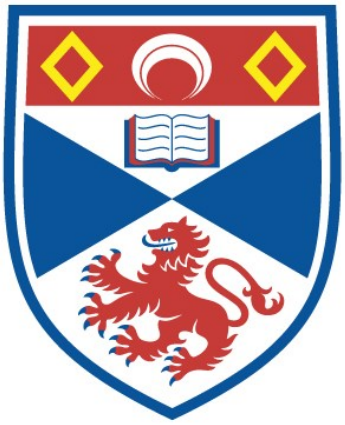
BORON-CONTAINING COMPOUNDS AS INHIBITORS OF HIV PROTEINASE

Donald Carles McNab

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Boron-Containing Compounds

as Inhibitors of HIV Proteinase

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Donald Charles McNab

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ABSTRACT

HIV Proteinase (HIV PR) has proved to be an excellent target for the development of anti-AIDS drugs. Four inhibitors of this enzyme are now approved for clinical use but they, like others, suffer from shortcomings associated with their size and the fact they are peptides. In this thesis the development of small non-peptidic cyclic compounds is described. They were designed to inhibit HIV PR principally by targeting its unique structural features rather than by mimicking its natural substrates.

The designed compounds all contained the borinic acid functional group which it was anticipated would interact with the two critical aspartic acid residues of HIV PR. A heteroatom incorporated into these compounds was positioned in such a way that a water molecule which plays a pivotal role in the binding of the enzyme's substrates was displaced. Finally, two benzyl groups were incorporated; these were designed to mimic the side-chains of phenylalanine and tyrosine frequently found in the substrates of HIV PR.

The borinic acid functional group has not previously been incorporated into HIV PR inhibitors. Therefore, an analogous series of five 2,6-dibenzylated-4-heterocyclohexanols, where the borinic acid group had been replaced by a hydroxyl group, were prepared and evaluated against the enzyme. These were prepared through *bis* aldol condensations followed by reduction of the carbonyl group in the *bis*enones that resulted from dehydration. Although low solubility prevented the analysis of three of these compounds and thereby their effectiveness, two of them were found to be moderately active. Having validated the design of the heterocyclic template, attempts were then made to synthesise borinic acid-containing analogues of these 4-heterocyclohexanols.

The attempted syntheses of a directly comparable series of compounds, through application of both *bis*metallation and *bis*hydroboration strategies, was unsuccessful. Instead, two acyclic diphenyl borinic acids were synthesised. Additionally, several related cyclic borinic acids and acyclic borinic and boronic acids consistent with the design strategy were prepared, from diphenyl sulfone, 2-bromodiphenyl ether and 2-bromodiphenyl sulfide, through the appropriate lithiated species formed by either lithium-hydrogen or lithium-halogen exchange. During the attempted syntheses of a cyclic borinic acid by selective oxidative cleavage of an organoborane derived from diphenyl sulfone, two highly novel borane-amine adducts were synthesised.

None of the boron-containing compounds assayed against HIV PR were found to be inhibitors of the enzyme. This is thought to be as a result of the phenyl groups being directly attached to the boron atom, rather than being present in benzyl substituents as had been originally planned.

CONTENTS

NON-AMINO ACID ABBREVIATIONS

ABBREVIATION	MEANING
Ac	Acetyl
AIDS	Acquired immune deficiency syndrome
Aliquot 336	Methyltri-*n*-octylammonium chloride
CA	Capsid protein
Cbz	Benzyloxycarbonyl
CI	Chemical ionisation
d.e.	diastereomeric excess
DIBAL-H	Di-*iso*butyl aluminium hydride
DMF	*N*,*N*-Dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EI	Electron impact
eq.	equivalent(s)
ES	Electrospray
ether	diethyl ether
gp	glycoprotein. The number that follows denotes its mass in kiloDaltons, as determined by electrophoresis.
HIV	Human immunodeficiency virus
HMPA	Hexamethylphosphoromide
HRMS	High resolution mass spectrometry
IC_{50}	The concentration of inhibitor required to reduce an enzyme's activity by 50%.
IN	Integrase enzyme

IR	Infra-red
IUPAC	International Union of Pure and Applied Chemistry
kD	kiloDaltons
K_d	Monomer-dimer dissociation constant
K_i	Enzyme inhibition constant
LDA	Lithium di*iso*propylamide
LTR	Long terminal repeat: identical base sequences found at the termini of a linear nucleic acid.
MA	Matrix protein
MCBS	Monochloroborane-methyl sulfide complex
*m*CPBA	*meta*-Chloroperbenzoic acid
n-BuLi	*n*-Butyllithium solution
NC	Nucleocapsid protein
NMR	Nuclear magnetic resonance
NPI	Non-peptidic inhibitor
ORF	Open reading frame: regions of DNA between stop codons that code for a substantial amount of protein.
p or Pr	protein. The number that follows denotes its mass in kiloDaltons, determined by electrophoresis.
PCC	Pyridinium chlorochromate
PR	Proteinase enzyme
RT	Reverse transcriptase enzyme
RNA	Ribonucleic acid
s-BuLi	*s*-Butyllithium solution
SU	Surface glycoprotein
TBDMS	*tert*-butyldimethylsilyl
THF	Tetrahydrofuran
TBAF	Tetra-*n*-butylammonium fluoride
t-BuLi	*t*-Butyllithium solution

TLC	Thin layer chromatography
TM	Transmembrane glycoprotein
TMEDA	*N,N,N',N'*-Tetramethylethylenediamine
TMS	Trimethylsilyl
UV	Ultraviolet

AMINO ACID ABBREVIATIONS

ABBREVIATION	AMINO ACID
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Nle	Norleucine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Tyr	Tyrosine
Val	Valine
Xaa	Any amino acid

CHAPTER ONE

INTRODUCTION

Introduction

1.1 The Realities of AIDS Today

The human immunodeficiency virus (HIV) that has been shown to cause AIDS,[1,2] is one of the principal threats to human life and well being today. The Global AIDS Policy Coalition estimated that the number of people infected worldwide at the end of 1987 was around 7 million.[3] Ten years later, estimates suggest that 25 million people are infected with the virus and that 6 million people have died from AIDS-related diseases.[4] The number of cases of HIV infection expected to have occurred by the year 2000 range from 40-110 million.[3]

It is now 16 years since AIDS sprang into medical and public awareness as a fatal disease of the immune system.[5,6] HIV is the most intensively studied virus in history. Despite this, many aspects of HIV infection and its pathogenesis remain unclear. Whilst treatments exist to control HIV in the body (these will be discussed through this chapter), there is no cure. It is true that there are other alarming diseases; 40% of the world's population are exposed to malaria and 2 million people die from it each year.[7] However, the rapid spread of HIV and lack of any vaccine to prevent AIDS make it particularly alarming. Although therapies will help to control the pandemic, HIV resistance to these treatments continues to increase and has been extensively examined.[8,9] Also, it is important to emphasise in this context that 90% of people with HIV infection live in societies without the financial resources to afford such treatments.[10] The unprecedented effort in the search for a cure will therefore continue.

1.2 General Introduction to HIV

After the identification of HIV as the primary aetiological agent for AIDS,[1,2] remarkable progress has been made in elucidating many of the aspects of the biological structure of HIV. It is a member of a large group of viruses known as the *retroviridae* or

retroviruses.[11] Retroviruses (*retro* and *virus*, respectively, are Latin for "backwards" and "poison") are defined as viruses with RNA genomes, the replication of which takes place by a DNA intermediate. Within this family HIV is classed as a lentivirus (*lentus* is Latin for "slow"); infection is followed by a subclinical latent phase which can last for several years. Such a delay in the pathogenesis enables HIV to survive in nature; as the major modes of transmission of HIV are either vertical or by intimate contact, it is paramount that it should not kill its host before it replicates otherwise it will not survive.[12]

Two genetically distinct but related HIV strains have been identified. The first, HIV-1,[1,2] is highly virulent and is primarily responsible for the pandemic throughout Europe, the USA, Africa, India and Asia. The second strain, HIV-2,[13] is virtually confined to regions of West Africa and is less pathogenic than HIV-1.[14] Within these two strains, however, there are many different subtypes. For example, ten discrete strains constitute the group M (major) of HIV-1 viruses.[15] The base sequences that comprise their genomes vary by 21-31% but each strain is know to contain a plethora of variants, whose genomes' base sequences vary by an average of 11%.[15] Whilst the organisations of the genomes of HIV-1 and HIV-2 are very similar, they differ by more than 55% in their primary nucleotide sequences.[16] In fact, HIV-2 is more closely related to simian immunodeficiency virus (SIV), which infects African monkeys with no pathological consequences, than it is to HIV-1.[17] As HIV-1 is the pathogen of greater consequence,[14] the remainder of this discussion will focus on this strain.

1.2.1 Description of an HIV-1 Virion

Electron microscopy reveals HIV-1 (Fig. 1.1) to be a roughly spherical particle with a diameter of 110 nm.[3,18] Its surface is covered in a lipid bilayer similar to and taken from the membranes surrounding human cells. Protruding from this layer are 72 spikes. These spikes are composed of multimers (probably containing four each) of two viral glycoproteins: surface protein (SU) gp120 and transmembrane protein (TM) gp41. Noncovalently linked to gp41, gp120 is displayed at the surface of the virus where it

recognises cells which display an appropriate receptor. [Genetic nomenclature used in this text is according to Leis *et al.* (for example the denotation of surface protein gp120 as SU)].[19]

Beneath the lipid bilayer is a sphere of matrix protein (MA) p17. Beneath MA is a bullet-shaped core enclosed by a further layer of protein, the capsid (CA) p24. It measures 100 nm by 50 nm tapering to 40 nm.

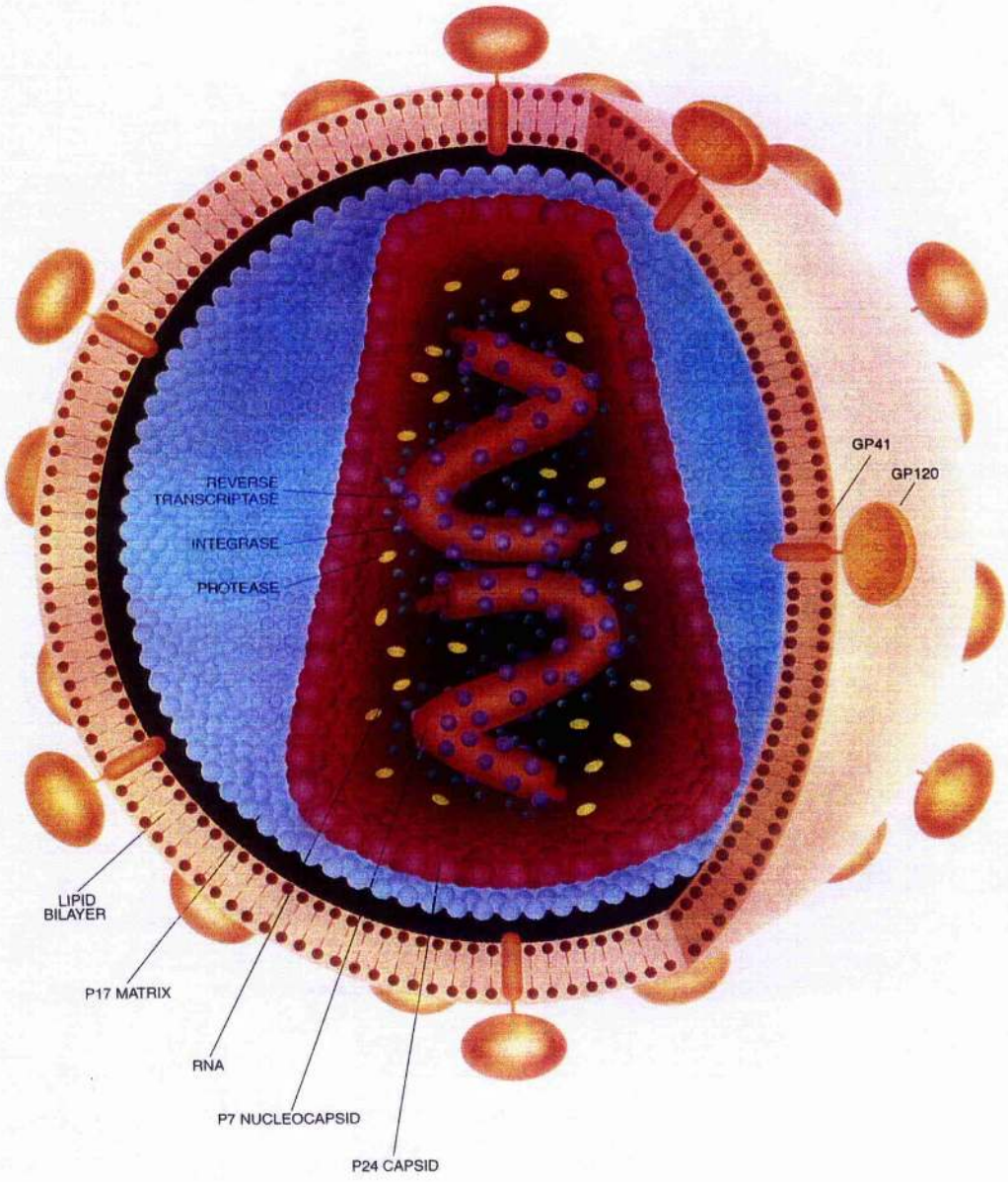


Figure 1.1: *A Single Virion of HIV-1 (reproduced from* Scientific American[3] *with the kind permission of the artist, Mr Kirk Moldoff)*

Contained within CA are the components required for replicating HIV,[20] as follows:

- two identical strands of the RNA genome (see section 1.2.2). This consists of 9,800 nucleotides which code for the entire structure and replication processes of HIV. Associated with the RNA are two further proteins, the nucleocapsid (NC) proteins p9 and p6.
- two cellular transfer RNAs captured during the budding process (see section 1.3.2.2)
- three virally encoded enzymes called reverse transcriptase (RT), integrase (IN) and proteinase (PR). The roles of these will be discussed later (section 1.3.2)

1.2.2 The Genome of HIV-1

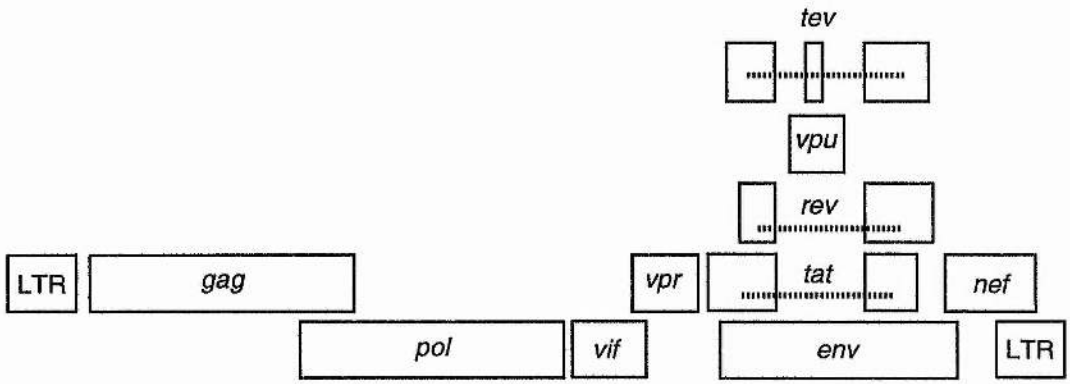


Figure 1.2: *The HIV-1 Genome*[21]

Despite its relatively small size (it is 100,000 times smaller than the human genome), sequencing of the HIV-1 genome revealed it to be astonishingly complex.[11] It exhibits the same basic organisation as all retroviruses (5'-LTR-*gag-pol-env*-LTR-3'),[22] but additionally carries ORFs for several regulatory proteins. As can be seen from Fig. 1.2 several of these genes overlap. This means that not all the proteins are encoded in the same ORF. However, all the viral proteins found in the intact virus described earlier are derived from just three genes (overleaf):[20,21,23,24]

gag • this encodes the structural proteins MA, CA and NC

pol • this encodes the viral enzymes required for replication: PR, RT and IN

env • this encodes the glycoproteins SU and TM

The remaining genes encode the regulatory proteins.[20,21,23-25] Two of these, *tat* (transactivator) and *rev* (regulator of virion expression) play essential roles in the replication cycle of HIV.[3,25] *Vpr* (viral protein R), *vpu* (viral protein U), *vif* (viral infectivity factor) and so-called *nef* (negative factor) are frequently referred to as non-essential or accessory proteins. This is because early studies,[25] suggested that these genes were dispensable for virus growth *in vitro* although important *in vivo* functions may remain to be elucidated. The remaining *tev* gene encodes a protein that appears to have both *tat* and *rev* activities, although its actual regulatory role is unknown.[21] At the termini of the genome are LTRs. Signals provided within the LTRs, which are unique to retroviruses,[12] stimulate and sometimes regulate the activity of the cellular transcription system.[3]

1.3 HIV Infection and Disease

The infection of humans with HIV is characterised by three phases. Within several weeks of infection there is an acute phase. This is typified by extensive viraemia and large numbers of CD4-bearing *T* cells in the blood (see section 1.3.1).[26] The onset of the immune response causes the amount of circulating virus to decline sharply, by a factor of 100 or more.[27] There then follows a clinically latent steady-state chronic phase when cells are infected (see section 1.3.2) and die in large numbers and at a high rate; during this time the total number of infected cells remains small but constant.[28,29] This phase lasts for about 10 years after which the number of CD4 cells have greatly declined (from an initial concentration of 1000 to less than 100 per mm^3).[3] The symptoms of AIDS (the final stage of HIV infection) then appear, coincident with sharply increasing viraemia and numbers of infected cells in circulation. At this point the immune system has been so badly damaged that the infected patient is prone to opportunistic infections such as pneumonia

(*Pneumocystis carinii*);[6] in the absence of an adequate defence mechanism, death usually results.

Many aspects of the progression of HIV-1 infection are extremely complex and poorly understood. The exact role that viral latency plays in HIV disease,[26,28] the mechanisms by which HIV-1 infection actually causes cell death and other aspects of HIV infection and disease are not understood fully.[30,31] However, much is known about the propagation of HIV-1 and this is discussed in section 1.3.2.

1.3.1 The Biological Targets of HIV-1

A major consequence of HIV-1 infection is the selective depletion of CD4-bearing *T*-lymphocytes (*T*4 cells),[6,32] the white blood cells that play a central role in regulating the immune system (CD4 is a 55 kD glycoprotein). Different cell types that display CD4 have also been shown to be infected.[33] However, other cells that display either low numbers of CD4 or none at all have also been shown to become infected,[24] suggesting that a second component, thought to be the "chemokine" receptor,[34] is required for binding events in the infection process. The mechanism of entry of HIV-1 is not clearly understood. It is a pH-independent process mediated by gp41,[35] in which the virion enters the cell by either endocytosis or cell fusion,[36] after initial recognition of (probably) CD4 by gp120.

1.3.2 The Replication Cycle of HIV

Once the virion is inside the host cell, the replication cycle may be divided into two sets of processes. The pre-integration phase, shown on the left of Fig. 1.3, consists of work performed almost exclusively by the enzymes contained within and encoded by the infecting virion. Collectively these processes result in the formation of the provirus, the term used to describe the virus once its two strands of DNA have been incorporated (integrated) into the chromosomes of the host. Thereafter, the enzymes and proteins of the

host cell perform the bulk of the work, shown on the right of Fig. 1.3. These processes comprise the post-integration phase.

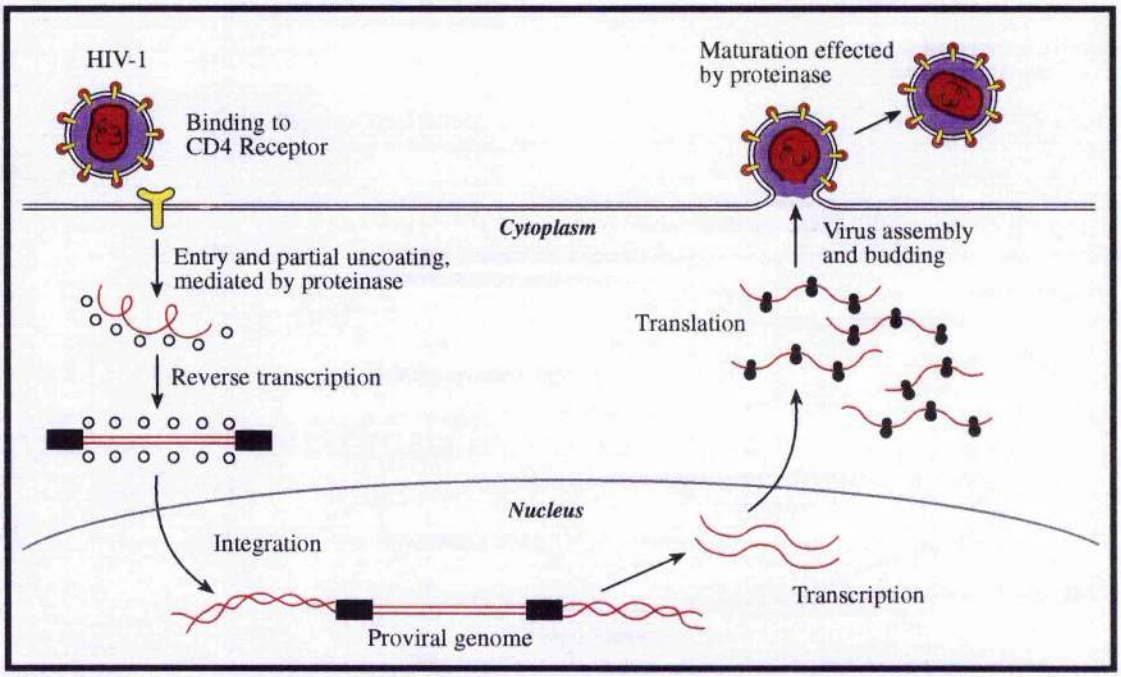


Figure 1.3: *The Replication Cycle of HIV-1*

1.3.2.1 The Pre-Integration Phase

Once the virion has entered the cytoplasm of a susceptible cell, it is "uncoated" by proteolysis. There is evidence both for,[37-39] and against,[40,41] the viral proteinase playing a role in this uncoating. The viral RNA is then transcribed to a DNA copy in a reaction catalysed by RT. RT is closely associated with the viral RNA nucleocapsid protein and is activated within 6 h of infection.[42,43] Initiation of transcription requires a host cell transfer RNA molecule, captured during the budding process (see section 1.3.2.2).[20] The role of RT is to synthesise a double stranded DNA genome which contains all the information originally held on the RNA genome. As part of a nucleoprotein complex comprising the MA, IN and perhaps other regulatory proteins, the proviral DNA is transported into the cell nucleus.[20] Once inside the nucleus, IN catalyses the insertion of the proviral genome into random regions of the host cell's chromatin. Because there is no

evidence to suggest that an integrated HIV provirus is ever excised from the host DNA, it is believed that infection is permanent from this point.

1.3.2.2 The Post-Integration Phase

The post-integration phase occurs when the provirus is expressed. Both transcription and translation are subject to complex regulation by both host cell proteins and by viral proteins such as *tat* and *rev*.[23,24] The initial task of the cell's replication apparatus is to transcribe RNA from the proviral DNA. This is done using cellular RNA polymerase II. Some of the RNA formed serves as the genetic material for the assembly of new virions and some is translated into viral "precursor" polyproteins, as is typical in the translation of viral genomes.[44] The actual viral proteins are synthesised during subsequent processing of these polyproteins. The significance of this delay shall be explained later.

