



PHD

## Synthetic and biological studies of antiparasitic natural product derivatives

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# SYNTHETIC AND BIOLOGICAL STUDIES OF ANTIPARASITIC NATURAL PRODUCT DERIVATIVES

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A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department of Pharmacy and Pharmacology

September 2009



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## ABSTRACT:

Trypanosomiasis and Leishmaniasis are tropical diseases caused by the parasites *Trypanosoma* and *Leishmania*, that cause severe medical and economical problems for millions of people in the developing world. Trypanosomiasis can be divided into African and American trypanosomiasis, which are caused by *Trypanosoma brucei* and *Trypanosoma cruzi* respectively. There are more than 20 different species of *Leishmania* worldwide that cause Leishmaniasis, but the most severe infection, visceral leishmaniasis, is caused by *Leishmania donovani*. Both diseases are transmitted by blood sucking insects like the tsetse fly and the sand fly.

The majority of existing drugs for trypanosomiasis and leishmaniasis are either too toxic or have low efficacy, and in some cases parasites have also developed resistance. There is therefore a pressing need to develop new chemotherapeutic agents, and in this context, the enzyme trypanothione reductase (TryR) has emerged as an attractive validated target for drug design. The natural product cadabicine, extracted from the plant *Cadaba farinosa*, is a diphenyl ether-containing macrocyclic spermidine alkaloid which has been identified as a potential inhibitor of TryR by virtual screening.

In order to investigate the potential of cadabicine as a TryR inhibitor, an efficient synthetic route to the natural product was delivered. This work was focused on the preparation and combination of three key synthetic units, namely an orthogonally protected spermidine derivative and two functionalised cinnamic acid units. This approach lead to the formation of the macrocycle by an intramolecular nucleophilic aromatic substitution followed by a convenient conversion to the natural product. In the same manner cyclic and non-cyclic analogues of cadabicine were prepared, in order to examine the structure-activity relationship of these alkaloids to TryR.

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## ABBREVIATIONS:

Ac	acetyl
AcOH	acetic acid
Ad	adamantyl
Aq	aqueous
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Boc	<i>tert</i> -butoxycarbonyl
DBAD	dibenzyl azodicarboxylate
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM	dichloromethane
Dde	1-(4,4-dimethyl-2,6-dioxohexylidene)-ethyl
DEAD	diethylazodicarboxylate
DIBAL	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMAP	4-(dimethylaminopyridine)
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNs	2,4-dinitro sulfonyl
DPPF	1,1'-Bis(diphenylphosphino)ferrocene
D <sup>t</sup> BPF	1,1'-bis(di- <i>tert</i> -butylphosphino)ferrocene
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Eq	equivalent(s)
ESMS	electrospray mass spectrometry
Et <sub>2</sub> O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	9-fluorenylmethoxycarbonyl
Fig	figure
g	grams
GR	glutathione reductase



h	hour
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HTS	high throughput screening
Hz	hertz
IC <sub>50</sub>	concentration required for 50% inhibition of activity
IR	infra red
<i>J</i>	coupling constant
KHDMS	potassium bis(trimethylsilyl) amide
<i>K<sub>i</sub></i>	inhibition constant
LDA	lithium diisopropylamide
Lit	literature
M	molar
m. p.	melting point
<i>m/z</i>	mass to charge ratio (mass spectrometry)
Me	methyl
MeCN	acetonitrile
MeOH	methanol
Min	minutes
mL	millilitres
mmol	millimoles
mol	moles
MsCl	methanesulfonyl chloride
nM	nanomolar
NMP	N-methylpyrrolidone
NMR	nuclear magnetic resonance
Ns	2-nitro sulfonyl
OAll	<i>O</i> -allyl
OBn	<i>O</i> -benzyl
Ph	Phenyl
ppm	parts per million
<i>p</i> -TsCl	<i>para</i> -toluenesulfonyl chloride
R <sub>f</sub>	retention factor
RP	reverse phase

SAR	structure activity relationship
S <sub>N</sub> Ar	nucleophilic aromatic substitution
TBAF	tetrabutyl ammonium fluoride
TCBoc	2,2,2-Trichloro-tert-butyloxycarbonyl Group
Tert	tertiary
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMHD	2,2,6,6-tetramethyl-3,5-heptanedionate
Troc	2,2,2-trichloroethoxycarbonyl
TryR	trypanothione reductase
UV	ultraviolet
Z	benzyloxycarbonyl

## 1. INTRODUCTION

### 1.1 KINETOPLASTID PROTOZOAN INFECTIONS.

Trypanosomiasis and leishmaniasis are parasitic diseases caused by the parasites *Trypanosoma* and *Leishmania* which are single cell eukaryotes of the kinetoplastid order. After malaria, they are the major cause of death in the undeveloped World. Human trypanosomiasis can be split into African trypanosomiasis or sleeping sickness, caused primarily by *Trypanosoma brucei* and American trypanosomiasis or Chagas' disease caused by *T.cruzi*. Leishmaniasis can be caused by 20 different species of *Leishmania*, but the most severe infection known as visceral leishmaniasis is caused by *Leishmania donovani*. Both trypanosomiasis and leishmaniasis are transmitted via the bite of a blood sucking insect that transfers the parasites from human to human. Once inside the human hosts, the parasites multiply and invade most tissues. Table 1.1 shows recent estimates of the occurrence and mortality rates of trypanosomiasis and leishmaniasis.<sup>1</sup>

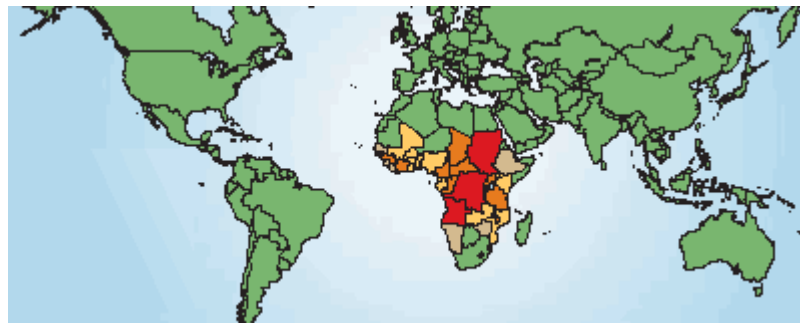
Disease	Cases Per year	Disease Burden DALYs (thousands)			Deaths (thousands) per year		
		Total	Male	Female	Total	Male	Female
African Trypanosomiasis	300-500 thousand	1,525	996	559	48	31	17
Chagas Disease	16-18 million	667	343	324	14	8	7
Leishmaniasis	500 thousand	2,090	1,24	840	51	30	21

**Table 1.1 : Number of cases of infections and deaths estimated by WHO tropical disease research. <sup>2</sup>**

#### 1.1.1 African trypanosomiasis

African trypanosomiasis can affect both humans and animals. Human African trypanosomiasis (HAT) is also known as sleeping sickness, and can be fatal if left untreated. In cattle it causes the infection known as nagana which restricts cattle

industry in many prime areas of Africa. The disease affects 36 countries in sub-Saharan Africa causing 50,000 deaths annually, with the increasing risk of major epidemics forming.



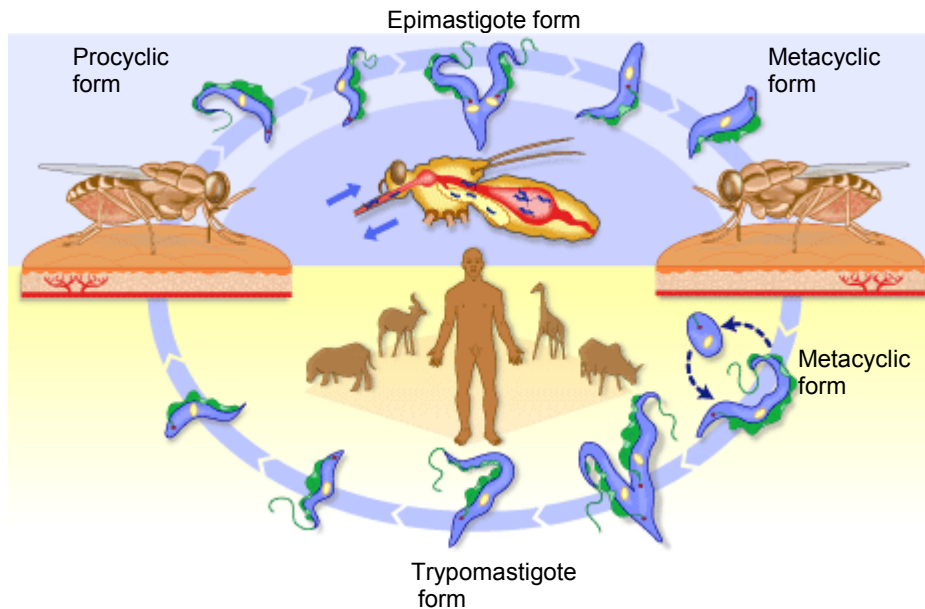
- Epidemic
- High endemicity
- Low endemicity
- At risk

**Figure 1.1: Map of the distribution of African trypanosomiasis.<sup>3</sup>**

Trypanosomiasis is caused by the parasite *Trypanosoma brucei*. There are three subspecies of *Trypanosoma brucei*; two of which cause infection to humans. These are *T.b.gambiense* which is found in West and Central Africa, and *T.b.rhodesiense* which is found in Eastern and South Africa. The species *Trypanosoma brucei brucei* does not affect humans, despite the fact that it is structurally and biochemically indistinguishable from *T.b.rhodesiense*, but along with the species *T.congolense* and *T.vivax*, it causes nagana in cattle, sheep and goats. *T.b.rhodesiense* is more virulent, but much less common in occurrence than is *T.b.gambiense*. *T.b.rhodesiense* causes acute infection that emerges within a few weeks of a fly's bite. As such, it is much easier to detect than *T.b.gambiense*, which may not show symptoms for years. Once *T.b.gambiense* does emerge, it is already in an advanced stage and difficult to treat.<sup>1</sup>

African trypanosomiasis is transmitted between vertebrate hosts via the tsetse fly of the *Glossina* species. When an infected fly is taking a blood meal, it transfers trypanosomes to the host; this is the first stage of the trypanosome infectious cycle, where the parasites exist in the metacyclic stage. At this stage the parasites multiply and cause painful indurate swelling at the site of the bite. The parasites then migrate from the lymph to the bloodstream where they develop to the slender trypomastigote form. Once in this form they multiply by asexual binary fission and spread to the

intracellular spaces of other tissues. They are then transferred back to the tsetse fly, when the insect is taking a blood meal, where they develop to the procyclic form in the insect's gut. The parasites then migrate to the insect's salivary glands where they take on the epimastigote form and later the metacyclic form.<sup>1</sup>



**Figure 1.2: Infectious life cycle of the *Trypanosoma brucei*.**<sup>3</sup>

The infection can also be transmitted accidentally by coming into contact with the blood of an infected person or animal.

Human African trypanosomiasis entails two main phases of the disease. At the early phase of the infection there are symptoms such as fever, joint pains, headaches, swollen tissues and itching. If the disease is diagnosed at this stage there is a high chance of cure. Once the parasite crosses the blood brain barrier, at the late stage of the disease and infects the central nervous system, the symptoms include confusion, sensory disturbances, loss of coordination, disturbance of the sleep cycle, coma and finally death.

The parasites (both *T.brucei* and *T.cruzi*) are remarkably well adapted in using the energy resources of both their insect and human hosts. Once in the insect mid-gut they use the citric acid cycle in order to metabolise intermediates, whilst in the mammalian

hosts they use glucose as the only form of energy and do not use the citric acid cycle. Unfortunately, no immunity against the parasites can be developed from the hosts, due to the parasites variable glycoprotein coat. Each metacyclic and bloodstream form of trypanosome are coated with a layer of glycoprotein known as a variable surface glycoprotein coat, which they change an unlimited number of times to immunologically distinct forms. This ability of the parasites makes the prospect of a vaccine unlikely, and thus chemotherapy remains the best available treatment.

The World Health Organisation is concerned that the social and economic impact of sleeping sickness is often underestimated. Epidemics have serious social and economic consequences and the disease has been the major cause of depopulation of large tracts of Africa. The fear it causes has led to abandonment of fertile lands and is an impediment to development.<sup>2</sup>

#### 1.1.2 American trypanosomiasis.

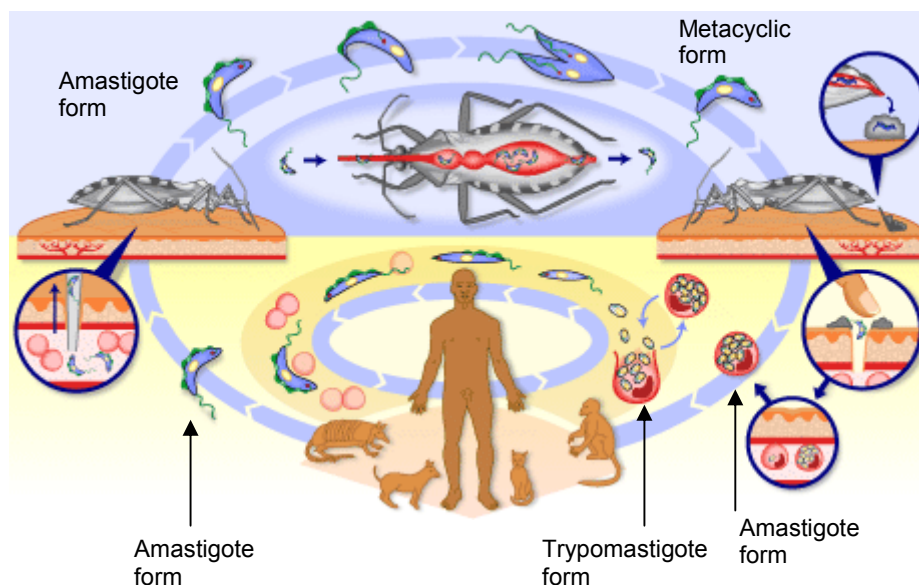
American trypanosomiasis or Chagas' disease infects both humans and small mammals which act as reservoir hosts. It affects 18 countries in Latin America, including Central America and Mexico, causing 13,000 deaths annually. Furthermore, due to recent, significant migrations from endemic countries towards developed countries, the threat of the disease is expanding to reach areas outside traditional geographic boundaries.



**Figure 1.3: Map of the distribution of Chagas' disease.**<sup>3</sup>

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*. The parasites are transmitted to human by blood-sucking bugs of variable genera such as assassin bugs, kissing bugs and reduviid bugs. The type of insect depends on the living conditions of humans. The faeces of the insects also contain parasites and they can

infect the host when they come in contact with broken skin. The parasite enters the host in the metacyclic phase, when the bug is taking a blood meal. It then multiplies at the site of bite causing local swelling or a skin nodule known as chagoma. At this stage the parasite invades host tissue cells and transforms to the amastigote phase where it multiplies to great numbers, filling the cell to its full capacity. The parasites then transform to the slender and mobile trypomastigote phase, where they burst the host cells and invade the bloodstream. The non-dividing extracellular trypomastigotes are then spread throughout the body where they invade the host's tissues and transform back to the intracellular proliferative amastigote form. The disease can also be transmitted through blood transfusions with infected blood and congenitally, from infected mother to foetus.<sup>1</sup>



**Figure 1.4: Infectious cycle of parasite.**<sup>3</sup>

Upon infection, the human host can experience two phases of symptoms. First, the sufferers enter the acute phase, which lasts from two weeks to months, where they experience fever, local swelling, skin rashes, myocarditis and hepatosplenomegaly. Approximately 10% of patients die in the acute phase of the infection, while the ones who survive enter the second chronic phase which can last for more than a decade. During the second phase the parasite invades most of the host's organs. The majority of the patients that enter the chronic phase do not experience any symptoms of the disease, while amongst the remainder, 32% develop cardiac and gastrointestinal symptoms that lead to death.

### 1.1.3 Leishmaniasis

Leishmaniasis affects humans, with animals acting as a reservoir for the parasites. It affects 88 countries, 16 of which are in Europe including France, Italy, Greece, Malta, Spain and Portugal, and it causes 59,000 deaths annually.

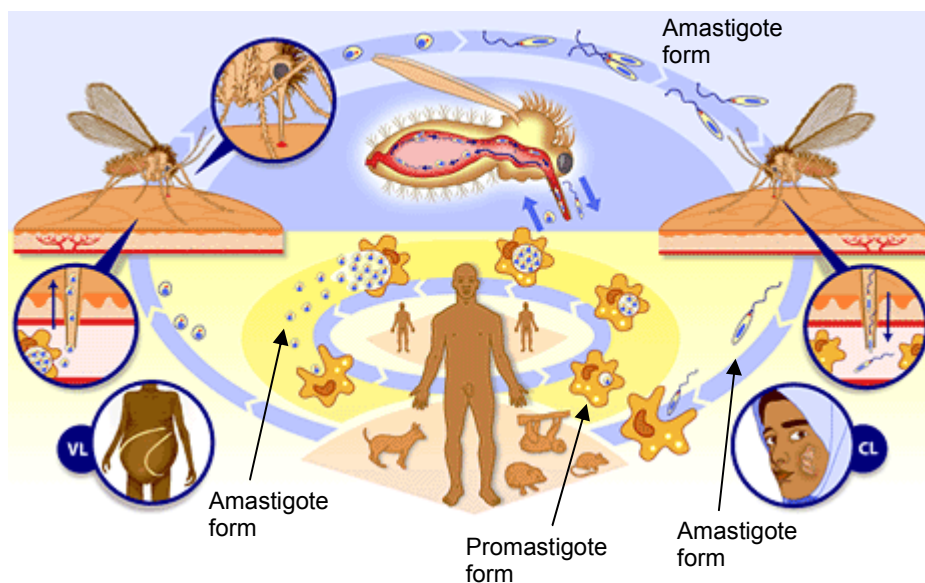


**Figure 1.5: Map of distribution of Leishmaniasis.<sup>3</sup>**

There are more than twenty species of Leishmaniasis which are known to be pathogenic to humans. The severity of the disease depends on the species of the parasite. Visceral leishmaniasis, otherwise known as Kala-azar (black fever) is the most severe infection, and is fatal if left untreated. It is caused by *Leishmania donovani*. The symptoms of visceral leishmaniasis are high fever, hyperpigmentation of the skin and swelling of the lymph nodes. The species *L.chagasi* found in South America and *L.infantum* found in the Mediterranean and the Middle East can also develop to visceral leishmaniasis. Cutaneous leishmaniasis is caused by *L.major* and *L.mexicana*. It is more common than visceral leishmaniasis but not as severe; it causes 1-200 skin lesions which self-heal within a few months but leave unsightly scars. The species *L.braziliensis* and *L.panamensis* have a high risk in developing to mucocutaneous leishmaniasis; where the infection begins with skin ulcers that spread to tissues especially the nose and mouth, leading to almost their complete destruction. Another form of the infection is diffuse cutaneous leishmaniasis which is quite difficult to treat. It causes disseminated and chronic skin lesions resembling those of leprosy.<sup>1</sup>



The infection is transmitted to humans via the bite of female sandflies of the family *Phlebotomus* found in Europe, Asia and Africa or *Lutzomyia* found in America. The sandfly gets infected from the parasite when it is feeding from a reservoir host. The parasite exists in the amastigote form inside the insect. When the sandfly is taking a blood meal from a human host, the parasite is transferred to the host via the insect's salivary glands. The amastigote form transforms to the promastigote form when it is ingested by the host's macrophage cells. The promastigote then transforms back to the amastigote and multiplies until the cell bursts. The amastigote form can only survive in the macrophage cells, once released it infects new cells and so spreads the infection. Sandflies then become infected with the amastigotes when they feed from infected hosts, thus completing the infectious cycle of the parasite.<sup>1</sup>



**Figure 1.6: Infectious cycle of leishmania parasites.<sup>3</sup>**

## **1.2 EXISTING CHEMOTHERAPIES.**

Numerous drugs have been developed to treat both leishmaniasis and trypanosomiasis, without giving promising results in reducing the spread of the infection. The problem of existing chemotherapies is that the majority of the drugs are toxic and have low efficacy. They are either very expensive to make, or difficult to

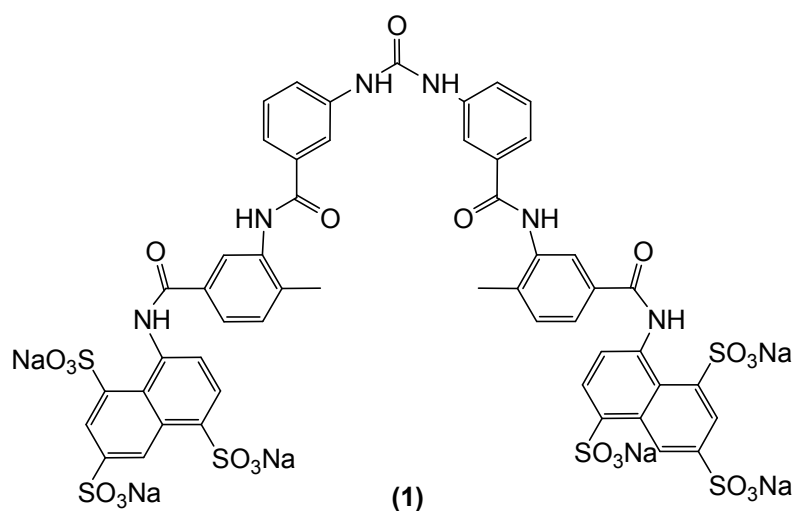
administer and parasites have already developed resistance to some of them. Furthermore the medical facilities and staff for long term treatment and follow up of patients do not exist in the affected regions.

### 1.2.1 African trypanosomiasis

Treating sleeping sickness has proven to be very difficult, especially when the parasites cross the blood-brain barrier, as few drugs are effective at this point.

#### i) Suramin Sodium

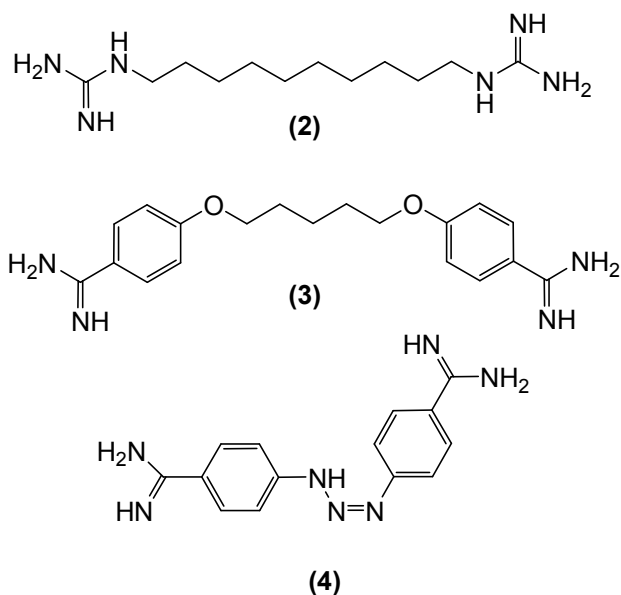
Suramin sodium (**1**) is a sulfonated naphthylamine polyanionic dyestuff introduced in the 1920s and is still used against sleeping sickness. It is chemically related to the dyes Trypan Red and Trypan blue, which also have anti-trypanosomal activity. Suramin is extremely polar and thus water soluble, and is administered by intravenous injection. It is very unstable in solution if the solution comes in contact with air, so it is dissolved immediately before administration. Due to its high polarity, it cannot cross the blood-brain barrier and so it is only used for the early phase of the infection. The selectivity of suramin is explained by the ability of trypanosome to accumulate the drug. However, its mode of action is not completely clear as it inhibits a number of trypanosomal enzymes, and thus multiple mechanisms are probably involved in its therapeutic effect. The use of suramin can cause a number of side effects including, vomiting, pruritus, urticaria, paresthesias, hyperesthesia of hands and feet, photophobia and peripheral neuropathy. Occasional side effects are kidney damage, blood dyscrasias, shock and optic atrophy.<sup>1</sup>



**Figure 1.7: Suramin Sodium (1).**

#### ii) Diamidines

Synthalin (**2**) was the first diamidine to show activity against *Trypanosoma*, but today pentamidine (**3**) and diminazene (**4**) are more commonly used. Pentamidine (**3**) was first developed as a hypoglycemic agent, but was introduced as an anti-trypanocidal agent in 1941. Pentamidine (**3**), as all diamidines, is almost always fully protonated, making it hard to be absorbed if it is orally administered. Therefore, pentamidine (**3**) like suramin sodium (**1**) has to be administered intravenously, with the drug solution made just before administration because it is otherwise unstable. Diamidines are polar, cannot cross the blood-brain barrier, and are thus used only in the first phase of the infection. The activity of pentamidine (**3**) and other diamidines are dependent on their active uptake by the parasite via the high affinity purine transporter P2.<sup>4</sup> The mechanism of action of pentamidine remains unclear, however Bailey *et al*,<sup>5</sup> have reported that pentamidine binds to nucleic acids in DNA and RNA, while Shapiro and Englund<sup>5</sup> reported that the drug promotes cleavage of the parasite's circular DNA in a manner similar to that of topoisomerase II inhibitors. Diminazene (**4**) is approved for veterinary use as it is a more toxic diamidine, and its safety has not been fully evaluated yet.<sup>6</sup> Some of the common side effects of pentamidine (**3**) include hypotension, hypoglycemia, vomiting, blood dyscrasia, renal damage, pain at the injection site and gastrointestinal disturbances. Rarer side effects include anaphylaxis, acute pancreatitis, hyperkalemia, and ventricular arrhythmias.



**Figure 1.8: Diamidines (2) Synthalin, (3) Pentamidine, (4) Diminazine.**

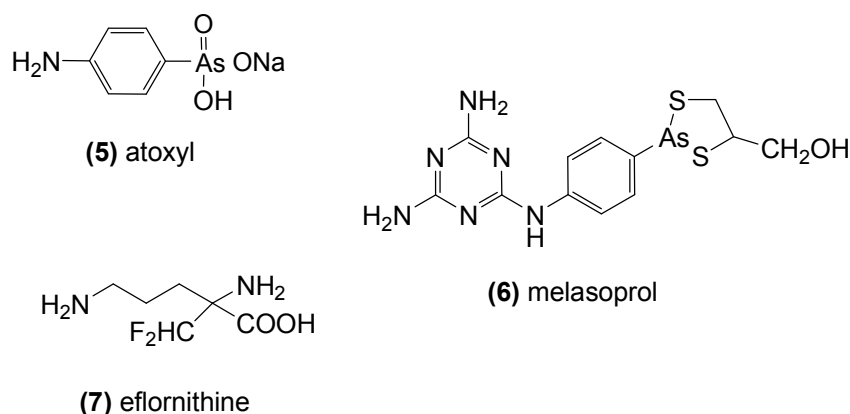
### iii) Arsenicals

One of the first arsenical drugs that was effective against sleeping sickness was Atoxyl (5).<sup>7</sup> Nevertheless, it was found to have serious side effects as it was highly toxic. Then in 1949 Melasoprol (6) was introduced, which was the only drug that was effective in the late stage of the infection. However, the parasites have since developed resistance to this drug, and now only eflornithine (7) is used for treatment of the late stage of the infection. Melasoprol (6) is actually a prodrug that converts to melarsen oxide. It has a relatively small half-life of 30 minutes and so it leaves the system within 24 hours.<sup>8</sup> There has been some controversy about the mechanism of action of melasoprol, but Fairlamb *et al.*<sup>9</sup> published that melarsoprol or melarsen oxide can form a stable adduct with trypanothione which is an essential substrate for redox homeostasis in trypanosomes. Melasoprol (6) can cause reactive encephalopathy in 5-10% of the patients treated and has a fatal outcome in 10-50% of these patients. The drug can also cause side effects such as hypertension, myocardial damage, vomiting and abdominal colic. It is a non-specific inhibitor of many different enzymes, which perhaps explains the many toxic side effects that it has.

### iv) Eflornithine (7)

Eflornithine (7) was originally developed as an anti-cancer agent. It was reported by Metcalf *et al.*<sup>10</sup> that it could block the biosynthesis of polyamines by inhibiting ornithine decarboxylase which is an enzyme vital for polyamine biosynthesis. Later in

the 1980s it was found to have anti-trypanosomal activity by Bacchi *et al.*<sup>11</sup> against the early and late stage of *T.b.gambiense* associated infections. However, it has no activity against other species of *Trypanosoma*, except *T.b.brucei*, for reasons not fully understood. It is suggested that in the mechanism of action of eflornithine (7), the cysteine 360 in the active site of ornithine decarboxylase, acts as a nucleophile and attacks the drug, which is then covalently bound to the enzyme.<sup>12</sup> It has also been reported that eflornithine (7) depletes putrescine and spermidine from *T.b.brucei* both *in vitro* and *in vivo*. As a result, the trypanosomes stop growing, and change to a short and stumpy form that cannot change their variable surface glycoprotein coat, and thus killed by the host's immune system.<sup>13</sup> The depletion of polyamines in eflornithine-treated trypanosomes causes a 40% decrease in trypanothione which possibly could contribute to eflornithine's mechanism of action as well as accounting for the synergistic effect with arsenical drugs *in vivo*.<sup>14</sup>



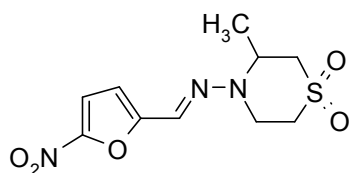
**Figure 1.9: Arsenicals & eflornithine.**

### 1.2.2 American trypanosomiasis

For American trypanosomiasis two drugs have been used for treatment of both the early and chronic phase of the disease. Unfortunately, the prospects of developing a vaccine against Chagas disease are slim, since *T.cruzi* antigens can cause autoimmunity.

### i) Nifurtimox (8)

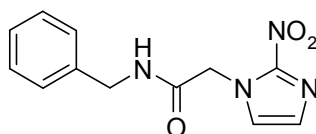
Nifurtimox (8) is a nitrofuran derivative that is effective against both *T.cruzi* and *T.brucei*<sup>15</sup> but is no longer in use. The full mechanism of action of the drug is not yet known, but it is clear that one electron transfer reaction is needed in order to form a nitro ion radical. In turn, the nitro radical is thought to produce oxygen radicals and hydrogen peroxide and thus induce oxidative stress in the parasites. Some of the side effects induced by this drug include nausea, vomiting, abdominal pain, anorexia, restlessness, insomnia, paresthesia, polyneuritis, and seizures, as well as allergic reactions such as dermatitis, icterus, pulmonary infiltrates and anaphylaxis.



**Figure 1.10: Nifurtimox (8)**

### ii) Benznidazole (9)

Benznidazole (9) is a nitroimidazole analogue and is now the drug of choice against Chagas disease. Unlike Nifurtimox, oxidative damage is not the key of action of Benznidazole, but it involves covalent and other bond interactions of nitroreduction intermediates with parasite components or binding to DNA lipids and proteins. Unfortunately some strains of *T.cruzi* have developed resistance to both drugs.



**Figure 1.11: Benznidazole (9)**

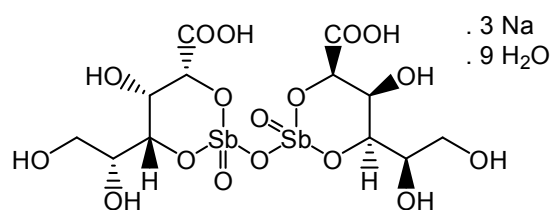
## 1.2.3 Leishmaniasis

Most cases of cutaneous leishmaniasis heal without treatment, but visceral leishmaniasis requires long courses of antimonial drugs or sodium stibogluconate. Recently, resistance to some drugs has been reported requiring the use of more toxic drugs such as amphotericin B.<sup>2</sup> Most available drugs are costly, require long treatment

regimes and are becoming more and more ineffective, necessitating the discovery of new drugs.<sup>2</sup>

### i) Antimonials

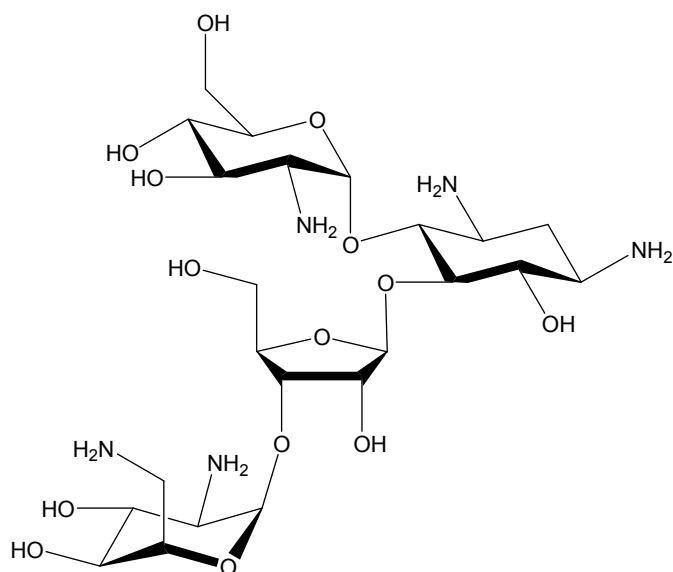
There are two main drugs that have been used in the past 50 years for the treatment of leishmaniasis. Sodium stibogluconate (**10**) and meglumine antimonate are both pentavalent derivatives of antimony. They are both prodrugs that need to be reduced from  $Sb^V$  to  $Sb^{III}$ , in order to be active against the amastigote stage of the parasite, and the treatment of both drugs is the same. Their mode of action is not yet completely understood, but it has been reported that they could inhibit glucose catabolism and fatty acid oxidation in amastigotes,<sup>16</sup> and also that they may inhibit trypanothione reductase.<sup>17</sup> However, resistance to antimonial drugs has been reported, causing problems in Bihar in India and in some parts of Bangladesh.<sup>2</sup> Side effects that can occur with pentavalent antimonial drugs include arrhythmias, transaminase elevations, muscle and joint pain, fatigue, nausea and pancreatitis.



**Figure 1.12: Sodium Stibogluconate (10).**

### ii) Paromycin (11)

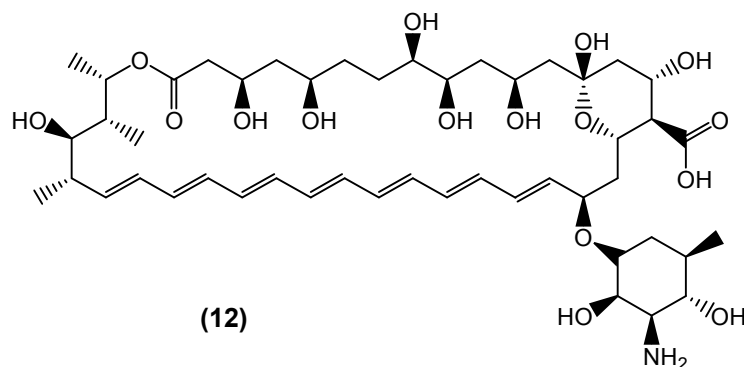
An alternative treatment to antimonial drugs is the antibiotic paromycin sulphate (**11**). It is effective against all forms of *Leishmania* and binds to the parasites' polysomes inhibiting protein synthesis by causing misreading and premature termination of translation of mRNA.<sup>18</sup> Unfortunately the drug is not currently commercially available. Some of the side effects of paromycin can cause are nephrotoxicity and damage to the eighth cranial nerve, resulting in hearing loss.



**Figure 1.13: Paromycin (11)**

### iii) Amphotericin B (12)

Amphotericin B (12) is also an antibiotic which is highly effective against antimony-resistant strains of *Leishmania*.<sup>19</sup> It is effective against all forms of leishmaniasis and has a cure rate close to 100%, which is much better than that of paromycin (11). Its mechanism of action involves the drug binding with ergosterol on the parasite's cell membrane. This causes pores to form on the cell membrane and an influx of ions into the cell leading to cell apoptosis.<sup>20</sup> Even though resistance to this drug is very rarely reported, it is not widely used because of its high cost, the difficulty of the paranteral administration needed and the long treatment periods required. The major side effect of the drug is nephrotoxicity, while other acute side effects include fever, chills, muscle spasms, vomiting, headache and anaphylaxis.



**Figure 1.14: Amphotericin B (12).**

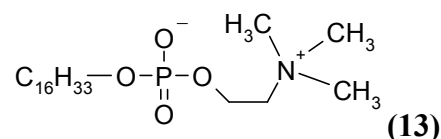


#### iv) Pentamidine (3)

Other than treating African sleeping sickness, pentamidine (3) is also used against leishmaniasis. It has serious side effects and resistance has been reported, therefore is only used as a back up alternative therapy to antimonial drugs and amphotericin B.

#### v) Miltefosine (13)

Miltefosine (13) was initially developed as an anti-cancer drug, but failed the screening process and was subsequently found to have antileishmanial activity. In 2004 the drug was in Phase IV trial in India. The trial involved 1200 patients, with 6 month follow up for efficacy and safety and a 1.5 year follow up for male reproductive function.<sup>2</sup> The significant advantage of using this drug is that it is orally administered unlike the other anti-leishmanial drugs. Up to now, it has shown very promising results with a cure rate of 98%. The exact mechanism of action of miltefosine is not known. The side effects of the drug are quite significant but tolerable; they include gastrointestinal distress and elevated aspartate aminotransferase levels.



**Figure 1.15: Miltefosine (13).**

### 1.3 NEW TARGETS FOR CHEMOTHERAPY.

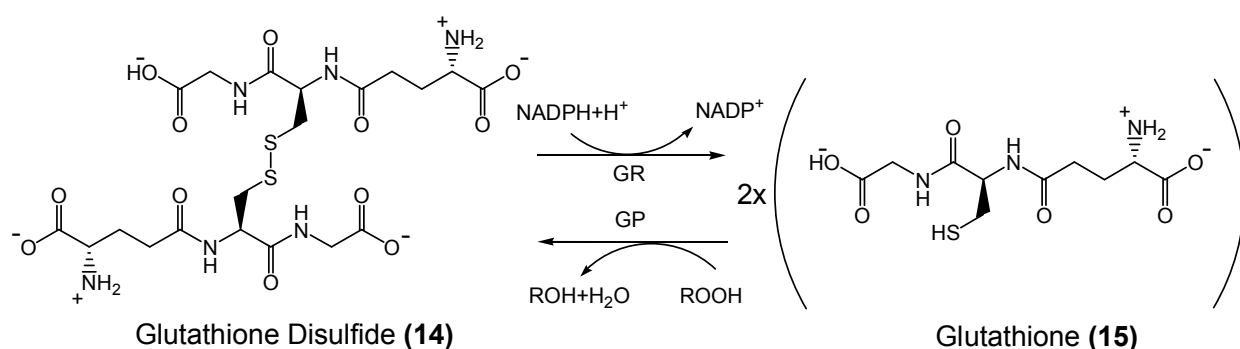
Despite the knowledge of many different enzyme pathways of the parasites and how they can be used as targets, not much development has taken place in terms of new chemotherapeutic agents. Ideally any new chemotherapeutic agent should have high efficacy and efficiency, low toxicity, low cost, and be easily administered.

### 1.3.1 Oxidative stress.

*Trypanosoma* and *Leishmania* parasites need a highly efficient thiol metabolism, to combat reactive oxygen species, making oxidative stress a new and very appealing chemotherapeutic strategy. Humans consume hundreds of litres of oxygen daily. Around 5% of that is converted to reactive oxygen species such as  $O_2^-$ ,  $HO_2^-$ ,  $ONOO^-$ ,  $HO^\cdot$ , and hydrogen peroxide. These species are highly toxic due to their ability to modify nucleic acids, thiol-containing proteins and membrane lipids. A cell is considered to be under oxidative stress, when the cell's mechanisms to combat oxidative stress are challenged. Oxidative stress can also be used by the host as a defence mechanism against invaders.<sup>21</sup>

### 1.3.2 Trypanothione and glutathione, TryR and GR.

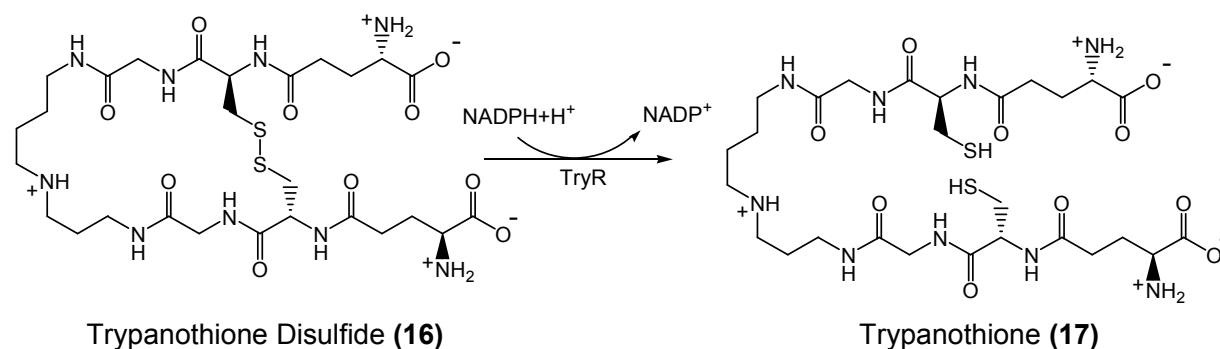
Humans depend on their glutathione redox cycle in order to combat oxidative stress. During this process the flavoenzyme glutathione reductase catalyses the NADPH-dependent reduction of glutathione disulfide (**14**) to glutathione (**15**). This causes an increase in glutathione (**15**) levels compared to glutathione disulfide (**14**). In parallel, the enzyme glutathione peroxidase along with two other antioxidative enzymes converts glutathione (**15**) back to glutathione disulfide (**14**), while reducing the radicals superoxide and hydrogen peroxide.



**Figure 1.16: Glutathione redox cycle.**

Trypanosomes lack these enzymes and substrates and depend instead on the analogous trypanothione redox system. In this system, the enzyme trypanothione

reductase catalyses the NADPH-dependent reduction of trypanothione disulphide (**16**) to trypanothione (*N*<sup>1</sup>,*N*<sup>8</sup>-bis(*L*- $\gamma$ -glutamyl-*L*-cysteinyl-glycyl)spermidine) (**17**).



**Figure 1.17: Trypanothione redox cycle.**

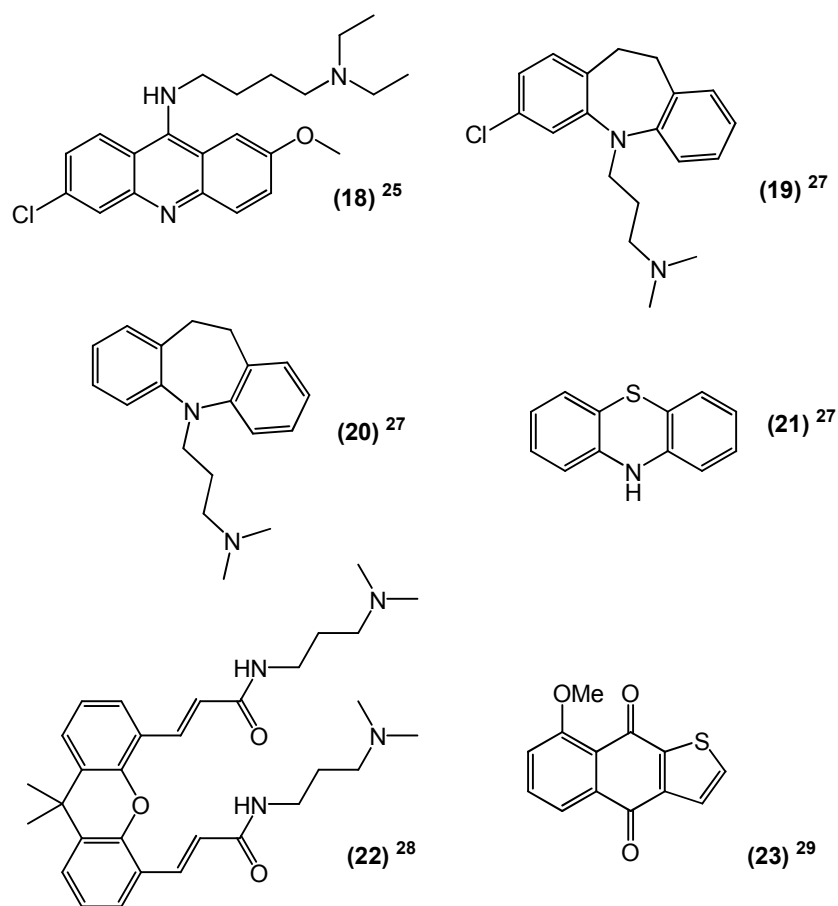
Trypanothione reductase has been found in all trypanosomatids so far examined. The two enzymes, TryR and GR both exist as homodimers and have three main domains, the FAD binding domain, NADPH binding domain and the interface domain. Despite the fact that TryR and GR are structurally and chemically similar,<sup>22</sup> the TryR active site is more open than GR, in order to accommodate the bulkier substrate of trypanothione disulfide (**16**), and also the TryR active site has an overall negative charge compared with GR that has a positive charge.<sup>23</sup> These differences are sufficient to account for why certain inhibitors are specific to TryR and do not affect GR. Targeted gene replacement studies have shown that TryR is vital for the survival of *Leishmania* and *Trypanosoma* when these organisms were put under oxidative stress conditions.<sup>24</sup> It would therefore seem that the inhibition of this enzyme could provide a worthy target for drug design. Inhibitors of TryR have been identified from a variety of sources and by different approaches including rational drug design and HTS, as outlined in the following sections.

## 1.4 EXISTING INHIBITORS OF TryR.

### 1.4.1 Tricyclic compounds.

One of the first tricyclic compounds to be identified as a selective competitive inhibitor of TryR and not GR was the drug mepacrine (**18**).<sup>25</sup> Since then more potent

inhibitors based on mepacrine, have been developed. Sulfonamide and urea derivatives of mepacrine containing an aromatic group in the N-9 side chain, are 10 times more potent than mepacrine.<sup>26</sup> Tricyclic compounds that have been used as antidepressants are also competitive inhibitors of TryR such as clomipramine **(19)**, imipramine **(20)**<sup>27</sup> and phenothiazene **(21)** (Fig. 1.18). The reason these compounds inhibit TryR is that their tricyclic core fits against a hydrophobic wall formed by Tyr21 and Met113, in the active site of the enzyme. Since then a second hydrophobic region in the active site, Z-site, formed by Phe395', Pro397', and Leu398' has also been shown to accommodate hydrophobic ligands (Fig. 1.18). Chibale *et al.*<sup>28</sup> also evaluated a small library of xanthene based tricyclic compounds containing polyamine chains, which displayed inhibitory activity against TryR comparable to that of the tricyclic antidepressants. Tricyclic inhibitors that display other modes of inhibition have also been identified. For example Zani and Fairlamb<sup>29</sup> identified 8-methoxy-naphtho[2,3-b]thiophen-4,9-quinone **(23)** as a non competitive inhibitor of TryR with a  $K_i = 5 \mu\text{M}$ .



**Fig 1.18: Tricyclic compounds, inhibitors of TryR.**

### 1.4.2 Aminodiphenylsulfides.

Due to the effectiveness of tricyclic antidepressants compounds as TryR inhibitors, a new class of aminodiphenylsulfide compounds was developed, based on the tricyclic core but with the middle ring open. This class of inhibitors are as powerful inhibitors as their tricyclic phenothazine analogues. Baillet *et al*<sup>30</sup> developed a series of diphenylsulfide derivatives with prolonged or branched polyamine chains (**24**)- (**31**), which were good inhibitors but toxic (Fig 1.19). The same group, solved the toxicity problem, by introducing compounds containing 2-aminodiphenylsulfide moieties linked by a spermidine bridge which were competitive inhibitors with a  $K_i$  value of 400 nM.<sup>31</sup> The most potent compound in this series with an  $IC_{50}$  of 200nM was a bis(2-aminodiphenylsulfide) (**32**) with an extra third bulky chain (Fig 1.19).<sup>32</sup>

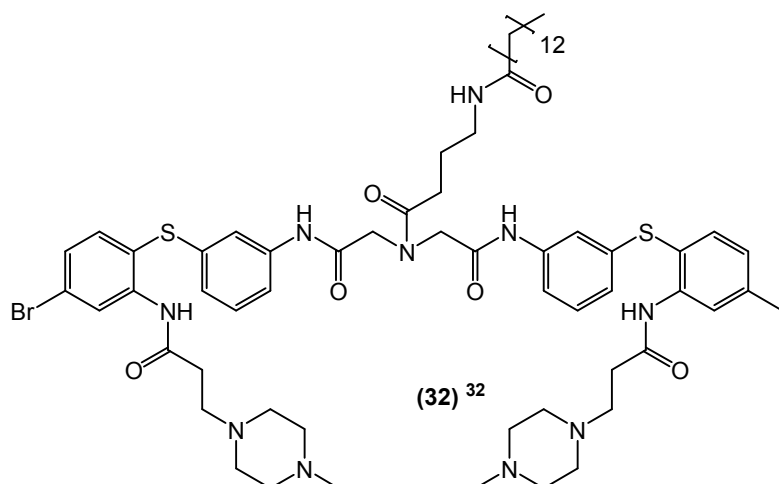
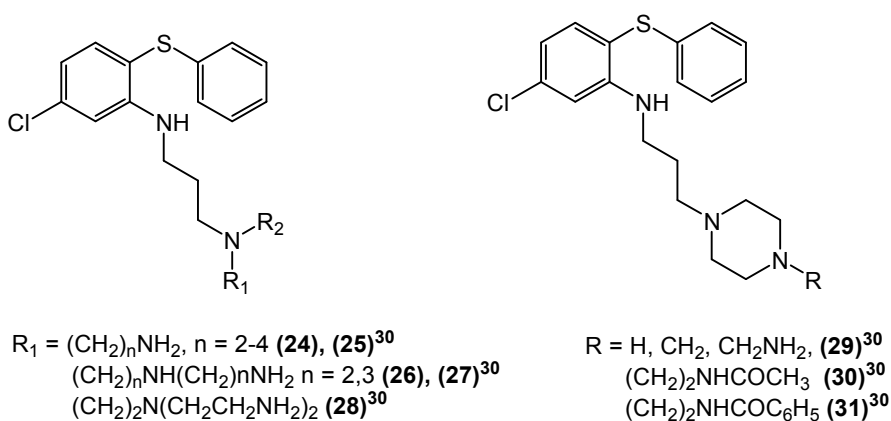
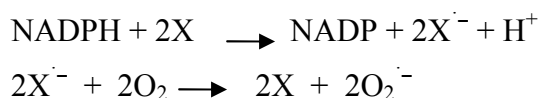


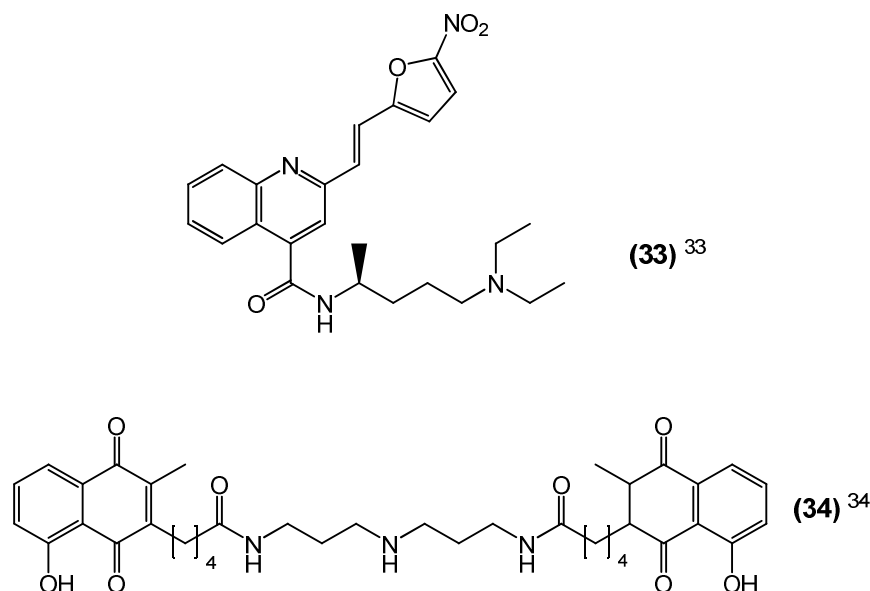
Figure 1.19: Aminodiphenylsulfides as TryR inhibitors

### 1.4.3 Subversive substrates.

Subversive substrates (X) are reduced in single-electron steps to their respective radicals, and then react with oxygen to give superoxide anion radicals. This action converts an antioxidative disulfide reductase enzyme like TryR into a prooxidative enzyme.



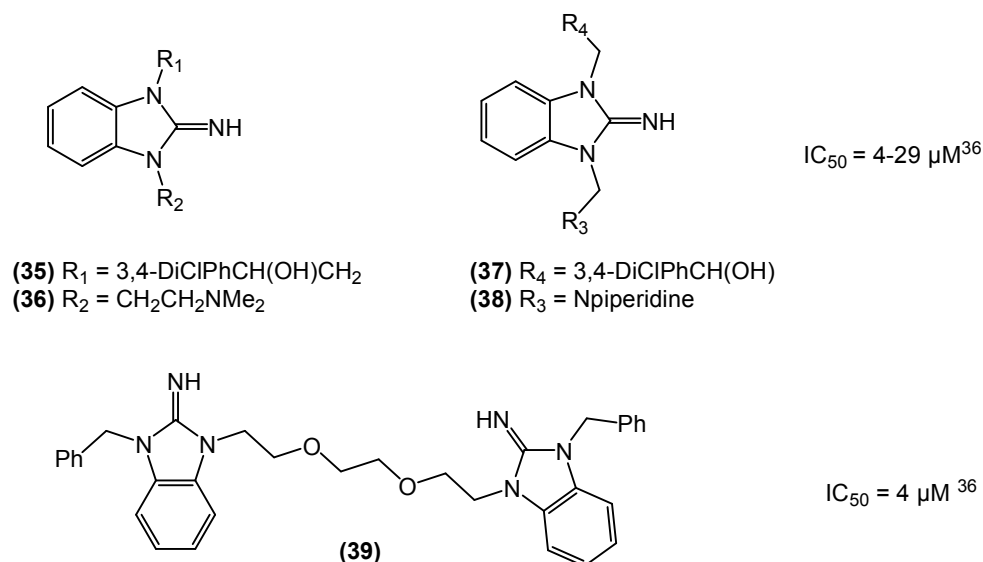
In TryR, NADPH and O<sub>2</sub> are used as the subversive substrate is regenerated from its radical intermediate, thus the compounds act as catalysts for oxidative stress, having a strong impact on the redox metabolism of the parasite.<sup>21</sup> Nitrofurans such as Chinifur **(33)** (Fig 1.20), a nitrofuran derivative with an aminoalkyl side chain is a TryR subversive inhibitor.<sup>33</sup> Naphthoquinones such as menadione, plumbagin, lapacol and other 1,4-naphthoquinones are also well known subversive substrates for TryR. The most potent derivative of naphthoquinones contains two 1,4-naphthoquinone moieties linked by a polyamine spacer **(34)** (Fig.1.20).<sup>34,35</sup>



**Figure 1.20: Subversive substrates.**

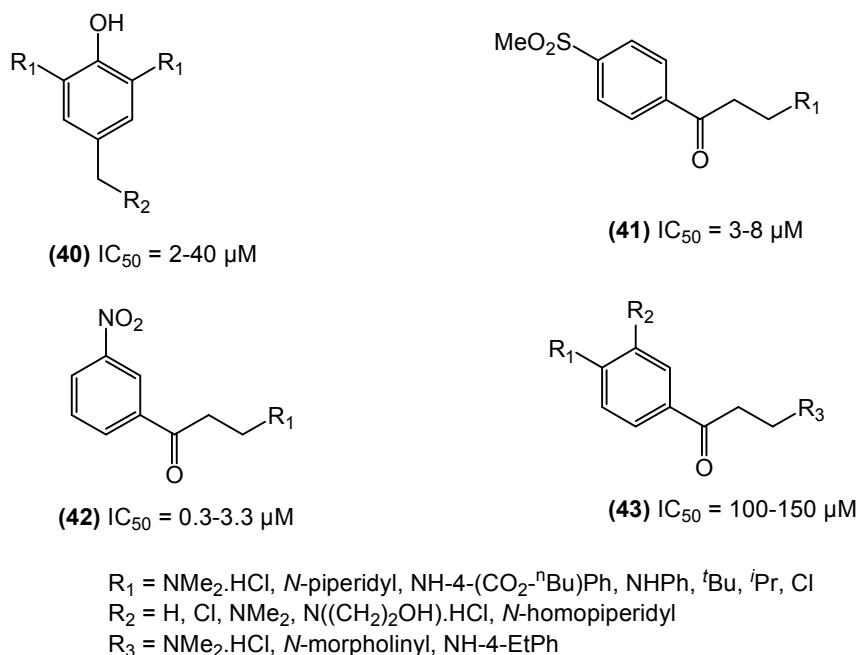
#### 1.4.4 Inhibitors derived by HTS.

High throughput screening a library of 100,000 lead-like compounds, identified 2-iminobenzimidazoles as a novel class of TryR inhibitors **(35)-(39)**.<sup>36</sup> They display potent trypanocidal activity, but do not inhibit human GR and have low cytotoxicity against mammalian cells. The most potent examples are shown in Figure 1.21.



**Figure 1.21 : 2-iminobenzimidazoles**

A number of other scaffolds have been identified as TryR inhibitors by HTS.<sup>37</sup> Figure 1.22, shows some recent examples.

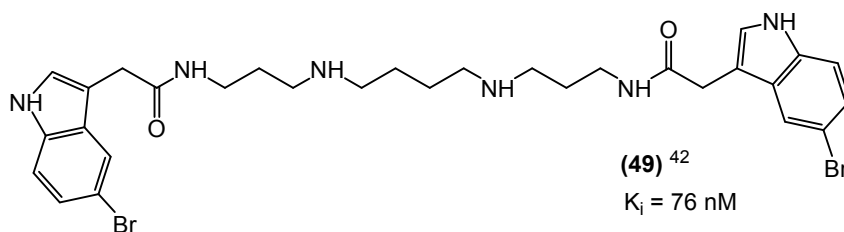
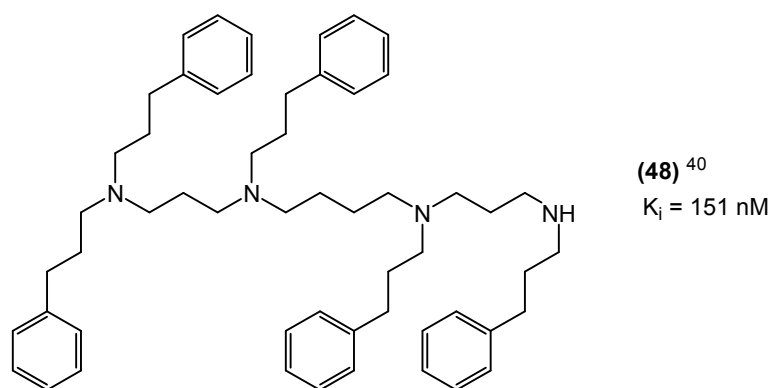
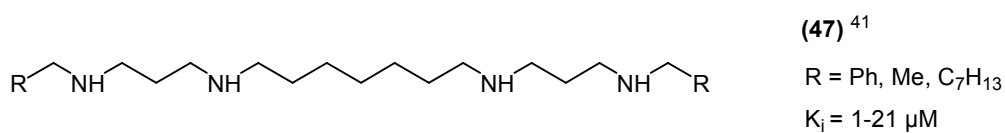
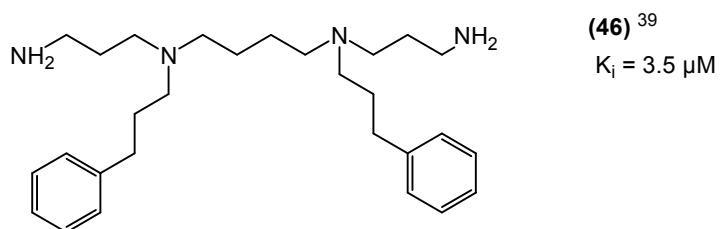
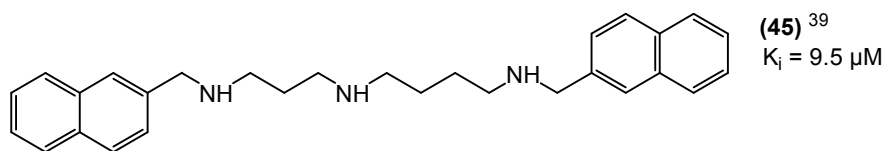
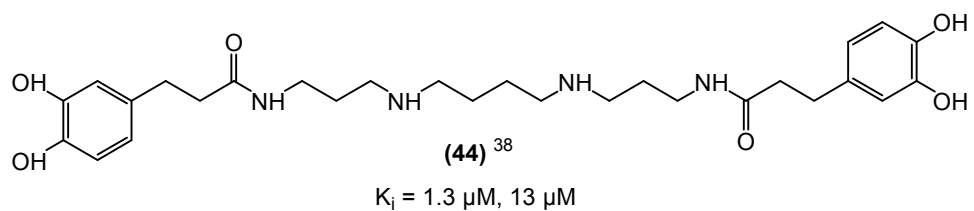


**Figure 1.22 : Examples reported by Martyn *et al.***<sup>37</sup>

### 1.4.5 Polyamine-based inhibitors.

One of the most studied type of inhibitors are polyamine based-compounds. Kukoamine A (**44**) is a natural spermine derivative, and is a mixed type inhibitor for TryR.<sup>38</sup> Its corresponding spermidine analogue  $N^1, N^8$ - bis(dihydrocaffeoyl) spermidine is a competitive inhibitor with  $K_i = 7.5 \mu\text{M}$ . Ponasik *et al.*<sup>38</sup> proposed that, both the aryl moieties in the molecules interact with the hydrophobic region of the enzyme, so that these spermine and spermidine derivatives would adopt a non-extended bound conformation. Due to the effectiveness of Kukoamine A as a TryR inhibitor, a lot of the resulting synthetic polyamine inhibitors were based on this structure, a polyamine unit with 2 or more hydrophobic substituents. Interestingly, spermine derivatives were found to be more effective than the corresponding spermidines.<sup>31</sup> O'Sullivan *et al.*<sup>39</sup> tested a small library of alkyl aryl polyamine compounds, the most potent (**45**)-(47) are shown in Figure 1.23. Further investigation showed that the potency of the inhibitor was proportional to the degree of substitution; however the penta-substituted derivative was more effective than the hexa-substituted, suggesting that the active site had reached its capacity.<sup>40</sup> The same group more recently screened a series of *N*-(3-phenylpropyl)-substituted spermines and spermidine derivatives, with the highly substituted  $N^1, N^1, N^4, N^8, N^{12}$ -penta(3-phenylpropyl)spermidine (**48**) as the most effective competitive inhibitor.<sup>40</sup> Woster *et al.*<sup>41</sup> have also prepared a series of polymamine analogues, but these did not contain a spermine or spermidine backbone. Instead these had a 3-3-3 or 3-7-3 backbone with the 3-7-3 analogues showing potent antiparasitic activity. The most potent polyamine-type inhibitors so far were reported by Bradley *et al.*<sup>42</sup> They prepared polyamine derivatives using indole aryl acids as capping groups. The bromoindole spermidine derivative (**49**) was the most potent inhibitor with  $K_i = 76 \text{ nM}$ .

