ether (60 mL) under a nitrogen atmosphere. ⁸³N,N,N',N'-Tetramethylethylenediamine (TMEDA) (16.34 mL, 107.14 mmol) was added and the solution stirred at room temperature for 10 minutes. The solution was cooled to -78 °C (dry ice/acetone) and stirred for 20 minutes. *n*-Butyllithium (2.5M solution in diethyl

ether, 51.43 mL, 128.58 mmol) was added drop wise. The mixture was warmed slowly to room temperature (i.e. keep flask in acetone solution until ambient temperature is achieved) and stirred for 21 hours. In a separate flask, dissolve diiodomethane (9.0 mL, 111.44 mmol) in dry diethyl ether (20 mL) and cool to 0 °C. The dimethylxanthene dianion solution prepared above is cooled to -78 °C and the diiodomethane solution transferred to this solution *via* cannula. After complete addition, the reaction mixture is warmed to room temperature and stirred for 12 hours. Distilled water (100 mL) was added to quench the reaction and stirred until 2 clear layers were observed. The layers are filtered through

Watman no.1 filter paper and the layers separated. Extract the water layer with diethyl ether (2 x 50 mL) and concentrate the combined ether layers. Dissolve the resulting residue in acetone and filter to remove inorganic salts. Evaporate the solvent and dry under reduced pressure. A cream coloured solid was obtained (13.25 g, 67 %), which was used without further purification: R_f 0.51 (5 % EtOAc/Petroleum ether); ^1H NMR (CDCl_3 , 300 MHz) δ 7.73 (2H, dd, J = 1.4 and 7.7 Hz, aromatic), 7.38 (2H, dd, J = 1.6 and 7.9 Hz, aromatic), 6.86 (2H, t, J = 7.7 Hz, aromatic), 1.61 (6H, s, 9-(CH_3)₂); ^{13}C NMR (CDCl_3 , 75 MHz) δ 150.2, 137.8, 131.5, 125.9, 125.2, 84.4, 35.2, 32.0; [HRMS (EI) Found M^+ 461.8987. $\text{C}_{15}\text{H}_{12}\text{OI}_2$ requires M , 461.8977].

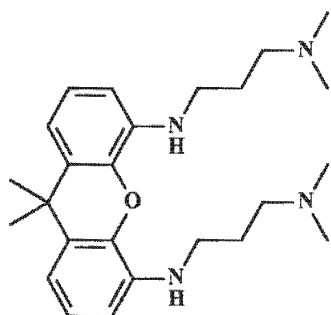


***N'*-(5-Iodo-9,9-dimethyl-9*H*-xanthen-4-yl)-*N,N*-dimethylpropane-1,3-**

diamine (38a) – A dry 2-necked flask was charged with iodide **57** (0.43 g, 0.93 mmol), sodium *tert*-butoxide (0.25 g, 2.60 mmol), 3-dimethylamino-1-propylamine (0.20 g, 0.24 mL, 1.96 mmol), tris(dibenzylideneacetone)

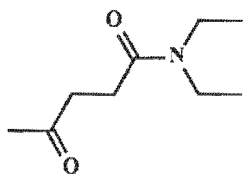
dipalladium (0) (Pd_2dba_3) (4.26 mg, 0.5 mol %), (*R*)-(+)-2,2'-

bis(diphenylphosphino)1,1'-binaphthyl (BINAP) (8.72 mg, 1.5 mol %) in 2 mL dry toluene under nitrogen.⁸⁶ The solution was heated to 80 °C and stirred for 48 hours. After cooling to room temperature, 20 mL of ether was added and the slurry filtered through Celite. The filtrate was concentrated and purified by silica gel chromatography (SiO_2) eluting with 20% MeOH/ CH_2Cl_2 followed by 10 % $\text{NH}_3(\text{aq})/\text{MeOH}$ to give 15.8 mg of the starting diiodide, 78.5 mg (21.3 %) of the mono-product (**38a**) and 98.4 mg (26.7 %) of the bis-amine **38**. Experimental data for **38a**: R_f 0.49 (20 % MeOH/ CH_2Cl_2); ^1H NMR (200 MHz, CDCl_3) δ 7.64 (1H, dd, J = 1.4 and 7.8 Hz, aromatic), 7.39 (1H, dd, J = 1.4 and 7.8 Hz, aromatic), 7.00 (1H, t, J = 7.9 Hz, aromatic), 6.83 (1H, t, J = 7.8 Hz, aromatic), 6.71 (1H, dd, J = 1.2 and 7.9 Hz, aromatic), 6.57 (1H, dd, J = 1.2 and 7.9 Hz, aromatic), 4.62 (1H, broad s, NH), 3.29 (2H, t, J = 6.7 Hz, -N- CH_2 -C-), 2.60 (2H, t, J = 7.2 Hz, -C- CH_2 -NMe₂), 2.35 (6H, s, -N(CH_3)₂), 1.97 (2H, quintet, J = 7.7 Hz, -C- CH_2 -C-), 1.59 (6H, s, 9-(CH_3)₂); IR (CHCl_3 film) ν/cm^{-1} 3663, 3588 (NH); [HRMS (EI) Found M^+ 436.1009. $\text{C}_{20}\text{H}_{25}\text{N}_2\text{OI}$ requires M , 436.1011].



***N,N*-Bis(3-dimethylaminopropyl)-9,9-dimethyl-9*H*-xanthene-4,5-diamine (38)** - Iodide **57** (1.0 g, 2.17 mmol), 3-dimethylamino-1-propylamine (0.55 g, 0.68 mL, 5.43 mmol), sodium *tert*-butoxide (0.58 g, 6.08 mmol), tris(dibenzylideneacetone) dipalladium (0) (Pd₂dba₃) (39.7 mg, 2 mol %) and (R)-(+)-2,2'-bis(diphenylphosphino)1,1'-binaphthyl (BINAP) (81.1 mg, 6 mol %) was combined in dry toluene (5 mL) under nitrogen.

