Synthesis and Biolgical Screening of Potential *Plasmodium Falciparum* DXR Inhibitors

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ABSTRACT

The non-mevalonate isoprenoid pathway, also known as the 1-deoxy-D-xylulose-5-phosphate DXP pathway, is absent in humans, but present in the anopheles mosquito responsible for the transmission of malaria. DXP reductoisomerase – a key enzyme in the DXP pathway in *Plasmodium falciparum* (*Pf*DXR) has been identified as a target for the design of novel anti-malarial drugs. Fosmidomycin and its acetyl analogue (FR900098) are known to be inhibitors of *Pf*DXR and, in this study, synthetic variations of the fosmidomycin scaffold have led to four series of novel analogues. Particular attention has been centred on the introduction of various substituted benzyl groups in each of these series in order to occupy a recently discovered vacant pocket in the *Pf*DXR active-site and thus enhance ligand-enzyme binding. In the process 160 ligands and precursors have been prepared, no less than 119 of them novel.

Fistly, a series of *C*-benzylated phosphonate esters and phosphonic acids were synthesised, in which the fosmidomycin hydroxamate Mg^{2+}– coordinating moiety was replaced by an amide funtionality and the number of methylene groups in the "hydrophobic patch" between the phosphonate and the hydroxamate moiety was decreased from two to one. Several approaches were explored for this series, the most successful involving reaction of 3-substituted anilines with α-bromo propanoic acid in the presence of the coupling agent 1,1'-carbonyldiimidazole (CDI), followed by Michaelis-Arbuzov phosphonation using triethyl phosphite. Reaction of the resulting chiral phosphonate esters with bromotrimethylsilane gave the corresponding phosphonic acids in good yields.

In order to obviate chirality issues, a second series of potential "reverse" fosmidomycin analogues was synthesised by replacing the methylene group adjacent to the the phosphonate moiety with a nitrogen atom. Deprotonation, alkylation and phosphorylation of various amines gave diethyl *N*-benzylphosphoramidate ester intermediate. Aza-Michael addition of these intermediates, followed by hydrolysis gave the corresponding carboxylic acids which could be reacted with different hydroxylamine hydrochloride derivatives to afford the novel hydroxamic acid derivatives in good yields.

Thirdly, a series of a novel *N*-benzylated phosphoramidate derivatives were prepared as aza-FR900098 analogues. Alkylation of different amines using bromoacetalde-hyde diethylacetal

gave a series of *N*-benzyl-2,2-diethoxyethylamine compounds, which were then elaborated *via* a futher six steps to afford novel *N*-benzylated phosphoramidate derivatives.

Finally, in order to ensure *syn*-orientation of the donor atoms in the Mg^{2+} – coordinating group and, at the same time, introduce conformational constraints in the ligand, the hydrophobic patch and the hydroxamate moiety were replaced by cyclic systems. Several approaches towards the synthesis of such conformationally constrained phosphoramidate analogues from maleic anhydride led to the unexpected isolation of an unprecedented acyclic furfuryl compound, and ^{1}H NMR and DFT-level theoretical studies have been initiated to explore the reaction sequence. A series of *N*-benzylated phosphoramidate derivatives containing dihydroxy aromatic rings (as the conformationally constrained groups) to replace the hydroxamate moiety, were successfully obtained in six steps from the starting material, 3,4-dihydroxylbenzaldehyde.

While *in vitro* assays have been conducted on all of the synthesised compounds, and some of the ligands show promising anti-malarial inhibitory activity – most especially the conformationally constrained cyclic *N*-benzylated phosphoramidate series. Interestingly, a number of these compounds has also shown activity against *T.brucei* – the causative agent of sleeping sickness. *In silico* docking studies of selected compounds has revealed the capacity of some of the ligands to bind effectively in the *Pf*DXR active-site with the newly introduced benzyl group occupying the adjacent vacant pocket. The physico-chemical properties of these ligands were also explored in order to predict the oral-bioavailability. Most of the ligands obeyed the Lipinski rule of 5, while QSAR methods have been used in an attempt to correlate structural variations and calculated molecular properties with the bioassay data.

DEDICATION

To my Lovely Parents

Elder and Mrs M.D Adeyemi

and

My Amazing Siblings

(Olufunke, Feyisetan, Adewale, Engr. Gbolahan and Emmanuel)

For nurturing me with so much love, encouragement and affection,
Your continuous dedicated partnership for my success in life can never be under-estimated

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All Thanks to Almighty God; who alone controls time, chance and fortune, for providing me the opportunity at all times to proceed and accomplish successfully."You always cause my lamp to be lighted and to shine; and illumines my darkness. With YOUR help, I can advance against a troop and by my God, I can climb over the walls". I dare not take this kudos/pre-eminence, for only YOU can do this.

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1. INTRODUCTION

1.1. MALARIA

1.1.1. The Malaria parasite

One of the most life-threatening diseases is "*Malaria*". For many years, the cause of malaria was unknown. The release of poisonous gases from marshy areas in which the disease was contracted led to the name '*mal aria*', meaning "bad air". We are now aware that malaria is a parasitic disease, which can be transmitted through a bite by an infected female anopheles mosquito – the insect vector. The malaria parasite is carried by several species of *Plasmodium*, namely; *P.ovale*, *P.malariae*, *P.vivax* and the species responsible for 95% of malaria deaths, *P.falciparum*.[1,2]

The *Plasmodium* life-cycle (Figure 1)[3] involves both the human host and the insect vector. When it bites a human, the infected female anopheles mosquito deposits malarial sporozoites into the human host from its salivary gland.[3,4] The sporozoites migrate into the liver where they undergo asexual replication, with each sporozoite developing into thousands of merozoites.[3,5] The merozites migrate from the liver into the bloodstream and penetrate into the red blood cells to begin asexual reproduction, producing several generations of newly-formed merozites in the red blood cells over the course of more than 48 hours.[3-5] The merozites that have not been replicated undergo sexual differentiation into female and male gametocytes, which may then be ingested by mosquitoes when they bite an infected human. The gametocytes then develop in the mid-gut of the mosquito to form male and female gametes;[3-6] through sexual reproduction these gametes form a zygote which matures into an ookinete. The ookinetes move through the mid-gut epithelium and develop into oocysts under the external membrane. The oocysts rupture and produce thousands of sporozoites, which migrate into the salivary gland where they may be injected and infect humans during subsequent blood-meals ingested by the mosquito.[7]

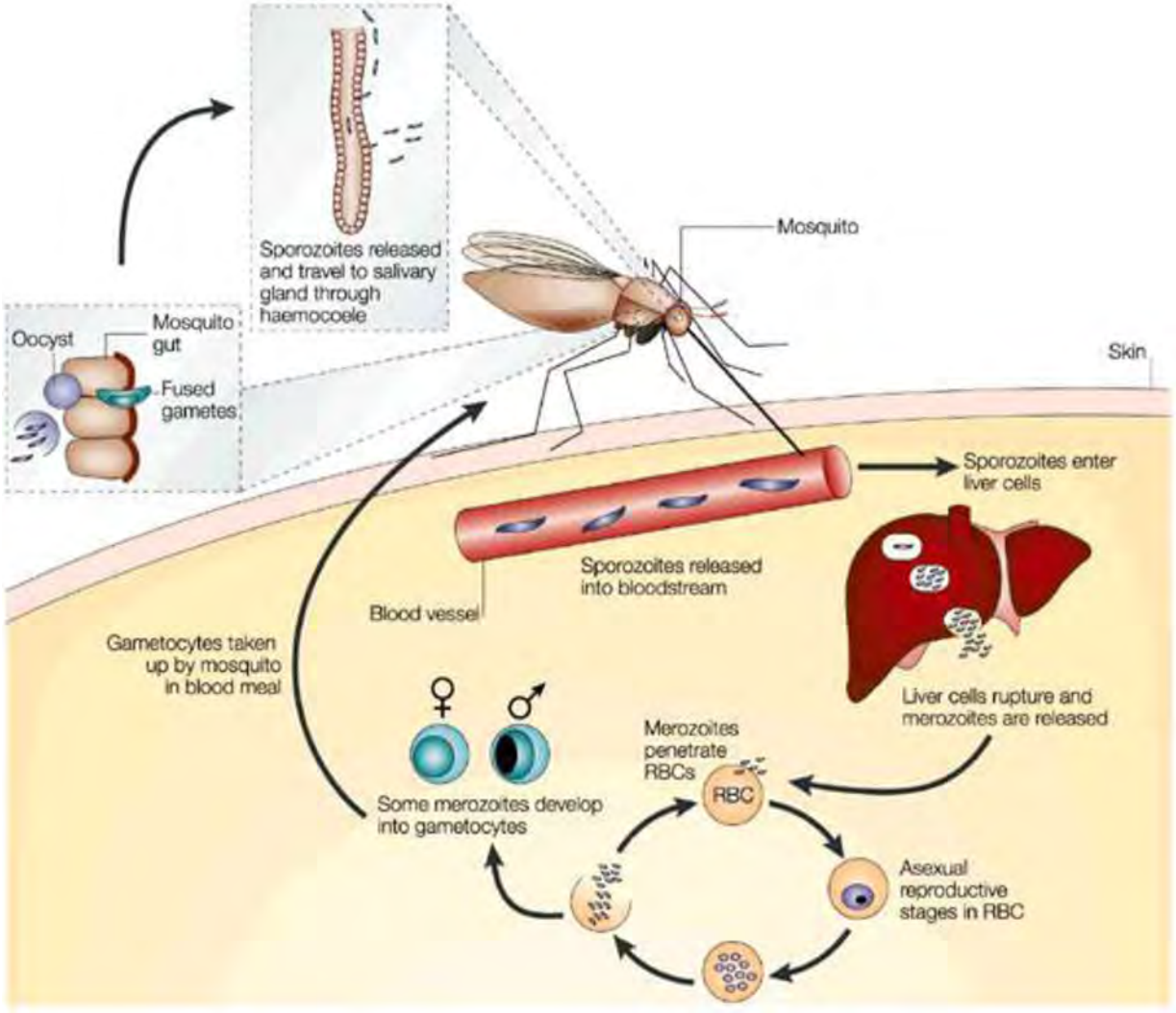


Figure 1. The life-cycle of *Plasmodium* parasite. Reproduced by permission from *Nature Reviews*.[3]

Malaria symptoms, which can vary from mild to severe,[8] occur during the asexual reproduction stage in the red blood cells 9-14 days after an infected mosquito bite. Symptoms include weakness, headache, high fever, sweating, vomiting, chills, diarrhoea, muscular and abdominal pain. When untreated, the disease may become life-threatening.[8] The parasite clogs the blood-carrying capillaries into the brain (cerebral malaria) and the destruction of red blood cells (anaemia) results in damage to other vital organs including the lungs, kidney and spleen-damage which might eventually cause death.[8,9]

1.1.2. Malaria burden

The World Health Organization (WHO) regularly publishes global estimates of malarial mortality rates[10] which show it's prevalence in South-East Asia, the Eastern Mediterranean, the Western Pacific and Africa. Malaria was previously more widespread, but effective mosquito eradication programmes have helped to reduce the number of malaria cases. Europe and North America have the lowest burden of malaria transmission with very few cases and no records of death (Figure 2).[10] Africa has been the most affected region with 90% of reported mortalities,[10] Pregnant women suffering from the disease give birth to low-weight babies,[10] and it is estimated that a child younger than 5 years dies from malaria every minute,[10,11] although it is difficult to obtain reliable statistical data to confirm this. During the past 15 years, however, there has been a huge improvement, with the number of deaths declining from 839,000 in 2000 to 438,000 in 2015, and most of this progress has been achieved in Africa with deaths decreasing from 764,000 to 395,000 (Figure 2).[10]

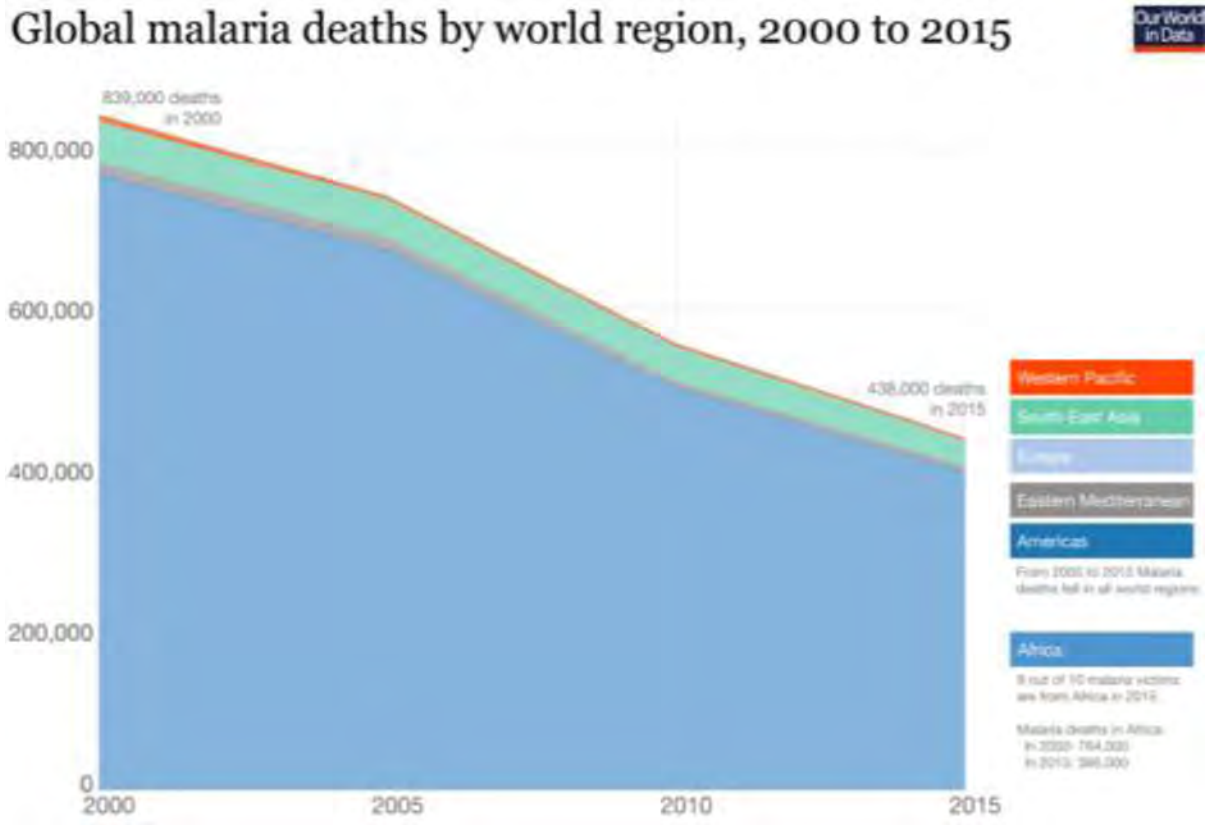


Figure 2. Estimated worldwide deaths (in millions) from malaria during the period 2000 to 2015. Reproduced by permission from WHO.[10]

The statistical data obtained since 2000 thus reveals a significant decrease in malaria transmission, with African countries experiencing a 75% reduction following serious attempts to meet the Millennium Development Goals (MDG) that were adopted in September, 2000.[12] One of these goals is to combat malaria and other diseases, with the main intention being to drastically reduce malaria infection and child mortality; the success of the initiative is clearly evidenced by the data in Figure 2.

1.1.3. Malaria prevention and eradication efforts

The most obvious measure for preventing malaria is to avoid contact with mosquitoes carrying the parasite. Over the years, there have been anti-malarial campaigns conducted by Malaria Control Programmes on encouraging the use of insecticides and mosquito nets, especially during the night.[1]

Immunization through vaccination is an alternative approach to the prevention of malaria, and research in this area on-going.[13] Unlike the other diseases for which effective vaccines have been developed, malaria presents a particular challenge due to the high rate of *Plasmodium falciparum* replication in various targets in the life-cycle of the parasite, thus affecting the development of vaccines.[14] Vaccination trials on selected children have shown 35.9% prevention against infection compared to the control group, but after 7 years immunity fell drastically to 4.4%, precluding explicit conclusions being drawn concerning the long-term efficacy of the malaria vaccine.[13,14]

Numerous drugs have been developed for the treatment of malaria and some of these are considered to have prophylactic properties. Such chemotherapeutic agents will be considered in the next section.

1.2. ANTIMALARIAL DRUGS

Antimalarial drugs can be classified depending on the developmental phases of the parasite being targeted, *viz.*, (i) tissue schizonticides, which inhibit the parasite's growth at the liver stage; (ii) blood schizonticides, which inhibit development at the erythrocytic stage; and (iii) gametocytocides, which prevent the maturation of the gametes formed during sexual reproduction.[19] Another classification involves the efficacy of antimalarial drugs based on different compartments where specific antimalarial drugs act (Figure 3).

Figure 3 shows a red blood cell infected by trophozoites giving an insight into potential drug targets and the corresponding established and new antimalarial drugs.[19]

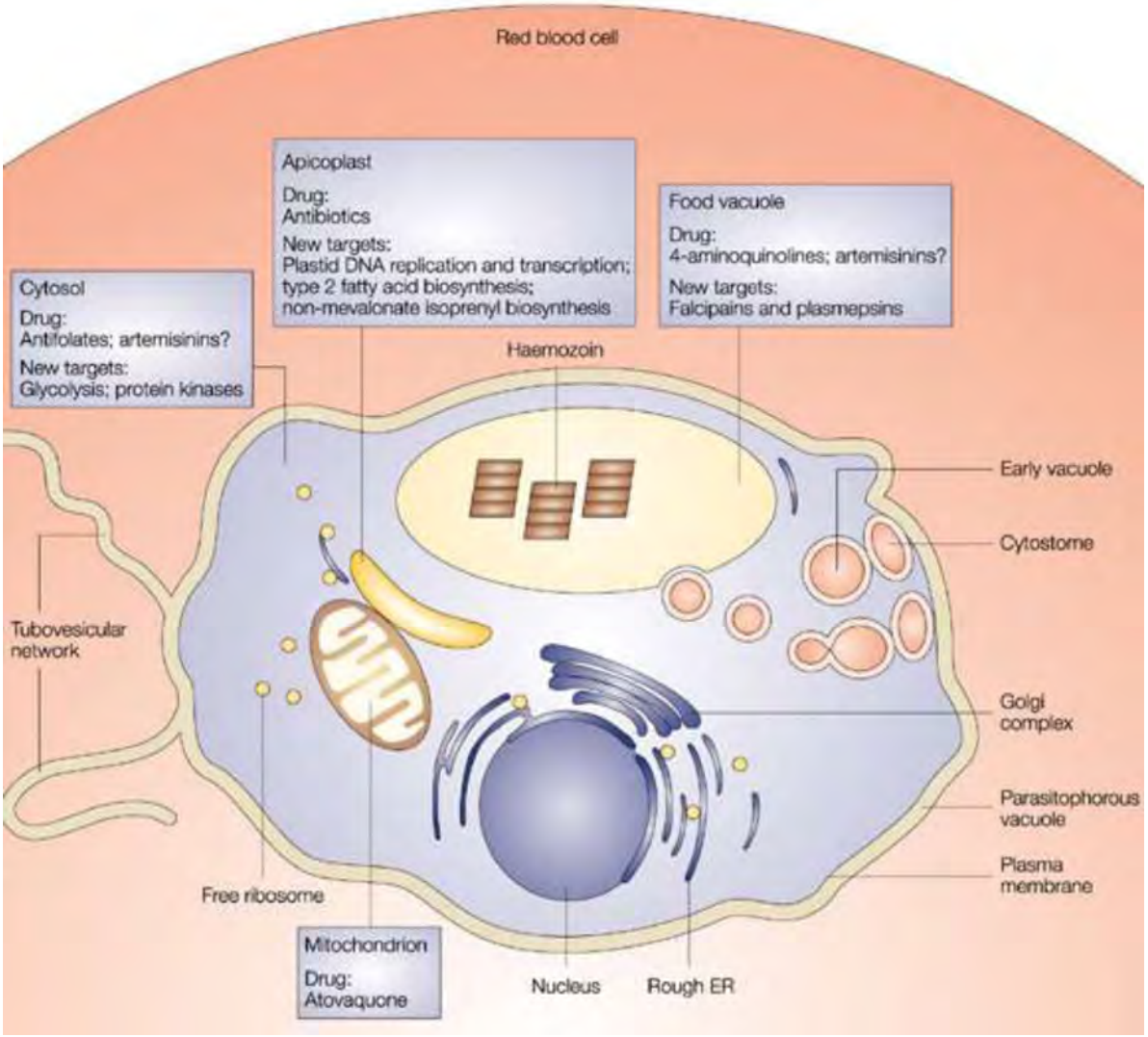


Figure 3. Several biological compartments in a red blood cell and the mode of action of major classes of existing antimalarial drugs. Reproduced by permission.[19]

1.2.1. Quinoline antimalarials

The natural product quinine **1**, which is extracted from the bark of *Cinchona* trees, has been widely used for the treatment of malaria for many years, and the isomer, quinidine, has been used occasionally to cure cerebral malaria.[20] Although the efficacy of these natural compounds have been compromised due to the development of resistance in many parts of the world, quinine proved to be the lead compound for the emergence of the synthetic drugs shown in Figure 4.

Some of these compounds contain the quinoline core, a heterocyclic aromatic organic compound that was extracted from coal tar.[21] Quinoline comprises a pyridine ring fused to a benzene ring, and derivatives are also be referred to as benzopyridines.[22] They have served as the centre-piece in antimalarial chemotherapy for decades. Other drugs, while not containing the quinoline core do contain an arylamino alcohol group.

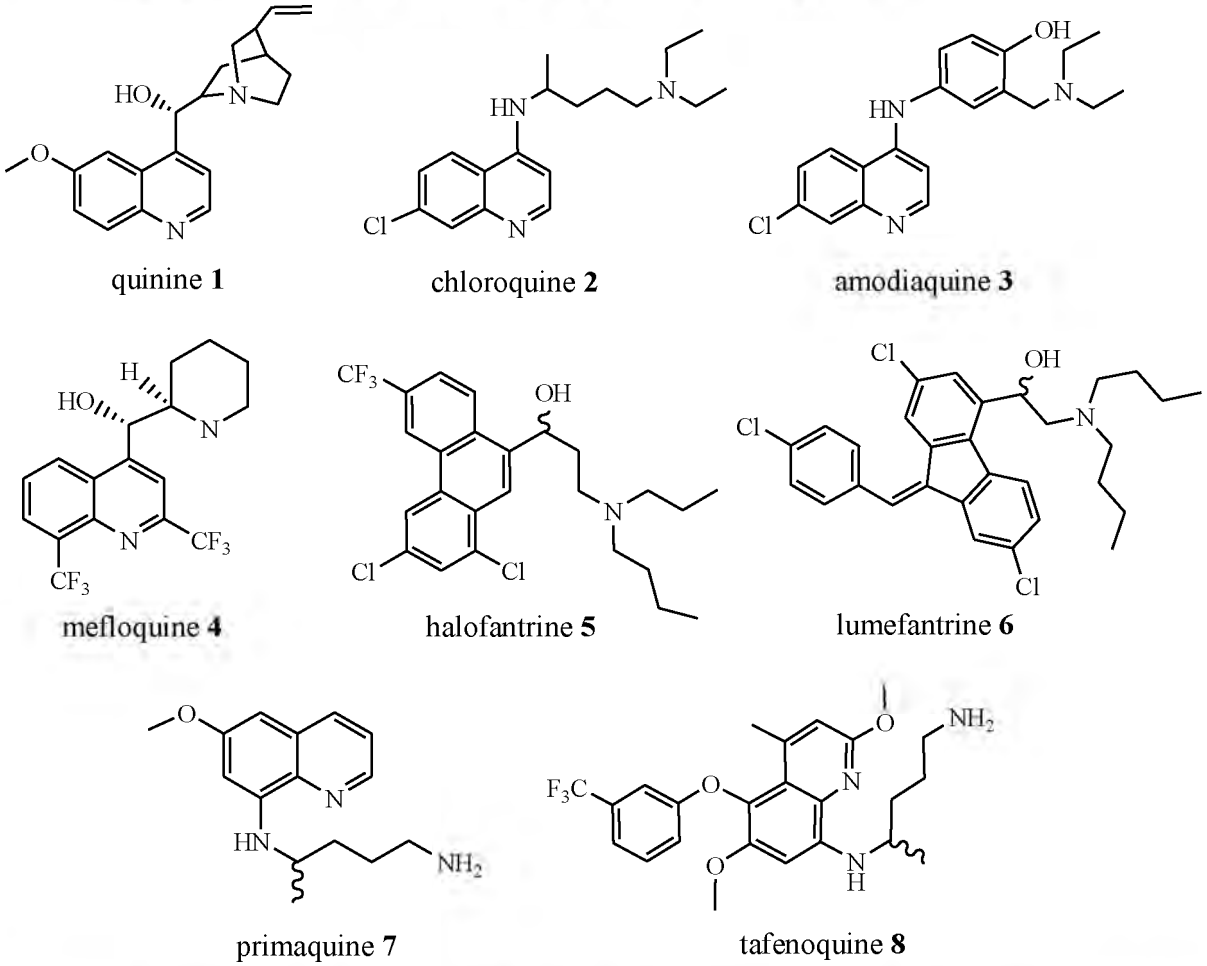


Figure 4. Structures of antimalarial drugs containing quinoline or arylamino alcohol moieties.

Chloroquine **2**, acts rapidly against the blood stages and has been used extensively, due to its affordability, low toxicity and good bioavailability.[20] However, *Plasmodium falciparum* resistance to it developed in the 1960's due to its frequent use.[23] Amodiaquine **3**, which can also be classified as a 4-aminoquinoline, has a 4-hydroxyaniline group and was regarded as suitable for prophylaxis because of its long half-life and lower susceptibility to resistance when compared with chloroquine **2**. However, the clinical use of amodiaquine **3** drastically decreased due to reported cases of hepatotoxicity and agranulocytosis of patients in the mid-1980s.[22-23]

The arylaminoalcohol antimalarial drugs mefloquine **4**, halofantrine **5** and lumefantrine **6** have a different mode of action from that of the 4-aminoquinolines; they prevent the fusion of haemoglobin and the shuttling of vesicles in the food vacuole.[24] Mefloquine **4** has a similar half-life to amodiaquine **3** making it useful as a prophylactic drug. Like chloroquine **2**, mefloquine **4** induces the formation of toxic haeme complexes within the parasite food vacuole.[23-26] Regrettably, the reported side-effects are neuropsychiatric (psychosis) and, therefore, mefloquine **4** cannot be used by pregnant women. Halofantrine **5**, a substituted phenanthrene, is highly effective, and acts against the gametocytes. Its use has been restricted due to frequent reports of cardiac problems.[27] Lumefantrine **6** is structurally similar to halofantrine **5** and, while having a weaker antimalarial activity, it has the advantage of lower cardio-toxic side-effects.[28]

The 8-aminoquinolines, primaquine **7** and tafenoquine **8** also find use as antimalarial drugs. Primaquine **7** is used to inhibit the parasite at the liver stage,[29] and is an "anti-relapse" agent since it interrupts the dormant hyponozoite stage responsible for the relapse of malaria infections. The major disadvantage of primaquine **7** is the short half-life. Tafenoquine **8** is an analogue of primaquine **7** which exhibits improved anti-parasitic activity and a longer half-life, but has the disadvantage of toxicity, *e.g.* gastrointestinal effects in patients.[30]

1.2.1.1. Mechanism of action of 4-aminoquinoline antimalarial drugs

The 4-aminoquinoline antimalarial drugs generally have the same mode of action when they accumulate in the parasite food vacuole during the erythrocytic stage of the *Plasmodium falciparum* life-cycle.[23-26] The breakdown of haemoglobin within the parasite food vacuole provides nutrients which are essential for the growth of the parasite. The unwanted haem is metabolised into ferriprotoporphyrin moieties (toxic haem) which are then converted into non-toxic haemozoin (malaria pigment crystals) which accumulate in the food vacuole (Figure 5).[31] The 4-aminoquinoline antimalarials inhibit the haem polymerization process, thereby permitting accumulation of harmful heam metabolites which destroy the membrane of the parasite. The subsequent accumulation of the toxic haem molecules in the food vacuole leads to the death of the parasite.[23-25]

Resistance against chloroquine **2** is due to mutations in the p*f*crt gene and changes in the transport proteins,[32] which reduce the binding affinity of the antimalarial drugs and the

mutation of the *pf*mdr 1 gene causing gene amplification, thus limiting the accumulation of the toxic haem molecules in the food vacuole.[33]

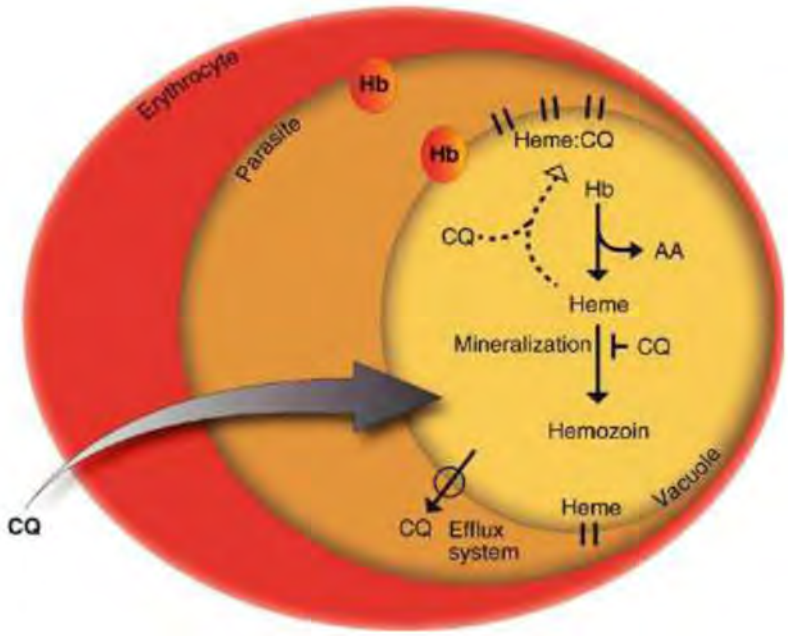
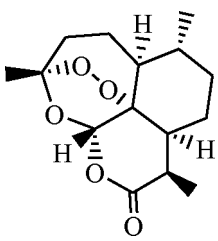
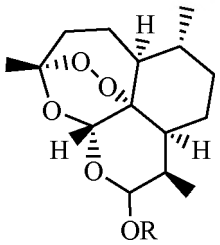


Figure 5. Mode of action and resistance of quinoline antimalarial drugs in the human host. Reproduced with permission.[33]

1.2.2. Artemisinin antimalarials



artemisinin **9**



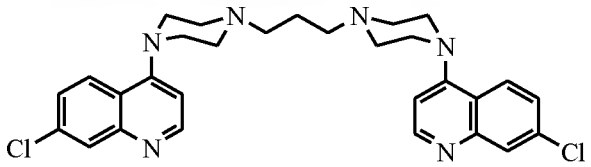
10	R = H	dihydroartemisinin
11	R = Me	artemether
12	R = Et	arteether
13	R = $CO(CH_2)_2COOH$	artesunate

Figure 6. Structures of artemisinin **9** and its derivatives.

Artemisia annua, a Chinese herbal plant which has been used for at least 2000 years, contains the sesquiterpene lactone, artemisinin **9**, that is responsible for its anti-plasmodial activity at the gametocyte stage.[23] Chemical modification of artemisinin to improve solubility and bioavailability has afforded semi-synthetic artemisinin derivatives,[23,34] which

have been used for over 20 years. Dihydroartemisinin **10**, produced by the reduction of artemisinin, is reported to be more potent than artemisinin **9**.[23,34] Replacement of the hydroxyl proton in dihydroartemisinin **10** gives other derivatives as shown in Figure 6. These include the oil-soluble artemether **11** and arteether **12**, which can only be given to patients by intramuscular injection, and the water-soluble artesunate **13**, which is the hemisuccinate ester of dihydroartemisinin **10** and can be administered orally or through intravenous injection to infected patients.[23,27]

Artemisinin and its derivatives are the most active antimalarial drugs available at present but, unfortunately, the development of low efficacy has been observed.[35] Consequently, artemisinin-based combination therapies (ACT's) are now recommended to reduce the risk of resistance and improve potency.[35,36] The ACT formulations available involve combinations of: artesunate **13** with amodiaquine **3**; artemether **12** with lumefantrine **6**; and dihydroartemisinin **10** with piperaquine **14**.[37] The obvious disadvantage of combination therapy is that it is much more expensive than mono-therapy.[36]



piperaquine **14**

1.2.2.1. Mechanism of action of artemisinin antimalarial drugs

The artemisinins' mode of action involves attack against the gametocytes in the *Plasmodium falciparum* life-cycle,[22,23] the mechanism depending on reductive cleavage of the endo-peroxide bridge following contact with haem Fe^{2+} in the food vacuole (Figure 7). The resulting carbon-centred radicals alkylate the haem molecule, thereby inhibiting detoxification of the free β-haematin,[23] and thus resulting in the destruction of the essential parasite membranes (mitochondria, endoplasmic reticulum and plasma membranes). These effects are largely responsible for the antimalarial activity of the artemisinins.[22,23]

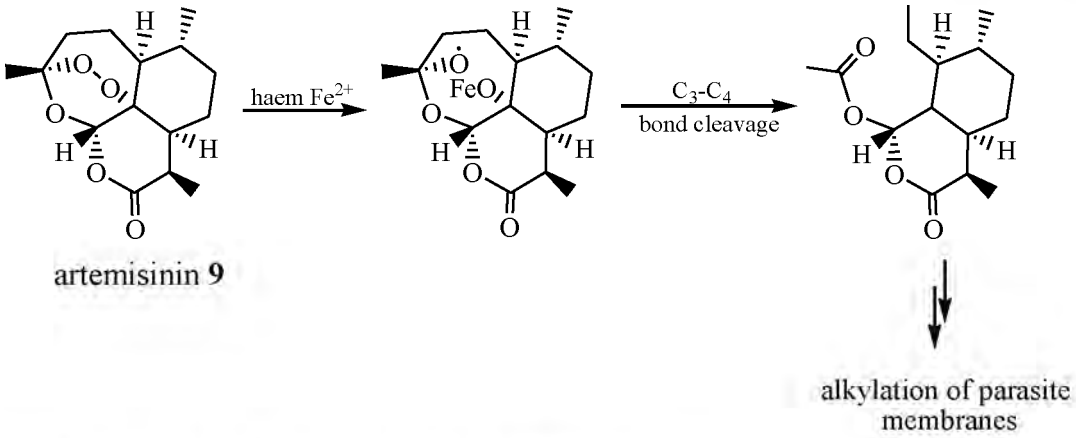


Figure 7. Proposed mechanism of action of artemisinin.

Another proposed mechanism of action involves the activation of artemisinin by Fe^{2+} in the endoplasmic reticulum, which contains an ATP-dependent calcium pump (*Pf*ATP6).[38] This pump is blocked by the binding of activated artemisinin, thus restraining the growth of the parasite. However, the mechanism of action by which *Pf*ATP6 inhibition leads to the death of the parasite remains unclear.[38] There are at present, few clinically reported cases of parasite resistance to artemisinin derivatives, with the feedback of the drugs reduced efficacy, which may be due to mutations in the *Pf*ATP6 gene.[39]

1.2.3. Antifolate antimalarials

The antifolate antimalarial drugs target the pre-erythrocytic and erythrocytic stages of the parasite life-cycle, acting as both prophylactic and therapeutic agents.[23] Several antifolate derivatives have been developed, including proguanil **15**, cycloguanil **16**, pyrimethamine **17** and sulfadoxine **18** (Figure 8).[40-43] These drugs act against malaria by interfering with biosynthetic pathways that are essential to parasite survival. Proguanil **15** undergoes oxidative ring closure with cytochrome P_{450} to yield the cyclic triazine cycloguanil **16** which is an active metabolite. Pyrimethamine **17** and sulfadoxine **18** act on enzymes in the same biosynthetic pathway, thus giving a synergistic effect, and are used in a combination therapy which is known as SP.[42]

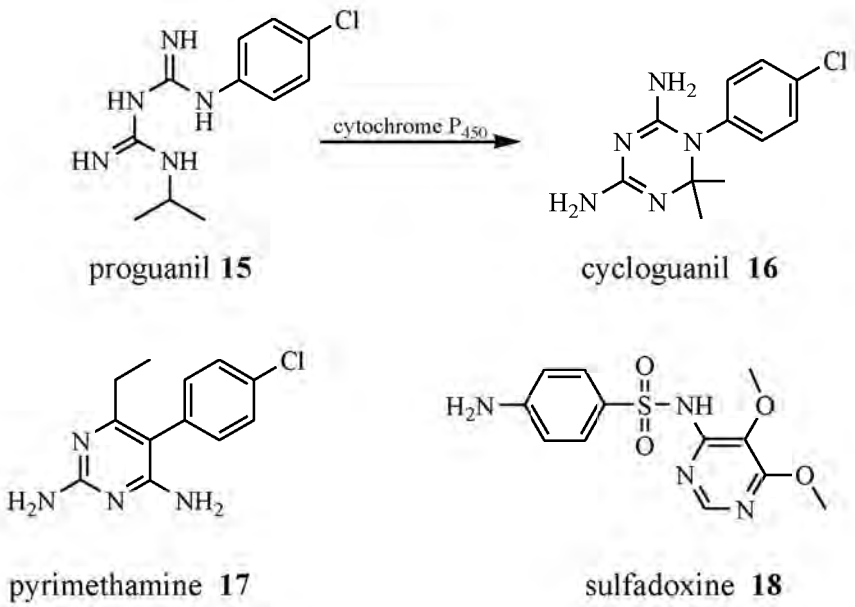


Figure 8. Structures of antifolate drugs.

1.2.3.1. Mechanism of action of antifolate antimalarial drugs

While tetrahydrofolic acid is a precursor in the human biosynthesis of thymidine, purine nucleotides and several amino acids including histidine, serine, methionine and glycine, the *Plasmodium* parasite also uses dihydrofolic acid as a precursor in biochemical transformations, but these are catalysed by dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) – enzymes which are absent in humans. Proguanil **15** and pyrimethamine **17** are competitive inhibitors of DHFR,[23,40] which catalyses the reduction of 7,8-dihydrofolate to tetrahydrofolate, while sulfadoxine **18** competitively inhibits DHPS which catalyses the conversion of 2-amino-4-hydroxy-6-hydroxymethyl-7-8-dihydropteridine into 7,8-dihydropteroate as shown in Figure 9.[41] Unfortunately, the antifolates have proven susceptible to resistance by the malaria parasite[24,25] due to genetic polymorphism caused by the mutations of the two essential enzymes in the folate biosynthesis pathway (DHPS and DHFR).

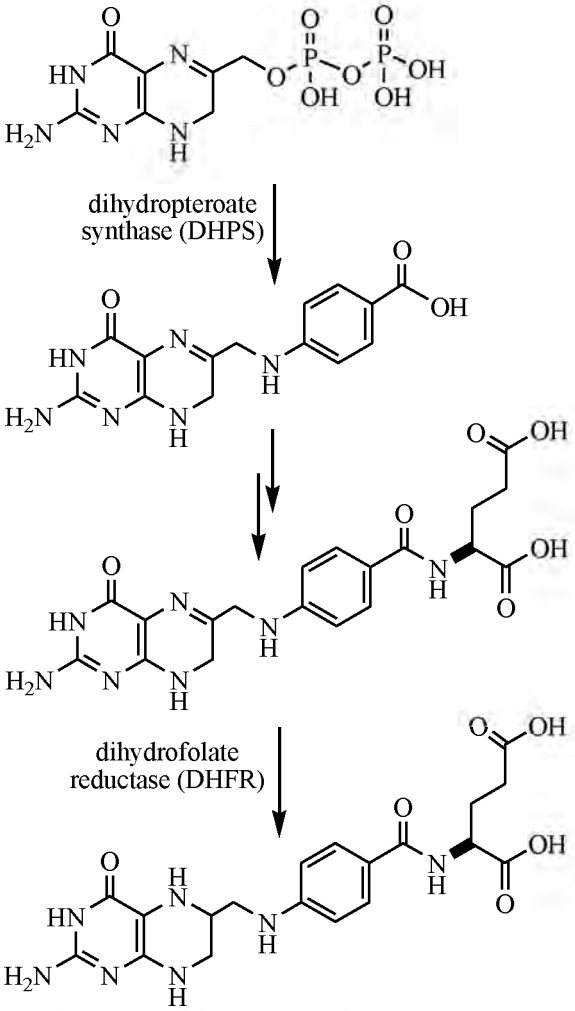
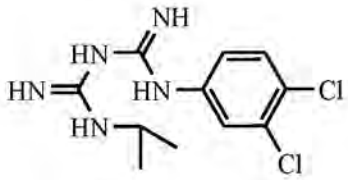
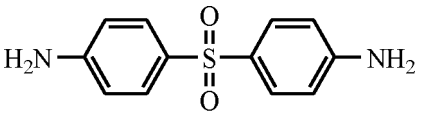


Figure 9. Enzyme targets of the antifolate antimalarial drugs proguanil **15**, cycloguanil **16**, pyrimethamine **17** and sulfadoxine **18**.[41]

Due to the reported resistance against antifolates, a combination of sulfadoxine **18** and pyrimethamine **17** (SP) has been used, but resistance has also developed against this combination.[42] However, a new combination of chlorproguanil-dapsone, known as Lap-Dap®, was recently introduced, replacing SP (Figure 10).[43] Cytochrome P_{450} catalyses the conversion of chlorproguanil **19** into chlorcycloguanil which inhibits the DHFR enzyme, while dapsone **20** acts similarly to sulfadoxine **18** as an inhibitor of DHPS. This combination has shown good potency across Africa, but not in South Asia due to mutation of the DHFR gene in that part of the world.[44]



chlorproguanil **19**

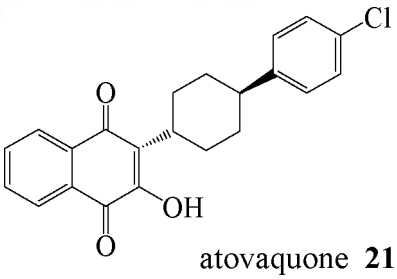


dapsone **20**

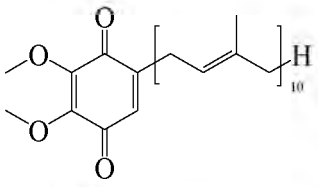
Figure 10. Compounds used in the Lap-Dap® combination.

1.2.4. Hydroxyquinone antimalarials

Atovaquone **21** {2-[*trans*-4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthaquinone} and ubiquinone **22** (Figure 11) are hydroxyquinone derivatives which can be used for uncomplicated malaria and chemo-prophylaxis.[27] Following reported cases of resistance with the use of these drugs, higher antimalarial efficacy has been achieved using a combination of atovaquone **21** with proguanil **15**.[18,27]



atovaquone **21**



ubiquinone **22**

Figure 11. Structures of hydroxyquinone derivatives.

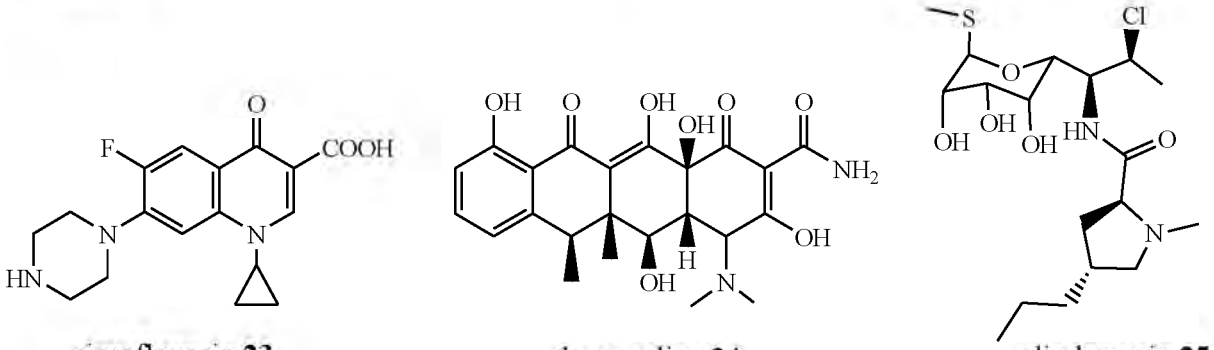
1.2.4.1. Mechanism of action of hydroxyquinone derivatives

The mode of action of hydroxyquinones involves the interruption of mitochondrial electron transport due to the collapse of the parasite membrane by binding of the drugs to the cytochrome *bc*1 complex. [27] Resistance of the *Plasmodium* parasites towards atovaquone **21** is attributed to point mutations of the cytochrome *bc*1 gene, thus affecting the drug-binding affinity.[23,45]

1.2.5. Antibiotic antimalarials

Several antibiotics, such as ciprofloxacin **23**, tetracyclines, azithromycin and spectinomycin (Figure 12), exhibit antimalarial activity by inhibiting biosynthesis and DNA replication.[23,45-46] While the most frequently used antibiotic for antimalarial prophylaxis is doxycycline **24**,

antibiotics can also be used for multi-resistant malaria treatment in combination with chloroquine **2** or artesunate **13**.[47] However, doxycycline **24** cannot be used by children and pregnant women, due to side-effects during bone formation.[48] A valuable alternative to doxycycline **24** is clindamycin **25**,[18,23] which inhibits the prokaryote-like protein synthesis of the apicoplast by binding to ribosome sub-units, thus preventing replication of the organelle.[49]



ciprofloxacin **23** doxycycline **24** clindamycin **25**

Figure 12. Structures of the most frequently used antibiotics.

1.3. THE DISCOVERY of NEW ANTIMALARIAL DRUGS

Antimalarial drug resistance has become a major problem in tackling malaria, besides the resistance to existing drugs, resistance to various combination therapies of existing antimalarial drugs, have also become apparent. The chart in Figure 13 summarizes the date of introduction of several existing antimalarial drugs, combined therapies and the development of resistance to them between 1940 and 2005. Resistance to some of the combination therapies developed almost immediately after their introduction, *e.g.*, SP (sulfadoxine **18** and pyrimethamine **17**) and Lap-Dap® (chlorproguanil **19** and dapsone **20**), known as, while artemisinin-combinations have thus far remained efficient for the treatment of uncomplicated malaria as recommended by WHO.[50]

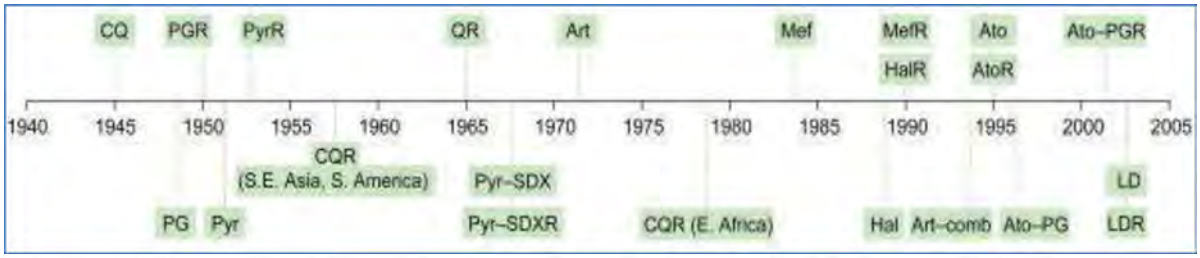


Figure 13. Summary of dates of the introduction of antimalarial drugs and the development of resistance.[50]

The development of drug resistance is due to several factors:- (i) adaptability of the parasite at genomic and metabolic levels; (ii) over-use of a particular antimalarial drug; (iii) ineffective therapeutic treatment of infections; and (iv) the rapid proliferation of the parasite and concomitant mutation (monogenic resistance) decreasing the efficacy of the antimalarial drug.[15] An additional, interesting factor is endemicity, which is the immunity of various individuals based in a particular location.

The problem of resistance which the existing antimalarial drugs face, has prompted the synthesis of novel antimalarial drugs that can act as chemo-therapeutic and prophylactic agents. There are several approaches to the developmemt of these synthetic compounds.[17] *viz*,: (i) modification of existing drugs while retaining the essential pharmacophores so as to maintain or improve their antimalarial activity; (ii) discovering new natural products with antimalarial activity; (iii) the identification of new biological targets and the design of appropriate inhibitors.

1.3.1. Synthesis of new 4-aminoquinoline derivatives

In developing new quinoline-based antimalarial drugs with greater potency and pharmacological activity, a number of structural features have been explored. These include:- (i) the 4-aminoquinoline nucleus **26** (Figure 14) which is required for hematin binding and the consequent anti-plasmodial activity;[23-24] (ii) the length of the carbon chain between the 4-amino nitrogen and a further amino group is important for the activity against resistant parasite strains[24,51] *e.g.*, shortening the carbon length from three to two in AQ13 **27** and dimerization as in piperaquine **14** have been reported to overcome chloroquine resistance;[52,53] (iii) incorporation of an aromatic group in the chain as in naphthoquine **28** and isoquine **29** to improve activity, thereby overcoming resistance;[24,52-54] (iv) incorporation of a ferrocene moiety as in ferroquine **30** and compound **31**;[55-57] and (v) the conjugation of two active molecules *via* an ester linkage as in compound **32**, which cleaves into two active compounds when used as an antimalarial drug.[58]

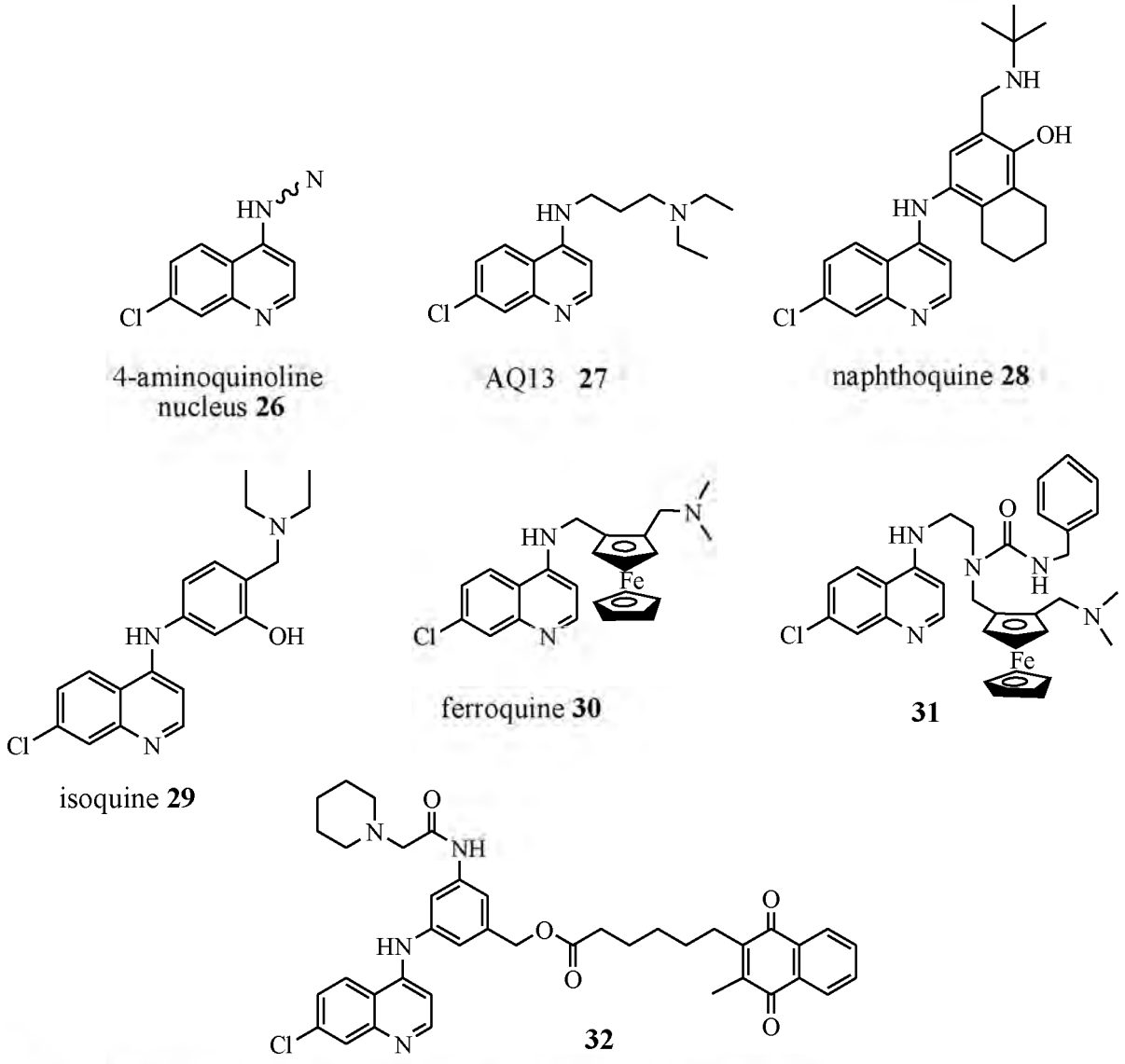


Figure 14. Structures of new synthetic 4-aminoquinoline derivatives.

1.3.2. New synthetic artemisinin derivatives

The major draw-back of the existing artemisinin drugs is their susceptibility to hydrolysis, which is responsible for their short half-lives. New synthetic drugs with improved bioavailability have been developed by focusing on variations of the 'R' substituent (Figure 15) to give compounds **33**, **34**, **35** and **36** using alkyl, aryl and heteroaryl groups, respectively.

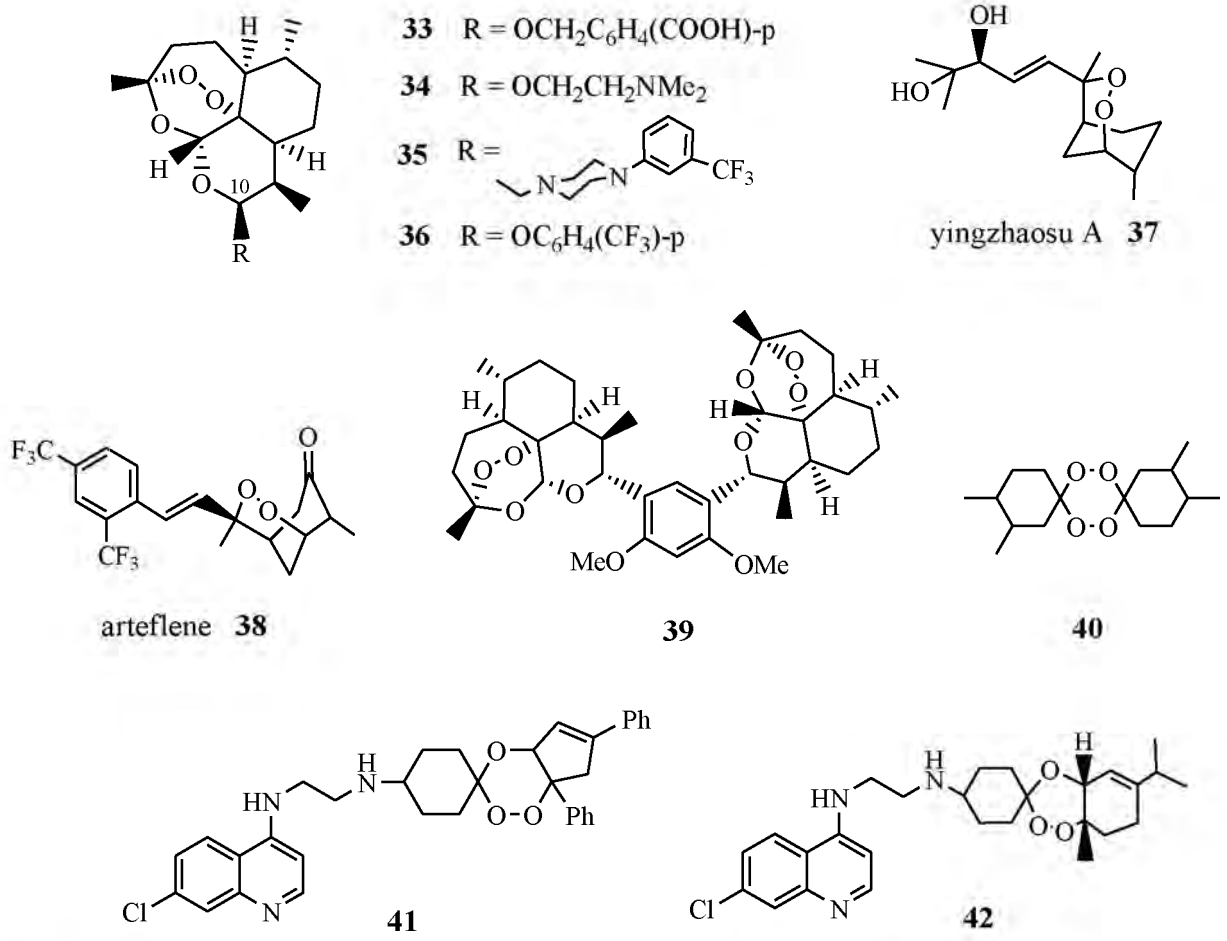


Figure 15. Structures of new synthetic artemisinin analogues.

Compounds **33** and **34** are both water soluble thus facilitating administration,[59,60] while the weakly basic piperazine ring in compound **35** has led to the enrichment of the drug in the food vacuole.[61] Modification of the C-10 substituent has led to the fluorinated artemisinin analogue **36**, which has proved to be more efficient than artesunate **13** on a malaria-infected mouse.[62]

Since the mode of action of the artemisinins depends on the generation of free radicals, other endoperoxide-generating compounds, not related to the artemisinin structure, have been considered, and both yingzhaosuA **37** and arteflene **38** exhibit antiplasmodial activity and have been used to treat uncomplicated malaria in some parts of Africa.[63,64] The "dimeric" compounds, *e.g.*, the trioxane dimers **39** and **40** containing 1,2,4,5-tetraoxane moieties have good activity when compared to artemisinin **9** but are not as effective as arteether **12**.[65-67] The design of the hybrid compounds **41** and **42** combines the 4-aminoquinoline and peroxide

moieties,[68] and are referred to as "dual inhibitors" with the expectation that they act against more than one target, thus improving the activity against resistant strains.

1.3.3. Identification of new antimalarial targets

Natural compounds, obtained from plants, marine organisms and bacteria, have been a major source of drugs for different diseases and infections. However, due to extraction and purification challenges, synthetic methods have often been developed to access these compounds. The pharmacophores present in natural products have also provided a basis for developing new drugs. Such drugs need to be efficacious, affordable and exhibit low toxicity.[40,69]

Biochemical advances have permitted the identification of organelles, pathways and particular enzymes as novel targets for therapeutic intervention. Table 1 summarises specific targets for the development of new antimalarial agents and includes: (i) the mitochondria due to the presence of cytochrome *C*-oxidoreductase (complex III), which is involved in electron transport;[70,71] (ii) the food vacuole, which accumulates toxic haem; and (iii) the apicoplast which accommodates isoprenoid precursor synthesis,[72] generally involving 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR).[70,73] The enzyme DXR, which catalyses a non-mevalonate pathway absent in humans and which is, therefore a good target for inhibition without causing harm to the human host,[74,76]is the focus of this research.

An important strategy in drug design is the use of computational methods to understand the details of the active-sites of various biological targets thus facilitating the design of suitable synthetic ligands. Computer modelling techniques, docking studies, quantitative structure activity relationships (QSAR) and quantitative structure property relationships (QSPR), which correlate structural changes with bioassay data are crucial for the rational design and development of new drugs.

Table 1. Summary of different new biological targets and the respective antimalarial drugs used as inhibitors.

Enzyme and Functions	Location	Antimalarial drug (Inhibitor)
DNA gyrase - DNA synthesis	Apicoplast	Quinolones (ciprofloxacin)
FAS I, FAS II - fatty acid biosynthesis		Thiolactomycin, triclosan
1-deoxy-D-xylulose-5-phosphate reductoisomerase DXR	Apicoplast (*Pf*DXR)	Fosmidomycin
Choline transporter-acetylcholine synthesis	Cell membrane	G-25
Cytochrome *C* oxidoreductase	Mitochondria	Hydroxynaphthoquinone
Plasmepsins	Parasite proteases	Leupeptin, pepstatin
Thymidylate synthase - DNA synthesis	H35 hepatoma cells	Fluorouracil
5-enolpyruvylshikimate-3-phosphate synthase (EPSP) – aminoacids biosynthesis	Shikimate pathway	Glyphosate (*N* (phosphono-methyl)glycine)
Thioredoxin reductase – thioredoxin reduction	Redox system	5,8-Dihydroxy-1,4-naphthoquinone
Farnesyl transferase	Plasma membrane	Biphenyl derivative FTI-2135

1.4. ISOPRENOID BIOSYNTHESIS

Isoprenoids are the widest range of natural biologically active products found in all living organisms. They comprise primary and secondary metabolites which perform diverse biological roles as pigments, metabolites, vitamins, plant hormones, carotenoids, sterols and chlorophyll.[72,77] All of these biological products perform vital functions such as: photoprotection of plants; electron transport carriers; regulation of growth and development; modulation of eukaryote and eubacteria membranes; and plant pollination.

In spite of their variation in structure, the biosynthesis of isoprenoid precursors always involves the phosphorylation of five-carbon blocks of isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) as described by Ruzicka's isoprene rule.[78,79]

In the 1980's, it was discovered that all animals, including humans, synthesise their isoprenoid precursors through the mevalonate pathway shown in Figure 16a.[80-82] This pathway starts with the condensation of two molecules of acetyl-CoA **43** to form acetoacetyl-CoA **44**, which condenses with another acetyl-CoA leading to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) **45**; the conversion of **44** to **45** is catalysed by HMG-CoA synthase (HMGS).[80-84] In the next step, HMG-CoA is reduced irreversibly to mevalonic acid (MVA) **46** by HMG-CoA reductase (HMGR),[80] and MVA then undergoes two successive phosphorylation steps, yielding mevalonic-5-monophosphate (MVA-P) **47** and mevalonic-5-diphosphate (MVA-PP) **48**, which is finally converted into IPP **49** by an ATP-dependent decarboxylation. Isomerisation of IPP to DMAPP **50** is catalysed by IPP isomerase.[80-84]

1.4.1. The 1-deoxy-D-xylulose-5-phosphate (DXP) pathway or non-mevalonate pathway

In 1993, Arigoni and Rohmer reported that the isoprenoid precursors (IPP and DMAPP) can be formed through another metabolic route, called the non-mevalonate or the 1-deoxy-D-xylulose-5-phosphate (DXP) pathway [or 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway] comprising seven enzymatic steps (Figure 16b).[80,85-87] Firstly, pyruvate **51** is condensed with glyceraldehyde-3-phosphate **52** in a transketolase-type reaction catalysed by 1-deoxy-D-xylulose-5-phosphate synthase (DXS) to produce 1-deoxy-D-xylulose-5-phosphate DOXP **53**.[80,87] DOXP is then converted into MEP **54** in a reaction mediated by DOXP-reductoisomerase (DXR) in a single step involving an intramolecular rearrangement and NADPH-mediated reduction in the presence of a co-factor and a divalent cation (Mn^{2+}, Mg^{2+} or Co^{2+}).[88] MEP **54** is transformed into 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDP-ME) **55** by the cytidine triphosphate (CTP)-dependent action of CDP-ME synthase. CDP-ME is phosphorylated at the *C*-hydroxyl group by CDP-ME kinase,[80,89] which is ATP dependent, forming 4-diphosphocytidyl-2-*C*-methyl-D-erythritol-2-phosphate (CDP-MECP) **56** and adenosine diphosphate (ADP).[80,90,91]

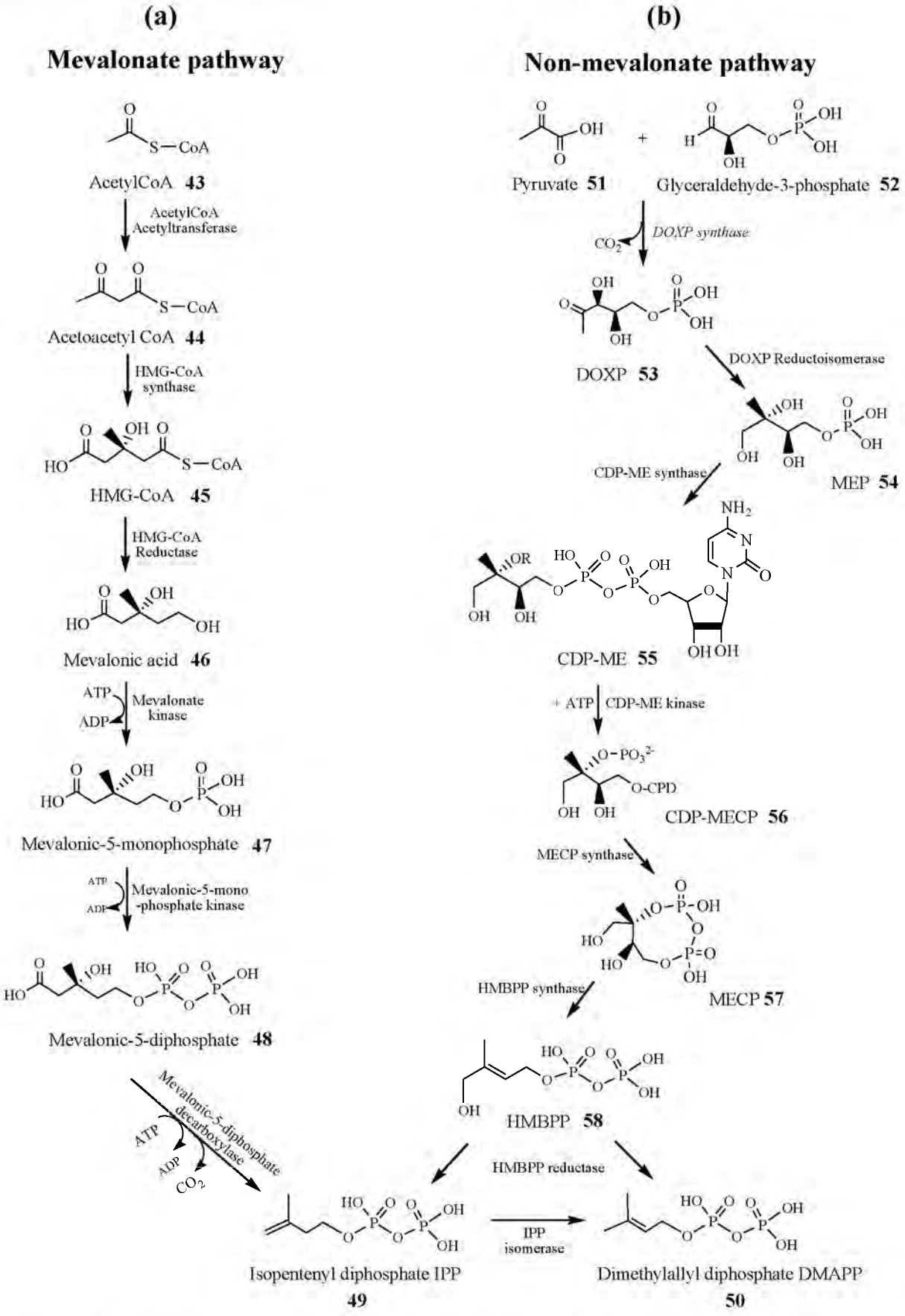


Figure 16. The two isoprenoid pathways (**a** and **b**) showing the enzymes involved.

Using CTP as a co-factor, CDP-MECP **56** is converted into 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate (MECP) **57** and cytidine monophosphate (CMP) in a reaction catalysed by MECP synthase. 1-Hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate synthase (HMBPP synthase) then converts MECP **57** into 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate HMBPP **58**.[80,92,93] Finally, the conversion of HMBPP **58** into IPP **49** and DMAPP **50** is catalysed by HMBPP reductase catalyses.[94,95]

The non-mevalonate pathway can only be found in plastids, the cytoplasm of higher plants and in bacteria, but is absent in humans. The *Plasmodium falciparum* enzymes in this pathway have been identified as antimalarial targets for the rational design of novel antimalarial drugs.[80,84]

1.4.2. 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (DXR)

1.4.2.1. Mechanistic studies of DXR

The mechanism of the conversion of DOXP **53** to MEP **54** in the non-mevalonate pathway is catalysed by DXR and a divalent metal ion (Co^{2+}, Mn^{2+} or Mg^{2+}) and has been carefully studied.[96,97] This reaction involves two steps (Figure 17); firstly, DOXP **53** is isomerised into 2-*C*-methyl-D-erythrose-4-phosphate, which is subsequently converted into MEP **54**. This step involves cleaving the DOXP C3-C4 bond and the formation of a C-2 – C-4 bond to produce the 2-*C*-methyl-D-erythrose-4-phosphate intermediate, which is later reduced to MEP **54** by NADPH.[96,97] Three different mechanisms have been proposed by Rohmer *et al.*, for this intramolecular arrangement, *viz.*, (i) a 1,2-hydride shift involving hydride migration from C-3 to C-2 of DOXP **53**, leading to the intermediate 2-*C*-methyl-D-erythrose-4-phosphate **59** from which NADPH reduction affords MEP **54**; (ii) an α-ketol rearrangement, involving deprotonation of the C-3 hydroxyl group of DOXP **53** to form a ketone, followed by migration of the phosphate-bearing C-2 sub-unit to afford the common intermediate 2-*C*-methyl-D-erythrose-4-phosphate **59**; and (iii) a retro-aldol mechanism involving the deprotonation of the C-4-hydroxyl group in DOXP **53**[95] and cleavage of the C-3 – C-4 bond to give glycoaldehyde phosphate **60** and the hydroxyacetone enol(ate) **61**. Combination of these two products *via* a new bond between C-2 and C-4 gives 2-*C*-methyl-D-erythrose-4-phosphate **59** as the common aldehyde intermediate.[82,96,97]

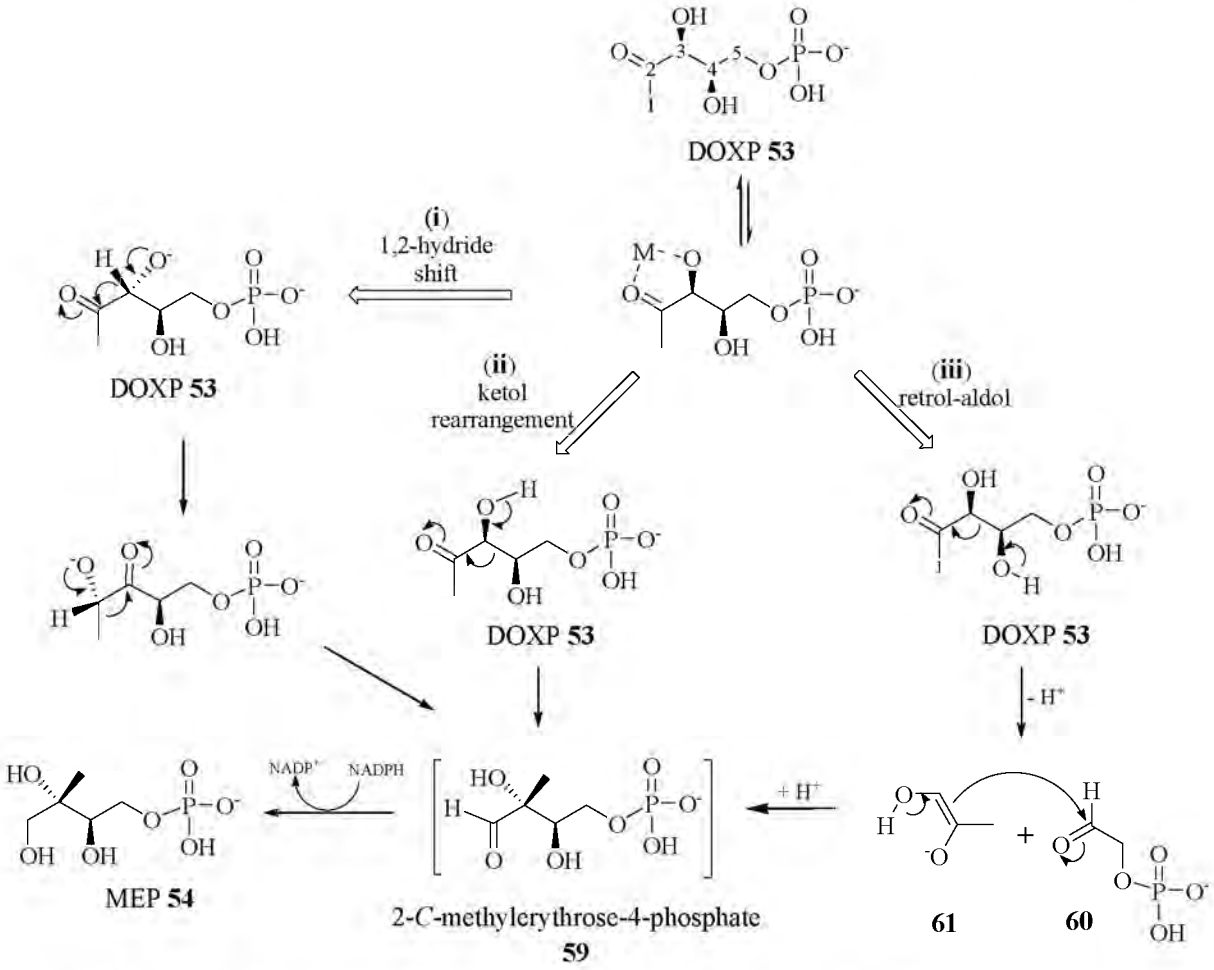


Figure 17. Mechanisms for the conversion of DOXP **53** into MEP **54**.

The final reduction step, was studied by Proteau and Arigoni *et al.*,[98,99] and involves an NADPH-mediated DXR-catalysed reaction, which is illustrated in (Figure 18).[98,100]

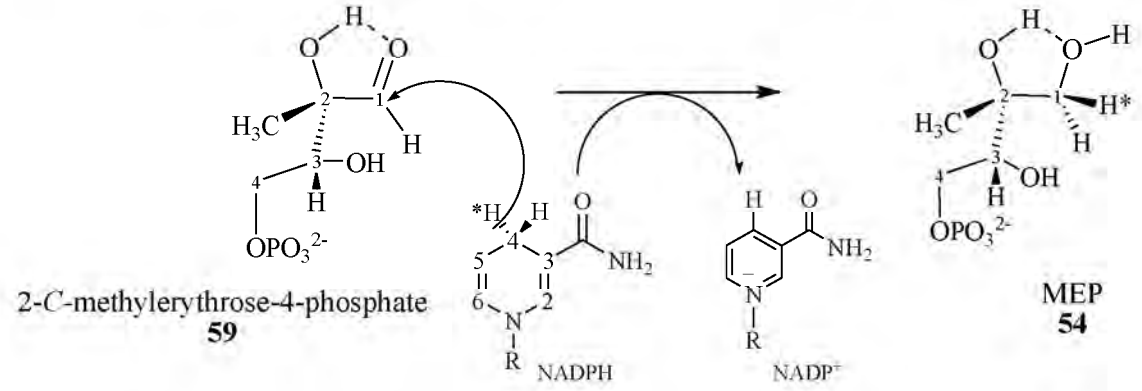


Figure **18**. Stereochemical effects in the DXR-catalysed conversion of intermediate **59** to MEP **54**; H* is the pro-*S*-hydrogen of NADPH involved in the rearrangement.[98,100]

1.4.2.2. Structure of DXR

Several DXR structures for various species have been determined crystallographically, including *E.coli* (*Ec*DXR),[101-104] *Mycobacterium tuberculosis* (*Mt*DXR),[105,106] and *Plasmodium falciparum* (*Pf*DXR),[80] permitting detailed investigation of the structure and functions of the enzymes.[101,105-109] *Ec*DXR was the first DXR enzyme structure to be determined, and was shown to comprise a 86 kDa homodimer unit and a 43 kDa monomer unit. The structure reveals a binary complex with sulphate ion and NADPH at the active-site, but provided no useful information about the interaction between the enzyme and the ligand.[101-104]

The *Ec*DXR homodimer has a V-shaped structure and is composed of 3 main domains, namely, (i) the amino-terminal (or NADPH-binding) domain containing amino acids 1-150 and a β sheet with 7 parallel strands (β1-β7) connected by α1-α6 helices, which are crucial for NADPH co-factor binding; (ii) the connective domain which comprises amino acids 150-285 and contains the active-site and the catalytic / flexible loop; and (iii) the carboxyl-terminal (or *C*-terminal domain) responsible for substrate binding which contains a β sheet (β8-β11), which is connected to the NADPH-binding domain by α-helices (α7-α11), giving structural support to the enzyme. The active-site of the enzyme accommodates the divalent metal ion and phosphate moiety of the natural substrate. In addition, there are two possible conformations of the catalytic loop. In the first conformation, also known as the active conformation, the flexible loop acts as a lid folding over the active-site and the connected NADPH binding domain, thus shielding the active-site from the solvent environment. In the second conformation, which is very important for the substrate binding, the flexible loop remains in the active-site of the *Ec*DXR.[101,102,104,110]

Fortunately, the X-ray structures of both *Mt*DXR[105-106] and *Pf*DXR have also been published. The latter, reported by Tomonobu Umeda *et al.*[80] in 2011 (Figure 19), shows great similarities with the structures of *Ec*DXR and *Mt*DXR.[104]

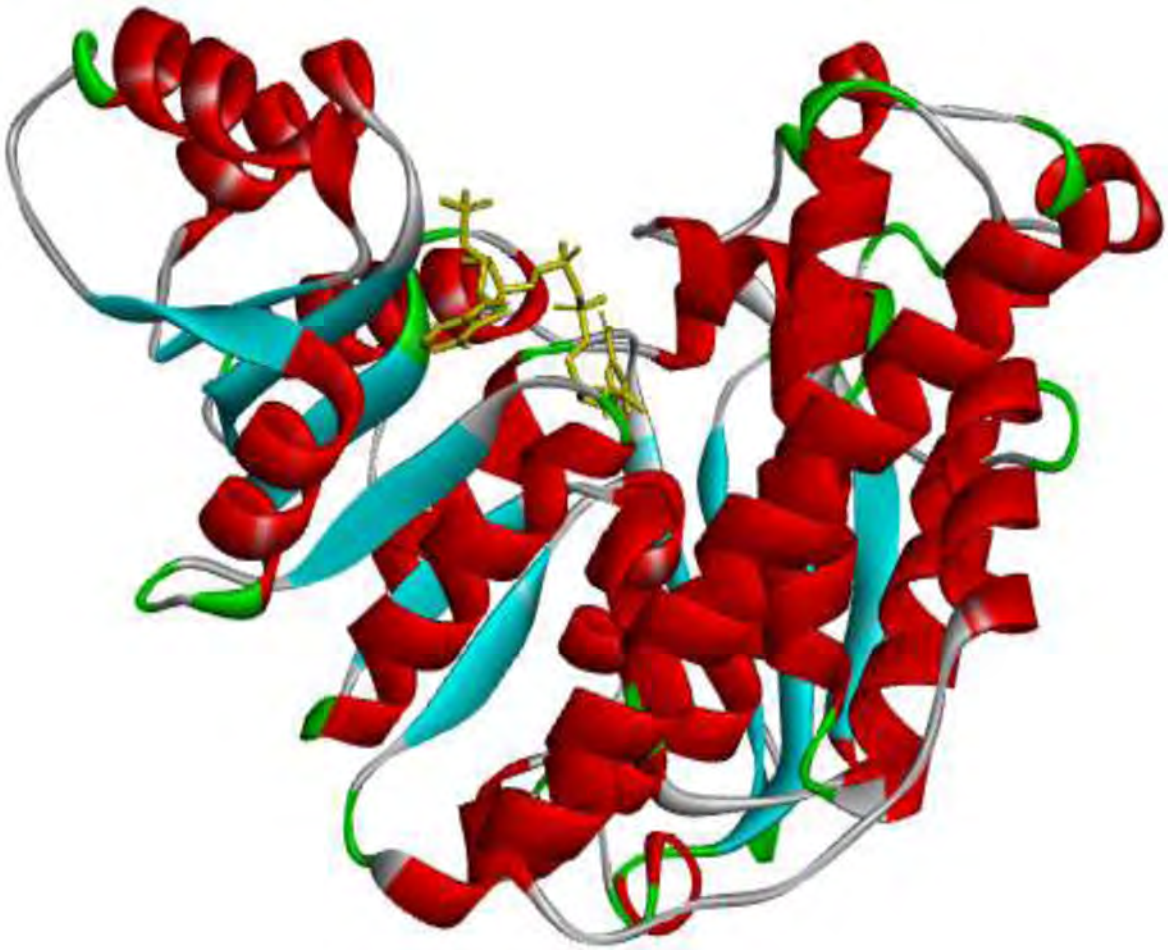


Figure 19. Ribbon representation of the *Pf*DXR crystal structure showing various domains: the *N*-terminal domain (red); the connective domain (cyan); and the catalytic loop (green) and NADPH (yellow). (Generated using Discovery Studio Visualizer 4.0).[111]

1.4.2.3. The DXR active-site: DOXP and fosmidomycin interaction with the proximal amino acid residues.

The structure of *Ec*DXR containing DOXP in the active-site as shown in Figure 20 reveals and interaction (a) between the DOXP carbon backbone and the β-indole ring of Trp212. The negatively charged phosphonate moiety is anchored (b) by Lys228, Ser222, Asn227, Glu152, His209 and Ser186 residues.[101] The C-2 carbonyl oxygen is hydrogen-bonded (c) to Asp150 and Ser151; the C-3 hydroxyl group (d) interacts with Glu231 and Lys125, and the C-4 hydroxyl group (e) is hydrogen-bonded to Glu152, Asn227 and Lys228. Mac Sweeney *et al.*[101] reported the interaction of NADPH with the amino acid residues Gly11, Ser12, Gly14, Ala35, Gly36 and Ala105 of the *N*-terminal domain, which contribute to the stabilization and the tight binding of the hydrophobic pocket in the active-site. The preferred co-factor needed

for substrate binding into the active-site is Mg^{2+} due to its greater activity when compared with other divalent metal ions (Mn^{2+} or Co^{2+}).[96] Studies have revealed the interaction of DOXP with the metal ion through bi-dentate chelation involving the hydroxamate group, by forming an octahedral complex with water molecules and the residues Glu231, Asp150 and Glu152.[102,104,110]

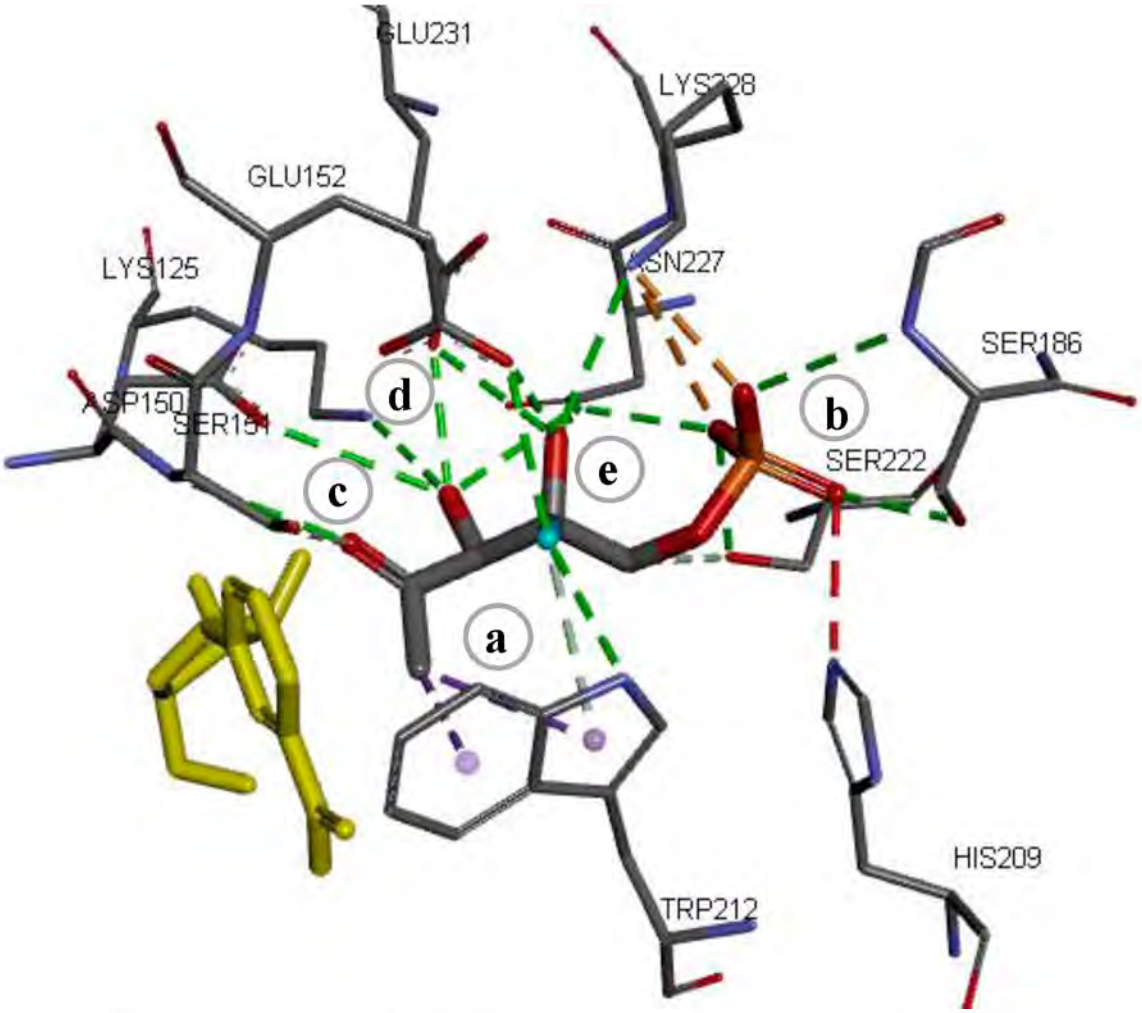


Figure 20. The natural substrate, DOXP, binding in the active-site of *Ec*DXR, and its interaction with the proximal amino acid residues. Fosmidomycin is shown in ball-and-stick format and coloured by element, NADPH is coloured in yellow and the amino acid residues are in wireframe format. Generated using Discovery Studio Visualizer 4.0.[111]

The structure of *Pf*DXR-fosmidomycin complex recently reported by Umeda *et al.*,[80] showed a similar mode for the binding of fosmidomycin **62** in the active-site as that of DOXP in the *Ec*DXR active-site, with several hydrogen-bonding interactions between fosmidomycin and proximal amino acid residues in the binding site, indicating fosmidomycin's potential as an inhibitor. The carbon backbone of fosmidomycin also lies parallel to the β-indole ring of

Trp212 (a; Figure 21). The *N*-hydroxyl oxygen (b) also interacts with Glu233 and Asp150 while the formyl oxygen hydrogen-bonds (c) with Ser232 and Asp150. The phosphonate group exhibits hydrogen-bonding interactions (d) with Lys312, Ser269, Ser270, Asn227 and Asn311 (which also interact with the formyl oxygen of the hydroxamate group).

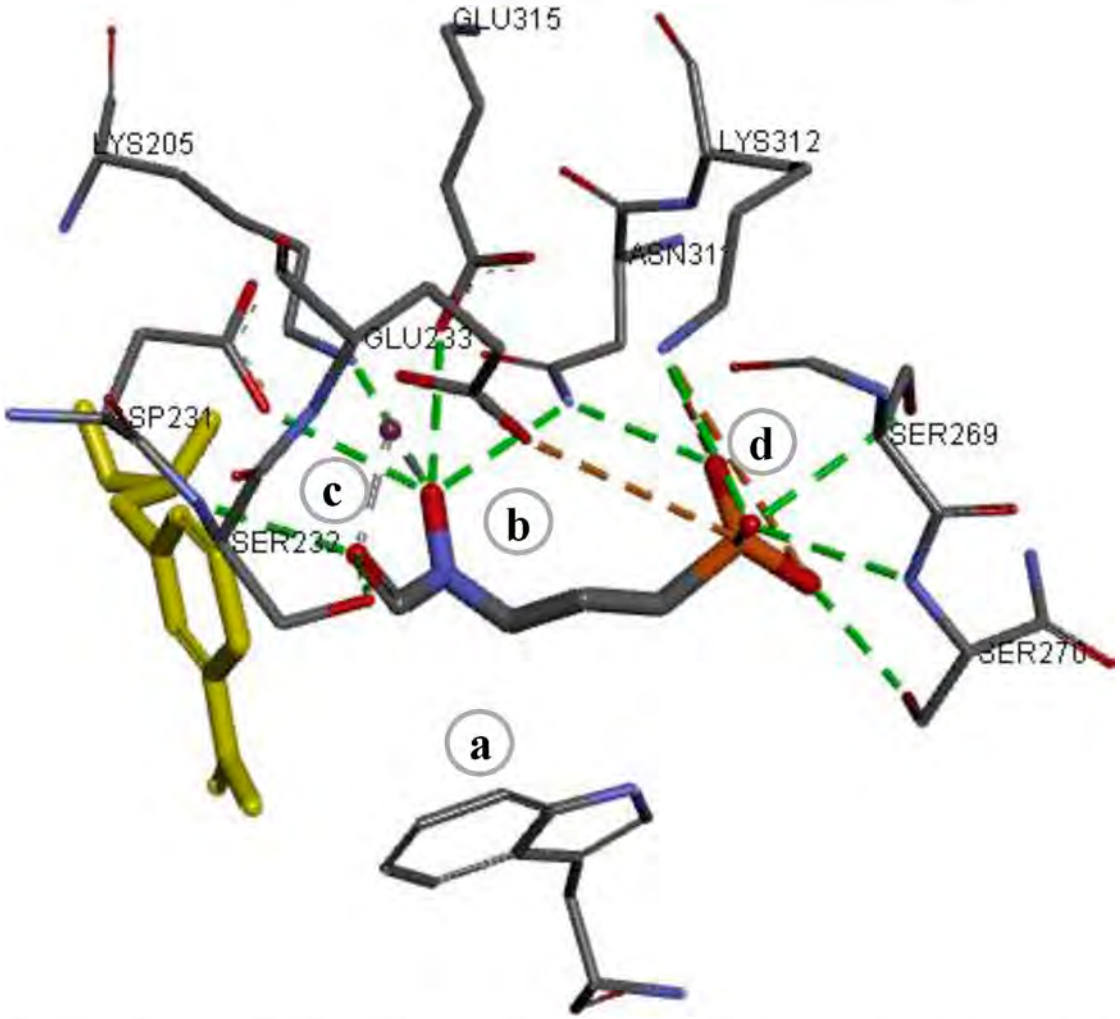


Figure 21. Fosmidomycin **62** binding into the active-site of *Pf*DXR and its interaction with the proximal amino acid residues. Fosmidomycin is shown in ball-and-stick format and coloured by element, NADPH is coloured in yellow and the amino acid residues are in wireframe format. Generated using Discovery Studio Visualizer 4.0.[111]

The presence of NADPH in the active-site also contributes to the binding in the active-site,[101] while the Mg^{2+} co-factor interacts with the hydroxamate formyl and hydroxyl oxygens of fosmidomycin **62**, forming a co-ordination complex as illustrated in Figure 22. The Figure also shows the similarity between the Mg^{2+} DOXP octahedral complex **I** with water and the active-site amino acid residues Glu231, Asp150 and Glu152 and the Mg^{2+}-fosmidomycin complex **II** in which the hydroxamate moiety chelates the divalent metal ion.

The presence of the divalent cation is crucial for the reduction of DOXP **53** into MEP **54** and inhibition depends on the correct positioning of the inhibitor in the *Pf*DXR active-site.[80,102]

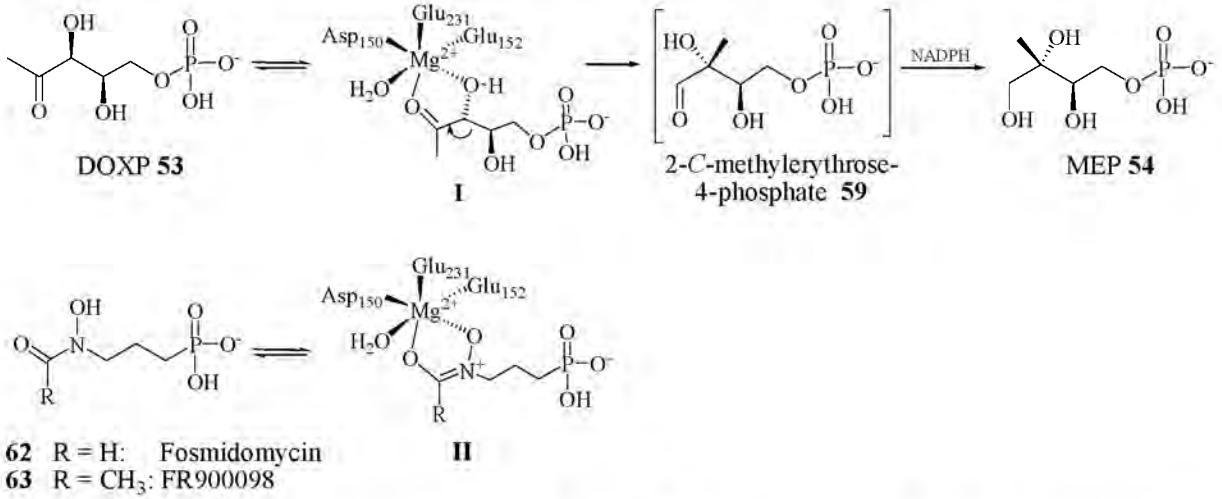


Figure 22. DXR-catalysed conversion of DOXP **53** into MEP **54** and the formation of octahedral Mg^{2+} complexes with proximal amino acids, water molecules and DOXP (complex **I**) and fosmidomycin (complex **II**).

1.4.2.4. Kinetic characterization of DXR

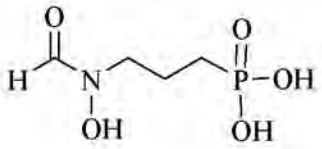
Enzyme activity can be characterised using the *Michaelis* dissociation constant K_m, the maximum velocity (V_{max}), the specificity constant (K_{cat}/K_m) and the second-order rate constant parameters,[81,108,112] which reveal the correlation between steady velocity and the concentration of the substrate.[108] The *Michaelis* constant (K_m) helps to determine the binding affinity of the substrate on the enzyme and can be defined as the inverse measure of the substrate's affinity for the enzyme. A decrease in K_m shows high binding affinity between the enzyme and the substrate. The major factors influencing K_m are the enzyme, temperature, pH and the substrate.[81] DXR exhibits optimal catalytic activity in a temperature range of 50-60 °C and a pH range of 7.0-8.5. Studies have also shown that DXR has a preference for the co-factor NADPH rather than NADH and a greater affinity for Mg^{2+} compared to other divalent metals.[110,113] The reported kinetic values for *Mt*DXR showed the following variation of K_m with different divalent metal cations; Mg^{2+} = 100 ± 4 μM; Co^{2+} = 42 ± 7 μM; and Mn^{2+} = 4.0 ± 0.3 μM. The preference for Mg^{2+} is thus reflected by the highest K_m value. However, no values have yet been reported for *Pf*DXR. Reported K_m values for *M.tuberculosis* and *E.coli* range from 3-250 μM and 4-250 μM respectively.[113] The catalytic constant K_{cat}, also referred to as "turnover number", reflects the maximum rate of the

substrate binding,[81,113] while the specificity constant (K_{cat}/K_m) or kinetic efficiency is a measure of the efficiency of an enzyme.[113]

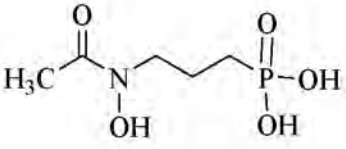
1.5. FOSMIDOMYCIN: a LEAD COMPOUND in DRUG DESIGN

1.5.1. Fosmidomycin discovery and clinical trials

Fosmidomycin **62** and its acetyl analogue FR900098 **63** are phosphonic acid derivatives which exhibit both antibiotic and herbicidal activity.[80] In the late 1970's, these compounds were isolated from *Streptomyces lavendulae* and *S. rubellomurinus sp. nov.*, respectively,[114,115] by Fujisawa Pharmaceutical Co., Ltd. They differ from each other in the nature of the *N*-acyl groups – a formyl group in fosmidomycin **63** and an acetyl group in FR900098 (Figure 23). In the mid-1980's, the use of fosmidomycin was reported for the treatment of urinary tract infections (UTI) with good efficacy, and there are no records of significant side-effects.[116] Since 1999, fosmidomycin **62** and FR900098 **63** have been the focus of great interest in antimalarial drug discovery due to their inhibition of the non-mevalonate (or DXR) pathway in the apicoplast of *P.falciparum*. As mentioned earlier, this pathway is absent in human biochemistry.[117]



Fosmidomycin **62**



FR900098 **63**

Figure 23. Structures of fosmidomycin **62** and its acetyl analogue FR900098 **63**.

Clinical trials in Thailand and Gabon showed promise in the treatment of uncomplicated malaria in humans using fosmidomycin **62** at a dosage of 1200 mg 3 times a day for 7 days.[118] However, low absorption, short serum half-life[80,119] and low bioavailability due to the polar nature of their structural parts have proved problematic.[120] Attempts made to improve the efficacy of fosmidomycin has led to successful combination therapies with artesunate[121] or clindamycin.[122-124] The clindamycin-fosmidomycin combination therapy has been used in the treatment of children aged 7-14 years, and shows efficacy superior to the existing combination therapy of sulfadoxine-pyrimethamin (SP).[122,123,125] Recently, there have been studies directed at developing fosmidomycin analogues with improved efficacy.

These studies have focused on modifications of the three main structural components of fosmidomycin **62** as shown in Figure 24., *viz*., (i) the hydroxamate moiety; (ii) the carbon spacer; and (iii) the phosphonic acid group.

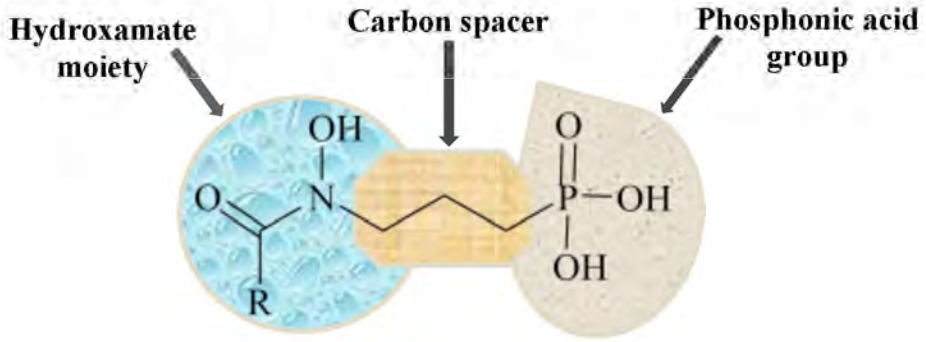


Figure 24. Critical structural features in fosmidomycin **62**.

1.5.2. Modifications of fosmidomycin's critical structural features

(i) *The hydroxamate moiety*. This group has strong metal chelating properties, forming an octahedral complex with the divalent cation in the *Pf*DXR active-site as shown in Figure 22. Such chelation requires that the N-OH and carbonyl oxygens of the hydroxamate group adopt a *syn*-conformation. Mercklé *et al*.[126] showed that 3-acetamidopropylphosphonate **64**, propylphosphonate **65** and aminopropylphosphonate **66**, have no hydroxamate group and have no inhibitory activity (Figure 25). Compound **67** which contains a cyclic analogue of the hydroxamate moiety, shows good inhibitory activity, but the activity of the hydroxyurea derivative **68** has yet to be demonstrated.[126,127] Woo *et al*., proved the importance of the NOH group by showing that both compound **69** and compound **70**, which contain formyl and acetyl groups corresponding to fosmidomycin and FR900098, respectively, exhibit decreased inhibitory activity.[128] The reduced inhibitory activity of compound **71** may be due to a preference for the *anti*-arrangement of the hydroxamate moiety; there was an even greater decrease in activity when the group was replaced by a hydroxyurea moiety.[129]

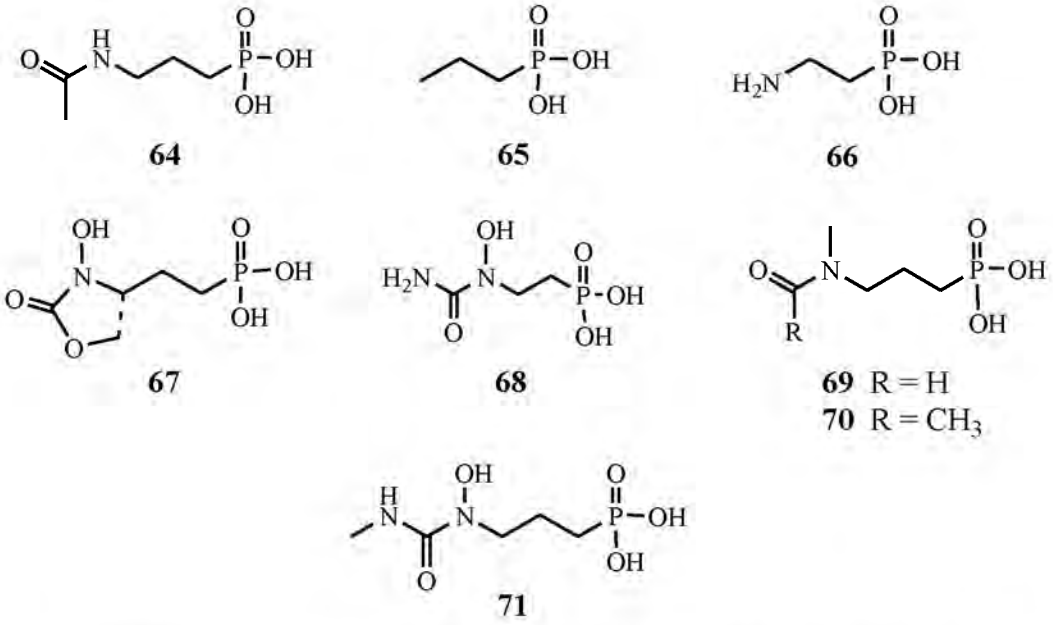


Figure 25. Structures of fosmidomycin analogues with modifications of the hydroxamate moiety.

(ii) *The carbon spacer*. This is the carbon chain between the hydroxamate and phosphonic acid groups in fosmidomycin **62** and FR900098 **63**, and is sometimes referred to as the "hydrophobic patch". Shortening the number of methylene groups from three in fosmidomycin **62** to two (in compound **72**) or one (in compound **73**) caused drastic decreases in the activity.[80,127] The reason is that the shorter compounds are too short to bind effectively in the active-site; the presence of longer carbon chains, on the other hand, prevents ideal binding.[80,127] Devreux *et al.*,[130] synthesised compound **74** which is a conformationally-restricted cyclopropyl analogue with two chiral centres, and found it to be as active as fosmidomycin **62** but less active than FR900098 **63**.[130] Compound **75** and its analogue **76**, which contain a hydroxyl group in the middle of a three-carbon spacer, also showed no inhibitory activity (Figure 26).[130] Replacement of the central methylene group in fosmidomycin by an ether oxygen as in compound 77 (Figure 26) also decreased the inhibitory activity due, possibly, to the reduction of the hydrophobicity of the spacer and hindrance of the interaction with the β-indole ring of Trp212 in the active-site.[80,131-133]

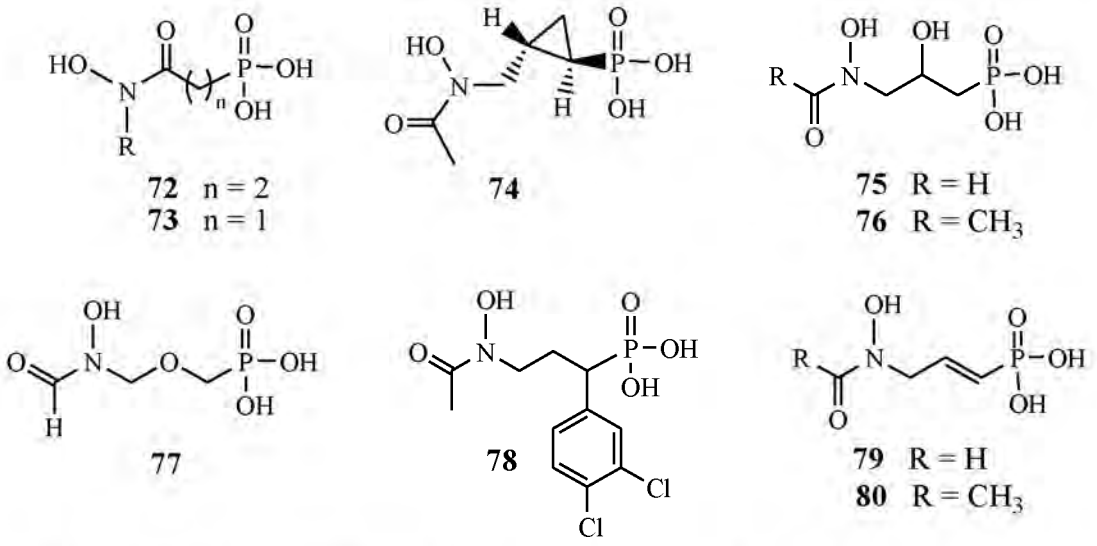


Figure 26. Structures of fosmidomycin analogues with modifications of the carbon spacer.

Identification of an additional hydrophobic pocket adjacent to the active-site has prompted the development of analogues containing additional aromatic or alkyl hydrophobic groups, of which the 3,4-dichlorophenyl substituted analogue **78**, has shown good activity against DXR.[80,131,133] *N*-Acyl derivatives of 3-(*N*-hydroxyamino)-1-*trans*-propenylphosphonic acid, *e.g.*, compound **79** and its analogue **80**, have exhibited little if any antimalarial activity (Figure 26).[134]

(iii) *The phosphonate moiety.* The phosphonic acid group is responsible for hydrogen-bonding with the amino acid residues Lys312, Ser269, Ser270, Asn227 and Asn311, resulting in a tightly bound network in the DXR active-site. Modifications of the phosphonate moiety by Woo *et al.*,[128] and Kurz *et al.*,[129] involved replacement of the phosphonate group with isosteric groups as in the carboxylic acid derivative **81** and the sulfamate **82** (Figure 27). However, none of these analogues showed activity against DXR. The hydroxamate-carboxylic acid analogues **83** and **84** have shown a 3000-fold decrease in DXR inhibition.[128,129] The failure of the carboxylic acid analogues has been attributed to the fact that the phosphonate moiety has a pyramidal structure while the carboxylate has a planar structure.[135] In order to mimic the pyramidal structure of the phosphonate group in fosmidomycin **62**, compounds containing sulfonic acid groups (**85** and **86**) and a series of alkyl or aryl sulfones (**87-92**) were synthesised, but none of these analogues showed antimalarial activity when tested on *Ec*DXR.[135,136] The absence in activity was attributed to the C-P and P-O bonds being shorter than the C-S and S-O bonds, thus preventing tight

binding at the active-site.[80,135] The diphenyl phosphonate analogue **93** was also synthesised, but also showed a decrease in DXR inhibitory activity due, it was suggested, to the presence of the bulky phenyl rings (Figure 27).[137]

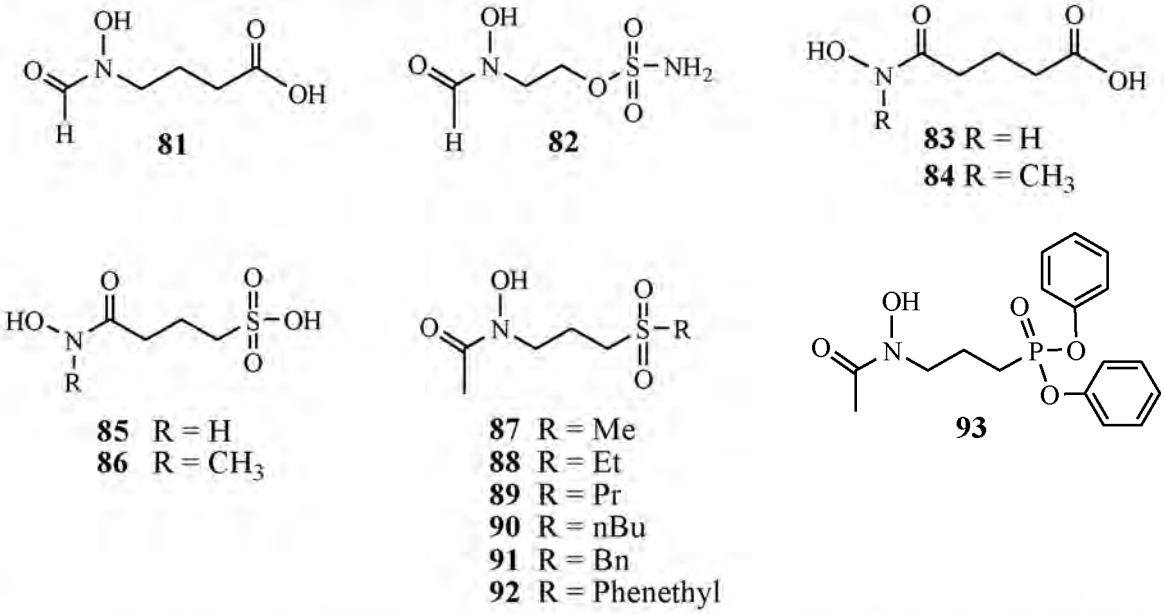


Figure 27. Structures of fosmidomycin analogues with modifications of the phosphonate moiety.