The *gag* gene is translated to produce a polyprotein called $Pr55^{gag}$. Independent translation of the *pol* gene, however, is not known; its translation has only been observed in conjunction with *gag*, to form a fusion polyprotein called $Pr160^{gag\text{-}pol}$.[45] As *gag* and *pol* are in different ORFs, such a translation may occur only by way of a ribosomal frameshift (when translation moves to a different ORF) to the *pol* ORF. The *gag* stop codon is, therefore, not read and translation continues until the *pol* stop codon is encountered. This frameshift occurs infrequently (11% of the time) and ensures that the structural proteins coded by *gag* are synthesised in an 8:1 excess to the enzymes coded by *pol*.[45] The *env* gene is translated as an 88 kD polyprotein containing the proteins necessary for both gp41 and gp120. The translation of the regulatory and accessory genes is complex and will not be discussed here.

The above polyproteins next undergo a series of post-translational modifications. The *env*-associated polyprotein is glycosylated before it is cleaved into gp41 and gp120 by a cellular proteinase.[46] These two glycoproteins are then transported to the cell membrane. Post-translational modification of both $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ is subtly regulated. The N-terminal amino acid, glycine, of both these polyproteins is the

same. This amino acid is recognised by a cellular acylase which adds a myristoyl (tetradecanoyl) unit to its amino group, an addition which directs both polyproteins to the cell membrane.[47,48]

The penultimate stage of the replication cycle is the assembly of viral components into particles and their budding from the cell. It appears that this is a self-assembly process in which the lipophilic N-myristoylated $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ are directed to and are anchored into the cytoplasmic side of the host cell membrane.[48] Strands of $Pr55^{gag}$ then bind both viral RNA and cellular transfer RNA and a small portion of the cellular membrane evaginates to form an immature viral particle.

The immature progeny virus is not infectious at this point.[49] Maturity and thus the ability to infect is conferred on the virus particle by the action of PR.[49] However, PR is active only as a homodimer (discussed in detail below in sections 1.5.2 and 1.5.3).[50] Each strand of $Pr160^{gag\text{-}pol}$ only contains "half" a PR and must therefore associate with another for an active PR to be generated. Such association is achieved during the budding process when the polyproteins are forced together.[51-53] Firstly, the anchoring of N-myristoylated $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ to the membrane increases the local concentration of PR monomer (contained within $Pr160^{gag\text{-}pol}$). Secondly, as the linear membrane buds into a spherical particle, the concentrations of $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ increase still further. At some point, two PR monomers contained within two separate strands of $Pr160^{gag\text{-}pol}$ are forced so close to one another that an active dimer is formed and PR catalyses its own release. This is thought to proceed in an *inter*molecular manner; two domains of PR fold and associate to form an active dimer which acts on a processing site at the PR region in a third strand of $Pr160^{gag\text{-}pol}$ (see section 1.5.3).[53] Once free the dimer performs the cascade of proteolytic cleavages discussed below, thereby effecting the maturation of the virion and the completion of the replication cycle. Maturation may be observed under an electron microscope: the doughnut-like centre of the immature virus is transformed to the diagnostic bullet shape of CA.[48]

It is not possible to overemphasise the critical nature and consequential timing of proteinase self-activation. Delay in the proteolysis of the viral polyproteins containing

the structural and enzymatic proteins ensures that they are all packaged into the budding virus particle. If PR was active before budding, proteolysis of $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ would occur in the cytoplasm of the host cell. This would leave only the N-terminal protein (MA, Fig. 1.6) of both $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ with an attached myristoyl group. Only it would, therefore, concentrate at the cell membrane; the other proteins would have no driving force for doing so. The consequences of premature proteolysis have been demonstrated by Kräusslich,[54] who transfected a gene encoding a tethered dimer of HIV-1 PR into a provirus. Expression of the linked dimer (two copies of the PR region linked by a structurally flexible hinge region) as a component of $Pr160^{gag\text{-}pol}$ by *in vitro* translation causes rapid autocatalytic processing. This phenomenon had previously been reported by a second group.[55] What Kräusslich showed was that this premature processing prevented virion formation and, therefore, the ability to infect. This contrasts directly with the behaviour of the wild-type polyprotein which was stable after prolonged incubation.

1.4 Molecular Targets for Antiviral Chemotherapies

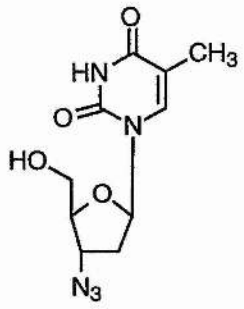
The challenges involved in the development of a prophylactic vaccine against HIV in the near future are considerable; they, and the disappointments experienced so far, have been extensively documented.[56-62] Just one of the many problems is the presence of a seemingly limitless number of strains of HIV-1 (see section 1.2).[15] This is a direct consequence of two apparent flaws of RT: it is highly error-prone and has no "proof-reading" abilities. This means that when it synthesises DNA, it fails to correct any mistakes that it makes. Unfortunately, it has been estimated that it makes an error of up to 1 base in 1,700, and as a consequence, each cycle of replication causes an average of up to five erroneous bases to be incorporated into the transcribed DNA.[63] Because of this, individuals tend to be infected with a viral inoculum composed of a variety of strains. It has therefore proved difficult to find an invariant region of gp120 on which to design a vaccine. As a cure has been impossible to find so far, a great deal of work has been

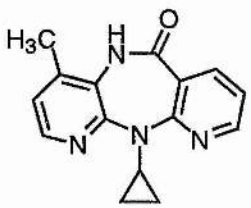
undertaken to develop effective treatments as independent as possible from the heterogeneity imparted by RT.

Knowledge of the array of molecular processes that occur in the replication cycle of HIV-1 (see section 1.3.2) has enabled the identification of numerous virus-specific processes, each of which is a potential target for antiviral chemotherapy. In principle, each stage of the replicative cycle can be envisaged as a viable target. To categorise broadly, these are adsorption, fusion, uncoating, reverse transcription, integration, transcription, translation, assembly and maturation.[64]

Up until the end of 1995, the only available treatments for AIDS were directed against the reverse transcriptase enzyme. As reverse transcription is unique to retroviruses, it was hoped that inhibitors of RT would not interfere with enzymes present naturally in the body. Azidothymidine (**1**, AZT) was approved by the Food and Drug Administration of the USA for the treatment of AIDS in March 1987. It and a number of drugs approved subsequently act against RT by being processed as alternative substrates (nucleoside analogues). They are incorporated by the enzyme into the growing DNA chain but do not permit any further elongation of it. The principal drawbacks of AZT are its toxicity (caused by interaction with other enzymes) and resistance, a problem frequently encountered with HIV therapies.[8,9] Resistant strains of HIV are rapidly found to most drugs as a consequence of the infidelity of RT. These mutants that evolve naturally by base mismatching survive by natural selection because they are drug-resistant. The extremely rapid evolution of HIV (it has recently been estimated that up to 10 billion HIV-1 virions may be produced daily),[28,29] compounds the problem of natural selection of its drug-resistant strains. Consequently, the benefits of drugs that inadvertently stimulate the evolutionary selection of resistant mutants are only temporary.

As a result of the toxicity of some of the nucleoside analogues, non-substrate analogues have been investigated.[65] One such inhibitor that has recently been licensed for clinical use, nevirapine (**2**),[66] is more active than any nucleoside analogue but results in a greater problem with the emergence of resistant mutants than do any of the nucleoside analogues.[9]

1, AZT 

 **2**, Nevirapine

The majority of clinical trials to date have involved drugs targeted against RT and PR. Because of its relevance here, HIV-1 PR is considered separately (see below), but space does not permit the inclusion of the many others enzymes and processes that have been studied. The reader is referred to an excellent review concerning the broad range of strategies that have been and are being investigated.[64] In particular much work has focused on the role and control of the regulatory proteins.[25]

1.5 The HIV-1 Proteinase

1.5.1 HIV-1 PR as a Target for Antiviral Intervention

HIV-1 PR was proposed as a target for therapeutic intervention in the treatment of AIDS as early as 1986.[67] As has been discussed earlier, PR is essential for the production of infectious virions. This was first proved in 1988 when uninfectious virions were produced following site-directed mutagenesis of a single base in the PR-coding region of *pol*.[49] The role of PR in the post-translational maturation step presents an immediate advantage in its inhibition over that of RT. Because RT plays only a pre-translational role, inhibitors of it have no effect against cells already infected.

A second, pre-integrational role of PR, (alluded to in section 1.3.2.1),[37-38] is thought to be the uncoating of the virion after it is brought into the cell. Inhibition of PR may therefore be effective in both infected and uninfected cells. Furthermore, cleavages mediated by HIV-1 PR are not mediated by cellular proteinases (see below).[44,68] This suggests that HIV-1 PR is sufficiently different from cellular proteinases to allow its preferential inhibition.

Finally, very early in the investigation of the enzyme there existed some practical advantages in this approach. Firstly, important technical advances came quickly on the scene:[69] a sufficient and safe source of the enzyme was provided through the application of recombinant DNA technology and convenient *in vitro* assays applicable to the mass screening of potential inhibitors were developed. Secondly, a vast bank of knowledge to help in the design of inhibitors already existed. HIV-1 PR turned out to be in many ways related to renin (an enzyme involved in blood pressure regulation), pepsin (a digestive enzyme) and other extensively studied non-viral proteinases.[70] This greatly sped up initial investigations into the design of inhibitors for the enzyme; indeed, some renin inhibitors were found to inhibit HIV-1 PR.[71]

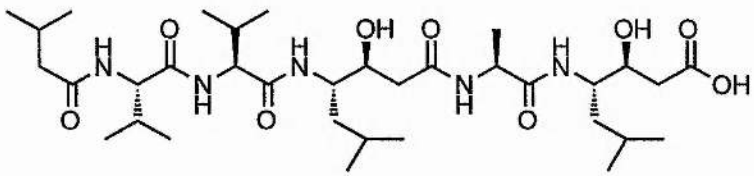
1.5.2 Classification and Structure of HIV-1 PR

The first task in the study of the proteinase was to determine its class: whether it was a cysteine, serine, metallo- or aspartic acid proteinase. This was achieved in a number of laboratories towards the end of the 1980s. After sequencing the entire genome of HIV-1, Ratner *et al.* postulated that the *pol* ORF encoded a proteinase similar to those found in other retroviruses.[11] However, the classes of all of these were unclear. It was subsequently observed that all those regions of retroviral genes thought to encode the proteinase contained the well conserved sequence Asp-Thr(Ser)-Gly.[72] This triad was known to be characteristic of the aspartic acid proteinases (so-called because of two highly conserved aspartic acid residues in the active site) such as renin and pepsin,[73] and it was proposed that the retroviral proteinases were of this class.[72]

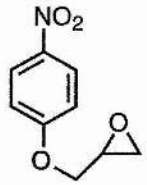
At the time, the known aspartic acid proteinases were two-domain enzymes more than 300 residues in length and contained two Asp-Thr(Ser)-Gly sequences. The retroviral proteinases whose sequences had been determined contained no more than 130 residues. Furthermore, they had only one Asp-Thr(Ser)-Gly sequence. It was proposed that they must act in dimeric forms.[74]

Three types of investigation confirmed both the mechanistic classification and the dimeric nature of the enzyme. Firstly, the enzyme was inhibited by pepstatin A (**3**) ,[71]

a known pentapeptide inhibitor of aspartic acid proteinases and inactivated by 1,2-epoxy-3-(4-nitrophenoxy)propane (**4**, EPNP),[50] which had previously been shown to esterify one or both of the active site aspartic acid residues of pepsin. Secondly, the experiment described in section 1.5.2 showed that the replacement of Asp25 with Asn produced inactive PR.[49] Finally, determination of the crystal structures of both native PR,[75-77] and PR complexed with inhibitor HIV-1 proteinase,[78] confirmed both its mechanistic class and its homodimeric architecture, with each monomer related to the other by a C_2 axis of symmetry (see Fig. 1.4), associated through non-covalent interactions.



3, pepstatin A



4, EPNP

1.5.3 Structural Features of HIV-1 PR

From the crystal structures obtained of the homodimeric HIV-1 PR, its overall molecular architecture and active site have been shown to be remarkably similar to the classical monomeric aspartic acid proteinases, yet they are encoded by only half the genetic information. In fact, it has been proposed that HIV-1 PR and the other viral aspartic acid proteinases are genetic "fossils". It had long been postulated that the primordial form of aspartic acid proteinases was a homodimer which had evolved into a heterodimer by gene duplication and fusion.[73] This is supported by the internal twofold symmetry exhibited by the secondary and tertiary structures of the current monomeric bilobal aspartic acid proteinases. Each lobe of the current monomeric proteinases contributes one Asp-Thr(Ser)-Gly sequence to the active site. In an entirely analogous manner, each 99-amino acid, 11 kD subunit of HIV-1 PR does the same (see Fig. 1.4).

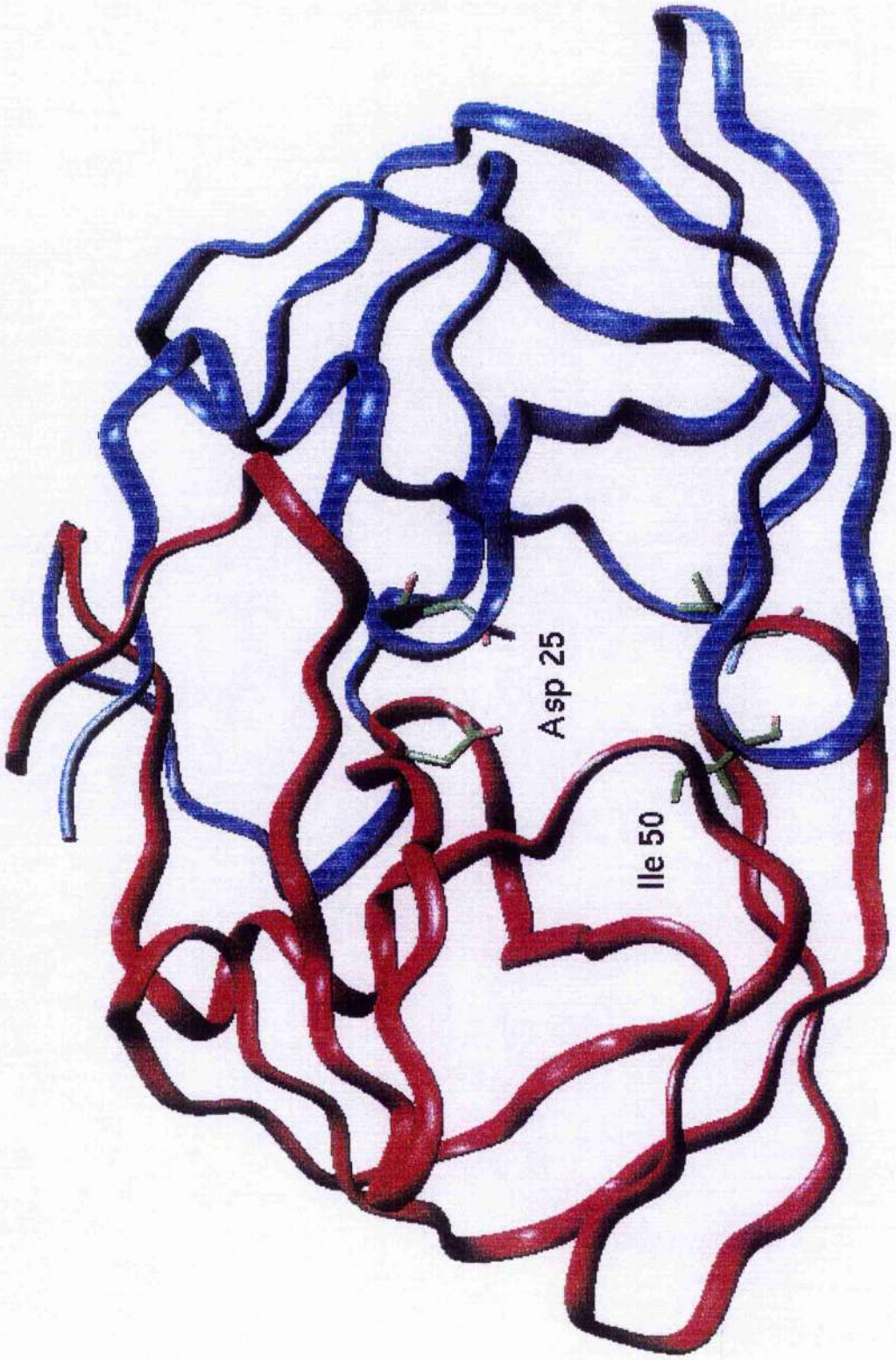


Figure 1.4: *Ribbon drawing of the α-carbon backbone of HIV-1 PR showing its C_2 tertiary structure, the catalytic aspartic acids, 25 and 25' and the isoleucine residues, 50 and 50', present in the flaps (see text)*

The N- and C- termini of both monomers are arranged in an interdigitating network of four antiparallel β-sheets which serve to hold the dimer together through hydrogen bonds.[79] These rigidly held terminal strands are (a) remote from the active site and (b) by virtue of being at the end of the protein chain, form two separate halves of a cleavage site acted on by PR. Consideration of these facts led to the proposal that the autoprocessing of PR was intermolecular (see section 1.3.2.2), involving three (or possibly more) but not two PR domains.[53]

The similarity of HIV-1 PR to the monomeric aspartic acid proteinases is further exemplified by the manner in which it embraces its substrates. HIV-1 PR contains two identical glycine-rich β-hairpin loops (flaps) which open to let substrate in, then close to form a binding pocket around it. This binding pocket is large enough to accommodate six or seven residues of the substrate. Once the cleavage is complete, products are released by the opening of the flaps, which move by up to 7 Å during the processing of an inhibitor.[78] The presence of such flaps is reminiscent of the single flap of the monomeric aspartic acid proteinases.

Unlike these monomeric aspartic acid proteinases however, there exists in most inhibitor complexes a water molecule (henceforth called the flap water molecule) which links the enzyme flaps to the inhibitor (or substrate during proteolysis) through hydrogen bonding interactions (Fig. 1.5). Normally located within 0.2 Å of the C_2 axis of the enzyme, it exhibits approximately tetrahedral coordination by bridging the amide hydrogen atoms of Ile50 and Ile50' [prime (') is employed to distinguish the two chains] and the P_2 and $P_{1'}$ carbonyl oxygen atoms of the inhibitor. (The P_n, $P_{n'}$ terminology of Schechter and Berger,[80] is adopted here. It prescribes that amino acids are numbered P_1, P_2,....from the scissile bond to the N-terminus and $P_{1'}$, $P_{2'}$,....from the scissile bond to the C-terminus. Furthermore, the pocket into which the P_n residue fits is known as S_n). A schematic diagram of the active site is shown overleaf, illustrating the terminology of Schechter and Berger, the hydrogen bonding network around the tetrahedral water molecule, *viz.* the catalytic aspartic acid residues and the scissile bond (boxed).

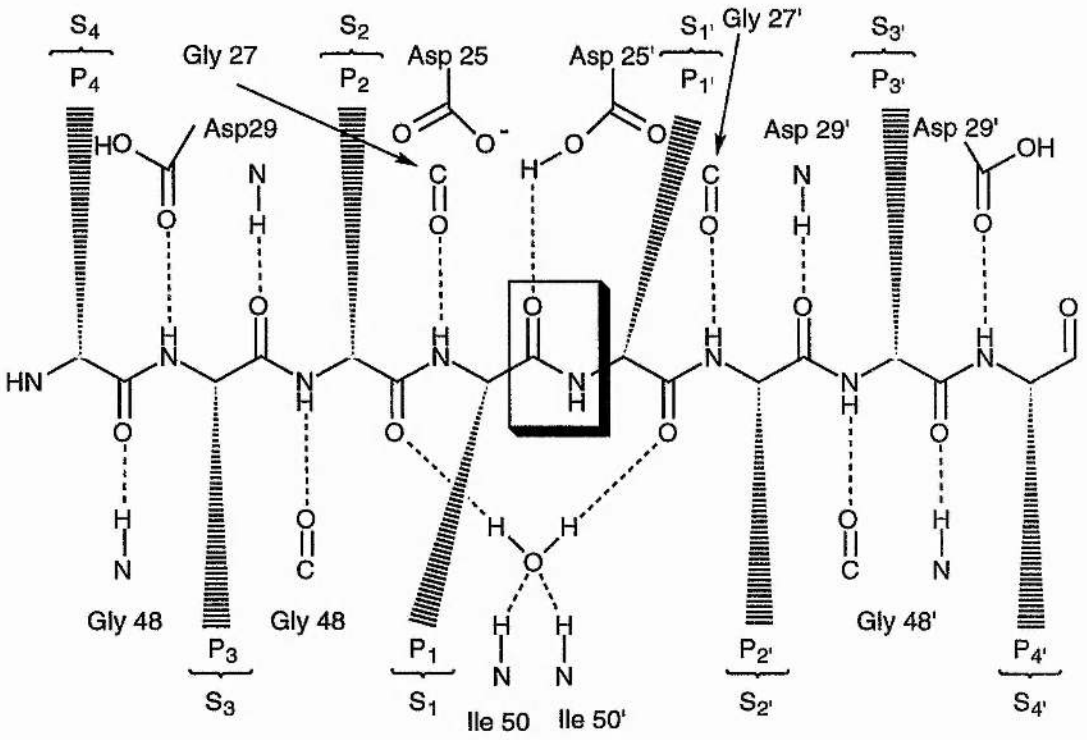


Figure 1.5: *Scheme of the hydrogen bonding between HIV-PR and a putative substrate*

It has been proposed that the flaps serve roles in both binding and catalysis (discussed in section 1.5.5 below). Overall, HIV-1 PR is a very hydrophobic enzyme and its substrates (see Fig. 1.7, page 18) are in general also hydrophobic. The flaps cover four substrate clefts (S_2-$S_{2'}$), increasing the hydrophobic environment around the active site; this is consistent with the hydrophobic nature of P_1 and $P_{1'}$. It is thought that, in addition to binding the substrate through hydrogen bonding interactions with a water molecule, the side chains of Ile50 and Ile50' "capture" the hydrophobic side chains of the P_1 and $P_{1'}$ residues, thereby adding a further distinctive feature to the specificity of the cleavage positions.[81]

1.5.3 Function HIV-1 PR

When processing the viral polyproteins $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$, the enzyme has been shown to perform eight specific cleavages (Figs. 1.6 and 1.7).[23] The scissile bonds of the cleavage sites are henceforth indicated with asterisks.