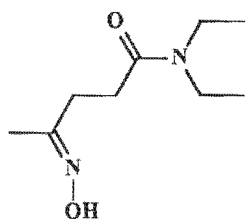
The suspension was heated to 100 °C and stirred at this temperature for 18 hours. The reaction mixture was cooled to room temperature and diethyl ether (20 mL) added. After stirring for 1 hour, the solution is filtered through Celite and the filtrate concentrated. The resulting dark oil was purified by silica gel column chromatography, by first eluting with 20 % MeOH/CH₂Cl₂ and switching to 10 % NH₃ (aq) - MeOH to give 0.79 g (89 %) of the aryl amine **38** as a brown oil: *R*_f 0.30 (5 % NH₃ (aq) - MeOH); ¹H NMR (300 MHz, CDCl₃) δ 6.95 (2H, t, *J* = 7.8 Hz, aromatic), 6.73 (2H, dd, *J* = 1.5 & 8.1 Hz, aromatic), 6.52 (2H, dd, *J* = 1.2 & 7.5 Hz, aromatic), 3.36 (4H, t, *J* = 6.6 Hz, Ar-N-CH₂), 5.10 (2H, broad s, NH), 2.57 (4H, t, *J* = 6.6 Hz, C-CH₂NMe₂), 2.36 (12H, s, NMe₂), 1.89 (4H, t, *J* = 6.3 Hz, -C-CH₂-C-NMe₂), 1.59 (6H, s, 9-Me₂); ¹³C NMR (75 MHz, CDCl₃) δ 137.9, 136.7, 129.5, 123.0, 113.1, 107.8, 57.3, 45.0, 41.6, 34.1, 32.0, 26.1; IR (CHCl₃ film) ν/cm⁻¹ 3663, 3588 (NH); [HRMS (EI) Found *M*⁺ 410.3048. C₂₅H₃₈N₄O requires *M*, 410.3045].



4-Oxopentanoic acid diethylamide (70) – Levulinic acid (2.0 g, 17.2 mmol) and diethylamine (1.95 mL, 18.9 mmol) was dissolved in dry dichloromethane (10 mL) under nitrogen and the solution cooled to 0 °C. 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC.HCl) (3.62 g, 18.9 mmol) and *N,N*-

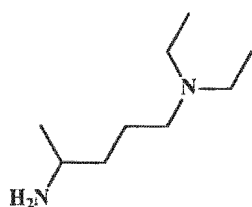
diisopropylethylamine (3.29 mL, 18.9 mmol) was added and the solution stirred at 0 °C for 1 hour. The reaction mixture was allowed to warm to room temperature and stirred for 18 hours. The solution was diluted with CH₂Cl₂ (150 mL), transferred to a separating funnel and washed with water (2 x 100 mL), saturated sodium hydrogen carbonate (100 mL), 10 % potassium hydrogen sulphate (100 mL) and brine (100 mL). The organic phase was dried over MgSO₄, filtered and concentrated to give 1.76 g (60 %) of the amide as yellow oil. The compound was sufficiently pure to be used in the next step: *R*_f 0.55 (5 % MeOH/CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 3.34 (4H, quintet, CON(CH₂CH₃)₂), 2.78 (2H, t, *J* = 6.8 Hz, -CH₂CH₂CON), 2.58 (2H, t, *J* = 6.4 Hz, -CH₂CH₂CON), 2.20 (3H, s, CH₃COC-), 1.19 (3H, t, *J* = 7.1 Hz, CONCH₂CH₃), 1.09 (3H, t, *J* = 7.1 Hz, CONCH₂CH₃); ¹³C NMR (100 MHz, CDCl₃) δ

208.0, 170.6, 41.8, 40.3, 38.2, 30.1, 27.0, 14.1, 13.0; IR (CHCl₃ film) ν/cm^{-1} 1710 (C=O of amide), 1624 (C=O of ketone); [HRMS (EI) Found M^+ 171.1245. C₉H₁₇NO₂ requires M , 177.1259].



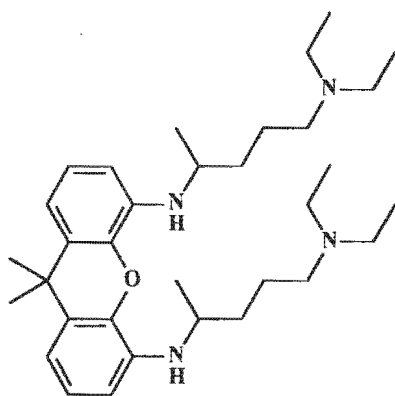
4-Hydroxyimino pentanoic acid diethylamide (71) – Ketone **70** (3.15 g, 18.42 mmol) and hydroxylamine hydrochloride (2.05 g, 29.47 mmol) was combined in denatured ethanol (30 mL).⁹⁶ Sodium hydroxide (2.43 g, 60.80 mmol) dissolved in 15 mL water was added drop wise to the ethanol solution with stirring. The solution was refluxed with stirring for 2 hours and quenched with water (40 mL).

The resulting solution was neutralized by treatment with CO₂ gas and extracted with dichloromethane (2 x 30 mL). The combined organic fractions were dried over Na₂SO₄, filtered and concentrated. The compound was purified by column chromatography (SiO₂, 5 % MeOH/CH₂Cl₂) to give 3.16 g (92 %) of oxime **71** as a clear oil: R_f 0.5 (5 % MeOH/ CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 8.62 (1H, broad s, OH), 4.13 (4H, q, J = 7.2 Hz, -N(CH₂CH₃)₂), 2.52 (2H, m, -CH₂-CON-), 1.88 (2H, m, -CH₂-C-CON-), 1.86 (3H, s, CH₃-C=N-), 1.24 (6H, t, J = 6.8 Hz, -N(CH₂CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ 172.7, 156.5, 44.7, 30.9, 24.2, 14.1, 13.8; IR (CHCl₃ film) ν/cm^{-1} 3665, 3589, 3294 (OH), 1728 (C=O); [HRMS (EI) Found M^+ 186.1373. C₉H₁₈N₂O₂ requires M , 186.1368].