Research on modifications of the main components of the fosmidomycin structure seem to indicate the necessity of keeping the phosphonic group intact, having three methylene groups in the spacer between the phosphonic acid and hydroxamate groups and having a *syn*-arrangement of the N-OH and the carbonyl oxygen in the hydroxamate group.

1.6. STRUCTURAL ANALOGUES of DOXP and NADPH as POTENTIAL INHIBITORS.

NADPH, acting as a co-factor together with a divalent metal cation are located within the *Pf*DXR active-site, thereby enhancing its stability. Therefore, attention has been given to developing composite analogues of DOXP and NADPH, with research focusing on the mechanism for the conversion of DOXP **53** to MEP **54** (See Figure 17). Compound **94** (Figure 28), is a DOXP analogue which contains a fluoromethyl group capable of stabilizing a negative charge in the enolate of the enediol **61** (Figure 17), thus favouring the retro-aldol mechanism; in the case of an α-ketol rearrangement mechanism, the electron-withdrawing fluoromethyl group would destabilize the positive aldehyde on carbon. Compound **94** has, in

fact, exhibited good inhibitory activity.[138,139] The NADPH analogue **95**, has shown moderate inhibitory activity against *Ec*DXR, but no evidence of the binding of this compound in the NADPH binding domain of the active-site has been reported. Rohmer and co-workers synthesised compound **96** which can be converted into the MEP analogue **97**, but neither compound showed significant inhibitory activity.[140,141] Other DOXP analogues containing a di- or triflouromethyl groups (**98** and **99**) were reported by Fox *et al.*, but the low inhibitory activity observed for these analogues was attributed to steric hindrance at the metal binding site.[139]

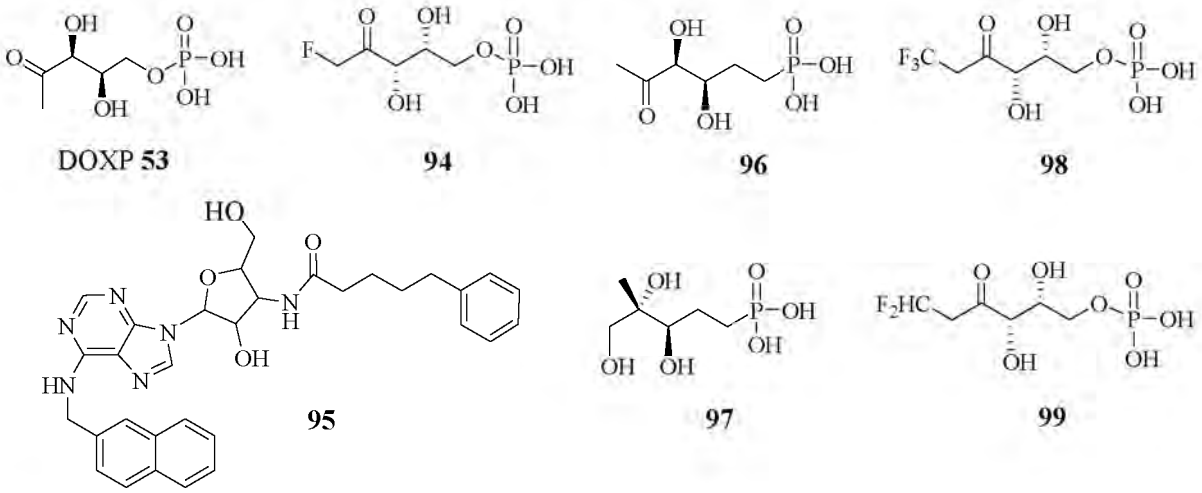
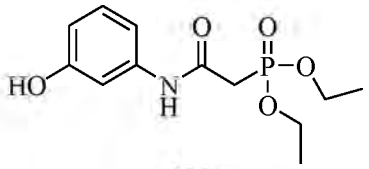


Figure 28. Reported analogues of DOXP **53**, MEP **54** and NADPH.

1.7. PREVIOUS WORK IN THE GROUP

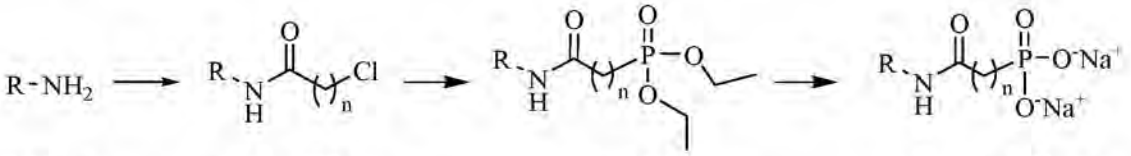
During research on the development of novel adenosine triphosphate (ATP) analogues as potential glutamine synthetase (GS) inhibitors for the treatment of tuberculosis, compound **100** (Figure 29)[142-144] was found to bind with *Ec*DXR in a Saturation Transfer Difference (STD) protein-NMR experiment. Its promising *in vitro* inhibition of *Ec*DXR prompted its adoption as a potential lead compound for the development of novel compounds as DXR inhibitors. In further studies in our group,[142-147] a series of novel compounds has been designed and synthesized, and a number of them have exhibited minimal to moderate *Ec*DXR and, subsequently, *Pf*DXR inhibitory activity when evaluated *in vitro*.



100

Figure 29. Lead compound for the group.

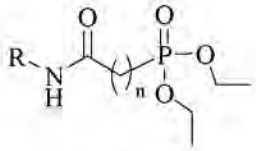
Conibear,[145,146] reacted heterocyclic amines **101a-e** (2-aminopyridine, furfurylamine, 2-aminothiazole, 3-aminoisothiazole and 5-acetyl-2-amino-4-methylthiazole) with chloroacetyl chloride or 3-chloropropionyl chloride to afford the corresponding chloroamides **102a-e** and **103a-e** (Scheme 1), ligands having one- or two-carbon spacers. Using the Michaelis-Arbuzov reaction, the chloroamides were converted to the diethyl phosphonate esters **104a-e** and **105a-e**. Hydrolysis of the esters was carried out using bromotrimethylsilane and basification gave the acid salts **106a-e** and **107a-e** some of which showed moderate DXR inhibitory activity.[145,146]



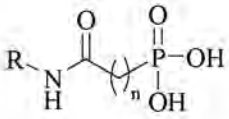
101a-e	n = 1; **102a-e**	n = 1; **104a-e**	n = 1; **106a-e**
R= heterocyclic	n = 2; **103a-e**	n = 2; **105a-e**	n = 2; **107a-e**

Scheme 1. Synthesis of phosphonate esters and their corresponding sodium salts by Conibear.

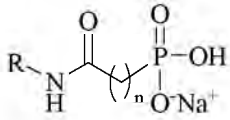
Mutorwa followed this study by replacing the heterocyclic systems with various 3-substituted aniline derivatives to obtain the phosphonate esters **108a-g**, phosphonic acids **109a-g** and their corresponding monosodium salts **110a-g** (Figure 30). These compounds contained 1, 2, 3 or 4 methylene carbon spacers corresponding to the variations on the hydrophobic patch in fosmidomycin **62**, and were accessed by reacting the anilines with different acid chlorides (chloroacetyl chloride, 3-chloropropionyl chloride, 4-chlorobutanoyl chloride and 5-chloropentanoyl chloride).[144,147]



108a-g



109a-g



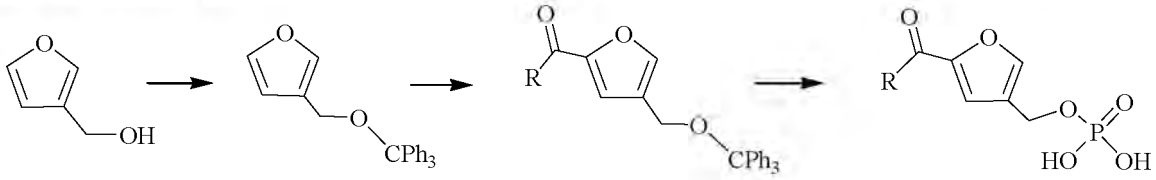
110a-g

n = 1, 2, 3 and 4

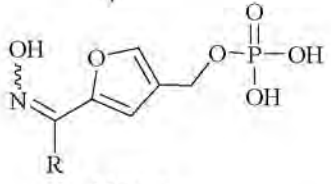
	R
a	3-(Hydroxylmethyl)phenyl
b	3-bromophenyl
c	3-Methoxylphenyl
d	3-Cyanophenyl
e	3-Fluorophenyl
f	3-Nitrophenyl
g	3-Hydroxyphenyl

Figure 30. Synthesis of 3-substituted aniline-derived phosphonate esters **108a-g**, phosphonic acids **109a-g** and their corresponding monosodium salts **110a-g** (n = 1-4).

The furan-derived phosphonate esters **114a-c** and the corresponding oximes acids **115a-c** were synthesised by Mutorwa in several steps as conformationally-restricted DOXP or fosmidomycin analogues (Scheme 2). Thus, 3-furanmethanol **111** was reacted with triphenylmethyl chloride in the presence of excess triethylamine and a catalytic amount of 4-dimethylaminopyridine (DMAP) under an inert atmosphere to afford 3-(trityloxymethyl)-furan **112** (Scheme 2).[147]



111 **112** **113a-c** **114a-c**



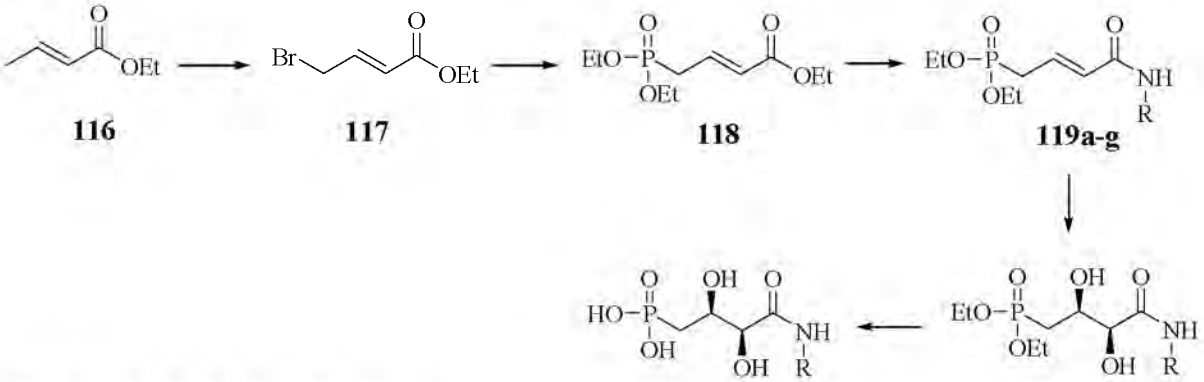
115a-c

R = H; Me; $CH_2C(CH_3)_3$

Scheme 2. Furan-derived phosphonate esters **114a-c** and their corresponding oximes **115a-c** as conformationally-restricted DOXP analogues.

Compound **112** was lithiated using butylithium at -30 °C and reacted with DMF, acetyl anhydride or *tert*-butylacetyl chloride to give the acylated derivatives **113a-c**. Compounds **113a-c** were sequentially de-tritylated and phosphorylated using phosphoric acid to provide access to the dihydrogen phosphates **114a-c**. The corresponding phosphorylated furanyl oximes **115a-c** were prepared using hydroxylamine hydrochloride as shown in Scheme 2.

Mutorwa also prepared a series of dihydroxy-amido phosphonate esters and phosphonic acid derivatives as novel DXR inhibitors by mimicking the alkyl backbone of DOXP **53** so that its binding specificity is retained. He achieved this using Wohl-Ziegler bromination, reacting ethyl (*E*)-crotonate **116** with *N*-bromosuccinimide to afford compound **117**, which was subsequently reacted with triethyl phosphite to give compound **118** (Scheme 3). The ester **118** was hydrolysed using potassium hydroxide and the resulting carboxylic acid was reacted with different amines using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), as the coupling agent, in the presence of hydroxybenzotriazole (HOBt) to give the amides **119a-g**, dihydroxylation of which was effected using the ruthenium-based catalyst system ($RuCl_3/CeCl_3/NaIO_4$). The resulting dihydroxy phosphonate esters **120a-g** were hydrolysed to afford phosphonic acids **121a-g** as shown in Scheme 3.[144,147]



116 **117** **118** **119a-g** **120a-g** **121a-g**

R = Me, Et, Pr, Pr^i, But, Ph, CH_2Ph

Scheme 3. Syntheisis of dihydroxy-amido phosphonic acid derivatives **121a-g**.

Conibear undertook careful *in silico* exploration studies of the *Pf*DXR active-site,[145,146] thus revealing additional, adjacent binding pockets. The presence of the additional binding pocket identified in Figure 31 prompted the *de novo design* of benzylated phosphonate esters. A synthetic pathway to ligands capable of exploiting this additional binding opportunity was designed and initiated by Conibear and completed by Mutorwa.[146,147]

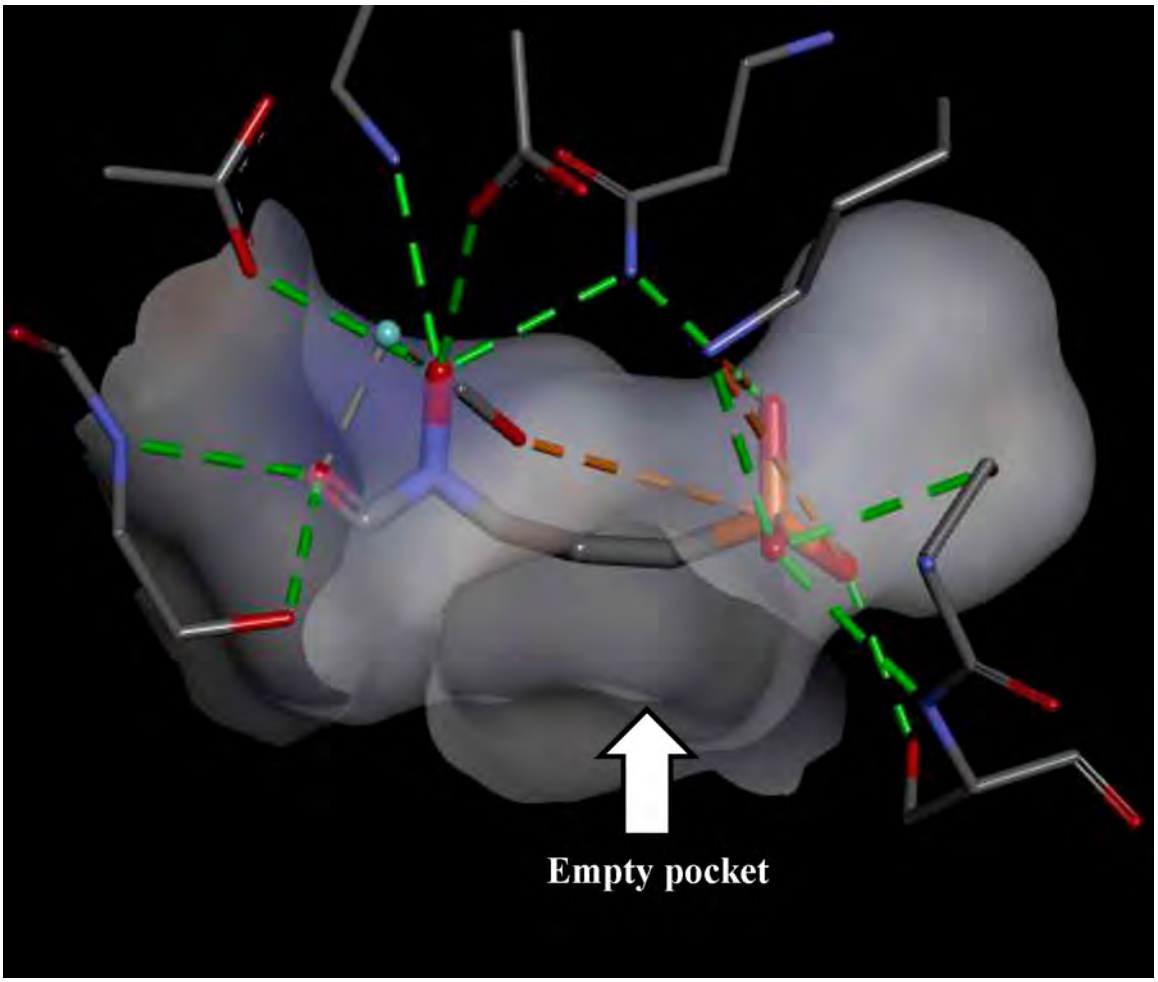
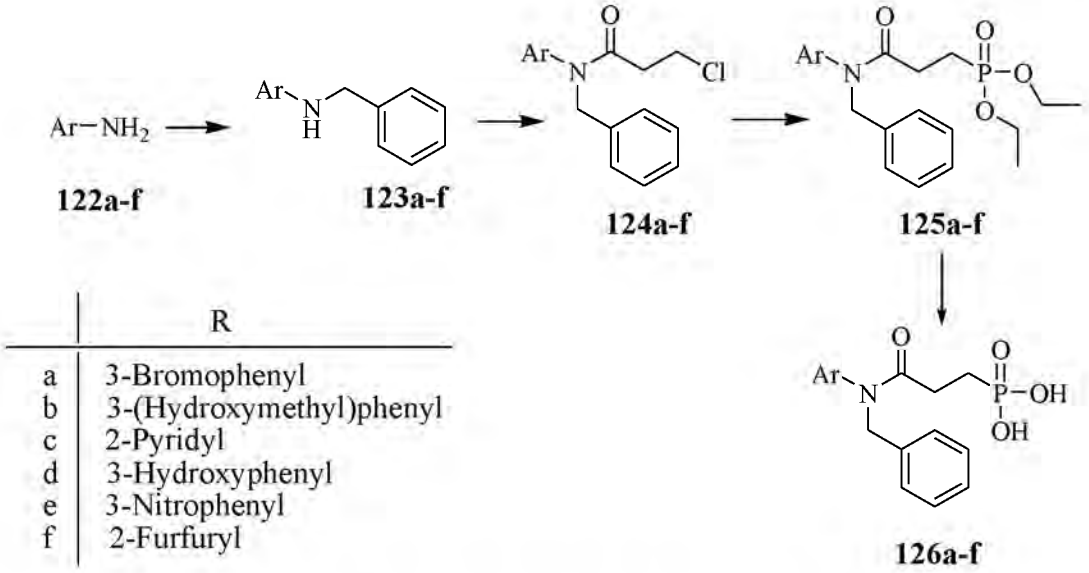
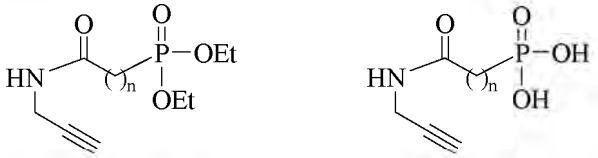


Figure 31. Revealing the additional binding pocket adjacent to the *Pf*DXR active-site.

In an MSc project,[146,147] we extended earlier work by synthesising various *N*-benzylated phosphonate esters and the corresponding acids by reacting different anilines **122a-f** with benzyl bromide to afford the *N*-benzylated amines **123a-f**. Acylation of the *N*-benzylated amines **123a-f** was effected by reaction with chloropropanoyl chloride to afford compounds containing a two-methylene group hydrophobic patch. Michaelis-Arbuzov reactions of each of the 3-chloropanamides **124a-f** with triethyl phosphite afforded the series of novel phosphonate esters **125a-f** and, following hydrolysis, the phosphonic acids **126a-f** (Scheme 4). Propagylamine was also reacted with chloroacetyl chloride or 3-chloropropanoyl chloride and the resulting chloroamides were reacted with triethyl phosphite to afford corresponding phosphonate esters **127a** and **127b**, and, following hydrolysis, their phosphonic acids **128a** and **128b** (Figure 32). All of these compounds were tested *in vitro*, and some of them exhibited encouraging antimalarial activity.[148,149]



Scheme 4. Synthesis of *N*-benzylated phosphonated esters **125a-f** and its acids **126a-f**.



n = 1; **127a**
n = 2; **127b**

n = 1; **128a**
n = 2; **128b**

Figure 32. Synthesis of propagryl phosphonated esters and its acids.

1.8. OBJECTIVES of the CURRENT INVESTIGATION

As indicated earlier, the development of resistance to existing antimalarial drugs presents ongoing challenges and underlines the need for the discovery of new antimalarial drugs. An understanding of the topology of the *Pf*DXR active-site has helped to design novel fosmidomycin analogues as potential DXR inhibitors. Particular attention has been given to the availability of the additional binding pocket of in the *Pf*DXR active-site, and the need to incorporate hydrophobic groups to occupy this pocket in the design of new compounds as potential DXR inhibitors. It was decided that: (i) the phosphonic moiety should be kept intact; (ii) the carbon length of the hydrophobic patch would be varied; (iii) a benzyl group should be attached to the hydrophobic patch; (iv) the hydroxamate group atoms could be transposed to afford both "***normal***" and "***reverse***" fosmidomycin analogues; (v) attention would be given to replacing the carbon anchor for the benzyl group by nitrogen to avoid

issues of chirality; and (vi) replacement of the hydroxamate moiety by structurally constrained isosteres to ensure *syn*-orientation of the metal cation chelating groups would be explored (Figure 33).

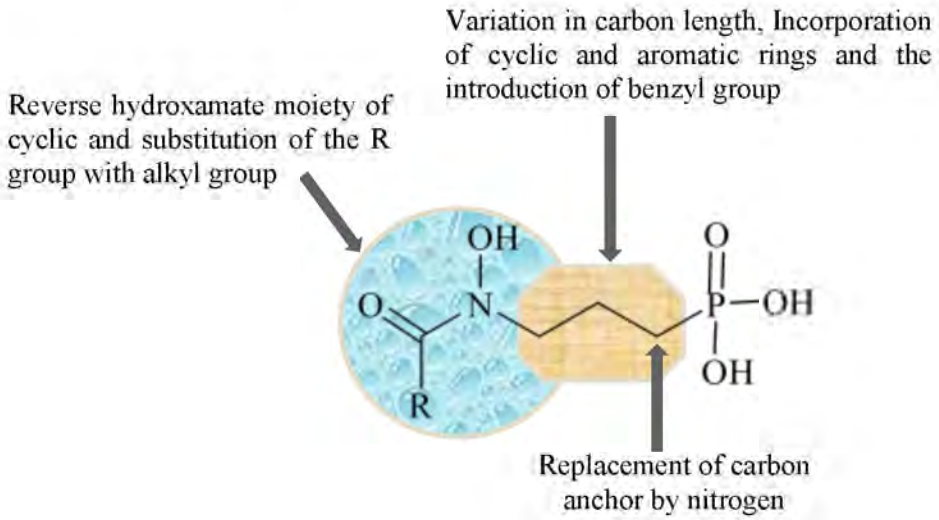


Figure 33. Overview of proposed modifications of fosmidomycin **62** structural features.

More specifically, the objectives of this project have included the following objectives;

1. The synthesis and characterization of:-
   (i) *C*-benzylated (*N*-phenylcarbamoyl)alkylphosphonate esters and their corresponding phosphonic acids as fosmidomycin analogues;
   (ii) *N*-substituted benzylphosphoramidic acid derivatives as fosmidomycin analogues;
   (iii) *N*-substituted benzylphosphoramidic acid derivatives as reverse fosmidomycin analogues; and
   (iv) *N*-benzylated benzylphosphoramidic acid containing structurally constrained cyclic hydroxamate isosteres.
2. *In vitro* bioassay of the synthesised compounds to access their *Pf*DXR inhibition and antimalarial potential.
3. Molecular modelling studies, including *in silico* docking and evaluation of QSAR parameters.

2. RESULTS AND DISCUSSION

In line with the aims and objectives in Section 1.8., this discussion will focus, as illustrated in Figure 34, on the following areas: (i) the synthesis of *C*-benzylated analogues **II** of the *N*-benzylated (*N*-arylcarbamoyl)alkylphosphonate esters and their corresponding phosphonic acids **I** (Section 2.1.); (ii) the synthesis of *N*-benzylphosphoramidic acid derivatives **III** as "reverse" fosmidomycin analogues (Section 2.2.); (iii) the synthesis of *N*-substituted benzylphosphoramidic acid derivatives **IV** (Section 2.3.) as "normal" fosmidomycin analogues; (iv) the synthesis of *N*-benzylated phosphoramidic acid derivatives **V** and **VI** containing conformationally constrained Mg^{2+} chelating moieties (Section 2.4.); (v) the biological evaluation of selected compounds as *Pf*DXR inhibitors (Section 2.5.); and (vi) computer modelling and docking studies (Section 2.6).

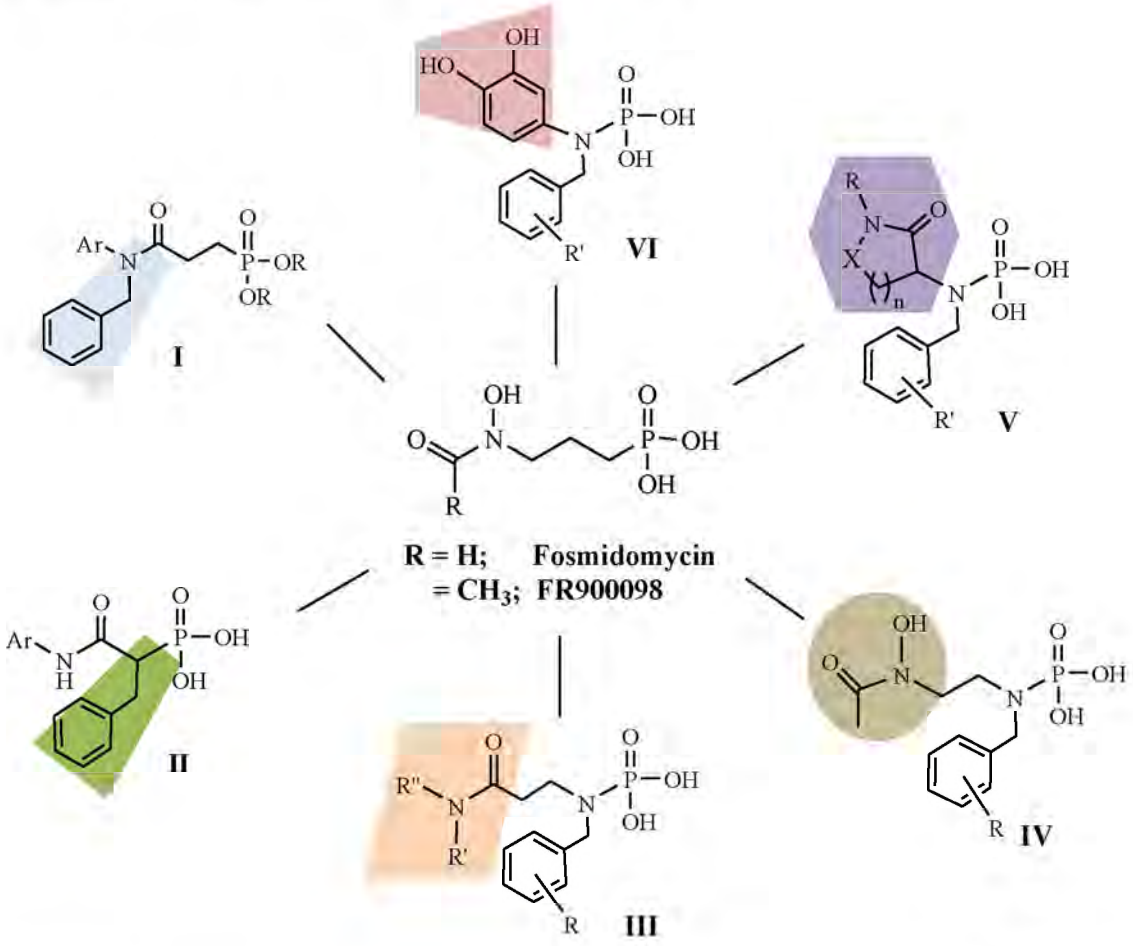
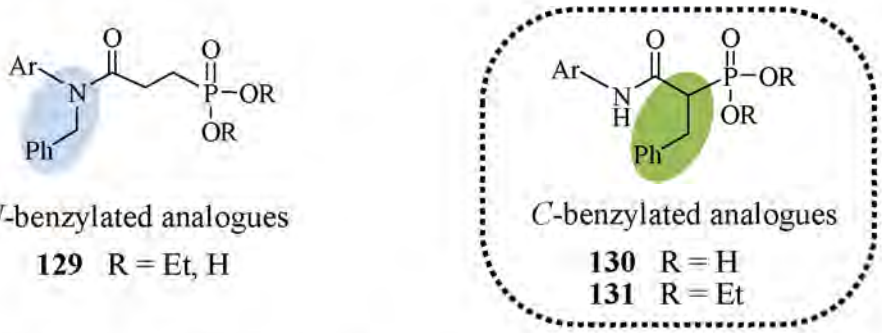


Figure 34. Overview of the proposed series of synthetic compounds as *Pf*DXR inhibitors.

2.1. SYNTHESIS of *C*-BENZYLATED (*N*-ARYLCARBAMOYL)ALKYL-PHOSPHONATE ESTERS and their CORRESPONDING PHOSPHONIC ACIDS

The fact that certain *N*-benzylated phosphonate esters **129** and their phosphonic acids, had been shown to exhibit promising *Pf*DXR inhibitory activity,[148,149] prompted the synthesis of the *C*-benzylated analogues **131** and their corresponding phosphonic acids **130** (Figure 35). In the series of compounds **131** the number of sp^3 carbons in the spacer between the hydroxamate group and phosphonate moiety is decreased and a benzyl group is attached to a spacer carbon to occupy the empty pocket adjacent to the *Pf*DXR active-site and thus enhance hydrophobicity.[150]



N-benzylated analogues
129 R = Et, H

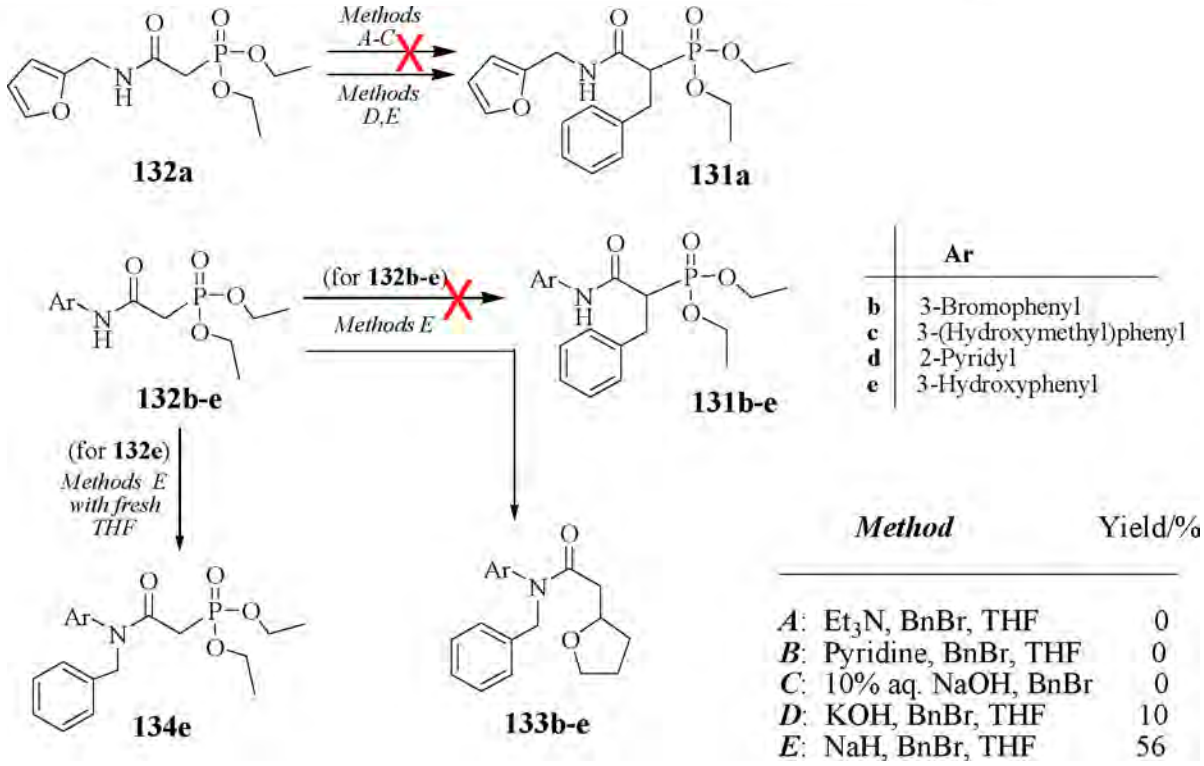
C-benzylated analogues
130 R = H
131 R = Et

Figure 35. Structures of the *N*-benzylated and *C*-benzylated analogues.

2.1.1. Direct benzylation approaches

We envisaged that access to the *C*-benzylated phosphonate esters **131a-e** could be achieved by simple base-catalysed benzylation of (*N*-arylcarbamoyl)alkylphosphonate esters **132** which had been synthesised previously.[148,149] In an earlier study, (MSc) the exploration of several benzylation methods (***A-E***; Scheme 5), *viz.*, reaction in dry THF using pyridine or triethylamine (*method **A** or **B***) as mild bases for the deprotonation of the (*N*-arylcarbamoyl)-phosphonate esters **132a**, failed to give the desired product **131a** when stirred at room temperature and at reflux. Boiling a mixture of the (*N*-arylcarbamoyl)phosphonate ester **132a** with benzyl bromide in 10% aqueous NaOH under reflux for *ca* 2 h (*method **C***) also failed.

The use of KOH in dry THF (*method **D***) finally afforded the desired *C*-benzylated phosphonate ester **131a** in 10% yield.



Scheme 5. Different methods explored to access the *C*-benzylated phosphonate esters **131**.

Attention was finally turned to an approach described by Uladzimir *et al.*,[151] to obtain *N*-benzylated analogues. This involved the reaction of amides with benzyl bromide in dry THF using sodium hydride as base (*method **E***). Conseqently, a solution of phosphonate ester **132a** in dry THF was refluxed with sodium hydride as a base for *ca* 6 h, and benzyl bromide (2.4 equivalents) was then added in portions. After refluxing the mixture for a further 4 h, work-up and chromatography afforded the desired *C*-benzylated phosphonate **131a** in 56% yield (Scheme 5) – not the *N*-benzylated analogue which might have been expected using Uladzimir's method. However, application of this method to the *N*-aryl substituted analogues **132b-e** failed to afford the desired *C*-benzylated derivatives **131b-e**.[152] Instead, of the THF derivatives **133b-e**, the *N*-benzylated **134e** was obtained, due to enhancement of the relative acidity of the amide proton by the electron withdrawing *N*-aryl substituents to favour formation of the resonance-stabilised anion **II** and, hence, formation of the *N*-benzylated derivative **134e** (Figure 36).

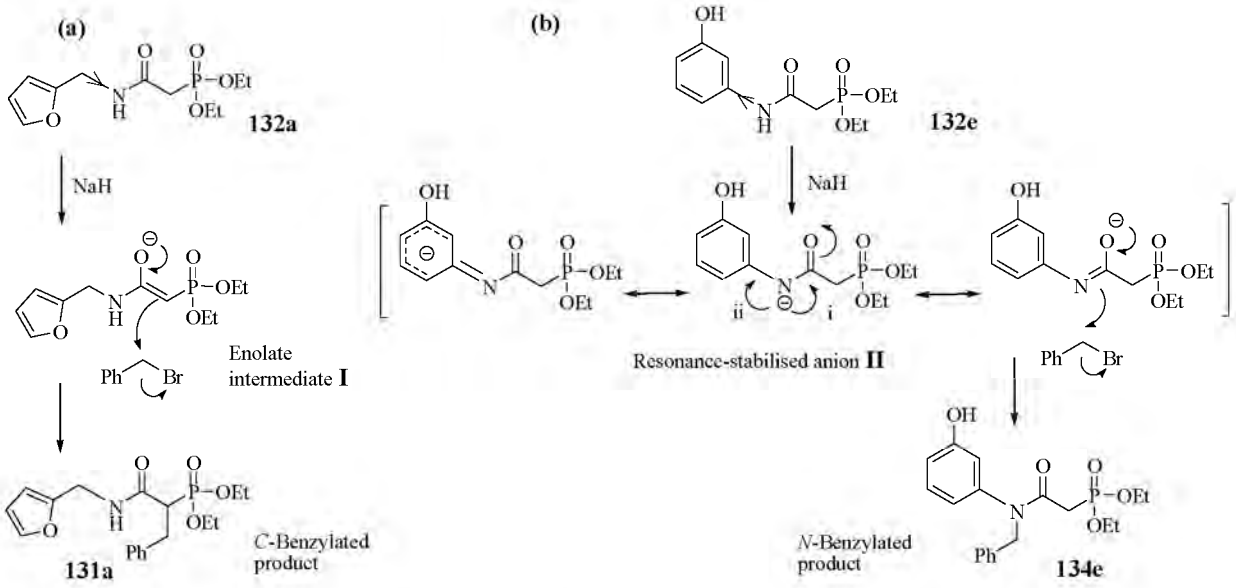


Figure 36. a) *C*-Benzylation of the *N*-furfuryl derivative **132a**. b) *N*-Benzylation of the *N*-(3-hydroxyphenyl) derivative **132e**.

The ^{1}H NMR spectrum (Figure 37) of compound **134e** clearly shows the *P*-methylene proton doublet at δ 2.99 ppm, while the COSY NMR spectrum (Figure 38) confirms the formation of the *N*-benzylated product since there is no correlation between the *P*-methylene and the benzylic protons.

Given these difficulties, another synthetic "approach" was explored to access the *C*-benzylated analogues **131**.

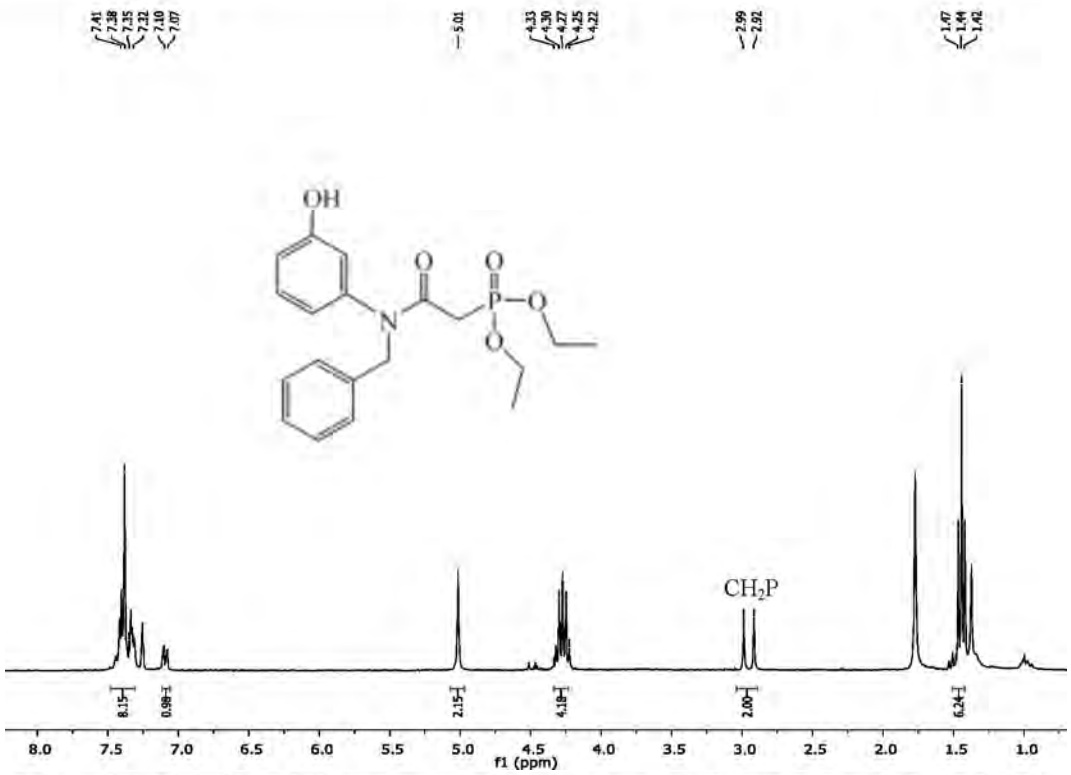


Figure 37. 300 MHz ^{1}H NMR spectrum of the *N*-benzylated compound **134e** in $CDCl_3$.

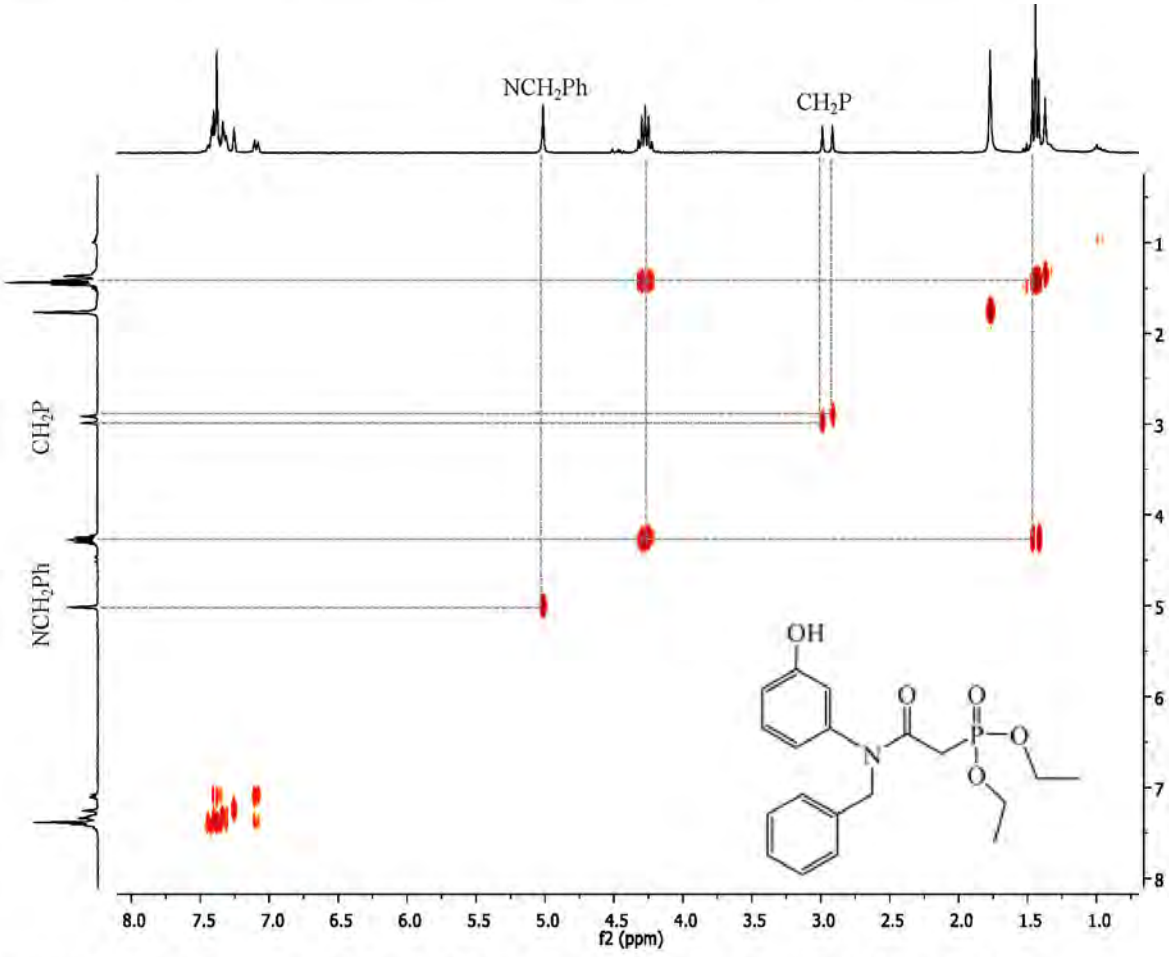
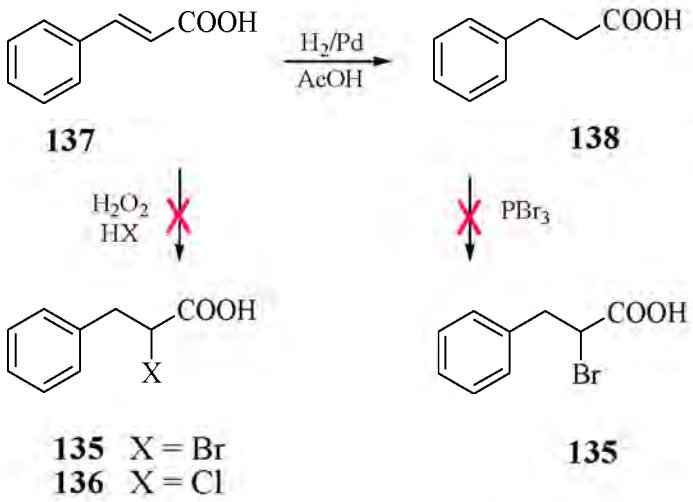


Figure 38. COSY NMR spectrum of the *N*-benzylated compound **134e** in $CDCl_3$.

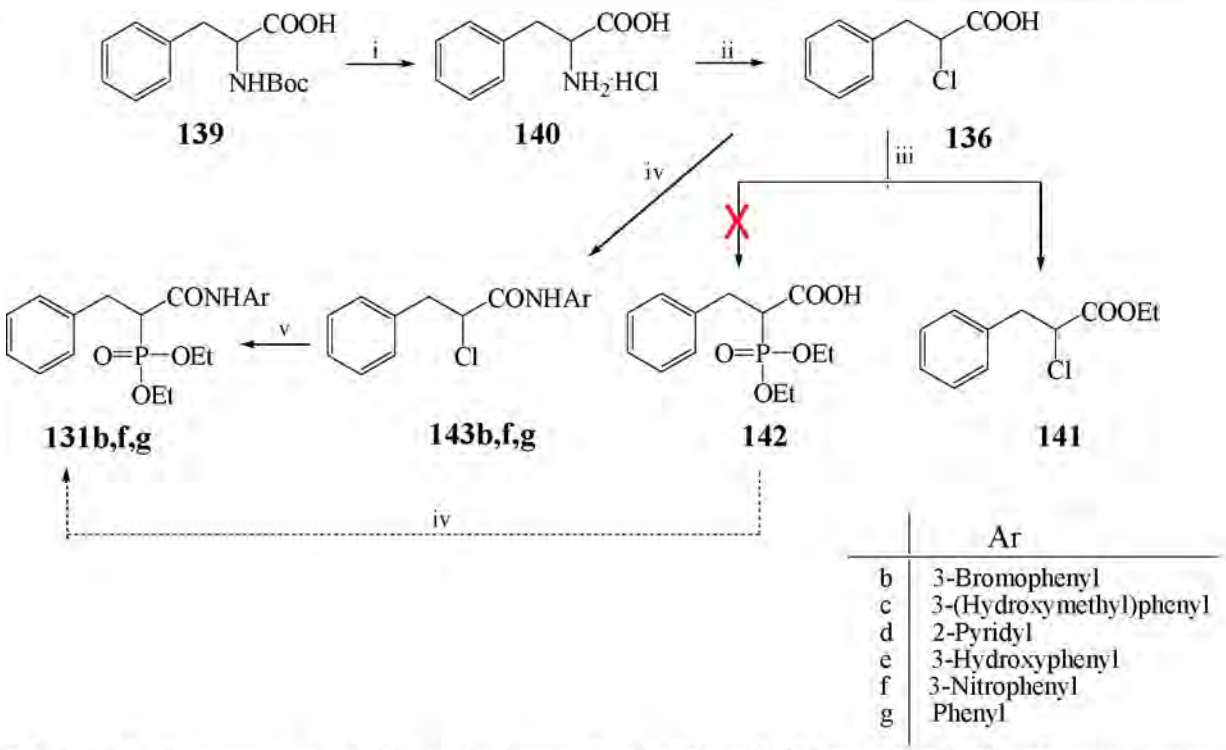
2.1.2. Alternative approaches to the synthesis of the *C*-benzylated analogues

In view of the difficulties encountered in attempting to prepare the *C*-benzylated derivatives **131b-e**, alternative approaches were explored. The α-halogenated phenylpropanoic acids **135** or **136** were identified as possible intermediates in a completely different path to the desired *C*-benzylated phosphonate esters **131b-e** (Scheme 7). Cinnamic acid **137** was subjected to catalytic hydrogenation using a palladium-on-carbon catalyst in acetic acid, under a hydrogen atmosphere to give 3-phenylpropanoic acid **138** (Scheme 7).[153] Bromination of the acid **138** using the Hell-Volhard-Zelinsky reaction,[154] was attempted using PBr_3 on 3-phenylpropanoic acid **138**, but this approach to 2-bromo-3-phenylpropanoic acid **135** proved unsuccessful.
The hydrochlorination or hydrobromination was attempted using HCl or HBr and H_2O_2 to afford either of the α-halogenated acids **135** or **136**, but this similarly failed.



Scheme 7. Attempted synthesis of the intermediates **135** and **136**.

Attention was then turned to the use of *N*-boc-phenylalanine **139**, which was deprotected by stirring with 4M-HCl in dioxane for 2 h at room temperature; removal of the solvent and trituration of the residue with EtOAc gave phenylalanine hydrochloride **140** in quantitative yield (Scheme 8).[156,157] The amino group in compound **140** was diazotised upon treatment with 6M-HCl and sodium nitrite in water at 0 °C and later at room temperature to afford 2-chloro-3-phenylpropanoic acid **136** (Scheme 8).[158,159] The ^{1}H NMR spectrum of compound **136** (Figure 39) shows the diastereotopic benzylic protons resonating as a pair of multiplets at 3.20 and 3.40 ppm due to coupling with each other and the α-methine proton which resonates at 4.50 ppm.



Scheme 8. Proposed synthetic route towards the *N*-aryl *C*-benzylated phosphonate esters **131b-g**.

Reagents and conditions:- (i) 4M-HCl, dioxane, r.t., 2 h; (ii) 6M-HCl, $NaNO_2$, H_2O, 0 °C, then r.t., 24 h; (iii) triethyl phosphite, 120-150 °C, 5 h; (iv) $SOCl_2$, DMF, 70 °C, 1 h, then $ArNH_2$, DMF, overnight; and (v) triethyl phosphite, 120-150 °C, 10 h.

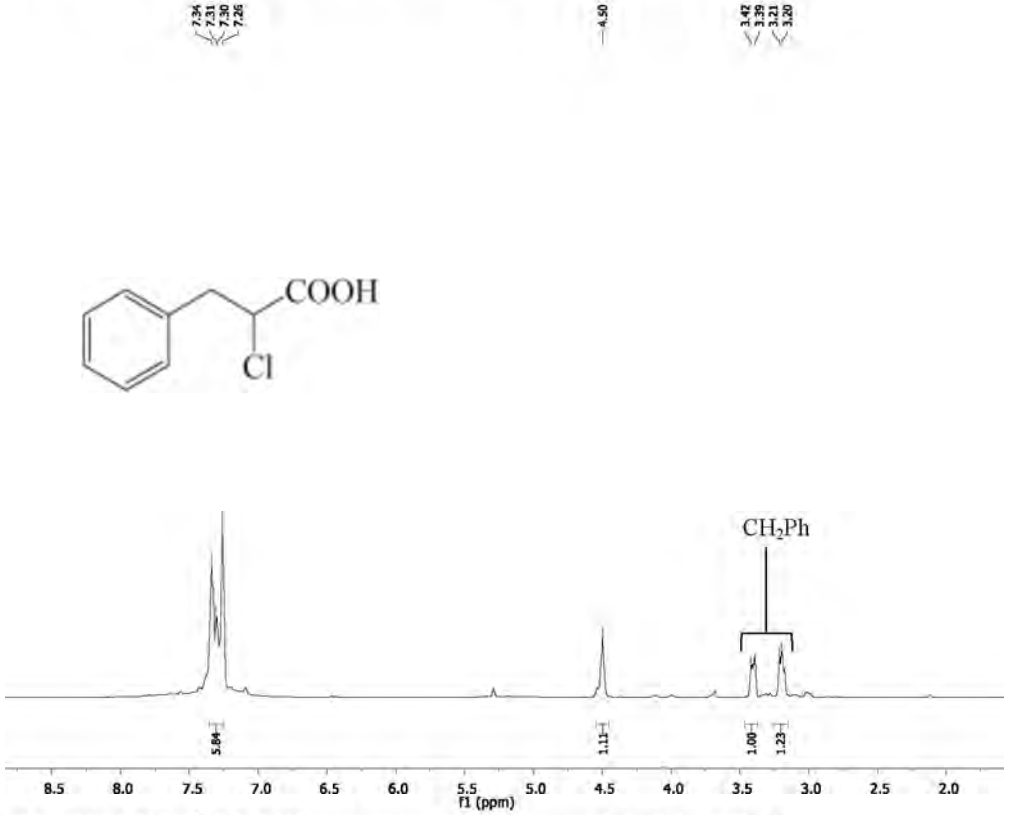


Figure 39. 300 MHz ^{1}H NMR spectrum of compound **136** in $CDCl_3$.

The phosphonate esters are crucial for the synthesis of the fosmidomycin analogues and several methods have been used for the introduction of the C-P bond.[160-162] We anticipated that application of the Michaelis-Arbuzov reaction[163,164] to 2-chloro-3-phenylpropanoic acid **136**, would be a useful approach to the envisaged phosphonate ester intermediate **142**. However, refluxing compound **136** with 2 equivalents of triethyl phosphite, under N_2 at 120-150 °C (Scheme 8) failed to afford the expected phosphonate ester **142**; instead, we obtained the corresponding ethyl ester **141**. Conseqently, the amidation of 2-chloro-3-phenylpropanoic acid **136** was effected first by converting compound **136** into an acid chloride, using thionyl chloride in DMF at 0 °C, and then reacting the acid chloride directly with the aromatic amines **144b,f,g** (3-bromoaniline, 3-nitroaniline and aniline) at room temperature overnight.[165] Purification by flash chromatography gave the corresponding, novel *N*-aryl-2-chloro-3-phenylpropanamides **143b**, **143f** and **143g** in moderate yields (Table 2, P. 56). Evidence for the formation of the amide products was provided by the ^1H and ^{13}C NMR data. Thus, the ^1H NMR spectrum of 2-chloro-*N*-phenyl-3-phenylpropanamide **143g** (Figure 40) revealed the amide proton resonating as a broad signal at 8.16 ppm and the presence of additional signals in the aromatic region. The ^{13}C NMR spectrum (Figure 41) shows the presence of the amide carbonyl signal at 166.5 ppm, and the requiste total of 11 ^{13}C signals.

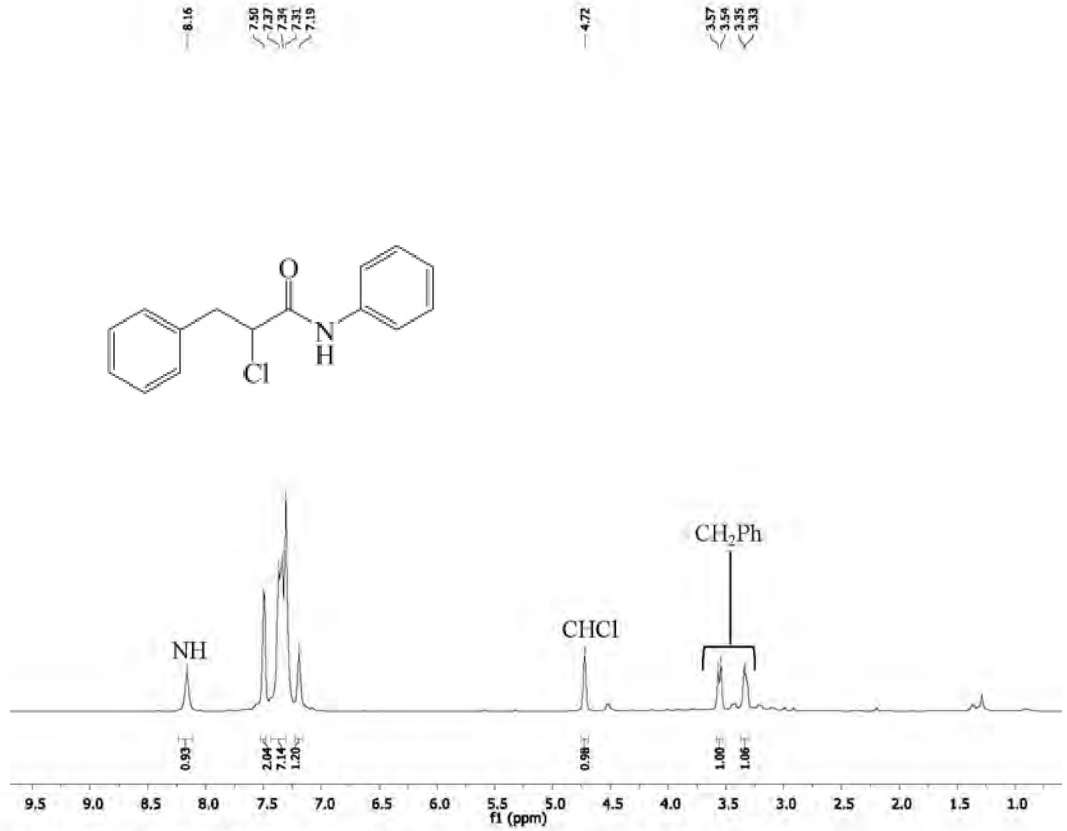


Figure 40. 300 MHz ^1H NMR spectrum of compound **143g** in $CDCl_3$.

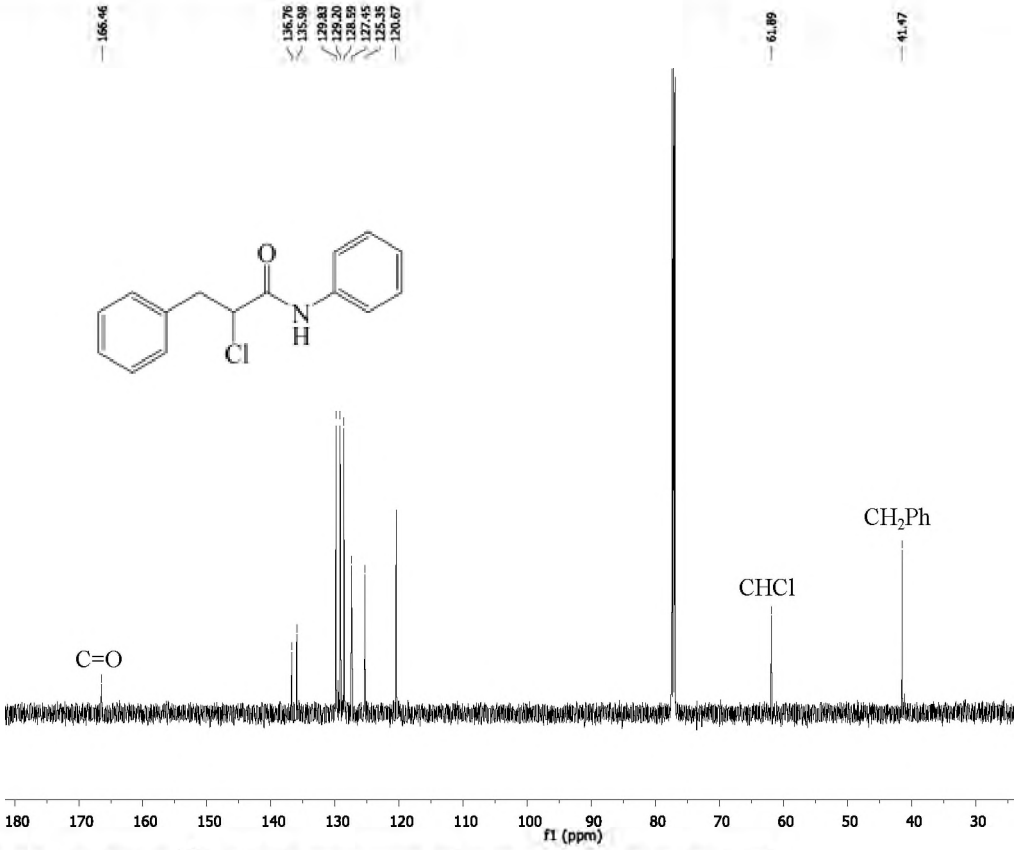


Figure 41. 75 MHz ^{13}C NMR spectrum of compound **143g** in $CDCl_3$.

Michaelis-Arbuzov reactions of compounds **143b**, **143f** and **143g** in refluxing triethyl phosphite (3 eq.) led to the targeted *C*-benzylated (*N*-arylcarbamoyl)phosphonate esters **131b**, **131f** and **131g** in low yields (25-35%). The low yields were due to the repeated attempts to remove the excess triethyl phosphite. Formation of the C-P bond is supported by the characteristic splitting of the *P*-methine proton signal at 3.15-3.44 ppm in the ^{1}H NMR spectrum of compound **131g** (Figure 42), reflecting the coupling with ^{31}P and the adjacent diastereotopic benzylic protons. However, the presence of unreacted triethyl phosphite is evidenced by the overlapping of the phosphite ester methyl and methylene signals with the corresponding phosphonate ester signals at *ca* 1.3 and 4.1 ppm, respectively.

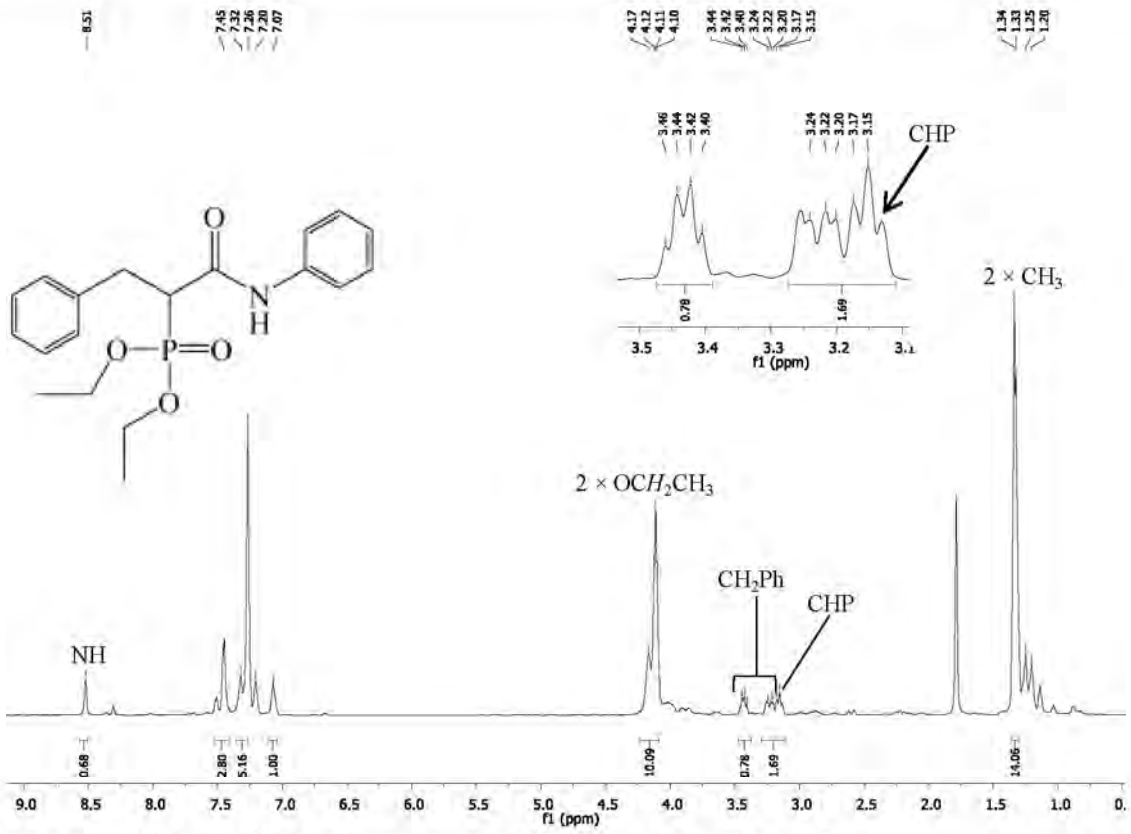
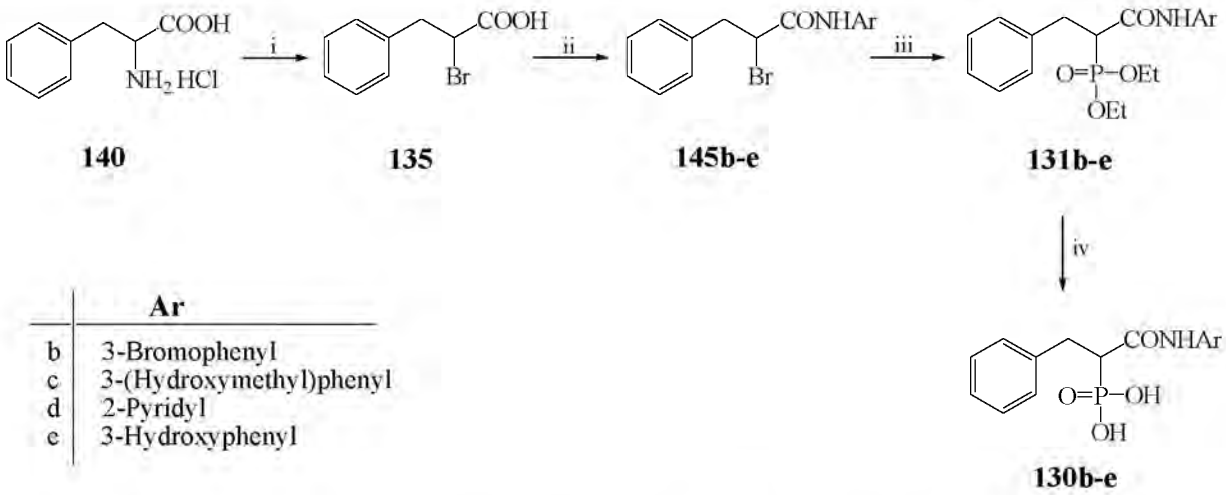


Figure 42. 300 MHz ^{1}H NMR spectrum of compound **131g** in $CDCl_3$.

In view of the low yields obtained for the thre phosphonate esters (**131b**, **131f** and **131g**) and the purification difficulties encountered using 2-chloro-3-phenylpropanoic acid **136**, we envisaged that 2-bromo-3-phenylpropanoic acid **135** could be used more effectively as the critical intermediate, since bromide is a better leaving group than chloride. Therefore, the diazotisation conditions were changed and following reported methods,[166-168] 4M-H_2SO_4 was used in place of 6M-HCl together with 3.5 equivalents of NaBr and 1.25 equivalents of $NaNO_2$ in water at 0 °C to produce 2-bromo-3-phenylpropanoic acid **135**, from phenylalanine hydrochloride **140** in 72% yield without the need for further purification (Scheme 9). Not surprisingly, the ^{1}H NMR spectrum of 2-bromo-3-phenylpropanoic acid **135** (Figure 43) shows close similarity with that of 2-chloro-3-phenylpropanoic acid **138** (Figure 39).



Scheme 9. Synthetic route to *C*-benzylated phosphonate esters and phosphonic acids.

Reagents and conditions:- (i) NaBr, $NaNO_2$, 4M-H_2SO_4, H_2O, 0 °C and then r.t.; (ii) CDI, DCM, $ArNH_2$; (iii) triethyl phosphite, 120-150 °C, 10 h; and (iv) TMSBr, dry DCM, N_2.

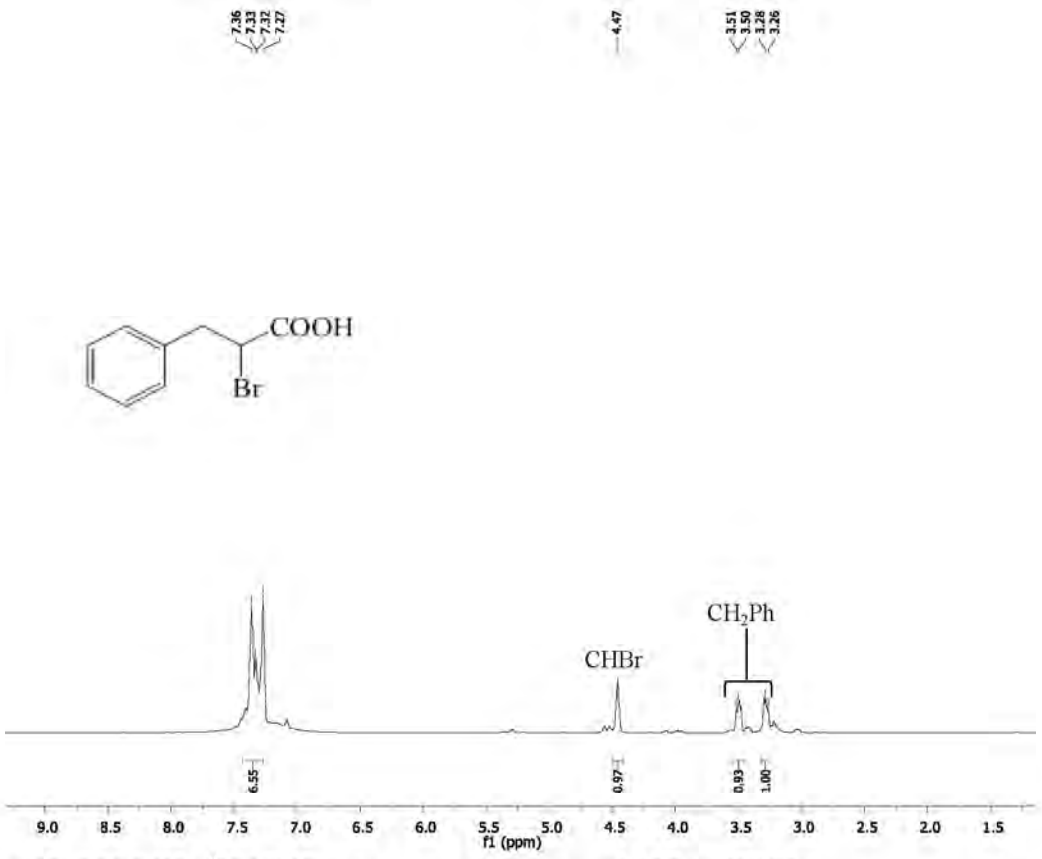


Figure 43. 300 MHz ^{1}H NMR spectrum of compound **135** in $CDCl_3$.

Instead of using the acid chloride to access the *N*-substituted 2-bromopropanamides **145b-e**, as was the case with the 2-chloropropanamide analogues **143b,f,g**, 2-bromo-3-phenylpropanoic

acid **135** was reacted directly with the corresponding 3-substituted aromatic amines **144b-e** in the presence of the coupling agent, 1,1'-carbonyldiimidazole (CDI), in DCM.[169] The crude products were chromatographed to afford the desired novel *N*-substituted 2-bromopropanamides **145b-e** in 60-65% yield (Table 2). The ^{1}H NMR spectrum of 2-bromo-*N*-(3-nitrophenyl)-3-phenylpropanamide **145b** (Figure 44) shows a broad singlet at 7.84 ppm corresponding to the amide proton and a pair of double doublets at 3.34 and 3.61 ppm corresponding to the diastereotopic benzylic protons coupling with the adjacent methine proton and with each other. The ^{13}C NMR spectrum of compound **145b** (Figure 45) reveals the benzylic carbon signal at 41.7 ppm and the expected number of aromatic carbon signals.

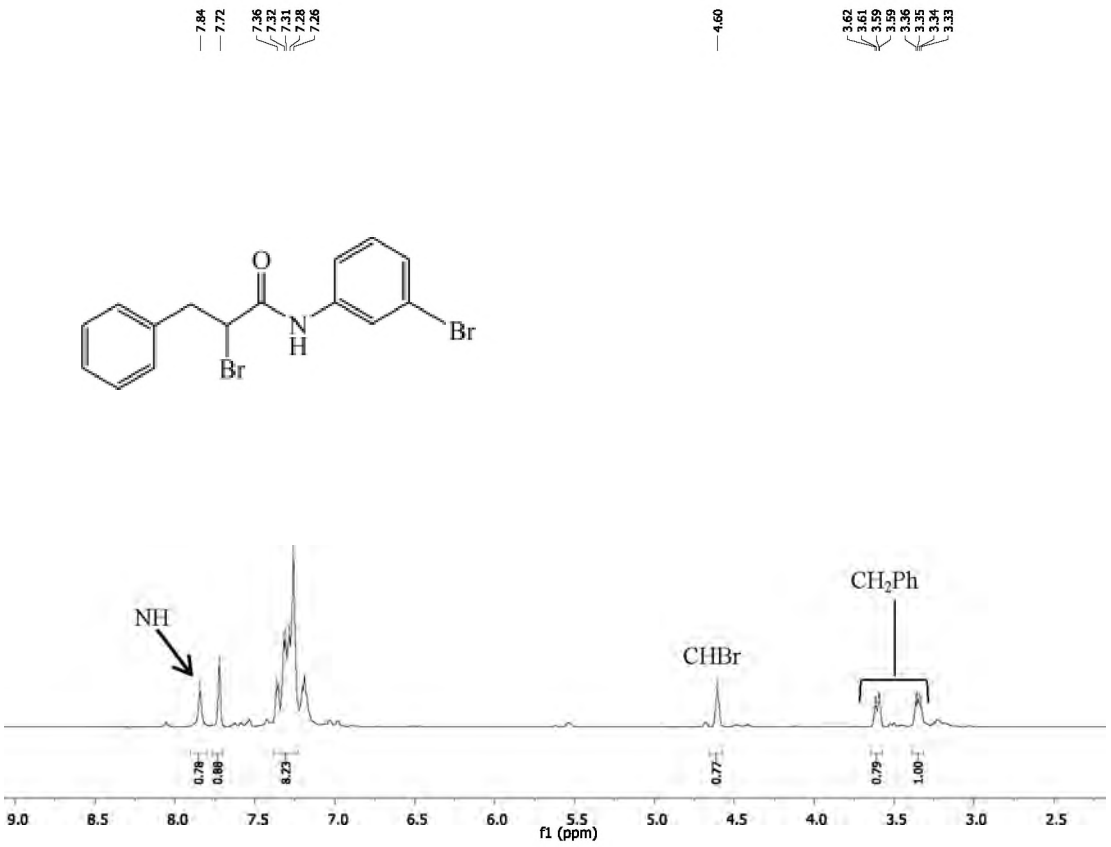


Figure 44. 300 MHz ^{1}H NMR spectrum of compound **145b** in $CDCl_3$.

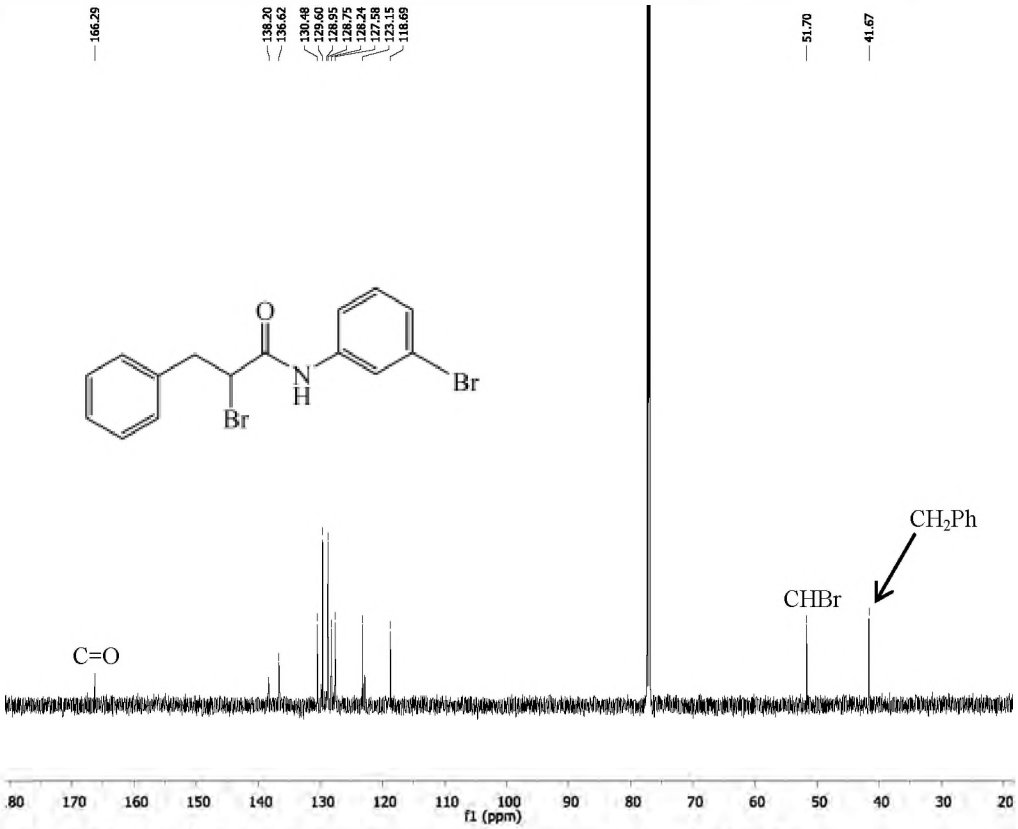
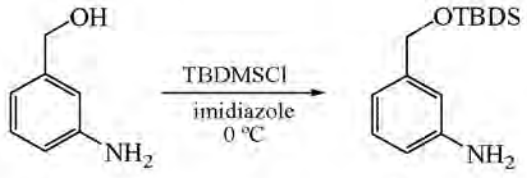


Figure 45. 75 MHz ^{13}C NMR spectrum of compound **145b** in $CDCl_3$.

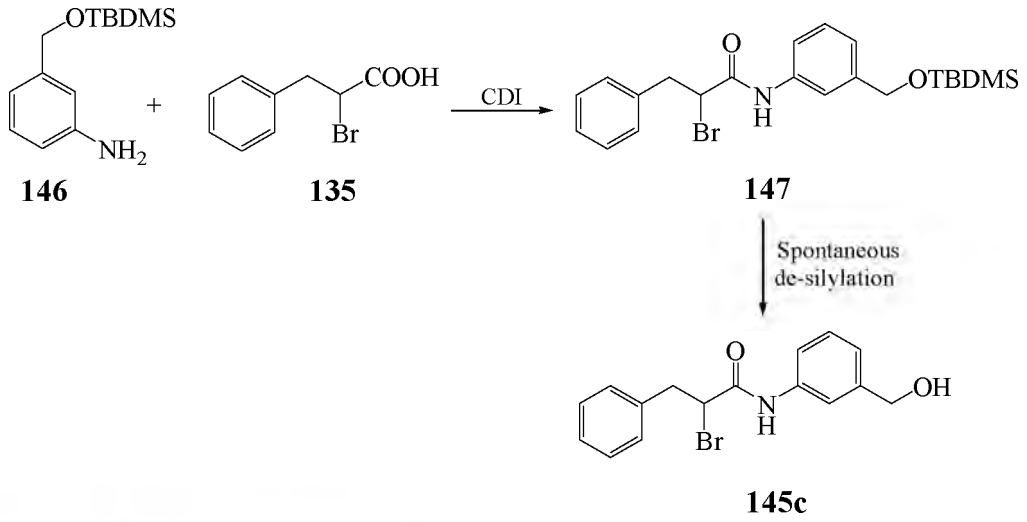
Use of 3-(hydroxymethyl)aniline **144c**, however, afforded not only the desired amide **145c** (42%) but also the undesired *N,O*-diacylated analogue (58%). Consequently, it was necessary to protect the hydroxyl group prior to amidation. As shown in Scheme 10, 3-(hydroxymethyl)aniline **144c** and 1.5 equivalents of imidazole were dissolved in dichloromethane (DCM) at 0 °C, and 1.1 equivalents of *tert*-butyldimethylsilyl chloride (TBDMSCl) were added in portions. The mixture was stirred at room temperature overnight,[170-172] and work-up afforded the TBDMS ether-protected compound **146** in quantitative yield.



144c **146**

Scheme 10. Protection of the benzylic hydroxyl group in compound **144c**.

The TBDMS ether-protected compound **146** was then reacted with CDI and 2-bromo-3-phenylpropanoic acid **135** to afford 2-bromo-*N*-{3-[(*tert*-butyldimethylsilyloxy)methyl]-phenyl}-3-phenylpropanamide **147** (Scheme 11); *in situ* cleavage of the silyl ether **147** occured overnight at room temperature to give 2-bromo-*N*-[3-(hydroxymethyl)phenyl]-3-phenylpropanamide **145c**. The formation of these compounds was confirmed using HRMS, NMR and IR analysis. In the ^{1}H NMR spectrum of compound **147** (Figure 46), the presence of the silyl ether group is evidenced by the methyl and *t*-butyl singlets at 0.04 and 0.79 ppm, respectively. The ^{13}C NMR spectrum of compound **147** (Figure 47) also confirms the presence of the silyl ether groups and the amide carbonyl carbon which resonates at 166.1 ppm. The decomposition of compound **147** is clearly supported by the significant decrease in the intensity of the silyl ether signals in the ^{1}H NMR spectrum of compound **145c** (Figure 48).



146 **135** **147** **145c**

Scheme 11. Synthesis of compound **145c**.

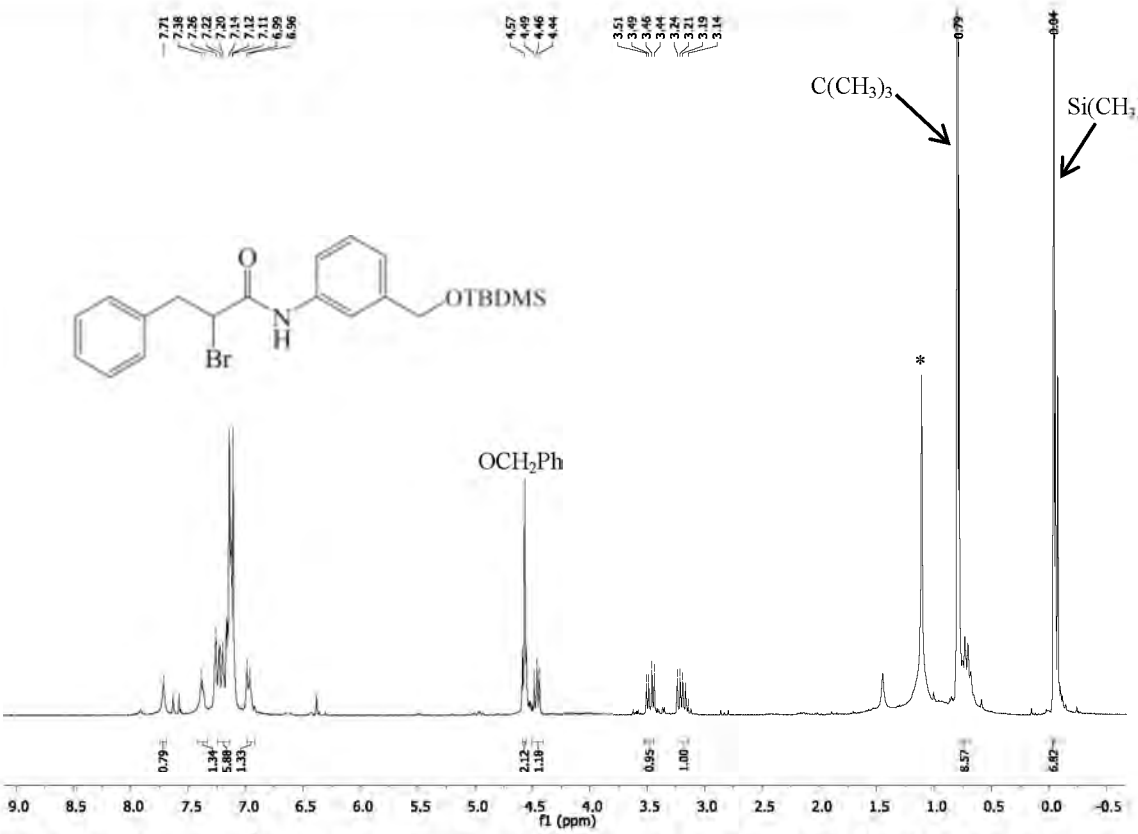


Figure 46. 300 MHz ^{1}H NMR spectrum of compound **147** in $CDCl_3$. $^{*}H_2O$ in $CDCl_3$.

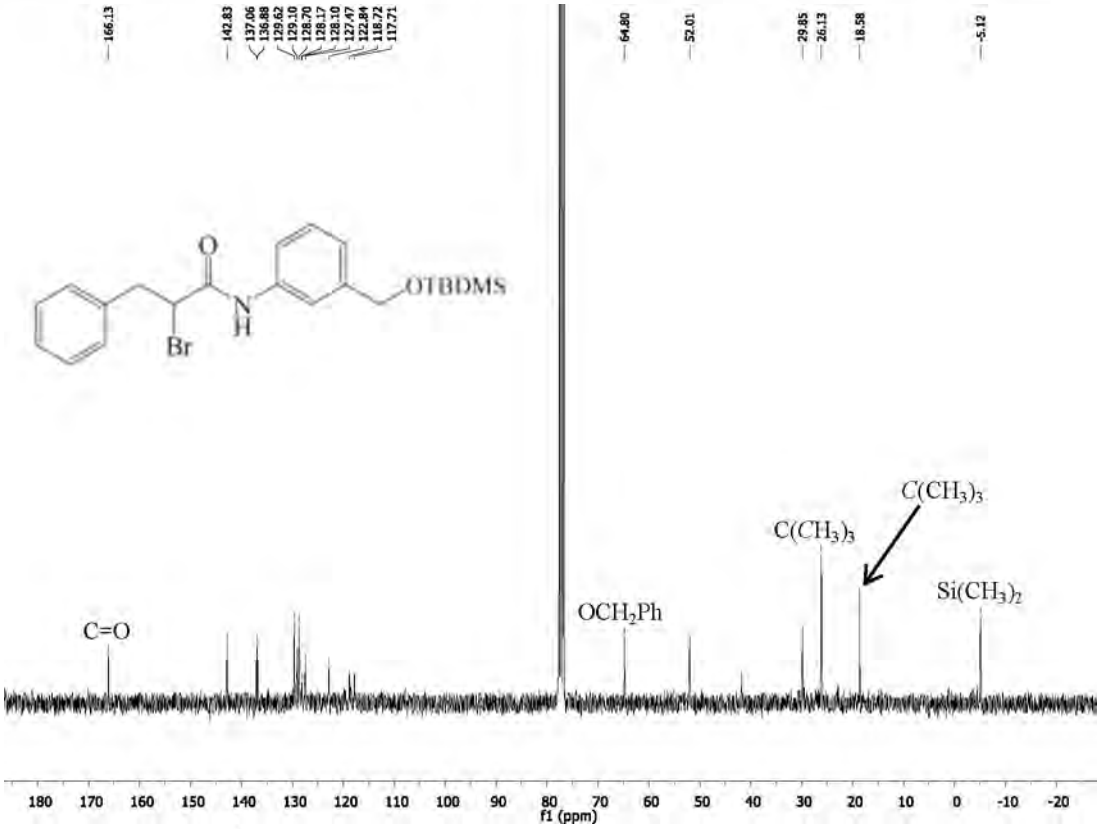


Figure 47. 300 MHz ^{1}H NMR spectrum of compound **147** in $CDCl_3$.

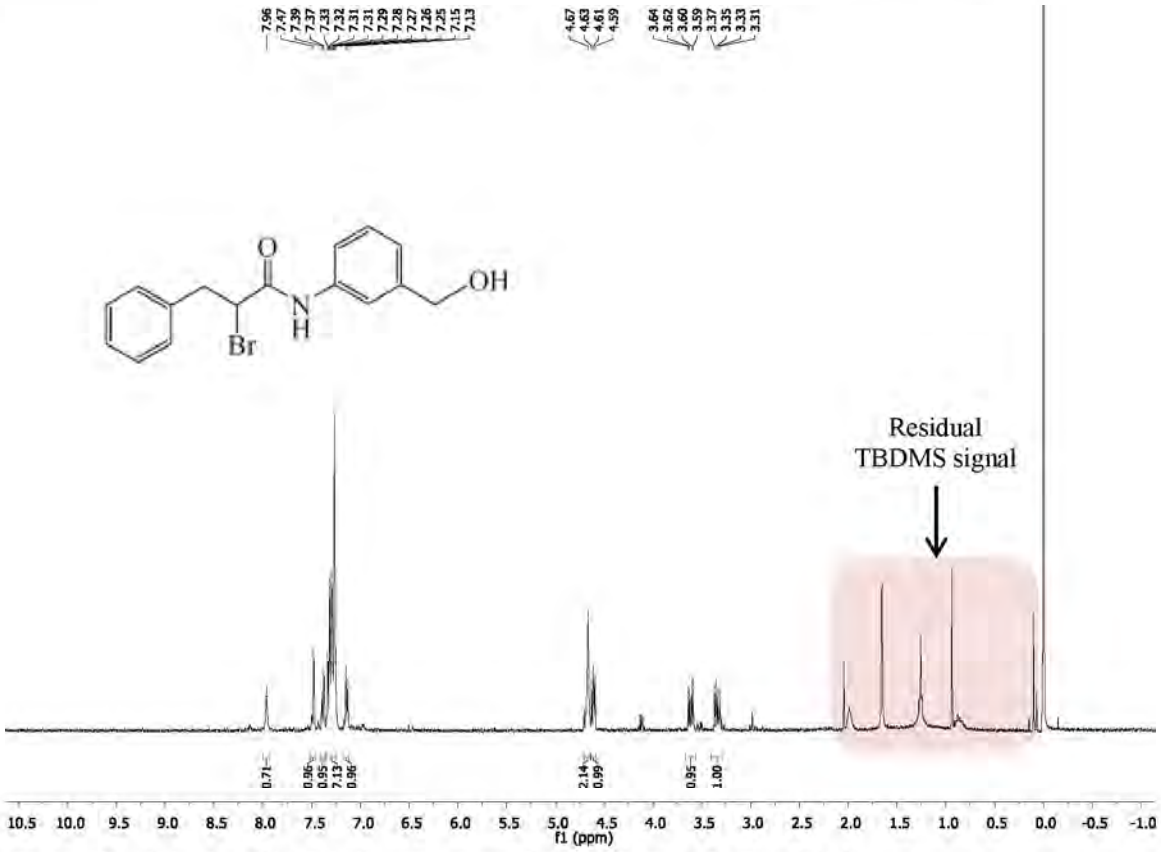


Figure 48. 300 MHz ^{1}H NMR spectrum of compound **145c** in $CDCl_3$.

Having successfully obtained the *N*-substituted 2-bromopropanamides **145b-e**, the phosphonation step was accomplished using the Michaelis-Arbuzov reaction (Scheme 9).[163,164] Thus, the *N*-substituted 2-bromopropanamides **145b-e** were reacted with triethyl phosphite under reflux in an inert atmosphere. Purification by flash column chromatography gave the phosphonate esters **131b-e** in yields of 32-61% (Table 2).

Table 2. Isolated yields of compounds **145b-e**, **143b,f,g** and **131b-f**

Compound	Yield (%)	Compound	Yield (%)
145b	69[a]	**131b**	61
145c	60	**131c**	49
145d	63	**131d**	32
145e	63	**131e**	59
143f	65	**131f**	36
143g	65	**131g**	25

[a] % yield of compound **143b** = 60%

The ^{1}H NMR spectra (illustrated for compound **131b** in Figure 49) of the phosphonate esters **131b-f** generally show a triplet and a multiplet at *ca* 1.2 and 4.1 ppm, respectively, corresponding to the phosphonate ester methyl and *O*-methylene protons. Formation of the desired compounds is further supported by the presence of the *P*-methine signal at *ca* 3.1 ppm (Figure 50). The products were all characterized using HRMS, NMR and IR analysis. The ^{13}C NMR spectrum of compound **131b** (Figure 50) also reveals a methyl carbon doublet at 16.5 ppm and a *pair* of *O*-methylene carbon doublets, at 63.0 and 63.7 ppm (Figure 50). The doublets reflect ^{13}C-^{31}P coupling, but the observation of a *pair* of such doublets reflects the inherent diastereotopicity of the *O*-methylene carbons which not only have different chemical shifts but also slightly different coupling constants ($J_{P,C}$ = 33.6 and 34.1 Hz). The DEPT 135 NMR spectrum (Figure 51) clearly confirms the critical assignments of the benzylic methylene and *P*-methine signals, while the COSY NMR spectrum (Figure 52) shows the critical proton-proton correlations.

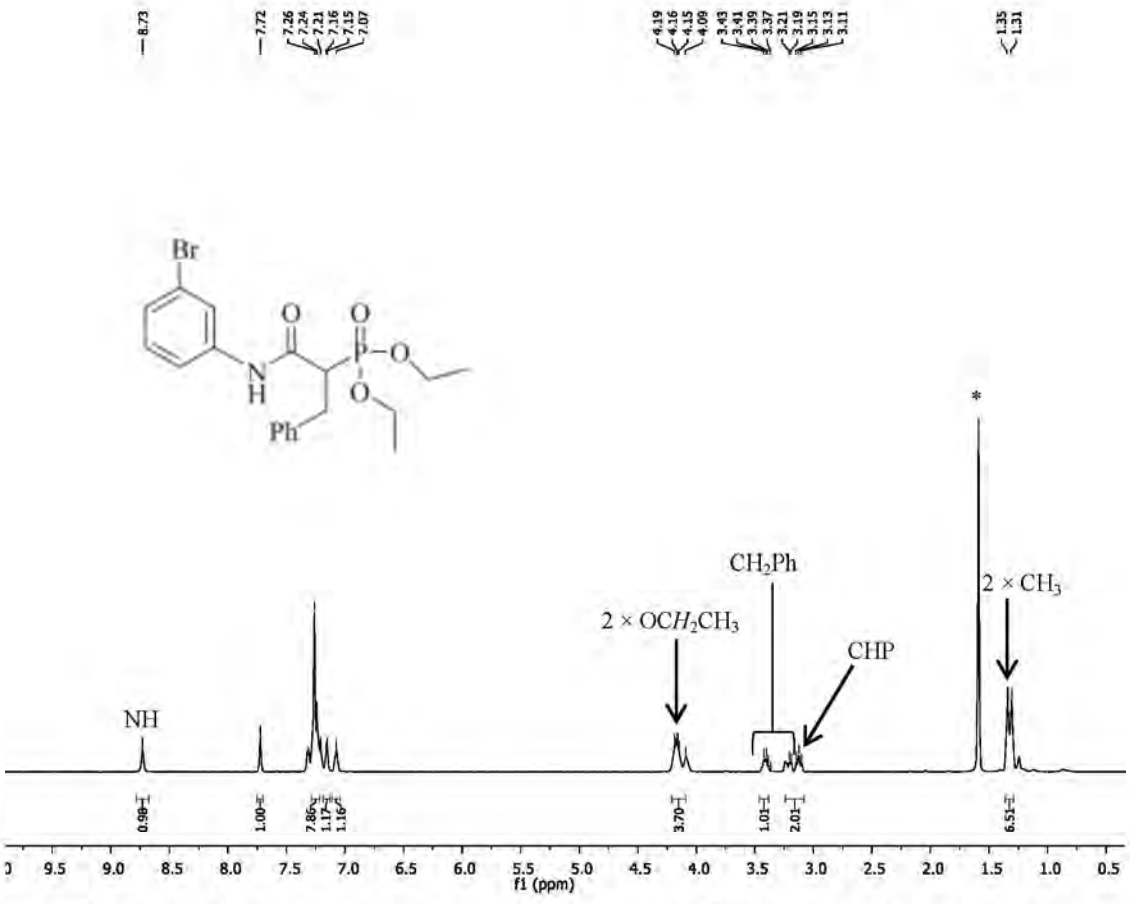


Figure 49. 300 MHz ^{1}H NMR spectrum of compound **131b** in $CDCl_3$. $^{*}H_2O$ in $CDCl_3$.

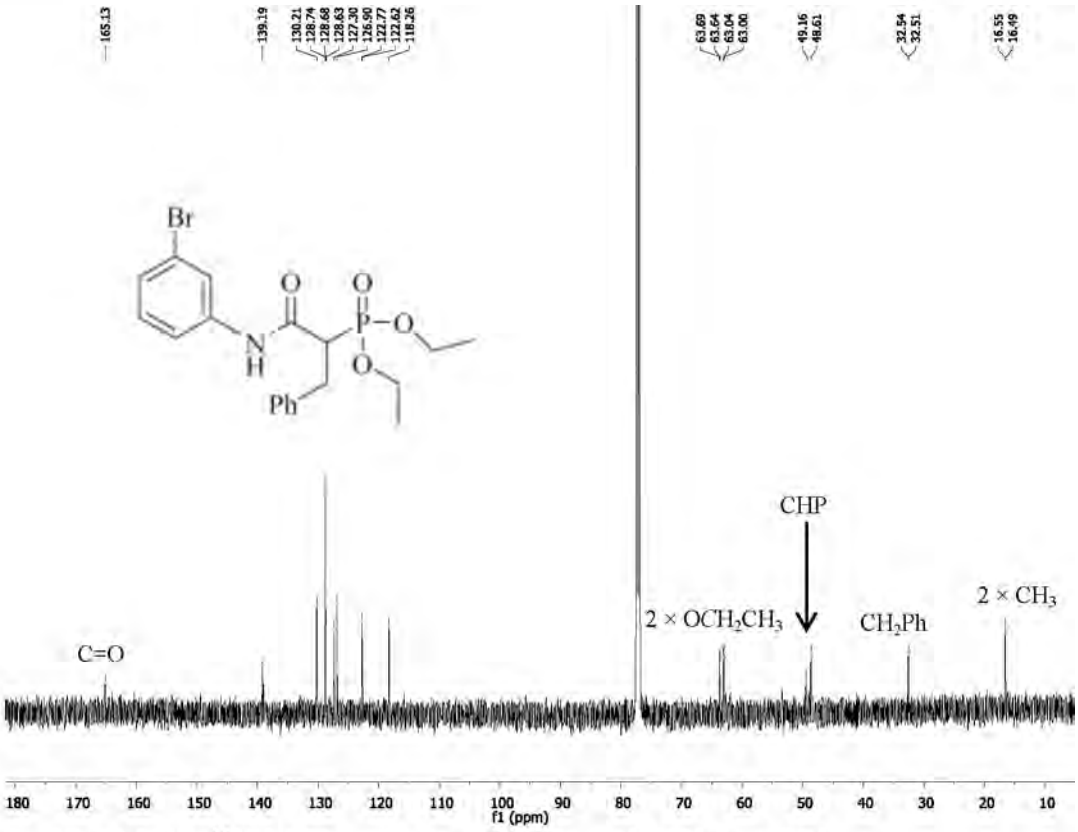


Figure 50. 75 MHz ^{13}C NMR spectrum of compound **131b** in $CDCl_3$.

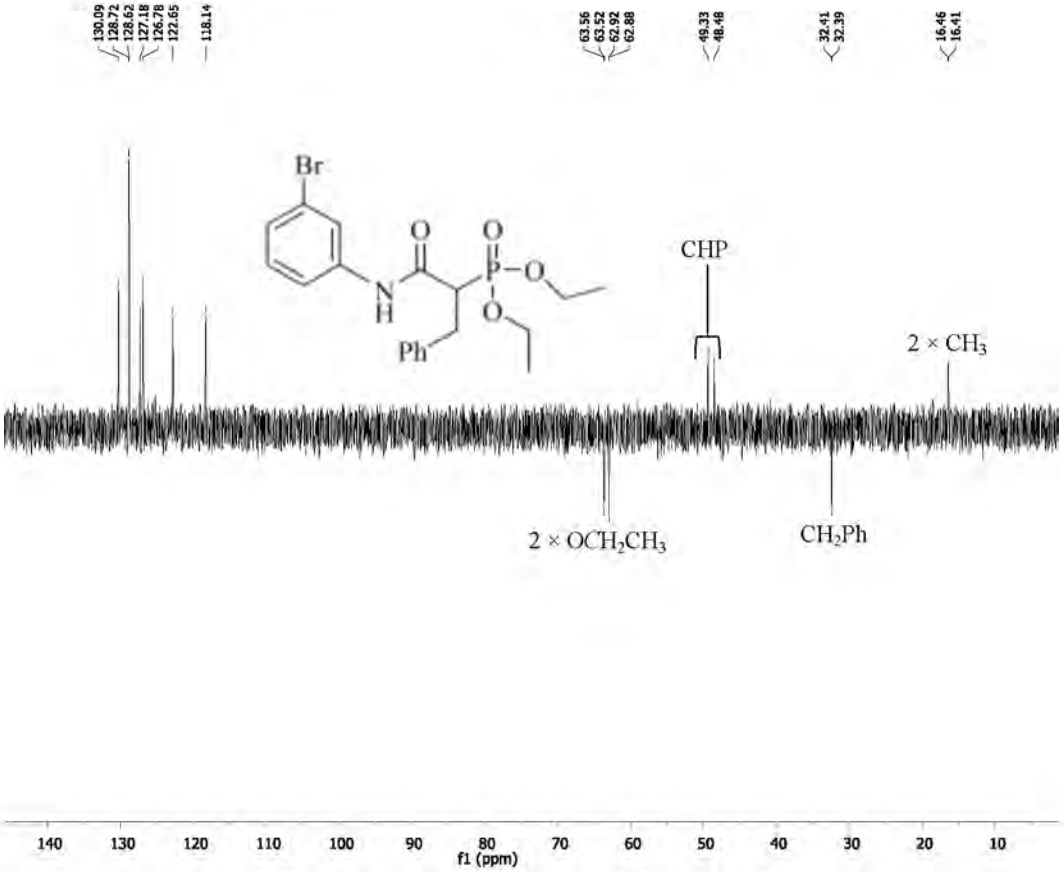


Figure 51. DEPT 135 NMR spectrum of compound **131b** in $CDCl_3$.

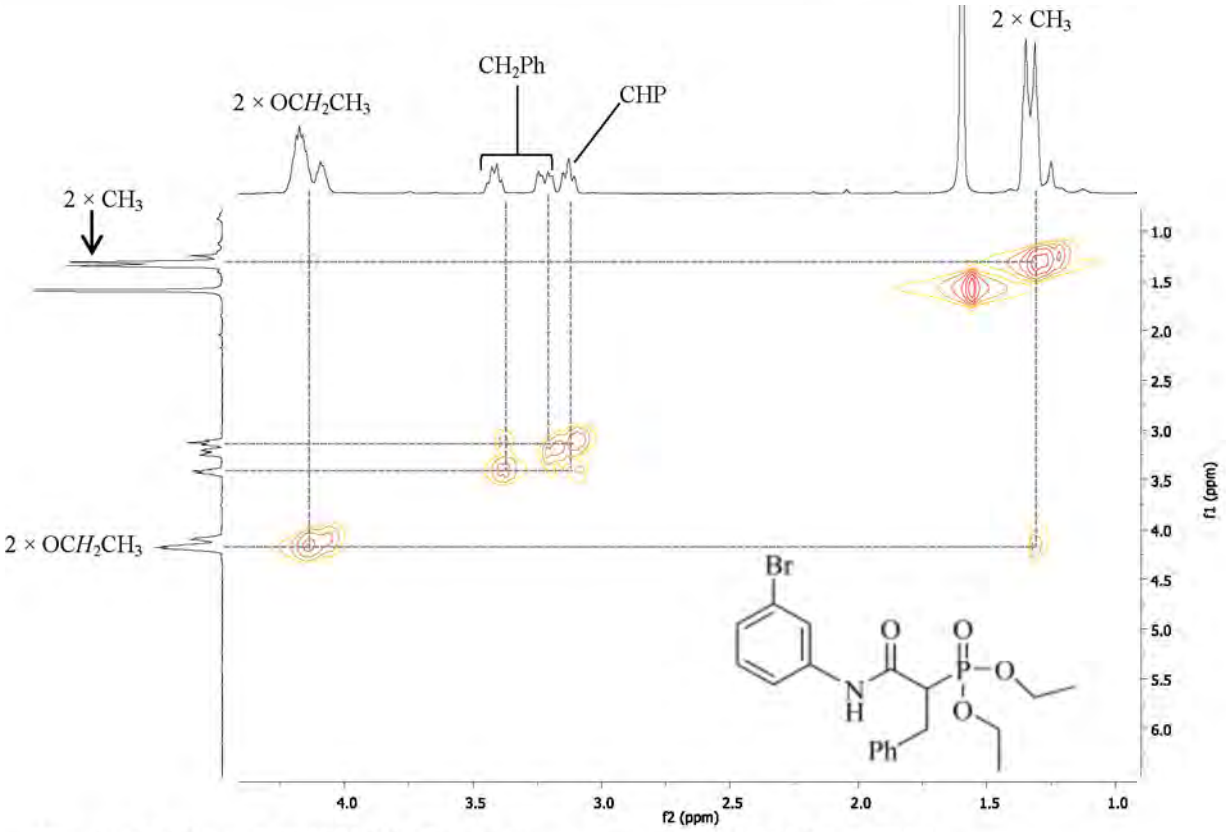


Figure 52. Partial COSY NMR spectrum of compound **131b** in $CDCl_3$.

Although, we envisaged that the purified phosphonate esters could serve directly as possible pro-drugs undergoing enzymic hydrolysis in the *P.falciparum* parasite (*p*LDH) bioassays, *in vitro* evaluation of specific *Pf*DXR inhibition potential was expected to require the use of the phosphonic acid analogues. Consequently, two of the phosphonate esters **131b** and **131e** were reacted with bromotrimethylsilane in dry DCM to form the corresponding bis-trimethylsilyl phosphonic acid diesters; hydrolysis upon treatment with H_2O gave the corresponding representative acids **130b** and **130e** in reasonable yields.[169,173,174] These acids are also new and were fully characterised using spectroscopic analysis. The ^{1}H and ^{13}C NMR spectra of phosphonic acid **132e** (Figures 53 and 54) confirm the absence of the phosphonate ethyl ester groups.

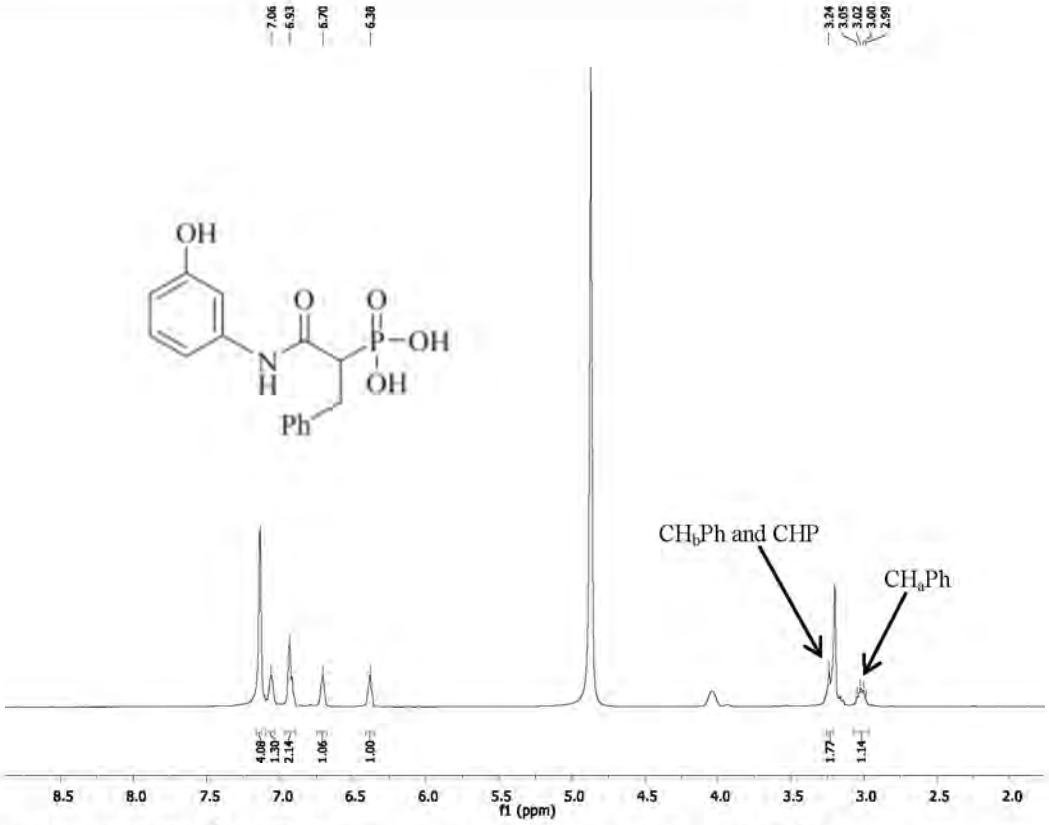


Figure 53. 300 MHz ^{1}H NMR spectrum of compound **130e** in CD_3OD.