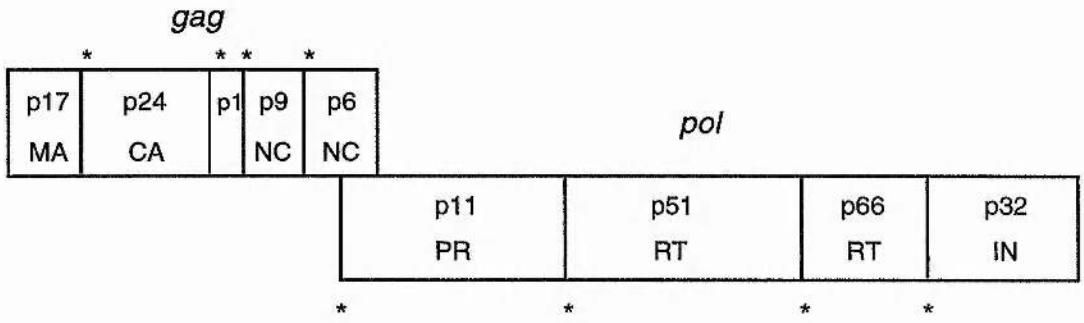


Figure 1.6: *The overlapping* gag *and* pol *ORFs of HIV-1 showing the proteolyses performed by HIV-1 PR once translation to* $Pr55^{gag}$ *and* $Pr160^{gag\text{-}pol}$ *has occurred. The* gag *protein between CA and NC (p9) consists of 14 amino acids and is designated p1.*[23]

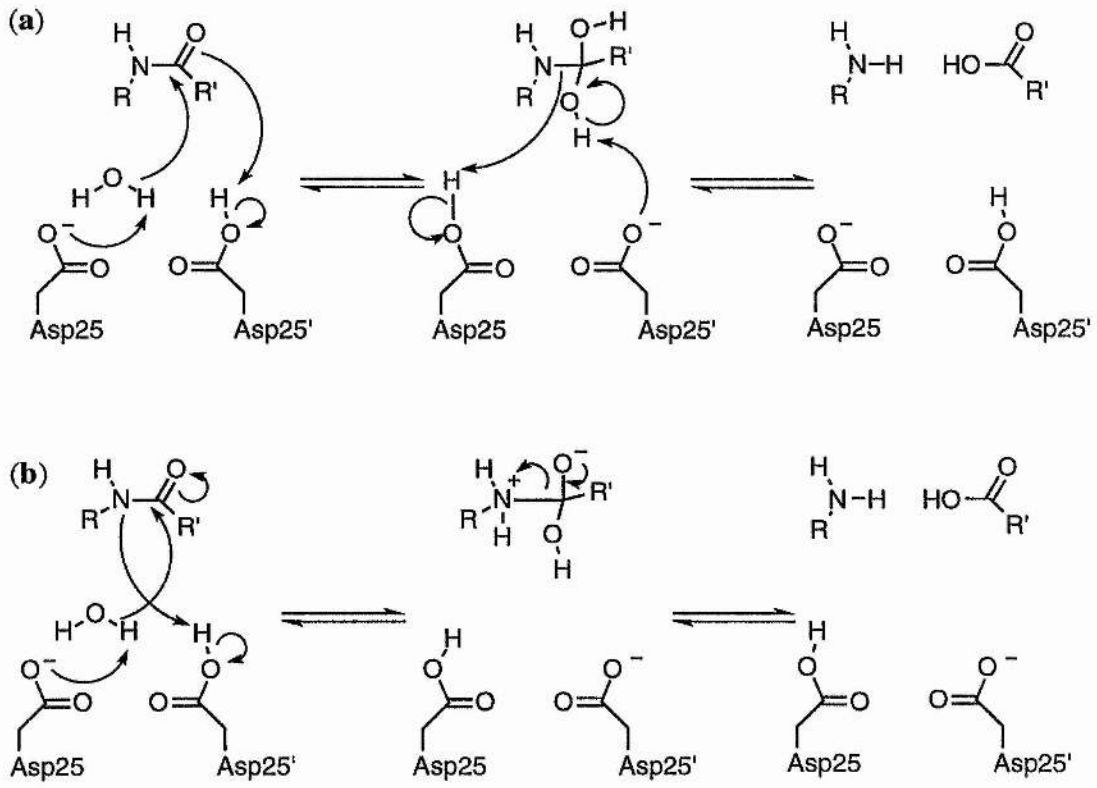
	$(P_4\text{-}P_1)$		$(P_{1'}\text{-}P_{4'})$
1.	Ser-Gln-Asn-Tyr	*	Pro-Ile-Val-Gln
2.	Ala-Arg-Val-Leu	*	Ala-Glu-Ala-Met
3.	Ala-Thr-Ile-Met	*	Met-Gln-Arg-Gly
4.	Pro-Gly-Asn-Phe	*	Leu-Gln-Ser-Arg
5.	Ser-Phe-Asn-Phe	*	Pro-Gln-Ile-Thr
6.	Thr-Leu-Asn-Phe	*	Pro-Ile-Ser-Pro
7.	Ala-Glu-Thr-Phe	*	Tyr-Val-Asp-Gly
8.	Arg-Lys-Ile-Leu	*	Phe-Leu-Asp-Gly

Figure 1.7: *The Cleavage Sites of HIV-1 PR*

At first sight, there is no obvious similarity between these sequences (shown in Fig. 1.7) suggesting that the primary sequence is not the dominant feature behind the specificity towards these particular substrates. In fact there is an apparent dichotomy between the adaptability of PR to tolerate the considerable variability in P_4-$P_{4'}$ and the selective cleavages that it performs. On closer inspection however, some similarities in the sequences can be identified; for example, in sites 1, 5 and 6 the similar sequence Ser/Thr-Xaa-Yaa-Phe/Tyr*Pro-Zaa (X/Y/Zaa represent any amino acid) appears. This sequence has been observed in some other retroviral Gag-Pol polyproteins and at the present time has only been found in retroviruses (no known mammalian or proteinase can hydrolyse Phe/Tyr*Pro).[84] The other residues show an overall hydrophobicity, particularly in the P_1 and $P_{1'}$ positions. In studies aimed at probing the specificities of the subsites with artificial substrates, it was shown that seven residues were required to bind in the enzyme in order for cleavage to occur. Smaller peptides were not processed.[85-87] Recognising these trends was important, and the information gleaned was invaluable in the design of inhibitors.

1.5.5 Mechanism of HIV-1 PR

There is a high degree of sequence homology and a similarity in the structural configurations of the catalytic aspartic acid residues that occupy the active site in the aspartic acid proteinases. This and their sensitivity to generic inhibitors such as pepstatin A implies that they might proceed by the same mechanism. Numerous mechanisms which fall into two broad categories, general acid-general base catalysis and catalysis by means of a covalently bound intermediate, have been proposed. Aspartic acid-catalysed proteolysis was long thought most likely to proceed through direct nucleophilic attack by one of the two aspartates, followed by subsequent hydrolysis of the covalent intermediate.[88] So far there has been no experimental evidence to support this. Furthermore, investigations into the mechanism of the cleavage of four oligopeptide substrates performed in the presence of ^{18}O-labelled water gave products (and reformed substrate) with $H_2{}^{18}O$ incorporation; this would not have been possible had the reaction proceeded by direct attack with aspartate.[89]



Scheme 1.1: *Alternative general acid-general base catalytic mechanisms proposed for HIV-1 PR, illustrating neutral (a) and zwitterionic (b) intermediates*

The consensual verdict of many recent kinetic and theoretical studies directed at HIV-1 PR is that the most likely mechanism involves general acid-general base catalysis, with either a neutral or zwitterionic intermediate (Scheme 1.1). These mechanisms require a water molecule (the lytic water molecule) to take part directly in the reaction. Implicit in the general acid-general base mechanism is that only one of the two aspartic acid residues is protonated. This is in agreement with studies of the pH dependence of the reaction rate.[90] In further studies with oligopeptides, plots of log (V/K) *vs* pH were bell-shaped,[90] indicating that (a) these two acids have remarkably different pK_a values (3.4 - 3.7 and 5.5 - 6.5) and (b) for catalysis to occur, it is required that one acid is protonated and one is not. However, because of their proximity and ability to share a hydrogen atom, it is has been proposed that the proton transfer between Asp25 and Asp25' is facile.[91]

In mechanism a of Scheme 1.1, Asp25 acts as a general base activating the lytic water molecule which then attacks the amide carbonyl group. Asp25', which is protonated, stabilises the negative charge built up on the oxygen atom of the carbonyl group and then protonates the oxygen atom. In the second step, Asp25 and Asp25' reverse roles; Asp25 protonates the scissile nitrogen atom and Asp25' abstracts a proton from the diol. In mechanism b depicted in Scheme 1.1, Asp25' provides a proton to the nitrogen atom leaving a zwitterion which undergoes internal collapse to form the products. In both of these mechanisms, the nucleophilic attack and proton transfer processes may be concerted or occur in separate steps, resulting in reaction intermediates with slightly different structures.

Although the flap water molecule is thought to play a part in the catalytic process because it is located near the active site in many crystal structures, its precise role is unclear. Its importance is thought to be in stabilising the transition state and in aiding the straining of the peptide linkage away from coplanarity, thereby weakening the bond and promoting cleavage.[91] However, it has been suggested that the flap water and lytic water molecules are one and the same. If the two are different, it has been proposed that an appropriately designed inhibitor would enable the detection of a lytic water in its crystal structure with the enzyme, in addition to the flap water molecule.[88]

Recently, a number of NMR and crystallographic studies have attempted to clarify the exact nature of the mechanism, in particular to establish information about the ionisation states of the catalytic aspartic acid groups. Such information is difficult to obtain in a direct fashion from X-ray data because protons are not observed in protein electron density maps. In addition, calculations using molecular dynamics have predicted that these ionisation states will vary with the interactions experienced as a result of each individual inhibitor bound in the active site.[92] For the complex formed between HIV-1 PR and the cyclic urea DMP-323 (**37,** page 42), NMR and X-ray evidence show that both aspartic acids are protonated.[93] In similar complexes formed with KNI-272 (**14**, page 27),[94] and pepstatin A,[95] NMR evidence indicates that just one aspartic acid is protonated.

Most pertinent to catalysis are the chemical properties of the aspartic acid residues in the unbound enzyme. The bell-shaped curve discussed above,[90] had been thought to confirm the presence of one protonated and one unprotonated aspartic acid residue in unbound HIV-1 PR at the optimal pH (5.5 - 6) of the enzyme. However, a recent report has been published with NMR evidence that contradicts this.[95] The authors prepared a sample of HIV-1 PR in which Asp25 and Asp25' were 96% ^{13}C-enriched at the quaternary centre of the side-chain carboxyl group. The chemical shift of this carbon atom is sensitive to the ionisation state of the acid and, on the basis of the presence of a single resonance in the ^{13}C NMR spectrum at pH 5.93, it was suggested that *both* aspartic acids are ionised at this pH. The authors proposed that a rapid equilibrium exists between all three ionised forms of the catalytic diad, in which the diprotonated form is predominant and the monoprotonated form is catalytically active.

1.6 Inhibitors for HIV-1 PR

Since it was proposed that HIV-1 PR was an appropriate target for antiviral intervention,[67] an enormous amount of work has been devoted to developing inhibitors. There are a number of complementary strategies that have been adopted in this search. These include random screening, the incorporation of transition state mimetics into suitable peptide sequences and the use of computer modelling both to assist in the *de novo* design of inhibitors and to evaluate the inhibitory potential of known compounds. The employment of all these strategies to discover HIV-1 PR inhibitors will be described below.

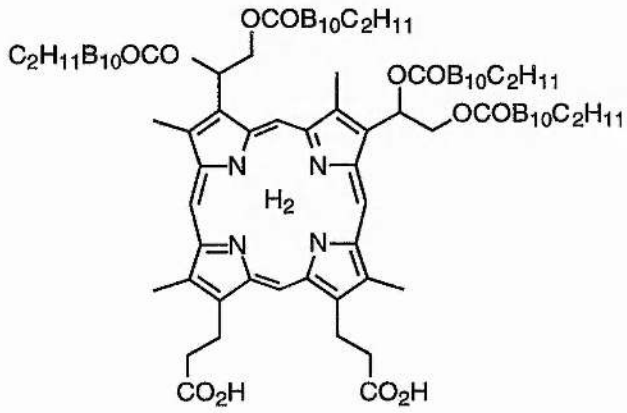
1.6.1 Random Screening

Many groups have set up high throughput screening assays to enable the testing of large numbers of compounds which originate from either natural sources or from

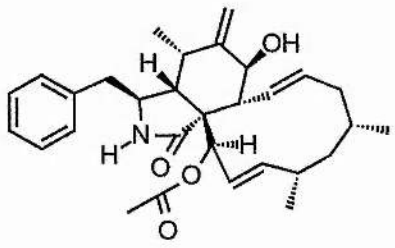
collections of synthetic compounds. As early as 1988, the US National Cancer Institute set up a mass screening programme to test 10,000 compounds a year for anti-HIV activity.[96] There are two principal advantages of this process. Natural products are a continuous and proven source of new lead compounds in drug discovery programmes. The other is the possibility of discovering inhibitors without the pharmacological problems inherent to rationally discovered peptide mimetics (see section 1.6.4)

Natural products have proved useful as lead compounds. Whilst such compounds have diverse structures and none have become drugs, it is interesting nevertheless to consider some of them briefly.

An examination of porphyrins revealed that they are active against HIV-1 PR at micromolar concentrations. In particular, the availability of a series of carborane ester derivatives of porphyrins synthesised as experimental boron neutron-capture therapeutics led to the discovery that compound **5** (the *tetrakis*carborane carboxylate ester of a porphyrin) had an IC_{50} of 185 nM.[97] The hydrophobic nature of the carborane cages were thought to be responsible for most of the binding interaction. However, the antiviral activity of this and similar compounds was severely impaired by their binding by the albumin present in the blood stream.



5

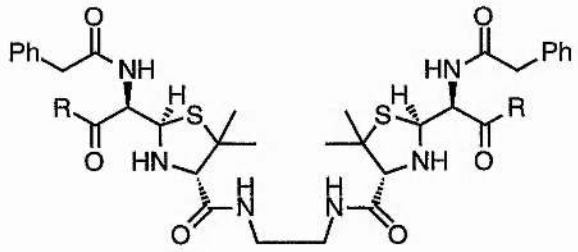


6

Another novel PR inhibitor (**6**) was purified from *Hypoxylon fragiforme*, a fungus isolated from the bark of the American beech tree. Unfortunately, **6** (IC_{50} = 3 μM)

also inhibited pepsin.[98] Studies are currently in progress to improve both the potency and the selectivity of this compound.[99,100]

A screening programme of existing compounds identified a crude sample of penicillin dimer with enzyme activity. Purification revealed the penicillin dimer to be inactive; the inhibition was shown to be caused by **7** (a C_2-symmetrical dimer; IC_{50} = 60 nM) which was an artifact of the purification process.[101] Development work on **7** gave more potent inhibitors **8** - **10** (IC_{50} = 0.9 - 4.8 nM).[101] Analogue **9** was selected as a candidate for drug development but poor pharmacokinetics ended the interest in this series of compounds.[102]



7: R = OMe
8: R = NH_2
9: R = NHEt
10: R = $NHCH_2PH$

A final and extremely successful series of inhibitors found through random screening shall be discussed in depth later (see section 1.6.5.1).

1.6.2 Peptidomimetic Inhibitors: Use of Transition State Surrogates

Armed with the knowledge of both the classification of HIV-1 PR as an aspartic acid proteinase and of natural and synthetic substrates, the design of inhibitors was initiated by applying transition state mimicry. This concept, originally proposed by Pauling,[103] is based on the premise that an enzyme has a higher affinity for the transition state than for either product(s) or reactant(s) and proved extremely effective when designing renin inhibitors.[70] In this approach, a P_1*$P_{1'}$ pair are replaced with a proteolytically stable dipeptide surrogate. The task of developing the first inhibitors was considerably facilitated by the vast body of information accumulated from 20 years work into the design of inhibitors for renin.[70] Inhibitors of HIV-1 PR were first reported in 1989, designed by

"tailoring" renin inhibitors "to fit" HIV-1 PR.[71] These inhibitors were very potent with little residual renin-inhibitory properties.

Some successful examples of dipeptide mimetics (with the actual transition state analogue of the scissile bond boxed) are shown below (Fig. 1.8).

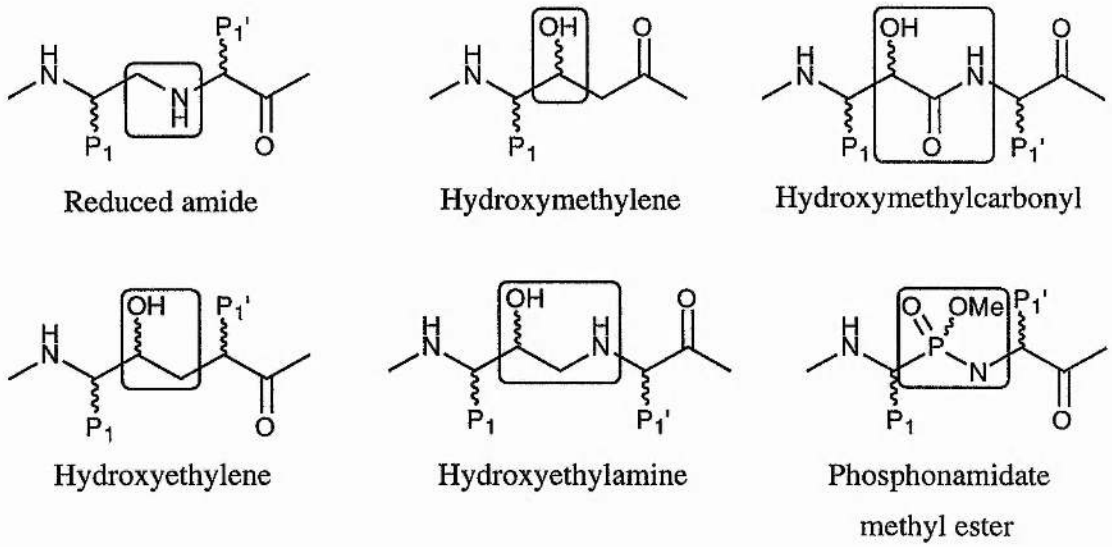


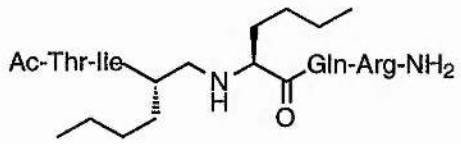
Figure 1.8: *Some dipeptide mimetics incorporated into HIV-1 PR inhibitors*

Each mimetic incorporates some but not all of the features of the transition state. They all possess tetrahedral geometry at the position designed to mimic the carbonyl group of the scissile peptide bond. However, other required characteristics cannot be met on chemical grounds. Some mimetics (*e.g.* hydroxymethylcarbonyl and hydroxyethylamine derivatives) contain more backbone atoms and some contain fewer atoms (*e.g.* hydroxymethylene derivatives). Intuitively, these mimetics might be expected to lead to poor inhibitors as their peptidic portions should be out of synchronisation with the pockets into which they are designed to fit. As will be described, however, some have made very good inhibitors.

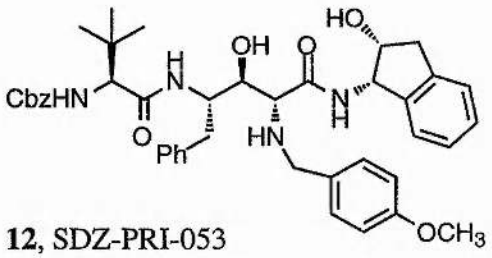
It is useful to review some of the highlights of the work in this field, particularly as all four HIV-1 PR inhibitors currently on the market,[104] resulted directly or indirectly from this approach. Furthermore, whilst describing the inhibitors, comments

will be made about the medicinal chemistry that led to their development. This should give some idea as to how much the state of the art has advanced over the last decade.

The earliest reported inhibitors incorporated the reduced amide surrogate. Many of the early compounds were of only modest potency (although inhibitors effective at subnanomolar concentrations have since been reported). However a co-crystal of an inhibitor containing this mimetic (**11**, MVT-101) and HIV-1 PR provided the first detailed three dimensional structure of HIV-1 PR in its inhibited form.[78] The modest inhibition of HIV-1 PR by most members of this series has been attributed to its comparative lack of functionality; this prevents effective hydrogen bonding to the catalytic aspartic acid residues.



11, MVT-101

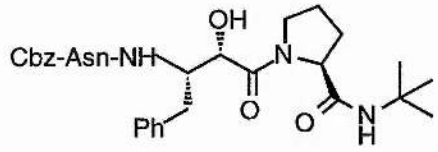


12, SDZ-PRI-053

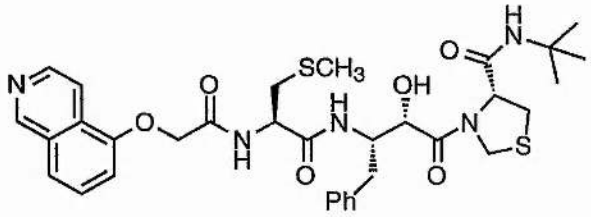
The archetypal aspartic acid PR inhibitor pepstatin A (**3**), contains the hydroxymethylene (statine) surrogate and is a moderate inhibitor of HIV-1 PR (IC_{50} ~ 1 μM). Originally, however, use of this surrogate did not deliver potent inhibitors, probably because it failed to project a residue into $S_{1'}$. This problem was solved through the development of a different synthetic route, which enabled the incorporation of substituents at the 2-position. This led to the development of a highly potent series of inhibitors culminating in **12** (SDZ-PRI-053; K_i = 9.5 nM).[105]

Hydroxymethylcarbonyl (norstatine) derivatives had previously been used as renin inhibitors. In contrast to renin inhibitors, potency was only achieved with the amino acid proline in the P_1 position (*e.g.* **13**, KNI-102; IC_{50} = 89 nM).[106] An extensive structure-activity study was undertaken, in which the pyrrolidine ring of proline was exchanged for heterocyclic 5-membered rings.[107] One exceptionally potent analogue

containing a thiazolidine ring (**14**, KNI-272; K_i = 5.5 pM) is active against a wide spectrum of HIV-1 strains and is currently undergoing phase II clinical trials.[107,108]



13, KNI-102



14, KNI-272

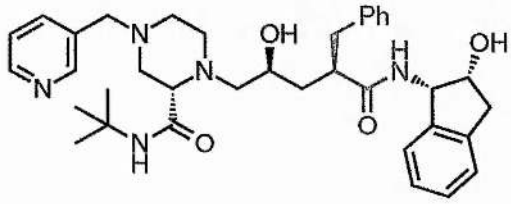
The extremely high potency of **14** is attributed in part to conformational preorganisation of this compound caused by the norstatine-thioproline bond being locked in the *trans* configuration. This is supported both by quantum mechanical calculations and by the conformations seen in crystal structures: both the free and PR-bound forms of **14** are very similar.[108] The binding of **14** in the active site of HIV-1 PR is therefore thought to be more favourable entropically than most other linear peptidomimetic inhibitors. Conformational preorganisation is an important factor behind the potency of the smaller non-peptidic compounds and will be discussed later (see section 1.6.5.2).