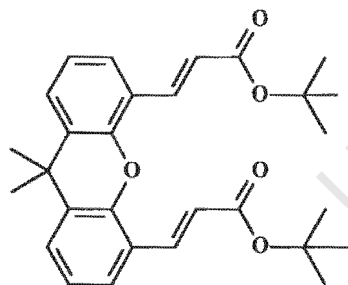
N',N'-Diethyl pentane-1,4-diamine (72) – Lithium aluminium hydride (0.31 g, 8.06 mmol) was combined with 5 mL dry tetrahydrofuran under nitrogen and cooled to 0 °C.⁹⁶ Oxime-amide **71** (0.5 g, 2.67 mmol) was dissolved in 3 mL dry tetrahydrofuran and added slowly to the lithium aluminium hydride slurry. The

solution was warmed to ambient temperature and heated under reflux for 6 hours. After cooling, the reaction is quenched by adding excess diethyl ether. The solution is transferred to a separating funnel and washed with brine (20 mL). The organic layer was dried over K₂CO₃ and evaporated under reduced pressure to give 0.28 g (67 %) of the amine **72** as pale yellow oil: R_f 0.60 (5 % NH₃(aq) – MeOH); ¹H NMR (300 MHz, CDCl₃) δ 2.86 (1H, sextet, J = 6.2 Hz, -CH), 2.49 (4H, q, J = 7.1 Hz, -N(CH₂CH₃)₂), 2.37 (2H, t, J = 7.3 Hz, -CH₂NEt₂), 1.44 (2H, q, J = 7.1 Hz, -CH-CH₂-), 1.60 (2H, broad s, NH₂ – disappears in D₂O), 1.28 (2H, quintet, J = 7.7 Hz, -C-CH₂-C-), 1.00 (9H, m, CH₃-CH- + -N(CH₂CH₃)₂); ¹³C NMR (75 MHz, CDCl₃) δ 53.0, 52.5, 46.9, 38.1, 30.7, 23.9, 11.5; IR (CHCl₃ film) ν/cm^{-1} 3406 (NH stretch); [HRMS (EI) Found M^+ 158.1791. C₉H₂₂N₂ requires M , 158.1783].



***N,N*-Bis (4-diethylamino-1-methylbutyl)-9,9-dimethyl-9*H*-xanthene-4,5-diamine (39).** – Iodide **57** (1.0 g, 2.17 mmol), amine **72** (0.86 g, 5.43 mmol), sodium *tert*-butoxide (0.58 g, 6.08 mmol), tris(dibenzylideneacetone) dipalladium (0) (Pd₂dba₃) (39.7 mg, 2 mol %) and (R)-(+)-2,2'-bis(diphenylphosphino)1,1'-binaphthyl (BINAP) (81.1 mg, 6 mol %) was combined in dry toluene (5 mL) under nitrogen. The solution is heated to 100 °C and stirred for 6 hours. After cooling to ambient temperature, the reaction mixture is diluted with

excess diethyl ether and filtered through Celite. The filtrate is concentrated and purified on silica gel chromatography eluting with 20 % MeOH/CH₂Cl₂ then switching to 5 % NH₃(aq)/MeOH to give 0.68 g (60 %) of the bis-amine **39** as a dark brown oil: *R_f* 0.45 (5 % NH₃(aq) – MeOH); ¹H NMR (300 MHz, CDCl₃) δ 6.93 (2H, t, *J* = 6.0 Hz, aromatic), 6.72 (2H, dd, *J* = 1.8 and 6.0 Hz, aromatic), 6.54 (2H, dd, *J* = 1.8 and 6.0 Hz, aromatic), 3.57 (2H, sextet, *J* = 5.8 Hz, -CH), 3.03 (12 H, m, -CH₂N(CH₂CH₃)₃), 1.69 – 1.92 (8H, m, -CHCH₂CH₂-C), 1.54 (6H, s, 9-(CH₃)₂), 1.26 (18H, m, CH₃-CH- + -N(CH₂CH₃)₂); ¹³C NMR (75 MHz, CDCl₃) δ 139.1, 135.8, 130.9, 123.4, 113.2, 109.5, 51.6, 50.6, 48.6, 46.5, 34.7, 30.9, 30.3, 21.2, 8.4; IR (CHCl₃ film) ν /cm⁻¹ 3650, 3567 (NH stretching); [HRMS (EI) Found *M*⁺ 522.4287. C₃₃H₅₄N₄O requires *M*, 522.4297].

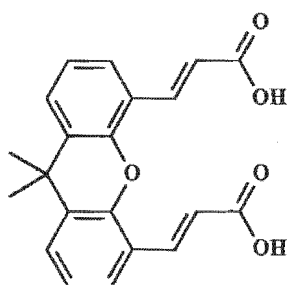


3-[5-(2-*tert*-Butoxycarbonylvinyl)-9,9-dimethyl-9*H*-xanthen-4-yl]

acrylic acid *tert* butyl ester (55) – Iodide **57** (4.0 g, 8.66 mmol), palladium acetate (0.2 g, 10 mol %) and triphenylphosphine (0.46 g, 20 mol %) was combined in 75 mL anhydrous DMF in a dry 250 mL round bottom flask.⁸³ *tert*-Butyl acrylate (12.3 g, 14.0 mL, 96.0 mmol) and 2.5 mL triethylamine was added. The reaction mixture is heated to 75 °C and

stirred for 12 hours. After cooling to ambient temperature, the reaction is quenched by adding 100 mL distilled water followed by 100 mL diethyl ether. The mixture was filtered through Celite and flushed with diethyl ether. The filtrate was concentrated and purified by chromatography (SiO₂, 10 % EtOAc/Hexane) to give 3.75 g (94 %) of the ester as a cream coloured solid: mp 147-149 °C; *R_f* 0.38 (30 % EtOAc/Hexane); ¹H NMR (300 MHz, CDCl₃) δ 8.19 (2H, d, *J* = 16.0 Hz, -CH=C-), 7.47 (4H, m, aromatic), 7.10 (2H, t, *J* = 7.2 Hz, aromatic), 6.43 (2H, d, *J* = 16.0 Hz, -C=CH), 1.62 (6H, s, 9-(CH₃)₂), 1.58 (18H, s, -C(CH₃)₃); ¹³C NMR (75 MHz, CDCl₃) δ 165.8, 148.4, 137.0, 130.9, 127.3, 125.4, 123.4,

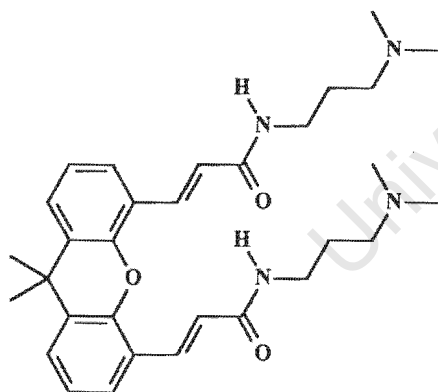
123.3, 122.1, 80.4, 34.3, 31.7, 28.1; IR (CHCl₃ film) ν/cm^{-1} 2988 (olefenic CH), 1691 (C=O), 1639 (C=C); [HRMS (EI) Found M^+ 462.2415. C₂₉H₃₄O₅ requires M , 462.2406].