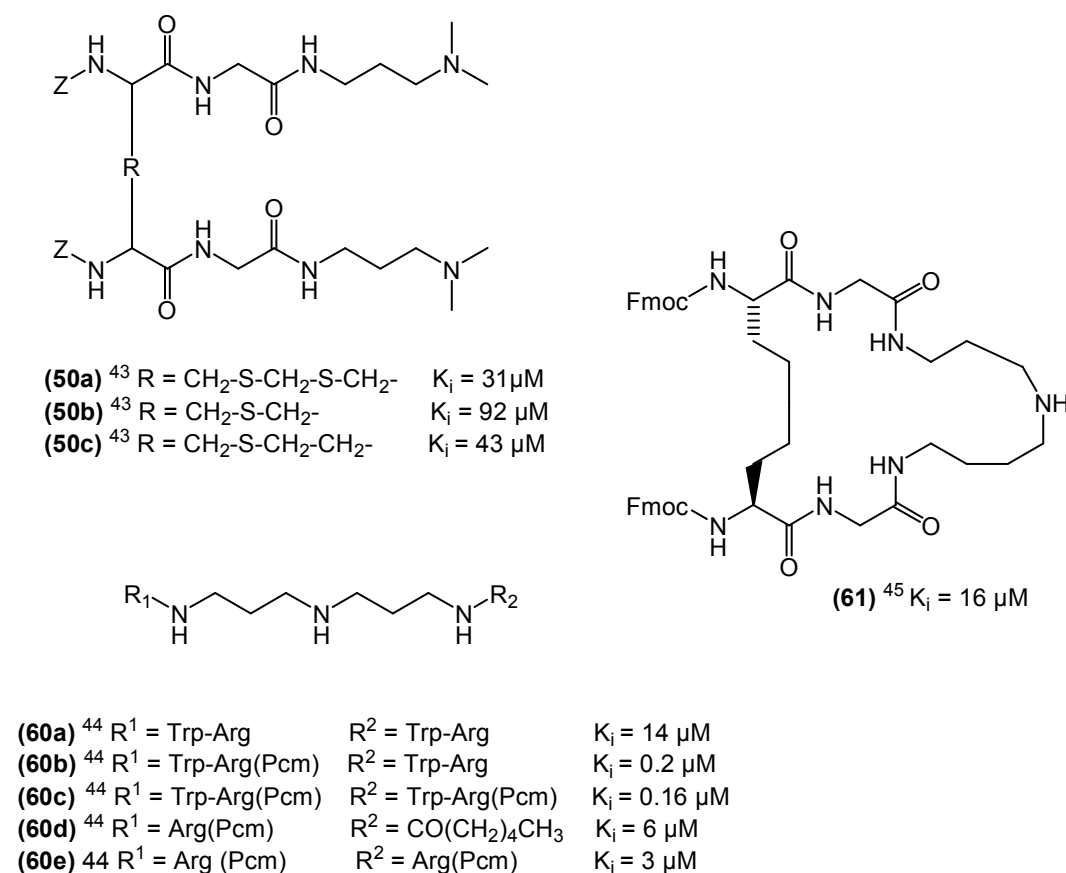




**Figure 1.23 : Polyamine-based inhibitors of TryR.**

### 1.4.6 TryR peptide-mimetic substrates.

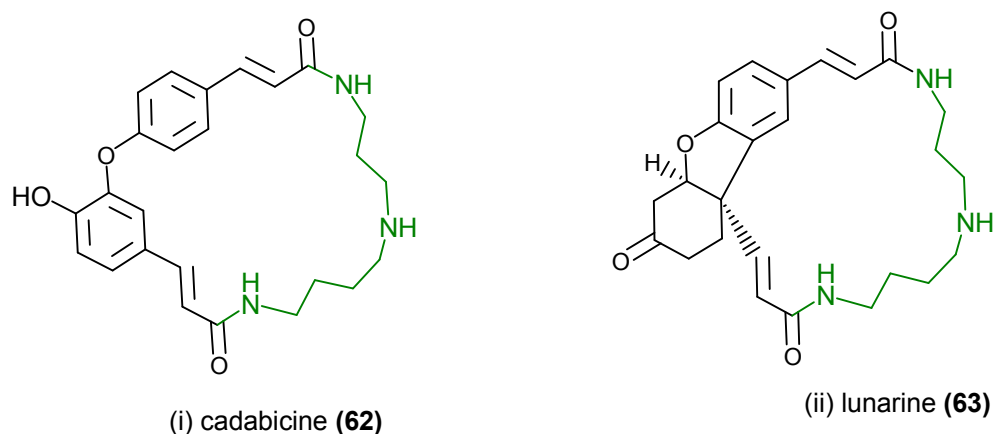
This class of inhibitors involves compounds that mimic the structure of the TryR natural inhibitor, trypanothione disulfide. Tromelin *et al*<sup>43</sup> developed a good substrate-mimetic inhibitor for TryR that had been modified at the disulfide bridge to obtain non reducible derivatives. The cysteine residues containing the disulfide bridge were replaced by 3 natural amino diacids: djenkolic acid (**50a**), lanthionine (**50b**) and cystanthione (**50c**), with the djenkolic acid derivative being the best inhibitor as seen in Figure 1.24. Similarly Dixon *et al*<sup>44</sup> prepared a small focused library of polyamine-peptide conjugates, that contained both competitive and non-competitive inhibitors of TryR. Examples of Dixon's<sup>44</sup> best inhibitors are shown in Fig 1.24 (compounds **(60a)**-**(60e)**). Recently Czechowicz *et al*<sup>45</sup> also prepared structural analogues of the natural substrate. Compound **(61)**, the best inhibitor in the series ( $K_i = 16 \mu\text{M}$ ), is an Fmoc protected analogue, which contains all the structural elements of the natural substrate except the redox-active disulfide moiety, which has been replaced by a pair of methylene groups.



**Figure 1.24: TryR peptide-mimetic substrates.**

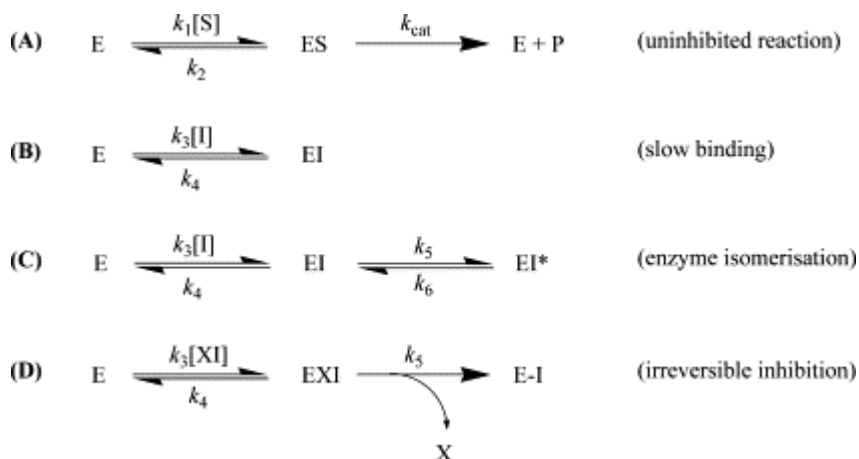
### 1.4.7 Polyamine Alkaloids

A further class of natural product inhibitors that are potential mimetics of the TryR substrate are macrocyclic polyamine alkaloids. The spermidine alkaloids lunarine (**63**) (isolated from *Lunaria annua*) and cadabicine (**62**) (isolated from *Cadaba farinosa*) were identified by virtual screening as potential inhibitors by Bond *et al.*<sup>46</sup> This analysis was based on their overall similarity to the cyclised trypanothione disulfide, which is the principal natural substrate of TryR, since both natural products contain spermidine in their macrocycle structure (highlighted in green).



**Figure 1.25: Natural alkaloid inhibitors of TryR.**

Initial kinetic studies with optically pure lunarine from natural sources confirmed that it is a modest competitive inhibitor of TryR.<sup>46</sup> It was subsequently shown<sup>47</sup> that lunarine inhibits TryR in a time-dependent manner, with an observed inhibition constant  $K_i = 114 \mu\text{M}$ .<sup>46,48</sup> In general; time dependent inhibition can arise from three different mechanisms (Fig. 1.26):<sup>47</sup>



**Figure 1.26 : mechanisms of time-dependent inhibition.**

In classical inhibition mechanisms, the steady state equilibrium between enzyme and inhibitor is rapidly established (usually within milliseconds). Simple slow binding (mechanism B) occurs when the inhibitor binds to the enzyme like a classical inhibitor but with a slower binding rate such that the steady state is attained over a much longer timescale of several seconds or even min. With an enzyme isomerisation mechanism (C), the Michaelis–Menten complex (EI) is rapidly formed followed by slower conformational changes in either the enzyme or inhibitor resulting in a more tightly bound complex (EI\*). Irreversible inhibition proceeds by the same rapid binding step followed by a rate-limiting covalent modification of the enzyme (D). Studies with lunarine and related compounds suggest that that mechanism C, involving formation of a covalent enzyme inhibitor species, is involved.<sup>49</sup> Bond *et al.*,<sup>46</sup> suggested that this may result from conjugate addition of a redox-active cysteine residue in the TryR active site onto either one of the two  $\alpha,\beta$ -unsaturated double bonds of lunarine. This is supported by the observation that time-dependent inhibition is associated with the NADPH-reduced form of the enzyme only<sup>46</sup> and the detection of a possible covalent TryR-lunarine species by mass spectrometry.<sup>49</sup> Time-dependent inhibitors such as lunarine offer an attractive alternative to many competitive inhibitors reported so far. It can be shown,<sup>1</sup> that to maintain a potentially lethal level of inhibition of TryR in the presence of accumulating millimolar levels of trypanothione, competitive inhibitors of low nanomolar potency are required. Inhibitors that form a covalent enzyme-inhibitor species, where accumulating substrate cannot reverse inhibition should not require such high affinity to be effective. This idea is supported by the fact that synthetic racemic lunarine and derivatives, based on the tricyclic core of lunarine are moderately potent ( $K_i \sim 4 \mu\text{M}$ ) TryR inhibitors, and also show activity against *T.brucei* trypomastigotes *in vitro*.<sup>49</sup> Although cadabicine was originally identified in the same virtual screening study as lunarine, so far no detailed analysis of its potential as a TryR inhibitor has been possible, owing to the lack of availability of the natural product. Its structural similarity to lunarine and results with other lunarine-like molecules suggest that it too may be a time-dependent inhibitor and an interesting lead for drug development.

## 1.5 AIMS

The aims of this project were as follows:

- Complete the first synthesis of the natural product cadabicine, applying procedures recently developed for related alkaloids.
- To investigate in detail the inhibition of TryR by cadabicine, using a spectrophotometric assay.
- The synthesis and evaluation as TryR inhibitors, of other potential inhibitors based on the cadabicine diphenyl ether scaffold.

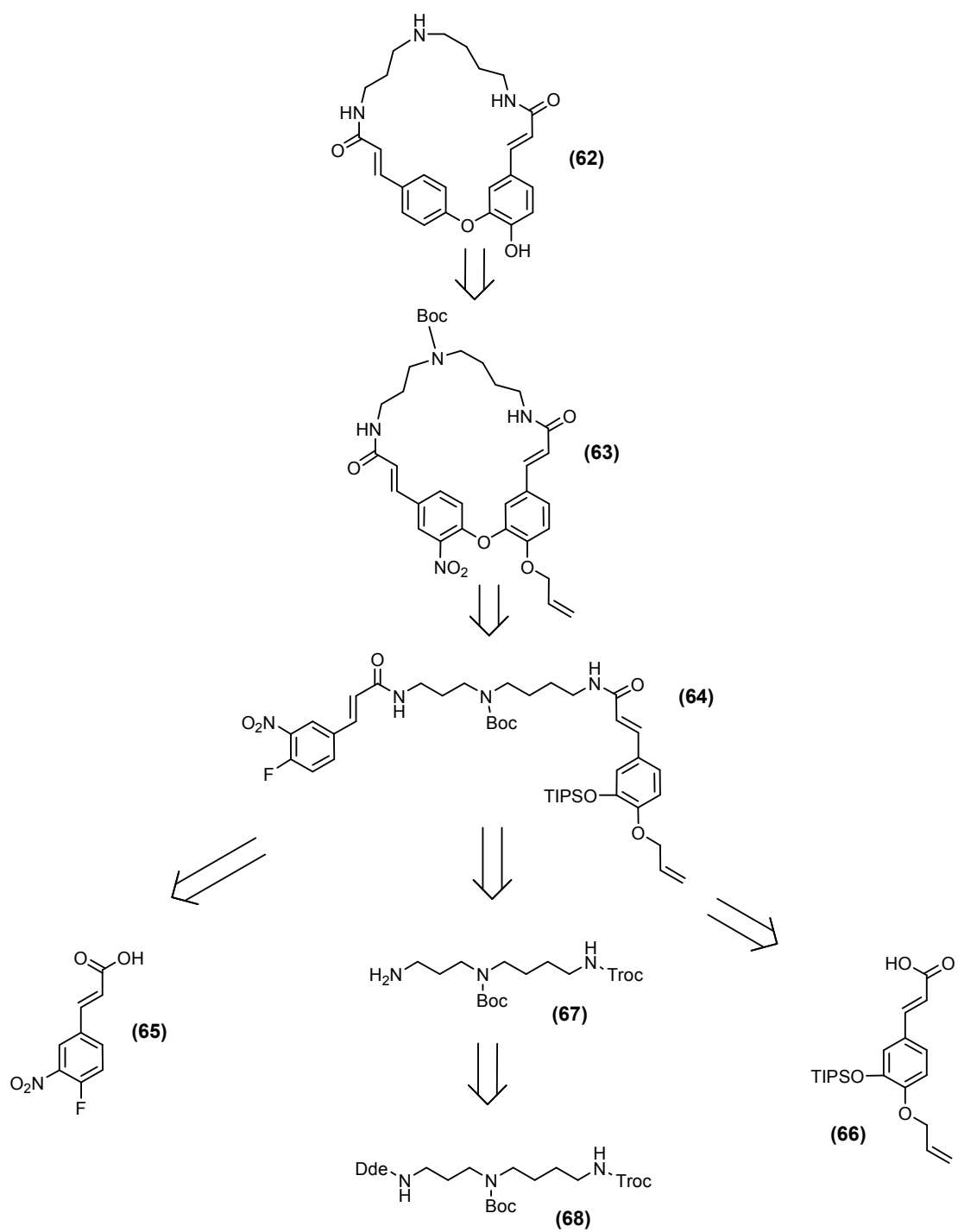
## 2. RESULTS AND DISCUSSIONS

### 2.1 RETROSYNTHESIS OF CADABICINE

The proposed synthetic approach to cadabicine is outlined in Scheme 2.1. The key features are formation of the macrocycle by a nucleophilic aromatic substitution reaction, and the preparation of two functionalised cinnamic acid units (**65**) and (**66**), for coupling to a novel orthogonally protected spermidine unit, derived from the triply protected compound (**68**).

The aim of this synthesis was to generate a fully protected spermidine derivative (**64**) that could be conveniently converted to the natural product. The choice of the protecting groups need to be compatible with the fluoronitrophenyl unit since it is potentially sensitive to both reduction and nucleophiles, and also the cinnamic acid units which are reducible.

Boc protection for the N<sup>4</sup> of spermidine and allyl ester protection for the phenol group were chosen with this in mind and to provide a simple final deprotection strategy. With N<sup>4</sup> protected with an acid-labile group, a protecting group that can be selectively cleaved by nucleophiles (in the presence of Boc) was suggested for N<sup>1</sup>, such as the Dde or phthaloyl group. Troc protection was initially chosen for N<sup>8</sup>, since it can be removed in the presence of phthaloyl and Boc with simple and cheap chemistry.



**Scheme 2.1: Retrosynthetic strategy for the synthesis of cadabicine**

## 2.2 SYNTHESIS OF ORTHOGONALLY PROTECTED SPERMIDINE DERIVATIVES

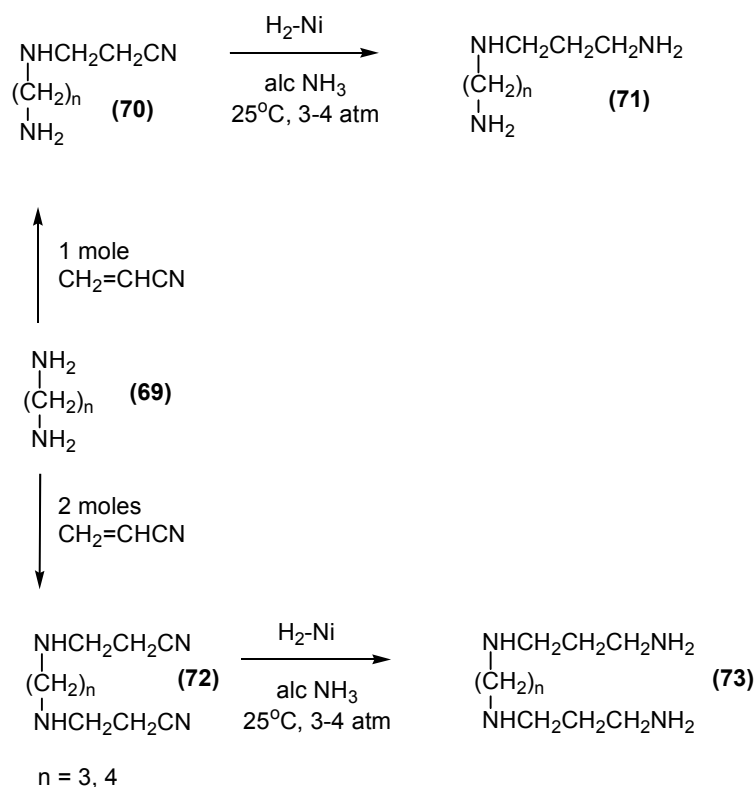
Aliphatic polyamines are widely found in a variety of biological systems having many interesting functions. These range from being part of toxins in spiders and wasps, through neurotransmission in humans and fruit ripening in plants, to ligands in inorganic chemistry; which makes them compounds of high synthetic interest. A problem often encountered in the synthesis is the selective protection of the amino groups. There are two ways to synthesise regioselectively derivatised polyamines. Either the existing primary and secondary amino functions in the polyamine may be selectively protected, or the desired polyamine skeleton can be assembled from scratch using protected building blocks. For the preparation of cadabacine, both approaches were followed. In one case, the polyamine skeleton was assembled via Mitsunobu chemistry using appropriately protected building blocks. Alternatively, the primary and secondary amino functions were differentiated via regioselective formation of a hexahydropyrimidine ring intermediate. In both cases, selective protection of the spermidine component allows regioselective acylation with the cinnamic acid units.

### 2.2.1 Fragment synthesis

In this approach the polyamine skeleton is assembled to the desired length by combining selectively protected alkylamine derivatives with other nitrogen containing compounds with the formation of new C-N bonds. This type of synthesis potentially offers a greater degree of flexibility in the design and preparation of a variety of complex polyamine analogues and conjugates.

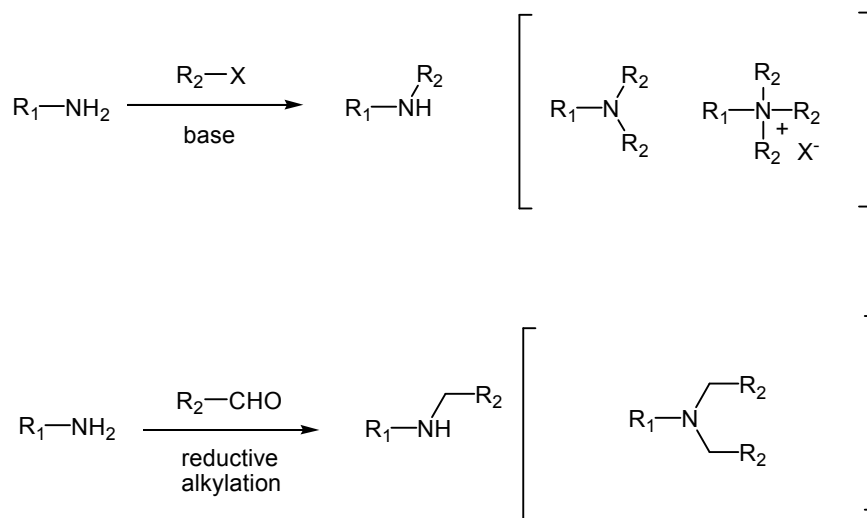
Conjugate addition of amino components to acrylonitrile, is one of the oldest and more frequently used methods for extending a polyamine chain by one or two aminopropyl moieties. This approach has been applied by Israel,<sup>50,51</sup> reacting diaminoalkanes (**69**) with one or two molecules of acrylonitrile, followed by catalytic hydrogenation of the nitrile group using Ni, or with LiAlH<sub>4</sub>. Either route provides easy access to various spermine (**73**) or spermidine-type molecules (**71**) (Scheme 2.2).





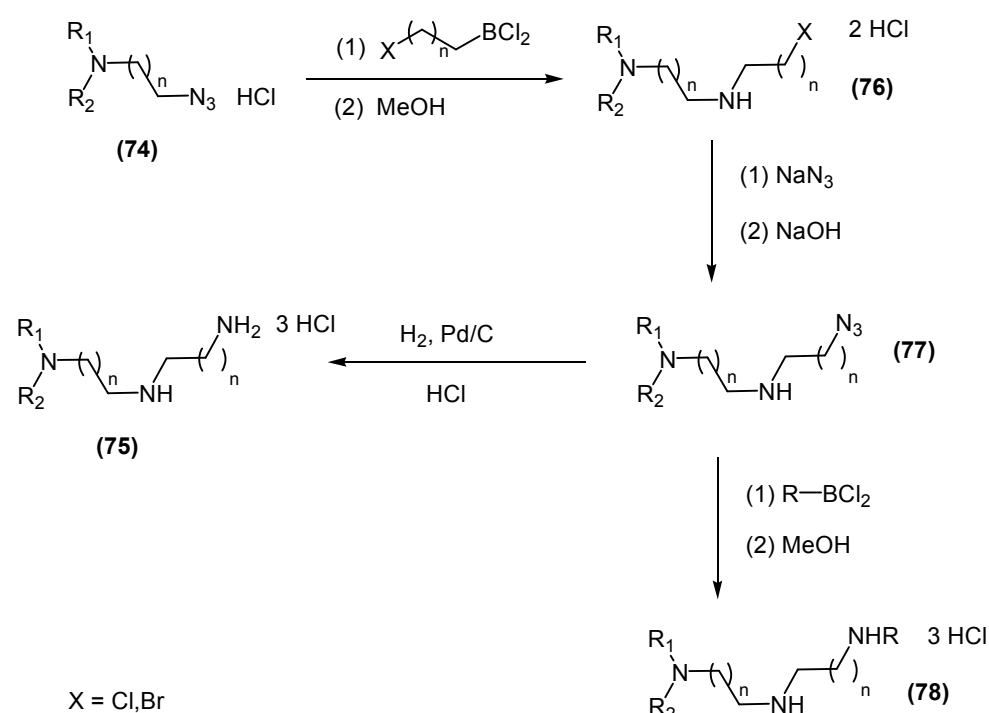
**Scheme 2.2: Israel's approach to polyamine synthesis.**<sup>50,51</sup>

Conversion of primary amines to the corresponding secondary amines appears deceptively simple. However, alkylation of primary amines with alkyl halides or sulfonates frequently leads to the formation of the undesired tertiary amine and/or quaternary ammonium salts. Furthermore, reductive alkylation with aldehydes or ketones using  $\text{NaBH}_3\text{CN}$  often produces tertiary amines, unless the desired secondary amine is sterically hindered (Scheme 2.3).<sup>52</sup>



**Scheme 2.3: Conversion of primary amines to the corresponding secondary amines.**

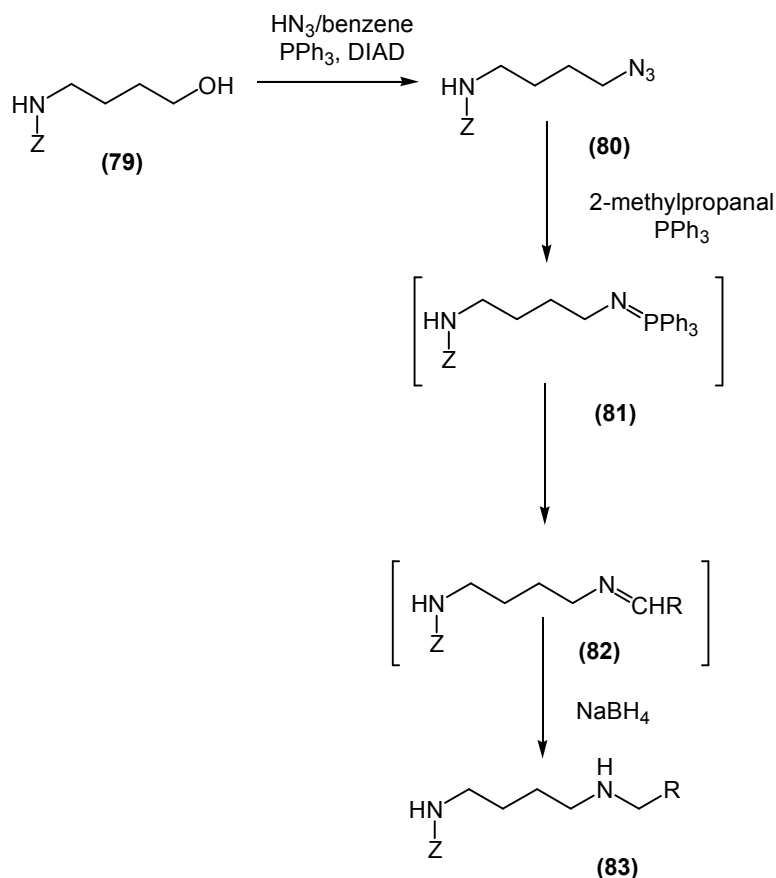
However, the reductive alkylation of an azide by a borane, provides an efficient chemoselective synthesis of secondary amines as discovered by H.C. Brown.<sup>53</sup> An extension of this work has been employed by Carboni,<sup>54</sup> who used aliphatic amino azides as key building blocks for polyamine synthesis (Scheme 2.4). The amino alkyl azide hydrochlorides were converted to diamine dihydrochlorides, by reductive alkylation using the appropriate dichloroboranes, followed by methanolysis. The diamine dihydrochlorides were then reacted with sodium azide followed by treatment with sodium hydroxide to give the corresponding azide, and hence increase the chain length. The azide was then either reduced by catalytic hydrogenation in order to give the primary amine, or by reductive alkylation followed by methanolysis in order to increase the chain further.



**Scheme 2.4: Aliphatic amino azides in polyamine syntheses**

Golding et al<sup>55</sup> has also used azides as synthons in polyamine synthesis (Scheme 2.5). This approach begins with Z-protected aminobutanol, which is converted to the azide (**80**) via a Mitsunobu reaction. The azide is then reacted with triphenylphosphine in a Staudinger reaction to give the iminophosphorane (**81**) which is in turn reacted with

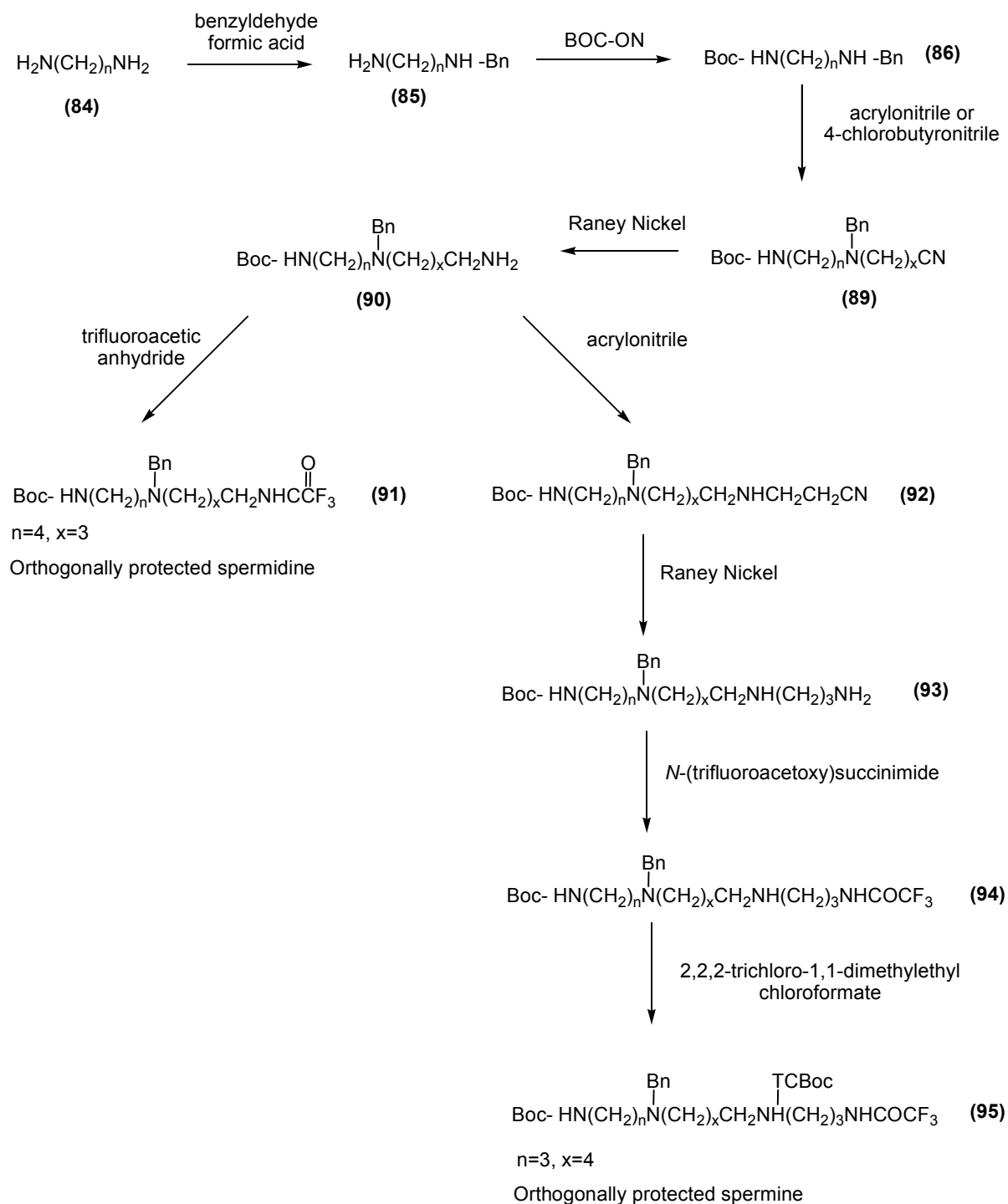
an aldehyde in an aza-Wittig reaction to give an imine (**82**) which is finally reduced *in situ* to give a secondary amine (**83**).



**Scheme 2.5: Golding's** <sup>55</sup> **approach to polyamine synthesis.**

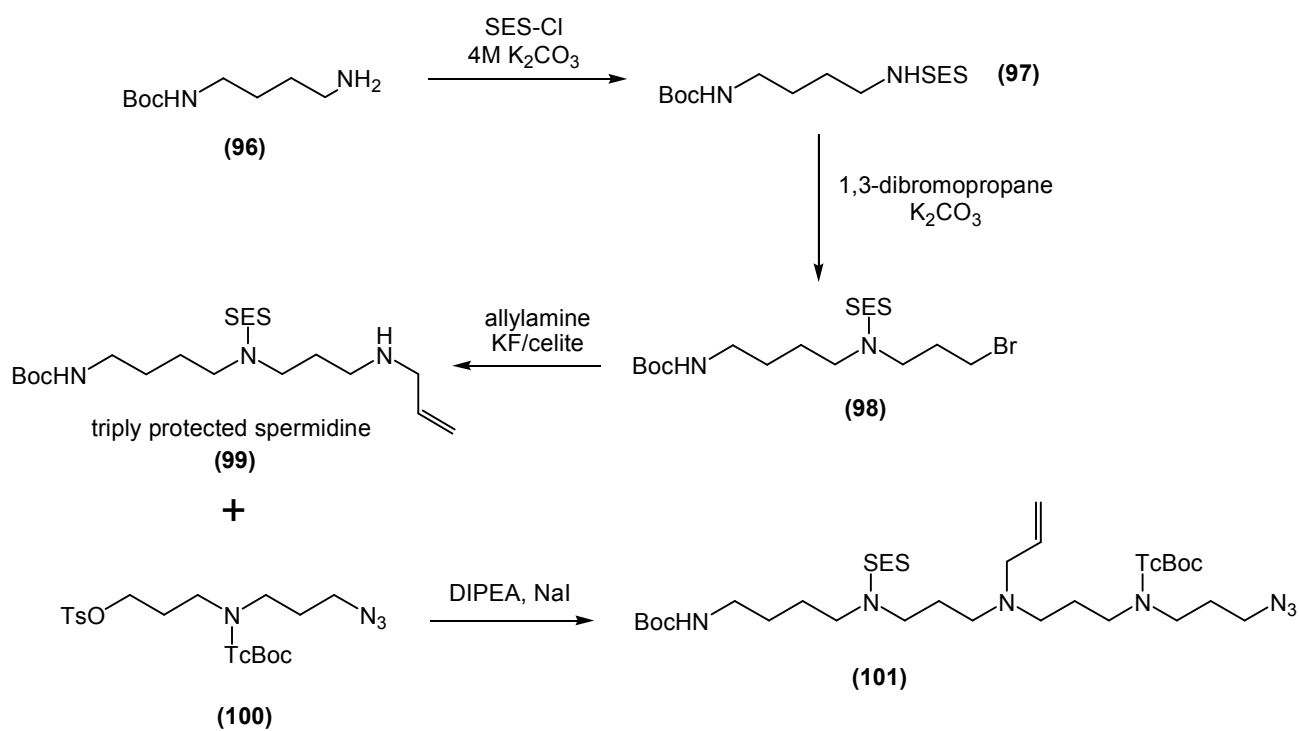
Bergeron<sup>56</sup> has taken Israel's approach one step further in order to generate an orthogonally protected spermidine and spermine. He successfully and in high yield monobenzylated putrecine (**84**) with benzaldehyde under reductive amination conditions. The monoprotected diamine (**85**) was further Boc protected to give the doubly protected (**86**). In order to increase the length of the chain, cyanoethylation of (**86**) with acrylonitrile was employed to give the nitrile (**89**) which was subsequently reduced with Raney Ni to give (**90**). Finally acylation of the primary amino group in (**90**) with trifluoroacetic anhydride according to the method described by O'Sullivan<sup>57</sup> gave the desired orthogonally protected spermidine (**91**). As for the synthesis of spermine, he started with spermidine precursor (**90**), in which  $\text{N}^8$  is free, where cyanoethylation and further reduction provided the spermine skeleton (**93**). The primary amino  $\text{N}^{12}$  was then acylated with trifluoroacetoxy succinimido ester in order to selectively protect the primary over the secondary amine. The remaining  $\text{N}^8$

secondary amine was then protected with the 2,2,2-trichloro-*tert*-butoxycarbonyl (TCBoc) group to give **(95)** which is stable to acidic and basic conditions and can be removed without affecting any of the other protecting groups by mild metal reduction (Scheme 2.6).



**Scheme 2.6 : Bergeron synthesis of orthogonally protected spermidine and spermine derivatives.**

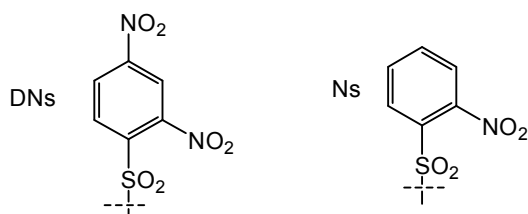
Hesse<sup>58</sup> has also described the synthesis of an orthogonally protected spermine by fragment synthesis as a building block for a fully *N*-functionalised pentamine, homocaldopentamine (**101**). This approach begins with mono-Boc protected 1,4-diaminobutane (**96**), then the remaining primary amine was further protected with a trimethylsilylethylsulfonyl group. The chain was then elongated by alkylation using 1,3-dibromopropane and potassium carbonate as base, creating the bromide which was finally converted to the spermidine reagent (**99**) using allylamine. This triply protected spermidine (**99**) was then coupled to the building block (**100**) to give the fully penta *N*-protected homocaldopentamine (**101**), where all five groups can be independently removed. TFA was used to remove Boc, CsF to remove SES, mild Zn reduction to remove TcBoc, (Ph<sub>3</sub>P)<sub>4</sub>Pd to remove the allyl group, and mild reduction using Ph<sub>3</sub>P with water to reduce the azido group.



**Scheme 2.7:** Synthesis of penta *N*-protected homocaldopentamine.

### 2.2.2 Spermidine by Fragment Synthesis

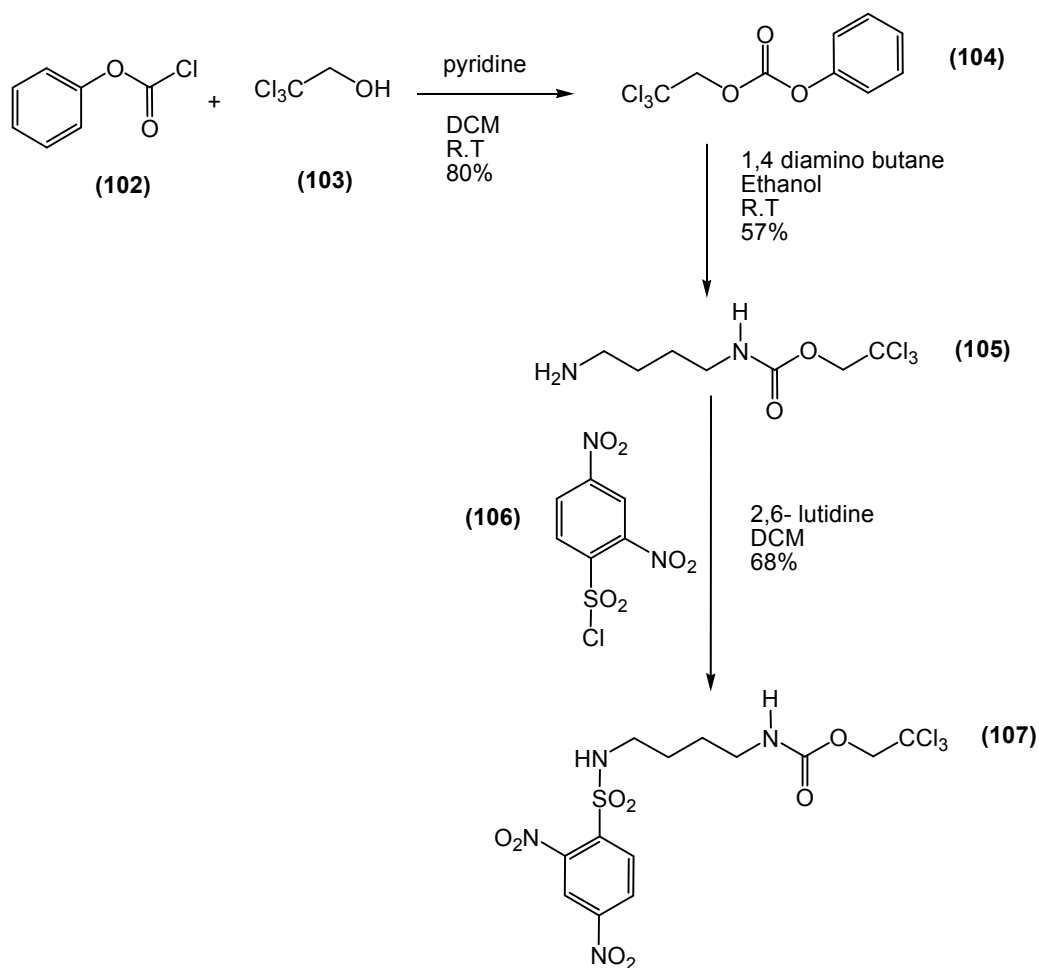
For the synthesis of cadabacine we chose to apply chemistry developed by Fukuyama,<sup>59</sup> which uses the 4- or 2,4-dinitrophenylsulfonyl group (Ns or DNs) as both a protecting and activating group in the synthesis of amines (Figure 2.1).



**Figure 2.1: 2,4-dinitrobenzenesulfonyl and 2-nitrobenzenesulfonyl protecting groups**

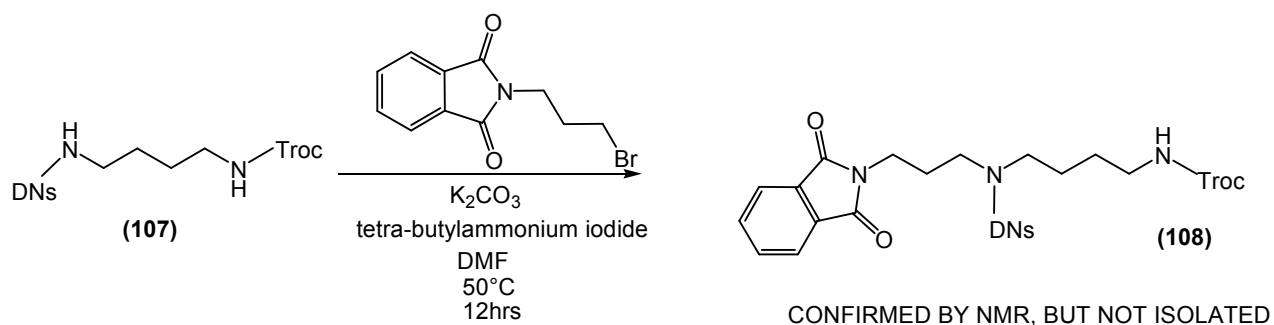
The alkylation of sulfonamides of this type under conventional or Mitsunobu conditions with another nitrogen-containing unit allows a polyamine skeleton to be constructed. In this case, the initial plan of synthesis adopted was to take a diaminoalkane and protect one primary amine function with a nitrophenylsulfonyl group. The remaining primary amino function would then be protected with a compatible protecting group, and then the formation of a new C-N bond would be achieved by an alkylation or Mitsunobu reaction of the doubly protected diaminoalkane with a suitably orthogonally protected amino component, in order to make a triply protected spermidine derivative.

Troc-phenyl carbamate (**104**) was synthesised in order to selectively protect just one of the primary amino groups of the diamine,<sup>60</sup> to give compound (**105**) in 57% yield. Compound (**105**) was then reacted with 2,4-dinitrobenzenesulfonyl chloride, to make the desired doubly protected diamine<sup>61</sup> (**107**) in 68% yield (Scheme 2.8).



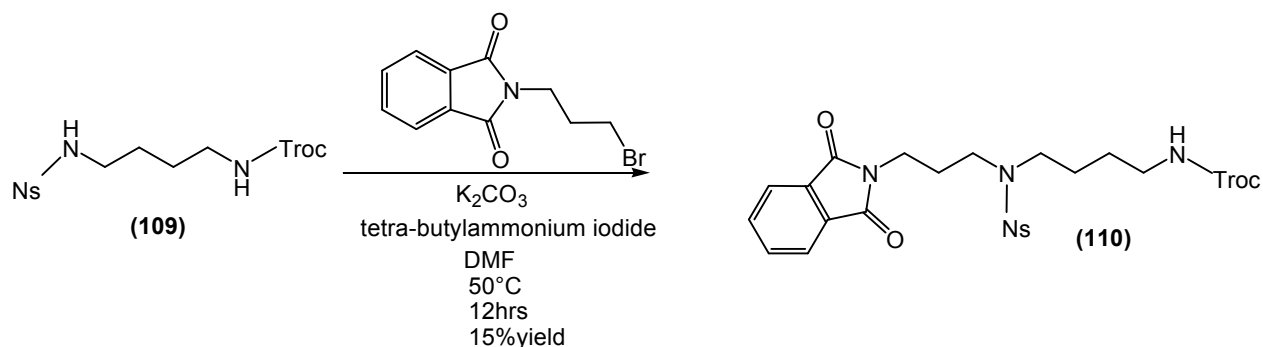
**Scheme 2.8: Preparation of doubly protected diamine.**

In order to make the orthogonally protected spermidine, a number of displacement reactions were tried.<sup>62</sup> The doubly protected diamine was reacted with 3-bromopropyl phthalimide, potassium carbonate and tetrabutylammonium iodide (Scheme 2.9). This reaction gave a complex mixture of products, and although the formation of the desired product (**108**) was confirmed by NMR, it was not possible to isolate it.



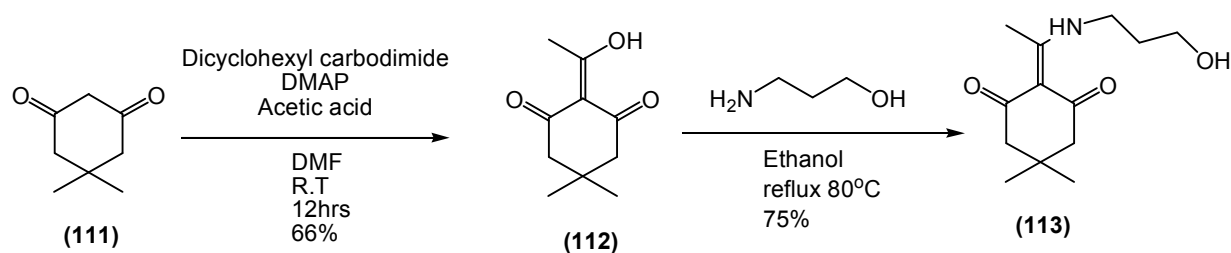
**Scheme 2.9: Displacement reaction with DN-protected diamine.**

The reaction was repeated, using the 2-nitrobenzenesulfonyl protected diamine (**109**) (prepared in the same way as (**107**)), which resulted in the desired product (**110**) but in low yield (15% - Scheme 2.10). The reaction was repeated using caesium carbonate, but although (**110**) was successfully obtained, there was no change in the yield.



**Scheme 2.10: Displacement reaction with Ns-protected diamine**

It was suspected that the bulk of the phthalimido group in the three carbon unit was hindering alkylation reactions. Therefore, a different protecting group was used, which can be removed under the same conditions as phthaloyl. The Dde protecting group was adopted. The reagent for the introduction of Dde (**112**) was synthesised by reacting equal amounts of dimedone with DCC and acetic acid, using DMAP as a catalyst. This gave (**112**) in 66% yield. (**112**) was then used to protect 3-aminopropanol<sup>63</sup> and give (**113**), one of the protected building blocks needed for the triply protected spermidine (Scheme 2.11).

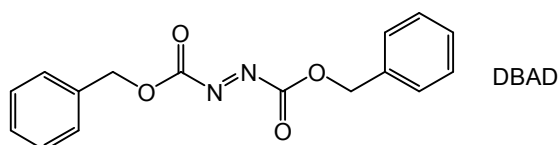
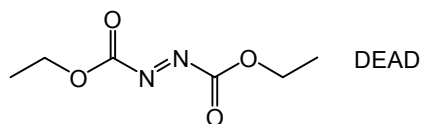


**Scheme 2.11: Preparation of Dde-protected amino propanol (113) .**

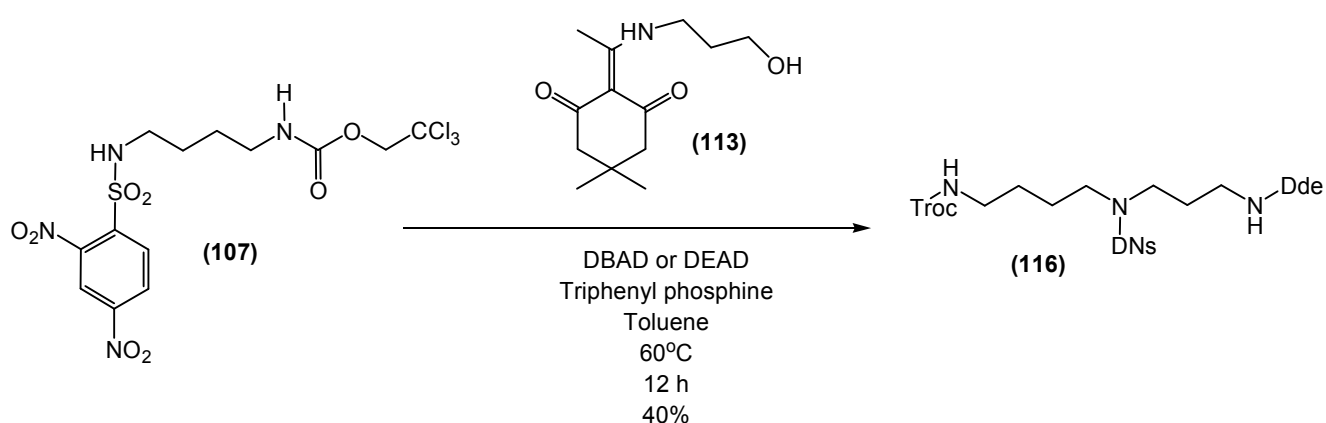
The doubly protected diamine (**107**), was now reacted with Dde-amino propanol (**113**), via a Mitsunobu reaction, to give the desired triply protected spermidine (**116**). The reaction was successful, but triphenylphosphine oxide, which is a by-product of the reaction was found to co-elute with the desired material. To overcome this



problem, the Mitsunobu reaction was repeated using polymer-supported triphenylphosphine,<sup>64</sup> which gave the desired product (**116**) in 40% yield, without contamination (Scheme 2.12). The use of different Mitsunobu reagents was also investigated, such as diethyl azodicarboxylate (DEAD) and dibenzyl azodicarboxylate (DBAD), but there was no difference in the yield of (**116**).

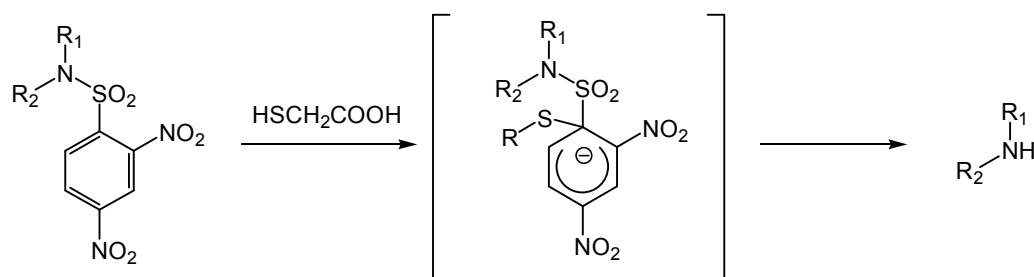


**Figure 2.2: Structure of Mitsunobu reagents DEAD, DBAD.**



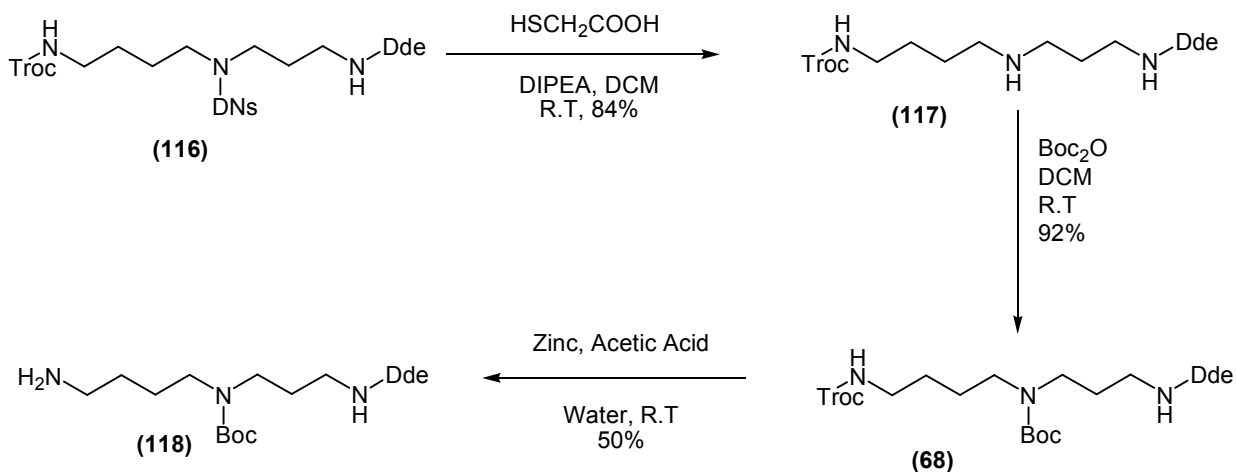
**Scheme 2.12: Mitsunobu Reaction.**

The DNs group in the triply protected spermidine (**116**) was removed using thioglycolic acid<sup>65</sup> presumably via the Meisenheimer complex (Scheme 2.13) to give the desired DNs-deprotected material (**117**) in 84% yield.



**Scheme 2.13: DNs deprotection via the Meisenheimer complex.**

(117) was Boc-protected using Boc-anhydride, to give the orthogonally protected spermidine (68) in 92% yield. The Troc group was then removed using zinc and acetic acid,<sup>66</sup> which gave the desired *N*-8 deprotected product (118) in 50% yield (Scheme 2.14).

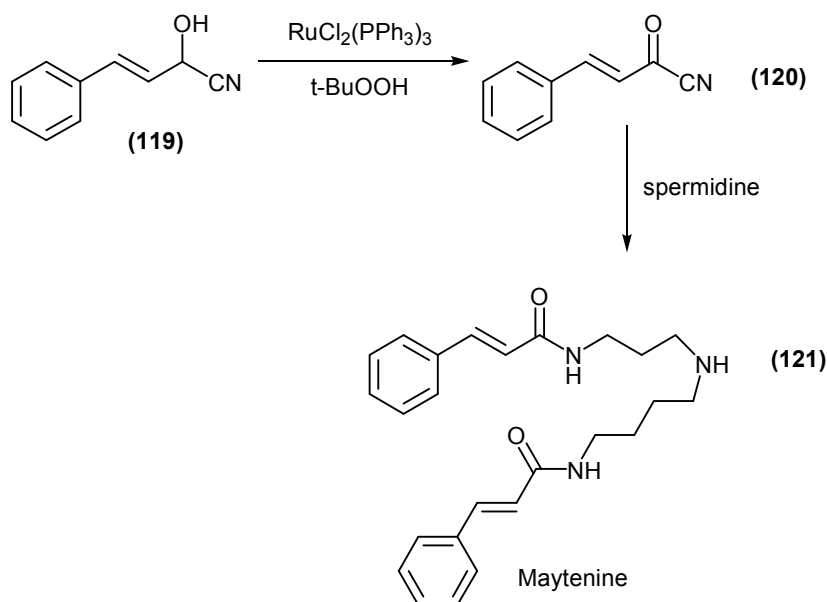


**Scheme 2.14: Fragment synthesis of triply protected spermidine.**

### 2.2.3 Selective Functionalisation.

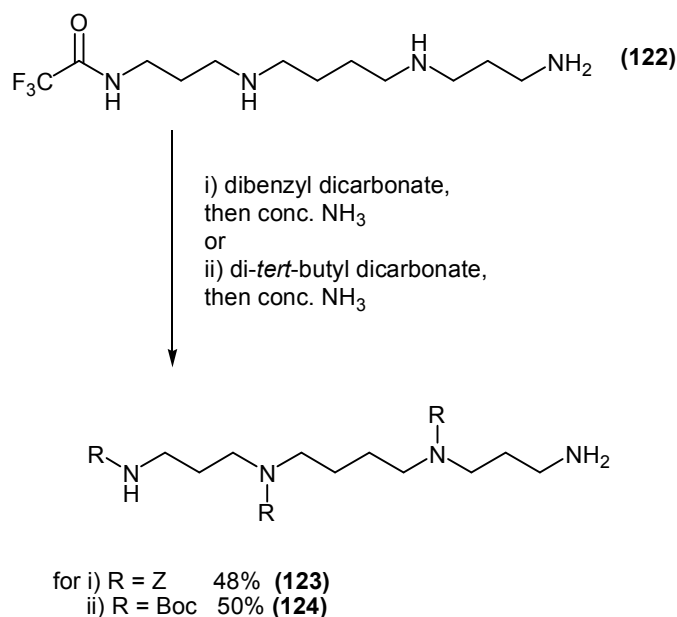
In selective functionalisation synthesis starts with an intact spermidine. Primary amines can be selectively protected in the presence of secondary functions with a number of different protecting groups, such as the phthaloyl (using PhthN-CO<sub>2</sub>Et), benzyloxycarbonyl (using Z-CN)<sup>67</sup>, *tert*-butoxycarbonyl (using Boc-Osu), trifluoroacetyl (using Tfa-OEt)<sup>57</sup>, the Trt (using Trt-Cl)<sup>68</sup>, the Mtr (using Mtr-Cl)<sup>69</sup> and the *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene) (using 2-acetyl dimedone)<sup>70</sup> groups. Reagents like Z-CN give selectivity for primary over secondary because they are less reactive than related reagents like Z-Cl. The same is true for ethyl trifluoroacetate, which is used in O'Sullivan's<sup>57</sup> approach – this is less reactive than trifluoroacetyl chloride or trifluoroacetic acid anhydride. These protecting groups may be introduced to polyamines in high yields and can be removed using relatively mild conditions, such as hydrazinolysis (Phth, Dde), acidolysis (Z, Boc, Trt, Mtr), catalytic hydrogenolysis (Z, Trt) and hydrolysis with weak bases (Tfa).<sup>71</sup>

For example Murahashi<sup>67</sup> has protected spermidine with benzoyl cyanide (**120**) to give *N*<sup>1</sup>, *N*<sup>8</sup>-bisbenzoylspermidine. Since a variety of acyl cyanides can easily be prepared, he also directly acylated spermidine with trans-cinnamoyl cyanide to obtain an excellent yield of the alkaloid maytenine (**121**).



**Scheme 2.15: Murahashi's synthesis of Maytenine.**<sup>67</sup>

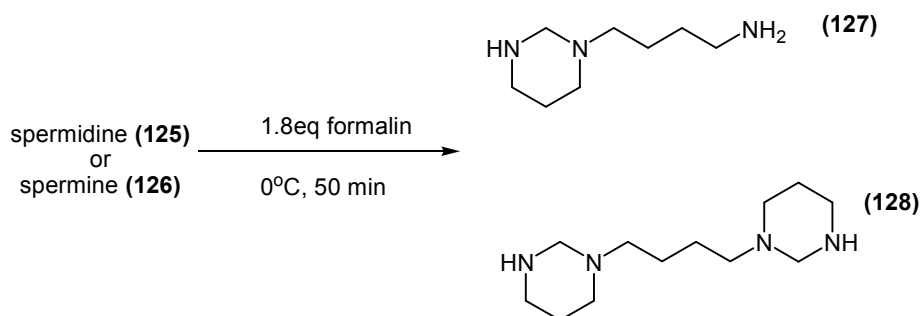
Following protection of primary amino functions, the secondary amino functions may be derivatised, or blocked with different (orthogonal) protecting groups, followed by deprotection of the primary amino functions if necessary. For example, Blagbrough<sup>72</sup> selectively differentiated the two secondary and one primary amino group of spermine, by first preparing *N*<sup>1</sup>-Tfa-spermine using  $\text{CF}_3\text{CO}_2\text{Et}$  at low temperatures. *N*<sup>1</sup>,*N*<sup>4</sup>,*N*<sup>9</sup>-tris(benzyloxycarbonyl)spermine and *N*<sup>1</sup>,*N*<sup>4</sup>,*N*<sup>9</sup>-tris(-tert-butoxycarbonyl)spermine, were obtained in moderate yields after chromatographic purification.