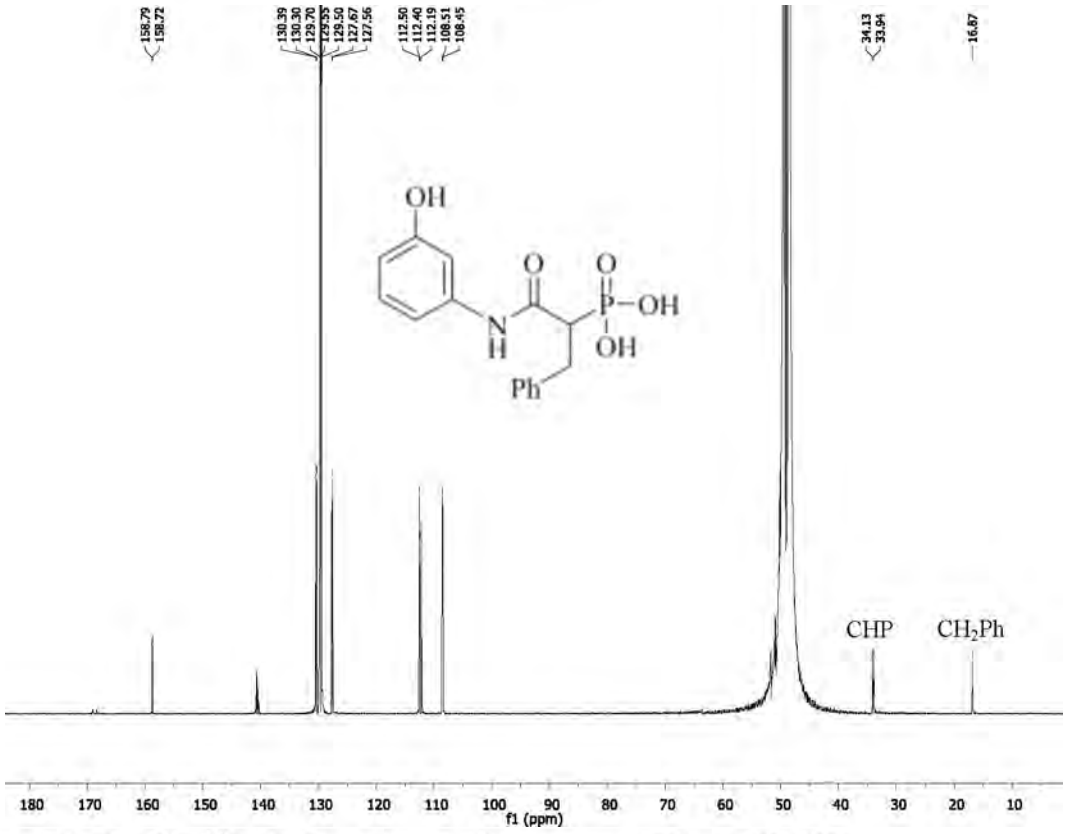


Figure 54. 75 MHz ^{13}C NMR spectrum of compound **130e** in CD_3OD.

2.2. SYNTHESIS of *N*-SUBSTITUTED PHOSPHORAMIDIC ACID DERIVATIVES as "REVERSE" FOSMIDOMYCIN ANALOGUES

Research on several fosmidomycin analogues has led to the preparation of promising ligands **148** which Brücher *et al.*, showed to have high inhibitory activity against *Pf*DXR (Figure 55).[175,176] In addition to the reverse arrangement of the hydroxamate moiety, these compounds contained an α-phenyl substituent capable of occupying the hydrophobic pocket adjacent to the *Pf*DXR active-site. Since our project has focused on the introduction of such hydrophobic moieties, we envisaged the development of phosphoramidic acid analogues **149** and **150** in which the hydrophobic aryl group was attached to nitrogen rather than an sp^3 carbon (as in compound **148**), thus obviating chirality issues.

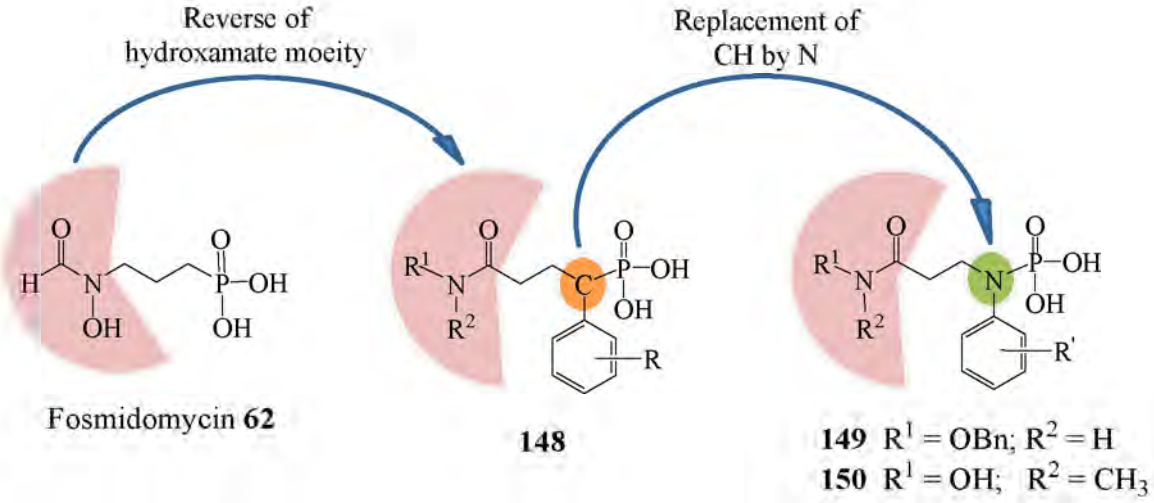
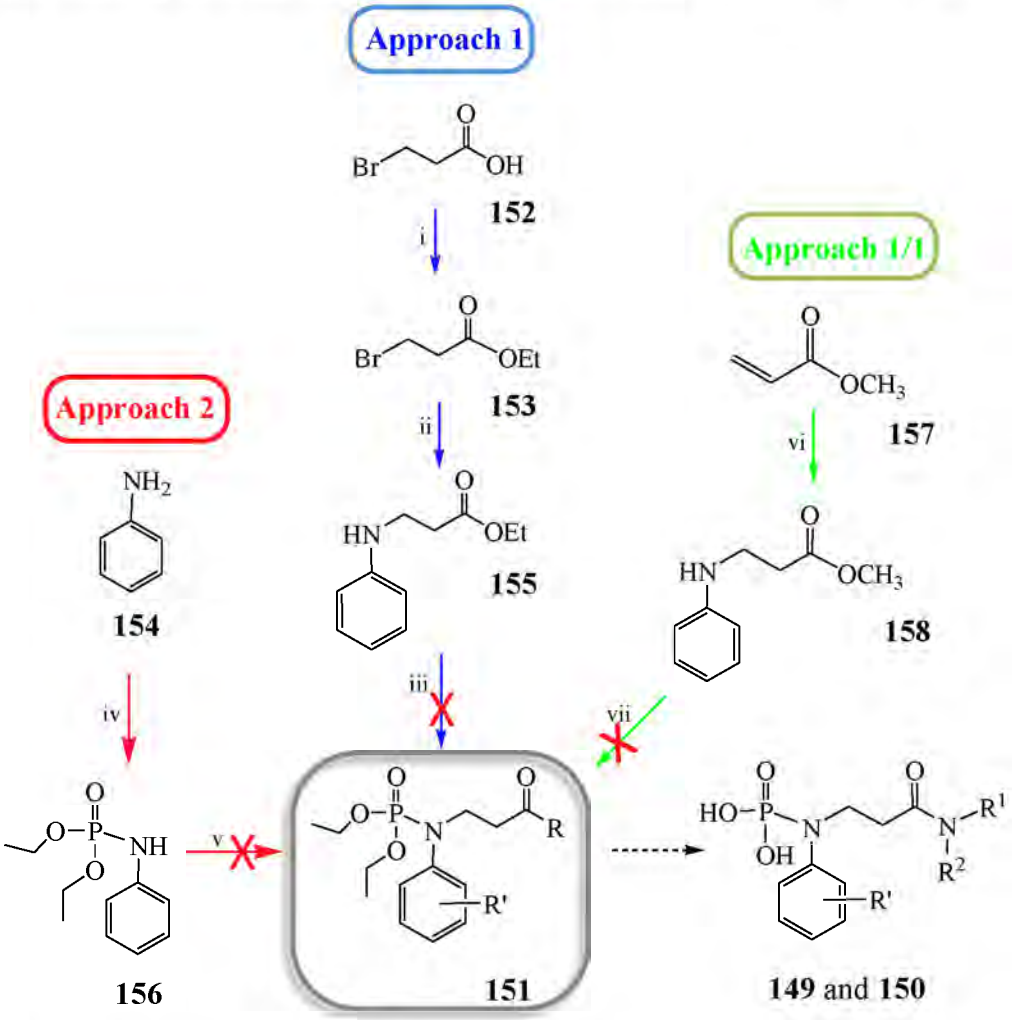


Figure 55. Novel *N*-aryl analogues **149** and **150** of ligands **148**.

In designing the synthesis of these reverse hydroxamate analogues, we identified compound **151** as the critical intermediate and several approaches to this compound were explored (Scheme 12). Our first attempt started from 3-bromopropanoic acid **152**, as it contains the bimethylene moiety required in our target ligands and the functionality needed to generate the reverse hydroxamate moiety. Ethyl 3-bromopropanoate **153**,[177-180] obtained by acid-catalysed esterification of the acid **152**, was reacted with aniline **154** and NaH (as a base to deprotonate aniline) in dry THF to furnish compound **155**. Introduction of the phosphonyl moiety by reacting the aniline derivative **155** with diethyl chlorophosphate and triethylamine in DCM was expected to afford to desired intermediate **151**. However, this approach failed and attempts to activate the amine nitrogen by deprotonation using sodium hydride and BuLi also failed to provide access to compound **151** (Scheme 12).

In the second approach, aniline **154** was first phosphonylated to give compound **156**, but attempts to alkylate this compound with ethyl 3-bromoethylpropanoate **153** using either NaH in dry THF (stirring for 2 days and then refluxing for 5 h) or BuLi were similarly unsuccessful.

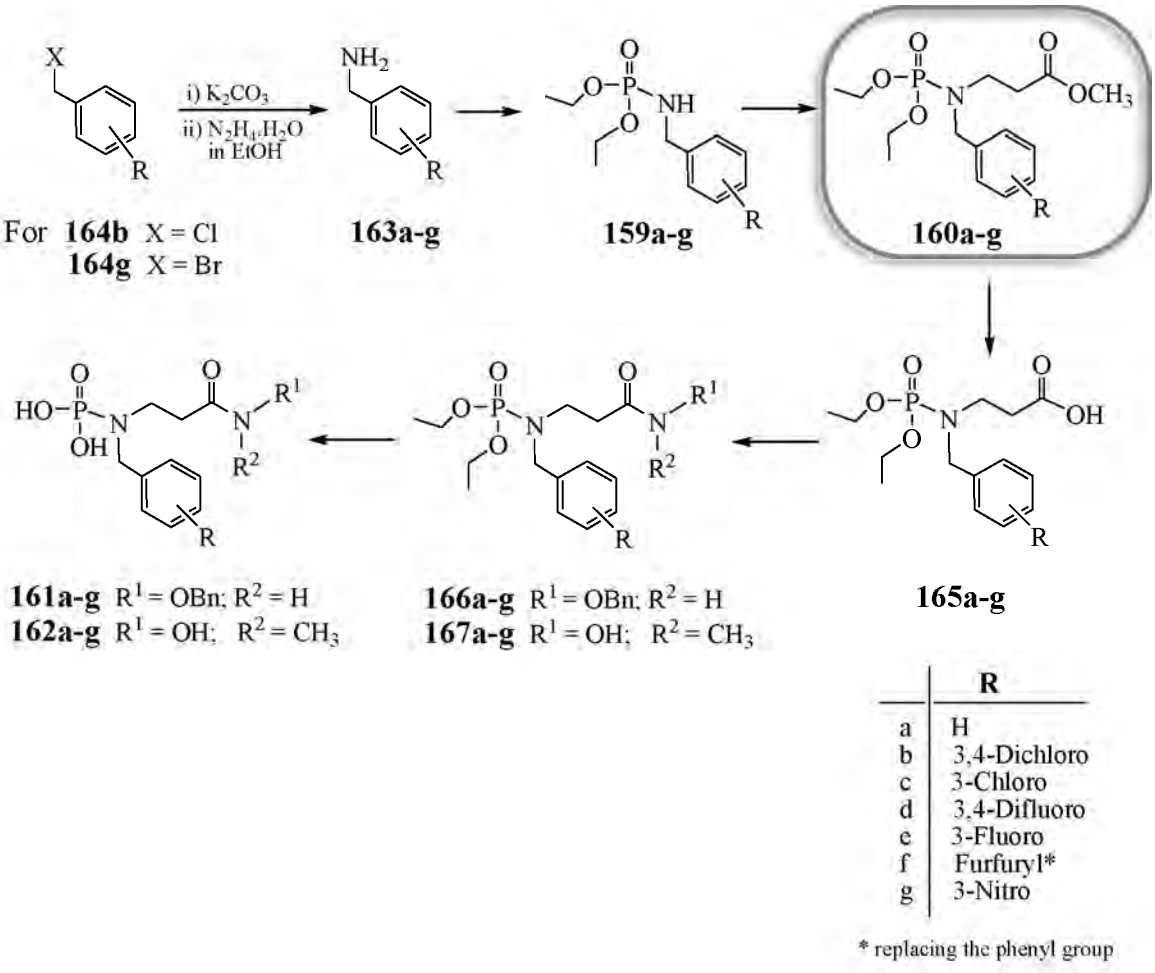


Scheme 12. Attempted synthesis of the intermediate **151**.

Reagents and conditions:- (i) H_2SO_4, EtOH, reflux, overnight; (ii) NaH, dry THF; (iii) diethyl chlorophosphate, triethylamine, DCM; or NaH or BuLi, dry THF; (iv) diethyl chlorophosphate, NaH, dry THF; (v) NaH or BuLi, dry THF; (vi) toluene, aniline refluxed overnight; and (vii) diethyl chlorophosphate, NaH or BuLi, dry THF.

In a variation on approach 1, a solution of methyl acrylate **157** and aniline **154** in toluene was refluxed overnight (Scheme 12) to afford the methyl ester **158** directly.[181-182] Attempts to phosphorylate the aza-Michael addition product **158** using diethyl chlorophosphate and triethylamine in dry DCM also failed even after using NaH or BuLi as base on different occassions. However, a similar aza-Michael reaction was to prove useful later.

Given the difficulties encountered in the synthesis of intermediate **151**, we decided to change the approach and generate the *N*-benzylated phosphoramidates (**159**) first and then attach the (alkyloxycarbonyl)ethyl group to give the critical intermediates (**160**) in the proposed synthesis of the *N*-benzylated "reverse" fosmidomycin analogues (**161** and **162**) as outlined in Scheme 13. While a number of amines (**163**) were available, 3,4-dichlorobenzylamine **163b** and 3-nitrobenzylamine **163g** were obtained by reacting the corresponding benzyl halides **164b,g** with potassium phthalimide,[183-184] followed by hydrazinolysis of the resulting *N*-benzylphthalimides.[185-187]

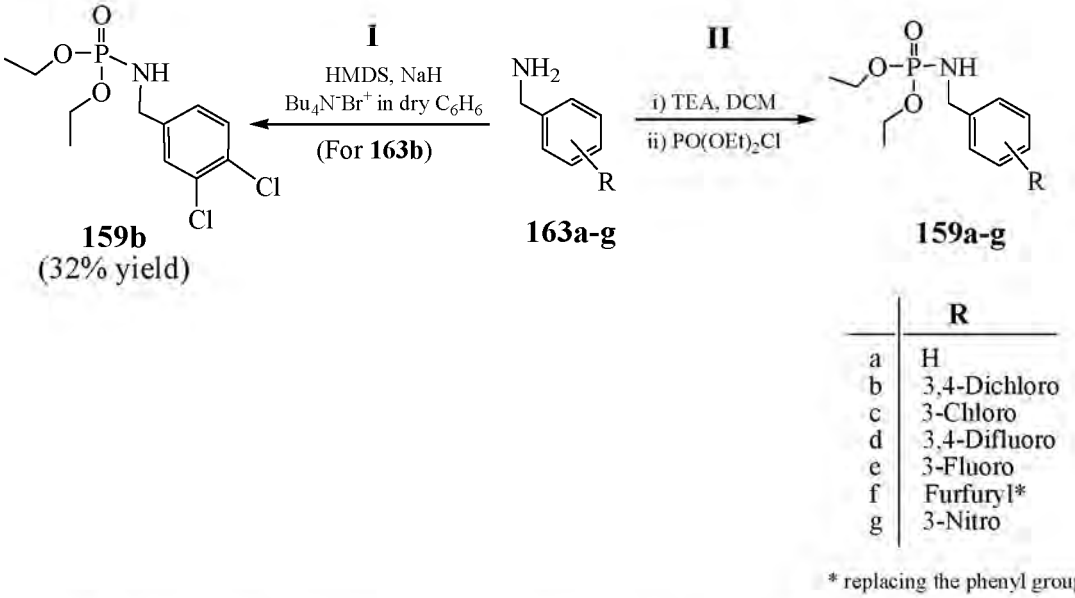


Scheme 13. Third proposed pathway to the *N*-benzylated "reverse" fosmidomycin analogues.

2.2.1. Synthesis of *N*-benzylphosphoramidate esters

Following the method described by Zwierzak,[188] 3,4-dichlorobenzyl chloride **164b** was reacted with diethyl phosphoramidate **168** using sodium hexamethyldisilazide as a base to give compound **159** in low yield together with side products (Path I; Scheme 14). In an alternative approach (Path II), the *N*-benzylated compounds **159a-g** were prepared by treating the amines

163a-g with triethylamine in DCM and then with diethyl chlorophosphate to furnish the *N*-benzylphosphoramidate **159a-g** in yields of up to 95% (Table 3) without the need for further purification.[189-191]



	R
a	H
b	3,4-Dichloro
c	3-Chloro
d	3,4-Difluoro
e	3-Fluoro
f	Furfuryl*
g	3-Nitro

* replacing the phenyl group

Scheme 14. Formation of the *N*-benzylphosphoramidate esters **159a-g**.

The ^{1}H NMR spectra of compounds **159a-g** showed the presence of the characteristic diethyl phosphoramidate ester triplet at *ca* 1.2 ppm (J = 7.1 Hz) and multiplet at *ca* 3.9 ppm; the benzylic protons resonate as a doublet at *ca* 4.0 ppm due to coupling with ^{31}P, as shown for compound **159c** in Figure 56. The DEPT 135 NMR spectrum of compound **159c** (Figure 57) confirms the presence of the benzylic methylene signal at 44.6 ppm and four aromatic methine signals.

Table 3. Yields obtained for the diethyl *N*-benzylphosphoramidate esters **159a-g**.

Compound	Yield (%)
159a	90
159b	93
159c	87
159d	95
159e	93
159f	92
159g	93

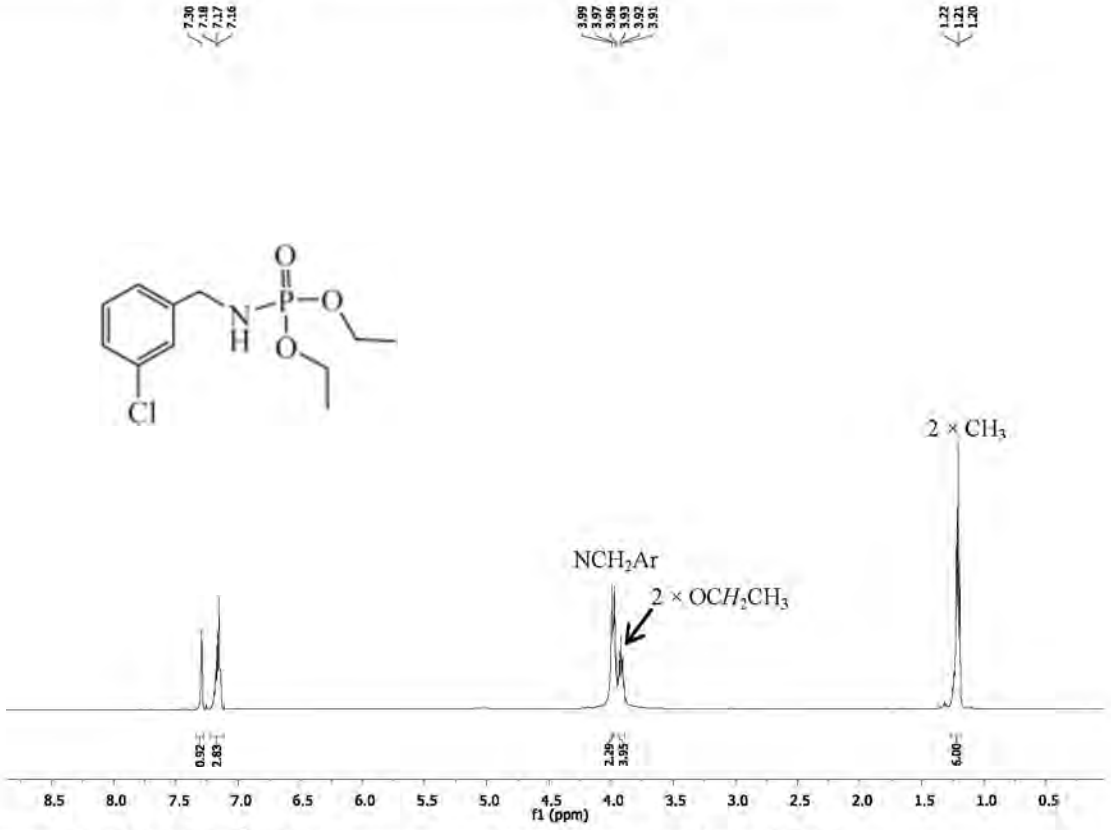


Figure 56. 600 MHz ^{1}H NMR spectrum of compound **159c** in $CDCl_3$.

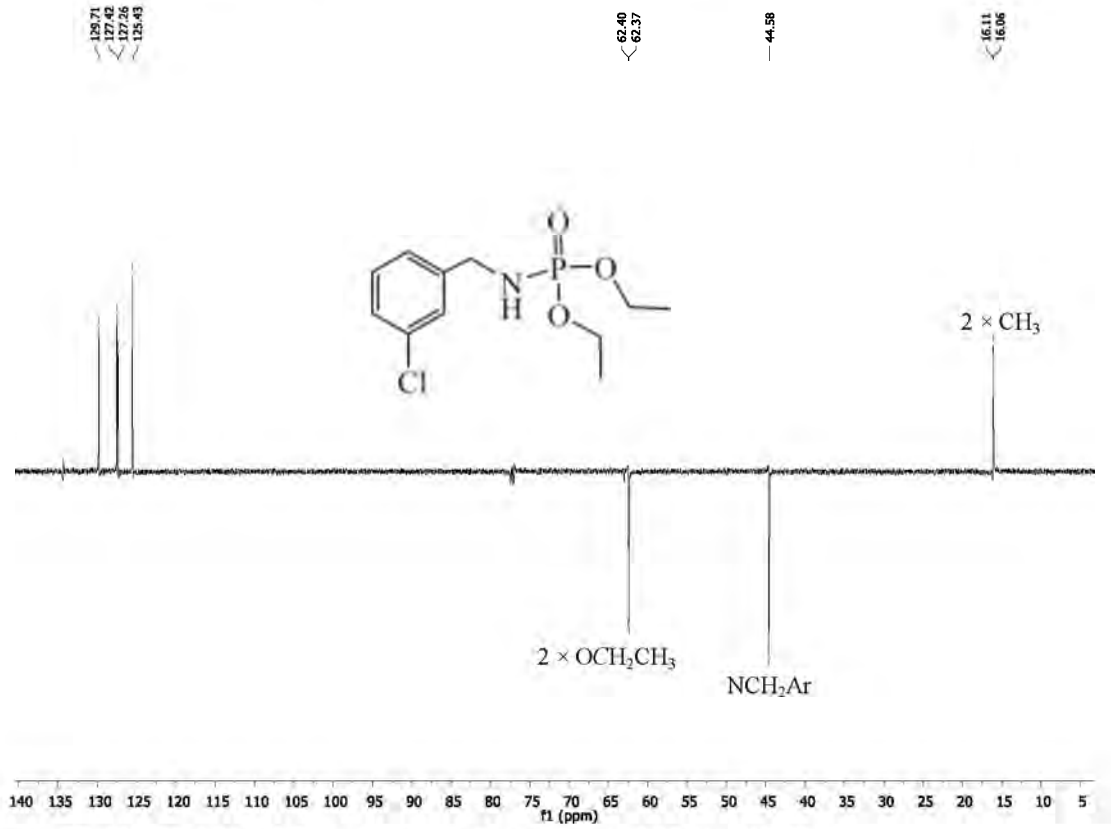
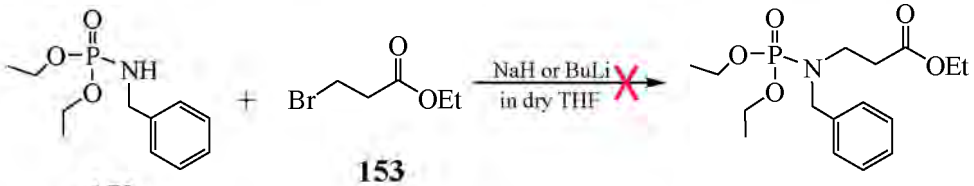


Figure 57. DEPT 135 NMR spectrum of compound **159c** in $CDCl_3$.

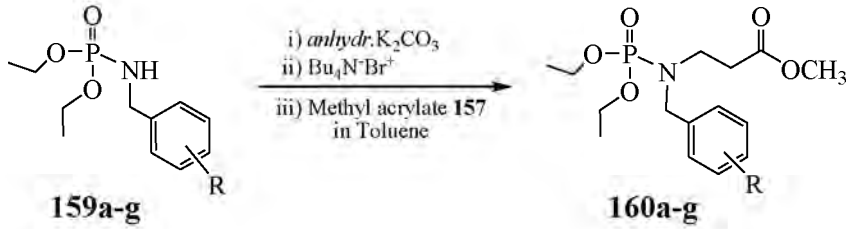
2.2.2. Aza-Michael addition reactions of diethyl *N*-benzylphosphoramidate esters to α,β-unsaturated esters

Several approaches to the synthesis of the intermediates **169** (Scheme 15), were also attempted. Reaction of ethyl 3-bromopropanoate **153** with diethyl *N*-benzylphosphoramidate **159a** (following deprotonation of the amide proton using NaH or BuLi in dry THF) failed to give the desired product **169**. Attention was therefore turned from an S_N approach to aza-Michael addition, as reported by Zwierzak *et al.*[192] and, as used earlier (Approach 1/1; Scheme 12).



Scheme 15. Attempted synthesis of intermediate **169**.

Thus, solutions of the *N*-benzylated phosphoramidate esters **159a-g** in toluene were each heated under reflux for 8 h with 2 equivalents of finely powdered anhydrous K_2CO_3, 0.1 equivalent of $Bu_4N^+Br^-$ and 1.2 equivalents of methyl acrylate (Scheme 16). Work-up and flash chromatography afforded the diethyl *N*-benzylphosphoramidate esters **160a-g** in yields of 56-84% (Table 4).

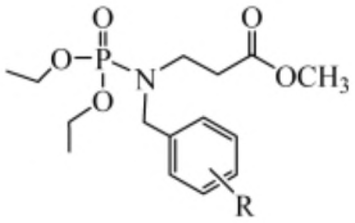


	R
a	H
b	3,4-Dichloro
c	3-Chloro
d	3,4-Difluoro
e	3-Fluoro
f	Furfuryl*
g	3-Nitro

* replacing the phenyl group

Scheme 16. Synthesis of the *N*-benzylated phosphoramidate esters **160a-g**.

Table 4. Yields obtained for the diethyl *N*-benzylphosphoramidate esters **160a-g**.



R	Compound	Yield (%)
H	**160a**	57
3,4-Dichloro	**160b**	84
3-Chloro	**160c**	77
3,4-Difluoro	**160d**	78
3-Fluoro	**160e**	69
Furfuryl*	**160f**	76
3-Nitro	**160g**	83

In the ^{1}H NMR spectrum of compound **160a**, for example (Figure 58), there are, in addition to the phosphoramidate diethyl ester multiplets at 1.31 and 4.02 ppm, a singlet at 3.62 ppm corresponding to the methyl ester and the two new multiplets at 2.49 and 3.23 ppm, reflecting successful introduction of the bimethylene spacer. The ^{13}C NMR spectrum of compound **160a** (Figure 59) confirms the presence of the expected ester carbonyl carbon resonating at 172.2 ppm. The methyl, methylene and diethyl phosphoramidate signals were assigned using the DEPT 135 NMR (Figure 60) and COSY NMR (Figure 61) spectra which show the correlations between relevant nuclei.

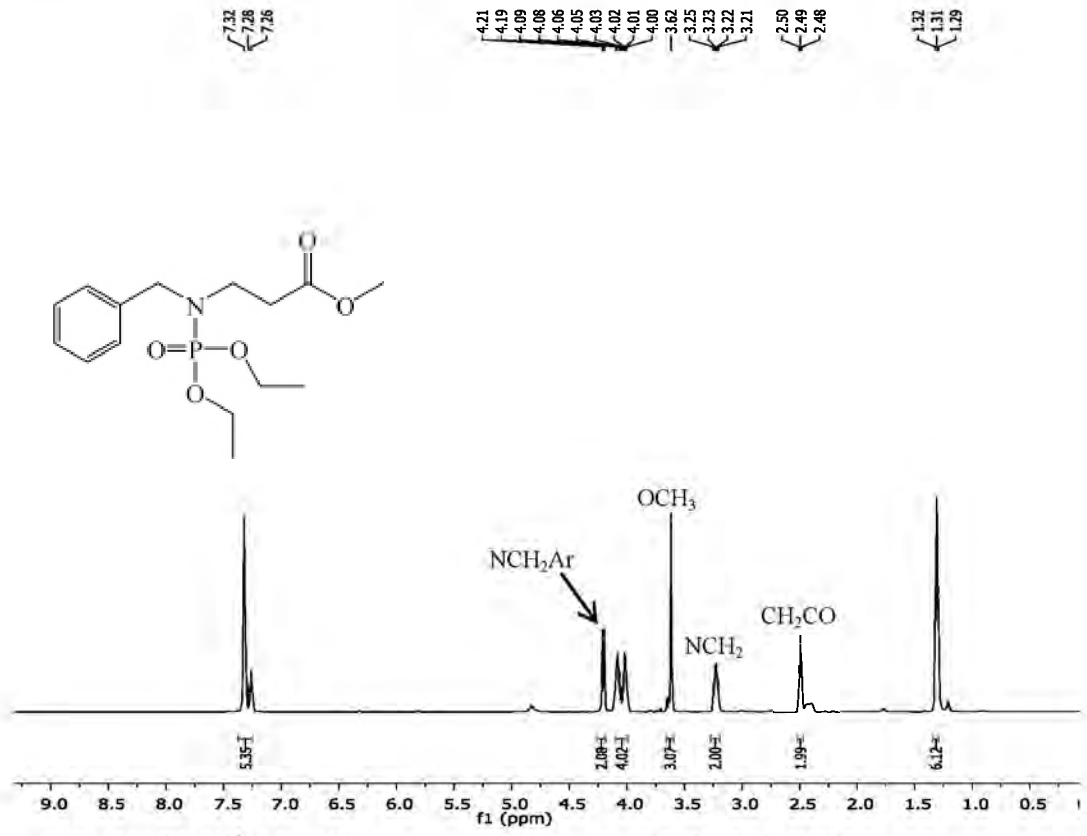


Figure 58. 600 MHz ^{1}H NMR spectrum of compound **160a** in $CDCl_3$.

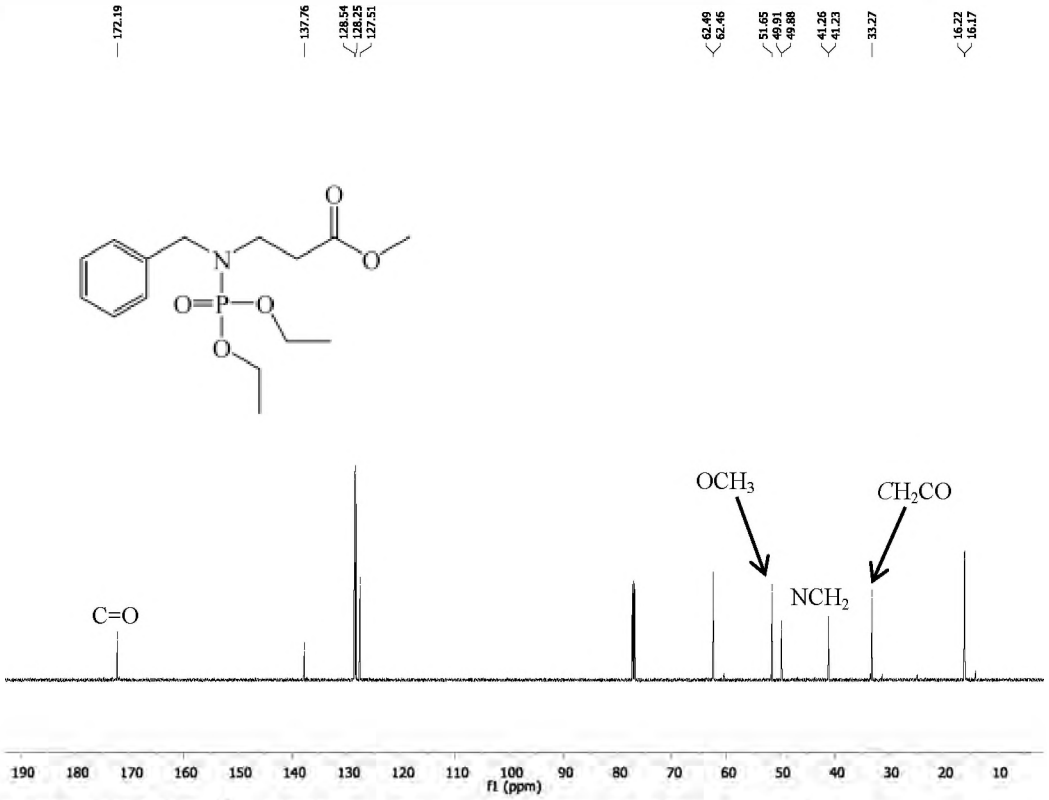


Figure 59. 150 MHz ^{13}C NMR spectrum of compound **160a** in $CDCl_3$.

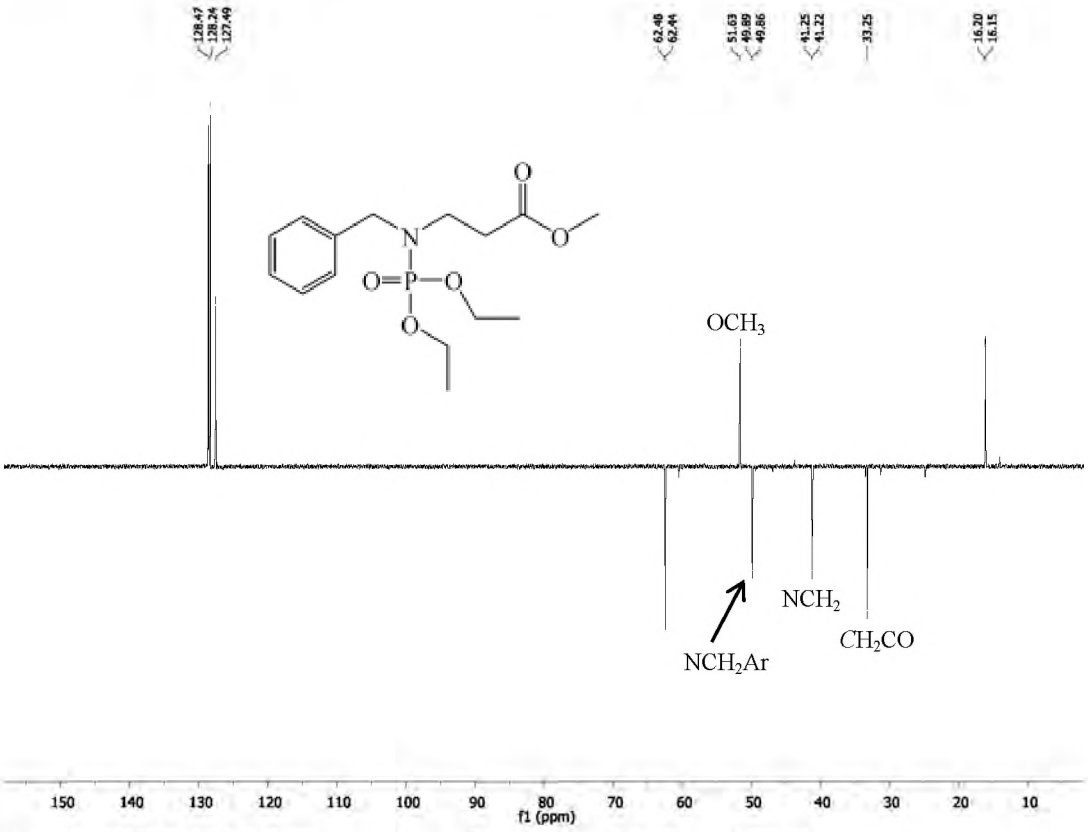


Figure 60. DEPT 135 NMR spectrum of compound **160a** in $CDCl_3$.

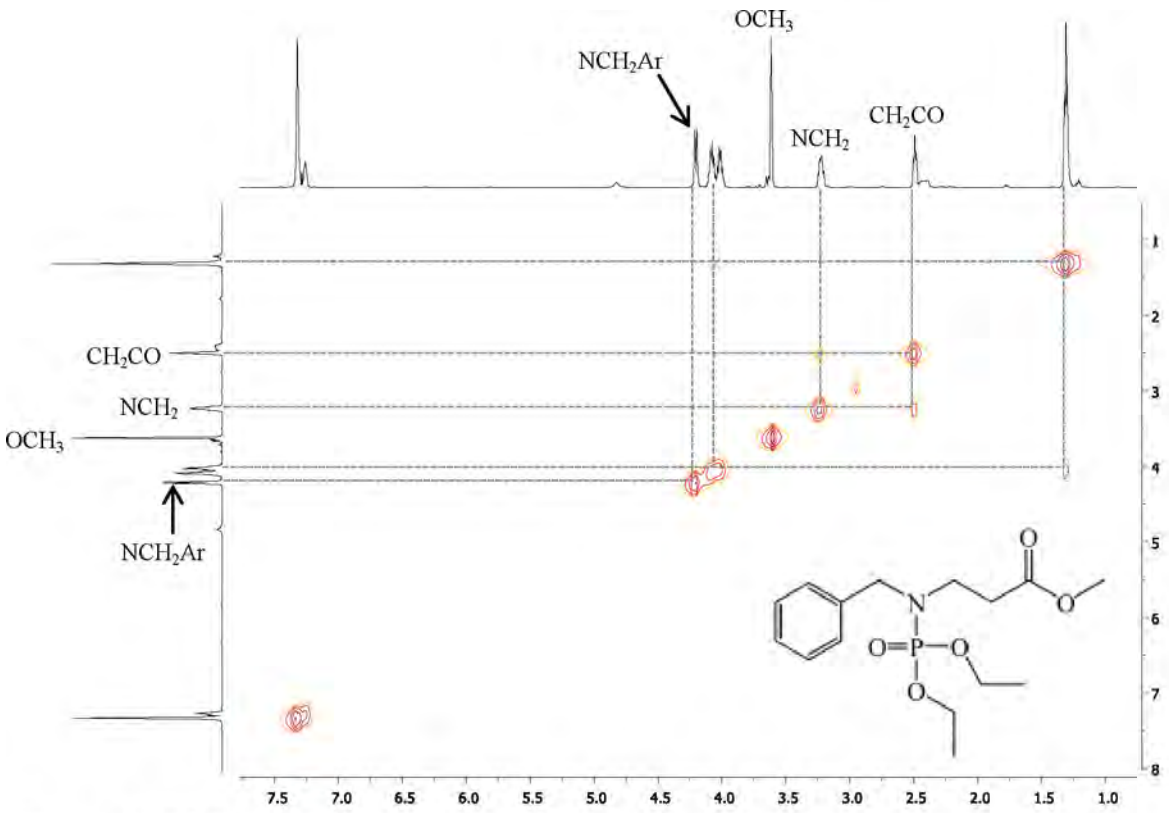
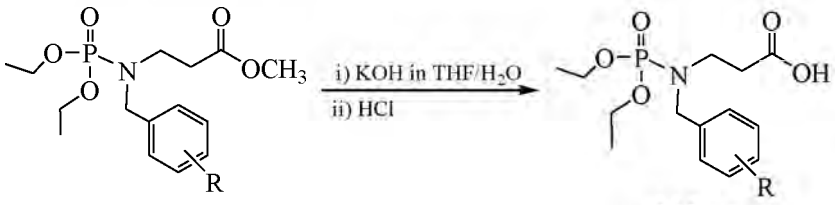


Figure 61. COSY NMR spectrum of compound **160a** in $CDCl_3$.

2.2.3. Hydrolysis of the methyl ester group in compounds 160a-g

The next step involved hydrolysis of the methyl ester group of compounds **160a-g** by reacting them with 1.2 equivalents of KOH in THF and water at 0 °C (Scheme 17); the reaction mixture was acidified using 1M-HCl, and the resulting mixture saturated with NaCl and then extracted with EtOAc to obtain the products in yields of up to 94% (Table 5).[193]



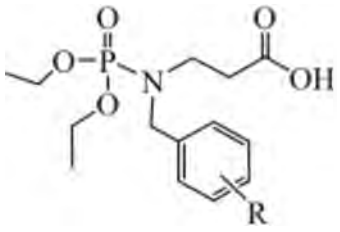
160a-g **165a-g**

	R
a	H
b	3,4-Dichloro
c	3-Chloro
d	3,4-Difluoro
e	3-Fluoro
f	Furfuryl*
g	3-Nitro

* replacing the phenyl group

Scheme 17. Formation of carboxylic acids **165a-g** from the esters **160a-g**.

Table 5. Yields of the carboxylic acid derivatives **165a-g**.



R	Compound	Yield (%)
H	**165a**	88
3,4-Dichloro	**165b**	94
3-Chloro	**165c**	89
3,4-Difluoro	**165d**	71
3-Fluoro	**165e**	70
Furfuryl*	**165f**	85
3-Nitro	**165g**	92

These products are all new and were fully characterised with the molecular formulae being supported by the HRMS data. The ^{1}H NMR spectrum of the furfuryl derivative **165f**, for example (Figure 62), confirms the absence of the methyl ester signal at *ca.* 3.6 ppm.

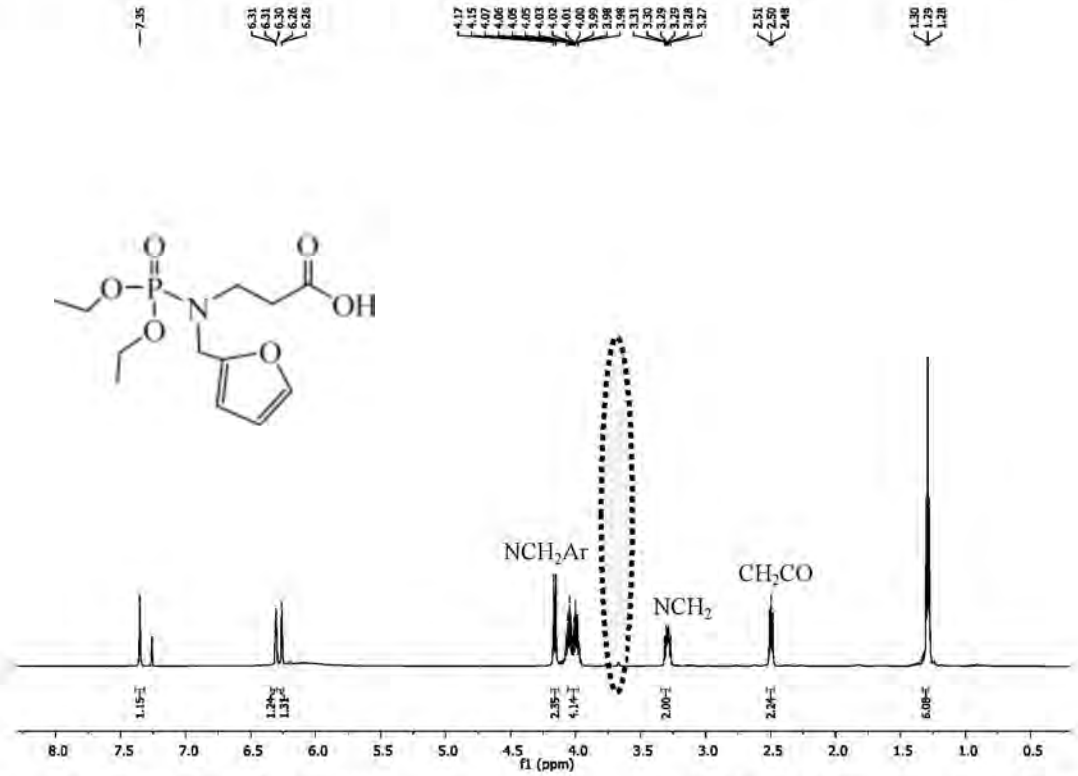
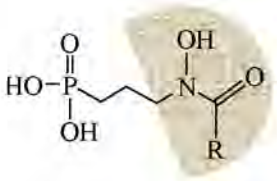


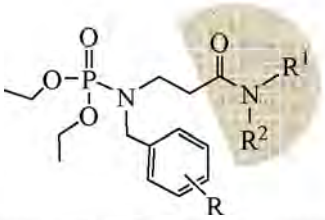
Figure 62. 600 MHz ^{1}H NMR spectrum of compound **165f** in $CDCl_3$, confirming the hydrolysis of the methyl ester **160f**.

2.2.4. Conversion of the carboxylic acids 165 to the hydroxamic acid derivatives 166 and 167.

The hydroxamate moiety which is a critical structural feature of fosmidomycin **62** and its acetyl analogue **63**, due to its coordination with a divalent metal cation, such as Mg^{2+}, in the active-site. In this aspect of our research, the hydroxamate moiety was reversed as in compounds **166** and **167** (Figure 63). This is a strategy which has been used by other groups with some success.[175] It was also our intention to vary the substituents on the hydroxamate moiety in order to explore the effect on the antimalarial activity of the compounds. We explored the possibility of replacing the hydroxyl group on the nitrogen by a benzyloxy giving compounds of type **166**, with enhanced hydrophobicity, whereas in compounds **167** the substituents on the hydroxamate moiety in FR900098 **63** (OH and CH_3) are both placed on the amide nitrogen atom.



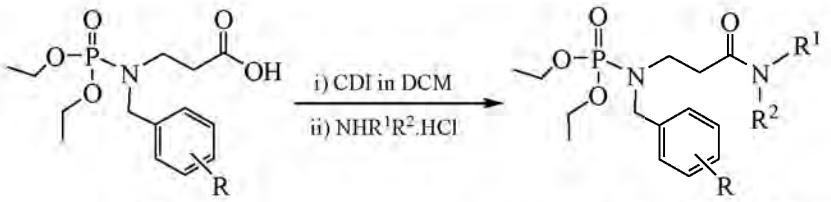
Fosmidomycin **62** R = H
FR900098 **63** R = CH_3



Compound containing a "reverse" hydroxamate moiety
166 R^1 = OBn; R^2 = H
167 R^1 = OH; R^2 = CH_3

Figure 63. Comparison of compounds **166** and **167** with fosmidomycin **62** and its acetyl analogue **63**.

The preparation of the *N*-benzyloxy and *N*-hydroxyl derivatives **166** and **167** was accomplished as shown in Scheme 19, starting from the carboxylic acids **165a-g**. Amidation of the carboxylic acids **165a-g** was carried out by reacting them with different hydroxylamine hydrochloride derivatives, using the coupling agent CDI in dry DCM and stirring at room temperature for 48 hours with *O*-benzylhydroxylamine hydrochloride, but for 7 days with *N*-methylhydroxylamine hydrochloride (Scheme 18).[174] After purification by flash chromatography, compounds **166a-g** and **167a-g** were obtained generally in good yields (Table 6).



165a-g

166a-g $R^1 = OBn$; $R^2 = H$
167a-g $R^1 = OH$; $R^2 = CH_3$

	R
a	H
b	3,4-Dichloro
c	3-Chloro
d	3,4-Difluoro
e	3-Fluoro
f	Furfuryl*
g	3-Nitro

* replacing the phenyl group

Scheme 18. Synthesis of hydroxamic acids **166a-g** and **167a-g**.

Table 6. Percentage yield of compounds **166a-g** and **167a-g**.

Compound	Yield (%)	Compound	Yield (%)
166a	78	**167a**	67
166b	66	**167b**	70
166c	83	**167c**	70
166d	70	**167d**	55
166e	72	**167e**	71
166f	70	**167f**	82
166g	78	**167g**	66

All of these compounds are new and were fully characterized using HRMS, NMR and IR analysis. The significant features in the ^{1}H NMR spectrum of compound **166f** (Figure 64) include a singlet integrating for two protons at 4.88 ppm corresponding to the *O*-benzylic protons, a multiplet integrating for the five additional protons in the aromatic region and the amide proton signal at 9.67 ppm. The DEPT 135 NMR spectrum of compound **166f** (Figure 65) confirms the presence of the *O*-benzylic methylene carbon signal at 78.0 ppm and the expected six aromatic methine carbon signals. The ^{13}C NMR spectrum of compound **166f** (Figure 66) confirms the presence of the two quaternary carbon signals and the carbonyl carbon resonating at 168.9 ppm.

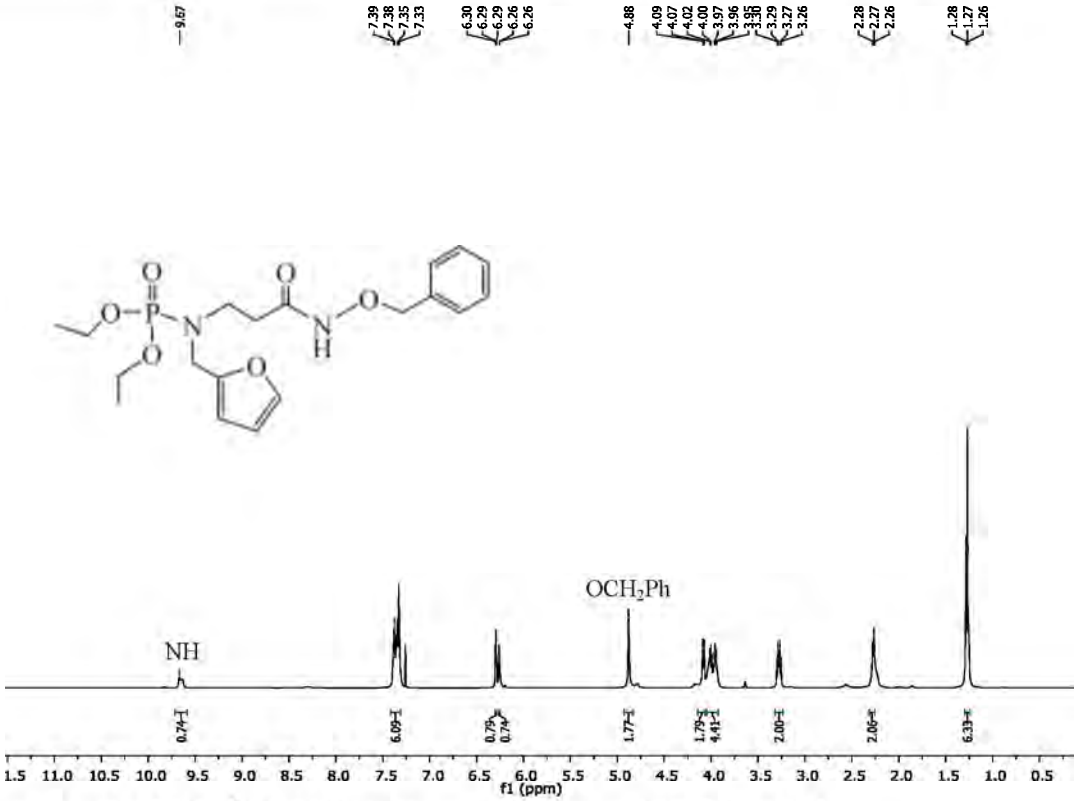


Figure 64. 600 MHz ^{1}H NMR spectrum of compound **166f** in $CDCl_3$.

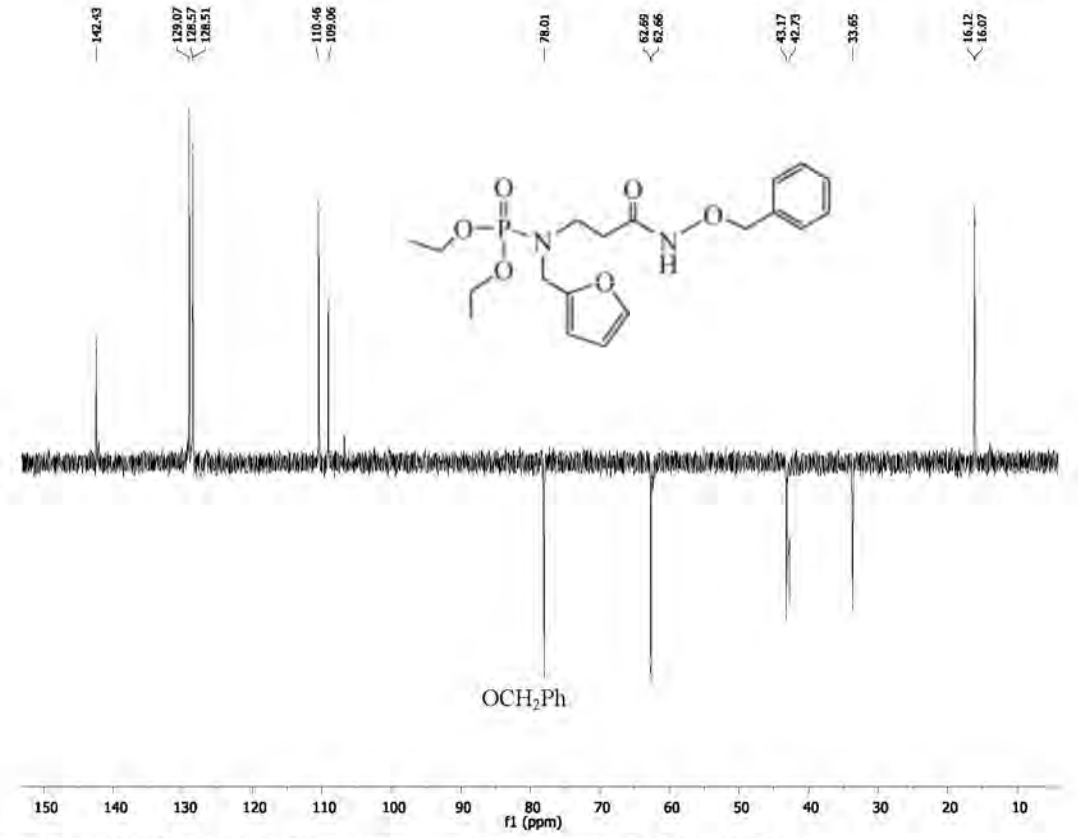


Figure 65. DEPT 135 NMR spectrum of compound **166f** in $CDCl_3$.

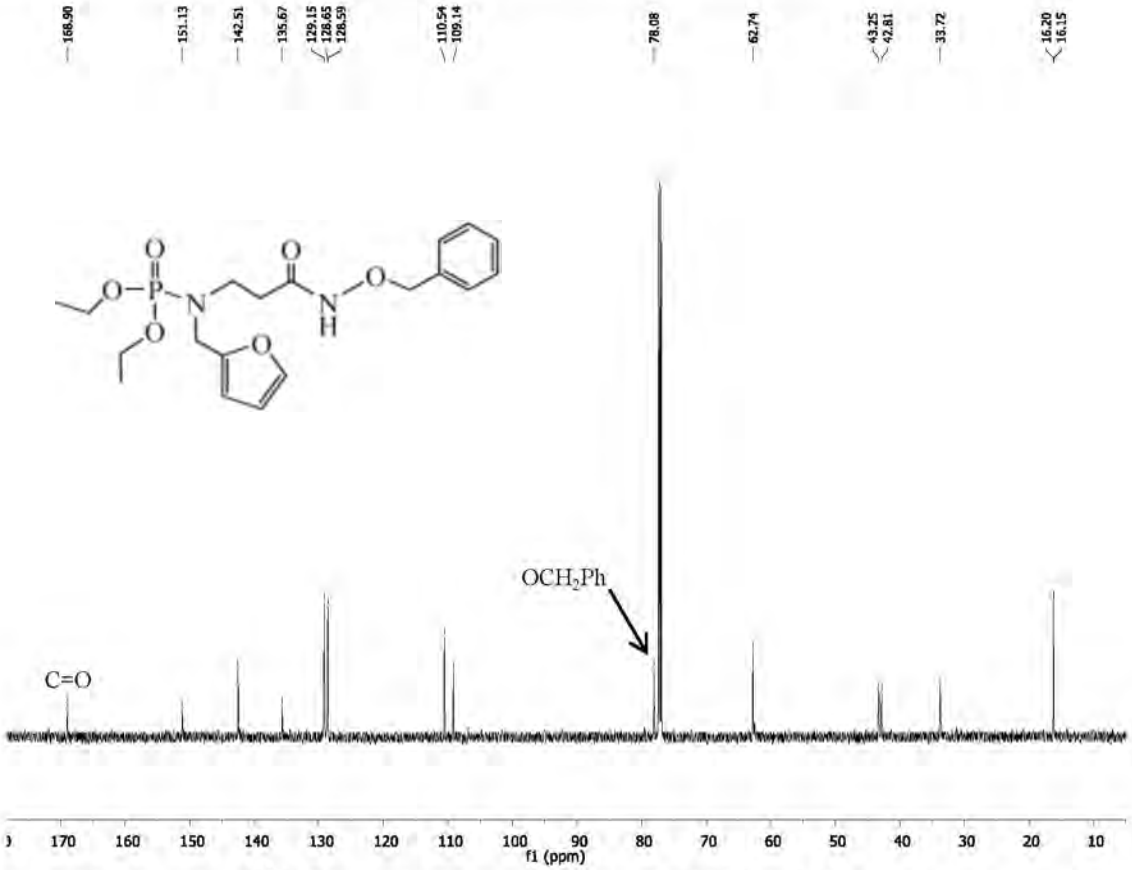


Figure 66. 150 MHz ^{13}C NMR spectrum of compound **166f** in $CDCl_3$.

The ^{1}H NMR spectrum (Figure 67) of *N*-methylhydroxylamine derivative **167g** reveals the *N*-methyl singlet at 3.19 ppm, while the diethyl phosphoramidate group remains intact with the expected triplet and multiplet signals at 1.12 and 4.06 ppm, respectively. The DEPT 135 NMR spectrum (Figure 68) clearly confirms the presence of the *N*-methyl signal at 35.9 ppm together with the four expected methylene carbon signals at 33.0, 41.3 and 48.6 ppm. Analysis of the COSY (Figure 69) and HSQC (Figure 70) NMR spectra of compound **167g** confirmed the correlations between the assigned protons, and between carbons and their attached protons.

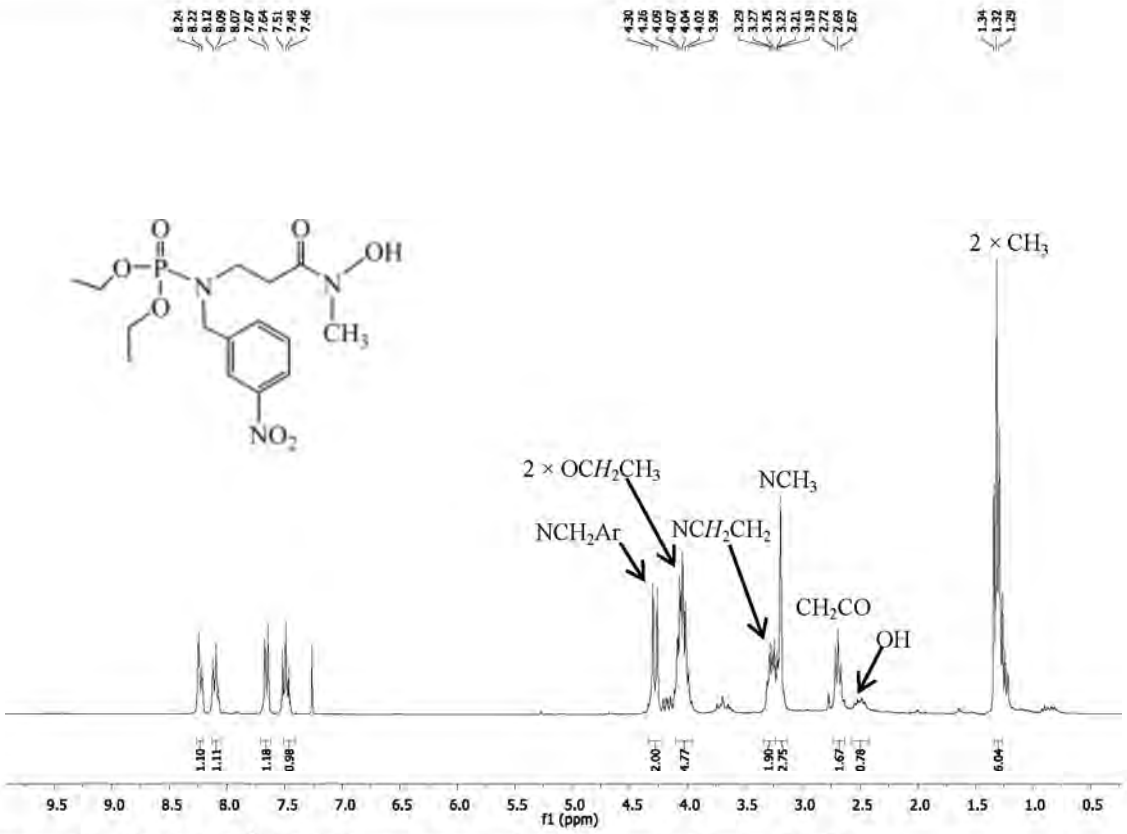


Figure 67. 300 MHz ^{1}H NMR spectrum of compound **167g** in $CDCl_3$.

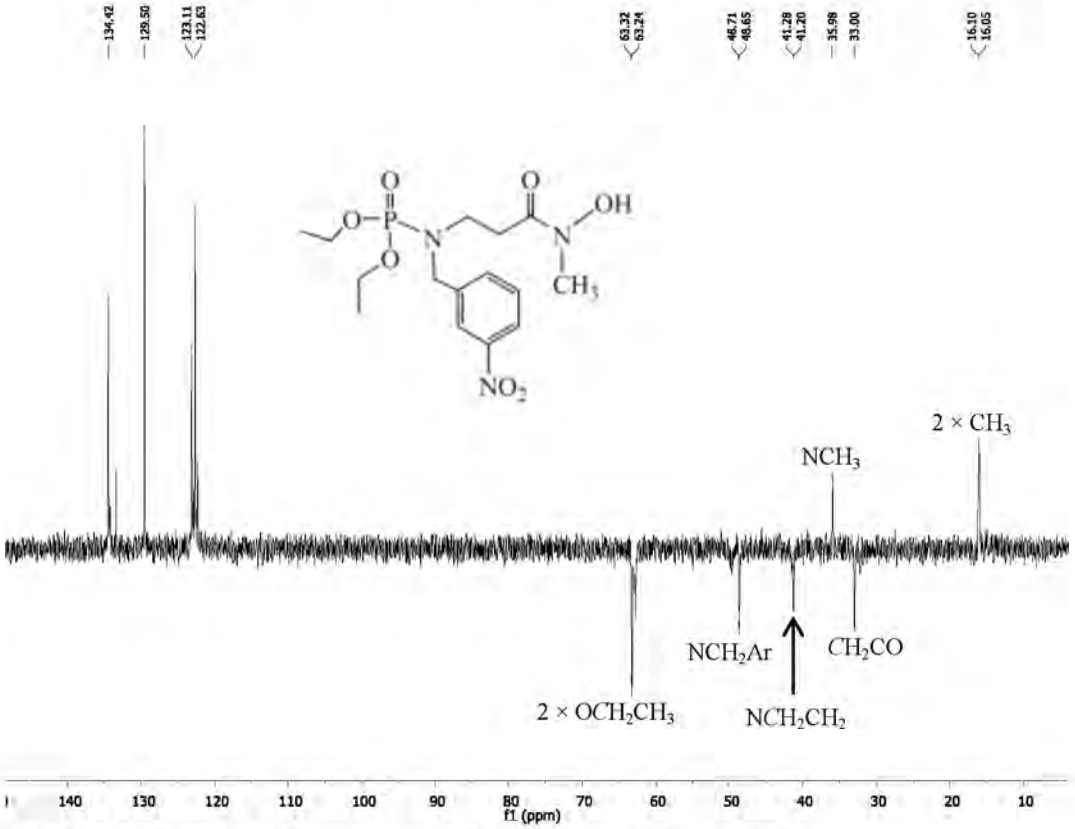


Figure 70. DEPT 135 NMR spectrum of compound **167g** in $CDCl_3$.

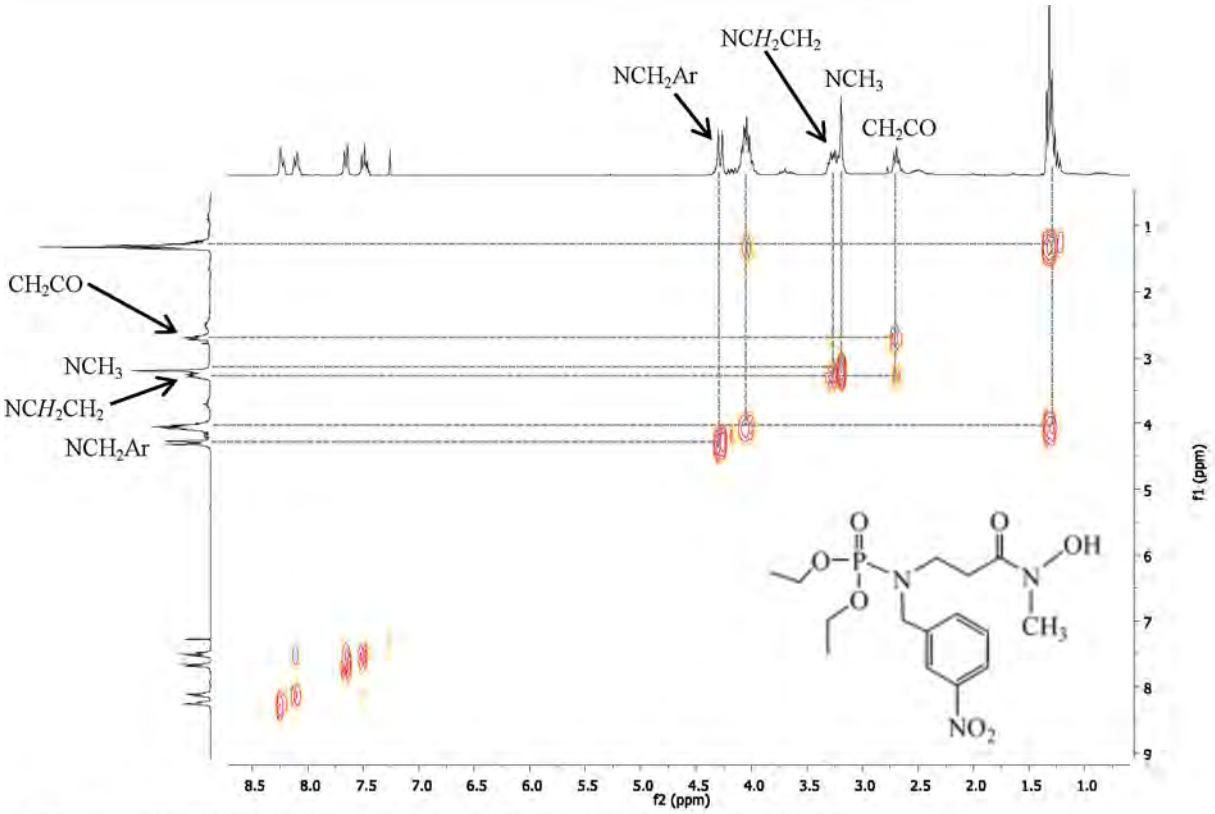


Figure 69. COSY NMR spectrum of compound **167g** in $CDCl_3$.

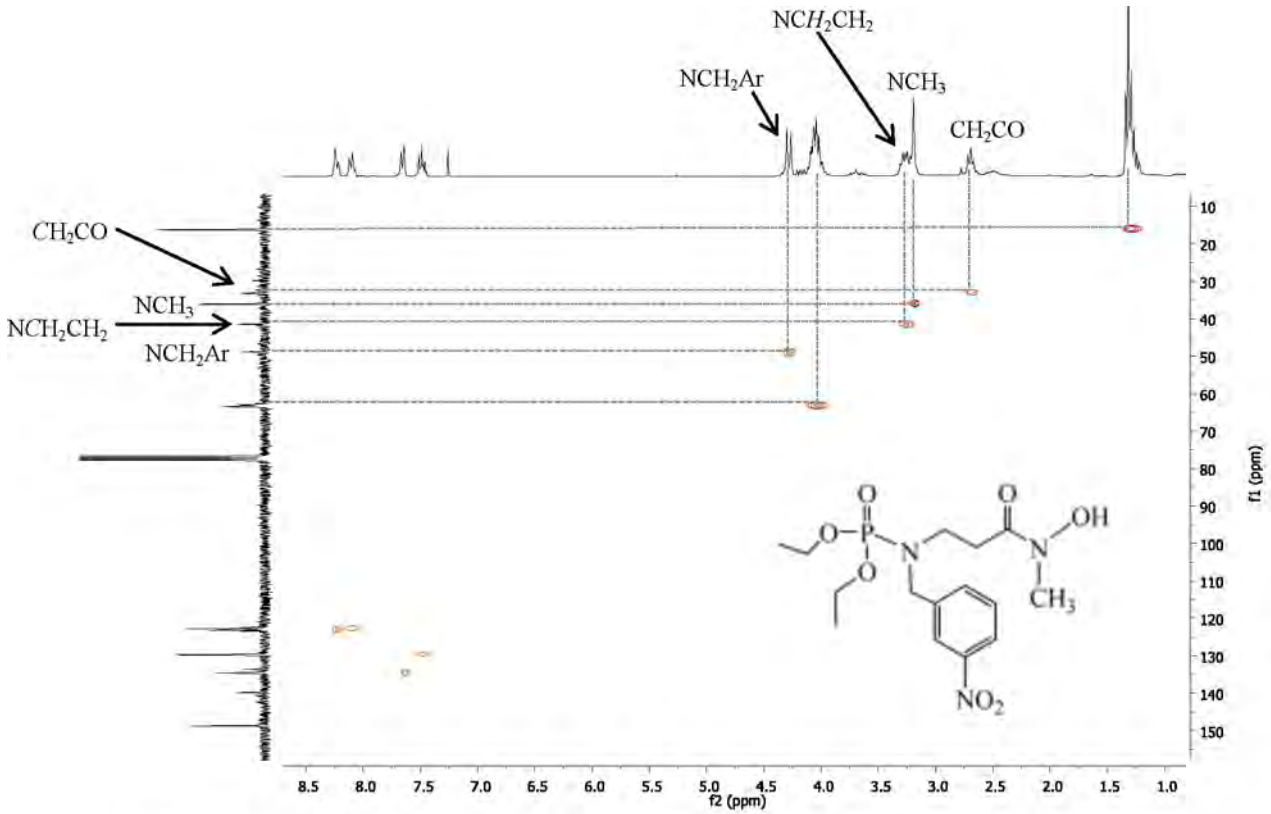
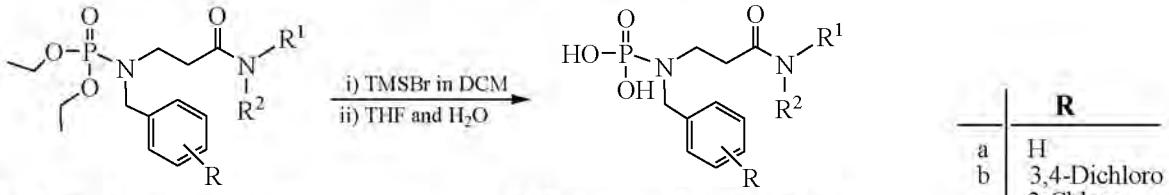


Figure 70. HSQC NMR spectrum of compound **167g** in $CDCl_3$.

2.2.5. Synthesis of the phosphoramidic acid derivatives 161 and 162

The diethyl phosphoramidate esters **166a-g** and **167a-g** were expected to have the lipophilicity necessary to cross phospholipid membranes, thereby possibly acting as ester pro-drugs which undergo *in vivo* hydrolysis by *esterases* to afford the phosphoramidic acid derivatives capable of binding in the *Pf*DXR active-site.[190,194-196] However, since the synthetic ligands were to be subjected to *in vitro* bioassay, we considered it important to prepare the corresponding phosphoramidic acids. This was achieved through the hydrolysis of the series of compounds **166** and **167** using the method which ealier proved successful in cleaving the phosphonate ester groups (Section 2.1). Compounds (**166a-g** and **167a-g**) were therefore reacted with trimethylsilyl bromide (TMSBr) at room temperature in dry DCM, under N_2 for 24 h and, following evaporation, stirred with THF and water to effect complete hydrolysis. Evaporation of the solvent and trituration using EtOAc gave the compounds **161a-g** as solids in good yields (Scheme 19, **Table 7**). After trituration, the products **162a-c,f,g**, however, were obtained as oils. Unfortunately, the hydrolysis of the fluorinated phosphoramidate esters **167d,e** failed to afford the desired phosphoramidic acids due to degradation.



166a-g $R^1 = OBn$; $R^2 = H$
167a-e $R^1 = OH$; $R^2 = CH_3$

161a-g $R^1 = OBn$; $R^2 = H$
162a-c,f,g $R^1 = OH$; $R^2 = CH_3$

	R
a	H
b	3,4-Dichloro
c	3-Chloro
d	3,4-Difluoro
e	3-Fluoro
f	Furfuryl*
g	3-Nitro

* replacing the phenyl group

Scheme 19. Formation of the phosphoramidic acids **161a-g** and **162a-c,f,g**.

Table 7. Percentage yields of compounds **161a-g** and **162a-c,f,g**.

Compound	Yield (%)	Compound	Yield (%)
161a	75	**162a**	75
161b	76	**162b**	70
161c	80	**162c**	79
161d	79	**162d**	-
161e	79	**162e**	-
161f	74	**162f**	62
161g	81	**162g**	74

All of the compounds **161a-g** and **162a-c,f,g** are new and were fully characterised. The ^{1}H NMR spectrum of compound **161b** (Figure 71) confirms the hydrolysis of the phosphoramidate ester in that the ethyl ester triplet and multiplet signals usually present *ca* 1.3 and 4.0 ppm, respectively are absent. The ^{13}C NMR spectrum of compound **161b** (Figure 72) also confirms the cleavage of the phosphoramidate ethyl ester group, with only the methylene, aromatic and the quaternary carbon signals being present.

Similar patterns were observed for compounds **162a-c,f,g**. For example, the ^{1}H NMR spectrum of compound **162g** (Figure 73) also reveals the absence of the ethyl ester signals and thus confirmsrolysis of the phosphoramidate ester.

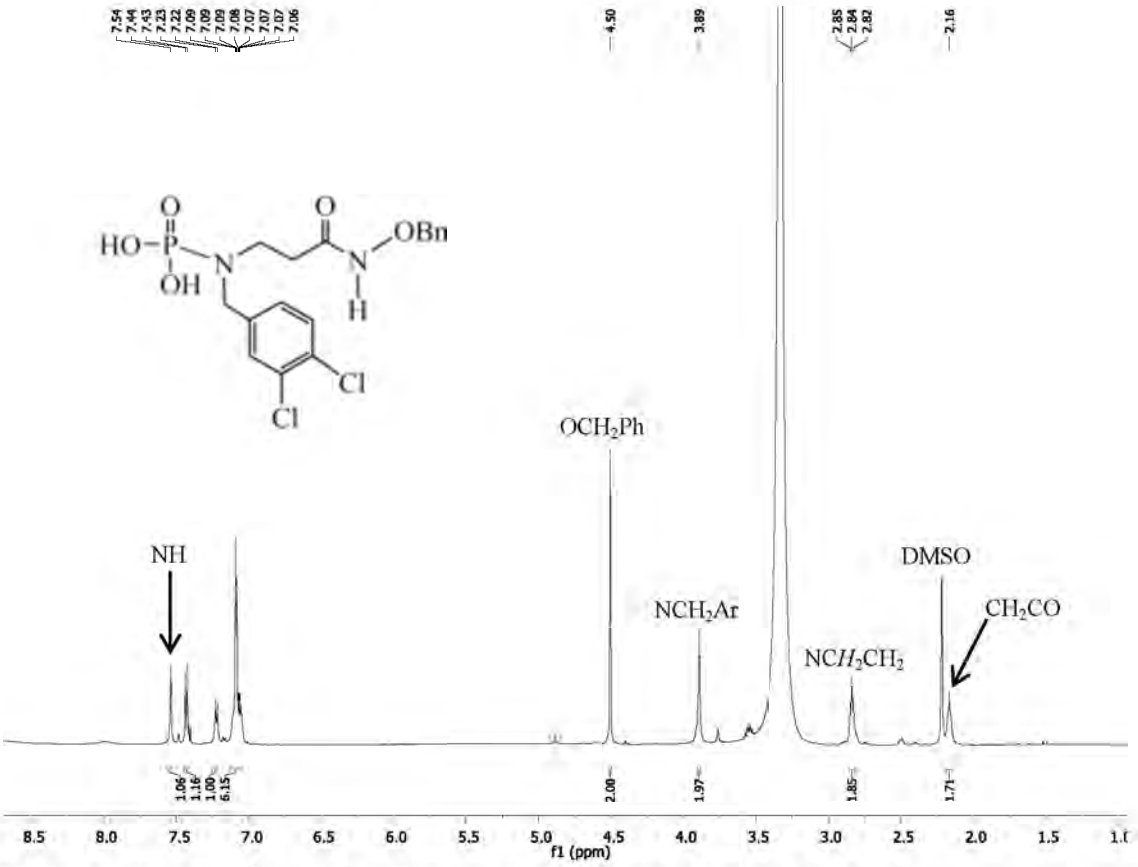


Figure 71. 600 MHz ^{1}H NMR spectrum of compound **161b** in DMSO-d_6.

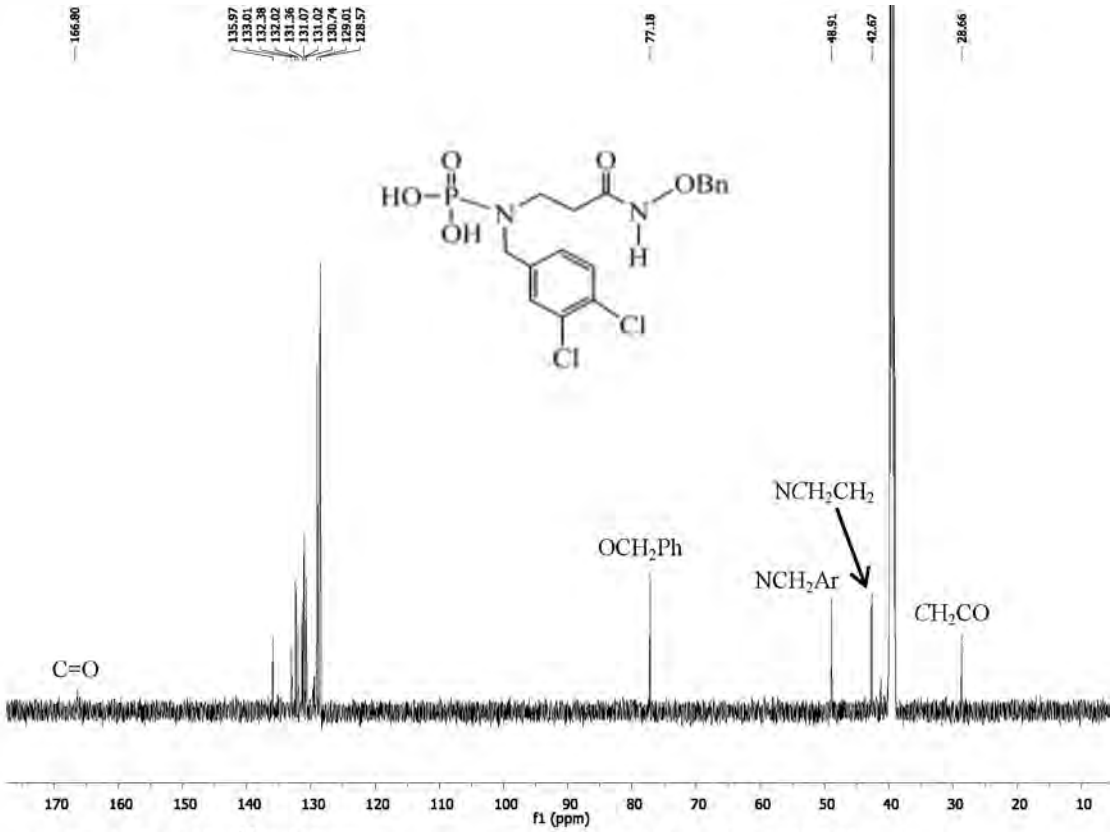


Figure 72. 150 MHz ^{13}C NMR spectrum of compound **161b** in DMSO-d_6.

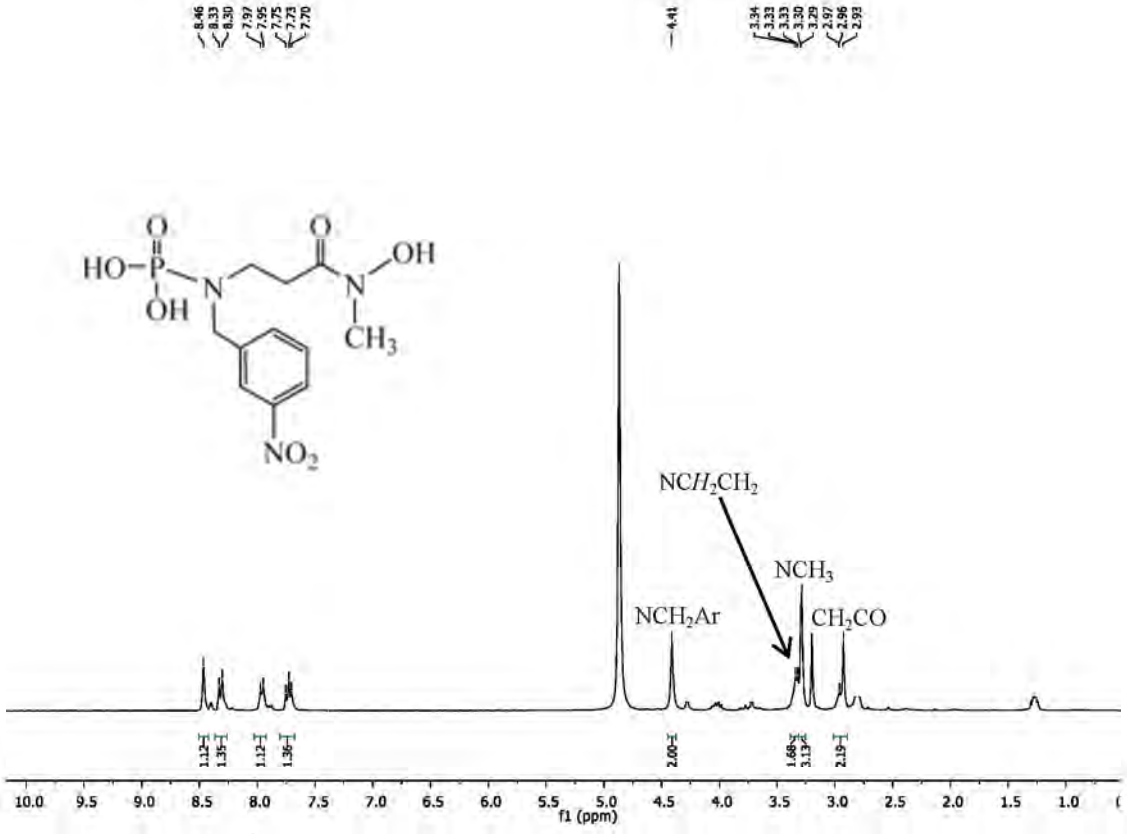


Figure 73. 600 MHz ^{1}H NMR spectrum of compound **162g** in CD_3OD.

2.3. SYNTHESIS of *N*-BENZYLATED PHOSPHORAMIDIC DERIVATIVES as FR900098 ANALOGUES BASED on a *DE NOVO* DESIGN STRATEGY.

Careful analysis of the 3-D topology of the *Pf*DXR active-site carried out in our group by Conibear,[145,146] revealed the presence of three, adjacent, hydrophobic binding pockets (**I**, **II** and **III**) as shown in Figure 74. Two of these pockets are close to the phosphonate moiety binding pocket reported by Perruchon and Deng[136,197] and is presumed to account for the increased activity of compound **78** with the aryl group occupying one of the empty pockets.

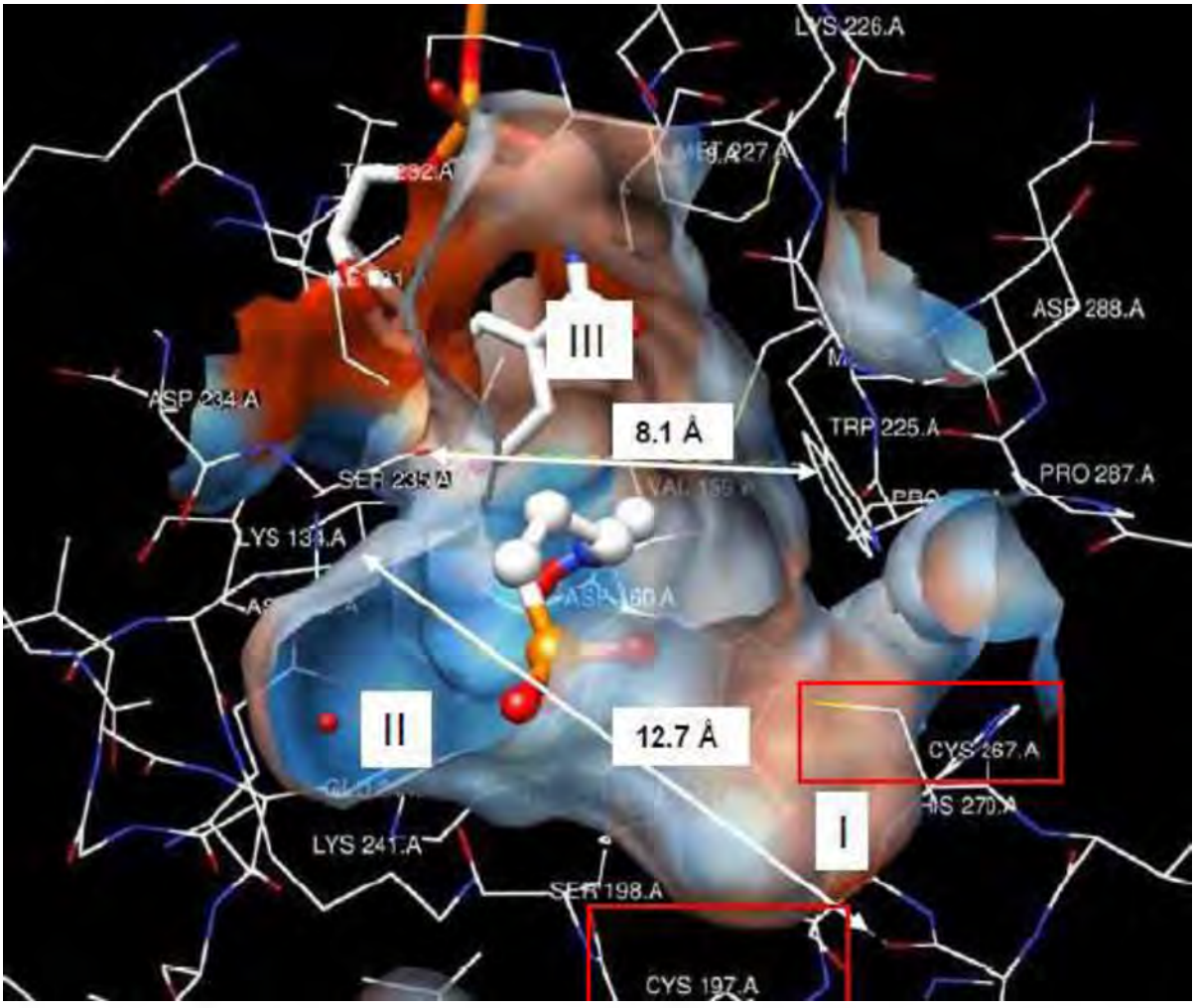


Figure 74. The *Pf*DXR active-site revealing the three additional binding pockets (**I**, **II** and **III**). The surface zone at 8.0 Å from fosmidomycin **62** is shown with 30% transparency, non-polar (white), coloured by hydrophobicity polar (blue), hydrophobic (orange) and clipped in front. Protein residues coloured by atom type, and residues less than 8.0 Å away from fosmidomycin **62** are labelled. Fosmidomycin **62** is shown in ball-and-stick format, coloured by atom type, NADPH as sticks coloured by atom type and water molecules as red spheres (Reproduced with permission from Conibear).[146]

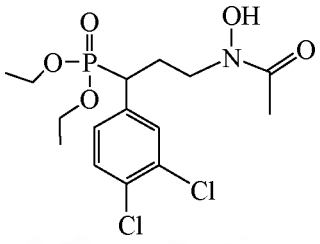
The work led to the *de novo* design of a series of *Pf*DXR inhibitors illustrated in Figure 75 containing:-

(i) a phosphoramidate moiety [**A**] in which the α-methylene group in phosphonate ligands, such as fosmidomycin and FR900098, is replaced by nitrogen;

(ii) an *N*-benzyl substituent [**B**] to occupy a hydrophobic pocket adjacent to the *Pf*DXR active-site, while obviating the chirality issues associated with *C*-benzylation;

(iii) a three-atom aminoethyl (N-C-C) chain [**C**] replacing the trimethylene hydrophobic patch in fosmidomycin and FR900098; and

(iv) the *N*-acetyl hydroxamate moiety [**D**] present in FR900098.



Compound **78**

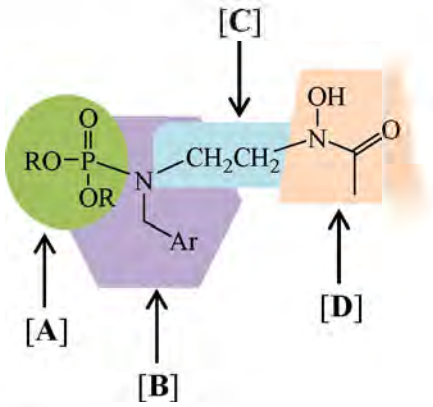
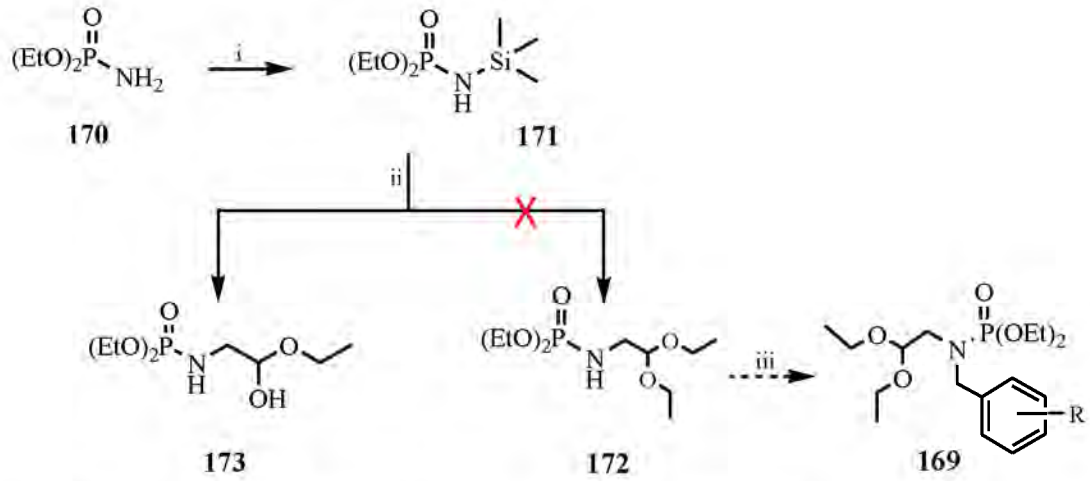


Figure 75. Design features of novel series of *Pf*DXR inhibitors.

The synthetic strategy to access this series of compounds was intiated by Conibear an earlier member of the group, while Mutorwa,[147] and Nokalipa[198] contributed to this programme with Mutorwa completing the synthesis of a number of compounds in the series. In the present study, attention has focused on repeating and extending the series of compounds prepared by Mutorwa and, importantly, obtaining sufficient quantities of the final products for undertaking biological screening.

The first approach to the targeted ligands **169a-f** was to use the method reported by Conibear in the preliminary studies, which was also was used by Mutorwa,[146-147] by reacting diethyl phosphoramidate **170** with hexamethyldisilazane in dry THF under an inert atmosphere at 80 °C to obtain compound **171** (Scheme 20). Treatment of the silylated derivated **171** with bromoacetaldehyde diethylacetal in the presence of NaH and a catalytic amount of tetra-

butylammonium bromide was expected to furnish the protected acetal **172**, but the hemiacetal **173** was isolated instead of the desired compound **169** (Scheme 20).

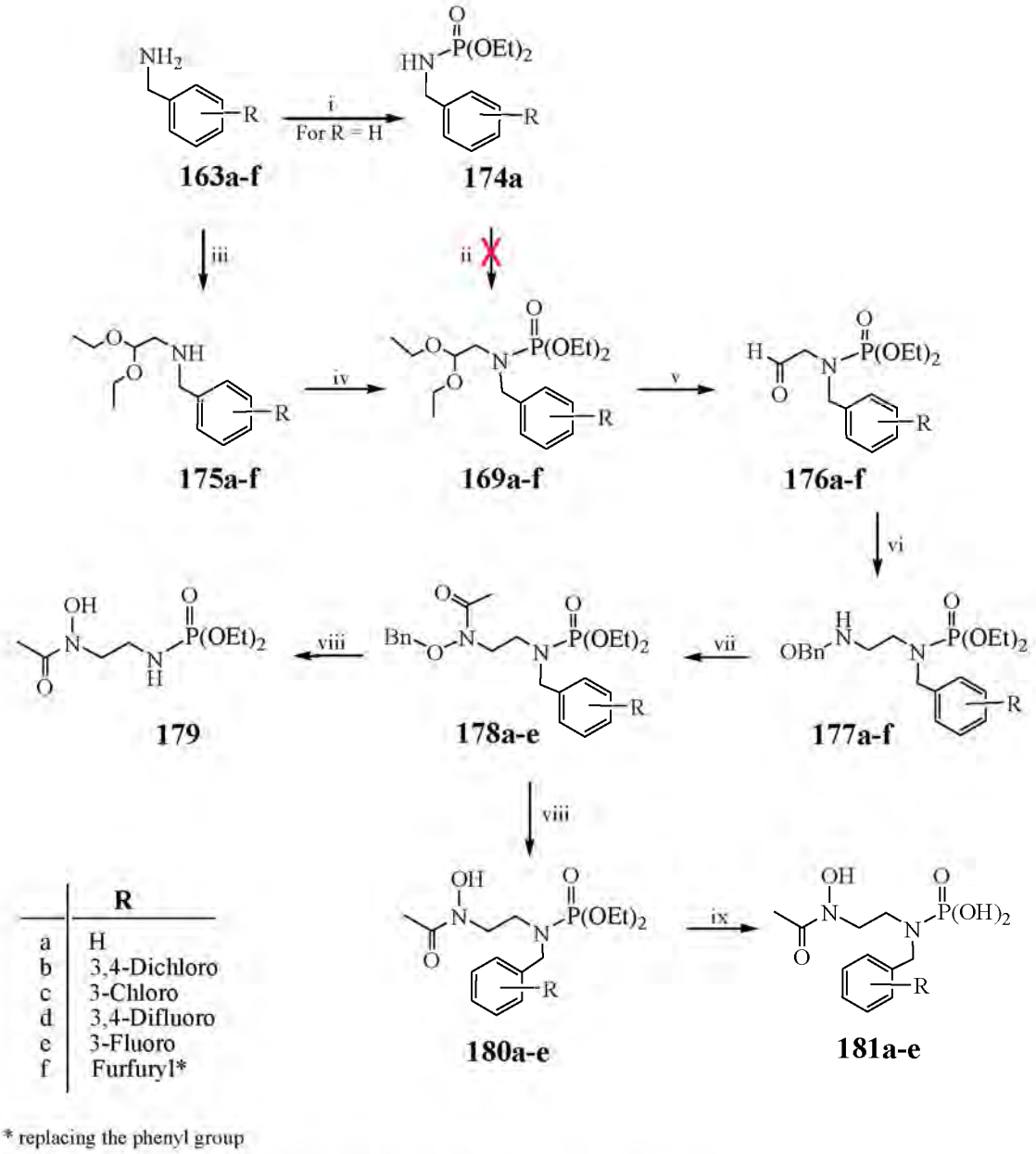


Scheme 20. Attempted synthesis of ligands using Mutorwa's method.

Reagents and conditions:- (i) Hexamethyldisilizane, dry THF, 80 °C, 3 h; (ii) NaH, dry THF, r.t., then bromoacetaldehyde diethylacetal, $Bu_4N^+Br^-$, dry THF, 80 °C 4 h and then EtOH, reflux, 1 h; and (iii) NaH, benzyl halides, dry THF, r.t., 24 h, N_2.

Although, Mutorwa[147] had completed the synthesis of some of these novel phosphoramidic acid derivatives. Nokalipa[198] had encountered difficulties with the stability of some of the intermediates and it was apparent that different strategies needed to be developed. A completely different approach was conseqently explored and this is outlined in (Scheme 21).

Benzylamine **163a** was reacted with diethyl chlorophosphate in the presence of triethylamine as a base to furnish compound **174** but attempts to react this compound with bromoacetaldehyde diethylacetal, in the presence of sodium hydride to remove the phosphoramidate proton, proved unsuccessful. Consequently, attention was given to introducing the diethyl acetal moiety first. All of these compounds appeared unstable at room temperature, thus bioassay screening was carried out promptly.

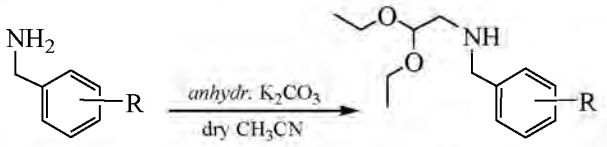


Scheme 21. New method for the synthesis of compounds **181a-e**.

Reagents and conditions:- (i) diethyl chlorophosphate, Et_3N, DCM; (ii) NaH, dry THF, r.t., 24 h, N_2; (iii) anhydr. K_2CO_3, dry CH_3CN, 24 h, 85 °C; (iv) diethyl chlorophosphate, Et_3N, DCM, r.t., 24 h; (v) 2.5M-HCl, dioxane, r.t, 6 h; (vi) $NH_2OBn \cdot HCl$, MeOH, 40 °C, 5 h and then $NaBH_3CN$, MeOH, conc. HCl, r.t.; (vii) Et_3N, acetyl chloride, dry DCM, 24 h, N_2; (viii) 10% Pd/C, dry MeOH, H_2; and (ix) TMSBr in dry DCM, N_2.

2.3.1. Reaction of the benzylamine derivatives 163a-f with bromoacetaldehyde diethylacetal

The amines **163a-f** were reacted with bromoacetaldehyde diethylacetal in the presence of anhydrous K_2CO_3 under reflux at 85 °C for 24 h (Scheme 22).[199] Work-up, followed by flash chromatography of the crude products afforded the pure compounds **175a-f** in low to moderate yield (Table 8).



163a-f **175a-f**

	R
a	H
b	3,4-Dichloro
c	3-Chloro
d	3,4-Difluoro
e	3-Fluoro
f	Furfuryl*

* replacing the phenyl group

Scheme 22. Alkylation of amines **163a-f**.

Table 8. Isolated yields of the products **175a-f**.

Compound	Yield (%)
175a	61
175b	50
175c	23
175d	56
175e	50
175f	40

While two of these compounds (**175a** and **175f**) are known, compounds **175b-e** are new and were fully characterised. The ^{1}H NMR spectrum of the dichloro analogue **175b** (Figure 76), shows a triplet at 1.21 ppm which corresponds to the methyl groups of the diethyl acetal moiety, while the multiplets at *ca* 3.5 ppm and 3.7 ppm correspond to the diastereotopic germinal protons in each of the *O*-methylene groups – an observation which reflects the inherent magnetic non-equivalence of the germinal methylene protons in such synmmetrically substituted achiral molecules, the acetal methine proton resonates as a triplet at 4.59 ppm. The ^{13}C NMR spectrum of compound **175b** (Figure 77) shows the methyl, methylene and methine carbons of the diethyl acetal group resonating at 15.5, 62.7 and 102.2 ppm, respectively.

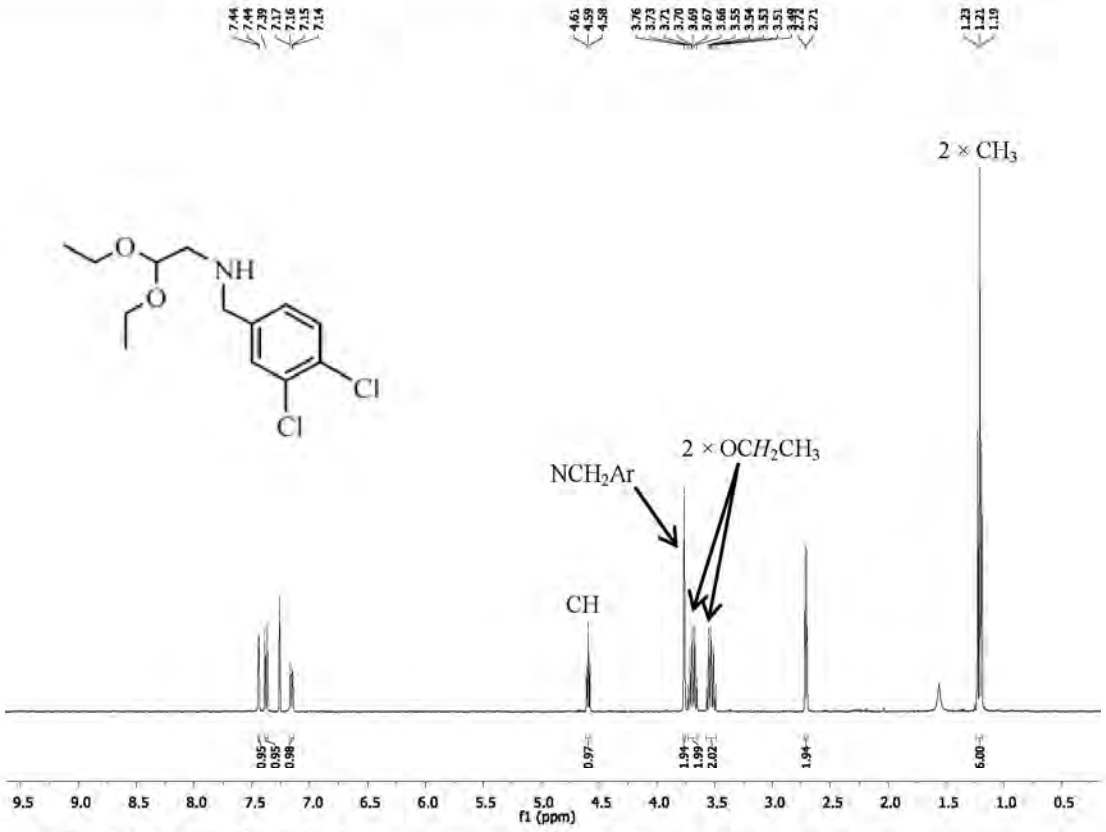


Figure 76. 600 MHz ^{1}H NMR spectrum of compound **175b** in $CDCl_3$.

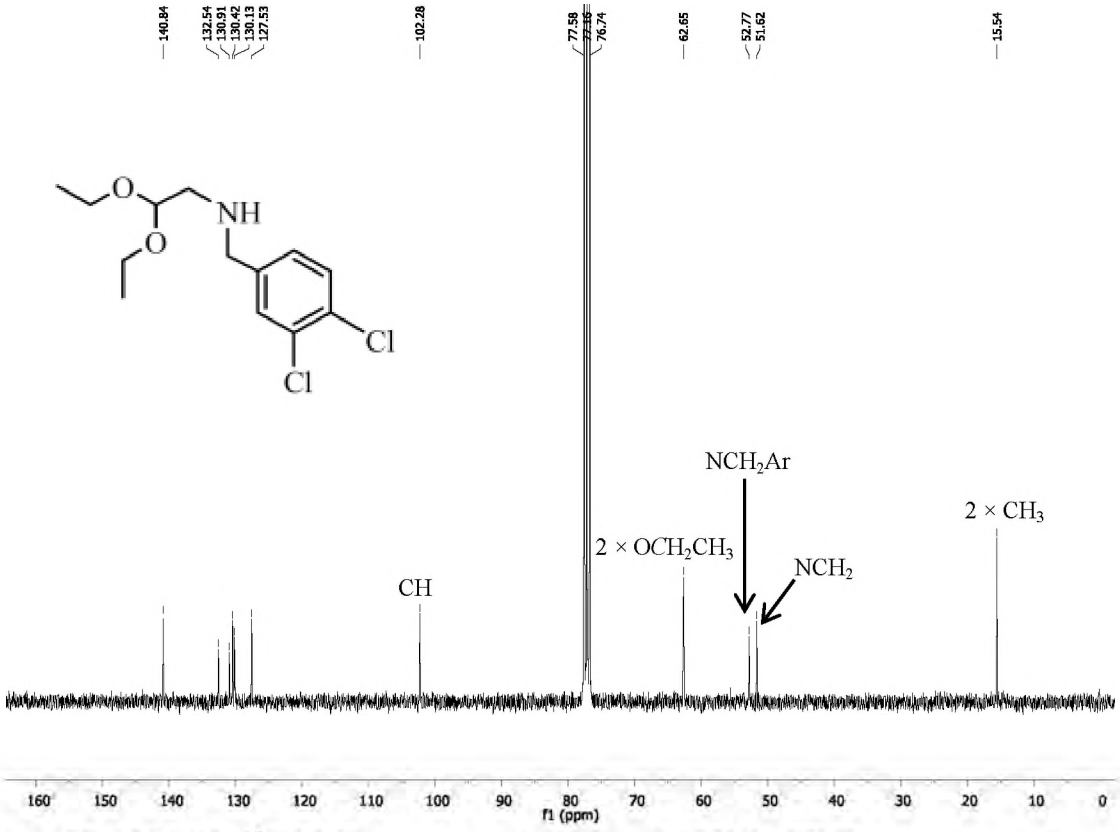


Figure 77. 150 MHz ^{13}C NMR spectrum of compound **175b** in $CDCl_3$.

2.3.2. Phosphonation of the *N*-benzyl acetal derivatives 175a-f

Compounds **175a-f** were each reacted with 1 eq. of triethylamine, and then with 1 eq. of diethyl chlorophosphate in DCM at room temperature for 24 h (Scheme 23).[189-191] Work-up, including washing with 10% HCl to remove excess triethylamine, followed by flash chromatography afforded the pure products **169a-f** in excellent yield (Table 9).

175a-f **169a-f**

	R
a	H
b	3,4-Dichloro
c	3-Chloro
d	3,4-Difluoro
e	3-Fluoro
f	Furfuryl*

* replacing the phenyl group

Scheme 23. Synthesis of phosphoramidate esters **169a-f**.

Table 9. Yields of the phosphoramidate ester derivatives **169a-f**

Compound	Yield (%)
169a	87
169b	77
169c	86
169d	88
169e	94
169f	71

With the exception of compound **169a**, all of these compounds are new and were fully characterised. The ^{1}H NMR spectrum of compound **169b** (Figure 78), for example, shows a pair of high field triplets corresponding to the phosphoramidate ester and acetal groups with each integrating for six protons. The *N*-methylene signal at *ca* 3.0 ppm appears as a double doublet reflecting coupling to both the vicinal methine proton and the ^{31}P nucleus. Even though the molecule is achiral, the *O*-methylene protons again resonate as a pair of multiplets at 3.49 and 3.68 ppm (expanded in Figure 78). The benzylic protons resonate as a doublet at 4.29 ppm due to coupling to the ^{31}P nucleus, clearly supporting the presence of the phosphonate moiety. In the ^{13}C NMR spectrum of compound **169b** (Figure 79) ^{13}C-^{31}P coupling is responsible for the doublets at 16.2 ppm and 62.5 ppm permitting their assignment to the phosphoramidate methyl and *O*-methylene carbons, and assignment of the signals at 15.3 ppm and 63.0 ppm to the corresponding groups in the acetal moiety. The COSY NMR

(Figure 80) and HSQC NMR data (Figure 81) permitted unambiguous assignment of all the proton and carbon signals.