3-[5-(2-Carboxyvinyl)-9,9-dimethyl-9H-xanthen-4-yl] acrylic acid (56) –

Ester **55** was dissolved in 2 mL dichloromethane and cooled to 0 °C. Trifluoroacetic acid (1 mL) was added slowly to the cooled solution and stirred at 0 °C for 20 minutes. Thereafter, the reaction is allowed to warm to room temperature and stirred for 2 hours. The product precipitates and the solvent is removed by evaporation. The resulting white powder was subjected to high vacuum to

remove excess TFA to give 0.3 g (99 %) of the acid that did not dissolve in most organic solvents: mp 312-314 °C; R_f 0.72 (5 % NH₃(aq) – MeOH); ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.07 (2H, d, J = 16.2 Hz, -CH=C-), 7.71 (2H, dd, J = 1.2 and 7.8 Hz, aromatic), 7.64 (2H, dd, J = 1.5 and 7.8 Hz, aromatic), 7.20 (2H, t, J = 7.8 Hz, aromatic), 6.58 (2H, d, J = 15.9 Hz, -C=CH-), 1.60 (6H, s, 9-(CH₃)₂); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 167.2, 147.5, 136.7, 130.7, 128.4, 125.4, 123.8, 122.2, 121.2, 33.9, 31.8; IR (nujol mull) ν/cm^{-1} 3862, 3748, 3669 (OH), 2928 (olefenic CH), 1676 (C=O); [HRMS (EI) Found M^+ 350.1156. C₂₁H₁₈O₅ requires M , 350.1154].

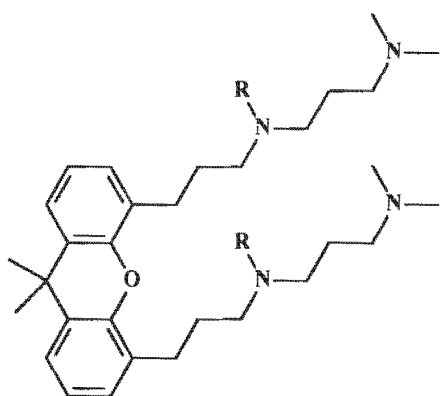


N-(3-Dimethylaminopropyl)-3-{5-[2-(3-dimethylamino propylcarbamoyl) vinyl]-9,9-dimethyl-9H-xanthen-4-yl}

acrylamide (36) – Acrylic acid **56** (0.2 g, 0.57 mmol) was suspended in dry CH₂Cl₂ (5 mL) under nitrogen. Oxalyl chloride (0.30 mL, 3.42 mmol) and a catalytic amount of *N,N*-dimethylformamide were added. The solution is refluxed while stirring for 3 hours. After cooling to room temperature, the solvent is removed by evaporation and the yellow residue re-dissolved in

dry dichloromethane (10 mL) and cooled to 0 °C. 3-Dimethylamino-1-propylamine (0.36 mL, 2.85 mmol) was added slowly while stirring. The reaction is warmed to room temperature for 1 hour and the solvent evaporated. Purification by silica gel chromatography, eluting with 20 % MeOH - CH₂Cl₂ then switching to 5 % NH₃(aq) – MeOH, gave 0.22 g (75 %) of the amide as yellow oil: R_f 0.34 (5 % NH₃(aq) – MeOH); ¹H NMR (400 MHz, CDCl₃) δ 7.99 (2H, d, J = 15.6 Hz, -CH=C-), 7.96 (2H, s, -CO-NH-), 7.39 (4H, td, J = 1.6, 7.6 and 16.8 Hz, aromatic), 7.08 (2H, t, J = 7.6 Hz, aromatic), 6.73 (2H, d, J = 15.6 Hz, -C=CH-), 3.47 (4H, q, J = 6.8 Hz, -CO-N-CH₂-), 2.41 (4H, t, J = 7.2 Hz, -CH₂NMe₂),

2.24 (12H, s, -N(CH₃)₂), 1.79 (4H, quintet, $J = 6.8$ Hz, -C-CH₂-C-), 1.61 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ 166.3, 149.0, 136.6, 130.5, 128.9, 127.2, 123.7, 123.3, 123.2, 58.2, 45.4, 39.1, 34.0, 32.5, 26.7; IR (CHCl₃ film) ν /cm⁻¹ 3391 (NH), 2976 (olefinic CH), 1647 (C=O); [HRMS (EI) Found M⁺ 518.3278. C₃₁H₄₂N₄O₃ requires M , 518.3256].



Preparation of derivatives based on polyamine **35**:

Arylsulphonyl chloride and aryl isocyanate (0.63 mmol) were added to a solution of amine **35** (0.15 g, 0.30 mmol) in 2 mL dry dichloromethane at room temperature. For sulphonyl chlorides, polymer supported morpholine (0.19 g, 0.66 mmol) was added. The reaction mixture was shaken at room temperature for 5 hours (ureas) and 24 hours (sulphonamides). The solutions containing polymers were filtered through a sintered funnel and polymer-supported tris(2-aminoethyl) amine (1.2 mmol) added to the filtrate. The suspensions were shaken at room temperature for a further 12 hours and polymers removed by filtration. The compounds were purified by silica gel chromatography, eluting with 10% MeOH in CH₂Cl₂. For the urea products, a second elution of 5 % NH₃(aq) – MeOH was required. Yields ranged from 50 to 83 %.

Sulphonyl chlorides and isocyanates used were toluene sulphonyl chloride, naphthalenesulphonyl chloride, *p*-tolyl isocyanate, benzyl isocyanate, 4-chlorophenyl isocyanate and 4-fluorophenyl isocyanate.

N-(3-Dimethylaminopropyl)-*N*-[3-(5-{3-[(3-dimethylaminopropyl)-4-methyl

benzenesulphonylamino] propyl}-9,9-dimethyl-9*H*-xanthen-4-yl) propyl]-4-methyl

benzenesulphonamide (**61**) – R_f 0.15 (10 % MeOH – CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.71 (4H, dd, $J = 2.0$ and 8.4 Hz, aromatic), 7.66 (4H, d, $J = 8.4$ Hz, aromatic), 7.51 (6H, m, aromatic), 3.04 (12H, m, -CH₂-N-CH₂- and -CH₂NMe₂), 2.76 (4H, m, Ar-CH₂-), 2.73 (12H, s, -N(CH₃)₂), 2.68 (6H, s, CH₃ on toluene rings), 2.06 (4H, quintet, $J = 8.4$ Hz, Ar-C-CH₂-C-), 1.92 (4H, m, -N-C-CH₂-C-), 1.61 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ 148.3, 143.6, 135.8, 130.2, 129.9, 128.2, 127.2, 125.9, 124.1, 122.9, 55.9, 49.0, 46.1, 43.8, 43.5, 40.6, 32.2, 28.6, 24.8, 21.5; IR (CHCl₃ film) ν /cm⁻¹ 1172 (SO₂-N); [MS Found M⁺ 803.4 C₄₅H₆₂N₄O₅S₂ requires M , 803.1].