**Scheme 2.16: Indirect functionalisation of spermine.**<sup>72</sup>

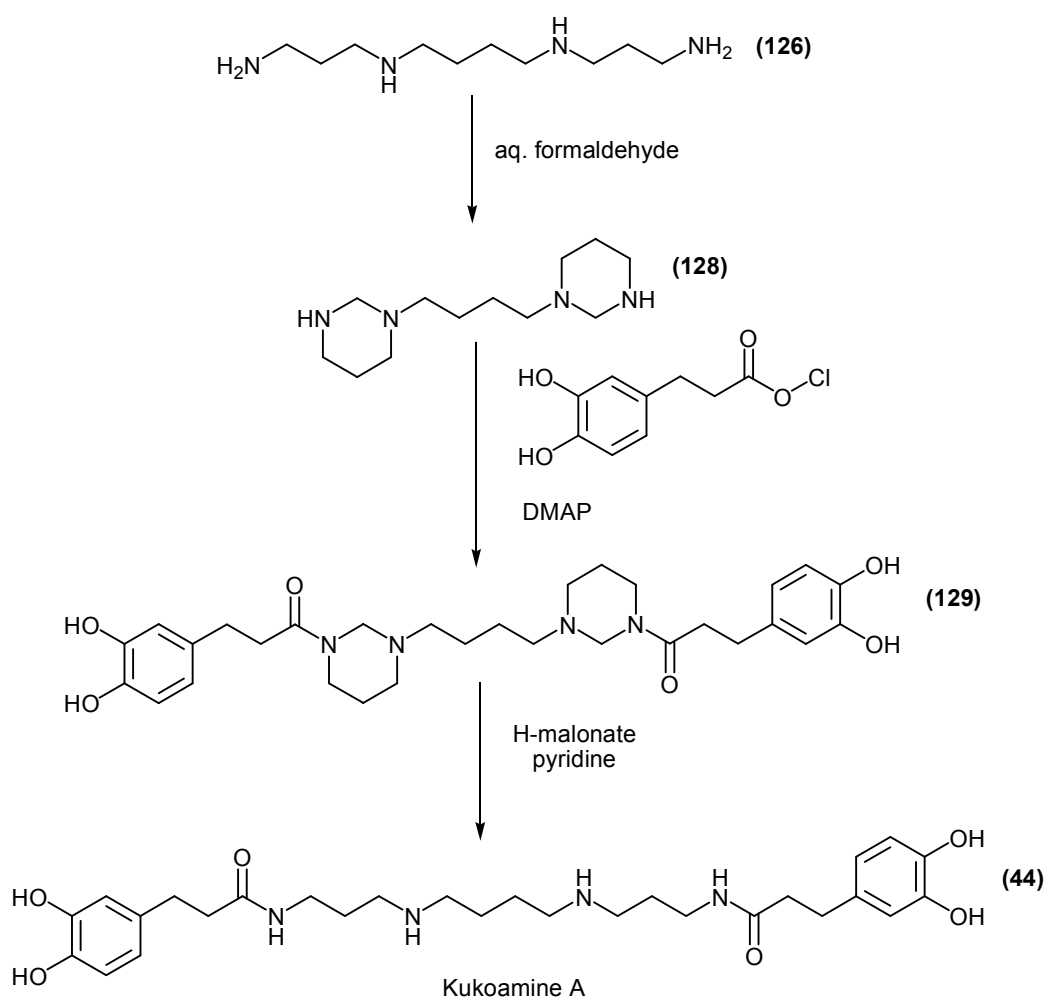
Macrocyclic polyamines, have been synthesised the same way, starting with N<sup>1</sup>,N<sup>8</sup>-Tfa spermidine.<sup>73</sup> Golding *et al.*<sup>74</sup> has used a similar approach, to reliably and efficiently prepare N<sup>1</sup>,N<sup>8</sup>-disubstituted spermidine derivatives, from spermidine either directly or via intermediate protection at N<sup>4</sup> with the 4-azidobenzyloxycarbonyl group, and/or at N<sup>1</sup> and N<sup>8</sup> with the trifluoroacetyl group. Similar methodology was applied in the synthesis of N<sup>4</sup>,N<sup>9</sup>-bis(trityl)spermine starting from N<sup>1</sup>,N<sup>12</sup>-bis(trifluoroacetyl)spermine.<sup>75</sup>

The classical and most often used method for the protection of the secondary amino groups in polyamines that bear the N-monosubstituted 1,3-diamine moiety like spermidine and spermine, has been developed by Ganem *et al.*<sup>76,77</sup> and involves their reaction with formaldehyde. Reaction of spermidine and spermine with formaldehyde leads to the corresponding mono- (**127**) and bis(hexahydropyrimidine (**128**) derivatives (Scheme 2.17).



**Scheme 2.17: Ganem's hexahydropyrimidine polyamine derivatives.**<sup>77</sup>

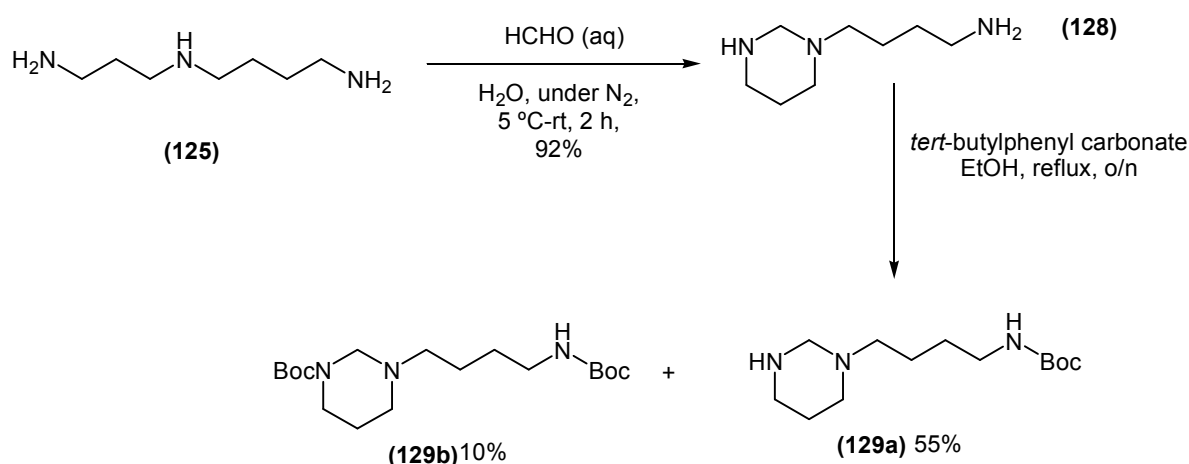
The mono-hexahydropyrimidine is a very interesting intermediate, as it can be selectively acylated at the primary amino function to deliver N<sup>8</sup> functionalised or protected spermidine, which in turn can be used in the synthesis of N<sup>1</sup> functionalised spermidine. Alternatively amino functions can be acylated to provide a N<sup>1</sup>, N<sup>8</sup>-functionalised spermidine. In either case the methylene protective group can easily be removed via a Knoevenagel condensation using ethylhydrogen malonate and pyridine. This methodology has been used for the synthesis of the antihypertensive agent Kukoamine A<sup>78</sup> (**44**) and related spermine and spermidine derivatives.<sup>38</sup> (Scheme 2.18)



**Scheme 2.18: Synthesis of Kukoamine A.**

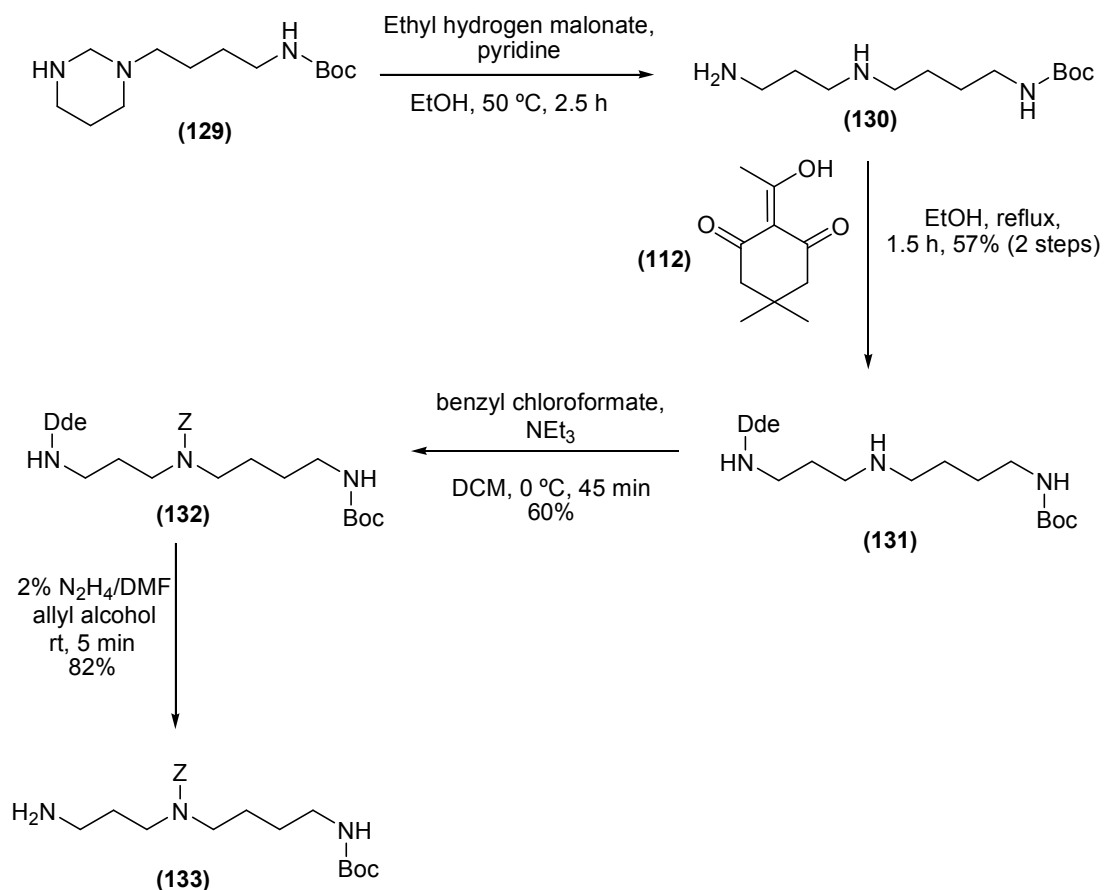
## 2.2.4 Spermidine synthesis by Selective Functionalisation

Ganem's methodology was applied here for the synthesis of cadabicine (**62**). Spermidine (**125**) was reacted with formaldehyde, to give the tetrahydropyrimidine derivative (**128**) in 92% yield. The primary amino group of compound (**128**) could now be selectively Boc protected using *tert*-butylphenyl carbonate<sup>60</sup> to give 55% of the monoprotected hexahydropyrimidine compound (**129a**) and 10% of the doubly protected (**129b**).



**Scheme 2.19: Selective functionalisation of spermidine.**

The methylene protecting group was removed via Knoevenagel condensation, by reacting (**129a**) with mono-ethylmalonate and pyridine, to give the N<sup>8</sup>-Boc protected spermidine (**130**). Without any further purification (**130**) was used directly in the next step, where N<sup>1</sup> primary amino group was selectively protected using 2-acetyl dimedone (Dde)<sup>70</sup> to produce the N<sup>1</sup>, N<sup>8</sup> bi-functionalised spermidine (**131**) in 57% yield over the two steps. The remaining N<sup>4</sup> secondary amine was then protected with the benzyloxycarbonyl group using benzyl chloroformate to give the orthogonally protected spermine (**132**) in 80% yield. Any of the three protecting groups, Boc, Z, Dde, can be independently removed from one another. For the synthesis of cadabicine N<sup>1</sup> was initially deprotected using hydrazine in DMF and allyl alcohol to give the N<sup>1</sup> deprotected spermidine (**133**) in 82% yield. Even though in this instance there are no such moieties, if N<sup>8</sup> was to be acylated first with the cinnamic acid derivative, there would be the double bond from the cinnamoyl group to consider, and in this case allyl alcohol would prevent its reduction (Scheme 2.20). (See Section 2.3).

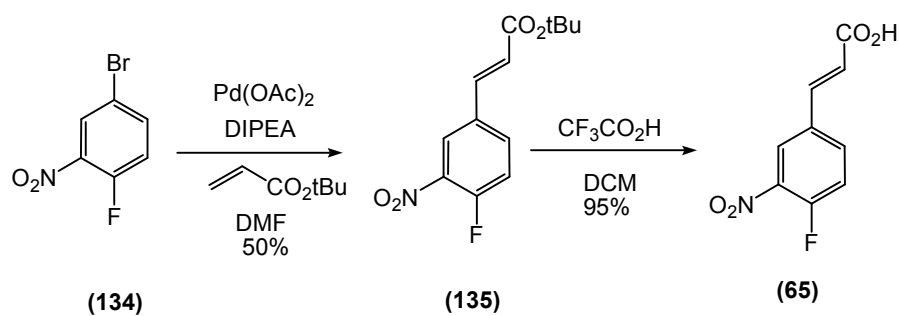


**Scheme 2.20: Synthesis of orthogonally protected spermidine**

## 2.3 SYNTHESIS OF CINNAMIC ACID DERIVATIVES AND COUPLING TO SPERMIDINE DERIVATIVES.

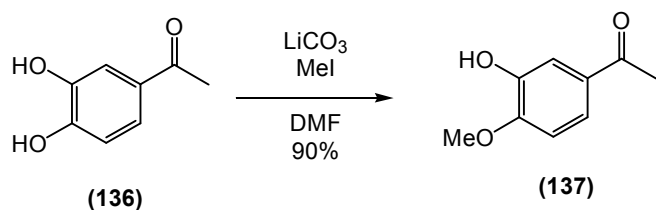
### 2.3.1 Synthesis of cinnamic acid derivatives.

Compound **(65)** was prepared via Heck coupling of 4-bromo-1-fluoro-2-nitrobenzene with *tert*-butyl acrylate to give the corresponding cinnamic acid ester **(135)** in 50% yield as shown in Scheme 2.21. DIPEA was used as base, instead of the more used triethylamine, since it had previously been observed that adducts from  $S_NAr$  reaction of the latter with the Heck product were also obtained. This was avoided by the use of a more bulky tertiary amine. The cinnamic acid ester **(135)** was then reacted with trifluoroacetic acid to cleave the *tert*-butyl ester and give compound **(65)** in 95% yield.



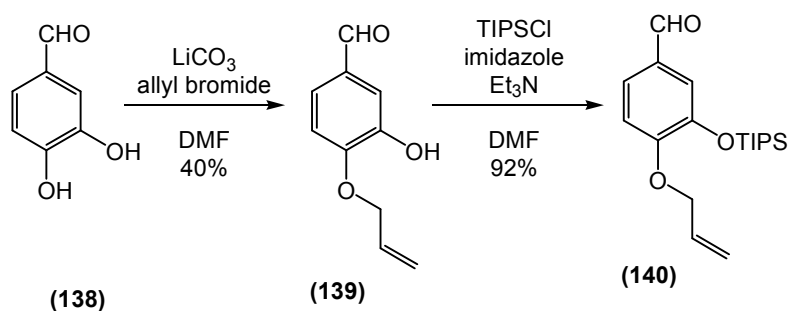
**Scheme 2.21: Preparation of compound (65)**

For the preparation of compound (66), 3,4-dihydroxybenzaldehyde was first regioselectively alkylated with allyl bromide and lithium carbonate (Scheme 2.23) to give 4-allyloxy-3-hydroxybenzaldehyde (139) in 40% yield. It was published by Pfister<sup>79</sup> that regioselectivity can be achieved in the alkylation of compounds of this type because of the greater acidity of the 4-hydroxyl compared to the 3-hydroxyl, due to the presence of an electron withdrawing 1-substituent (Scheme 2.22.).



**Scheme 2.22: Pfister's selective alkylation.**

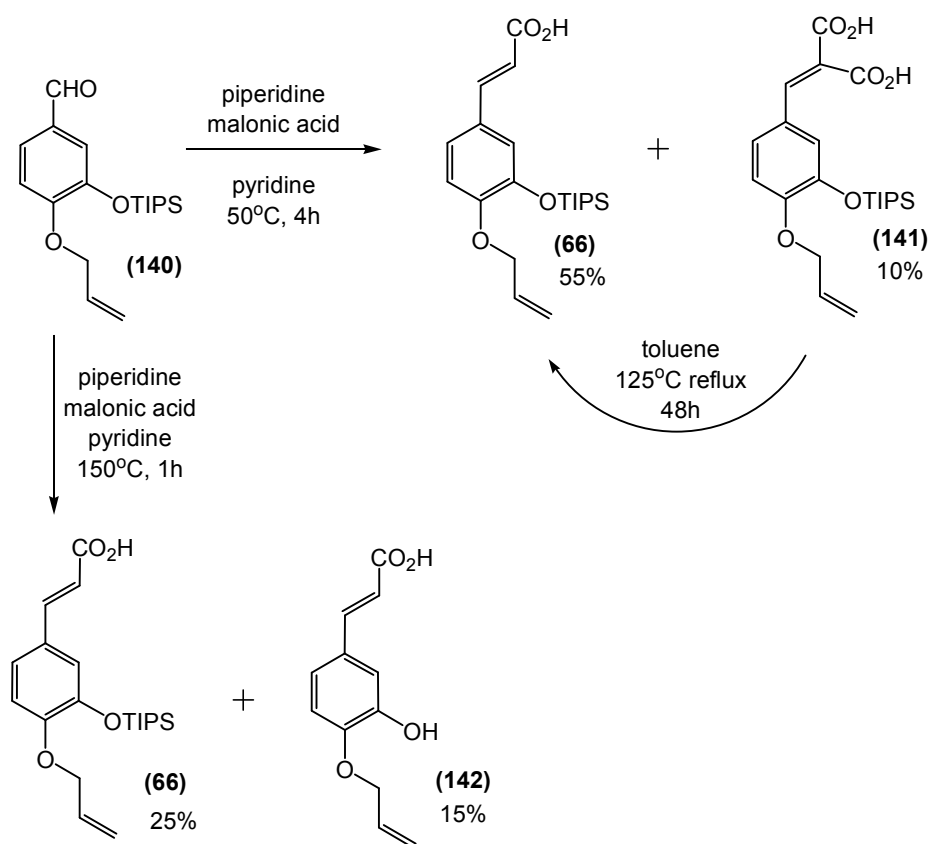
Other products obtained from this reaction were the 3-allyloxy-4-hydroxybenzaldehyde in 5% yield as well as traces of the dialkylated product. The free hydroxyl group of compound (139) was protected with a triisopropylsilyl group in 92% yield.



**Figure 2.23: Selective alkylation and TIPS protection.**



Finally, the doubly protected benzaldehyde derivative (**140**) was converted to (**66**) by a Doebner condensation with malonic acid in the presence of pyridine and piperidine. When the reaction was carried out at 150°C for 1 h, the desired cinnamic acid was obtained in only 25% yield, along with the desilylated derivative (**142**). Lowering the temperature to 50° avoided cleavage of the TIPS group, giving the desired product (**66**) in acceptable yield (55%). However under these conditions, decarboxylation of the diacid intermediate was incomplete and this derivative was also isolated. Fortunately, the diacid (**141**) could be readily converted to (**66**) by prolonged heating in an inert solvent (Scheme 2.24).<sup>80</sup>

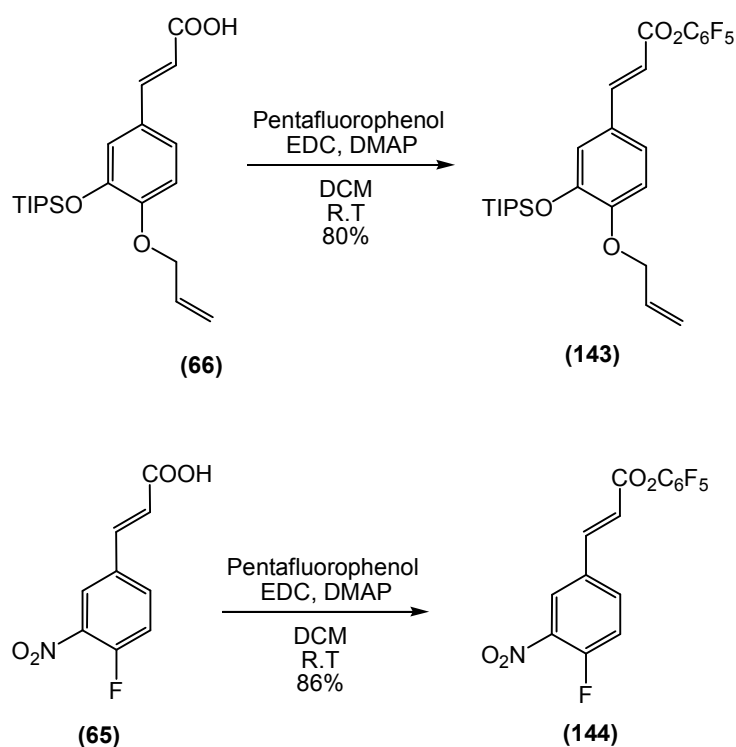


**Scheme 2.24: Preparation of compound (66).**

### 2.3.2 Activation of cinnamic acids.

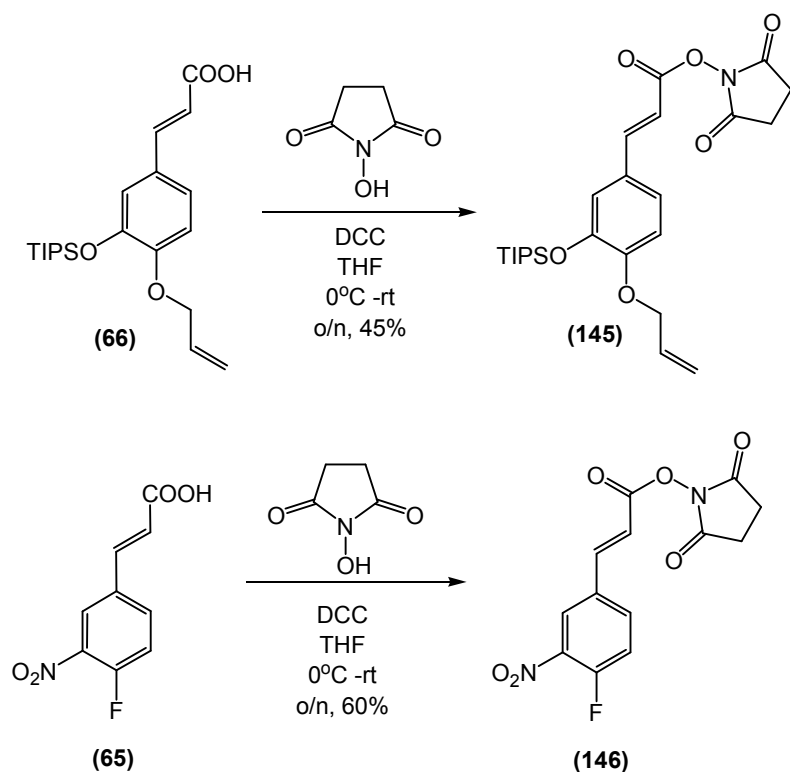
For coupling to a suitably protected spermidine derivative, both cinnamic acids were converted to their pentafluorophenyl and succinimido ester derivatives.

The activated pentafluorophenyl cinnamic acid esters (**143**) and (**144**) were derived by reacting the cinnamic acid derivatives (**66**) and (**65**) with pentafluorophenol and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, in the presence of DMAP as catalyst.



**Scheme 2.25: Preparation of pentafluorophenyl activated cinnamic acid esters (144) and (143).**

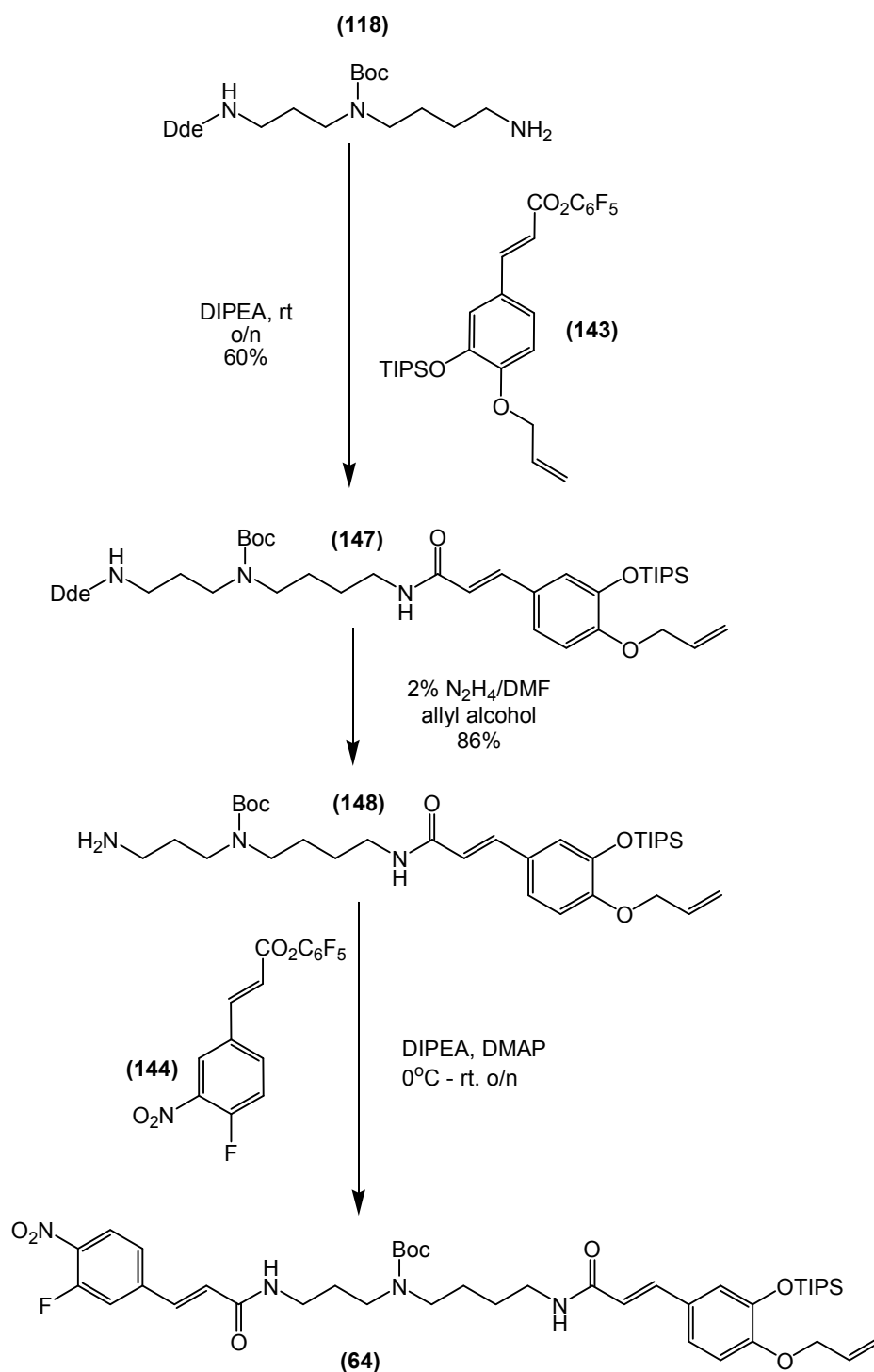
The preparation of the cinnamic acid succinimido esters (**145**), and (**146**) was achieved successfully by coupling the cinnamic acids (**66**) and (**65**) with *N*-hydroxy succinimide in the presence of DCC.



**Scheme 2.26: Preparation of succinimido activated cinnamic esters (145) and (146).**

### 2.3.3 Coupling activated cinnamic esters to spermidine derivatives.

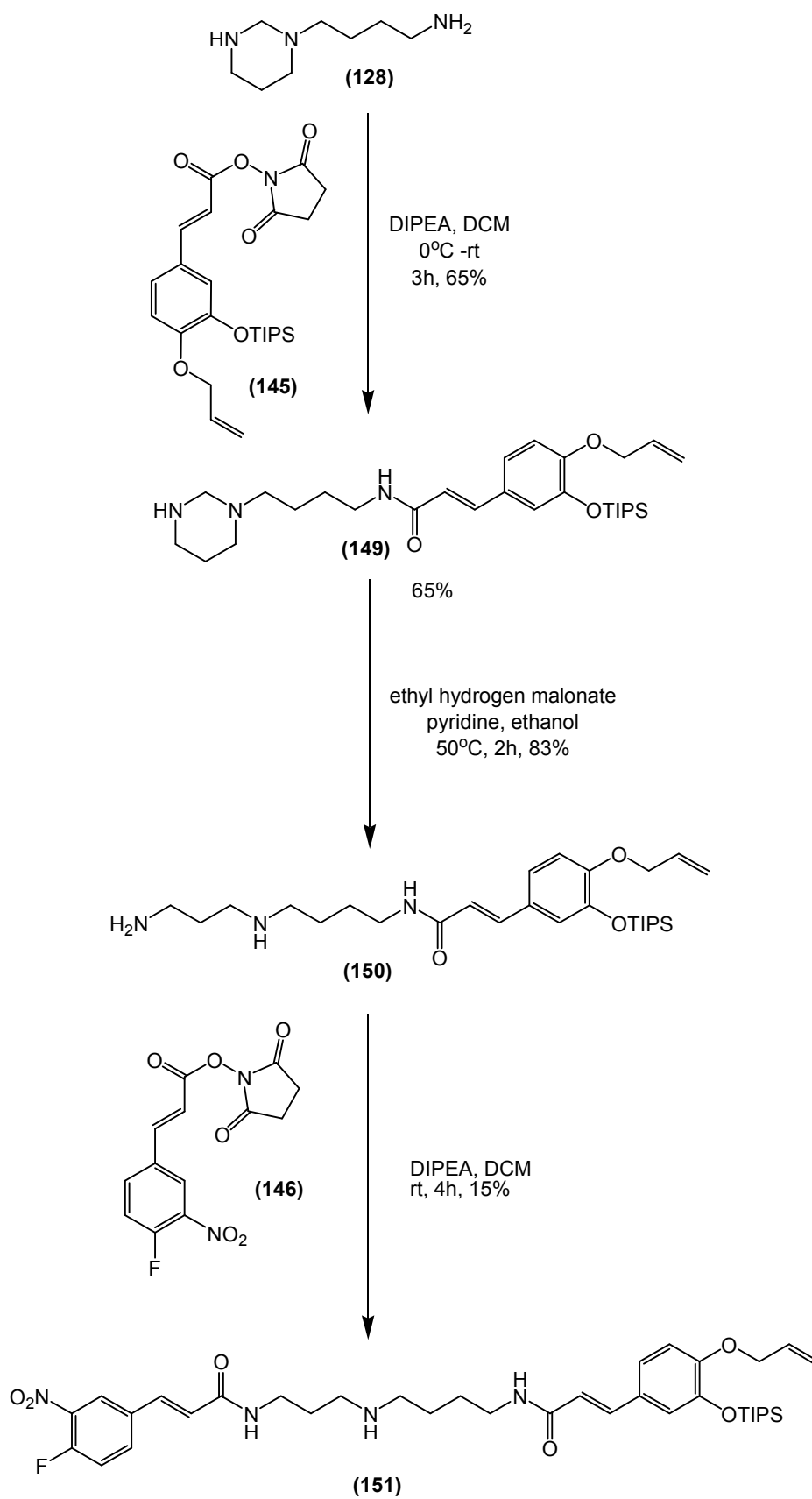
Starting with the orthogonally protected spermidine (**118**) derived by fragment synthesis, the pentafluorophenyl activated cinnamic acid ester (**143**) was coupled to the free N<sup>8</sup> amino group, to give the monoacylated compound (**147**) in 60% yield (Scheme 2.27). The selective removal of the N-1 Dde group was then attempted. Using hydrazine and allyl alcohol, the N<sup>1</sup> deprotected derivative (**148**) was obtained in 86% yield. According to Rohwedder<sup>81</sup>, the use of allyl alcohol and shorter reaction times for Dde deprotection, ensures that the possible side reaction of partial reduction of C=C double bonds is significantly reduced. Competing reduction is due to contamination of commercial hydrazine by diimide, which is scavenged by adding allyl alcohol. In this case the reaction was monitored by TLC, and was stopped when the starting material was consumed, after less than 10 minutes. No reduction of either the allyl ether or cinnamoyl unit was observed. Compound (**148**) was then coupled with activated cinnamic acid (**144**) to give the diacylated spermidine derivative (**64**), which was ready to undergo intramolecular cyclisation as seen in Scheme 2.1.



**Scheme 2.27: Synthesis of doubly acylated spermidine (64).**

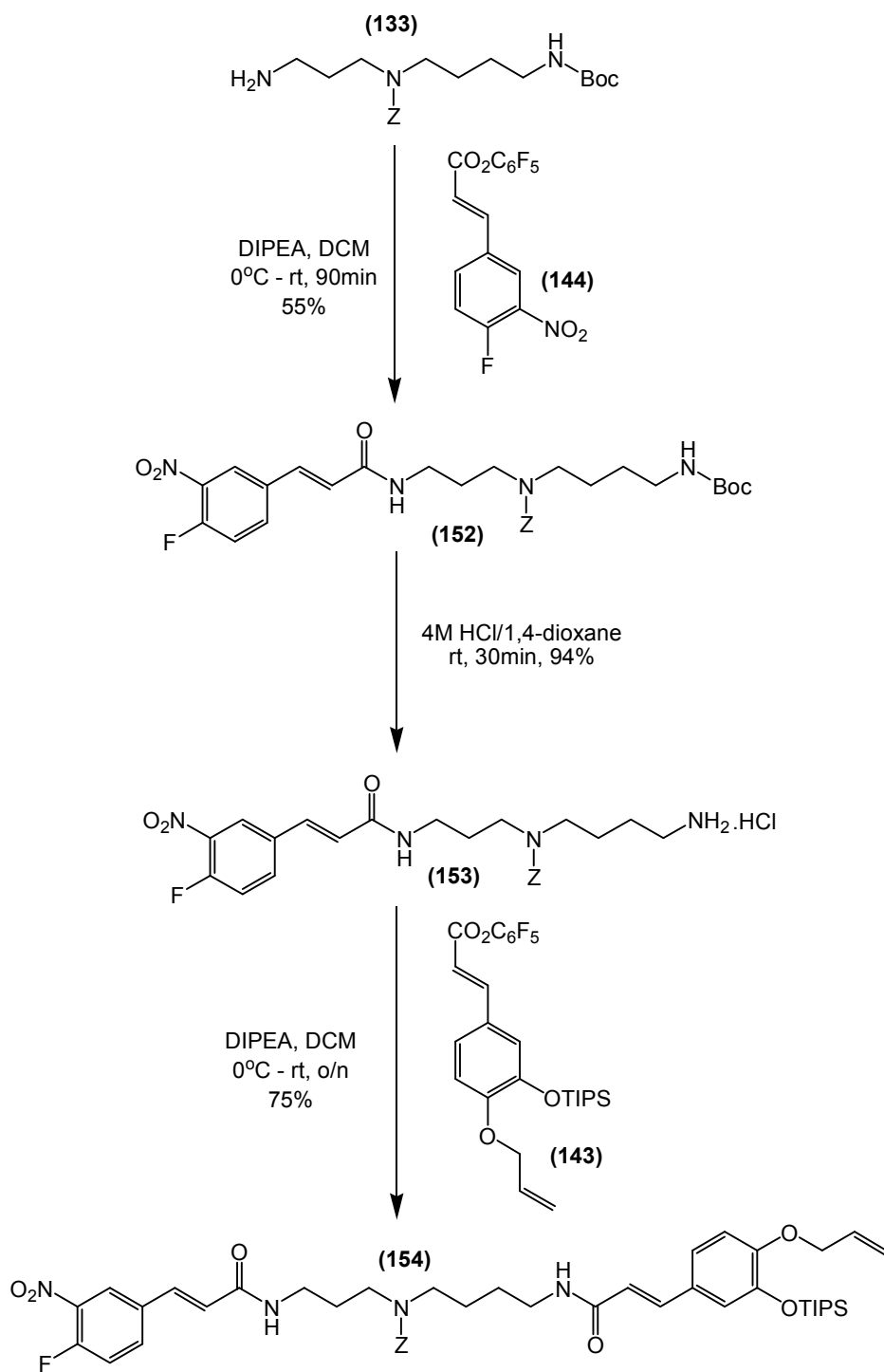
Meanwhile, coupling the activated cinnamic acid (143) directly to the hexahydropyrimidine derivative (128) was investigated. The first attempt to acylate the hexahydropyrimidine (128) with the pentafluorophenyl activated cinnamic ester

(143) was unsuccessful due to the lack of selectivity of the pentafluorophenyl activating group for the primary amino function over the secondary. Therefore activation via the succinimido ester was used which has been reported by Garnelis *et al*<sup>82</sup> to be selective for primary amines. Direct selective acylation of the primary amino function of the hexahydropyrimidine derivative (128), with the activated cinnamic ester (145) in the presence of base, gave the desired monoacylated product (149) in 65% yield, as well as the diacylated product in 4% yield. In order to obtain the desired spermidine derivative for reaction with activated ester (146), it was then necessary to open the hexahydropyrimidine ring of (149). This was achieved via a Knoevenagel reaction with ethyl hydrogen malonate in pyridine, which gave the desired compound (150) in 83% yield. This type of reaction has previously been described by Ponasik *et al*<sup>38</sup> for the synthesis of Kukoamine A (44) derivatives, and also by Husson *et al*<sup>83</sup> in the preparation of the spermidine natural product maytenine (121). In both these cases the Knoevenagel reaction was performed at significantly higher temperatures and for longer reaction times. As it was preferred here to avoid any possible cleavage of the TIPS protecting group in (149), the temperature was kept at 50°C, which appeared to have no effect on the efficiency of the ring opening. Direct selective acylation of the primary amino function of the spermidine derivative (150), with the activated cinnamic ester (146) was then attempted. The desired doubly acylated spermidine (151) was successfully isolated, but in a disappointing yield of 15%, with several other products being observed.



**Scheme 2.28: Doubly acylated spermidine (151).**

Since the yield for the second acylation reaction was inadequate, a new strategy was adopted. It was felt that the bulk of the triisopropyl silyl protecting group could be the reason why the second acylation was not very successful. One way this problem could be investigated, was to couple the pentafluorophenyl activated cinnamic acid (**144**), on the protected spermidine (**133**) first, followed by the coupling of the TIPS containing activated cinnamic acid (**143**). To do this, the N1 free amine of the orthogonally protected spermidine (**133**) synthesised by selective functionalisation in section 2.2.4, was coupled to activated cinnamic acid (**144**), to give the monoacylated compound (**152**) in 92% yield. In turn the Boc group was then cleaved, to give compound (**153**). This was finally ready to be coupled to the other activated cinnamic acid (**143**) to give the diacylated compound (**154**) in 75% yield. This method is much more efficient compared to the previous two and was adopted in generating more diacylated material for the continuation of the project (Scheme 2.29).



**Scheme 2.29: Synthesis of diacylated spermidine derivative (154).**



## 2.4 INTRAMOLECULAR CYCLISATION OF CADABICINE AND FINAL FUNCTIONALISATION.

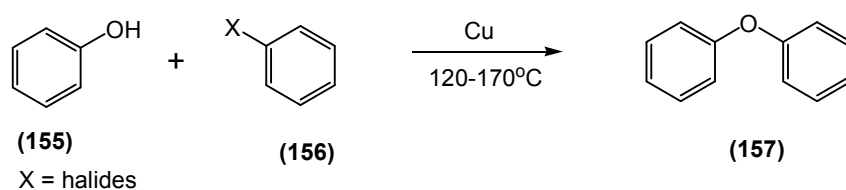
### 2.4.1 Diphenyl Ether Synthesis

Diphenyl ethers occur widely in a number of organic compounds throughout the life sciences and material chemistry. A number of compounds containing diphenyl ethers are biologically active, such as the antibiotic vancomycin<sup>84</sup>, antitumour compounds bouvardin and bastadin<sup>85</sup>, antimitotic agents like combretastatins<sup>86</sup> to name but a few.

### 2.4.2 Ullmann and Buchwald-Hartwig Diphenyl Ether Reactions.

#### i) Intermolecular Coupling reactions.

The classic synthesis of diphenyl ethers is under Ullmann conditions, which involve the coupling of aryl halides with phenols in the presence of copper powder or copper salts (Scheme 2.30). However it requires harsh conditions, with at least stoichiometric amounts of copper, large excess of phenols and high temperatures. This is due to the poor nucleophilicity of the phenoxide ion and the usually low reactivity of the aryl halides involved.<sup>87</sup>

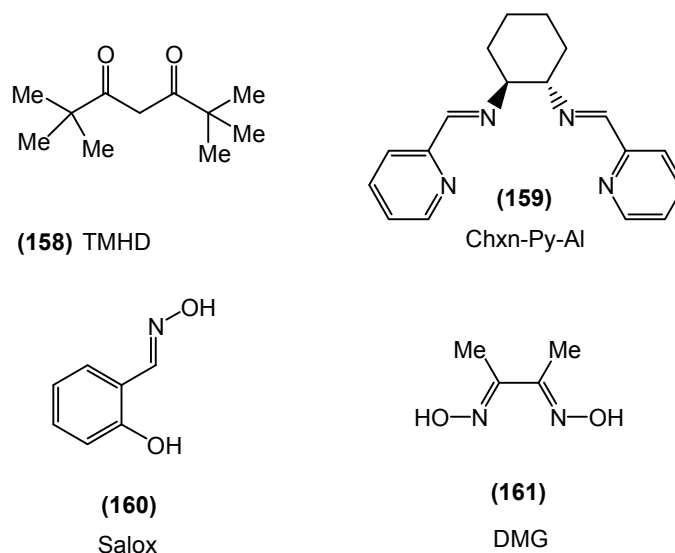


**Scheme 2.30: General scheme for Ullmann type coupling.**

Several ligands have been reported to improve the solubility of the cuprous ions which are the catalytic species in the Ullmann reaction, and when used along with a base this can improve the efficiency of the coupling, even at low temperatures.<sup>88</sup>

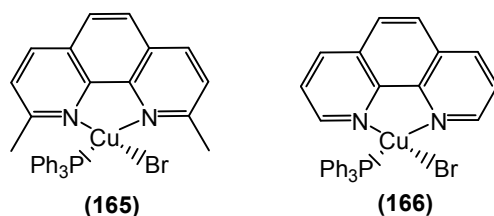
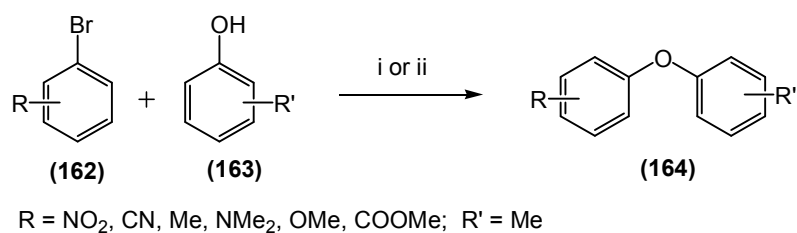
Examples include 2,2,6,6-tetramethylheptane-3,5-dione (TMHD)<sup>89</sup>, suitable for coupling various aryl bromides with phenols; *N,N*-di-methylglycine<sup>90,91</sup> suitable for both electron rich aryl halides and reaction at lower temperatures; Hauptman's<sup>92</sup>

variety of bi-dentate pyridine containing ligands; 1,10-phenanthrolines<sup>24</sup> suitable for aryl iodides and Cristau's<sup>93</sup> multidentate chelating ligands Chxn-Py-Al (**159**), salicylaldehyde oxime (Salox) (**160**) and dimethylglyoxime (DMG) (**161**), which allow reaction at the lowest so far reported temperature for Ullmann diphenyl ether synthesis (Figure 2.3).



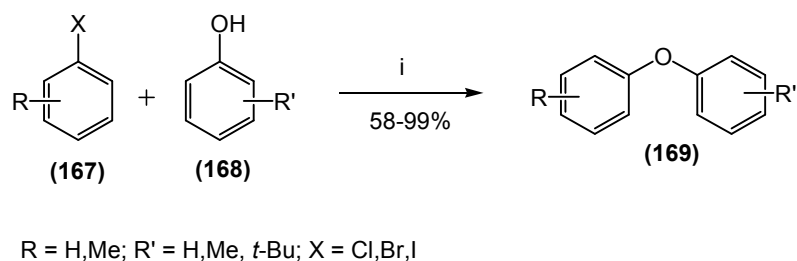
**Figure 2.3: Examples of ligands used in Ullmann diphenyl ether synthesis.**

Other research groups instead of adding a ligand in order to improve the solubility of the catalytic cuprous ions, modify the catalyst itself. Gujaghur and Venkataraman<sup>94,95</sup> have used air and moisture stable  $\text{Cu}(\text{PPh}_3)\text{Br}$  and copper phenanthroline complexes (**165**), (**166**) to successfully couple electron deficient aryl bromides to phenols (Scheme 2.31). Using  $\text{Cu}(\text{PPh}_3)\text{Br}$  gave good yields (55-88%) in the coupling of electron rich aryl bromides with electron rich phenols, however electron deficient phenols failed to react. While use of copper-phenanthroline complexes, was also not successful for aryl bromides with ortho substituents.



**Scheme 2.31: Reaction and conditions: i) Cu(PPh<sub>3</sub>)<sub>3</sub>Br, Cs<sub>2</sub>CO<sub>3</sub> (3 equiv), NMP, 100°C, 48h. ii) Cu(neocup)(PPh<sub>3</sub>) (10mol%), CsCO<sub>3</sub>, toluene, 110°C, 36 h.**

Diphenyl ether synthesis was also reported to be successful without the use of ligands by Xia et al,<sup>96</sup> by using Raney Nickel-Aluminium alloy along with various copper salts as illustrated in Scheme 2.32. This method was only suitable for coupling *ortho* and *para* substituted phenols with inactivated bromides and iodides.



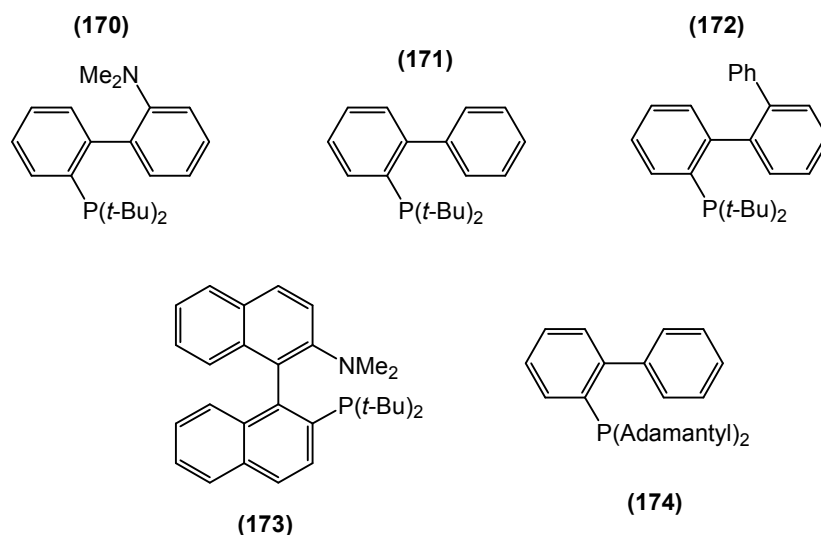
**Scheme 2.32: Reagents and conditions: i) CuI (10mol%), K<sub>2</sub>CO<sub>3</sub>, Raney Ni-Al alloy (10-50 mg/mmol halide), dioxane, 110°C, 24 h.**

Solid phase methods also have been used for the synthesis of diphenyl ethers, by using a triazene group as a linker.<sup>97</sup> The couplings were performed under mild conditions using a soluble copper complex CuBr·SMe<sub>2</sub>. Using this method a number of highly substituted diphenyl ethers were obtained in high purity and good yields.

Another way to avoid the harsh Ullmann reaction conditions, was employed by Buchwald<sup>98</sup> and Hartwig<sup>99</sup> by using palladium-catalysed diphenyl ether synthesis

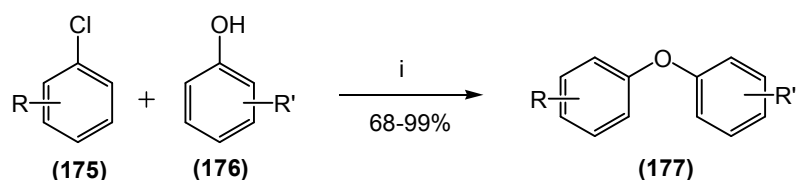
with the aid of ligands, such as (S)-(-)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP), 1,1'-bis(diphenylphosphino) ferrocene (DPPF), P(*o*-tolyl)<sub>3</sub> and 1,1'-bis(di-*tert*-butylphosphino)ferrocene (D<sup>t</sup>BPF). Using this method diphenyl ethers could be obtained at significantly lower temperatures compared to the Ullmann method, even as low as room temperature. The only major disadvantage is the relative expense of the palladium reagent.

A number of different ligands have since been reported, to facilitate the process. Buchwald *et al*<sup>98</sup> developed a variety of sterically bulky ligands containing two *tert*-butyl (170)-(173) or adamantyl groups (174) (Figure 2.4), suitable for coupling a wide range of electron-deficient, electronically neutral and electron-rich aryl halides or triflates with a variety of phenols in high yields.

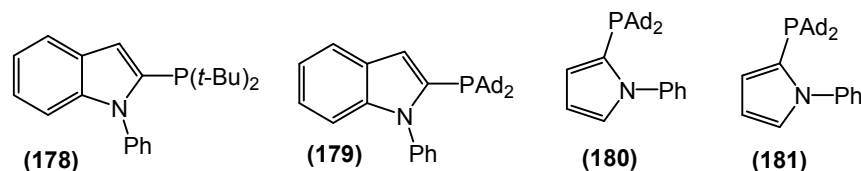


**Figure 2.4: Buchwald's sterically bulky ligands.**

Other examples include phosphine ligands based on 2-phosphino *N*-aryl pyrrole and indole structures (scheme 2.33), developed by Beller *et al.*<sup>100</sup> This variation of the Pd-catalysed method, produces diphenyl ethers in excellent yields using low catalyst concentrations with high turnover numbers.



R = Me, CF<sub>3</sub>, OMe, CN; R' = H, Me

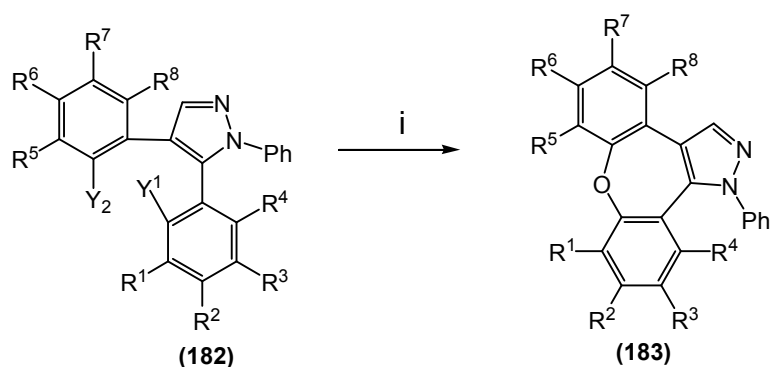


Beller's novel monophosphine ligands

**Scheme 2.33: Reagents and conditions: i) Pd(OAc)<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, ligand (1mol%), toluene, 120°C, 20 h.**

ii) Intramolecular Coupling Reactions.

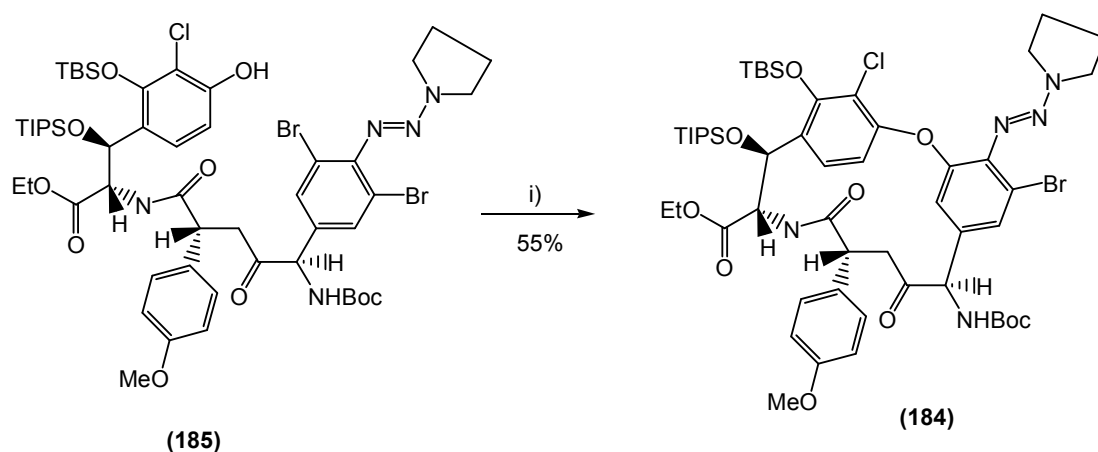
Dominguez *et al*<sup>101, 102</sup> has successfully used CuBr.SMe<sub>2</sub> in pyridine at 120°C in order to achieve the intramolecular coupling of 4,5-(*o,o'*-halohydroxy) arylpyrazoles (**182**) to give dibenzoxepines (**183**) (scheme 2.34). In contrast with intermolecular couplings, variation of substituents with different electronic properties appeared to have little influence and hence did not affect the isolated yields of dibenzoxepines, indicating the efficiency of this reaction in dibenzoxepine ring closure.



Y<sup>1</sup>, Y<sup>2</sup> = OH or halogen

**Scheme 2.34: Reagents and conditions: i) CuBr.SMe<sub>2</sub>, NaH, pyridine, 120°C.**

The synthesis of one of the most important antibiotics, vancomycin, has been approached using this chemistry. For example, Nicolaou and Boddy<sup>103,104</sup> used a soluble CuBr.SMe<sub>2</sub> complex in order to obtain the diphenyl ether intermediate (**184**) from its non cyclic precursor (**185**) (Scheme 2.34).



**Scheme 2.34:** Reagents and conditions: i) CuBr.SMe<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, pyridine.

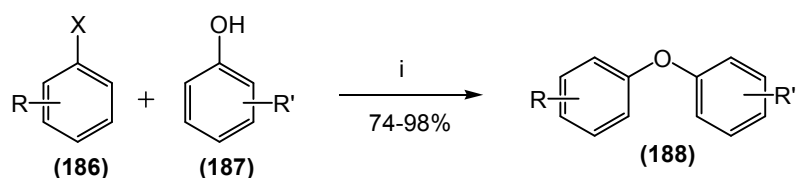
Wang and Harvey<sup>105</sup> have also used palladium-catalysed intramolecular diphenyl ether formation to successfully obtain polycyclic xanthenes and furans.

#### 2.4.3 Synthesis of Diphenyl Ethers via S<sub>N</sub>Ar.

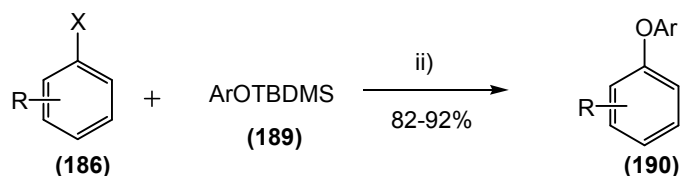
Direct nucleophilic coupling of phenols to aryl halides is best achieved within electron deficient skeletons, as seen mainly in the synthesis of natural products such as vancomycin, piperazinomycin and combrestatins. Nitro, triazenyl and methylcarbonyl are groups most commonly used to activate the aryl halides for S<sub>N</sub>Ar with phenols, with the nitro group being the most favourable due to its strong electron withdrawing effect. This makes reactions possible under relatively mild conditions which are compatible with peptide bonds and other potentially sensitive moieties occurring in natural products.

##### i) Intermolecular S<sub>N</sub>Ar

Wang *et al*<sup>106,107</sup> successfully and in high yields, synthesised a number of diphenyl ethers from electron deficient phenols and aryl halides using microwave activation. The group also developed an efficient method for direct coupling of aryl *tert*-butylmethylsilyl (TBDMS) ethers to electron deficient aryl halides or aryl triflates, using cesium carbonate (Scheme 2.35). Interestingly this method was selective for phenolic TBDMS esters in the presence of aliphatic TBDMS ethers.



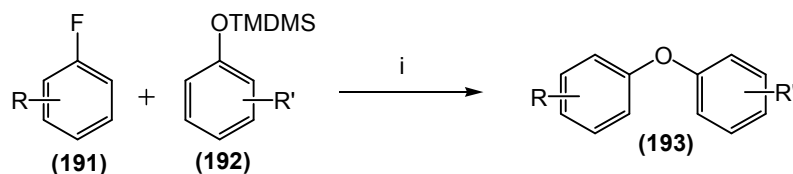
X = F, Cl, Br; R = CN, NO<sub>2</sub>; R' = CF<sub>3</sub>, Cl, OMe, NO<sub>2</sub>, CN



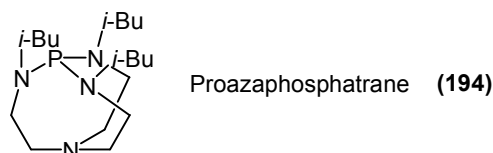
X = F, Cl, Br, OTf; R = COMe, C<sub>6</sub>H<sub>4</sub>, NO<sub>2</sub>, SO<sub>2</sub>Me; Ar = Ph, C<sub>6</sub>H<sub>4</sub>-*p*-Me, quinolin-8-yl

**Scheme 2.35: Reagents and conditions: i) K<sub>2</sub>CO<sub>3</sub>, DMSO, MW (300W), 5-10min; ii) Cs<sub>2</sub>CO<sub>3</sub> (1 equiv), DMF, 100°C, 8h.**

Similarly Urgaonkar and Verkade<sup>108</sup>, efficiently coupled electron poor aryl fluorides to aryl trimethylsilyl or TBDMS ethers, using proazaphosphatrane. Even though this method gave diphenyl ethers in high yields using milder conditions compared to Wang's methodology, unfortunately it only works on aryl fluorides.

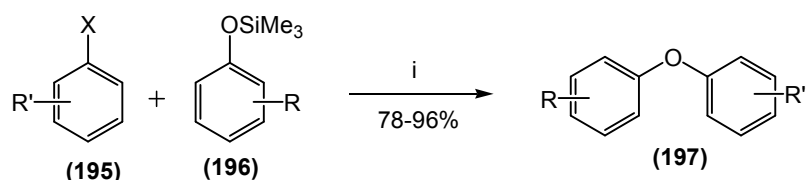


R = CO<sub>2</sub>Et, CHO, CN, NO<sub>2</sub>; R' = CN, Cl, Br, OMe



**Scheme 2.36: Reagents and conditions: i) 10 mol%, proazaphosphatrane, toluene or DMF, r.t. or 80°C, 1 h.**

In the same fashion Zhu *et al.*<sup>109</sup> and more recently Zhao and Wang<sup>110</sup> have reported using tetrabutylammonium fluoride in order to couple trimethylsilyl ethers with electron deficient aryl halides to form diphenyl ethers (Scheme 2.37).

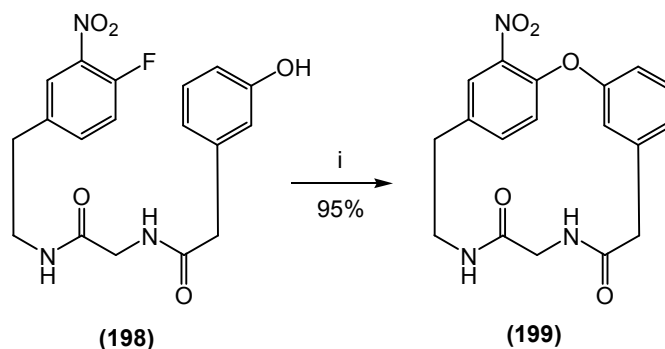


R = CHO, COMe, COPh, NO<sub>2</sub>; R' = H, Me; X = F, Cl, Br

**Scheme 2.37: Reagents and conditions: i) TBAF, Et<sub>3</sub>N, EtOH, DMF, 100°C, 12h**

ii) Intramolecular S<sub>N</sub>Ar.

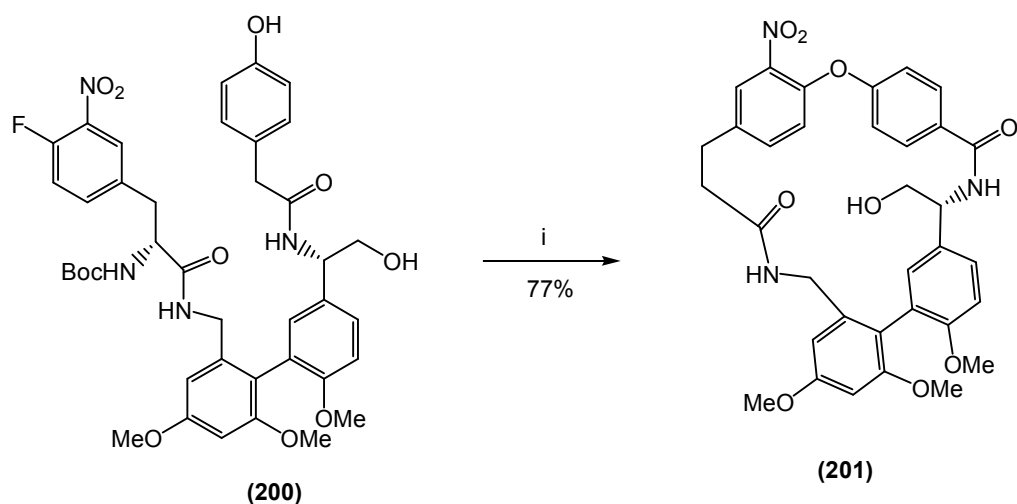
In an early example of the intramolecular S<sub>N</sub>Ar approach with an activated halide, Beugelmans *et al.*,<sup>111</sup> described the synthesis of a vancomycin ring analogue, using potassium carbonate as base in DMF (Scheme 2.38). Interestingly, it was noted that the reaction can be performed without high dilution of the reaction mixture, which is commonly the case for intramolecular cyclisation, in order to favour intra- over inter-molecular cyclisations.



**Scheme 2.38: Agents and conditions i) K<sub>2</sub>CO<sub>3</sub>, DMF.**

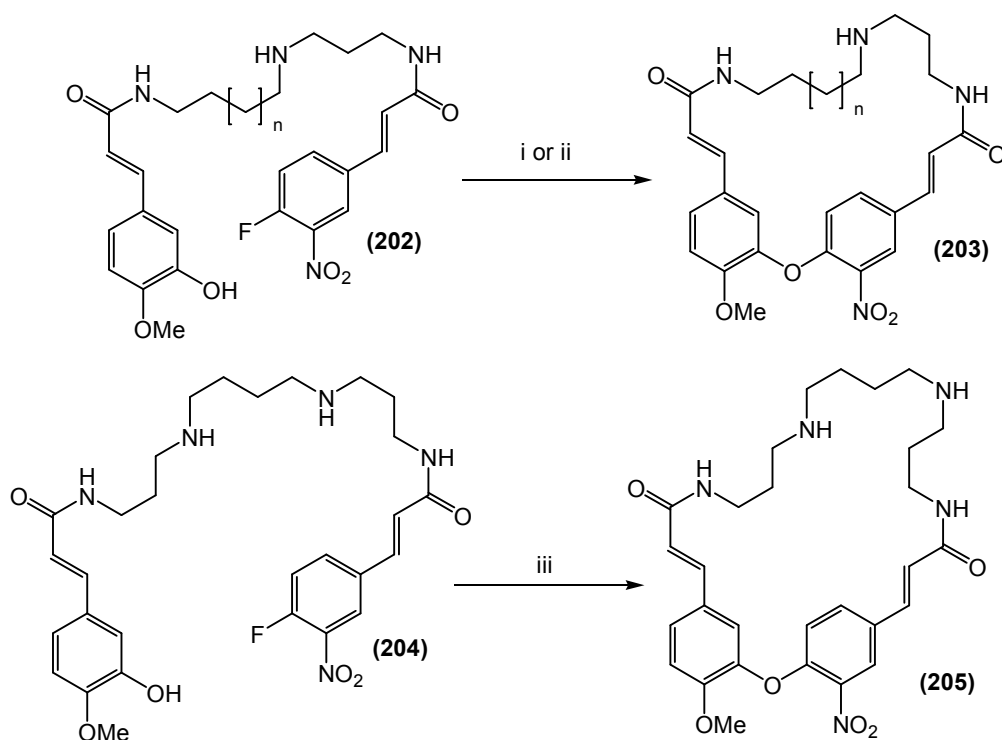
One of the most noteworthy applications of intramolecular S<sub>N</sub>Ar is in the synthesis of vancomycin as reported by Zhu *et al.*,<sup>112</sup> where the macrocycle AB-C-O-D was cyclised as a whole, rather than synthesising the component rings AB and C-O-D individually. This was successfully achieved using potassium carbonate and crown ether 18-C-6 in THF (Scheme 2.39). Zhu<sup>113</sup> also described intramolecular S<sub>N</sub>Ar on solid support using potassium carbonate, where the precursor is bound to Wang resin via an ester linkage.