A general point worth raising about the KNI series of norstatine inhibitors is that they all incorporate an unnatural amino acid, phenylnorstatine [(2R,3S)-3-amino-2-hydroxy-4-phenylbutyric acid]. Unnatural amino acids have been widely employed to stabilise peptidomimetic HIV-PR inhibitors towards proteolysis. This is because they are not recognised by cellular proteinases. A similar strategy employed to evade metabolic degradation has been the use of D-amino acids in place of the natural L-amino acids. Compounds that contain such surrogates have been shown to retain inhibitory activity, although modifications to side chains need to be engineered on account of the reversal of the stereochemistry.[109-110]

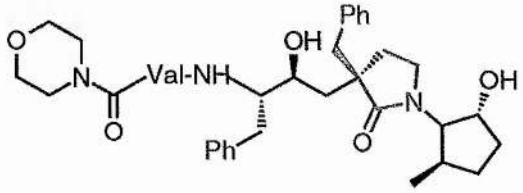
Five different transition state mimetics were directly compared in a study conducted by Dreyer *et al.* in which the surrogates were incorporated into the substrate Ser-Ala-Ala-Phe*Pro/Gly-Val-Val-OCH_3.[111] The reduced amide analogue gave the

poorest inhibition (K_i = 19 μM). The hydroxyethylene-containing compound gave the greatest inhibition (K_i = 62 nM).[111] This outcome guaranteed an extensive investigation of the hydroxyethylene surrogate and has ultimately yielded an inhibitor (**15**, indinavir; K_i = 0.34 nM),[112] which is now in clinical use.[104]

Modifications to the hydroxyethylene surrogate have led to other novel inhibitors. Through computer modelling studies,[113] it was calculated that an analogue of **15** bound to HIV-1 PR such that the aminoindan NH and the α-CH were nearly coplanar (the comparable atoms and bonds are shown in red in **15**). Incorporation of these atoms into a 5-membered lactam ring (shown in red in **16**) combined with additional structural features has delivered a series of potent inhibitors including **16** (IC_{50} = 5 nM).[113] These γ-lactams have shown significant oral bioavailability in dogs,[114] no doubt as a result of the elimination of the indan amide bond.



15, indinavir

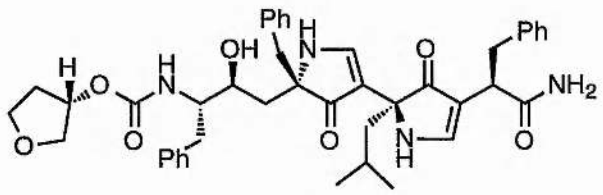


16

Compound **16** is another example of the constraining of an inhibitor into its bioactive conformation similar to that described previously for **14**. Such conformationally constrained peptidomimetic inhibitors have been extensively reported. There are many publications which describe the advantages of macrocycles over the acyclic peptidic inhibitors from which they are derived. Benefits include the protection of amide bonds from proteolysis, increased water solubility (and lipid solubility),[115] and the entropic benefits of conformational preorganisation already described.

In a highly novel approach to the construction of rigid molecular structures, the Amos B. Smith III group have developed β-strand peptidomimetic inhibitors, analogues of which have recently been shown to have good bioavailabilities.[116] These compounds were

designed with the knowledge that HIV-1 PR binds its substrates and inhibitors by generating β-pleated sheets.[117] Flanking the hydroxyethylene surrogate with linked pyrrolinones as β-strand mimetics enabled a reduction in the number of amide bonds. Compound **17** (IC_{50} = 1.3 nM) was more active in a *cell*-based anti-HIV assay than corresponding peptidic inhibitor **18** (IC_{50} = 0.4 nM), despite the *in vitro* IC_{50} values indicating the reverse. This suggested that the premise that lowering the number of peptide bonds aids the transport of these inhibitors into cells is correct.[117,118]



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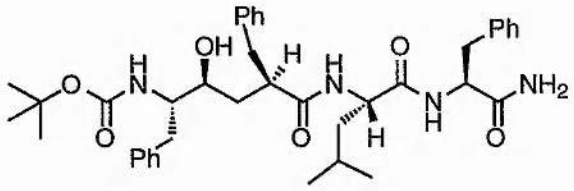


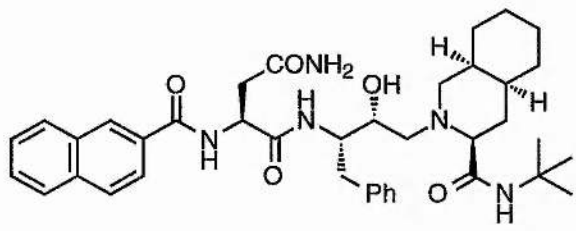
18

A second type of compound, developed by the Gani group at St Andrews, has also been shown to aid in cell-uptake properties. By incorporating the phosphonamidate methyl ester transition state surrogate in a series of compounds derived from one of the HIV-1 cleavage sequences, moderate inhibitors of PR were derived. All the analogues prepared had a marked ability to enter cells, as demonstrated by the approximate equivalence of IC_{50} values found for both solution- and cell-based assays.[119]

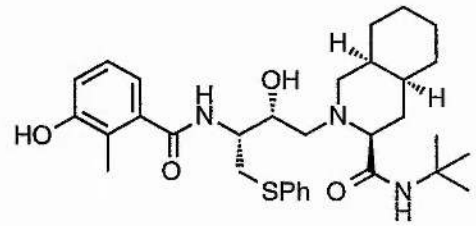
Finally, the hydroxyethylamine surrogate has arguably proved to be the most successful of the transition state mimetics in generating potent HIV-1 PR inhibitors. Extensive structure-activity studies of inhibitors containing the hydroxyethylamine analogue of the Phe-Pro scissile bond have shown that inhibitors could be truncated to occupy only S_3-$S_{2'}$.[120]

Further modification of the $P_{1'}$ proline to the decahydroisoquinoline group led to the discovery of **19** (saquinavir; K_i = 0.1 nM),[120] the first HIV-1 PR inhibitor to be approved for clinical use.[104] Further manipulation enabled truncation of the N-terminal

end to just P_1 and P_2 and led to the identification of **20** (nelfinavir; K_i = 2 nM),[121] which has improved bioavailability compared to **19** and is itself now approved for clinical use.[104]



19, saquinavir



20, nelfinavir

1.6.3 Exploitation of the C_2-Symmetry of HIV-1 PR

An elegant approach to the design of HIV-1 PR inhibitors exploits the C_2-symmetrical nature of the enzyme. Such a design approach has never been adapted for any other enzyme target. As this has been discussed already (see sections 1.5.2 and 1.5.3), it is sufficient to summarise that as the symmetry-related pockets S_n and $S_{n'}$ should be indistinguishable in the native enzyme, duplication of either the N- or C-terminus of a substrate or inhibitor by a C_2-operation should produce inhibitors with novel structural features.[122] Four C_2-symmetrical inhibitors have already been mentioned (compounds **7** - **10**). Their discovery was serendipitous, in contrast to virtually every other C_2 inhibitor. The majority of such compounds have been designed by performing C_2 operations (Fig. 1.9) on the transition state of the Phe*Pro cleavage site and have duplicated the N-terminal side.[122] This is partly because this side, the "P region", had been shown to be of greater importance in the binding of renin inhibitors.[123] Also, the P_1 residue in Phe*Pro is Phe. As may be seen from cleavage sequences, P_1 and $P_{1'}$ are often aromatic residues.

As with those discussed earlier, the initial symmetrical inhibitors were still highly peptidic. An enormous number have been synthesised and reviewed.[124-126] After the modification of an initial symmetrical compound identified by rational design, **21** (ritonavir; K_i = 20 pM), which does not display perfect C_2 symmetry was identified,[127] and is currently licensed for clinical use.[104]

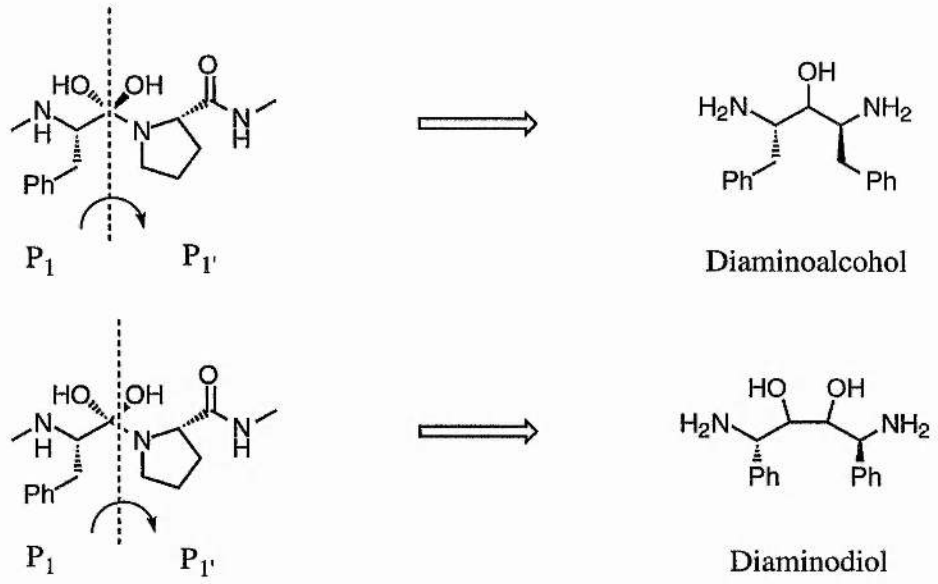
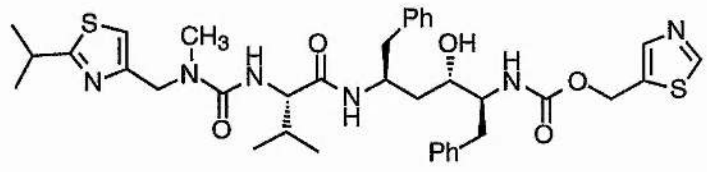


Figure 1.9: *Symmetry operations on Phe*Pro giving C_2-symmetrical transition state mimetics: after defining a hypothetical axis of symmetry based on the C_2-symmetry of the enzyme, one half is deleted and a C_2 operation on the other half provides two distinct chemically stable core units.*[18]



21, ritonavir

1.6.4 Problems with Substrate-Based Peptidomimetic Inhibitors

Desirable properties in drugs are the combination of good bioavailability with pharmacological efficacy, long duration of action and minimal toxicity. Such a valuable portfolio of properties is not generally bestowed upon the peptide-based or large inhibitors described above. Antiviral potencies of these compounds frequently do not parallel their

ability to inhibit an enzyme *in vitro*. They suffer from a number of problems outlined below:[115,128]

- Their unique structural features mean that the peptides are poorly soluble in the water present in most parts of the body and are poorly transported across cellular membranes.
- Whilst the gastrointestinal (GI) tract provides amply for the digestion of food, notably protein, the onslaught of degradative enzymes (particularly proteinases) ensures that relatively few intact peptides enter the portal vein.
- Those drugs that survive the hostile environment of the GI tract quickly encounter the liver through which the portal blood supply must pass before more widespread distribution. Removal of alien substances here is known as the "first pass effect" and can prevent their further distribution. The propensity of a compound towards biliary excretion is governed most notably by its molecular weight. Excretion is favourable for compounds whose molecular weight exceeds 500 although other factors such as lipophilicity, solubility and ionic charge also affect this property.
- Once through the liver, drugs are subject to degradation in the kidney, blood and in various other tissues. Furthermore, HIV-1 PR inhibitors have been shown to tightly bind to plasma proteins (> 98%), reducing still further their antiviral activity.[128]

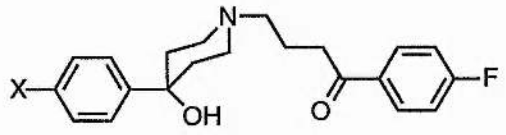
Although no class of drugs is exempt from the problems of oral bioavailability, tissue distribution, metabolism and excretion, and protein binding, these problems have been a particular problem with peptide-based drugs. In particular, these and other problems specific to the pharmacokinetics associated with HIV-1 PR inhibitors have been reviewed.[128] As a result of the above problems, many groups have departed from the traditional peptidomimetic routes described earlier and instead have sought to discover small non-peptidic inhibitors (NPIs).

1.6.5 Non-Peptidic Inhibitors (NPIs)

Two complementary strategies have been employed in the search for novel PR NPIs. These are the rational design of new compounds and the screening of known compounds. Both these approaches have been successful insofar as two series of highly potent inhibitors have been discovered, both containing central rigid cyclic structures from which groups are projected into the central (S_2-$S_{2'}$) subsites. Rather than diluting the following discussion by covering the large numbers and types of NPIs so far identified, ranging from metal ions,[129-132] to fullerene derivatives,[133-135] focus will be directed primarily on these two series.

1.6.5.1 NPIs Found by the Screening of Known Compounds

The omission of the adjective "random" from the above title seeks to emphasise a sense of direction prevalent in many approaches to the screening of large numbers of compounds. This has been achieved by the use of computers to assist in the lead discovery process. One of the first examples of this was the use of the computer programme DOCK to identify potential inhibitors of HIV-1 PR. DOCK searched 10,000 compounds in the Cambridge Crystallographic Database for a shape complementary to the enzyme's active site.[136] Using this technique, bromoperidol (**22**) was identified and a closely related analogue, the known antipsychotic drug, haloperidol (**23**) was found to be a weakly active inhibitor, albeit at toxic concentrations (K_i = 100 mM).[136]



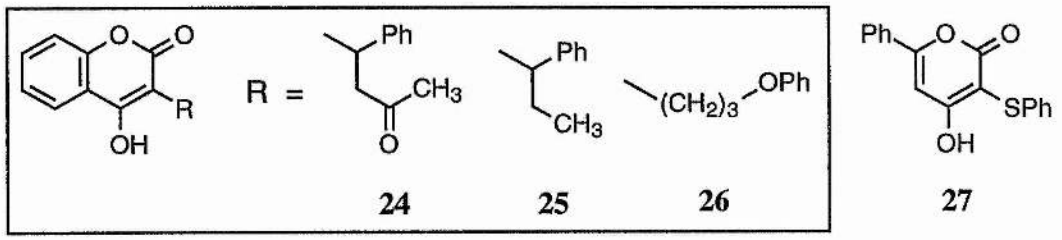
22, bromoperidol: X = Br
23, haloperidol: X = Cl

Since the application of X-ray crystallography to HIV-1 PR-inhibitor complexes, it has been possible to construct pharmacophores mimicking key features of enzyme-inhibitor interactions. These include the binding of the central hydroxy group in

both hydroxyethylamine and hydroxyethylene inhibitors to the active site aspartic acid residues and the interactions with the flap water molecule. Such pharmacophores may then be used in a more directed search of databases containing the three-dimensional structures of known compounds. This and similar approaches have proved useful in generating lead compounds quickly, as all major pharmaceutical companies maintain databases of proprietary compounds. In a recent publication, the construction and application of such a pharmacophore led to the identification of fifteen previously unknown NPIs.[137] Furthermore, careful analysis of X-ray data obtained from co-crystals of NPI-PR complexes have greatly advanced the rate at which drug discovery programmes have progressed. This will become evident later.

In addition to computer-assisted screening of known compounds before their biological evaluation, there is of course random screening. Whilst not exactly a testament to the intellectual basis driving modern medicinal chemistry, such testing has delivered one of the most followed-up NPI lead-compounds to date.

4-Hydroxypyranones and 4-hydroxycoumarins were first observed independently by two groups (at Parke-Davis Pharmaceutical Research and at The Upjohn Company) to inhibit weakly HIV-1 PR.[138,139] The 4-hydroxycoumarin, warfarin (**24**) had been reported to demonstrate anti HIV-1 activity in 1993, but its mechanism of action was not known.[140] In 1994, however, the same compound was found to have anti-HIV-1 PR activity in two separate high throughput screening programmes.[138,139] Further screening work identified several related compounds including two 4-hydroxycoumarins (**25**,[139] and **26**,[141]) and the 4-hydroxypyrone **27**.[141]



24 **25** **26** **27**

Subsequent analysis of the crystal structures of HIV-1 PR complexed with compounds **25**,[139] and **26**,[142] showed that both bound in a similar fashion to the active site and that the 4-hydroxypyrone ring was functioning as the pharmacophore (Fig. 1.10)

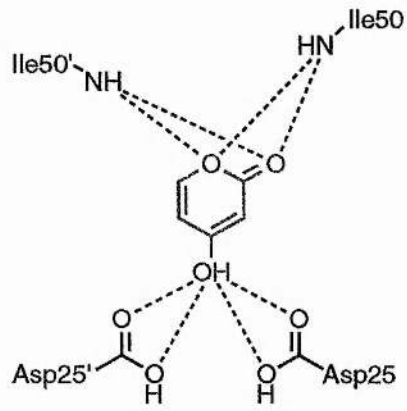


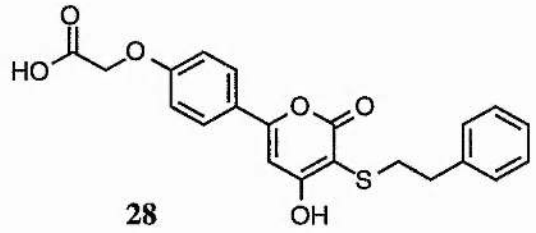
Figure 1.10: *Hydrogen bonds formed between the 4-hydroxypyrone pharmacophore and the Asp25, Asp25', Ile50 and Ile50' residues of the active site of PR*

The establishment of the mode of binding of the 4-hydroxypyrones served as a springboard from which a number of series of potent inhibitors have evolved. One of the most important observations made of the crystal structures was that the flap water molecule had been displaced by the lactone oxygen atoms of the pyrone ring. Such a displacement has been part of the design strategy for NPIs of a number of groups and is discussed later (see section 1.6.5.2). Additionally, the X-ray crystal structures showed the importance of the C-3 residue, *viz.* that it was able to interact with S_1 and S_2 and/or S_3.[139,142]

Medicinal chemists chose next to synthesise analogues of compounds **25** and **26** by varying the nature and length of the C-3 substituent, and by investigating the effect of substitution patterns on both aromatic rings.[142,143] Most of these derivatives were good inhibitors. It was noted, however, that a decrease in activity was observed in both series of compounds when there was no branching at the α-position of the C-3 substituent.

Whilst the C-3 substituent was interacting with S_1-S_3, the position and rigidity of the fused benzene ring posed a serious obstacle to any further development of the

potential of the 4-hydroxypyrone pharmacophore. Although there was an interaction between the benzene ring of the inhibitor and $S_{1'}$, it was limited and so attention at Parke-Davis turned to the development of the 4-hydroxypyrone lead **27**. Insertion of one methylene group between the sulfur atom and phenyl group of the C-3 substituent led to a modest improvement in activity (IC_{50} = 1670 nM *vs* 3000 nM).[144] Insertion of two carbon atoms in the same position together with the attachment of a carboxylic acid group to the *para* position of the C-6 phenyl group (thought to interact with an arginine residue in $S_{3'}$) gave compound **28**, which had significantly enhanced inhibitory activity (IC_{50} = 160 nM).[144]



28

Unfortunately, as a result of the presence of the sulfur atom, this and other analogues failed to achieve any interaction with both S_1 and S_2 (the probable cause of the benefits arising as a result of branching at the α-position of the C-3 substituent described earlier). Incorporating the sulfur atom in the β-position enabled the introduction of a number of substituents at the α-position.[145] A variety of analogues were prepared, the most potent being compound **29** (Fig. 1.11; IC_{50} of 58 nM). An X-ray crystal structure of a **29**-HIV-1 PR co-complex revealed the same substituent binding pattern (S_2 - $S_{1'}$) that had been predicted by modelling (Fig. 1.11); differences were seen, however, in the hydrogen bonding network from that previously found (Fig. 1.10). Only one flap isoleucine amide bond was hydrogen bonded to the oxygen atoms of the lactone and only one catalytic aspartic acid formed a direct hydrogen bond to the enol of the inhibitor. The second aspartic acid was indirectly bonded through a bridging water molecule. This had not been observed previously in any other crystal structure and may provide evidence for the existence of separate lytic and flap water molecules (see section 1.5.5, page 21).

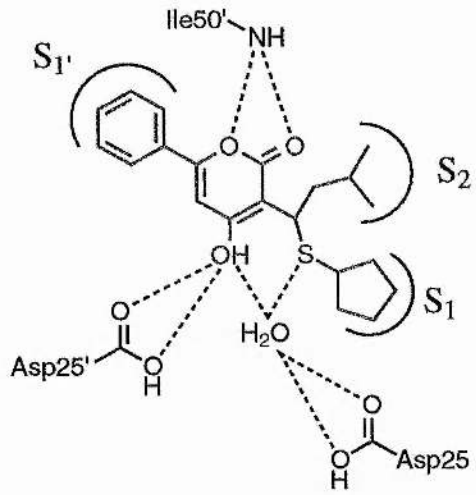
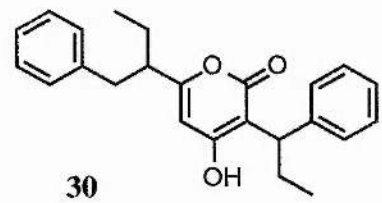


Figure 1.11: *Hydrogen bonds and subsite interactions derived from a crystal structure of a* ***29****-HIV-1 PR complex*

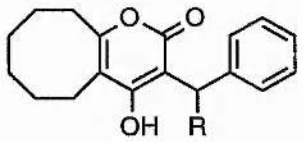
Elsewhere (at Upjohn), the potential of 4-hydroxypyrone analogues was also being explored.[139] Based around 4-hydroxycoumarin **25**, the fused benzene ring was replaced with a flexible and substituted C-6 tether in the pyrone template to yield compound **30**. The substituents pendant from the central pyrone ring interacted with S_2-$S_{2'}$ and its enzyme inhibition (K_i = 38 nM) and antiviral activity against clinical isolates of HIV-1 enabled it to be the first 4-hydroxypyrone analogue to enter clinical trials. It displayed good oral bioavailability in animal models and all four constituent diastereoisomers demonstrated good K_i values (14 - 109 nM).[139] Whilst it proved to be well tolerated by humans, phase I clinical trials were discontinued because of the identification of more potent inhibitors.[146]



30

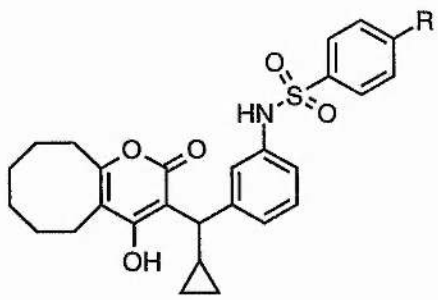
An alternative strategy to overcome the limitations imposed by the fused benzene ring in compound **25** was also pursued at Upjohn.[147] By replacement with more

flexible cycloalkyl rings, compounds much more potent than **25** (K_i = 1000 nM) were discovered. It transpired than an 8-membered ring was the optimum size (**31**; K_i = 75 nM); through X-ray crystallography this was shown to be because the cyclo-octyl ring effectively filled the $S_{1'}$ pocket.[147] Through iterative substitution of the α-carbon of the C-3 substituent of **31**, a cyclopropyl ring (**32**) was found to be the most potent inhibitor (K_i = 15 nM) of a series of compounds.[147]



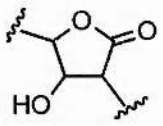
31: R = ethyl
32: R = cyclopropyl

Previously substitution of an amide group at the *meta*-position of the free phenyl group in analogues of **25** had resulted in a significant increase in activity.[143] Similarly, amide-containing substituents were attached to the *meta*-position of the phenyl group in compound **32**. Although, several of these proved to be potent inhibitors (K_i < 15 nM), they later proved to have very little antiviral activity.[148] Subsequently, other analogues of compound **32** were prepared and a series of sulfonamides gave very encouraging results.[149] Whilst simple alkyl substituted sulfonamides had activity little better than the parent compound **32**, their phenyl analogues (**33**; K_i = 3 nM and **34**; K_i = 0.8 nM) proved much better. A crystal structure of a **33**-PR complex showed that the sulfonamide functionality was forming hydrogen bonds with the Gly48 and Asp29 residues and that the phenyl group was filling S_3.[149] Furthermore, the antiviral activities of sulfonamide derivatives have turned out to be the best of the 4-hydroxypyrone series discovered so far. Consequently, compound **34** was selected for clinical trials.[150]



33: R = H
34: R = CN

Modelling studies suggested two further structures upon which to attach substituents. Both the tetronic,[146] acid and dihydropyrone rings,[151,152] contain sp^3 centres at their C-5 and C-6 atoms respectively (Fig. 1.12). They provide novel opportunities for investigating binding interactions with the little explored $S_{2'}$ and $S_{3'}$ in addition to $S_{1'}$, both by enabling the ability to substitute twice at these positions and through the difference in bond angles exhibited by sp^2 and sp^3 carbon atoms.