***N*-(3-Dimethylaminopropyl)-*N*-[3-(5-{3-[(3-dimethylaminopropyl)-2-naphthalenesulphonylamino]-propyl}-9,9-dimethyl-9*H*-xanthen-4-yl) propyl]-2-naphthalenesulphonamide (62)** – R_f 0.20 (10 % MeOH – CH₂Cl₂) ¹H NMR (300 MHz, CDCl₃) δ 8.34 (2H, s, aromatic), 7.86 (10H, m, aromatic), 7.59 (6H, m, aromatic), 7.26 (2H, m, aromatic), 3.13 (8H, m, -CH₂-N-CH₂-), 2.76 (20 H, m, Ar-CH₂- and -CH₂N(CH₃)₂), 2.16 (4H, quintet, J = 6.9 Hz, Ar-C-CH₂-C-), 1.95 (4H, quintet, J = 7.5 Hz, -N-C-CH₂-C-), 1.59 (6H, s, 9-(CH₃)₂); ¹³C NMR (75 MHz, CDCl₃) δ 148.2, 135.6, 134.7, 132.2, 130.1, 129.3, 128.2, 128.0, 127.8, 127.1, 124.0, 122.9, 122.4, 55.7, 49.0, 46.0, 43.2, 40.0, 32.1, 28.6, 27.2, 24.6; [MS Found M^+ 875.3 C₅₁H₆₂N₄O₅S₂ requires M , 875.2].

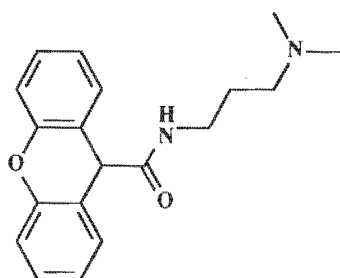
3-Benzyl-1-[3-(5-{3-[3-benzyl-1-(3-dimethylaminopropyl) ureido]-propyl}-9,9-dimethyl-9*H*-xanthen-4-yl)-propyl-1-(3-dimethylaminopropyl) urea (63) – R_f 0.39 (8:2:0.1 MeOH:CH₂Cl₂:Et₃N); ¹H NMR (400 MHz, CDCl₃) δ 7.17 – 7.28 (12H, m, aromatic), 7.05 (2H, dd, J = 1.6 and 7.3 Hz, 3-H and 6-H), 7.00 (2H, t, J = 7.5 Hz, 2-H and 7-H), 6.48 (2H, broad s, CONH), 4.32 (4H, s, CONHCH₂Ph), 3.37 – 3.44 (8H, m, -CH₂-N-CH₂-), 2.77 – 2.86 (8H, m, Ar-CH₂- and -CH₂-NMe₂), 2.53 (12H, s, -N(CH₃)₂), 1.96 – 1.99 (8H, m, -CH₂-C-N-C-CH₂-), 1.59 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ 158.54, 148.26, 140.33, 130.15, 128.70, 128.35, 127.40, 126.80, 123.92, 122.84, 55.30, 53.39, 46.52, 44.70, 43.21, 34.30, 32.15, 28.70, 27.32, 24.27; [HRMS (EI) Found M^+ 760.5030. C₄₇H₆₄N₆O₃ requires M , 760.5036].

1-(3-Dimethylaminopropyl)-1-[3-(5-{3-[1-(3-dimethylaminopropyl)-3-(4-methylbenzene)-ureido]-propyl}-9,9-dimethyl-9*H*-xanthen-4-yl)-propyl]-3-(4-methylbenzene) urea (64) - R_f 0.52 (8:2:0.1 MeOH:CH₂Cl₂:Et₃N); ¹H NMR (400 MHz, CDCl₃) δ 7.82 (2H, broad s, CONH), 7.29 (2H, dd, J = 1.6 and 7.6 Hz, 1-H and 8-H), 7.22 (4H, d, J = 8.0 Hz, Ar-H on toluene rings), 7.09 (2H, dd, J = 2.0 and 7.6 Hz, 3-H and 6-H), 6.99 – 7.05 (6H, m, 2-H, 7-H and Ar-H on toluene rings), 3.44 – 3.51 (8H, m, CH₂NCH₂), 2.82 – 2.91 (8H, m, Ar-CH₂ and CH₂-NMe₂), 2.61 (12H, s, N(CH₃)₂), 2.25 (6H, s, CH₃ on toluene rings), 1.97 – 2.04 (8H, m, -CH₂-C-N-CH₂-), 1.60 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ 156.35, 148.26, 137.06, 132.16, 130.21, 129.09, 128.64, 127.49, 124.02, 122.93, 120.65, 55.33, 46.75, 43.95, 43.40, 32.19, 30.87, 28.66, 27.29, 24.13, 20.69; [HRMS (EI) Found M^+ 760.5042. C₄₇H₆₄N₆O₃ requires M , 760.5036].

1-(3-Dimethylaminopropyl)-1-[3-(5-{3-[1-(3-dimethylaminopropyl)-3-(4-chlorophenyl)-ureido]-propyl}-9,9-dimethyl-9H-xanthen-4-yl)-propyl]-3-(4-chlorophenyl) urea (65) – R_f 0.63 (8:2:0.1 MeOH:CH₂Cl₂:Et₃N); ¹H NMR (400 MHz, CDCl₃) δ 8.22 (2H, broad s, CONH), 7.33 (4H, d, J = 8.8 Hz, Ar-H on chlorophenyl ring), 7.29 (2H, dd, J = 1.6 and 7.7 Hz, 1-H and 8-H), 7.14 (4H, d, J = 8.8 Hz, Ar-H on chlorophenyl ring), 7.09 (2H, dd, J = 1.6 and 7.3 Hz, 3-H and 6-H), 7.03 (2H, t, J = 7.5 Hz, 2-H and 7-H), 3.46 – 3.53 (8H, m, -CH₂-N-CH₂-), 2.81 – 2.88 (8H, m, Ar-CH₂- and CH₂-NMe₂), 2.63 (12H, s, N(CH₃)₂), 1.98 – 2.03 (8H, m, -CH₂-C-N-C-CH₂-), 1.60 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ 156.12, 148.23, 138.56, 130.21, 128.60, 128.45, 127.52, 124.06, 122.94, 121.62, 55.29, 46.77, 43.92, 43.54, 34.31, 32.21, 28.64, 27.35, 24.10; IR (CHCl₃ film) ν /cm⁻¹ 3392 (NH), 1652 (C=O); [HRMS (EI) Found M^+ 800.3933. C₄₅H₅₈N₆O₃Cl₂ requires M , 800.3944].