**Scheme 2.39:** Agents and conditions: i)  $K_2CO_3$ , 18-C-6, THF,  $50^\circ C$ .

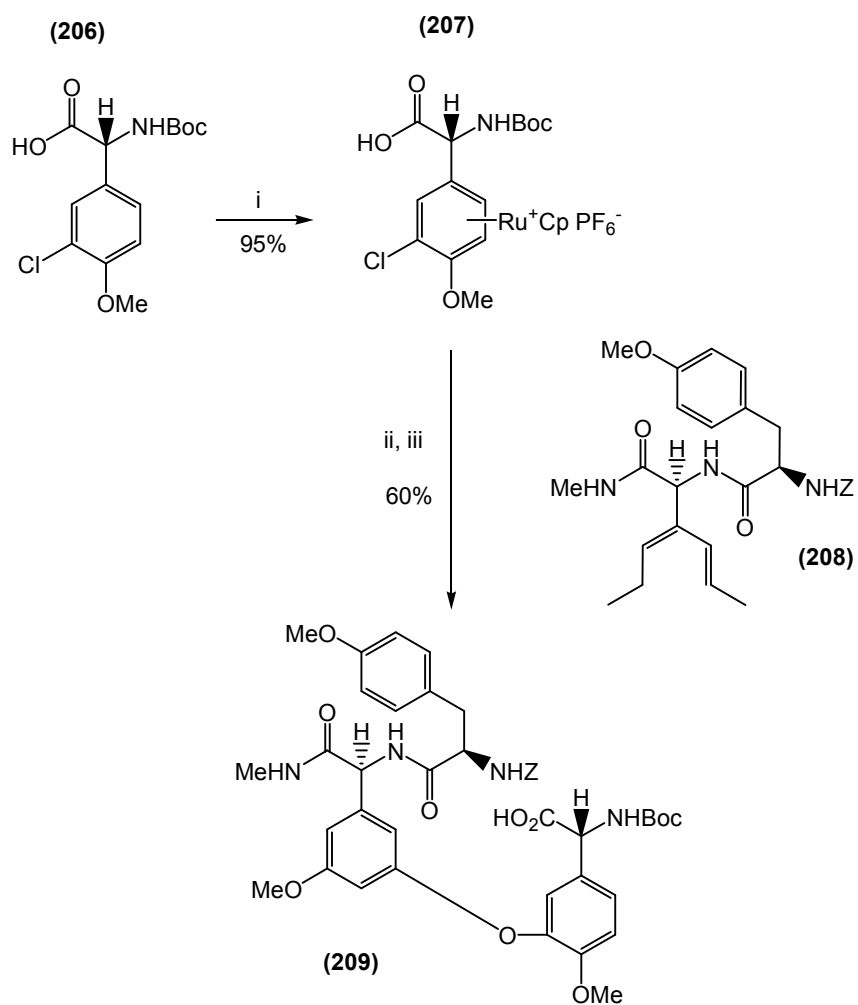
Intramolecular  $S_NAr$  has been also used by Blagbrough<sup>73</sup> for the diphenyl ether closure of 23-, 24- (codonocarpine and nitrocadabine), and 28-membered macrocyclic polyamine lactams (Scheme 2.40). It has been proposed that the two aryl rings come in close proximity during the cyclisation reaction, due to  $\pi$ -orbital stacking interactions between the electron rich phenol ring and the deficient *o*-fluoro-nitrophenyl ring<sup>84</sup>. However macrocycle formations of this type, have also been successfully achieved when the aryl hydroxyl group is replaced by an alkyl hydroxyl group, suggesting that  $\pi$ - $\pi$  interactions are not necessary for cyclisations to occur.<sup>114</sup>



**Scheme 2.40:** When  $n=0$  i) 5 eq CsF, DMF, 18 h, 79%. When  $n=1$  ii) 3 eq CsF, DMF, 18 h, 71%. iii) 3 eq  $\text{K}_2\text{CO}_3$ , 10 eq 18-crown-6, DMSO,  $50^\circ\text{C}$ , 5 h, 66%.

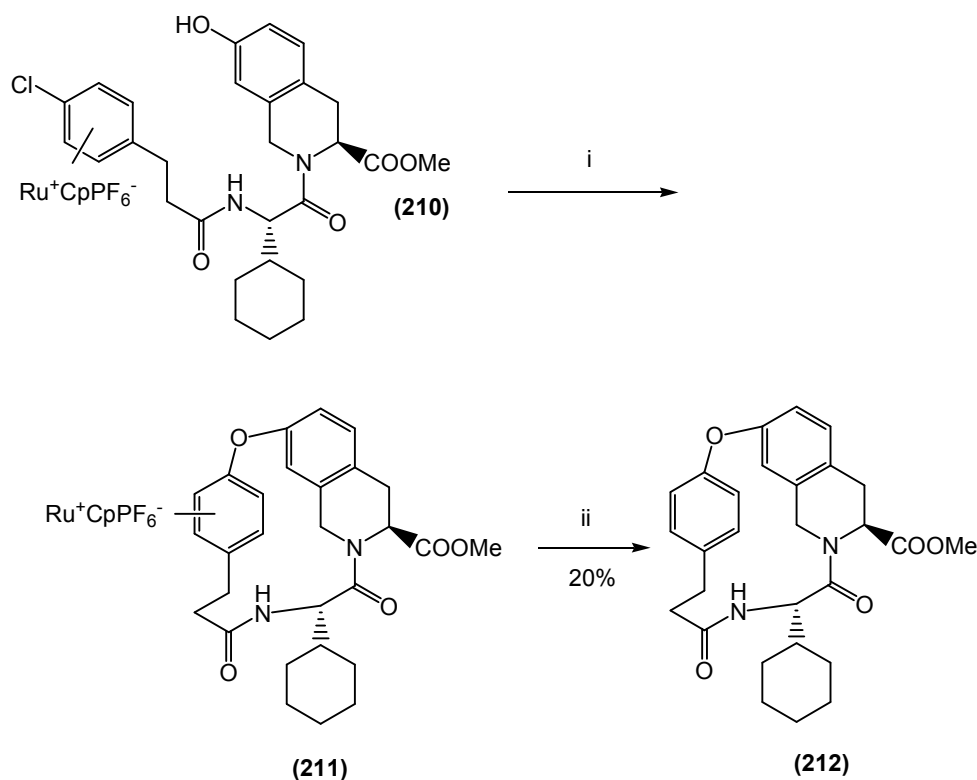
#### 2.4.4 $\text{S}_{\text{N}}\text{Ar}$ additions to Metal-Arene Complexes.

$\text{S}_{\text{N}}\text{Ar}$  addition using metal-arene complexes is a mild method to synthesise diphenyl ethers. A normally non-reactive and non-activated aromatic halide component for the  $\text{S}_{\text{N}}\text{Ar}$  is activated by forming a metal arene complex. An example of  $\text{S}_{\text{N}}\text{Ar}$  additions to metal-arene complexes is reported by Pearson and Belmont,<sup>115</sup> where peptide aryl ether rings of ristocetin and teiplanin molecules are synthesised via a ruthenium complex (Scheme 2.41). The toxic ruthenium complex can be efficiently recovered and recycled.



**Scheme 2.41:** Agents and conditions i)  $\text{Cp}(\text{MeCN})_3\text{RuPF}_6$ , DCE, reflux; ii)  $(t\text{-Bu})_2\text{C}_6\text{H}_3\text{O}^- \text{K}^+$ , 18-C-6, THF; iii) MeCN, hv.

Ruthenium-induced intramolecular cyclisation has also been successfully used for the construction of the diphenyl ether macrocyclic compound (212) (Scheme 2.42), as described by Venkatraman *et al*<sup>116</sup>.



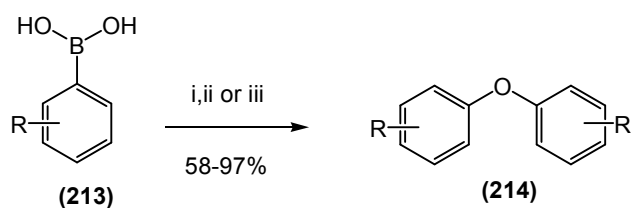
**Scheme 2.42: Reagents and conditions i) Cs<sub>2</sub>CO<sub>3</sub>, DMF; ii) MeCN, hv, 20% (over two steps)**

Similar reactions using ruthenium complexes have been also used by Lindel and Schmid<sup>117</sup>.

#### 2.4.5 Miscellaneous methods for diphenyl ether formation.

##### i) Coupling of Phenols with Arylboronic Acids.

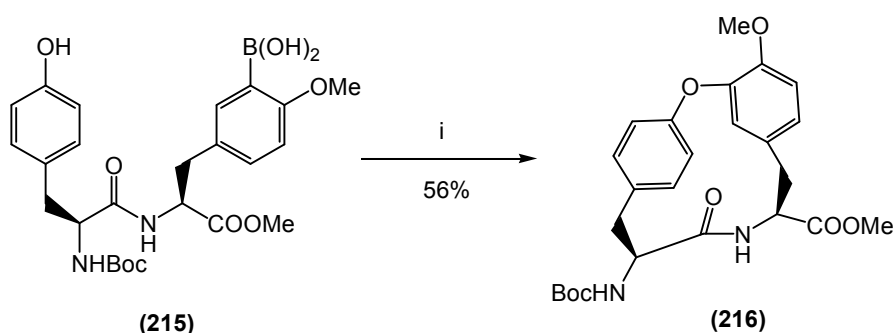
Chan<sup>118</sup> and Evans<sup>119</sup> were first to report the synthesis of diphenyl ethers by copper (II)-promoted cross-coupling of phenols with arylboronic acids in the presence of molecular sieves. The major advantage of this method is that the reaction proceeds at low temperature, hence tolerating a wide variety of substituents on both reactants as well as achieving high yields. Petasis *et al.*,<sup>120</sup> and later Sagar *et al.*,<sup>121</sup> described a one-pot reaction for the conversion of arylboronic acids to phenols, and subsequent intramolecular coupling of both species to form symmetrical diphenyl ethers (Scheme 2.43).



R = Me, *t*-Bu, OMe, COMe, Cl, F, Br, NO<sub>2</sub>

**Scheme 2.43: Reagents and conditions i) H<sub>2</sub>O<sub>2</sub> (30%), DCM; ii) Cu(OAc)<sub>2</sub>, Et<sub>3</sub>N, DCM, MS, 25°C; iii) Cu(OAc)<sub>2</sub>, Et<sub>3</sub>N, H<sub>2</sub>O, DCM-MeCN, 25°C.**

Hitotsuyanagi et al.<sup>122</sup> applied this approach for the efficient intramolecular coupling of the dipeptide precursor (215), for the synthesis of the macrocycle L,L-cycloisodityrosine (216) (Scheme 2.44).



**Scheme 2.44: Reagents and conditions i) Cu(OAc)<sub>2</sub>, Et<sub>3</sub>N, DCM, MS, r.t., 48h.**

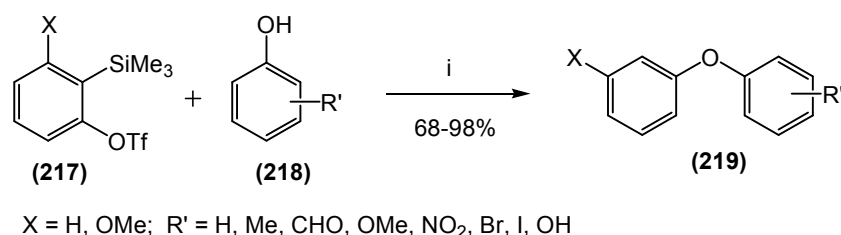
### ii) Diphenyl Ether Synthesis via Oxidative Coupling

The synthesis of diphenyl ethers can also be accomplished by oxidative coupling of 2,6-dihalophenols. This method has been used in the synthesis of vancomycin<sup>123</sup> using thallium nitrate as an oxidant, and more recently in the synthesis of bastadin (a cyclic tetramer of brominated tyrosine derivatives containing two diphenyl ether moieties) using cerium(IV) diammonium nitrate as an oxidant.<sup>85</sup>

### iii) Diphenyl Ether Synthesis via Benzyne Mechanism.

This method involves the synthesis of diphenyl ethers from silylaryl triflates with phenols<sup>124</sup> (Scheme 2.45). The advantage of this reaction is that it occurs at room

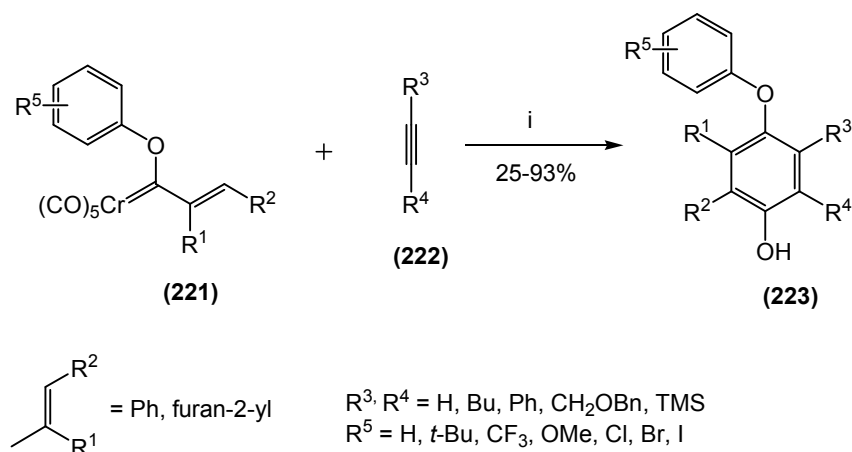
temperature, and phenols with electron withdrawing groups such as formyl and nitro as well as halides are tolerated.



**Scheme 2.45: Reagents and conditions i) CsF, MeCN, 25°C, 2.5 d.**

#### iv) Diphenyl Ether Synthesis by Fischer Chromium Carbene Mediated Benzannulation.

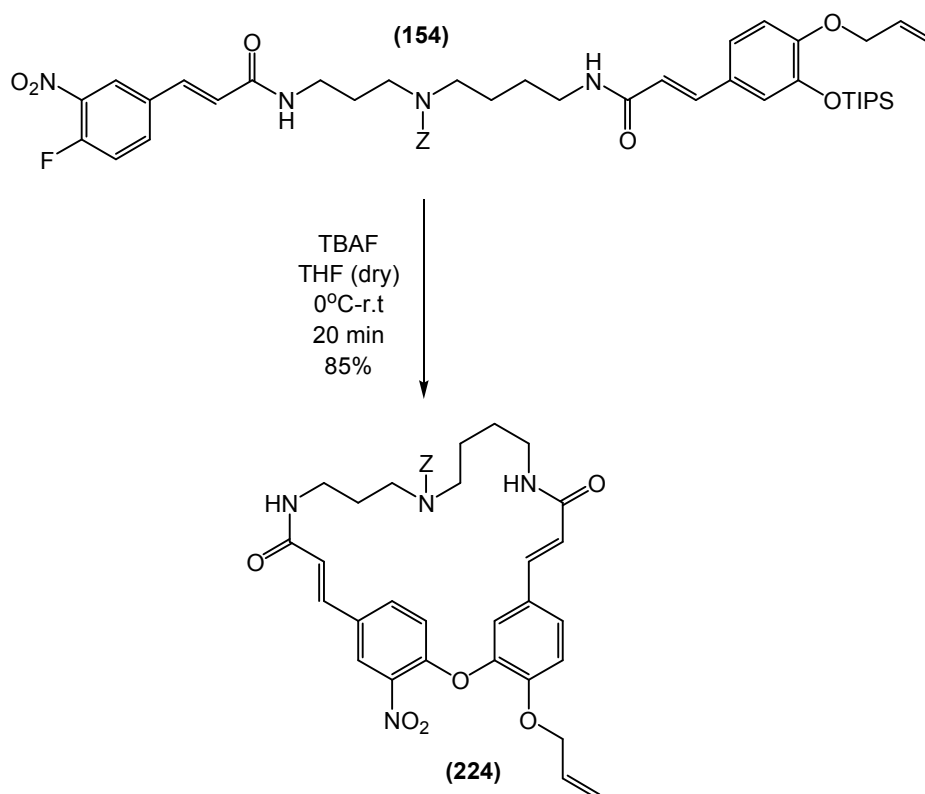
A less common method is to build one of the aromatic rings of the diphenyl ether via a 3+2+1 cycloaddition between an aryloxy- or alkylamino- substituted Fischer carbene, and an alkyne. A recent example by Pulley *et al*<sup>125</sup> describes the benzannulation between Fischer chromium carbene complexes (**221**) and alkynes (**222**), giving diphenyl ethers (**223**) as shown in scheme 2.46. The main advantage of this approach is that it allows for a wide range of substituents on both aryl components.



**Scheme 2.46: Reagents and conditions i) 50-55°C, 16-36 h.**

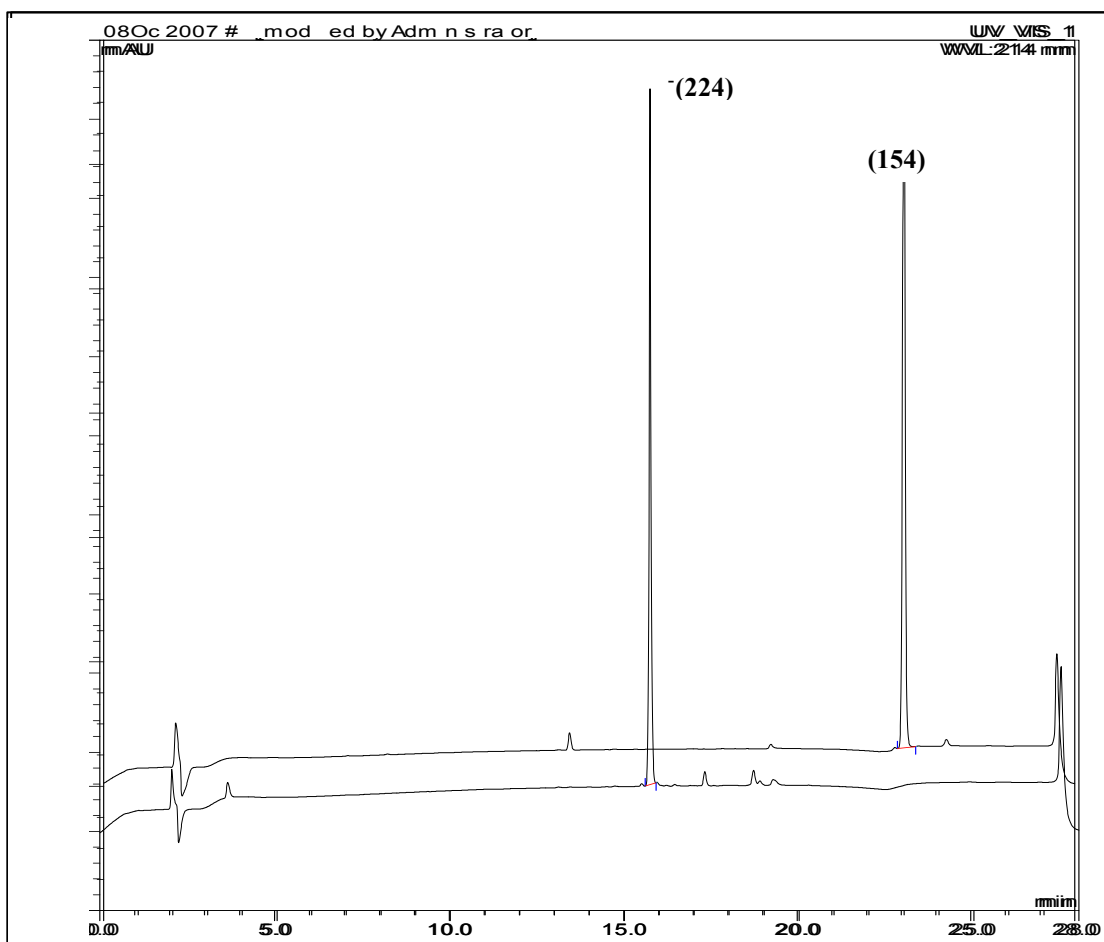
## 2.4.6 Intramolecular $S_NAr$ cyclisation for Cadabicine

As highlighted in the retrosynthesis (section 2.1), our synthetic strategy for cadabicine was based on an intramolecular  $S_NAr$  reaction with a spermidine intermediate derivatised with suitable activated halide and phenol containing units. As noted in the previous section, this approach should be compatible with a range of protecting groups on both spermidine and aromatic components as required here. Following from section 2.3, the linear compound (**154**) was cyclised via  $S_NAr$  intramolecular coupling, using TBAF in THF (Scheme 2.47). The reaction was performed in dilute conditions (1 mM/mL), in order to favour intra over inter molecular coupling. In this reaction the TIPS group was cleaved and compound (**154**) was cyclised in one step to give the macrocycle (**224**).<sup>110</sup>



**Scheme 2.47:  $S_NAr$  Cyclisation**

The reaction was monitored by HPLC, confirming that the reaction was fast, clean and efficient (Figure 2.5).



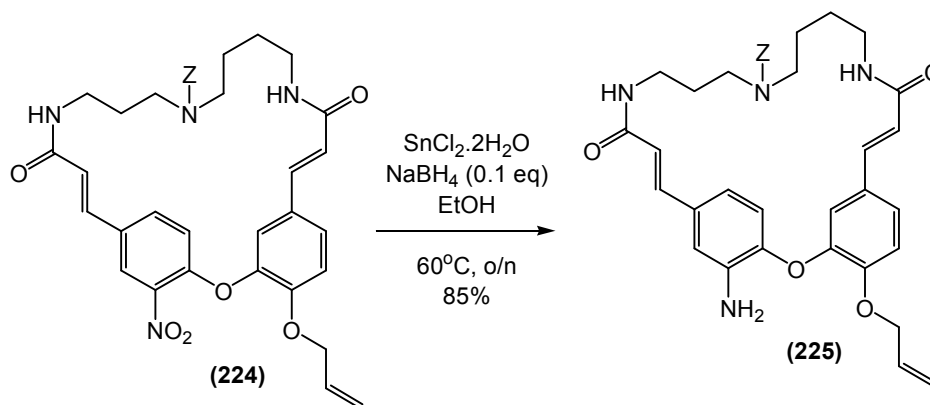
**Figure 2.5:  $S_NAr$  macrocyclisation compound (224). Upper trace linear compound (154). Lower trace cyclised compound (224).**

The reaction was also repeated at a 10-fold higher concentration and was again monitored by HPLC. There was no difference in the yield obtained of the macrocycle (224), or in the efficiency of the reaction.

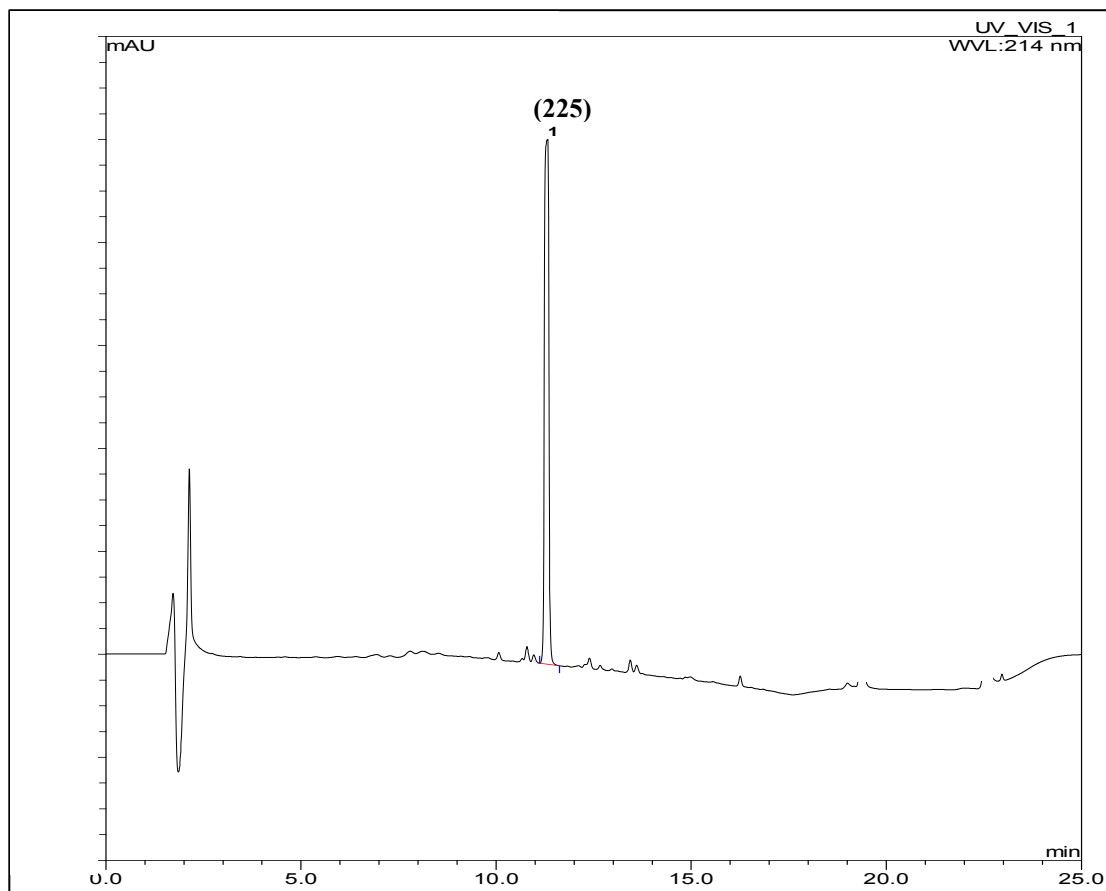
Having successfully obtained (224), the final steps required in order to obtain cadabicine, were to remove the nitro group and cleave the allyl and Z protecting groups. The standard procedure for the reduction of the nitro group involves catalytic hydrogenation using palladium on carbon<sup>126</sup> or Raney nickel.<sup>127</sup> However this method was not suitable, as it would reduce the double bonds of the macrocycle and the allyl and Z protecting groups. In contrast, the use of  $\text{SnCl}_2$  for mild and chemoselective reduction of aromatic nitro groups to anilines has been well documented.<sup>128</sup> The reduction of the nitro group in the macrocycle (224), was initially attempted using tin chloride in DMF.<sup>129</sup> This method was monitored by TLC and MS, but unfortunately



failed, giving multiple products, and the desired aniline was not observed. Therefore the method was modified according to Satoh's<sup>130</sup> protocol, which uses SnCl<sub>2</sub>, and 0.1 eq NaBH<sub>4</sub> in EtOH at 60°C. This method is reported to be compatible with  $\alpha,\beta$ -unsaturated alkenes and successfully reduced compound **(224)** to the corresponding amine **(225)** in high yield (Scheme 2.48). The reduction was monitored by HPLC, which showed complete disappearance of starting material and the formation of a single reduced product (Figure 2.6).

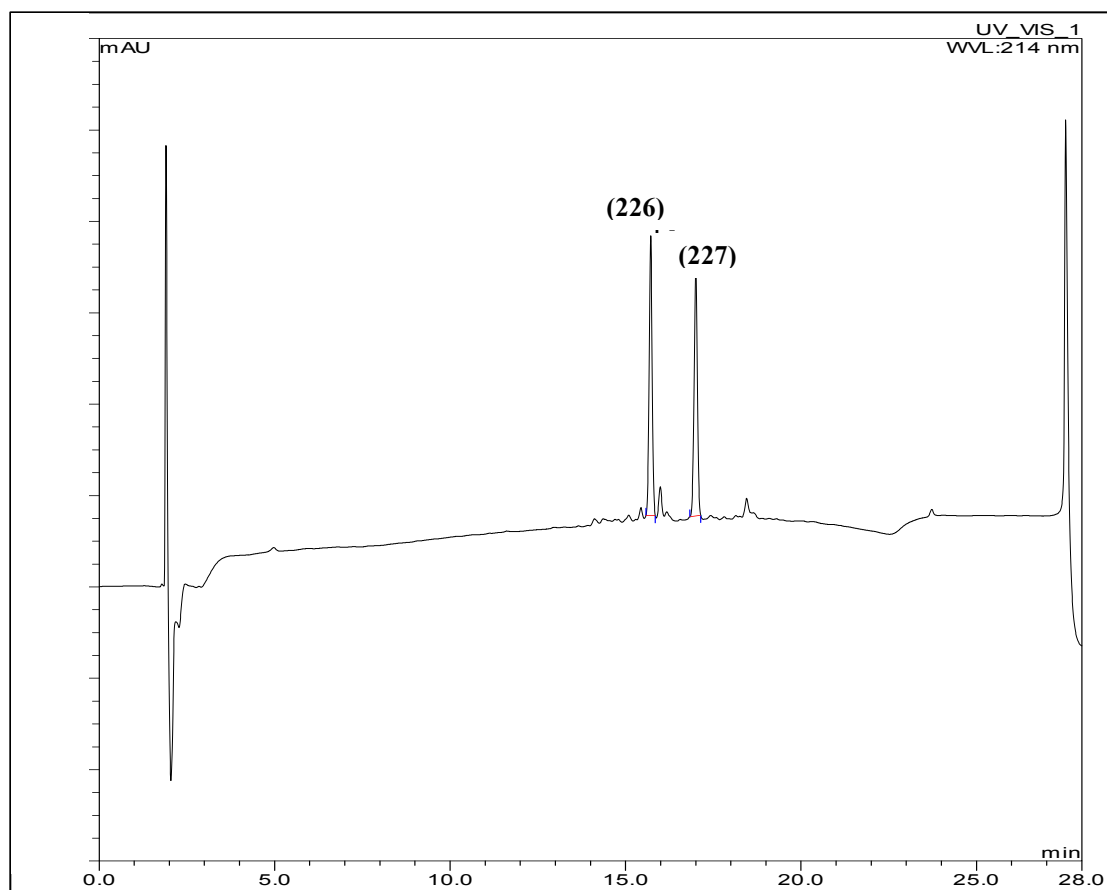


**Scheme 2.48: Reduction of aromatic nitro group.**



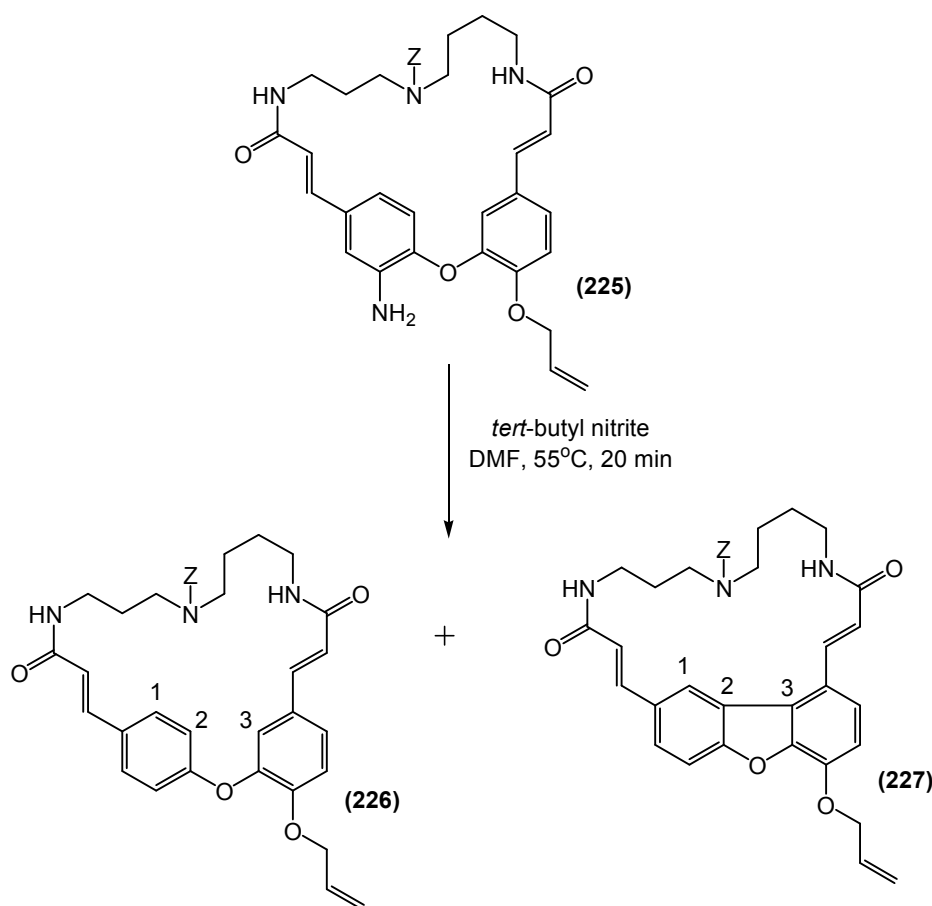
**Figure 2.6: Reduction of (224) using Satoh's protocol.**

Deamination of **(225)** proved to be a critical and very challenging step. Such transformations of aromatic amines have usually only been carried out in simple systems using relatively harsh conditions. The transformation is typically achieved in two steps, involving diazotization followed by reduction, in a ‘one-pot’ diazotisation-dediazotisation reaction using sodium nitrite in hypophosphorus acid. These reaction conditions cannot tolerate sensitive moieties or protecting groups. Furthermore the aryldiazonium salts are unstable, and many of these reaction proceed via radicals, and thus lead to side reaction such as dimerisation. Doyle et al<sup>131</sup> developed a method for rapid deamination of arylamines by *tert*-butyl nitrite, using DMF as a solvent and hydrogen donor. A number of other solvents were investigated showing that DMF is by far the best H donor thus facilitating these types of reaction. It was therefore decided to attempt the deamination of macrocycle **(225)** in the same manner. The reaction was monitored by HPLC, and gave rise to two main products in a ratio of 1:1; one of which was the desired intermediate **(226)** (Figure 2.7).



**Figure 2.7:** HPLC trace of Deamination reaction using *tert*-butyl nitrite.

The two peaks were isolated via HPLC purification, and further analysed by MS, 1D and 2D NMR studies in order to identify the unknown product (**227**). Following MS analysis the unknown compound had a mass 2 units lower than the desired compound (**226**). It was thought that this compound could correspond to a product formed by Pschorr type ring closure. This hypothesis was confirmed by NMR studies. 2-D NMR studies including COSY, and carbon-hydrogen (HMBC, HSQC) showed the distinct difference between the two. The COSY for desired compound (**226**) showed a clear interaction between H<sup>1</sup> and H<sup>2</sup>, while this interaction was not present in the Pschorr intermediate (**227**) spectra. Moreover the carbon-hydrogen correlation showed an interaction between C-2 and H-2, as well as C-3 and H-3, while this interaction was not present for compound (**227**).

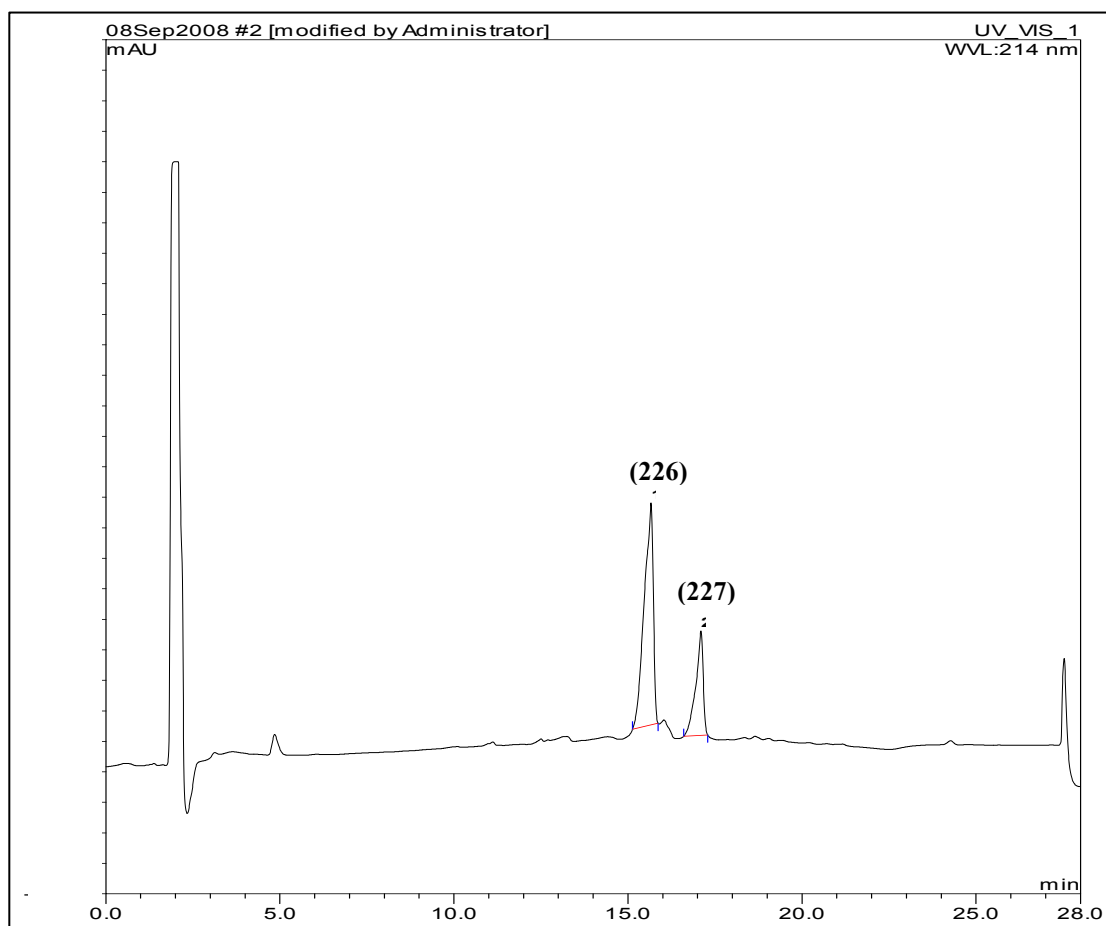


**Scheme 2.49: Deamination reaction using *tert*-butyl nitrite.**

During repetitions of this reaction it was observed that the addition time of the aniline (**225**), to the DMF/*tert*-butyl nitrite, was very important in determining the resulting ratio of the two products. If the addition was fast (under 1 min) the reaction gave predominately the Pschorr product (**227**), while if the addition was slow over 20 min,

this gave a ratio of approximately 1:1 by HPLC. Increasing the addition time beyond 20 min, did not change the ratio in preference of the desired product; it remained 1:1.

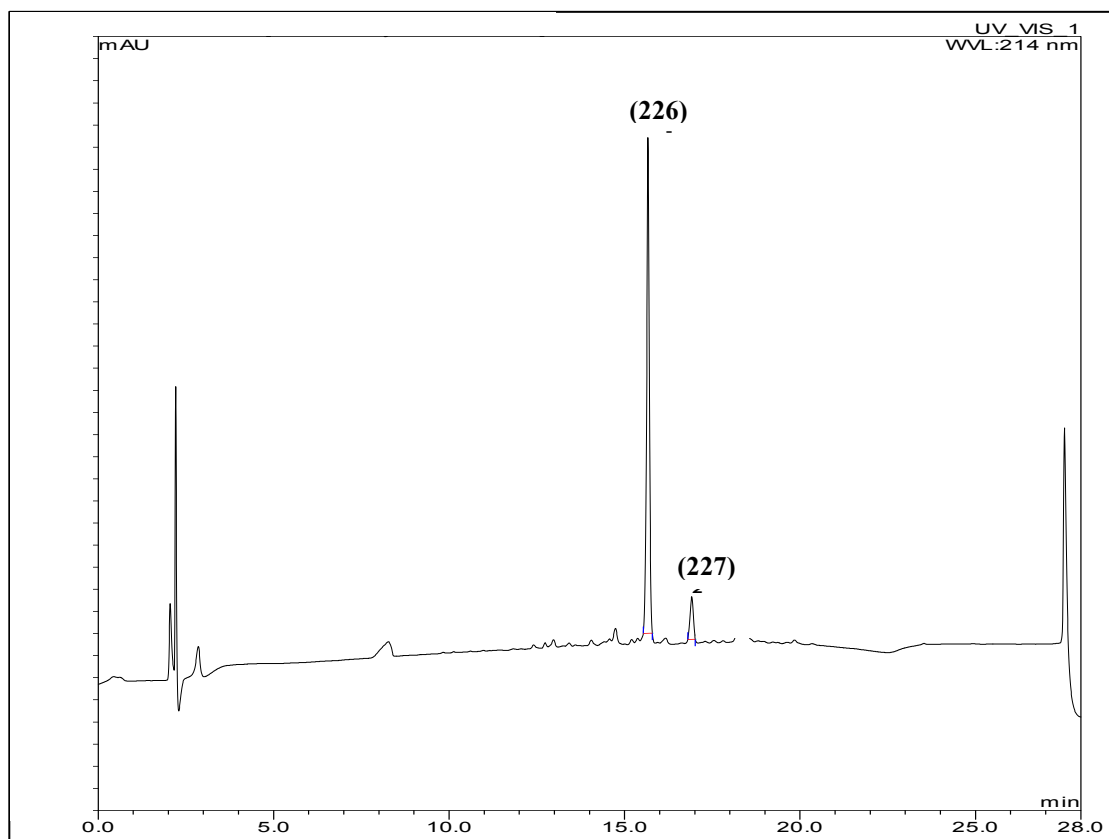
In order to improve the yield of the desired deaminated material a recently reported milder deamination reaction was attempted. This involves a mild diazotisation using sodium nitrite and acetic acid followed by *in situ* reduction by bisulfite, a mild reducing agent, and its corresponding conjugate acid, a weak acid.<sup>132</sup> The reaction was initially attempted using EtOH and water as solvents, as suggested in the literature. This generated the desired derivative (**226**) in a 3:1 ratio to the Pschorr derivative (**227**) as shown in Figure 2.8.



**Figure 2.8: Deamination using EtOH/H<sub>2</sub>O as solvents.**

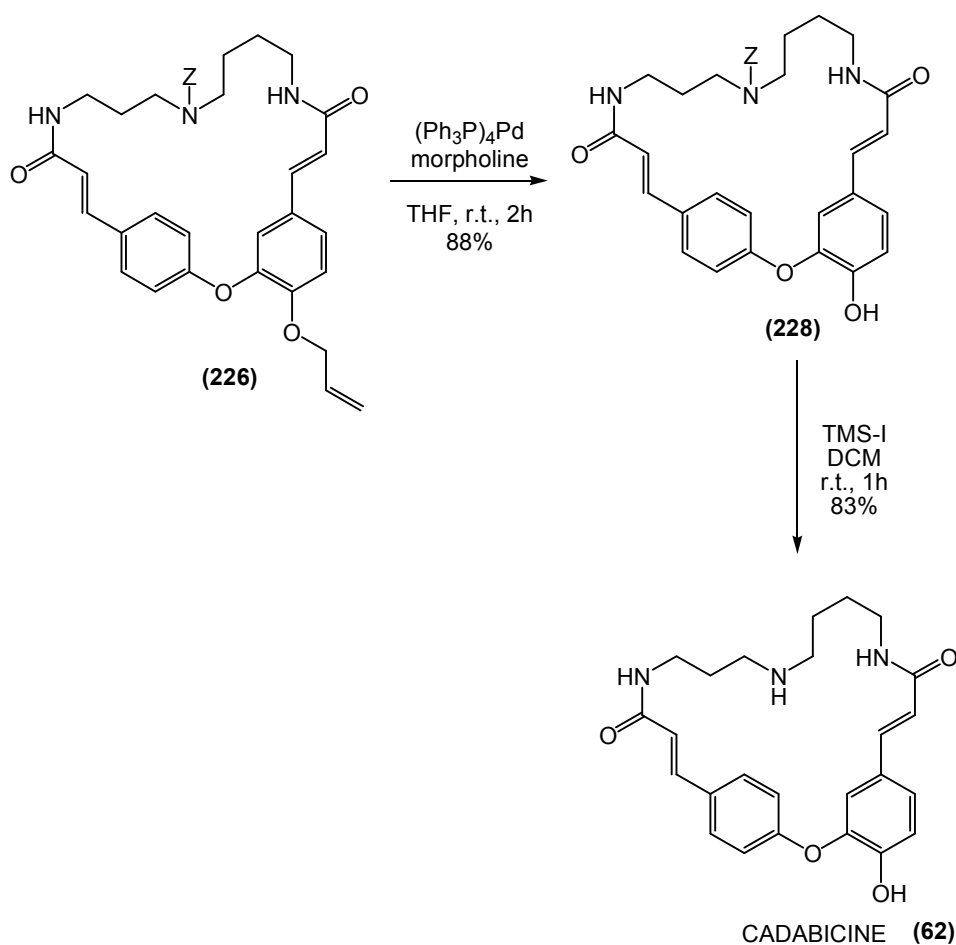
In order to improve the ratio towards the desired deaminated compound (**226**), a mixture of EtOH/DMF was used as a solvent, as it has been previously suggested that DMF is the best H-donor in these types of reaction. Thus, the aniline (**225**) was dissolved in a solution of ethanol/DMF, water and acetic acid at room temperature,

and a solution of sodium nitrite in water was added followed immediately by the addition of aqueous solution of sodium bisulfite. This method now gave predominantly the desired deaminated compound (**226**), as it shown in Figure 2.9.



**Figure 2.9:** HPLC trace form  $\text{NaNO}_2$  deamination using EtOH/DMF as solvent.

The deaminated material (**226**) was in turn allyl deprotected using  $(\text{Ph}_3\text{P})\text{Pd}^{133}$  to give the deprotected material (**228**). This was in turn subjected to final deprotection of the Z group. Deprotection of a Z group is usually performed by hydrogenolysis, however this method would also reduce the double bonds in the molecule. Therefore compound (**228**) was Z deprotected using TMS-I<sup>69</sup> which is a mild method compatible with  $\alpha,\beta$  unsaturated alkenes (Scheme 2.50). Following purification by column chromatography and HPLC, cadabcine (**62**) was isolated in 83% yield as the trifluoroacetate salt.



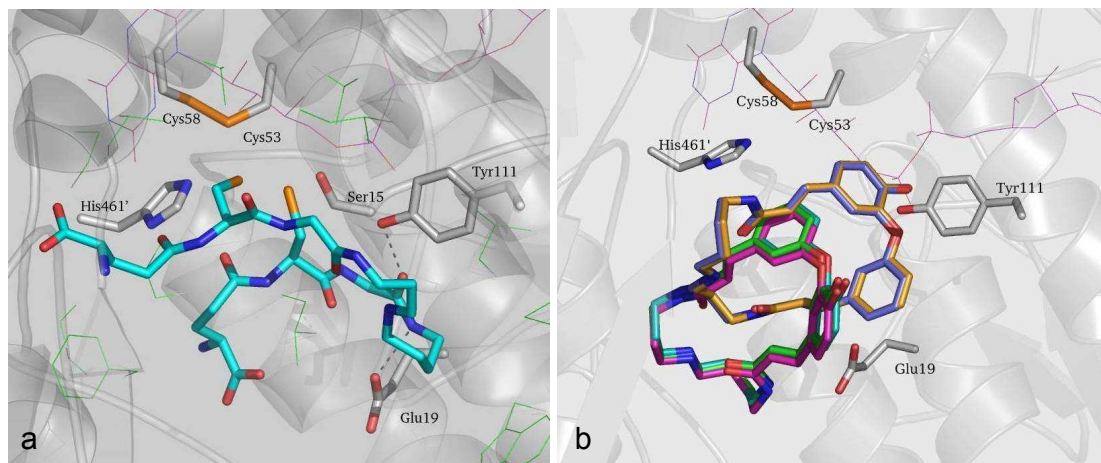
**Scheme 2.50: Allyl and Z deprotection**

## 2.5 SYNTHESIS OF CADABICINE ANALOGUES

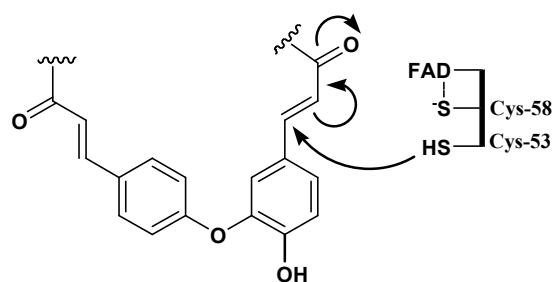
### 2.5.1 Cyclic analogues

Having established the synthesis of cadabicine (**62**), a small group of analogues was prepared in order to investigate structure-activity relationship. Macrocyclic polyamine alkaloids closely related to cadabicine, such as lunarine (**63**) have been shown to inhibit TryR in a competitive, time-dependent manner, resulting from conjugate addition of a redox-active cysteine residue in the TryR active site onto an  $\alpha,\beta$ -unsaturated carbonyl unit.<sup>48,47</sup> In principle cadabicine could also inhibit TryR in a similar fashion. Docking experiments suggested that cadabicine could bind with its diphenyl ether unit in a hydrophobic region of the TryR active site normally occupied by the spermidine unit of the substrate. One such high scoring structure also placed an

activated double bond on one side of the molecule, 4.9Å from one of the redox active Cys residues of the enzyme, providing support for the proposed interaction shown in Figure 2.11.

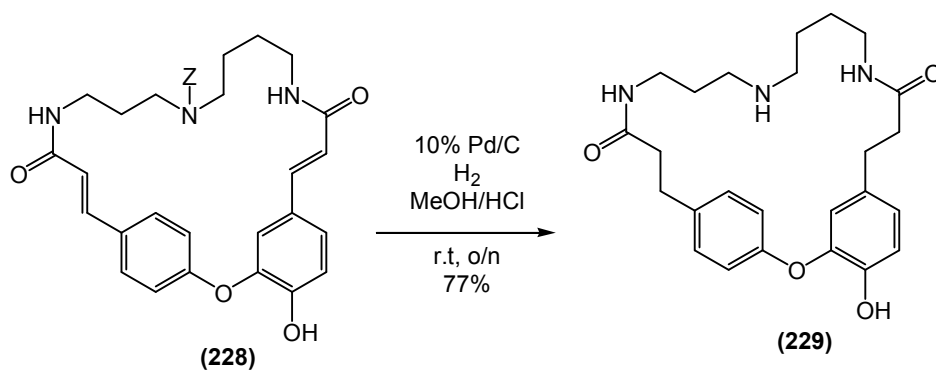


**Figure 2.10:** a) T[SH]<sub>2</sub> bound in TryR active site. b) Selected results of GOLD docking calculations for cadabicine and TryR.



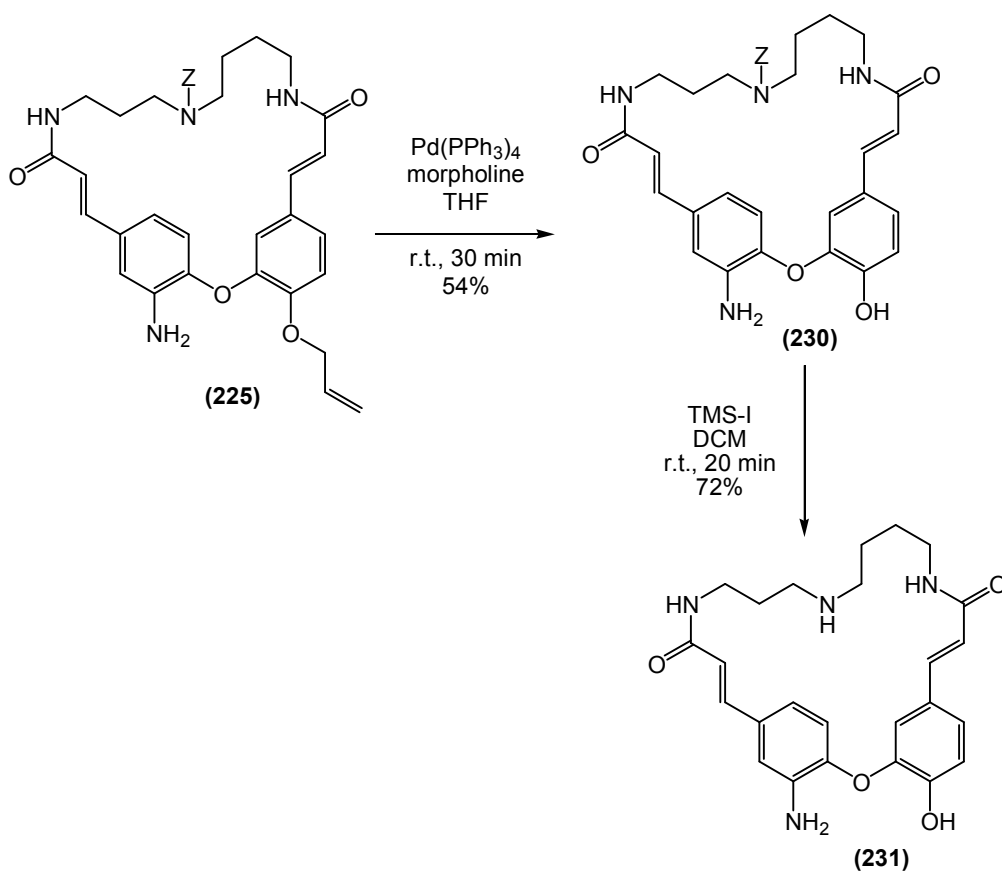
**Figure 2.11:** Possible time-dependent inactivation of TryR by cadabicine.

In order to investigate the significance of the  $\alpha,\beta$ -unsaturated alkene units, and whether they could contribute to time dependent inhibition, a saturated tetrahydro analogue of cadabicine was synthesised. Allyl deprotected compound (**228**), was subjected to hydrogenolysis using Pd/C, which removed the Z group while reducing the double bonds to give compound (**229**) in 77% yield (Scheme 2.51).



**Scheme 2.51: Synthesis of tetrahydro analogue of cadabicine.**

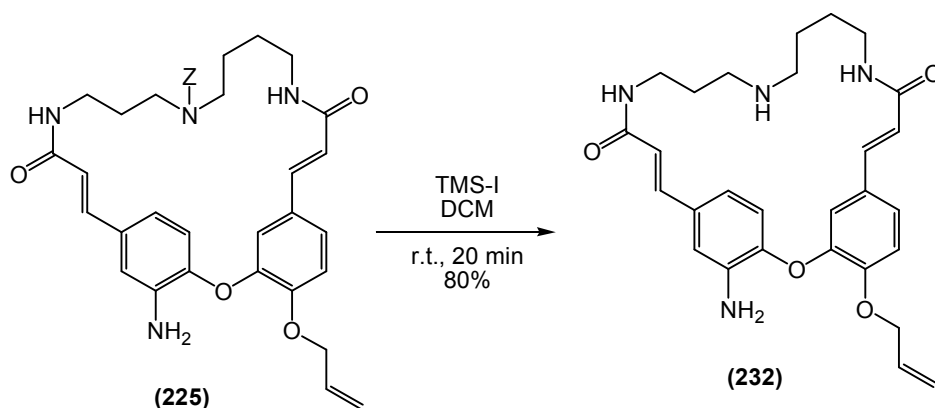
Based on the macrocyclic template another four analogues were synthesised, featuring changes in the substituents of the diphenyl ether unit in order to examine possible electronic/steric effect upon the enzyme interaction. The amino cadabicine analogue (231) was synthesised by allyl deprotecting the reduced intermediate (225), to give compound (230) which was then treated with TMS-I to remove the Z group (Scheme 2.52).



**Scheme 2.52: Synthesis of the amino cadabicine.**

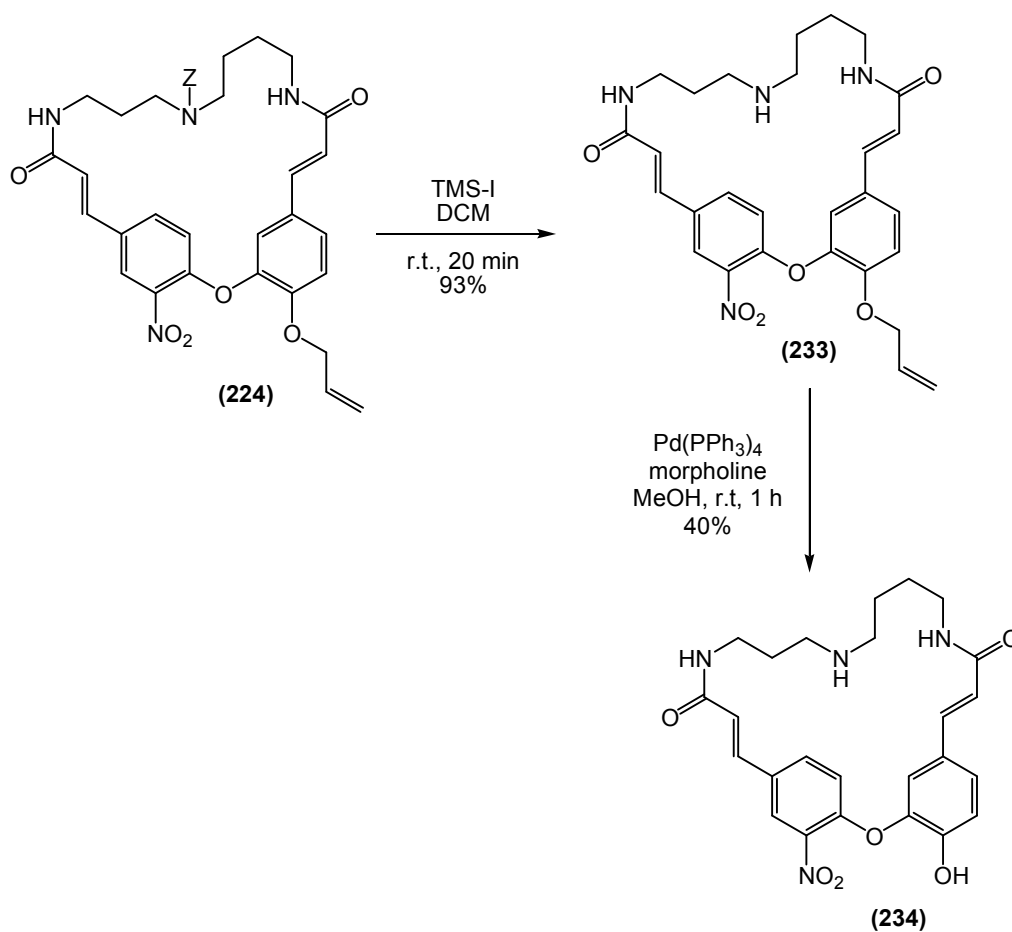


The amino-allyl cadabicine analogue (**232**) was then synthesised by Z deprotecting compound (**225**), using TMS-I in 80% yield (Scheme 2.53).



**Scheme 2.53: Synthesis of amino-allyl cadabicine.**

In a similar fashion, the nitro cadabicine (**234**) and nitro-allyl cadabicine (**233**) analogues were synthesised (Scheme 2.54). In this instance though, the Z group was removed prior to the allyl group, thus illustrating the orthogonality of these protecting groups.