Tetronic acid: 

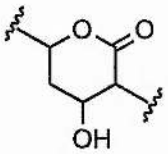
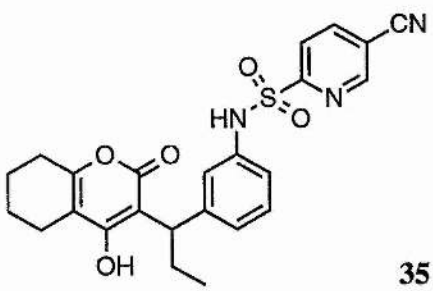
Dihydropyrone: 

Figure 1.12: *Molecular structures related to 4-hydroxypyrone and 4-hydroxycoumarin*

Outside the paper in which it was originally suggested, there appears to have been little work reported on inhibitors based on the tetronic acid template.[146] However, the dihydropyrone ring has prompted further efforts to invent ever more potent inhibitors.

The saturation of the 5,6-double bond which distinguishes the 4-hydroxypyrone and 4-hydroxydihydropyrone templates was shown to alter the optimum cycloalkyl ring size from eight to six carbon atoms in compounds analogous to **33** and **34**.[153] Structure-activity studies have also proved that 2-pyridyl sulfonamides have better antiviral activity than phenyl sulfonamides.[153] Through such studies, cyclohexyl-dihydropyrone **35** (K_i = 50 pM),[153] and more complex chiral C-6-disubstituted dihydropyrone **36** (K_i = 8 pM),[154,155] proved to be potent NPIs. Currently, compound **36** is being evaluated prior to its probable entrance into clinical trials.[154]



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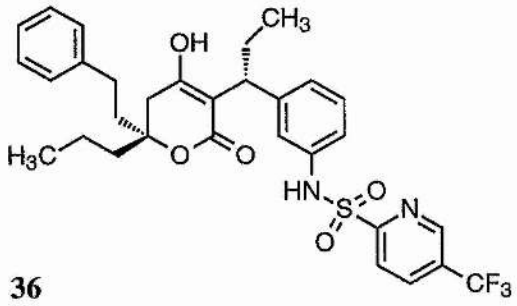

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1.6.5.2 The Rational Design of Non-Peptidic Inhibitors

Whilst structure-based design was used to deliver the clinical candidates described above, the initial lead was discovered by random screening. A second highly successful series of cyclic compounds have evolved from an initial lead invented by the application of an entirely *de novo* design strategy.[156] These were developed after initial consideration of the structural features believed to be essential to the development of any NPI. These features (outlined below) were then incorporated into appropriate molecular templates (Fig. 1.13).

- An initial pharmacophore was generated which contained a hydroxyl group. It was expected that this would bind to the catalytic aspartic acid residues.
- An entirely novel idea was to incorporate an atom or atoms capable of displacing the flap water molecule. It was reasoned that such a displacement would be favourable on entropic grounds.[157]
- Such an entropic benefit was in danger of being offset by the entropy lost upon the binding of such inhibitors. To counter this, it was proposed that cyclic inhibitors be designed. The entropy "toll" charged by a cyclic inhibitor (as opposed to a flexible linear inhibitor) could thus be "prepaid" during synthesis and would ensure positive effects from the displacement of the flap water.
- Finally, it was desired to build these structural features within a framework that would allow the attachment of C_2-symmetrically related P_1 and $P_{1'}$ and possibly P_2 and $P_{2'}$ groups. Through incorporation of all these features, it was hoped to depart from the multisubsite interactions made by peptidomimetic inhibitors. This would enable a reduction in the molecular weight of inhibitors thereby increasing their bioavailability.[115]

Fig. 1.13 shows how these structures were refined to generate the cyclic urea structure. A cyclohexanone ring (**A**) was chosen as the initial hypothetical cyclic structure

with the ketone oxygen atom designed to displace the flap water molecule. This was expanded to a seven-membered ring to allow the introduction of a diol (**B**). Diaminodiols had proved to be superior inhibitors to diamino alcohols.[124-126] Further elaboration to the cyclic urea (**C**) was based on two considerations. Firstly, there was precedent for cyclic ureas as excellent hydrogen bond acceptors.[158-160] Secondly, it was realised that the seven membered cyclic urea was synthetically accessible by cyclisation of a phenylalanine derived diaminodiol with carbonyl diimidazole (CDI). This places a phenyl group in both S_1 and $S_{1'}$ and closely mimics the aromatic amino acids phenylalanine and tyrosine commonly found in these pockets. Finally conformational analysis predicted the optimal stereochemistry to be that shown in **D**. This skeleton is in fact derived from (D)-phenylalanine.

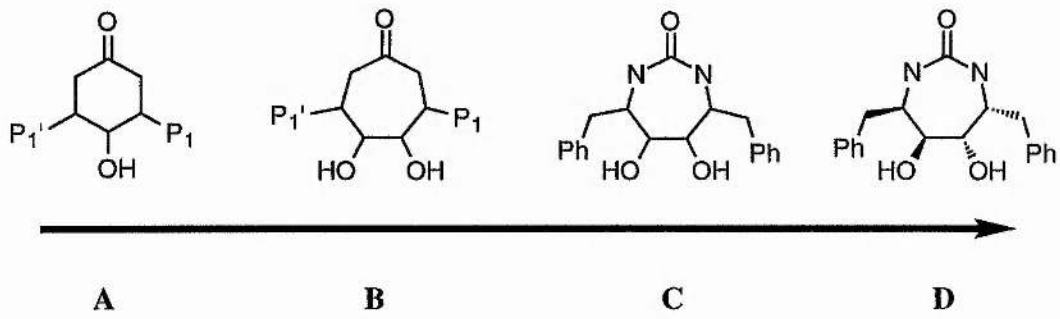


Figure 1.13: *Evolution of a template for cyclic non-peptidic HIV-1 PR inhibitors*

The design process was now complete. Synthetic chemists began to corroborate both the hypothesis of cyclic urea inhibitors and the conformational analysis. Simple cyclic ureas derived from both enantiomers of phenylalanine were tested against HIV-1 PR. This showed that the (D) phenylalanine-derived urea was indeed 1000-fold more potent than the (L) phenylalanine-derived analogue.[156]

By attaching a number of different hydrophobic substituents to the urea nitrogen atoms of **D**, interactions with S_2 and $S_{2'}$ were optimised. The optimal substituent was found to be a *para*-hydroxymethylbenzyl group. This gave compound **37** (DMP 323; K_i = 0.23 nM) which exhibited sufficient bioavailability and selectivity and had antiviral

properties that permitted it to enter clinical trials.[156] NMR and crystallographic studies showed that amongst the interactions it had been designed to make, it had indeed displaced the flap water molecule; the urea oxygen atom was shown to be coordinated to the amide bonds of Ile50 and 50' (Fig. 1.14).[161]

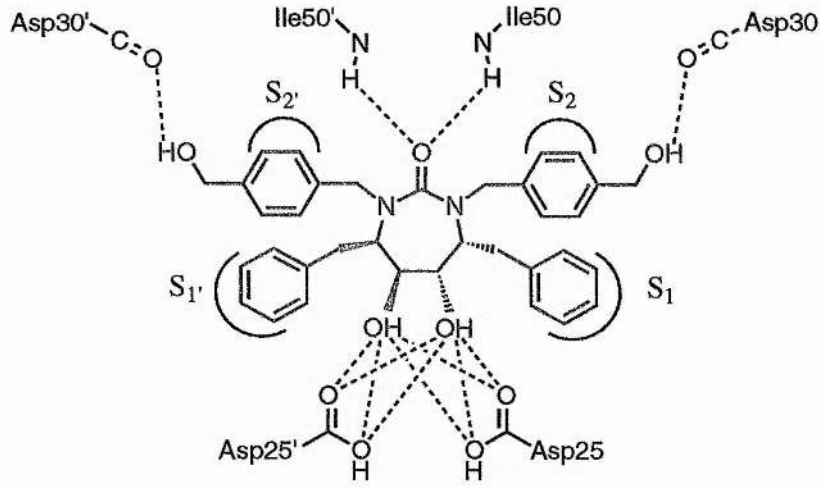
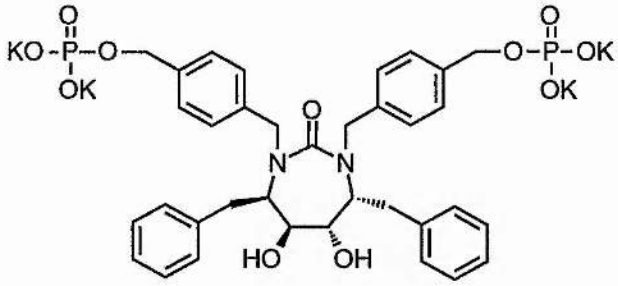


Figure 1.14: *Hydrogen bonds and subsite interactions derived from a crystal structure of a 37-HIV-1 PR complex*[161]

Unfortunately compound **37** was withdrawn from clinical trials as a result of variable bioavailability in humans, caused primarily by its poor solubility in both aqueous and lipid media.[162] Attempts were made to circumvent this problem by transforming it into a water soluble phosphate ester (**38**).[163] This prodrug was shown to be hydrolysed to the parent drug by phosphatase enzymes in the gastrointestinal tract. However, it transpired that the benzylic hydroxymethyl groups of the parent drug were metabolically unstable and this line of development was discontinued.[163]

A second more soluble analogue (**39**, DMP 450; K_i = 0.28 nM) was developed.[162] Examination of an X-ray crystal structure of a **39**-HIV-1 PR co-crystal confirmed the same general pattern of binding as is shown for compound **37** above. The first pK_a of the anilino residue (4.6) is high enough for the amine to be protonated in the stomach and for formulation as salts of strong acids, but low enough to be neutral in the

cytosol. As a result, the greatly improved bioavailability of compound **39** permitted its entrance into clinical trials.[162]



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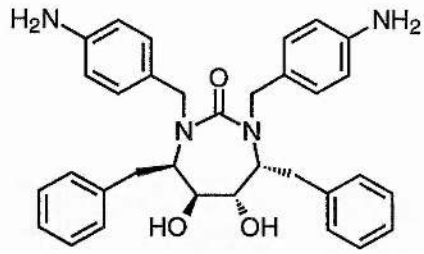
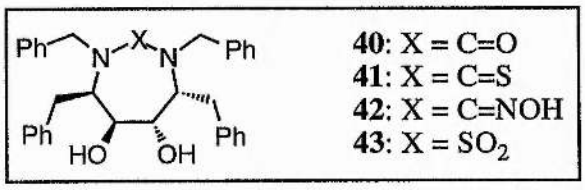


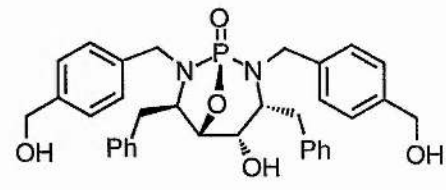
39, DMP 450

The development of the cyclic ureas has prompted a great deal of work related to these initial compounds. Both analogues of the 7-membered cyclic ureas themselves and related cyclic compounds whose design is either based on the cyclic ureas or on the principles from which they were designed (see pages 40-41) have been synthesised and tested against HIV-1 PR. Some of representative compounds of the many that have been found to be potent inhibitors of PR shall be discussed below.

Many alternatives for the surrogate that displaces the flap water have been investigated. By the use of an alternative cyclisation reagents to CDI, the thiourea **41** (K_i = 3.1 nM) was synthesised.[164] Although the C=S bond is 40% longer than the C=O bond of the parent urea **40** (K_i = 3.6 nM),[156] and thus shortens the length of the hydrogen bonds to the flap Ile residues, such hydrogen bonds are weaker and the K_i stays approximately the same. Through the use of a guanidinating agent, compound **42** (K_i = 42 nM) was synthesised.[164] By a different route, cyclic sulfamide **43** (K_i = 15 nM) has also been prepared.[165] The related phosphordiamidate **44** (K_i = 0.6 nM) was found to be a potent inhibitor although it was found to be unstable to hydrolysis.[166]



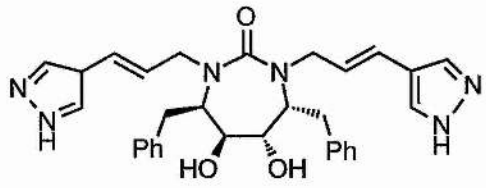
40: X = C=O
41: X = C=S
42: X = C=NOH
43: X = SO_2



44

Much work has been reported on modifications to the phenyl rings of P_1 and $P_{1'}$ in urea **40**,[167] through the addition of substituents onto the phenyl ring. Whilst such modification has not made drastic differences to the potency of such analogues (no inhibitors had a lower K_i than **37**), it was seen, as with compounds **38** and **39**, that a superior cellular antiviral activity profile could be created.

Similarly, work has been done to augment favourable interactions made by P_2 and $P_{2'}$. As with the 4-hydroxypyrone series, incorporation of heterocycles in $P_2/P_{2'}$ has been reported. Representative of their class of heterocyclic $P_2/P_{2'}$-containing cyclic ureas are **45** (XU348; K_i = 0.25 nM),[168] and its structurally constrained analogue **46** (K_i = 18 pM),[169] which exhibit excellent antiviral activity. Compound **46** was selected for *in vitro* bioavailability studies. Unfortunately, whilst its enzyme inhibitory properties translated well to a cell-based assay, poor aqueous solubility has been shown to be responsible for disappointing bioavailability. Work is in progress to improve the aqueous solubility of compound **46** and similar analogues.[169]



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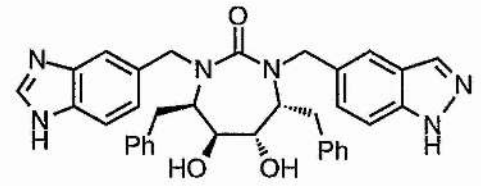
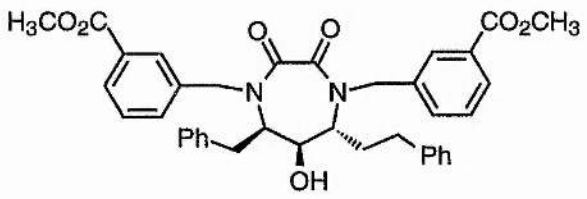


46

As has already been mentioned, a number of inhibitors closely related to the above series of seven-membered ring cyclic ureas have been reported. Several representative examples are discussed below.

Cyclic oxamide **47** (K_i = 40 nM) was synthesised because it possessed a structural core complementary to **37** in which the number carbonyl groups and hydroxyl groups were reversed.[170] In a series of azacyclic ureas exemplified by compound **48** (K_i = 5 pM) deletion of two stereogenic centres and one of the hydroxyl groups possessed by the cyclic ureas showed that an element of structural simplification could be achieved

with retention of excellent activity. Unfortunately, the compounds of this series proved not to be orally bioavailable.[171,172]



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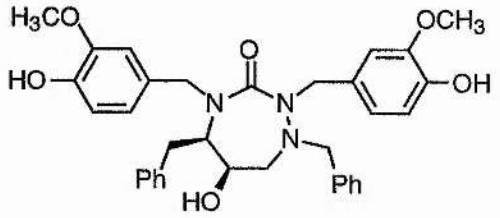
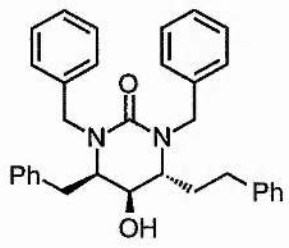


48

Two series of compounds have been synthesised through ring contraction reactions of **40**.[173,174] These reactions generate inhibitors lacking either one hydroxyl-bearing carbon atom such as **49** (K_i = 15 nM),[173] or both hydroxyl-bearing carbon atoms such as **50** (K_i = 55 nM),[174] possessed by the parent compound **40**. The potency of compound **50** is remarkable considering that it contains no hydroxyl group capable of hydrogen bonding to Asp25 and Asp25'. It would appear that the phenethyl groups are critical for maintaining effective interaction with P_1 and $P_{1'}$ from the condensed five-membered ring as the analogue containing benzyl groups at P_1 and $P_{1'}$ (**51**); (K_i = 15,500 nM) is a considerably weaker inhibitor.



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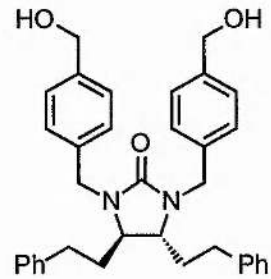

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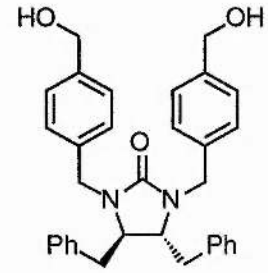
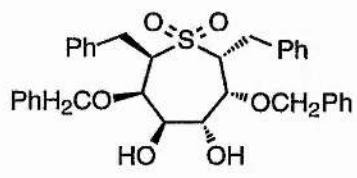


51

Finally, there are many examples of alternative cyclic structures that attempt to incorporate the flap water molecule into inhibitor design. Three of these are shown below. A series of seven-membered cyclic sulfones typified by compound **52** (K_i = 0.6 nM) have

been shown to be potent NPIs.[175] A combination of database searching and design work gave sulfoxide **53** (K_i = 7000 nM) and similar analogues.[176] The naturally occurring cyclic urea biotin was substituted to give modest inhibitors such as **54** (K_i = 570 nM).[177]



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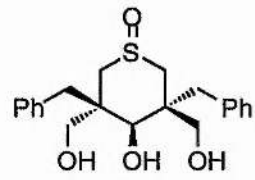

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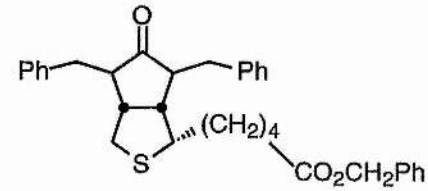


54

1.6.6 Other methods of Inhibiting HIV-1 PR

All the inhibitors described so far in section 1.6 are examples of reversible competitive inhibitors of HIV-1 PR, *i.e.* the inhibition may be overcome by the presence of excess substrate and the inhibitor competes with substrate for the same target (the active site of the enzyme). It is possible to inhibit enzymes in other ways and two such strategies will now be briefly discussed.

EPNP (**4**) is an example of an irreversible inhibitor, that is to say it reacts with the catalytic aspartic acid residues to form covalent ester bonds. There have been a number of reports of irreversible inhibitors that show greater specificity towards PR than EPNP does.[178-181] Even so, the specificity of these inhibitors for HIV-1 PR is still derived principally from their reaction with the catalytic aspartic acid residues. Thus irreversible inhibitors can circumvent the problems of resistance associated with the great majority of anti-HIV drugs;[8,9] if the catalytic aspartic acids mutate PR will not function.[49] However the reactivity of the epoxides and other electrophilic groups such as α,β-unsaturated ketones incorporated into irreversible inhibitors is such that the *in vivo* efficacy of inhibitors containing such reactive chemical groups is greatly diminished. Consequently irreversible inhibitors have not received as much attention as the reversible competitive inhibitors described earlier.

The target of both reversible and irreversible inhibitors discussed above is the active site of HIV-1 PR. However, an alternative approach to the inhibition of multiple subunit enzymes is possible. The importance of the dimeric structure of HIV-1 PR to its activity has already been stressed. Prevention of the association of the two subunits should abolish catalytic activity. As the interfacial β-sheet portion is highly conserved amongst HIV-1 isolates, this approach may have an advantage in delivering inhibitors less susceptible to point mutations than many of the inhibitors discussed above. It has been noted that 3000 $Å^2$ of the combined monomers' surface area is buried upon dimer formation and therefore 1500 $Å^2$ could be the potential target area to which dimerisation inhibitors could bind.[79]

One of the "inhibitors" chosen to try to complex with this vast target area was HIV-2 PR. There have been several reports concerning the study of heterodimers formed from one subunit each of HIV-1 PR and HIV-2 PR. In one study, in which these two different monomers were mixed, a reduction in the expected activity of the heterodimeric enzyme was observed.[182] This was thought to be caused by the formation of a disordered dimer interface. In a second experiment, one subunit of HIV-1 PR was covalently linked to one subunit of HIV-2 PR. The hybrid was fully active suggesting that in contradiction to the earlier report, the different subunits folded in a similar fashion, despite the difference in sequence homology.[183] This conclusion was supported by a third study which investigated the activity of two single chain tethered HIV-1 PR/HIV-2 PR heterodimers.[184] They were found to be just as active towards two distinct substrates as were two other tethered HIV-1 PR and HIV-2 PR homodimers.

Despite these ambiguous results, it is clear that disruption of the dimer interface is a possible way to inactivate HIV-1 PR. An alternative "protein engineering" strategy has been reported. Amino acid substitutions are identified at the dimer interface of HIV-1 PR that should prevent self-association of defective monomers and facilitate preferential association of heterodimers.[185] Following the *in vitro* expression of such HIV-1 PR monomers containing such substitutions, the infective capability of HIV-1 was reduced.