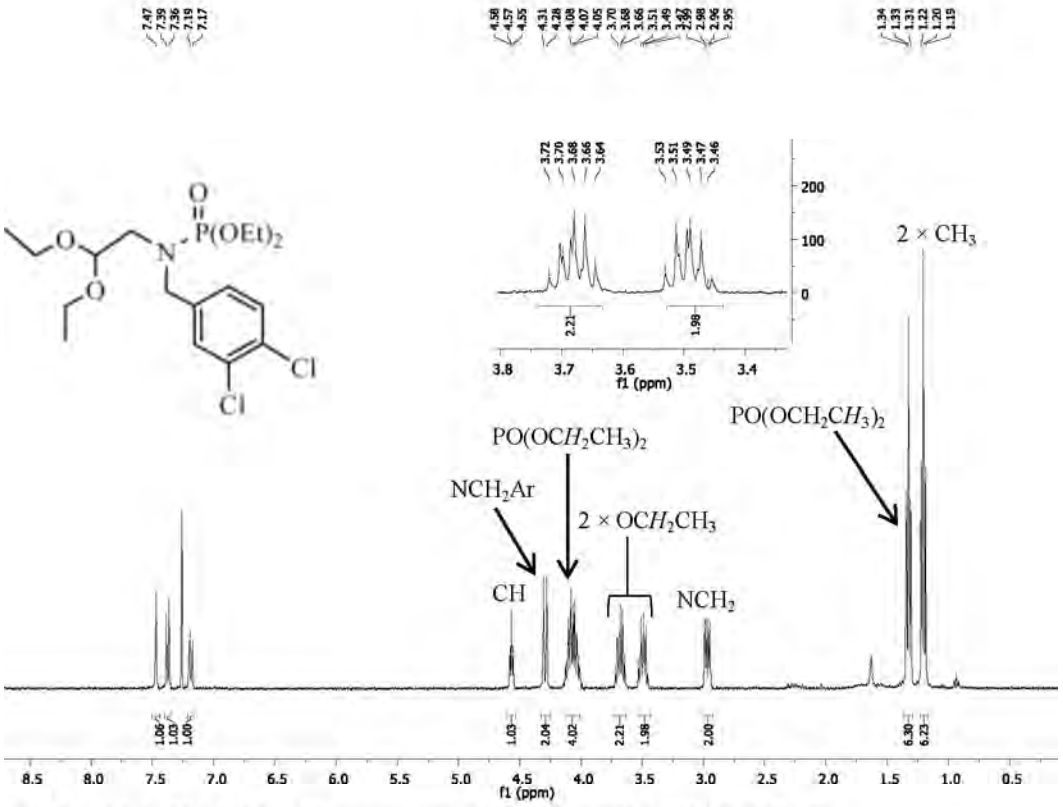


Figure 78. 600 MHz ^{1}H NMR spectrum of compound **169b** in $CDCl_3$.

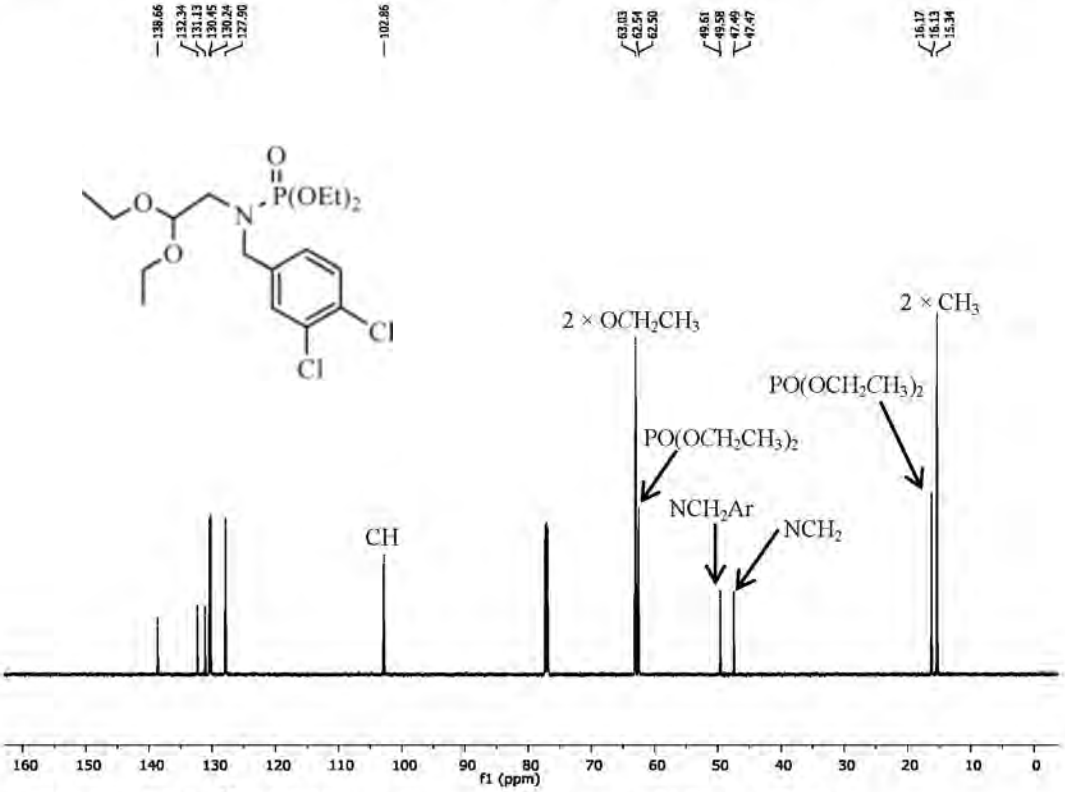


Figure 79. 150 MHz ^{13}C NMR spectrum of compound **169b** in $CDCl_3$.

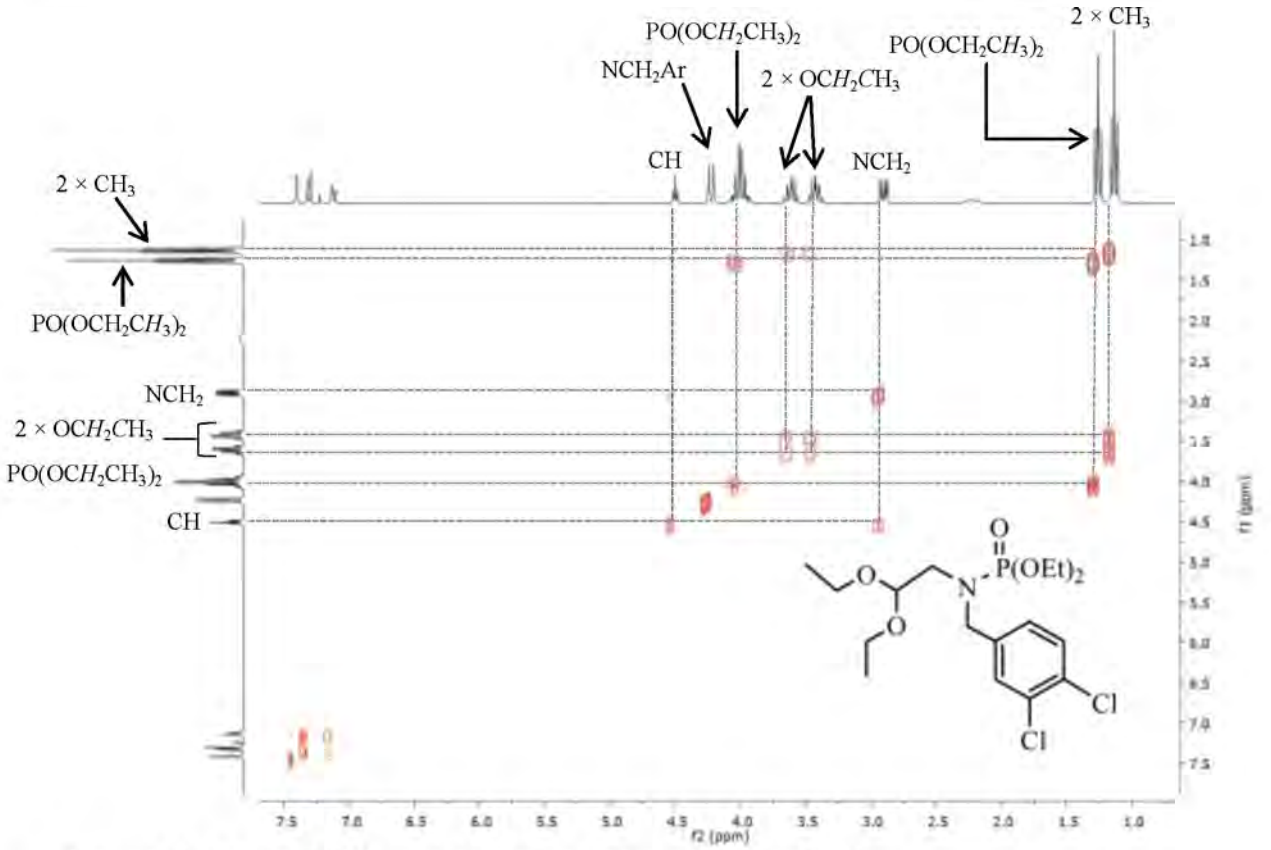


Figure 80. COSY NMR spectrum of compound **169b** in $CDCl_3$.

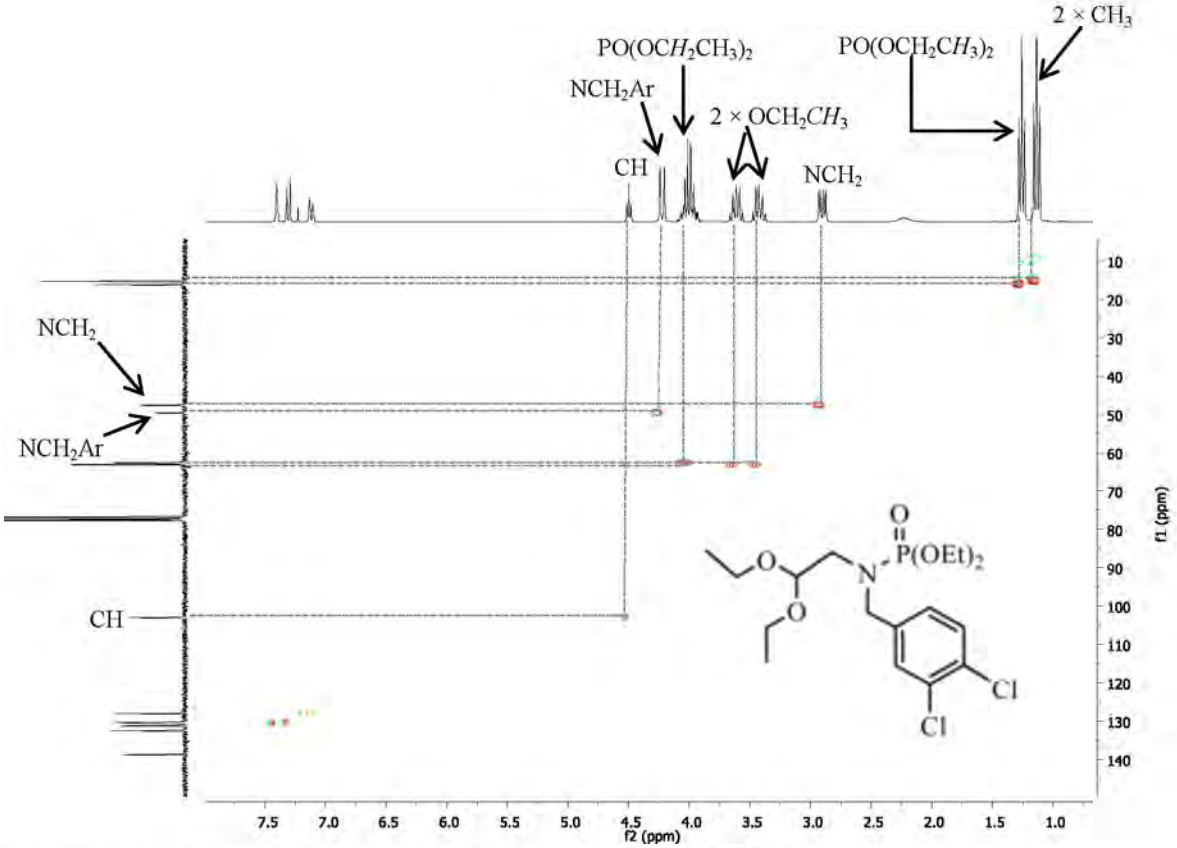
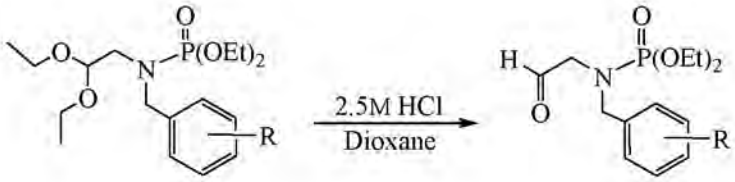


Figure 81. HSQC NMR spectrum of compound **169b** in $CDCl_3$.

2.3.3. Formation of the aldehydes 176a-f

The hydrolysis of compounds **169a-f** was carried out initially using Mutorwa's procedure (*Method A*), by stirring with 2M-HCl at room temperature for 24 h to afford the aldehydes **176a,b,f** (30-60%; Table 10).[144,200,201] We later discovered that using dioxane as a solvent with 2.5M-HCl (*Method B*) afforded the aldehydes **176a,c-e** in higher yield percentage yields (64-74%) in shorter time (5 h) and without the need for further purification (Scheme 24).[202] All of the aldehydes (**176**), however, proved to be unstable at room temperature and had to be used promptly.



169a-f **176a-f**

Scheme 24. Formation of aldehydes **176a-f**.

Table 10. Yields of the aldehyde derivatives **176a-f**.

R	Compound	Method	Yield (%)
H	**176a**	**A**	45
H	**176a**	**B**	64
3,4-Dichloro	**176b**	**A**	60
3-Chloro	**176c**	**B**	70
3,4-Difluoro	**176d**	**B**	67
3-Fluoro	**176e**	**B**	74
Furfuryl*	**176f**	**A**	30

Method A: 2M-HCl, 24 h. *Method B*: 2.5M-HCl, dioxane, 5 h. *replacing the phenyl group

These novel compounds were fully characterised. As shown in Figure 82, the ^{1}H NMR spectrum of compound **176c** confirmed the successful cleavage of the acetal protecting group; the acetal methyl proton triplet, the pair of acetal *O*-methylene doublets and the methine triplet signals at *ca* 1.3, 3.5 and 4.3 ppm, respectively, are all absent. The new aldehydic proton signal is evident at 9.48 ppm. The characteristic phosphoramidate ester signals at 1.35 and 4.13 ppm, however, are present and indicate that this moiety was not affected under the hydrolytic conditions. In the ^{13}C NMR spectrum of compound **176c** (Figure 83), the aldehyde carbonyl carbon resonates at 199.2 ppm and the methylene carbon adjacent to the aldehyde group at 56.6 ppm. The phosphoramidate ethyl ester group signal at 16.3 ppm and 63.1 ppm

are split due to coupling to the ^{31}P nucleus, as is the benzylic carbon signal at 55.5 ppm, while all the aromatic carbons are all accounted for.

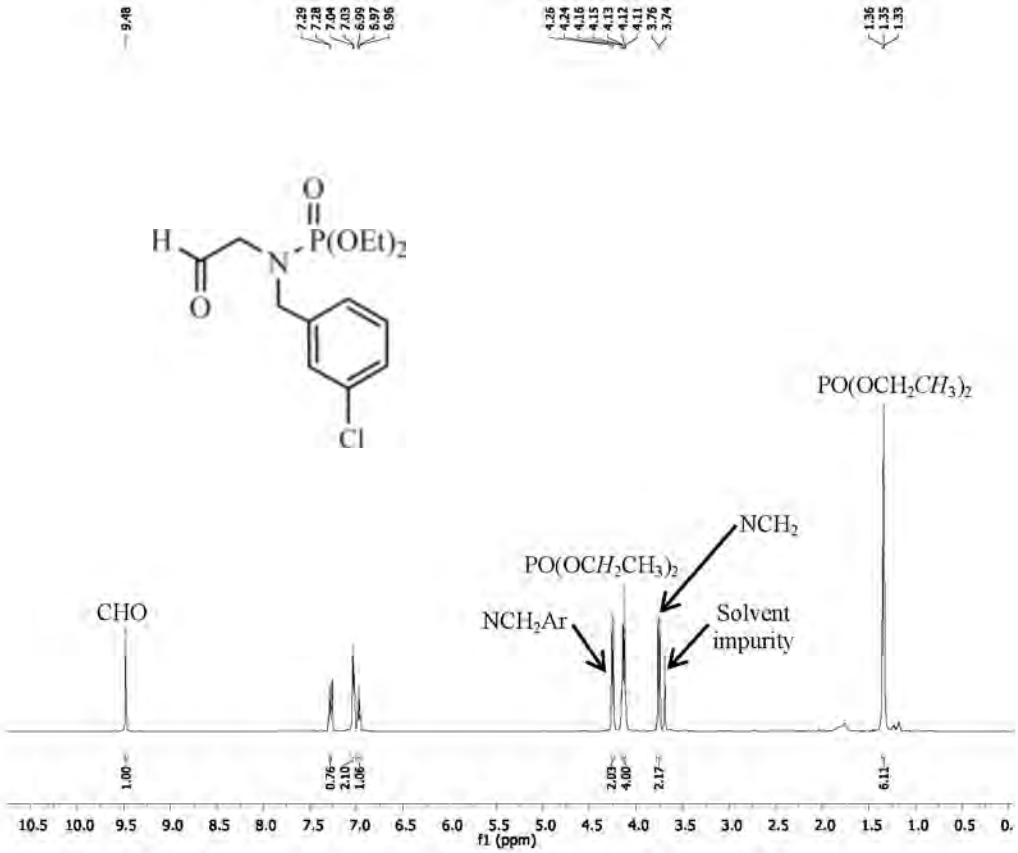


Figure 82. 600 MHz ^{1}H NMR spectrum of compound **176c** in $CDCl_3$.

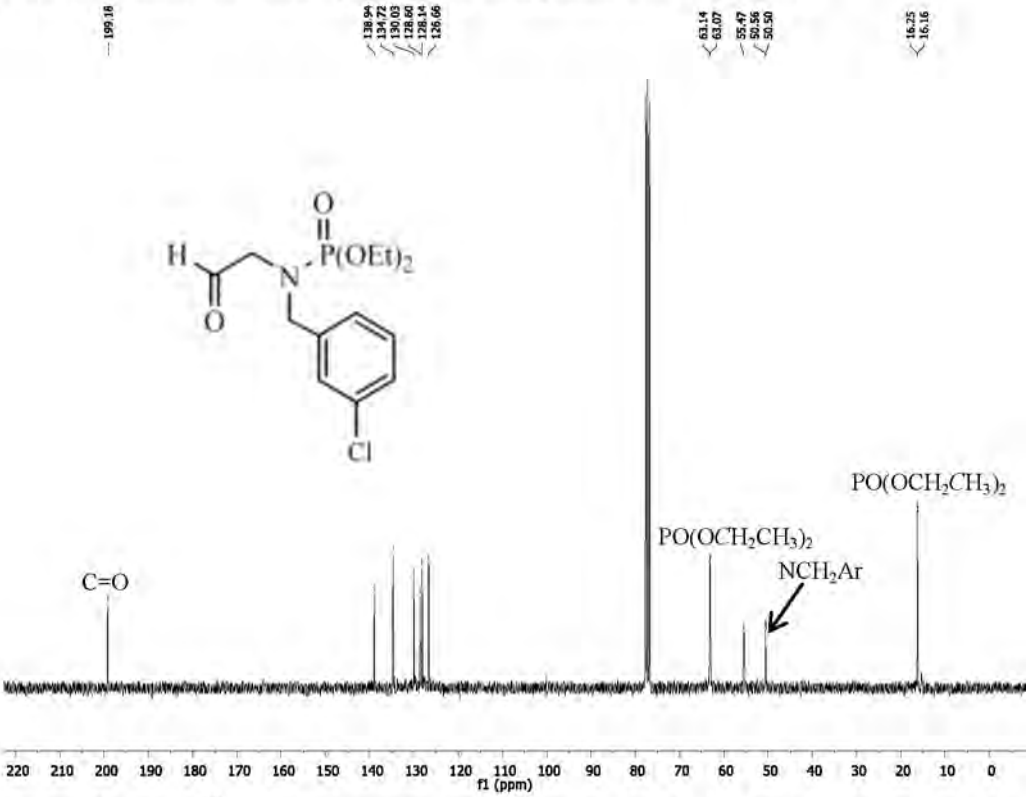
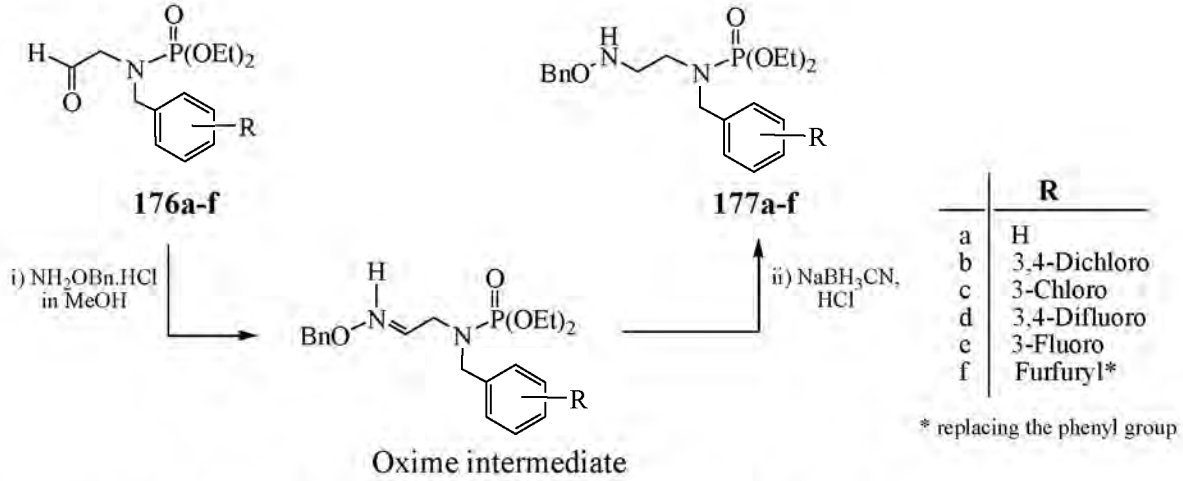


Figure 83. 150 MHz ^{13}C NMR spectrum of compound **176c** in $CDCl_3$.

2.3.4. Formation of *O*-benzylhydroxylamine derivatives 177a-f.

The *O*-benzylhydroxyamino compounds **177a-f** were obtained in a one-pot synthesis by reacting each of the aldehydes **176a-f** with *O*-benzylhydroxylamine hydrochloride in MeOH, followed by *in situ* reduction of the intermediate oximes (Scheme 25), using sodium cyanoborohydride and conc. HCl, to furnish the *O*-benzylhydroxylamines **177a-f**.[203-204] The purification of these compounds, however, was quite frustrating, due to their decomposition on silica gel resulting in generally low overall yields (Table 11) and the presence of an impurity evidenced by unwanted signals in the NMR spectra.



Scheme 25. Synthesis of *O*-benzylhydroxyamino compounds **177a-f**.

Table 11. Percentage overall yields of compounds **177a-f**

Compound	Yield (%)
177a	21
177b	38
177c	39
177d	57
177e	42
177f	35

For example, the ^{1}H NMR spectrum of compound **177a** (Figure 84) confirms the success of the reductive amination step by revealing the aromatic signals as a broad signal between 7.26 and 7.31 ppm integrating for the expected ten protons present in the two benzyl groups. The new singlet at 4.63 ppm corresponds to the *O*-benzylic protons. The ^{13}C NMR spectrum illustrated in Figure 85 also confirms formation of the *O*-benzylhydroxylamine derivative

177a, showing the new *O*-benzyl signal at 76.2 ppm and the additional four aromatic carbon signals.

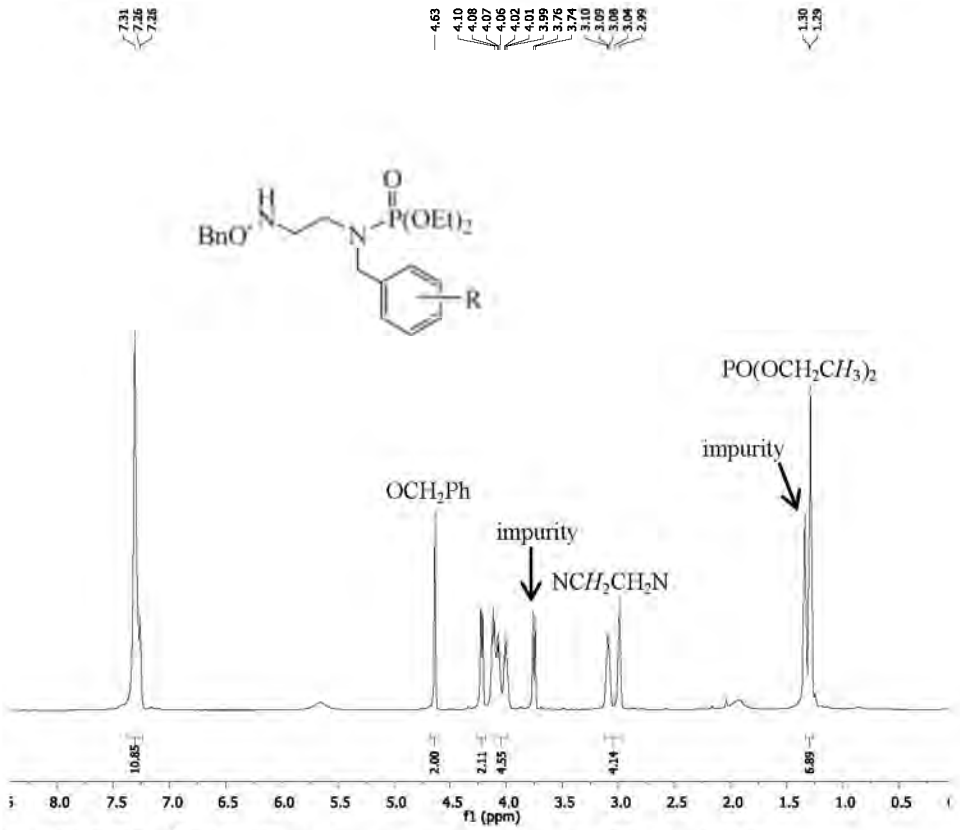


Figure 84. 600 MHz ^{1}H NMR spectrum of compound **177a** in $CDCl_3$.

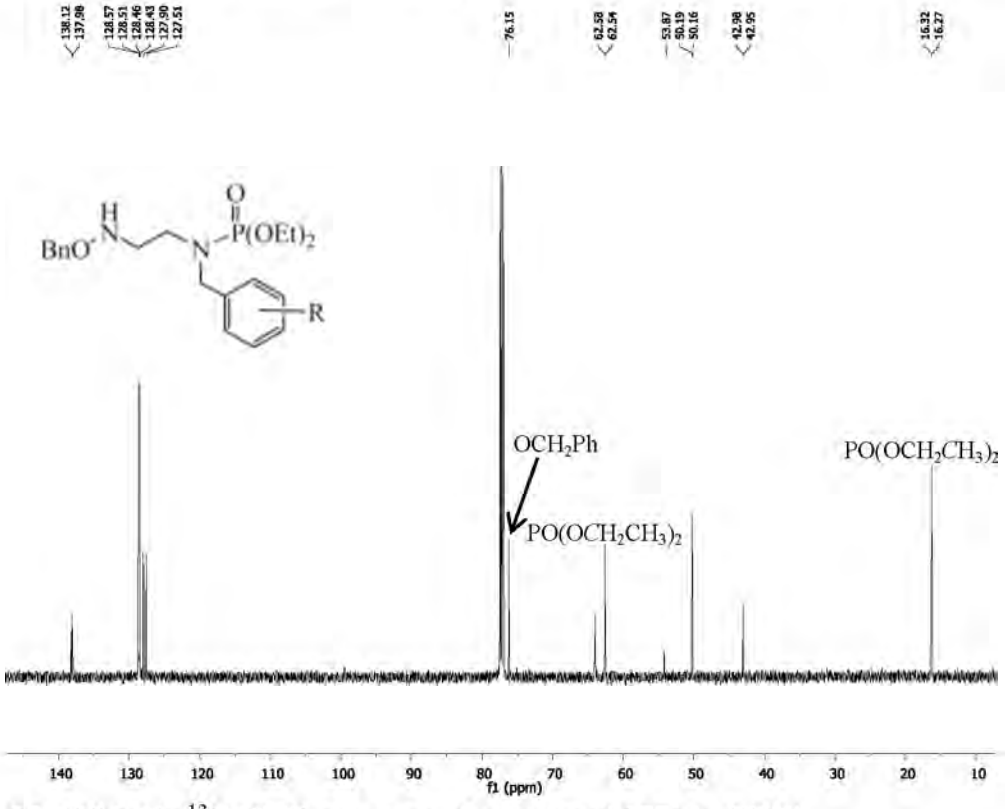
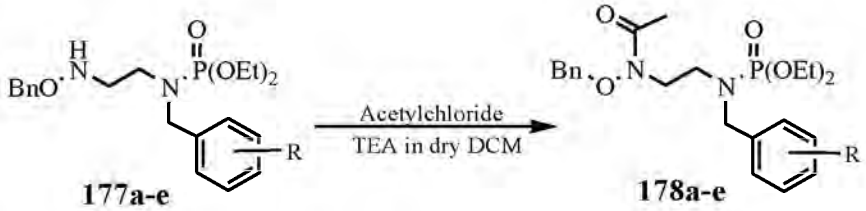


Figure 85. 150 MHz ^{13}C NMR spectrum of compound **177a** in $CDCl_3$.

2.3.5. Acetylation of the *O*-benzylhydroxylamine derivatives 177a-e

The formyl hydrogen in fosmidomycin **62** is replaced by an acetyl group in FR900098 **63**, a change which is responsible for the higher inhibitory activity of the latter compound. Introduction of an acetyl group in this study was considered crucial in order to mimic the FR900098 **63** structure as closely as possible. Acetylation of the hydroxylamine nitrogen was achieved by reacting each of the compounds **177a-e**, firstly, with 2 equivalents of triethylamine, using dry DCM as a solvent under an inert atmosphere and, then, with 1.5 equivalents of acetyl chloride, added dropwise followed by stirring at room temperature for 24 h (Scheme 26).[205-207] Compounds **178a-e** were obtained in good purity and in moderate to high yields as shown in Table 12.



Scheme 26. Formation of compounds **178a-e**.

Table 12. Yields of the acetylated compounds **178a-e**.

R	Compound	Yield (%)
H	**178a**	52
3,4-Dichloro	**178b**	61
3-Chloro	**178c**	86
3,4-Difluoro	**178d**	61
3-Fluoro	**178e**	72

These new compounds were fully characterised. The ^{1}H NMR spectrum for compound **178e** (Figure 86) confirms the absence of the unwanted signals (impurities) and the formation of the expected product. The signal at 2.06 ppm integrates for the three acetyl protons and the inherent diastereotopicity of the germinal protons in each of the phosphoramidate *O*-methylene groups is again evident in the presence of a pair of multiplets at 4.04 and 4.08 ppm. Evidence for acetylation is also provided by the ^{13}C NMR spectrum of 3-fluoro analogue **178e** (Figure 87) which clearly shows the acetyl methyl signal at 20.5 ppm. The complex coupling observed in the aromatic region of this ^{13}C NMR spectrum between 114.5 ppm and 130.1 ppm, as expanded in Figure 87, is worthy of further discussion. The C-2, and C-4 nuclei resonate as

doublets at 114.5 and 115.3 ppm due to coupling to the ^{19}F nucleus (J = 21.1 and 21.6 Hz), in addition to coupling to both the C-5 (J = 2.7 Hz) and C-1 resonaling at 140.7 ppm as a doublet of doublet (dd), while the carbon attached directly to flourine (C-3) has the largest coupling (J = 244.5 Hz). Analysis of the COSY and HSQC data supported these assignments.

The DEPT 135 NMR spectrum (Figure 88) reveals the presence of the new acetyl methyl signal at 20.4 ppm and the absence of a signal for the carbonyl carbon as it no longer has an attached proton.

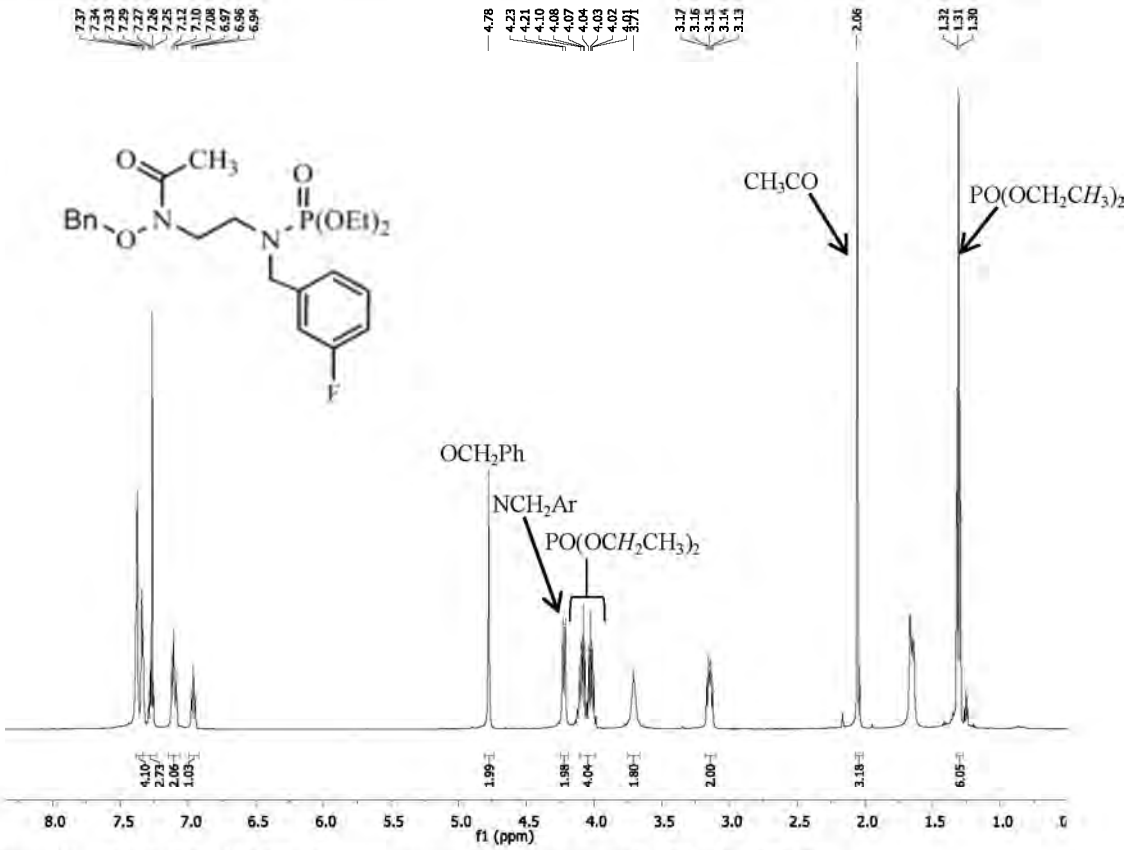


Figure 86. 600 MHz ^1H NMR spectrum of compound **178e** in $CDCl_3$.

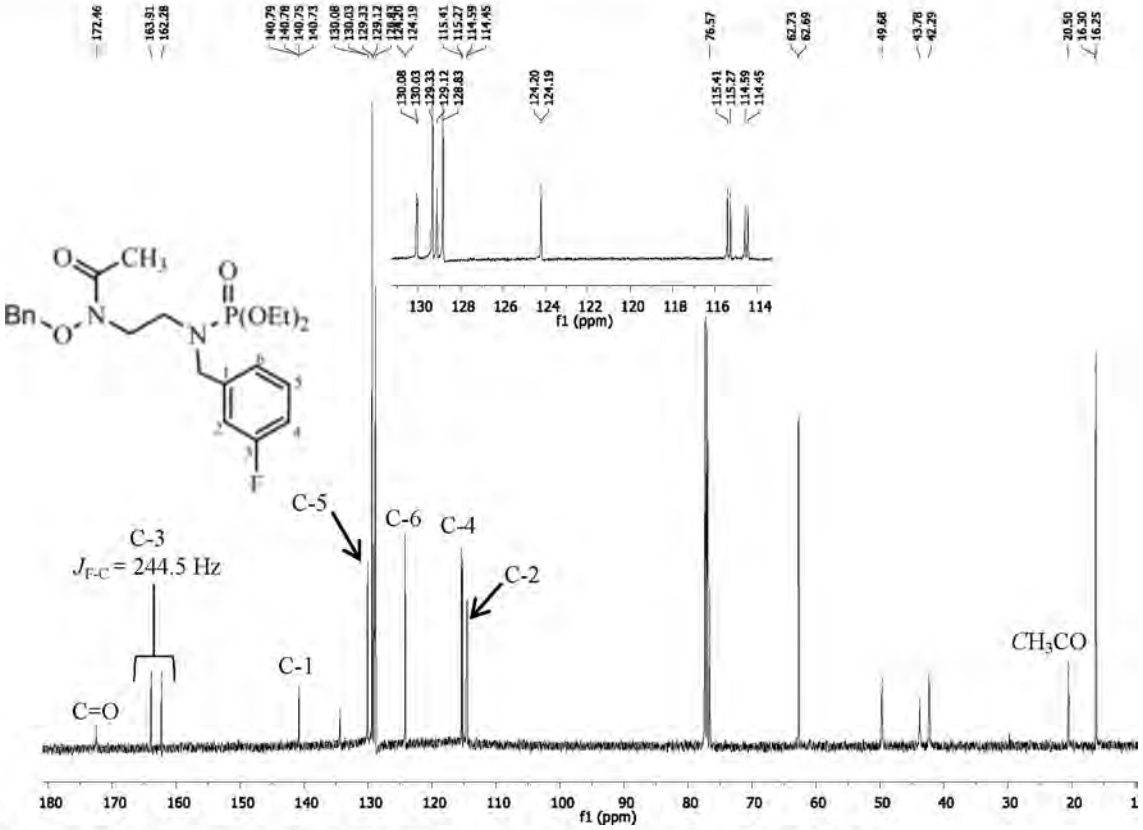


Figure 87. 150 MHz ^{13}C NMR spectrum of compound **178e** in $CDCl_3$.

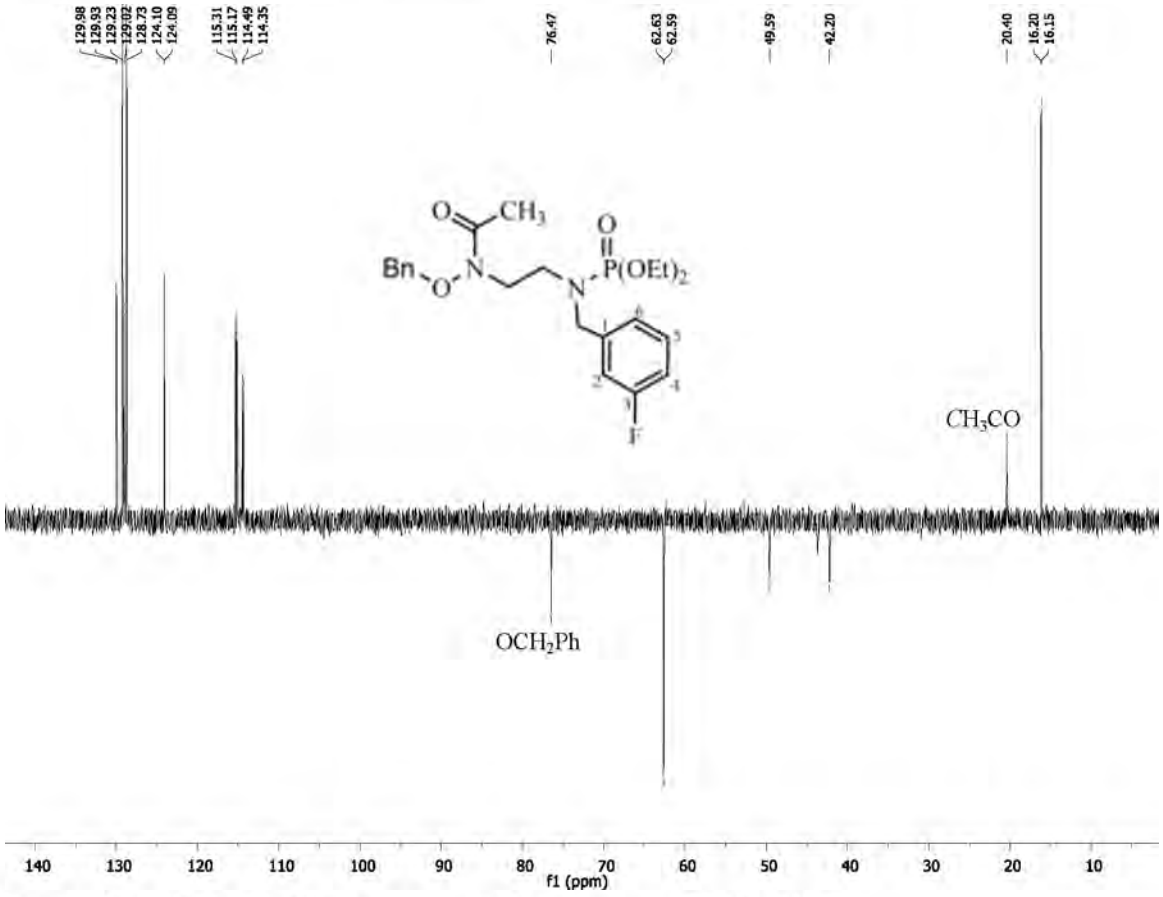
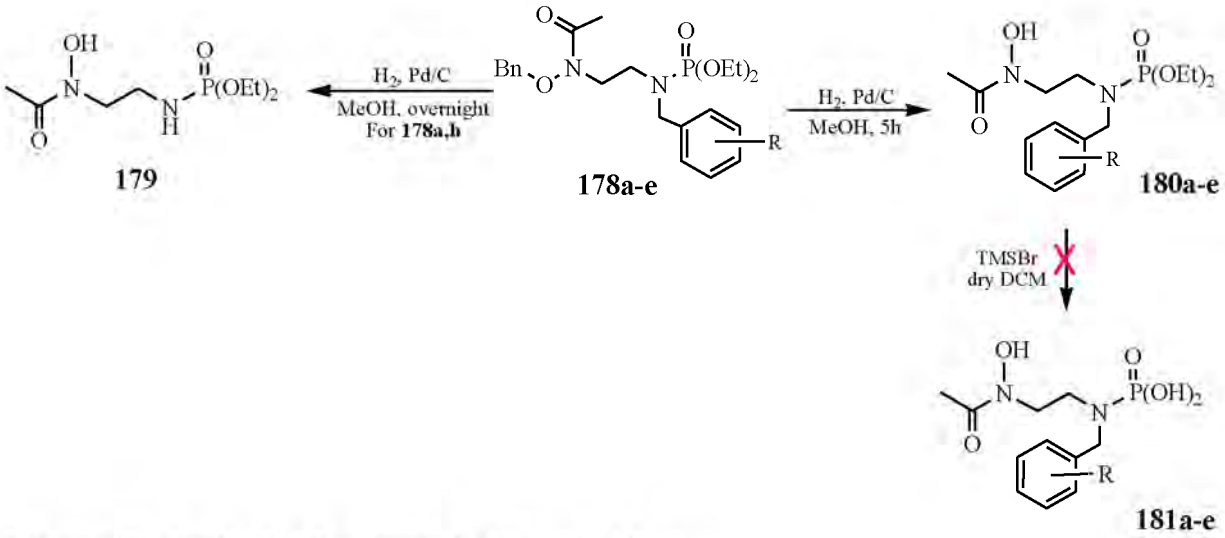


Figure 88. DEPT 135 NMR spectrum of compound **178e** in $CDCl_3$.

2.3.6. Selective deprotection of the *O*-benzylated hydroxylamine derivatives 178a-e.

Deprotection of the *N*-benzylated hydroxylamines **178a-e** was accomplished by catalytic hydrogenation using 10% palladium catalyst on carbon in dry MeOH at room temperature. This method proved to be problematic at first, since, when compound **178a,b** were stirred overnight under these condition compound **179** was obtained (Scheme 27). The procedure was non-selective resulting in the cleavage of both the *O*- and *N*-benzyl rings. The product, compound **179** is, in fact, very similar to FR900098 **63**, but with the amino group in the hydrophobic patch replacing a methylene group in the latter compounds. The method was then repeated and the reaction carefully monitored by TLC to ensure selective removal of the *O*-benzyl group alone,[208] and furnish the desired *N*-benzylated compounds **180a-e** after 5 hours in excellent yield, (Scheme 27, Table 13).



Scheme 27. Synthesis of compounds **179** and **180a-e**.

Table 13. Yields of the compounds **180a-e**

R	Compound	Yield (%)
H	**180a**	91
3,4-Dichloro	**180b**	95
3-Chloro	**180c**	97
3,4-Difluoro	**180d**	89
3-Fluoro	**180e**	90

The ^{1}H NMR spectrum of compound **179** (Figure 89) confirms the absence of all aromatic protons but the presence of a broad hydroxyl signal at 9.70 ppm; signals for both sets benzylic protons are also absent. However, the characteristic phosphonate ester moiety signals are still evident at 1.28 and 4.00 ppm. Compounds **180a-e** were fully characterised and the molecular formulae were supported by the HRMS data. The ^{1}H NMR spectrum of compound **180a**, for example (Figure 90), reveals a decrease in the number of aromatic protons from 10 to 5 and the disappearance of the *O*-benzylic proton signal, while the ^{13}C and HSQC NMR spectra illustrated in Figures 91 and 92, respectively, provide unambiguous support for the structural assignment.

Attempts to synthesise their corresponding phosphoramidic acids **181a-e** using TMSBr in dry DCM failed, due to concomitant loss of the *N*-benzyl group (Scheme 27).

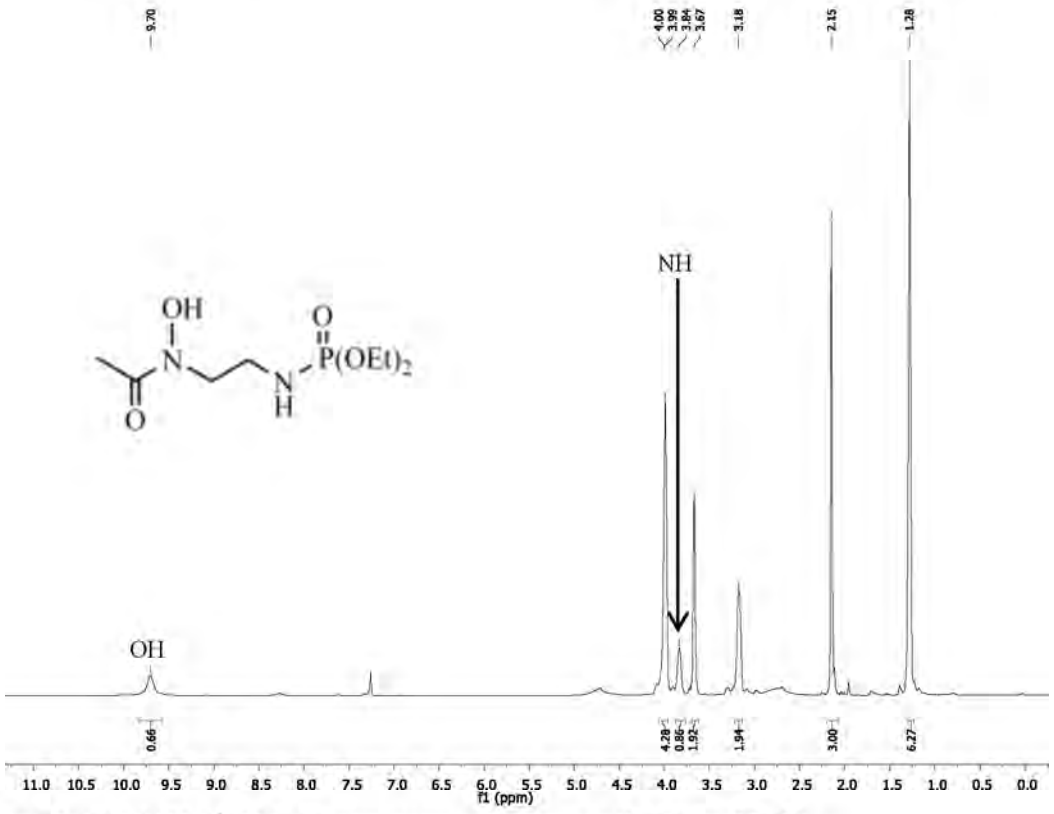


Figure 89. 600 MHz ^{1}H NMR spectrum of compound **179** in $CDCl_3$.

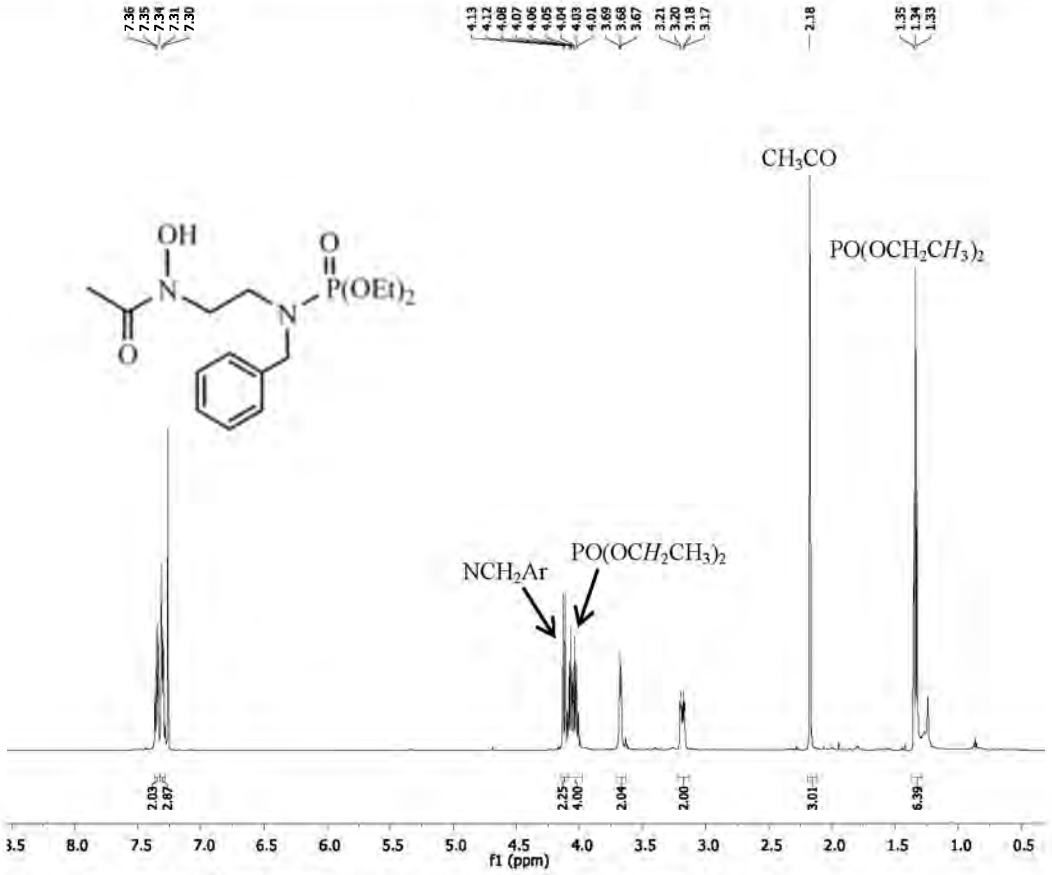


Figure 90. 600 MHz ^{1}H NMR spectrum of compound **180a** in $CDCl_3$.

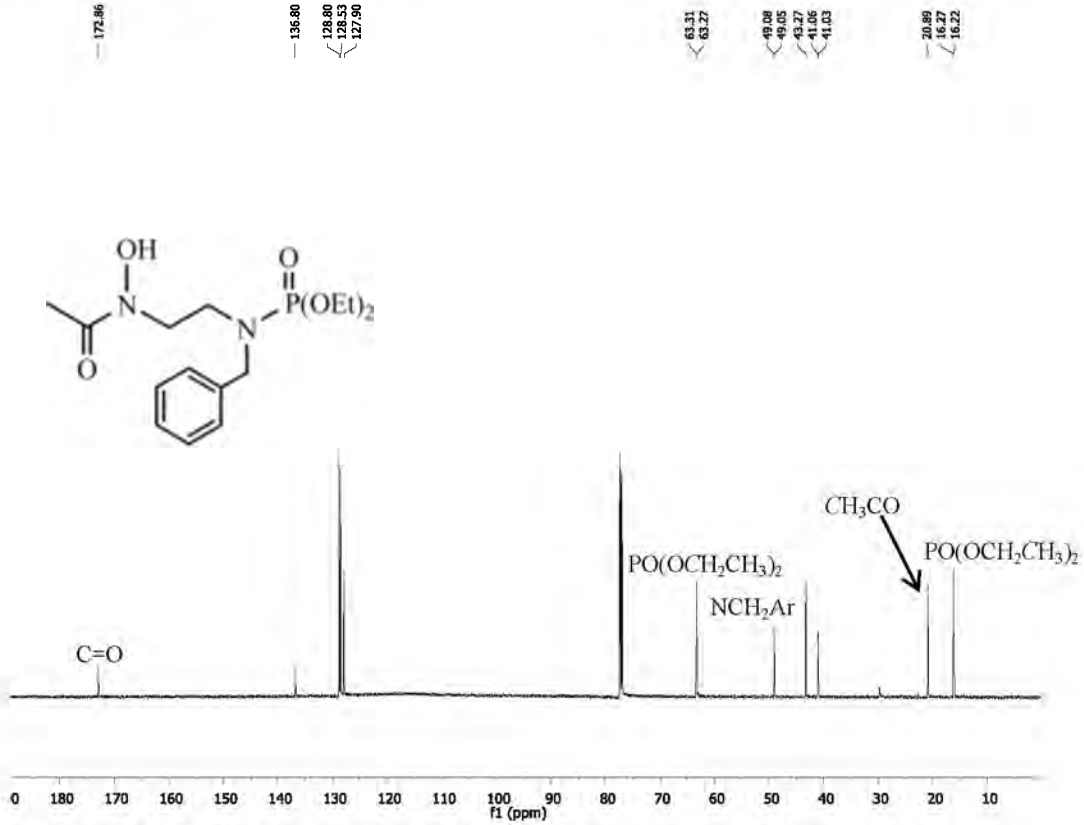


Figure 91. 150 MHz ^{13}C NMR spectrum of compound **180a** in $CDCl_3$.

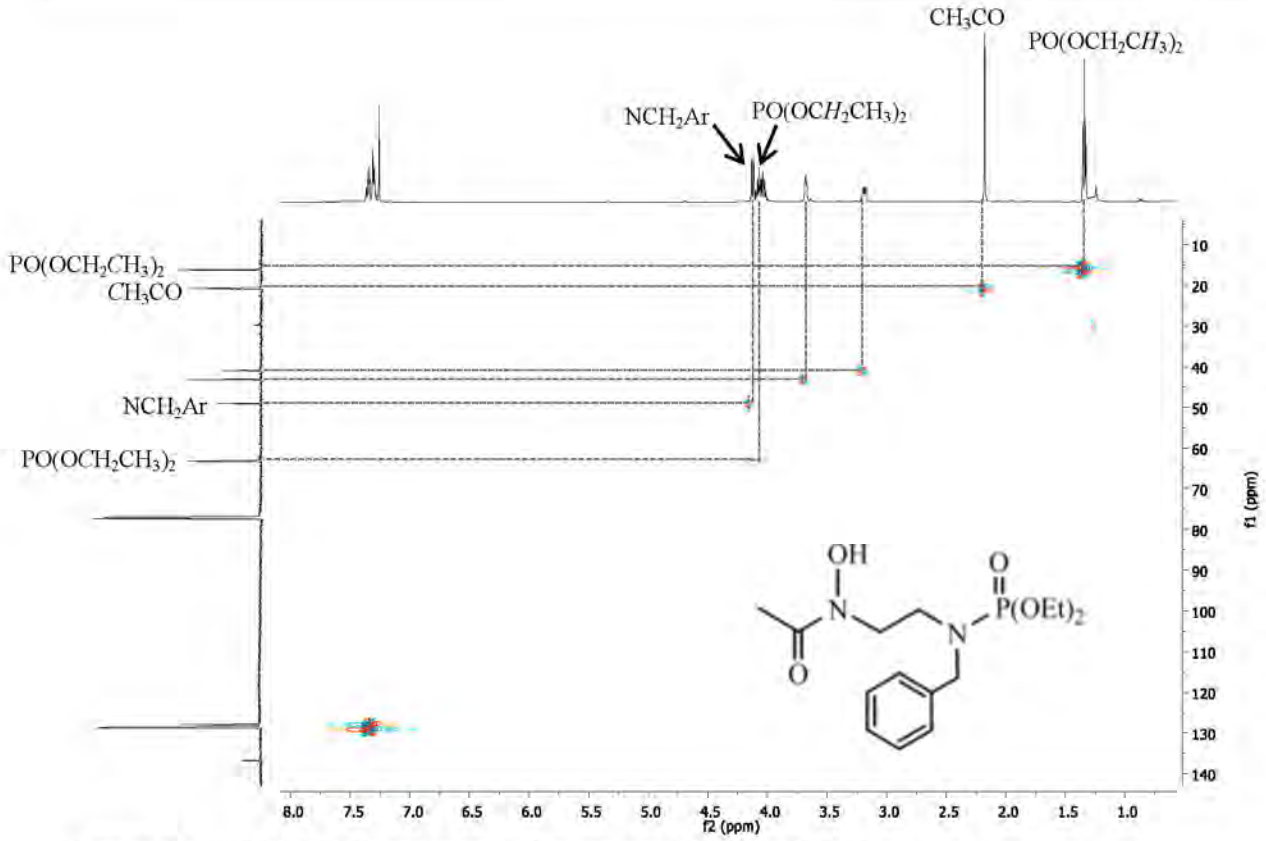


Figure 92. HSQC NMR spectrum of compound **180a** in $CDCl_3$.

2.4. SYNTHESIS of *N*-BENZYLATED CONFORMATIONALLY CONSTRAINED Mg^{2+} CHELATING LIGANDS

With an understanding of the importance of various structural features in fosmidomycin **62**,[209] we became interested in the possibility of introducing conformational constraints in the hydrophobic patch and, particularly, in facilitating a *syn*-orientation of the Mg^{2+} coordinating donor atoms in the ligand. We envisaged that this could be achieved by the introduction of a cyclic system between the hydroxamate moiety and the phosphonate group. Our aim of occupying the vacant hydrophobic pocket adjacent to the active-site was also incorporated by introducing an *N*-benzyl group close to the phosphonate moiety, thus extending our work on *N*-benzyl phosphoramidates in this project (Figure 93).

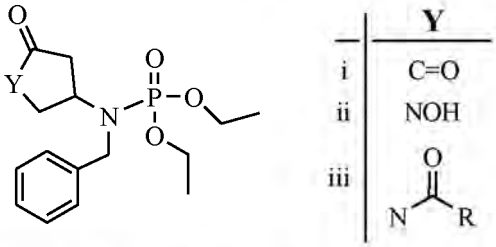
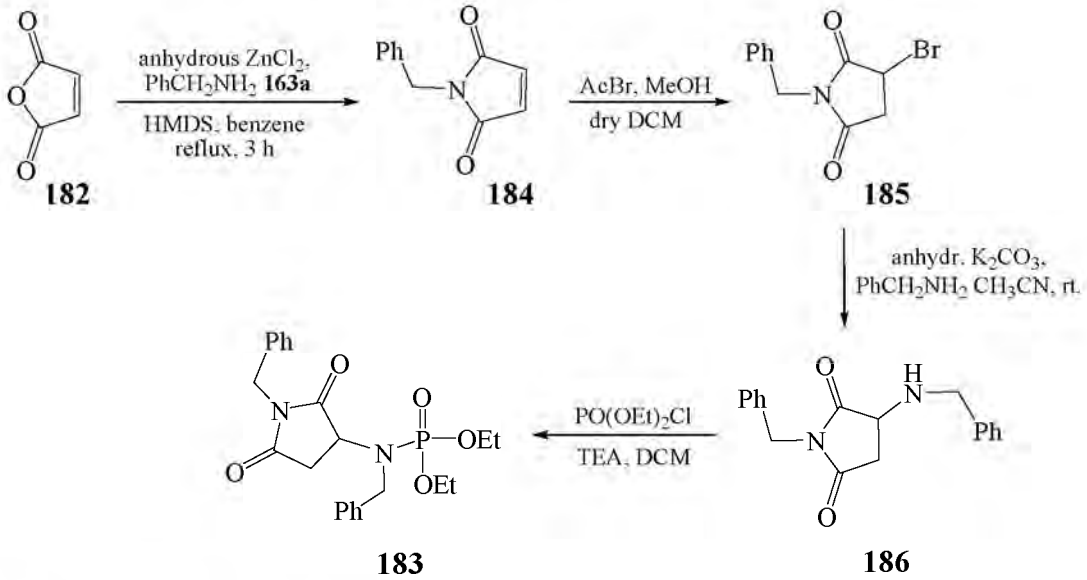


Figure 93. Structure of proposed analogues of cyclic systems.

2.4.1. Approaches towards the synthesis of conformationally constrained phosphoramidate ester analogues from maleic anhydride 182.

Several approaches to cyclic systems were explored in which attention was focused mainly on the replacement of the hydroxamate hydroxyl group by various other groups with metal coordinating properties. Firstly, the feasibility of using a maleimide scaffold to access suitable ligands was investigated, and using the *N*-benzylated system **184** as a model to establish the overall synthetic methodology. It was expected that other substituents, containing an oxygen atom for the purpose of coordinating with Mg^{2+} ion in the *Pf*DXR active-site, would then be prepared.

For the synthesis of compound **184**, a mixture of maleic anhydride **182** and anhydrous $ZnCl_2$ in dry benzene was refluxed, before adding benzylamine **163a**, followed by the treatment with hexamethyldisilazane, to afford the product *N*-benzyl malemide **184** (Scheme 28).[210] The ^1H NMR spectrum of compound **184** (Figure 94) revealed the presence of two vinylic protons resonating as singlet at 7.01 ppm, the benzylic protons as a signlet at 4.58 ppm and the expected number of aromatic protons resonating between 7.19 and 7.32 ppm.



Scheme 28. Synthesis of compound **183**.

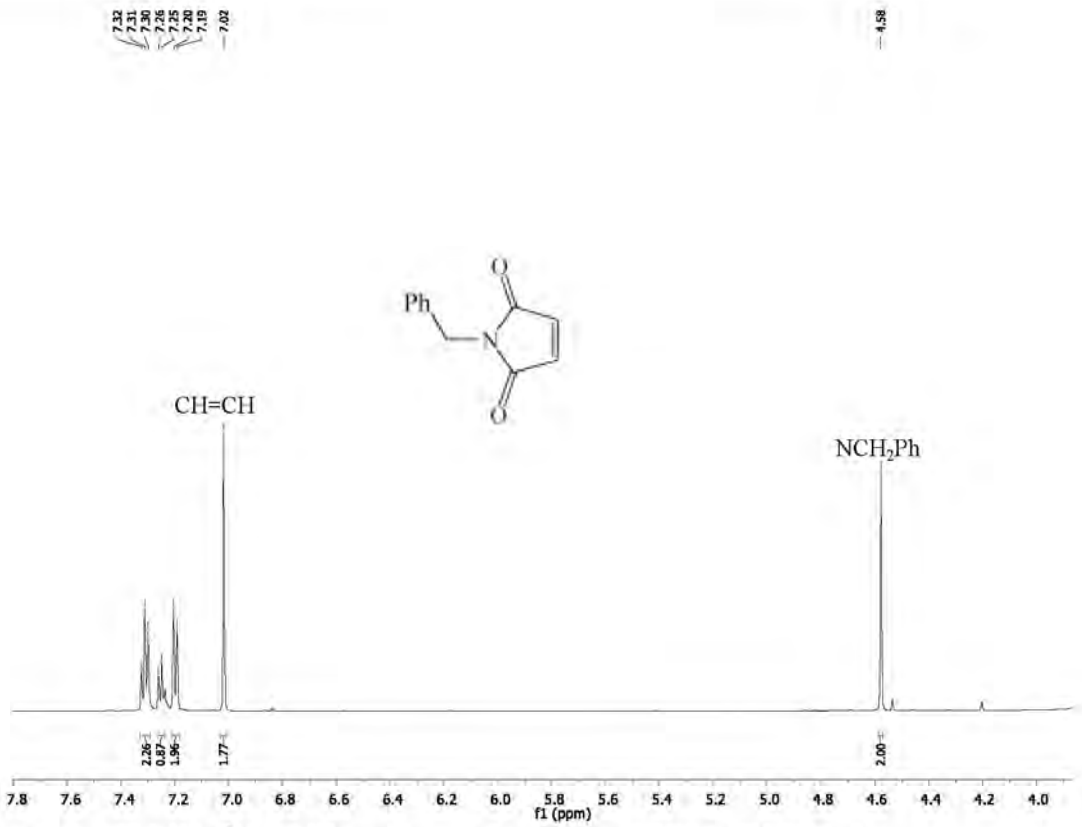


Figure 94. 600 MHz ^{1}H NMR spectrum of compound **184** in DMSO-d_6.

Hydrobromination of the vinylic protons of compound **184** was carried out by generating HBr *in situ* by reacting one equivalent of acetyl bromide (CAUTION-exothermic reaction!) with dry MeOH in cold, dry DCM as the solvent; compound **184** was then added and the mixture was stirred at room temperature overnight to furnish compound **185** in excellent yield without the need for further purification.[211]

The benzylamino derivative **186** was prepared by reacting the brominated compound **185** with benzylamine **163a** and anhydrous K_2CO_3 in dry acetonitrile at room temperature for 4 hours. Treatment of compound **186** with Et_3N and diethyl chlorophosphate at room temperature overnight then furnished the diethyl phosphoramidate ester **183** in 62% yield after column chromatography. The success of the phosphonation reaction is clearly supported by the ^{1}H NMR spectrum of compound **183** (Figure 95). The phosphonate ethyl ester methyl triplet appears at 1.27 ppm and the *O*-methylene multiplet at 4.10 ppm. In addition to this, the DEPT 135 NMR spectrum (Figure 96) reveals coupling between the ^{31}P nucleus and the methine and benzylic carbons which resonate as doublets at 55.5 and 63.0 ppm, respectively.

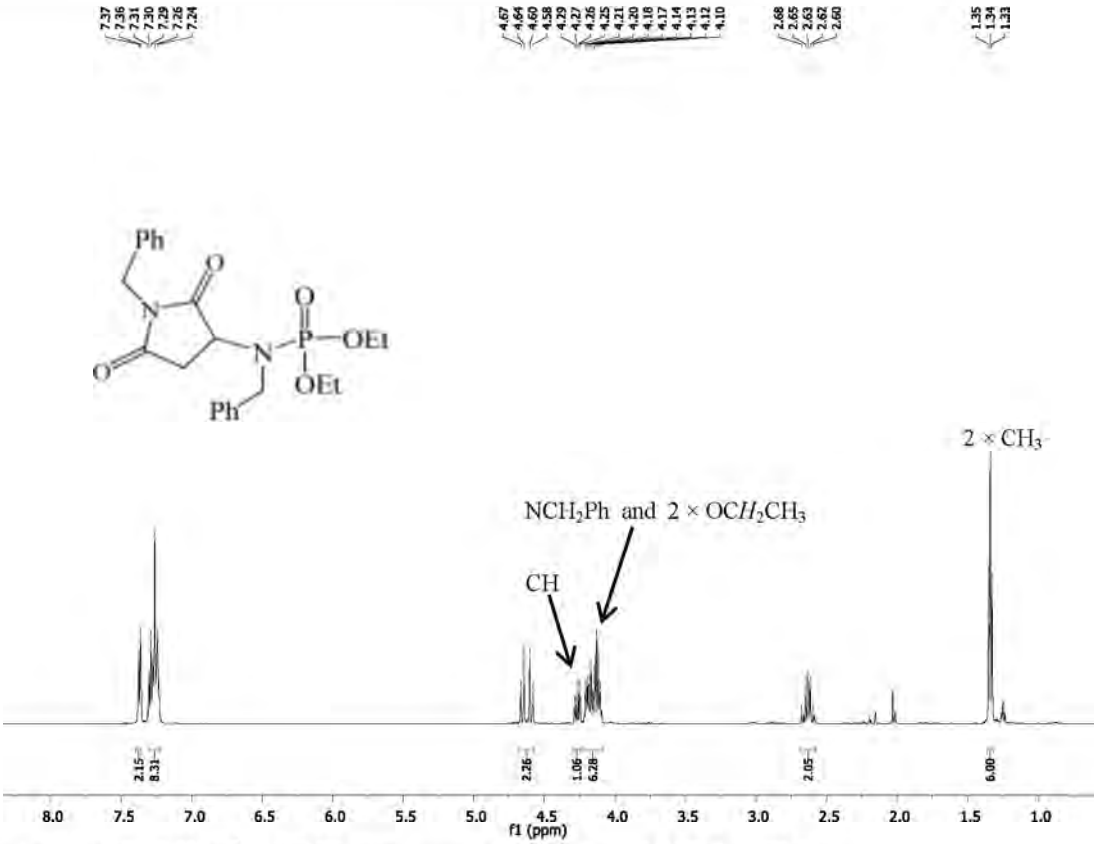


Figure 95. 600 MHz ^{1}H NMR spectrum of compound **183** in $CDCl_3$.

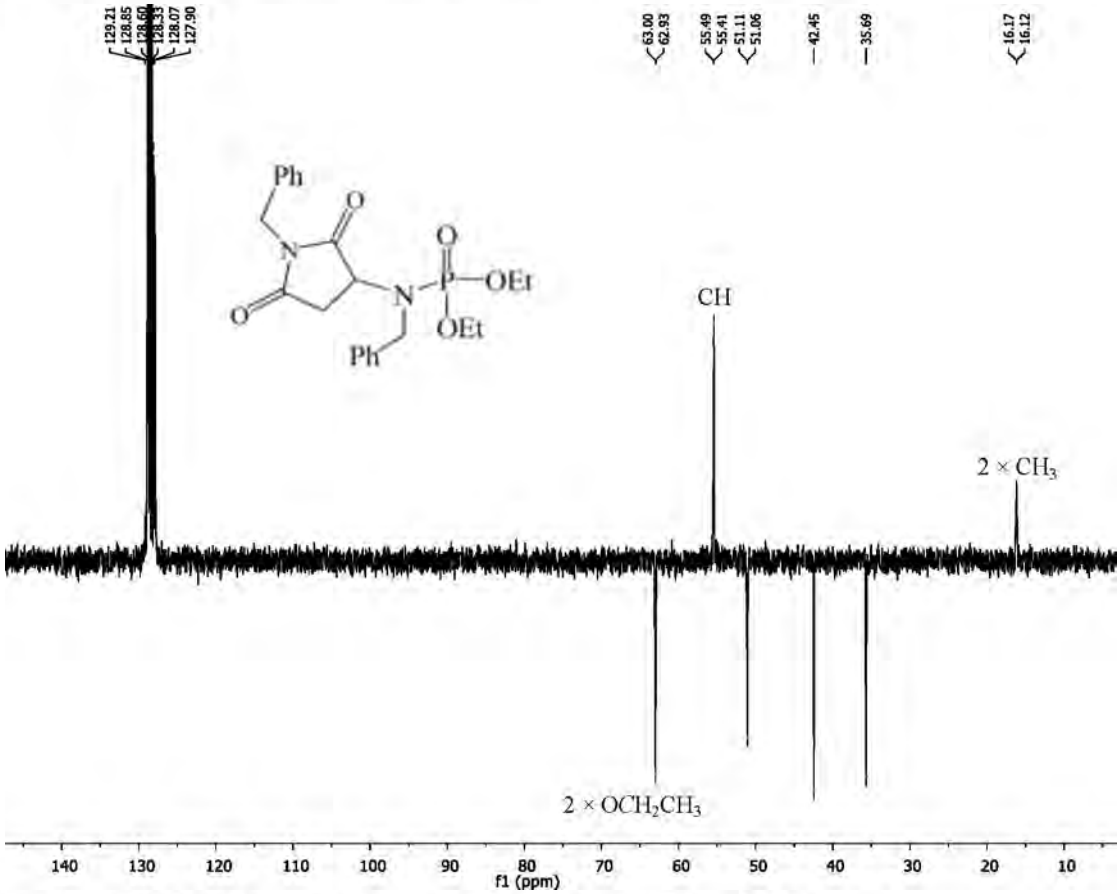
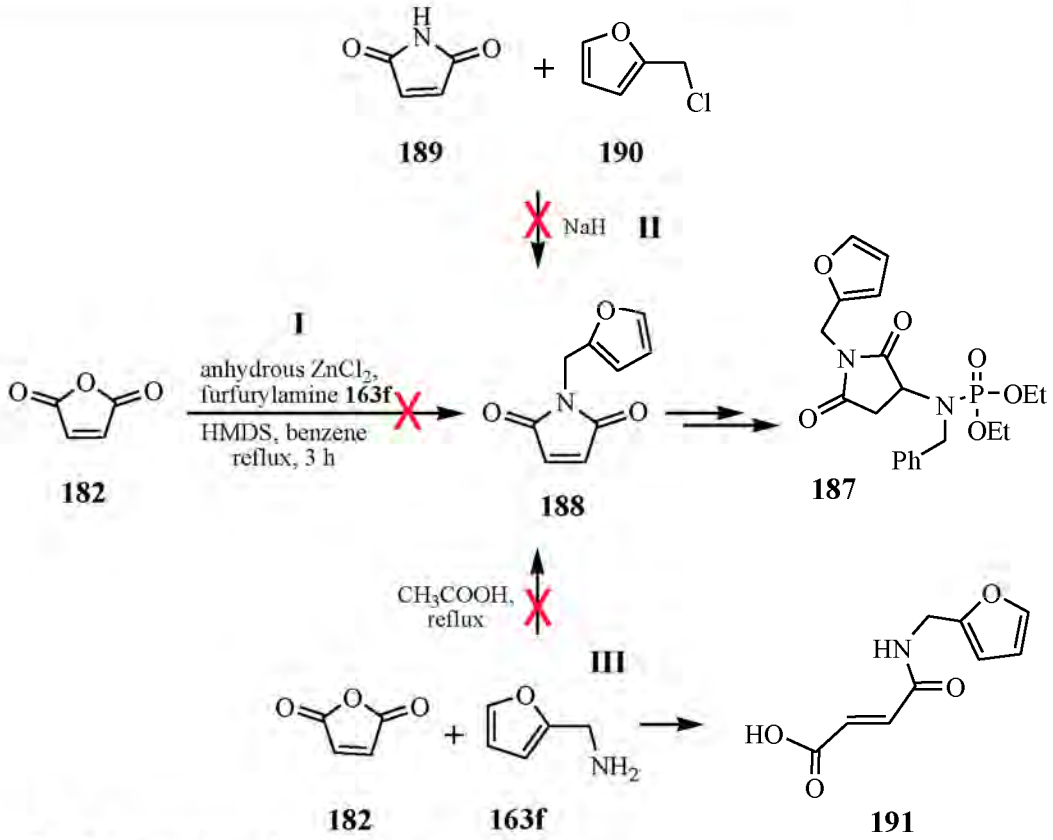


Figure 96. DEPT 135 NMR spectrum of compound **183** in $CDCl_3$.

Following the successful preparation of the *N*-benzylsuccinimide derivative **183**, attention was turned to the *N*-furfuryl analogue **187** (Scheme 29) which would provide an oxygen donor to coordinate with the divalent metal ion in the active-site. The established method for the synthesis of compound **183** (Scheme 28), was applied by reacting maleic anhydride **182** with furfurylamine **163f**, HMDS and anhydrous $ZnCl_2$ in dry benzene (Path I, Scheme 29).[210] Unfortunately, this failed to give the desired product **188**, and the reaction was repeated with careful monitoring of each step of the reaction using ^{1}H NMR analysis, but this failed to afford compound **188**. Deprotonation of the amide proton of maleimide **189** using NaH prior to the addition of furfuryl chloride **190** was similarly unsuccessful (Path II). On refluxing furfurylamine **163f** and maleic anhydride **182** in acetic acid (Path III),[212-213] the formation of a product was detected by TLC. However, the ^{1}H NMR spectrum (Figure 97) suggested this to be the acyclic intermediate **191**.

Further NMR, IR and HRMS analysis confirmed the product to be the acyclic intermediate **191**. In the ^{1}H NMR spectrum of compound **191**, the vinylic protons resonate as a pair of doublets at 6.27 ppm and 6.50 ppm, with a common coupling constant of 12.8 Hz, instead of the singlet expected for the cyclised intermediate **188**; a broad signal at 8.37 ppm corresponds to the amide proton. The ^{13}C NMR spectrum of compound **191** (Figure 98) shows a signal at 37.2 ppm corresponding to the *N*-methylene group, while the two carbonyl signals at 166.2 and 166.3 ppm correspond to the carboxylic and amide carbonyl carbons in the acyclic compound **191**. The HSQC NMR spectrum (Figure 99) permitted correlation of the proton and carbon signals and, especially, the vinylic signals in the opened ring.



Scheme 29. Attempted synthesis of compound **187**.

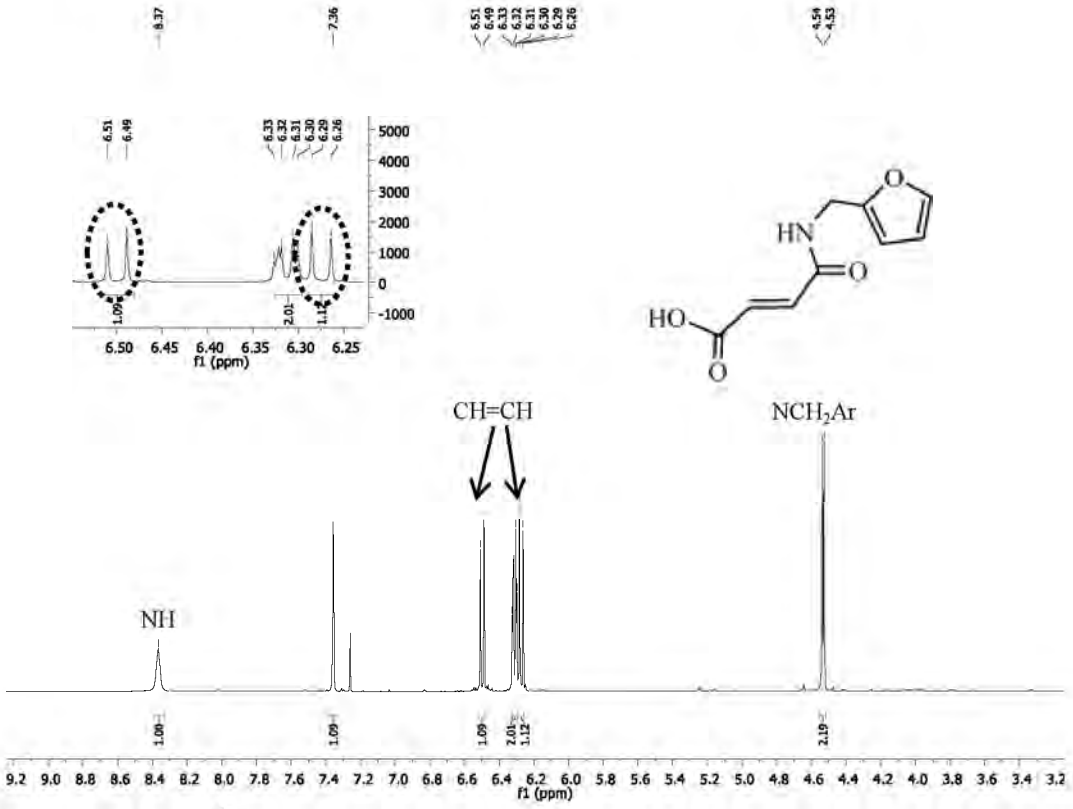


Figure 97. 600 MHz ^{1}H NMR spectrum of compound **191** in $CDCl_3$, with inset showing expansion of the spectrum in the aromatic region.

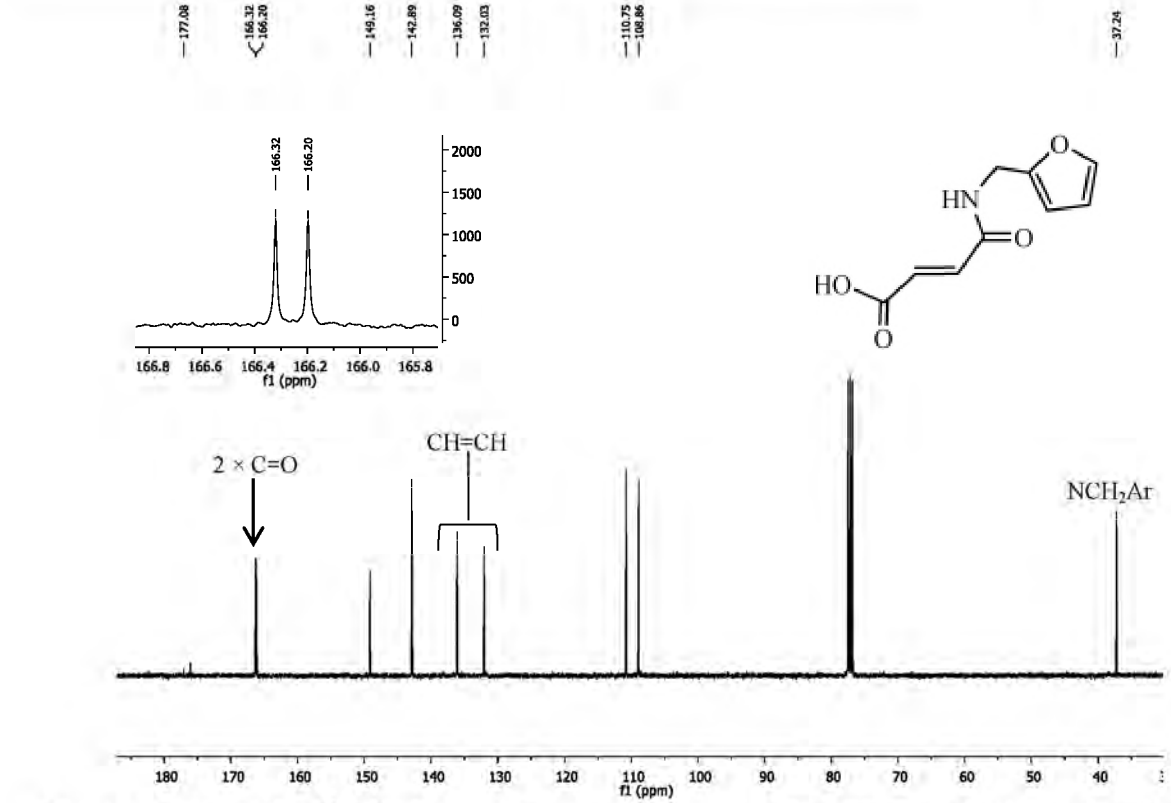


Figure 98. 100 MHz ^{13}C NMR spectrum of compound **191** in $CDCl_3$, with inset showing expansion of the carbonyl region.

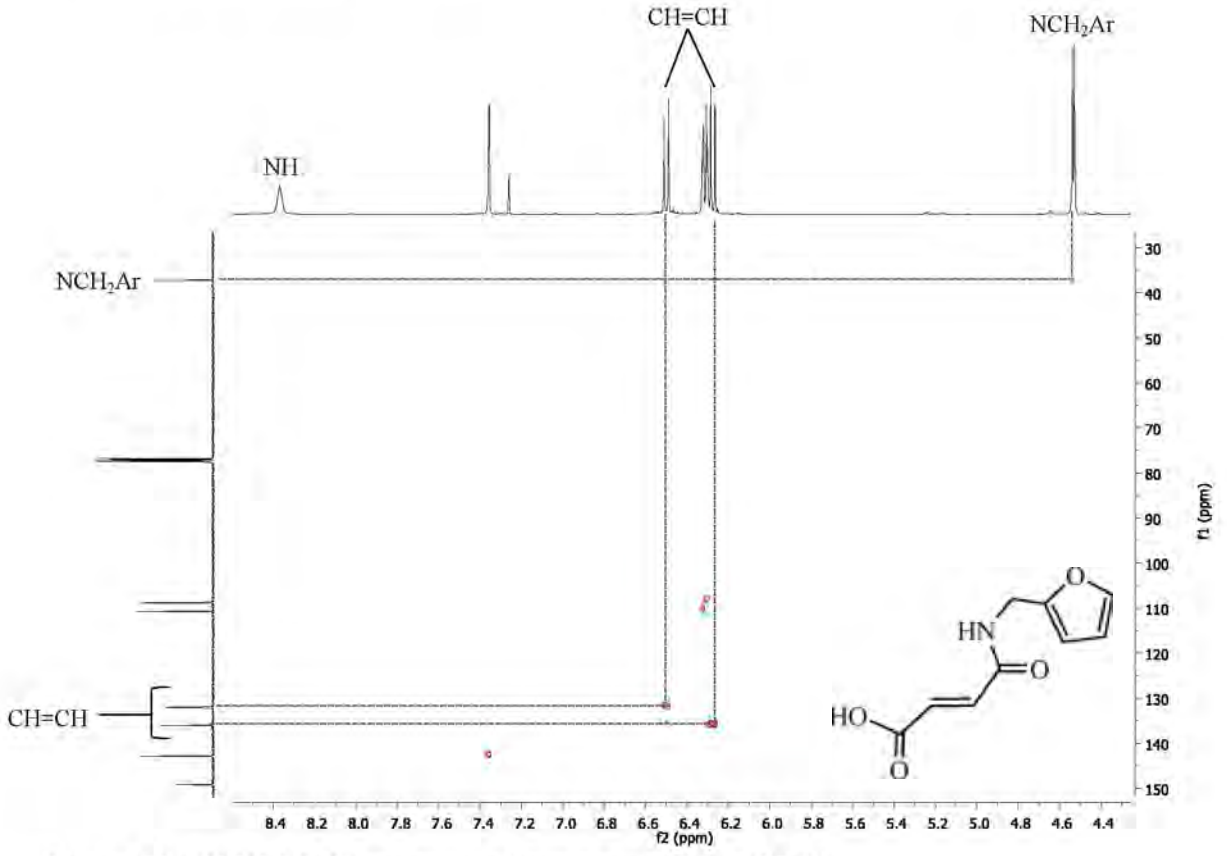
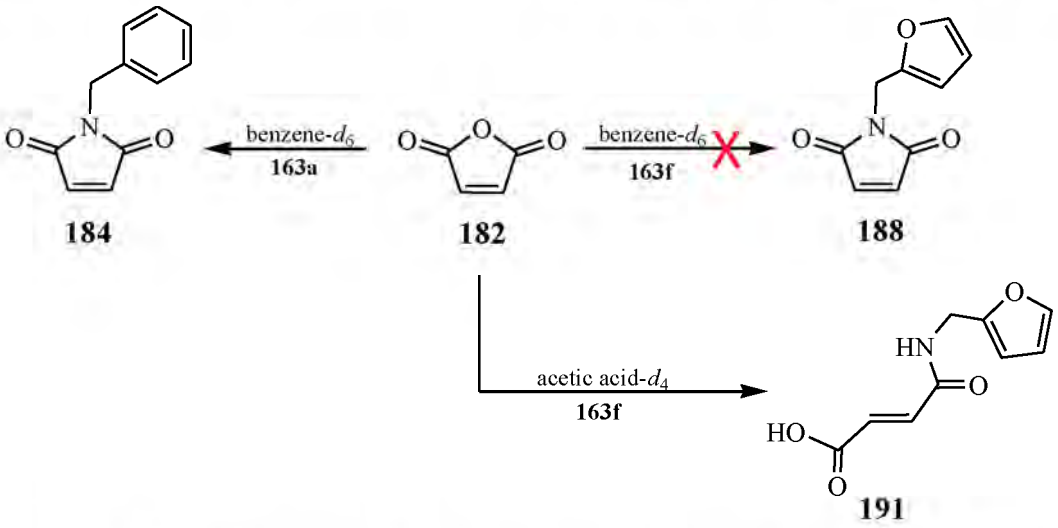


Figure 99. HSQC NMR spectrum of compound **191** in $CDCl_3$.

2.4.2. Preliminary NMR and theoretical studies of the reactions of maleic anhydride 182 with benzylamine 163a and furfurylamine 163f.

Although the method reported by Reddy *et al.*,[210] successfully provided access to *N*-benzylmaleimide **184** (Scheme 28), it unfortunately failed to afford the *N*-furfurylmaleimide analogue **188**. Given the difficulties encountered with the reaction with furfurylamine **163f**, it was decided to undertake a careful investigation of the reaction leading to compound **191**.



Scheme 30. Synthesis of compounds **184** and **191** in the presence of HMDS and $ZnCl_2$.

The reaction with furfurylamine **163f** (Scheme 30) was monitored by ^{1}H NMR spectroscopy at each point at which a reagent was added, thereby providing the following observations:

a. A slight change was observed in the ^{1}H NMR spectrum after anhydrous $ZnCl_2$ was added to the solution of maleic anhydride **182** in deuterated benzene (C_6D_6) and the mixture refluxed for 20 min. (Figure 100a).

b. When furfurylamine **163f** was added to the reaction mixture, the maleic anhydride **182** vinyl signal shifted upfield by *ca.* 4 ppm, indicating some form of association between the reactants and a change in the hybridisation state of the maleic anhydride vinylic carbons from sp^2 to sp^3 (Figure 100b).

c. Traces of product were evident from TLC analysis after hexamethyldisilazane (HMDS) was added and the reaction mixture had been heated under reflux; no essential change on ^{1}H NMR spectrum except the presence of the hexamethyldisilizane at 0.2 ppm. (Figure 100c).

d. After work-up, the "trace products" became predominant, with TLC analysis revealing many spots with closely related R_f values making isolation impossible. (Figure 100d). In contrast, ^{1}H NMR analysis of the *N*-benzylated analogue **184** after work-up confirmed formation of the product in excellent yield.

e. NMR studies of the reaction of maleic anhydride **182** and furfurylamine **163f** in refluxing acetic acid-d_4 revealed formation of the undesired acyclic product **191** early in the course of the reaction.

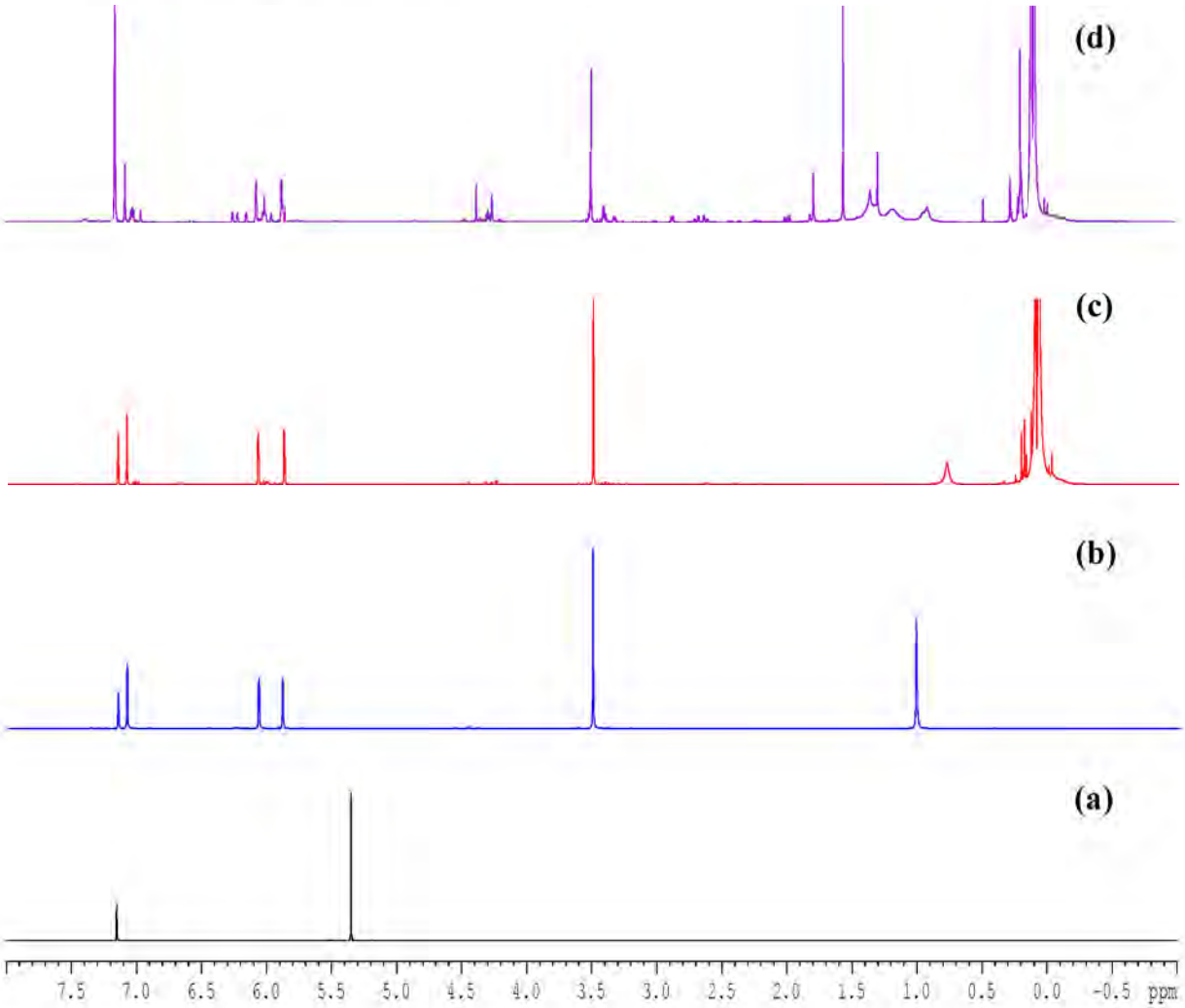
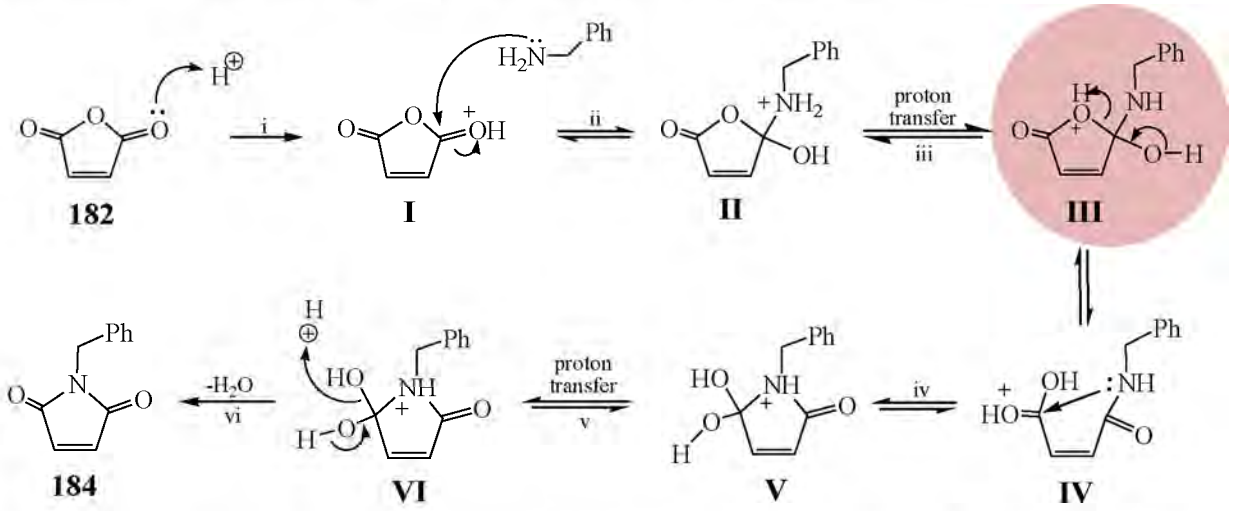


Figure 100a-d. ^{1}H NMR spectra of observations of the maleic anhydride **182** and furfurylamine **163f** reaction in benzene-d_6.

Given the marked differences in the reaction of maleic anhydride **182** with benzylamine **163a** and with furfurylamine **163f**, it seemed reasonable to intiate a theoretical study to elucidate the differences between the two systems.

Attention has been given to unraveling the expected mechanistic sequence for the reaction with benzylamine (Scheme 31) assuming a tetrahedral acyl substitution mechanism for the reaction at each of the carbonyl centres in maleic anhydride **182**. This mechanism involves: (i) protonation of a carbonyl oxygen in maleic anhydride **182**; (ii) attack by the nucleophile, benzylamine **163a**, on the protonated substrate **I** producing the tetrahedral intermediate **II**; (iii) proton transfer **III** and ring opening; (iv) nucleophilic attack by nitrogen on the second carbonyl **II** to form the tetrahedral intermediate **V**; (v) proton transfer; and (vi) dehydration **VI**.

Thus far, geometry-optimised structures have been located for various minima and one of the transition-state complexes in the benzylamine reaction at the B3LYP[216] 6-31G(d) level using Gaussian 09.[217] This work is ongoing and will be reported in due course.



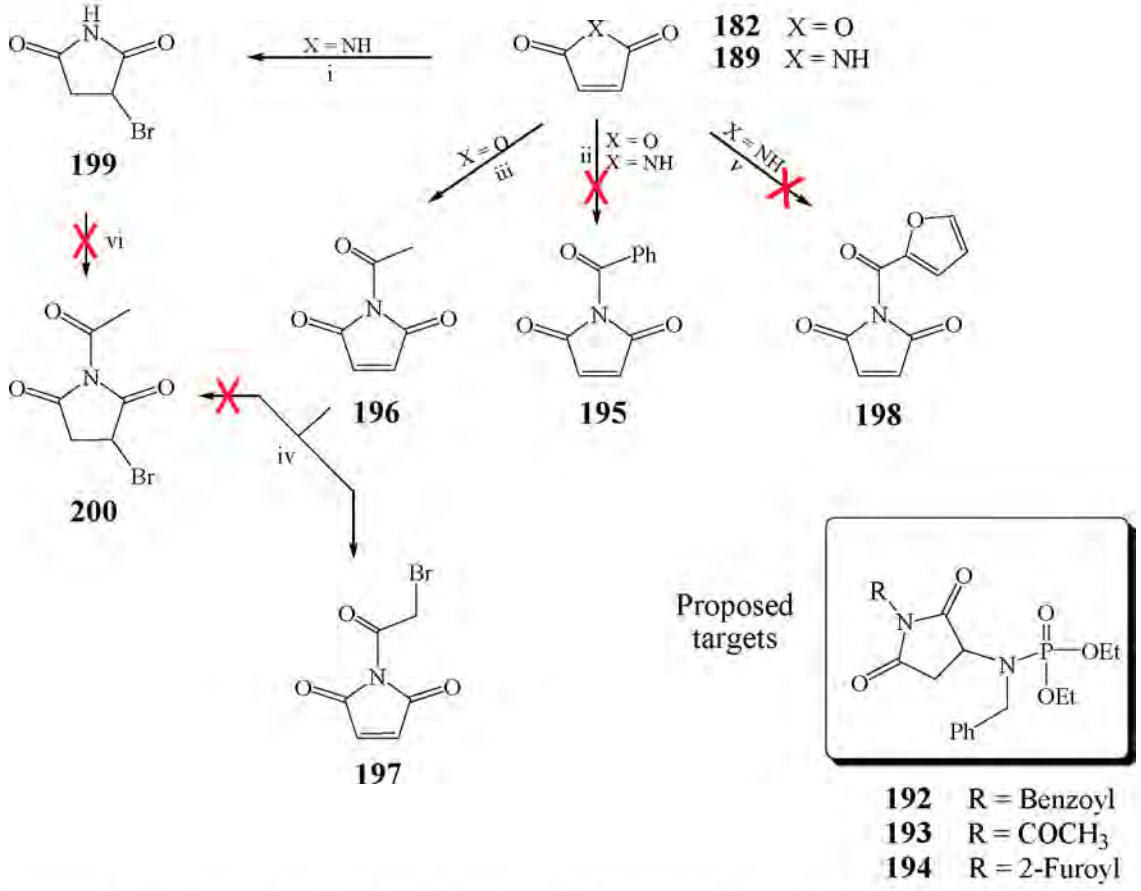
Scheme 31. Expected steps in the transformation of maleic anhydride **182** into *N*-benzyl-maleimide **184** *via* sequential tetrahedral mechanisms, (H^+ from AcOH or representing the lewis acid $ZnCl_2$)

2.4.3. Exploration of *N*-acylated maleimide derivatives

Given the difficulty encountered in the synthesis of compound **188**, access to *N*-acylated maleimide derivatives was investigated – specifically the *N*-benzoyl, *N*-acetyl and *N*-2-furoyl derivatives **192**, **193** and **194**, respectively (Scheme 32). The formation of the intermediate compounds **195** and **196** failed when maleic anhydride **182** (X = O) was treated with benzamide or acetamide, using anhydrous $ZnCl_2$ and HMDS in dry benzene.[210] Refluxing a mixture of maleic anhydride **182** and benzamide in acetic acid also failed to afford compound **195**, giving instead a ring-opened product after column chromatography. The expected

intermediate **196** was however obtained by refluxing maleic anhydride **182** with acetamide in acetic acid, but an attempt to hydrobrominate the double bond was unsuccessful, and led to the α-brominated derivative **197** instead.

A different approach was then explored using maleimide **189** (X = NH) instead of maleic anhydride **182** (X = O). The reactions of maleimide **189** with benzoyl chloride and 2-furoyl chloride using triethylamine in dry DCM, or NaH in dry THF, failed to furnish compounds **195** and **198** respectively (Scheme 32). Hydrobromination of maleimide **189** afforded compound **199**, but the reaction of 3-bromomaleimide **199** with acetyl chloride failed to afford the desired *N*-acetyl derivative **200** (Scheme 32).



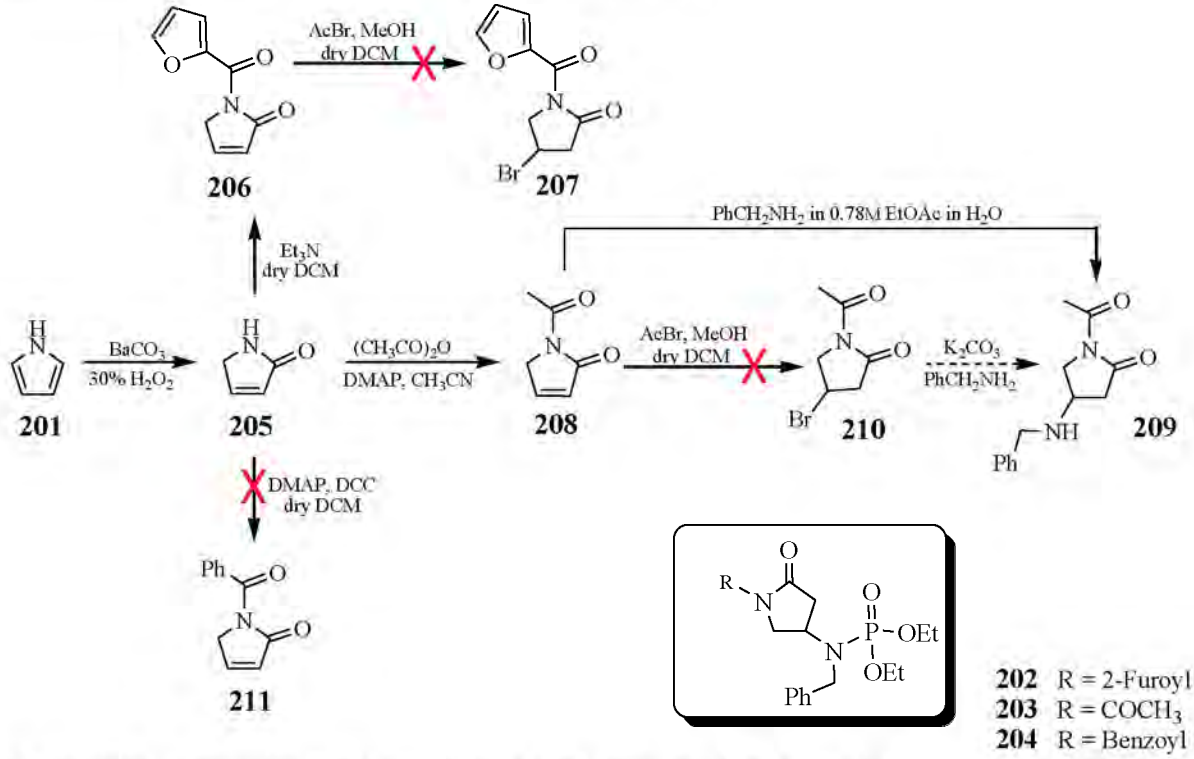
Scheme 32. Attempted synthesis of intermediate compounds **195**, **196** and **198**.

Reagents and conditions for reactions of maleic anhydride **182** (X = O) *as starting material*: (ii) benzamide, anhydr. $ZnCl_2$, HMDS, dry benzene; (iii) a) acetamide, anhydr. $ZnCl_2$, HMDS, dry benzene, b) acetamide, acetic acid; and iv) acetyl bromide, dry MeOH, dry DCM.

Reagents and conditions for reactions of maleimide **189** (X = NH) *as starting material*: (i) acetyl bromide, dry MeOH, dry DCM; (ii) benzoyl chloride, triethylamine, dry DCM or NaH, dry THF; (v) 2-furoyl chloride, triethylamine, DCM or NaH, dry THF; and (vi) acetyl chloride, Et_3N, dry DCM.

In view of these difficulties, we decided to explore the use of pyrrole **201** to synthesise compounds **202**, **203** and **204** as outlined in Scheme 33. Pyrrole **201** was reacted with 30% hydrogen peroxide in the presence of barium carbonate in refluxing distilled water to give 2*H*-pyrrol-2-one **205** in 32% yield.[219,220] 2-Furoyl chloride was then reacted with 2*H*-pyrrol-2-one **205** using triethylamine, as a mild base, in dry DCM to give compound **206**, but attempted hydrobromination failed to afford the 4-bromopyrrolidin-2-one **207**.

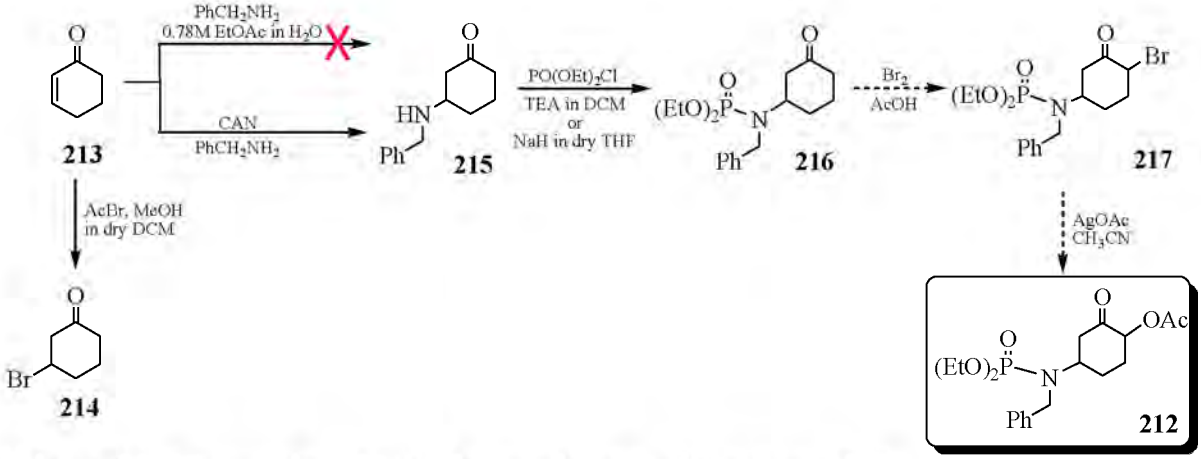
Acetylation of compound **205** with acetic anhydride and a catalytic quantity of DMAP gave compound **208** in 60% yield,[221] but an attempt to hydrobrominate compound **208** was unsuccessful. The amine **209** was therefore obtained by direct conjugate addition with benzylamine in a 0.78M dispersion of EtOAc in H_2O (Scheme 33).[222] The ^1H NMR spectrum of the crude product confirms the formation of the compound **209**, but attempted purification using both column and preparative layer chromatography led to the decomposition of the product. Treatment of 2*H*-pyrrol-2-one **205** with benzoic acid using DCC and DMAP in dry DCM under argon[223] also failed to give compound **211**.