## Scheme 2.54: Synthesis of nitro cadabicine analogues.

### 2.5.2 Non-cyclic analogues.

Two non-cyclic analogues of cadabicine were synthesised. This type of analogues was chosen since bis-polyamine functionalised disulfides such as the naturally occurring  $N^1$ -glutathionylspermidine disulfide, and the synthetic bis-dimethylaminopropyl- and bis- $N^4$ -methylpiperazinyl amides of Ellman's reagent (DTNB)<sup>134</sup>, are known TryR substrates. Moreover in previous research on lunarine (**63**), non-cyclic analogues were investigated; while retaining the tricyclic scaffold, they featured spermidine or dimethylaminopropylamine side chains and were found to be time dependent inhibitors and significantly more active than natural lunarine (Figure 2.12).<sup>49</sup>

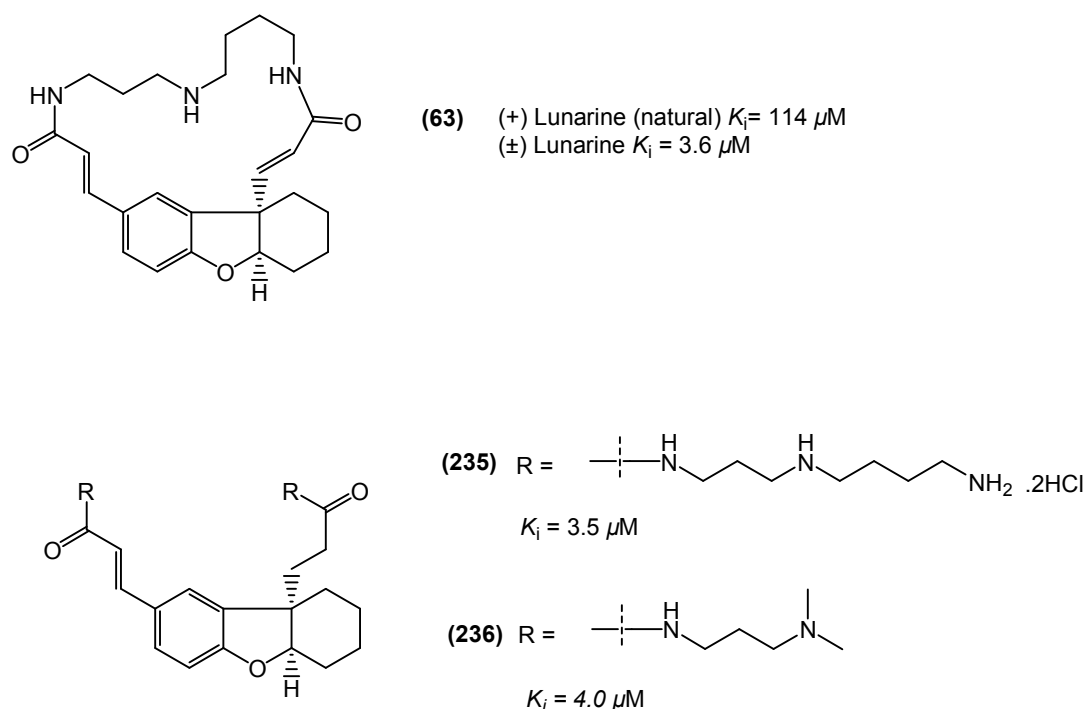
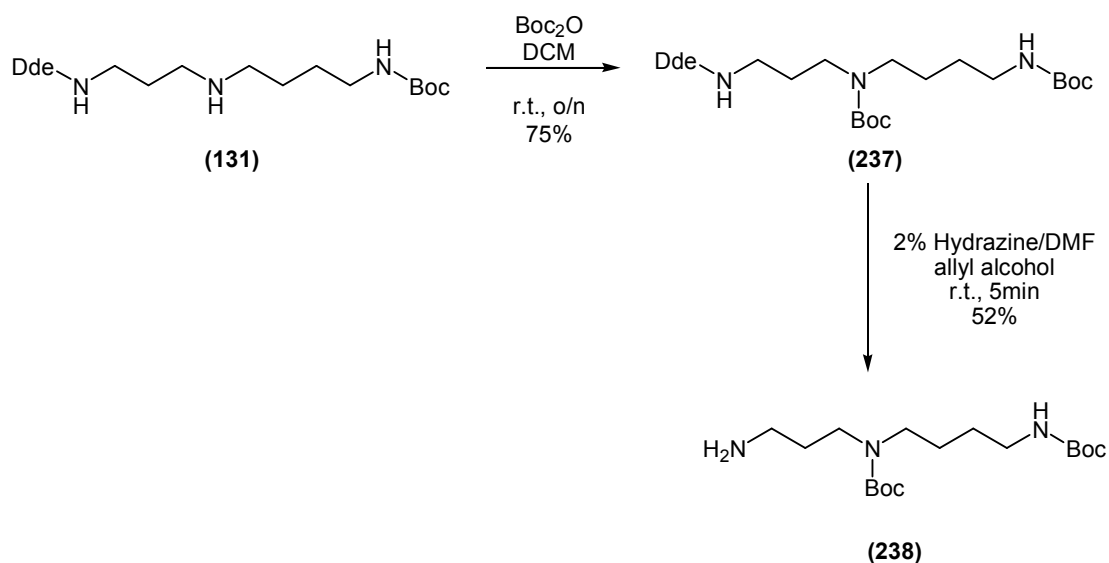


Figure 2.12: Lunarine and non-cyclised polymanime derivatives.

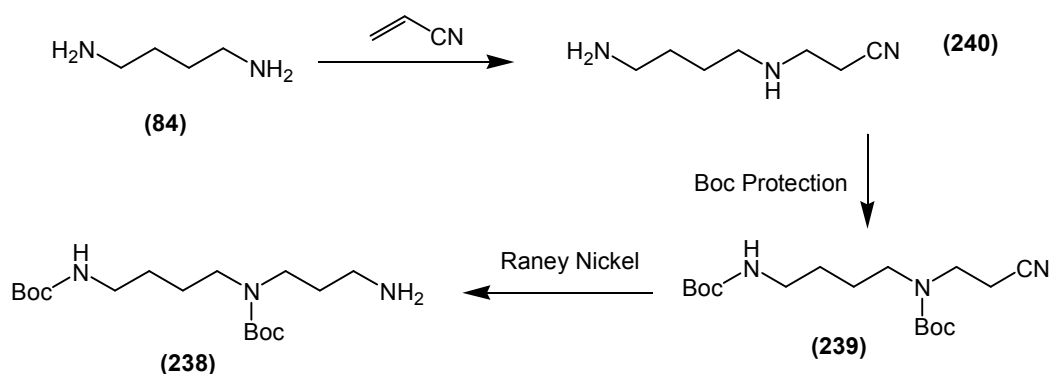
Using the same concept, two non-cyclic cadabicine analogues containing two spermidine chains were synthesised. Starting with the doubly protected spermidine (**131**),  $N^4$  was Boc protected using Boc anhydride. In this case there was no need for three orthogonal protecting groups, since both Boc protecting groups would be removed at the same time (Scheme 2.55).  $N^1$  was then Dde deprotected with 2%

hydrazine to yield N<sup>1</sup> free amine spermidine compound (**238**) in 52% yield (scheme 2.55).



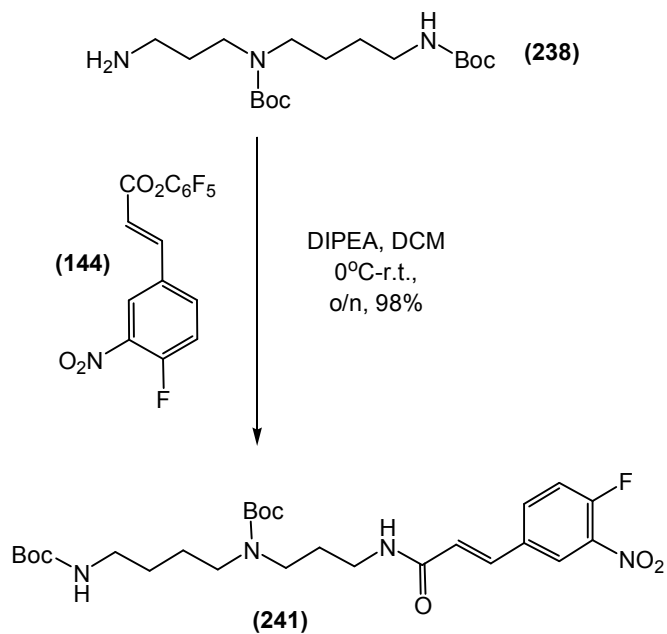
**Scheme 2.55: Synthesis of the spermidine unit for the acyclic analogues.**

The standard way of synthesising compounds such as (**238**), is by reacting 1,4 diamino butane with acrylonitrile, Boc protecting the two amino functions and then reducing the nitrile group using Raney Nickel . The reason the doubly Boc protected spermidine (**238**), was synthesised as in scheme 2.55, is because compound (**238**) was available from the previous synthetic work (section 2.2.4) and also allows the potentially difficult and low yielding Raney Nickel reduction to be avoided.<sup>51</sup>

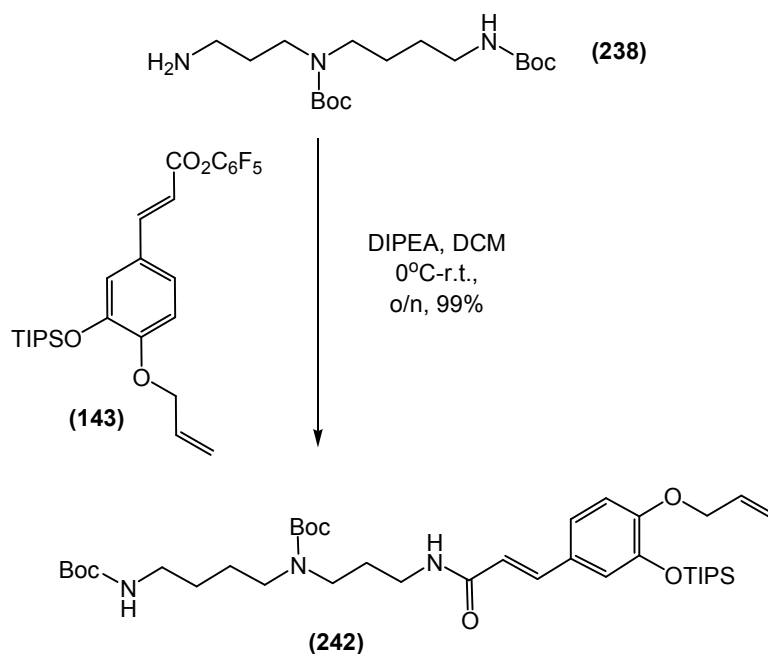


**Scheme 2.56: Fractional synthesis of spermidine derivatives.**

Compound **(238)** was in turn coupled to activated cinnamic acid derivative **(144)** and separately also to activated cinnamic acid derivative **(143)** to give the two spermidine-cinnamic acid compounds **(241)** and **(242)** in excellent yields (Schemes 2.57 and 2.58).

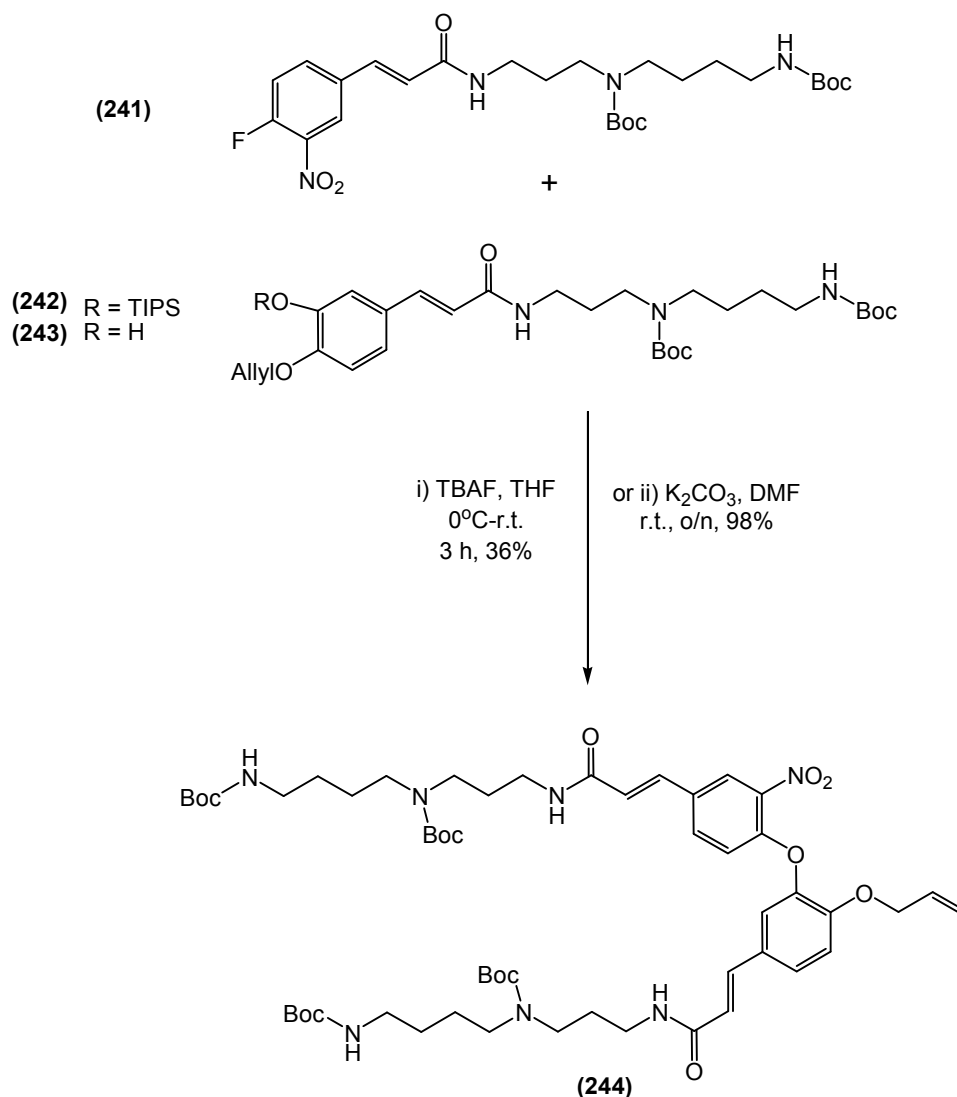


Scheme 2.57: Preparation of compound **(241)**.



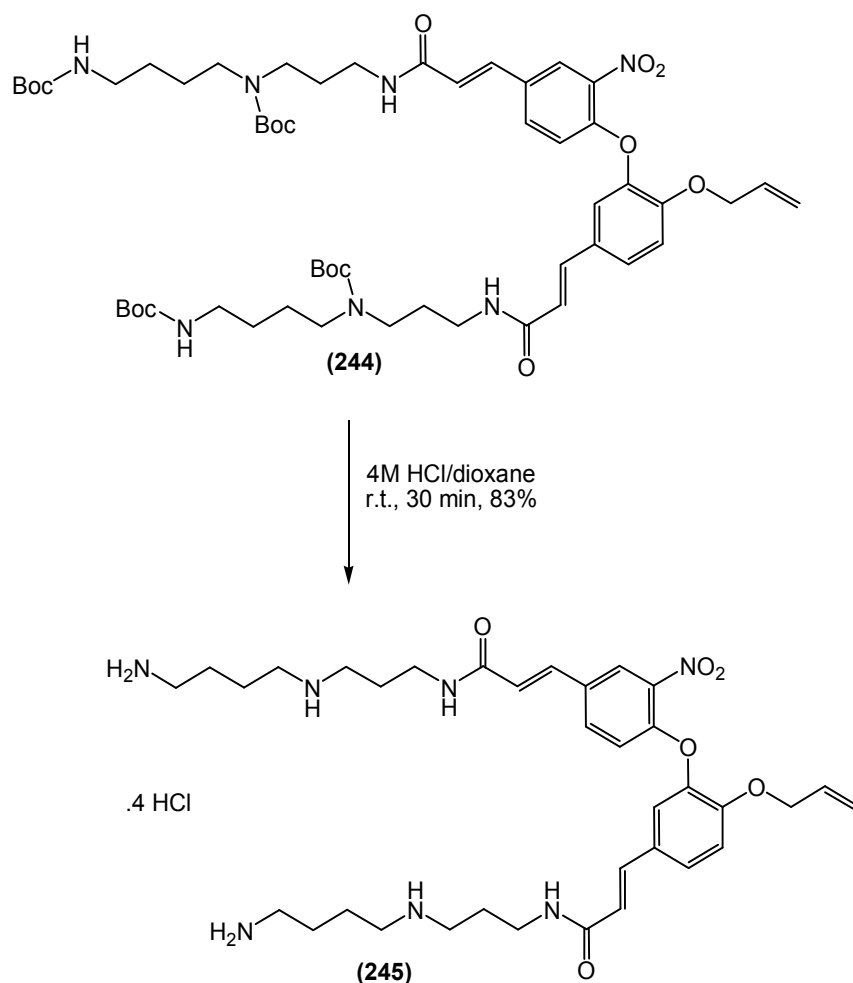
Scheme 2.58: Preparation of compound **(242)**.

The two cinnamic amides (**242**) and (**241**) were treated with TBAF to form the diphenyl ether via intermolecular  $S_NAr$ . This gave the desired diphenyl ether (**244**), however the reaction did not proceed to completion. (**244**) was isolated in 36% yield together with unchanged (**241**) (32%) and desilylated derivative (**243**) (29%). Therefore the starting materials (**241**) and (**243**) were recycled and reacted again using  $K_2CO_3$ , which proceeded to completion and gave the desired ether (**244**) in almost quantitative yield (Scheme 2.59).



**Scheme 2.59: Intermolecular  $S_NAr$  route to (**244**).**

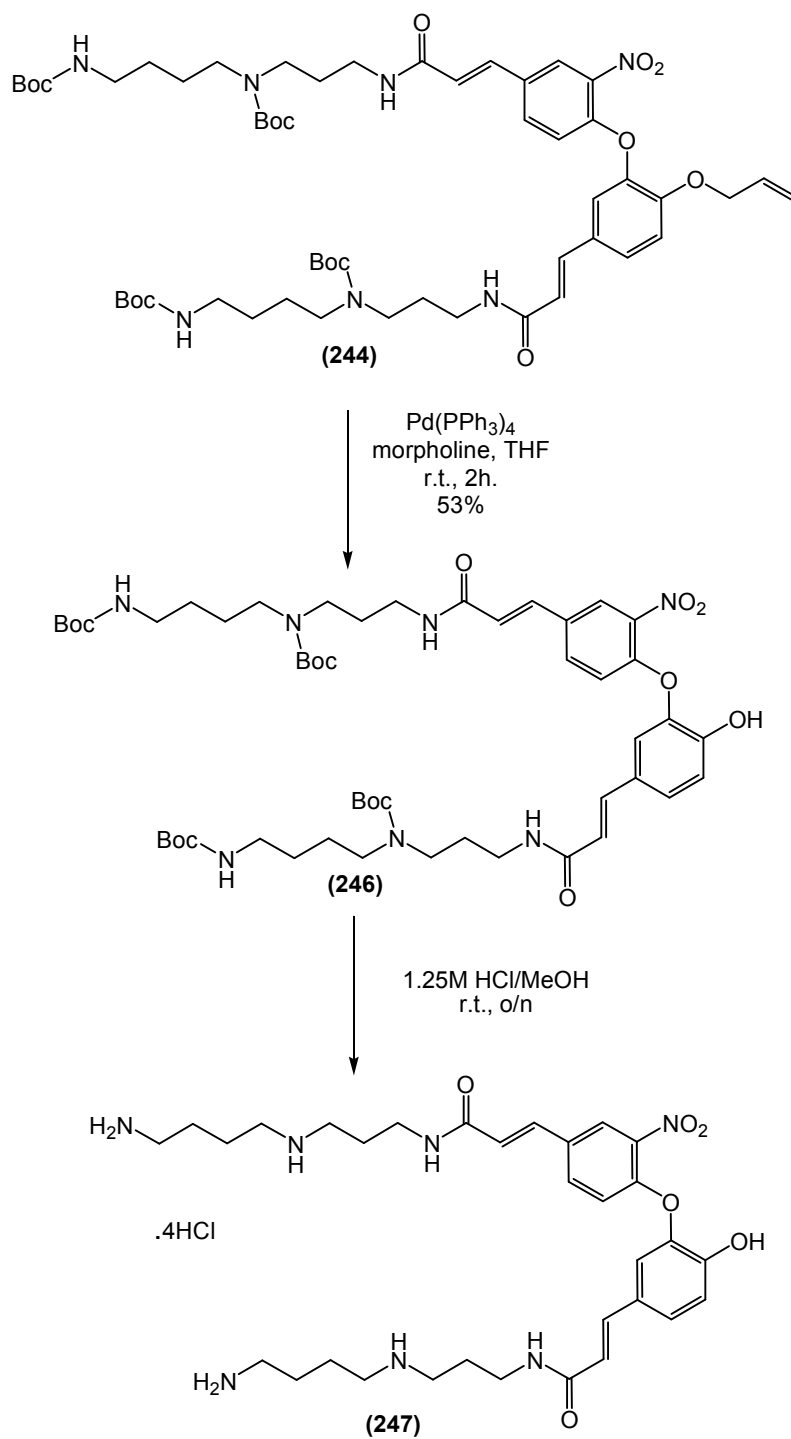
The bis spermidine-containing diphenyl ether (**244**) was then Boc deprotected (4M HCl/dioxane) to give analogue (**245**) as seen in Scheme 2.60.



**Scheme 2.60: Preparation of analogue (245).**

An interesting point about the non-cyclic analogues is that unlike the macrocyclic derivatives the spermidine-related peaks in the NMR are very broad, probably due to restricted rotation about the diphenyl ether linkage (atropisomerism).

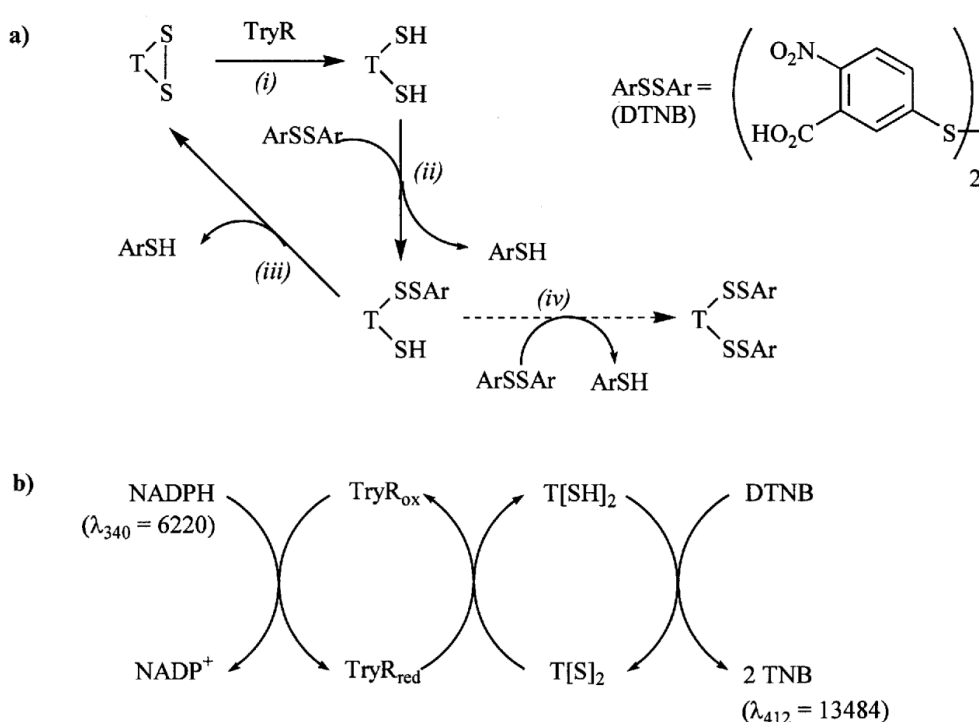
As for the allyl deprotected analogue, diphenyl analogue (**244**) was allyl deprotected first using  $(\text{Ph}_3\text{P})_4\text{Pd}$  in 53% yield. Boc protection was carried out using 1.25 M HCl in MeOH (Scheme 2.61). When the deprotection was attempted in dioxane as previously, the reaction was incomplete. This was due to the precipitation of partly deprotected intermediates, hence methanol was needed to improve solubility and avoid this problem.



**Scheme 2.61: Synthesis of acyclic cadabicine analogue (247).**

## 2.6 INHIBITION ASSAYS

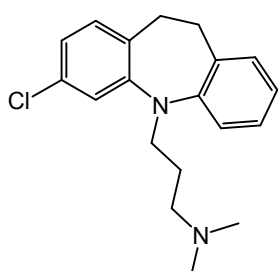
Cadabicine, its various analogues and clomipramine, a well known competitive inhibitor of TryR, were assayed against TryR using the DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)] coupled assay, which has been developed by Hamilton *et al.*<sup>48</sup> DTNB is included in the assay mixture to mediate the rapid re-oxidation of the product  $T[SH]_2$  back to  $T[S]_2$ . Therefore  $T[SH]_2$  is continually recycled, thus the concentration of the substrate remains constant for the duration of these prolonged assays, so that any decrease in enzyme activity over time would be assigned to time-dependent inhibition rather than substrate depletion (Scheme 2.62b). Reaction progress in the assay can be monitored by measuring the absorbance of the TNB anion which is yellow coloured at 412 nm. (Scheme 2.62a).



**Scheme 2.62:** (a) Mechanism of DTNB-mediated re-oxidation of  $T[SH]_2$  : i) TryR-catalysed reduction of  $T[S]_2$ ; ii) mixed-disulfide formation by reaction with DTNB *in situ*; iii) rapid intramolecular disulfide exchange reaction regenerates  $T[S]_2$ ; iv) possible reaction of the mixed-disulfide intermediate with DTNB (when in too large an excess) to give the bis-disulfide. (b) Schematic of the DTNB-coupled assay.

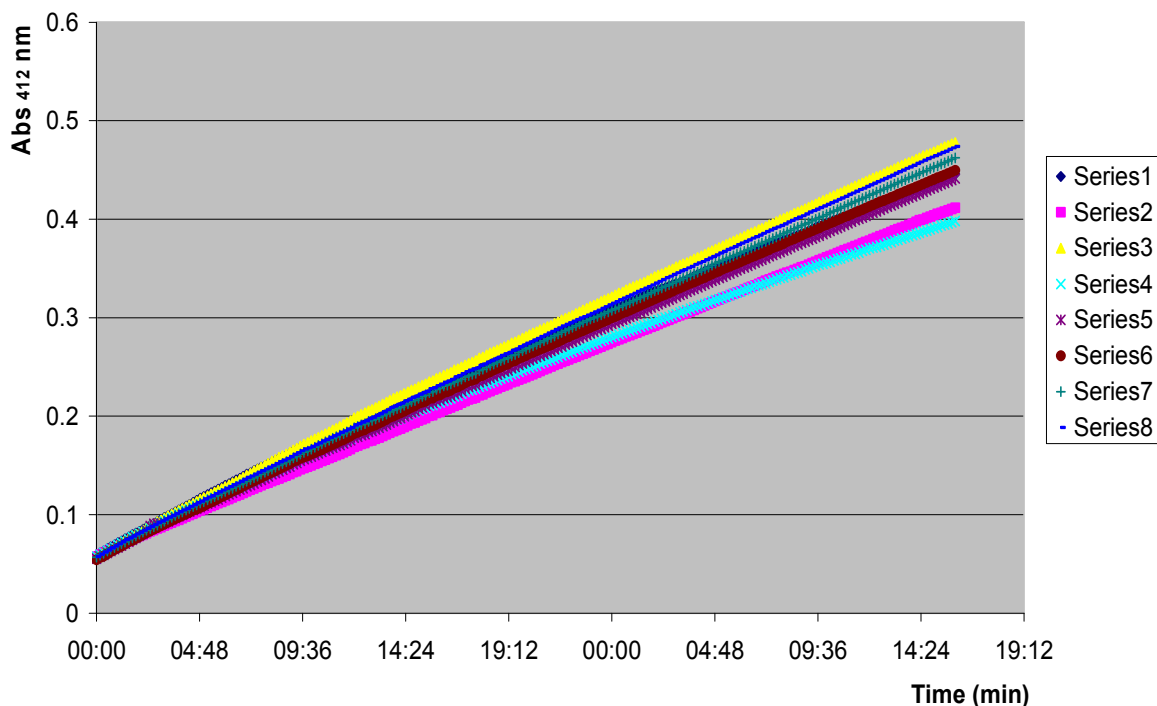


In order to determine whether cadabicine and analogues were reversible or irreversible inhibitors, an assay that determined the decrease in enzyme activity over time was devised. This was done by pre-incubating the enzyme with the inhibitor along with NADPH, for 10 min; enough time to ensure binding, before adding the substrate, DTNB and NADPH mixture. The increasing absorbance of TNB at 412 nM over time (40 min) was then measured. Clomipramine was assayed along with cadabicine and the analogues. Clomipramine is a well known reversible inhibitor of TryR, and was assayed for comparison.



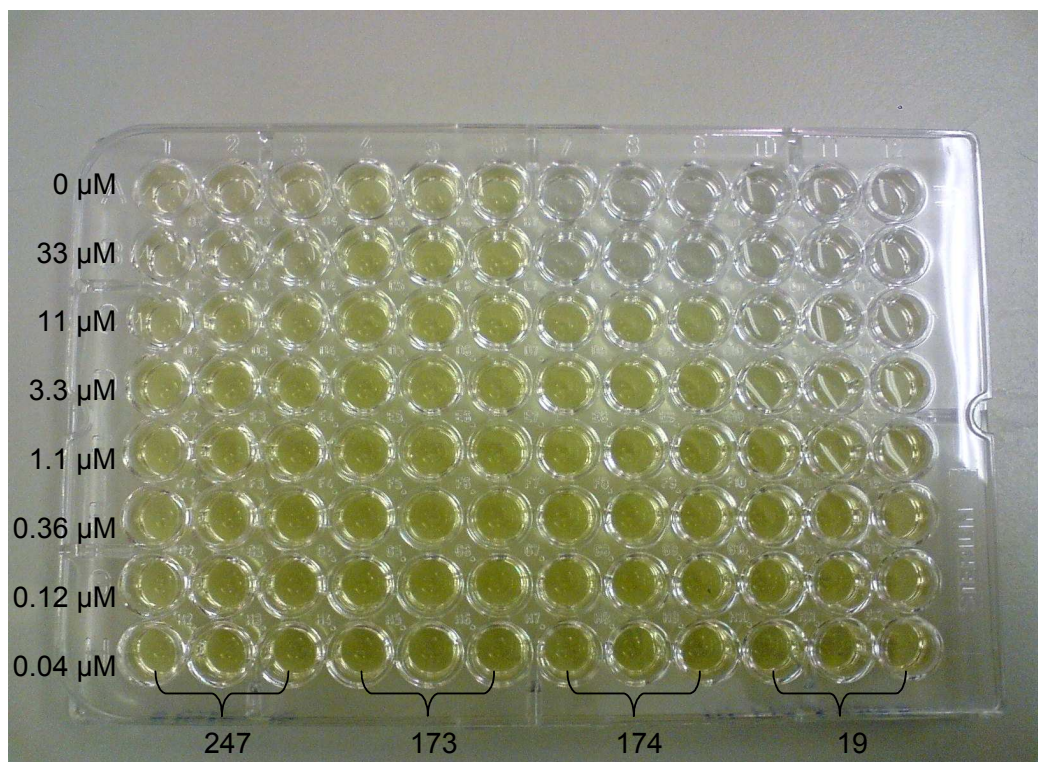
**Figure 2.13: Clomipramine (19)**

For all the new components studied, the increase in absorbance at 412 nM was linear with time, as observed for clomipramine (Figure 2.14). Thus in this assay, no time-dependent inhibition was observed. Cadabicine and analogues follow clomipramine's profile, as reversible inhibitors.

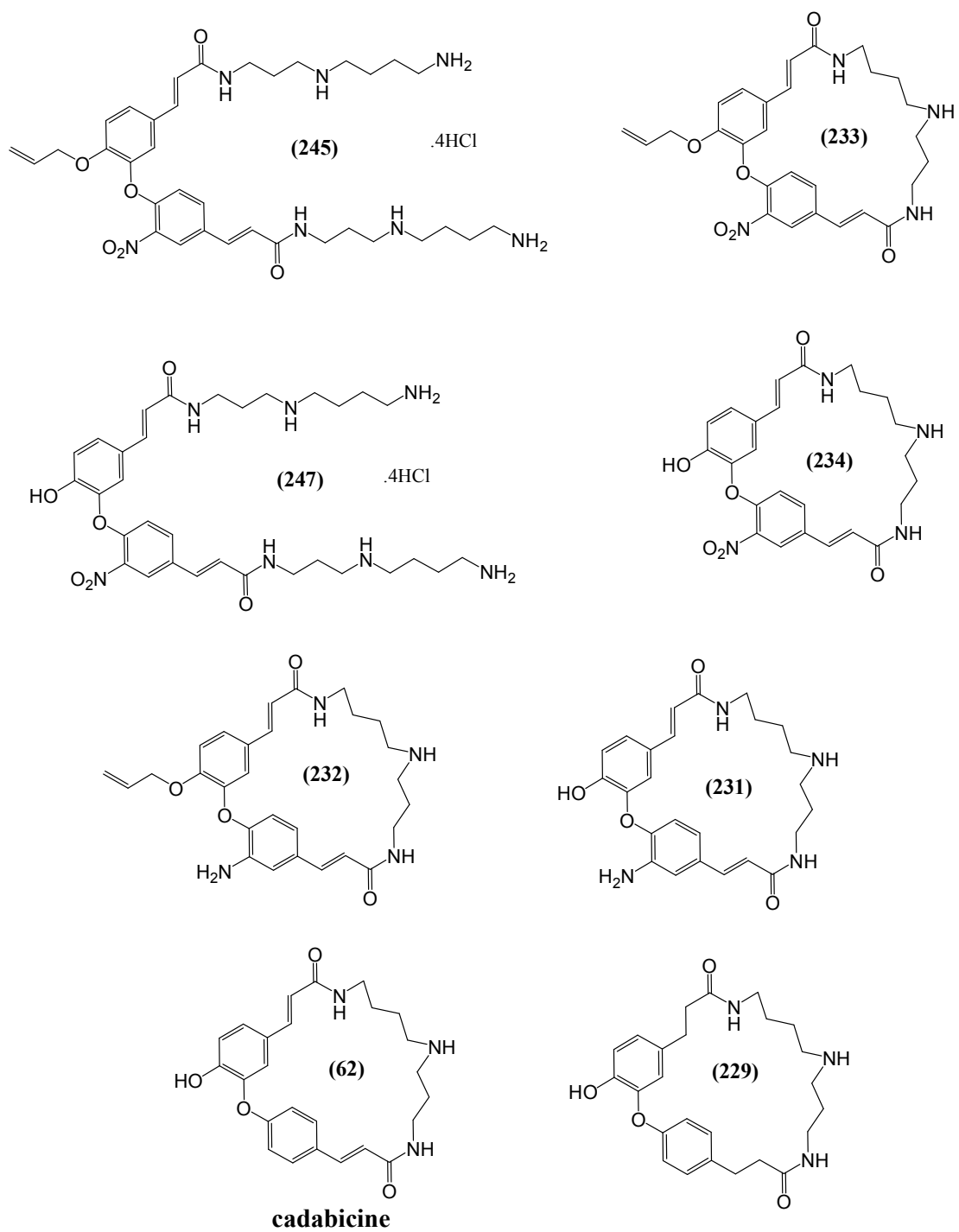


**Figure 2.14: Series 1 : Clomipramine, Series 2: (150), Series 3: (152), Series 4: (154), Series 5 : (155), Series 6: (172), Series 7: Cadabicine, Series 8: (174)**

In order to measure the  $IC_{50}$  values of cadabicine and analogues, a similar assay was devised. The enzyme TryR was pre-incubated with the inhibitor (at various concentrations) and NADPH for 5 min to ensure sufficient binding. The substrate was then added, and absorbance at 412 nm was measured over time. Each concentration of inhibitor was analysed in triplicate. An example of the microplate assay is shown in Figure 2.15. The assay mixtures contained 1% (v/v) DMSO, so as not to interfere significantly with the enzyme activity, with the exception of one clomipramine standard and cadabicine, which both contained 10% (v/v) DMSO. The results obtained in forms of  $IC_{50}$  values in Table 2, standard deviation and Hill's coefficient are summarised.



**Figure 2.15: Picture of the DTNB microassay plate- colour formation. Inhibitors tested in triplicate at various concentrations.**



**Figure 2.16: Cadabicine and analogues.**

Compound	IC <sub>50</sub> (μM)	SD (μM)	Hill coefficient
Clomipramine (19) Standard	5.5	0.15	-1.6503
Clomipramine (19) Standard 10% (v/v) DMSO	15.8	3.79	-0.8344
DMSO	N/D	N/D	N/D
(233)	Data failed to fit- No inhibition		
(234)	29.6	7.90	-2.1932
(232)	9.3	7.23	-1.3191
(247)	Data failed to fit- No inhibition		
(245)	4.7	2.37	-2.5207
(231)	Data failed to fit- No inhibition		
Cadabicine (62)	446.9	195.65	-0.8318
(229)	20.6	5.08	-2.3307

**Table 2: Assay results.**

The Hill coefficient is a measure of cooperativity in a binding process. A coefficient of 1 indicates completely independent binding, regardless of how many additional ligands are already bound. Numbers greater than one indicate positive cooperativity, while numbers less than one indicate negative cooperativity.

As seen from the Table, cadabicine is a very weak inhibitor. Even though the computer modelling (Figure 2.10) suggests that there is a possibility that one of the active double bonds is positioned in a favourable orientation for nucleophilic attack, close to an active Cys residue, this does not appear to occur in practice. That is, inhibition is reversible; no time-dependent inhibition effects that might be associated with covalent modification of the enzyme (as observed with lunarine) are apparent. Although the precise mode of inhibition cannot be deduced, cadabicine is clearly a very weak inhibitor. However compound (229), the tetrahydro analogue of cadabicine, is as potent as the standard clomipramine, but again the mode of inhibition cannot be deduced from this preliminary study. The absence of the double

bonds in **(229)**, would improve its conformational flexibility, and hence allow **(229)** to mould more efficiently in the TryR active site. The TryR active site is quite rigid,<sup>46</sup> therefore relatively flexible inhibitors are more likely to be favoured. This scaffold is an interesting inhibition motif for further study. A variety of tetrahydro analogues can potentially be synthesised, with different groups present in the diphenyl ether, or changes in the polyamine chain, or even acyclic tetrahydro analogues.

Compound **(233)** does not inhibit TryR, while the phenol containing compound **(234)** is a moderate reversible inhibitor. Compound **(232)** is a good reversible inhibitor, the best in the series, but its allyl deprotected analogue **(231)** has negligible inhibition.

Despite the fact that lunarine-based non-cyclic inhibitors were found to be time dependent inhibitors, cadabicine based non-cyclic analogue **(247)** does not inhibit TryR, but **(245)** has an apparent  $IC_{50}$  of 4.7. However since the compound is yellow coloured and DTNB is also yellow, this could be a false positive. Even though these structures are more flexible than cadabicine, the nature of their flexibility could be to their disadvantage. The spermidine chains would make the molecules too flexible to readily adapt the appropriate configuration in order to fit in the enzyme's active site.

To summarise, cadabicine is not a time-dependent TryR inhibitor as proposed, but a weak reversible inhibitor. None of the analogues showed time-dependent inhibition but compounds **(234)**, **(232)** and **(229)** are good reversible inhibitors. Overall, the changes to the substitution pattern of the diphenyl ether moiety of cadabicine examined here, do not seem to have any significant effect on TryR.

## 2.7 CONCLUSIONS & FUTURE WORK

The total synthesis of the natural alkaloid cadabicine has been successfully completed. This was achieved by developing an efficient route to triply orthogonally protected spermidine, both by fragment synthesis, and selective functionalisation. Furthermore a key step to the synthesis was the efficient and facile cyclisation of the functionalised linear spermidine derivative (**154**) to the macrocycle (**224**). This methodology can be generally applied in the synthesis of alkaloid macrocycles. We used methods and techniques that are mild and sensitive to moieties most commonly found in polyamine alkaloids.

A small series of cyclic and non-cyclic analogues of cadabicine analogues were synthesised, and evaluated as TryR inhibitors. Cadabicine based on its structural similarity to lunarine a known time-dependent TryR inhibitor, and from docking experiments, was expected to show time-dependent inhibition. From the TryR inhibition assays, performed in this study, it appears that cadabicine is a weak reversible inhibitor of TryR. However the tetrahydro analogue (**229**) showed some promising results. The double bonds present in cadabicine could cause the compound to be more rigid, and affect its flexibility in properly fitting into the TryR active site. The tetrahydro analogue (**229**) lacks the double bonds making it more flexible hence the better inhibition results. The scaffold of (**229**) makes a promising template for inhibitor design. Future work would include the synthesis of both cyclic and non-cyclic analogues based on (**229**) scaffold, as potential TryR inhibitors. It would also be worth exploring further, to determine the mode of inhibition of these types of compounds as well as testing their biological activity against *Trypanosoma* and *Leishmania* species.

Another scaffold worth investigating is the Pschorr tricyclic derivative (**227**) that we came across in the deamination studies. The scaffold of (**227**) is very closely related to those of tricyclic antidepressant inhibitors of TryR. It would be interesting to examine if cyclic or non-cyclic analogues based on that motif would inhibit TryR, and their mode of inhibition, both retaining and not retaining the double bonds.

### 3. EXPERIMENTAL

#### 3.1 GENERAL

Chemical reagents were purchased from Sigma, Aldrich, Fluka, Acros, Lancaster and Novabiochem. Anhydrous DCM was obtained by distillation over calcium hydride, anhydrous THF and Et<sub>2</sub>O over sodium/benzophenone. All other solvents were purchased from Fisher Scientific. Analytical TLC was performed using silica gel 60 F<sub>254</sub> pre-coated on aluminium sheets (0.25 mm thickness) and reverse phase analytical TLC was performed with RP-18 F<sub>254</sub> pre-coated on aluminium sheets (0.27 mm thickness). Column chromatography was performed on silica gel 60 (35-70 micron) from Fisher Scientific. Melting points were recorded on a Reichert-Jung Kofler block apparatus and are uncorrected. <sup>1</sup>H and <sup>13</sup>C NMR were recorded using a Bruker Advance DPX 500MHz FT, JEOL JMN GX-270MHz or EX-400MHz spectrometers. *J* values are given in Hz. High resolution mass spectrometry was performed using a Bruker MicroTOF autospec electrospray ionisation mass spectrometer. Analytical RP-HPLC was performed on a Dionex HPLC system (system 1) equipped with a Dionex Acclaim 3 μm C-18 (150 x 4.6mm) column with a flow rate of 1 mL/min or a Dionex UltiMate 3000 HPLC system (system 2) equipped with a Phenomenex Gemini 5μm C-18 (150 x 4.6 mm) column with a flow rate of 1 mL/min. Preparative RP-HPLC was performed on a Dionex HPLC system equipped with a Phenomenex Gemini 5μm C-18 (250 x 30 mm) with a flow rate of 22.5 mL/min. Mobile phase A was 0.1% TFA in water, mobile phase B was 0.1% TFA in acetonitrile, mobile phase C was 0.1% FA in water, mobile phase D was 0.1% FA in acetonitrile unless specified.

Analytical HPLC gradient: *T* = 0 min, *D* = 5%; *T* = 20 min, *D* = 95%; *T* = 20.1 min, *D* = 5%; *T* = 28.1 min, *D* = 5%.

Preparative HPLC gradient : *T* = 0 min, *B* = 5%; *T* = 20 min, *B* = 95%; *T* = 20.1 min, *B* = 5%; *T* = 28.1 min, *B* = 5%.



## 3.2 DOCKING CALCULATIONS

Methods for the generation of Ligands and Performance of Docking Calculations:

The molecules were created in Sybyl 7.1 and all preparation calculations were performed within the Sybyl 7.1 molecular modelling package. The molecules were built using the molecule building tools from the provided structures and were then minimised using the MMFF94s force field. Each compound was subjected to 10 pico seconds of simulated annealing of heating to 350 K followed by cooling to 150 K. This was repeated for 20 cycles, then the lowest energy conformation was then re-minimised using the MMFF94s force field as implemented within Sybyl 7.1. The docking calculations were performed in the GOLD<sup>135</sup> docking program from the CDCC. The docking site was described by a 12 angstrom radius encompassing the active site from the PDB crystal structure of TryR using the bound ligand as a reference. Each ligand was docked in a total of 25 times.

## 3.3 INHIBITION ASSAYS

*T. cruzi* was obtained from Prof. Alan Fairlamb (University of Dundee). The enzyme was supplied as 70% ammonium sulfate precipitates.<sup>48</sup>

TryR molecular weight calculated as 53867.8 Da based on the Swissprot sequence calculated using protparam (<http://www.expasy.ch/tools/protparam.html>). Protein concentrations were determined using the Bio-Rad DC assay in a microtitre plate with 1.5, 0.75, 0.325, 0.1625 mg/mL BSA as standard. This assay measures the protein main-chain. The stock solution of TryR was determined to be  $0.971 \pm 0.039$  mg/mL ( $18 \pm 0.72$   $\mu$ M).

The standard assay mixture contained TryR, buffer, NADPH, T[S]<sub>2</sub>, DTNB and various concentrations of inhibitor. Inhibitor stock solutions were made up using buffer and DMSO, so that the final assay mixture contained no more than 1% (v/v) DMSO, so as not to interfere significantly with enzyme activity. Enzyme activity was

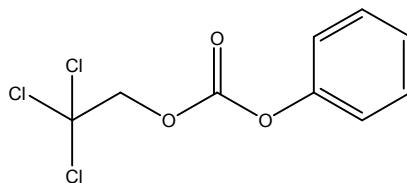
measured by the increase in absorbance at 412nm due to the formation of TNB; using a P.E. Lamda EZ201 UV-visible recording microplate spectrophotometer.

IC<sub>50</sub> determinations using 5  $\mu$ M substrate, used 3.6 nM of enzyme in the assay. All absorbances read at 40 minutes incubation. Determinations of clomipramine and Compound **(62)** used dilutions of compound in 50% DMSO/buffer. DMSO concentration was held constant at 10% (v/v) in addition to the small amount of DMSO in the buffer. Protein concentrations in these experiments were 10.8 nM and absorbances were read at 30 minutes. In all determinations control experiments showed that longer incubation times resulted in higher absorbances, *i.e.* DTNB was not limiting. Data was analysed with SigmaPlot 11.0 using the four parameter logistic equation using y-replicatants and log<sub>10</sub> concentration scale.

### 3.4 COMPOUND DATA

#### Carbamic acid phenyl ester 2,2,2-trichloroethyl ester

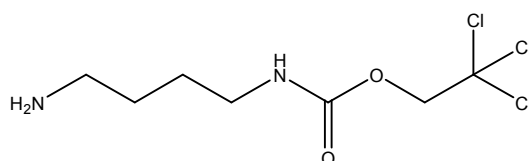
[104]



A stirred solution of trichloroethanol (7.5 g, 0.05 mol) and pyridine (5 mL) in DCM (8 mL) was treated with phenyl chloroformate (7.83 g, 0.05 mol) added over 1 h. The reaction mixture was stirred for an additional 3 h. Water (15 mL) was added. The organic phase was washed with aqueous sulfuric acid (2 M, 2x15 mL), dried (MgSO<sub>4</sub>), filtered and evaporated to dryness to give **104** as a white solid (13.0 g, 96%); mp: 29-31°C;  $\delta_{\text{H}}$  (500MHz, CDCl<sub>3</sub>): 4.80 (2H, s, CH<sub>2</sub>), 7.15 (2H, d, *J* 7.5, Ar), 7.21 (2H, t, *J* 7.5, Ar), 7.34 (1H, t, *J* 7.5, Ar);  $\delta_{\text{C}}$  (125MHz, CDCl<sub>3</sub>): 77.31 (CH<sub>2</sub>), 94.17(CCl<sub>3</sub>), 120.95 (Ar), 126.55 (Ar), 129.67 (Ar), 150.86 (Ar), 152.63 (C=O); [Found: (ES<sup>+</sup>) 290.9355 [M+Na]<sup>+</sup>, C<sub>9</sub>H<sub>7</sub>C<sub>13</sub>O<sub>3</sub>Na requires 267.9353].

#### (4-Amino-butyl)-carbamic acid 2,2,2-trichloroethyl ester<sup>60</sup>

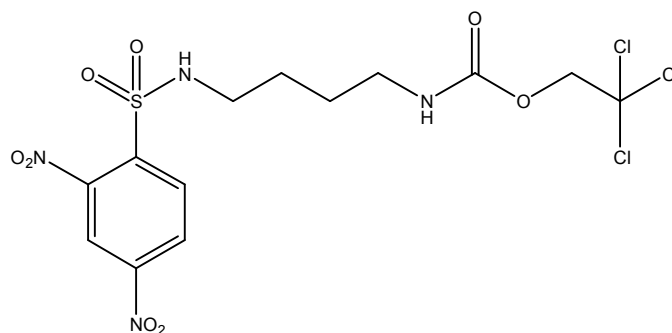
[105]



A stirred solution of **104** (15.52 g, 0.0576 mol) in absolute ethanol (200 mL), was treated with 1,4-diaminobutane (5.07 g, 0.0576 mol). The mixture was stirred overnight at room temperature. Water was added (200 mL) and the pH was adjusted to 3 by addition of aqueous HCl (1M), followed by extraction with DCM (2 x 400 mL). The aqueous phase was then made strongly basic by addition of aqueous NaOH (1M) and extracted with DCM (2 x 400 mL). The organic phase was dried (MgSO<sub>4</sub>), filtered and evaporated to dryness to give **105** as a yellow oil (9.62g, 63%);  $\delta_{\text{H}}$

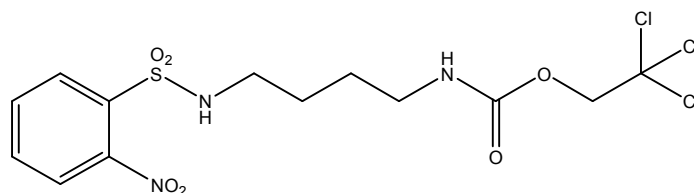
**(500MHz, DMSO):** 1.30-1.36 (2H, m, CH<sub>2</sub>CH<sub>2</sub>), 1.41-1.47(2H, m, CH<sub>2</sub>CH<sub>2</sub>), 1.51-1.54 (1H, br, NH), 1.73-2.00 (2H, br, NH<sub>2</sub>), 2.52 (2H, t, *J* 6.8, CH<sub>2</sub>N), 3.01(2H, q, *J* 6.8, CH<sub>2</sub>N), 4.78(2H, s, CH<sub>2</sub>CCl<sub>3</sub>). **δ<sub>C</sub> (100MHz, DMSO):** 26.54 (CH<sub>2</sub>), 28.95(CH<sub>2</sub>), 40.20 (CH<sub>2</sub>-NH), 42.18 (CH<sub>2</sub>-NH), 75.29 (CH<sub>2</sub>CCl<sub>3</sub>), 96.37 (CCl<sub>3</sub>), 165.85 (C=O). [Found: (ES<sup>+</sup>) 263.0104 [M+H]<sup>+</sup>, C<sub>7</sub>H<sub>14</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>2</sub> requires 263.0115].

**[4-(2,4-Dinitro-benzenesulfonylamino)-butyl]-carbamic acid 2,2,2-trichloroethyl ester**<sup>61</sup> **[107]**



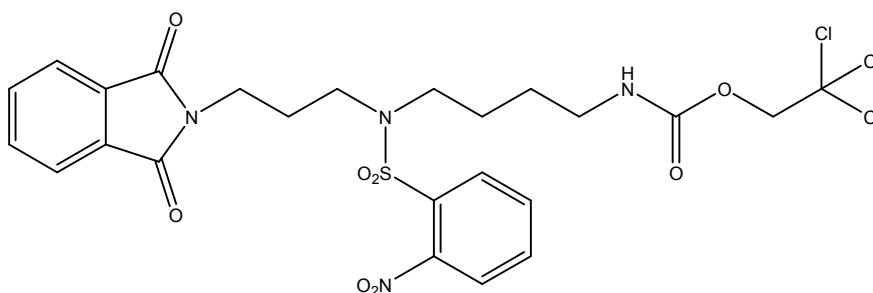
A stirred solution of **105** (0.5 g, 0.0018 mol) in DCM (8 mL), was treated with 2,6-lutidine (0.204 g, 0.0018 mol), followed by 2,4-dinitrosulfonyl benzene chloride (0.506 g, 0.0018 mol). The mixture was stirred at 0°C for 10 min, and then stirred for a further 3 h at room temperature. Purification by silica gel chromatography (0-50% ethyl acetate/hexane) gave **107** as a yellow oil (0.654 g, 70%); **δ<sub>H</sub> (500MHz, CDCl<sub>3</sub>):** 1.54 (4H, m, 2xCH<sub>2</sub>), 3.13 (4H, q, *J* 6.5, CH<sub>2</sub>NH), 4.62 (2H, s, CH<sub>2</sub>CCl<sub>3</sub>), 5.03 (1H, t, *J* 5.7, NH), 5.51 (1H, t, *J* 6.0, NH), 8.29 (1H, d, *J* 8.6, Ar), 8.49 (1H, dd, *J* 8.6, 2.5, Ar), 8.60 (1H, d, *J* 2.5, Ar); **δ<sub>C</sub> (125MHz, CDCl<sub>3</sub>):** 21.11 (CH<sub>2</sub>), 26.92 (CH<sub>2</sub>), 40.41 (CH<sub>2</sub>NH), 43.44 (CH<sub>2</sub>NH), 74.46 (CH<sub>2</sub>CCl<sub>3</sub>), 95.58 (CCl<sub>3</sub>), 120.85 (Ar-CH), 127.23 (Ar-CH), 132.63, (Ar-CH), 139.12 (Ar-CSO<sub>2</sub>), 148.22 (Ar-CNO<sub>2</sub>), 149.86 (Ar-CNO<sub>2</sub>), 154.75 (C=O). [Found: (ES<sup>+</sup>) 492.9752 [M+H]<sup>+</sup>, C<sub>13</sub>H<sub>16</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>8</sub>S requires 492.9749].

**[4-(2-Nitro-benzenesulfonylamino)-butyl]carbamic acid 2,2,2-trichloro-ethyl ester**<sup>61</sup> [109]



Preparation as for compound **107** on a 7.7mmol scale but using 2-nitro sulfonyl chloride, gave **109** (0.8g, 24%) as a yellow oil.  $\delta_{\text{H}}$  (500MHz,  $\text{CDCl}_3$ ): 1.51-1.56 (4H, m,  $\text{CH}_2\text{CH}_2$ ), 3.065 (2H, q,  $J$  6.3,  $\text{NHCH}_2$ ), 3.16 (2H, q,  $J$  6.3,  $\text{NHCH}_2$ ), 4.64 (2H, s,  $\text{CH}_2\text{CCl}_3$ ), 7.66-7.70 (2H, m, Ar H-4, Ar H-5), 7.78-7.82 (1H, m, Ar-6), 8.05-8.09 (1H, m, Ar-3).  $\delta_{\text{C}}$  (67.5MHz,  $\text{CDCl}_3$ ): 21.38 ( $\text{CH}_2$ ), 26.87 ( $\text{CH}_2$ ), 40.62 ( $\text{NH-CH}_2$ ), 43.37 ( $\text{NH-CH}_2$ ), 74.54 ( $\text{CH}_2\text{CCl}_3$ ), 100.00 ( $\text{CCl}_3$ ), 125.56 (Ar-3), 131.20 (Ar-6), 132.98 (Ar-5), 133.76 (Ar-4), 138.79 (Ar- $\text{SO}_2$ ), 148.50 (Ar-  $\text{NO}_2$ ), 154.74 ( $\text{C=O}$ ); [Found: ( $\text{ES}^+$ ) 447.9894 [ $\text{M}+\text{H}$ ]<sup>+</sup>,  $\text{C}_{13}\text{H}_{17}\text{Cl}_3\text{N}_3\text{O}_6\text{S}$  requires 447.9898].

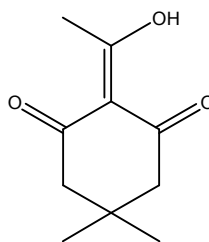
**[4-[[3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-propyl-(2-nitro-benzenesulfonyl)-amino]-butyl]-carbamic acid 2,2,2-trichloro-ethyl ester**<sup>63</sup> [110]



A stirred solution of **109** (0.204 g, 0.4 mmol) in DMF (25 mL), was treated with 3-bromopropyl phthalimide (0.11 g, 0.4 mmol) and potassium carbonate (0.167 g, 1.2 mmol). The reaction mixture was heated at 50°C overnight. The solvent was evaporated and the residue was taken up in ethyl acetate (25 mL) and washed sequentially with 10% citric acid (2 x 25 mL), saturated  $\text{NaHCO}_3$  (2 x 25 mL) and brine (2 x 25 mL). The organic layer was dried ( $\text{MgSO}_4$ ), and purified by silica gel chromatography (10-50% EtOAc/Hex) to give **110** (0.023 g, 9%), as a yellow oil.  $\delta_{\text{H}}$

(500MHz, CDCl<sub>3</sub>): 1.55-1.72 (4H, m, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>N), 1.918 (2H, quintet, *J* 7.3, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH), 3.33-3.41 (4H, m, Ns-N(CH<sub>2</sub>)<sub>2</sub>), 3.70 (2H, t, *J* 7.4, Dde-NCH<sub>2</sub>), 3.71 (2H, q, *J* 6.5, HNCH<sub>2</sub>), 4.70 (2H, s, CH<sub>2</sub>CCl<sub>3</sub>), 7.60-7.72 (3H, m, Ar-4,5,6(Ns)), 7.72-7.74 (2H, m, Ar<sub>3,4</sub>(Phth)), 7.84-7.87 (2H, m, Ar<sub>2,6</sub>(Phth)), 7.97 (1H, dd, *J* 1.5, 7.6, Ar-3(Ns)). δ<sub>C</sub> (67.5MHz, CDCl<sub>3</sub>): 25.89 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.87 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 27.49 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 35.54 (NCH<sub>2</sub>), 40.69 (NCH<sub>2</sub>), 45.74 ((Ns)-NCH<sub>2</sub>), 47.77 ((Ns)-NCH<sub>2</sub>), 74.49 (CH<sub>2</sub>-CCl<sub>3</sub>), 123.48 ((Ns)-Ar<sub>3</sub>), 124.32 ((Dde)-Ar-2), 130.81 ((Dde)-Ar-6), 131.79 ((Dde)-Ar-1), 132.02 ((Dde)-Ar-3), 133.13 ((Ns)-Ar-4), 133.70 ((Ns)-Ar-5), 134.22 ((Ns)-Ar-1), 168.37 ((Dde)-C=O); [Found: (ES<sup>+</sup>) 657.0366 [M+Na]<sup>+</sup>, C<sub>24</sub>H<sub>25</sub>C<sub>13</sub>N<sub>4</sub>NaO<sub>8</sub>S requires 657.0351].

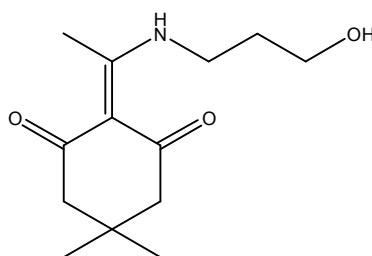
**2-(1-Hydroxy-ethylidene)-5,5-dimethyl-cyclohexane-1,3-dione**<sup>70</sup> [112]



A solution of dimedone (6.0 g, 42.8 mmol), dicyclohexylcarbodiimide (8.83 g, 42.8 mmol), acetic acid (2.56 g, 42.8 mmol), and dimethylaminopyridine (0.523 g, 42.8 mmol) in DMF (70 ml) was stirred overnight at room temperature. The precipitate that formed was removed by filtration, and the filtrate was poured into water (100 mL) and extracted with ethyl acetate (4x60 mL). The organic phase was dried (MgSO<sub>4</sub>) and evaporated to dryness. Purification by silica gel chromatography (0-5% MeOH/DCM) gave **112** as a yellow oil (7.88 g, 66%). δ<sub>H</sub> (500MHz, CDCl<sub>3</sub>): 1.10 (6H, s, C(CH<sub>3</sub>)<sub>2</sub>), 2.37 (2H, s, CH<sub>2</sub>), 2.54 (2H, s, CH<sub>2</sub>), 2.61 (3H, s, C=CCH<sub>3</sub>). δ<sub>C</sub> (125MHz, CDCl<sub>3</sub>): 28.26 (C(CH<sub>3</sub>)<sub>2</sub>), 28.67 (C(CH<sub>3</sub>)<sub>2</sub>), 30.73 (C(CH<sub>3</sub>)<sub>2</sub>), 46.94 (CH<sub>2</sub>), 52.52 (CH<sub>2</sub>), 112.41 (C=COH), 195.29 (C=O), 197.99 (COH), 202.57 (C=O).

**2-[1-(3-Hydroxy-propylamino)-ethylidene]-5,5-dimethyl-cyclohexane-1,3-dione**<sup>63</sup>

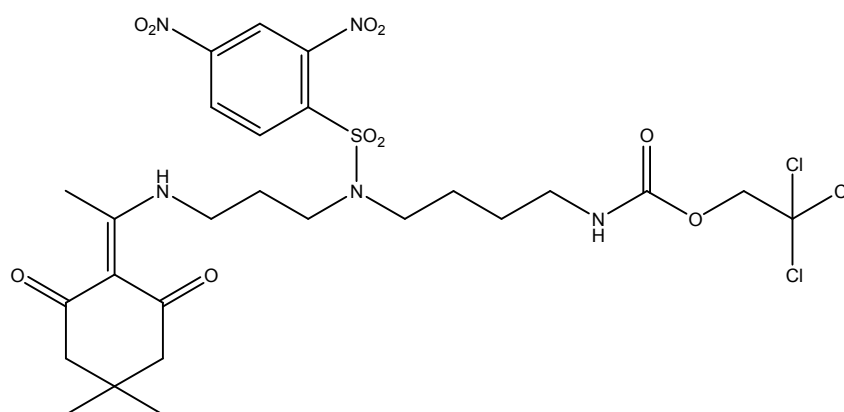
[113]



A stirred solution of **112** (2.00 g, 0.0109 mol) in ethanol (3 mL), was treated with 3-amino propanol (0.816 g, 0.0109 mol). The mixture was heated at 80°C under reflux for 30 min. The mixture was cooled, and the solvent was evaporated. Recrystallisation from ethyl acetate-hexane gave **113** as white crystals (2.103 g, 87%); **mp**: 106-108°C;  **$\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>)**: 0.98 (6H, s, C(CH<sub>3</sub>)<sub>2</sub>), 1.88 (2H, quintet, *J* 6.4, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.31 (4H, s, CH<sub>2</sub>-Dde), 2.52 (3H, s, C=CCH<sub>3</sub>), 3.09 (1H, br, NH), 3.53 (2H, q, *J* 6.4, CH<sub>2</sub>NH), 3.72 (2H, t, *J* 5.8, CH<sub>2</sub>OH), 13.63 (1H, br, OH).  **$\delta_{\text{C}}$  (67.5MHz, CDCl<sub>3</sub>)**: 18.05 (C=CCH<sub>3</sub>), 28.33 (C(CH<sub>3</sub>)<sub>2</sub>), 30.20 (HNCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH), 31.63 (C(CH<sub>3</sub>)<sub>2</sub>), 38.70 (CH<sub>2</sub>NH), 40.37 (CH<sub>2</sub>), 59.14 (CH<sub>2</sub>OH), 107.95 (C=CCH<sub>3</sub>), 173.88 (C=CCH<sub>3</sub>), 198.10 (C=O).

**{4-[3-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino]-propyl}-(2,4-dinitro-benzenesulfonyl)-amino]-butyl}-carbamic acid 2,2,2-trichloro-ethyl ester**

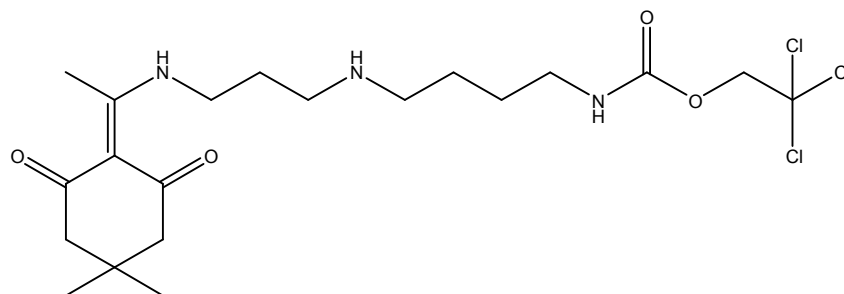
[116]



A suspension of **107** (1.150g, 2.32mmol), **7** (0.0556g, 2.32mmol), diethylazodicarboxylate (0.405g, 2.32mmol), and polymer-supported triphenylphosphine (2.117 g, 2.32 mmol), in toluene (5 mL) was reacted at room temperature overnight.