Although the inactivation of HIV-1 PR has been achieved through heterodimer formation, this has been reported quite recently (1996). Much earlier, attempts were made to inactivate HIV-1 PR through the use of small peptides mimicking the unique sequences of its C- and N-termini . This is because it had been calculated that the formation of the β-sheet (see section 1.5.3) is the main driving force behind the association of monomers, accounting for around 50% of the interfacial area of the homodimer.[79] The synthesis and testing of peptide fragments corresponding to these terminal sequences have been reported by a number of groups and some cause a reduction of HIV-1 PR activity *in vitro*.[186-188] For example, the tetrapeptide Ac-Thr-Leu-Asn-Phe-CO_2H was found to moderately inhibit the dimerisation of HIV-1 PR. The complex formed between it and monomeric HIV-1 PR had an monomer-dimer equilibrium constant (K_d) of 45 μM.[186] It is difficult to compare this inhibition favourably with the K_d of dimeric HIV-1 PR (approximately 4 nM).[186] This extremely low value is caused by the two domains of HIV-1 PR forming such a tight complex themselves. However, a recent report describes the further improvement in the inhibitory power of such peptides such that they are effective inhibitors at sub-micromolar concentrations (comparable to the inhibition caused by pepstatin A).[188] The authors also found poorer inhibition from peptides derived from N-terminal segments relative to their C-terminal counterparts. This was anticipated as such peptides, like the N-terminal segments that they mimic, are attached to the outside of the β-sheet and form only one hydrogen bond per peptide linkage. This may be seen by examination of Fig. 1.15. In all cases, kinetic studies have identified at least one mode of inhibition, that of the prevention of dimerisation. It was therefore possible to show that some of the peptides tested exhibited additional *competitive* (active site) inhibition, sometimes giving rise to the synergistic inhibition of HIV-1 PR.[188]

Whilst the number of reports of research into this aspect of HIV-1 PR inhibition is small compared to the number of active site-targeted inhibitors, the strategy holds great promise. Work reported within a single paper documented how a series of compounds had been improved 1000-fold in potency.[188] Encouraging results have recently described the use of crosslinked peptides containing the N- and C-terminal regions of HIV-1 PR

(Fig. 1.16).[189] There has even been a report describing the synergistic effects encountered from the tethering of an inhibitor targeted towards the active site to one directed towards the interfacial domain.[190] This is similar to an exceptionally ambitious strategy in which peptidonucleosides (a peptidic HIV PR inhibitor tethered to a nucleoside HIV RT inhibitor) were found to exhibit weak levels of synergism when evaluated for antiviral activity against both PR and RT.[191]

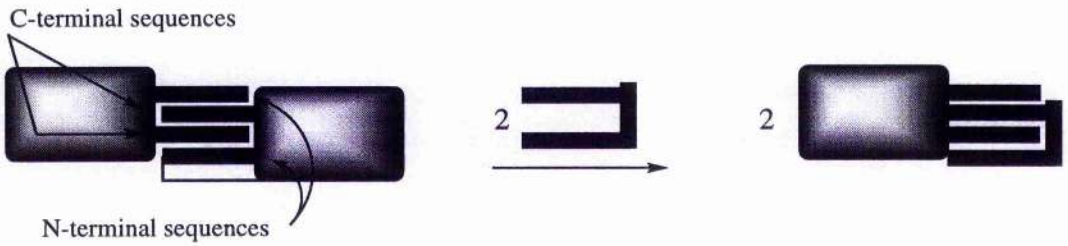


Figure 1.15: *Representation of the use of cross-linked interfacial peptides in the inhibition of dimerisation. The HIV-1 PR homodimer is shown on the left. On the right is a complex of monomeric HIV-1 PR complexed with an inhibitor comprising C- and N-terminal sequences, linked by a tether of varying length.*

Finally and most encouragingly, is both the report of an NPI of HIV-1 PR dimerisation and the manner in which it was discovered. Consideration of the structures of several peptidic inhibitors led to the derivation of a pharmacophore. By searching the Cambridge Crystallographic Database, polycyclic triterpenes were identified as potential inhibitors. They were subsequently assayed and found to have IC_{50} values of approximately 1 μM.[192] This avenue of investigation must therefore be considered to still be in its infancy, with the promise of much more to come.

1.7 Conclusion and Prospects for the future

Whilst the discovery of an effective vaccine is still a long way away, research has recently demonstrated that attenuated vesicular stomatitus virus, which causes a mouth infection in cattle, kills HIV-infected cells *in vitro*.[34] However, a cure is still remote. In the meantime, pharmacotherapeutic advances in the treatment of AIDS have delivered an armoury of treatments: nucleoside and non-nucleoside reverse transcriptase inhibitors and proteinase inhibitors have been approved for use both individually and in combination in antiviral chemotherapy.[104] Combination therapy is currently in use in the clinic, and has a number of advantages over monotherapy. Through the use of combinations of drugs directed towards a single target, the different spectrum of escape mutations produced may prevent cross-resistance. Furthermore, the use of two or more drugs directed towards the same or different target enzymes (*e.g.* PR and RT) in therapeutic regimes normally foster at the least additive and sometimes synergistic effects greatly enhancing efficacy over toxicity. Unfortunately, such multiple therapy regimes have given rise to increasing concerns about their cost to public and private health insurance programmes and thus patients' access to pharmaceutical innovation.[10]

The cost of manufacture of the complex HIV-1 PR inhibitors currently approved for clinical use is a serious problem. However, the few steps that are required to produce most of the NPIs discussed in section 1.6.5 should allow companies to avoid some of the supply problems that have plagued earlier peptide-based HIV-1 PR inhibitors.[193] These "second generation" inhibitors address some of the fundamental problems associated with large peptide-like inhibitors (see section 1.6.4). Although some of these were discovered through serendipity, others have been discovered by the searching of 3-dimensional databases; others have been discovered through rational design. Whatever the origin of the lead, subsequent structure-based design has been used to optimise the hydrophobic and hydrogen bonding interactions that these inhibitors make with the active site, and is continuing to deliver ever more potent inhibitors of HIV-1 proteinase.

CHAPTER TWO

RESULTS AND DISCUSSION

Results and Discussion

2.1 Aim of Project and Design of Potential Inhibitors

As a result of the problems encountered *in vivo* with large and peptidic HIV-1 PR inhibitors (see section 1.6.4),[115] and renin inhibitors,[194] it was decided to develop small compounds in an attempt to try to overcome these difficulties. Therefore, a design similar to that which led to the development of the cyclic urea series of inhibitors was undertaken.

When the research described here was started, there were no published reports concerning the preparation of small NPIs of HIV-1 PR. The cyclic ureas discussed earlier (see section 1.6.5.2) were only actually published in 1994,[156] following a poster presentation in June of the previous year.[195]

2.1.1 General Considerations in the Design Strategy

The features that were considered when designing our target compounds were broadly similar to those that led to the discovery of the cyclic ureas. These are as follows:

- The best inhibitors, including those approved for clinical use (**15**, **19**, **20** and **21**), contain secondary hydroxyl groups which replace the nucleophilic water molecule and hydrogen bond to Asp25 and Asp25'. We wished, therefore, to incorporate a hydroxyl group into our potential inhibitors.
- We also reasoned that both entropic benefit and specificity towards HIV-1 PR over other aspartic proteinases could be derived from the displacement of the flap water. It was anticipated that this displacement could be achieved by the presence of a heteroatom, which would hydrogen bond to the amide NH group of the flap residues Ile50 and Ile50'.

- We wished to build these structural features within a framework that would allow the attachment of C_2-symmetrically related P_1 and $P_{1'}$ groups. However, we wanted to investigate whether effective inhibitors could be developed in the absence of other subsite interactions. It was hoped that both the displacement of the two water molecules critical to the proteinase enzyme and the presence of symmetrically related P_1 and $P_{1'}$ groups would confer sufficient specificity towards the PR. By minimising the reliance of inhibition on extensive subsite interactions, both the molecular weight and the complexity of synthesising potential inhibitors could be reduced.
- Finally, it was hoped that any inhibitors would not be susceptible to resistance resulting from point mutations.[8,9] However, because the interactions with the catalytic apparatus of the enzyme were to be made through hydrogen bonds, these inhibitors would avoid the problems associated with irreversible inhibitors (see section 1.6.6, page 46).

2.1.2 Design of the Basic Template for the Target Compounds

With these structural features in mind, we set out to design appropriate cyclic compounds. This process was facilitated through molecular modelling of the interior of the enzyme's active site and was performed with the aid of Dr Wilkie, a computational chemist in the Gani group. The enzyme's active site is shown schematically overleaf (Fig. 2.1).

Fig. 2.1 shows the catalytic aspartic acids and flap isoleucine residues, and the amino acids that are close to them in the active site. The line drawn between the oxygen atoms of the two water molecules forms an axis of C_2-symmetry which almost exactly relates each Xaa to its partner Xaa'. Critical to the ability of any cyclic compound to displace both water molecules from the active site is the exact position and orientation of its water molecule-surrogates. Specifically, these should be located approximately 5.3 -5.6 Å from each other to enable effective hydrogen bonding with the catalytic aspartic acids and the flap isoleucine residues. There is, perhaps, an element of variability in this distance

caused by the flexibility of the flaps (see section 1.5.3, page 16). The precise orientation of the heteroatom lone pairs is crucial, however, as the isoleucine residues are disposed to one another through an axis perpendicular to that which relates Asp25 and Asp25'.

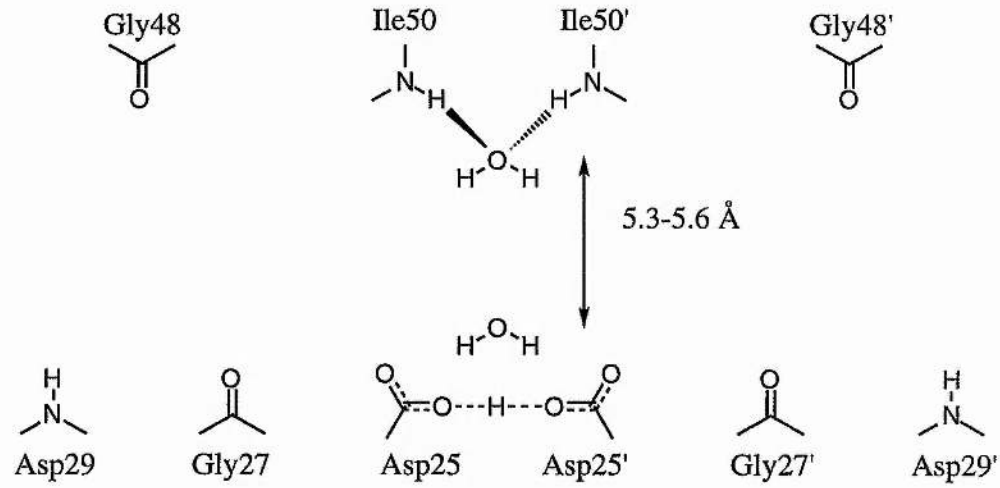


Figure 2.1: *Schematic representation of the active site of the enzyme, showing the positions of both structural and nucleophilic water molecules*

Taking all these factors into consideration, appropriate cyclic structures were designed and refined (Fig. 2.2).

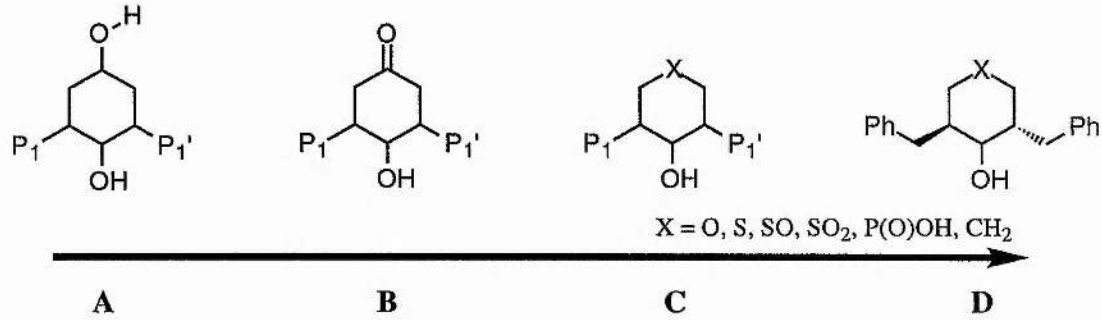


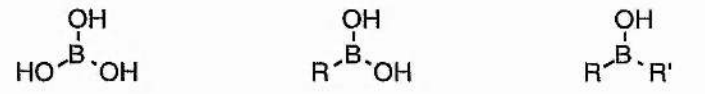
Figure 2.2: *Refinement of the template for non-peptidic inhibitors*

All the structures initially considered were based on a cyclohexane ring containing a secondary hydroxyl group designed to interact with the flap isoleucine

residues. Attention was then given to the heteroatom(s) with which to displace the flap water molecule. Both the hydroxyl (**A**) and carbonyl (**B**) groups were deemed unsuitable because they do not place the lone pairs of the oxygen atoms in the correct orientation for hydrogen bonding to the amide bonds of Ile50 and Ile50'. Consideration was therefore given to the inclusion of a heteroatom within the ring itself (**C**). Incorporation of an oxygen atom was examined first; however, modelling studies suggested that the adjacent methylene groups would interact adversely with the residues of the flap. As the C-S bond is 0.4 Å longer than the C-O bond (1.8 Å *vs* 1.4 Å), we hoped that the introduction of a sulfur atom might alleviate this problem. Additionally, the presence of a sulfur atom would enable the displacement potential of the synthetically accessible sulfoxide and sulfone analogues to be investigated. The phosphinic acid analogue was not pursued because of the focus that subsequently developed on sulfur-containing compounds (see later). The manner in which such a phosphinic acid-containing compound might have bound to the proteinase enzyme would have proved interesting; peptide-based phosphinic acids are potent HIV-1 PR inhibitors, as a result of the strong interaction between the phosphinic acid and the aspartic acids.[196-199] Finally, we decided to include the methylene-containing analogue because it could not hydrogen bond to the amide bonds of Ile50 and Ile50'. This would allow the strengths of the interactions which the oxygen- and sulfur-containing analogues were making with the flaps to be determined qualitatively.

2.1.3: Borinic and Boronic Acids Inhibit Serine Proteinases

At this stage in the design process, our attention was drawn to the well-known inhibition of serine proteinase inhibitors by boronic and borinic acids (Fig. 2.3).[200-203]



Boric Acid Boronic Acids Borinic Acids

Figure 2.3: *The Boron Acids*

Serine proteinases operate by a different mechanism to any of those proposed for HIV-1 PR; in the proteolysis that they catalyse, the scissile peptide bond is directly attacked by an active site serine residue. A significant feature of the inhibition of serine proteinases by boronic and borinic acids is the formation of a tetrahedral boronates and borinates ("ate" complexes) between the electrophilic boron atom and nucleophilic serine residue (Fig. 2.4).[202] It is thought that "ate" complexes are good mimics of the transition state in amide bond hydrolysis.

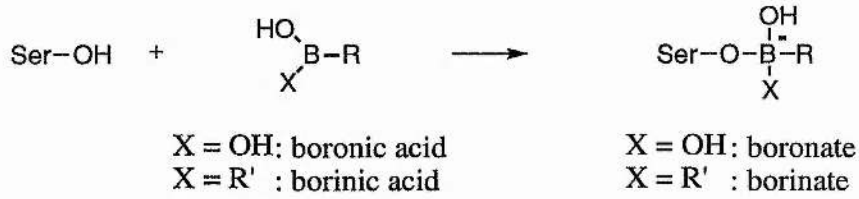


Figure 2.4: *Formation of "ate" complexes between boronic and borinic acids and a serine residue*

Whilst there are very few reports of borinic acids acting as inhibitors of serine proteinases,[200-203] possibly as a result of their susceptibility to atmospheric oxidation,[204] they are generally better than their boronic acid counterparts. For example, compared to phenyl boronic acid, diphenyl borinic acid is 30-times more potent an inhibitor of α-chymotrypsin and 60-times more potent as an inhibitor of bovine trypsin.[205] This difference has been attributed, at least in part, to the higher electrophilicity of the boron centre in borinic than in boronic acids. This is reflected in their pKa values (Fig. 2.5).

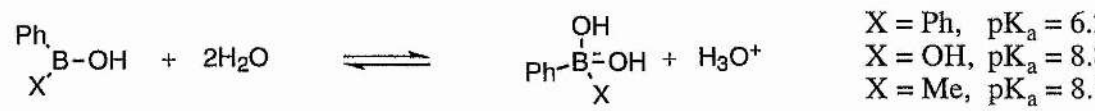


Figure 2.5: *Comparison of the acidities of borinic and boronic acids*

As the boron atom in diphenyl borinic acid is more electrophilic, the equilibrium of the reaction between it and water lies further to the right and hence the pK_a is lower. If methyl phenyl borinic acid (X = Me) and phenyl boronic acid (X = OH) are compared, it appears that the replacement of a methyl with a hydroxyl group results in greater stabilisation of the *acid* than its conjugate base. It might have been thought that a greater stabilisation of the negative charge in the conjugate *base* by the hydroxyl group would make phenyl boronic acid the stronger acid.

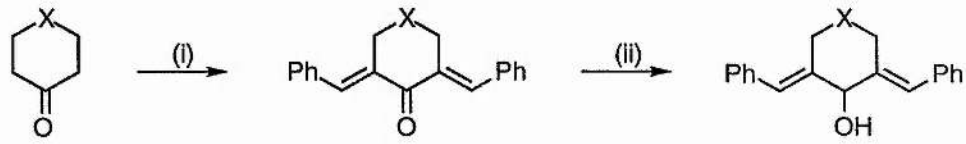
2.1.4 Incorporation of the Borinic Acid Functionality into the Template for the Target Molecules

Although the serine and aspartic proteinases operate by different mechanisms, we hoped that the electrophilicity of the boron centre in borinic acids particularly would enable such compounds to function as inhibitors by forming "ate" adducts with the catalytic aspartic acids of HIV-1 PR and we sought to test this hypothesis. In order to do this we wished to synthesise a series of cyclohexanols based on structure **D** in Fig. 2.2. After testing them against the proteinase enzyme in an *in vitro* assay, it was aimed to establish what effect the borinic acid had on inhibition by incorporating it in exchange for the hydroxyl group in a directly comparable series of compounds.

2.2 Synthesis of Cyclic Heteroatom-substituted Cyclohexanols

It was envisaged that the desired alcohols could be prepared by the reduction of the C=C bonds present in cyclic alcohols **61-63** (see Scheme 2.1, overleaf). The ketones **58-60** were all synthesised using the simple base-catalysed aldol reaction (Scheme 2.1) developed by Camp,[206] a procedure which gave better yields than the acid-catalysed syntheses previously reported.[207,208] Novel alcohols **62** and **63**, which had previously been made by Camp,[206] through reduction of the carbonyl groups in **59** and **60** with sodium borohydride in ethanol, were re-prepared and fully characterised. It was not

possible, however, to isolate the only known alcohol in this series, **61**,[209] without it decomposing almost immediately. It could be synthesised by reducing ketone **58** [the reduction of all three ketones could be monitored by both colour change (bright yellow to colourless) and by TLC] with subsequent precipitation from ethanol by the addition of water. However, when the white precipitate was left to dry for less than 5 minutes, either at the pump or under reduced pressure (the procedure used to produce **62** and **63**), it was found to decompose consistently. ^{1}H and ^{13}C NMR spectroscopic analysis of the partially dry solid revealed that it was the desired product. The spectra contained, amongst other resonances, a ^{13}C NMR signal at $\delta = 81.04$ ppm, indicative of the hydroxyl-bearing carbon atom, and a ^{1}H NMR signal corresponding to the phenylmethylene protons, upfield from its position in the spectrum of its parent ketone **58** [δ_H (CH=C) = 7.84 ppm in **58** and 6.61 ppm in **61**]. The failure to isolate alcohol **61** was a disappointment as it reduced our ability to determine how effective the heteroatom present in other analogues was at interacting with the flap isoleucine residues.



X = CH_2:	**55**	**58**	**61**
X = O:	**56**	**59**	**62**
X = S:	**57**	**60**	**63**

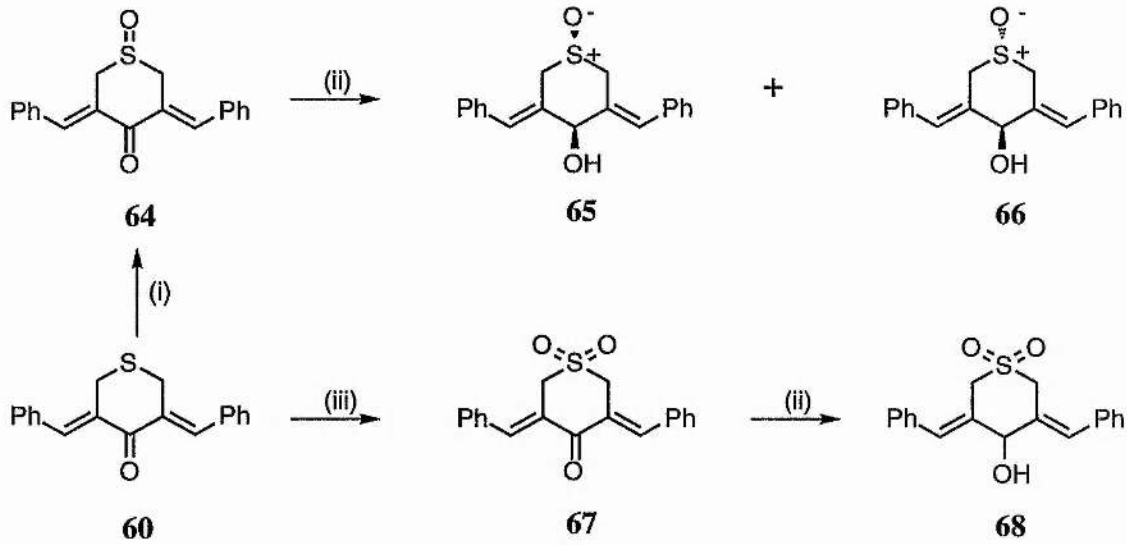
Reagents and Conditions: (**i**) Benzaldehyde, NaOH, H_2O-EtOH, 0→25 °C, 1-16 h, 62-68%; (**ii**) $NaBH_4$, EtOH, 0→25 °C, then H_2O, 92-94%

Scheme 2.1: *Preparation of 4-heterosubstituted cyclohexanols **61-63***

Attention was then directed towards the syntheses of the novel alcohols **65**, **66** and **68** (Scheme 2.2). Sulfoxide **64** and sulfone **67** were prepared by modifications to published procedures.[210,211] Compound **64** had previously been made by the oxidation of sulfide **60** with sodium metaperiodate,[210] but the use of *m*CPBA was found to result in a

higher yield. Sulfone **67** could also be synthesised by the oxidation of **60** with *m*CPBA but the reaction gave a higher yield when hydrogen peroxide was used as the oxidant, over a period of 3 days.

The novel alcohols **65**, **66** and **68** were prepared in excellent yields by the reduction of ketones **64** and **67** with sodium borohydride. Column chromatography using 2% ethanol in dichloromethane as the eluant enabled the diastereoisomers **65** ($R_F = 0.12$) and **66** ($R_F = 0.08$) to be separated (Scheme 2.2).



Reagents and Conditions: (**i**) *m*CPBA, CH_2Cl_2, 25 °C, 2 h, 77%; (**ii**) $NaBH_4$, EtOH, 0→25 °C, 20 min, then H_2O, 89-90%; (**iii**) H_2O_2, AcOH, 0→25 °C, 72 h, 59%

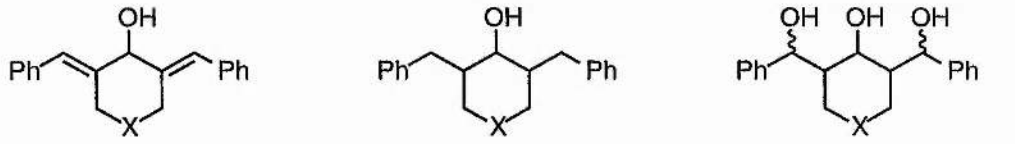
Scheme 2.2: *Preparation of 4-heterosubstituted cyclohexanols **65**, **66** and **68***

Attempts both to crystallise these alcohols and to form potentially more crystalline derivatives by reaction with 4-bromobenzoyl chloride were unsuccessful. The assignment of stereochemistry was made, therefore, on the basis of their IR spectra. There exists a sharp absorption at 3358 cm^{-1} in the spectrum of **65** in contrast to the broader absorption at 3367 cm^{-1} in that of **66**. This suggests the presence of an intramolecular hydrogen bond in the solid state.[212] There is also a difference between the two alcohols in

the stretching frequencies of their C-O bonds. The absorption occurs at 1001 cm^{-1} in **65**, and at 1035 cm^{-1} in **66**. It has been demonstrated,[213] that the former is characteristic of an axial hydroxyl group and the latter of an equatorial. The presence of an axially oriented hydroxyl group would permit the formation of an intramolecular hydrogen bond between it and the oxygen atom of the sulfoxide if both groups are on the same face of the six membered ring. This stereochemical requirement is met in **65**.