1-(3-Dimethylaminopropyl)-1-[3-(5-{3-[1-(3-dimethylaminopropyl)-3-(4-fluorophenyl)-ureido]-propyl}-9,9-dimethyl-9H-xanthen-4-yl)-propyl]-3-(4-fluorophenyl) urea (66) – R_f 0.54 (8:2:0.1 MeOH:CH₂Cl₂:Et₃N); ¹H NMR (400 MHz, CDCl₃) δ 7.83 (2H, s, CONH), 7.32 – 7.36 (4H, m, Ar-H on fluorophenyl rings), 7.28 (2H, dd, J = 1.6 Hz and 7.7 Hz, 1-H and 8-H), 7.08 (2H, dd, J = 1.6 and 7.3 Hz, 3-H and 6-H), 7.02 (2H, t, J = 7.5 Hz, 2-H and 7-H), 6.88 (4H, t, J = 8.8 Hz, Ar-H on fluorophenyl rings), 3.46 – 3.54 (8H, m, CH₂-N-CH₂), 3.00 (4H, t, J = 7.0 Hz, Ar-CH₂-C), 2.82 (4H, t, J = 7.3 Hz, CH₂N(CH₃)₂), 2.70 (12H, s, N(CH₃)₂), 1.98 – 2.03 (8H, m, -CH₂-C-N-C-CH₂-), 1.58 (6H, s, 9-(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃) δ 159.86, 157.46, 156.17, 148.21, 135.66, 130.21, 128.58, 127.50, 124.08, 122.72, 114.98, 55.54, 46.86, 43.92, 43.26, 34.30, 32.19, 28.71, 27.25, 23.86; [HRMS (EI) Found M^+ 768.4546. C₄₅H₅₈F₂N₆O₃ requires M , 768.4535].

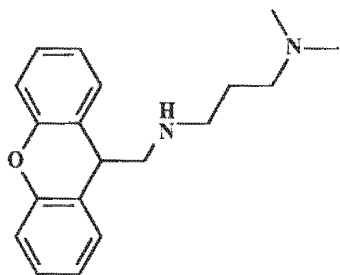
6.3 PROCEDURES FOR 9-MONOSUBSTITUTED XANTHENE ANALOGUES



9H-Xanthene-9-carboxylic acid 3-(dimethylamino) propylamide (73) -

Oxalyl chloride (2.32 cm³, 26.55 mmol) and a catalytic amount of *N,N*-dimethylformamide were added to a solution of xanthene-9-carboxylic acid (2.0 g, 8.85 mmol) in dry CH₂Cl₂ (50 cm³). After 3 hours of reflux, the solution was concentrated under reduced pressure. The residue was redissolved in dry dichloromethane and cooled to 0°C. 3-Dimethylamino-1-propylamine (1.33 cm³, 10.62 mmol) was added drop wise and the reaction mixture stirred at room temperature for 1 hour. Subsequent column chromatography (SiO₂, 10 % NH₃(aq) - MeOH) gave 2.68 g

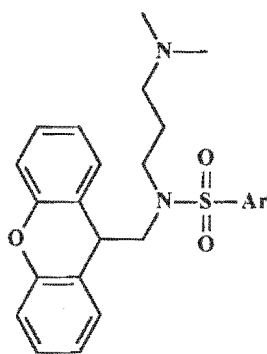
(98 %) of amide **73** as a white solid: mp 190-194 °C; R_f 0.53 (5 % $\text{NH}_3(\text{aq})$ - MeOH); ^1H NMR (400 MHz; $\text{DMSO}-d_6$) δ 8.70 (1H, t, $J = 5.7$ Hz, -CO-NH), 7.28 (4H, m, aromatic), 7.08 (4H, m, aromatic), 4.97 (1H, s, 9-H), 3.07 (2H, q, $J = 6.6$ Hz, -CONH- CH_2 -C), 2.66 (2H, t, $J = 7.5$ Hz, -C- CH_2 -N(CH_3) $_2$), 2.44 (6H, s, -C-N(CH_3) $_2$), 1.68 (2H, quintet, $J = 7.78$ Hz, -C- CH_2 -C); ^{13}C NMR (100 MHz; $\text{DMSO}-d_6$) δ 171.89, 151.25, 129.03, 128.94, 123.65, 120.66, 116.72, 55.56, 45.35, 43.50, 36.77, 25.43; IR (CHCl_3 film) ν/cm^{-1} 3418 (NH), 1635 (C=O); [HRMS (FAB) Found M^+ 310.1670. $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_2$ requires M , 310.1679].



***N,N*-Dimethyl-*N'*-(9*H*-xanthen-9-ylmethyl)-propane-1,3-diamine (**74**) –**

Borane tetrahydrofuran complex (22.0 cm^3 of a 1M solution in THF, 22.0 mmol) was added to a solution of the amide **73** (2.73 g, 8.81 mmol) suspended in tetrahydrofuran at 0°C.⁹⁷ The solution was stirred at 0°C for 15 minutes and then refluxed for 18 hours. After cooling to room temperature, the reaction was quenched by addition of 3M hydrochloric

acid (40 cm^3). The apparatus was rearranged for distillation and tetrahydrofuran distilled off with hydrogen evolution from the borane-amine complex and the remaining aqueous solution cooled to 0°C. Sodium hydroxide pellets were added to this cooled solution until a pH of 6-7 was reached. The solution was then extracted with diethyl ether and the organic phase dried over sodium sulphate which yielded 1.71 g (66 %) of amine as a yellow oil after column chromatography (SiO_2 , 10 % $\text{NH}_3(\text{aq})$ - MeOH); R_f 0.51 (10% $\text{NH}_3(\text{aq})$ - MeOH); ^1H NMR (400 MHz; CDCl_3) δ 7.23 (4H, m, aromatic), 7.07 (4H, m, aromatic), 4.07 (1H, t, $J = 6.7$ Hz, 9-H), 2.77 (2H, d, $J = 6.9$ Hz, -C- CH_2 -NH), 2.58 (2H, t, $J = 6.9$ Hz, -NH- CH_2 -C), 2.19 (2H, t, $J = 7.08$ Hz, -C- CH_2 -N(CH_3) $_2$), 2.12 (6H, s, -N(CH_3) $_2$), 1.84 (1H, s, NH), 1.55 (2H, quintet, $J = 7.29$ Hz, NH-C- CH_2 -C-), ^{13}C NMR (100 MHz; CDCl_3) δ 152.3, 128.7, 127.8, 124.1, 123.2, 116.5, 58.2, 57.9, 47.9, 45.4, 39.6, 27.6; IR (CHCl_3 film) ν/cm^{-1} 3410 (NH); [HRMS (EI) Found M^+ 296.1880. $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}$ requires M , 296.1889].