The polymer-supported reagent was filtered off, and the filtrate was evaporated to dryness. Purification by silica gel chromatography (0-50% ethyl acetate/DCM) gave **116** as a yellow oil (0.679 g, 41%);  $\delta_{\text{H}}$  (500MHz,  $\text{CDCl}_3$ ): 1.05 (6H, s,  $\text{C}(\text{CH}_3)_2$ ), 1.55-1.61 (4H, m,  $2 \times \text{CH}_2$ ), 1.68-1.74 (2H, m,  $\text{CH}_2$ ), 2.36 (2H, br,  $\text{CH}_2$ ), 2.44 (2H, br,  $\text{CH}_2$ ), 2.56 (3H, s,  $\text{C}=\text{CCH}_3$ ), 3.28 (2H, q,  $J$  6.4,  $\text{CH}_2\text{NH}$ ), 3.39 (2H, t,  $J$  6.4,  $\text{CH}_2\text{NH}$ ), 3.43-3.52 (4H, m,  $2 \times \text{CH}_2\text{NH}$ ), 4.73 (2H, s,  $\text{CH}_2\text{CCl}_3$ ), 5.72 (NH), 8.33 (1H, d,  $J$  8.6, Ar), 8.49 (1H, d,  $J$  2.1, Ar), 8.53 (1H, dd,  $J$  2.1,  $J$  8.6, Ar), 13.45 (NH);  $\delta_{\text{C}}$  (67.5MHz,  $\text{CDCl}_3$ ): 18.02 ( $\text{CH}_3$ ), 25.21 ( $\text{CH}_2$ ), 26.70 ( $\text{CH}_2$ ), 27.79 ( $\text{CH}_2$ ), 28.21 ( $\text{CH}_3$ ), 30.09 ( $\text{C}(\text{CH}_3)_2$ ), 40.29 ( $\text{CH}_2\text{NH}$ ), 40.34 ( $\text{CH}_2\text{NH}$ ), 44.93 ( $\text{CH}_2\text{NH}$ ), 47.58 ( $\text{CH}_2\text{NH}$ ), 61.40 ( $\text{CH}_2$ ), 74.32 ( $\text{CH}_2\text{-CCl}_3$ ), 95.68 ( $\text{CCl}_3$ ), 108.07 ( $\text{C}=\text{CCH}_2$ ), 119.78 (Ar), 126.30 (Ar), 132.82 (Ar), 138.24 (Ar- $\text{SO}_2$ ), 147.93 (Ar- $\text{NO}_2$ ), 149.71 (Ar- $\text{NO}_2$ ), 154.80 ( $\text{C}=\text{O}$ ), 173.93 ( $\text{C}=\text{CCH}_2$ ), 198.02 ( $\text{C}=\text{O}$ ).

**(4-{3-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-ethylaminol]-propylamino}-butyl)-carbamic acid 2,2,2-trichloro-ethyl ester** [117]

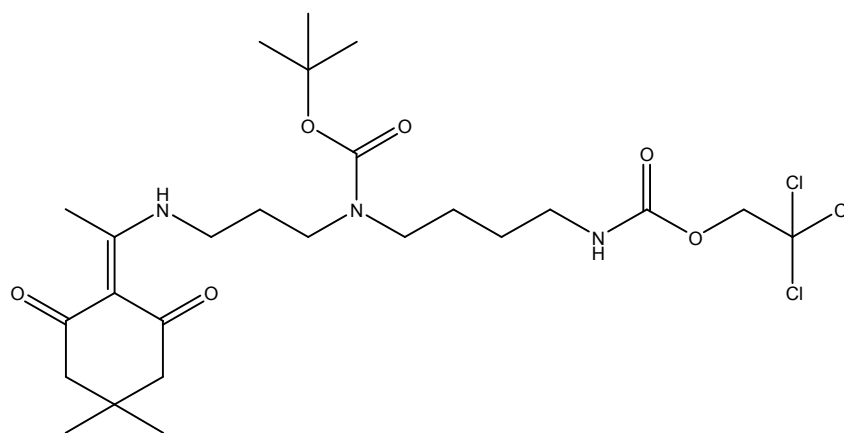


A stirred solution of **116** (0.64 g, 0.89 mmol) in DCM (10 mL), was treated with thioglycolic acid (0.13 mL, 1.8 mmol) and DIPEA (0.32 mL, 1.8 mmol). The reaction was stirred at room temperature for 3 h. The reaction mixture was diluted with DCM (15 mL) and washed with saturated  $\text{NaHCO}_3$  (2 x 25 mL) and brine (2 x 25 mL). The organic phase was dried ( $\text{MgSO}_4$ ) and evaporated to dryness to give **117** as a pale yellow oil (0.365 g, 84%).  $\delta_{\text{H}}$  (500MHz,  $\text{CDCl}_3$ ): 1.03 (6H, s,  $\text{C}(\text{CH}_3)_2$ ), 2.56 (3H, s,  $\text{C}=\text{CCH}_3$ ), 2.36 (4H, s,  $2 \times \text{CH}_2$ ), 1.53-1.66 (4H, m,  $2 \times \text{CH}_2$ ), 1.87 (2H, quintet,  $J$  6.8,  $\text{CH}_2$ ), 2.66 (2H, t,  $J$  6.7,  $\text{CH}_2\text{NH}$ ), 2.74 (2H, t,  $J$  6.7,  $\text{CH}_2\text{NH}$ ), 3.24 (2H, q,  $J$  5.9,  $\text{CH}_2\text{NH}$ ), 3.50 (2H, q,  $J$  4.6,  $\text{CH}_2\text{NH}$ ), 4.72 (2H, s,  $\text{CH}_2\text{CCl}_3$ ), 6.11 (1H, s, NH), 13.43 (1H, s, NH).  $\delta_{\text{C}}$  (67.5MHz,  $\text{CDCl}_3$ ): 17.94 ( $\text{C}=\text{CCH}_3$ ), 27.19 ( $\text{CH}_2$ ), 27.54 ( $\text{CH}_2$ ),



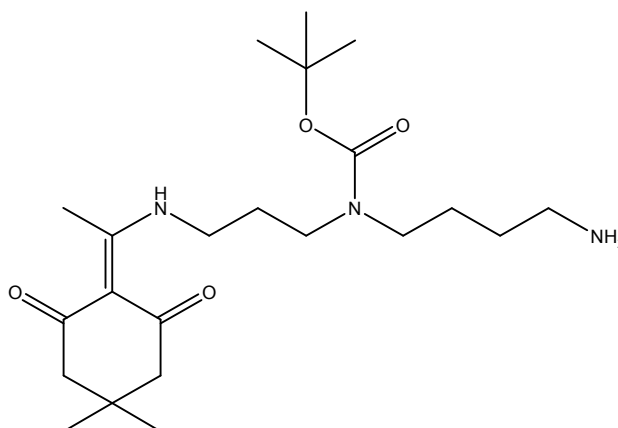
28.28 (CH<sub>2</sub>), 29.30 (C(CH<sub>3</sub>)<sub>2</sub>), 30.12 (C(CH<sub>3</sub>)<sub>2</sub>), 41.12 (NHCH<sub>2</sub>), 41.34 (NHCH<sub>2</sub>), 46.66 (NHCH<sub>2</sub>), 49.26 (NHCH<sub>2</sub>), 52.90 (CH<sub>2</sub>), 74.38 (CH<sub>2</sub>CCl<sub>3</sub>), 95.82 (CCl<sub>3</sub>), 107.90 (C=CCH<sub>2</sub>), 154.70 (C=O), 173.55 (C=CCH<sub>3</sub>), 200.04 (C=O). [Found: (ES<sup>+</sup>) 484.1517 [M+H]<sup>+</sup>, C<sub>20</sub>H<sub>33</sub>C<sub>13</sub>N<sub>3</sub>O<sub>4</sub> requires 484.1531].

**{3-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino]-propyl}-[4-(2,2,2-trichloro-ethoxycarbonylamino)-butyl]-carbamic acid *tert*-butyl ester** [68]



A stirred solution of **116** (0.32 g, 0.66 mmol) in DCM (5 mL), was treated with Boc anhydride (0.144 g, 0.66 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with DCM (20 mL) and extracted with 10% citric acid (2 x 25 mL), saturated NaHCO<sub>3</sub> (2 x 25 mL), and brine (2x 25 mL). The organic phase was dried (MgSO<sub>4</sub>) and evaporated to dryness to give **68** (0.356 g, 95%) as a yellow oil.  $\delta_{\text{H}}$  (500MHz, CDCl<sub>3</sub>): 0.99 (6H, s, C(CH<sub>3</sub>)<sub>2</sub>), 1.36 (9H, s, 3x CH<sub>3</sub>-Boc), 1.43-1.46 (4H, m, 2x CH<sub>2</sub>), 1.84 (2H, quintet, *J* 6.9, CH<sub>2</sub>), 2.29 (4H, s, 2x CH<sub>2</sub>), 2.48 (3H, s, C=CCH<sub>3</sub>), 3.14 (2H, br, CH<sub>2</sub>N), 3.18 (2H, q, *J* 6.4, CH<sub>2</sub>NH), 3.23 (2H, t, *J* 6.8, CH<sub>2</sub>N), 3.34 (2H, q, *J* 6.5, CH<sub>2</sub>NH), 4.65 (2H, s, CH<sub>2</sub>CCl<sub>3</sub>), 5.73 (1H, br, NH), 13.42 (1H, br, NH).  $\delta_{\text{C}}$  (67.5MHz, CDCl<sub>3</sub>): 17.93 (C=CCH<sub>3</sub>), 25.17 (CH<sub>2</sub>), 25.64 (CH<sub>2</sub>), 26.84 (C(CH<sub>3</sub>)<sub>2</sub>), 27.38 (CH<sub>2</sub>), 28.40 (CH<sub>3</sub>-Boc), 30.08 (C(CH<sub>3</sub>)<sub>2</sub>), 40.65 (NHCH<sub>2</sub>), 41.05 (NHCH<sub>2</sub>), 44.40 (NHCH<sub>2</sub>), 46.54 (NHCH<sub>2</sub>), 53.38 (CH<sub>2</sub>), 74.33 (CH<sub>2</sub>CCl<sub>3</sub>), 79.82 (C(CH<sub>3</sub>)<sub>3</sub>), 95.82 (CCl<sub>3</sub>), 107.89 (C=CCH<sub>2</sub>), 146.59 (C=O), 155.46 (C=O), 173.61 (C=CCH<sub>3</sub>), 200.04 (C=O). [Found: (ES<sup>+</sup>) 584.2059 [M+H]<sup>+</sup>, C<sub>25</sub>H<sub>41</sub>C<sub>13</sub>N<sub>3</sub>O<sub>6</sub> requires 584.2055].

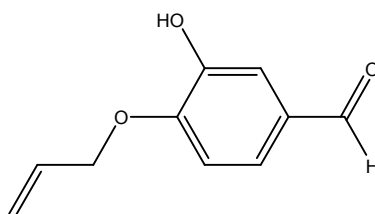
**(4-Amino-butyl)-{3-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)-ethylaminol]-propyl}-carbamic acid *tert*-butyl ester** [118]



A stirred solution of **68** (0.160 g, 2.739 mmol) in acetic acid (4.5 mL) and water (0.5 mL), was treated with activated zinc dust (0.54 g, 8.217 mmol). The mixture was stirred at room temperature for 3 h. The insoluble materials were filtered off through celite. The filtrate was evaporated to dryness and the residue was dissolved in diethyl ether (10 mL). The solution was extracted with water (5 x 5 mL). The extracts were combined and evaporated to dryness to give **118** (0.060 g, 47%) as a colourless oil.  $\delta_{\text{H}}$  (270MHz,  $\text{CDCl}_3$ ): 0.99 (6H, s,  $\text{C}(\text{CH}_3)_2$ ), 1.40 (4H, s, minor  $\text{CH}_3$ -Boc rotamer), 1.41(5H, s, minor  $\text{CH}_3$ -Boc rotamer), 1.55-1.72 (4H, m,  $\text{CH}_2$ ), 1.80-1.94 (2H, m,  $\text{CH}_2$ ), 2.32 (4H, s, 2x  $\text{CH}_2$ -Dde), 2.52 (3H, s,  $\text{C}=\text{CCH}_3$ ), 3.10-3.32 (4H, m, 2x  $\text{CH}_2$ -N), 3.34-3.59 (4H, m, 2x  $\text{CH}_2$ -N), 5.73 (2H, br,  $\text{NH}_2$ ), 13.46 (1H, br, NH).  $\delta_{\text{C}}$  (67.5MHz,  $\text{CDCl}_3$ ): 18.05 ( $\text{C}=\text{CCH}_3$ ), 25.17 ( $\text{CH}_2$ ), 25.64 ( $\text{CH}_2$ ), 28.34 ( $\text{C}(\text{CH}_3)_2$ ), 28.49 ( $\text{CH}_2$ ), 30.08 ( $\text{C}(\text{CH}_3)_2$ ), 30.19 ( $\text{C}(\text{CH}_3)_3$ ), 43.11 ( $\text{NCH}_2$ ), 43.19 ( $\text{NCH}_2$ ), 44.44 ( $\text{NCH}_2$ ), 44.48 ( $\text{NCH}_2$ ), 53.13 (2x  $\text{CH}_2$ ), 79.73 ( $\text{C}(\text{CH}_3)_3$ ), 107.99 ( $\text{C}=\text{CCH}_3$ ), 173.644 ( $\text{C}=\text{CCH}_3$ ). [Found: ( $\text{ES}^+$ ) 410.3010 [ $\text{M}+\text{H}$ ] $^+$ ,  $\text{C}_{22}\text{H}_{40}\text{N}_3\text{O}_4$  requires 410.3013].

**4-Allyloxy-3-hydroxy-benzaldehyde**

[139]

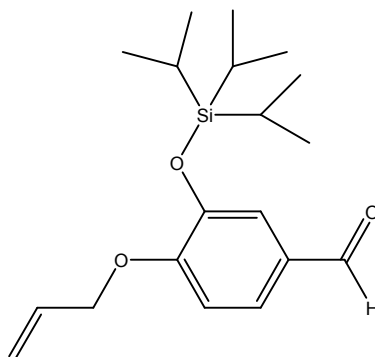


A 100 mL flask was equipped with a stirrer, 3,4-Dihydroxy benzaldehyde (2.004 g, 0.0145 mol) and lithium carbonate (2.692 g, 0.0365 mol). The flask was placed under

vacuum and flushed with argon three times. Anhydrous DMF (30 mL) was syringed in the reaction mixture, followed by freshly distilled dry allyl bromide (3.1 mL, 0.0358 mol) and the reaction mixture was heated at 55°C for 60 h. The solvent was then evaporated and the residue was taken up in ethyl acetate (40 mL) and washed sequentially with water (2 x 25 mL), 0.1M HCl (2 x 25 mL) and brine (2 x 25 mL). The organic layer was dried (MgSO<sub>4</sub>), and evaporated to dryness. The residue was purified by silica gel chromatography (5%-25% AcOEt/Hexane) to give **139** (1.02 g, 40%) as a white solid; **mp**: 57-59°C; **δ<sub>H</sub>** (500MHz, CDCl<sub>3</sub>): 4.67 (2H, dt, *J*5.5, 1.4, ArOCH<sub>2</sub>), 5.31-5.46 (2H, m, C=CH<sub>2</sub>), 5.95-6.11 (1H, m, HC=CH<sub>2</sub>), 6.93 (1H, d, *J*8.2, Ar-2), 7.35 (1H, d, *J*2.0, Ar-5), 7.37 (1H, dd, *J*8.2, 2.0, Ar-6), 9.80 (1H, s, CHO). **δ<sub>C</sub>** (67.5MHz, CDCl<sub>3</sub>): 70.12 (CH<sub>2</sub>HC=CH<sub>2</sub>), 111.44 (HC=CH<sub>2</sub>), 114.40 (Ar-5), 119.43 (Ar-2), 124.46 (Ar-6), 130.79 (Ar-1), 131.86 (HC=CH<sub>2</sub>), 146.30 (Ar-3), 150.79 (Ar-4), 191.17 (CHO). [Found: (ES<sup>+</sup>) 179.0699 [M+H]<sup>+</sup>, C<sub>10</sub>H<sub>11</sub>O<sub>3</sub> requires 179.0703].

#### 4-Allyloxy-3-triisopropylsilyloxy-benzaldehyde

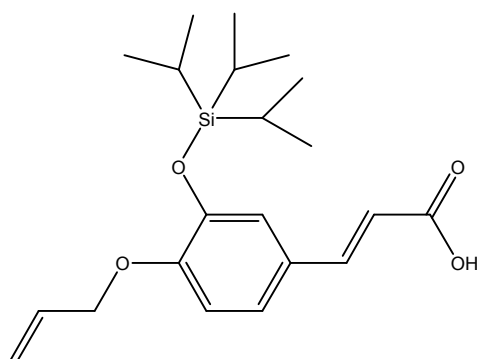
[140]



A stirred solution of **139** (1.037 g, 8.5 mmol) in DCM (15 mL), was treated sequentially with imidazole (0.02 g, 0.293 mmol), triethylamine (1.1 mL, 7.02 mmol) and triisopropyl silyl chloride (1.128 g, 5.85 mmol). The mixture was stirred under argon overnight, and then the reaction was quenched with water (15 mL). The organic layer was separated and washed with 0.1M NaOH (15 mL) and brine (15 mL). The organic phase was dried (MgSO<sub>4</sub>) and evaporated to dryness to give **140** (1.793 g, 90%) as a dark red oil. **δ<sub>H</sub>** (270MHz, CDCl<sub>3</sub>): 1.03 (18H, d, *J* 8.4, 6xCH<sub>3</sub>(TIPS)), 1.18-1.43 (3H, m, 3xCHCH<sub>3</sub>), 4.59 (2H, dt, *J* 5.5, 1.4, ArOCH<sub>2</sub>), 5.28 (1H, m, HC=CH<sub>2</sub>), 5.40 (1H, m, HC=CH<sub>2</sub>), 5.96-6.13 (1H, m, HC=CH<sub>2</sub>), 6.90 (1H, d, *J* 8.2, Ar-2), 7.36 (1H, d, *J* 1.98, Ar-6), 7.41 (1H, dd, *J* 8.2, 2.0, Ar-5), 9.78 (1H, s, CHO).

$\delta_C$  (67.5MHz, CDCl<sub>3</sub>): 12.87 (CH<sub>3</sub>), 17.98 (CH<sub>2</sub>(CH<sub>3</sub>)<sub>2</sub>), 69.58 (CH<sub>2</sub>CH=CH<sub>2</sub>), 112.41 (CH<sub>2</sub>CH=CH<sub>2</sub>), 118.46 (Ar-5), 119.40 (Ar-2), 126.11 (Ar-6), 130.18 (Ar-1), 132.54 (CH=CH<sub>2</sub>), 146.24 (Ar-3), 155.66 (Ar-4), 191.05 (CHO). [Found: (ES<sup>+</sup>) 357.1840 [M+Na]<sup>+</sup>, C<sub>19</sub>H<sub>30</sub>NaO<sub>3</sub>Si requires 357.1856].

**(E)-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-acrylic acid** [66]



Method A: A stirred solution of **140** (4.84 g, 14.5 mmol) in pyridine (20 mL), was treated with malonic acid (1.51 g, 14.5 mmol) and piperidine (0.5 mL). The reaction mixture was heated 50°C for 4 h, then the solvent was evaporated and the residue was re-dissolved in EtOAc (40 mL). The organic layer was washed with 5% citric acid (40 mL), water (40 mL) and brine (40 mL). The organic phase was dried (MgSO<sub>4</sub>) and evaporated to dryness. Purification via silica gel column chromatography 10%-50% EtOAc/Hex gave **66** (2.99 g, 55%) as a white solid along with the diacid **141** (620 mg, 10%).

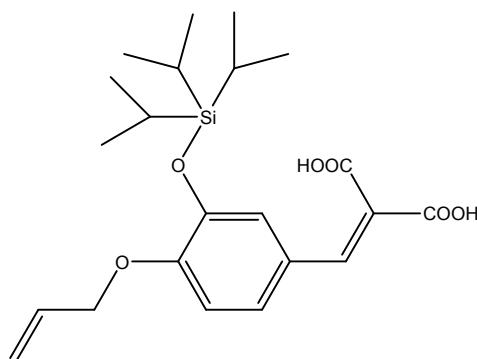
Method B: A stirred solution of **140** (1.63 g, 4.9 mmol) in pyridine (15 mL), was treated with malonic acid (0.51 g, 4.9 mmol) and piperidine (0.1 mL). The reaction mixture was heated 150°C for 4 hours, then the solvent was evaporated and the residue re-dissolved in EtOAc (30 mL). The organic layer was washed with 5% citric acid (30 mL), water (30 mL) and brine (30 mL). The organic phase was dried (MgSO<sub>4</sub>) and evaporated to dryness. Purification via silica gel column chromatography 10%-50% EtOAc/Hex gave desired product **66** (0.44 g, 25%) as a white solid along with deprotected material **142** (0.16 g, 15%).

Method C: A stirred solution of **141** (370 mg, 0.88 mmol) in toluene (7 mL) was heated under reflux (125°C) for 48 h. The solvent was evaporated to dryness and the residue was purified via silica gel column chromatography 0%-50% EtOAc/Hex to give **66** (170 mg, 52%).

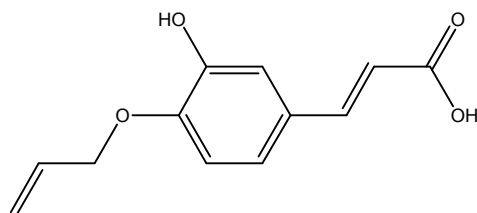
**mp:** 95-99°C;  $\delta_{\text{H}}$  (270MHz,  $\text{CDCl}_3$ ): 0.65(18H, d,  $J$  7.8, 3x $\text{CH}_3$ ), 0.73-0.93(3H, m, 3x $\text{CHCH}_3$ ), 4.50(2H, d,  $J$  6.01,  $\text{ArOCH}_2$ ), 5.26-5.49 (2H, m,  $\text{CH}=\text{CH}_2$ ), 6.09-6.24(1H, m,  $\text{CH}=\text{CH}_2$ ), 6.38(1H, d,  $J$  17.6,  $\text{ArCH}=\text{CH}$ ), 7.02(1H, d,  $J$  9.8, Ar-2), 7.28-7.32(2H, m, Ar-5,6), 7.96(1H, d,  $J$  17.6,  $\text{ArCH}=\text{CHCOOH}$ ).  $\delta_{\text{C}}$  (67.5MHz,  $\text{CDCl}_3$ ): 12.91 ( $\text{CH}(\text{CH}_3)_2$ ), 18.01 ( $\text{CH}(\text{CH}_3)_2$ ), 69.56 ( $\text{CH}_2$ ), 113.21 (Ar-5), 114.44 (Ar-2), 116.22 ( $\text{HC}=\text{CH}_2$ ), 119.59 ( $\text{HC}=\text{CHCOOH}$ ), 123.17 (Ar-6), 127.13 (Ar-1), 132.20 ( $\text{HC}=\text{CH}_2$ ), 138.80 (Ar-3), 146.01 ( $\text{HC}=\text{CHCOOH}$ ), 152.58 (Ar-4), 172.33 ( $\text{C}=\text{O}$ ). [Found: ( $\text{ES}^+$ ) 377.2153 [ $\text{M}+\text{H}$ ] $^+$ ,  $\text{C}_{21}\text{H}_{33}\text{O}_4\text{Si}$  requires 377.2143 ].

**2-(4-Allyloxy-3-triisopropylsilyloxy-benzylidene)-malonic acid**

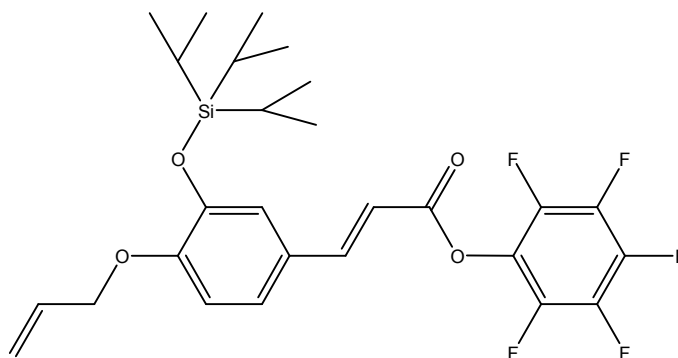
[141]



$\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ): 1.09 (18H, d,  $J$  7.6, 6x  $\text{CH}_3$ ), 1.21-1.32 (3H, m, 3x $\text{CH}(\text{CH}_3)_2$ ), 4.57 (2H, d,  $J$  5.2,  $\text{OCH}_2$ ), 5.35-5.46 (2H, m,  $\text{CH}=\text{CH}_2$ ), 5.90-6.09(1H, m,  $\text{CH}=\text{CH}_2$ ), 6.86 (1H, d,  $J$  8.8,  $\text{ArCH}=\text{C}$ ), 7.21-7.24 (2H, m, Ar-5,6), 7.86 (1H, s, Ar-2).  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ): 13.02 (3x $\text{CH}(\text{CH}_3)_2$ ), 18.12 (3x $\text{CH}(\text{CH}_3)_2$ ), 69.34 ( $\text{OCH}_2$ ), 112.89 (Ar), 118.15 ( $\text{HC}=\text{CH}_2$ ), 120.07 ( $\text{C}(\text{COOH})_2$ ), 121.83 (Ar), 124.78 (Ar- $\text{HC}=\text{C}$ ), 126.03 (Ar), 132.54 ( $\text{CH}=\text{CH}_2$ ), 145.72 (Ar- $\text{HC}=\text{C}$ ), 146.78 (Ar-O), 153.37 (Ar-O), 170.18 ( $\text{COOH}$ ), 172.19 ( $\text{COOH}$ ). [Found: ( $\text{ES}^+$ ) 421.2037 [ $\text{M}+\text{H}$ ] $^+$ ,  $\text{C}_{22}\text{H}_{33}\text{O}_6\text{Si}$  requires 421.2041].

**(E)-3-(4-Allyloxy-3-hydroxy-phenyl)-acrylic acid****[142]**

**mp:** 176-178°C;  **$\delta_{\text{H}}$  (270MHz, DMSO):** 4.56 (2H, dt,  $J$  5.7, 1.6, ArOCH<sub>2</sub>), 5.24-5.52 (2H, m, CH=CH<sub>2</sub>), 6.08-6.24 (1H, m, CH=CH<sub>2</sub>), 6.37 (1H, d,  $J$  17.6, ArCH=CH), 7.14 (1H, d,  $J$  9.0, Ar-2), 7.26-7.33 (2H, m, Ar-5,6), 7.71 (1H, d,  $J$  17.6, ArCH=CHCOOH), 9.54 (1H, br, Ar-OH).  **$\delta_{\text{C}}$  (100MHz, DMSO):** 68.90 (CH<sub>2</sub>-O), 113.52 (Ar-2), 114.40 (ArCH=CH), 116.36 (Ar-5), 117.61 (CH=CH<sub>2</sub>), 120.85 (Ar-6), 127.26 (Ar-1), 133.66 (CH=CH<sub>2</sub>), 144.17 (Ar-3), 146.90 (ArCH=CH), 148.64 (Ar-4), 167.81 (COOH); [Found: (ES<sup>+</sup>) 221.812 [M+H]<sup>+</sup>, C<sub>12</sub>H<sub>13</sub>O<sub>4</sub> requires 221.0808 ].

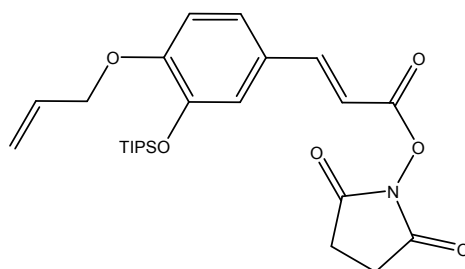
**(E)-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-acrylic acid pentafluorophenyl ester****[143]**

A stirred solution of **66** (1.5 g, 4 mmol) in DCM (15 mL) under argon, was treated sequentially with 4-dimethylaminopyridine (50 mg, 4 mmol), pentafluorophenol (75 mg, 4 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (78 mg, 4 mmol). The reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was then diluted with DCM (10 mL) and was washed with 5% NaHCO<sub>3</sub> (2 x 25 mL) and brine (25 mL). The organic phase was dried (MgSO<sub>4</sub>), evaporated to dryness and purified by silica gel chromatography (DCM) to give **143**

(1.7g, 80%) as a yellow oil.  $\delta_{\text{H}}$  (270MHz,  $\text{CDCl}_3$ ): 1.02 (18H, d,  $J$  7.8,  $3\times\text{CH}_3$ ), 1.20-1.31 (3H, m,  $3\times\text{CHCH}_3$ ), 4.50 (2H, d,  $J$  6.01,  $\text{ArOCH}_2$ ), 5.26-5.49 (2H, m,  $\text{CH}=\text{CH}_2$ ), 6.09-6.24 (1H, m,  $\text{CH}=\text{CH}_2$ ), 6.36 (1H, d,  $J$  17.6,  $\text{ArCH}=\text{CH}$ ), 6.82 (1H, d,  $J$  9.8, Ar-2), 7.28-7.32 (2H, m, Ar-5,6), 7.86 (1H, d,  $J$  17.6, ArCH). [Found:  $(\text{ES}^+)$  543.1988  $[\text{M}+\text{H}]^+$ ,  $\text{C}_{27}\text{H}_{32}\text{F}_5\text{O}_4\text{Si}$  requires 543.1985].

**(E)-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-acrylic acid succinimido ester**

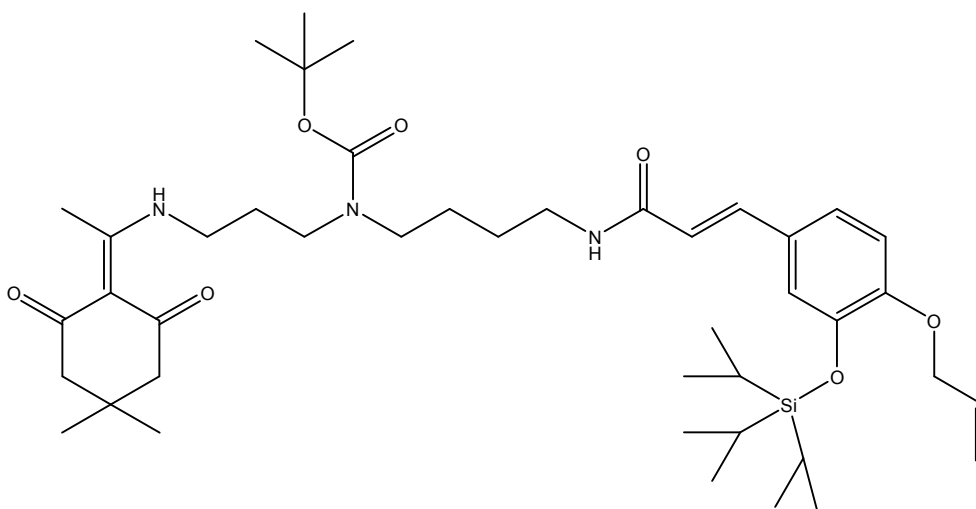
[145]



A stirred solution of **66** (0.21 g, 0.56 mmol) and N-hydroxysuccinimide (0.065 g, 0.056 mmol) in THF (5 mL), was cooled to  $0^\circ\text{C}$  and then treated with N,N'-dicyclohexylcarbodiimide (0.116 g, 0.056 mmol). The reaction mixture was allowed to attain to room temperature overnight. The precipitate that formed was removed by filtration, and the filtrate was evaporated to dryness. The residue was re-dissolved in EtOAc (15 mL), then filtered and the filtrate was washed sequentially with 5%  $\text{NaHCO}_3$  (15 mL), 5% citric acid (15 mL) and brine (15 mL). The organic phase was dried ( $\text{MgSO}_4$ ) and evaporated to dryness to give **145** as a yellow oil (0.119g, 43%).  $\delta_{\text{H}}$  (270MHz,  $\text{CDCl}_3$ ): 1.08 (18H, d,  $J$  6.9,  $3\times\text{CH}_3$ ), 1.19-1.33 (3H, m,  $3\times\text{CHCH}_3$ ), 2.87 (4H, s,  $2\times\text{CH}_2$ ), 4.56 (2H, d,  $J$  5.5,  $\text{ArOCH}_2$ ), 5.25-5.45 (2H, m,  $\text{CH}=\text{CH}_2$ ), 5.97-6.11 (1H, m,  $\text{CH}=\text{CH}_2$ ), 6.35 (1H, d,  $J$  16.0,  $\text{ArCH}=\text{CH}$ ), 6.83 (1H, d,  $J$  7.4, Ar-2), 7.07-7.13 (2H, m, Ar-5,6), 7.79 (1H, d,  $J$  16.0,  $\text{ArCH}=\text{CH}$ ).  $\delta_{\text{C}}$  (67.5MHz,  $\text{CDCl}_3$ ): 12.89 ( $\text{CH}(\text{CH}_3)_3$ ), 18.01 ( $\text{CH}_3$ ), 25.65 ( $\text{CH}_2$ ), 69.54 ( $\text{OCH}_2$ ), 108.54 (Ar), 113.08 (Ar), 118.28 ( $\text{C}=\text{CH}_2$ ), 119.54(Ar), 124.01 ( $\text{ArCH}=\text{CH}$ ), 126.56 (Ar), 132.80 ( $\text{CH}=\text{CH}_2$ ), 146.08 (Ar-O), 150.26 ( $\text{ArCH}=\text{CH}$ ), 153.42 (Ar-O), 162.45 ( $\text{OC}=\text{O}$ ), 169.63 ( $\text{NC}=\text{O}$ ). [Found:  $(\text{ES}^+)$  496.2122  $[\text{M}+\text{Na}]^+$ ,  $\text{C}_{25}\text{H}_{35}\text{N}_1\text{O}_6\text{SiNa}$  requires 496.2126].

**(E)-tert-butyl 4-(3-(4-(allyloxy)-3-(triisopropylsilyloxy)phenyl)acrylamido)butyl**  
**(3-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)propyl) carbamate**

[147]

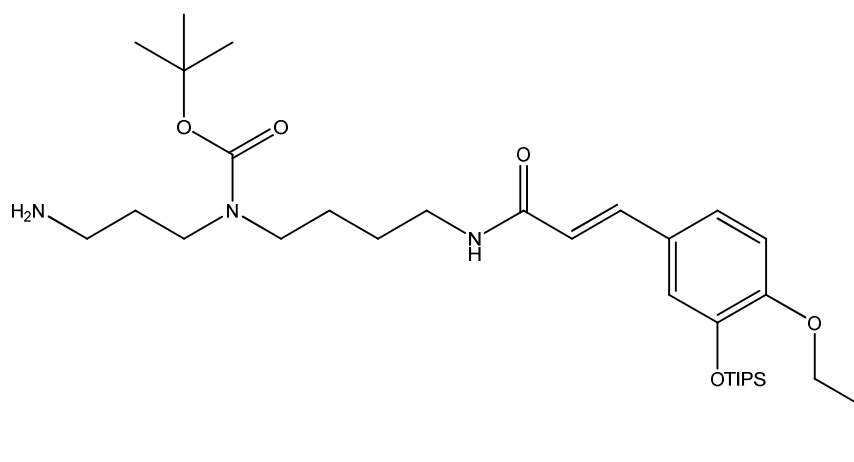


A stirred solution of **118** (60 mg, 12.78 mmol) in dry DCM (5 mL) under argon, was treated with **143** (94 mg, 17.36 mmol), followed by DIPEA (46  $\mu$ L, 25 mmol) and DIMAP (1.56 mg, 1.27 mmol). The mixture was stirred overnight at room temperature. The reaction mixture was then quenched with 5% citric acid (20 mL), and was extracted with DCM (20 mL) and washed with 5% NaCO<sub>3</sub> (20 mL), water (20 mL) and brine (20 mL). The organic phase was dried, concentrated *in vacuo* and purified by silica gel chromatography (0-5% methanol/ DCM), to give **147** (57 mg, 59%) as a yellow oil.  $\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>): 0.95 (6H, s, C(CH<sub>3</sub>)<sub>2</sub>), 1.02 (18H, d, *J* 7.4, Si(CH<sub>3</sub>)<sub>3</sub>(CH<sub>3</sub>)<sub>3</sub>), 1.15-1.25 (3H, m, Si(CH<sub>3</sub>)<sub>3</sub>(CH<sub>3</sub>)<sub>3</sub>), 1.37 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.47-1.58 (4H, m, 2xCH<sub>2</sub>-Dde), 1.77-1.88 (2H, m, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.25-2.32 (4H, m, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>N), 2.49 (3H, s, C=CCH<sub>3</sub>), 3.11-3.26 (4H, m, NCH<sub>2</sub>), 3.33 (4H, q, *J* 5.5, NCH<sub>2</sub>), 4.47 (2H, d, *J* 5.5, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.27 (2H, dd, *J* 1.2, 10.5, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.89 (1H, br, NH), 5.93-6.03 (1H, m, CH<sub>2</sub>CH=CH<sub>2</sub>), 6.14 (1H, d, *J* 15.6, ArCH=CH), 6.73 (1H, d, *J* 8.9, Ar-2), 6.95-6.98 (2H, m, Ar-5,6), 7.42 (1H, d, *J* 15.6, ArCH=CH), 13.50 (1H, br, NH).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 12.78 (C=CCH<sub>3</sub>), 13.07 (CH(CH<sub>3</sub>)<sub>2</sub>), 17.90 (CH<sub>3</sub>-TIPS), 25.93 (CH<sub>2</sub>). 28.21 (CH<sub>3</sub>-Boc), 28.36 (CH<sub>2</sub>), 28.40(CH<sub>2</sub>), 30.07 (CH<sub>3</sub>-Dde), 30.93 (C(CH<sub>3</sub>)<sub>2</sub>), 39.14 (CH<sub>2</sub>N), 43.02 (CH<sub>2</sub>N), 44.38 (CH<sub>2</sub>N), 46.36 (CH<sub>2</sub>N), 52.35 (CH<sub>2</sub>), 69.48 (OCH<sub>2</sub>), 80.01 (C-Boc), 107.88 (Ar), 113.26 (Ar), 113.29 (C=CCH<sub>3</sub>), 117.82 (CH=CH<sub>2</sub>), 119.35 (CH=CH), 121.66 (Ar),



128.04 (Ar), 133.08 (CH=CH<sub>2</sub>), 140.89 (Ar), 145.72 (CH=CH), 152.57 (Ar), 155.12 (C=O), 166.38 (NC=O), 173.55 (C=CCH<sub>3</sub>), 192.31 (C=O).

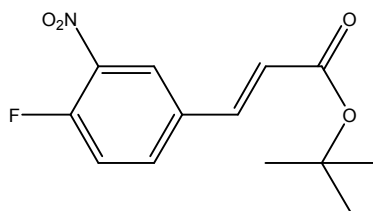
**(3-Amino-propyl)-{4-[(E)-3-(2-triisopropylsilyloxy-4-vinyloxy-phenyl)-acryloylamino]-butyl}-carbamic acid *tert*-butyl ester** [148]



A solution of **147** (57 mg, 0.0758  $\mu$ mol), in 2% hydrazine/ DMF (1 mL) was treated with allyl alcohol (1 mL, 0.152 mol). The reaction was stirred at room temperature for 5 minutes. The solvent was removed under vacuo and the mixture was purified by silica gel chromatography (1:5:44, NH<sub>3</sub>: MeOH : DCM) to give **148** (37 mg, 86%) as a yellow oil.  $\delta_{\text{H}}$  (500MHz, CDCl<sub>3</sub>): 1.04 (18H, t, *J* 7.5, 3xCH(CH<sub>3</sub>)<sub>2</sub>), 1.15-1.31 (3H, m, 3xCH(CH<sub>3</sub>)<sub>2</sub>), 1.38 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.42-1.54 (4H, m, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>N), 1.58-1.66 (2H, m, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.63 (2H, br, NH<sub>2</sub>), 3.08-3.24 (4H, m, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>N), 3.29-3.36 (4H, m, H<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 4.47 (2H, d, *J* 5.5, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.20 (1H, dd, *J* 10.5, 1.5, CH<sub>2</sub>CH=CH<sub>2</sub>) 5.32 (1H, dd, *J* 17.2, 1.5, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.94-6.03 (1H, m, CH<sub>2</sub>CH=CH<sub>2</sub>), 6.07-6.18 (1H, m, ArCH=CH), 6.74 (1H, d, *J* 9.0, Ar-2), 6.96-6.99 (2H, m, Ar-5,6), 7.42 (1H, d, *J* 15.5, ArCH=CH), 8.89 (1h, br, NH);  $\delta_{\text{C}}$  (100MHz, DMSO): 12.10 (CH-TIPS), 17.89 (CH<sub>3</sub>-TIPS), 25.60 (CH<sub>2</sub>), 25.81 (CH<sub>2</sub>), 26.63 (CH<sub>2</sub>), 28.09 (CH<sub>3</sub>-Boc), 38.35 (NCH<sub>2</sub>), 38.89 (NCH<sub>2</sub>), 40.15 (NCH<sub>2</sub>), 40.60 (NCH<sub>2</sub>), 68.94 (OCH<sub>2</sub>), 79.22 (C(CH<sub>3</sub>)<sub>3</sub>), 113.71 (Ar), 117.55 (CH=CH<sub>2</sub>), 119.72 (CH=CH), 128.07 (Ar), 133.79 (CH=CH<sub>2</sub>), 138.57 (Ar), 146.95 (HC=CH), 147.91 (Ar), 155.01 (C=O), 155.12 (Ar), 165.49 (C=O (Boc)); [Found: (ES<sup>+</sup>) 604.4146 [M+H]<sup>+</sup>, C<sub>33</sub>H<sub>58</sub>N<sub>3</sub>O<sub>5</sub>Si requires 604.4145].

**(E)-3-(4-Fluoro-3-nitro-phenyl)-acrylic acid tert-butyl ester**

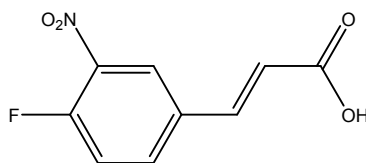
[135]



A stirred solution of 1-fluoro-4-bromo-2-nitrobenzene (3.0 g, 0.014 mol) in anhydrous DMF (30 mL) under argon, was sequentially treated with palladium acetate (31 mg, 0.0136 mol), triphenyl phosphine (0.170 g, 0.0648 mol), *tert*-butyl acrylate (2.15 mL, 0.015 mol) and DIPEA (2.3 mL, 0.0136 mol). The reaction mixture was heated at 100°C under reflux, and stirred overnight. The solvent was evaporated and the residue was redissolved in ethyl acetate and then washed with water (2 x 30 mL), and brine (2 x 30 mL). Purification by silica gel chromatography (5% EtOAc/ hexane) gave **135** (1.8g, 48%) as a red-orange solid; **mp**: 69-71°C;  $\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>): 1.57 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 6.43 (1H, d, *J* 15.9, ArCH=CH), 7.28-7.38 (1H, m, Ar-6), 7.56 (1H, d, *J* 15.9, ArCH=CH), 7.72-7.78 (1H, m, Ar-2), 8.23 (1H, d, *J* 6.9, Ar-5).  $\delta_{\text{C}}$  (67.5MHz, CDCl<sub>3</sub>): 28.20 (CH<sub>3</sub>), 81.40 (C(CH<sub>3</sub>)<sub>3</sub>), 114.76 (HC=CHCOO<sup>t</sup>Bu), 119.06 (Ar-5), 123.35 (Ar-2), 132.04 (Ar-1), 134.51 (Ar-6), 139.55 (Ar-3), 154.00 (HC=CHCOO<sup>t</sup>Bu), 157.96 (Ar-4), 165.38 (C=O); [Found: (ES<sup>+</sup>) 290.0800 [M+Na]<sup>+</sup>, C<sub>13</sub>H<sub>14</sub>FNNaO<sub>4</sub> requires 290.0799].

**(E)-3-(4-Fluoro-3-nitro-phenyl)-acrylic acid**

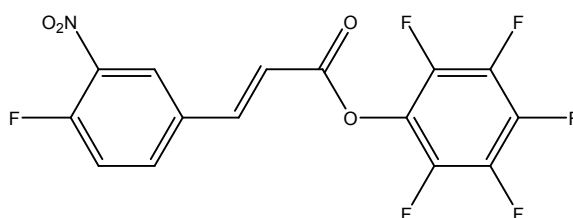
[65]



A stirred solution of **135** (1.7 g, 6.4 mmol) in DCM (14 mL), was treated with trifluoro acetic acid (14.7 mL, 0.19 mol). After 1h the solvent was evaporated and the residue co-evaporated several times with diethyl ether. Purification by crystallisation from EtOAc/ hexane gave **65** (1.05 g, 78%) as a yellow solid; **mp**: 215-217°C;  $\delta_{\text{H}}$  (270MHz, DMSO): 6.69 (1H, d, *J* 16.1, ArCH=CH), 7.62-7.70 (2H, m, Ar-5,6),

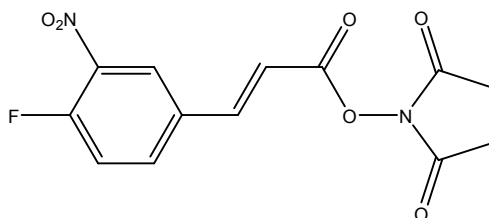
8.17-8.21 (1H, m, Ar-2), 8.50 (1H, d,  $J$  16.4, ArCH=CH).  $\delta_C$  (67.5MHz, DMSO): 119.82 (HC=CHCOOH), 122.57 (Ar-5), 126.39 (Ar-2), 132.45 (Ar-1), 135.96 (Ar-6), 141.20 (Ar-3), 153.68 (HC=CHCOOH), 154.38 (Ar-4), 167.74 (COOH); [Found: (ES<sup>+</sup>) 212.0353 [M+H]<sup>+</sup>, C<sub>9</sub>H<sub>7</sub>FNO<sub>4</sub> requires 212.0354].

**(E)-3-(4-Fluoro-3-nitro-phenyl)-acrylic acid pentafluorophenyl ester** [144]



A stirred solution of **65** (0.542 g, 2.57 mmol), in dry DCM (10 mL) under argon, was treated sequentially with DMAP (0.032 g, 0.257 mmol), pentafluorophenol (0.473 g, 2.57 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.398 g, 2.57 mmol). The reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was then diluted with DCM (10 mL) and was washed with 5% NaHCO<sub>3</sub> (2 x 15 mL) and brine (15 mL). The organic phase was dried (MgSO<sub>4</sub>), evaporated to dryness and purified by silica gel chromatography (DCM) to give **144** (0.831 g, 86%) as a red oil.

**(E)-3-(4-Fluoro-3-nitro-phenyl)-acrylic acid 2,5-dioxo-pyrrolidin-1-yl ester** [146]

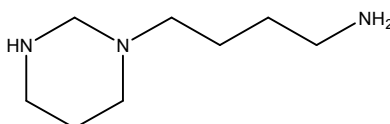


A stirred solution of **65** (140 mg, 0.66 mmol) in DMF (5 mL) at 0°C, was treated sequentially with DMAP (8 mg, 0.066 mmol), N-hydroxy succinimide (76 mg, 0.66mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (127

mg, 0.66 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was removed in vacuo and the residue was redissolved in DCM (10 mL) and washed sequentially with 10% citric acid (10 mL), sat. NaHCO<sub>3</sub> (10 mL), water (10 mL) and brine (10 mL). The organic phase was dried (MgSO<sub>4</sub>) and evaporated to dryness to give **146** (125 mg, 61%) as an orange oil.  $\delta_{\text{H}}$  (270MHz, DMSO): 2.51 (4H, s, 2x CH<sub>2</sub>), 7.23 (1H, d, *J* 15.9, ArCH=CH), 7.78-7.88 (1H, m, Ar-6), 8.12 (1H, d, *J* 15.9, ArCH=CH), 8.31-8.39 (1H, m, Ar-2), 8.73 (1H, d, *J* 6.9, Ar-5).  $\delta_{\text{C}}$  (67.5MHz, DMSO): 25.55 (2x CH<sub>2</sub>), 114.66 (HC=CH), 119.06 (Ar), 119.48 (Ar), 127.08 (Ar), 136.27 (Ar), 136.37 (Ar), 146.62 (HC=CH), 154.62 (Ar), 162.06 (2x C=O), 170.37 (C=O).

#### 4-(Tetrahydro-pyrimidin-1-yl)-butylamine<sup>77</sup>

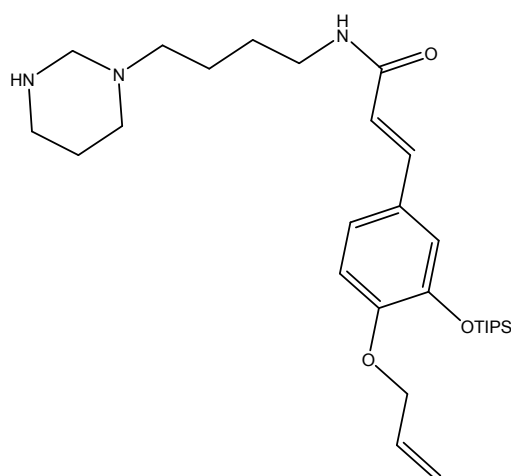
[128]



A solution of spermidine (2.08 g, 14.3 mmol) in distilled water (50 mL) was cooled to 5°C and stirred under nitrogen. Aqueous formaldehyde (0.4 g, 13.0 mmol) was then slowly added. The reaction mixture was stirred for 2 h allowing to reach room temperature. The aqueous layer was then saturated with solid NaCl and extracted with chloroform (6x 50 mL). The combined chloroform extracts were dried (MgSO<sub>4</sub>) and concentrated to dryness to give **128** (2.07g, 92%) as a clear oil.  $\delta_{\text{H}}$  (400MHz, CDCl<sub>3</sub>): 1.40-1.50 (4H, m, 2xCH<sub>2</sub>), 1.54-1.62 (2H, m, CH<sub>2</sub>), 1.63-1.70 (3H, br, NH<sub>2</sub>, NH), 2.22 (2H, t, *J* 7.2, NCH<sub>2</sub>), 2.50-2.56 (2H, m, NCH<sub>2</sub>), 2.67 (2H, t, *J* 6.6, NCH<sub>2</sub>), 2.78 (2H, t, *J* 5.6, NCH<sub>2</sub>), 3.35 (2H, s, NCH<sub>2</sub>N).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 24.27 (CH<sub>2</sub>), 27.13 (CH<sub>2</sub>), 31.77 (CH<sub>2</sub>), 42.00 (NCH<sub>2</sub>), 45.17 (NCH<sub>2</sub>), 53.08 (NCH<sub>2</sub>), 55.38 (NCH<sub>2</sub>), 69.93 (NCH<sub>2</sub>N).

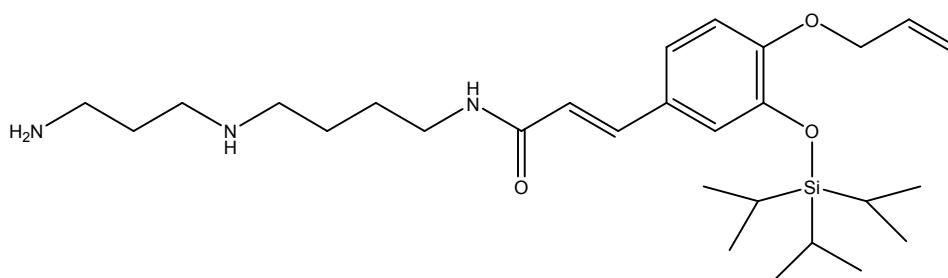
**(E)-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-N-[4-(tetrahydro-pyrimidin-1-yl)-butyl]-acrylamide**

[149]



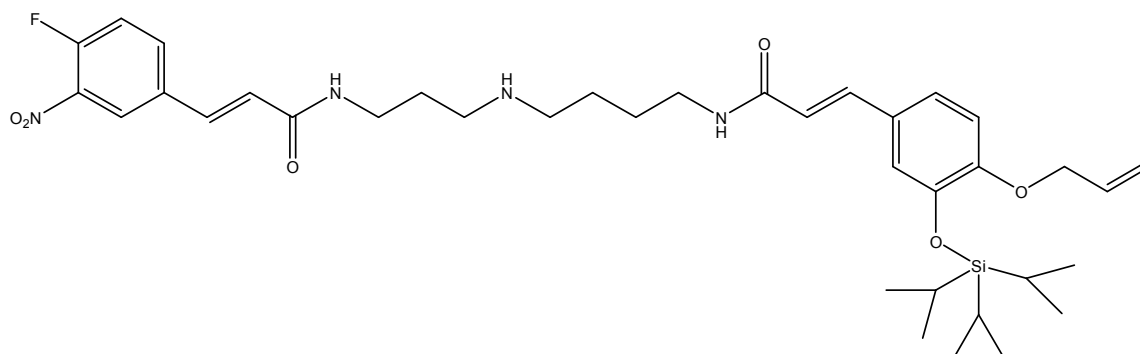
A stirred solution of **128** (190 mg, 1.2 mmol) in DCM (10 mL), was treated sequentially with DIPEA (0.21 mL, 1.2 mmol) and **145** (550 mg, 1.2 mmol). The reaction mixture was stirred for 3 h at 0°C. The solvent was removed *in vacuo*. Purification by silica gel chromatography (0%-10% MeOH/DCM, then 1:9:90 NH<sub>3</sub> (aq): MeOH: DCM) gave **149** as a yellow oil (255 mg, 40%).  $\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>): 1.05 (18H, d, *J* 7.1, 3xCH<sub>3</sub>), 1.11-1.31 (3H, m, 3xCHCH<sub>3</sub>), 1.45-1.7 (6H, m, 3XCH<sub>2</sub>), 2.15-2.32 (2H, m, NCH<sub>2</sub>), 2.49-2.62 (2H, m, NCH<sub>2</sub>), 2.74-2.85 (2H, m, CH<sub>2</sub>), 3.23-3.45 (4H, m, 2xNCH<sub>2</sub>), 4.41-4.54 (2H, m, ArOCH<sub>2</sub>), 5.18-5.4 (2H, m, CH=CH<sub>2</sub>), 5.92-6.80 (1H, m, CH=CH<sub>2</sub>), 6.22 (1H, d, *J* 15.7, ArCH=CH), 6.40-6.57 (1H, m, NH), 6.68-6.80 (1H, m, Ar-2), 6.92-7.04 (2H, m, Ar-5,6), 7.45 (1H, d, *J* 15.7, ArCH=CH).  $\delta_{\text{C}}$  (67.5MHz, CDCl<sub>3</sub>): 12.89 (CH(CH<sub>3</sub>)<sub>3</sub>), 18.02 (CH<sub>3</sub>), 24.29 (CH<sub>2</sub>), 26.81 (CH<sub>2</sub>), 27.61 (CH<sub>2</sub>), 39.51 (HNCH<sub>2</sub>), 45.08 (HNCH<sub>2</sub>), 53.05 (NCH<sub>2</sub>), 54.86 (NCH<sub>2</sub>), 69.55 (HNCH<sub>2</sub>N), 69.82 (OCH<sub>2</sub>), 113.33 (Ar), 117.94 (CH=CH<sub>2</sub>), 118.64 (Ar), 119.49 (Ar), 121.85 (ArCH=CH), 128.04 (ArCH=CH), 133.18 (OCH<sub>2</sub>CH=CH<sub>2</sub>), 140.53 (Ar-O), 145.81 (ArCH=CH), 151.43 (ArO), 166.39 (C=O). [Found: (ES<sup>+</sup>) 516.3610 [M+H]<sup>+</sup>, C<sub>29</sub>H<sub>50</sub>N<sub>3</sub>O<sub>3</sub>Si requires 516.3616].

**(E)-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-N-[4-(3-amino-propylamino)-butyl]-acrylamide** [150]



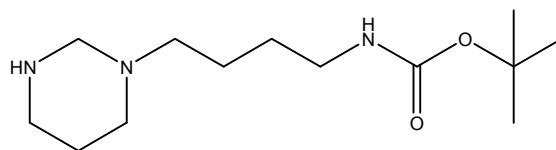
A solution of **149** (250 mg, 0.5 mmol) in absolute ethanol (8 mL), was treated with ethyl hydrogen malonate (321 mg, 2.5 mmol), and pyridine (0.157 mL, 2 mmol). The reaction mixture was stirred at 50°C for 2.5 hours. The solvent was then removed in vacuo and the residue was dissolved in saturated NaHCO<sub>3</sub> (5 mL), and extracted with DCM (9x 5 mL). The combined organic layers were dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo* to give **150** (192 mg, 79%) as a pale yellow oil.  $\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>): 1.06 (18H, d, *J* 7.1, 3xCH<sub>3</sub>), 1.17-1.30 (3H, m, 3x CHCH<sub>3</sub>), 1.47-1.73 (6H, m, CH<sub>2</sub>), 2.35-2.85 (8H, m, HNCH<sub>2</sub>) 3.22-3.45 (2H, m, NH<sub>2</sub>), 4.51 (2H, d, *J* 4.6, ArOCH<sub>2</sub>), 5.22-5.40 (2H, m, CH=CH<sub>2</sub>), 5.95-6.09 (1H, m, CHCH<sub>2</sub>), 6.18 (1H, d, *J* 15.7, ArCH=CH), 6.72-6.80 (1H, m, Ar-2), 6.92-7.11 (2H, m, Ar-5,6), 7.46 (1H, d, *J* 15.7, ArCH=CH).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 167.01 (C=O), 151.30 (Ar-O), 145.72 (ArCH=CH), 140.36 (Ar-O), 133.11 (CH=CH<sub>2</sub>), 127.99 (Ar), 121.57 (ArCH=CH), 119.56 (Ar), 118.66 (Ar), 117.79 (CH=CH<sub>2</sub>), 113.31 (Ar), 69.48 (OCH<sub>2</sub>), 48.97 (HNCH<sub>2</sub>), 48.58 (HNCH<sub>2</sub>), 39.29 (HNCH<sub>2</sub>), 38.65 (HNCH<sub>2</sub>), 29.67 (CH<sub>2</sub>), 27.14 (2xCH<sub>2</sub>), 17.90 (C(CH<sub>3</sub>)<sub>3</sub>), 12.79 (C(CH<sub>3</sub>)<sub>3</sub>). [Found: (ES<sup>+</sup>) 504.3618 [M+H]<sup>+</sup>, C<sub>28</sub>H<sub>50</sub>N<sub>3</sub>O<sub>3</sub>Si requires 504.3616].

**(E)-N-(3-{4-[(E)-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-acryloylamino]-butylamino}-propyl)-3-(4-fluoro-3-nitro-phenyl)-acrylamide** [151]



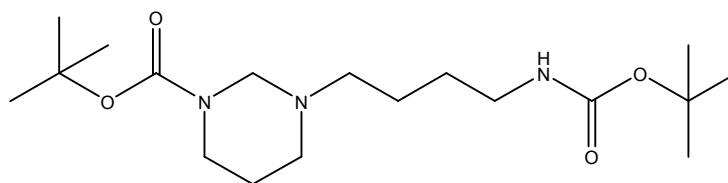
A stirred solution of **150** (50 mg, 0.1 mmol) in DCM (2 mL), was treated with **146** (31 mg, 0.1 mmol) in the presence of DIPEA (9  $\mu$ L, 0.1 mmol). The reaction mixture was stirred at 0°C for 2 h. At this stage no reaction had taken place; the pH of the reaction mixture was found to be 7, therefore another equivalent of DIPEA (9  $\mu$ L, 0.1 mmol) was added to ensure that the pH of the reaction mixture was 9. The reaction mixture was stirred for a further 1 hour, then it was evaporated to dryness and the residue purified by silica gel chromatography (0%-10% MeOH/DCM, then 1:9:90 NH<sub>3</sub> (aq): MeOH: DCM) to give **151** as a yellow oil (18 mg, 26%).  $\delta_{\text{H}}$  (400MHz, CDCl<sub>3</sub>): 1.06 (18H, d, *J* 7.2, 6xCH<sub>3</sub>), 1.21-1.35 (3H, m, 3xCH(CH<sub>3</sub>)<sub>2</sub>), 1.52 (1H, br, NH), 1.62-1.65 (4H, m, 2xCH<sub>2</sub>), 1.77-1.80 (2H, m, CH<sub>2</sub>), 2.68-2.79 (4H, m, 2xHNCH<sub>2</sub>), 3.09 (1H, br, NH), 3.28 (1H, br, NH), 3.36-3.50 (4H, m, 2xHNCH<sub>2</sub>), 4.51 (2H, d, *J* 5.2, OCH<sub>2</sub>), 5.24-5.40 (2H, m, HC=CH<sub>2</sub>), 5.99-6.06 (1H, m, HC=CH<sub>2</sub>), 6.20 (1H, d, *J* 15.2, ArCH=CH), 6.47(1H, d, *J* 15.6, ArCH=CH), 6.76 (1H, d, *J* 9.2, Ar-2), 6.96-6.99 (2H, m, Ar-5,6), 7.17-7.25 (1H, m, Ar-5), 7.44 (1H, d, *J* 15.2, ArCH=CH), 7.53 (1H, d, *J* 15.6, ArCH=CH), 7.67-7.70 (1H, m, Ar-6), 8.14 (1H, dd, *J* 2.0, 6.8, Ar-2).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 12.79 (3xCH(CH<sub>3</sub>)<sub>2</sub>), 17.90 (6xCH<sub>3</sub>), 26.58 (CH<sub>2</sub>), 27.32 (CH<sub>2</sub>), 28.16 (CH<sub>2</sub>), 38.79 (NCH<sub>2</sub>), 39.28 (NCH<sub>2</sub>), 47.80 (NCH<sub>2</sub>), 48.88 (NCH<sub>2</sub>), 69.47 (OCH<sub>2</sub>), 113.22 (Ar), 117.88 (Ar-CH=CH), 118.21 (C=CH<sub>2</sub>), 119.34 (Ar-CH=CH), 121.83 (Ar), 124.05 (Ar), 124.48 (Ar), 127.70 (Ar), 128.52 (Ar), 133.03 (HC=CH<sub>2</sub>), 134.69 (Ar), 136.59 (Ar), 140.78 (Ar-CH=CH), 145.58 (Ar), 151.50 (Ar), 154.04 (Ar), 158.25 (Ar), 166.52 (C=O), 167.81 (C=O). [Found: (ES<sup>+</sup>) 697.3798 [M+H]<sup>+</sup>, C<sub>37</sub>H<sub>54</sub>FN<sub>4</sub>O<sub>6</sub>Si requires 697.3791].