The five target 4-heterocyclohexanols (**62**, **63**, **65**, **66** and **68**) were tested against the proteinase enzyme using the *in vitro* assay described in Chapter 3. The testing turned out to be near impossible because of the extremely low solubility of most of the alcohols in the assay buffer (see also Appendix 2). In particular, **62**, **65** and **66** proved too insoluble to determine an IC_{50} value. Compounds **63** and **68** were found to be moderate inhibitors of the enzyme with IC_{50} values of approximately 40 and 80 μM respectively. Whilst these inhibitors were relatively poor compared with those discussed in Chapter 1, we were greatly encouraged because of their extremely small size and the relatively few interactions that they could be making with the enzyme.

The analogous benzyl analogues (Fig. 2.6) were our original targets as the benzyl group most closely mimics the side-chains of the common P_1 and $P_{1'}$ residues phenylalanine and tyrosine. However Camp had previously failed to synthesise the benzyl



62, **63**, **65**, **66** and **68** Benzyl Analogues Hydroxybenzyl Analogues

$X = O, S, SO, SO_2$

Figure 2.6: *Other series of cyclic alcohols that may inhibit HIV-1 PR*

analogues of alcohols **62** and **63** by reduction of the C=C bonds using hydrogen gas and 10% palladium on charcoal.[206] Employing this method to try to reduce the C=C bonds in olefinic ketones **64** and **67** also proved unsuccessful. Whilst the outcome of these experiments was disappointing, modelling studies had suggested that, in alcohols **63** and **68**, sp^2 character was forced upon the central six-membered rings by the presence of the phenylmethylene substituents. It was thought that the C=C bonds imparted rigidity to the central ring and helped align correctly the hydroxyl group and heteroatom(s) of these inhibitors with the catalytic aspartic acids and flap isoleucine residues of the enzyme (Figs. 2.7 and 2.8).

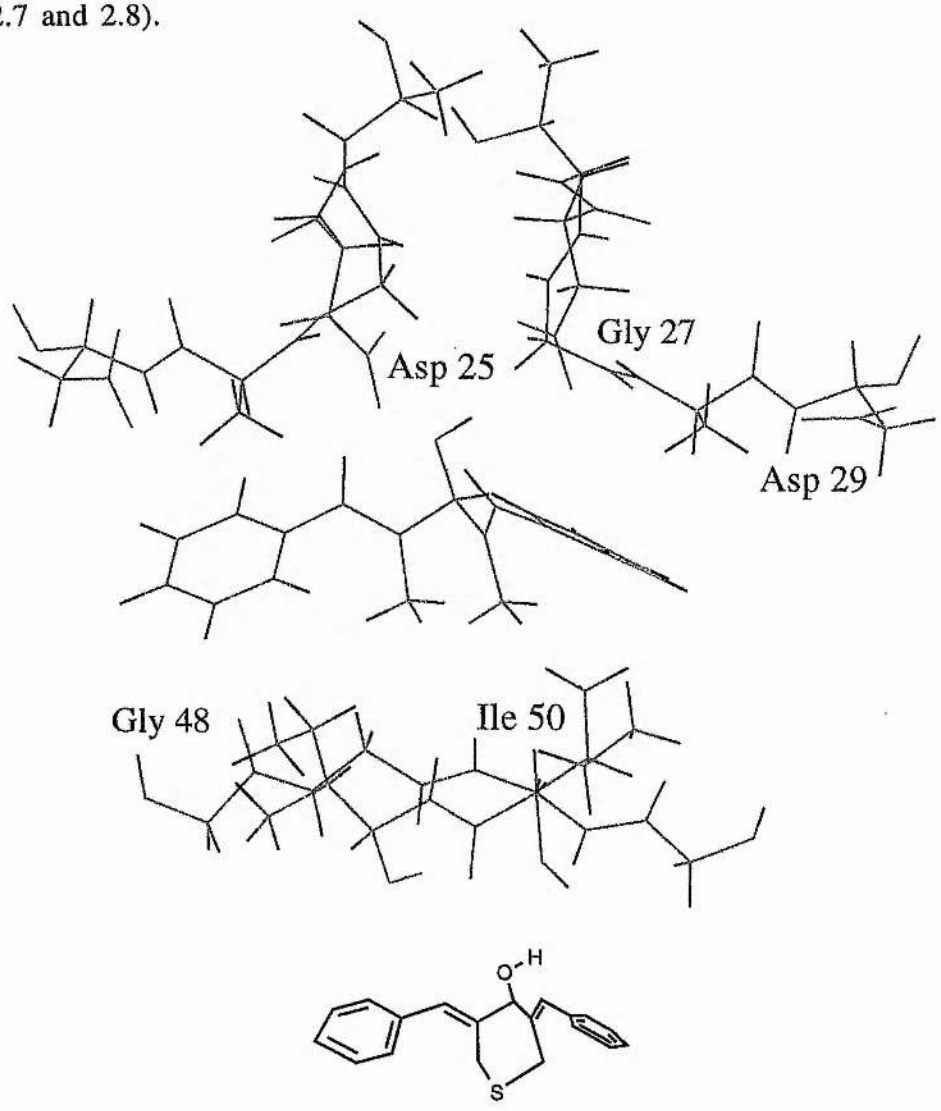


Figure 2.7: *Inhibitor **63** docked in the active site of HIV-1 PR, showing the interaction with the catalytic aspartic acids and the flap isoleucine residues*

Thus, because it appeared that the presence of the double bonds was beneficial to inhibitor potency, it was decided to discontinue attempts to synthesise the α,α'-dibenzyl series of alcohols. Modelling studies also helped explain the greater inhibition of the sulfone **68** over sulfide **63**. It appears (from a comparison of Figs. 2.7 and 2.8) that the oxygen atoms of sulfone **68** make for a better interaction with the isoleucine NH groups than does the sulfur atom of **63**.

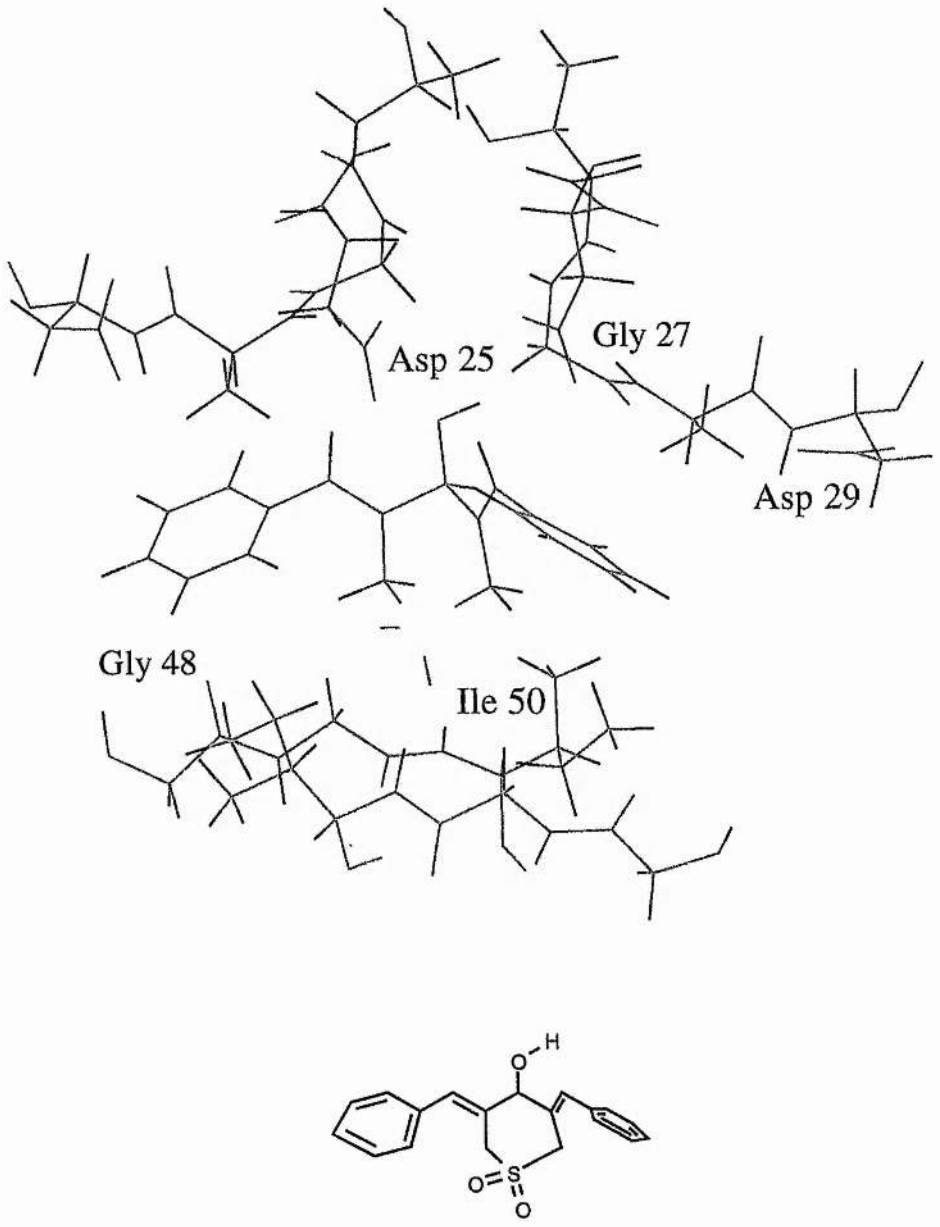


Figure 2.8: *Inhibitor **68** docked in the active site of HIV-1 PR, showing the interaction with the catalytic aspartic acids and the flap isoleucine residues*

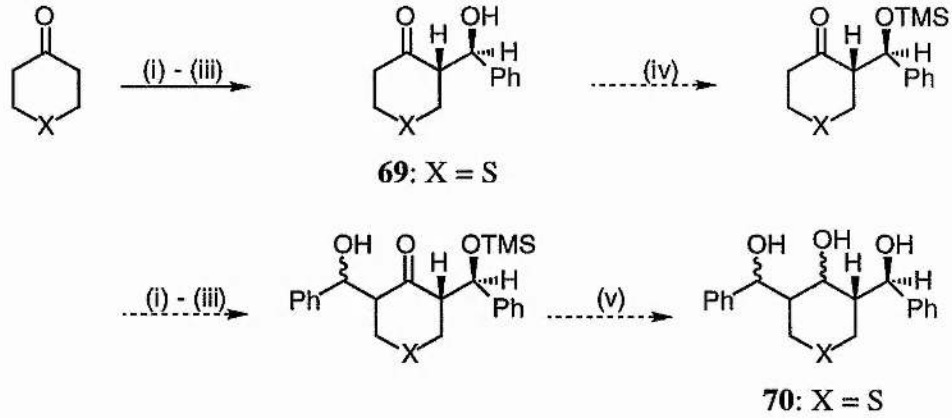
Whilst these studies were in progress, the first publication concerning the identification and synthesis of non-peptidic inhibitors appeared from SmithKline Beecham.[176] A number of compounds that are extremely closely related in structure to ours were disclosed, the most potent being compound **53** (page 46; $K_i = 7$ μM). Although disappointing, it was encouraging that our inhibitors had been discovered entirely through rational design, whilst the initial template at SmithKline Beecham had been identified by the use of a version of DOCK (page 33). Notably, compound **53** and other related compounds all contained hydroxyl groups which are capable of acting as hydrogen bond donors to the amide carbonyl groups of Gly27 and Gly27' (see Fig. 2.1). Also, an analogue in which the sulfoxide group was exchanged for a methylene group possessed no activity against the proteinase enzyme.

As a result of some of the conclusions drawn from this publication, and from modelling studies carried out by Dr Wilkie, we decided to attempt the synthesis of another series of cyclohexanols containing hydroxybenzyl side-chains (see Fig. 2.6), before embarking on the syntheses of borinic acid-containing compounds. It was predicted that the two hydroxyl groups, incorporated into benzyl substituents, would make strong interactions with Gly27 and Gly27' (see Fig. 2.1).

2.3 Attempted Synthesis of Cyclic *bis*Hydroxybenzylated Heteroatom-substituted Cyclohexanols

It was thought that the best route to obtaining the desired hydroxybenzyl analogues of alcohols **62**, **63**, **65**, **66** and **68** (Fig. 2.6) would be to follow the sequence of reactions outlined in Scheme 2.3. We did not intend to separate the diastereoisomers formed in any reaction until the (mixtures of) diastereoisomers of the target *bis*aldols had been assayed against HIV-1 PR. In the event of measurable activity, thereby making such separations necessary, the use of the TBDMS rather than the TMS group would have been employed as it is a less labile group when present during purification by column

chromatography. However, the TMS group was preferred as it is stable to base (in the second aldol reaction), but not to borohydride in the reduction step (Scheme 2.3).



69: X = S

70: X = S

Reagents and Conditions: (**i**) LDA, THF, -70 °C; (**ii**) benzaldehyde, -70 °C; (**iii**) $NH_4Cl_{(aq.)}$; (**iv**) Li_2S, MeCN, Me_3SiCl, 25 °C; (**v**) $NaBH_4$, EtOH, then H_2O, 25 °C.

Scheme 2.3: *Proposed synthesis of* α,α'-bis*(hydroxyphenylmethyl)-4-hetero-cyclohexanols*

The synthetic strategy to access the target *bis*-aldol compounds requires non-aqueous conditions in order for the aldols themselves to be prepared. (Previously, benzaldehyde was condensed with cyclohexanone and its 4-thio and 4-oxo derivatives using sodium hydroxide as base in aqueous ethanol, giving the dehydrated *bis* enone products). Fortunately, a published procedure existed for the preparation of aldol **69**,[214] in which it was claimed that it could be formed in 98% by the aldol reaction shown in Scheme 2.3. This showed high selectivity towards the *anti* diastereoisomer (80% *d.e.*), as expected from the Zimmerman-Traxler Model.[215] This model predicts that the most favourable 6-membered transition state is that in which the maximum number of substituents lie in the equatorial conformation (Fig. 2.9, overleaf).

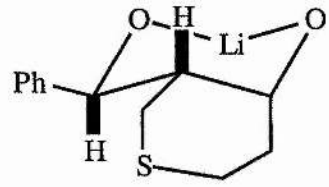


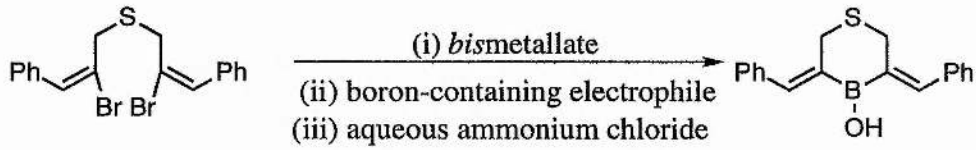
Figure 2.9: *Transition state that gives the anti-diastereoisomer* ***69*** *in Scheme 2.3*

Many careful repetitions of the published procedure for the preparation of **69**, in which a solution of benzaldehyde in THF is added to the lithium enolate of tetrahydrothiopyran-4-one, followed by the rapid (within 30 seconds) addition of saturated aqueous ammonium chloride failed to give any of the required products. Only on one occasion did a compound, thought to be the *bis* aldol **70** (Scheme 2.3), precipitate as a white solid from an ethereal solution following the reaction workup (in 24% yield). It was not, unfortunately, possible to obtain either a mass spectrum to corroborate the crude ^{1}H NMR spectrum or to repeat the synthesis.

Some time after the isolation of **70**, an air-sensitive product thought to be the aldol **69** (crude ^{1}H NMR spectrum) was isolated in 33% yield. However, because a considerable amount of time had been unsuccessfully devoted to the same capricious reaction, it was decided to start work on the borinic acid-containing target compounds.

2.4 Syntheses of the Borinic Acid-containing Compounds

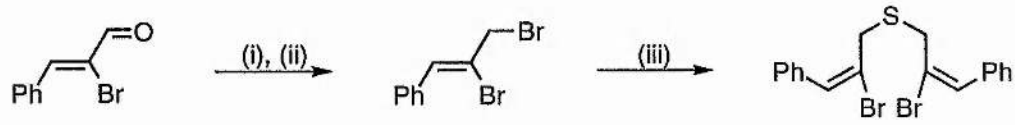
The synthesis of a series of borinic acid-containing analogues of inhibitors **63** and **68** required a completely different synthetic strategy to be developed. Initially it was hoped that it would be possible to react sulfide **71** with either two equivalents of *n*-BuLi or of magnesium metal to give a *bis*metallated intermediate which could then be reacted with a boron-containing electrophile, such as a boron trihalide or a trialkyl borate. This would give, after quenching with ammonium chloride solution, borinic acid **72** (Scheme 2.4).



71 **72**

Scheme 2.4: *Attempted preparation of 72 by a* bis*metallation strategy*

Although a mixture of geometrical isomers of sulfide **71** was prepared in a straightforward three-step synthesis from α-bromocinnamaldehyde (Scheme 2.5), it proved impossible to purify these isomers by column chromatography. Attempted distillation resulted in total decomposition, probably as a result of the elimination of HBr to form the highly conjugated and reactive compound, phenylallenyl sulfide.



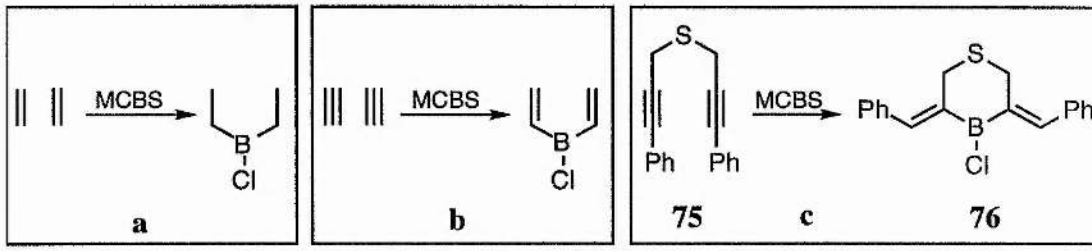
73 **74** **71**

Reagents and Conditions: **(i)** $NaBH_4$, MeOH, 25 °C, 2 h; **(ii)** PPh_3, Br_2, CCl_4, 0→77 °C, 1 h, 82% (from **73**); **(iii)** $Na_2S.9H_2O$, MeOH, 25 °C, Ar, 2 h, 94%

Scheme 2.5: *Synthesis of sulfide 71*

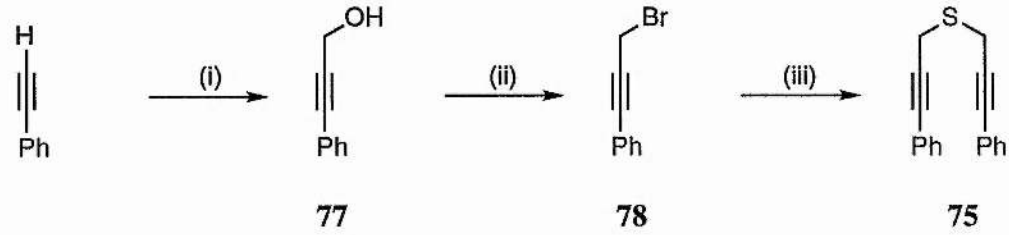
The presence of strong base in both of the *bis*metallation strategies attempted almost certainly caused the elimination of HBr from sulfide **71**. Both attempts to form a dianion of **71** failed. Even when the temperature of the solution of **71** in THF or ether was lowered to -100 °C, its colour turned black immediately the *n*-BuLi was added. After the subsequent additions of trimethylborate and then ice-cold saturated aqueous ammonium chloride, analysis by TLC indicated the presence of many inseparable products.

An alternative method of preparing **72** was, therefore, sought and it was decided to adopt the dihydroboration protocol developed by Brown *et al.*[216-220] This procedure uses the commercially available reagent monochloroborane, as its dimethyl sulfide complex (MCBS), to connect two double (Scheme 2.6a), or two triple (Scheme 2.6b) bonds together through a boron atom. It was hoped to extend this methodology and cyclise diyne **75**, thereby affording the intermediate cyclodialkenyl chloroborane (**76**; Scheme 2.6c), which would give borinic acid **72** on quenching with ammonium chloride solution.



Scheme 2.6: *Cyclisation reactions using MCBS*

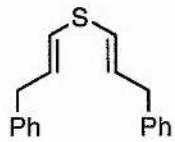
The known sulfide **75**,[221-223] was prepared as a substrate for such a cyclisation reaction (Scheme 2.7).



Reagents and Conditions: (**i**) *n*-BuLi, THF, -40→-10 °C, 1 h, then $(CH_2O)_n$ -10→25 °C, 3 h, then H_2O, 90%; (**ii**) pyridine, PBr_3, ether, 0→50 °C, 2 h, 65%; (**iii**) $Na_2S.9H_2O$, MeOH, 25 °C, Ar, 16 h, 99%

Scheme 2.7: *Synthesis of sulfide 75*

Unfortunately, several attempted cyclisations of sulfide **75** with MCBS showed no evidence for the expected borinic acid **72** but instead a large number of products when examined by TLC and ^{1}H NMR spectroscopy. The reaction was attempted using different ratios of **72** and MCBS and tried at different temperatures. It had been expected that cooling the reaction to -30 °C and below would have been beneficial as this has been shown to retard dihydroboration of internal alkynes.[224] Ether was always used as a solvent as it had proved to be far superior to THF, because of the strong ability of THF to complex to, and deactivate, monochloroborane.[216] This lack of success was disappointing; however, although the cyclisation has been reported using diene substrates,[219] such cyclisations have not, to the best of our knowledge, been performed on diynes. The failure of the reaction may arise as a result of both a propensity of the diyne to dihydroborate and a failure of it to react regioselectively with MCBS. The problem of regioselectivity may be appreciated by consideration of the transition state in a hydroboration reaction. In it, the carbon atom not being bonded to the boron atom has a slight partial positive charge. In reactions not governed solely by steric effects, the regiochemistry is influenced by the stabilisation of this charge. In the internal diyne **75**, such a partial positive charge may be stabilised by either the phenyl group or by the sulfur atom. Hence a number of potential products may be envisaged. In an attempt to counter the problem of products resulting from dihydroboration of the diyne, the known dienes **79**,[225,226] and **80**,[227] were reacted with MCBS.



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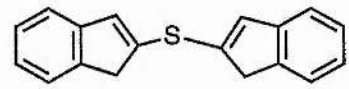


80

Sulfide **79** (cinnamyl sulfide) was prepared in a similar fashion to sulfides **71** and **75**, by the reaction of cinnamyl chloride with sodium sulfide nonahydrate. Sulfide **80** was prepared by refluxing indan-2-one in toluene with Lawesson's reagent; the initial

product of this reaction, indan-2-thione, then reacts with its tautomeric thioenol resulting in the elimination of hydrogen sulfide to give **80**. Unfortunately, neither of these products gave the desired borinic acids on treatment with MCBS. The failures of all of the cyclohydroboration reactions is both difficult to understand and very disappointing. Consequently, it was necessary to design alternative borinic acid-containing targets that might prove easier to synthesise.