Preparation of derivatives 75. Aryl sulphonyl chloride (0.384 mmol) was added to a suspension of amine **74** (0.1 g, 0.32 mmol) and polymer supported morpholine (0.14 g, 0.48 mmol) in 2 mL dry dichloromethane at room temperature. The reaction mixture was shaken at room temperature for 18 hours. The solutions were filtered through a sintered funnel and the filtrate concentrated. The compounds were purified by silica gel chromatography, eluting with 10% MeOH in CH_2Cl_2 .

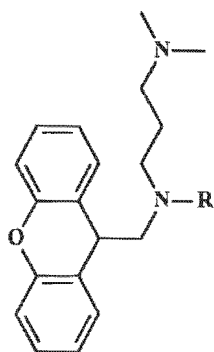
Aryl sulphonyl chlorides used were benzene sulphonyl chloride, toluene sulphonyl chloride and 2-naphthalene sulphonyl chloride.

***N*-(3-Dimethylaminopropyl)-*N*-(9*H*-xanthen-9-ylmethyl)-benzenesulphonamide (75a)** – R_f 0.23 (10 % MeOH - CH_2Cl_2); ^1H NMR (CDCl_3 , 300 MHz) δ 7.82 (2H, m, aromatic), 7.51 (3H, m, aromatic), 7.36 (2H, m, aromatic), 7.24 (2H, m, aromatic), 7.09 (4H, m, aromatic), 4.50 (1H, t, $J = 7.8$ Hz, 9-H), 3.10 (2H, d, $J = 7.8$ Hz, $-\text{CH}_2\text{-N-}$), 2.84 (2H, t, $J = 7.5$ Hz, $-\text{N-CH}_2\text{-C-}$), 2.14 (6H, s, $\text{N}(\text{CH}_3)_2$), 2.05 (2H, t, $J = 6.9$ Hz, $-\text{CH}_2\text{-NMe}_2$), 1.14 (2H, quintet, $J = 7.5$ Hz, $-\text{C-CH}_2\text{-C-}$); ^{13}C NMR (75 MHz, CDCl_3) δ 152.4, 138.5, 132.6, 129.6, 129.1, 128.2, 128.1, 127.3, 123.3, 116.5, 56.4, 50.7, 48.9, 44.6, 40.2, 24.9; IR (CHCl_3 film) ν/cm^{-1} 1182 ($\text{SO}_2\text{-N}$); [HRMS (EI) Found M^+ 436.1813. $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_3\text{S}$ requires M , 436.1820].

***N*-(3-Dimethylaminopropyl)-4-methyl-*N*-(9*H*-xanthen-9-ylmethyl)-benzenesulphonamide (75b)** – R_f 0.35 (10 % MeOH - CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3) δ 7.08 – 7.72 (12H, m, aromatic), 4.53 (1H, t, $J = 7.71$ Hz, 9-H), 3.03 (2H, d, $J = 7.71$ Hz, $-\text{CH}_2\text{-N}$), 2.84 (2H, t, $J = 7.17$ Hz, $\text{N-CH}_2\text{-C}$), 2.41 (3H, s, PhMe), 2.28 (6H, s, NMe_2), 2.21 (2H, t, $J = 7.18$ Hz, CH_2NMe_2), 1.21 (2H, q, $J = 7.18$ Hz, $\text{C-CH}_2\text{-C}$); ^{13}C NMR (CDCl_3 , 100 MHz) δ 152.4, 143.7, 134.9, 130.0, 129.8, 128.7, 127.5, 125.8, 123.4, 116.6, 57.1, 56.0, 49.1, 44.2, 40.2, 24.4, 21.5; [HRMS (EI) Found M^+ 450.1986. $\text{C}_{26}\text{H}_{30}\text{N}_2\text{O}_3\text{S}$ requires M , 450.1977].

Naphthalene-2-sulphonic acid (3-dimethylaminopropyl)-(9*H*-xanthen-9-ylmethyl)-amide (75c) – R_f 0.38 (10 % MeOH - CH_2Cl_2); ^1H NMR (CDCl_3 , 400 MHz) δ 7.07 – 8.40 (15H, m, aromatic), 4.54 (1H, t, $J = 7.44$ Hz, 9-H), 3.18 (2H, d, $J = 7.71$ Hz, $-\text{CH}_2\text{-N}$), 2.91 (2H, t, $J = 6.91$ Hz, $\text{N-CH}_2\text{-C}$), 2.09 (6H, s, NMe_2), 2.03 (2H, t, $J = 7.18$ Hz, CH_2NMe_2), 1.18 (2H, q, $J = 7.45$ Hz, $\text{C-CH}_2\text{-C}$); ^{13}C NMR (CDCl_3 ,

100 MHz) δ 152.5, 135.6, 134.8, 132.2, 130.0, 123.2-129.8 (m), 122.6, 116.6, 56.6, 56.2, 48.2, 44.7, 40.1, 25.0; [HRMS (EI) Found M^+ 486.1963. $C_{29}H_{30}N_2O_3S$ requires M , 486.1977].