**[4-(Tetrahydro-pyrimidin-1-yl)-butyl]-carbamic acid *tert*-butyl ester**<sup>60</sup> [129]



A stirred solution of **128** (1.53 g, 9.7 mmol) in absolute ethanol (12 mL), was treated with *tert*-butyl phenylcarbonate (2.09 g, 10.7 mmol). The reaction mixture was stirred under reflux overnight, followed by removal of volatiles under vacuum. Purification by silica gel column chromatography with 0-10% MeOH/DCM, then 0.1: 1: 9 NH<sub>3</sub> (aq): MeOH: DCM, gave **129** (1.3 g, 55%) as a yellow oil and the di-acylated side product [**129b**] (10%).  $\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>): 1.38 (9H, s, 3xCH<sub>3</sub>), 1.45-1.51 (4H, m, 2xCH<sub>2</sub>), 1.54-1.62 (2H, m, CH<sub>2</sub>), 2.19 (2H, t, *J* 6.75, NCH<sub>2</sub>), 2.46-2.57 (2H, m, NCH<sub>2</sub>), 2.76 (2H, t, *J* 5.5, NCH<sub>2</sub>), 3.02-3.06 (2H, m, NCH<sub>2</sub>), 3.33 (2H, s, NCH<sub>2</sub>).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 23.09 (CH<sub>2</sub>), 24.52 (CH<sub>2</sub>), 27.04 (CH<sub>2</sub>), 28.50 (C(CH<sub>3</sub>)<sub>3</sub>), 45.19 (NCH<sub>2</sub>), 53.14 (NCH<sub>2</sub>), 54.83 (NCH<sub>2</sub>), 55.29 (NCH<sub>2</sub>), 69.29 (HNCH<sub>2</sub>N), 78.80 (C(CH<sub>3</sub>)<sub>3</sub>), 156.05 (C=O).

**3-(4-*tert*-Butoxycarbonylamino-butyl)-tetrahydro-pyrimidine-1-carboxylic acid *tert*-butyl ester** [129b]

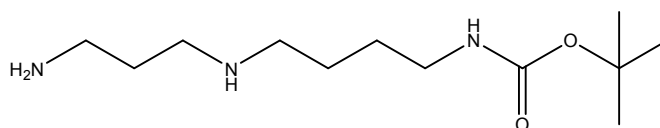


$\delta_{\text{H}}$  (400MHz, CDCl<sub>3</sub>): 1.44 (18H, t, *J* 2.0, 2xC(CH<sub>3</sub>)<sub>3</sub>), 1.48-1.55 (4H, m, 2xCH<sub>2</sub>), 1.58-1.62 (2H, m, CH<sub>2</sub>), 2.41 (2H, d, *J* 6.8, NCH<sub>2</sub>), 2.61-2.78 (2H, m, NCH<sub>2</sub>), 3.09-3.15 (2H, m, NCH<sub>2</sub>), 3.38 (2H, s, NCH<sub>2</sub>), 4.07 (2H, s, NCH<sub>2</sub>), 4.94 (1H, br, NH).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 22.26 (CH<sub>2</sub>), 24.64 (CH<sub>2</sub>), 28.09 (CH<sub>2</sub>), 28.69 (2x CH<sub>3</sub>), 52.10 (NCH<sub>2</sub>), 52.74 (NCH<sub>2</sub>), 53.93 (NCH<sub>2</sub>), 58.11 (NCH<sub>2</sub>), 74.11 (NCH<sub>2</sub>), 79.72 (2x C(CH<sub>3</sub>)<sub>3</sub>), 156.02 (C=O), 157.90 (C=O). [Found: (ES<sup>+</sup>) 358.2683 [M+H]<sup>+</sup>, C<sub>18</sub>H<sub>36</sub>N<sub>3</sub>O<sub>4</sub> requires 358.2700].



**[4-(3-Amino-propylamino)-butyl]-carbamic acid *tert*-butyl ester**

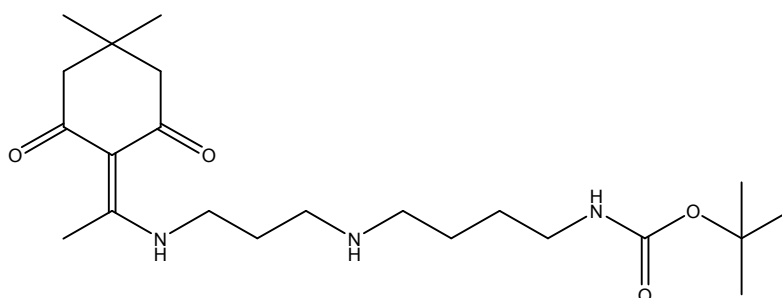
**[130]**



A stirred solution of **129** (4.15 g, 16 mmol), in absolute ethanol (80 mL), was treated with ethylhydrogen malonate (9.52 mL, 80 mmol), and pyridine (5.2 mL, 54 mmol). The reaction mixture was stirred for 2.5 h under reflux. The solvent was removed *in vacuo* and the residue **130** was used without further purification in the next step.

**(4-{3-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino]-propylamino}-butyl)-carbamic acid *tert*-butyl ester**

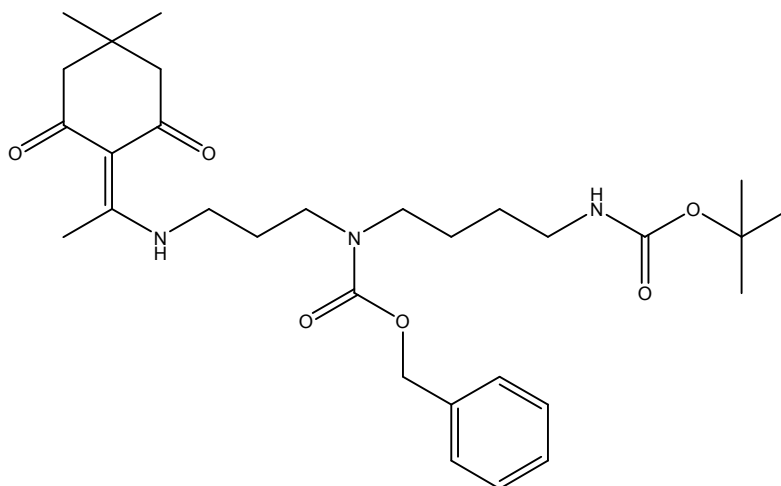
**[131]**



A stirred solution of crude **130** (3.93 g crude, 16 mmol) in absolute ethanol (20 mL), was treated with **112** (2.95 g, 16 mmol). The reaction mixture was heated under reflux for 1.5 h followed by removal of volatiles *in vacuo*. Purification by silica gel column chromatography with 0-10% MeOH/DCM, then 0.1: 1: 9 NH<sub>3</sub> (aq): MeOH: DCM, gave **131** (5.03g, 76%) as a yellow oil.  $\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>): 1.01 (6H, s, C(CH<sub>3</sub>)<sub>2</sub>), 1.41 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.48-1.53 (4H, m, 2xCH<sub>2</sub>), 1.79-1.91 (2H, m, CH<sub>2</sub>), 2.34 (4H, s, 2xCH<sub>2</sub>(Dde)), 2.55 (3H, s, CH<sub>3</sub> (Dde)), 2.59-2.65 (2H, m, NCH<sub>2</sub>), 2.69-2.76 (2H, m, NCH<sub>2</sub>), 3.01-3.15 (2H, m, NCH<sub>2</sub>), 3.47 (2H, q, *J* 5.5, NCH<sub>2</sub>), 4.85 (1H, br, NH), 12.04 (1H, br, NH), 13.40 (2H, br, NH<sub>2</sub>).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 17.96 (CH<sub>3</sub>-Dde), 26.46 (CH<sub>2</sub>), 27.65 (CH<sub>2</sub>), 28.29 (C(CH<sub>3</sub>)<sub>2</sub>), 28.46 (C(CH<sub>3</sub>)<sub>3</sub>), 28.73 (CH<sub>2</sub>), 29.96 (C(CH<sub>3</sub>)<sub>2</sub>), 41.26 (2xNCH<sub>2</sub>), 46.38 (NCH<sub>2</sub>), 49.03 (NCH<sub>2</sub>), 51.87 (2xCH<sub>2</sub>-Dde), 78.99

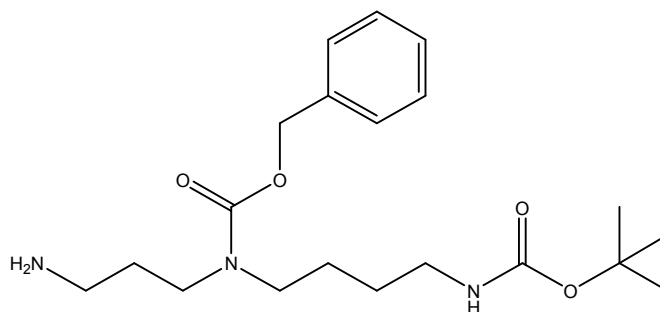
(C(CH<sub>3</sub>)<sub>3</sub>), 107.86 (C=CCH<sub>3</sub>), 156.05 (C=O), 173.59 (C=CCH<sub>3</sub>), 190.03 (2xC=O).  
[Found: (ES<sup>+</sup>) 410.2995 [M+H]<sup>+</sup>, C<sub>22</sub>H<sub>40</sub>N<sub>3</sub>O<sub>4</sub> requires 410.3013].

**(4-tert-Butoxycarbonylamino-butyl)-{3-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino]-propyl}-carbamic acid benzyl ester** [132]



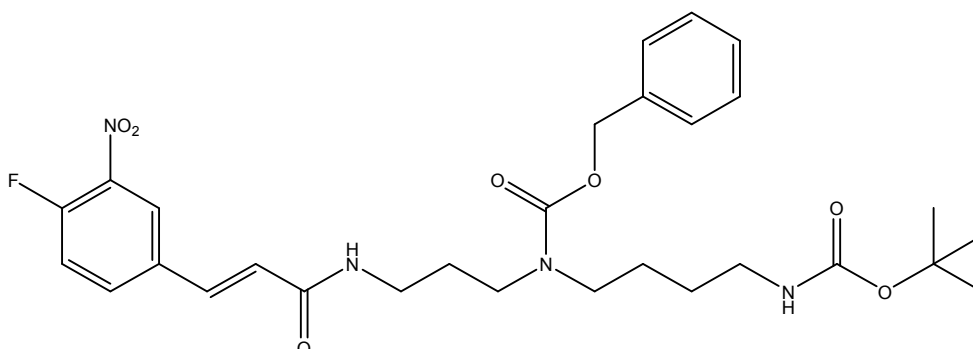
A stirred solution of **131** (1.40 g, 3.4 mmol) in dry DCM (8 mL) under nitrogen, was treated with triethylamine (0.5 mL, 3.4 mmol) followed by benzyl chloroformate (0.5 mL, 3.4 mmol). The reaction mixture was stirred at 0°C for 45 min and then diluted with DCM (10 mL) and washed sequentially with water (2x 20 mL) and brine (2x 20 mL), dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. Purification by silica gel column chromatography with 0-3% MeOH/DCM gave **132** (0.9 g, 60%) as a yellow oil.  $\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>): 1.00 (6H, s, C(CH<sub>3</sub>)<sub>2</sub>), 1.41 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.46-1.61 (4H, m, 2xCH<sub>2</sub>), 1.81-1.90 (2H, m, 2xCH<sub>2</sub>), 2.33 (4H, s, 2xCH<sub>2</sub>(Dde)), 2.53 (3H, s, CH<sub>3</sub>(Dde)), 2.95-3.15 (2H, m, NCH<sub>2</sub>), 3.16-3.43 (6H, m, 3xNCH<sub>2</sub>), 4.63-4.72 (1H, m, NH), 5.10 (2H, s, CH<sub>2</sub>-Ar), 7.31-7.34 (5H, m, Ar), 12.04 (1H, br, NH).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 18.12 (CH<sub>3</sub>-Dde), 25.97 (CH<sub>2</sub>), 27.50 (CH<sub>2</sub>), 28.15 (CH<sub>2</sub>), 28.50 (C(CH<sub>3</sub>)<sub>3</sub>), 28.66 (C(CH<sub>3</sub>)<sub>2</sub>), 30.05 (C(CH<sub>3</sub>)<sub>2</sub>), 41.25 (2xNCH<sub>2</sub>), 45.10 (NCH<sub>2</sub>), 47.44 (NCH<sub>2</sub>), 52.66 (2xCH<sub>2</sub>-Dde), 67.44 (CH<sub>2</sub>-Z), 79.01 (C(CH<sub>3</sub>)<sub>3</sub>), 107.86 (C=CCH<sub>3</sub>), 127.90 (Ar), 128.10 (2xAr), 128.51 (2x Ar), 136.56 (Ar), 155.97 (2xC=O), 173.50 (C=CCH<sub>3</sub>), 192.73 (C=O). [Found: (ES<sup>+</sup>) 544.3396 [M+H]<sup>+</sup>, C<sub>30</sub>H<sub>45</sub>N<sub>3</sub>O<sub>6</sub> requires 544.3381].

**(3-Amino-propyl)-(4-tert-butoxycarbonylamino-butyl)-carbamic acid benzyl ester** [133]



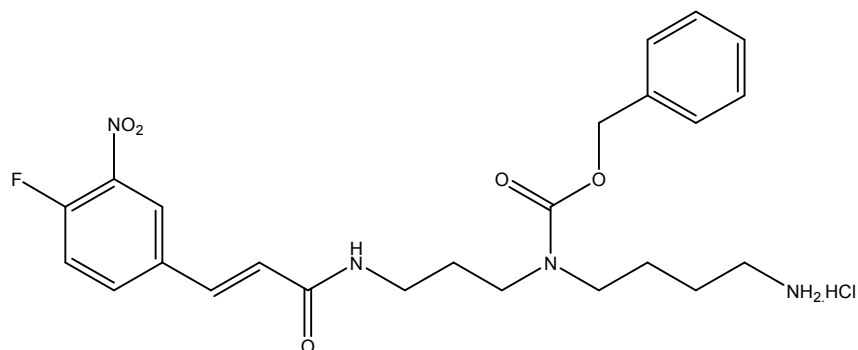
A 2% hydrazine/DMF (10 mL) solution, was treated with allyl alcohol (9.1 mL, 0.31 mol). This mixture was then added to **132** (1.69 g, 3.1 mmol). The reaction mixture was stirred at room temperature for 8 min. The volatiles were removed under vacuum. Purification by silica gel column chromatography with 0-10% MeOH/DCM, then 0.1: 1: 9 NH<sub>3</sub> (aq): MeOH: DCM gave **133** (805 mg, 82%) as a yellow oil.  $\delta_{\text{H}}$  (**270MHz**, **CDCl<sub>3</sub>**): 1.41 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.44-1.68 (2H, m, CH<sub>2</sub>), 1.98 (4H, br, 2xCH<sub>2</sub>), 2.58-2.74 (2H, m, NCH<sub>2</sub>), 2.94-3.38 (6H, m, 3xNCH<sub>2</sub>), 5.09 (2H, s, CH<sub>2</sub>-Ar), 7.31-7.35 (5H, m, Ar).  $\delta_{\text{C}}$  (**100MHz**, **CDCl<sub>3</sub>**): 25.71 (CH<sub>2</sub>), 27.25 (CH<sub>2</sub>), 28.38 (C(CH<sub>3</sub>)<sub>3</sub>), 29.44 (CH<sub>2</sub>), 37.96 (H<sub>2</sub>NCH<sub>2</sub>), 40.03 (HNCH<sub>2</sub>), 44.22 (NCH<sub>2</sub>), 46.43 (NCH<sub>2</sub>), 67.18 (CH<sub>2</sub>-Z), 79.11 (C(CH<sub>3</sub>)<sub>3</sub>), 127.87 (2xAr), 128.03 (2xAr), 128.49 (Ar), 136.59 (Ar), 155.99 (C=O), 156.63 (C=O). [Found: (ES<sup>+</sup>) 380.2526 [M+H]<sup>+</sup>, C<sub>20</sub>H<sub>34</sub>N<sub>3</sub>O<sub>4</sub> requires 380.2544].

**(4-tert-Butoxycarbonylamino-butyl)-[3-[(E)-3-(4-fluoro-3-nitro-phenyl)-acryloylamino]-propyl]-carbamic acid benzyl ester** [152]



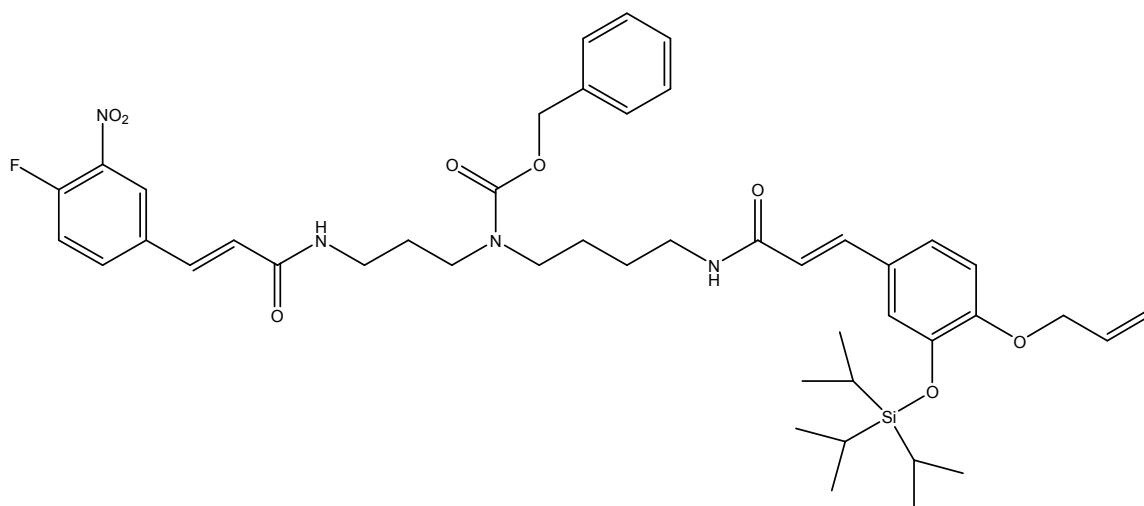
A stirred solution of **133** (460 mg, 1.21 mmol) in dry DCM (20 mL), was treated with DIPEA (0.2 mL, 1.21 mmol) and activated cinnamic acid **144** (456 mg, 1.21 mmol). The reaction was stirred at 0°C for 90 min. The solvent was removed *in vacuo*. Purification via silica gel column chromatography, with 0-5% MeOH/DCM gave **152** (380 mg, 55%) as a yellow oil.  $\delta_{\text{H}}$  (270MHz,  $\text{CDCl}_3$ ): 1.42 (9H, s, 3xCH<sub>3</sub>), 1.48-1.57 (4H, m, 2xCH<sub>2</sub>), 1.64-1.78 (2H, m, CH<sub>2</sub>), 3.04-3.15 (2H, m, NCH<sub>2</sub>), 3.21-3.40 (6H, m, 3xNCH<sub>2</sub>), 4.49 (1H, br, NH), 4.73 (1H, br, NH), 5.15 (2H, s, CH<sub>2</sub>-Z), 6.48 (1H, d, *J* 15.68, ArCH=CH), 7.17 (1H, m, Ar), 7.29-7.39 (5H, m, 5xAr), 7.55 (1H, d, *J* 15.68, ArCH=CH), 7.71-7.77 (1H, m, Ar), 8.18 (1H, d, *J* 6.62, Ar).  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ): 25.80 (CH<sub>2</sub>), 27.63 (CH<sub>2</sub>), 28.64 (3xCH<sub>3</sub>), 35.90 (CH<sub>2</sub>), 36.63 (CH<sub>2</sub>), 40.15 (CH<sub>2</sub>), 43.98 (CH<sub>2</sub>), 46.61 (CH<sub>2</sub>), 67.67 (CH<sub>2</sub>-Z), 79.32 (C(CH<sub>3</sub>)<sub>3</sub>), 118.85 (CH), 124.15 (Ar), 124.63 (Ar), 127.69(Ar-Z), 128.19 (Ar-Z), 128.60 (Ar-Z), 134.52 (Ar), 136.43 (Ar), 136.76 (CH), 137.53 (Ar-Z), 154.29 (Ar-NO<sub>2</sub>), 156.03 (Ar-F), 156.95 (C=O), 157.27 (C=O), 164.84 (C=O). [Found: (ES<sup>+</sup>) 573.2525 [M+H]<sup>+</sup>, C<sub>29</sub>H<sub>38</sub>FN<sub>4</sub>O<sub>7</sub> requires 573.2719].

**(4-Amino-butyl)-{3-[(E)-3-(4-fluoro-3-nitro-phenyl)-acryloylamino]-propyl}-**  
**carbamic acid benzyl ester hydrochloric salt** [153]



Compound **152** (1.11 g, 2 mmol), was treated with a 4M HCl solution in dioxane (20 mL). The reaction was stirred under N<sub>2</sub> at room temperature for 30 min. Solvents were removed *in vacuo* and the residue was co-evaporated several times with ether to give **163** (0.96 g, 94%) as a yellow oil. **153** was used in the next step without further purification. [Found: (ES<sup>+</sup>) 473.2181 [M+H]<sup>+</sup>, C<sub>24</sub>H<sub>30</sub>FN<sub>4</sub>O<sub>5</sub> requires 473.2195].

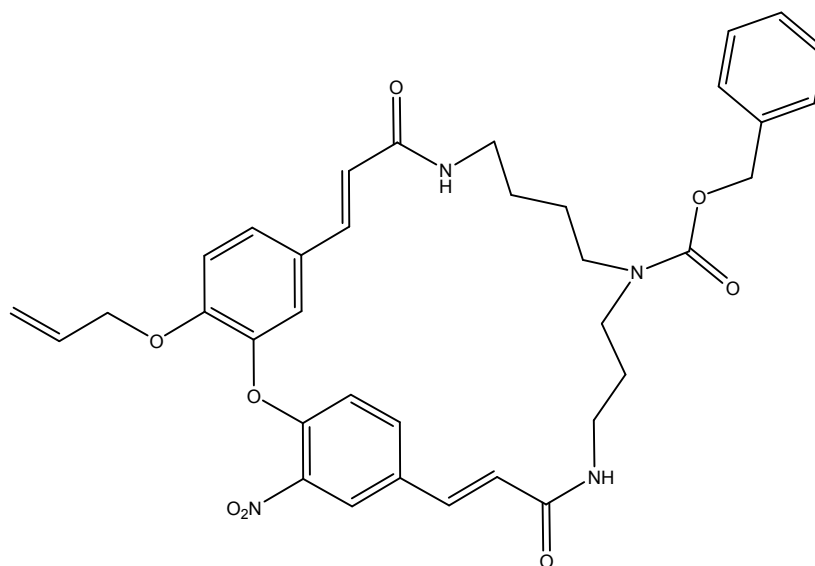
**{4-[(E)-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-acryloylamino]-butyl}-{3-**  
**[(E)-3-(4-fluoro-3-nitro-phenyl)-acryloylamino]-propyl}-carbamic acid benzyl**  
**ester** [154]



A stirred solution of **153** (0.96 g, 1.9 mmol) in dry DCM (20 mL), was treated with DIPEA (0.7 mL, 3.8 mmol) followed by **143** (1.03 g, 1.9 mmol). The reaction mixture was stirred under nitrogen at room temperature, overnight. Volatiles were removed *in vacuo*. Purification via silica gel column chromatography with 0%-3% MeOH/DCM, gave **154** (1.17 g, 75%), as a yellow oil.  $\delta_{\text{H}}$  (270MHz, CDCl<sub>3</sub>): 1.06 (18H, d, *J* 7.2, 3xCH(CH<sub>3</sub>)<sub>2</sub>), 1.16-1.28 (3H, m, 3xCH(CH<sub>3</sub>)<sub>2</sub>), 1.45-1.69 (4H, m, 2xCH<sub>2</sub>), 1.72-1.80

(2H, m, CH<sub>2</sub>), 3.18-3.50 (8H, m, 4xNCH<sub>2</sub>), 4.51 (2H, d, *J* 5.51, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.15 (2H, s, CH<sub>2</sub>-Z), 5.23-5.42 (2H, m, CH=CH<sub>2</sub>), 5.66 (1H, br, NH), 5.95-6.09 (1H, m, CH=CH<sub>2</sub>), 6.14 (1H, d, *J* 15.42, ArCH=CH), 6.51 (1H, d, *J* 15.96, ArCH=CH), 6.76 (1H, d, *J* 8.59, Ar), 6.98 (2H, s, 2xAr), 7.31-7.41 (5H, m, 5xAr), 7.45 (1H, d, *J* 15.42, ArCH=CH), 7.55 (1H, *J* 15.95, ArCH=CH), 7.65-7.72 (1H, m, Ar), 8.15 (1H, d, *J* 5.24, Ar).  $\delta_C$  (100MHz, CDCl<sub>3</sub>): 12.86 (3xCH(CH<sub>3</sub>)<sub>2</sub>), 17.89 (6xCH<sub>3</sub>), 25.74 (CH<sub>2</sub>), 27.03 (CH<sub>2</sub>), 27.55 (CH<sub>2</sub>), 36.16 (NCH<sub>2</sub>), 38.93 (NCH<sub>2</sub>), 44.25 (NCH<sub>2</sub>), 46.47 (NCH<sub>2</sub>), 67.39 (CH<sub>2</sub>-Z), 69.54 (CH<sub>2</sub>CH=CH<sub>2</sub>), 113.36 (CH), 117.86 (CH<sub>2</sub>CH=CH<sub>2</sub>), 118.77 (CH), 118.98 (CH), 119.29 (CH), 121.92 (CH), 124.92 (CH), 124.52 (CH), 127.72 (C), 128.21 (CH), 128.63 (CH), 132.33 (C), 133.08 (CH), 134.44 (CH), 136.53 (CH), 137.59 (CH), 137.66 (CH), 141.09 (CH), 145.92 (C), 151.63 (C), 154.25 (C), 156.91 (C), 165.01 (C=O), 166.64 (C=O). [Found: (ES<sup>+</sup>) 831.4173 [M+H]<sup>+</sup>, C<sub>45</sub>H<sub>60</sub>FN<sub>4</sub>O<sub>8</sub>Si requires 831.4159].

**4-Allyloxy-27-nitro-10,21-dioxo-2-oxa-11,16,20-triaza-tricyclo[22.2.2.1<sup>3,7</sup>] non  
acosa-1(27),3,5,7(29),8,22,24(28),25-octaene-16-carboxylic acid benzyl ester**  
[224]



A stirred solution of **154** (553 mg, 0.67 mmol) at 0°C in dry THF (670 mL), was treated with TBAF (67 mL, 0.67 mmol). The reaction was left stirring at room temperature for 2 h. The solvent was removed *in vacuo*. The residue was redissolved in EtOAc and was washed with 5% citric acid and brine. Purification via column chromatography 1-5% MeOH/DCM gave **224** (369 mg, 85%), as a yellow oil.  $\delta_H$

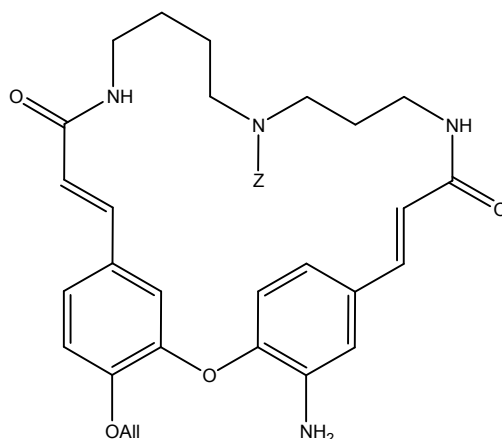
**(400MHz, CD<sub>3</sub>OD):** 1.35-1.47 (2H, m, CH<sub>2</sub>), 1.49-1.58 (2H, m, CH<sub>2</sub>), 1.72-1.82 (2H, m, CH<sub>2</sub>), 3.13-3.26 (6H, m, 3xCH<sub>2</sub>), 3.40-3.52 (2H, m, CH<sub>2</sub>), 4.75 (2H, d, *J* 5.2, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.11 (2H, s, CH<sub>2</sub>-Z), 5.41 (2H, dd, *J* 1.2, 10.4, 1.2, 17.2, CH=CH<sub>2</sub>), 5.94 (1H, d, *J* 15.6, ArCH=CH), 6.09-6.18 (1H, m, CH=CH<sub>2</sub>), 6.44 (1H, d, *J* 2.0, Ar), 6.67 (1H, d, *J* 15.6, ArCH=CH), 7.07-7.11 (2H, m, Ar), 7.31-7.38 (6H, m, 5 xAr, ArCH=CH), 7.43 (1H, d, *J* 8.8, Ar), 7.61 (1H, d, *J* 15.6, ArCH=CH), 8.05 (1H, dd, *J* 2.0, 8.0, Ar), 8.33 (1H, d, *J* 2.0, Ar). **δ<sub>C</sub> (100MHz, CD<sub>3</sub>OD):** 25.52 (CH<sub>2</sub>), 26.72 (CH<sub>2</sub>), 27.35 (CH<sub>2</sub>), 37.31 (NCH<sub>2</sub>), 37.62 (NCH<sub>2</sub>), 39.34 (NCH<sub>2</sub>), 46.34 (NCH<sub>2</sub>), 68.29 (CH<sub>2</sub>-Z), 71.02 (CH<sub>2</sub>CH=CH<sub>2</sub>), 111.52 (Ar), 115.67 (Ar), 117.94 (CH<sub>2</sub>CH=CH<sub>2</sub>), 120.09 (Ar), 125.86 (2xCH), 127.35 (Ar), 127.51 (Ar), 128.89 (Ar-Z), 129.17 (Ar-Z), 129.59 (Ar-Z), 134.31 (Ar), 134.55 (Ar), 135.55 (Ar-Z), 138.19 (Ar), 138.66 (Ar), 140.36 (2xCH), 144.92 (Ar-NO<sub>2</sub>), 150.39 (Ar-O), 150.81 (Ar-O), 151.09 (Ar-O), 158.25 (C=O), 167.49 (C=O), 168.20 (C=O). [Found: (ES<sup>+</sup>) 655.2753 [M+H]<sup>+</sup>, C<sub>36</sub>H<sub>39</sub>N<sub>4</sub>O<sub>8</sub> requires 655.2762].

**(8E,22E)-4-Allyloxy-27-amino-10,21-dioxo-2-oxa-11,16,20-triaza-tricyclo**

**[22.2.2.1<sup>3,7</sup>]nonacosa-1(27),3,5,7(29),8,22,24(28),25-octaene-16-carboxylic acid**

**benzyl ester**

**[225]**

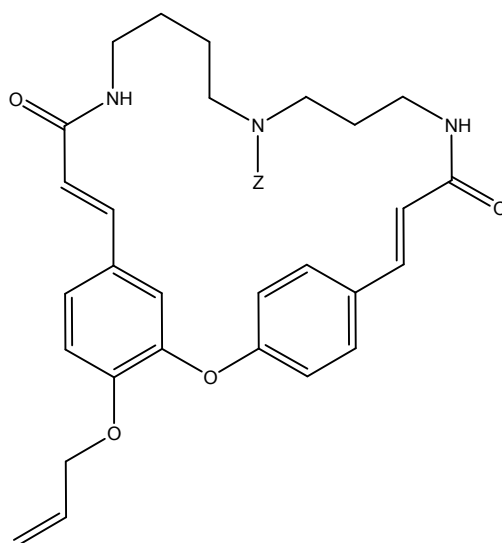


A solution of **224** (238 mg, 0.36 mmol) in EtOH (10 mL) at 60°C, was treated with SnCl<sub>2</sub>·2H<sub>2</sub>O (411 mg, 1.82 mmol), followed by slow addition of NaBH<sub>4</sub> (7 mg, 0.182 mmol). The reaction was stirred at 60°C overnight. The reaction mixture was cooled over an ice bath; water (15 mL) was added, and basified with 1M NaOH to pH 9. The

ethanol was then evaporated off, and the aqueous layer was continuously extracted with  $\text{CHCl}_3$ . The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), and evaporated to dryness. Purification via silica gel chromatography (0%-10% MeOH/ $\text{CHCl}_3$ , then 0.1:1:9  $\text{NH}_3(\text{aq})$ :MeOH: $\text{CHCl}_3$ ) gave **225** (196 mg, 85%) as a yellow solid.  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ): 1.40-1.48 (4H, m,  $2 \times \text{CH}_2$ ), 1.73-1.78 (2H, m,  $\text{CH}_2$ ), 3.11-3.14 (2H, m,  $\text{NCH}_2$ ), 3.24-3.28 (4H, m,  $2 \times \text{NCH}_2$ ), 3.49-3.51 (2H, m,  $\text{NCH}_2$ ), 4.68 (2H, d,  $J$  5.2,  $\text{OCH}_2$ ), 5.10 (2H, s,  $\text{CH}_2\text{-Z}$ ), 5.32-5.49 (2H, m,  $\text{HC}=\text{CH}_2$ ), 6.07-6.15 (1H, m,  $\text{HC}=\text{CH}_2$ ), 6.26 (1H, d,  $J$  14.8,  $\text{HC}=\text{CHAr}$ ), 6.40 (1H, s, Ar-H), 6.85-6.96 (5H, m,  $5 \times \text{Ar-H}$ ), 7.30 (5H, m,  $5 \times \text{ArH(Z)}$ ), 7.40 (1H, d,  $J$  15.4,  $\text{HC}=\text{CHAr}$ ), 7.53 (1H, d,  $J$  15.4,  $\text{HC}=\text{CHAr}$ ).  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ): 25.72 ( $\text{CH}_2$ ), 26.32 ( $\text{CH}_2$ ), 27.83 ( $\text{CH}_2$ ), 37.31 ( $\text{NCH}_2$ ), 39.34 ( $\text{NCH}_2$ ), 46.34 ( $\text{NCH}_2$ ), 67.26 ( $\text{CH}_2\text{-Z}$ ), 69.89 ( $\text{OCH}_2$ ), 109.70 (Ar-H), 113.43 (Ar-H), 115.38 (Ar-H), 118.62 ( $\text{HC}=\text{CH}_2$ ), 118.88 (Ar-H), 121.57 ( $\text{HC}=\text{CHAr}$ ), 123.82 (Ar-H), 125.24 (Ar-H), 127.81 (ArH-Z), 127.87 (Ar-C), 128.16 (ArH-Z), 128.57 (ArH-Z), 132.67 ( $\text{HC}=\text{CH}_2$ ), 133.43 (Ar-C), 136.49 (Ar-C), 139.87 ( $\text{HC}=\text{CHAr}$ ), 140.01 (Ar-C), 140.75 ( $\text{HC}=\text{CHAr}$ ), 142.46 (Ar-C), 148.54 (Ar-C), 149.16 (Ar-C), 156,17 (C=O), 165.81 (C=O(Z)). [Found: ( $\text{ES}^+$ ) 625.3050  $[\text{M}+\text{H}]^+$ ,  $\text{C}_{36}\text{H}_{41}\text{N}_4\text{O}_4$  requires 625.3061].

**(8E,22E)-4-Allyloxy-10,21-dioxo-2-oxa-11,16,20-triaza-tricyclo**

**[22.2.2.1<sup>3,7</sup>]nonacosa-1(27),3,5,7(29),8,22,24(28),25-octaene-16-carboxylic acid benzyl ester** [226]

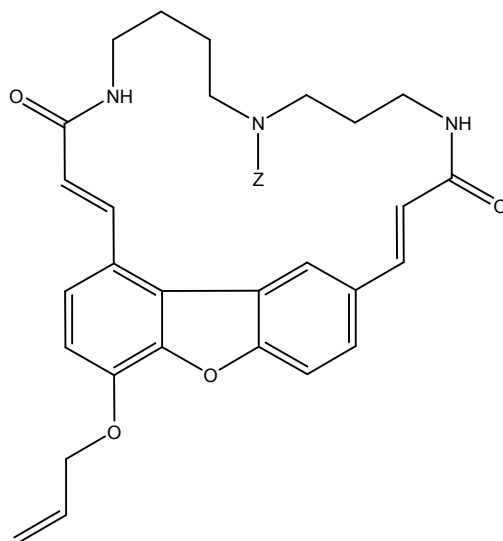




*Method A:* In a two neck 25 mL r.b. flask a solution of *t*-butylnitrite (23  $\mu$ L) in DMF (3.5 mL), at 65°C was stirred vigorously. Once the desired temperature was reached compound **225** (80 mg, 0.13 mmol) in DMF (1.2 mL) was added slowly over 15 min via a syringe pump. The reaction was stirred for a further 30 min. The reaction mixture was then cooled in an ice bath and once cooled, EtOAc (20 mL) was added along with 0.1M HCl (20 mL). The layers were separated and the organic phase was washed once more with 0.1M HCl (20 mL). The organic phase was dried with MgSO<sub>4</sub> and evaporated to dryness. The resulting crude material was separated by HPLC in MeCN/H<sub>2</sub>O to give the side product compound **227** (14.1mg, 15%) and the desired compound **226** (7.2mg, 7.5%).

*Method B:* Compound **225** (185 mg, 0.3 mmol) was dissolved in DMF/EtOH (4 mL/4 mL) and acetic acid (0.8 mL). A solution of NaNO<sub>2</sub> (205.5mg, 0.3mmol in water (1 mL) and NaHSO<sub>3</sub> (308.2 mg, 0.3 mmol) in water (1 mL) were added sequentially to the stirred aniline solution. The reaction was stirred overnight at room temperature. The reaction mixture was evaporated to dryness and redissolved in CHCl<sub>3</sub> (30 mL) and washed with water (30 mL). The organic layer was dried with MgSO<sub>4</sub> and evaporated to dryness. The crude material was purified via silica gel column chromatography (0%-5% MeOH/DCM) to give the desired product **226** (30.2 mg, 20% yield) and the side product **227** (6 mg, 3%).  $\delta_{\text{H}}$  (400MHz, CD<sub>2</sub>Cl<sub>2</sub>): 1.38-1.48 (4H, m, 2xCH<sub>2</sub>), 1.52-1.73 (2H, m, CH<sub>2</sub>), 3.11-3.51 (8H, m, 4xNCH<sub>2</sub>), 4.65 (2H, dd, *J* 5.2, OCH<sub>2</sub>), 5.08 (2H, s, CH<sub>2</sub>-Z), 5.30-5.49 (2H, m, HC=CH<sub>2</sub>), 5.67 (1H, d, *J* 14.4, HC=CHAr), 6.08-6.17 (1H, m, HC=CH<sub>2</sub>), 6.30 (1H, d, *J* 15.2, HC=CHAr), 6.32 (1H, s, ArH), 6.89-6.91 (2H, m, 2x ArH), 7.07 (2H, d, *J* 8.4, 2x ArH), 7.31 (5H, s, 5xArH-Z), 7.36 (1H, d, *J* 15.2, HC=CHAr), 7.56 (1H, d, *J* 15.2, HC=CHAr), 7.57 (2H, d, *J* 8.4, 2xArH).  $\delta_{\text{C}}$  (100MHz, CD<sub>2</sub>Cl<sub>2</sub>): 26.47 (CH<sub>2</sub>), 26.74 (CH<sub>2</sub>), 28.27 (CH<sub>2</sub>), 37.25 (NCH<sub>2</sub>), 37.37 (NCH<sub>2</sub>), 39.67 (NCH<sub>2</sub>), 46.71 (NCH<sub>2</sub>), 67.26 (CH<sub>2</sub>-Z), 70.23 (OCH<sub>2</sub>), 111.08 (ArCH), 113.87 (ArCH), 118.58 (HC=CH<sub>2</sub>), 119.32 (HC=CHAr), 119.37 (ArCH), 123.01 (ArCH), 123.51 (HC=CHAr), 125.36 (ArCH), 127.99 (ArCH-Z), 128.07 (ArCH-Z), 128.27 (ArCH-Z), 128.82 (ArC), 129.81 (ArCH), 133.28 (ArCH), 133.32 (HC=CH<sub>2</sub>), 137.46 (ArCH), 139.75 (HC=CHAr), 139.81 (HC=CHAr), 149.76 (ArC), 150.99 (ArC), 156.32 (ArC), 156.65 (C=O), 165.55 (C=O), 165.64 (C=O). [Found: (ES<sup>+</sup>) 610.2917 [M+H]<sup>+</sup>, C<sub>36</sub>H<sub>40</sub>N<sub>3</sub>O<sub>6</sub> requires 608.2912].

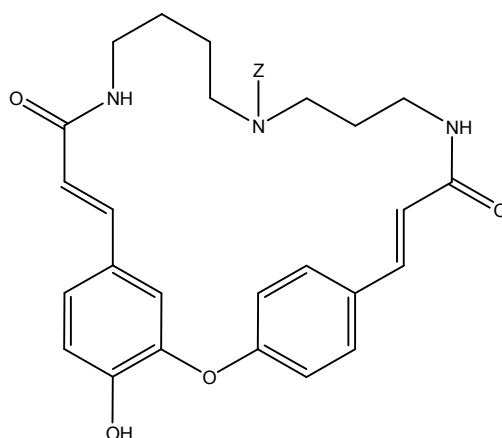
## Compound 227



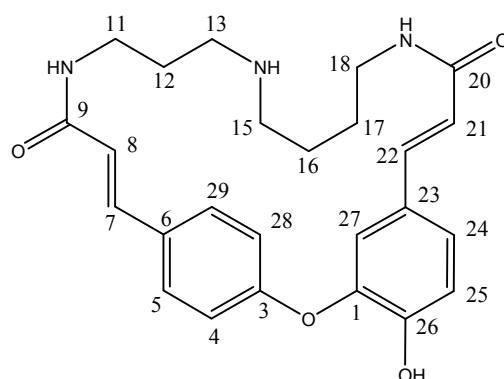
$\delta_{\text{H}}$  (400MHz,  $\text{CD}_2\text{Cl}_2:\text{MeOD}$  8:1): 1.57-1.68 (4H, m,  $2\times\text{CH}_2$ ), 1.79-1.83 (2H, m,  $\text{CH}_2$ ), 3.26-3.37 (8H, m,  $4\times\text{NCH}_2$ ), 4.78 (2H, dt,  $J$  1.4, 5.2,  $\text{OCH}_2$ ), 5.05 (2H, s,  $\text{CH}_2\text{-Z}$ ), 5.32-5.50 (2H, m,  $\text{HC}=\text{CH}_2$ ), 6.09-6.17 (1H, m,  $\text{HC}=\text{CH}_2$ ), 6.44 (1H, d,  $J$  15.2,  $\text{HC}=\text{CHAr}$ ), 6.72 (1H, d,  $J$  15.6,  $\text{HC}=\text{CHAr}$ ), 7.00 (1H,  $J$  8.0, Ar-H), 7.29 (5H, s,  $5\times\text{ArH-Z}$ ), 7.32 (1H, d,  $J$  8.4, ArH), 7.49 (1H, dd,  $J$  1.4, 8.4, ArH), 7.58 (1H, d,  $J$  8.4, ArH), 7.59 (1H, d,  $J$  16.0,  $\text{HC}=\text{CHAr}$ ), 8.30 (1H, d,  $J$  16.0,  $\text{HC}=\text{CHAr}$ ), 8.39 (1H, s, ArH).  $\delta_{\text{C}}$  (100MHz,  $\text{CD}_2\text{Cl}_2:\text{MeOD}$  8:1): 26.33 ( $\text{CH}_2$ ), 28.58 ( $\text{CH}_2$ ), 30.06 ( $\text{CH}_2$ ), 37.34 ( $\text{NCH}_2$ ), 39.42 ( $\text{NCH}_2$ ), 45.76 ( $\text{NCH}_2$ ), 47.68 ( $\text{NCH}_2$ ), 67.41 ( $\text{CH}_2\text{-Z}$ ), 70.52 ( $\text{OCH}_2$ ), 112.02 (ArCH), 112.40 (ArCH), 118.68 ( $\text{HC}=\text{CH}_2$ ), 120.24 (ArCH), 121.95 ( $\text{HC}=\text{CHAr}$ ), 123.00 (ArCH), 123.05 ( $\text{HC}=\text{CHAr}$ ), 124.08 (ArC), 124.68 (ArC), 125.17 (ArC), 128.02 (ArCH-Z), 128.26 (ArCH-Z), 128.80 (ArCH-Z), 129.86 (ArCH), 131.18 (ArC), 133.15 ( $\text{HC}=\text{CH}_2$ ), 137.34 (ArC), 138.78 ( $\text{HC}=\text{CHAr}$ ), 139.43 ( $\text{HC}=\text{CHAr}$ ), 145.58 (ArC), 146.25 (ArC), 156.83 (ArC), 157.42 ( $\text{C}=\text{O}$ ), 167.11 ( $\text{C}=\text{O}$ ), 167.63 ( $\text{C}=\text{O}$ ). [Found: ( $\text{ES}^+$ ) 608.2769  $[\text{M}+\text{H}]^+$ ,  $\text{C}_{36}\text{H}_{38}\text{N}_3\text{O}_6$  requires 608.2755].

**(8E,22E)-4-Hydroxy-10,21-dioxo-2-oxa-11,16,20-triaza-tricyclo[22.2.2.1<sup>3,7</sup>]nonacosa-1(27),3,5,7(29),8,22,24(28),25-octaene-16-carboxylic acid benzyl ester**

[228]

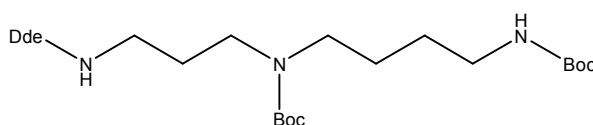


A solution of **226** (34 mg, 0.05 mmol) in dry THF (2.5 mL) was treated with Pd(PPh<sub>3</sub>)<sub>4</sub> (21 mg, 0.018 mmol), followed by dropwise addition of dry morpholine (50 mg, 0.5 mmol). The reaction was stirred under N<sub>2</sub> at room temperature overnight. Volatiles were then removed and the crude material was purified by silica gel column chromatography 0-5% MeOH/DCM to give **228** (28 mg, 88%) as a yellow oil.  $\delta_{\text{H}}$  (400 MHz, CD<sub>3</sub>OD): 1.35-1.39 (2H, m, CH<sub>2</sub>), 1.48-1.51 (2H, m, CH<sub>2</sub>), 1.73-1.83 (2H, m, CH<sub>2</sub>), 3.17-3.25 (8H, m, 4NCH<sub>2</sub>), 5.11 (2H, s, CH<sub>2</sub>-Z), 5.83 (1H, d, *J* 15.6, HC=CHAr), 6.46 (1H, s, ArH), 6.51 (1H, d, *J* 16.0, HC=CHAr), 6.88 (2H, s, 2xAr-H), 7.18 (2H, d, *J* 8.6, 2xAr-H), 7.29 (1H, d, *J* 15.6, HC=CHAr), 7.34-7.36 (5H, m, 5xArH-Z), 7.56 (1H, d, *J* 16.0, HC=CHAr), 7.69 (2H, d, *J* 8.6, 2xArH).  $\delta_{\text{C}}$  (100 MHz, CD<sub>3</sub>OD): 27.75 (CH<sub>2</sub>), 37.62 (NCH<sub>2</sub>), 40.04 (NCH<sub>2</sub>), 46.69 (NCH<sub>2</sub>), 68.32 (CH<sub>2</sub>-Z), 112.46 (ArCH), 117.58 (ArCH), 119.15 (ArCH=CH), 123.81 (ArCH=CH), 124.06 (ArCH), 126.85 (ArCH), 127.60 (ArC), 128.12 (ArCH), 128.86 (ArCH-Z), 129.13 (ArCH-Z), 129.57 (ArCH-Z), 130.61 (ArCH), 130.87 (ArCH), 134.78 (ArC), 138.25 (ArC), 140.92 (ArCH=CH), 141.05 (ArCH=CH), 150.01 (ArC), 150.45 (ArC), 157.87 (ArC), 158.59 (C=O), 168.33 (C=O), 168.59 (C=O).



A solution of **228** (15.9 mg, 0.028 mmol) in dry DCM (2.5 mL), was treated with TMS-I (10  $\mu$ L, 0.042 mmol) added dropwise. The solution was stirred under  $N_2$  for 20 min at room temperature. MeOH (5 mL) was added, and the mixture was stirred for a further 15 min. The solvent was then removed under vacuo and the crude material was purified. Purification by silica gel chromatography 5-10% MeOH/DCM gave **62** the free base as yellow oil (10.3 mg, 83%). Further purification by preparative HPLC gave **62** as the TFA salt in 18% yield.  $\delta_H$  (400MHz,  $D_2O$ ): 1.40-1.65 (4H, m, 2 x  $CH_2$ ), 1.85-1.97 (2H, m,  $CH_2$ ), 2.90-2.99 (4H, m, 2 x  $NCH_2$ ), 3.17-3.22 (2H, m,  $NCH_2$ ), 3.43 (2H, t,  $J$  5.7,  $NCH_2$ ), 5.82 (1H, d,  $J$  15.7, H-8 or H-21), 6.33 (1H, s, H-27), 6.52 (1H, d,  $J$  15.9, H-8 or H-21), 6.90 (2H, s, H-24, H-25), 7.13 (2H, d,  $J$  8.6, H-4, H-28), 7.15 (1H, d,  $J$  15.7, H-7 or H-22), 7.50 (1H, d,  $J$  15.9, H-7 or H-22), 7.68 (2H, d,  $J$  8.8, H-5, H-29);  $\delta_C$  (100MHz,  $D_2O$ ): 22.8 ( $CH_2$ ), 24.9 ( $CH_2$ ), 25.5 ( $CH_2$ ) 36.5 ( $NCH_2$ ), 39.5 ( $NCH_2$ ), 44.6 ( $NCH_2$ ), 47.3 ( $NCH_2$ ), 111.2 (C-27), 116.5 (C-25), 118.0 (C-8 or C-21), 121.3 (C-8 or C-21), 123.3 (C-4, C-28), 126.1 (C-24), 127.3 (C-6 or C-23), 130.1 (C-5, C-29), 132.6 (C-6, C-23), 139.7 (C-7 or C-22), 140.8 (C-7 or C-22), 147.0 (C-26), 149.0 (C-1 or C-3), 156.4 (C-1 or C-3), 163.0, 163.4 (C=O, two rotamers), 168.7, 168.9 (C=O, two rotamers).

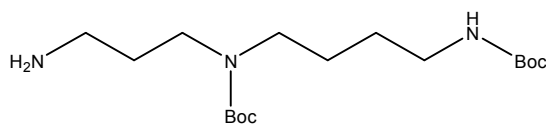
**(4-tert-Butoxycarbonylamino-butyl)-{3-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino]-propyl}-carbamic acid tert-butyl ester** [237]



A solution of **131** (1.02 g, 2.5 mmols), in dry DCM (10 mL), was treated with di-*tert*-butyl dicarbonate (0.55 g, 2.5 mmols). The reaction mixture was stirred at room

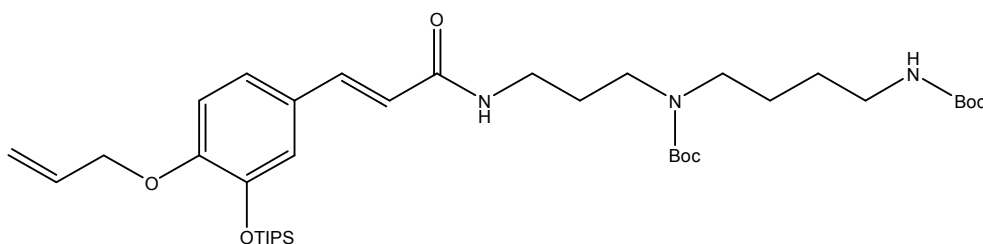
temperature overnight. The reaction mixture was diluted with DCM (20 mL) and washed consequentially with 10% citric acid (2x 20 mL), saturated NaHCO<sub>3</sub> (2x 20 mL) and brine (2x 20 mL). The organic layer was dried over MgSO<sub>4</sub> and evaporated to dryness. Purification by silica gel column chromatography with 0%-3% MeOH/DCM gave **237** (0.95 g, 75%) as a clear oil.  $\delta_{\text{H}}$  (400MHz, CDCl<sub>3</sub>): 0.99 (6H, s, C(CH<sub>3</sub>)<sub>2</sub>), 1.41 (18H, s, 2x C(CH<sub>3</sub>)<sub>3</sub>), 1.44-1.52 (4H, m, 2xCH<sub>2</sub>), 1.82-1.92 (2H, m, CH<sub>2</sub>), 2.34 (4H, s, 2xCH<sub>2</sub>), 2.53 (3H, s, CH<sub>3</sub>), 3.04-3.18 (4H, m, NCH<sub>2</sub>), 3.27 (2H, t, *J* 6.5, NCH<sub>2</sub>), 3.37 (2H, q, *J* 6.5, NCH<sub>2</sub>), 4.72 (1H, br, NH), 13.45 (1H, br, NH).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 17.82 (CH<sub>3</sub>), 25.71 (CH<sub>2</sub>), 25.95 (CH<sub>2</sub>), 27.30 (CH<sub>2</sub>), 28.20 (2xCH<sub>3</sub>), 28.37 (6xCH<sub>3</sub>), 30.03 (CH<sub>2</sub>C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>), 40.01 (CH<sub>2</sub>), 41.01 (CH<sub>2</sub>), 44.33 (CH<sub>2</sub>), 46.91 (CH<sub>2</sub>), 52.31 (CH<sub>2</sub>), 53.41 (CH<sub>2</sub>), 79.02 (2xC(CH<sub>3</sub>)<sub>3</sub>), 108.05 (C=C), 155.51 (C=O), 155.96 (C=O), 173.49 (C=C), 197.06 (C=O), 200.01 (C=O). [Found: (ES<sup>+</sup>) 510.3528 [M+H]<sup>+</sup>, C<sub>27</sub>H<sub>48</sub>N<sub>3</sub>O<sub>6</sub> requires 510.3535].

**(3-Amino-propyl)-(4-tert-butoxycarbonylamino-butyl)-carbamic acid tert-butyl ester** [238]



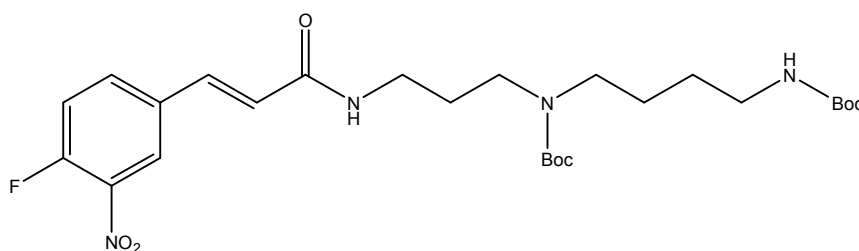
**237** (0.94 g, 1.85 mmols) was treated with 2% Hydrazine/DMF solution (10 mL). The reaction mixture was stirred at room temperature for 10 minutes. Volatiles were removed under vacuo. Purification via silica gel column chromatography with 5%-10% MeOH/DCM then 0.5:10:89.5 NH<sub>3</sub>(aq):MeOH:DCM gave **45** (0.33 g, 52%) as a yellow oil.  $\delta_{\text{H}}$  (400MHz, CDCl<sub>3</sub>): 1.43 (18H, s, 2xC(CH<sub>3</sub>)<sub>3</sub>), 1.53-1.59 (2H, m, CH<sub>2</sub>), 1.62-1.66 (2H, m, CH<sub>2</sub>), 1.82-1.92 (2H, m, CH<sub>2</sub>), 3.09-3.11 (4H, m, 2xNCH<sub>2</sub>), 3.12-3.14 (4H, m, 2xNCH<sub>2</sub>), 4.65 (1H, br, NH).  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>): 25.78 (CH<sub>2</sub>), 27.39 (CH<sub>2</sub>), 28.38 (CH<sub>3</sub>), 28.42 (CH<sub>3</sub>), 31.46 (CH<sub>2</sub>), 38.95 (CH<sub>2</sub>), 40.15 (CH<sub>2</sub>), 43.83 (CH<sub>2</sub>), 46.48 (CH<sub>2</sub>), 79.06 (C(CH<sub>3</sub>)<sub>3</sub>), 79.39 (C(CH<sub>3</sub>)<sub>3</sub>), 155.97 (C=O). [Found: (ES<sup>+</sup>) 346.2694 [M+H]<sup>+</sup>, C<sub>17</sub>H<sub>35</sub>N<sub>3</sub>O<sub>4</sub> requires 346.2700].

**{3-[E]-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-acryloylamino]-propyl}-**  
**(4-tert-butoxycarbonylamino-butyl)-carbamic acid tert-butyl ester** [242]



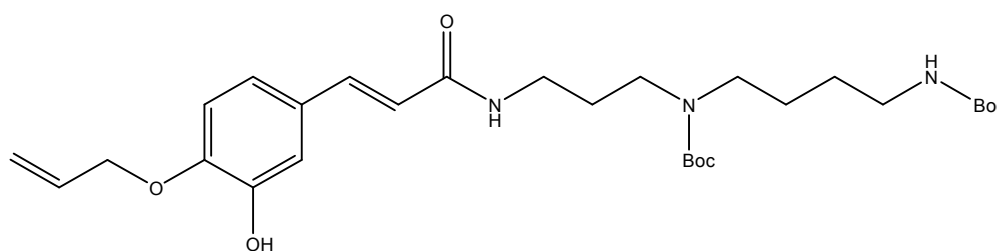
A stirred solution of **238** (190 mg, 0.55 mmol) in dry DCM (4 mL) at 0°C, was treated with DIPEA (0.1mL, 0.55mmol) and activated cinnamic acid **17** (300 mg, 0.55 mmol). The reaction was stirred at room temperature overnight. Volatiles were removed in vacuo. Purification via silica gel column chromatography, with 0-5% MeOH/DCM gave **46** (380 mg, 98%) as a clear oil.  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ): 1.12 (21H, d,  $J$  6.8, 3xCH(CH<sub>3</sub>)<sub>2</sub>), 1.27-1.34 (2H, m, CH<sub>2</sub>), 1.51 (18H, d,  $J$  14.4, 2x C(CH<sub>3</sub>)<sub>3</sub>), 1.55-1.61 (2H, m, CH<sub>2</sub>), 1.72-1.80 (2H, m, CH<sub>2</sub>), 3.10-3.23 (4H, m, 2xNCH<sub>2</sub>), 3.35-3.46 (4H, m, 2xNCH<sub>2</sub>), 4.58 (2H, d,  $J$  5.6, OCH<sub>2</sub>), 5.29-5.45 (2H, m, HC=CH<sub>2</sub>), 6.04-6.12 (1H, m, HC=CH<sub>2</sub>), 6.30 (1H, d,  $J$  15.6, HC=CHAr), 6.84 (1H, d,  $J$  8.4, Ar), 7.07-7.10 (2H, m, Ar), 7.52 (1H, d,  $J$  15.6, HC=CHAr).  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ): 12.80 (3xCH(TIPS)), 17.89 (6xCH<sub>3</sub>(TIPS)), 25.64 (CH<sub>2</sub>), 27.38 (CH<sub>2</sub>), 27.56 (CH<sub>2</sub>), 28.36 (3xCH<sub>3</sub>(Boc)), 28.41 (3xCH<sub>3</sub>(Boc)), 35.95 (NCH<sub>2</sub>), 40.05 (NCH<sub>2</sub>), 43.31 (NCH<sub>2</sub>), 46.60 (NCH<sub>2</sub>), 69.48 (OCH<sub>2</sub>), 79.33 (C(CH<sub>3</sub>)<sub>3</sub>), 79.99 (C(CH<sub>3</sub>)<sub>3</sub>), 113.28 (CH-Ar), 117.81 (HC=CH<sub>2</sub>), 118.38 (HC=CHAr), 119.60 (CH-Ar), 121.83 (CH-Ar), 127.87 (ArC-HC=CH), 133.10 (HC=CH<sub>2</sub>), 140.89 (HC=CHAr), 145.75 (ArC-OTIPS), 151.46 (ArC-OAll), 156.06 (C=O), 156.68 (C=O), 166.99 (C=O). [Found: (ES<sup>+</sup>) 726.4508 [M+Na]<sup>+</sup>, C<sub>38</sub>H<sub>65</sub>N<sub>3</sub>O<sub>7</sub>SiNa requires 726.4484].

**(4-tert-Butoxycarbonylamino-butyl)-[3-[(E)-3-(4-fluoro-3-nitro-phenyl)-acryloylamino]-propyl]-carbamic acid tert-butyl ester** [241]



A stirred solution of **238** (170 mg, 0.49 mmol) in dry DCM (4 mL) at 0°C, was treated with DIPEA (0.1 mL, 0.49 mmol) and activated cinnamic acid **144** (185 mg, 0.49 mmol). The reaction was stirred at room temperature overnight, and volatiles were removed in vacuo. Purification via silica gel column chromatography, with 0-5% MeOH/DCM gave **241** (260 mg, 98%) as a yellow oil.  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ): 1.47 (18H, d,  $J$  16.8, 2x  $\text{C}(\text{CH}_3)_3$ ), 1.51-1.58 (2H, m,  $\text{CH}_2$ ), 1.62-1.71 (4H, m, 2x $\text{CH}_2$ ), 3.10-3.15 (4H, m, 2x $\text{NCH}_2$ ), 3.31-3.56 (4H, m, 2x $\text{CH}_2$ ), 6.50 (1H, d,  $J$  15.6,  $\text{HC}=\text{CHAr}$ ), 7.27-7.32 (2H, m, 2xArH), 7.45 (1H, br, NH), 7.56 (1H, d,  $J$  15.6,  $\text{HC}=\text{CHAr}$ ), 7.72 (1H, br, NH), 8.18 (1H, dd,  $J$  7.2, 4.8, ArH).  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ): 25.63 ( $\text{CH}_2$ ), 27.46 ( $\text{CH}_2$ ), 28.38 (3x $\text{CH}_3(\text{Boc})$ ), 28.41 (3x $\text{CH}_3(\text{Boc})$ ), 35.73 ( $\text{NCH}_2$ ), 43.09 ( $\text{NCH}_2$ ), 46.09 ( $\text{NCH}_2$ ), 46.65 ( $\text{NCH}_2$ ), 79.33 ( $\text{C}(\text{CH}_3)_3$ ), 80.12 ( $\text{C}(\text{CH}_3)_3$ ), 118.85 ( $\text{CH}(\text{Ar})$ ), 124.27 ( $\text{HC}=\text{CHAr}$ ), 132.25 ( $\text{CNO}_2(\text{Ar})$ ), 134.54 ( $\text{ArC}-\text{HC}=\text{CH}$ ), 136.68 ( $\text{HC}=\text{CHAr}$ ), 137.62 ( $\text{CF}(\text{Ar})$ ), 154.28 ( $\text{C}=\text{O}$ ), 156.05 ( $\text{C}=\text{O}$ ), 164.90 ( $\text{C}=\text{O}$ ). [Found: ( $\text{ES}^+$ ) 561.2690 [ $\text{M}+\text{Na}$ ] $^+$ ,  $\text{C}_{26}\text{H}_{39}\text{FN}_4\text{O}_7\text{Na}$  requires 561.2695].