Scheme 33. Attempted synthesis towards analogues **202**, **203** and **204**.

Following the difficulties encountered in the use of 5-membered cyclic scaffolds, attention was turned to the synthesis of the 6-membered ring system **212** with the commercially available 2-cyclohexen-1-one **213** being used as the starting material (Scheme 34).

Hydrobromination of 2-cyclohexen-1-one **213** was attempted using acetyl bromide and dry methanol in DCM, but the 3-bromo derivative **214** decomposed during workup. 2-Cyclohexen-1-one **213** was then reacted with benzylamine using a dispersion of EtOAc in H_2O but this method failed to give the desired aza-Michael product **215**. However, use of cerium ammonium nitrate (CAN) as a catalyst afforded the desired product **215**, but this decomposed during purification.[224] Attempted phosphonation of the impure compound **215** using diethyl chlorophosphate and triethylamine in DCM, or sodium hydride in THF, was unsuccessful (Scheme 34).



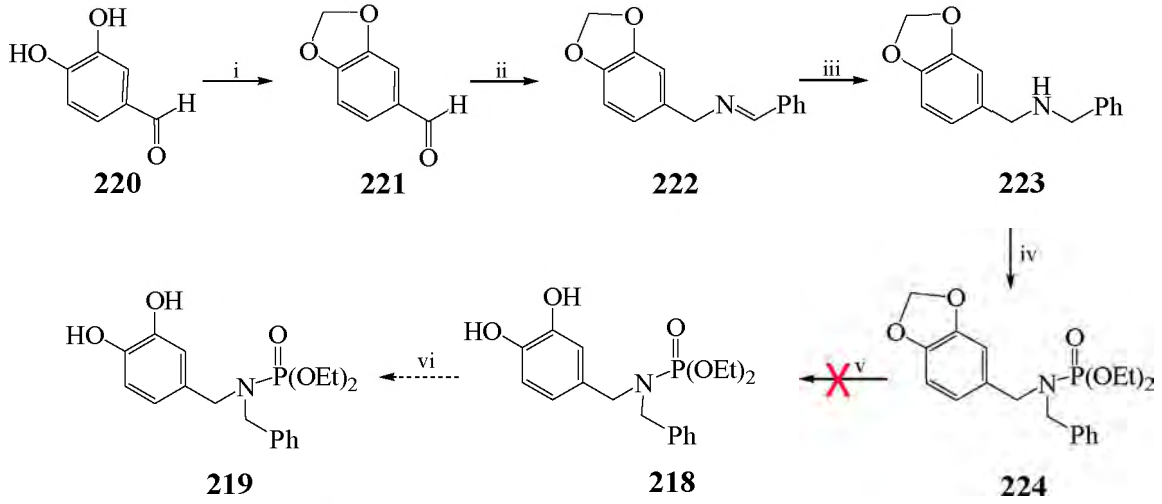
Scheme 34. Attempted synthesis of 6-membered cyclic analogue **212**.

2.4.4. Exploration of *ortho*-dihydroxyphenyl derivatives

Having encountered difficulties in accessing the cyclic phosphoramidate esters **187, 192-194**, **202-204** and **212**, we envisaged the possibility of using a pair of *ortho* hydroxyl groups attached to an aromatic ring to coordinate the Mg^{2+} divalent cation in the DXR active-site. The proposed route to the corresponding phosphoramidate ester (**218**) and acid (**219**) ligands containing the *ortho*-dihydroxyphenyl group is outlined in Scheme 35.

Using the protection strategy reported by Zelle and Moreau,[225,226] the commercially available 3,4-dihydroxybenzaldehyde **220** was reacted with bromochloromethane and caesium carbonate under reflux for 7 hours in dry acetonitrile to obtain the pure protected aldehyde **221** in excellent yield (Scheme 35). In the ^1H NMR spectrum of compound **221**, the presence of the methylenedioxy protons resonating as a singlet at 6.07 ppm, confirmed the success of the protection reaction. The expected signals for the three aromatic protons between 6.91 and 7.41 ppm and the aldehydic proton signal at 9.80 ppm were also present.

The protected aldehyde was treated with benzylamine in the presence of 2 equivalents of anhydrous $MgSO_4$ in dry DCM to give the imine **222**,[227,228] which was reduced *in situ* using sodium cyanoborohydride and conc. HCl to furnish the amine **223** (Scheme 35). The ^{1}H NMR spectrum of compound **223** (Figure 101) shows the two benzylic methylene singlets at 3.75 and 3.82 ppm.



Scheme 35. Proposed synthesis of ligands **218** and **219**.

Reagents and conditions: (i) $BrCH_2Cl$, Cs_2CO_3, dry CH_3CN, reflux, 7 h; (ii) anhydr. $MgSO_4$, $PhCH_2NH_2$, dry DCM, r.t, 3 h; (iii) $NaBH_3CN$, conc. HCl, MeOH, rt, 3 h; (iv) $PO(OEt)_2Cl$, triethylamine, DCM; (v) anhydr. $AlCl_3$, dry DCM, N_2, r.t; and (vi) TMSBr, dry DCM, N_2, r.t.

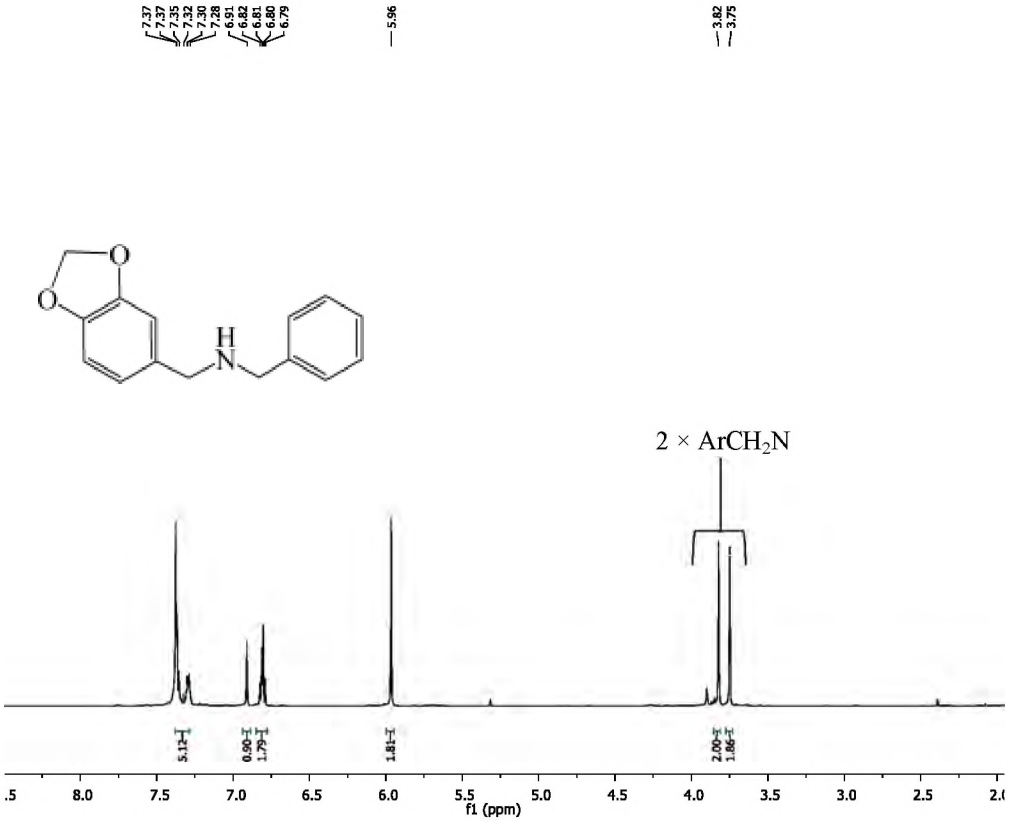


Figure 101. 600 MHz ^{1}H NMR spectrum of the amine **223** in $CDCl_3$.

The next step involved phosphonation of the amine **223** using diethyl chlorophosphate and triethylamine in DCM, to give the phosphoramidate ester **224** which was purified by flash chromatography. The ^{1}H NMR spectrum of compound **224** (Figure 102) reveals the characteristic phosphoramidate ester methyl triplet at 1.35 ppm. The ester *O*-methylene protons and the two pairs of benzylic protons resonate at *ca* 4.1 ppm, and their coupling with the nearby ^{31}P nucleus is evident in the expanded portions of the DEPT 135 and HSQC NMR spectra (Figures 103 and 104). Unfortunately, attempts to deprotect the *ortho*-dihydroxy group in compound **224** using anhydrous aluminium chloride in dry DCM, as reported by Stivers *et al.*,[229] or with conc. HCl or conc. H_2SO_4, resulted in decomposition. Consequently, another protection stategy was needed to be explored.

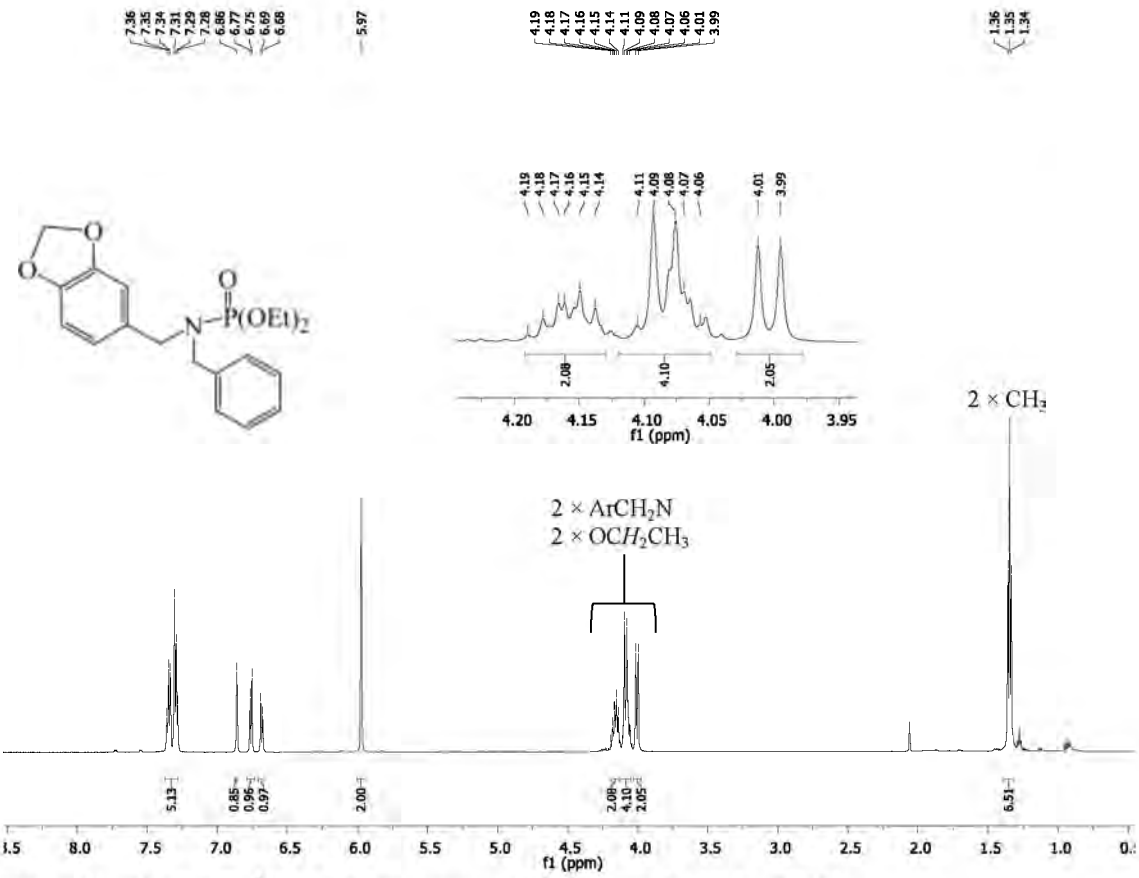


Figure 102. 600 MHz ^{1}H NMR spectrum of compound **224** in $CDCl_3$.

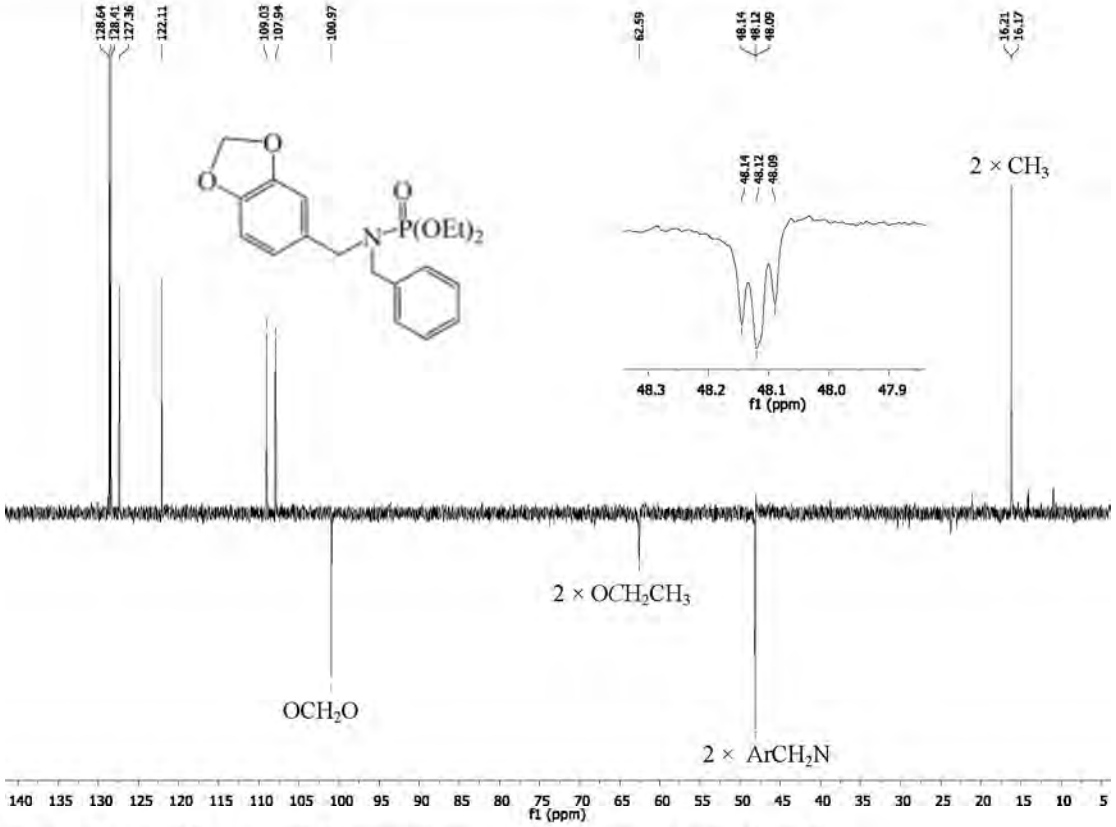


Figure 103. DEPT 135 NMR spectrum of compound **224** in $CDCl_3$.

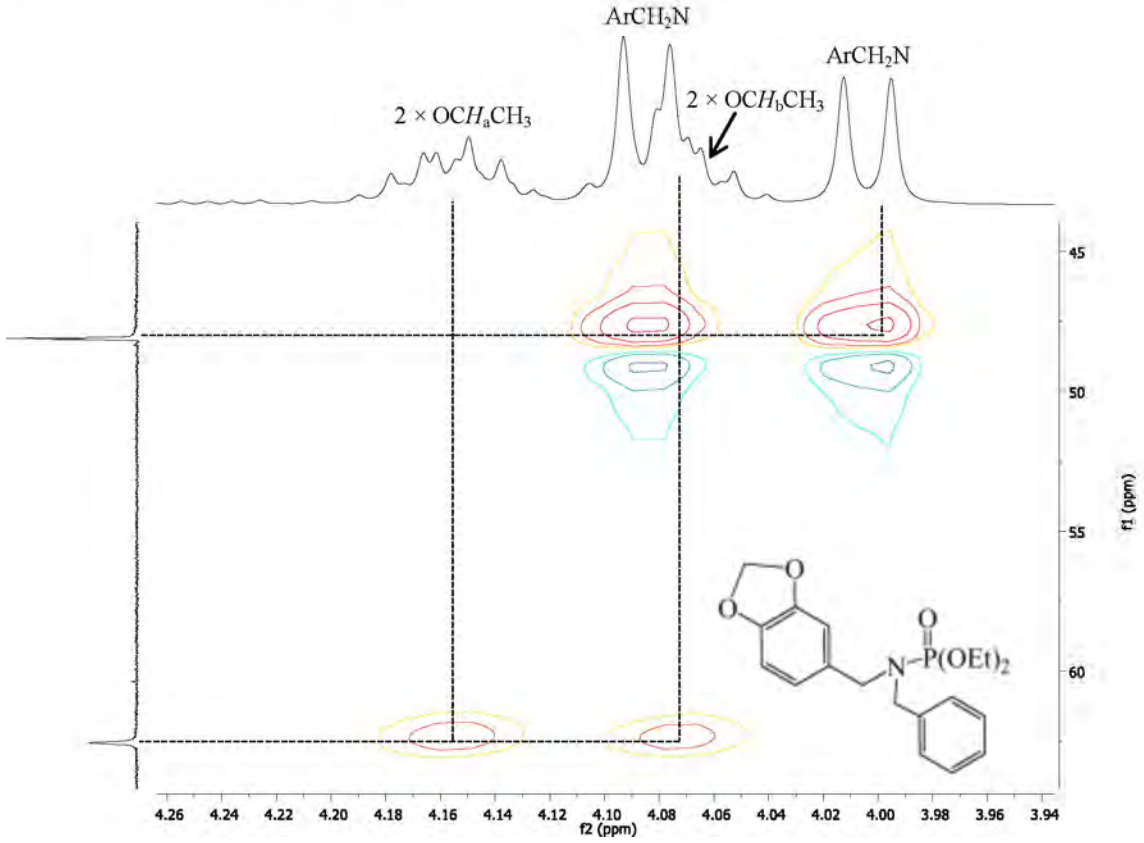
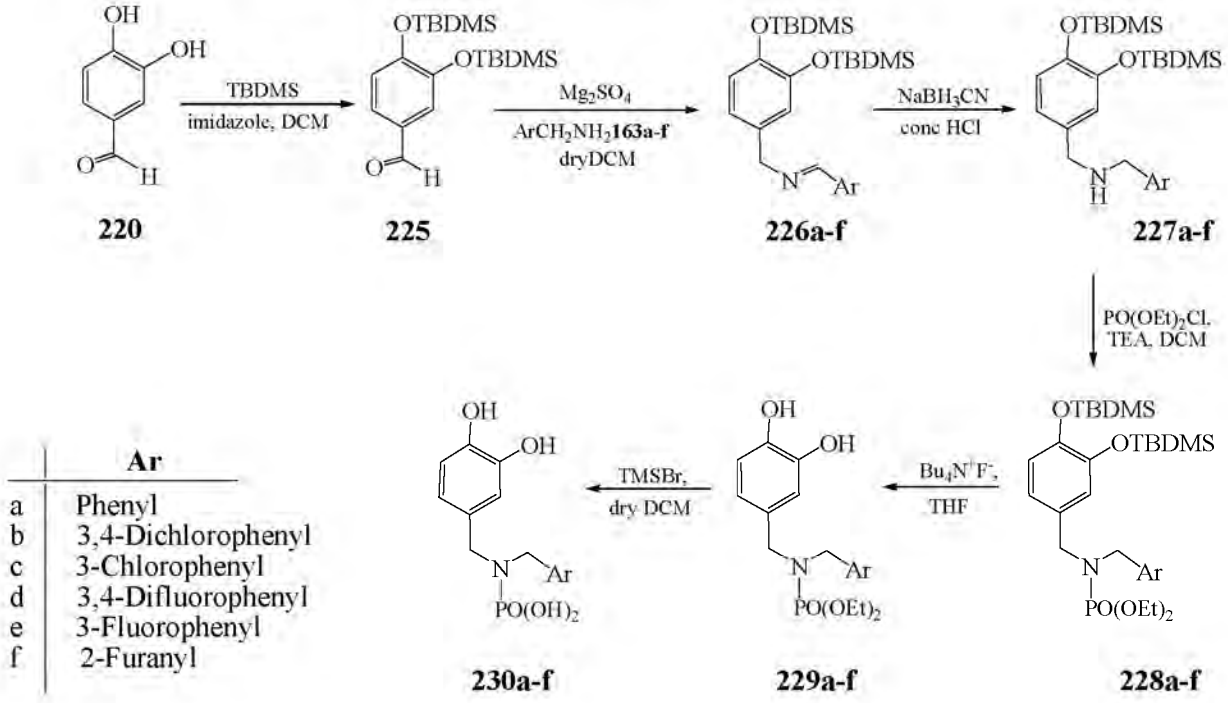


Figure 104. Partial HSQC NMR spectrum of compound **224** in $CDCl_3$.

Using the approach reported by Faler and Lawrence *et al.*,[230-231] 3,4-dihydroxybenzaldehyde **220** was reacted with *tert*-butyldimethylsiyl chloride in the presence of imidazole to furnish the protected derivative **225** (Scheme 36).[230] The ^{1}H NMR spectrum of this compound (**225**) showed two, 6-proton methyl singlets at 0.23 and 0.25 ppm and two singlets at 0.99 and 1.0 ppm integrating for the 18 protons corresponding to the pair of *tert*-butyl groups attached to silicon. Given the success in obtaining the bis-TBDMS-protected aldehyde **225**, the next step involved the formation of the series of imines **226a-f** using the amines **163a-f**,[229-230] (Scheme 36). The imines **226a-f** were reduced using sodium cyanoborohydride and conc. HCl to give the corresponding amines **227a-f** in good yields (Table 14). All of these compounds **226a-f** and **227a-f** are new and were fully characterised. Comparison of the ^{1}H NMR spectra of the imine **226f** (precursor) in Figure 105 and of the amine **227f** (product) in Figure 105 shows the disappearance of the imine proton signal at 7.98 ppm, and the appearance of an additional benzylic proton singlet signals at *ca.* 3.7 ppm corresponding to the furfuryl methylene group. The ^{13}C NMR spectrum of compound **227f** (Figure 106) confirms formation of the product by revealing the two benzylic carbon signals at 45.2 and 52.3 ppm with the silyl protecting group signals resonating at -3.92 (Me) and 26.1 ppm (Bu^{t}).



	Ar
a	Phenyl
b	3,4-Dichlorophenyl
c	3-Chlorophenyl
d	3,4-Difluorophenyl
e	3-Fluorophenyl
f	2-Furanyl

Scheme 36. Strategic plan for the synthesis of ligands **229** and **230**.

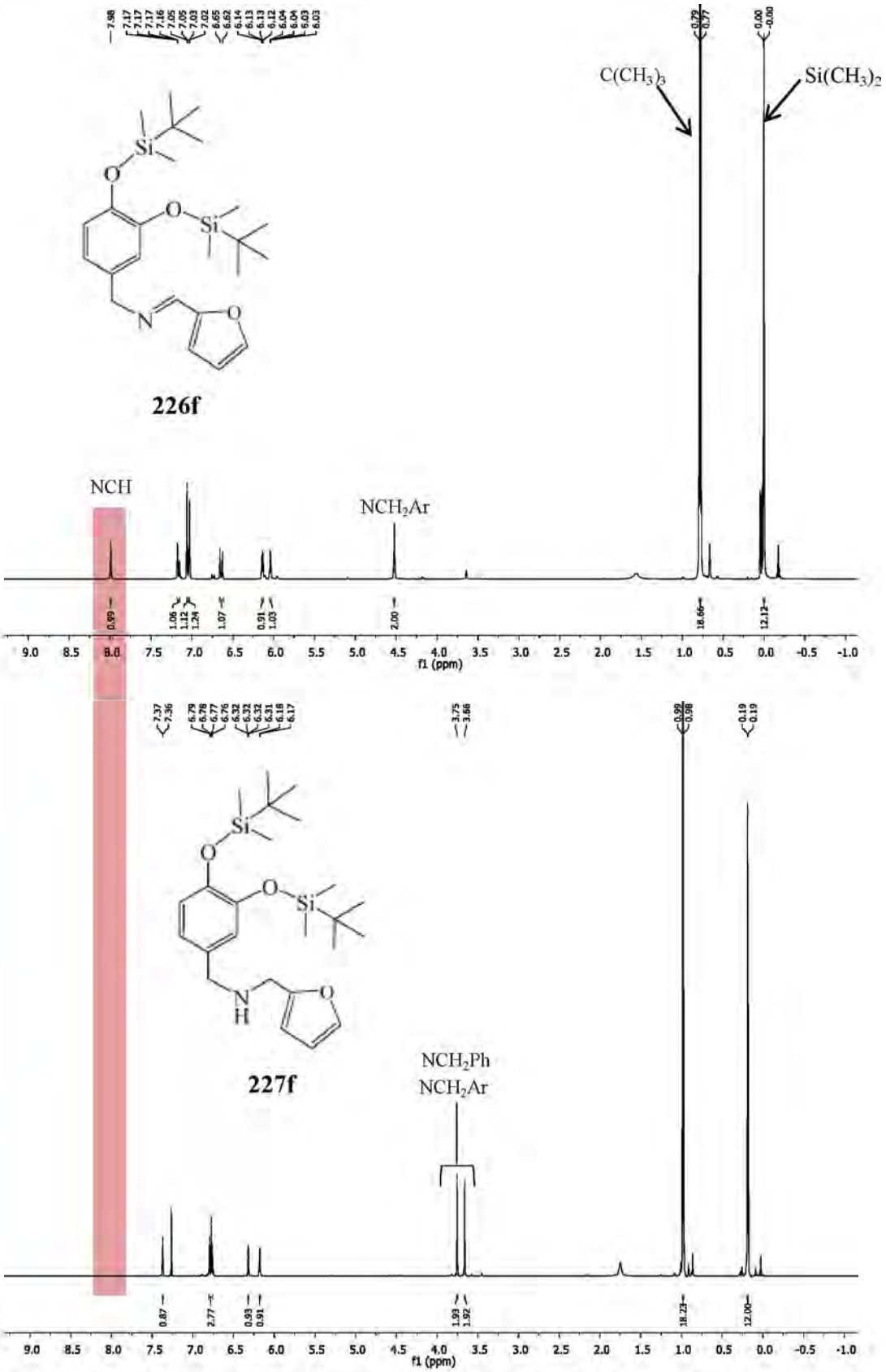


Figure 105. 600 MHz ^{1}H NMR spectra of compound **226f** (precursor) and compound **227f** (product) in $CDCl_3$.

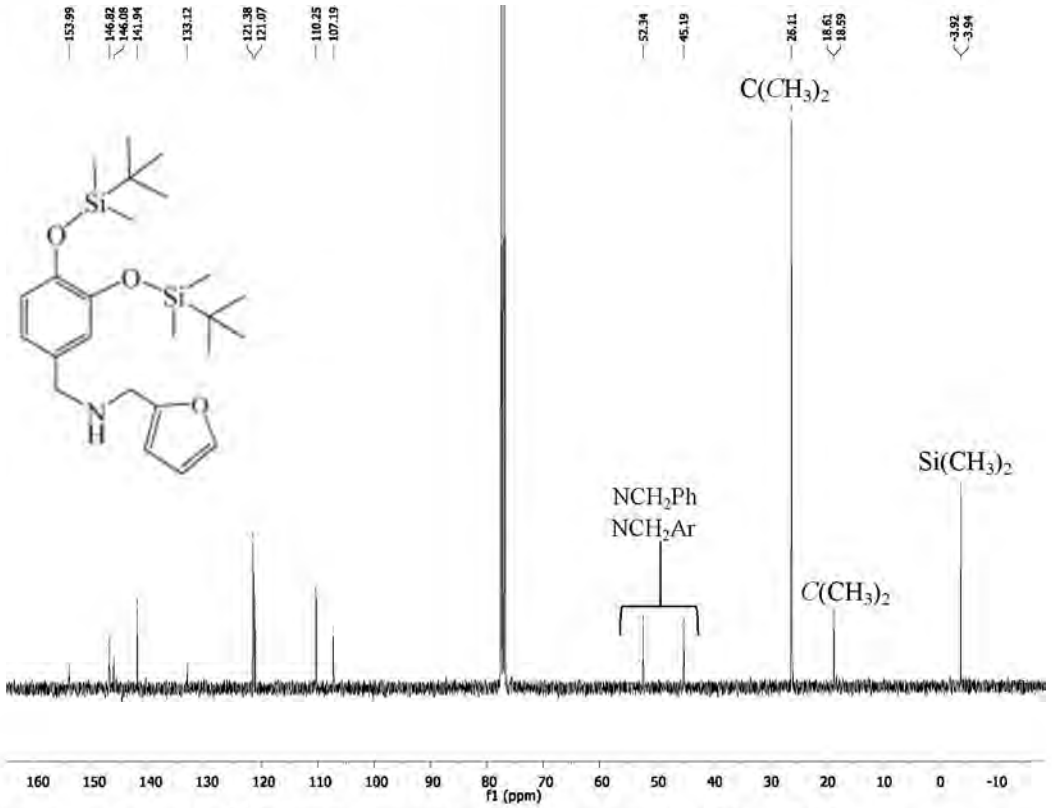


Figure 106. 100 MHz ^{13}C NMR spectrum of compound **227f** in $CDCl_3$.

Phosphonation of each of the amines **227a-f** afforded the novel phosphoramidate esters **228a-f** in excellent yields, ranging from 80% to 86% (Table 14). NMR spectroscopic analysis confirmed the formation of the phosphoramidate esters **228a-f**, as illustrated in the ^{1}H NMR spectrum of the 3-fluorophenyl derivative **228e** in Figure 107; the ester methyl protons resonate as a triplet at 1.30 ppm and the *O*-methylene protons as a multiplet at 4.05 ppm due to coupling with vicinal methyl protons and the ^{31}P nucleus. The ^{13}C NMR spectrum (Figure 108) reveals the signals at 16.3 and 62.6 ppm, respectively, and some of the aromatic signals as doublets due to coupling to the ^{19}F nucleus.

The silyl protecting group was cleaved from each of the phosphonated compounds **228a-f** using 2.5 equivalents of 1M-*tert*-butylammonium fluoride (TBAF) in THF and stirring at room temperature for 3 h to furnish the novel dihydroxy compounds **229a-f** in excellent yields (Table 14) after flash column chromatography;[232-233] all of these compounds were fully characterised. The ^{1}H NMR spectrum of compound **229b** (Figure 109) reveals two distinct doublets for the benzylic protons at 3.93 and 3.99 ppm, and the absence of the *Si-tert*-butyl signals confirming the successful deprotection of the silyl group. Significantly, the phosphoramidate ester moiety remains intact as evidenced by the ester methyl and methylene signals at 1.32 and *ca.* 4.1 ppm, respectively. The DEPT 135 NMR spectrum (Figure 110)

reveals that each of the benzylic carbons resonates as a doublet, due to ^{31}P-^{13}C coupling as do the phosphoramidate ester signals at 16.1 and 63.3 ppm. Several attempts were made to hydrolyse the phosphoramidate ester **229a-f** using TMSBr in dry DCM or conc HCl, but these attempts to access the corresponding acids **230a-f** proved unsuccessful.

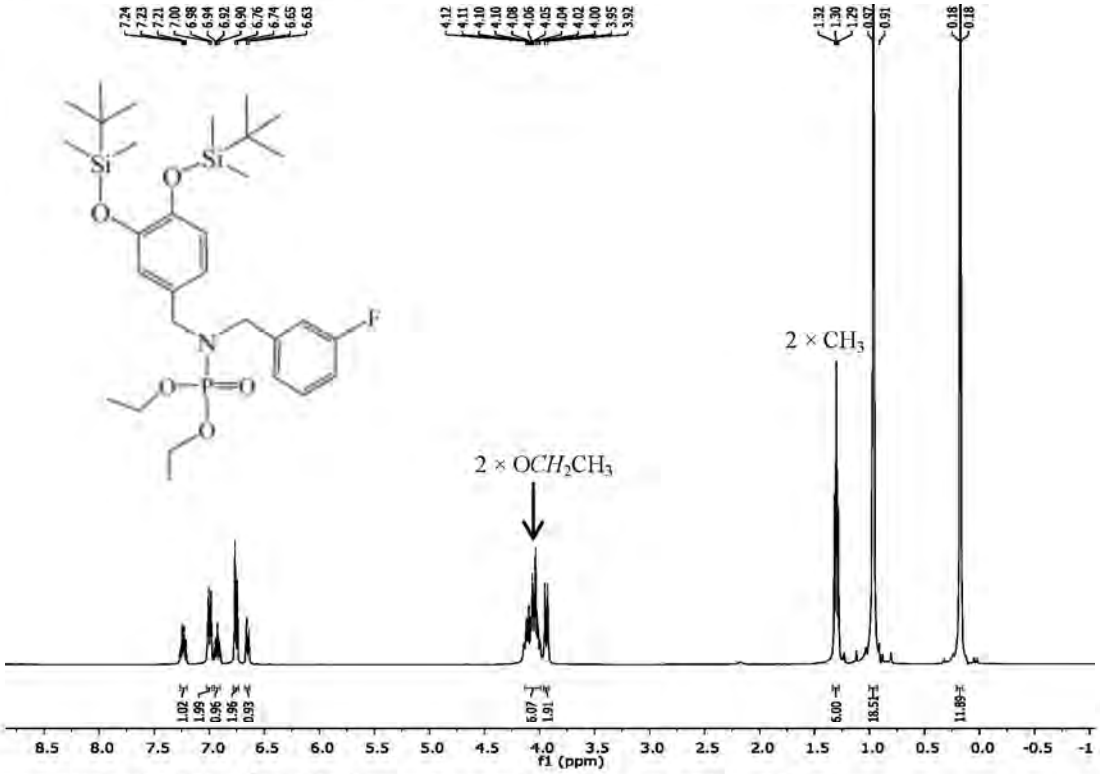


Figure 107. 400 MHz ^{1}H NMR spectrum of compound **228e** in $CDCl_3$.

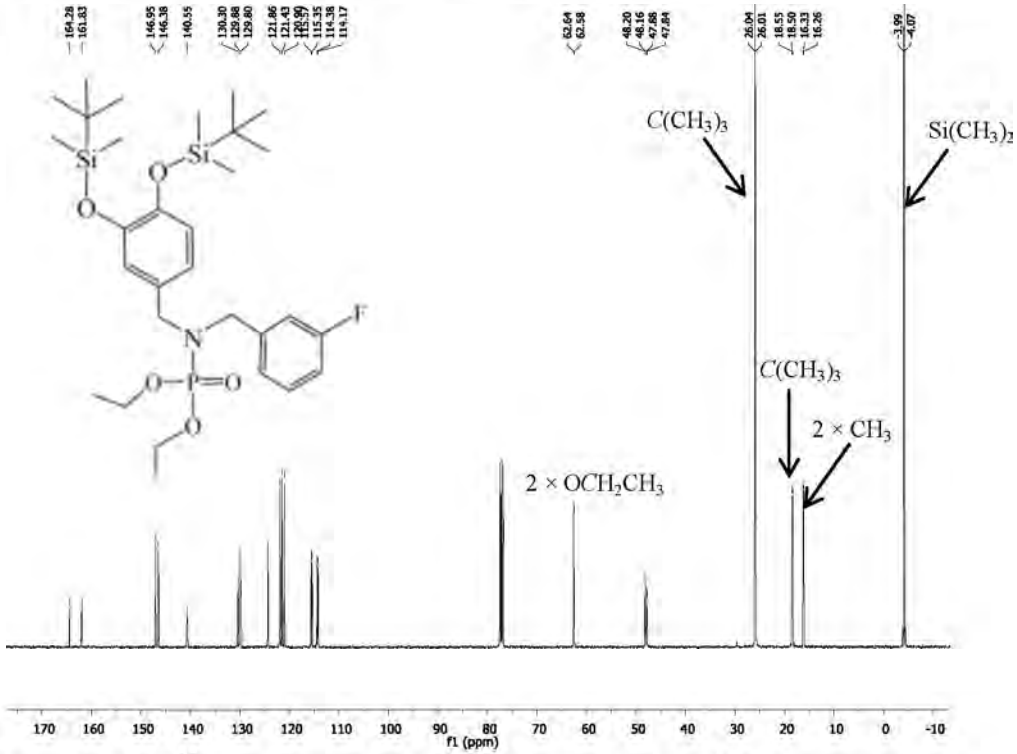


Figure 108. 100 MHz ^{13}C NMR spectrum of compound **228e** in $CDCl_3$.

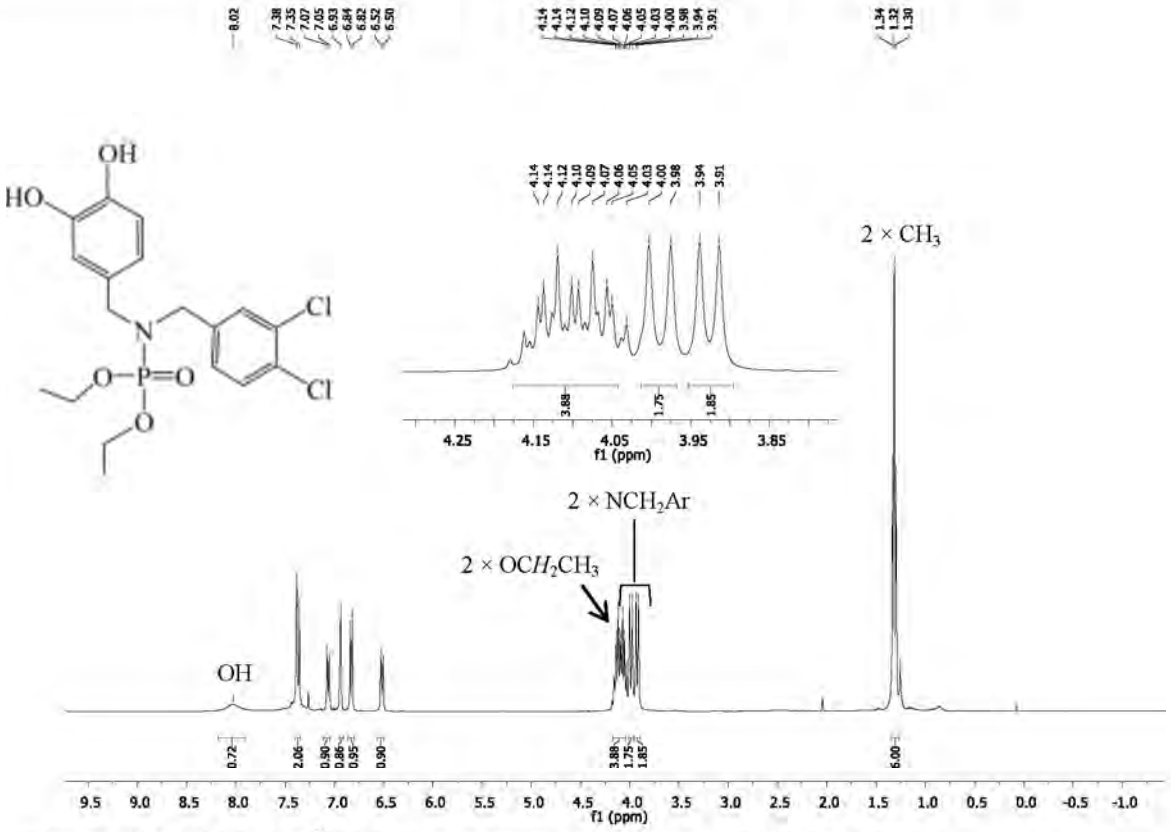


Figure 109. 400 MHz ^{1}H NMR spectrum of compound **229b** in $CDCl_3$.

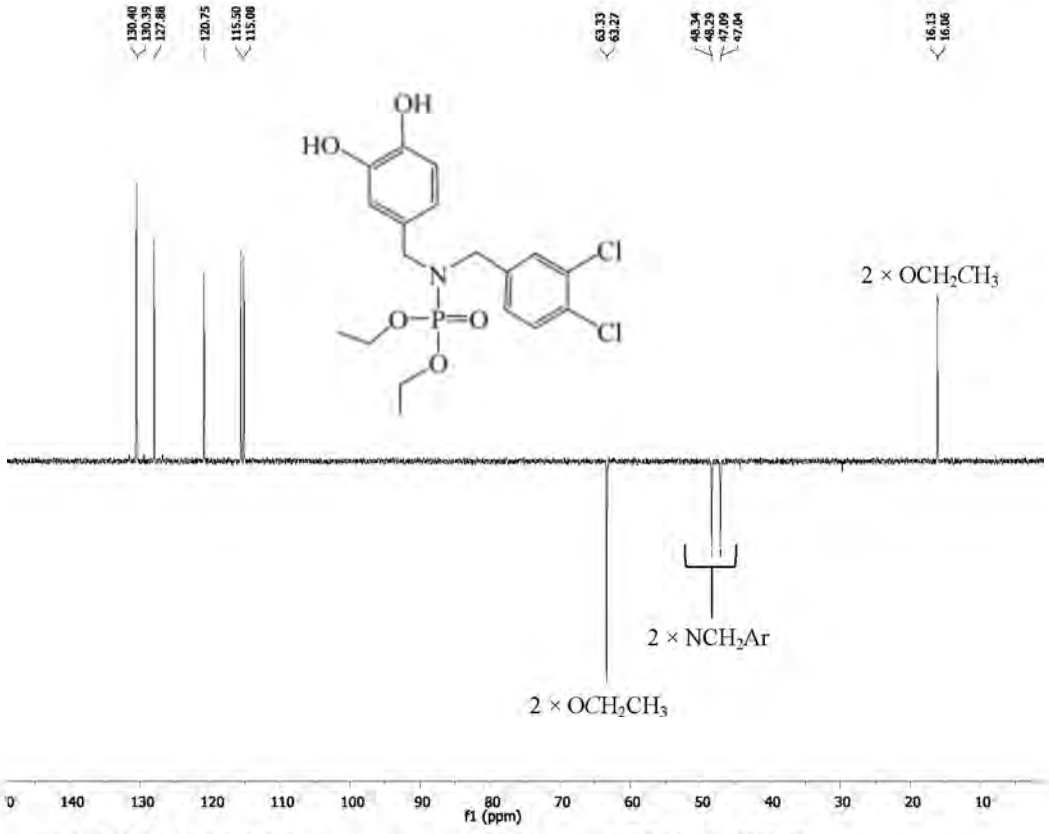
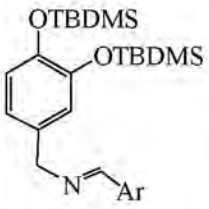
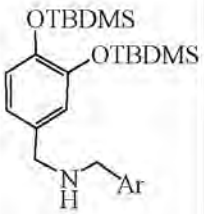
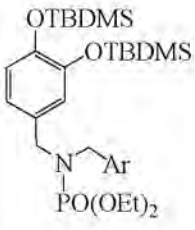
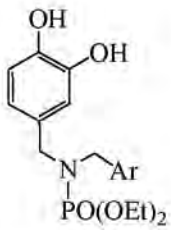


Figure 110. DEPT 135 NMR spectrum of compound **229b** in $CDCl_3$.

Table 14. Yields (%), following purification when necessary of protected imines, amines and phosphoramidate esters and the deprotected phosphoramidate esters (Scheme 36).

Ar								
Phenyl	**226a**	72	**227a**	80	**228a**	82	**229a**	83
3,4-Dichlorophenyl	**226b**	78	**227b**	88	**228b**	86	**229b**	87
3-Chlorophenyl	**226c**	86	**227c**	80	**228c**	83	**229c**	88
3,4-Difluorophenyl	**226d**	83	**227d**	85	**228d**	80	**229d**	84
3-Fluorophenyl	**226e**	84	**227e**	86	**228e**	88	**229e**	85
2-Furanyl	**226f**	80	**227f**	84	**228f**	83	**229f**	85

2.5. BIOLOGICAL ASSAYS

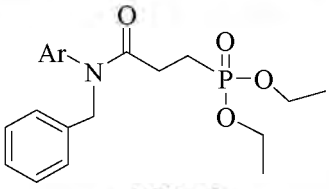
In vitro bioassays were undertaken to explore the enzyme inhibition potential and toxicity of selected compounds, the synthesis of which has been discussed in Sections **2.1**, **2.2**, **2.3** and **2.4**. The various bioassays were conducted for the author at Rhodes University.[234,235]

Bioassays were conducted to determine toxicity against human embryonic kidney (HEK 293) or Henrietta Lacks (HeLa) cells and the anti-malarial (*Pf*LDH and *Pf*DXR) and anti-trypanosomal (*T.brucei*) activity of these selected ligands using emetine, chloroquine, FR900098 and pentamine, respectively, as the positive control standards. When the percentage enzyme viability was less than 25%, the IC_{50} value was also calculated.

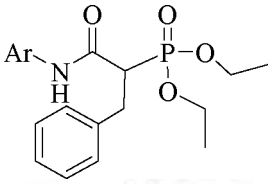
2.5.1. Evaluation of the *C*-benzylated analogues 131b-g and 130b,e

The cytotoxicity of the *C*-benzylated phosphonate esters **131b-g** and phosphonic acids **130b,e** was examined using HEK 293 cells and the results are summarised in Table 15. None of these compounds showed significant toxicity at 25 μM as high percentage cell viabilities were observed in all cases.

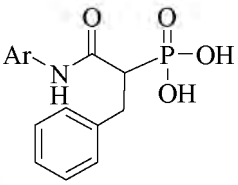
Table 15. Results for the HEK 293 cell toxicity and PLDH, *Pf*DXR and *T.brucei* assays of compounds **131b-g** and **130b,e**.



129b-f



131b-g



130b,e

Ar	Compound	HEK 293 % viability at 25 μM	PLDH[e] % activity at 20 μM		*Pf*DXR % activity at 20 μM	*T.brucei* % activity at 20 μM
3-Bromophenyl	**131b**	104.7±8.9	66.2±2.6	*35.2*	86.7±9.1	92.6±1.6
3-(Hydroxymethyl)phenyl	**131c**	104.0±4.2	84.7±3.2	*55.4*	104.4±9.1	52.9±2.4
2-Pyridyl	**131d**	115.8±7.6	117.0±7.1	*-5.3*	97.0±3.0	104.5±2.1
3-Hydroxylphenyl	**131e**	90.9±4.9	82.7±7.1	*-8.1*	83.7±9.1	107.9±3.4
3-Nitrophenyl	**131f**	107.4±6.0	104.4±3.2	*59.6*	99.3±1.5	100.9±0.7
Phenyl	**131g**	114.9±3.9	119.5±3.9	-	92.6±3.0	96.7±2.3
3-Bromophenyl	**130b**	100.3±9.1	84.9±9.9	-	93.3±13.6	110.7±0.9
3-Hydroxylphenyl	**130e**	91.0±3.0	100.0±12	-	86.7±21.2	106.3±4.9
	Control	0[a]	0[b]	0[b]	0[c]	0[d]

Positive control standards: [a]Emetine (25 μM), [b]Chloroquine, [c]FR900098, [d]Pentamine and [e]Data in italics refer to the corresponding *N*-benzylated bimethylene analogues.[148-149]

Compounds **131b-g** and **130b,e** all showed > 25% enzyme activity in the PLDH, *Pf*DXR and *T.brucei* assays and, therefore IC_{50} values were not necessary. It should be noted, however, that at 20 μM concentration compound **131b** exhibit encouraging inhibition of the parasite at *ca.* 66% residual *P*LDH activity, while compounds **131c,e** and **130b** showed weaker activity. The corresponding *N*-benzylated analogues **129b-f**,[148,149] contain a bimethylene linker which exhibited significantly better PLDH inhibition (data cited in italics in Table 15). The compounds (**131** and **130**) exhibit little if any inhibitory activity against the *Pf*DXR enzyme itself.

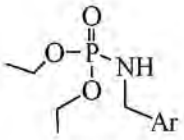
Compound **131c** clearly shows some anti-trypanosomal activity against *T.brucei* (52.9% residual enzyme activity).

2.5.2. Evaluation of *N*-substituted phosphoramidic acid derivatives 161-162a-g and their precursors 159a-g, 160a-g and 165-167a-g as "reverse" fosmidomycin analogues

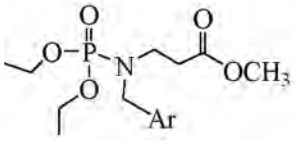
As indicated in Table 16, with the exception of compound **160c**, none of the compounds exhibit significant toxicity, with the percentage viability being > 80% at 25 μM using HEK 293 cells. These compounds show little (74-83%) or no inhibitory potency when tested against PLDH. Those compounds showing some inhibition are highlighted in Table 16. Nonetheless, a number of weak structure-activity relationship patterns may be discerned in the PLDH assay data. (i) The *N*-benzyloxy derivatives (compounds **166a-g**) have slightly better activities compared with the *N*-hydroxyl derivatives (**167a-g**), perhaps due to their greater hydrophobicity; (ii) the *N*-benzyloxy phosphoramidic acid compounds **161a-g** are similarly more active than their *N*-hydroxy analogues **162a-c,f-g**; and (iii) generally, the 3-nitrophenyl derivatives (marked with an asterisk) were observed to have the best antimalarial inhibition potential (*ca.* 74% PLDH activity) compared with the other derivatives.

The mono-substituted chloro analogue **167c** was the only compound shown to be active against *T.brucei* (*ca.* 49% enzyme activity at 20 μM).

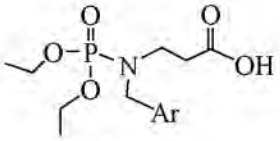
Table 16. Results for the HEK 293 cell toxicity and PLDH and *T.brucei* assays of compounds **159a-g**, **160a-g**, **165a-g**, **166a-g**, **167a-g**, **161a-g** and **162a-c,f,g**.



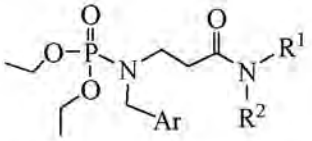
159a-g



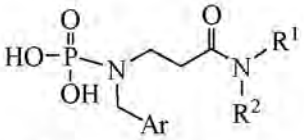
160a-g



165a-g



166a-g R^1 = OBn; R^2 = H
167a-g R^1 = OH; R^2 = CH_3



161a-g R^1 = OBn; R^2 = H
162a-g R^1 = OH; R^2 = CH_3

Ar	Compound	HEK 293 % viability at 25 µM	PLDH % activity at 20 µM	*T.brucei* % activity at 20 µM
Phenyl	**159a**	90.1±13.0	93.8±5.1	102.8±0.6
3,4-Dichlorophenyl	**159b**	98.2±13.0	93.9±10.2	105.8±0.0
3-Chlorophenyl	**159c**	103.2±30.3	80.9±4.4	95.4±11
3,4-Difluorophenyl	**159d**	81.2±11.2	80.9±4.0	105.1±0.4
3-Fluorophenyl	**159e**	95.4±0.7	88.5±2.3	104.8±2.0
2-Furanyl	**159f**	100.8±7.4	93.8±0.0	103.6±0.1
3-Nitrophenyl	**159g**	104.2±9.5	75.9±2.3*	107.7±1.5
Phenyl	**160a**	126.2±9.2	90.5±3.3	105.0±0.5
3,4-Dichlorophenyl	**160b**	94.4±22.6	92.4±9.5	105.5±0.4
3-Chlorophenyl	**160c**	33.9±0.4	79.0±6.5	101.6±4.2
3,4-Difluorophenyl	**160d**	87.1±8.6	90.9±6.0	106.3±0.9
3-Fluorophenyl	**160e**	93.0±7.1	89.4±1.0	98.6±6.7
2-Furanyl	**160f**	96.6±3.7	96.3±0.4	105.1±1.1
3-Nitrophenyl	**160g**	100.9±9.3	75.8±2.2*	108.6±0.4
Phenyl	**165a**	140.7±1.3	89.2±1.4	108.3±1.6
3,4-Dichlorophenyl	**165b**	116.2±2.1	93.7±2.6	105.7±0.6
3-Chlorophenyl	**165c**	112.4±23.8	81.4±1.1	100.4±3.5
3,4-Difluorophenyl	**165d**	89.6±15.8	85.6±2.7	104.8±0.5
3-Fluorophenyl	**165e**	128.2±10.9	83.4±1.0	103.9±1.5
2-Furanyl	**165f**	125.0±12.9	90.3±1.1	103.7±5.5
3-Nitrophenyl	**165g**	112.6±11.6	73.8±4.8*	106.0±2.5
Phenyl	**166a**	97.5±0.3	81.8±3.2	107.0±2.4
3,4-Dichlorophenyl	**166b**	99.8±9.8	98.8±1.7	96.4±0.5
3-Chlorophenyl	**166c**	99.1±1.8	78.5±5.5	95.3±1.2
3,4-Difluorophenyl	**166d**	89.9±0.43	91.2±1.7	102.8±0.4
3-Fluorophenyl	**166e**	104.6±40.4	74.6±4.6	106.8±1.0
2-Furanyl	**166f**	115.1±13.7	88.2±4.7	108.7±0.1
3-Nitrophenyl	**166g**	109.7±8.6	80.3±6.1	108.7±3.2
Phenyl	**167a**	101.4±7.7	95.5±5.7	100.3±1.6
3,4-Dichlorophenyl	**167b**	92.9±2.0	103.5±1.2	105.0±1.6

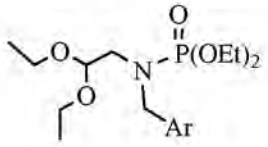
3-Chlorophenyl	**167c**	90.2±1.7	97.2±1.2	48.5±0.5
3,4-Difluorophenyl	**167d**	84.5±10.9	109.8±4.2	111.1±4.3
3-Fluorophenyl	**167e**	100.4±3.7	102.5±4.6	105.6±0.8
2-Furanyl	**167f**	93.5±2.1	95.7±2.2	105.7±4.5
3-Nitrophenyl	**167g**	102.1±3.8	101.9±0.0	100.1±0.1
Phenyl	**161a**	94.3±10.5	78.8±1.2	106.4±0.8
3,4-Dichlorophenyl	**161b**	89.6±11.2	88.0±0.6	102.2±0.3
3-Chlorophenyl	**161c**	97.4±5.7	84.0±7.7	102.6±4.7
3,4-Difluorophenyl	**161d**	97.5±5.7	89.7±5.3	96.8±4.4
3-Fluorophenyl	**161e**	88.0±11.9	83.1±1.1	106.0±0.1
2-Furanyl	**161f**	105.2±7.2	88.2±3.7	107.5±0.3
3-Nitrophenyl	**161g**	109.8±14.9	78.0±4.3*	108.1±1.0
Phenyl	**162a**	113.2±5.0	93.5±23.2	99.2±4.0
3,4-Dichlorophenyl	**162b**	100.7±5.4	91.1±1.9	103.3±2.1
3-Chlorophenyl	**162c**	98.3±0.7	104.2±13.6	104.4±0.6
2-Furanyl	**162f**	100.9±2.0	114.5±9.6	100.7±1.6
3-Nitrophenyl	**162g**	95.6±4.0	99.3±7.3	104.2±0.0
	Control	0[a]	0[b]	0[c]

Positive control standards: [a]Emetine , [b]Chloroquine and [c]Pentamine

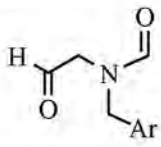
2.5.3. Evaluation of *N*-benzylated phosphoramidate derivatives as FR900098 analogues

Compounds **169a-e, 176-180a-e** are FR900098 analogues, and were all found to have essentially insignificant toxicity at a concentration of 20 μM (Table 17). A number of these compounds show some inhibition of both PLDH and *Pf*DXR, with the dichlorophenyl derivatives **169b** and **178b** showing the best PLDH inhibition (53.9 and 66.5% enzyme activity) and the di-substituted chloro analogues **177b** and **178b** exhibiting the most encouraging *Pf*DXR inhibition (*ca.* 70 and 67% residual enzyme activity, respectively). Some of these compounds (**169b**, **177b**, **178b** and **178c**) were observed to have significant activity against *T.brucei*, and promising IC_{50} values were obtained (Figure 111); three of these are dichloro analogues (**169b**, **177b** and **178b**). Surprisingly, compound **179** which has a very similar structure to FR900098 shows no promising activity against PLDH or *Pf*DXR.

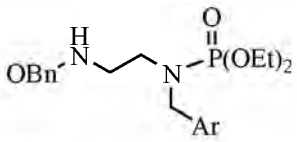
Table 17. Results for the HeLa cell toxicity and PLDH, *Pf*DXR and *T.brucei* assays compounds **169a-e**, **176a-e**, **177a-e**, **178a-e**, **179a-e** and **180a-e**.



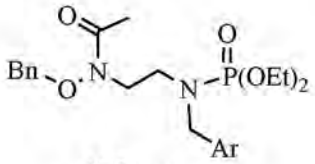
169a-e



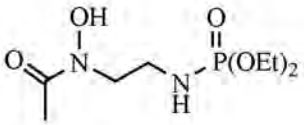
176a-e



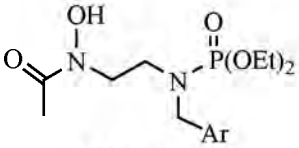
177a-e



178a-e



179



180a-e

Ar	Compound	HeLa cell % viability at 20 µM	PLDH % activity at 20 µM	*Pf*DXR % activity at 20 µM	*T.brucei* % activity at 20 µM
Phenyl	**169a**	109.7±4.3	106.2±0.5	90.6±39.8	97.1±0.4
3,4-Dichlorophenyl	**169b**	101.0±2.1	53.9±6.5	96.9±4.4	2.6±0.3
3-Chlorophenyl	**169c**	98.2±5.1	90.5±3.9	90.6±13.2	101.7±9.4
3,4-Difluorophenyl	**169d**	119.1±4.9	127.9±3.8	100.0±17.7	102.8±1.1
3-Fluorophenyl	**169e**	80.9±10.7	121.3±2.8	81.3±8.8	112.0±4.7
Phenyl	**176a**	113.9±0.3	106.8±6.7	93.8±0.0	102.9±4.4
3,4-Dichlorophenyl	**176b**	91.1±2.1	85.4±5.0	109.4±13.2	96.2±0.8
3-Chlorophenyl	**176c**	114.0±6.7	113.9±2.6	93.8±0.0	90.0±2.7
3,4-Difluorophenyl	**176d**	95.8±13.5	95.5±8.9	96.9±4.4	102.2±16.7
3-Fluorophenyl	**176e**	103.9±11.1	106.8±0.1	75.0±8.8	97.4±10.3
Phenyl	**177a**	114.0±9.9	81.6±7.1	100.0±8.8	60.7±10.1
3,4-Dichlorophenyl	**177b**	101.1±4.9	69.8±7.9	165.6±4.4	2.1±0.1
3-Chlorophenyl	**177c**	115.5±0.6	85.5±10.7	78.1±13.3	26.5±31.2
3,4-Difluorophenyl	**177d**	93.5±11.5	119.9±8.5	87.5±17.7	112.2±1.6
3-Fluorophenyl	**177e**	96.0±13.4	101.2±6.8	100.0±0.4	118.1±1.9
Phenyl	**178a**	101.0±4.1	115.8±4.3	96.9±22.1	97.2±1.0
3,4-Dichlorophenyl	**178b**	98.7±0.5	66.5±12.5	162.5±26.5	1.2±0.3
3-Chlorophenyl	**178c**	115.8±6.4	93.6±4.0	103.1±4.4	9.2±0.2
3,4-Difluorophenyl	**178d**	98.1±6.9	85.5±4.3	81.3±8.8	31.8±0.1
3-Fluorophenyl	**178e**	90.4±0.2	102.5±2.0	109.4±30.9	60.2±2.8
-	**179**	105.6±5.5	101.3±0.2	100.0±8.8	106.4±3.2
Phenyl	**180a**	91.3±8.1	114.4±9.0	100.0±17.8	110.6±3.8
3,4-Dichlorophenyl	**180b**	119.8±8.4	103.1±7.6	96.9±13.3	103.9±2.6
3-Chlorophenyl	**180c**	89.6±5.9	104.1±2.8	96.9±4.4	117.8±2.9
3,4-Difluorophenyl	**180d**	99.6±11.2	100.0±1.4	96.9±4.4	104.4±10.7
3-Fluorophenyl	**180e**	117.2±0.3	115.9±0.4	115.6±4.4	108.2±0.7
	Control	0[a]	0[b]	0[c]	0[d]

Positive control standards: [a]Emetine, [b]Chloroquine, [c]FR900098 and Pentamine

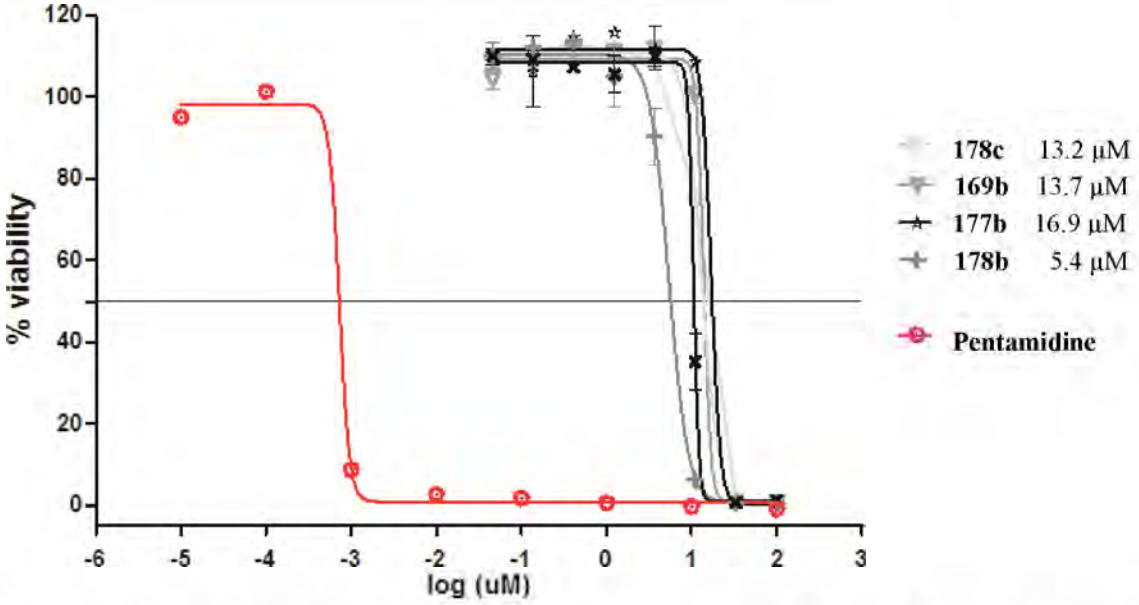


Figure 111. The inhibitory effects of selected compounds, showing IC_{50} values for *T.brucei* inhibition.

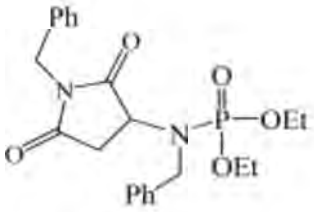
2.5.4. Evaluation of the *N*-benzylated phosphoramidate derivatives 183, 224, 228a-f and 229a-f containing conformationally constrained Mg^{2+} chelating moieties

The compounds in this series were designed to optimise Mg^{2+} chelation potential. Quantification of the inhibitory effects of all of these compounds as potential antiparasitic agents (Table 18) was performed using different assays (toxicity, PLDH, *Pf*DXR and *T.brucei*). Generally, these compounds exhibited much more promising anti-malarial (PLDH) activity than the compounds reported in Sections **2.5.1.**, **2.5.2.** and **2.5.3**. The HeLa cell toxicity assays of all of the compounds confirmed their essential non-toxicity at 20 µM. Interestingly, compounds **183** and **224** show insignificant activity against PLDH, probably due to the absence of *two* donor atoms to chelate with Mg^{2+}.

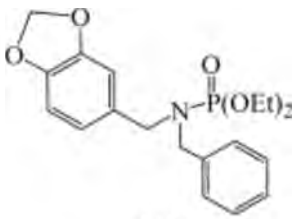
The silyl-protected compounds **228a-f** were observed to show promising antimalarial inhibitory activity in the PLDH assay with the furfuryl derivative **228f** showing the lowest percentage viability (-1.3%). However, the inhibitory activity decreased somewhat for most of the compounds when the protecting group was cleaved to give compounds **229a-f**. Figure 112 shows the PLDH IC_{50} values determined for the compounds producing < 25% residual enzyme activity. The results reveal that unprotected furfuryl analogue **229f** show the best IC_{50} value of 5.6 µM.

Compounds **228a,d-f** were observed to exhibit a dual inhibitory role showing promising activity in both the PLDH and *T.brucei* assays, with the furfuryl derivative **228f** exhibiting the best inhibitory activity in both assays. The deprotected compounds **229a-f**, however, showed no significant activity against *T.brucei*. Surprisingly, compound **183** appeared to inhibit *T.brucei*. *T.brucei* IC_{50} values were determined for a number of the active compounds (Figure 113).

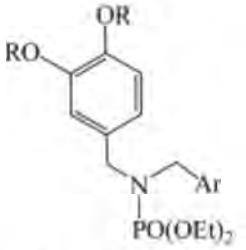
Table 18. Results for the HeLa cell toxicity, PLDH, *Pf*DXR and *T.brucei* assays of the compounds **183**, **224**, **228a-f** and **229a-f**.



183



224



228a-f R = TBDMS
229a-f R = H

Ar	Compound	HeLa cell % viability at 20 µM	PLDH % activity at 20 µM	*Pf*DXR % activity at 20 µM	*T.brucei* % activity at 20 µM
-	**183**	98.4±8.7	81.9±2.2	137.5±35.4	2.1±0.2
-	**224**	89.2±2.1	88.7±4.1	84.1±16.6	98.1±6.0
Phenyl	**228a**	102.5±0.9	12.9±1.6	93.2±6.9	8.7±3.6
3,4-Dichlorophenyl	**228b**	101.6±2.6	49.7±1.1	84.8±8.0	33.8±0.7
3-Chlorophenyl	**228c**	104.2±2.7	28.0±3.2	87.5±6.4	34.2±17.7
3,4-Difluorophenyl	**228d**	106.7±10.8	15.6±0.5	88.6±3.7	7.8±0.0
3-Fluorophenyl	**228e**	98.2±7.9	20.4±2.4	92.8±3.2	5.4±0.1
Furfuryl	**228f**	103.1±7.0	-1.3±0.6	98.1±9.6	1.4±0.1
Phenyl	**229a**	114.8±0.4	58.8±8.3	89.8±11.8	97.6±0.3
3,4-Dichlorophenyl	**229b**	110.4±6.9	16.5±4.5	101.1±9.6	62.6±21.9
3-Chlorophenyl	**229c**	99.1±1.6	31.4±0.0	97.3±7.5	91.9±7.5
3,4-Difluorophenyl	**229d**	86.1±5.4	23.9±5.2	89.0±10.7	90.0±5.1
3-Fluorophenyl	**229e**	95.7±0.7	48.2±14.7	89.4±7.0	85.9±10.1
Furfuryl	**229f**	104.6±11.7	53.4±17.6	85.6±11.2	87.2±16.4
	Control	0[a]	0[b]	0[d]	0[c]

Positive control standards: [a]Emetine, [b]Chloroquine, [c]FR900098 and [d]Pentamine

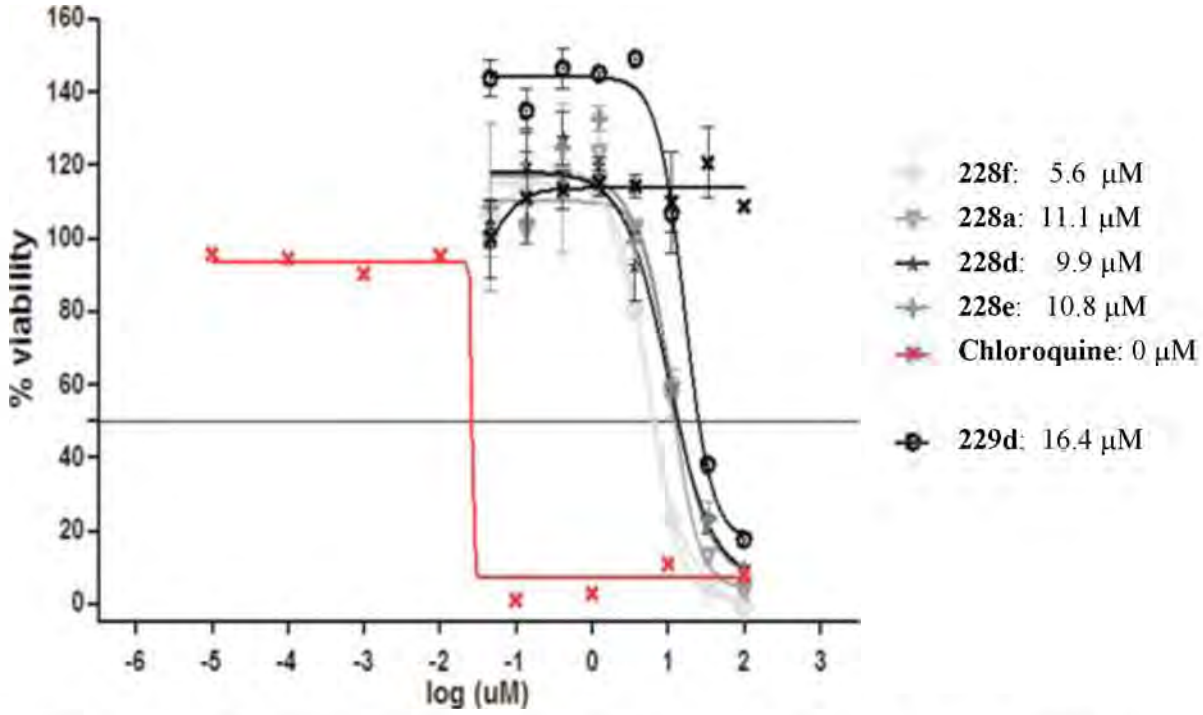


Figure 112. The anti-malarial activity of selected compounds, showing PLDH IC_{50} values.

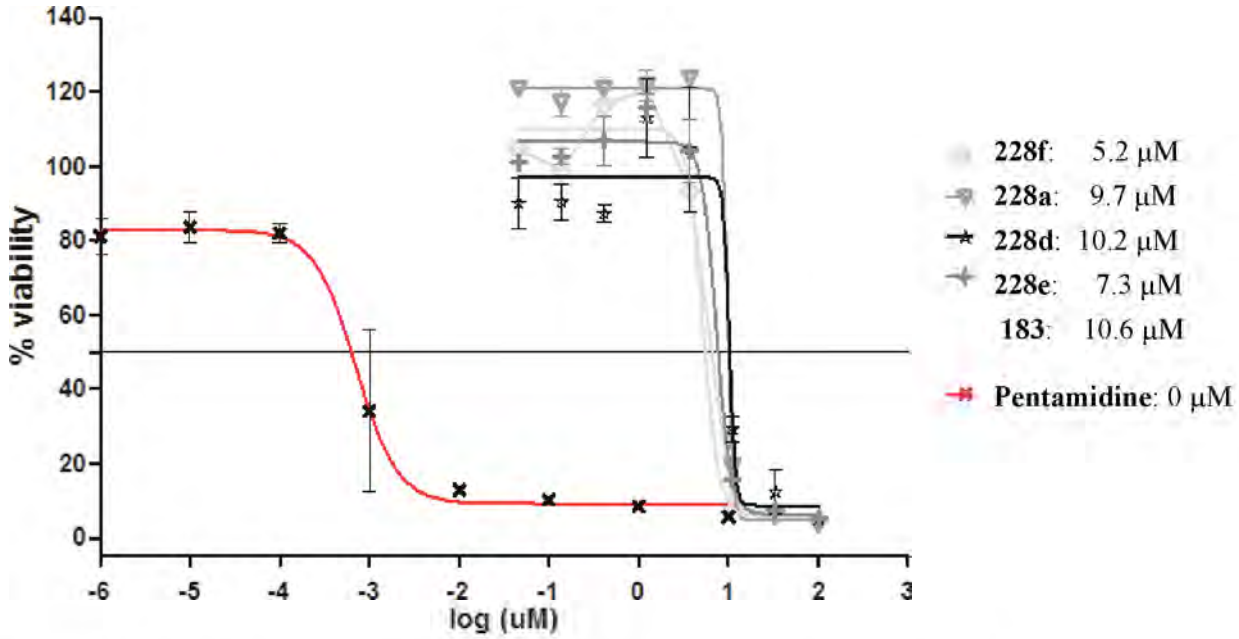


Figure 113. The anti-trypanosomal effects of selected compounds, showing *T.brucei* IC_{50} values.

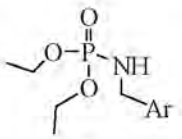
2.6. COMPUTER MODELLING and DOCKING STUDIES

Molecular docking is a useful aid in drug design, helping to elucidate the level of structural complementarity between the ligands and the active-site, and optimising the interaction between the receptor cavity and the proposed ligand in order to minimise the free energy of the system.[236] *In silico* docking studies give insight into the ligand binding orientation, thereby permitting predictions concerning the binding affinity and likely activity of the proposed ligands. In this study, computer modelling studies were undertaken in order to explore the interactions between the synthesised ligands and the *Pf*DXR active-site.

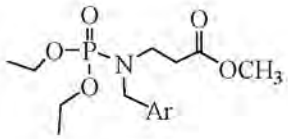
Using Discovery Studio Visualizer,[111] the *Pf*DXR crystal structure[111] used in these modelling studies was accessed from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank, and all water molecules were removed from the original PDB file. The selected ligands were constructed *in silico* as their negatively charged mono-deprotonated phosphoramidic acid species, since the phosphoramidate esters (acting as pro-drugs) were expected to be hydrolysed *in vivo* before reaching the active-site. Gaussian 03 and 09[217,237] were used to perform geometry optimization of the structure of each compound at the hybrid density functional theory DFT [B3LYP/6–31G(d)] level. To validate the reliability of the bioassay results, the protein active-site and surrounding residues were also mapped using Discovery Studio Visualizer,[111] and the optimised ligand structures were docked using Autodock Vina[238] with a flexible approach into the *Pf*DXR receptor cavity, with the proximal active-site amino acid residues Ser270, Ser306, Asn311, Lys312 and Glu315 rendered flexible, and the Mg^{2+} ion still present. For visual analysis the docked ligands were overlayed with the crystal structure containing fosmidomycin **62** within the active site.

2.6.1. Modelling studies of "reverse" and "normal" *N*-substituted *N*-benzylated phosphoramidate compounds

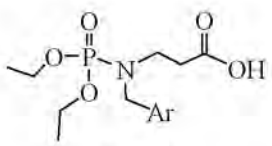
The *C*-benzylated derivatives **131a-f** were explored following the promising anti-malarial activity exhibited by certain *N*-benzylated analogues, the design of which was based on an earlier modelling study.[146]



159a-g

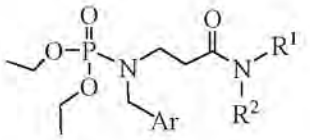


160a-g

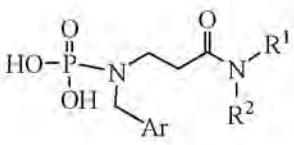


165a-g

	Ar
a	Phenyl
b	3,4-Dichlorophenyl
c	3-Chlorophenyl
d	3,4-Difluorophenyl
e	3-Fluorophenyl
f	2-Furanyl
g	3-Nitrophenyl



166a-g R^1 = OBn; R^2 = H
167a-g R^1 = OH; R^2 = CH_3



161a-g R^1 = OBn; R^2 = H
162a-g R^1 = OH; R^2 = CH_3

Note: Ligands modelled and docked as their negatively charged mono-deprotonated phosphoramidic acids

Docking of the "reverse'' *N*-substituted compounds ligands **159a-g**, **160a-g**, **165-167a-g** and **161-162a-g** into the *Pf*DXR active-site is illustrated in Figure 114. Although, these ligands exhibit some hydrogen-bonding interaction with the active-site amino acid residues, they appear to be too bulky to fit into the *Pf*DXR active-site cavity. Figure 114 shows the distance between fosmidomycin **62** and some of the compounds in this series which have docked outside the active-site. These results are consistent with the enzyme-inhibition assay data discussed in Table 16 (Section **2.5.2.**), which revealed that many of the compounds exhibited poor activity. Interestingly, the docking conformation of compound **161g** – the nitro derivative in the *Pf*DXR active-site (Figure 118) reveals its close similarity to fosmidomycin **62** in binding to the protein, which corresponds to the somewhat better bioassay result (Table 16). This unexpected activity was attributed to the presence of the oxygen atoms of the nitro group interacting with Ser232, which follows the same binding as the C-2 carbonyl oxygen of fosmidomycin. The phosphonic acids of fosmidomycin and compound **161g** occupy the appropriate binding-region, and they were also observed to interact with amino acid residues (Ser269, Ser270, Glu272 and Ser309). It should be noted that a *preference* for binding at another site outside the active-site does not necessarily preclude the possibility of a smaller population of ligands molecules binding at the active-site or that binding at the other site might induce an allosteric effect.

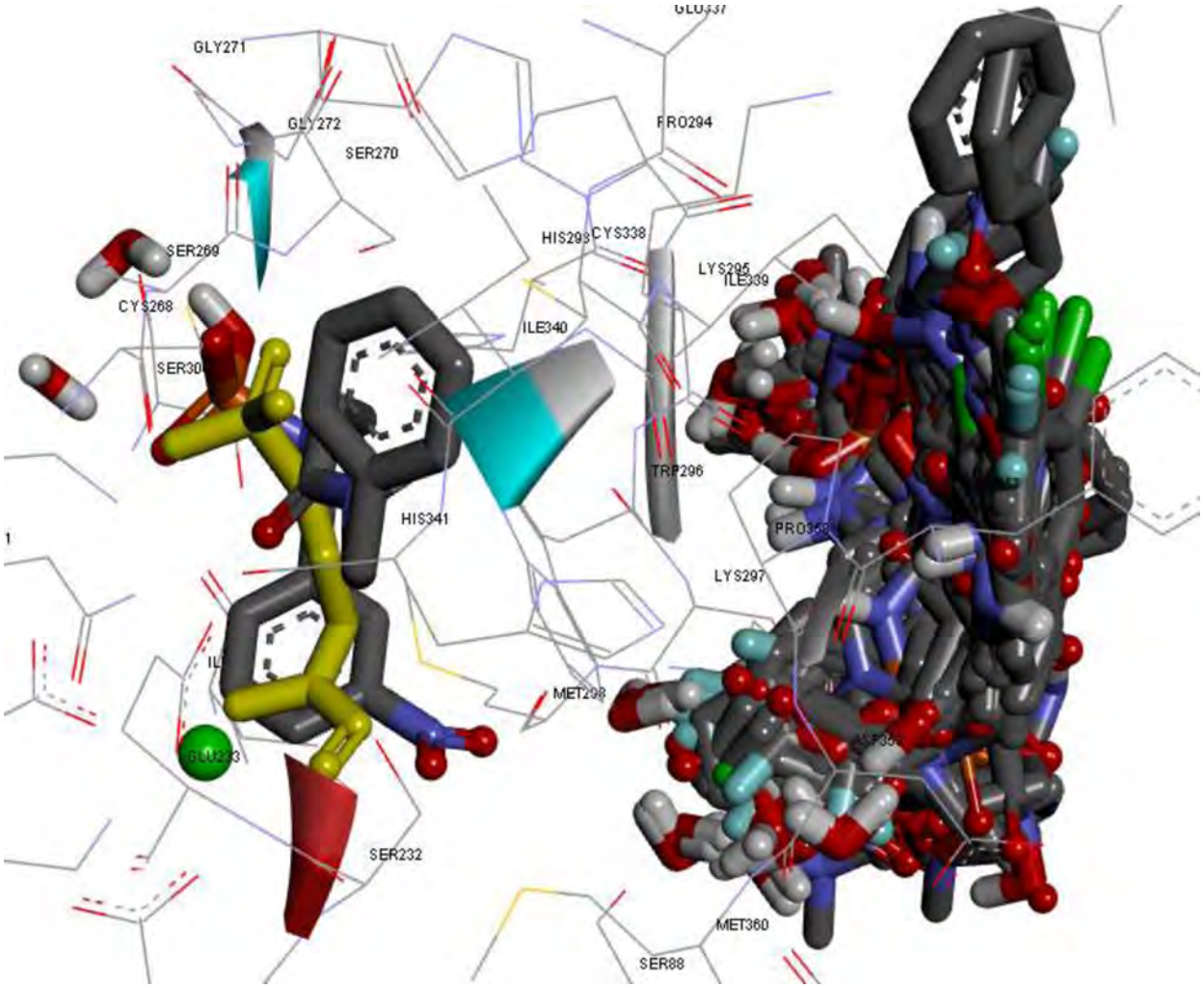
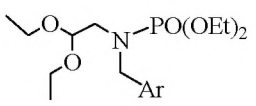


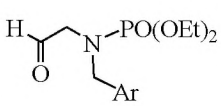
Figure 114. Docked conformations of "reverse'' *N*-substituted compounds **159a-g**, **160a-g**, **165-167a-g** and **161-162a-g** as their negatively charged mono-deprotonated acids in the *Pf*DXR active-site (3AU9)[111] illustrating, on the left-hand side, the alignment of ligand **161g** with fosmidomycin and hydrogen-bonding of the ligand with active-site residues. The cluster of ligands on the right-hand side of the figure reflects their binding in a different location. The crystal structure conformation of fosmidomycin **62** is shown in stick format coloured yellow and Mg^{2+} as a green sphere. Protein active-site amino acid residues are shown in wire-frame coloured by atom type, the ligand in stick format coloured by atom type.

The docking studies of the *N*-substituted benzylphosphoramidic derivatives **169, 176-180a-e** (Figure 115) also reveal the effect of the bulky ligands preventing the binding into the *Pf*DXR active-site. However, there were interactions with proximal amino acid residues in the enzyme receptor cavity. The failure of these ligands to dock into the active-site presumably accounts for their low inhibitory activity results obtained in the enzyme inhibition assay (Table 17). The few compounds that did show some inhibition potential may be acting *via* an allosteric effect or acting at a yet to be determined target.

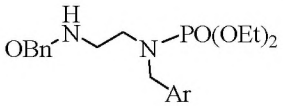
	Ar
a	Phenyl
b	3,4-Dichlorophenyl
c	3-Chlorophenyl
d	3,4-Difluorophenyl
e	3-Fluorophenyl
f	2-Furanyl



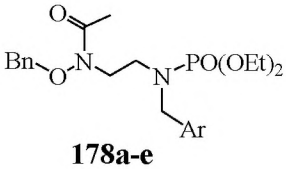
169a-e



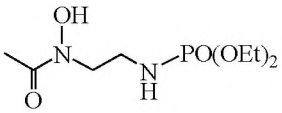
176a-e



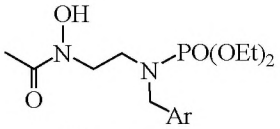
177a-e



178a-e



179



180a-e

Note: Ligands modelled and docked as their negatively charged mono-deprotonated phosphoramidic acids

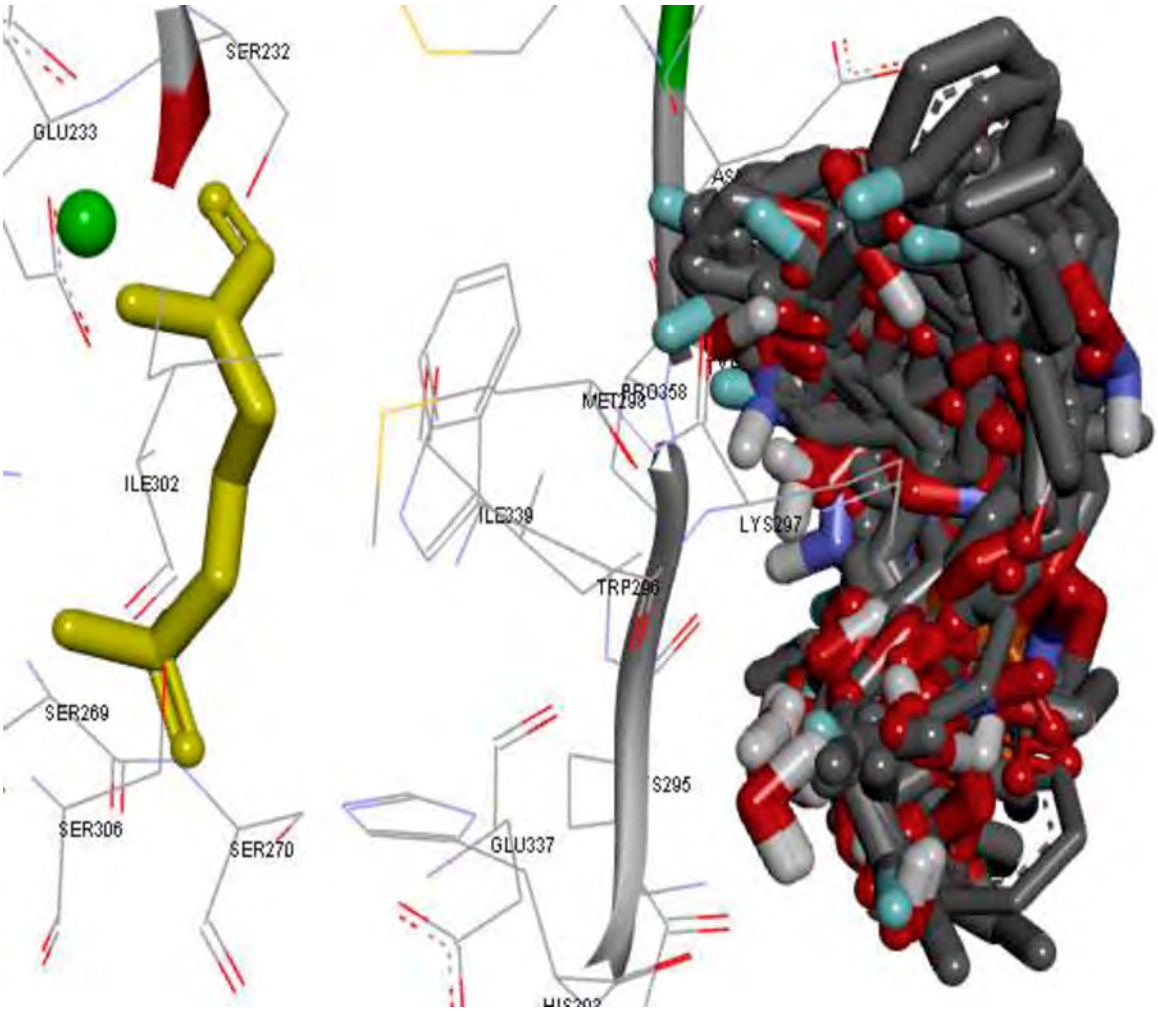
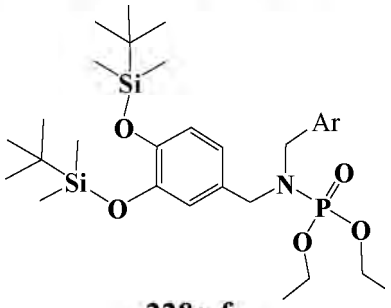


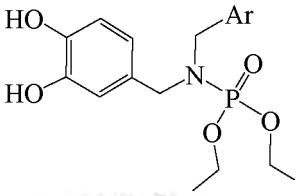
Figure 115. Docked conformations of *N*-benzyphosphoramidic acid derivatives **169a-e, 176-180a-e** as their negatively charged mono-deprotonated acids in the *Pf*DXR active-site (3AU9)[111] illustrating the distance between the ligands and fosmidomycin **62**. The crystal structure conformation of fosmidomycin **62** is shown in stick format coloured yellow and Mg^{2+} as a green sphere. Protein active-site amino acid residues are shown in wire-frame coloured by atom type, the ligand in stick format coloured by atom type.

2.6.2. Modelling studies of *N*-benzylated phosphoramidate derivatives containing the *ortho*-dihydroxyphenyl - Mg^{2+} chelating moiety

	Ar
a	Phenyl
b	3,4-Dichlorophenyl
c	3-Chlorophenyl
d	3,4-Difluorophenyl
e	3-Fluorophenyl
f	2-Furanyl



228a-f



229a-f

Note: Ligands modelled and docked as their negatively charged mono-deprotonated phosphoramidic acids

Docking of the silyl ether-protected compounds **228** was not possible using Autodock Vina[238] due to the absence of atom parameters for Si atom types. Therefore, using AD4 Si parameters, the Autodock tools 4.2 programme[239] was used to explore the binding mode of the ligands **228a-f** and **229a-f**. Using a flexible docking approach, Gasteiger partial charges[240] were used with a +2 charge assigned to the Mg^{2+} cation. The active-site interactions were mapped using the AutoGrid 4.2 algorithm and a genetic algorithm was applied for conformational search purposes. Docking was performed using a Lamarckian genetic algorithm, together with a starting population size of 150, a maximum of 25,000,000 generations and 100 concurrent runs. With this protocol, each of the docking experiments generated 100 docked conformers, and the best conformer, in each case was selected for further analysis based on:- (i) the reproducibility of the docked conformation; (ii) hydrogen-bonding interactions; and (iii) the estimated free binding energy.

The docking stimulations of these ligands reflected low free binding energies (< -9.7 Kcal.mol^{-1}) with low predicted inhibition concentrations (nM) compared with the ligands in the previous (Section **2.6.1**). The binding of these ligands (**228** and **229**) to the *Pf*DXR active-site (Figure 116) revealed that all of them were in close alignment with fosmidomycin, fitted into the *Pf*DXR active-site receptor cavity and exhibited potential hydrogen-bonding interactions with the proximal amino acid residues. Most importantly, these ligands fill the adjacent vacant pocket (previously described) of the *Pf*DXR active-site. The phosphoramidic acid moiety occupies the usual region for this group and interacts with amino acid residues Ser306, Asn311, His293, Gly272 and Ser269. The binding also permits hydrogen-bonding interactions between the Si-O atoms and the Cys268 and Glu233 resides (Figure 116). The chelation of fosmidomycin **62** and some of the ligands with Mg^{2+} is also evident.

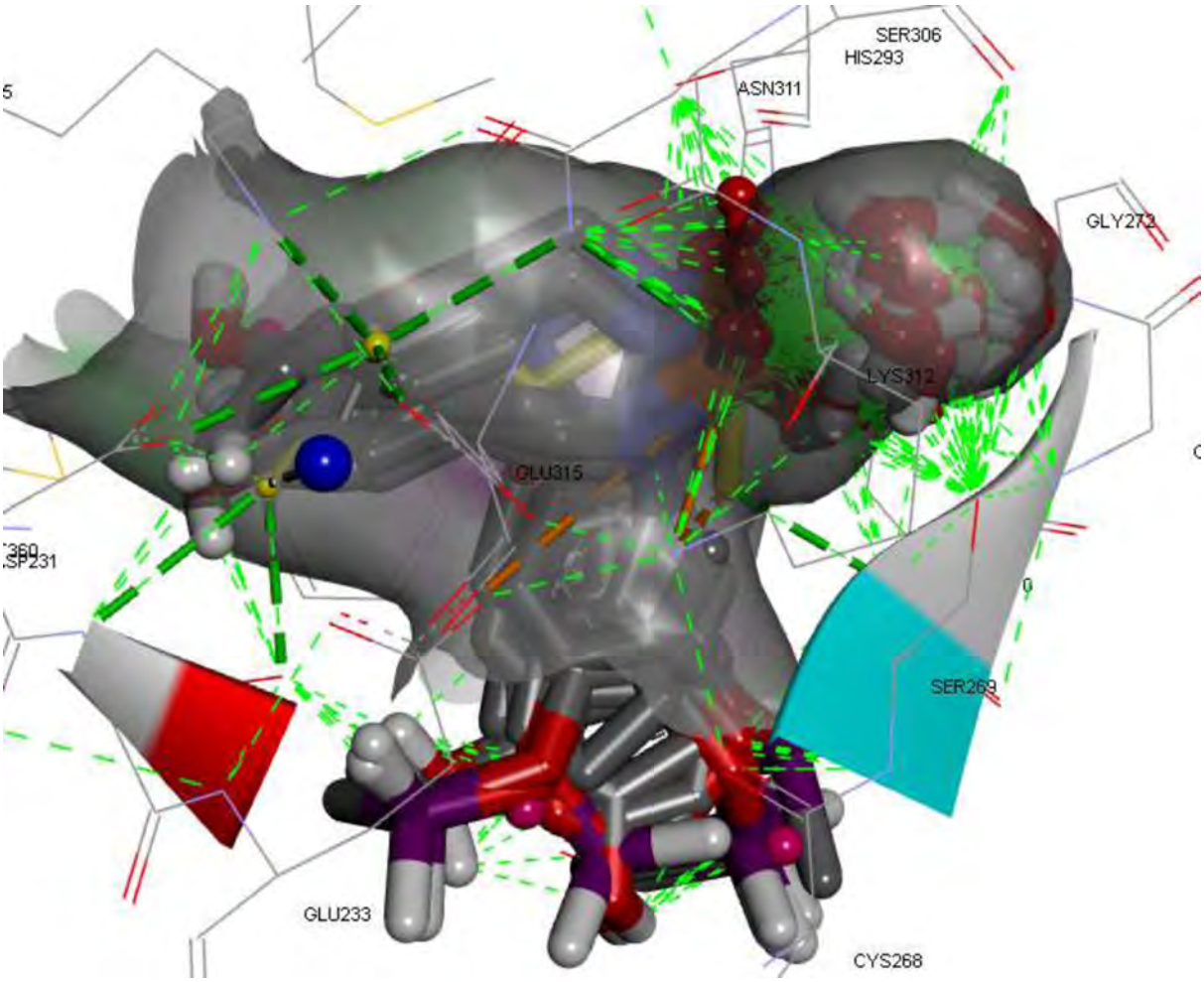


Figure 116. Docked conformations of the *N*-benzylated phosphoramidic acid derivatives **228a-f** and **229a-f**, as their negatively charged mono-deprotonated acids, containing the conformationally constrained *ortho*-dihydroxyphenyl Mg^{2+} chelating moiety in the *Pf*DXR receptor cavity (3AU9),[111] illustrating the close alignment of the ligands with fosmidomycin **62**. The crystal structure conformation of fosmidomycin is shown in stick format coloured yellow, Mg^{2+} as a blue sphere and the ligand in stick format coloured by atom type. Protein active-site amino acid residues are shown in wire-frame coloured by atom type. Hydrogen bonds are shown as green dashed lines.

Examination of the enzyme inhibition data (Table 18) reveals that while the ligands **228a-f** and **229a-f** all show promising activity against malarial cells, their activity against *Pf*DXR is negligible. This apparent discrepancy is readily resolved by the fact that the bioassays were conducted using the phosphoramidate *esters*, which can be hydrolysed *in situ* by *esterases* in the malarial cells but not by the *Pf*DXR enzyme. The *in silico* docking, however, has involved the use of the mono-deprotonated phosphoramidic acids.

Interestingly, as shown in Figure 117, removal of the silyl ether protecting groups, leads to a very different interaction of the *N*-furfuryl ligand **229f** with the Cys268 and Glu233 residues

when compared with the silylated precursor **228f**. The furan ring rather than the hydrophobic benzyl group in ligand **229f** is found to occupy the empty pocket, thus reducing the binding efficacy.

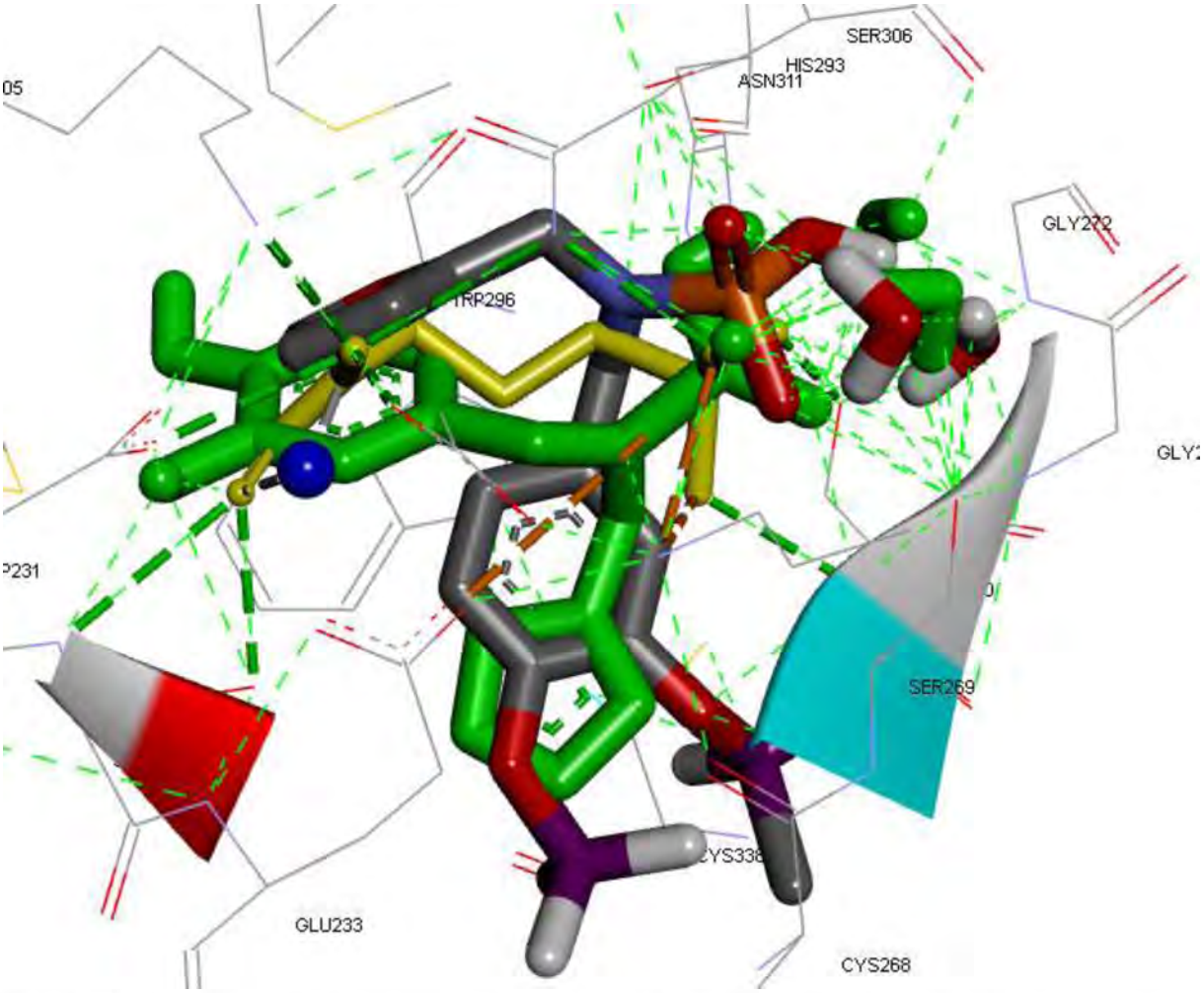


Figure 117. Overlay of the docked conformations of compound **228f** (coloured by element) and its phosphoramidic acid derivative **229f** (in green) as their negatively charged mono-deprotonated acids, in close alignment with fosmidomycin **62** (yellow) in the *Pf*DXR active-site (3AU9).[111] The crystal structure conformation of fosmidomycin **62** is shown in stick format coloured yellow and Mg^{2+} as a blue sphere and the ligand in stick format coloured by atom type. Protein active-site amino acid residues are shown in wire-frame coloured by atom type. Hydrogen bonds are shown as green dashed lines.

2.6.3. Exploratory *in silico* studies of the physico-chemical properties of the ligands

2.6.3.1. CDK Descriptors

With the intention assessing "druggability" of the synthesised compounds, their physical chemical property profiles were reviewed to estimate the solubility and oral-bioavailability of these compounds. Having constructed 3-D structures for a library of the ligands, CDK Descriptor Calculator[241] was used to identify calculable parameters that are related to drug permittivity and permeability. The most important of the parameters are the "Lipinski (rule of 5) failures"[242-243] which predict absorption/permeation of the drug using the following criteria.

(i) The number of hydrogen-bond donors (nHBDon), for instance OH and NH, must not exceed 5. As illustrated in Table 19, in none of the compounds examined does nHBDon exceed 5; however, compounds **179**, **229a** and **229b** have a higher number (4), compared to the other compounds.

(ii) The number of hydrogen-bond acceptors (nHBDAcc), *e.g.*, N and O must be < 10, since the presence of a high number of acceptors hinders permeability across a membrane bi-layer. All of the compounds obey this rule, as they have less than 10 hydrogen-bond acceptors, but compound **183** has the highest number of acceptors.

(iii) The molecular weight must be less than 500 $g.mol^{-1}$. All of the compounds obey the rule except compound **178b** which has a molecular weight of 512 $g.mol^{-1}$; compound **166b** is close to failing this rule having a molecular weight of 488 $g.mol^{-1}$.

(iv) The partition coefficient XLogP must not exceed 5.0. Compound **177b** just fails the rule due to having an XlogP value of 5.02 .

Table 19 summarises the calculated properties for the series of compounds, with various parameters, *viz.*, partition coefficients (ALogP and XlogP), Adaptive Multi-Rate (AMR), topological polar surface area (TopoPSA) and Molecular weight (MW).

Table 19. Physcio-chemical property parameters of the ligands.

Compd.	ALogP	ALogp2	AMR	nHB Don	nHB Acc	Lipinski Failure	TopoPSA	MW	XLogP
159a	1.282	1.645	66.58	1	4	0	57.37	243.10	1.67
159b	2.020	4.080	76.02	1	4	0	57.37	311.02	2.91
159c	1.651	2.726	71.30	1	4	0	57.37	277.06	2.29
159d	1.102	1.214	66.84	1	4	0	57.37	279.08	1.99
159e	1.192	1.421	66.72	1	4	0	57.37	261.09	1.83
159f	-0.382	0.146	56.37	1	4	0	70.51	233.08	0.31
159g	0.789	0.623	68.8	1	4	0	103.19	288.09	1.56
160a	1.340	1.796	87.84	0	6	1	74.88	329.13	1.77
160b	2.077	4.316	97.27	0	6	1	74.88	397.06	3.01
160c	1.709	2.920	92.55	0	6	1	74.88	363.10	2.39
160d	1.160	1.345	88.09	0	6	1	74.88	365.12	2.09
160e	1.250	1.562	87.97	0	6	1	74.88	347.13	1.93
160f	-0.324	0.105	77.62	0	6	1	88.02	319.12	0.41
160g	0.847	0.717	90.05	0	6	1	120.70	374.12	1.66
165a	1.089	1.186	83.07	1	6	0	85.88	315.12	1.45
165b	1.826	3.336	92.5	1	6	0	85.88	383.05	2.69
165c	1.458	2.125	87.78	1	6	0	85.88	349.08	2.07
165d	0.909	0.826	83.32	1	6	0	85.88	351.10	1.77
165e	0.999	0.998	83.20	1	6	0	85.88	333.11	1.61
165f	-0.305	0.093	74.16	1	6	0	99.02	305.10	0.15
165g	0.596	0.355	85.28	1	6	1	131.70	360.11	1.34
166a	1.759	3.095	119.36	1	5	1	86.90	420.18	3.11
166b	2.497	6.234	128.79	1	5	1	86.91	488.10	4.35
166c	2.128	4.528	124.07	1	5	1	86.91	454.14	3.72
166d	1.579	2.493	119.61	1	5	1	86.91	456.16	3.43
166e	1.669	2.786	119.49	1	5	1	86.91	438.17	3.27
166f	0.365	0.133	110.45	1	5	1	100.05	410.16	1.80
166g	1.266	1.602	121.57	1	5	1	132.73	465.17	2.99
167a	0.336	0.112	91.50	1	5	0	89.12	344.15	0.69
167b	1.073	1.152	100.98	1	5	0	89.12	412.07	1.94
167d	0.156	0.024	91.81	1	5	0	89.12	380.13	1.02

167e	0.246	0.060	91.68	1	5	0	89.12	362.14	0.86
167f	-1.058	1.120	82.64	1	5	0	102.26	334.13	-0.61
167g	-0.157	0.025	93.77	1	5	1	134.94	389.14	0.59
161a	0.757	0.572	101.23	3	5	0	108.91	364.12	1.22
161b	1.494	2.232	110.66	3	5	0	108.91	432.04	2.47
161c	1.125	1.266	105.95	3	5	0	108.91	398.08	1.84
161d	0.576	0.332	101.49	3	5	0	108.91	400.10	1.54
161e	1.494	2.231	110.66	3	5	0	108.91	432.04	2.47
161f	-0.193	0.037	86.32	3	5	0	122.05	340.08	0.86
161g	0.263	0.069	103.45	3	5	0	154.73	409.10	1.11
162a	-0.667	0.445	73.43	3	5	0	111.12	288.09	-1.19
162b	0.071	0.005	82.86	3	5	0	111.12	356.10	0.05
162f	-2.061	4.247	64.52	3	5	0	124.26	278.07	-2.49
162g	-1.160	1.346	75.64	3	5	0	156.94	333.07	-1.29
169a	1.823	3.325	97.67	0	6	1	67.04	359.19	2.55
169b	2.561	6.558	107.13	0	6	1	67.04	427.11	3.79
169c	2.192	4.805	102.41	0	6	1	67.04	393.15	3.17
169d	1.643	2.699	97.95	0	6	1	67.04	395.17	2.87
169e	1.733	3.004	97.83	0	6	1	67.04	377.17	2.71
176a	0.888	0.789	77.44	0	5	0	65.65	285.11	1.71
176b	1.626	2.643	86.88	0	5	0	65.65	353.04	2.95
176c	1.257	1.580	82.16	0	5	0	65.65	319.07	2.33
176d	0.708	0.501	77.70	0	5	0	65.65	321.09	2.03
176e	0.798	0.637	77.57	0	5	0	65.65	303.10	1.87
177a	1.951	3.804	115.38	1	4	1	69.84	392.19	3.77
177b	2.688	7.225	124.81	1	4	2	69.84	460.11	5.02
177c	2.319	5.379	120.10	1	4	1	69.84	426.14	4.40
177d	1.771	3.133	115.63	1	4	1	69.84	428.16	4.10
177e	1.860	3.461	115.51	1	4	1	69.84	410.18	3.94
178a	1.746	3.049	124.95	0	5	1	78.12	434.20	3.17
178b	2.483	6.167	134.38	0	5	1	78.12	502.12	4.41
178c	2.115	4.472	129.66	0	5	1	78.12	468.16	3.79
178d	1.566	2.451	125.20	0	5	1	78.12	470.18	3.49
178e	1.656	2.742	125.07	0	5	1	78.12	452.19	3.33

179	-2.002	4.007	38.87	4	5	0	119.91	198.04	-2.97
180a	-0.782	0.612	73.32	3	5	0	111.12	288.09	-1.02
180b	-0.045	0.002	82.75	3	5	0	111.12	356.01	0.23
180c	-0.963	0.927	73.57	3	5	0	111.12	324.07	-0.70
180d	-0.414	0.171	78.03	3	5	0	111.12	322.05	-0.40
180e	-0.873	0.761	73.44	3	5	0	111.12	306.08	-0.86
183	-0.059	0.003	100.89	1	7	0	110.79	373.10	0.64
224	0.325	0.106	87.59	2	4	0	71.97	321.07	1.22
228a	0.547	0.300	94.01	2	4	0	71.97	397.09	2.42
228b	0.584	0.341	102.61	1	4	0	91.87	464.01	3.66
228c	0.215	0.046	97.89	1	4	0	91.87	430.06	3.04
228d	-0.334	0.111	93.43	1	4	0	91.87	432.07	2.74
228e	0.457	0.209	94.14	2	4	0	71.97	415.08	2.57
228f	-0.847	0.717	85.10	2	4	0	85.11	387.07	1.11
229a	0.396	0.157	84.68	4	4	0	93.97	309.08	1.08
229b	1.134	1.285	94.11	4	4	0	93.97	377.00	2.33
229c	0.064	0.004	88.56	3	4	0	113.87	342.03	1.71
229d	-0.485	0.236	84.10	3	4	0	113.87	344.05	1.41
229e	-0.395	0.156	83.97	3	4	0	113.87	326.06	1.25
229f	-1.699	2.887	74.93	3	4	0	127.01	298.05	-0.22

2.6.3.2. Quantitative structure-activity relationship (QSAR) studies

QSAR is a computational analytical method to correlate structural changes with experimental bioassay data obtained for synthesized compounds.[244] The percentage activity obtained from the bioassay data for each series was included in the Syntax Definition Formalism (sdf) file for the best docked conformation of each ligand. Open 3D-QSAR[245] was used to open the collective sdf files. A grid box having a 1Å step size was set around the ligands for the calculation of electrostatic and van der Waals' field type parameters at each point within the grid. Figures 118-120 show the orientation of the ligand within the box containing the grid for each of the three series.