General procedure for synthesis of compounds 75 - 77. – Polymer supported morpholine (Aldrich 2.5 mmol/g, 0.14 g, 0.34 mmol) was added to amine **74** (0.30 mmol) in a VARIAN Bond Elute tube followed by 0.9 mmol of the building blocks (PS-Morpholine was not added to isocyanates). Dry dichloromethane (2 mL) was added and the suspension shaken at room temperature for 2 hours. Macroporous *p*-toluene sulphonic acid (MP-TsOH) (Argonaut 1.4 mmol/g, 0.65 g, 0.90 mmol) and an additional 2 mL dichloromethane was added. The suspension is then shaken at room temperature for 4 hours and filtered. The polymer residue is washed with small amounts of CH_2Cl_2 and 2M ammonia in methanol (2 mL) added. The polymer is shaken in this solution for 15 hours. The methanolic solution is removed and the polymer washed with small amounts of methanol. The filtrate is concentrated under reduced pressure to give the products. Yields were between 63-96%. Pure compounds were obtained by preparative TLC (SiO_2) using 10 % MeOH/ CH_2Cl_2 as tank solvent. Compounds were extracted from the silica by sonication in a 4:1 MeOH/ CH_2Cl_2 solution for 15 minutes.

Building blocks used were benzene sulphonyl chloride, 4-chlorobenzenesulphonyl chloride, benzoyl chloride, 4-chlorobenzoyl chloride, phenyl isocyanate, 4-chlorophenyl isocyanate.

4-Chloro-*N*-(3-dimethylaminopropyl)-*N*-(9*H*-xanthen-9-ylmethyl)-benzenesulphonamide (75d) – R_f 0.23 (10 % MeOH - CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (2H, m, aromatic), 7.44 (2H, m, aromatic), 7.33 (2H, m, aromatic), 7.26 (2H, m, aromatic), 7.09 (4H, m, aromatic), 4.45 (1H, t, J = 7.6 Hz, 9-H), 3.14 (2H, d, 7.6 Hz, $-CH_2-N-S-$), 2.86 (2H, t, J = 7.6 Hz, $-N-CH_2-C-$), 2.06 (6H, s, $-N(CH_3)_2$), 1.60 (2H, t, J = 7.2 Hz, $-CH_2-NMe_2$), 1.15 (2H, quintet, J = 8.0 Hz, $-C-CH_2-C-$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.6, 139.3, 137.7, 129.7, 129.5, 128.9, 128.5, 123.6, 123.2, 116.7, 56.6, 56.4, 48.8, 45.2, 40.4, 25.5; [HRMS (EI) Found M^+ 470.1419. $C_{25}H_{27}N_2O_3SCl$ requires M , 470.1430].

***N*-(3-Dimethylaminopropyl)-*N*-(9*H*-xanthen-9-ylmethyl)-benzamide (76a) – R_f 0.17 (10 % MeOH - CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.11 (13H, m, aromatic), 4.69 (1H, m, 9-H), 3.42 (2H, d, J = 7.6 Hz, $-CH_2-N-CO-$), 2.78 (2H, m, $-CO-N-CH_2-$), 2.43 (2H, m, $-CH_2NMe_2$), 2.31 (6H, s, $-N(CH_3)_2$), 1.26 (2H, m, $-C-CH_2-C-$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 152.4, 136.5, 129.5, 128.6, 128.5,**

128.4, 126.8, 126.4, 123.6, 116.7, 56.4, 54.4, 45.1, 43.6, 37.4, 26.4; [HRMS (EI) Found M^+ 400.2146. $C_{26}H_{28}N_2O_2$ requires M , 400.2151].

4-Chloro-*N*-(3-dimethylaminopropyl)-*N*-(9*H*-xanthen-9-ylmethyl)-benzamide (76b) – R_f 0.17 (10 % MeOH - CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.12 (12H, m, aromatic), 4.64 (1H, m, 9-H), 3.45 (2H, m, $-CH_2N-CO-$), 2.75 (4H, m, $-CO-N-CH_2-$ + $-CH_2NMe_2$), 2.55 (6H, s, $-N(CH_3)_2$), 1.28 (2H, m, $-C-CH_2-C-$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 152.3, 134.6, 130.8, 128.9, 128.4, 128.0, 127.7, 123.5, 121.8, 116.7, 56.2, 54.0, 44.1, 42.9, 37.2, 25.6; IR ($CHCl_3$ film) ν/cm^{-1} 1625 (C=O); [HRMS (EI) Found M^+ 434.1761. $C_{26}H_{27}N_2O_2Cl$ requires M , 434.1761].

1-(3-Dimethylaminopropyl)-3-phenyl-1-(9*H*-xanthen-9-ylmethyl)-urea (77a) – R_f 0.27 (10 % MeOH - CH_2Cl_2); ^1H NMR (200 MHz, $CDCl_3$) δ 7.84 (1H, broad s, NH), 7.60 (9H, m, aromatic), 7.18 (4H, m, aromatic), 4.73 (1H, t, $J = 8.0$ Hz, 9-H), 3.24 (2H, d, $J = 7.7$ Hz, $-CH_2-N-CO$), 2.67 (2H, m, $-N-CH_2-C-$), 1.97 (2H, t, $J = 5.9$ Hz, $-CH_2-NMe_2$), 1.88 (6H, s, $-N(CH_3)_2$), 1.22 (2H, quintet, $J = 5.7$ Hz, $-C-CH_2-C-$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 152.3, 140.4, 133.1, 128.6, 128.5, 128.3, 126.8, 124.4, 123.2, 116.1, 56.1, 53.8, 45.7, 44.9, 38.1, 25.2; IR ($CHCl_3$ film) ν/cm^{-1} 3418, 3244 (NH), 1631 (C=O); [HRMS (EI) 429.2414. $C_{26}H_{29}N_3O_2$ requires M , 429.2416].

3-(4-Chlorophenyl)-1-(3-dimethylaminopropyl)-1-(9*H*-xanthen-9-ylmethyl)-urea (77b) – R_f 0.27 (10 % MeOH - CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 9.82 (1H, broad s, NH), 7.42 (2H, m, aromatic), 7.28 (6H, m, aromatic), 7.09 (4H, m, aromatic), 4.80 (1H, t, $J = 7.9$ Hz, 9-H), 3.26 (2H, d, $J = 8.1$ Hz, $-CH_2-N-CO$), 2.77 (2H, t, $J = 5.7$ Hz, $-N-CH_2-C-$), 2.17 (6H, s, $-N(CH_3)_2$), 2.07 (2H, t, $J = 6.0$ Hz, $-CH_2-NMe_2$), 1.33 (2H, quintet, $J = 5.7$ Hz, $-C-CH_2-C-$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6, 152.6, 139.8, 129.5, 128.9, 128.1, 126.8, 124.5, 123.6, 120.2, 116.5, 55.7, 53.7, 46.1, 44.8, 36.1, 24.9; [HRMS (EI) Found M^+ 449.1859. $C_{26}H_{28}N_3O_2Cl$ requires M , 449.1870].

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