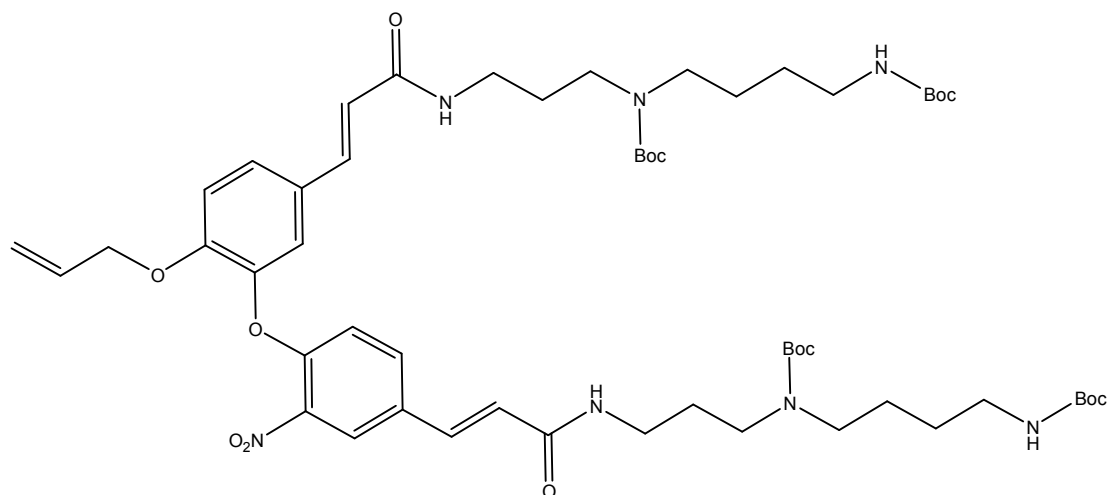
**{3-[(E)-3-(4-Allyloxy-3-triisopropylsilyloxy-phenyl)-acryloylamino]-propyl}-**  
**(4-tert-butoxycarbonylamino-butyl)-carbamic acid tert-butyl ester** [243]



A stirred solution of **238** (190 mg, 0.55 mmol) in dry DCM (4 mL) at 0°C, was treated sequentially with DIPEA (0.1 mL, 0.55 mmol) followed by activated cinnamic acid **17** (300 mg, 0.55 mmol). The reaction was stirred at room temperature overnight, and volatiles were removed in vacuo and residue was purified by silica gel column chromatography 0%-5% MeOH/DCM to give **243** (380 mg, 98%) as a yellow oil.  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ): 1.42 (18H, d,  $J$  13.6, 9x $\text{CH}_3$ ), 1.47-1.54 (4H, m,  $\text{CH}_2$ ), 1.64-1.68 (2H, m,  $\text{CH}_2$ ), 3.08-3.18 (4H, m, 2x $\text{NCH}_2$ ), 3.20-3.34 (2H, m, 2x $\text{NCH}_2$ ), 4.57 (2H, d,  $J$  5.6,  $\text{OCH}_2$ ), 4.65 (1H, br, NH), 5.26-5.40 (2H, m,  $\text{HC}=\text{CH}_2$ ), 5.97-6.06 (1H, m,  $\text{HC}=\text{CH}_2$ ), 6.27 (1H, d,  $J$  15.6,  $\text{HC}=\text{CHAr}$ ), 6.77 (1H, d,  $J$  8.2, Ar-H), 6.92 (1H, d,  $J$  8.2, Ar-H), 7.04 (1H, br, NH), 7.11 (1H, s, Ar-H), 7.48 (1H, d,  $J$  15.6,  $\text{HC}=\text{CHAr}$ ).  $\delta_{\text{C}}$  (100 MHz,  $\text{CDCl}_3$ ): 25.62 ( $\text{CH}_2$ ), 27.35 ( $\text{CH}_2$ ), 27.61 ( $\text{CH}_2$ ), 28.33 (3x $\text{CH}_3$ ), 28.37 (3x $\text{CH}_3$ ), 35.75 ( $\text{NCH}_2$ ), 39.99 ( $\text{NCH}_2$ ), 43.32 ( $\text{NCH}_2$ ), 46.54 ( $\text{NCH}_2$ ), 69.69 ( $\text{OCH}_2$ ), 79.10 ( $\text{C}(\text{CH}_3)_3$ ), 79.78 ( $\text{C}(\text{CH}_3)_3$ ), 112.00 ( $\text{CH-Ar}$ ), 113.16 ( $\text{CH-Ar}$ ), 118.48 ( $\text{HC}=\text{CH}_2$ ), 119.47 ( $\text{HC}=\text{CHAr}$ ), 120.99 ( $\text{CH-Ar}$ ), 128.69 ( $\text{ArC-HC}=\text{CH}$ ), 132.47 ( $\text{HC}=\text{CH}_2$ ), 140.08 ( $\text{HC}=\text{CHAr}$ ), 146.00 ( $\text{ArC-OAll}$ ), 146.96 ( $\text{ArC-OH}$ ), 156.00 ( $\text{C}=\text{O}$ ), 156.44 ( $\text{C}=\text{O}$ ), 166.25 ( $\text{C}=\text{O}$ ). [Found: ( $\text{ES}^+$ ) 548.3323 [ $\text{M}+\text{H}$ ] $^+$ ,  $\text{C}_{26}\text{H}_{46}\text{N}_3\text{O}_7$  requires 548.3330].



**[3-((E)-3-{4-Allyloxy-3-[4-((E)-2-{3-[tert-butoxycarbonyl-(4-tert-butoxycarbonylamino-butyl)-amino]-propylcarbamoyl}-vinyl)-2-nitro-phenoxy]-phenyl}-acryloylamino)-propyl]-[4-tert-butoxycarbonylamino-butyl]-carbamic acid tert-butyl ester** [244]

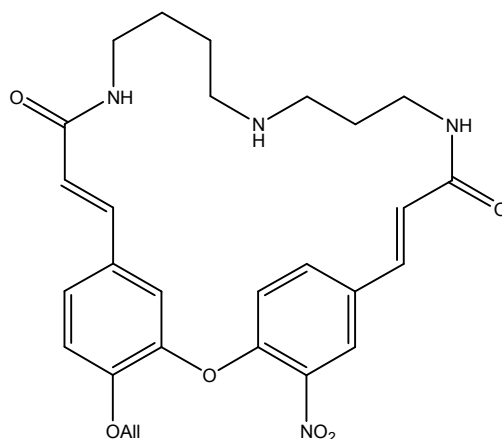


*Method A:* A solution of **242** (328 mg, 0.47 mmol) and **241** (251 mg, 0.47 mmol) in dry THF (30 mL), was treated with TBAF (0.47 mL, 0.47 mmol). The reaction was stirred at room temperature for 5 h (until no further change was observed by TLC). Solvent was removed *in vacuo* and residue was redissolved in 5% citric acid (20 mL) and extracted with EtOAc (3x 20 mL). The organic extracts were combined and washed with brine, dried (MgSO<sub>4</sub>), and concentrated *in vacuo*. Purification via silica gel column chromatography 0-5% MeOH/DCM gave the desired product **244** (180 mg, 36%) as a yellow oil, as well as the TIPS deprotected cinnamic acid **243** (150 mg), and unreacted **241** (135 mg).

*Method B:* A solution of **241** (136 mg, 0.24 mmol) and **243** (132 mg, 0.24 mmol) in DMF (15 mL), was treated with K<sub>2</sub>CO<sub>3</sub> (130 mg, 0.83 mmol) and stirred at room temperature overnight. Volatiles were removed *in vacuo* and the residue was redissolved in EtOAc (25 mL) and washed sequentially with 5% citric acid (25 mL), water (20 mL) and brine (20 mL). The organic phase was dried (MgSO<sub>4</sub>) and evaporated to dryness. Purification via silica gel column chromatography 0-5% MeOH/DCM, gave **49** (252 mg, 98%) as a yellow oil.

$\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ): 1.45 (36H, t,  $J$  7.6, 9xCH<sub>3</sub>), 1.57 (12H, s, 6xCH<sub>2</sub>), 3.13 (8H, br, 4xNCH<sub>2</sub>), 3.31 (8H, br, 4xNCH<sub>2</sub>), 4.51 (2H, dt,  $J$  4.8, 1.6, OCH<sub>2</sub>), 5.12-5.20 (2H, m, HC=CH<sub>2</sub>), 5.75-5.85 (1H, m, HC=CH<sub>2</sub>), 6.31 (1H, d,  $J$  14.4, HC=CHAr), 6.42 (1H, d,  $J$  15.2, HC=CHAr), 6.80 (1H, d,  $J$  8.8, Ar-H), 6.97 (1H, d,  $J$  8.4, Ar-H), 7.31-7.36 (2H, m, 2xAr-H), 7.50 (1H, d,  $J$  15.2, HC=CHAr), 7.51 (1H, s, Ar-H), 7.54 (1H, d,  $J$  14.4, HC=CHAr), 8.10 (1H, d,  $J$  2.0, Ar-H).  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ): 25.55 (2xCH<sub>2</sub>), 27.31 (2xCH<sub>2</sub>), 27.51 (2xCH<sub>2</sub>), 28.28 (2x(CH<sub>3</sub>)<sub>3</sub>), 28.30 (2x(CH<sub>3</sub>)<sub>3</sub>), 35.71 (2xNCH<sub>2</sub>), 39.91 (2xNCH<sub>2</sub>), 43.16 (2xNCH<sub>2</sub>), 46.49 (2xNCH<sub>2</sub>), 69.93 (OCH<sub>2</sub>), 79.01 (2xC(CH<sub>3</sub>)<sub>3</sub>), 79.73 (2xC(CH<sub>3</sub>)<sub>3</sub>), 114.53 (CH-Ar), 117.65 (CH-Ar), 117.80 (HC=CH<sub>2</sub>), 120.62 (Ar-CH), 120.74 (HC=CHAr), 122.64 (HC=CHAr), 124.19 (CH-Ar), 126.97 (CH-Ar), 128.99 (ArC-HC=CH), 129.52 (ArC-HC=CH), 131.86 (HC=CH<sub>2</sub>), 133.03 (CH-Ar), 137.11 (HC=CHAr), 138.59 (HC=CHAr), 139.61(Ar), 143.00 (Ar), 150.92 (Ar), 151.95 (Ar), 155.96 (2xC=O(Boc)), 156.43 (2xC=O(Boc)), 165.15 (C=O), 165.71 (C=O). [Found: (ES<sup>+</sup>) 1088.5864 [M+Na]<sup>+</sup>, C<sub>55</sub>H<sub>83</sub>N<sub>7</sub>O<sub>14</sub>Na requires 1088.5890].

**(8E,22E)-4-Allyloxy-27-nitro-2-oxa-11,16,20-triaza-tricyclo[22.2.2.1<sup>3,7</sup>]nonacosan-1(27),3,5,7(29),8,22,24(28),25-octaene-10,21-dione** [233]



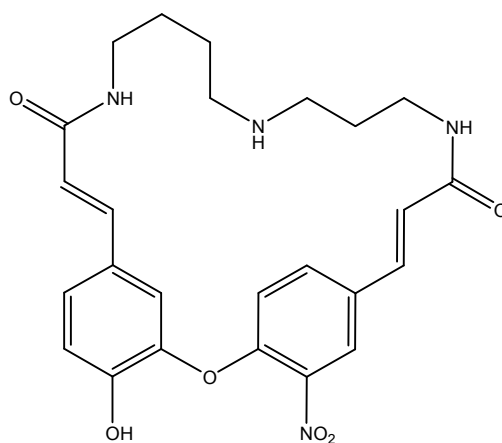
A solution of **224** (60 mg, 0.09 mmol) in dry DCM (2 mL) was treated dropwise with iodotrimethylsilane (20  $\mu\text{L}$ , 0.135 mmol). The reaction mixture was stirred at room temperature for 20 min. MeOH (5 mL) was added and the mixture was stirred for a further 15 min. Volatiles were removed *in vacuo* and the residue was purified via silica gel chromatography 0%-10%MeOH/DCM to give **233** (44 mg, 93%) as a white

solid.  $\delta_{\text{H}}$  (400MHz,  $\text{CD}_3\text{OD}$ ): 1.53-1.57 (2H, m, 2x $\text{CH}_2$ ), 1.74-1.84 (2H, m, 2x $\text{CH}_2$ ), 1.92-2.02 (2H, m, 2x $\text{CH}_2$ ), 3.02-3.15 (6H, m, 3x $\text{NCH}_2$ ), 3.46-3.56 (2H, m,  $\text{NCH}_2$ ), 4.73 (2H, dt,  $J$  5.6, 1.6,  $\text{OCH}_2$ ), 5.29-5.32 (2H, m,  $\text{HC}=\text{CH}_2$ ), 6.09-6.18 (1H, m,  $\text{HC}=\text{CH}_2$ ), 6.21 (1H, d,  $J$  15.6,  $\text{HC}=\text{CHAr}$ ), 6.53 (1H, d,  $J$  2.0, Ar-H), 6.63 (1H, d,  $J$  16.0,  $\text{HC}=\text{CHAr}$ ), 7.01-7.09 (2H, m, 2xAr-H), 7.32 (1H, d,  $J$  15.6,  $\text{HC}=\text{CHAr}$ ), 7.41 (1H, d,  $J$  8.8, Ar-H), 7.57 (1H, d,  $J$  16.0,  $\text{HC}=\text{CHAr}$ ), 8.06 (1H, dd,  $J$  8.8, 2.4, Ar-H), 8.30 (1H, d,  $J$  2.0, Ar-H).  $\delta_{\text{C}}$  (100MHz,  $\text{CD}_3\text{OD}$ ): 24.84 ( $\text{CH}_2$ ), 26.41 ( $\text{CH}_2$ ), 27.01 ( $\text{CH}_2$ ), 37.41 ( $\text{NCH}_2$ ), 40.00 ( $\text{NCH}_2$ ), 46.36 (2x $\text{NCH}_2$ ), 71.05 ( $\text{OCH}_2$ ), 111.83 (Ar-CH), 115.62 (Ar-CH), 118.19 ( $\text{HC}=\text{CH}_2$ ), 120.68 ( $\text{HC}=\text{CHAr}$ ), 126.36 (Ar-CH), 127.16 (Ar-CH), 127.24 (Ar-CH), 127.28 ( $\text{HC}=\text{CHAr}$ ), 129.47 (Ar-C), 134.37 (Ar-CH), 135.48 (Ar-C), 138.47 ( $\text{HC}=\text{CHAr}$ ), 140.30 ( $\text{HC}=\text{CHAr}$ ), 144.67 (Ar-C), 150.12 (Ar-C), 150.91 (Ar-C), 150.98 (Ar-C), 168.09 (C=O), 168.58 (C=O). [Found: ( $\text{ES}^+$ ) 521.2380 [ $\text{M}+\text{H}$ ] $^+$ ,  $\text{C}_{28}\text{H}_{33}\text{N}_4\text{O}_6$  requires 521.2395].

**(8E,22E)-4-Hydroxy-27-nitro-2-oxa-11,16,20-triaza-tricyclo[22.2.2.1<sup>3,7</sup>**

**nonacosa-1(27),3,5,7(29),8,22,24(28),25-octaene-10,21-dione**

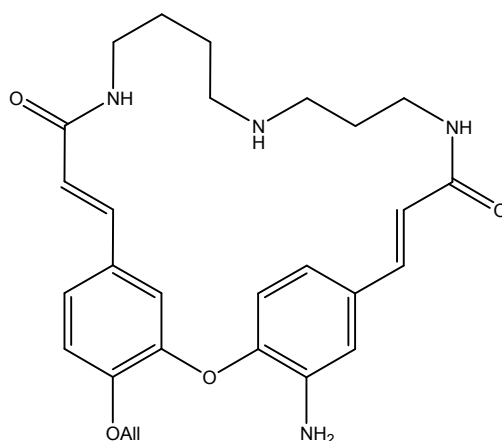
[234]



A solution of **233** (22 mg, 0.04 mmol) in dry MeOH (2 mL) was treated sequentially with tetrakis(triphenylphosphine)palladium (6 mg, 0.004 mmol) and morpholine (40  $\mu\text{L}$ , 0.4 mmol). The reaction mixture was stirred for 30 min. The volatiles were removed *in vacuo* and the residue was purified via silica gel chromatography 0%-10% MeOH/DCM then 0.5:10:89.5  $\text{NH}_3(\text{aq})$ :MeOH:DCM to give **234** (8 mg, 40%) as white solid.  $\delta_{\text{H}}$  (400MHz,  $\text{CD}_3\text{OD}$ ): 1.45-1.56 (2H, m, 2x $\text{CH}_2$ ), 1.74-1.78 (2H, m, 2x $\text{CH}_2$ ), 1.93-1.98 (2H, m, 2x $\text{CH}_2$ ), 3.01-3.28 (4H, m, 2x $\text{NCH}_2$ ), 3.34-3.61 (4H, m,

2xNCH<sub>2</sub>), 6.10 (1H, d, *J* 15.6, HC=CHAr), 6.48 (1H, d, *J* 1.6, Ar-H), 6.86-6.97 (3H, m, 2xAr-H, HC=HCAr), 7.30 (1H, d, *J* 15.6, HC=CHAr), 7.43 (1H, d, *J* 8.4, Ar-H), 7.58 (1H, d, *J* 15.6, HC=CHAr), 8.05 (1H, dd, *J* 2.4, 8.4, Ar-H), 8.30 (1H, d, *J* 2.4, Ar-H).  $\delta_C$  (100MHz, CD<sub>3</sub>OD): 22.79 (CH<sub>2</sub>), 24.48 (CH<sub>2</sub>), 25.09 (CH<sub>2</sub>), 35.39 (NCH<sub>2</sub>), 38.00 (NCH<sub>2</sub>), 44.29 (NCH<sub>2</sub>), 109.87 (Ar-H), 115.87 (HC=CHAr), 117.59 (HC=CHAr), 124.25 (Ar-H), 124.99 (Ar-C), 125.41 (Ar-H), 125.77 (Ar-H), 126.15 (Ar-H), 132.78 (Ar-H), 133.44 (Ar-C), 136.58 (HC=CHAr), 138.70 (HC=CHAr), 142.70 (Ar-C), 147.41 (Ar-C), 148.04 (Ar-C), 148.38 (Ar-C), 166.03 (C=O), 166.76 (C=O).

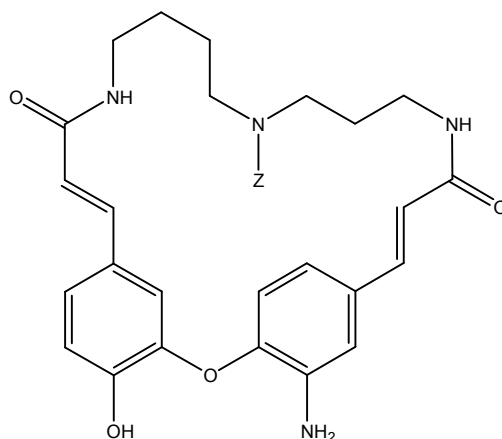
**(8E,22E)-4-Allyloxy-27-amino-2-oxa-11,16,20-triaza-tricyclo[22.2.2.1<sup>3,7</sup>]nonacosa -1(27),3,5,7(29),8,22,24(28),25-octaene-10,21-dione** [232]



A solution of **225** (35 mg, 0.056 mmol) in dry DCM (5 mL) was treated dropwise with iodotrimethylsilane (20  $\mu$ L, 0.135 mmol). The reaction mixture was stirred at room temperature for 20 min. MeOH (5 mL) was added and mixture was stirred for a further 15 min. Volatiles were removed *in vacuo* and the residue was purified via silica gel chromatography 0%-10%MeOH/DCM to give **232** (21 mg, 80%) as a yellow oil.  $\delta_H$  (400MHz, CD<sub>3</sub>OD): 1.48-1.55 (4H, m, 2xCH<sub>2</sub>), 1.78-1.1.82 (2H, m, CH<sub>2</sub>), 2.64-2.72 (4H, m, NCH<sub>2</sub>), 3.18-3.28 (2H, m, NCH<sub>2</sub>), 3.35-3.47 (2H, m, NCH<sub>2</sub>), 4.72 (2H, d, *J* 5.6, OCH<sub>2</sub>), 5.31-5.53 (2H, m, HC=CH<sub>2</sub>), 5.92 (1H, d, *J* 15.6, HC=CHAr), 6.12-6.22 (1H, m, HC=CH<sub>2</sub>), 6.47 (1H, d, *J* 16.0, HC=CHAr), 6.58 (1H, s, Ar-H), 6.88 (1H, d, *J* 8.8, Ar-H), 6.96-7.07 (2H, m, 2xAr-H), 7.10 (1H, s, Ar-H),

7.26 (1H, d, *J* 8.8, Ar-H), 7.33 (1H, d, *J* 15.6, HC=CHAr), 7.46 (1H, d, *J* 16.0, HC=CHAr).  $\delta_C$  (100MHz, CD<sub>3</sub>OD): 27.54 (CH<sub>2</sub>), 29.33 (CH<sub>2</sub>), 30.75 (CH<sub>2</sub>), 38.78 (NCH<sub>2</sub>), 40.60 (NCH<sub>2</sub>), 47.93 (NCH<sub>2</sub>), 49.07 (NCH<sub>2</sub>), 71.01 (OCH<sub>2</sub>), 111.07 (Ar-CH), 115.06 (Ar-CH), 116.97 (Ar-CH), 118.30 (HC=CH<sub>2</sub>), 118.73 (Ar-CH), 119.92 (HC=CHAr), 122.99 (HC=CHAr), 124.40 (Ar-CH), 126.33 (Ar-CH), 129.44 (Ar-C), 129.63 (Ar-C), 134.71 (Ar-C), 135.11 (Ar-C), 140.85 (Ar-C), 141.89 (Ar-C), 150.17 (Ar-C), 150.89 (Ar-C), 168.62 (C=O), 168.68 (C=O). [Found: (ES<sup>+</sup>) 491.3000 [M+H]<sup>+</sup>, C<sub>28</sub>H<sub>35</sub>N<sub>4</sub>O<sub>4</sub> requires 491.2658].

**(8E,22E)-27-Amino-4-hydroxy-10,21-dioxo-2-oxa-11,16,20-triazatricyclo[22.2.2.1<sup>3,7</sup>]nonacosa-1(27),3,5,7(29),8,22,24(28),25-octaene-16-carboxylic acid benzyl ester** [230]

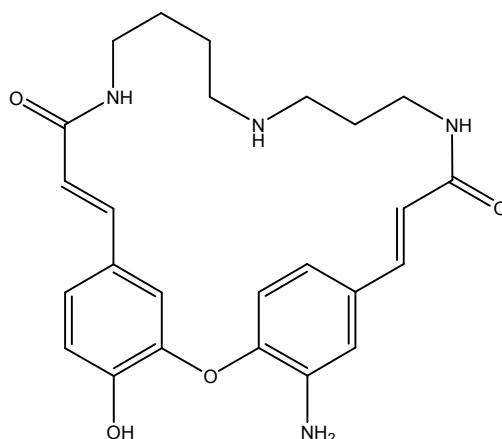


A solution of **232** (35 mg, 0.056 mmol) in dry THF (5 mL), was treated sequentially with tetrakis(triphenylphosphine)palladium (6.5 mg, 0.0056 mmol) and morpholine (50  $\mu$ L, 0.56 mmol). The reaction was stirred at room temperature for 30min. Volatiles were removed *in vacuo*, and the residue was purified by silica gel column chromatography 0%-10% MeOH/DCM then 0.5:10:89.5 NH<sub>3</sub>(aq):MeOH:DCM to give **230** (17mg, 54%) as a white solid.  $\delta_H$  (400MHz, CD<sub>3</sub>OD): 1.31-1.38 (2H, m, CH<sub>2</sub>), 1.40-1.54 (2H, m, CH<sub>2</sub>), 1.75-1.1.80 (2H, m, CH<sub>2</sub>), 2.80-3.20 (4H, m, 2xNCH<sub>2</sub>), 3.12-3.26 (4H, m, 2xNCH<sub>2</sub>), 5.11 (2H, s, CH<sub>2</sub>(Z)), 5.86 (1H, d, *J* 15.4, HC=CHAr), 6.46 (1H, d, *J* 16.0, HC=CHAr), 6.53 (1H, s, CH(Ar)), 6.87 (2H, s,, 2xCH(Ar)), 6.96-7.01 (2H,m, 2xCH(Ar)), 7.10 (1H, d, *J* 1.6, CH(Ar)), 7.31 (1H, d, *J*

15.4, HC=CHAr), 7.35 (5H, br, 5x CH(Z)), 7.46 (1H, d, *J* 16.0, HC=CHAr). ).  $\delta_C$  (100MHz, CD<sub>3</sub>OD): 27.61 (CH<sub>2</sub>), 27.71 (CH<sub>2</sub>), 37.49 (NCH<sub>2</sub>), 40.05 (NCH<sub>2</sub>), 46.59 (NCH<sub>2</sub>), 68.17 (CH<sub>2</sub>-Z), 110.85 (Ar-CH), 117.02 (Ar-CH), 117.47 (Ar-CH), 118.55 (Ar-CH), 118.84 (HC=CHAr), 122.84 (HC=CHAr), 124.52 (Ar-CH), 126.84 (Ar-CH), 128.00 (Ar-C(Z)), 128.85 (Ar-CH(Z)), 129.14 (Ar-CH(Z)), 129.57 (Ar-CH(Z)), 135.02 (Ar-C), 138.19 (Ar-C), 141.36 (HC=CHAr), 141.95 (HC=CHAr), 142.46 (Ar-C), 143.87 (Ar-C), 148.87 (Ar-C), 150.02 (Ar-C), 157.78 (C=O), 168.56 (C=O), 168.69 (C=O). [Found: (ES<sup>+</sup>) 585.2686 [M+H]<sup>+</sup>, C<sub>33</sub>H<sub>37</sub>N<sub>4</sub>O<sub>6</sub> requires 585.2708].

### Amino-Cadabicine

[231]

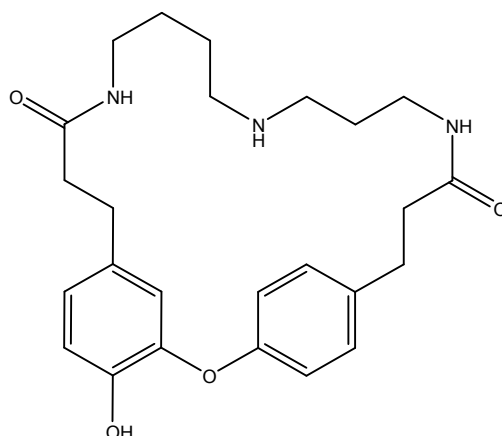


A solution of **230** (14.5 mg, 0.025mmol) in dry DCM (2.5 mL) was treated dropwise with iodotrimethylsilane (10  $\mu$ L, 0.037 mmol). The reaction mixture was stirred at room temperature for 20 min. MeOH (5 mL) was added and the mixture was stirred for a further 15 min. Volatiles were removed under vacuo and the residue was purified via silica gel chromatography 0%-10%MeOH/DCM to give **231** (8 mg, 72%) as a yellow oil.  $\delta_H$  (400MHz, CD<sub>3</sub>OD): 1.42-1.63 (4H, m, 2xCH<sub>2</sub>), 1.79-1.89 (2H, m, CH<sub>2</sub>), 2.68-2.78 (4H, m, 2xNCH<sub>2</sub>), 3.22-3.29 (2H, m, NCH<sub>2</sub>), 3.53-3.50 (2H, m, NCH<sub>2</sub>), 5.89 (1H, d, *J* 15.2, HC=CHAr), 6.50 (1H, d, *J* 15.6, HC=CHAr), 6.56 (1H, s, Ar-H), 6.87 (2H, s, 2xAr-H), 6.99 (2H, s, 2xAr-H), 7.10 (1H, s, Ar-H), 7.30 (1H, d, *J* 16.0, HC=CHAr), 7.46 (1H, d, *J* 16.0, HC=CHAr). (100MHz, CD<sub>3</sub>OD): 27.07 (CH<sub>2</sub>), 27.17 (CH<sub>2</sub>), 28.97 (CH<sub>2</sub>), 38.62 (NCH<sub>2</sub>), 40.50 (NCH<sub>2</sub>), 47.74 (NCH<sub>2</sub>), 50.24 (NCH<sub>2</sub>), 110.91 (ArCH), 116.99 (ArCH), 117.56 (ArCH), 118.65 (ArCH), 118.81

(HC=CHAr), 123.08 (HC=CHAr), 124.49 (ArCH), 126.83 (ArCH), 127.83 (ArC), 135.09 (ArC), 141.32 (HC=CHAr), 141.88 (HC=CHAr), 142.45 (ArC), 143.93 (ArC), 148.92 (ArC), 150.37 (ArC), 168.74 (C=O), 168.89 (C=O).

### Tetrahydro-cadabicine

[229]



A stirred mixture of **228** (11 mg,) and Pd/C (11 mg) in MeOH/HCl (2 mL), was reacted under H<sub>2</sub> overnight. The mixture was then filtered through celite and concentrated to dryness. The resulting crude material was purified via silica gel column chromatography 0%-3% MeOH/DCM to give **229** (6.5 mg, 77%) as a white solid. **mp**: 171°C,  **$\delta_{\text{H}}$  (400MHz, CD<sub>3</sub>OD)**: 1.39-1.48 (4H, m, 2xCH<sub>2</sub>), 1.73 (2H, t, *J* 6.0, CH<sub>2</sub>), 2.43-2.47 (2H, m, COCH<sub>2</sub>), 2.55 (4H, t, *J* 6.8, 2xNCH<sub>2</sub>), 2.64-2.67 (2H, m, COCH<sub>2</sub>), 2.83-2.87 (2H, m, ArCH<sub>2</sub>), 2.92-2.95 (2H, m, ArCH<sub>2</sub>), 3.12 (2H, t, *J* 6.2, NCH<sub>2</sub>), 3.26 (2H, t, *J* 6.2, NCH<sub>2</sub>), 6.80 (1H, d, *J* 2.0, Ar-H), 6.84-6.87 (3H, m, 3xAr-H), 6.91 (1H, dd, *J* 2.2, 8.2, Ar-H), 7.18 (1H, d, *J* 8.4, Ar-H). **(100MHz, CD<sub>3</sub>OD)**: 24.16 (CH<sub>2</sub>), 27.49 (CH<sub>2</sub>), 27.65 (CH<sub>2</sub>), 31.13 (ArCH<sub>2</sub>), 31.99 (ArCH<sub>2</sub>), 36.10 (NCH<sub>2</sub>), 36.82 (COCH<sub>2</sub>), 38.91 (NCH<sub>2</sub>), 39.03 (COCH<sub>2</sub>), 45.64 (NCH<sub>2</sub>), 48.45 (NCH<sub>2</sub>), 117.53 (ArCH), 118.34 (ArCH), 123.31 (ArCH), 126.62 (ArCH), 130.57 (ArCH), 133.86 (ArC), 135.44 (ArC), 143.89 (ArC), 148.62 (ArC), 157.99 (ArC).

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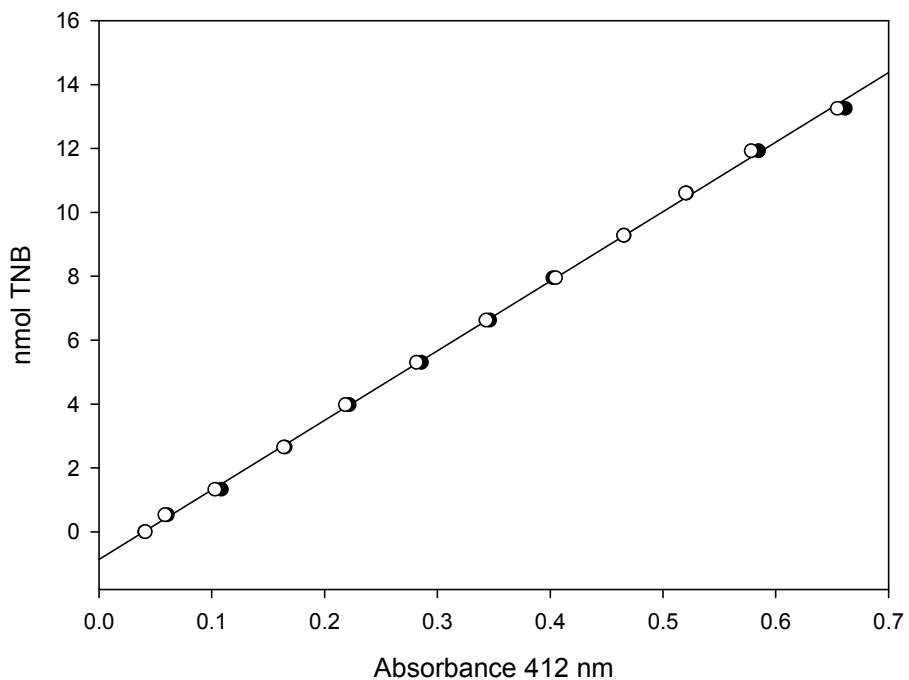
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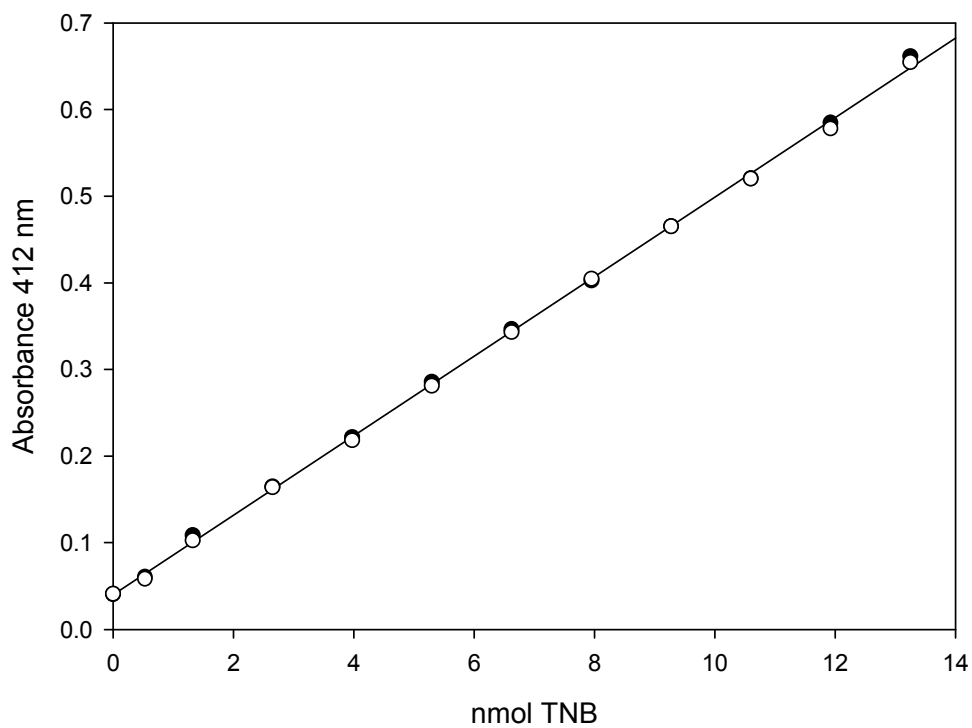
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## 5. APPENDIX

Standard curve of 2-nitro-5-thiobenzoic acid

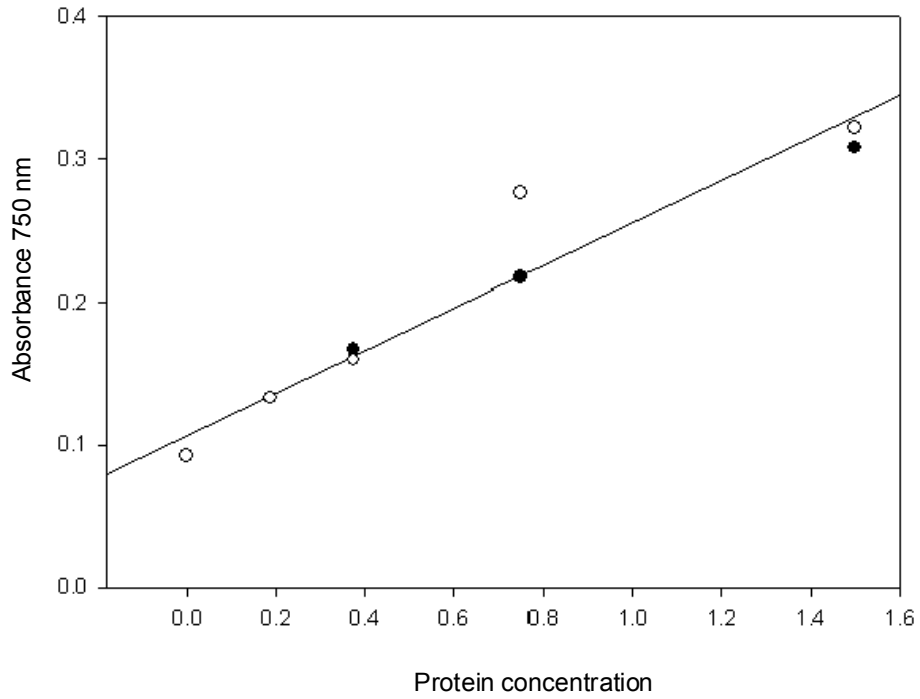


Standard curve of 2-nitro-5-thiobenzoate

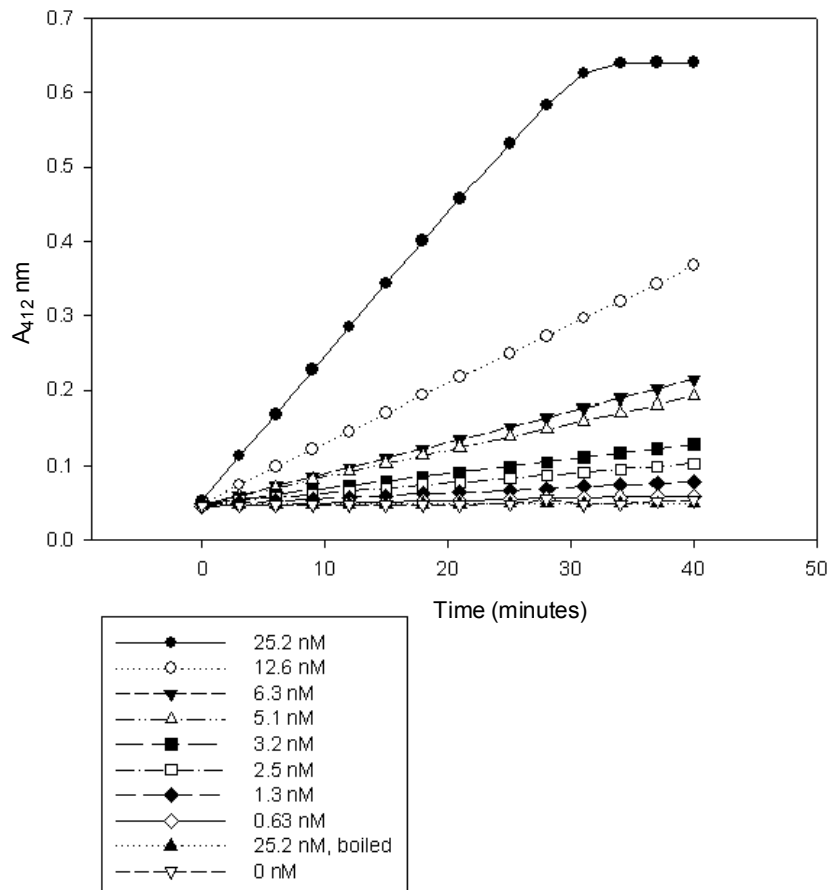




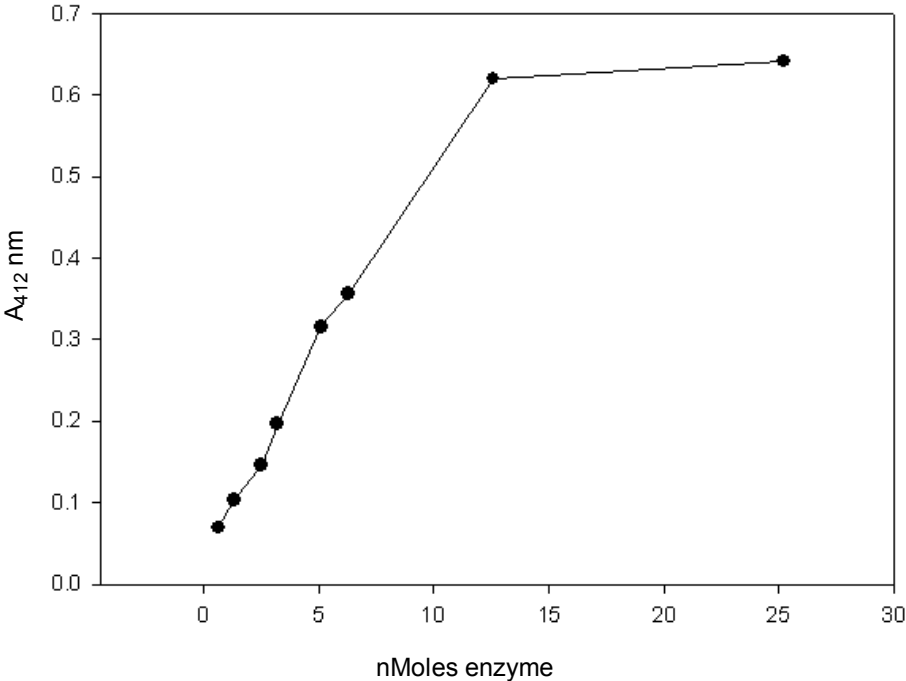
### Determination of protein concentrations by Lowry assay



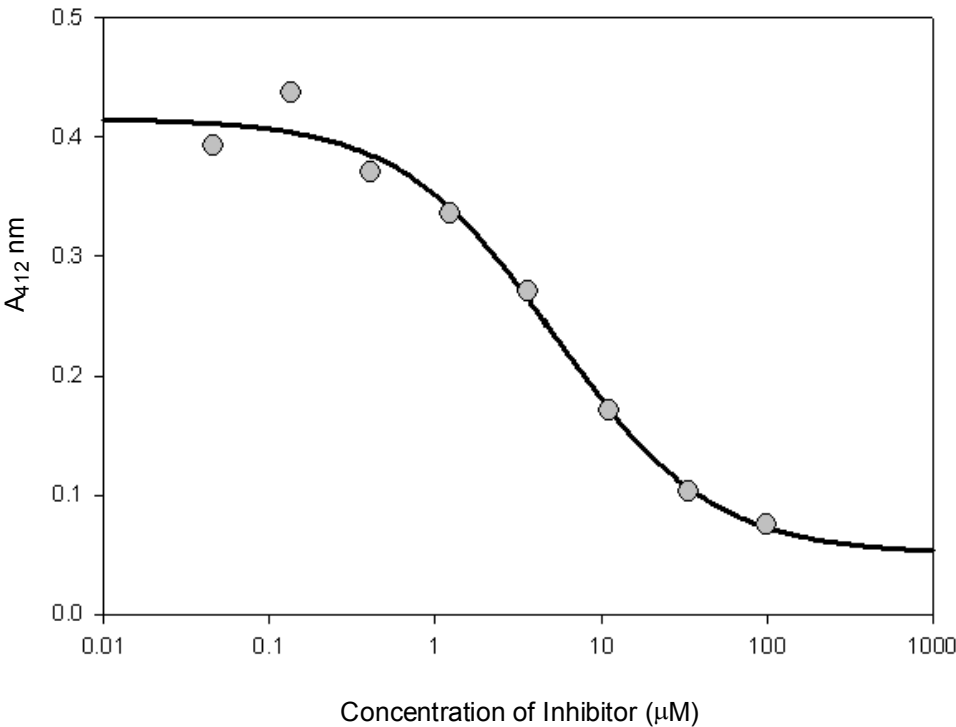
### Product formation at various concentrations of enzyme



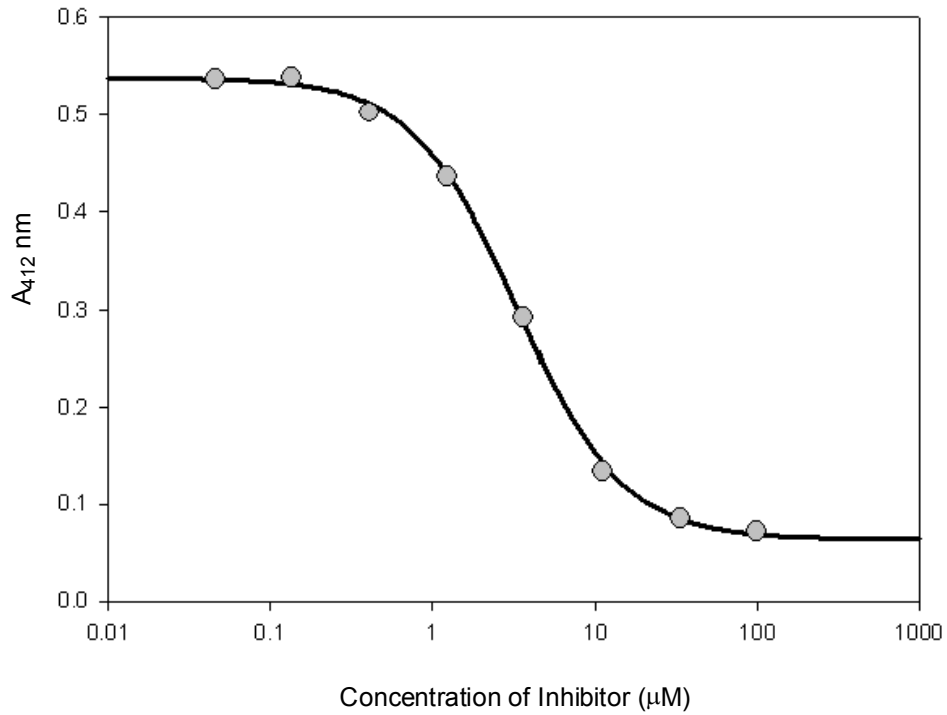
**Product vs. amount of enzyme**



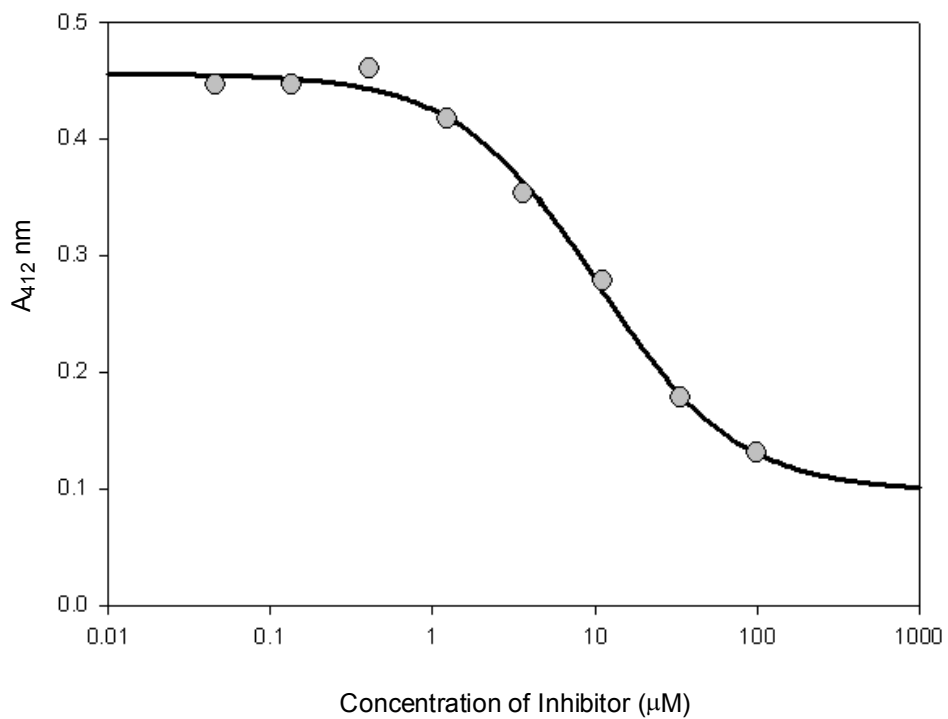
**Cloripramine inhibition at 1  $\mu$ M typanothione  
- determination on 12-11-2008**



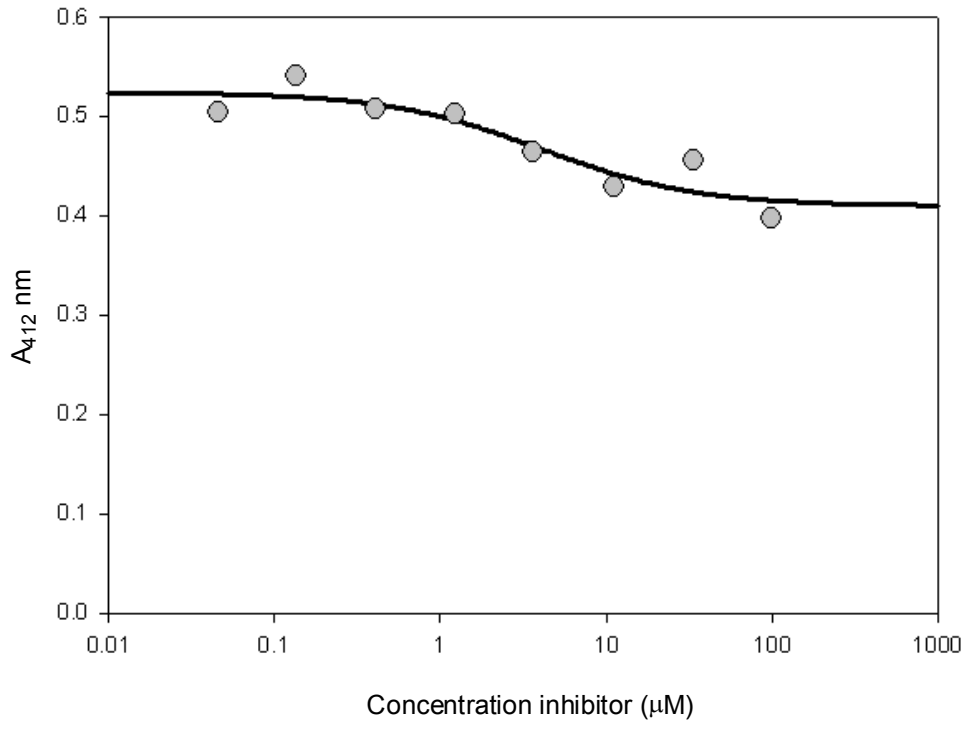
**Cloripramine inhibition at 1  $\mu$ M typanothione  
- repeat determination on 14-11-2008**



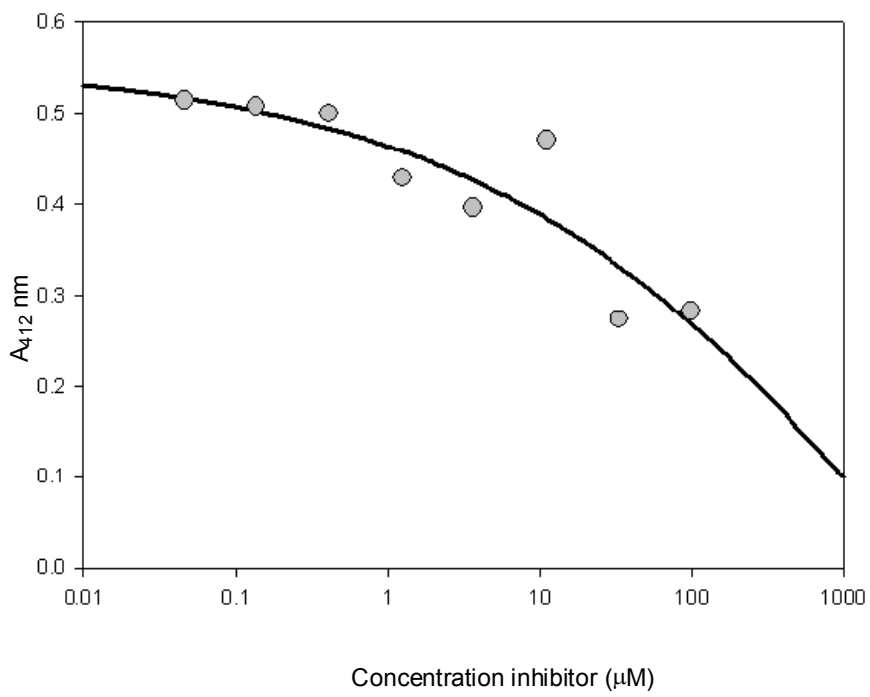
**Cloripramine inhibition at 5  $\mu$ M typanothione**



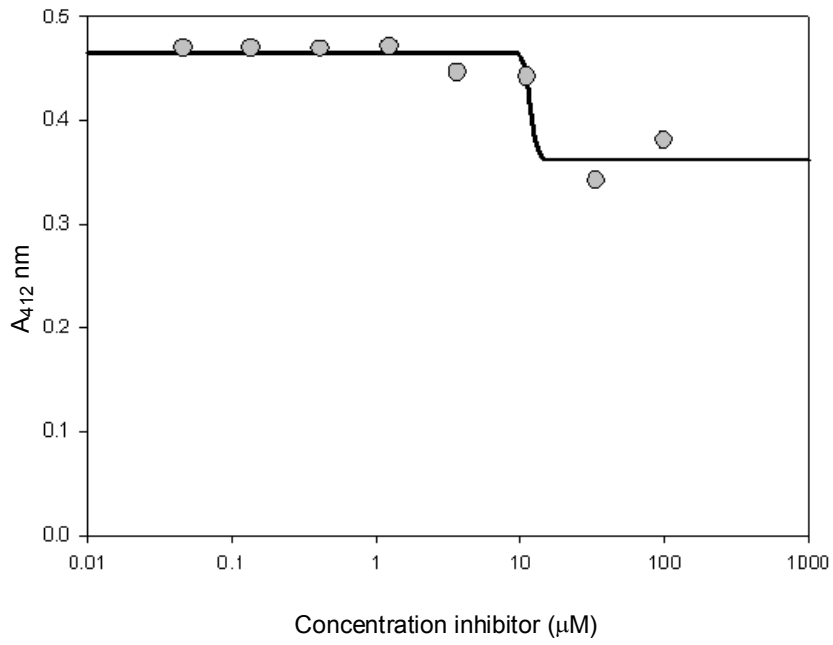
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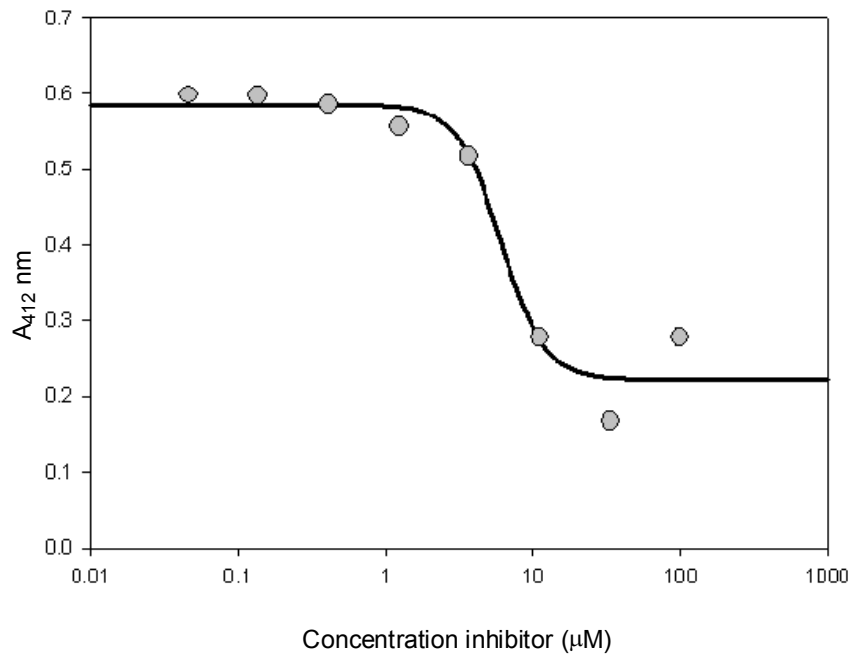
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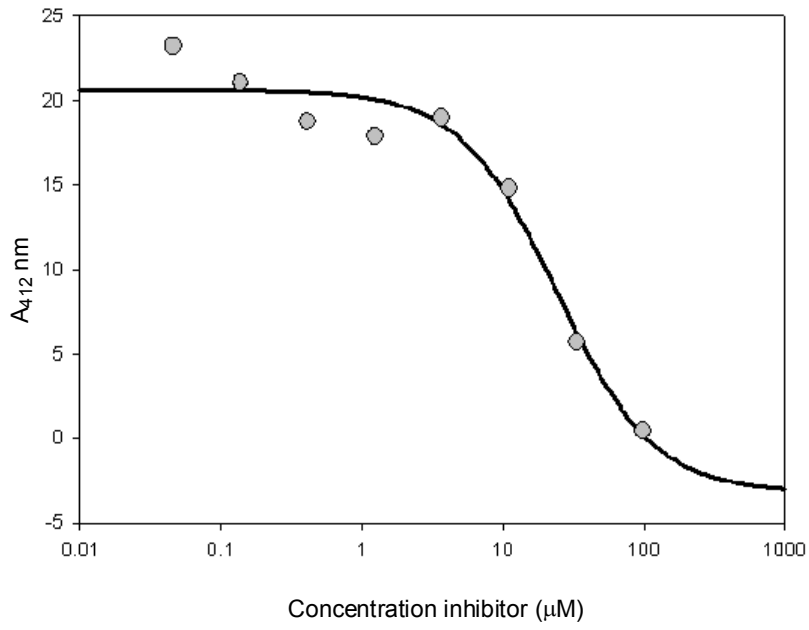
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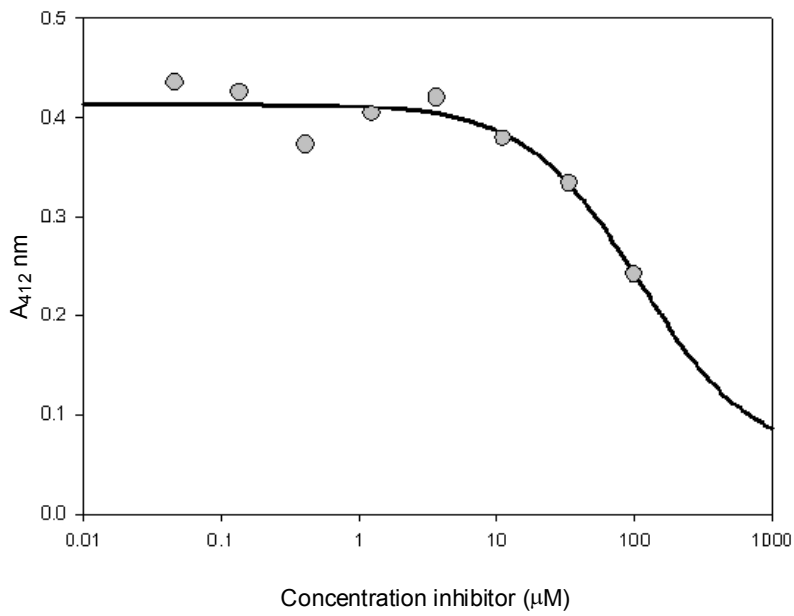
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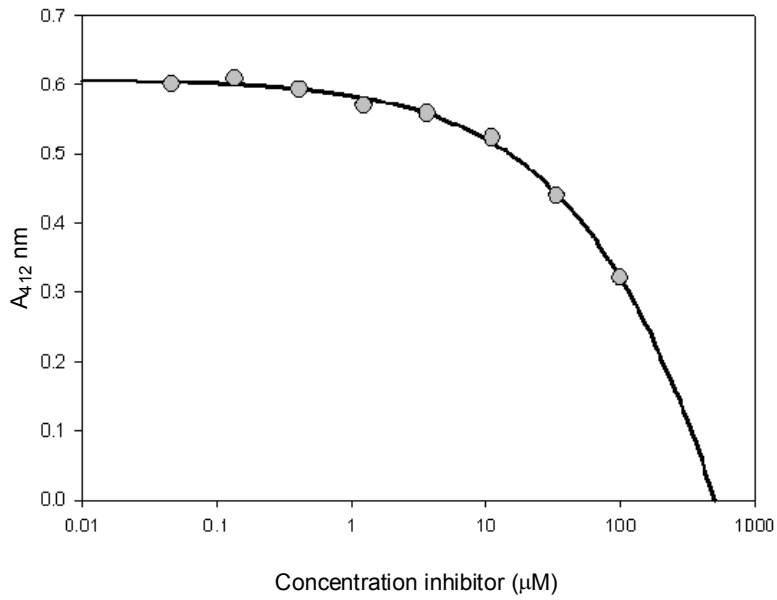
245



231



62



229 inhibition at 1  $\mu\text{M}$  typanothione  
- determination on 14-11-2008