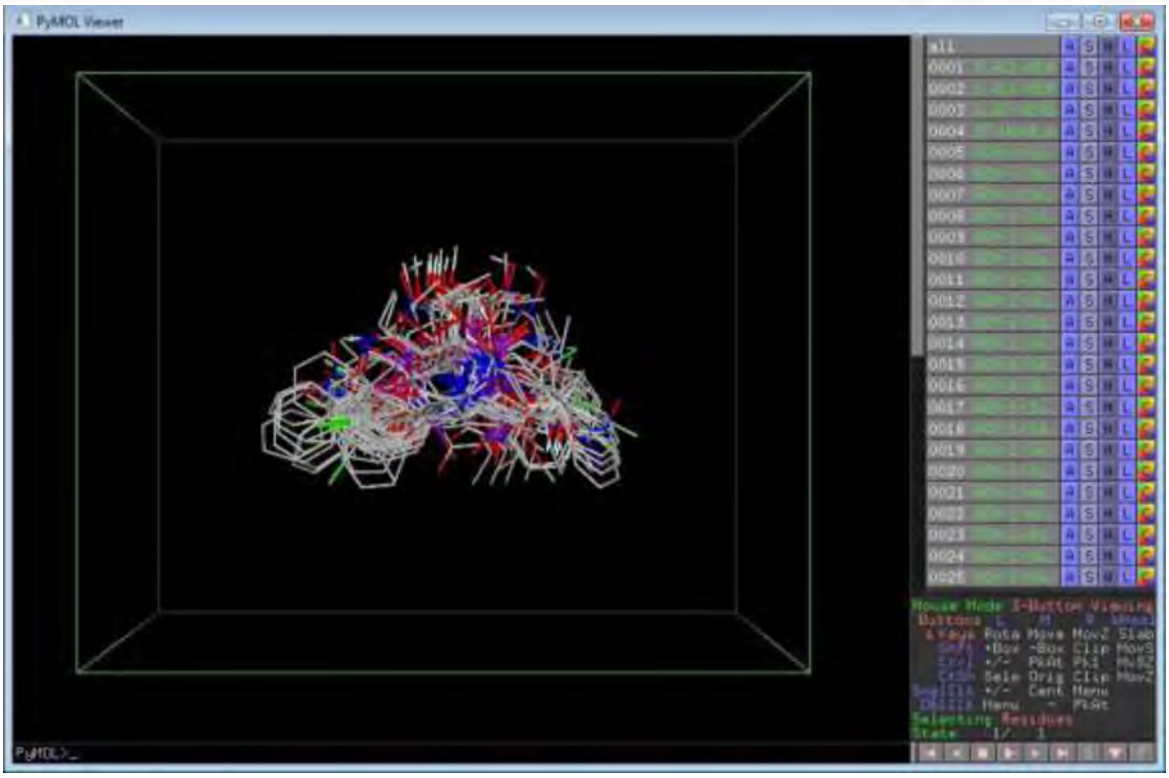


Figure 118. Series of *N*-substituted phosphoramidic derivatives **159a-g**, **160a-g**, **165-167a-g** and **161-162a-g** as "reverse" *N*-substutited fosmidomycin analogues in the 3DQSAR grid box.

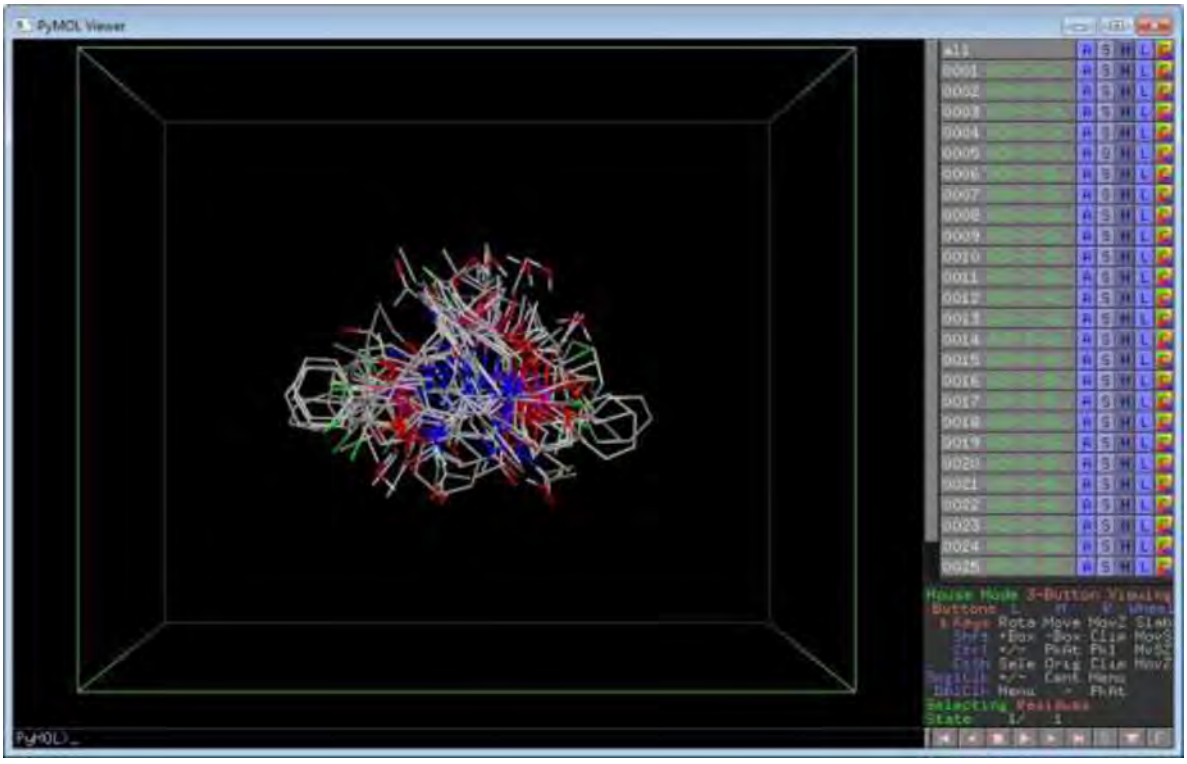


Figure 119. Series of *N*-benzylated phosphoramidic derivatives **169a-e, 176-180a-e** as FR900098 analogues in the 3DQSAR grid box.

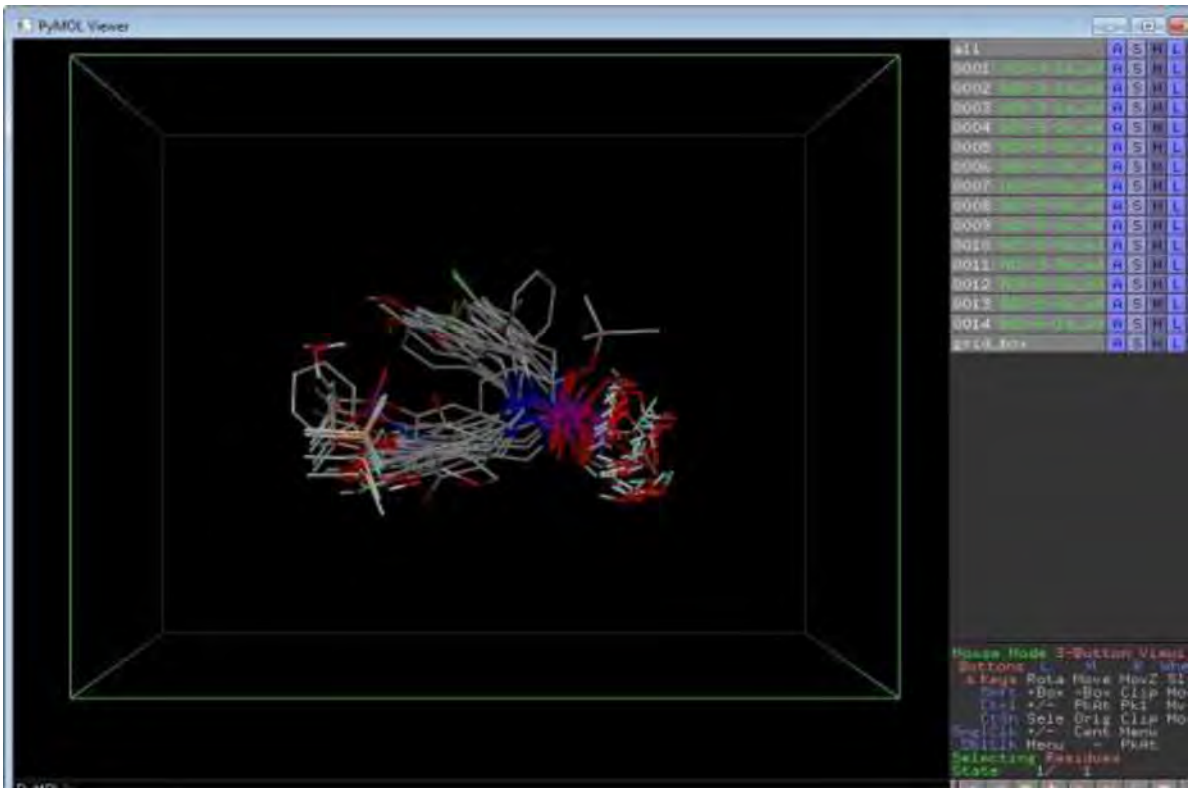
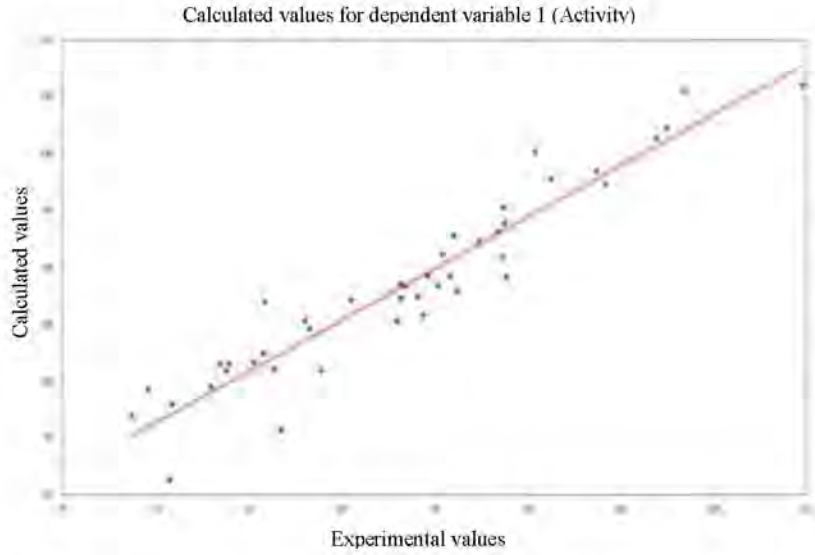


Figure 120. Series of *N*-benzylated benzylphosphoramidic acid derivatives **183**, **224**, **228a-f** and **229a-f** containing conformationally constrained Mg^{2+} chelating moieties in the 3DQSAR grid box.

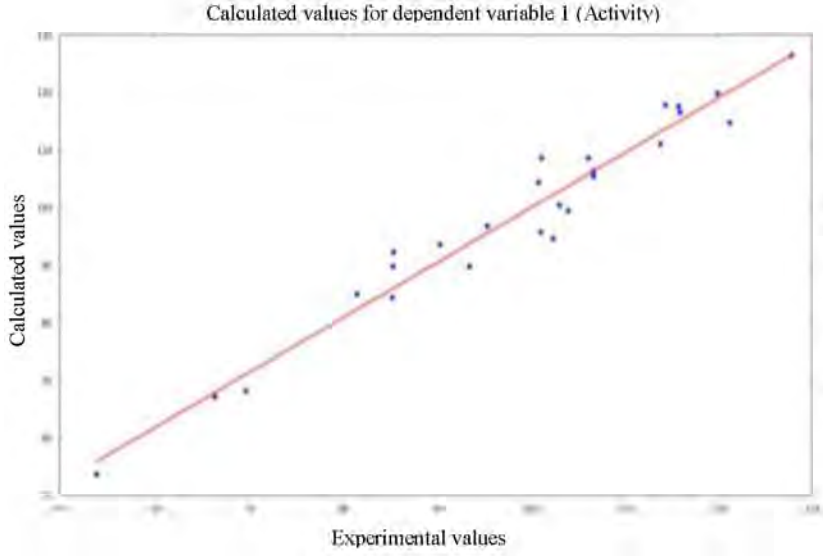
After calculation of the field parameters, cutoffs for these parameters were applied, with minimum and maximum levels set to 30.0 and -30.0, respectively. A partial least squares (PLS) model was then generated with respect to the principal components.

By default, in the partial least squares (PLS) method used 5 principle components and resulted:- high value of r^2 (0.9018-0.9958); Figure 121-123) for each of the series. Gnuplot[246] was used to obtain the calculated values *vs* experimental values plot. However, in the plots for the first and second series (Figures 121 and 122), the points are somewhat scattered, while the points in the cyclic series plot are well aligned (Figure 123), this is consistent with the fact that their bioassay activities of these compounds are quite high.



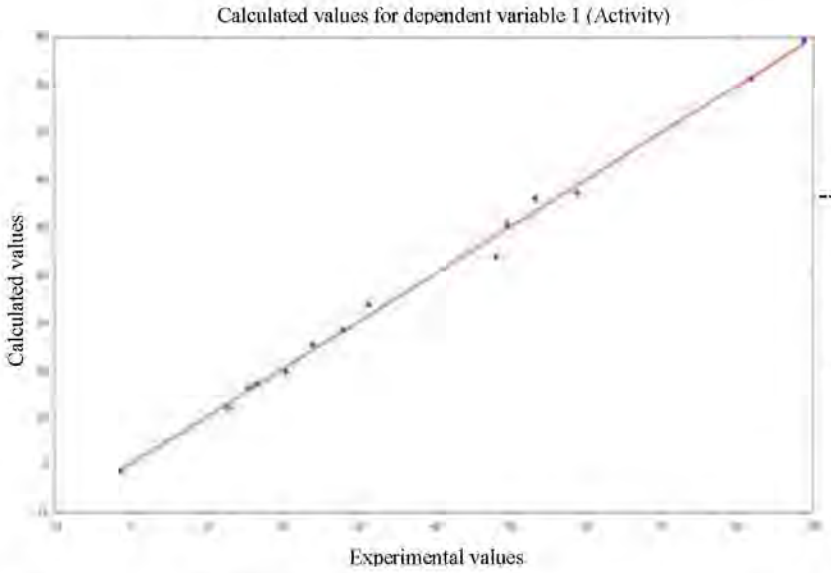
PC	SDEC	r^2	F-test
0	8.4211	0.0000	-
1	7.1713	0.2748	15.5359
2	5.3816	0.5916	28.9711
3	4.5648	0.7062	31.2427
4	3.6367	0.8135	41.4378
5	2.6391	0.9018	67.9438

Figure 121. Gnuplot of *N*-substituted phosphoramidic derivatives **159a-g**, **160a-g**, **165-167a-g** and **161-162a-g** as "reverse" fosmidomycin analogues, showing the variation of calculated values and experimental values.



PC	SDEC	r^2	F-test
0	17.4902	0.0000	-
1	15.4711	0.2176	6.6730
2	10.2547	0.6562	21.9537
3	6.9089	0.8440	39.6634
4	4.6551	0.9292	68.8627
5	3.7610	0.9538	82.5059

Figure 122. Gnuplot of *N*-benzylated phosphoramidic derivatives **169a-e, 176-180a-e** as FR900098 analogues, showing the variation of calculated values and experimental values.



PC	SDEC	r^2	F-test
0	25.6241	0.0000	-
1	13.7818	0.7107	29.4828
2	7.7233	0.9092	55.0418
3	5.7600	0.9495	62.6352
4	4.0979	0.9744	85.7254
5	1.6622	0.9958	378.6272

Figure 123. Gnuplot of *N*-benzylated benzylphosphoramidic acid derivatives **183**, **224**, **228a-f** and **229a-f** containing conformationally constrained Mg^{2+} chelating moieties, showing the variation of calculated values and experimental values.

The results were then challenged using an internal validation method,[245] which was carried out by leaving out one (LOO) and leaving many out (LMO) in different cases in order to test the predictive power of the model. Unfortunately, the results raise concerns about the validity of the correlation, and further work is required to optimise the methodology.

2.7. CONCLUSIONS

The study has successfully achieved the stated aims of synthesising four series of novel analogues of fosmidomycin and FR900098 – established *Pf*DXR inhibitors and active anti-malarial agents. Particular attention has been focused on the introduction of hydrophobic benzyl groups designed to occupy a vacant pocket adjacent to the *Pf*DXR active-site. Over 115 new compounds have been synthesised and characterised using high resolution mass spectrometric (HRMS) and spectroscopic (IR and 1- and 2D-NMR) analysis.

The synthesis of *C*-benzylated analogues of *N*-benzylated amidophosphonates, which had previously been shown to exhibit encouraging anti-malarial activity, was challenging, with a variety of synthetic approaches proving unsuccessful. Attempted *C*-benzylation of the *N*-arylcarbamoylalkylphosphonate esters using benzyl bromide in the presence of base was only successful in the case of the furfuryl derivative. Finally, a series of six *C*-benzylated phosphonate esters was successfully obtained by the CDI coupling of 2-chloro- and 2-bromo-3-phenylpropanoic acid with various 3-substituted anilines followed by phosphorylation using triethyl phosphite. Access to representative phosphonic acids was achieved by hydrolysis using TMSBr and water.

In order to avert issues of chirality, attention was given to the introduction of an *N*-benzyl moiety as part of the hydrophobic patch thereby generating phosphoramidate analogues. After several unproductive approaches, access to the series of "reverse" fosmidomycin analogues was successfully initiated reacting various benzylamine derivatives with diethyl chloro-phosphate. Subsequent aza-Michael addition and hydrolysis steps afforded the corresponding carboxylic acids which were then reacted with CDI and *O*-benzylhydroxylamine or *N*-methylhydroxylamine hydrochloride to give two series phosphoramidic acid derivatives containing different hydroxamate moieties.

The *N*-benzylated phosphoramidate FR900098 analogues were successfully synthesised in a six-step sequence incorporating several important improvements on previously developed approaches. Modification of the acid-catalysed hydrolysis of critical acetal intermediates afforded the corresponding aldehydes in shorter time, better yields and without the need for further purification. Careful monitoring permitted selective de-protection of the *O*-benzyl group without affecting the integrity of the phosphoramidate ester moiety to give the targeted

compounds. However, attempts to hydrolyse the phosphoramidate esters to afford the corresponding acids proved unsuccessful.

Attention was then turned to forming conformationally constrained fosmidomycin analogues in which a cyclic system was to ensure a *syn*-orientation of the Mg^{2+}– coordinating donor atoms. A maleimide scaffold was formed by reacting maleic anhydride with benzylamine in the presence of anhydrous $ZnCl_2$ and hexamethylsilazane; a subsequent three-step sequence gave the *N*-benzylmaleimide phosphoramidate model compound. However, attempts to extend the method to access *N*-furfurylmaleimide (to provide a second donor atom) using furfurylamine instead of benzylamine proved to be frustrating, and led to a number of undesired products. Noteworthy amongst these was an unexpected acyclic furfuryl derivative, which was formed *via* ring opening of maleic anhydride **182** in acetic acid, and theoretical studies at the DFT [B3LYP 6/31G(d)] level of the reaction of maleic anhydride with benzylamine and furfurylamine have been initiated.

The use of the 3,4-dihydroxybenzaldehyde to generate *N*-benzylated *ortho*-dihydroxyphenyl phosphoramidate compounds containing a conformationally constrained Mg^{2+} co-ordinating moiety was then explored. This resulted into protection of the hydroxyl groups in the starting material, 3,4-dihydroxybenzaldehyde. Unfortunately, the acetal protecting group used initially proved difficult to cleave at the final stage in the synthetic sequence, and attention was turned to a TBDMS protection strategy. The desired *N*-benzylated (3,4-dihydroxyphenyl)methyl-phosphoramidate derivatives were finally obtained *via* an overall six-step pathway.

The *in vitro* evaluation of the toxicity of the synthetic compounds was undertaken and, generally, these compounds exhibited no significant toxicity against HeLa and HEK 293 cell lines. With the exception of the furfuryl analogue the PLDH inhibitory activity of the *C*-benzylated phosphonate esters was generally significantly lower than that of the *N*-benzylated phosphonate ester analogues. The anti-malarial (PLDH) assays of most of the "reverse" fosmidomycin analogues revealed some inhibitory activity but, the corresponding phosphoramidic acids showed much lower *Pf*DXR inhibition levels, suggesting that the ligands were acting at a different and, as yet unknown, malarial target. The TMBDS-protected *ortho*-dihydroxyphenyl derivatives exhibited significant anti-malarial (PLDH) activity with IC_{50} values as low as 5.6 μM but, on removal of the TBDMS protecting groups, a decrease in activity was observed. All of the compounds were also tested against *T.brucei* for anti-

trypanosomal (sleeping sickness) activity and, interestingly, a number of these compounds show promising activity.

In silico modelling studies have revealed that most of synthesised ligands (docked as their negatively charged monoprotonated phosphonic or phosphoramidic acids) tend to bind preferentially to a site adjacent to the *Pf*DXR active-site. Their observed levels of *Pf*DXR inhibition may be due to an allosteric binding effect to a statistically small proportion also binding at the energetically less attractive active-site. The enhanced inhibition activity of ligands containing the conformationally constrained dihydroxyphenyl Mg^{2+} – chelating moieties is clearly supported by the docking studies as these ligands fit well into the *Pf*DXR cavity and, most importantly, occupy the empty pocket adjacent to the active-site. *In silico* physico-chemical studies of the ligands have also revealed their druggability potential.

While the stated aims and objectives of this present study have been achieved, future work in this area is expected to include the following objectives.

1. Further exploration of methods for accessing the *N*-acyl maleimide derived phosphoramidic acids.
2. Extension of the development of the *ortho*-dihydroxyphenyl derivatives which showed such promising antimalarial potential.
3. Completion of the theoretical analysis of the reactions of benzylamine and furfurylamine with maleic anhydride.
4. Investigating the design of novel anti-trypanosomal agents based on the data obtained in this study.
5. Further exploration of the application QSAR analysis methods.

3. EXPERIMENTAL

3.1. GENERAL

Reagents supplied by Sigma-Aldrich or Fluka were used without further purification. Solvents were re-distilled and dried under nitrogen as prescribed by Perrin and Armarego.[247] THF was distilled from Na wire in the presence of benzophenone under nitrogen. DCM was first stored over $CaCl_2$ and then distilled from CaH_2 under nitrogen. Methanol was dried by storing over 3Å molecular sieves for 72 hours. When considered necessary, reactions that required an inert atmosphere were conducted under nitrogen or argon.

Pre-coated Merck silica gel PF_{254} plates were used for thin layer chromatography (TLC) and spots were examined under UV light at 254 / 365 nm or revealed by iodine vapour. Flash chromatography was performed on Merck silica gel 60 [230-400 mesh (particle size 0.040-0.063 mm)]. Preparative layer chromatography (PLC) was carried out using Merck silica gel 60 PF_{254} as the stationary phase.

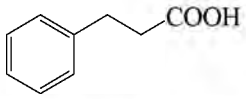
NMR spectra were obtained on Bruker 300 MHz, Bruker 400 MHz, or Bruker Biospin 600 MHz spectrometers. Chemical shifts are given in parts per million (ppm) [relative to residual solvent signals ($CDCl_3$: 7.26 ppm for ^1H and 77.4 ppm for ^{13}C; DMSO-d_6 : 2.50 ppm for ^1H and 39.5 ppm for ^{13}C; and CD_3OD : 4.80 ppm for ^1H and 49.0 ppm for ^{13}C)] and coupling constants are expressed in Hertz (Hz). Abbreviations used are: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br s = broad singlet. Structural assignments were confirmed, when necessary, using DEPT, COSY and HSQC data.

Melting points were determined using a hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer, analysing the compounds neat on a diamond window. High-resolution mass spectra (HRMS) were recorded on a Waters API Q-TOF Ultima spectrometer at the University of Stellenbosch, South Africa, and elemental analysis was done on a Vario MICRO VI.6.2 elemental analyser system GmbH at Rhodes.

Names of all the new compounds are italicized and literature references are cited for known compounds.

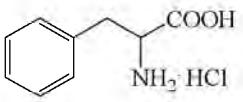
3.2. SYNTHESIS of *C*-BENZYLATED (*N*-PHENYLCARBAMOYL)ALKYL-PHOSPHONATE ESTERS and their CORRESPONDING PHOSPHONIC ACID ANALOGUES.

3-Phenylpropanoic acid 138[153]



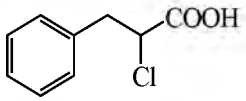
Cinnamic acid **137** (0.50 g, 3.4 mmol) in acetic acid (5 mL) and a catalytic amount of 10% palladium-on-carbon catalyst was refluxed at 100 °C for 5 h, whilst stirring under H_2. The resulting mixture was filtered through a pad of celite and then precipitated by the addition of crushed ice. Filtration of the precipitated solid under suction afforded 3-phenylpropanoic acid **138** as a white crystalline solid (0.50 g, 92%), m.p. 105-107 °C (Lit.[153] 107 °C); ν_{max}/cm^{-1} 3350-2990 (br, OH) and 1753 (C=O); δ_H/ppm (400 MHz; DMSO-d_6) 2.52 (2H, t, J = 7.7 Hz, CH_2CO), 2.81 (2H, t, J = 7.6 Hz, CH_2Ph), 7.16-7.29 (5H, overlapping m, Ar-H) and 12.1 (1H, br s, OH); δ_C/ppm (100 MHz; DMSO-d_6) 30.3 (CH_2Ph), 35.2 (CH_2CO), 126.0, 128.2, 128.3 and 148.9 (Ar-C) and 173.7 (C=O).

2-Amino-3-phenylpropanoic acid hydrochloride 140[156,157]



A mixture of Boc-phenylalanine **139** (2.0 g, 7.6 mmol) and 4M-HCl in dioxane (25 mL) was stirred at r.t. for 2 h. The mixture was concentrated *in vacuo*, and the residue was titrurated with EtOAc and then filtered to afford 2-amino-3-phenylpropanoic acid hydrochloride **140** as a white solid (1.5 g, 96%), m.p. 148-150 °C (Lit. melting point not reported); ν_{max}/cm^{-1} 3348-3100 (br, OH and NH) and 1760 (C=O); δ_H/ppm (300 MHz; DMSO-d_6) 3.15 (2H, dd, J = 2.0 and 6.2 Hz, CH_2Ph), 4.12 (1H, m, CH), 7.27-7.32 (5H, overlapping m, Ar-H), 8.56 (3H, br s, NH_3^+); δ_C/ppm (75 MHz; DMSO-d_6) 35.7 (CH_2Ph), 53.3 (CH), 127.3, 128.6, 129.7 and 135.1 (Ar-C) and 170.4 (C=O).

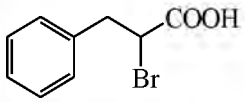
2-Chloro-3-phenylpropanoic acid 136[158,159]



A solution of $NaNO_2$ (0.99 g, 14 mmol) in water (1.3 mL) was added dropwise to a solution of 2-amino-3-phenylpropanoic acid hydrochloride **140** (1.5 g, 7.5 mmol) in 6M-HCl at 0 °C,

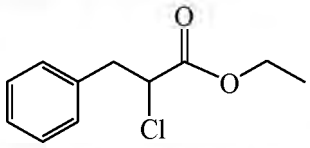
and the resulting mixture was stirred at r.t. for 24 h. The crude product was extracted with EtOAc and the organic layer was washed sequentially with distilled water and brine and then dried with anhydrous $MgSO_4$, filtered, concentrated *in vacuo* and flash chromatographed [on silica gel; elution with EtOAc-hexane (7:3)] to afford 2-chloro-3-phenylpropanoic acid **136** as a yellow oil (1.1 g, 78%); ν_{max}/cm^{-1} 3313-3012 (br, OH) and 1757 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 3.21 (1H, m, CH_aPh), 3.40 (1H, m, CH_bPh), 4.50 (1H, m, CHCl), 7.26-7.34 (5H, overlapping m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 40.9 (CH_2Ph), 57.5 (CHCl), 127.6, 128.8, 129.5 and 135.7 (Ar-C) and 174.4 (C=O).

2-Bromo-3-phenylpropanoic acid 135[166-168]



2-Amino-3-phenylpropanoic acid hydrochloride **140** (0.60 g, 3.0 mmol) was dissolved in 4M-H_2SO_4 (7 mL); NaBr (1.1 g, 10 mmol) was added at 0 °C followed by the drop-wise addition of a solution of $NaNO_2$ (0.26 g 3.7 mmol) in water (1.2 mL) to the chilled solution. After stirring at r.t. for *ca.* 24 h, the product was extracted repeatedly with EtOAc. The combined organic extracts were washed with water and brine. The aqueous washings were extracted with EtOAc and the organic solutions were combined and dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was isolated as a yellow oil (0.53 g) and purified by column chromatography [on silica gel; elution with EtOAc-hexane (7:3)] to afford 2-bromo-3-phenylpropanoic acid **135** as a yellow oil (0.49 g, 72%); ν_{max}/cm^{-1} 3318-3020 (br, OH) and 1736 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 3.27 (1H, m, CH_aPh), 3.51 (1H, m, CH_bPh), 4.47 (1H, m, CHBr), 7.27-7.36 (5H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 40.9 (CH_2Ph), 45.0 (CHBr), 127.6, 128.9, 129.3 and 136.5 (Ar-C) and 174.8 (C=O).

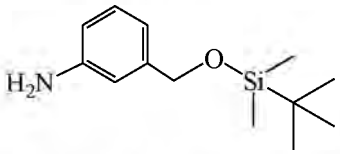
Ethyl 2-chloro-3-phenylpropanoate 141



To 2-chloro-3-phenylpropanoic acid **136** (1.0 g, 5.4 mmol) was added triethyl phosphite (1.9 mL, 11 mmol), and the mixture was boiled under reflux at 165 °C for *ca* 5 h. Upon completion of the reaction, as monitored by TLC, the crude product was purified by flash

chromatography [on silica gel; elution with EtOAc-hexane (6:4)] to afford ethyl 2-chloro-3-phenylpropanoate **141** as a yellow oil (1.2 g, 76%); ν_{max}/cm^{-1} 1698 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.25 (3H, m, CH_3), 3.21 (1H, m, CH_aPh), 3.39 (1H, m, CH_bPh), 4.21 (2H, br s, OCH_2CH_3), 4.48 (1H, m, CHCl) and 7.26-7.34 (5H, overlapping m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 14.0 (CH_3), 41.2 (CH_2Ph), 57.5 (CHCl), 62.1 (OCH_2CH_3), 127.4, 128.7, 129.5 and 136.0 (Ar-C) and 169.3 (C=O).

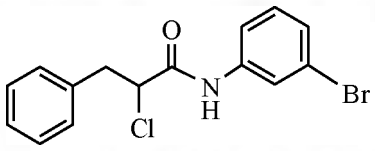
The *tert*-butyldimethylsilyl ether-protected 3-aminobenzyl alcohol 146



Imidazole (0.48 g, 7.1 mmol) was added to a solution of 3-aminobenzyl alcohol **144c** (0.59 g, 4.8 mmol) in dry DCM at 0 °C. After stirring for 15 min., TBDMSCl (0.79 g, 5.2 mmol) was added in portions and the reaction mixture was stirred overnight at r.t. The solution was diluted with DCM, washed with water and brine, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to afford the tert-*butyldimethylsilyl ether-protected 3-aminobenzyl alcohol* **146** as a yellow oil (1.1 g, 95%) [HRMS: *m/z* calculated for $C_{13}H_{24}NOSi$ (MH^+) 238.1627. Found 238.1632]; ν_{max}/cm^{-1} 3340 (NH); δ_H/ppm (300 MHz; $CDCl_3$) 0.11 [6H, s, $Si(CH_3)_2$], 0.95 [9H, s, $C(CH_3)_3$], 4.67 (2H, s, CH_2Ph), 6.53 (1H, d, J = 8.7 Hz, Ar-H), 6.69-6.72 (2H, overlapping m, Ar-H) and 7.11 (1H, t, J = 8.7 Hz, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) -5.10 [$Si(CH_3)_2$], 18.6 [$C(CH_3)_3$], 26.2 [$C(CH_3)_3$], 65.1 (CH_2Ph), 112.9, 113.8, 116.4, 129.3, 142.9 and 146.5 (Ar-C).

3.2.1. General procedure for amidation reactions

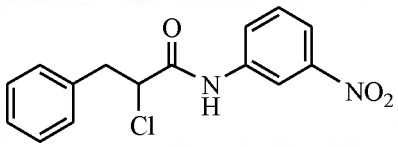
***N*-(3-Bromophenyl)-2-chloro-3-phenylpropanamide 143b**



A mixture of 2-chloro-3-phenylpropanoic acid **136** (0.1 g, 0.5 mmol), thionyl chloride (0.08 mL, 1.1 mmol) and a catalytic amount of DMF (3 drops) was heated at 70 °C for 1 h. The resulting 2-chloro-3-phenylpropanoic acid chloride was added dropwise to a stirred chilled solution of 3-bromoaniline **144b** (0.060 mL, 0.54 mmol) in DMF (1 mL) and the resulting mixture was stirred at r.t. overnight. Water was added and the mixture extracted with EtOAc

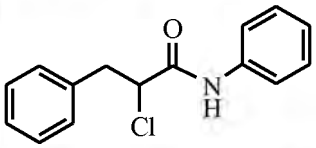
(3 × 2 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford N-*(3-bromophenyl)-2-chloro-3-phenylpropanamide* **143b** as a brown oil (0.12 g, 65%); [HRMS: *m/z* calculated for $C_{15}H_{14}{}^{79}Br^{35}ClNO$ (MH^+) 337.9947. Found 337.9933]; ν_{max}/cm^{-1} 3134 (NH) and 1668 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 3.30 (1H, m, CH_aPh), 3.51 (1H, m, CH_bPh), 4.68 (1H, m, CHCl), 7.21-7.36 (8H, overlapping m, Ar-H), 7.73 (1H, s, Ar-H) and 8.05 (1H, s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 41.5 (CH_2Ph), 61.9 (CHCl), 118.8, 122.8, 123.3, 127.6, 128.3, 128.7, 129.9, 130.5, 135.8 and 138.0 (Ar-C) and 166.4 (C=O).

2-Chloro-*N*-(3-nitrophenyl)-3-phenylpropanamide 143f



The general procedure for the synthesis of *N*-(3-bromophenyl)-2-chloro-3-phenylpropanamide **143b** was employed, using 2-chloro-3-phenylpropanoic acid **136** (0.31 g, 1.2 mmol), thionyl chloride (0.18 mL, 2.5 mmol) and 3-nitroaniline **144f** (0.17 g, 1.2 mmol) in DMF (3 mL). Chromatography [PLC on silica gel; elution with EtOAc-hexane (5:5)] afforded *2-chloro-N-(3-nitrophenyl)-3-phenylpropanamide* **143f** as a pale-yellow oil (0.31 g, 65%) [HRMS: *m/z* calculated for $C_{15}H_{12}{}^{79}BrN_2O_3$ $(M-H)^-$ 303.0536. Found 303.0534]; ν_{max}/cm^{-1} 3486 (NH) and 1675 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 3.28 (1H, dd, J = 6.0 and 9.0 Hz, CH_aPh), 3.46 (1H, dd, J = 6.0 and 6.0 Hz, CH_bPh), 4.66 (1H, m, CHCl), 7.19-7.23 (5H, overlapping m, Ar-H), 7.45 (1H, m, Ar-H), 7.78 (1H, d, J = 9.0 Hz, Ar-H), 7.94 (1H, d, J = 7.3 Hz, Ar-H), 8.15 (1H, br s, NH) and 8.27 (1H, m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 41.4 (CH_2Ph), 61.7 (CHCl), 115.3, 120.0, 120.1, 127.7, 128.7, 128.9, 129.6, 130.1, 135.6 and 138.0 (Ar-C) and 166.8 (C=O).

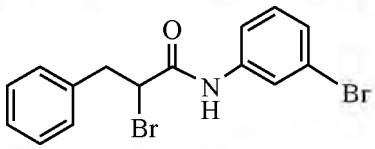
2-Chloro-*N*-phenyl-3-phenylpropanamide 143g[165]



The general procedure for the synthesis of *N*-(3-bromophenyl)-2-chloro-3-phenyl-propanamide **143b** was employed, using 2-chloro-3-phenylpropanoic acid **136** (0.30 g, 1.6 mmol), thionyl chloride (0.24 mL, 3.3 mmol) and aniline **144g** (0.15 mL, 1.6 mmol) in DMF

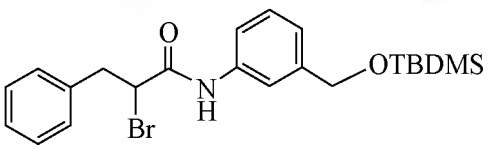
(3 mL) to afford 2-chloro-*N*-phenyl-3-phenylpropanamide **143g** as a yellow oil (0.42 g, 65%); ν_{max}/cm^{-1} 3440 (NH) and 1670 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 3.34 (1H, d, J = 13.0 Hz, CH_aPh), 3.51 (1H, d, J = 14.2 Hz, CH_bPh), 4.72 (1H, m, CHCl), 7.19 (1H, s, Ar-H), 7.31-7.50 (9H, overlapping m, Ar-H) and 8.16 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 41.5 (CH_2Ph), 61.9 (CHCl), 120.7, 125.4, 127.5, 128.6, 129.2, 129.8, 136.0 and 136.8 (Ar-C) and 166.5 (C=O).

2-Bromo-*N*-(3-bromophenyl)-3-phenylpropanamide 145b

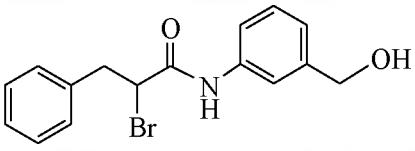


2-bromo-3-phenylpropanoic acid **135** (0.20 g, 0.89 mmol) was dissolved in dry DCM (5 mL) and CDI (0.16 g, 0.98 mmol) was added in portions. The mixture was allowed to stir for 1 h before 3-bromoaniline **144b** (0.10 mL, 0.89 mmol) was added and the reaction mixture was stirred at r.t. for 48 h. The solution was concentrated and the residue chromatographed [PLC on silica gel; elution with EtOAc-hexane (7:3)] to afford *2-bromo*-N-*(3-bromophenyl)-3-phenylpropanamide* **145b** as a yellow solid (0.24 g, 69%), m.p. 116-118 °C [HRMS: *m/z* calculated for $C_{15}H_{14}{}^{79}Br_2NO$ (MH^+) 381.9442. Found 381.9430]; ν_{max}/cm^{-1} 3490 (NH) and 1670 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 3.35 (1H, m, CH_aPh), 3.60 (1H, m, CH_bPh), 4.60 (1H, m, CHBr), 7.26-7.36 (8H, overlapping m, Ar-H), 7.72 (1H, s, Ar-H) and 7.84 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 41.7 (CH_2Ph), 51.7 (CHBr), 118.7, 123.2, 127.6, 128.2, 128.8, 129.0, 129.6, 130.5, 136.6 and 138.2 (Ar-C) and 166.3 (C=O).

2-bromo-*N*-{[3-(*tert*-butyldimethylsilyloxy) methyl]phenyl}-3-phenylpropanamide 147



2-Bromo-*N*-[3-(hydroxymethyl)phenyl] -3-phenylpropanamide 145c



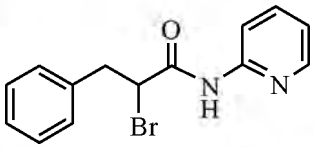
The general procedure for the synthesis of 2-bromo-*N*-(3-bromophenyl)-3-phenyl-propanamide **145b** was employed, using 2-bromo-3-phenylpropanoic acid **135** (0.19 g, 0.81 mmol), CDI (0.15 g, 0.89 mmol) and the *tert*-butyldimethylsilyl ether-protected amine **146** (0.19 g, 0.81 mmol) in dry DCM (5 mL). Chromatography [PLC on silica gel; elution with EtOAc-hexane (7:3)] afforded *2-bromo*-N-*{[3-(*tert-*butyldimethylsilyloxy)methy]phenyl}-3-*

phenylpropanamide **147** as a yellow oil (0.16 g, 60%) [HRMS: *m/z* calculated for $C_{22}H_{31}{}^{79}BrNO_2Si$ (MH^+) 448.1307. Found 448.1301]; ν_{max}/cm^{-1} 3481 (NH) and 1678 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 0.04 [6H, s, $Si(CH_3)_2$], 0.79 [9H, s, $C(CH_3)_3$], 3.19 (1H, dd, *J* = 9.0 and 15.0 Hz, CH_bPh), 3.49 (1H, dd, *J* = 6.0 and 15.0 Hz, CH_aPh), 4.46 (1H, m, CHBr), 4.57 (2H, s, CH_2Ph), 7.98 (1H, d, *J* = 8.4 Hz, Ar-H), 7.11-7.26 (7H, overlapping m, Ar-H), 7.38 (1H, s, Ar-H) and 7.71 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) -5.12 [$Si(CH_3)_2$], 18.6 [$C(CH_3)_3$], 26.1 [$C(CH_3)_3$], 29.9 (CH_2Ph), 52.0 (CHBr), 64.8 (CH_2OSi), 117.7, 118.7, 122.8, 127.5, 128.2, 128.7, 129.1, 129.6, 137.1, 142.8 (Ar-C) and 166.1 (C=O).

Compound **147** was unstable and decomposed to afford:

2-bromo-*N*-[3-(hydroxymethyl)phenyl]-3-phenylpropanamide 145c as a yellow oil (0.16 g, 60%) [HRMS: *m/z* calculated for $C_{16}H_{17}{}^{79}BrNO_2$ (MH^+) 334.0443. Found 334.0429]; ν_{max}/cm^{-1} 3492 (NH) and 1669 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 3.34 (1H, dd, *J* = 3.0 and 6.0 Hz, CH_aPh), 3.61 (1H, dd, *J* = 3.0 and 6.0 Hz, CH_bPh), 4.61 (1H, m, CHBr), 4.67 (2H, s, OCH_2Ph), 7.14 (1H, d, *J* = 7.5 Hz, Ar-H), 7.25-7.31 (7H, overlapping m, Ar-H and OH), 7.37 (1H, d, *J* = 8.2 Hz, Ar-H), 7.47 (1H, s, Ar-H) and 7.96 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 41.7 (CH_2Ph), 52.0 (CHBr), 65.1 (OCH_2Ph), 118.7, 119.4, 123.6, 124.1, 125.0, 127.5, 128.7, 129.0, 129.6 and 130.2 (Ar-C) and 166.4 (C=O).

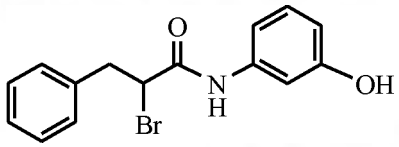
2-Bromo-3-phenyl-*N*-(2-pyridyl)propanamide 145d



The general procedure for the synthesis of 2-bromo-*N*-(3-bromophenyl)-3-phenyl-propanamide **145b** was employed, using 2-bromo-3-phenylpropanoic acid **135** (0.25 g, 1.1 mmol), CDI (0.19 g, 1.2 mmol) and 2-aminopyridine **144d** (0.10 g, 1.1 mmol) in DCM (5 mL). Chromatography [PLC on silica gel; elution with EtOAc-hexane (6:4)] afforded *2-bromo-3-phenyl-*N*-(2-pyridyl)propanamide* **145d** as a yellow oil (0.21 g, 63%) [HRMS: *m/z* calculated for $C_{14}H_{14}{}^{79}BrN_2O$ (MH^+) 305.0290. Found 305.0294]; ν_{max}/cm^{-1} 3482 (NH) and 1671 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 3.24 (1H, dd, *J* = 6.0 and 9.0 Hz, CH_aPh), 3.56 (1H, dd, *J* = 6.0 and 9.0 Hz, CH_bPh), 4.48 (1H, m, CHBr), 7.02 (1H, m, Ar-H), 7.17-7.24 (5H, overlapping m, Ar-H), 7.67 (1H, t, *J* = 8.7 Hz, Ar-H), 8.12 (1H, d, *J* = 4.9 Hz, Ar-H), 8.21 (1H, d, *J* = 8.3 Hz, Ar-H) and 8.58 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 41.3

(CH_2Ph), 49.8 (CHBr), 127.5, 128.8, 129.1, 129.5, 129.7, 129.9, 136.8, 140.4, 143.3 and 150.4 (Ar-C) and 167.6 (C=O).

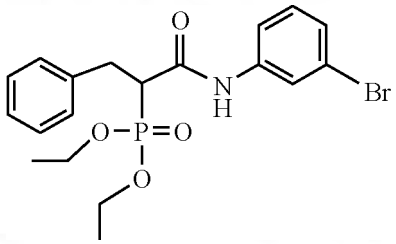
2-Bromo-*N*-(3-hydroxyphenyl)-3-phenylpropanamide 145e



The general procedure for the synthesis of 2-bromo-*N*-(3-bromophenyl)-3-phenyl-propanamide **145b** was employed, using 2-bromo-3-phenylpropanoic acid **135** (0.21 g, 0.90 mmol), CDI (0.16 g, 0.99 mmol) and 3-aminophenol **144e** (0.10 g, 0.90 mmol) in DCM (5 mL). Chromatography [PLC on silica gel; elution with EtOAc-hexance (7:3)] afforded *2-bromo-*N*-(3-hydroxyphenyl)-3-phenylpropanamide* **145e** as a brown solid (0.18 g, 63%), m.p. 118-120 °C [HRMS: *m/z* calculated for $C_{15}H_{15}{}^{79}BrNO_2$ (MH^+) 320.0286. Found 320.0275]; ν_{max}/cm^{-1} 3495 (NH) and 1677 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 3.36 (1H, dd, J = 6.0 and 9.0 Hz, CH_aPh), 3.61 (1H, dd, J = 6.0 and 9.0 Hz, CH_bPh), 4.61 (1H, m, CHBr), 6.68 (2H, m, Ar-H), 7.17 (1H, t, J = 8.1 Hz, Ar-H), 7.28-7.32 (5H, overlapping m, Ar-H) and 7.45 (1H, m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 41.9 (CH_2Ph), 51.9 (CHBr), 107.6, 111.9, 112.7, 127.6, 128.8, 129.6, 130.1, 136.6, 138.0 and 156.9 (Ar-C) and 166.8 (C=O).

3.2.2. General procedure for Arbuzov reactions of propanamides 131b-g

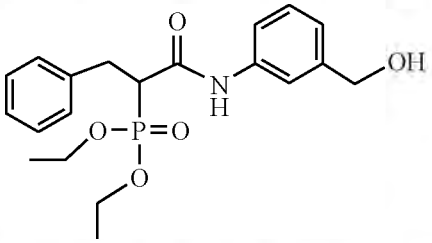
Diethyl 1-[*N*-(3-bromophenyl)carbamoyl]-2-phenylethylphosphonate 131b



2-Bromo-*N*-(3-bromophenyl)-3-phenylpropanamide **145b** (0.12 g, 0.32 mmol) was dissolved in triethyl phosphite (0.16 mL, 0.97 mmol) and the resulting solution was refluxed for 9 h under an inert atmosphere. The crude product was purified using column chromatography [on silica gel; elution with EtOAc-hexane (6:4)] to afford *diethyl 1-[*N*-(3-bromophenyl)-carbamoyl]-2-phenylethylphosphonate* **131b** as an off-white solid (0.086 g, 61%), m.p 108-110 °C [HRMS: *m/z* calculated for $C_{19}H_{24}{}^{79}BrNO_4P$ (MH^+) 440.0626. Found 440.0634]; ν_{max}/cm^{-1} 3490 (NH) and 1671 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.33 (6H, d, J = 12.0 Hz,

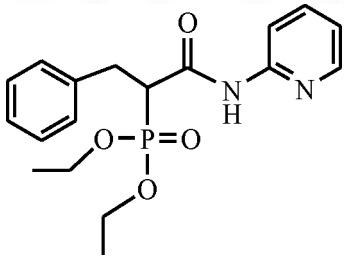
2 × CH_3), 3.13 and 3.20 (2H, 2 × m, CH_2Ph), 3.43 (1H, m, CHP), 4.16 (4H, m, 2 × OCH_2CH_3), 7.07 (1H, m, Ar-H), 7.15 (1H, m, Ar-H), 7.21-7.26 (6H, overlapping m, Ar-H), 7.72 (1H, s, Ar-H) and 8.73 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 16.5 (d, $J_{P\text{-}C}$ = 3.5 Hz, 2 × CH_3), 32.5 (d, $J_{P\text{-}C}$ = 9.0 Hz, CH_2Ph), 48.6 (d, $J_{P\text{-}C}$ = 63.1 Hz, CHP), 62.9 (d, $J_{P\text{-}C}$ = 33.6 Hz, $OC_aH_2CH_3$), 63.2 (d, $J_{P\text{-}C}$ = 34.1 Hz, $OC_bH_2CH_3$), 118.3, 122.6, 122.8, 126.9, 127.3, 127.6, 128.6, 128.7, 130.2 and 139.2 (Ar-C) and 165.1 (C=O).

Diethyl 1-{*N*-[3-(hydroxymethyl)phenyl]carbamoyl}-2-phenylethylphosphonate 131c*



The general procedure for the synthesis of diethyl 1-[*N*-(3-bromophenyl)carbamoyl]-2-phenylethylphosphonate **131b** was employed, using 2-bromo-*N*-[3-(hydroxymethyl)phenyl]-3-phenylpropanamide **145c** (0.12 g, 0.35 mmol) and triethyl phosphite (0.20 mL, 1.1 mmol). Flash chromatography [on silica gel; elution with EtOAc-hexane (8:2)] afforded *diethyl 1-{*N*-[3-(hydroxymethyl)phenyl]carbamoyl}-2-phenylethylphosphonate* **131c** as a yellow oil (0.068 g, 49%); ν_{max}/cm^{-1} 3495 (NH) and 1694 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.32 (6H, t, *J* = 6.0 Hz, 2 × CH_3), 3.28 and 3.34 (2H, 2 × m, CH_2Ph), 3.62 (1H, m, CHP), 4.10 (4H, m, 2 × OCH_2CH_3), 5.04 (2H, s, CH_2OH), 7.26-7.37 (7H, overlapping m, Ar-H), 7.50 (2H, m, Ar-H) and 8.14 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 (d, $J_{P\text{-}C}$ = 3.3 Hz, 2 × CH_3), 29.9 (d, $J_{P\text{-}C}$ = 9.0 Hz, CH_2Ph), 41.5 (d, $J_{P\text{-}C}$ = 63.5 Hz, CHP), 64.1 (d, $J_{P\text{-}C}$ = 33.8 Hz, $OC_aH_2CH_3$), 64.2 (d, $J_{P\text{-}C}$ = 34.3 Hz, $OC_bH_2CH_3$), 68.7 (CH_2OH), 119.5, 120.2, 120.3, 124.5, 127.5, 128.6, 128.7, 129.6, 129.9 and 130.9 (Ar-C) and 166.4 (C=O).

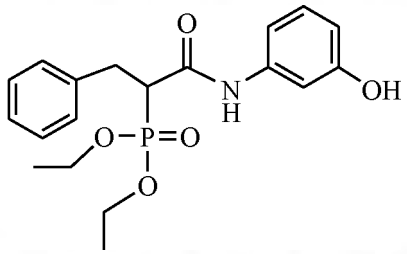
Diethyl 1-[*N*-(2-pyridyl)carbamoyl]-2-phenylethylphosphonate 131d*



Not possible to obtain satisfactory HRMS data

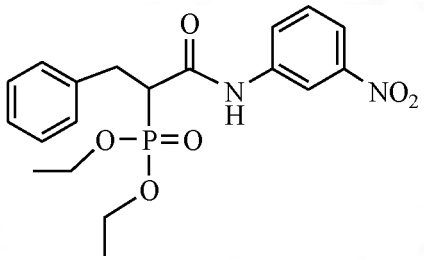
The general procedure for the synthesis of diethyl 1-[*N*-(3-bromophenyl)carbamoyl]-2-phenylethylphosphonate **131b** was employed, using 2-bromo-3-phenyl-*N*-(2-pyridyl)-propanamide **145d** (0.12 g, 0.40 mmol) and triethyl phosphite (0.20 mL, 1.2 mmol). Chromatography [PLC on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl 1-[N-(2-pyridyl)carbamoyl]-2-phenylethylphosphonate* **131d** as a pale-yellow oil (0.048 g, 32%); ν_{max}/cm^{-1} 3492 (NH) and 1675 (C=O); δ_H/ppm (400 MHz; $CDCl_3$) 1.28 (6H, t, J = 6.0 Hz, 2 × CH_3), 3.08 and 3.14 (2H, 2 × m, CH_2Ph), 3.36 (1H, m, CHP), 4.11 (4H, m, 2 × OCH_2CH_3) and 7.02-7.67 (9H, overlapping m, Ar-H) and 8.68 (1H, s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 16.7 (d, J_{P-C} = 3.5 Hz, 2 × CH_3), 32.8 (d, J_{P-C} = 9.0 Hz, CH_2Ph), 48.8 (d, J_{P-C} = 63.3 Hz, *C*HP), 63.2 (d, J_{P-C} = 33.0 Hz, $OC_aH_2CH_3$), 63.9 (d, J_{P-C} = 33.7 Hz, $OC_bH_2CH_3$), 118.5, 122.8, 123.0, 127.1, 128.8, 128.9, 129.0, 129.1, 130.4 and 139.4 (Ar-C) and 165.3 (C=O).

Diethyl 1-[*N*-(3-hydroxyphenyl)carbamoyl]-2-phenylethylphosphonate 131e



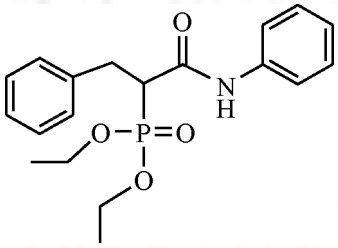
The general procedure for the synthesis of diethyl 1-[*N*-(3-bromophenyl)carbamoyl]-2-phenylethylphosphonate **131b** was employed, using 2-bromo-*N*-(3-hydroxyphenyl)-3-phenylpropanamide **145e** (0.14 g, 0.43 mmol) and triethyl phosphite (0.22 mL, 1.2 mmol). Purification using column chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl 1-[N-(3-hydroxyphenyl)carbamoyl]-2-phenylethylphosphonate* **131e** as a white solid (0.095 g, 59%), m.p 158-160 °C [HRMS: *m/z* calculated for $C_{19}H_{24}NO_5P$ (MH^+) 378.1470. Found 378.1468]; ν_{max}/cm^{-1} 3496 (NH) and 1677 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.28 (6H, m, 2 × CH_3), 3.13 (1H, m, CHP), 3.30 and 3.36 (2H, 2 × m, CH_2Ph), 4.13 (4H, m, 2 × OCH_2CH_3), 6.56 (1H, d, J = 7.6 Hz, Ar-H), 6.84 (1H, d, J = 7.3 Hz, Ar-H) 7.16-7.26 (6H, overlapping m, Ar-H), 7.47 (1H, s, Ar-H) and 8.47 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 16.5 (d, J_{P-C} = 3.0 Hz, 2 × CH_3), 32.8 (d, J_{P-C}= 8.5 Hz, CH_2Ph), 49.9 (d, J_{P-C} = 63.8 Hz, CHP), 63.3 (d, J_{P-C} = 33.8 Hz, $OC_aH_2CH_3$), 63.7 (d, J_{P-C} = 34.5 Hz, $OC_bH_2CH_3$), 105.9, 107.2, 111.3, 112.1, 126.9, 128.7, 128.8, 123.0, 138.7 and 157.2 (Ar-C) and 165.6 (C=O).

Diethyl 1-[*N*-(3-nitrophenyl)carbamoyl]-2-phenylethylphosphonate 131f*



The general procedure for the synthesis of diethyl 1-[*N*-(3-bromophenyl)carbamoyl]-2-phenylethylphosphonate **131b** was employed, using *2-chloro-*N*-(3-nitrophenyl)-3-phenyl-propanamide* **143f** (0.094 g, 0.27 mmol) and triethyl phosphite (0.14 mL, 0.81 mmol). Purification using flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl 1-[*N*-(3-nitrophenyl)carbamoyl]-2-phenylethylphosphonate* **131f** as a yellow oil (0.040 g, 3%); ν_{max}/cm^{-1} 3490 (NH) and 1667 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.31 (6H, m, 2 × CH_3), 3.24 and 3.26 (2H, 2 × m, CH_2Ph), 3.48 (1H, m, CHP), 4.19 (4H, m, 2 × OCH_2CH_3), 7.13 (1H, s, Ar-H), 7.26-7.38 (5H, overlapping m, Ar-H), 7.51 (3H, m, Ar-H) and 8.6 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 3.0 Hz, 2 × CH_3), 29.9 (CH_2Ph), 39.9 (d, J_{P-C} = 63.3 Hz, CHP), 63.3 (d, J_{P-C} = 33.2 Hz, $OC_aH_2CH_3$), 63.7 (d, J_{P-C} = 33.9 Hz, $OC_bH_2CH_3$), 115.0, 119.5, 125.8, 127.3, 127.4, 128.6, 129.8, 130.0, 135.4 and 138.7 (Ar-C) and 167.8 (C=O).

Diethyl 1-[*N*-phenylcarbamoyl]-2-phenylethylphosphonate 131g



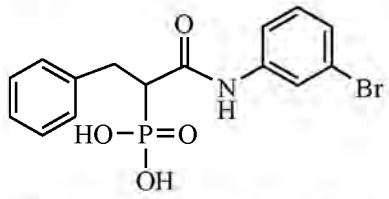
The general procedure for the synthesis of diethyl 1-[*N*-(3-bromophenyl)carbamoyl]-2-phenylethylphosphonate **131b** was employed, using *N*-phenyl-2-chloro-3-phenyl-propanamide **143g** (0.094 g, 0.27 mmol) and triethyl phosphite (0.14 mL, 0.81 mmol). Purification using column chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded diethyl 1-(*N*-phenylcarbamoyl)-2-phenylethylphosphonate **131g** as a yellow oil (0.080 g, 25%) [HRMS: *m/z* calculated for $C_{19}H_{25}NO_4P$ (MH^+) 362.1521. Found 362.1519]; ν_{max}/cm^{-1} 3497 (NH) and 1678 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.33 (6H, m, 2 × CH_3), 3.17 and 3.22 (2H, 2 × m, CH_2Ph), 3.42 (1H, m, CHP), 4.12 (4H, m, 2 × OCH_2CH_3), 7.07

Not possible to obtain satisfactory HRMS data

(2H, m, Ar-H), 7.20-7.32 (6H, overlapping m, Ar-H), 7.45 (2H, m, Ar-H) and 8.51 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 16.2 (d, $J_{P\text{-}C}$ = 3.0 Hz, 2 × CH_3), 32.5 (CH_2Ph), 48.6 (d, $J_{P\text{-}C}$ = 63.5 Hz, CHP), 63.3 (d, $J_{P\text{-}C}$ = 33.6 Hz, $OC_aH_2CH_3$), 63.7 (d, $J_{P\text{-}C}$ = 34.5 Hz, $OC_bH_2CH_3$), 119.7, 120.0, 124.3, 126.7, 127.9, 128.8, 137.8 and 139.0 (Ar-C) and 165.0 (C=O)

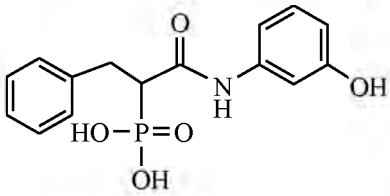
3.2.3. Hydrolysis of the diethyl phosphonate esters

1-{*N*-[(3-bromophenyl)carbamoyl]}-2-phenylethylphosphonic acid 130b*



To a solution of diethyl 1-{*N*-[(3-bromophenyl)carbamoyl]}-2-phenylethylphosphonate **131b** (0.10 g, 0.23 mmol) in dry DCM (5 mL) at 0 °C was added bromotrimethylsilane (0.15 mL, 1.1 mmol). After 1 h, the solution was allowed to warm to r.t. and stirred for an additional 24 h. The solvent was removed under reduced pressure and the residue was dissolved THF (0.5 mL) and H_2O (0.05mL) and stirred for 1 h. The resulting solution was concentrated *in vacuo* and the residue was digested with EtOAc at -10 °C to afford *1-{N-[(3-bromophenyl)carbamoyl]}-2-phenylethylphosphonic acid* **130b** as a yellow oil (0.060 g, 71 %) [HRMS: *m/z* calculated for $C_{15}H_{16}{}^{79}BrNO_4P$ (MH^+) 383.9993. Found 383.9991]; ν_{max}/cm^{-1} 3501 (NH) and 1670 (C=O); δ_H/ppm (300 MHz; CD_3OD) 2.86 and 2.88 (2H, 2 × m, CH_2Ph), 3.09 (1H, m, CHP), 6.23 (1H, s, Ar-H), 6.56-6.77 (3H, m, Ar-H) and 6.78-6.91 (5H, overlapping m, Ar-H); δ_C/ppm (75 MHz; CD_3OD) 17.2 (CH_2Ph), 34.4 (d, $J_{P\text{-}C}$ = 15 Hz, CHP), 108.7, 112.7, 112.8, 127.8, 128.0, 128.7, 128.8, 130.0, 130.6 and 130.7 (Ar-C) and 159.1 (C=O)

1-{*N*-[(3-hydroxyphenyl)carbamoyl]}-2-phenylethylphosphonic acid 130e



The general procedure for the synthesis of 1-{*N*-[(3-bromophenyl)carbamoyl]}-2-phenylethylphosphonic acid **132b** was employed, using diethyl 1-{*N*-[(3-hydroxyphenyl)carbamoy-

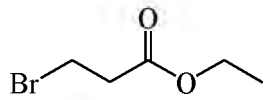
Not possible to obtain satisfactory HRMS data

l]}-2-phenylethylphosphonate **131e** (0.13 g, 0.35 mmol) in dry DCM (5 mL) and bromotrimethylsilane (0.25 mL, 1.7 mmol). Work-up afforded *1-{N-[(3-hydroxyphenyl)-carbamoyl]}-2-phenylethylphosphonic acid* **130e** as a yellow oil (0.090 g, 86%) [HRMS: *m/z* calculated for $C_{15}H_{17}NO_5P$ (MH^+) 322.0844. Found 322.0836]; ν_{max}/cm^{-1} 3450-3220 (br, OH) and 1674 (C=O); δ_H/ppm (300 MHz; CD_3OD) 2.99 and 3.02 (2H, 2 × m, CH_2Ph), 3.24 (1H, m, CHP), 6.38 (1H, s, Ar-H), 6.70-6.93 (3H, m, Ar-H) and 7.06 (5H, overlapping m, Ar-H); δ_C/ppm (75 MHz; CD_3OD) 16.9 (CH_2Ph), 34.1 (d, J_{P-C} = 14.3 Hz, CHP), 108.4, 108.5, 112.4, 127.6, 127.7, 129.5, 129.6, 129.7, 130.3 and 130.4 (Ar-C) and 158.8 (C=O).

3.3. SYNTHESIS of *N*-SUBSTITUTED PHOSPHORAMIDIC ACID DERIVATIVES as "REVERSE" FOSMIDOMYCIN ANALOGUES

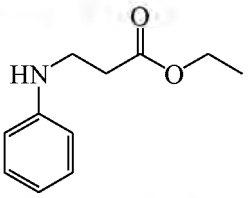
3.3.1. Approaches to the synthesis of phosphoramidate compounds

Ethyl 3-bromopropanoate 153[177-180]



To a solution of 3-bromopropanoic acid **152** (0.50 g, 3.3 mmol) in EtOH (20 mL) was added a few drops of conc. H_2SO_4. After refluxing for 12 h, the reaction mixture was neutralised with saturated $NaHCO_3$ until effervescence ceased. The product was extracted with EtOAc (2 × 20 mL), and the organic solutions were combined, dried with anhydrous $MgSO_4$ and concentrated *in vacuo* to afford ethyl 3-bromopropanoate **153** as a yellow oil (0.54 g, 91%); ν_{max}/cm^{-1} 1743 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.28 (3H, t, J = 7.1 Hz, CH_3), 2.91 (2H, t, J = 6.9 Hz, CH_2CH_2Br), 3.58 (2H, t, J = 6.9 Hz, CH_2Br) and 3.58 (2H, q, J = 7.1 Hz, $COOCH_2CH_3$); δ_C/ppm (150 MHz; $CDCl_3$) 14.3 (CH_3), 26.1 (CH_2Br), 37.9 (CH_2CH_2Br), 61.2 (OCH_2CH_3) and 170.3 (C=O).

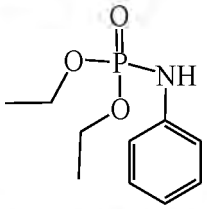
Ethyl 3-(phenylamino)propanoate 155[248]



Aniline (0.70 mL, 0.76 mmol) was dissolved in dry THF (3 mL) and NaH (60% dispersion in mineral oil; (0.036 g, 1.5 mmol) was added in small portions (controlling the evolution of

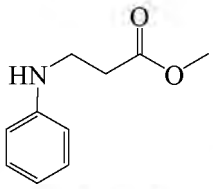
H_2) and the mixture was stirred for 30 min. Ethyl 3-bromopropanoate **153** was then added slowly to the resulting mixture and stirring was continued at r.t. for 48 h. The product was extracted using EtOAc and the combined organic solutions were washed sequentially with saturated aq. $NaHCO_3$, water and brine. The solvent was evaporated *in vacuo* and the crude product, which was flash chromatographed [on silica gel; elution with EtOAc-hexane (6:4)] to afford ethyl 3-(phenylamino)propanoate **155** as a yellow oil (0.11 g, 64%); ν_{max}/cm^{-1} 3325 (NH) and 1739 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.26 (3H, t, J = 7.1 Hz, CH_3), 2.62 (2H, t, J = 6.4 Hz, CH_2CH_2Br), 3.45 (2H, t, J = 6.4 Hz, CH_2Br), 3.58 (2H, q, J = 6.9 Hz, $COOCH_2CH_3$), 6.64 (2H, d, J = 10.6 Hz, Ar-H), 6.73 (1H, t, J = 7.3 Hz, Ar-H) and 7.19 (2H, m, Ar-H).

Diethyl *N*-phenylphosphoramidate 156[249]



The procedure used for the synthesis of ethyl 3-(phenylamino)propanoate **155** was employed using aniline (0.46 mL, 5.0 mmol), NaH (0.12 g, 5.0 mmol) and diethyl chlorophosphate (0.72 mL, 5.0 mmol) in dry THF (10 mL). Work-up and chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded diethyl *N*-phenylphosphoramidate **156** as a yellow oil (0.11 g, 64%); ν_{max}/cm^{-1} 3340 (NH); δ_H/ppm (300 MHz; $CDCl_3$) 1.31 (6H, t, J = 7.1 Hz, 2 × CH_3), 3.29 (4H, m, 2 × OCH_2CH_3), 6.96 (2H, m, Ar-H) and 7.24 (3H, m, Ar-H).

Methyl 3-(phenylamino)propanoate 158[181-182]

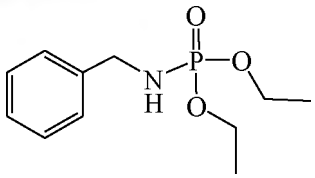


Aniline (0.22 mL, 2.4 mmol) was dissolved in toluene (10 mL), methyl acrylate (0.22 mL, 2.4 mmol) was added and the resulting mixture was refluxed for 24 h. The mixture was concentrated *in vacuo* and the residue flash chromatographed [on silica gel; elution with EtOAc-hexane (6:4)] to afford methyl 3-(phenylamino)propanoate **158** as a brown oil (0.22 g, 52%); ν_{max}/cm^{-1} 3335 (NH) and 1741 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 2.65 (2H, t, J = 6.4 Hz, $NHCH_2CH_2$), 3.48 (2H, J = 6.4 Hz, $NHCH_2CH_2$), 3.72 (3H, s, OCH_3), 6.56 (2H, d, J

= 7.7 Hz, Ar-H), 6.75 (1H, t, J = 7.3 Hz, Ar-H) and 7.21 (2H, m, Ar-H).

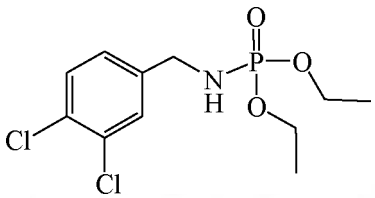
3.3.2. Reaction of benzylamine derivatives with diethyl chlorophosphate

Diethyl *N*-benzylphosphoramidate 159a[191]



A mixture of benzylamine **163a** (2.5 mL, 23 mmol), triethylamine (3.2 mL, 23 mmol) and diethyl chlorophosphate (3.3 mL, 23 mmol) in DCM (15 mL) was stirred in a stoppered reaction flask at r.t. for 6 h. The mixture was then washed sequentially with 10% HCl (2 × 20 mL) and water (2 × 20 mL) and the aqueous layers were re-extracted with DCM (2 × 25 mL). The organic layers were combined, dried with anhydrous $MgSO_4$ and concentrated *in vacuo* to afford diethyl *N*-benzylphosphoramidate **159a** as a pale-yellow oil (5.0 g, 90%); ν_{max}/cm^{-1} 1205 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.27 (6H, t, J = 7.1 Hz, 2 × CH_3), 3.98-4.07 (6H, m, 2 × OCH_2CH_3 and NCH_2Ph) and 7.24-7.32 (5H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.2 Hz, 2 × CH_3), 45.4 (NCH_2Ph), 62.5 (d, J_{P-C} = 5.2 Hz, 2 × OCH_2CH_3), 127.4, 128.6, 129.1 and 139.7 (Ar-C).

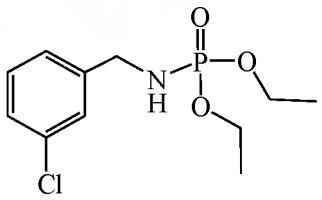
Diethyl *N*-(3,4-dichlorobenzyl)phosphoramidate 159b



The general procedure for the synthesis of diethyl *N*-benzylphosphoramidate **159a** was employed, using 3,4-dichlorobenzylamine **163b** (0.86 g, 4.9 mmol), triethylamine (0.68 mL, 4.9 mmol) and diethyl chlorophosphate (0.71 mL, 4.9 mmol) in DCM (10 mL). Work-up afforded *diethyl N-(3,4-dichlorobenzyl)phosphoramidate* **159b** as a pale-yellow oil (1.4 g, 93%) [HRMS: *m/z* calculated for $C_{11}H_{17}{}^{35}Cl_2NO_3P$ (MH^+) 312.0323. Found 312.0320]; ν_{max}/cm^{-1} 1200 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.27 (6H, t, J = 7.1 Hz, 2 × CH_3), 3.96-4.05 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar), 7.15 (1H, dd, J = 2.0 and 8.2 Hz, Ar-H), 7.37 (1H, d, J = 8.2 Hz, Ar-H) and 7.44 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 (d, J_{P-C} =

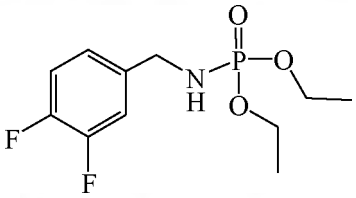
7.1 Hz, 2 × CH_3), 44.3 (NCH_2Ar), 62.7 (d, J_{P-C} = 5.3 Hz, 2 × OCH_2CH_3), 126.7, 129.4, 130.5, 131.3, 132.6 and 140.3 (Ar-C).

Diethyl *N*-(3-chlorobenzyl)phosphoramidate 159c



The general procedure for the synthesis of diethyl *N*-benzylphosphoramidate **159a** was employed, using 3-chlorobenzylamine **163c** (0.43 mL, 3.5 mmol), triethylamine (0.49 mL, 3.5 mmol) and diethyl chlorophosphate (0.51 mL, 3.5 mmol) in DCM (10 mL). Work-up afforded *diethyl* N-*(3-chlorobenzyl)phosphoramidate* **159c** as a pale-yellow oil (0.86 g, 87%) [HRMS: *m/z* calculated for $C_{11}H_{18}{}^{35}ClNO_3P$ (MH^+) 278.0713. Found 278.0707]; ν_{max}/cm^{-1} 1205 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.21 (6H, t, *J* = 7.1 Hz, 2 × CH_3), 3.91-3.99 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar), 7.16-7.18 (3H, overlapping m, Ar-H), 7.30 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.1 (d, J_{P-C} = 7.2 Hz, 2 × CH_3), 44.6 (NCH_2Ar), 62.4 (d, J_{P-C} = 5.2 Hz, 2 × OCH_2CH_3), 125.4, 127.3, 127.4, 129.7, 134.2 and 142.1 (Ar-C).

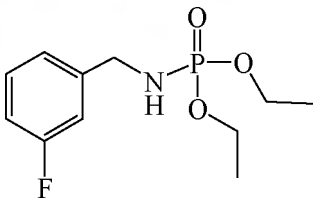
Diethyl *N*-(3,4-difluorobenzyl)phosphoramidate 159d



The general procedure for the synthesis of diethyl *N*-benzylphosphoramidate **159a** was employed, using 3,4-difluorobenzylamine **163d** (0.49 mL g, 4.2 mmol), triethylamine (0.58 mL, 4.2 mmol) and diethyl chlorophosphate (0.60 mL, 4.2 mmol) in DCM (10 mL). Work-up afforded *diethyl* N-*(3,4-difluorobenzyl)phosphoramidate* **159d** as a pale-yellow oil (1.1 g, 95%) [HRMS: *m/z* calculated for $C_{11}H_{17}{}^{19}F_2NO_3P$ (MH^+) 280.0914. Found 280.0916]; ν_{max}/cm^{-1} 1226 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 (6H, t, *J* = 7.4 Hz, 2 × CH_3), 3.11 (1H, br s, NH), 4.01-4.11 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar), 7.03 (1H, d, *J* = 8.3 Hz, Ar-H), 7.11 (1H, dd, J_{H-H} = 9.2 and J_{F-H} = 12.3 Hz, Ar-H), 7.16-7.20 (1H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.1 Hz, 2 × CH_3), 45.0 (NCH_2Ar), 62.6 (d, J_{P-C} = 5.2 Hz, 2

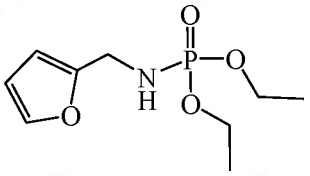
× OCH_2CH_3), 114.2 (d, J_{F-C} = 21.5 Hz), 114.4 (d, J_{F-C} = 6.5 Hz), 122.9 (d, J_{F-C} = 2.8 Hz), 130.2 (d, J_{F-C} = 8.2 Hz), 142.5 and 163.9 (d, J_{F-C} = 244.5 Hz) (Ar-C).

Diethyl *N*-(3-fluorobenzyl)phosphoramidate 159e



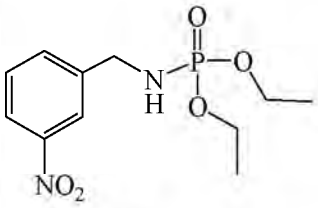
The general procedure for the synthesis of diethyl *N*-benzylphosphoramidate **159a** was employed, using 3-fluorobenzylamine **163e** (0.72 mL, 6.4 mmol), triethylamine (0.88 mL, 6.4 mmol) and diethyl chlorophosphate (0.92 mL, 6.4 mmol) in DCM (20 mL). Work-up afforded *diethyl* N-*(3-fluorobenzyl)phosphoramidate* **159e** as a pale-yellow oil (1.5 g, 93%) [HRMS: *m/z* calculated for $C_{11}H_{18}{}^{19}FNO_3P$ (MH^+) 262.1008. Found 262.1016]; ν_{max}/cm^{-1} 1208 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 (6H, t, *J* = 7.1 Hz, 2 × CH_3), 4.01-4.10 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar), 7.03 (1H, s, Ar-H), 7.10 (2H, m, Ar-H), 7.18 (1H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 44.4 (NCH_2Ar), 62.6 (d, J_{P-C} = 5.3 Hz, 2 × OCH_2CH_3), 116.3 (d, J_{F-C} = 17.5 Hz), 117.3 (d, J_{F-C} = 17.1 Hz), 123.1 (dd, J_{F-C} = 4.5 and 6.0 Hz), 124.5, 142.6 and 162.9 (d, J_{F-C} = 240.0 Hz) (Ar-C).

Diethyl *N*-furfurylphosphoramidate 159f[250]



The general procedure for the synthesis of diethyl *N*-benzylphosphoramidate **159a** was employed, using furfurylamine **163f** (2.3 mL, 26 mmol), triethylamine (3.6 mL, 26 mmol) and diethyl chlorophosphate (3.7 mL, 26 mmol) in DCM (15 mL). Work-up afforded diethyl *N*-furfurylphosphoramidate **159f** as an orange oil (5.5 g, 92%); ν_{max}/cm^{-1} 1217 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.26 (6H, t, *J* = 7.4 Hz, 2 × CH_3), 3.34 (1H, br s, NH), 3.96-4.03 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar), 6.18 (1H, d, *J* = 3.6 Hz, Ar-H), 6.27 (1H, m, Ar-H) and 7.31 (1H, d, *J* = 1.1 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.2 Hz, 2 × CH_3), 38.3 (NCH_2Ar), 62.4 (d, J_{P-C} = 5.2 Hz, 2 × OCH_2CH_3), 106.8, 110.4, 142.1 and 153.0 (Ar-C).

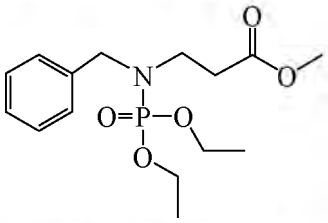
Diethyl *N*-(3-nitrobenzyl)phosphoramidate 159g



The general procedure for the synthesis of diethyl *N*-benzylphosphoramidate **159a** was employed, using 3-nitrobenzylamine **163g** (2.8 g, 18 mmol), triethylamine (2.6 mL, 18 mmol) and diethyl chlorophosphate (2.7 mL, 18 mmol) in DCM (15 mL). Work-up afforded *diethyl* N-*(3-nitrobenzyl)phosphoramidate* **159g** as pale-yellow oil (3.5 g, 93%) [HRMS: *m/z* calculated for $C_{11}H_{18}N_2O_5P$ (MH^+) 289.0953. Found 289.0949]; ν_{max}/cm^{-1} 1207 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.07 (6H, t, J = 7.4 Hz, 2 × CH_3), 3.78 (4H, m, 2 × OCH_2CH_3), 4.01 (2H, d, $J_{P\text{-}H}$ = 12.6 Hz, NCH_2Ar), 7.33 (1H, t, J = 7.9 Hz, Ar-H), 7.55 (1H, d, J = 7.6 Hz, Ar-H), 7.89 (1H, d, J = 8.1 Hz, Ar-H), 8.07 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.7 (d, $J_{P\text{-}C}$ = 7.1 Hz, 2 × CH_3), 44.0 (NCH_2Ar), 62.2 (d, $J_{P\text{-}C}$ = 5.2 Hz, 2 × OCH_2CH_3), 122.8, 129.1, 133.3, 135.5, 142.3 and 147.9 (Ar-C).

3.3.3. Aza-Michael addition reactions of diethyl *N*-benzylphosphoramidate esters

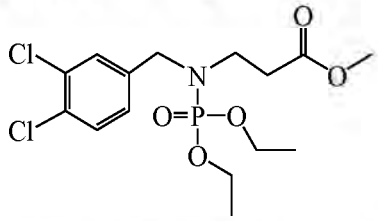
Diethyl *N*-benzyl-*N*-[2-(methoxycarbonyl)ethyl]phosphoramidate 160a



To a solution of diethyl *N*-benzylphosphoramidate **159a** (1.6 g, 6.5 mmol) in toluene (20 mL) was added anhydrous K_2CO_3 (1.8 g, 13 mmol), tetrabutylammonium bromide (0.21g, 0.65 mmol) and methyl acrylate (0.71 mL, 7.8 mmol). The reaction mixture was refluxed for 8 h at 120 °C, and allowed to cool to r.t. Benzene (10 mL) and water (20 mL) were added and the stirred mixture was stirred for 15 min., and then washed with cold water. The organic layer was dried with anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] to afford *diethyl* N-*benzyl*-N-*[2-(methoxycarbonyl)ethyl]phosphoramidate* **160a** as a yellow oil (1.5 g, 57%) [HRMS: *m/z* calculated for $C_{15}H_{25}NO_5P$ (MH^+) 330.1470. Found 330.1475]; ν_{max}/cm^{-1} 1734 (C=O) and 1243 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.31 (6H, t, J = 7.5 Hz, 2 × CH_3),

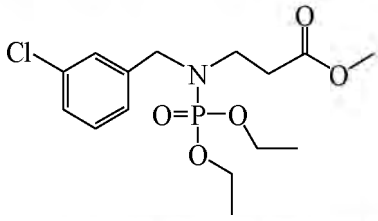
2.49 (2H, t, J = 7.3 Hz, CH_2CO), 3.23 (2H, m, NCH_2), 3.62 (3H, s, OCH_3), 4.02 (4H, m, 2 × OCH_2CH_3), 4.20 (2H, d, $J_{P\text{-}H}$ = 9.6 Hz, NCH_2Ph) and 7.26-7.32 (5H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, $J_{P\text{-}C}$ = 7.2 Hz, 2 × CH_3), 33.3 (CH_2CO), 41.3 (d, $J_{P\text{-}C}$ = 4.5 Hz, NCH_2), 49.9 (d, $J_{P\text{-}C}$ = 4.9 Hz, NCH_2Ph), 51.7 (OCH_3), 62.5 (d, $J_{P\text{-}C}$ = 5.7 Hz, 2 × OCH_2CH_3), 127.5, 128.3, 128.5 and 137.8 (Ar-C) and 172.2 (C=O).

Diethyl *N*-(3,4-dichlorobenzyl)-*N*-[2-(methoxycarbonyl)ethyl]phosphoramidate 160b



The general procedure for the synthesis of diethyl *N*-benzyl-*N*-[2-(methoxycarbonyl)ethyl]-phosphoramidate **160a** was employed, using diethyl *N*-(3,4-dichlorobenzyl)phosphoramidate **159b** (1.4 g, 4.5 mmol), anhydrous K_2CO_3 (1.2 g, 8.9 mmol), tetrabutylammonium bromide (0.14 g, 0.45 mmol) and methyl acrylate (0.49 mL, 5.4 mmol) in toluene (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl N-(3,4-dichlorobenzyl)-N-[2-(methoxycarbonyl)ethyl]phosphoramidate* **160b** as a yellow oil (1.5 g, 84%) [HRMS: *m/z* calculated for $C_{15}H_{23}{}^{35}Cl_2NO_5P$ (MH^+) 398.0691. Found 398.0687]; ν_{max}/cm^{-1} 1736 (C=O) and 1243 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.28 (6H, t, J = 7.3 Hz, 2 × CH_3), 2.46 (2H, t, J = 7.3 Hz, CH_2CO), 3.17 (2H, m, NCH_2), 3.60 (3H, s, OCH_3), 4.04 (4H, m, 2 × OCH_2CH_3), 4.13 (2H, d, $J_{P\text{-}H}$ = 10.0 Hz, NCH_2Ar), 7.14 (1H, d, J = 8.3 Hz, Ar-H), 7.35 (1H, d, J = 8.2 Hz, Ar-H) and 7.41 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, $J_{P\text{-}C}$ = 7.2 Hz, 2 × CH_3), 33.4 (CH_2CO), 41.5 (d, $J_{P\text{-}C}$ = 4.3 Hz, NCH_2), 49.1 (d, $J_{P\text{-}C}$ = 5.0 Hz, NCH_2Ar), 51.8 (OCH_3), 62.7 (d, $J_{P\text{-}C}$ = 5.7 Hz, 2 × OCH_2CH_3), 127.5, 130.0, 130.5, 131.5, 132.6 and 138.5 (Ar-C) and 172.1 (C=O).

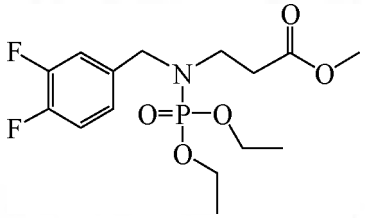
Diethyl *N*-(3-chlorobenzyl)-*N*-[2-(methoxycarbonyl)ethyl]phosphoramidate 160c



The general procedure for the synthesis of diethyl *N*-benzyl-*N*-[2-(methoxycarbonyl)ethyl]-phosphoramidate **160a** was employed, using diethyl *N*-(3-chlorobenzyl)phosphoramidate

159c (0.70 g, 2.5 mmol), anhydrous K_2CO_3 (0.70 g, 5.1 mmol), tetrabutylammonium bromide (0.08 g, 0.25 mmol) and methyl acrylate (0.27 mL, 3.0 mmol) in toluene (10 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*(3-chlorobenzyl)*-N-*[2-(methoxycarbonyl)ethyl]phosphoramidate* **160c** as a pale-yellow oil (0.70 g, 77%) [HRMS: *m/z* calculated for $C_{15}H_{24}{}^{35}ClNO_5P$ (MH^+) 364.1081. Found 364.1070]; ν_{max}/cm^{-1} 1736 (C=O) and 1244 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.31 (6H, t, J = 7.2 Hz, 2 × CH_3), 2.49 (2H, m, CH_2CO), 3.22 (2H, m, NCH_2), 3.62 (3H, s, OCH_3), 4.04 (4H, m, 2 × OCH_2CH_3), 4.18 (2H, d, J_{P-C} = 10.0 Hz, NCH_2Ar), 7.20-7.28 (3H, overlapping m, Ar-H) and 7.33 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.2 Hz, 2 × CH_3), 33.4 (CH_2CO), 41.5 (d, J_{P-C} = 4.3 Hz, NCH_2), 49.6 (d, J_{P-C} = 5.1 Hz, NCH_2Ar), 51.8 (OCH_3), 62.7 (d, J_{P-C} = 5.8 Hz, 2 × OCH_2CH_3), 126.3, 127.7, 128.2, 129.8, 134.5 and 140.2 (Ar-C) and 172.2 (C=O).

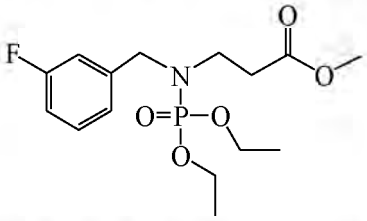
Diethyl *N*-(3,4-difluorobenzyl)-*N*-[2-(methoxycarbonyl)ethyl]phosphoramidate 160d



The general procedure for the synthesis of diethyl *N*-benzyl-*N*-[2-(methoxycarbonyl)ethyl]-phosphoramidate **160a** was employed, using diethyl *N*-(3,4-difluorobenzyl)phosphoramidate **159d** (0.90 g, 3.2 mmol), anhydrous K_2CO_3 (0.89 g, 6.4 mmol), tetrabutylammonium bromide (0.10 g, 0.32 mmol) and methyl acrylate (0.35 mL, 3.9 mmol) in toluene (10 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*(3,4-difluorobenzyl)*-N-*[2-(methoxycarbonyl)ethyl]phosphoramidate* **160d** as a yellow oil (0.92 g, 78%) [HRMS: *m/z* calculated for $C_{15}H_{23}{}^{19}F_2NO_5P$ (MH^+) 366.1282. Found 366.1286]; ν_{max}/cm^{-1} 1737 (C=O) and 1240 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.33 (6H, t, J = 7.3 Hz, 2 × CH_3), 2.50 (2H, m, CH_2CO), 3.24 (2H, m, NCH_2), 3.65 (3H, s, OCH_3), 4.06 (4H, m, 2 × OCH_2CH_3), 4.18 (2H, d, J_{P-H} = 10.2 Hz, NCH_2Ar), 7.09 (1H, s, Ar-H) and 7.14-7.23 (2H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.1 (d, J_{P-C} = 7.1 Hz, 2 × CH_3), 33.3 (CH_2CO), 41.3 (d, J_{P-C} = 4.2 Hz, NCH_2), 49.2 (d, J_{P-C} = 5.2 Hz, NCH_2Ar), 51.7 (OCH_3), 62.4 (d, J_{P-C} = 5.3 Hz, 2 × OCH_2CH_3), 116.2 (d, J_{F-C} = 18.0 Hz), 117.1 (dd, J_{F-C} = 17.1, 29.2 Hz), 124.1 (dd, J_{F-C} = 3.0 and 7.5 Hz), 135.2 (m), 149.1 (dd, J_{F-C} = 13.5 and 246.0 Hz) and 150.7 (ddd, J_{F-C} = 9.0, 12.0 and 246.0 Hz) (Ar-C) and 172.1

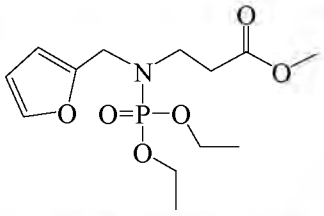
(C=O).

Diethyl *N*-(3-fluorobenzyl)-*N*-[2-(methoxycarbonyl)ethyl]phosphoramidate 160e



The general procedure for the synthesis of diethyl *N*-benzyl-*N*-[2-(methoxycarbonyl)ethyl]-phosphoramidate **160a** was employed, using diethyl *N*-(3-fluorobenzyl)phosphoramidate **159e** (1.3 g, 5.2 mmol), anhydrous K_2CO_3 (1.5 g, 11 mmol), tetrabutylammonium bromide (0.16 g, 0.52 mmol) and methyl acrylate (0.56 mL, 6.4 mmol) in toluene (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl N-(3-fluorobenzyl)-N-[2-(methoxycarbonyl)ethyl]phosphoramidate* **160e** as a yellow oil (1.3 g, 69%) [HRMS: *m/z* calculated for $C_{15}H_{24}{}^{19}FNO_5P$ (MH^+) 348.1376. Found 348.1372]; ν_{max}/cm^{-1} 1738 (C=O) and 1235 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.28 (6H, t, J = 7.2 Hz, 2 × CH_3), 2.46 (2H, t, J = 7.5 Hz, CH_2CO), 3.20 (2H, m, NCH_2), 3.59 (3H, s, OCH_3), 3.99 (4H, m, 2 × OCH_2CH_3), 4.17 (2H, d, $J_{P\text{-}H}$ = 9.8 Hz, NCH_2Ar), 6.90 (1H, d, J = 8.0 Hz, Ar-H) and 7.04-7.23 (3H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.1 (d, $J_{P\text{-}C}$ = 7.3 Hz, 2 × CH_3), 33.3 (CH_2CO), 41.4 (d, $J_{P\text{-}C}$ = 4.0 Hz, NCH_2), 49.5 (d, $J_{P\text{-}C}$ = 5.0 Hz, NCH_2Ar), 51.6 (OCH_3), 62.4 (d, $J_{P\text{-}C}$ = 5.3 Hz, 2 × OCH_2CH_3), 114.0 (d, $J_{F\text{-}C}$ = 18.2 Hz), 114.9 (d, $J_{F\text{-}C}$ = 18.8 Hz), 123.7 (d, $J_{F\text{-}C}$ = 2.5 Hz), 130.0 (dd, $J_{F\text{-}C}$ = 9.0 and 12.0 Hz), 142.6 and 162.9 (d, $J_{F\text{-}C}$ = 234.9 Hz) (Ar-C) and 172.1 (C=O).

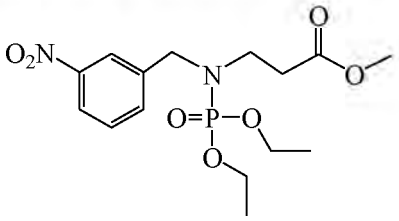
Diethyl *N*-furfuryl-*N*-[2-(methoxycarbonyl)ethyl]phosphoramidate 160f



The general procedure for the synthesis of diethyl *N*-benzyl-*N*-[2-(methoxycarbonyl)ethyl]-phosphoramidate **160a** was employed, using diethyl *N*-furfurylphosphoramidate **159f** (3.0 g, 13 mmol), anhydrous K_2CO_3 (3.6 g, 26 mmol), tetrabutylammonium bromide (0.42 g, 1.3 mmol) and methyl acrylate (1.7 mL, 19 mmol) in toluene (20 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*furfuryl-*

N-*[2-(methoxycarbonyl)ethyl]phosphoramidate* **160f** as a yellow oil (3.1 g, 76%) [HRMS: *m/z* calculated for $C_{13}H_{23}NO_6P$ (MH^+) 320.1263. Found 320.1260]; ν_{max}/cm^{-1} 1735 (C=O) and 1244 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.27 (6H, t, J = 7.5 Hz, 2 × CH_3), 2.48 (2H, t, J = 6.2 Hz, CH_2CO), 3.26 (2H, m, NCH_2), 3.63 (3H, s, OCH_3), 4.04 (4H, m, 2 × OCH_2CH_3), 4.16 (2H, d, J_{P-H} = 9.7 Hz, NCH_2Ar), 6.24-6.29 (2H, overlapping m, Ar-H) and 7.34 (1H, d, J = 1.6 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.3 Hz, 2 × CH_3), 33.5 (CH_2CO), 41.9 (d, J_{P-C} = 4.4 Hz, NCH_2), 43.0 (d, J_{P-C} = 5.7 Hz, NCH_2Ar), 51.8 (OCH_3), 62.4 (d, J_{P-C} = 5.4 Hz, 2 × OCH_2CH_3), 108.8, 110.4, 142.4 and 151.6 (Ar-C) and 172.3 (C=O).

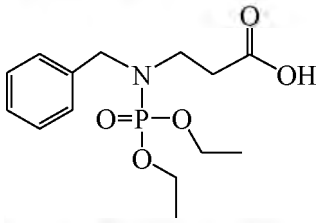
Diethyl *N*-[2-(methoxycarbonyl)ethyl]-*N*-(3-nitrobenzyl)phosphoramidate 160g



The general procedure for the synthesis of diethyl *N*-benzyl-*N*-[2-(methoxycarbonyl)ethyl]-phosphoramidate **160a** was employed, using diethyl *N*-(3-nitrobenzyl)phosphoramidate **159g** (3.4 g, 12 mmol), anhydrous K_2CO_3 (3.3 g, 24 mmol), tetrabutylammonium bromide (0.38 g, 1.2 mmol) and methyl acrylate (1.3 mL, 14 mmol) in toluene (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl* N-*[2-(methoxycarbonyl)ethyl]*-N-*(3-nitrobenzyl)phosphoramidate* **160g** as a yellow oil (3.7 g, 83%) as a yellow oil [HRMS: *m/z* calculated for $C_{15}H_{24}N_2O_7P$ (MH^+) 375.1321. Found 375.1328]; ν_{max}/cm^{-1} 1734 (C=O) and 1244 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.25 (6H, t, J = 7.2 Hz, 2 × CH_3), 2.45 (2H, t, J = 7.5 Hz, CH_2CO), 3.15 (2H, m, NCH_2), 3.54 (3H, s, OCH_3), 4.00 (4H, m, 2 × OCH_2CH_3), 4.26 (2H, d, J_{P-H} = 10.5 Hz, NCH_2Ar), 7.44 (1H, t, J = 7.8 Hz, Ar-H), 7.61 (1H, d, J = 6.6 Hz, Ar-H), 8.03 (1H, d, J = 6.3 Hz, Ar-H) and 8.16 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.0 (d, J_{P-C} = 7.1 Hz, 2 × CH_3), 33.3 (CH_2CO), 41.6 (d, J_{P-C} = 4.0 Hz, NCH_2), 49.4 (d, J_{P-C} = 5.0 Hz, NCH_2Ar), 51.7 (OCH_3), 62.7 (d, J_{P-C} = 5.3 Hz, 2 × OCH_2CH_3), 122.4, 122.7, 129.5, 134.1, 140.4 and 148.3 (Ar-C) and 171.9 (C=O).

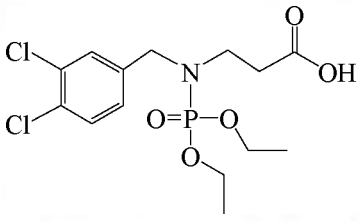
3.3.4. Hydrolysis of the methyl ester group in compounds 160a-g.

Diethyl *N*-benzyl-*N*-(2-carboxyethyl)phosphoramidate 165a



A solution of diethyl *N*-benzyl-*N*-[2-(methoxycarbonyl)ethyl]phosphoramidate **160a** (1.1 g, 3.3 mmol) in THF (10 mL) was diluted with water (50 mL), and then cooled to 0 °C in an ice bath. A solution of dil. aq. KOH in water (12 mL) was added dropwise followed by stirring at 0 °C for 1 h, and then at r.t. for 3 h. The reaction mixture was acidified to pH = 2 by the dropwise addition of 1M-HCl and the solution was then saturated with NaCl and extracted with EtOAc (2 × 20 mL). The organic solutions were combined, dried with anhydrous $MgSO_4$, filtered and evaporated *in vacuo* to afford *diethyl* N-*benzyl*-N-*(2-carboxyethyl)-phosphoramidate* **165a** as a yellow oil (0.90 g, 88%) [HRMS: *m/z* calculated for $C_{14}H_{23}NO_5P$ (MH^+) 316.1314. Found 316.1319]; ν_{max}/cm^{-1} 3530-3250 (br, OH), 1723 (C=O) and 1235 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.29 (6H, t, J = 7.2 Hz, 2 × CH_3), 2.48 (2H, m, CH_2CO), 3.20 (2H, m, NCH_2), 4.04 (4H, m, 2 × OCH_2CH_3), 4.19 (2H, d, J_{P-H} = 9.8 Hz, NCH_2Ph) and 7.25-7.30 (5H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.4 Hz, 2 × CH_3), 33.3 (CH_2CO), 41.2 (d, J_{P-C} = 4.6 Hz, NCH_2), 49.8 (d, J_{P-C} = 4.9 Hz, NCH_2Ph), 62.8 (d, J_{P-C} = 5.8 Hz, 2 × OCH_2CH_3), 127.6, 128.3, 128.6 and 137.6 (Ar-C) and 175.0 (C=O).

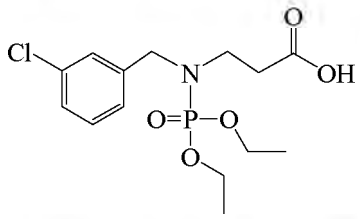
Diethyl *N*-(2-carboxyethyl)-*N*-(3,4-dichlorobenzyl)phosphoramidate 165b



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2-carboxyethyl)-phosphoramidate **165a** was followed, using diethyl *N*-(3,4-dichlorobenzyl)-*N*-[2-(methoxy-carbonyl)ethyl]phosphoramidate **160b** (1.4 g, 3.6 mmol) in THF (10 mL), H_2O (40 mL) and dil. aq. KOH (13 mL). Work-up afforded *diethyl* N-*(2-carboxyethyl)*-N-*(3,4-dichlorobenzyl)-phosphoramidate* **165b** as a yellow oil (1.3 g, 94%) [HRMS: *m/z* calculated for

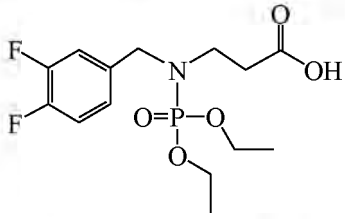
$C_{14}H_{21}{}^{35}Cl_2NO_5P$ (MH^+) 384.0534. Found 384.0531]; ν_{max}/cm^{-1} 3526-3216 (br, OH), 1724 (C=O) and 1233 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.29 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.48 (2H, t, J = 7.2 Hz, CH_2CO), 3.18 (2H, m, NCH_2), 4.03 (4H, m, 2 × OCH_2CH_3), 4.15 (2H, d, $J_{P\text{-}H}$ = 10.5 Hz, NCH_2Ar), 7.15 (1H, d, J = 8.2 Hz, Ar-H), 7.36 (1H, d, J = 8.2 Hz, Ar-H) and 7.42 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, $J_{P\text{-}C}$ = 7.2 Hz, 2 × CH_3), 33.5 (CH_2CO), 41.4 (d, $J_{P\text{-}C}$ = 4.4 Hz, NCH_2), 49.0 (d, $J_{P\text{-}C}$ = 5.1 Hz, NCH_2Ar), 63.1 (d, $J_{P\text{-}C}$ = 5.8 Hz, 2 × OCH_2CH_3), 127.6, 130.1, 130.5, 131.6, 132.7 and 138.3 (Ar-C) and 174.9 (C=O).

Diethyl *N*-(2-carboxyethyl)-*N*-(3-chlorobenzyl)phosphoramidate 165c



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2-carboxyethyl)-phosphoramidate **165a** was followed, using diethyl *N*-(3-chlorobenzyl)-*N*-[2-(methoxy-carbonyl)ethyl]phosphoramidate **160c** (1.4 g, 3.8 mmol) in THF (10 mL), H_2O (40 mL) and dil. aq. KOH (18 mL). Work-up afforded *diethyl* N-*(2-carboxyethyl)*-N-*(3-chlorobenzyl)-phosphoramidate* **165c** as a yellow oil (1.2 g, 89%) [HRMS: *m/z* calculated for $C_{14}H_{22}{}^{35}ClNO_5P$ (MH^+) 350.0924. Found 350.0912]; 3550-3230 (br, OH), 1726 (C=O) and 1230 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.32 (6H, t, J = 7.3 Hz, 2 × CH_3), 2.52 (2H, m, CH_2CO), 3.24 (2H, m, NCH_2), 4.06 (4H, m, 2 × OCH_2CH_3), 4.18 (2H, d, $J_{P\text{-}H}$ = 10.1 Hz, NCH_2Ar), 7.20 (1H, s, Ar-H), 7.25 (2H, m, Ar-H) and 7.33 (1H, d, J = 6.4 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 (d, $J_{P\text{-}C}$ = 7.2 Hz, 2 × CH_3), 33.5 (CH_2CO), 41.5 (d, $J_{P\text{-}C}$ = 4.5 Hz, NCH_2), 49.6 (d, $J_{P\text{-}C}$ = 5.0 Hz, NCH_2Ar), 63.0 (d, $J_{P\text{-}C}$ = 5.8 Hz, 2 × OCH_2CH_3), 125.4, 126.3, 127.8, 128.3, 129.9 and 140.0 (Ar-C) and 175.0 (C=O).

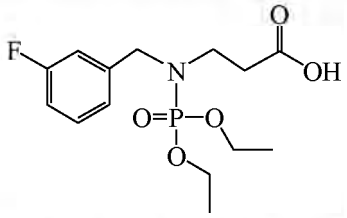
Diethyl *N*-(2-carboxyethyl)-*N*-(3,4-difluorobenzyl)phosphoramidate 165d



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2-carboxyethyl)-phosphoramidate **165a** was followed, using diethyl *N*-(3,4-difluorobenzyl)-*N*-[2-(methoxy-

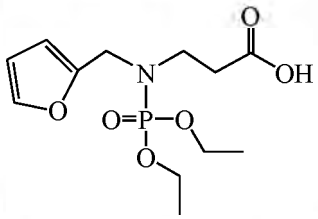
carbonyl)ethyl]phosphoramidate **160d** (0.90 g, 2.5 mmol) in THF (7.5 mL), H_2O (35 mL) and dil. aq. KOH (9 mL). Work-up afforded *diethyl* N-*(2-carboxyethyl)*-N-*(3,4-difluoro-benzyl)phosphoramidate* **165d** as a yellow oil (0.60 g, 71%) [HRMS: *m/z* calculated for $C_{14}H_{21}{}^{19}F_2NO_5P$ (MH^+) 352.1125. Found 352.1126]; ν_{max}/cm^{-1} 3545-3228 (br, OH), 1734 (C=O) and 1229 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.31 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.50 (2H, t, J = 7.5 Hz, CH_2CO), 3.20 (2H, m, NCH_2), 4.05 (4H, m, 2 × OCH_2CH_3), 4.16 (2H, d, J_{P-H} = 10.2 Hz, NCH_2Ar), 7.03 (1H, s, Ar-H), 7.09-7.18 (2H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.2 Hz, 2 × CH_3), 33.6 (CH_2CO), 41.4 (d, J_{P-C} = 4.2 Hz, NCH_2), 49.5 (d, J_{P-C} = 5.1 Hz, NCH_2Ar), 63.9 (d, J_{P-C} = 5.5 Hz, 2 × OCH_2CH_3), 116.3 (d, J_{F-C} = 17.7 Hz), 117.3 (d, J_{F-C} = 16.9 Hz), 123.1 (d, J_{F-C} = 6.0 Hz), 124.2 (m), 148.9 (dd, J_{F-C} = 13.5 and 246.0 Hz) and 151.2 (dd, J_{F-C} = 14.0 and 245.2 Hz) (Ar-C) and 174.9 (C=O).

Diethyl *N*-(2-carboxyethyl)-*N*-(3-fluorobenzyl)phosphoramidate 165e



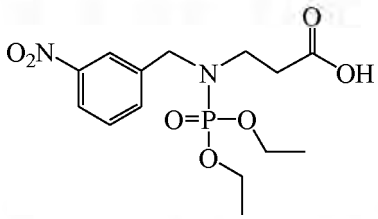
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2-carboxyethyl)-phosphoramidate **165a** was followed, using diethyl *N*-(3-fluorobenzyl)-*N*-[2-(methoxy-carbonyl)ethyl]phosphoramidate **160e** (1.2 g, 3.4 mmol) in THF (10 mL), H_2O (50 mL) and dil. aq. KOH (13 mL). Work-up afforded *diethyl* N-*(2-carboxyethyl)*-N-*(3-fluorobenzyl)-phosphoramidate* **165e** as a yellow oil (0.68 g, 70%) [HRMS: *m/z* calculated for $C_{14}H_{22}{}^{19}FNO_5P$ (MH^+) 334.1220. Found 334.1216]; ν_{max}/cm^{-1} 3535-3219 (br, OH), 1724 (C=O) and 1232 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.31 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.50 (2H, t, J = 7.5 Hz, CH_2CO), 3.22 (2H, m, NCH_2), 4.07 (4H, m, 2 × OCH_2CH_3), 4.20 (2H, d, J_{P-H} = 10.1 Hz, NCH_2Ar), 6.95 (1H, t, J = 7.5 Hz, Ar-H), 7.06 (2H, m, Ar-H) and 7.28 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 33.5 (CH_2CO), 41.5 (d, J_{P-C} = 4.2 Hz, NCH_2), 49.6 (d, J_{P-C} = 5.1 Hz, NCH_2Ar), 63.2 (d, J_{P-C} = 5.5 Hz, 2 × OCH_2CH_3), 114.2 (d, J_{F-C} = 21.7 Hz), 115.1 (d, J_{F-C} = 21.7 Hz), 123.9 (d, J_{F-C} = 2.7 Hz), 130.1 (t, J_{F-C} = 8.2 Hz), 142.4 and 163.1 (d, J_{F-C} = 239.0 Hz) (Ar-C) and 174.8 (C=O).

Diethyl *N*-(2-carboxyethyl)-*N*-furfurylphosphoramidate 165f



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2-carboxyethyl)-phosphoramidate **165a** was followed, using diethyl *N*-furfuryl-*N*-[2-(methoxycarbonyl)-ethyl]phosphoramidate **160f** (1.2 g, 3.8 mmol) in THF (12 mL), H_2O (45 mL) and dil. aq. KOH (14 mL). Work-up afforded *diethyl* N-*(2-carboxyethyl)*-N-*furfurylphosphoramidate* **165f** as a brown oil (0.98 g, 85%) [HRMS: *m/z* calculated for $C_{12}H_{21}NO_6P$ (MH^+) 306.1106. Found 306.1110]; ν_{max}/cm^{-1} 3561-3236 (br, OH), 1730 (C=O) and 1230 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.29 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.50 (2H, m, CH_2CO), 3.29 (2H, m, NCH_2), 4.02 (4H, m, 2 × OCH_2CH_3), 4.16 (2H, d, $J_{P\text{-}H}$ = 9.7 Hz, NCH_2Ar), 6.26 (1H, d, J = 3.0 Hz, Ar-H), 6.30 (1H, m, Ar-H) and 7.35 (1H, d, J = 1.0 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, $J_{P\text{-}C}$ = 7.4 Hz, 2 × CH_3), 33.7 (CH_2CO), 41.9 (d, $J_{P\text{-}C}$ = 4.8 Hz, NCH_2), 43.0 (d, $J_{P\text{-}C}$ = 5.6 Hz, NCH_2Ar), 62.8 (d, $J_{P\text{-}C}$ = 5.5 Hz, 2 × OCH_2CH_3), 109.0, 110.5, 142.5 and 151.4 (Ar-C) and 175.3 (C=O).

Diethyl *N*-(2-carboxyethyl)-*N*-(3-nitrobenzyl)phosphoramidate 165g



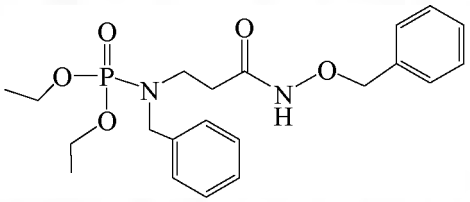
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2-carboxyethyl)-phosphoramidate **165a** was followed, using diethyl *N*-[2-(methoxycarbonyl)ethyl]-*N*-(3-nitrobenzyl)phosphoramidate **160g** (3.4 g, 9.2 mmol) in THF (20 mL), H_2O (100 mL) and dil. aq. KOH (33 mL). Work-up afforded *diethyl* N-*(2-carboxyethyl)*-N-*(3-nitrobenzyl)-phosphoramidate* **165g** as a yellow oil (3.0 g, 92%) [HRMS: *m/z* calculated for $C_{14}H_{22}N_2O_7P$ (MH^+) 361.1165. Found 361.1163]; ν_{max}/cm^{-1} 3535-3218 (br, OH), 1734 (C=O) and 1235 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.29 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.50 (2H, m, CH_2CO), 3.19 (2H, m, NCH_2), 4.07 (4H, m, 2 × OCH_2CH_3), 4.31 (2H, d, $J_{P\text{-}H}$ = 10.7 Hz, NCH_2Ar), 7.47 (1H, t, J = 7.9 Hz, Ar-H), 7.65 (1H, d, J = 7.6 Hz, Ar-H), 8.09 (1H, d, J = 7.0 Hz, Ar-H)

and 8.20 (1H, s, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.2 (d, $J_{P\text{-}C}$ = 7.1 Hz, 2 × CH_3), 33.5 (CH_2CO), 41.7 (d, $J_{P\text{-}C}$ = 4.3 Hz, NCH_2), 49.6 (d, $J_{P\text{-}C}$ = 5.1 Hz, NCH_2Ar), 63.2 (d, $J_{P\text{-}C}$ = 5.9 Hz, 2 × OCH_2CH_3), 122.6, 123.0, 129.6, 134.2, 140.4 and 148.5 (Ar-C) and 174.8 (C=O).

3.3.5. Conversion of the carboxylic acids 165 to the hydroxamic acid derivatives 166 and 167

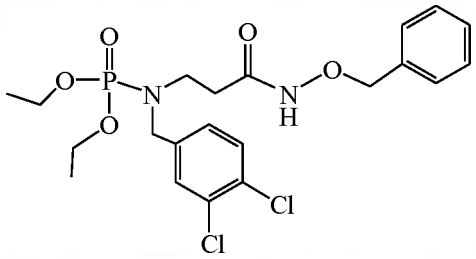
3.3.5.1. Reaction of carboxylic acids 165 *with* O*-benzylhydroxylamine hydrochloride*

Diethyl *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)ethyl]phosphoramidate 166a



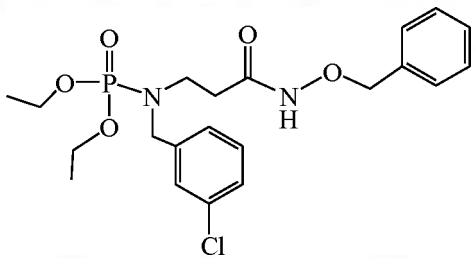
To a solution of diethyl *N*-benzyl-*N*-(2-carboxyethyl)phosphoramidate **165a** (0.83 g, 2.6 mmol) in dry DCM (10 mL) was added CDI (0.47 g, 2.9 mmol) in portions and the solution was stirred at r.t. for 1 h. *O*-Benzylhydroxylamine hydrochloride (0.42 g, 2.6 mmol) was added at once and the resulting mixture was stirred for 48 h. The solvent was evaporated *in vacuo* and the residue was dissolved in EtOAc. The organic layer was sequentially washed with 10% citric acid and saturated $NaHCO_3$, dried with anhydrous $MgSO_4$ and filtered and the solvent was evaporated *in vacuo*. The crude product was flash chromatographed [on silica gel; elution with EtOAc-hexane (6:4)] to afford *diethyl* N-*benzyl*-N-*[2-(*N*-benzyloxy-carbamoyl)ethyl]phosphoramidate* **166a** as a pale-yellow oil (0.86 g, 78%) [HRMS: *m/z* calculated for $C_{21}H_{30}N_2O_5P$ (MH^+) 421.1892. Found 421.1904]; ν_{max}/cm^{-1} 1667 (C=O) and 1227 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.29 (6H, t, J = 7.3 Hz, 2 × CH_3), 2.28 (2H, m, CH_2CO), 3.22 (2H, m, NCH_2CH_2), 4.02 (4H, m, 2 × OCH_2CH_3), 4.13 (2H, d, $J_{P\text{-}H}$ = 9.7 Hz, NCH_2Ph), 4.88 (2H, s, OCH_2Ph) and 7.31-7.37 (10H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 (d, $J_{P\text{-}C}$ = 7.1 Hz, 2 × CH_3), 33.5 (CH_2CO), 42.3 (NCH_2CH_2), 50.2 (d, $J_{P\text{-}C}$= 3.2 Hz, NCH_2Ph), 62.9 (d, $J_{P\text{-}C}$ = 5.3 Hz, 2 × OCH_2CH_3), 78.1 (OCH_2Ph), 127.4, 127.7, 128.4, 128.6, 129.1, 135.7, 137.5 and 139.7 (Ar-C) and 169.0 (C=O).

Diethyl *N*-[2-(*N*-benzyloxycarbamoyl)ethyl]-*N*-(3,4-dichlorobenzyl)phosphoramidate 166b



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-benzyloxy-carbamoyl)ethyl]phosphoramidate **166a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3,4-dichlorobenzyl)phosphoramidate **165b** (1.2 g, 3.2 mmol), CDI (0.57 g, 3.5 mmol) and *O*-benzylhydroxylamine hydrochloride (0.51 g, 3.2 mmol) in dry DCM (10 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl* N-*[2-(*N-*benzyloxycarbamoyl)ethyl]*-N-*(3,4-dichlorobenzyl)phosphoramidate* **166b** as a yellow oil (1.0 g, 66%) [HRMS: *m/z* calculated for $C_{21}H_{28}{}^{35}Cl_2N_2O_5P$ (MH^+) 489.1113. Found 489.1107]; ν_{max}/cm^{-1} 1667 (C=O) and 1229 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.27 (6H, t, J = 7.2 Hz, 2 × CH_3), 2.26 (2H, m, CH_2CO), 3.13 (2H, m, NCH_2CH_2), 3.97 (4H, m, 2 × OCH_2CH_3), 4.05 (2H, d, J_{P-H} = 10.5 Hz, NCH_2Ar), 4.82 (2H, s, OCH_2Ph), 7.13 (1H, d, J = 7.4 Hz, Ar-H), 7.26-7.31 (6H, overlapping m, Ar-H) and 7.41 (1H, s, Ar-H) and 10.43 (1H, br s, NH); δ_C/ppm (150 MHz; $CDCl_3$) 16.0 (d, J_{P-C} = 7.1 Hz, 2 × CH_3), 32.9 (CH_2CO), 42.0 (NCH_2CH_2), 48.8 (NCH_2Ar), 62.7 (d, J_{P-C} = 5.3 Hz, 2 × OCH_2CH_3) 77.8 (OCH_2Ph), 127.7, 128.4, 129.0, 129.2, 130.0, 131.3, 131.4, 132.3, 135.4 and 138.0 (Ar-C) and 168.6 (C=O).

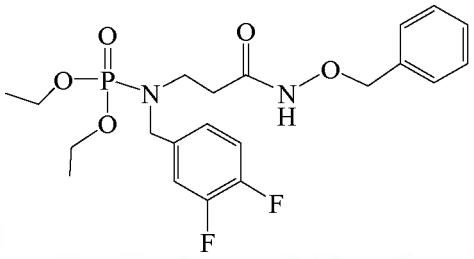
Diethyl *N*-[2-(*N*-benzyloxycarbamoyl)ethyl]-*N*-(3-chlorobenzyl)phosphoramidate 166c



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-benzyloxy-carbamoyl)ethyl]phosphoramidate **166a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3-chlorobenzyl)phosphoramidate **165c** (0.56 g, 1.6 mmol), CDI (0.29 g, 1.8 mmol) and *O*-benzylhydroxylamine hydrochloride (0.25 g, 1.6 mmol) in dry DCM (5 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl* N-*[2-(*N-*benzyloxycarbamoyl)ethyl]*-N-*(3-chlorobenzyl)phosphoramidate* **166c** as a yellow oil

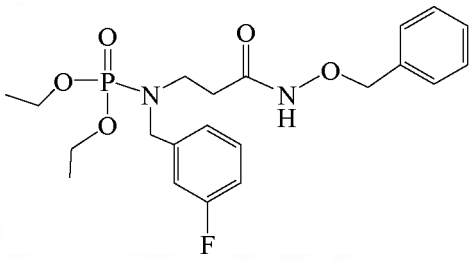
(0.60 g, 83%) [HRMS: *m/z* calculated for $C_{21}H_{29}{}^{35}ClN_2O_5P$ (MH^+) 455.1503. Found 455.1498]; ν_{max}/cm^{-1} 1658 (C=O) and 1214 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.31 (6H, t, J = 7.2 Hz, 2 × CH_3), 2.32 (2H, m, CH_2CO), 3.24 (2H, m, NCH_2CH_2), 4.02-4.13 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar), 4.87 (2H, s, OCH_2Ph), 7.18-7.37 (9H, overlapping m, Ar-H) and 9.10 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.2 Hz, 2 × CH_3), 33.2 (CH_2CO), 44.7 (NCH_2CH_2), 49.6 (NCH_2Ar), 62.5 (d, J_{P-C} = 5.3 Hz, 2 × OCH_2CH_3), 78.0 (OCH_2Ph), 125.5, 126.5, 127.5, 127.7, 128.3, 129.1, 129.8, 134.4, 135.6 and 139.8 (Ar-C) and 168.7 (C=O).

Diethyl *N*-[2-(*N*-benzyloxycarbamoyl)ethyl]-*N*-(3,4-difluorobenzyl)phosphoramidate 166d



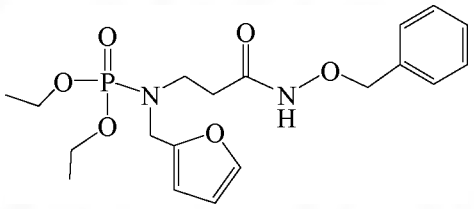
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-benzyloxy-carbamoyl)ethyl]phosphoramidate **166a** was followed, diethyl *N*-(2-carboxyethyl)-*N*-(3,4-difluorobenzyl)phosphoramidate **165d** (0.58 g, 1.7 mmol), CDI (0.30 g, 1.8 mmol) and *O*-benzylhydroxylamine hydrochloride (0.26 g, 1.7 mmol) in dry DCM (5 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl* N-*[2-(*N-*benzyloxycarbamoyl)ethyl]*-N-*(3,4-difluorobenzyl)phosphoramidate* **166d** as a yellow oil (0.53 g, 70%) [HRMS: *m/z* calculated for $C_{21}H_{28}{}^{19}F_2N_2O_5P$ (MH^+) 457.1704. Found 457.1702]; ν_{max}/cm^{-1} 1672 (C=O) and 1233 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.27 (6H, t, J = 7.3 Hz, 2 × CH_3), 2.28 (2H, m, CH_2CO), 3.18 (2H, m, NCH_2CH_2), 3.96-4.08 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar), 4.87 (2H, s, OCH_2Ph) and 7.02-7.36 (8H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 33.4 (CH_2CO), 44.4 (NCH_2CH_2), 49.4 (NCH_2Ar), 62.6 (d, J_{P-C} = 5.4 Hz, 2 × OCH_2CH_3), 78.1 (OCH_2Ph), 116.4 (d, J_{F-C} = 17.5 Hz), 117.3 (d, J_{F-C} = 17.3 Hz), 123.2, 124.6, 128.6, 128.7, 129.1, 137.0 (d, J_{F-C} = 211.5 Hz), 149.4 (dd, J_{F-C} = 13.6 and 244.5 Hz) and 150.9 (ddd, J_{F-C} = 6.8, 9.1 and 247.5 Hz) (Ar-C) and 168.9 (C=O).

Diethyl *N*-[2-(*N*-benzyloxycarbamoyl)ethyl]-*N*-(3-fluorobenzyl)phosphoramidate 166e



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)ethyl]phosphoramidate **166a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3-fluorobenzyl)phosphoramidate **165e** (0.39 g, 1.2 mmol), CDI (0.21 g, 1.3 mmol) and *O*-benzylhydroxylamine hydrochloride (0.19 g, 1.2 mmol) in dry DCM (10 mL). Work-up and chromatography [PLC on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl* N-*[2-(*N-*benzyloxycarbamoyl)ethyl]*-N-*(3-fluorobenzyl)phosphoramidate* **166e** as a yellow oil (0.37 g, 72%) [HRMS: *m/z* calculated for $C_{21}H_{29}{}^{19}FN_2O_5P$ (MH^+) 439.1798. Found 439.1791]; ν_{max}/cm^{-1} 1674 (C=O) and 1231 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.33 (6H, t, *J* = 7.3 Hz, 2 × CH_3), 2.33 (2H, m, CH_2CO), 3.26 (2H, m, NCH_2CH_2), 4.03-4.16 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar), 4.92 (2H, s, OCH_2Ph), 6.98 (2H, m, Ar-H), 7.10 (3H, overlapping m, Ar-H) and 7.29-7.41 (4H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 33.6 (CH_2CO), 45.0 (NCH_2CH_2), 49.9 (NCH_2Ar), 62.7 (d, J_{P-C} = 5.4 Hz, 2 × OCH_2CH_3), 78.2 (OCH_2Ph), 114.4 (d, J_{F-C} = 21.9 Hz), 115.1 (d, J_{F-C} = 21.6 Hz), 122.9 (d, J_{F-C} = 2.4 Hz), 124.0, 128.7, 129.2, 130.2, 135.6, 142.5 and 163.1 (d, J_{F-C} = 246.5 Hz) (Ar-C) and 174.8 (C=O).

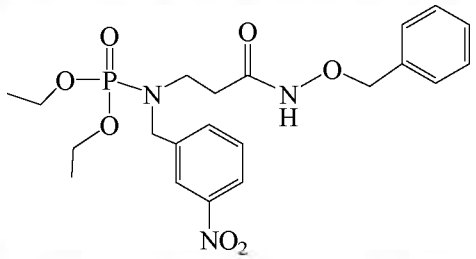
Diethyl *N*-[2-(*N*-benzyloxycarbamoyl)ethyl]-*N*-furfurylphosphoramidate 166f



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)ethyl]phosphoramidate **166a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-furfurylphosphoramidate **165f** (0.50 g, 1.6 mmol), CDI (0.29 g, 1.8 mmol) and *O*-benzylhydroxylamine hydrochloride (0.26 g, 1.6 mmol) in dry DCM (5 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl* N-*[2-(*N-*benzyloxycarbamoyl)ethyl]*-N-*furfurylphosphoramidate* **166f** as a yellow oil (0.47 g,

70%) [HRMS: *m/z* calculated for $C_{19}H_{28}N_2O_6P$ (MH^+) 411.1685. Found 411.1674]; ν_{max}/cm^{-1} 1667 (C=O) and 1226 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.27 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.27 (2H, m, CH_2CO), 3.28 (2H, m, NCH_2CH_2), 3.98 (4H, m, 2 × OCH_2CH_3), 4.08 (2H, d, J_{P-H} = 9.5 Hz, NCH_2Ar), 4.88 (2H, s, OCH_2Ph), 6.26 (1H, d, J = 2.3 Hz, Ar-H), 6.30 (1H, d, J = 2.4 Hz, Ar-H), 7.33-7.39 (6H, overlapping m, Ar-H) and 9.67 (1H, br s, NH); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.3 Hz, 2 × CH_3), 33.7 (CH_2CO), 42.8 (NCH_2CH_2), 43.2 (NCH_2Ar), 62.7 (d, J_{P-C} = 5.5 Hz, 2 × OCH_2CH_3), 78.1 (OCH_2Ph), 109.1, 110.5, 127.6, 128.7, 129.2, 135.7, 142.5 and 151.1 (Ar-C) and 168.9 (C=O).

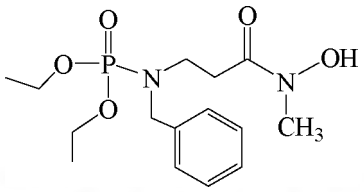
Diethyl *N*-[2-(*N*-benzyloxycarbamoyl)ethyl]-*N*-(3-nitrobenzyl)phosphoramidate 166g



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)ethyl]phosphoramidate **166a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3-nitrobenzyl)phosphoramidate **165g** (1.8 g, 4.1 mmol), CDI (0.73 g, 4.9 mmol) and *O*-benzylhydroxylamine hydrochloride (0.65 g, 4.1 mmol) in dry DCM (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl N-[2-(N-benzyloxycarbamoyl)ethyl]-N-(3-nitrobenzyl)phosphoramidate* **166g** as a yellow oil (1.9 g, 78%) [HRMS: *m/z* calculated for $C_{21}H_{29}N_3O_7P$ (MH^+) 466.1743. Found 466.1754]; ν_{max}/cm^{-1} 1668 (C=O) and 1229 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 (6H, t, J = 7.3 Hz, 2 × CH_3), 2.33 (2H, m, CH_2CO), 3.21 (2H, m, NCH_2CH_2), 4.03 (4H, m, 2 × OCH_2CH_3), 4.25 (2H, d, J_{P-H} = 10.2 Hz, NCH_2Ar), 4.87 (2H, s, OCH_2Ph), 7.31-7.36 (5H, overlapping m, Ar-H), 7.49 (1H, m, Ar-H), 7.66 (1H, m, Ar-H) and 7.41 (2H, m, Ar-H), 10.0 (1H, s, NH); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 33.3 (CH_2CO), 42.4 (NCH_2CH_2), 49.5 (NCH_2Ar), 63.1 (d, J_{P-C} = 5.7 Hz, 2 × OCH_2CH_3), 78.0 (OCH_2Ph), 122.2, 122.6, 123.0, 128.5, 129.0, 129.6, 134.4, 135.5, 140.1 and 148.4 (Ar-C) and 168.7 (C=O).

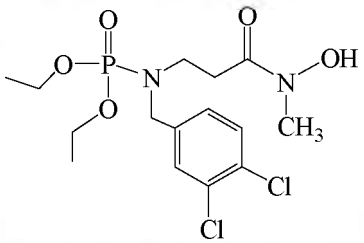
3.3.5.2. Reaction of carboxylic acids 165 *with* N*-methylhydroxylamine hydrochloride*

Diethyl *N*-benzyl-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]phosphoramidate 167a



N-Methylhydroxylamine hydrochloride (0.017 g, 0.20 mmol) was added to a mixture of diethyl *N*-benzyl-*N*-(2-carboxyethyl)phosphoramidate **165a** (0.13 g, 0.20 mmol) and CDI (0.039 g, 0.24 mmol) in dry DCM (5 mL) and the mixture was left to stir at r.t. for 7 days. The solvent was evaporated *in vacuo* and the residue dissolved in EtOAc. The organic solution was sequentially washed with 10% citric acid and saturated aq. $NaHCO_3$, dried with anhydrous $MgSO_4$ and filtered. The solvent was evaporated *in vacuo* and the crude product was flash chromatographed [on silica gel; elution with EtOAc-hexane (6:4)] to afford *diethyl* N-*benzyl*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)ethyl]phosphoramidate* **167a** as a pale-yellow oil (0.093 g, 67%) [HRMS: *m/z* calculated for $C_{15}H_{26}N_2O_5P$ (MH^+) 345.1579. Found 345.1577]; ν_{max}/cm^{-1} 3325 (OH), 1634 (C=O) and 1227 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.26 (6H, m, 2 × CH_3), 2.28 (2H, m, CH_2CO), 3.17-3.24 (5H, overlapping m, NCH_3 and NCH_2CH_2), 3.98 (4H, m, 2 × OCH_2CH_3), 4.13 (2H, m, NCH_2Ph) and 7.20-7.28 (5H, overlapping m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.2 Hz, 2 × CH_3), 29.8 (CH_2CO), 33.7 (NCH_3), 36.1 (d, J_{P-C} = 4.8 Hz, NCH_2CH_2), 41.5 (d, J_{P-C} = 5.1 Hz, NCH_2Ph), 62.9 (d, J_{P-C} = 6.0 Hz, 2 × OCH_2CH_3), 127.4, 128.4, 128.7 and 137.8 (Ar-C) and 174.4 (C=O).

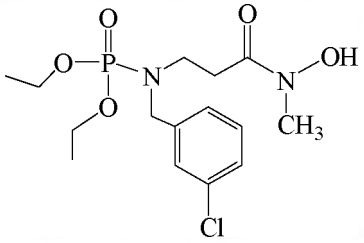
Diethyl *N*-(3,4-dichlorobenzyl)-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]-phosphoramidate 167b



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-hydroxy-*N*-methyl-carbamoyl)ethyl]phosphoramidate **167a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3,4-dichlorobenzyl)phosphoramidate **165b** (0.15 g, 0.38 mmol), CDI (0.072 g, 0.48 mmol) and *N*-methylhydroxylamine hydrochloride (0.032 g, 0.38 mmol) in dry DCM (5 mL).

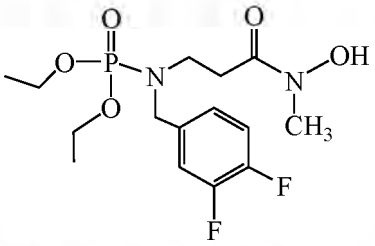
Work-up afforded *diethyl* N-*(3,4-dichlorobenzyl)*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)-ethyl]phosphoramidate* **167b** as a brown oil (0.11 g, 70%) [HRMS: *m/z* calculated for $C_{15}H_{24}{}^{35}Cl_2N_2O_5P$ (MH^+) 413.0799. Found 413.0797]; ν_{max}/cm^{-1} 3334 (OH), 1638 (C=O) and 1229 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.27 (6H, m, 2 × CH_3), 2.66 (2H, m, CH_2CO), 3.17-3.27 (5H, overlapping m, NCH_3 and NCH_2CH_2), 3.95-4.10 (6H, m, 2 × OCH_2CH_3 and NCH_2Ar) and 7.10 (1H, d, J = 10.2 Hz, Ar-H) and 7.37 (2H, m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 6.9 Hz, 2 × CH_3), 29.8 (CH_2CO), 33.6 (NCH_3), 36.1 (d, J_{P-C} = 4.6 Hz, NCH_2CH_2), 41.1 (d, J_{P-C} = 5.3 Hz, NCH_2Ar), 62.9 (d, J_{P-C} = 5.9 Hz, 2 × OCH_2CH_3), 127.6, 127.9, 130.2, 130.6, 131.7 and 137.5 (Ar-C) and 171.2 (C=O).

Diethyl *N*-(3-chlorobenzyl)-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]-phosphoramidate 167c



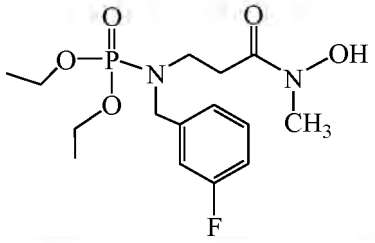
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-hydroxy-*N*-methyl-carbamoyl)ethyl]phosphoramidate **167a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3-chlorobenzyl)phosphoramidate **165c** (0.12 g, 0.33 mmol), CDI (0.060 g, 0.38 mmol) and *N*-methylhydroxylamine hydrochloride (0.029 g, 0.33 mmol) in dry DCM (5 mL). Work-up afforded *diethyl* N-*(3-chlorobenzyl)*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)ethyl]phosphoramidate* **167c** as a yellow oil (0.090 g, 70%) [HRMS: *m/z* calculated for $C_{15}H_{25}{}^{35}ClN_2O_5P$ (MH^+) 379.1190. Found 379.1189]; ν_{max}/cm^{-1} 3329 (OH), 1642 (C=O) and 1231 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.27 (6H, t, J = 7.0 Hz, 2 × CH_3), 2.65 (2H, m, CH_2CO), 3.17-3.28 (5H, overlapping m, NCH_3 and NCH_2CH_2), 3.95 (4H, m, 2 × OCH_2CH_3), 4.10 (2H, d, J_{P-H} = 10.3 Hz, NCH_2Ar), 7.15-7.20 (3H, overlapping m, Ar-H) and 7.28 (1H, d, J = 7.9 Hz, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 6.9 Hz, 2 × CH_3), 29.8 (CH_2CO), 33.6 (NCH_3), 36.1 (d, J_{P-C} = 4.9 Hz, NCH_2CH_2), 41.1 (d, J_{P-C}= 5.0 Hz, NCH_2Ar), 62.7 (d, J_{P-C} = 5.4 Hz, 2 × OCH_2CH_3), 126.7, 127.6, 128.0, 128.6, 129.9 and 137.5 (Ar-C) and 171.2 (C=O).

Diethyl *N*-(3,4-difluorobenzyl)-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]-phosphoramidate 167d



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-hydroxy-*N*-methyl-carbamoyl)ethyl]phosphoramidate **167a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3,4-difluorobenzyl)phosphoramidate **165d** (0.14 g, 0.39 mmol), CDI (0.070 g, 0.42 mmol) and *N*-methylhydroxylamine hydrochloride (0.032 g, 0.39 mmol) in dry DCM (5 mL). Work-up afforded *diethyl* N-*(3,4-difluorobenzyl)*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)-ethyl]phosphoramidate* **167d** as a pale-yellow oil (0.090 g, 55%) [HRMS: *m/z* calculated for $C_{15}H_{24}{}^{19}F_2N_2O_5P$ (MH^+) 381.1391. Found 381.1397]; ν_{max}/cm^{-1} 3351 (OH), 1626 (C=O) and 1227 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.29 (6H, m, 2 × CH_3), 2.69 (2H, m, CH_2CO), 3.22-3.31 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.07 (4H, m, 2 × OCH_2CH_3), 4.10 (2H, d, J_{P-H} = 10.3 Hz, NCH_2Ar) and 7.03-7.18 (3H, overlapping m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 29.8 (CH_2CO), 33.6 (NCH_3), 36.1 (d, J_{P-C} = 4.9 Hz, NCH_2CH_2), 41.1 (d, J_{P-C} = 5.2 Hz, NCH_2Ar), 62.7 (d, J_{P-C} = 5.4 Hz, 2 × OCH_2CH_3), 116.4 (d, J_{F-C} = 17.7 Hz), 117.3 (d, J_{F-C} = 16.9 Hz), 123.2, 136.9 (m), 148.6 (dd, J_{F-C} = 13.4 and 243.0 Hz) and 151.9 (d, J_{F-C} = 13.1 and 239.2 Hz) (Ar-C) and 168.9 (C=O).

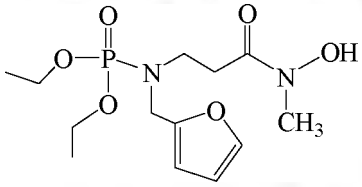
Diethyl *N*-(3-fluorobenzyl)-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]-phosphoramidate 167e



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-hydroxy-*N*-methyl-carbamoyl)ethyl]phosphoramidate **167a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3-fluorobenzyl)phosphoramidate **165e** (0.13 g, 0.39 mmol), CDI (0.075 g, 0.46 mmol) and *N*-methylhydroxylamine hydrochloride (0.039 g, 0.39 mmol) in dry DCM (5 mL). Work-up afforded *diethyl* N-*(3-fluorobenzyl)*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)ethyl]phosphoramidate* **167e** as a pale-yellow oil (0.10 g, 71%) [HRMS: *m/z* calculated for $C_{15}H_{25}{}^{19}FN_2O_5P$

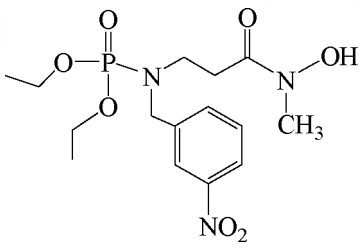
(MH^+) 363.1485. Found 363.1479]; ν_{max}/cm^{-1} 3334 (OH), 1638 (C=O) and 1229 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.29 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.70 (2H, t, J = 7.1 Hz, CH_2CO), 3.22-3.31 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.05 (4H, m, 2 × OCH_2CH_3), 4.18 (2H, d, J_{P-H} = 10.2 Hz, NCH_2Ar), 6.95 (2H, m, Ar-H) and 7.08 (2H, m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 33.7 (CH_2CO), 36.1 (NCH_3), 40.9 (d, J_{P-C} = 4.9 Hz, NCH_2CH_2), 44.9 (d, J_{P-C} = 5.0 Hz, NCH_2Ar), 62.7 (d, J_{P-C} = 5.4 Hz, 2 × OCH_2CH_3), 114.3 (d, J_{F-C} = 19.0 Hz), 114.9 (d, J_{F-C} = 18.7 Hz), 122.9 (d, J_{F-C} = 2.6 Hz), 130.2 (d, J_{F-C} = 8.0 Hz), 142.5 and 163.1 (d, J_{F-C} = 246.2 Hz) (Ar-C) and 171.2 (C=O).

Diethyl *N*-furfuryl-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]phosphoramidate 167f



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]phosphoramidate **167a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-furfurylphosphoramidate **165f** (0.13 g, 0.41 mmol), CDI (0.080 g, 0.49 mmol) and *N*-methylhydroxylamine hydrochloride (0.034 g, 0.41 mmol) in dry DCM (5 mL). Work-up afforded *diethyl* N-*furfuryl*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)ethyl]phosphoramidate* **167f** as a yellow oil (0.11 g, 82%) [HRMS: *m/z* calculated for $C_{13}H_{24}N_2O_6P$ (MH^+) 335.1372. Found 335.1367]; ν_{max}/cm^{-1} 3334 (OH), 1632 (C=O) and 1232 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.29 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.73 (2H, t, J = 7.1 Hz, CH_2CO), 3.21-3.37 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.02-4.15 (6H, overlapping m, 2 × OCH_2CH_3 and NCH_2Ar), 6.30 (2H, m, Ar-H) and 7.36 (1H, s, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.3 Hz, 2 × CH_3), 29.8 (CH_2CO), 33.4 (NCH_3), 36.1 (d, J_{P-C} = 5.0 Hz, NCH_2CH_2), 41.8 (d, J_{P-C} = 5.2 Hz, NCH_2Ar), 62.8 (d, J_{P-C} = 5.6 Hz, 2 × OCH_2CH_3), 109.0, 109.3, 110.5 and 142.6 (Ar-C) and 171.5 (C=O).

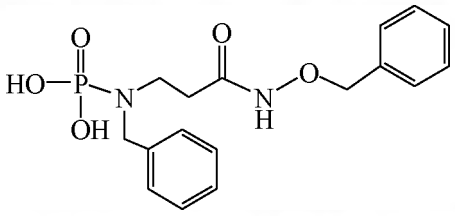
Diethyl *N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]-*N*-(3-nitrobenzyl)-phosphoramidate 167g



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[2-(*N*-hydroxy-*N*-methyl-carbamoyl)ethyl]phosphoramidate **167a** was followed, using diethyl *N*-(2-carboxyethyl)-*N*-(3-nitrobenzyl)phosphoramidate **165g** (0.16 g, 0.46 mmol), CDI (0.081 g, 0.50 mmol) and *N*-methylhydroxylamine hydrochloride (0.038 g, 0.46 mmol) in dry DCM (5 mL). Work-up afforded *diethyl* N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)ethyl]*-N-*(3-nitrobenzyl)phosphoramidate* **167g** as a pale-yellow oil (0.12 g, 66%) [HRMS: *m/z* calculated for $C_{15}H_{25}N_3O_7P$ (MH^+) 390.1430. Found 390.1439]; ν_{max}/cm^{-1} 3329 (OH), 1636 (C=O) and 1235 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.32 (6H, t, J = 7.2 Hz, 2 × CH_3), 2.50 (1H, br s, OH) 2.69 (2H, m, CH_2CO), 3.19-3.29 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.04 (4H, m, 2 × OCH_2CH_3), 4.28 (2H, d, J_{P-H} = 10.1 Hz, NCH_2Ar), 7.49 (1H, t, J = 7.9 Hz, Ar-H), 7.66 (1H, d, J = 7.6 Hz, Ar-H), 8.11 (1H, d, J = 8.1 Hz, Ar-H) and 8.23 (1H, s, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.1 Hz, 2 × CH_3), 33.0 (CH_2CO), 36.0 (NCH_3), 41.3 (d, J_{P-C} = 4.9 Hz, NCH_2CH_2), 48.7 (d, J_{P-C} = 5.1 Hz, NCH_2Ar), 63.3 (d, J_{P-C} = 5.5 Hz, 2 × OCH_2CH_3), 122.6, 123.1, 129.5, 134.4, 139.6 and 148.5 (Ar-C) and 171.2 (C=O).

3.3.6. Synthesis of the phosphoramidic acid derivatives 161 and 162

N-Benzyl-N-[2-(N-benzyloxycarbamoyl)ethyl]phosphoramidic acid 161a*

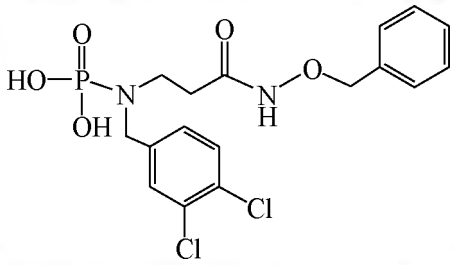


Bromotrimethylsilane (0.34 mL, 4.9 mmol) was added to a chilled stirred solution of diethyl *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)ethyl]phosphoramidate **166a** (0.40 g, 0.98 mmol) in dry DCM (5 mL) at 0 °C. After 1 h, the solution was allowed to warm to r.t and stirred for an additional 24 h. The solvent was removed under reduced pressure and the residue was dissolved in THF (3 mL) and H_2O (0.04 mL) and then stirred for 1 h. The resulting mixture was concentrated *in vacuo* and the residue was triturated with EtOAc at -10 °C to afford N-*benzyl*-N-*[2-(*N-*benzyloxycarbamoyl)ethyl]phosphoramidic acid* **161a** as a white solid (0.26 g, 75%), m.p. 122-124 °C; ν_{max}/cm^{-1} 1646 (C=O); δ_H/ppm (600 MHz; DMSO-d_6) 2.43 (2H, m, CH_2CO), 3.12 (2H, m, NCH_2CH_2), 4.15 (2H, s, NCH_2Ph), 4.78 (2H, s, OCH_2Ph), 7.37 (5H, m, Ar-H), 7.44 (3H, m, Ar-H), 7.49 (2H, d, J = 6.6 Hz, Ar-H); δ_C/ppm (150 MHz;

*Not possible to obtain satisfactory HRMS data

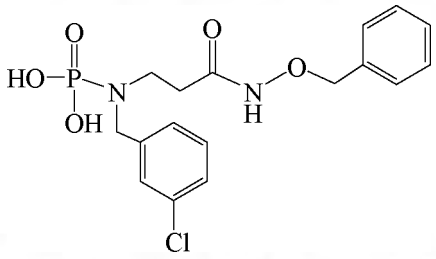
DMSO-d_6) 28.9 (CH_2CO), 42.7 (NCH_2CH_2), 50.4 (NCH_2Ph), 77.33 (OCH_2Ph), 128.7, 128.8, 129.1, 129.5, 129.6, 130.3, 132.0 and 136.1 (Ar-C) and 171.9 (C=O).

_N_-[2-(_N_-Benzyloxycarbamoyl)ethyl]-_N_-(3,4-dichlorobenzyl)phosphoramidic acid 161b*



The procedure described for the synthesis of _N_-benzyl-_N_-[2-(_N_-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl _N_-[2-(_N_-benzyloxy-carbamoyl)ethyl]-_N_-(3,4-dichlorobenzyl)phosphoramidate **166b** (0.50 g, 1.0 mmol), bromo-trimethylsilane (0.70 mL, 5.1 mmol) and dry DCM (5 mL). Work-up afforded N-*[2-(*N-*benzyloxycarbamoyl)ethyl]*-N-*(3,4-dichlorobenzyl)phosphoramidic acid* **161b** as an off-white solid (0.15 g, 76 %), m.p. 108-110 °C; ν_{max}/cm^{-1} 1661 (C=O); δ_H/ppm (600 MHz; DMSO-d_6) 2.16 (2H, m, CH_2CO), 2.84 (2H, t, J = 7.1 Hz, NCH_2CH_2), 3.89 (2H, s, NCH_2Ar), 4.50 (2H, s, OCH_2Ph), 7.06-7.09 (5H, overlapping m, Ar-H), 7.23 (1H, d, J = 8.1 Hz, Ar-H), 7.43 (1H, d, J = 8.2 Hz, Ar-H) and 7.54 (1H, s, Ar-H); δ_C/ppm (150 MHz; DMSO-d_6) 28.7 (CH_2CO), 42.7 (NCH_2CH_2), 48.9 (NCH_2Ar), 77.2 (OCH_2Ph), 128.6, 129.0, 130.7, 131.0, 131.1, 131.4, 132.0, 132.4, 133.0 and 136.0 (Ar-C) and 166.8 (C=O).

_N_-[2-(_N_-Benzyloxycarbamoyl)ethyl]-_N_-(3-chlorobenzyl)phosphoramidic acid 161c*

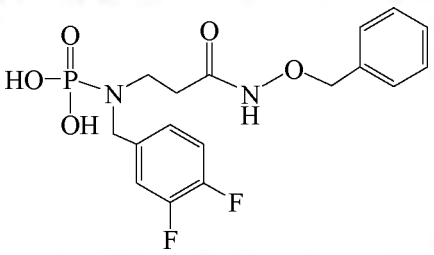


The procedure described for the synthesis of _N_-benzyl-_N_-[2-(_N_-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl _N_-[2-(_N_-benzyloxy-carbamoyl)ethyl]-_N_-(3-chlorobenzyl)phosphoramidate **166c** (0.57 g, 1.2 mmol), bromo-trimethylsilane (0.8 mL, 6.2 mmol) and dry DCM (5 mL). Work-up afforded N-*[2-(*N-*benzyloxycarbamoyl)ethyl]*-N-*(3-chlorobenzyl)phosphoramidic acid* **161c** as a white solid (0.40 g, 80%), m.p. 154-156 °C; ν_{max}/cm^{-1} 1659 (C=O); δ_H/ppm (300 MHz; DMSO-d_6) 2.43

*_Not possible to obtain satisfactory HRMS data_

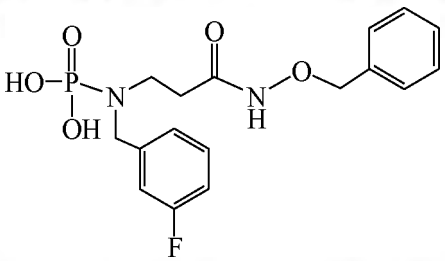
(2H, m, CH_2CO), 3.10 (2H, m, NCH_2CH_2), 4.16 (2H, s, NCH_2Ar), 4.77 (2H, s, OCH_2Ph), 7.34-7.55 (8H, overlapping m, Ar-H), 7.61 (1H, s, Ar-H) and 8.91 (2H, br s, 2 × OH); δ_C/ppm (75 MHz; DMSO-d_6) 28.2 (CH_2CO), 42.7 (NCH_2CH_2), 49.4 (NCH_2Ar), 77.0 (OCH_2Ph), 128.4, 128.8, 129.0, 129.9, 130.5, 130.6, 133.2, 134.2, 135.8 and 136.3 (Ar-C) and 166.3 (C=O).

_N_-[2-(_N_-Benzyloxycarbamoyl)ethyl]-_N_-(3,4-difluorobenzyl)phosphoramidic acid 161d*



The procedure described for the synthesis of _N_-benzyl-_N_-[2-(_N_-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl _N_-[2-(_N_-benzyloxy-carbamoyl)ethyl]-_N_-(3,4-difluorobenzyl)phosphoramidate **166d** (0.41 g, 0.90 mmol), bromo-trimethylsilane (0.60 mL, 4.5 mmol) and dry DCM (5 mL). Work-up afforded N-*[2-(N-benzyloxycarbamoyl)ethyl]*-N-*(3,4-difluorobenzyl)phosphoramidic acid* **161d** as an off-white solid (0.42 g, 79%), m.p. 178-180 °C; ν_{max}/cm^{-1} 1654 (C=O); δ_H/ppm (300 MHz; DMSO-d_6) 2.37 (2H, m, CH_2CO), 3.06 (2H, m, NCH_2CH_2), 3.99 (2H, s, NCH_2Ar), 4.13 (1H, s, NH), 4.74 (2H, s, OCH_2Ph), 7.32-7.62 (8H, overlapping m, Ar-H) and 8.18 (2H, br s, 2 × OH); δ_C/ppm (150 MHz; DMSO-d_6) 28.8 (CH_2CO), 41.7 (NCH_2CH_2), 49.3 (NCH_2Ar), 77.5 (OCH_2Ph), 118.2 (d, J_{F-C} = 18.0 Hz), 118.8 (d, J_{F-C} = 17.5 Hz), 119.9 (d, J_{F-C} = 12.1 Hz), 126.8, 128.0, 128.8, 129.3, 136.3 (d, J_{F-C} = 210.4 Hz), 149.4 (dd, J_{F-C} = 13.9 and 238.5 Hz) and 150.9 (d, J_{F-C} = 14.0 and 239.2 Hz) (Ar-C) and 166.6 (C=O).

_N_-[2-(_N_-Benzyloxycarbamoyl)ethyl]-_N_-(3-fluorobenzyl)phosphoramidic acid 161e*

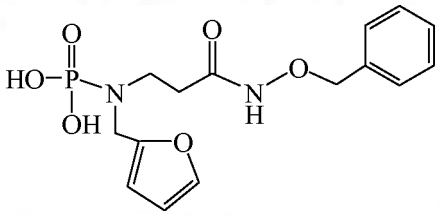


The procedure described for the synthesis of _N_-benzyl-_N_-[2-(_N_-benzyloxycarbamoyl)ethyl]-phosphoramidic acid **161a** was followed, using diethyl _N_-[2-(_N_-benzyloxycarbamoyl)ethyl]-

Not possible to obtain satisfactory HRMS data

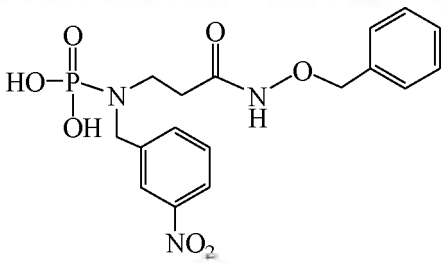
N-(3-fluorobenzyl)phosphoramidate **166e** (0.24 g, 0.54 mmol), bromotrimethylsilane (0.36 mL, 2.7 mmol) and dry DCM (5 mL). Work-up afforded N-*[2-(*N*-benzyloxycarbamoyl)-ethyl]*-N-*(3-fluorobenzyl)phosphoramidic acid* **161e** as an off-white solid (0.42 g, 79%), m.p. 150-152 °C; ν_{max}/cm^{-1} 1674 (C=O); δ_H/ppm (400 MHz; DMSO-d_6) 2.44 (2H, m, CH_2CO), 3.14 (2H, m, NCH_2CH_2), 4.07 (2H, s, NCH_2Ar), 4.20 (1H, s, NH), 4.80 (2H, s, OCH_2Ph), 7.27-7.49 (9H, overlapping m, Ar-H) and 8.22 (2H, br s, 2 × OH); δ_C/ppm (150 MHz; DMSO-d_6) 28.5 (CH_2CO), 41.8 (NCH_2CH_2), 49.5 (NCH_2Ar), 77.1 (OCH_2Ph), 115.5 (d, J_{F-C} = 21.9 Hz), 115.8 (d, J_{F-C} = 20.7 Hz), 125.1 (d, J_{F-C} = 2.6 Hz), 126.3, 128.5, 128.9, 130.9, 136.7 (d, J_{F-C} = 7.9 Hz), 140.9 and 162.1 (d, J_{F-C} = 244.4 Hz) (Ar-C) and 166.4 (C=O).

_N_-[2-(_N_-Benzyloxycarbamoyl)ethyl]-_N_-furfurylphosphoramidic acid 161f*



The procedure described for the synthesis of *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl *N*-[2-(*N*-benzyloxy-carbamoyl)ethyl]-*N*-furfurylphosphoramidate **166e** (0.24 g, 0.58 mmol), bromotrimethyl-silane (0.4 mL, 2.8 mmol) and dry DCM (3 mL). Work-up afforded N-*[2-(*N*-benzyloxy-carbamoyl)ethyl]*-N-*furfurylphosphoramidic acid* **161f** as a dark-brown oil (0.15 g, 74%); ν_{max}/cm^{-1} 1646 (C=O); δ_H/ppm (600 MHz; DMSO-d_6) 2.59 (2H, m, CH_2CO), 2.98 (2H, m, NCH_2CH_2), 4.25 (2H, s, NCH_2Ar), 4.79 (2H, s, OCH_2Ph), 6.53 (1H, d, J = 2.3 Hz, Ar-H), 6.61 (1H, d, J = 2.4 Hz, Ar-H) and 7.31-7.44 (6H, overlapping m, Ar-H); δ_C/ppm (150 MHz; DMSO-d_6) 30.5 (CH_2CO), 35.2 (NCH_2CH_2), 42.5 (NCH_2Ar), 76.1 (OCH_2Ph), 111.3, 112.6, 128.6, 128.6, 128.9, 129.5, 133.7 and 144.6 (Ar-C) and 172.2 (C=O).

_N_-[2-(_N_-Benzyloxycarbamoyl)ethyl]-_N_-(3-nitrobenzyl)phosphoramidic acid 161f*



The procedure described for the synthesis of *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)ethyl-

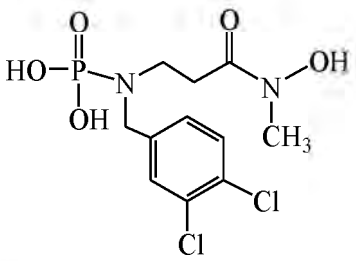
Not possible to obtain satisfactory HRMS data

phosphoramidic acid **161a** was followed, using diethyl *N*-[2-(*N*-benzyloxycarbamoyl)-ethyl]-*N*-(3-nitrobenzyl)phosphoramidate **166g** (0.60 g, 1.3 mmol), bromotrimethylsilane (0.86 mL, 6.4 mmol) and dry DCM (5 mL). Work-up afforded N-*[2-(*N-*benzyloxy-carbamoyl)ethyl]*-N-*(3-nitrobenzyl)phosphoramidic acid* **161g** as a cream solid (0.42 g, 81%), m.p. 158-160 °C; ν_{max}/cm^{-1} 1664 (C=O); δ_H/ppm (600 MHz; DMSO-d_6) 2.46 (2H, t, J = 7.1 Hz, CH_2CO), 3.17 (2H, t, J = 7.2 Hz, NCH_2CH_2), 4.34 (2H, s, NCH_2Ar), 4.80 (2H, s, OCH_2Ph), 7.36-7.39 (5H, overlapping m, Ar-H), 7.76 (1H, t, J = 8.0 Hz, Ar-H), 7.98 (1H, d, J = 7.5 Hz, Ar-H), 8.29 (1H, d, J = 7.8 Hz, Ar-H) and 8.47 (1H, s, Ar-H); δ_C/ppm (150 MHz; DMSO-d_6) 28.5 (CH_2CO), 42.6 (NCH_2CH_2), 49.2 (NCH_2Ar), 77.1 (OCH_2Ph), 123.6, 124.0, 125.1, 128.4, 128.9, 130.4, 134.2, 136.1, 137.1 and 147.8 (Ar-C) and 166.0 (C=O).

_N_-Benzyl-_N_-[2-(_N_-hydroxy-_N_-methylcarbamoyl)ethyl]phosphoramidic acid 162a*

The procedure described for the synthesis of *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl *N*-benzyl-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]phosphoramidate **167a** (0.080 g, 0.24 mmol), bromotrimethylsilane (0.15 mL, 1.2 mmol) and dry DCM (3 mL). Work-up afforded N-*benzyl*-N-*[2-(*N-*hydroxy-*N-*methylcarbamoyl)ethyl]phosphoramidic acid* **162a** as a yellow oil (0.052 g, 75%); ν_{max}/cm^{-1} 1646 (C=O); δ_H/ppm (600 MHz; CD_3OD) 3.21 (2H, m, CH_2CO), 3.26-3.30 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.25 (2H, s, NCH_2Ph) and 7.45-7.52 (5H, overlapping m, Ar-H); δ_C/ppm (75 MHz; CD_3OD) δ_C/ppm (75 MHz; CD_3OD) 31.1 (CH_2CO), 36.2 (NCH_3), 43.9 (NCH_2CH_2), 52.4 (NCH_2Ph), 130.0, 130.3, 131.0 and 132.3 (Ar-C) and 172.3 (C=O).

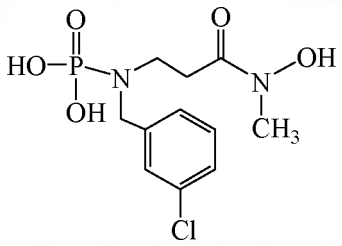
_N_-(3,4-Dichlorobenzyl)-_N_-[2-(_N_-hydroxy-_N_-methylcarbamoyl)ethyl]phosphoramidic acid 162b*



Not possible to obtain satisfactory HRMS data

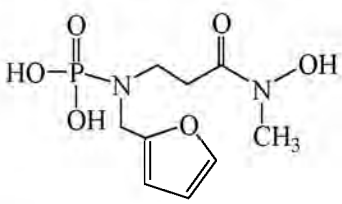
The procedure described for the synthesis of *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl *N*-(3,4-dichlorobenzyl)-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]phosphoramidate **167b** (0.074 g, 0.20 mmol), bromotrimethylsilane (0.12 mL, 0.89 mmol) and dry DCM (3 mL). Work-up afforded N-*(3,4-dichlorobenzyl)*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)ethyl]phosphoramidic acid* **162b** as a yellow oil (0.046 g, 70%); ν_{max}/cm^{-1} 1640 (C=O); δ_H/ppm (600 MHz; CD_3OD) 2.84 (2H, m, CH_2CO), 3.19-3.24 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.23 (2H, s, NCH_2Ar) and 7.30-7.34 (3H, overlapping m, Ar-H); δ_C/ppm (75 MHz; CD_3OD) 30.1 (CH_2CO), 37.7 (NCH_3), 44.5 (NCH_2CH_2), 51.5 (NCH_2Ar), 129.0, 130.2, 130.8, 131.2, 132.6 and 135.4 (Ar-C) and 173.1 (C=O).

_N_-(3-Chlorobenzyl)-_N_-[2-(_N_-hydroxy-_N_-methylcarbamoyl)ethyl]phosphoramidic acid 162c*



The procedure described for the synthesis of *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl *N*-(3-chlorobenzyl)-*N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]phosphoramidate **167c** (0.12 g, 0.36 mmol), bromotrimethylsilane (0.24 mL, 1.8 mmol) and dry DCM (3 mL). Work-up afforded N-*(3-chlorobenzyl)*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)ethyl]phosphoramidic acid* **162c** as a yellow oil (0.075 g, 79%); ν_{max}/cm^{-1} 1640 (C=O); δ_H/ppm (600 MHz; CD_3OD) 2.86 (2H, m, CH_2CO), 3.21-3.24 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.19 (2H, s, NCH_2Ar), 7.36 (1H, s, Ar-H) 7.40-7.45 (3H, overlapping m, Ar-H) and 7.53 (1H, br s, OH); δ_C/ppm (75 MHz; CD_3OD) 31.1 (CH_2CO), 38.0 (NCH_3), 44.1 (NCH_2CH_2), 51.6 (NCH_2Ar), 130.0, 130.8 131.1, 131.9, 134.6 and 136.0 (Ar-C) and 173.6 (C=O).

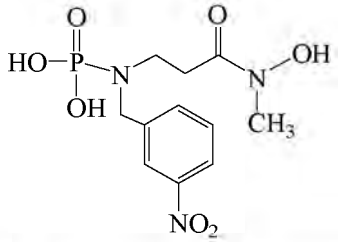
_N_-Furfuryl-_N_-[2-(_N_-hydroxy-_N_-methylcarbamoyl)ethyl]phosphoramidic acid 162f*



*Not possible to obtain satisfactory HRMS data

The procedure described for the synthesis of *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl *N*-furfuryl-*N*-[2-(*N*-hydroxy-N-methylcarbamoyl)ethyl]phosphoramidate **167f** (0.11 g, 0.32 mmol), bromotrimethylsilane (0.24 mL, 1.6 mmol) and dry DCM (3 mL). Work-up afforded N-*furfuryl*-N-*[2-(*N-*hydroxy*-N-*methylcarbamoyl)ethyl]phosphoramidic acid* **162f** as a yellow oil (0.075 g, 62%); ν_{max}/cm^{-1} 1646 (C=O); δ_H/ppm (600 MHz; CD_3OD) 2.93 (2H, m, CH_2CO), 3.26-3.31 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.33 (2H, s, NCH_2Ar), 6.49 (1H, d, J = 2.7 Hz, Ar-H), 6.67 (1H, d, J = 2.9 Hz, Ar-H) and 7.61 (1H, m, Ar-H); δ_C/ppm (75 MHz; CD_3OD) 29.1 (CH_2CO), 31.0 (NCH_3), 38.0 (NCH_2CH_2), 43.5 (NCH_2Ar), 112.1, 113.9, 120.5 and 145.9 (Ar-C) and 172.3 (C=O).

_N_-[2-(_N_-Hydroxy-_N_-methylcarbamoyl)ethyl]-_N_-(3-nitrobenzyl)phosphoramidic acid 162g*

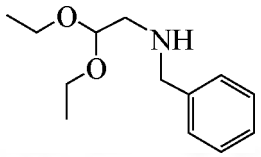


The procedure described for the synthesis of *N*-benzyl-*N*-[2-(*N*-benzyloxycarbamoyl)-ethyl]phosphoramidic acid **161a** was followed, using diethyl *N*-[2-(*N*-hydroxy-*N*-methyl-carbamoyl)ethyl]-*N*-(3-nitrobenzyl)phosphoramidate **167g** (0.082 g, 0.21 mmol), bromo-trimethylsilane (0.14 mL, 1.1 mmol) and dry DCM (3 mL). Work-up afforded *N*-[2-(*N*-hydroxy-*N*-methylcarbamoyl)ethyl]-*N*-(3-nitrobenzyl)phosphoramidic acid **162g** as a yellow oil (0.052 g, 74%); ν_{max}/cm^{-1} 1630 (C=O); δ_H/ppm (600 MHz; CD_3OD) 2.86 (2H, m, CH_2CO), 3.21-3.24 (5H, overlapping m, NCH_3 and NCH_2CH_2), 4.19 (2H, s, NCH_2Ar), 7.73 (1H, t, J = 7.3 Hz, Ar-H), 7.96 (1H, d, J = 6.6 Hz, Ar-H), 8.32 (1H, d, J = 7.8 Hz, Ar-H) and 8.47 (1H, s, Ar-H); δ_C/ppm (75 MHz; CD_3OD) 29.3 (CH_2CO), 38.1 (NCH_3), 44.5 (NCH_2CH_2), 51.3 (NCH_2Ar), 125.5, 126.0, 131.6, 134.5, 137.4 and 150.0 (Ar-C) and 171.8 (C=O).

Not possible to obtain satisfactory HRMS data

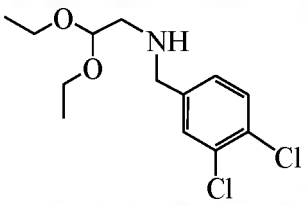
3.4. SYNTHESIS of *N*-BENZYLATED PHOSPHORAMIDATE DERIVATIVES

N-Benzyl-2,2-diethoxyethanamine 175a*[251]



Bromoacetaldehyde diethylacetal (4.2 mL, 28 mmol) was added to a solution of benzylamine **163a** (3.0 mL, 28 mmol) and anhydrous K_2CO_3 (3.9 g, 28 mmol) in dry acetonitrile (15 mL) and the mixture was refluxed for 24 h. The mixture was diluted with EtOAc (20 mL) and washed sequentially with water (3 × 25 mL) and dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] to afford *N*-benzyl-2,2-diethoxyethanamine **175a** as a yellow oil (3.8 g, 61%) ν_{max}/cm^{-1} 3399 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 1.20 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.75 (2H, d, J = 5.6 Hz, NCH_2), 3.53 (2H, m, 2 × OCH_aCH_3), 3.68 (2H, m, 2 × OCH_bCH_3), 3.81 (2H, s, NCH_2Ph), 4.62 (1H, t, J = 5.6 Hz, CH), 7.25 (1H, m, Ar-H) and 7.32-7.33 (4H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.6 (2 × CH_3), 51.7 (NCH_2), 54.0 (NCH_2Ph), 62.5 (2 × OCH_2CH_3), 102.3 (CH), 127.1, 128.3, 128.5 and 140.3 (Ar-C).

N-(3,4-Dichlorobenzyl)-2,2-diethoxyethanamine 175b*

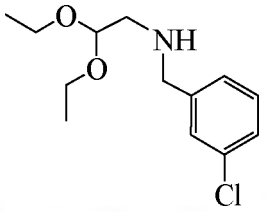


The procedure described for the synthesis of *N*-benzyl-2,2-diethoxyethanamine **175a** was followed, using 3,4-dichlorobenzylamine **163b** (2.3 mL, 17 mmol), anhydrous K_2CO_3 (2.4 g, 17 mmol) and bromoacetaldehyde diethylacetal (2.6 mL, 17 mmol) in dry acetonitrile (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded N-*(3,4-dichlorobenzyl)-2,2-diethoxyethanamine* **175b** as a yellow oil (2.5 g, 50%) [HRMS: *m/z* calculated for $C_{13}H_{20}{}^{35}Cl_2NO_2$ (MH^+) 292.0871. Found 292.0880]; ν_{max}/cm^{-1} 3333 (NH); δ_H/ppm (400 MHz; $CDCl_3$) 1.21 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.71 (2H, d, J = 5.5 Hz, NCH_2), 3.53 (2H, m, 2 × OCH_aCH_3), 3.69 (2H, m, 2 × OCH_bCH_3), 3.76 (2H, s, NCH_2Ar), 4.59 (1H, t, J = 5.6 Hz, CH), 7.16 (1H, dd, J = 2.0 and 8.2 Hz, Ar-H), 7.38 (1H, d,

*Compound decomposes at r.t.,

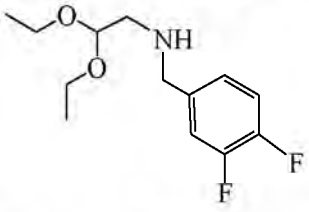
J = 8.2 Hz, Ar-H) and 7.44 (1H, d, J = 2.3 Hz, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 15.5 (2 × CH_3), 51.6 (NCH_2), 52.8 (NCH_2Ar), 62.7 (2 × OCH_2CH_3), 102.3 (CH) 127.5, 130.1, 130.4, 130.9, 132.5 and 140.8 (Ar-C).

_N_-(3-Chlorobenzyl)-2,2-diethoxyethanamine 175c*



The procedure described for the synthesis of _N_-benzyl-2,2-diethoxyethanamine **175a** was followed, using 3-chlorobenzylamine **163c** (3.4 mL, 28 mmol), anhydrous K_2CO_3 (3.9 g, 28 mmol) and bromoacetaldehyde diethylacetal (4.2 mL, 28 mmol) in dry acetonitrile (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (5:5)] afforded N-*(3-chlorobenzyl)-2,2-diethoxyethanamine* **175c** as a yellow oil (1.7 g, 23%) [HRMS: *m/z* calculated for $C_{13}H_{21}{}^{35}ClNO_2$ (MH^+) 258.1261. Found 258.1260]; ν_{max}/cm^{-1} 3325 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 1.21 (6H, t, J = 7.0 Hz, 2 × CH_3), 2.73 (2H, d, J = 5.5 Hz, NCH_2), 3.53 (2H, m, 2 × OCH_aCH_3), 3.68 (2H, m, 2 × OCH_bCH_3), 3.79 (2H, s, NCH_2Ar), 4.60 (1H t, J = 5.5 Hz, CH), 7.20 (1H, t, J = 7.0 Hz, Ar-H), 7.23 (2H, m, Ar-H) and 7.33 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.4 (2 × CH_3), 51.5 (NCH_2), 53.2 (NCH_2Ar), 62.2 (2 × OCH_2CH_3), 102.1 (CH) 126.2, 127.1, 128.2, 129.6, 134.2 and 142.4 (Ar-C).

2,2-Diethoxy-_N_-(3,4-difluorobenzyl)ethanamine175d*

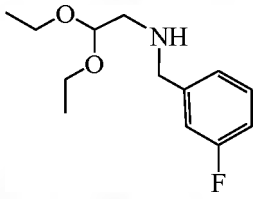


The procedure described for the synthesis of _N_-benzyl-2,2-diethoxyethanamine **175a** was followed, using 3,4-difluorobenzylamine **163d** (3.3 mL, 28 mmol), anhydrous K_2CO_3 (3.9 g, 28 mmol) and bromoacetaldehyde diethylacetal (4.2 mL, 28 mmol) in dry acetonitrile (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *2,2-diethoxy*-N-*(3,4-difluorobenzyl)ethanamine* **175d** as a yellow oil (4.1 g, 56%) [HRMS: *m/z* calculated for $C_{13}H_{20}{}^{19}F_2NO_2$ (MH^+) 260.1462. Found 260.1469]; ν_{max}/cm^{-1}

*Compound decomposes at r.t.,

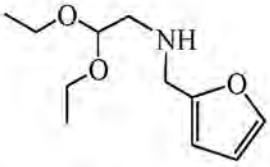
3329 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 1.21 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.71 (2H, d, J = 5.5 Hz, NCH_2), 3.53 (2H, m, 2 × OCH_aCH_3), 3.69 (2H, m, 2 × OCH_bCH_3), 3.76 (2H, s, NCH_2Ar), 4.59 (1H, t, J = 5.5 Hz, CH), 7.01 (1H, d, J_{F-H} = 9.8 Hz, Ar-H), 7.09 (1H, dd, J_{F-H} = 8.2 and 18.4 Hz, Ar-H) and 7.16 (1H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.4 (2 × CH_3), 51.4 (NCH_2), 52.7 (NCH_2Ar), 62.5 (2 × OCH_2CH_3), 102.2 (CH), 116.8 and 117.0 (2 × d, J_{F-C} = 17.2 Hz), 123.8 (m), 137.6, 149.0 (dd, J_{F-C} = 13.5 and 277.0 Hz) and 151.0 (dd, J_{F-C} = 13.5 and 246.0 Hz) (Ar-C).

2,2-Diethoxy-*N*-(3-fluorobenzyl)ethanamine 175e*



The procedure described for the synthesis of *N*-benzyl-2,2-diethoxyethanamine **175a** was followed, using 3-fluorobenzylamine **163e** (3.2 mL, 28 mmol), anhydrous K_2CO_3 (3.9 g, 28 mmol) and bromoacetaldehyde diethylacetal (4.2 mL, 28 mmol) in dry acetonitrile (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *2,2-diethoxy-*N*-(3-fluorobenzyl)ethanamine* **175e** as a yellow oil (3.4 g, 50%) [HRMS: *m/z* calculated for $C_{13}H_{21}{}^{19}FNO_2$ (MH^+) 242.1556. Found 242.1564]; ν_{max}/cm^{-1} 3280 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 1.21 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.73 (2H, d, J = 5.5 Hz, NCH_2), 3.53 (2H, m, 2 × OCH_aCH_3), 3.69 (2H, m, 2 × OCH_bCH_3), 3.81 (2H, s, NCH_2Ar), 4.61 (1H, t, J = 5.5 Hz, CH), 6.93 (1H, t, J = 8.5 Hz, Ar-H), 7.07 (2H, dd, J_{H-H} = 8.7 and J_{F-H} = 17.0 Hz, Ar-H) and 7.26 (1H, d, J_{F-H} = 13.8 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.3 (2 × CH_3), 51.4 (NCH_2), 53.2 (NCH_2Ar), 62.4 (2 × OCH_2CH_3), 102.1 (CH), 113.7 (d, J_{F-C} = 21.2 Hz), 114.8 (d, J_{F-C} = 21.2 Hz), 123.5 (d, J_{F-C} = 2.6 Hz), 129.7 (d, J_{F-C} = 8.2 Hz), 139.2 and 129.7 (d, J_{F-C} = 246.5 Hz) (Ar-C).

2,2-Diethoxy-*N*-(2-furfuryl)ethanamine 175f*[251]

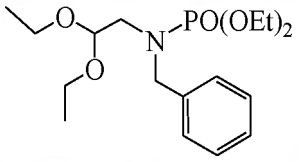


The procedure described for the synthesis of *N*-benzyl-2,2-diethoxyethanamine **175a** was followed, using furfurylamine **163f** (2.7 mL, 31 mmol), anhydrous K_2CO_3 (4.3 g, 31 mmol)

**Compound decomposes at r.t.,*

and bromoacetaldehyde diethylacetal (4.6 mL, 31 mmol) in dry acetonitrile (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded 2,2-diethoxy-*N*-(2-furfuryl)ethanamine **175f** as a yellow oil (3.9 g, 40%); ν_{max}/cm^{-1} 3280 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 1.22 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.76 (2H, d, J = 5.5 Hz, NCH_2), 3.55 (2H, m, 2 × OCH_aCH_3), 3.64 (2H, m, 2 × OCH_bCH_3), 3.86 (2H, s, NCH_2Ar), 4.65 (1H, t, J = 5.5 Hz, CH), 6.61 (1H, d, J = 2.5 Hz, Ar-H), 6.70 (1H, d, J = 2.3 Hz, Ar-H) and 7.36 (1H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.5 (2 × CH_3), 51.3 (NCH_2), 52.9 (NCH_2Ar), 63.1 (2 × OCH_2CH_3), 102.8 (CH), 106.7, 110.5, 143.1 and 155.0 (Ar-C).

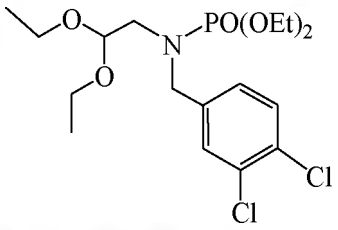
Diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)phosphoramidate 169a*



A mixture of *N*-benzyl-2,2-diethoxyethanamine **175a** (3.8 g, 17 mmol), triethylamine (2.4 mL, 17 mmol) and diethyl chlorophosphate (2.5 mL, 17 mmol) in DCM (20 mL), was stirred overnight. The mixture was washed with water (2 × 20 mL). The organic layer was dried with anhydrous $MgSO_4$ and filtered, and the solvent was removed *in vacuo*. The crude material was purified by flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] to afford to *diethyl* N-*benzyl*-N-*(2,2-diethoxyethyl)phosphoramidate* **169a** as a yellow oil (5.3 g, 87%) [HRMS: *m/z* calculated for $C_{17}H_{31}NO_5P$ (MH^+) 360.1940. Found 360.1943]; ν_{max}/cm^{-1} 1240 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.20 (6H, t, J = 7.0 Hz, 2 × CH_3), 1.31 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.00 (2H, dd, $J_{H\text{-}H}$ = 5.4 and $J_{P\text{-}H}$ = 11.2 Hz, NCH_2), 3.48 (2H, m, 2 × OCH_aCH_3), 3.67 (2H, m, 2 × OCH_bCH_3), 4.07 [4H, m, $PO(OCH_2CH_3)_2$], 4.33 (2H, d, $J_{P\text{-}H}$ = 10.5 Hz, NCH_2Ph), 4.59 (1H, t, J= 5.4 Hz, CH), 7.25 (1H, d, J = 7.4 Hz, Ar-H), 7.31 (2H, t, J = 7.5 Hz, Ar-H) and 7.35 (2H, d, J = 7.5 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.5 (2 × CH_3), 16.3 [d, $J_{P\text{-}C}$ = 7.4 Hz, $PO(OCH_2CH_3)_2$], 47.4 (d, $J_{P\text{-}C}$ = 4.6 Hz, NCH_2), 50.6 (d, $J_{P\text{-}C}$ = 4.6 Hz, NCH_2Ph), 62.4 [d, $J_{P\text{-}C}$ = 5.6 Hz, $PO(OCH_2CH_3)_2$], 62.9 (2 × OCH_2CH_3), 102.8 (CH), 127.4, 128.5, 128.8 and 138.2 (Ar-C).

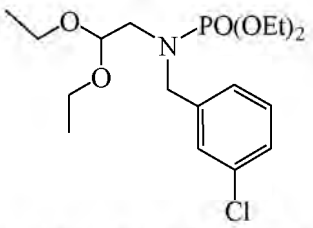
**Compound decomposes at r.t.,*

Diethyl *N*-(3,4-dichlorobenzyl)-*N*-(2,2-diethoxyethyl)phosphoramidate 169b*



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)-phosphoramidate **169a** was followed, using *N*-(3,4-dichlorobenzyl)-2,2-diethoxyethanamine **175b** (2.5 g, 8.4 mmol), triethylamine (1.2 mL, 8.4 mmol) and diethyl chlorophosphate (1.2 mL, 8.4 mmol) in DCM (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*(3,4-dichlorobenzyl)*-N-*(2,2-diethoxyethyl)-phosphoramidate* **169b** as a yellow oil (2.8 g, 77%) [HRMS: *m/z* calculated for $C_{17}H_{29}{}^{35}Cl_2NO_5P$ (MH^+) 428.1160. Found 428.1162]; ν_{max}/cm^{-1} 1247 (P=O); δ_H/ppm (400 MHz; $CDCl_3$) 1.20 (6H, t, J = 7.0 Hz, 2 × CH_3), 1.33 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.92 (2H, d, $J_{P\text{-}H}$ = 11.5 Hz, NCH_2), 3.48 (2H, m, 2 × OCH_aCH_3), 3.68 (2H, m, 2 × OCH_bCH_3), 4.07 [4H, m, $PO(OCH_2CH_3)_2$], 4.29 (2H, d, $J_{P\text{-}H}$ = 10.7 Hz, NCH_2Ar), 4.57 (1H, t, J = 5.3 Hz, CH), 7.18 (1H, d, J = 8.2 Hz, Ar-H), 7.38 (1H, d, J = 8.2 Hz, Ar-H) and 7.47 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.3 (2 × CH_3), 16.2 [d, $J_{P\text{-}C}$ = 7.3 Hz, $PO(OCH_2CH_3)_2$], 47.5 (d, $J_{P\text{-}C}$ = 4.3 Hz, NCH_2), 49.6 (d, $J_{P\text{-}C}$ = 4.7 Hz, NCH_2Ar), 62.5 [d, $J_{P\text{-}C}$ = 5.7 Hz, $PO(OCH_2CH_3)_2$], 63.0 (2 × OCH_2CH_3), 102.9 (CH), 127.9, 130.2, 130.5, 131.1, 132.3 and 138.7 (Ar-C).

Diethyl *N*-(3-chlorobenzyl)-*N*-(2,2-diethoxyethyl)phosphoramidate 169c*

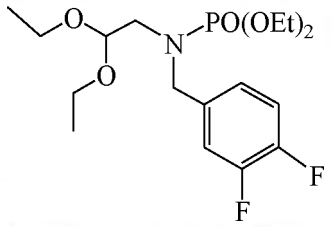


The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)-phosphoramidate **169a** was followed, using *N*-(3-chlorobenzyl)-2,2-diethoxyethanamine **175c** (1.7 g, 6.5 mmol), triethylamine (0.91 mL, 6.5 mmol) and diethyl chlorophosphate (0.93 mL, 6.5 mmol) in DCM (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (5:5)] afforded *diethyl* N-*(3-chlorobenzyl)*-N-*(2,2-diethoxy-ethyl)phosphoramidate* **169c** as a yellow oil (2.2 g, 86%) [HRMS: *m/z* calculated for

*Compound decomposes at r.t.,

$C_{17}H_{30}{}^{35}ClNO_5P$ (MH^+) 394.1550. Found 394.1553]; ν_{max}/cm^{-1} 1239 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.20 (6H, t, J = 7.0 Hz, 2 × CH_3), 1.32 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.99 (2H, dd, $J_{H\text{-}H}$ = 5.3 and $J_{P\text{-}H}$ = 12.0 Hz, NCH_2), 3.48 (2H, m, 2 × OCH_aCH_3), 3.67 (2H, m, 2 × OCH_bCH_3), 4.06 [4H, m, $PO(OCH_2CH_3)_2$], 4.31 (2H, d, $J_{P\text{-}H}$ = 10.5 Hz, NCH_2Ar), 4.57 (1H, t, J = 5.3 Hz, CH), 7.20-7.26 (3H, overlapping m, Ar-H) and 7.36 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.4 (2 × CH_3), 16.2 [d, $J_{P\text{-}C}$ = 7.3 Hz, $PO(OCH_2CH_3)_2$], 47.7 (d, $J_{P\text{-}C}$ = 4.5 Hz, NCH_2), 50.3 (d, $J_{P\text{-}C}$ = 4.7 Hz, NCH_2Ar), 62.6 [d, $J_{P\text{-}C}$ = 5.7 Hz, $PO(CH_2CH_3)_2$], 63.0 (2 × OCH_2CH_3), 102.9 (CH), 126.7, 127.5, 128.7, 129.7, 134.3 and 140.5 (Ar-C).

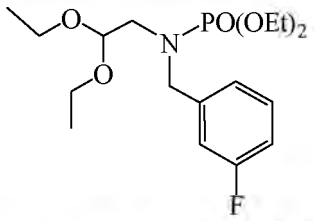
Diethyl *N*-(2,2-diethoxyethyl)-*N*-(3,4-difluorobenzyl)phosphoramidate 169d*



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)-phosphoramidate **169a** was followed, using 2,2-diethoxy-*N*-(3,4-difluorobenzyl)ethanamine **175d** (4.1 g, 16 mmol), triethylamine (2.2 mL, 16 mmol) and diethyl chlorophosphate (2.3 mL, 16 mmol) in DCM (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*(2,2-diethoxyethyl)*-N-*(3,4-difluorobenzyl)-phosphoramidate* **169d** as a yellow oil (5.5 g, 88%) [HRMS: *m/z* calculated for $C_{17}H_{29}{}^{19}F_2NO_5P$ (MH^+) 396.1754. Found 396.1758]; ν_{max}/cm^{-1} 1249 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.20 (6H, t, J = 7.0 Hz, 2 × CH_3), 1.32 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.96 (2H, dd, $J_{H\text{-}H}$ = 5.3 and $J_{P\text{-}H}$ = 12.0 Hz, NCH_2), 3.49 (2H, m, 2 × OCH_aCH_3), 3.68 (2H, m, 2 × OCH_bCH_3), 4.06 [4H, m, $PO(OCH_2CH_3)_2$], 4.29 (2H, d, $J_{P\text{-}H}$ = 10.5 Hz, NCH_2Ar), 4.56 (1H, t, J = 5.3 Hz, CH), 7.08 (2H, m, Ar-H) and 7.21 (1H, t, J = 7.5 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.5 (2 × CH_3), 16.3 [d, $J_{P\text{-}C}$ = 7.3 Hz, $PO(OCH_2CH_3)_2$], 47.5 (d, $J_{P\text{-}C}$ = 4.4 Hz, NCH_2), 49.8 (d, $J_{P\text{-}C}$ = 3.7 Hz, NCH_2Ar), 62.6 [d, $J_{P\text{-}C}$ = 5.7 Hz, $PO(OCH_2CH_3)_2$], 62.9 (2 × OCH_2CH_3), 103.1 (CH), 117.1 (d, $J_{F\text{-}C}$ = 17.2 Hz), 117.6 (d, $J_{F\text{-}C}$ = 16.1 Hz), 124.6 (d, $J_{F\text{-}C}$ = 2.4 Hz), 135.5, 148.4 (dd, $J_{F\text{-}C}$ = 12.0 and 245.3 Hz) and 151.7 (dd, $J_{F\text{-}C}$ = 12.8 and 246.0 Hz) (Ar-C).

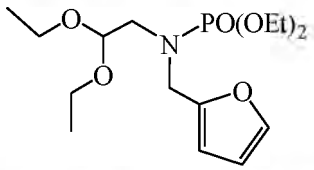
**Compound decomposes at r.t.,*

Diethyl *N*-(2,2-diethoxyethyl)-*N*-(3-fluorobenzyl)phosphoramidate 169e[*‡]



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)-phosphoramidate **169a** was followed, using 2,2-diethoxy-*N*-(3-fluorobenzyl)ethanamine **175e** (3.4 g, 14 mmol), triethylamine (1.9 mL, 14 mmol) and diethyl chlorophosphate (2.0 mL, 14 mmol) in DCM (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*(2,2-diethoxyethyl)*-N-*(3-fluorobenzyl)-phosphoramidate* **169e** as a brown oil (4.9 g, 94%) [HRMS: *m/z* calculated for $C_{17}H_{30}{}^{19}FNO_5P$ (MH^+) 378.1846. Found 378.1848]; ν_{max}/cm^{-1} 1229 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.19 (6H, t, J = 7.0 Hz, 2 × CH_3), 1.32 [6H, t, J = 6.9 Hz, $PO(OCH_2CH_3)_2$], 2.99 (2H, dd, $J_{H\text{-}H}$ = 5.4 and $J_{P\text{-}H}$ = 12.1 Hz, NCH_2), 3.47 (2H, m, 2 × OCH_aCH_3), 3.67 (2H, m, 2 × OCH_bCH_3), 4.07 [4H, m, $PO(OCH_2CH_3)_2$], 4.32 (2H, d, $J_{P\text{-}H}$ = 10.4 Hz, NCH_2Ar), 4.58 (1H, t, J = 5.3 Hz, CH), 6.93 (1H, t, J = 8.3 Hz, Ar-H), 7.09 (2H, t, $J_{F\text{-}H}$ = 9.6 Hz, Ar-H) and 7.25 (1H, s, Ar-H).

Diethyl *N*-(2,2-diethoxyethyl)-*N*-furfurylphosphoramidate 169f[¥*]



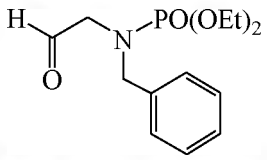
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)-phosphoramidate **169a** was followed, using 2,2-diethoxy-*N*-(2-furfuryl)ethanamine **175f** (3.7 g, 17 mmol), triethylamine (2.4 mL, 17 mmol) and diethyl chlorophosphate (2.5 mL, 17 mmol) in DCM (15 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded diethyl *N*-(2,2-diethoxyethyl)-*N*-furfuryl-phosphoramidate **169f** as a yellow oil (4.0 g, 71%); ν_{max}/cm^{-1} 1225 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.21 (6H, t, J = 7.1 Hz, 2 × CH_3), 1.30 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.06 (2H, dd, $J_{H\text{-}H}$ = 5.5 and $J_{P\text{-}H}$ = 11.8 Hz, NCH_2), 3.51 (2H, m, 2 × OCH_aCH_3), 3.71 (2H, m, 2 × OCH_bCH_3), 4.03 [4H, m, $PO(OCH_2CH_3)_2$], 4.30 (2H, d, $J_{P\text{-}H}$ = 10.2 Hz, NCH_2Ar), 4.59 (1H, t, J = 5.5

¥*Compound decomposes precluding HRMS analysis*

**Compound decomposes at r.t., ^{13}C data not cited due to decomposition*

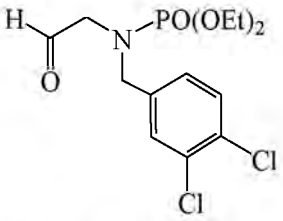
Hz, CH), 6.25 (1H, d, J = 3.1 Hz, Ar-H), 6.25 (1H, d, J = 3.0 Hz, Ar-H) and 7.34 (1H, s, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.5 (2 × CH_3), 16.3 [d, J_{P-C} = 7.6 Hz, $PO(OCH_2CH_3)_2$], 43.7 (d, J_{P-C} = 5.3 Hz, NCH_2), 47.6 (d, J_{P-C} = 4.4 Hz, NCH_2Ar), 62.3 [d, J_{P-C} = 5.3 Hz, $PO(OCH_2CH_3)_2$], 63.1 (2 × OCH_2CH_3), 103.0 (CH), 109.0, 110.3, 142.3 and 152.0 (Ar-C).

Diethyl *N*-benzyl-*N*-(2-oxoethyl)phosphoramidate 176a* (*Method A*)



A mixture of diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)phosphoramidate **169a** (5.2 g, 14 mmol) and 2M-HCl (45 mL) was stirred at r.t. for 24 h. $CHCl_3$ (40 mL) was added and the resulting mixture was washed with water (3 × 20 mL). The aqueous layers were combined and was re-extracted with $CHCl_3$. The organic layers were combined, washed with $NaHCO_3$ (3 × 20 mL), brine (3 × 20 mL), dried with anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was flash chromatographed [on silica gel; elution with EtOAc-hexane (6:4)] to afford *diethyl* N-*benzyl*-N-*(2-oxoethyl)phosphoramidate* **176a** as a yellow oil (1.9 g, 45%) [HRMS: *m/z* calculated for $C_{13}H_{21}NO_4P$ (MH^+) 286.1208. Found 286.1218]; ν_{max}/cm^{-1} 1695 (C=O) and 1230 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.38 [6H, t, J = 7.0 Hz, $PO(OCH_2CH_3)_2$], 3.77 (2H, d, J_{P-H} = 11.6 Hz, NCH_2), 4.16 [4H, m, $PO(OCH_2CH_3)_2$], 4.28 (2H, d, J_{P-H} = 8.0 Hz, NCH_2Ph), 7.29-7.35 (5H, overlapping m, Ar-H) and 9.46 (1H, s, CHO); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 [d, J_{P-C} = 7.2 Hz, $PO(OCH_2CH_3)_2$], 51.1 (d, J_{P-C} = 3.8 Hz, NCH_2), 55.4 (d, J_{P-C} = 4.7 Hz, NCH_2Ph), 63.0 [d, J_{P-C} = 5.5 Hz, $PO(OCH_2CH_3)_2$], 128.1, 128.8, 128.9 and 136.7 (Ar-C) and 199.9 (CHO).

Diethyl *N*-(3,4-dichlorobenzyl)-*N*-(2-oxoethyl)phosphoramidate 176b* (*Method A*)

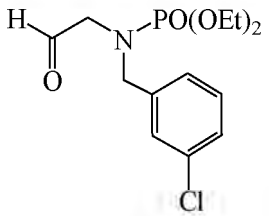


The general procedure (*Method A*) used for the synthesis of diethyl *N*-benzyl-*N*-(2-oxoethyl)phosphoramidate **176a** was employed, using diethyl *N*-(3,4-dichlorobenzyl)-*N*-(2,2-diethoxyethyl)phosphoramidate **169b** (2.7 g, 6.4 mmol) in 2M-HCl (20 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl*

**Compound decomposes at r.t.*

N-*(3,4-dichlorobenzyl)*-N-*(2-oxoethyl)phosphoramidate* **176b** as a pale-yellow oil (1.4 g, 60%) [HRMS: *m/z* calculated for $C_{13}H_{19}{}^{35}Cl_2NO_4P$ (MH^+) 354.0429. Found 354.0435]; ν_{max}/cm^{-1} 1698 (C=O) and 1231 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.27 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.67 (2H, d, $J_{P\text{-}H}$ = 11.8 Hz, NCH_2), 4.07 [4H, m, $PO(OCH_2CH_3)_2$], 4.19 (2H, d, $J_{P\text{-}H}$ = 8.5 Hz, NCH_2Ar), 7.15-7.27 (3H, overlapping m, Ar-H) and 9.37 (1H, s, CHO); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 [d, $J_{P\text{-}C}$ = 7.2 Hz, $PO(OCH_2CH_3)_2$], 51.2 (d, $J_{P\text{-}C}$ = 3.8 Hz, NCH_2), 55.5 (d, $J_{P\text{-}C}$ = 4.8 Hz, NCH_2Ar), 63.0 [d, $J_{P\text{-}C}$ = 5.4 Hz, $PO(OCH_2CH_3)_2$], 126.9, 128.1, 128.7, 128.8, 136.7 and 136.8 (Ar-C) and 199.8 (CHO).

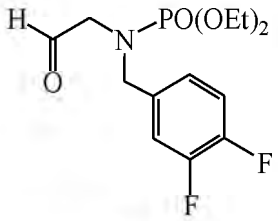
Diethyl *N*-(3-chlorobenzyl)-*N*-(2-oxoethyl)phosphoramidate 176c* (*Method B*)



A mixture of diethyl *N*-(3-chlorobenzyl)-*N*-(2,2-diethoxyethyl)phosphoramidate **169c** (2.2 g, 5.5 mmol) in 2.5M-HCl (20 mL) and dioxane (5 mL) was stirred at r.t. for 5 h. $CHCl_3$ (40 mL) was added and the resulting mixture was washed with water (3 × 20 mL). The aqueous layers were combined and was re-extracted with $CHCl_3$. The organic layers were combined, washed with $NaHCO_3$ (3 × 20 mL), brine (3 × 20 mL), dried with anhydrous $MgSO_4$ and concentrated *in vacuo* afforded *diethyl* N-*(3-chlorobenzyl)*-N-*(2-oxoethyl)phosphoramidate* **176c** as a pale-yellow oil (1.2 g, 70%) [HRMS: *m/z* calculated for $C_{13}H_{20}{}^{35}ClNO_4P$ (MH^+) 320.0819. Found 320.0818]; ν_{max}/cm^{-1} 1699 (C=O) and 1231 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.35 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.75 (2H, d, $J_{P\text{-}H}$ = 11.7 Hz, NCH_2), 4.13 [4H, m, $PO(OCH_2CH_3)_2$], 4.25 (2H, d, $J_{P\text{-}H}$ = 8.9 Hz, NCH_2Ar), 6.96 (1H, t, J = 8.4 Hz, Ar-H), 7.03 (2H, m, Ar-H), 7.29 (1H, d, J = 6.3 Hz, Ar-H) and 9.48 (1H, s, CHO); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 [d, $J_{P\text{-}C}$ = 7.1 Hz, $PO(OCH_2CH_3)_2$], 50.4 (d, $J_{P\text{-}C}$ = 3.7 Hz, NCH_2), 55.5 (d, $J_{P\text{-}C}$ = 4.7 Hz, NCH_2Ar), 63.1 [d, $J_{P\text{-}C}$ = 5.5 Hz, $PO(OCH_2CH_3)_2$] 126.7, 128.0, 128.5, 129.1, 134.6 and 138.9 (Ar-C) and 199.2 (CHO).

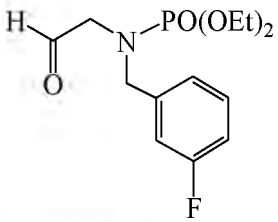
**Compound decomposes at r.t.*

Diethyl *N*-(3,4-difluorobenzyl)-*N*-(2-oxoethyl)phosphoramidate 176d* (*Method B*)



The general procedure (*Method B*) used for the synthesis of diethyl *N*-(3-chlorobenzyl)-*N*-(2-oxoethyl)phosphoramidate **176c** was employed, using diethyl *N*-(2,2-diethoxyethyl)-*N*-(3,4-difluorobenzyl)phosphoramidate **169d** (5.5 g, 14 mmol) in 2.5M-HCl (20 mL) and dioxane (5 mL). Work-up afforded *diethyl* N-*(3,4-difluorobenzyl)*-N-*(2-oxoethyl)phosphoramidate* **176d** as a pale-yellow oil (3.0 g, 67%) [HRMS: *m/z* calculated for $C_{13}H_{19}{}^{19}F_2NO_4P$ (MH^+) 322.1020. Found 322.1029]; ν_{max}/cm^{-1} 1733 (C=O) and 1231 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.33 [6H, t, J = 7.2 Hz, $PO(OCH_2CH_3)_2$], 3.74 (2H, d, $J_{P\text{-}H}$ = 11.5 Hz, NCH_2), 4.11 [4H, m, $PO(CH_2CH_3)_2$], 4.20 (2H, d, $J_{P\text{-}H}$ = 9.1 Hz, NCH_2Ar), 6.96 (1H, d, J = 8.3 Hz, Ar-H), 7.07-7.15 (2H, overlapping m, Ar-H) and 9.47 (1H, s, CHO); δ_C/ppm (75 MHz; $CDCl_3$) 15.8 [d, $J_{P\text{-}C}$ = 7.0 Hz, $PO(OCH_2CH_3)_2$], 49.9 (d, $J_{P\text{-}C}$ = 3.5 Hz, NCH_2), 55.1 (d, $J_{P\text{-}C}$ = 4.4 Hz, NCH_2Ar), 62.8 [d, $J_{P\text{-}C}$ = 5.6 Hz, $PO(OCH_2CH_3)_2$], 117.1 (d, $J_{F\text{-}C}$ = 17.3 Hz), 117.6 (d, $J_{F\text{-}C}$ = 16.9 Hz), 124.4 (d, $J_{F\text{-}C}$ = 2.6 Hz), 135.9, 150.6 (dd, $J_{F\text{-}C}$ = 12.0 and 246.8 Hz) and 150.6 (dd, $J_{F\text{-}C}$ = 12.8 and 247.5 Hz) (Ar-C) and 198.8 (CHO).

Diethyl *N*-(3-fluorobenzyl)-*N*-(2-oxoethyl)phosphoramidate 176e* (*Method B*)

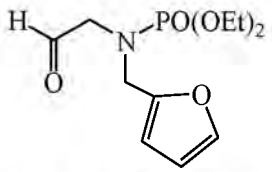


The general procedure (*Method B*) used for the synthesis of diethyl *N*-(3-chlorobenzyl)-*N*-(2-oxoethyl)phosphoramidate **176c** was employed, using diethyl *N*-(2,2-diethoxy-ethyl)-*N*-(3-fluorobenzyl)phosphoramidate **169e** (4.9 g, 13 mmol) in 2.5M-HCl (40 mL) and dioxane (5 mL). Work-up afforded *diethyl* N-*(3-fluorobenzyl)*-N-*(2-oxoethyl)phosphoramidate* **176e** as a pale-yellow oil (2.9 g, 74%) [HRMS: *m/z* calculated for $C_{13}H_{20}{}^{19}FNO_4P$ (MH^+) 304.1114. Found 304.1114]; ν_{max}/cm^{-1} 1732 (C=O) and 1232 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.35 [6H, t, J = 7.0 Hz, $PO(OCH_2CH_3)_2$], 3.76 (2H, d, $J_{P\text{-}H}$ = 11.7 Hz, NCH_2), 4.13 (4H, m, $PO(OCH_2CH_3)_2$], 4.23 (2H, d, $J_{P\text{-}H}$ = 9.1 Hz, NCH_2Ar), 7.16 (1H, d, J = 10.6 Hz, Ar-

*Compound decomposes at r.t.

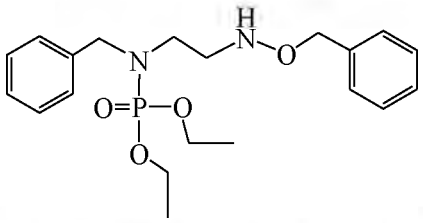
H), 7.25 (2H, m, Ar-H), 7.31 (1H, s, Ar-H) and 9.49 (1H, s, CHO); δ_C/ppm (75 MHz; $CDCl_3$) 16.1 [d, J_{P-C} = 7.1 Hz, $PO(OCH_2CH_3)_2$], 51.1 (d, J_{P-C} = 3.9 Hz, NCH_2), 55.3 (d, J_{P-C} = 4.6 Hz, NCH_2Ar), 63.0 [d, J_{P-C} = 5.6 Hz, $PO(OCH_2CH_3)_2$], 114.8 (d, J_{F-C} = 21.2 Hz), 115.2 (dd, J_{F-C} = 21.6 Hz), 124.0 (d, J_{F-C} = 2.8 Hz), 130.2 (d, J_{F-C} = 8.1 Hz), 139.4 (d, J_{F-C} = 3.4 and 6.8 Hz), 162.6 (d, J_{F-C} = 240.0 Hz) (Ar-C) and 199.1 (CHO).

Diethyl *N*-furfuryl-*N*-(2-oxoethyl)phosphoramidate 176f[†*] (*Method A*)



The general procedure (*Method A*) used for the synthesis of diethyl *N*-benzyl-*N*-(2-oxoethyl)phosphoramidate **176a** was employed, using diethyl *N*-(2,2-diethoxyethyl)-*N*-furfurylphosphoramidate **169f** (3.7 g, 11 mmol) in 2M-HCl (20 mL). Work-up afforded diethyl *N*-furfuryl-*N*-(2-oxoethyl)phosphoramidate **176f** as a pale-yellow oil (0.89 g, 30%); ν_{max}/cm^{-1} 1709 (C=O) and 1235 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.34 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.82 (2H, d, J_{P-H} = 11.1 Hz, NCH_2), 4.10 [4H, m, $PO(OCH_2CH_3)_2$], 4.23 (2H, d, J_{P-H} = 7.7 Hz, NCH_2Ar), 6.23-6.31 (2H, m Ar-H), 7.37 (1H, s, Ar-H) and 9.42 (1H, s, CHO); δ_C/ppm (150MHz; $CDCl_3$) 16.3 [d, J_{P-C} = 7.5 Hz, $PO(OCH_2CH_3)_2$], 43.9 (d, J_{P-C} = 4.0 Hz, NCH_2), 56.0 (d, J_{P-C} = 4.8 Hz, NCH_2Ar), 62.9 [d, J_{P-C} = 5.2 Hz, $PO(OCH_2CH_3)_2$], 109.7, 110.6, 142.9 and 150.6 (Ar-C) and 199.9 (CHO).

Diethyl *N*-benzyl-*N*-[2-(benzyloxyamino)ethyl]phosphoramidate 177a[*]

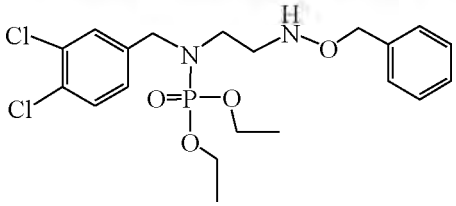


To a stirred solution of diethyl *N*-benzyl-*N*-(2-oxoethyl)phosphoramidate **176a** (1.8 g, 6.5 mmol) in MeOH (15 mL) was added *O*-benzylhydroxylamine hydrochloride (1.4 g, 8.4 mmol). The solution was refluxed at 65 °C for 5 h and, after cooling. sodium cyanoborohydride (1.2 g, 19 mmol) was added in portions; conc. HCl (1.7 mL) was later added dropwise and the mixture was allowed to stir at r.t. for 1.5 h. Sodium cyanoborohydride (0.41 g, 0.48 mmol) was again added in portions, and the reaction mixture was stirred for 1h. The solvent

[†]*Compound decomposes precluding HRMS analysis,* *Compound decomposes at r.t.*

was evaporated *in vacuo*, and the residue was dissolved in MeOH (30 mL), treated with ice water (20 mL) and the pH of the solution was adjusted to pH 10 using aq. KOH. The solution was extracted using DCM (3 × 25 mL) and the organic layers were combined, washed with saturated aq. $NaHCO_3$ (50 mL) and brine (50 mL) and then dried with anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] to yield *diethyl* N-*benzyl*-N-*[2-(benzyloxyamino)ethyl]phosphoramidate* **177a** as a yellow oil (0.54 g, 21%) [HRMS: *m/z* calculated for $C_{20}H_{30}N_2O_4P$ (MH^+) 393.1943. Found 393.1946]; ν_{max}/cm^{-1} 3408 (NH) and 1237 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.29 [6H, d, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.06 (4H, m, NCH_2 and CH_2NH), 4.05 [4H, m, $PO(OCH_2CH_3)_2$], 4.22 (2H, d, $J_{P\text{-}H}$ = 9.7 Hz, NCH_2Ph), 4.63 (2H, s, OCH_2Ph) and 7.26-7.31 (10H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 [d, $J_{P\text{-}C}$ = 7.1 Hz, $PO(OCH_2CH_3)_2$], 43.0 (d, $J_{P\text{-}C}$ = 4.0 Hz, NCH_2), 50.2 (d, $J_{P\text{-}C}$ = 5.0 Hz, CH_2NH), 53.9 (NCH_2Ph), 62.6 [d, $J_{P\text{-}C}$ = 5.7 Hz, $PO(CH_2CH_3)_2$], 76.2 (OCH_2Ph), 127.5, 127.9, 128.3, 128.4, 128.5, 128.6, 138.0 and 138.1 (Ar-C).

Diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-(3,4-dichlorobenzyl)phosphoramidate 177b*

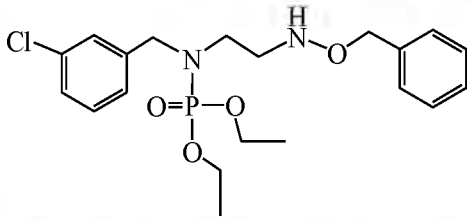


The general procedure used for the synthesis of diethyl *N*-benzyl-*N*-[2-(benzyloxyamino)-ethyl]phosphoramidate **177a** was employed, using diethyl *N*-(3,4-dichlorobenzyl)-*N*-(2-oxoethyl)phosphoramidate **176b** (1.3 g, 3.7 mmol) and *O*-benzylhydroxylamine hydrochloride (0.80 g, 5.0 mmol) and MeOH (10 mL). Sodium cyanoborohydride (0.70 g, 11 mmol), conc. HCl (2 mL) and, later, further sodium cyanoborohydride (0.26 g, 4.1 mmol) was added. Work-up and purification by flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] yielded *diethyl* N-*[2-(benzyloxyamino)ethyl]*-N-*(3,4-dichlorobenzyl)-phosphoramidate* **177b** as a yellow oil (0.65 g, 38%) [HRMS: *m/z* calculated for $C_{20}H_{28}{}^{35}Cl_2N_2O_4P$ (MH^+) 461.1164. Found 461.1165]; ν_{max}/cm^{-1} 3453 (NH) and 1241 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.30 [6H, m, $PO(OCH_2CH_3)_2$], 3.02 (4H, m, NCH_2 and CH_2NH), 4.04 [4H, m, $PO(OCH_2CH_3)_2$], 4.18 (2H, d, $J_{P\text{-}H}$ = 10.3 Hz, NCH_2Ar), 4.65 (2H, s, OCH_2Ph), 7.15 (1H, d, J = 7.9 Hz, Ar-H) and 7.26-7.37 (7H, overlapping m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 [d, $J_{P\text{-}C}$ = 7.3 Hz, $PO(OCH_2CH_3)_2$], 43.2 (d, $J_{P\text{-}H}$ = 3.8 Hz, NCH_2),

**Compound decomposes at r.t.*

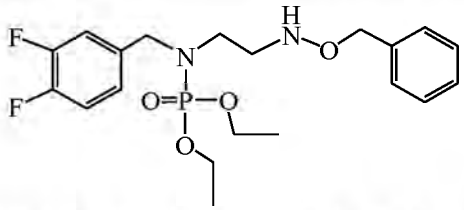
49.4 (d, $J_{P\text{-}C}$ = 5.2 Hz, CH_2NH), 50.3 (NCH_2Ar), 62.8 [d, $J_{P\text{-}C}$ = 5.7 Hz, $PO(OCH_2CH_3)_2$], 76.3 (OCH_2Ph), 128.0, 128.4, 128.5, 131.0, 130.3, 130.6, 131.5, 132.7, 137.9 and 138.8 (Ar-C).

Diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-(3-chlorobenzyl)phosphoramidate 177c*



The general procedure used for the synthesis of diethyl *N*-benzyl-*N*-[2-(benzyloxyamino)-ethyl]phosphoramidate **177a** was employed, using diethyl *N*-(3-chlorobenzyl)-*N*-(2-oxo-ethyl)phosphoramidate **176c** (1.9 g, 6.0 mmol) and *O*-benzylhydroxylamine hydrochloride (1.2 g, 7.8 mmol) and MeOH (20 mL). Sodium cyanoborohydride (1.1 g, 18 mmol), conc. HCl (2 mL) and, later, further sodium cyanoborohydride (0.42 g, 6.6 mmol) was added. Work-up and purification by flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] yielded *diethyl* N-*[2-(benzyloxyamino)ethyl]*-N-*(3-chlorobenzyl)phosphoramidate* **177c** as a yellow oil (1.0 g, 39%) [HRMS: *m/z* calculated for $C_{20}H_{29}{}^{35}ClN_2O_4P$ (MH^+) 427.1553. Found 427.1556]; ν_{max}/cm^{-1} 3452 (NH) and 1241 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.00 (2H, t, J = 6.3 Hz, NCH_2), 3.08 (2H, m, CH_2NH), 4.07 [4H, m, $PO(OCH_2CH_3)_2$], 4.20 (2H, d, $J_{P\text{-}H}$ = 10.4 Hz, NCH_2Ar), 4.65 (2H, s, OCH_2Ph), 7.19 (1H, d, J = 4.2 Hz, Ar-H), 7.24 (2H, dd, J = 2.1 and 3.8 Hz, Ar-H) and 7.28-7.34 (6H, overlapping m, Ar-H).

Diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-(3,4-difluorobenzyl)phosphoramidate 177d*¥

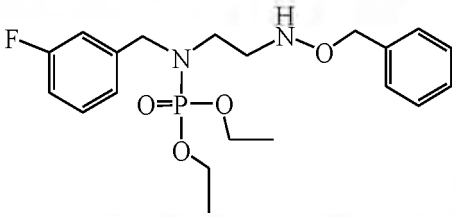


The general procedure used for the synthesis of diethyl *N*-benzyl-*N*-[2-(benzyloxyamino)-ethyl]phosphoramidate **176a** was employed, using diethyl *N*-(3,4-difluorobenzyl)-*N*-(2-oxo-ethyl)phosphoramidate **177d** (2.3 g, 7.2 mmol), *O*-benzylhydroxylamine hydrochloride (1.5 g, 9.3 mmol) and MeOH (20 mL). Sodium cyanoborohydride (1.4 g, 22 mmol), conc. HCl (3 mL) and, later, further sodium cyanoborohydride (0.50 g, 7.9 mmol) were added. Work-up

**Compound decomposes at r.t., ¥^{13}C data not cited due to decomposition*

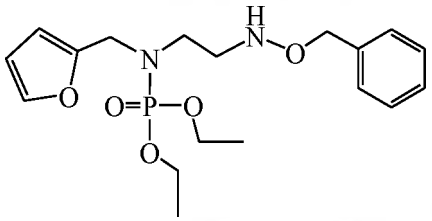
and purification by flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] yielded *diethyl* N-*[2-(benzyloxyamino)ethyl]*-N-*(3,4-difluorobenzyl)phosphoramidate* **177d** as a yellow oil (1.7 g, 57%) [HRMS: *m/z* calculated for $C_{20}H_{28}{}^{19}F_2N_2O_4P$ (MH^+) 429.1755. Found 429.1761]; ν_{max}/cm^{-1} 3463 (NH) and 1242 (P=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.33 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.97 (2H, m, NCH_2), 3.06 (2H, m, CH_2NH), 4.05 [4H, m, $PO(OCH_2CH_3)_2$], 4.17 (2H, d, J_{P-H} = 10.4 Hz, NCH_2Ar), 4.64 (2H, s, OCH_2Ph), 7.07 (2H, m, Ar-H), 7.17 (2H, m, Ar-H) and 7.29-7.33 (4H, overlapping m, Ar-H).

Diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-(3-fluorobenzyl)phosphoramidate 177e*



The general procedure used for the synthesis of diethyl *N*-benzyl-*N*-[2-(benzyloxyamino)-ethyl]phosphoramidate **177a** was employed, using diethyl *N*-(3-fluorobenzyl)-*N*-(2-oxo-ethyl)phosphoramidate **176e** (2.2 g, 7.2 mmol), *O*-benzylhydroxylamine hydrochloride (1.5 g, 9.4 mmol) and MeOH (20 mL). Sodium cyanoborohydride (1.4 g, 21 mmol), conc. HCl (2 mL) and, later, further sodium cyanoborohydride (0.50 g, 7.9 mmol) was added. Work-up and purification by flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] yielded *diethyl* N-*[2-(benzyloxyamino)ethyl]*-N-*(3-fluorobenzyl)phosphoramidate* **177e** as a brown oil (1.2 g, 42%) [HRMS: *m/z* calculated for $C_{20}H_{29}{}^{19}FN_2O_4P$ (MH^+) 411.1849. Found. 411.1862]; ν_{max}/cm^{-1} 3456 (NH) and 1243 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.01 (2H, m, NCH_2), 3.09 (2H, m, CH_2NH), 4.07 [4H, m, 2 × $PO(OCH_2CH_3)_2$], 4.22 (2H, d, J_{P-H} = 10.3 Hz, CH_2Ar), 4.64 (2H, s, OCH_2Ph), 6.96 (1H, t, J = 8.3 Hz, Ar-H), 7.07 (3H, m, Ar-H) and 7.26-7.33 (5H, overlapping m, Ar-H).

Diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-furfurylphosphoramidate 177f ¥*

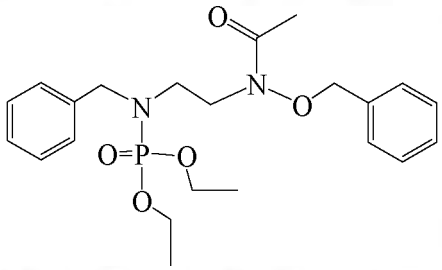


The general procedure used for the synthesis of diethyl *N*-benzyl-*N*-[2-(benzyloxyamino)-

¥*Compound decomposes precluding HRMS analysis*
**Compound decomposes at r.t. and ^{13}C data not cited due to decomposition*

ethyl]phosphoramidate **177a** was employed, using diethyl *N*-furfuryl-*N*-(2-oxoethyl)-phosphoramidate **176f** (0.50 g, 1.8 mmol), *O*-benzylhydroxylamine hydrochloride (0.38 g, 2.4 mmol) and MeOH (10 mL). Sodium cyanoborohydride (0.34 g, 5.4 mmol), conc. HCl (1 mL) and, later, further sodium cyanoborohydride (0.13 g, 2.2 mmol) was added. Work-up and purification by flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] yielded diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-furfurylphosphoramidate **177f** as a pale-yellow oil (0.24 g, 35%); ν_{max}/cm^{-1} 3466 (NH) and 1247 (P=O); δ_H/ppm (400 MHz; $CDCl_3$) 1.27 [6H, m, $PO(OCH_2CH_3)_2$], 2.98-3.19 (4H, overlapping m, NCH_2 and CH_2NH), 4.01 [4H, m, $PO(OCH_2CH_3)_2$], 4.10 (2H, d, J_{P-H} = 10.3 Hz, NCH_2Ar), 4.67 (2H, s, OCH_2Ph), 6.27 (2H, m, Ar-H) and 7.29-7.35 (6H, m, Ar-H).

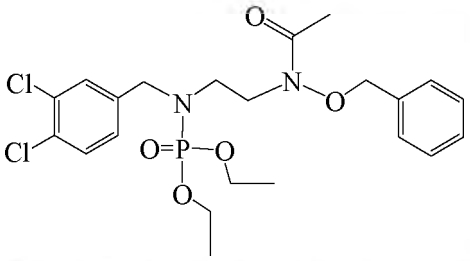
Diethyl *N*-benzyl-*N*-{2-[*N*-benzyloxy)acetamido]ethyl}phosphoramidate 178a*



Acetyl chloride (0.24 mL, 3.4 mmol) was added dropwise to a chilled, stirred solution of diethyl *N*-benzyl-*N*-[2-(benzyloxyamino)ethyl]phosphoramidate **177a** (0.66 g, 1.7 mmol) and triethylamine (0.36 mL, 2.5 mmol) in dry DCM (10 mL) under N_2. The reaction mixture was allowed to stir at 0 °C for 1 h, and then at r.t for 24 h. The solvent was removed *in vacuo* and the residue was dissolved in diethyl ether (15 mL). The solution was washed sequentially with aq. K_2CO_3, 0.5M-HCl and water. The organic solution was dried with anhydrous $MgSO_4$ and the solvent evaporated *in vacuo* to yield *diethyl* N-*benzyl*-N-*{2-[*N-*(benzyloxy)acetamido]ethyl}phosphoramidate* **178a** as a yellow oil (0.38 g, 52%) [HRMS: *m/z* calculated for $C_{22}H_{32}N_2O_5P$ (MH^+) 435.2049. Found 435.2046]; ν_{max}/cm^{-1} 1662 (C=O) and 1245 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 [6H, t, J = 6.7 Hz, $PO(OCH_2CH_3)_2$], 2.04 (3H, s, CH_3CO), 3.14 (2H, m, NCH_2), 3.71 (2H, m, CH_2NHCO), 4.04 [4H, m, $PO(OCH_2CH_3)_2$], 4.23 (2H, d, J_{P-H} = 9.1 Hz, NCH_2Ph), 4.75 (2H, s, OCH_2Ph) and 7.27-7.36 (10H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 [d, J_{P-C} = 7.2 Hz, $PO(OCH_2CH_3)_2$], 20.5 (CH_3CO), 42.0 (NCH_2), 43.6 (CH_2NCO), 50.0 (NCH_2Ph), 62.6 [d, J_{P-C} = 5.7 Hz, $PO(OCH_2CH_3)_2$], 76.4 (OCH_2Ph), 127.6, 128.6, 128.7, 128.8, 129.1, 129.3, 134.4 and 137.9 (Ar-C) and 172.4 (C=O).

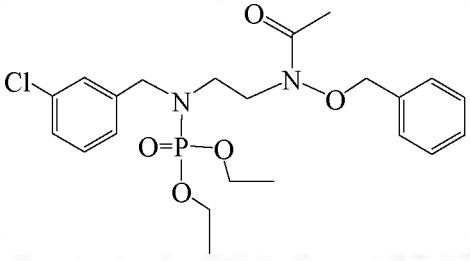
**Compound decomposes at r.t.*

Diethyl *N*-{2-[*N*-(benzyloxy)acetamido]ethyl}-*N*-(3,4-dichlorobenzyl)phosphoramidate 178b*



The general procedure used for the synthesis of diethyl *N*-benzyl-*N*-{2-[*N*-(benzyloxy)-acetamido]ethyl}phosphoramidate **178a** was employed, using diethyl *N*-[2-(benzyloxy-amino)ethyl]-*N*-(3,4-dichlorobenzyl)phosphoramidate **177b** (0.49 g, 1.1 mmol), acetyl chloride (0.15 mL, 2.1 mmol) and triethylamine (0.22 mL, 1.6 mmol) in dry DCM (10 mL). Work-up afforded *diethyl* N-*{2-[*N*-(benzyloxy)acetamido]ethyl}*-N-*(3,4-dichlorobenzyl)-phosphoramidate* **178b** as a yellow oil (0.32 g, 61%) [HRMS: *m/z* calculated for $C_{22}H_{30}{}^{35}Cl_2N_2O_5P$ (MH^+) 503.1269. Found 503.1274]; ν_{max}/cm^{-1} 1652 (C=O) and 1243 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.29 [6H, m, $PO(OCH_2CH_3)_2$], 2.05 (3H, s, CH_3CO), 3.09 (2H, m, NCH_2), 3.68 (2H, m, CH_2NCO), 4.02 [4H, m, $PO(OCH_2CH_3)_2$], 4.16 (2H, d, J_{P-H} = 10.1 Hz, NCH_2Ar), 4.77 (2H, s, OCH_2Ph), 7.16 (1H, s, Ar-H) and 7.26-745 (7H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.2 Hz, $PO(OCH_2CH_3)_2$], 20.5 (CH_3CO), 42.0 (NCH_2), 43.6 (CH_2NCO), 48.9 (NCH_2Ar), 62.7 [d, J_{P-C} = 5.7 Hz, $PO(OCH_2CH_3)_2$], 76.6 (OCH_2Ph), 128.0, 128.5, 128.8, 129.1, 129.3, 130.5, 131.5, 132.6, 134.2 and 138.4 (Ar-C) and 172.5 (C=O).

Diethyl *N*-{2-[*N*-(benzyloxy)acetamido]ethyl}-*N*-(3-chlorobenzyl)phosphoramidate 178c*

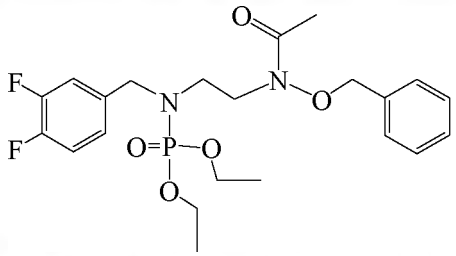


The general procedure used for the synthesis of *N*-benzyl-*N*-{2-[*N*-(benzyloxy)acetamido]-ethyl}phosphoramidate **178a** was employed, using diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-(3-chlorobenzyl)phosphoramidate **177c** (0.61 g, 1.4 mmol), acetyl chloride (0.20 mL, 2.9 mmol) and triethylamine (0.30 mL, 2.1 mmol) in dry DCM (10 mL). Work-up afforded *diethyl* N-*{2-[*N*-(benzyloxy)acetamido]ethyl}*-N-*(3-chlorobenzyl)phosphoramidate* **178c** as a yellow oil (0.57 g, 86%) [HRMS: *m/z* calculated for $C_{22}H_{31}{}^{35}ClN_2O_5P$ (MH^+) 469.1659.

**Compound decomposes at r.t.*

Found 469.1661]; ν_{max}/cm^{-1} 1660 (C=O) and 1243 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.31 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.06 (3H, s, CH_3CO), 3.14 (2H, m, NCH_2), 3.71 (2H, m, CH_2NCO), 4.04 [4H, m, $PO(OCH_2CH_3)_2$], 4.20 (2H, d, J_{P-H} = 10.4 Hz, NCH_2Ar), 4.78 (2H, s, OCH_2Ph), 7.22-7.26 (3H, overlapping m, Ar-H) and 7.33-7.37 (6H, overlapping m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 [d, J_{P-C} = 7.2 Hz, $PO(OCH_2CH_3)_2$], 20.5 (CH_3CO), 42.3 (NCH_2), 43.8 (CH_2NCO), 49.7 (NCH_2Ar), 62.7 [d, J_{P-C} = 5.8 Hz, $PO(OCH_2CH_3)_2$], 76.7 (OCH_2Ph), 126.5, 126.7, 127.7, 128.6, 128.8, 129.1, 129.3, 129.8, 134.5 and 140.2 (Ar-C) and 172.7 (C=O).

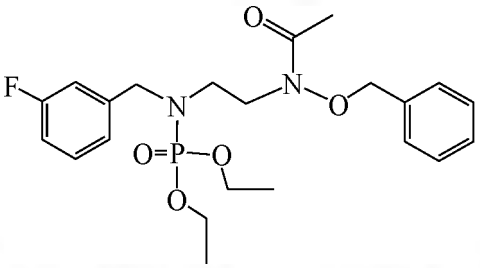
Diethyl *N*-{2-[*N*-(benzyloxy)acetamido]ethyl}-*N*-(3,4-difluorobenzyl)phosphoramidate 178d*



The general procedure used for the synthesis of *N*-benzyl-*N*-{2-[*N*-(benzyloxy)acetamido]-ethyl}phosphoramidate **178a** was employed, using diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-(3,4-difluorobenzyl)phosphoramidate **177d** (1.0 g, 2.4 mmol), acetyl chloride (0.35 mL, 4.9 mmol) and triethylamine (0.51 mL, 3.7 mmol) in dry DCM (10 mL). Work-up afforded *diethyl* N-*{2-[*N*-(benzyloxy)acetamido]ethyl}*-N-*(3,4-difluorobenzyl)phosphoramidate* **178d** as a yellow oil (0.32 g, 61%) [HRMS: *m/z* calculated for $C_{22}H_{30}{}^{19}F_2N_2O_5P$ (MH^+) 471.1860. Found 471.1869]; ν_{max}/cm^{-1} 1659 (C=O) and 1239 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.31 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.06 (3H, s, CH_3CO), 3.11 (2H, m, NCH_2), 3.69 (2H, m, CH_2NCO), 4.04 [4H, m, $PO(OCH_2CH_3)_2$], 4.17 (2H, d, J_{P-H} = 10.5 Hz, NCH_2Ar), 4.79 (2H, s, OCH_2Ph), 7.06 (2H, m, Ar-H), 7.20 (1H, m, Ar-H) and 7.33-7.38 (5H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 [d, J_{P-C} = 7.1 Hz, $PO(OCH_2CH_3)_2$], 20.5 (CH_3CO), 42.2 (NCH_2), 43.7 (CH_2NCO), 49.3 (NCH_2Ar), 62.8 [d, J_{P-C} = 5.7 Hz, $PO(OCH_2CH_3)_2$], 76.7 (OCH_2Ph), 116.9 (d, J_{F-C} = 17.5 Hz), 117.3 (d, J_{F-C} = 16.9 Hz), 124.6 (d, J_{F-C} = 12.1 Hz), 128.9, 129.2, 129.3, 134.4, 135.2, 148.5 (dd, J_{F-C} = 12.7 and 42.0 Hz) and 151.8 (dd, J_{F-C} = 12.6 and 42.8 Hz) (Ar-C) and 172.5 (C=O).

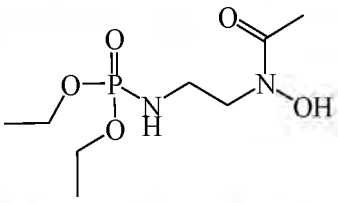
**Compound decomposes at r.t.*

Diethyl *N*-{2-[*N*-(benzyloxy)acetamido]ethyl}-*N*-(3-fluorobenzyl)phosphoramidate 178e*



The general procedure used for the synthesis of *N*-benzyl-*N*-{2-[*N*-(benzyloxy)acetamido]-ethyl}phosphoramidate **178a** was employed, using diethyl *N*-[2-(benzyloxyamino)ethyl]-*N*-(3-fluorobenzyl)phosphoramidate **177e** (0.60 g, 1.5 mmol), acetyl chloride (0.21 mL, 2.9 mmol) and triethylamine (0.31 mL, 2.2 mmol) in dry DCM (5 mL). Work-up afforded *diethyl* N-*{2-[*N-*(benzyloxy)acetamido]ethyl}*-N-*(3-fluorobenzyl)phosphoramidate* **178e** as a yellow oil (0.47 g, 72%) [HRMS: *m/z* calculated for $C_{22}H_{31}{}^{19}FN_2O_5P$ (MH^+) 453.1955. Found 453.1957]; ν_{max}/cm^{-1} 1661 (C=O) and 1243 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.31 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.06 (3H, s, CH_3CO), 3.15 (2H, m, NCH_2), 3.71 (2H, m, CH_2NCO), 4.04 [4H, m, $PO(OCH_2CH_3)_2$], 4.22 (2H, d, J_{P-H} = 10.3 Hz, NCH_2Ar), 4.78 (2H, s, OCH_2Ph), 6.96 (1H, t, J = 9.4 Hz, Ar-H), 7.10 (2H, t, J = 9.9 Hz, Ar-H) and 7.25-7.37 (6H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 [d, J_{P-C} = 7.1 Hz, $PO(OCH_2CH_3)_2$], 20.5 (CH_3CO), 42.3 (NCH_2), 43.8 (CH_2NCO), 49.7 (NCH_2Ar), 62.7 [d, J_{P-C} = 5.8 Hz, $PO(OCH_2CH_3)_2$], 76.6 (OCH_2Ph), 114.5 (d, J_{F-C} = 21.1 Hz), 115.3 (d, J_{F-C} = 21.6 Hz), 124.2 (d, J_{F-C} = 2.7 Hz), 128.8, 129.3, 130.1 (d, J_{F-C} = 8.2 Hz), 134.4, 140.8 (dd, J_{F-C} = 2.7 and 6.8 Hz), 162.3 and 163.9 (d, J_{F-C} = 240.0 Hz), (Ar-C) and 172.5 (C=O).

Diethyl *N*-{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate 179*

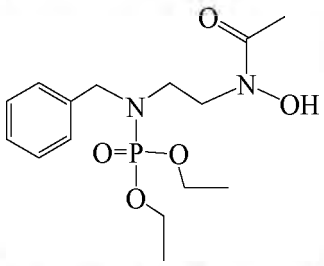


A solution of diethyl *N*-benzyl-*N*-{2-[*N*-(benzyloxy)acetamido]ethyl}phosphoramidate **178a** (0.12 g, 0.28 mmol) in dry MeOH (2 mL) was added to a dispersion of 10% Pd/C (0.28 g) in dry MeOH (1 mL) saturated with H_2, and the mixture was stirred overnight at r.t. The mixture was filtered through celite pad and the filtrate was concentrated *in vacuo* to afford *diethyl* N-*{2-(*N-*hydroxyacetamido)ethyl}phosphoramidate* **179** as a pale-yellow oil (0.063 g, 90%) [HRMS: *m/z* calculated for $C_8H_{20}N_2O_5P$ (MH^+) 255.1110. Found 255.1103]; ν_{max}/cm^{-1}

**Compound decomposes at r.t.*

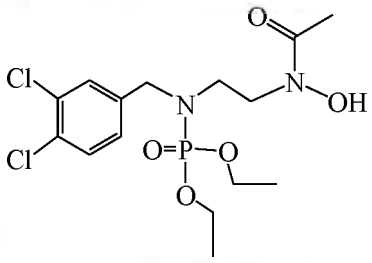
3222 (OH), 1623 (C=O) and 1209 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.28 [6H, m, $PO(OCH_2CH_3)_2$], 2.15 (3H, s, CH_3CO), 3.18 (2H, m, NCH_2), 3.67 (2H, m, CH_2NCO), 3.84 (1H, s, NH), 3.99 [4H, m, $PO(OCH_2CH_3)_2$] and 9.70 (1H, s, OH); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.1 Hz, $PO(OCH_2CH_3)_2$], 20.7 (CH_3CO), 37.9 (NCH_2), 48.3 (CH_2NCO), 63.1 [d, J_{P-C} = 5.7 Hz, $PO(OCH_2CH_3)_2$] and 173.2 (C=O).

Diethyl *N*-benzyl-*N*-{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate 180a*



The general procedure used for the synthesis of diethyl *N*-{2-(*N*-hydroxyacetamido)ethyl}-phosphoramidate **179** was employed, using diethyl *N*-benzyl-*N*-{2-[*N*-(benzyloxy)-acetamido]ethyl}phosphoramidate **178a** (0.12 g, 0.28 mmol) and Pd/C (10%, 0.28 g) in dry MeOH (2 mL), and the mixture was stirred at r.t for 5 h. Work-up afforded *diethyl* N-*benzyl*-N-*{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate* **180a** as a yellow oil (0.090 g, 91%) [HRMS: *m/z* calculated for $C_{15}H_{26}N_2O_5P$ (MH^+) 345.1579. Found 345.1584]; ν_{max}/cm^{-1} 3160 (OH) and 1634 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.34 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.18 (3H, s, CH_3CO), 3.19 (2H, m, NCH_2), 3.68 (2H, m, CH_2NCO), 4.05 [4H, m, $PO(OCH_2CH_3)_2$], 4.12 (2H, d, J_{P-H} = 9.3 Hz, NCH_2Ph), 7.31 (3H, m, Ar-H) and 7.35 (2H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.3 Hz, $PO(OCH_2CH_3)_2$], 20.9 (CH_3CO), 41.0 (d, J_{P-C} = 5.2 Hz, NCH_2), 43.3 (CH_2NCO), 49.1 (d, J_{P-C} = 3.6 Hz, NCH_2Ph), 63.3 [d, J_{P-C} = 6.1 Hz, $PO(OCH_2CH_3)_2$], 127.9, 128.5, 128.8 and 136.8 (Ar-C) and 172.9 (C=O).

Diethyl *N*-(3,4-dichlorobenzyl)-*N*-{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate 180b*

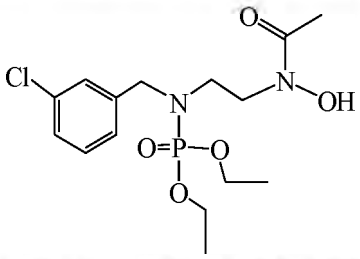


The procedure described for the synthesis of diethyl *N*-benzyl-*N*-{2-(*N*-hydroxyacetamido)-ethyl}phosphoramidate **180a** was employed, using diethyl *N*-{2-[*N*-(benzyloxy)acetamido]

**Compound decomposes at r.t.*

ethyl}-*N*-(3,4-dichlorobenzyl)phosphoramidate **178b** (0.10 g, 0.21 mmol) and Pd/C (10 %, 0.21 g) in dry MeOH (2 mL). The solvent was evaporated *in vacuo* to yield *diethyl* N-*(3,4-dichlorobenzyl)*-N-*{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate* **180b** as a brown oil (0.084 g, 95%) [HRMS: *m/z* calculated for $C_{15}H_{24}{}^{35}Cl_2N_2O_5P$ (MH^+) 413.0800. Found 413.0805]; ν_{max}/cm^{-1} 3215 (OH) and 1645 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.34 [6H, t, *J* = 7.2 Hz, $PO(OCH_2CH_3)_2$], 2.18 (3H, s, CH_3CO), 3.18 (2H, m, NCH_2), 3.69 (2H, m, CH_2NCO), 4.03-4.13 [6H, overlapping m, $PO(OCH_2CH_3)_2$ and NCH_2Ar], 4.87 (1H, br s, OH), 7.15 (1H, d, *J* = 7.9 Hz, Ar-H) and 7.33 (2H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.3 Hz, $PO(OCH_2CH_3)_2$], 20.9 (CH_3CO), 41.2 (d, J_{P-C} = 5.4 Hz, NCH_2), 43.1 (CH_2NCO), 48.2 (d, J_{P-C} = 3.9 Hz, NCH_2Ar), 63.3 [d, J_{P-C} = 6.2 Hz, 2 × $POCH_2CH_3)_2$], 127.9, 128.5, 130.3, 130.8, 132.0 and 133.0 (Ar-C) and 172.9 (C=O).

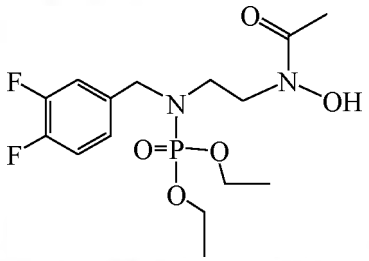
Diethyl *N*-(3-chlorobenzyl)-*N*-{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate 180c*



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-{2-(*N*-hydroxyacetamido)-ethyl}phosphoramidate **180a** was employed, using diethyl *N*-{2-[*N*-(benzyloxy)acetamido]-ethyl*}-N*-(3-chlorobenzyl)phosphoramidate **178c** (0.15 g, 0.32 mmol) and Pd/C (10 %, 0.32 g) in dry MeOH (2 mL). The solvent was evaporated *in vacuo* to yield *diethyl* N-*(3-chloro-benzyl)*-N-*{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate* **180c** as a yellow oil (0.12 g, 97%) [HRMS: *m/z* calculated for $C_{15}H_{25}{}^{35}ClN_2O_5P$ (MH^+) 379.1190. Found 379.1192]; ν_{max}/cm^{-1} 3465 (OH) and 1661 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.33 [6H, t, *J* = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.18 (3H, s, CH_3CO), 3.19 (2H, m, NCH_2), 3.68 (2H, m, CH_2CO), 4.04 [4H, m, $PO(OCH_2CH_3)_2$], 4.10 (2H, d, J_{P-H} = 8.9 Hz, NCH_2Ar) and 7.29-7.36 (4H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.2 Hz, $PO(OCH_2CH_3)_2$], 20.8 (CH_3CO), 41.1 (d, J_{P-C} = 5.2 Hz, NCH_2), 43.5 (CH_2NCO), 49.1 (d, J_{P-C} = 3.4 Hz, NCH_2Ar), 63.3 [d, J_{P-C} = 6.0 Hz, $PO(OCH_2CH_3)_2$], 127.9, 128.5, 128.8, 128.9, 129.9 and 136.7 (Ar-C) and 172.9 (C=O).

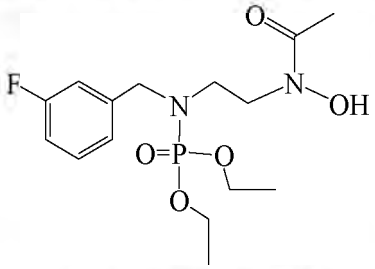
**Compound decomposes at r.t.*

Diethyl *N*-(3,4-difluorobenzyl)-*N*-{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate 180d*



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-{2-(*N*-hydroxyacetamido)-ethyl}phosphoramidate **180a** was employed, using diethyl *N*-{2-[*N*-(benzyloxy)acetamido]-ethyl}-*N*-(3,4-difluorobenzyl)phosphoramidate **178d** (0.11 g, 0.23 mmol) and Pd/C (10 %, 0.23 g) in dry MeOH (2 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*(3,4-difluorobenzyl)*-N-*{2-(*N-*hydroxyacetamido)-ethyl}phosphoramidate* **180d** as a brown oil (0.079 g, 89%) [HRMS: *m/z* calculated for $C_{15}H_{24}{}^{19}F_2N_2O_5P$ (MH^+) 381.1391. Found 381.1396]; ν_{max}/cm^{-1} 3170 (OH) and 1639 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 1.34 [6H, t, J = 6.9 Hz, $PO(OCH_2CH_3)_2$], 2.17 (3H, s, CH_3CO), 3.14 (2H, m, NCH_2), 3.68 (2H, m, CH_2NCO), 4.00-4.09 [6H, overlapping m, $PO(OCH_2CH_3)_2$ and NCH_2Ar], 7.03 (1H, s, Ar-H) and 7.08-7.19 (2H, overlapping m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.1 Hz, $PO(OCH_2CH_3)_2$], 20.8 (CH_3CO), 41.1 (d, J_{P-C} = 5.1 Hz, NCH_2), 43.2 (CH_2NCO), 48.3 (d, J_{P-C} = 3.6 Hz, NCH_2Ar), 63.5 [d, J_{P-C} = 6.1 Hz, $PO(OCH_2CH_3)_2$], 117.1 (d, J_{F-C} = 17.2 Hz), 117.6 (d, J_{F-C} = 16.8 Hz), 124.5 (dd, J_{F-C} = 3.4 and 5.9 Hz), 134.1 (d, J_{F-C} = 3.8 Hz), 148.7 (dd, J_{F-C} = 12.0 and 246.8 Hz) and 152.0 (dd, J_{F-C} = 12.8 and 247.5 Hz) (Ar-C) and 172.9 (C=O).

Diethyl *N*-(3-fluorobenzyl)-*N*-{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate 180e*



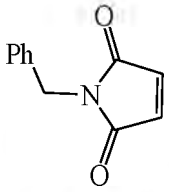
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-{2-(*N*-hydroxyacetamido)-ethyl}phosphoramidate **180a** was employed, using diethyl *N*-{2-[*N*-(benzyloxy)acetamido]-ethyl}-*N*-(3-fluorobenzyl)phosphoramidate **178e** (0.13 g, 0.28 mmol) and Pd/C (10 %, 0.28 g) in dry MeOH (2 mL). The solvent was evaporated *in vacuo* to yield *diethyl* N-*(3-fluoro-benzyl)*-N-*{2-(*N-*hydroxyacetamido)ethyl}phosphoramidate* **180e** as a yellow oil (0.092 g,

**Compound decomposes at r.t.*

90%) [HRMS: *m/z* calculated for $C_{15}H_{25}{}^{19}FN_2O_5P$ (MH^+) 363.1485. Found 363.1482]; ν_{max}/cm^{-1} 3160 (OH) and 1634 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.33 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.16 (3H, s, CH_3CO), 3.18 (2H, m, NCH_2), 3.69 (2H, m, CH_2NCO), 4.04 (4H, m, 2 × $POCH_2CH_3$), 4.10 (2H, d, J_{P-H} = 9.8 Hz, NCH_2Ar), 6.97 (1H, t, J = 8.4 Hz, Ar-H), 7.05 (2H, m, Ar-H), 7.30 (1H, dd, J_{H-H} = 7.8 and J_{F-H} = 13.9 Hz, Ar-H) and 9.41 (1H, s, OH); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.2 Hz, $PO(OCH_2CH_3)_2$], 20.8 (CH_3CO), 41.2 (d, J_{P-C} = 5.2 Hz, NCH_2), 43.2 (CH_2NCO), 48.6 (d, J_{P-C} = 3.2 Hz, NCH_2Ar), 63.4 (d, J_{P-C} = 6.1 Hz, 2 × $POCH_2CH_3$), 114.8 (d, J_{F-C} = 21.2 Hz), 115.2 (d, J_{F-C} = 21.7 Hz), 124.1 (d, J_{F-C} = 2.8 Hz), 130.3 (d, J_{F-C} = 8.2 Hz), 139.6 (dd, J_{F-C} = 2.8 and 6.8 Hz), 162.4 (d, J_{F-C} = 244.5 Hz) (Ar-C) and 172.8 (C=O).

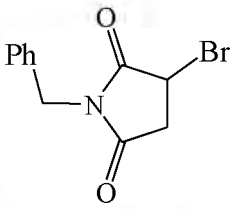
3.5. SYNTHESIS of *N*-BENZYLATED CONFORMATIONALLY CONSTRAINED Mg^{2+} CHELATING LIGANDS

_N_-benzylmaleimide 184[233]



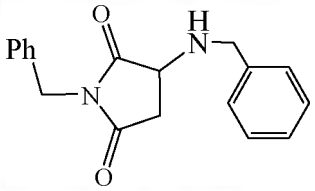
To a two-necked flask containing a solution of maleic anhydride **182** (2.0 g, 20 mmol) in dry benzene (20 mL) was added anhydrous $ZnCl_2$ (2.8 g, 20 mmol), and the mixture was refluxed at 70 °C under N_2. After 20 min, benzylamine **163a** (2.2 mL, 20 mmol) was added and the mixture was boiled for 1 h. A solution of hexamethyldisilazane (6.4 mL, 31 mmol) in dry benzene (5 mL) was added dropwise to the reaction mixture which was refluxed for 2 h. The mixture was allowed to cool and then washed with 0.5M-HCl and extracted with EtOAc (3 × 25 mL). The organic layers were combined, washed with saturated aq. $NaHCO_3$ and brine, and then dried with anhydrous $MgSO_4$, filtered and the solvent was evaporated *in vacuo* to give *N*-benzylmaleimide **184** as a brown solid (3.5 g, 93%), m.p. 66-68 °C (Lit.[233] 68-70 °C); ν_{max}/cm^{-1} 1688 (C=O); δ_H/ppm (600 MHz; DMSO-d_6) 4.58 (2H, s, NCH_2Ph), 7.02 (2H, s, 2 × CH), 7.19 (2H, d, J = 7.4 Hz, Ar-H), 7.25 (1H, t, J = 7.3 Hz, Ar-H) and 7.31 (2H, t, J = 7.5 Hz, Ar-H); δ_C/ppm (75 MHz; DMSO-d_6) 40.7 (NCH_2Ph), 127.4 (2 × CH), 127.7, 128.9, 134.9 and 136.9 (Ar-C) and 171.2 (2 × C=O).

1-Benzyl-3-bromopyrrolidine-2,5-dione 185[252]



Acetyl bromide (0.40 mL, 5.3 mmol) was slowly added to a solution of dry methanol (0.22 mL, 5.3 mmol) in dry DCM (10 mL) cooled in an ice-bath. After 1 h, *N*-benzylmaleimide **184** (1.0 g, 5.3 mmol) was added and the mixture left to stir at r.t. for 24 h. The crude mixture was washed with 5% aq. $NaHCO_3$ and brine. The solvent was evaporated from the organic layer *in vacuo* to afford 1-benzyl-3-bromopyrrolidine-2,5-dione **185** as a cream solid (1.1 g, 76%), m.p. 40-43 °C (Lit.[252] melting point not cited); ν_{max}/cm^{-1} 1698 (C=O); δ_H/ppm (600 MHz; DMSO-d_6) 3.02 (1H, dd, J = 3.1 and 19.0 Hz, CH_aCHBr), 3.51 (1H, m, CH_bCHBr), 4.59 (2H, s, NCH_2Ph), 5.03 (1H, dd, J = 3.1 and 8.3 Hz, CHBr), 7.23 (2H, d, J = 7.0 Hz, Ar-H), 7.33 (1H, t, J = 7.3 Hz, Ar-H) and 7.33 (2H, t, J = 7.3 Hz, Ar-H); δ_C/ppm (150 MHz; DMSO-d_6) 37.6 (CH_2CHBr), 39.73 (NCH_2Ph), 42.2 (CHBr), 127.7, 128.0, 129.0 and 135.9 (Ar-C) and 174.3 and 174.5 (2 × s, C=O).

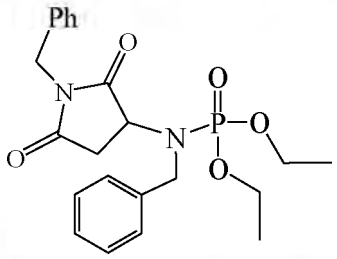
1-Benzyl-3-(benzylamino)pyrrolidine-2,5-dione 186



Anhydrous K_2CO_3 (0.30 g, 2.2 mmol) was added to a benzylamine **163a** (0.24 mL, 2.2 mmol) in dry acetonitrile (5 mL). After stirring for 20 min, 1-benzyl-3-bromopyrrolidine-2,5-dione **185** (0.60 g, 2.2 mmol) was added dropwise and and the mixture stirred at r.t. for 4 h. Ethyl acetate was added and the resulting solution was washed with water; the aqueous washings were extracted with EtOAc and the organic solutions were combined, dried with anhydrous $MgSO_4$ and filtered. The solvent was evaporated *in vacuo* to afford *1-benzyl-3-(N-benzylamino)pyrrolidine-2,5-dione* **186** as a brown solid (0.58 g, 88%), m.p. 38-40 °C [HRMS: *m/z* calculated for $C_{18}H_{19}N_2O_2$ (MH^+) 295.1447. Found 295.1445]; ν_{max}/cm^{-1} 3290 (NH) and 1689 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 2.06 (1H, s, NH), 2.39 (1H, dd, J = 5.0 and 17.9 Hz, CH_aCHNH), 3.51 (1H, dd, J = 8.3 and 17.9 Hz, CH_bCHNH), 3.62 (1H, dd, J = 5.0 and 8.3 Hz, CH), 3.74 (2H, d, J = 4.9 Hz, $NHCH_2Ph$), 4.54 (2H, s, NCH_2Ph) and 7.13-

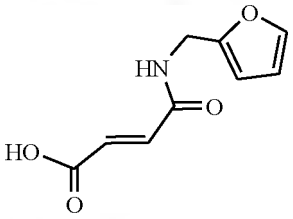
7.28 (10H, overlapping m, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 36.4 (CH_2CHNH), 42.4 (NCH_2Ph), 51.8 ($NHCH_2Ph$), 55.5 (CH), 127.6, 128.1, 128.3, 128.6, 128.7, 128.8, 135.6 and 138.8 (Ar-C) and 174.9 and 177.7 (2 × s, C=O).

Diethyl *N*-benzyl-*N*-(1-benzyl-2,5-dioxopyrrolidin-3-yl)phosphoramidate 183



A mixture of 1-benzyl-3-(*N*-benzylamino)pyrrolidine-2,5-dione **186** (0.40 g, 1.3 mmol), triethylamine (0.20 mL, 1.3 mmol) and diethyl chlorophosphate (0.20 mL, 1.3 mmol) in DCM (5 mL) was stirred in a stoppered flask at r.t. overnight. The mixture was washed with 10% HCl and water (2 × 10 mL). The organic layer was dried with anhydrous $MgSO_4$, and filtered, and the solvent was removed *in vacuo*. The crude mixture was flash chromatographed [on silica gel; elution with EtOAc-hexane (6:4)] to afford d*iethyl* N-*benzyl*-N-*(1-benzyl-2,5-dioxopyrrolidin-3-yl)phosphoramidate* **183** as a yellow oil (0.36 g, 62%) [HRMS: *m/z* calculated for $C_{22}H_{28}N_2O_5P$ (MH^+) 431.1736. Found 431.1725]; ν_{max}/cm^{-1} 1704 (C=O) and 1249 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.34 (6H, t, J = 7.2 Hz, 2 × CH_3), 2.63 (2H, m, CH_2CHN), 4.16 (6H, m, 2 × OCH_2CH_3 and $PNCH_2Ph$), 4.25 (1H, m, CH), 4.62 (2H, 2 × d, J = 18.0 and 18.0 Hz, NCH_2Ph), 7.23-7.30 (8H, overlapping m, Ar-H) and 7.37 (2H, d, J = 7.4 Hz, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 16.3 (dd, J = 3.8 and 7.1 Hz, 2 × CH_3), 35.8 (CH_2CHN), 42.5 (NCH_2Ph), 51.3 ($PONCH_2Ph$), 55.6 (CH), 63.1 (dd, J = 3.8 and 5.5 Hz, 2 × OCH_2CH_3), 128.0, 128.1, 128.4, 128.7, 128.9, 135.7, 137.1 and 137.2 (Ar-C), 174.0 and 176.2 (2 × s, C=O).

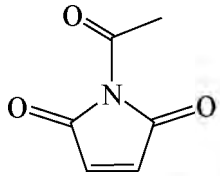
(2*Z*)-3-(*N*-Furfurylcarbamoyl)propenoic acid 191



Maleic anhydride **182** (1.0 g, 10 mmol) was dissolved in acetic acid (10 mL), and furfurylamine **163f** (0.90 mL, 10 mmol) was added dropwise and the mixture refluxed at 100

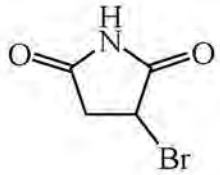
°C for 5 h. The crude mixture was diluted with EtOAc (15 mL) and washed with saturated aq. $NaHCO_3$ (2 × 25 mL) and water (2 × 25 mL). The solvent was removed from the organic solution *in vacuo* to afford (2*Z*)-3-(*N*-furfurylcarbamoyl)propenoic acid **191** as a yellow solid (1.5 g, 81%), m.p. 94-96 °C (Lit.[252] 113-114 °C) [HRMS: *m/z* calculated for $C_9H_{10}NO_4$ (MH^+) 196.0610. Found 196.0605]; ν_{max}/cm^{-1} 3242 (OH) and 1699 and 1628 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 4.53 (2H, d, J = 5.7 Hz, NCH_2Ph), 6.28 (1H, d, J = 12.8 Hz, C*H*=CH), 6.31 (2H, m, Ar-H), 6.50 (1H, d, J = 12.8 Hz, CH=C*H*), 7.36 (1H, s, Ar-H) and 8.37 (1H, br s, NH); δ_C/ppm (100 MHz; $CDCl_3$) 37.2 (NCH_2Ar), 108.9 (Ar-C), 110.8 and 132.0 (2 × s, *C*H=*C*H), 136.1, 142.9 and 149.2 (Ar-C) and 166.2 and 166.3 (2 × s, C=O).

1-Acetylmaleimide 196[253]



The procedure described for the synthesis of (2*Z*)-3-(*N*-furfurylcarbamoyl)propenoic acid **191** was employed, using maleic anhydride **182** (1.0 g, 10 mmol) and acetamide (0.60 g, 10 mmol) in acetic acid (10 mL). After refluxing for 24 h, work-up of the crude mixture afforded 1-acetylmaleimide **196** as a brown oil (1.3 g, 88%); ν_{max}/cm^{-1} 1653 (C=O); δ_H/ppm (600 MHz; DMSO-d_6) 1.89 (3H, s, CH_3CO) and 6.23 (2H, s, 2 × CH); δ_C/ppm (100 MHz; $CDCl_3$) 22.7 (*C*H_3CO), 131.3 (2 × CH) and 163.1 (2 × C=O).

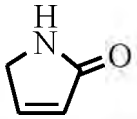
3-Bromopyrrolidine-2,5-dione 199[254]



Maleimide **189** (0.50 g, 5.2 mmol) was added to a stirred solution of HCl in MeOH [generated by the cautions addition of acetyl bromide (0.76 mL, 10 mmol) to ice-cold MeOH (0.42 mL, 10 mmol) with stirring] in dry DCM (5 mL) under N_2. The reaction mixture was stirred overnight at r.t. and the solvent was evaporated *in vacuo* to afford 3-bromo-pyrrolidine-2,5-dione **199** as a cream solid (0.89 g, 97%), m.p. 40-42 °C; ν_{max}/cm^{-1} 3503 (NH) and 1697 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 3.00 (1H, m, CH_aCO), 3.40 (1H, m,

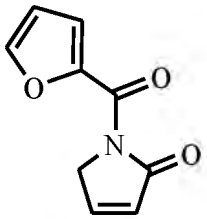
CH_bCO), 4.63 (1H, m, CH) and 6.50 (1H, br s, NH); δ_C/ppm (100 MHz; $CDCl_3$) 37.4 (CH_2CO), 45.8 (CH) and 175.0 and 176.1 (2 × s, C=O).

2-Oxo-3-pyrroline 205[217,218]



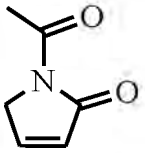
30% H_2O_2 (10 mL) was added to a mixture of pyrrole **201** (5.2 mL, 75 mmol) and $BaCO_3$ (1.6 g, 7.8 mmol) in water (300 mL). After refluxing at 100 °C for 5 h, the solution was allowed to cool to r.t., and PbO_2 was added (to quench excess H_2O_2) until effervescence ceased. The mixture was filtered and the filtrate concentrated *in vacuo* to afford the crude product. Dioxane (50 mL) was added, and the mixture was filtered and residue was washed sequentially with dioxane. The solvent was removed *in vacuo* to give 2-oxo-3-pyrroline **205** as a yellow oil (2.0 g, 32%); ν_{max}/cm^{-1} 3245 (NH) and 1653 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 4.06 (2H, dd, J = 1.8 and 3.1 Hz, CH_2NH), 6.16 (1H, m, $CHCH_2$), 7.16 (1H, m, CHCO) and 7.52 (1H, br s, NH); δ_C/ppm (75 MHz; $CDCl_3$) 49.1 (CH_2NH), 127.8 (*C*HCO), 146.1 ($CHCH_2$) and 175.6 (C=O).

1-(2-Furoyl)-2-oxo-3-pyrroline 206



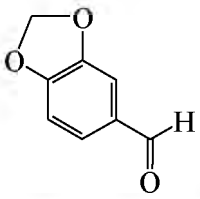
A solution of 2-oxo-3-pyrroline **205** (0.24 g, 2.9 mmol) in dry DCM (5 mL) containing triethylamine (0.80 mL, 5.7 mmol) was cooled in an ice-bath. 2-Furoyl chloride (0.56 mL, 5.7 mmol) was added to the stirred solution and, after 24 h the mixture was diluted with DCM (10 mL) and washed with water (15 mL). After drying the organic layer with anhydrous $MgSO_4$, the solvent was removed *in vacuo* and the residue was flash chromatographed [on silica gel; elution with EtOAc-hexane (6:4)], to afford 1-(2-furoyl)-2-oxo-3-pyrroline **206** as a yellow oil (0.21 g, 40%); ν_{max}/cm^{-1} 1654 (C=O); δ_H/ppm (300 MHz; $CDCl_3$) 4.30 (2H, t, J = 2.6 Hz, CH_2CH), 6.34 (1H, dt, J = 1.9 and 6.4 Hz, $CHCH_2$), 6.45 (3H, m, CHCO and Ar-H) and 7.32 (1H, s, Ar-H); δ_C/ppm (75 MHz; $CDCl_3$) 50.9 (CH_2CH), 111.6 (Ar-H), 113.7 (Ar-H), 127.8 (*C*HCO), 139.0 ($CHCH_2$), 146.3 and 147.1 (Ar-H), 170.1 and 170.6 (2 × s, C=O).

1-Acetyl-2-oxo-3-pyrroline 208[221]



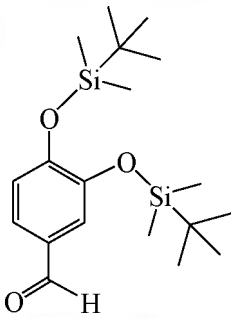
2*H*-pyrrol-2-one **205** (0.60 g, 7.2 mmol) and DMAP (0.35 g, 2.9 mmol) was dissolved in acetonitrile (5 mL) and stirred for 20 mins., acetic anhydride (1.04 mL, 10.8 mmol) was added dropwise and the reaction mixture was stirred at r.t. for 5 h. The solution was diluted with 2M-HCl and then extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine, dried with anhydrous $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)], and subsequent evaporation of the solvent *in vacuo* afforded 1-acetyl-2-oxo-3-pyrroline **208** as a yellow oil (0.54 g, 60%); ν_{max}/cm^{-1} 1683 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 2.55 (3H, s, CH_3CO), 4.40 (2H, t, J = 2.0 Hz, CH_2CH), 6.18 (1H, dt, J = 1.9 and 6.1 Hz, $CHCH_2$) and 7.30 (1H, dt, J = 2.0 and 6.1 Hz, CHCO); δ_C/ppm (75 MHz; $CDCl_3$) 29.8 (CH_3CO), 50.7 (CH_2CH), 127.8 (*C*HCO), 146.7 ($CHCH_2$) and 170.1 and 170.4 (2 × s, C=O).

3,4-(Methylenedioxy)benzaldehyde 221[225,226]



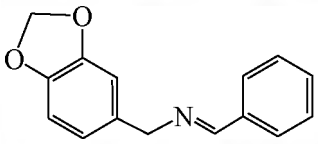
To a stirred solution of 3,4-dihydroxybenzaldehyde **220** (0.60 g, 4.3 mmol) in dry acetonitrile (10 mL) was added Cs_2CO_3 (2.1 g, 6.5 mmol), and the mixture was stirred for 15 min., at r.t. Bromochloromethane (0.40 mL, 6.5 mmol) was added and the reaction mixture was refluxed for 7 h at 85 °C under N_2. After cooling, the mixture was filtered through a pad of celite, which was washed with EtOAc, and the filtrate and washings were combined. The solvent was evaporated under reduced pressure to afford 3,4-(methylenedioxy)benzaldehyde **221** as a yellow oil (0.52 g, 79%); ν_{max}/cm^{-1} 1670 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 6.07 (2H, s, OCH_2O), 6.93 (1H, d, J = 7.9 Hz, Ar-H), 7.33 (1H, s, Ar-H), 7.41 (1H, d, J = 7.4 Hz, Ar-H) and 9.80 (1H, s, CHO); δ_C/ppm (150 MHz; $CDCl_3$) 102.2 (OCH_2O), 107.1, 108.5, 128.8, 132.0, 148.9 and 153.3 (Ar-C) and 190.4 (CHO).

3,4-Bis(*tert*-butyldimethylsilyloxy)benzaldehyde 225[230,231]



Tert-butyldimethylsilyl chloride (6.0 g, 38 mmol) was added in portions to a solution of 3,4-dihydroxybenzaldehyde **220** (2.5 g, 19 mmol) and imidazole (3.7 g, 55 mmol) in dry DCM (20 mL) at 0 °C. The solution was allowed to warm r.t. and stirred overnight. Water (10 mL) was added and the mixture was extracted with DCM (3 × 25 mL). The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvent was evaporated *in vacuo*. Flash chromatography of the residue [on silica gel; elution with EtOAc-hexane (5:5)] afforded 3,4-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **225** as a yellow solid (5.5 g, 90%), m.p. 38-40 °C (Lit.[228] 36-38 °C); ν_{max}/cm^{-1} 1669 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 0.23 and 0.25 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.99 and 1.00 [18H, 2 × s, 2 × $C(CH_3)_3$], 6.94 (1H, d, J = 8.0 Hz, Ar-H), 7.36 (2H, m, Ar-H) and 9.81 (1H, s, CHO); δ_C/ppm (150 MHz; $CDCl_3$) -4.0 and -3.9 [2 × s, 2 × $Si(CH_3)_2$], 18.6 and 18.7 [2 × s, 2 × $C(CH_3)_3$], 25.9 and 26.0 [2 × s, 2 × $C(CH_3)_3$], 120.7, 121.0, 125.4, 130.9, 147.8 and 143.5 (Ar-C) and 191.0 (CHO).

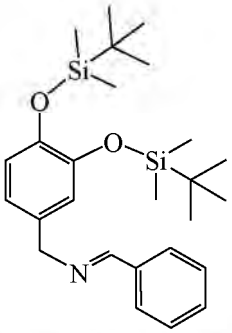
3,4-Methylenedioxy-1-[(phenylimino)methyl]benzene 222



A mixture of 3,4-(methylenedioxy)benzaldehyde **221** (0.46 g, 3.1 mmol), anhydrous $MgSO_4$ (0.74 g, 6.2 mmol) and benzylamine **163a** (0.34 g, 3.1 mmol) in dry DCM (10 mL) was stirred at r.t. for 24 h. The mixture was filtered and the solvent evaporated *in vacuo* to afford *3,4-methylenedioxy-1-[(phenylimino)methyl]benzene* **222** as a brown solid (0.60 g, 81%), m.p 34-36 °C [HRMS: *m/z* calculated for $C_{15}H_{14}NO_2$ (MH^+) 240.1025. Found 240.1025]; ν_{max}/cm^{-1} 1640 (N=C); δ_H/ppm (600 MHz; $CDCl_3$) 4.78 (2H, s, CH_2N), 6.00 (2H, s, OCH_2O), 6.83 (1H, d, J = 8.0 Hz, Ar-H), 7.15 (1H, d, J = 9.5 Hz, Ar-H), 7.31-7.35 (6H, overlapping m, Ar-H) and 8.27 (1H, s, N=CH); δ_C/ppm (150 MHz; $CDCl_3$) 63.5 (CH_2N),

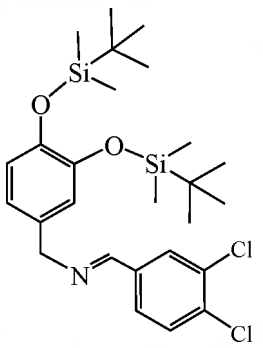
101.5 (OCH_2O), 107.1, 107.8, 123.8, 128.4, 129.1, 130.0, 135.7, 139.2, 148.2, 153.3 (Ar-C) and 162.6 (N=CH).

3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(phenylimino)methyl]benzene 226a



The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylimino)methyl]-benzene **222** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **225** (0.91 g, 2.5 mmol), anhydrous $MgSO_4$ (0.60 g, 4.9 mmol) and benzylamine **163a** (0.27 mL, 2.5 mmol) in dry DCM (10 mL). Work-up afforded *3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(phenylimino)methyl]benzene* **226a** as a yellow oil (0.81 g, 72%) [HRMS: *m/z* calculated for $C_{26}H_{42}NO_2Si_2$ (MH^+) 456.2754. Found 456.2760]; ν_{max}/cm^{-1} 1626 (N=C); δ_H/ppm (600 MHz; $CDCl_3$) 0.22 and 0.24 [12H, 2 × s, 2 × $Si(CH_3)_2$], 1.01 and 1.02 [18H, 2 × s, 2 × $C(CH_3)_3$], 4.80 (2H, s, CH_2N), 6.88 (1H, d, J = 8.1 Hz, Ar-H), 7.28 (2H, m, Ar-H), 7.33-7.36 (5H, overlapping m, Ar-H) and 8.26 (1H, s, N=CH); δ_C/ppm (150 MHz; $CDCl_3$) -3.50 and -3.45 [2 × s, 2 × $Si(CH_3)_2$], 18.10 and 18.13 [2 × s, 2 × $C(CH_3)_3$], 25.7 and 27.8 [2 × s, 2 × $C(CH_3)_3$], 63.3 (CH_2N), 127.2, 127.4, 128.0, 128.2, 128.3, 128.5, 128.6, 128.8, 128.9 and 130.0 (Ar-C) and 157.1 (N=CH).

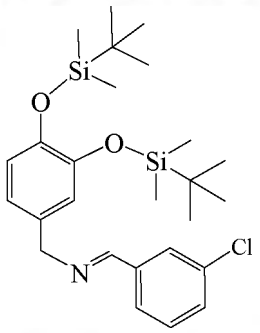
3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-dichlorophenylimino)methyl]benzene 226b



The procedure described for the synthesis 3,4-methylenedioxy-1-[(phenylimino)methyl]-benzene **222** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **225** (1.0 g, 2.7 mmol), anhydrous $MgSO_4$ (0.33 g, 5.5 mmol) and 3,4-dichlorobenzylamine **163b**

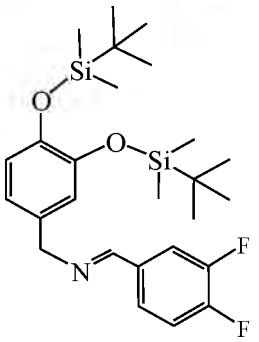
(0.36 mL, 2.7 mmol) in dry DCM (10 mL). Work-up afforded *3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-dichlorophenylimino)methyl]benzene* **226b** as a yellow solid (1.1 g, 78%), m.p. 38-40 °C [HRMS: *m/z* calculated for $C_{26}H_{40}{}^{35}Cl_2NO_2Si_2$ (MH^+) 524.1975. Found 524.1979]; ν_{max}/cm^{-1} 1633 (N=C); δ_H/ppm (600 MHz; $CDCl_3$) 0.22 and 0.23 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.99 and 1.00 [18H, 2 × s, 2 × $C(CH_3)_3$], 4.70 (2H, s, CH_2N), 6.87 (1H, d, J =8.2 Hz, Ar-H), 7.17 (1H, d, J = 8.2 Hz, Ar-H), 7.33 (2H, m, Ar-H), 7.39 (1H, d, J = 8.2 Hz, Ar-H), 7.44 (1H, s, Ar-H) and 8.23 (1H, s, N=CH); δ_C/ppm (75 MHz; $CDCl_3$) -4.0 and -3.9 [2 × s, 2 × $Si(CH_3)_2$], 18.6 and 18.7 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 63.5 (CH_2N), 120.8, 121.2, 122.3, 127.2, 129.9, 130.2, 130.4, 130.8, 132.5, 140.4, 147.4 and 150.1 (Ar-C) and 162.2 (N=CH).

3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(3-chlorophenylimino)methyl]benzene 226c



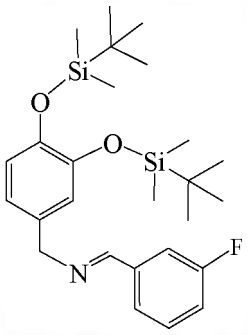
The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylimino)methyl]-benzene **222** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **225** (1.0 g, 2.7 mmol), anhydrous $MgSO_4$ (0.33 g, 5.5 mmol) and 3-chlorobenzylamine **163c** (0.33 mL, 2.7 mmol) in dry DCM (10 mL). Work-up afforded *3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3-chlorophenylimino)methyl]benzene* **226c** as a yellow oil (1.1 g, 86%) [HRMS: *m/z* calculated for $C_{26}H_{41}{}^{35}ClNO_2Si_2$ (MH^+) 490.2364. Found 490.2370]; ν_{max}/cm^{-1} 1634 (N=C); δ_H/ppm (600 MHz; $CDCl_3$) 0.24 and 0.25 [12H, 2 × s, 2 × $Si(CH_3)_2$], 1.01 and 1.03 [18H, 2 × s, 2 × $C(CH_3)_3$], 4.76 (2H, s, CH_2N), 6.89 (1H, d, J =8.2 Hz, Ar-H), 7.24-7.32 (5H, overlapping m, Ar-H), 7.36 (1H, s, Ar-H) and 8.26 (1H, s, N=CH); δ_C/ppm (75 MHz; $CDCl_3$) -3.92 and -3.91 [2 × s, 2 × $Si(CH_3)_2$], 18.6 and 18.7 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 63.2 (CH_2N), 120.9, 121.2, 122.2, 126.0, 127.1, 128.1, 129.8, 130.1, 134.4, 142.1, 147.3 and 150.0 (Ar-C) and 162.0 (N=CH).

3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-difluorophenylimino)methyl]benzene 226d



The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylimino)methyl]-benzene **222** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **225** (1.0 g, 2.7 mmol), anhydrous $MgSO_4$ (0.33 g, 5.5 mmol) and 3,4-difluorobenzylamine **163d** (0.32 mL, 2.7 mmol) in dry DCM (10 mL). Work-up afforded *3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-difluorophenylimino)methyl]benzene* **226d** as a yellow oil (1.1 g, 83%) [HRMS: *m/z* calculated for $C_{26}H_{40}{}^{19}F_2NO_2Si_2$ (MH^+) 492.2562. Found 492.2577]; ν_{max}/cm^{-1} 1645 (N=C); δ_H/ppm (600 MHz; $CDCl_3$) 0.24 and 0.25 [12H, 2 × s, 2 × $Si(CH_3)_2$], 1.01 and 1.03 [18H, 2 × s, 2 × $C(CH_3)_3$], 4.76 (2H, s, CH_2N), 6.89 (1H, d, J = 8.2 Hz, Ar-H), 6.69-7.20 (3H, overlapping m, Ar-H), 7.36 (2H, d, J = 14.7 Hz, Ar-H) and 8.25 (1H, s, N=CH); δ_C/ppm (75 MHz; $CDCl_3$) -4.0 and -3.9 [2 × s, 2 × $Si(CH_3)_2$], 18.6 and 18.7 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 63.7 (CH_2N), 116.8 (d, J_{F-C} = 17.6 Hz), 117.1 (d, J_{F-C} = 17.2 Hz), 120.8, 121.2, 121.4, 122.2, 123.6, 124.5, 130.0, 137.1, 147.3 and 150.1 (d, J_{F-C} = 245.0 Hz) (Ar-C) and 162.0 (N=CH).

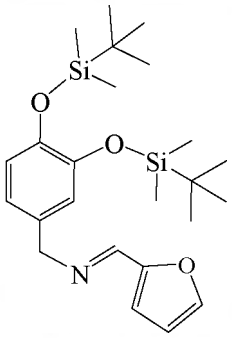
3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(3-fluorophenylimino)methyl]benzene 226e



The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylimino)methyl]-benzene **222** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **225** (1.0 g, 2.7 mmol), anhydrous $MgSO_4$ (0.33 g, 5.5 mmol) and 3-fluorobenzylamine **163e** (0.31 mL, 2.7 mmol) in dry DCM (10 mL). Work-up afforded *3,4-bis(*tert*-butyldimethylsilyloxy)-*

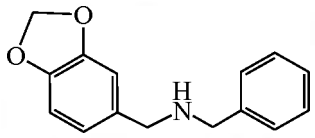
1-[(3-fluorophenylimino)methyl]benzene **226e** as a yellow oil (1.1 g, 84%) [HRMS: *m/z* calculated for $C_{26}H_{41}{}^{19}FNO_2Si_2$ (MH^+) 474.2660. Found 474.2657]; ν_{max}/cm^{-1} 1629 (N=C); δ_H/ppm (600 MHz; $CDCl_3$) 0.24 and 0.25 [12H, 2 × s, 2 × $Si(CH_3)_2$], 1.02 and 1.03 [18H, 2 × s, 2 × $C(CH_3)_3$], 4.79 (2H, s, CH_2N), 6.89 (1H, d, J = 8.2 Hz, Ar-H), 6.96 (1H, t, J = 9.6 Hz, Ar-H), 7.08 (1H, d, J = 9.8 Hz, Ar-H), 7.28-7.32 (3H, overlapping m, Ar-H) and 8.26 (1H, s, N=CH); δ_C/ppm (75 MHz; $CDCl_3$) -3.93 and -3.91 [2 × s, 2 × $Si(CH_3)_2$], 18.6 and 18.7 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 64.3 (CH_2N), 113.8 (d, J_{F-C} = 21.2 Hz), 114.8 (d, J_{F-C} = 21.6 Hz), 120.9, 121.2, 122.2, 123.3, 123.4, 130.1, 142.6 (d, J_{F-C} = 7.2 Hz), 147.3, 150.0, 161.5 (d, J_{F-C} = 239.0 Hz) (Ar-C) and 164.8 (N=CH).

3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(furfurylimino)methyl]benzene 226f



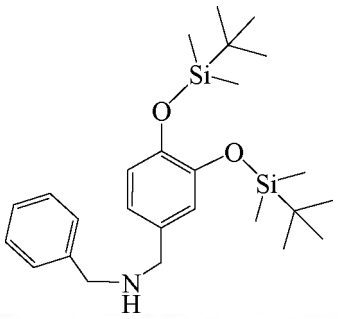
The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylimino)methyl]-benzene **222** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **225** (1.0 g, 2.7 mmol), anhydrous $MgSO_4$ (0.33 g, 5.5 mmol) and furfurylamine **163f** (0.24 mL, 2.7 mmol) in dry DCM (10 mL). Work-up afforded *3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(furfurylimino)methyl]benzene* **226f** as a yellow oil (0.97 g, 80%) [HRMS: *m/z* calculated for $C_{24}H_{40}NO_3Si_2$ (MH^+) 446.2547. Found 446.2558]; ν_{max}/cm^{-1} 1627 (N=C); δ_H/ppm (600 MHz; $CDCl_3$) -0.002 and 0.000 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.77 and 0.79 [18H, 2 × s, 2 × $C(CH_3)_3$], 4.52 (2H, s, CH_2N), 6.03 (1H, dd, J = 0.7 and 3.2 Hz, Ar-H), 6.13 (1H,dd, J = 1.9 and 3.2 Hz, Ar-H), 6.64 (1H, m, Ar-H), 7.02-7.06 (3H, overlapping m, Ar-H) and 7.98 (1H, s, N=CH); δ_C/ppm (75 MHz; $CDCl_3$) -4.0 and -3.9 [2 × s, 2 × $Si(CH_3)_2$], 18.6 and 18.7 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 57.3 (CH_2N), 107.3, 110.5, 120.9, 121.1, 122.2, 130.1, 147.2, 150.0, 150.4 and 153.0 (Ar-C) and 162.6 (N=CH).

3,4-Methylenedioxy-1-[(phenylamino)methyl]benzene 223



Sodium cyanoborohydride (0.51 g, 8.1 mmol) was added in portions to a solution of 3,4-methylenedioxy-1-[(phenylimino)methyl]benzene **222** (0.46 g, 1.9 mmol) in MeOH (10 mL). After 15 min., conc. HCl (0.5 mL) was slowly added and the reaction mixture was stirred at r.t. for 3 h. The solvent was evaporated *in vacuo*, and the residue was dissolved in MeOH (5 mL) and cold water (5 mL), and then the pH of the solution was adjusted to pH = 10 using aq. KOH followed by the extraction with DCM (3 × 20 mL). The combined organic layers were aq. washed with $NaHCO_3$ (2 × 50 mL) and brine (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford *3,4-methylene-dioxy-1-[(phenylamino)methyl]benzene* **223** as a pale-yellow oil (0.30 g, 64%) [HRMS: *m/z* calculated for $C_{15}H_{16}NO_2$ (MH^+) 242.1181. Found 242.1178]; ν_{max}/cm^{-1} 3217 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 3.75 and 3.82 (4H, 2 × s, 2 × NCH_2), 5.96 (2H, s, OCH_2O), 6.80 (2H, m, Ar-H), 6.91 (1H, s, Ar-H) and 7.30-7.42 (5H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 53.0 and 53.1 (2 × N CH_2), 101.0 (OCH_2O), 108.2, 108.8, 121.4, 127.1, 128.3, 128.5, 134.4, 140.3, 146.6 and 147.8 (Ar-C).

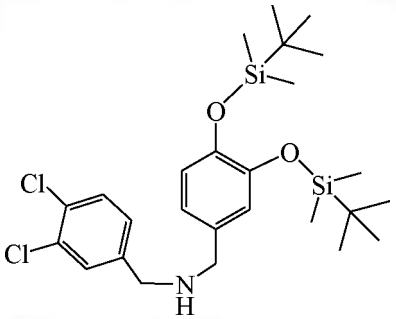
3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(phenylamino)methyl]benzene 227a



The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylamino)methyl]-benzene **223** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(phenylimino)-methyl]benzene **226a** (0.60 g, 1.3 mmol), sodium cyanoborohydride (0.35 g, 5.5 mmol) and conc. HCl (0.5 mL) in MeOH (10 mL). Work-up afforded *3,4-bis(*tert-*butyldimethyl-silyloxy)-1-[(phenylamino)methyl]benzene* **227a** as a yellow oil (0.48 g, 80%) [HRMS: *m/z* calculated for $C_{26}H_{44}NO_2Si_2$ (MH^+) 458.2911. Found 458.2908]; ν_{max}/cm^{-1} 2930 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 0.25 and 0.26 [12H, 2 × s, 2 × $Si(CH_3)_2$], 1.05 and 1.06 [18H, 2

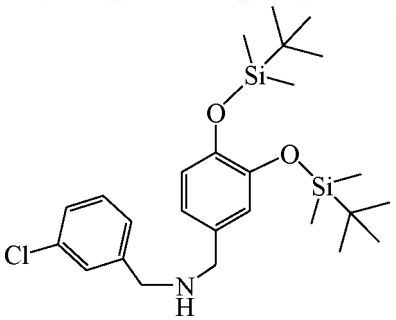
× s, 2 × $C(CH_3)_3$], 3.74 and 3.83 (4H, 2 × s, 2 × NCH_2), 6.86 (3H, m, Ar-H) and 7.28-7.38 (5H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) -4.0 and -3.9 [2 × s, 2 × $Si(CH_3)_2$], 18.6 and 18.7 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 52.5 and 52.8 (2 × NCH_2), 121.2, 121.4, 127.2, 128.6, 129.7, 134.2, 134.4, 142.6, 145.1 and 145.9 (Ar-C).

3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-dichlorophenylamino)methyl]benzene 227b



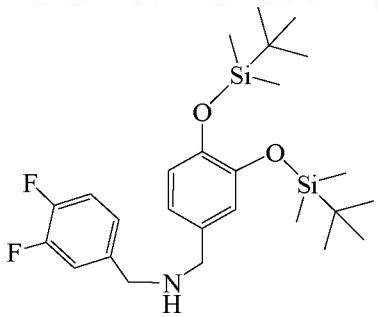
The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylamino)methyl]-benzene **223** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-dichlorophenyl-amino)methyl]benzene **226b** (0.84 g, 1.6 mmol), sodium cyanoborohydride (0.42 g, 6.7 mmol) and conc. HCl (0.5 mL) in MeOH (10 mL). Work-up afforded *3,4-bis(*tert-*butyldimethylsilyloxy)-1-[(3,4-dichlorophenylamino)methyl]benzene* **227b** as a yellow oil (0.74 g, 88%) [HRMS: *m/z* calculated for $C_{26}H_{42}{}^{35}Cl_2NO_2Si_2$ (MH^+) 526.2131. Found 526.2126]; ν_{max}/cm^{-1} 2929 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 0.19 and 0.20 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.98 and 0.99 [18H, 2 × s, 2 × $C(CH_3)_3$], 3.66 and 3.73 (4H, 2 × s, 2 × NCH_2), 6.76 (2H, m, Ar-H), 6.81 (1H, s, Ar-H), 7.18 (1H, d, J = 8.2 Hz, Ar-H), 7.38 (1H, d, J = 8.2 Hz, Ar-H) and 7.46 (1H, s, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -3.94 and -3.89 [2 × s, 2 × $Si(CH_3)_2$], 18.5 and 18.7 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 51.6 and 52.5 (2 × NCH_2), 121.1, 121.2, 127.4, 127.7, 127.9, 130.3, 130.4, 132.6, 132.9, 142.0, 142.9 and 145.9 (Ar-C).

3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(3-chlorophenylamino)methyl]benzene 227c



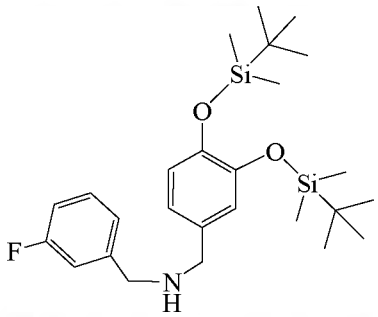
The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylamino)methyl]-benzene **223** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3-chlorophenyl-imino)methyl]benzene **226c** (1.1 g, 2.2 mmol), sodium cyanoborohydride (0.59 g, 9.4 mmol) and conc. HCl (0.5 mL) in MeOH (10 mL). Work-up afforded *3,4-bis(*tert*-butyldimethyl-silyloxy)-1-[(3-chlorophenylamino)methyl]benzene* **227c** as a yellow oil (0.88 g, 80%) [HRMS: *m/z* calculated for $C_{26}H_{43}{}^{35}ClNO_2Si_2$ (MH^+) 492.2521. Found 492.2524]; ν_{max}/cm^{-1} 2929 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 0.19 and 0.20 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.98 and 0.99 [18H, 2 × s, 2 × $C(CH_3)_3$], 3.67 and 3.75 (4H, 2 × s, 2 × NCH_2), 6.78 (3H, m, Ar-H), 7.20-7.25 (3H, overlapping m, Ar-H) and 7.36 (1H, s, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -3.94 and -3.89 [2 × s, 2 × $Si(CH_3)_2$], 18.5 and 18.6 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 52.4 and 52.7 (2 × NCH_2), 121.0, 121.9, 121.2, 126.4, 127.2, 128.4, 129.7, 133.2, 134.4, 142.6, 145.1 and 146.9 (Ar-C).

3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-difluorophenylamino)methyl]benzene 227d



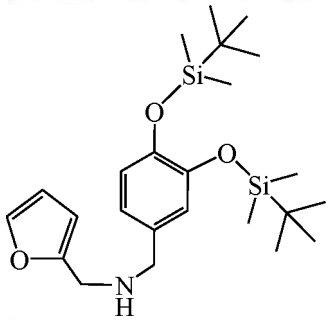
The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylamino)methyl]-benzene **223** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-difluorophenyl-imino)methyl]benzene **226d** (1.0 g, 2.1 mmol), sodium cyanoborohydride (0.54 g, 8.6 mmol) and conc. HCl (0.5 mL) in MeOH (10 mL). Work-up afforded *3,4-bis(*tert*-butyldimethyl-silyloxy)-1-[(3,4-difluorophenylamino)methyl]benzene* **227d** as a yellow oil (0.86 g, 85%) [HRMS: *m/z* calculated for $C_{26}H_{42}{}^{19}F_2NO_2Si_2$ (MH^+) 494.2722. Found 494.2719]; ν_{max}/cm^{-1} 3000 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 0.19 and 0.20 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.98 and 0.99 [18H, 2 × s, 2 × $C(CH_3)_3$], 3.66 and 3.72 (4H, 2 × s, 2 × NCH_2), 6.74-6.81 (3H, overlapping m, Ar-H), 7.03-7.11 (2H, overlapping m, Ar-H) and 7.19 (1H, t, J = 7.9 Hz, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -3.94 and -3.91 [2 × s, 2 × $Si(CH_3)_2$], 18.5 and 18.6 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 51.9 and 52.6 (2 × NCH_2), 117.0 (d, J = 21.0 Hz), 117.1 (d, $J_{F\text{-}C}$ = 19.8 Hz), 120.9, 121.0, 121.16, 121.19, 121.5, 123.9 (dd, $J_{F\text{-}C}$ = 3.5 and 6.0 Hz), 133.2, 134.9, 137.7 and 145.5 (dd, $J_{F\text{-}C}$ = 210.0 Hz) (Ar-C).

3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(3-fluorophenylamino)methyl]benzene 227e



The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylamino)methyl]-benzene **223** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3-fluorophenyl-imino)methyl]benzene **226e** (1.0 g, 2.1 mmol), sodium cyanoborohydride (0.56 g, 8.9 mmol) and conc. HCl (0.5 mL) in MeOH (10 mL). Work-up afforded *3,4-bis(*tert-*butyldimethy-lsilyloxy)-1-[(3-fluorophenylamino)methyl]benzene* **227e** as a yellow oil (0.87 g, 86%) [HRMS: *m/z* calculated for $C_{26}H_{43}{}^{19}FNO_2Si_2$ (MH^+) 476.2816. Found 476.2819]; ν_{max}/cm^{-1} 3015 (OH); δ_H/ppm (600 MHz; $CDCl_3$) 0.22 and 0.23 [12H, 2 × s, 2 × $Si(CH_3)_2$], 1.01 and 1.02 [18H, 2 × s, 2 × $C(CH_3)_3$], 3.70 and 3.80 (4H, 2 × s, 2 × NCH_2), 6.78-6.85 (3H, overlapping m, Ar-H), 6.96 (1H, t, J = 8.4 Hz, Ar-H), 7.11 (2H, m, Ar-H), and 7.19 (1H, dd, J = 2.0 and 9.9 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) -3.94 and -3.91 [2 × s, 2 × $Si(CH_3)_2$], 18.5 and 18.6 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 52.4 and 52.7 (2 × NCH_2), 113.9 (d, J_{F-C} = 21.1 Hz), 115.0 (d, J_{F-C} = 21.3 Hz), 121.0, 121.2 (d, J_{F-C} = 3.1 Hz), 122.7, 123.8 (d, J_{F-C} = 2.7 Hz), 129.0 (d, J_{F-C} = 8.2 Hz), 133.3, 143.3, 146.0, 146.9, 162.0 (d, J_{F-C} = 242.0 Hz) (Ar-C).

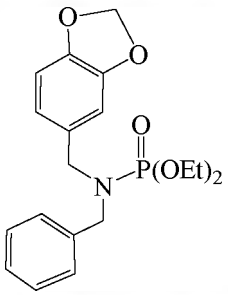
3,4-Bis(*tert*-butyldimethylsilyloxy)-1-[(furfurylamino)methyl]benzene 227f



The procedure described for the synthesis of 3,4-methylenedioxy-1-[(phenylamino)methyl]-benzene **223** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(furfurylimino)-methyl]benzene **226f** (0.86 g, 1.9 mmol), sodium cyanoborohydride (0.51 g, 8.1 mmol) and conc. HCl (0.5 mL) in MeOH (10 mL). Work-up afforded *3,4-bis(*tert-*butyldimethyl-*

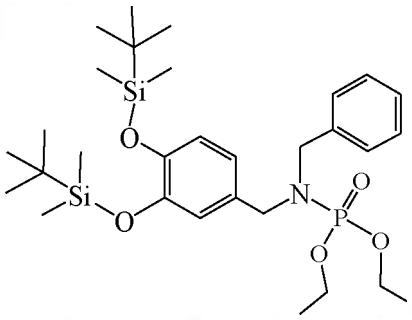
silyloxy)-1-[(furfurylamino)methyl]benzene **227f** as a yellow oil (0.73 g, 84%) [HRMS: *m/z* calculated for $C_{24}H_{42}NO_3Si_2$ (MH^+) 448.2703. Found 448.2710]; ν_{max}/cm^{-1} 2999 (NH); δ_H/ppm (600 MHz; $CDCl_3$) 0.18 and 0.19 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.98 and 0.99 [18H, 2 × s, 2 × $C(CH_3)_3$], 3.66 and 3.75 (4H, 2 × s, 2 × NCH_2), 6.17 (1H, d, J = 3.0 Hz, Ar-H), 6.32 (1H, t, J = 3.2 Hz, Ar-H), 6.76-6.79 (3H, overlapping m, Ar-H) and 7.36 (1H, s, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -3.94 and -3.92 [2 × s, 2 × $Si(CH_3)_2$], 18.58 and 18.61 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 45.2 and 52.3 (2 × NCH_2), 107.2, 110.3, 120.8, 121.1, 121.4, 133.1, 141.9, 146.1, 146.8 and 154.1 (Ar-C).

Diethyl *N*-benzyl-*N*-[(3,4-methylenedioxyphenyl)methyl]phosphoramidate 224



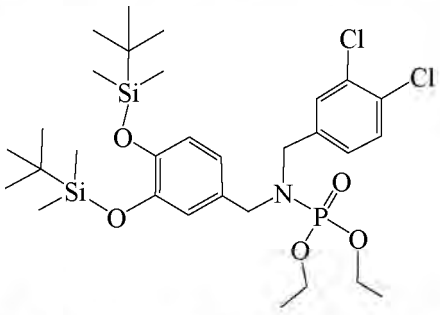
A mixture of 3,4-methylenedioxy-1-[(phenylamino)methyl]benzene **223** (0.20 g, 0.83 mmol), triethylamine (0.12 mL, 0.83 mmol) and diethyl chlorophosphate (0.12 mL, 0.83 mmol) in DCM (5 mL) was stirred at r.t overnight. The reaction mixture was subsequently washed with 10% HCl (5 mL) and water (5 mL), and re-extracted with DCM (2 × 20 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated *in vacuo*. The crude product was purified by flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)], to afford *diethyl* N-*benzyl*-N-*[(3,4-methylenedioxy-phenyl)methyl]phosphoramidate* **224** as a yellow oil (0.23 g, 73%) [HRMS: *m/z* calculated for $C_{19}H_{25}NO_5P$ (MH^+) 378.1470. Found 378.1463]; ν_{max}/cm^{-1} 1242 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.35 (6H, t, J = 7.1 Hz, 2 × CH_3), 4.00 (2H, d, J_{P-H} = 10.4 Hz, CH_2N), 4.08 (4H, m, 2 × OCH_aCH_3 and $ArCH_2N$), 4.16 (2H, m, 2 × OCH_bCH_3), 5.97 (2H, s, OCH_2O), 6.68 (1H, d, J = 7.8 Hz, Ar-H), 6.76 (1H, d, J = 7.9 Hz, Ar-H), 6.86 (1H, s, Ar-H) and 7.28-7.37 (5H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 (d, J_{P-C} = 7.3 Hz, 2 × CH_3), 48.2 (2 × overlapping d, 2 × NCH_2), 62.7 (2 × OCH_2CH_3), 101.1 (OCH_2O), 108.1, 109.2, 122.3, 127.5, 128.5, 128.8, 131.5, 137.6, 147.0 and 148.0 (Ar-C).

Diethyl *N*-benzyl-*N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}-phosphoramidate 228a



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-methylenedioxyphenyl)methyl]phosphoramidate **224** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(phenylamino)methyl]benzene **227a** (0.46 g, 1.0 mmol), triethylamine (0.14 mL, 1.0 mmol) and diethyl chlorophosphate (0.14 mL, 1.0 mmol) in DCM (5 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*benzyl*-N-*{[3,4-bis(*tert-*butyldimethylsilyloxy)phenyl]methyl}phosphoramidate* **228a** as a yellow oil (0.49 g, 82%) [HRMS: *m/z* calculated for $C_{30}H_{53}NO_5Si_2P$ (MH^+) 594.3200. Found 594.3210]; ν_{max}/cm^{-1} 1242 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) -0.00 and 0.00 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.78 and 0.79 [18H, 2 × s, 2 × $C(CH_3)_3$], 1.11 (6H, t, J = 7.0 Hz, 2 × CH_3), 3.74 (2H, d, $J_{P\text{-}H}$ = 9.9 Hz, CH_2N), 3.80-3.92 (6H, m, 2 × OCH_2CH_3 and NCH_2), 6.48 (1H, d, J = 8.2 Hz, Ar-H), 6.55 (2H, m, Ar-H) and 7.04-7.13 (5H, overlapping m, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -4.0 and -3.9 [2 × s, 2 × $Si(CH_3)_2$], 16.3 (d, $J_{P\text{-}C}$ = 7.1 Hz, 2 × CH_3), 18.56 and 18.60 [2 × s, 2 × $C(CH_3)_3$], 26.07 and 26.09 [2 × s, 2 × $C(CH_3)_3$], 48.0 (d, $J_{P\text{-}C}$ = 5.0 Hz, NCH_2), 48.2 (d, $J_{P\text{-}C}$ = 4.8 Hz, CH_2N), 62.5 (d, $J_{P\text{-}C}$ = 5.6 Hz, 2 × OCH_2CH_3), 120.9, 121.5, 121.9, 125.6, 127.4, 128.4, 128.7, 128.9, 146.3 and 146.9 (Ar-C).

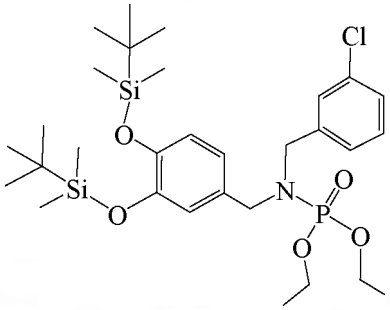
Diethyl *N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}-*N*-(3,4-dichlorobenzyl)-phosphoramidate 228b



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-methylenedioxyphenyl)methyl]phosphoramidate **224** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-dichlorophenylamino)methyl]benzene **227b** (0.71 g, 1.4 mmol), triethyl-

amine (0.19 mL, 1.4 mmol) and diethyl chlorophosphate (0.19 mL, 1.4 mmol) in DCM (10 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}*-N-*(3,4-dichlorobenzyl)phosphoramidate* **228b** as a yellow oil (0.77 g, 86%) [HRMS: *m/z* calculated for $C_{30}H_{51}{}^{35}Cl_2NO_5Si_2P$ (MH^+) 662.2420. Found 662.2415]; ν_{max}/cm^{-1} 1251 (P=O); δ_H/ppm (400 MHz; $CDCl_3$) 0.19 and 0.20 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.94 and 0.99 [18H, 2 × s, 2 × $C(CH_3)_3$], 1.33 (6H, t, J = 7.0 Hz, 2 × CH_3), 3.9.2 (2H, d, J = 9.2 Hz, CH_2N), 4.01-4.14 (6H, m, 2 × OCH_2CH_3 and NCH_2), 6.64 (1H, d, J = 8.1 Hz, Ar-H), 6.76 (2H, dd, J = 5.1 and 7.1 Hz, Ar-H), 7.07 (1H, d, J = 6.5 Hz, Ar-H) and 7.36 (2H, d, J = 8.5 Hz, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -4.0 and -3.9 [2 × s, 2 × $Si(CH_3)_2$], 16.4 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 18.56 and 18.61 [2 × s, 2 × $C(CH_3)_3$], 26.05 and 26.08 [2 × s, 2 × $C(CH_3)_3$], 47.4 (d, J_{P-C} = 5.1 Hz, NCH_2), 48.3 (d, J_{P-C} = 4.5 Hz, CH_2N), 62.8 (d, J_{P-C} = 5.7 Hz, 2 × OCH_2CH_3), 121.0, 121.4, 121.8, 128.1, 130.4, 130.7, 131.4, 132.6, 132.6, 138.4, 146.6 and 147.1 (Ar-C).

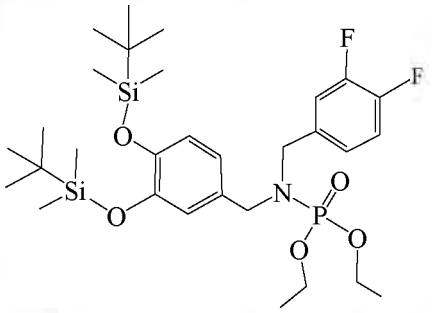
Diethyl *N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}-*N*-(3-chlorobenzyl)-phosphoramidate 228c



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-methylenedioxyphenyl)methyl]phosphoramidate **224** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3-chlorophenylamino)methyl]benzene **227c** (0.68 g, 1.4 mmol), triethylamine (0.19 mL, 1.4 mmol) and diethyl chlorophosphate (0.19 mL, 1.4 mmol) in DCM (10 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}*-N-*(3-chlorobenzyl)-phosphoramidate* **228c** as a yellow oil (0.72 g, 83%) [HRMS: *m/z* calculated for $C_{30}H_{52}{}^{35}ClNO_5Si_2P$ (MH^+) 628.2810. Found 628.2806]; ν_{max}/cm^{-1} 1252 (P=O); δ_H/ppm (400 MHz; $CDCl_3$) 0.19 and 0.20 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.94 and 0.99 [18H, 2 × s, 2 × $C(CH_3)_3$], 1.32 (6H, t, J = 7.1 Hz, 2 × CH_3), 3.94 (2H, d, J_{P-H} = 9.5 Hz, CH_2N), 4.01-4.15 (6H, m, 2 × OCH_2CH_3 and NCH_2), 6.66 (1H, dd, J = 2.0 and 8.2 Hz, Ar-H), 6.77 (2H, m, Ar-H), 7.11 (1H, t, J = 4.9 Hz, Ar-H), 7.22 (2H, m, Ar-H) and 7.29 (1H, s, Ar-H); δ_C/ppm

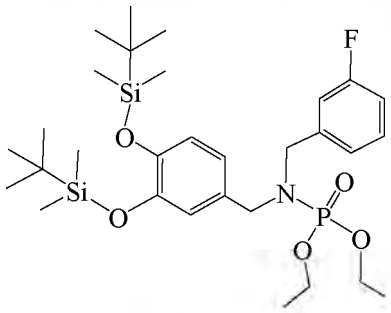
(100 MHz; $CDCl_3$) -4.04 and -3.95 [2 × s, 2 × $Si(CH_3)_2$], 16.3 (d, J_{P-C} = 7.1 Hz, 2 × CH_3), 18.3 and 18.4 [2 × s, 2 × $C(CH_3)_3$], 26.0 and 26.1 [2 × s, 2 × $C(CH_3)_3$], 47.8 (d, J_{P-C} = 5.1 Hz, NCH_2), 48.2 (d, J_{P-C} = 4.7 Hz, CH_2N), 62.7 (d, J_{P-C} = 5.8 Hz, 2 × OCH_2CH_3), 120.9, 121.4, 121.9, 126.9, 127.6, 128.8, 129.7, 130.3, 134.4, 140.0, 146.4 and 147.0 (Ar-C).

Diethyl *N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}-*N*-(3,4-difluorobenzyl)-phosphoramidate 228d



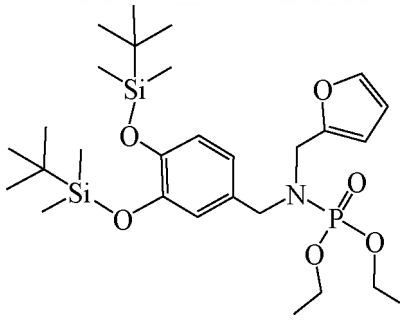
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-methylenedioxyphenyl)methyl]phosphoramidate **224** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3,4-difluorophenylamino)methyl]benzene **227d** (0.73 g, 1.5 mmol), triethylamine (0.20 mL, 1.5 mmol) and diethyl chlorophosphate (0.21 mL, 1.5 mmol) in DCM (10 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}*-N-*(3,4-difluorobenzyl)phosphoramidate* **228d** as a yellow oil (0.74 g, 80%) [HRMS: *m/z* calculated for $C_{30}H_{51}{}^{19}F_2NO_5Si_2P$ (MH^+) 630.3011. Found 630.3024]; ν_{max}/cm^{-1} 1256 (P=O); δ_H/ppm (400 MHz; $CDCl_3$) 0.18 and 0.19 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.91 and 0.98 [18H, 2 × s, 2 × $C(CH_3)_3$], 1.32 (6H, t, *J* = 7.1 Hz, 2 × CH_3), 3.92 (2H, d, J_{P-H} = 9.3 Hz, CH_2N), 4.00-4.15 (6H, m, 2 × OCH_2CH_3 and NCH_2), 6.64 (1H, dd, *J* = 1.9 and 8.1 Hz, Ar-H), 6.76 (2H, m, Ar-H), 6.93 (1H, s, Ar-H) and 7.08 (2H, m, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -4.01 and -3.98 [2 × s, 2 × $Si(CH_3)_2$], 16.3 (d, J_{P-C} = 7.0 Hz, 2 × CH_3), 18.5 and 18.6 [2 × s, 2 × $C(CH_3)_3$], 26.01 and 26.04 [2 × s, 2 × $C(CH_3)_3$], 47.5 (d, J_{P-C} = 5.0 Hz, NCH_2), 48.2 (d, J_{P-C} = 4.5 Hz, CH_2N), 62.7 (d, J_{P-C} = 5.8 Hz, 2 × OCH_2CH_3), 117.0 (d, J_{F-C} = 17.1 Hz), 117.6 (d, J_{F-C} = 17.3 Hz), 120.9, 121.4, 121.8, 124.6 (d, J_{F-C} = 3.6 and 6.2 Hz), 130.1, 135.1, 146.5, 147.0, 148.8 (dd, J_{F-C} = 12.0 and 246.0 Hz) and 151.3 (d, J_{F-C} = 13.0 and 247.0 Hz) (Ar-C).

Diethyl *N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}-*N*-(3-fluorobenzyl)-phosphoramidate 228e



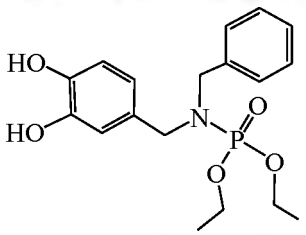
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-methylenedioxyphenyl)methyl]phosphoramidate **224** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(3-fluorophenylamino)methyl]benzene **227e** (0.81 g, 1.7 mmol), triethylamine (0.24 mL, 1.7 mmol) and diethyl chlorophosphate (0.24 mL, 1.7 mmol) in DCM (10 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*{[3,4-bis(*tert-*butyldimethylsilyloxy)phenyl]methyl}*-N-*(3-fluorobenzyl)-phosphoramidate* **228e** as a yellow oil (0.91 g, 88%) [HRMS: *m/z* calculated for $C_{29}H_{52}{}^{19}FNO_5Si_2P$ (MH^+) 612.3097. Found 612.3099]; ν_{max}/cm^{-1} 1256 (P=O); δ_H/ppm (400 MHz; $CDCl_3$) 0.176 and 0.183 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.91 and 0.97 [18H, 2 × s, 2 × $C(CH_3)_3$], 1.30 (6H, t, J = 7.0 Hz, 2 × CH_3), 3.90 (2H, d, $J_{P\text{-}H}$ = 9.6 Hz, CH_2N), 4.00-4.12 (6H, m, 2 × OCH_2CH_3 and NCH_2), 6.64 (1H, d, J = 8.1 Hz, Ar-H), 6.75 (2H, m, Ar-H), 6.92 (1H, t, J = 8.1 Hz, Ar-H), 6.99 (2H, d, J = 8.0 Hz, Ar-H) and 7.23 (1H, m, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -4.1 and -4.0 [2 × s, 2 × $Si(CH_3)_2$], 16.3 (d, $J_{P\text{-}C}$ = 7.0 Hz, 2 × CH_3), 18.5 and 18.6 [2 × s, 2 × $C(CH_3)_3$], 26.01 and 26.04 [2 × s, 2 × $C(CH_3)_3$], 47.9 (d, $J_{P\text{-}C}$ = 5.3 Hz, NCH_2), 48.2 (d, $J_{P\text{-}C}$ = 4.5 Hz, CH_2N), 62.6 (d, $J_{P\text{-}C}$ = 5.7 Hz, 2 × OCH_2CH_3), 114.3 (d, $J_{F\text{-}C}$ = 21.2 Hz), 115.5 (d, $J_{F\text{-}C}$ = 21.5 Hz), 120.9, 121.4, 121.9, 124.3, 129.8 (d, $J_{F\text{-}C}$ = 8.1 Hz), 130.3, 140.6, 146.4, 147.0, 161.8 (d, $J_{F\text{-}C}$ = 245.0 Hz) (Ar-C).

Diethyl *N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}-*N*-furfuryl-phosphoramidate 228f



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-methylenedioxyphenyl)methyl]phosphoramidate **224** was followed, using 3,4-bis(*tert*-butyldimethylsilyloxy)-1-[(furfuryl)methyl]benzene **227f** (0.68 g, 1.5 mmol), triethylamine (0.22 mL, 1.5 mmol) and diethyl chlorophosphate (0.22 mL, 1.5 mmol) in DCM (10 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*{[3,4-bis(*tert-*butyldimethylsilyloxy)phenyl]methyl}*-N-*furfurylphosphoramidate* **228f** as a yellow oil (0.73 g, 83%) [HRMS: *m/z* calculated for $C_{28}H_{51}NO_6Si_2P$ (MH^+) 584.2993. Found 584.2983]; ν_{max}/cm^{-1} 1252 (P=O); δ_H/ppm (400 MHz; $CDCl_3$) 0.19 and 0.20 [12H, 2 × s, 2 × $Si(CH_3)_2$], 0.92 and 0.99 [18H, 2 × s, 2 × $C(CH_3)_3$], 1.30 (6H, t, J = 7.0 Hz, 2 × CH_3), 3.99-4.10 (8H, m, 2 × CH_2N and 2 × OCH_2CH_3), 6.15 (1H, d, J = 3.0 Hz, Ar-H), 6.30 (1H, s, Ar-H), 6.76 (2H, m, Ar-H), 6.85 (1H, s, Ar-H) and 7.36 (1H, s, Ar-H); δ_C/ppm (100 MHz; $CDCl_3$) -4.04 and -3.95 [2 × s, 2 × $Si(CH_3)_2$], 16.3 (d, J_{P-C} = 7.1 Hz, 2 × CH_3), 18.5 and 18.6 [2 × s, 2 × $C(CH_3)_3$], 26.06 and 26.07 [2 × s, 2 × $C(CH_3)_3$], 41.0 (d, J_{P-C} = 5.6 Hz, NCH_2), 48.5 (d, J_{P-C} = 4.3 Hz, CH_2N), 62.4 (d, J_{P-C} = 5.7 Hz, 2 × OCH_2CH_3), 108.8, 110.3, 120.9, 121.5, 121.9, 130.6, 142.2, 146.3, 146.9 and 160.0 (Ar-C).

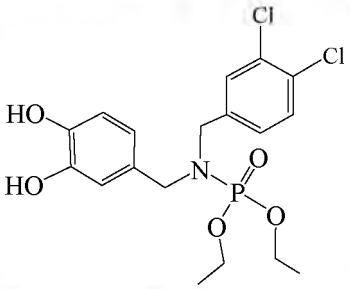
Diethyl *N*-benzyl-*N*-[(3,4-dihydroxyphenyl)methyl]phosphoramidate 229a



1M-*Tert*-butylammonium fluoride (TBAF; 0.20 g, 0.74 mmol) in THF was added dropwise to a chilled solution of diethyl *N*-benzyl-*N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]-methyl}phosphoramidate **228a** (0.20 g, 0.34 mmol) in THF (2 mL). After stirring at r.t. for 3 h, water (5 mL) was added and the mixture was extracted with EtOAc (2 × 20 mL). The organic layers were combined, dried using anhydrous $MgSO_4$, and filtered and the solvent was evaporated *in vacuo* to afford the crude product. Flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*benzyl*-N-*[(3,4-dihydroxyphenyl)-methyl]phosphoramidate* **229a** as a yellow oil (0.10 g, 83%) [HRMS: *m/z* calculated for $C_{18}H_{25}NO_5P$ (MH^+) 366.1470. Found 366.1468]; ν_{max}/cm^{-1} 3395 (OH); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 (6H, t, J = 7.0 Hz, 2 × CH_3), 3.95 (2H, d, J_{P-H} = 10.8 Hz, CH_2N), 4.05 (2H, d, J_{P-H} = 10.9 Hz, NCH_2), 4.07 (4H, m, 2 × OCH_2CH_3), 6.51 (1H, d, J = 7.7 Hz, Ar-H), 6.65 (1H, s, OH), 6.81 (1H, d, J = 7.9 Hz, Ar-H), 7.09 (1H, s, Ar-H), 7.26-7.34 (5H, overlapping

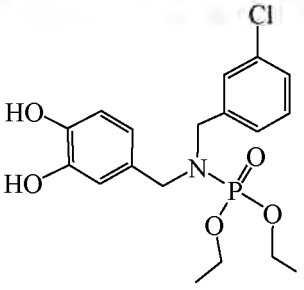
m, Ar-H) and 8.59 (1H, br s, OH); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.3 Hz, 2 × CH_3), 41.0 (d, J_{P-C} = 5.2 Hz, NCH_2), 48.5 (d, J_{P-C} = 4.8 Hz, CH_2N), 63.1 (d, J_{P-C} = 6.0 Hz, 2 × OCH_2CH_3), 114.6, 115.5, 121.0, 127.6, 128.6, 128.7, 129.2, 137.3, 144.4 and 144.9 (Ar-C).

Diethyl *N*-(3,4-dichlorobenzyl)-*N*-[(3,4-dihydroxyphenyl)methyl]phosphoramidate 229b



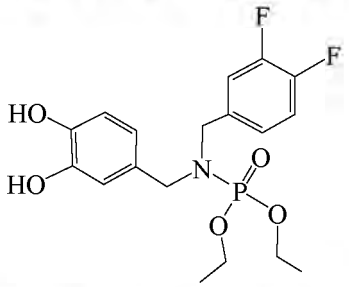
The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-dihydroxyphenyl)-methyl]phosphoramidate **229a** was followed, using diethyl *N*-{[3,4-bis(*tert*-butyldimethyl-silyloxy)phenyl]methyl}-*N*-(3,4-dichlorobenzyl)phosphoramidate **228b** (0.60 g, 0.91 mmol) and 1M-*tert*-butylammonium fluoride (TBAF; 0.59 g, 2.3 mmol) in THF (3 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl N-(3,4-dichlorobenzyl)-N-[(3,4-dihydroxyphenyl)methyl]phosphoramidate* **229b** as a cream solid (0.34 g, 87%), m.p. 104-106 °C [HRMS: *m/z* calculated for $C_{18}H_{23}{}^{35}Cl_2NO_5P$ (MH^+) 434.0691. Found 434.0690]; ν_{max}/cm^{-1} 3392 (OH); δ_H/ppm (400 MHz; $CDCl_3$) 1.24 (6H, t, *J* = 7.1 Hz, 2 × CH_3), 3.84 (2H, d, J_{P-H} = 9.9 Hz, CH_2N), 3.90 (2H, d, J_{P-H} = 11.1 Hz, NCH_2), 4.02 (4H, m, 2 × OCH_2CH_3), 6.43 (1H, d, *J* = 8.0 Hz, Ar-H), 6.75 (1H, d, *J* = 8.0 Hz, Ar-H), 6.86 (1H, s, Ar-H), 6.98 (1H, d, *J* = 8.1 Hz, Ar-H), 7.28 (2H, m, Ar-H) and 7.94 (1H, br s, OH); δ_C/ppm (100 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.3 Hz, 2 × CH_3), 47.2 (d, J_{P-C} = 5.0 Hz, NCH_2), 48.4 (d, J_{P-C} = 4.9 Hz, CH_2N), 63.4 (d, J_{P-C} = 6.1 Hz, 2 × OCH_2CH_3), 115.2, 115.6, 120.8, 128.0, 128.5, 130.5, 130.6, 131.6, 132.7, 137.7, 144.4 and 144.5 (Ar-C).

Diethyl *N*-(3-chlorobenzyl)-*N*-[(3,4-dihydroxyphenyl)methyl]phosphoramidate 229c



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-dihydroxyphenyl)methyl]phosphoramidate **229a** was followed, using diethyl *N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}-*N*-(3-chlorobenzyl)phosphoramidate **228c** (0.60 g, 0.95 mmol) and 1M-*tert*-butylammonium fluoride (TBAF; 0.62 g, 2.4 mmol) in THF (3 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] afforded *diethyl* N-*(3-chlorobenzyl)*-N-*[(3,4-dihydroxyphenyl)methyl]phosphoramidate* **229c** as a cream solid (0.34 g, 88%), m.p. 72-74 °C [HRMS: *m/z* calculated for $C_{18}H_{24}{}^{35}ClNO_5P$ (MH^+) 400.1081. Found 400.1077]; ν_{max}/cm^{-1} 3394 (OH); δ_H/ppm (400 MHz; $CDCl_3$) 1.31 (6H, t, J = 7.1 Hz, 2 × CH_3), 3.94 (2H, d, J_{P-H} = 10.3 Hz, CH_2N), 4.00-4.15 (6H, overlapping m, NCH_2 and 2 × OCH_2CH_3), 6.53 (1H, dd, J = 1.8 and 8.0 Hz, Ar-H), 6.83 (1H, d, J = 8.0 Hz, Ar-H), 6.98 (1H, s, Ar-H), 7.12 (1H, t, J = 8.0 Hz, Ar-H), 7.23-7.30 (4H, overlapping m, Ar-H and OH) and 8.18 (1H, br s, OH); δ_C/ppm (100 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.3 Hz, 2 × CH_3), 47.6 (d, J_{P-C} = 4.9 Hz, NCH_2), 48.4 (d, J_{P-C} = 5.0 Hz, CH_2N), 63.3 (d, J_{P-C} = 6.0 Hz, 2 × OCH_2CH_3), 115.0, 115.6, 120.9, 125.6, 126.8, 127.7, 128.7, 129.8, 134.5, 139.6, 144.4 and 144.9 (Ar-C).

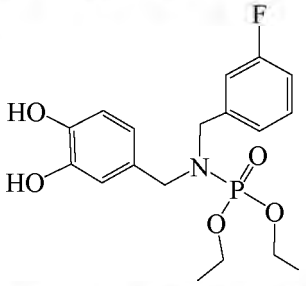
Diethyl *N*-(3,4-difluorobenzyl)-*N*-[(3,4-dihydroxyphenyl)methyl]phosphoramidate 229d



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-dihydroxyphenyl)methyl]phosphoramidate **229a** was followed, using diethyl *N*-{[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]methyl}-*N*-(3,4-difluorobenzyl)phosphoramidate **228d** (0.60 g, 0.95 mmol) and 1M-*tert*-butylammonium fluoride (TBAF; 0.62 g, 2.4 mmol) in THF (3 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl* N-*(3,4-difluorobenzyl)*-N-*[(3,4-dihydroxyphenyl)methyl]phosphoramidate* **229d** as a cream solid (0.32 g, 84%), m.p. 60-62 °C [HRMS: m/z calculated for $C_{18}H_{23}{}^{19}F_2NO_5P$ (MH^+) 402.1282. Found 402.1278]; ν_{max}/cm^{-1} 3359 (OH); δ_H/ppm (400 MHz; $CDCl_3$) 1.31 (6H, t, J = 7.1 Hz, 2 × CH_3), 3.92 (2H, d, J_{P-H} = 10.1 Hz, CH_2N), 3.99 (2H, d, J_{P-H} = 10.9 Hz, NCH_2), 4.08 (4H, m, 2 × OCH_2CH_3), 6.51 (1H, d, J = 8.0 Hz, Ar-H), 6.82 (1H, d, J = 8.0 Hz, Ar-H), 6.94 (2H, m, Ar-H) and 7.10 (2H, m, Ar-H); δ_C/ppm (100

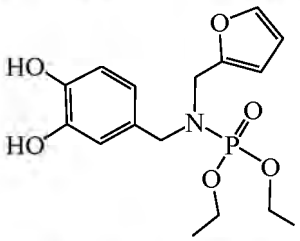
MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.2 Hz, 2 × CH_3), 47.2 (d, J_{P-C} = 5.0 Hz, NCH_2), 48.3 (d, J_{P-C} = 4.9 Hz, CH_2N), 63.3 (d, J_{P-C} = 6.0 Hz, 2 × OCH_2CH_3), 115.1, 115.6, 116.5, 117.3 (dd, J_{F-C} = 17.3 and 20.4 Hz), 120.8, 124.6 (d, J_{F-C} = 3.5 and 6.2 Hz), 128.6 (d, J_{F-C} = 3.0 Hz), 134.5, 144.4, 145.0, 148.9 (dd, J_{F-C} = 12.0 and 246.0 Hz) and 151.4 (dd, J_{F-C} = 13.0 and 247.0 Hz) (Ar-C).

Diethyl *N*-[(3,4-dihydroxyphenyl)methyl]-*N*-(3-fluorobenzyl)phosphoramidate 229e



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-dihydroxyphenyl)-methyl]phosphoramidate **229a** was followed, using diethyl *N*-{[3,4-bis(*tert*-butyldimethyl-silyloxy)phenyl]methyl}-*N*-(3-fluorobenzyl)phosphoramidate **228e** (0.60 g, 0.98 mmol) and 1M-*tert*-butylammonium fluoride (TBAF; 0.64 g, 2.5 mmol) in THF (3 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (3:7)] afforded *diethyl* N-*{[(3,4-dihydroxyphenyl)methyl]*-N-*(3-fluorobenzyl)phosphoramidate* **229e** as a cream solid (0.32 g, 85%), m.p. (52-54 °C) [HRMS: m/z calculated for $C_{18}H_{24}{}^{19}FNO_5P$ (MH^+) 384.1376. Found 384.1372]; ν_{max}/cm^{-1} 3278 (OH); δ_H/ppm (400 MHz; $CDCl_3$) 1.27 (6H, t, J = 7.1 Hz, 2 × CH_3), 3.91 (2H, d, J_{P-H} = 10.4 Hz, CH_2N), 3.98-4.12 (6H, m, NCH_2 and 2 × OCH_2CH_3), 6.49 (1H, d, J = 8.0 Hz, Ar-H), 6.79 (1H, d, J = 8.0 Hz, Ar-H), 6.90-6.99 (5H, overlapping m, Ar-H and OH), 7.22 (1H, t, J = 7.0 Hz, Ar-H) and 8.14 (1H, br s, OH); δ_C/ppm (100 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.3 Hz, 2 × CH_3), 47.7 (d, J_{P-C} = 5.0 Hz, NCH_2), 48.3 (d, J_{P-C} = 5.0 Hz, CH_2N), 63.2 (d, J_{P-C} = 6.0 Hz, 2 × OCH_2CH_3), 114.5 (d, J_{F-C} = 21.2 Hz), 115.1, 115.4 (d, J_{F-C} = 21.5 Hz), 115.7, 120.9, 124.2 (d, J_{F-C} = 2.7 Hz), 128.7 (d, J_{F-C} = 2.7 Hz), 130.0 (d, J_{F-C} = 8.2 Hz), 140.1 (dd, J_{F-C} = 2.6 and 6.8 Hz), 144.4, 144.9, 161.9 (d, J_{F-C} = 244.0 Hz) (Ar-C).

Diethyl *N*-[(3,4-dihydroxyphenyl)methyl]-*N*-furfurylphosphoramidate 229f



The procedure described for the synthesis of diethyl *N*-benzyl-*N*-[(3,4-dihydroxyphenyl)-methyl]phosphoramidate **229a** was followed, using diethyl *N*-{[3,4-bis(*tert*-butyldimethyl-silyloxy)phenyl]methyl}-*N*-furfurylphosphoramidate **228f** (0.60 g, 1.0 mmol) and 1M-*tert*-butylammonium fluoride (TBAF; 0.67 g, 2.6 mmol) in THF (3 mL). Work-up and flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] afforded *diethyl* N-*[(3,4-dihydroxyphenyl)methyl]*-N-*furfurylphosphoramidate* **229f** as a yellow oil (0.31 g, 85%) [HRMS: m/z calculated for $C_{16}H_{23}NO_6P$ (MH^+) 356.1263. Found 356.1255]; ν_{max}/cm^{-1} 3240 (OH); δ_H/ppm (400 MHz; $CDCl_3$) 1.29 (6H, t, J = 7.1 Hz, 2 × CH_3), 3.99-4.13 (8H, overlapping m, 2 × NCH_2 and 2 × OCH_2CH_3), 6.19 (1H, d, J = 3.0 Hz, Ar-H), 6.30 (dd, J = 1.9 and 3.1 Hz, Ar-H), 6.64 (1H, dd, J = 1.9 and 8.0 Hz, Ar-H), 6.84 (1H, d, J = 8.0 Hz, Ar-H), 7.00 (1H, d, J = 1.9 Hz, Ar-H), 7.24 (1H, br s, OH), 7.36 (1H, s, Ar-H) and 8.17 (1H, br s, OH); δ_C/ppm (100 MHz; $CDCl_3$) 16.2 (d, J_{P-C} = 7.4 Hz, 2 × CH_3), 40.9 (d, J_{P-C} = 5.6 Hz, NCH_2), 48.7 (d, J_{P-C} = 4.7 Hz, CH_2N), 63.0 (d, J_{P-C} = 5.9 Hz, 2 × OCH_2CH_3), 109.2, 110.4, 115.1, 115.7, 120.9, 129.0, 142.4, 144.4, 144.9 and 151.3 (Ar-C).

3.6. BIOASSAY PROCEDURES

The assays were conducted for the author by Ms Michelle Isaacs and Mr Dumisani Mnkandhla (Centre for Chemico- and Biomedicinal Research, Rhodes University.[234,235] 20 mM Solutions of the phosphonated esters / phosphoramidates / phosphonic acids / phosphoramidic acids in DMSO were prepared and kept in the freezer. The assays were conducted for each of the compounds in triplicate.[255]

a. **Cytotoxicity assay** – Using a sterile working area, the compounds were incubated at 48 h at fixed concentrations of 100 μM, 25 μM and 6.25 μM in a 96-well plate containing HeLa (human cervix adenocarcinoma) cells or HEK 293 (human embryonic kidney) (6.7×10^5 cells; 150 μL). The test compound solution (20 μL) and resazurin dye (20 μL) were then added to each well, and the plate was returned to the incubator

for 2 h. The plates were placed in the gyratory shaker to equalize distribution. The numbers of cells surviving drug exposure were determined using the resazurin-based reagent flurorometrically in a multiwall plate reader at a wavelength of 560 nm and 590 nm. Emetine was used as a standard control with an IC_{50} of 0.017μM.

b. **PLDH assay** – Malaria parasites were maintained in RPMI 1640 medium containing 2 mM L-glutamine and 25mM Hepes (Lonza) at 37 °C under an atmosphere of 5% CO_2, 5% O_2, 90% N_2 in sealed T25 or T75 culture flasks. The assays were carried out by further dilution of the 20 mM ligand solutions to 20 μM in DMSO; the diluted solution of each compound (100 μL) was added to the parasite cultures in a 96 well plate, followed by 2% of haematocrit (100 μL) and 2% parasitemia (100 μL) and the plates were incubated under CO_2 at 37 °C. After 48 h, 20 μL from each well was transferred to a fresh well plate, and mixed with Malstat and NBT/PES (125 μL). The mixtures were kept in a dark cupboard and the results quantified in a 96-well plate reader by absorbance at 620 nm (Abs_{620}) after 30 min. Chloroquine was used as a reference standard.

c. ***Pf*DXR assay** – The ligand soutions were diluted to 10 mM in DMSO, and 20 μM of each compound was added to 100 μL of a reaction mixture which contained 100 mM Tris-HCl (pH 7.5), 1 mM $MnCl_2$, 0.3 mM NADPH, 0.3 mM 1-deoxy-1-D-xylulose-5-phosphate (DOXP) and 20 μg/mL recombinant *Pf*DXR, and the plate was pre-incubated at 37 °C for 5 min before initiating the reactions by adding DOXP. The course of the reaction was monitored using a Synergy MX3 plate reader (Molecular Devices), measuring the decreasing absorbance at 340 nm. The *Pf*DXR inhibitor FR900098 (Sigma-Aldrich) was used as a positive control.

d. ***Trypanosoma brucei* assay** – *T. brucei* (strain 427) trypomastigotes were incubated with four-fold serial dilutions of the test compounds (final concentrations: 100 μM to 0.0061 μM) in 96-well plates in IMDM medium supplemented with foetal calf serum, HMI-9 supplement, hypoxanthine and penicillin/streptomycin in a 5% CO_2 incubator for 24 h. After incubation, 20 μL of resazurin toxicology reagent (Sigma-Aldrich) was added to each well and, after an additional 2-4 h incubation, the fluorescence was measured at 560 nm and 590 nm. Pentamidine was included as a trypanocidal standard compound.

3.7. COMPUTATIONAL ANALYSIS

a. **Energy level calculations** – The compounds were drawn using Discovery Studio Visualizer and saved in mol2 format.[111] The Gaussview 4.1 program was used to visualize the resulting structures. Transition state searches were conducted using redundant coordinate scans of 20 steps of -0.1Å. Calculations were done using the Gaussian calc setup in opt + freq job type, at the B3LYP/6-31+G(d) level of theory on the local cluster (perkin). Further calculations were conducted using the national CHPC facility,[218] and Chemcraft 8.1 was used to visualize the different energy levels for each scan. Transition states were characterised by the presence of a single imaginary frequency.

b. **Modelling studies** – The compounds were prepared in Discovery Studio Visualizer,[111] cleaned and saved as pdb files. The com files were accessed using the command.

babel -ipdb file.pdb -ogau file.com

The com files were edited in WinSCP and geometry optimisation and frequency calculation were conducted at the B3LYP/6-31G(d) levels; the charges on the atom were also edited. The edited com files were submitted on perkin or CHPC using Gaussian 03 or Gaussian 09 respectively.[217,237] The successfully optimized log files were converted to new pdb files depending on where the initial calculations were made using the command.

CHPC: *babel -ig09 file.log -opdb file.pdb*
Perkin: *babel -ig03 file.log -opdb file.pdb*

The X-ray crystal structure of *Pf*DXR taken from the RCSB Protein Data Base (PDB entry code 3AU9);[111] water molecules and inhibitor (fosmidomycin) were deleted, leaving NADPH and the Mg^{2+} cofactor in place. For flexible docking, Ser270, Ser306, Asn311, Lys312 and Glu315 residues were made flexible. The Autovina 4.2 programme[238] was used to explore the binding mode of selected compounds when docked in the binding site.

Since silicon atoms are not supported on Autovina, the AD4 parameter files for silicon atoms were obtained and used for the *in silico* docking on Autodock tools. As usual, the structures of the compound and the protein were prepared using Discovery Studio

Visualizer.[111] All water molecules were deleted from the pdb file, hydrogen atoms were added and each atom was assigned an Autodock Type using AutoDock Tools (ADT). Gasteiger partial charges[240] were assigned and the non-polar hydrogens were merged for each ligand and the protein model. All ligand torsions were permitted to rotate during the docking. Using the same flexible residues as that of Autovina docking approach, the AutoGrid 4.0 algorithm was employed to locate the *Pf*DXR active-site with a grid box of dimensions 40 × 40 × 40 units along the x-, y- and z-directions. The Mg atom was assigned a charge of +2 prior to docking using the AD4 parameter file value. A Larmarckian genetic algorithm was used to conduct the *in silico* docking of each of the ligands with the following parameters were used: runs of 100; population size of 150; allowing a maximum of 25,000,000 generations and 2.5×10^7 energy evaluations; mutation rate of 0.02 and a cross over rate. For each ligand docking, one hundred docked conformers were generated, and the best conformer was selected based on:- (i) the numbers of conformers; (ii) hydrogen-bonding interaction; and (iii) the estimated free binding energy. Visualization was done using Discovery Studio Visualizer.[111]

c. **CDK descriptors** - Using the CDK descriptor calculator, which is a java-based tool based on the chemical development kit (CDK), the sdf files were read and several physico-chemical properties calculated (ALogP, ALog2, AMR, Lipinski's rule of five, XLogP, hydrogen-bond acceptors, hydrogen-bond donors, TopoSA).

d. **QSAR** - Quantitative structure activity relationships studies were performed using the ligands constructed for the docking studies and the bioassay percent inhibition results. The ligand structures were merged into an sdf file format, and the bioassay results were included into the sdf file. This file was then used in the Open3DQSAR software for further analysis.

4. REFERENCES

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