2.5 Design of Alternative Borinic Acid-Containing Compounds

As the target borinic acids described in section 2.4 may have been too unstable to isolate, the logical next step, therefore, was to design of a series of potentially more stable target molecules. Diphenyl borinic acid, in addition to its ability to inhibit serine proteinases, is also reported to be relatively stable.[205] Because of these properties, it was decided to modify the first set of target borinic acids (**72** being the sulfide-containing analogue) to the cyclic diphenyl borinic acids **81-85** shown in Fig. 2.10.

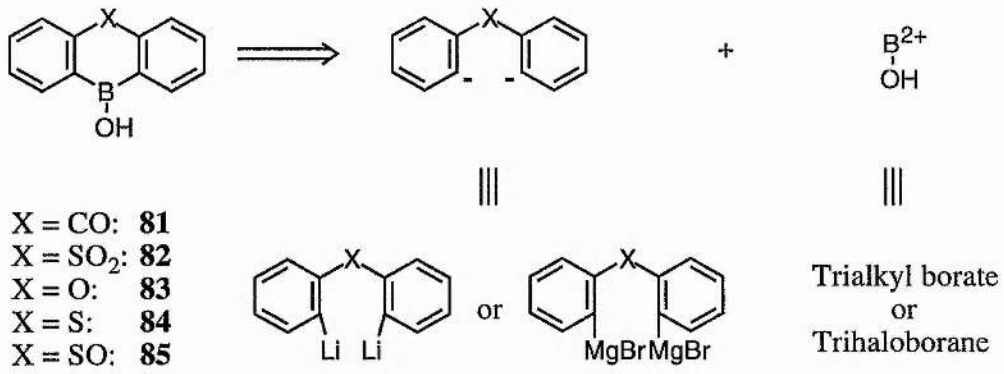


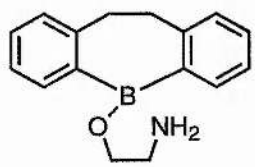
Figure 2.10: *Target diphenyl borinic acids, synthons and synthetic equivalents*

The synthetic strategy envisaged for these target compounds was more straightforward than those attempted in section 2.4. It required the *bis*metallation of compounds containing two phenyl groups joined through an atom or atoms designed to

mimic the flap water molecule, and reaction of these metallated compounds with one equivalent of a boron-containing electrophile, such as a trialkyl borate or a more reactive trihaloborane.

The syntheses of these compounds were predicted to be relatively easy. However, we were aware that the phenyl groups of these potential inhibitors were positioned very close to their centres and, therefore, to the borinic acid and flap water surrogates. Such clustering of all the important groups of these compounds threatened to endanger their interaction with their respective "partners" within the active site of HIV-1 PR (*i.e.* S_1, $S_{1'}$, the catalytic aspartic acids and the amide bonds of the flap isoleucine residues). However, the 4-hydroxycoumarin series of inhibitors (page 34) contain a benzene ring fused to the central six-membered ring. This reassured us that, whilst our template might not deliver potent inhibitors, it would be a valid one with which to test the inhibition of HIV-1 PR with borinic acids.

Only four cyclic diphenyl borinic acids have been reported; these were described between 1955 and 1961 (compounds **83** and **86-88**).[228-231] Just one of these (**83**) was actually characterised as the free borinic acid,[230] the remaining three being isolated as their ethanolamine esters. They were all prepared from the reaction of dilithiated precursors (because of the tendency of di-Grignard precursors to precipitate from solution),[230] and a variety of boron-containing electrophiles.



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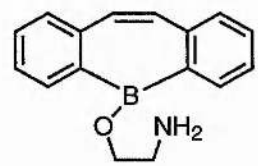

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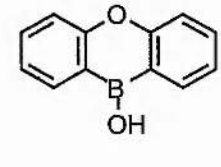

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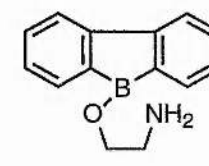


88

Ethanolamine is very useful for the stabilisation and characterisation of diaryl borinic acids.[204] It reacts readily with them, even in solutions that are not completely water-free, to give air-stable and sharp-melting ethanolamine esters. These derivatives are known to hydrolyse very rapidly to the parent acids in aqueous solution.[201,204] Indeed,

the actual ethanolamine esters of borinic acids have been assayed directly against serine proteinases as the equivalent of borinic acids.[201,203]

2.6 Syntheses of Cyclic Diphenyl Borinic Acids

The first cyclic diphenyl borinic acid (of the type illustrated in Fig. 2.10) whose synthesis was attempted was **81** (X = CO). It was decided to begin with this compound for several reasons. Firstly, the inhibition of HIV-1 PR by cyclic ureas had recently been disclosed. We had rejected the carbonyl group as a water-surrogate in our original design process (Fig. 2.2). It was of great interest, therefore, to synthesise borinic acid **81** and compare its potency against the proteinase enzyme with an analogous compound which contained a heteroatom or atoms that we thought would better interact with the flap isoleucine residues (either compound **82** or **85**). Although a diaryl ketone is a poorer hydrogen bond acceptor than a urea, it satisfied our principal requirement that it is located outwith the central six-membered ring, and should therefore experience fewer adverse interactions with flaps than might be predicted to occur with the known diaryl ether-containing analogue (**83**, X = O).

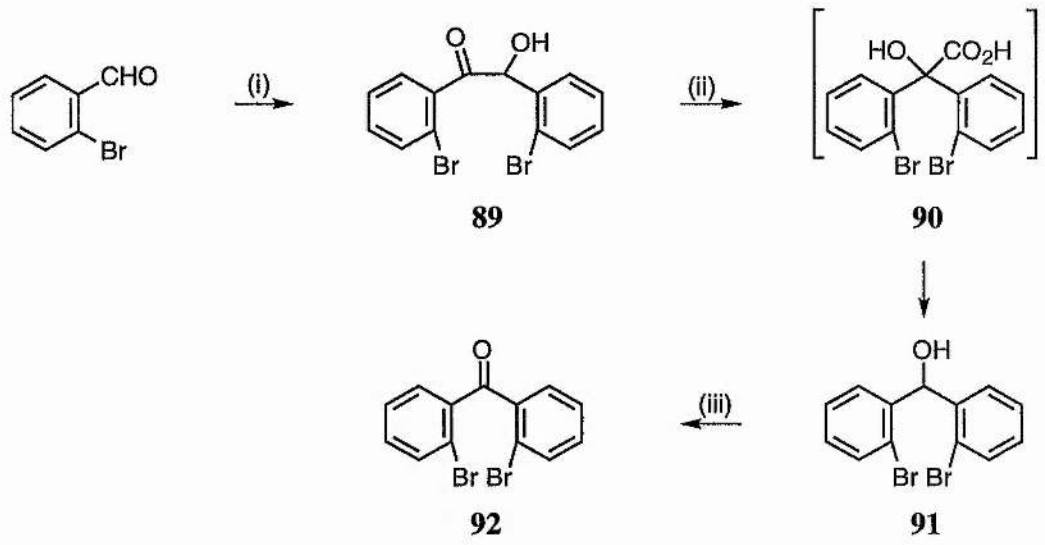
2.6.1 Attempted Synthesis of Borinic Acid 81

The retrosynthesis depicted in Fig. 2.10 revealed that a di-anionic synthon was required. In order to generate an appropriate synthetic equivalent of this, it was necessary to prepare a derivative of 2,2'-dibromobenzophenone (**92**; Scheme 2.8) in which the ketal group had been protected. The dilithiated intermediate could then be generated by lithium-halogen exchange.

2,2'-Dibromobenzophenone is a known compound, first synthesised in 1915.[232] The synthesis was extremely lengthy and gave a poor yield. Other syntheses have since been published. It seemed that the most expedient of these was that described by Lee *et al.* (Scheme 2.8).[233] In this procedure, a benzoin condensation gave **89** which was then treated with 0.3 equivalents of potassium bromate in aqueous alkali. Heating of

this solution under reflux was reported to cause an oxidation-benzilic acid rearrangement to give benzilic acid **90**, which spontaneously collapses to give benzhydrol **91**.

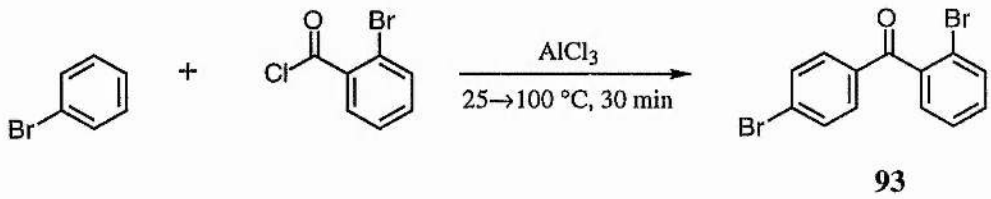
We decided to follow this procedure in order to obtain our desired starting material. Benzoin **89** was synthesised through an alternative benzoin reaction that uses a thiazolium salt as the catalyst, not the cyanide ion.[234] The use of these conditions to effect the benzoin condensation, gave, in addition to the desired product, a small (*ca.* 5%) but consistent amount of an additional product, benzil **94** (see Scheme 2.10, page 73), thought to result from oxidation of **89** *in situ*. All attempts to synthesise benzhydrol **91** from benzoin **89** failed. After modifying the reaction conditions, it was found that the use of a full equivalent of potassium bromate in this reaction enabled the synthesis of impure benzilic acid **90** in approximately quantitative yield. No evidence was ever observed for decarboxylation and thus production of benzhydrol **91**. This was confirmed by repeating these reactions after the independent preparation of **91** (by reduction of **92** with sodium borohydride). Comparison of the TLCs of these reaction solutions with that of authentic **91** showed that it was never formed under the conditions described by Lee *et al.*[233]



Reagents and Conditions: (**i**) KCN, DMF, 25 °C, 48 h; (**ii**) NaOH, $KBrO_4$, H_2O, 100 °C, 20 h; (**iii**) PCC, Celite, CH_2Cl_2, 25 °C, 6 h, 40% (over 3 steps).

Scheme 2.8: *The preparation of benzophenone **92** reported by Lee* et al.[233]

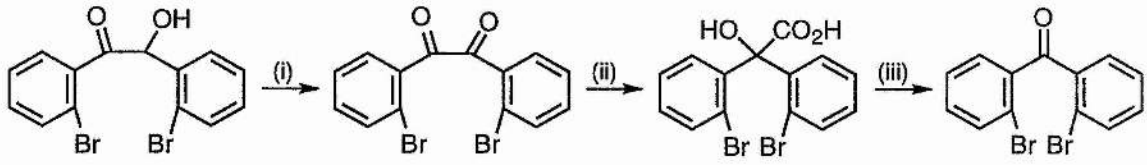
As a result of the difficulty in obtaining the desired benzophenone **92**, an alternative Friedel-Crafts approach was tried in which the acylation of bromobenzene with 2-bromobenzoyl chloride was attempted (Scheme 2.9); unfortunately, the acylation of bromobenzene only occurred at the *para* position to give the known 2,4'-dibromobenzophenone,[235,236] in 30% yield.



93

Scheme 2.9: *Friedel-Crafts acylation of bromobenzene*

As the Friedel-Crafts approach was being investigated, the other publications describing the synthesis of **92** were examined. The report from Bickelhaupt *et al.*[237] gave more experimental details than that from Lee *et al.*[233] and also described the isolation and characterisation of intermediates. Although the latter did not cite the publication of the former, Bickelhaupt *et al.* reported a very similar preparation of **92** (Scheme 2.10),[237] the principal difference being the greater reliability of their work. Additionally, it was reported that benzilic acid **90** could be synthesised from the benzil **94**, which in turn was prepared by the oxidation of the benzoin **89** with nitric acid (Scheme 2.10). Furthermore, it was also reported that benzilic acid **90** could be made by treating benzoin **89** with potassium bromate in aqueous alkali, as we had found, but in low (24%) yield. No mention was made of the benzhydrol **91**.



89 **94** **90** **92**

Reag*ents and Conditions*: (**i**) HNO_3, 100 °C, 7 h, 60%; (**ii**) KOH, EtOH-H_2O, 78 °C, 15 min, 72%; (**iii**) $NaBiO_3$, AcOH, 50-60 °C, 5 h, then H_3PO_4-H_2O, 25 °C, 16 h, 84%

Scheme 2.10: *The preparation of benzophenone* **92** *reported by of Bickelhaupt* et al.[237]

Each step described by Bickelhaupt *et al.* worked well and thus adequate quantities of benzophenone **92** were prepared. However, after all the effort had been made to produce **92**, a considerably more efficient synthesis was discovered. The purification of both products from the thiazolium-catalysed benzoin reaction (benzoin **89** and benzil **94**) was not easy; however, once a mixture of them was separated from the residue of the benzoin reaction, it proved possible to perform the oxidation-rearrangement reaction described above [Scheme 2.8 (**ii**) or Scheme 2.10 (**ii**)] on this mixture. The aqueous solution of the reaction had to be acidified in order to extract the benzilic acid from it. However, it transpired that if concentrated (rather than dilute) hydrochloric acid was used, the solution became (unsurprisingly) extremely hot and (surprisingly) was found to contain benzophenone **92**. This represented a pleasing 60% yield over the two steps. Unfortunately, the resynthesis of **92** that led to this discovery was necessitated by its decomposition during repeated attempts to protect its carbonyl group. The carbonyl group must be protected before exposure to organolithium agents, because its electrophilicity results in it being attacked during such reactions.[238] The attempted protection with ethylene glycol catalysed by *p*-toluene sulfonic acid in refluxing toluene, with azeotropic removal of water, was not successful. A second procedure developed specifically for the protection of diaryl ketones,[239] was attempted. This procedure, in which triflic acid (TfOH) is used as the acidic catalyst, had allowed the protection of 2,2'-dichlorobenzophenone (**95**) to give ketal **96** (Scheme 2.11).[239] However, the use of

this procedure to protect compound **92**, failed to deliver any of the desired product, even after heating under reflux for 1 week.

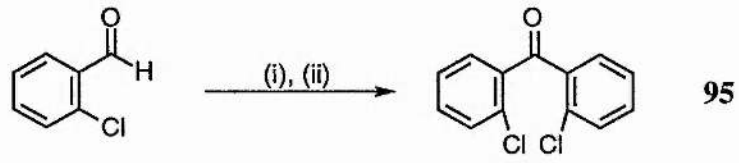
95 **96**

Reagents and Conditions: $(MeO)_3CH$, MeOH, $MeNO_2$, then TfOH, 5→101 °C, 36 h, 92%

Scheme 2.11: *Protection of the carbonyl group in a hindered diaryl ketone*

This was another considerable setback. The original reason for wanting to synthesise a compound in which the carbonyl group of **92** had been protected was to enable the formation of a dilithiated species by lithium-bromine exchange. This di-anion was to be reacted with an appropriate boron-containing electrophile. Thus, the most logical next step was to prepare ketal **96** and use it in the attempted synthesis of borinic acid **81**. As lithium-chlorine exchange is rarely successful, this approach required that **96** be used to prepare a di-Grignard reagent, rather than its dilithiated derivative.

Benzophenone **95** is a known compound, prepared in four steps from 2-chlorobenzaldehyde,[240] in a synthesis similar to that of **92** shown in Scheme 2.10. However, it proved possible to synthesise it in two steps (Scheme 2.12) by the shortened procedure described earlier for the synthesis of **95**.



95

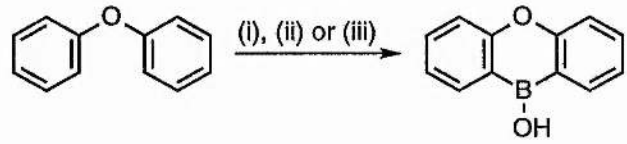
Reagents and Conditions: **(i)** 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, Et_3N, EtOH, 78 °C, 5 h; **(ii)** NaOH, $KBrO_4$, H_2O, 100 °C, 5 h, then conc. HCl, 63% (over two steps).

Scheme 2.12: *Synthesis of 2,2'-dichlorobenzophenone 95*

Unfortunately, the ketalisation of benzophenone **95** shown in Scheme 2.11 could not be repeated, despite several attempts in which the reaction was left to heat under reflux for up to a week, and in which differing amounts of triflic acid were used. Because of the failures to protect the carbonyl groups in either **92** or **95**, there was no alternative but to attempt the synthesis of the remaining target borinic acids **82-85**.

2.6.2 Synthesis of Borinic Acid 83

The principal target borinic acids were those which contained heteroatoms projected from the ring (*i.e.* ketone **81**, sulfone **82** and sulfoxide **85**). However, as no borinic acids had been made thus far, it was decided to attempt the synthesis of the known oxygen-containing borinic acid **83**.[230] It was first synthesised by the reaction of 2,2'-dilithiodiphenyl ether with either *n*-butyl metaborate or boron trifluoride etherate to afford crude **83** in yields of 42 and 23% respectively (Scheme 2.13).



83

Reagents and Conditions: (**i**) *n*-BuLi, ether, 35 °C, 72 h; (**ii**) *n*-butyl metaborate, 35 °C, 2 h, 42% [over (**i**) and (**ii**)]; (**iii**) $BF_3.OEt_2$, 35 °C, 1 h, 23% [over (**i**) and (**iii**)].

Scheme 2.13: *The preparation of borinic acid **83** reported by Davidson and French*[230]

It is useful at this point to discuss how the intermediate 2,2'-dilithiodiphenyl ether in Scheme 2.13 was produced. The lithiation took a considerable time, but occurred only at the positions *ortho* to the oxygen atom. Highly regiospecific lithiations such as these are known as directed or "*ortho*" lithiations. They arise from the ability of the "*ortho* directors" to withdraw electron density away from the point of lithiation, to co-ordinate

with the lithiating agent or both. Four classes of *ortho* directors that participate in the deprotonation shown in Fig. 2.11 have been described.[238] These are listed in ascending order of effectiveness.

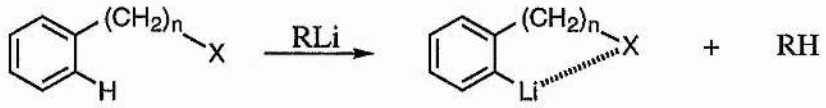


Figure 2.11: Ortho *lithiation; n = 0-2 and X = a directing group*

Class 1: Co-ordination only

When n > 0, the effect of the saturated carbon atom(s) on the acidity of the *ortho* proton is considered neutral. However, X is capable of co-ordinating to the alkyl lithium species if it possesses non-bonding electrons. Most effective in this class are amines; much less effective are alkoxides, acetals and ketals.

Class 2: Electron-withdrawing only

When X is a halogen, or a carbonyl or trifluoromethyl group, the acidity of the *ortho* proton is markedly increased. However, these groups have very little ability to co-ordinate to the lithiating agent.

Class 3: Electron-withdrawing groups with moderate co-ordination potential

In this class, most of the driving force towards deprotonation is the withdrawal of electron density from the acidic centre, an effect enhanced upon co-ordination to the lithiating agent. Examples of such *ortho* directors are $-NR^1R^2$, -SR << -OR << $-SO_2$-aryl.

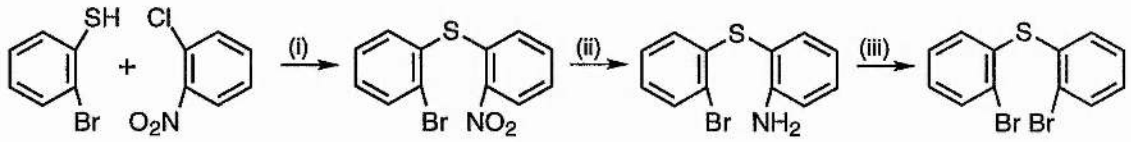
Class 4: Electron-withdrawing groups with good co-ordination potential.

Groups such as -$CONR^1R^2$ and 2-pyridines co-ordinate through the nitrogen lone pair in addition to their electron-withdrawing properties and are very effective *ortho* directors.

Returning to the preparation of borinic acid **83**, its known synthesis,[230] was modified to a procedure using trimethyl borate as the electrophile. This did afford the desired product, but in a yield of less than 5%. We thought that there were two reasons for this poor yield: (a) the extremely long time taken to effect the dilithiation of diphenyl ether and (b) the relative volatility (bp = 68 °C) of the borate. Consequently, the stronger base *s*-BuLi was used to reduce the time taken to dilithiate diphenyl ether, and a less volatile borate (tri-*n*-butyl borate, bp = 230 °C) was used. These efforts were successful and resulted in us obtaining the desired compound **83** in 61% yield.

2.6.3 Synthesis of Borinic Acid 84

The conditions successfully used to synthesise borinic acid **83** were used to try to make the sulfur-containing analogue **84**. Unfortunately, these proved unsuccessful, even when the base *t*-BuLi was used. Compared to diphenyl ether, the poorer reactivity of diphenyl sulfide towards organolithium compounds was not surprising as the sulfur atom is not able to co-ordinate to lithium as effectively as the oxygen atom. Additionally, its electron-withdrawing capability is poorer. Probably because of these reasons, there are no reports that claim that 2,2'-dilithiodiphenyl sulfide can be made by direct lithiation of diphenyl sulfide. Instead, it is synthesised by the treatment of 2,2'-dibromodiphenyl sulfide (**99**) with *n*-BuLi.[241-244] A thorough search revealed only one published synthesis of sulfide **99**, shown in Scheme 2.14 overleaf.

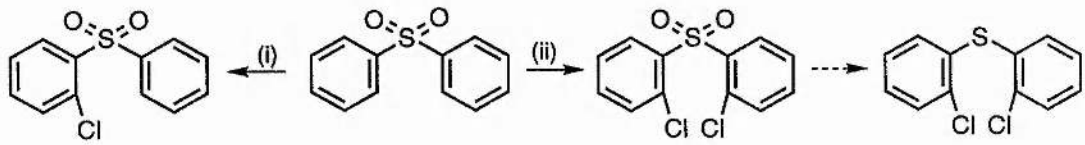


97 **98** **99**

Reagents and Conditions: (**i**) $NaHCO_3$, DMF, 70 °C, 4 h, 96%; (**ii**) $SnCl_2.2H_2O$, HCl-EtOH, 70-80 °C, 4 h, 70%; (**iii**) $HOSO_2ONO$, 16-20 °C, 0.5 h, then CuBr, 45% HBr in AcOH, 80 °C, 1 h, 35%

Scheme 2.14: *Synthesis of sulfide* ***99***[241]

As this seemed a rather involved synthesis for the complexity of **99**, several attempts were made to develop a simpler route. It was anticipated that it would be possible to synthesise a di-Grignard reagent from 2,2'-dichlorodiphenyl sulfide (**102**), which, it was hoped, could be synthesised by the reduction of 2,2'-dichlorodiphenyl sulfone (**101**). Sulfone **101** is a known compound which has been prepared by the oxidation of sulfide **102**,[245] which itself is made by a synthesis closely resembling that for **99** shown above (Scheme 2.14).[245] However, we hoped that a one step synthesis of 2-chlorodiphenyl sulfone (**100**; Scheme 2.15),[246] could be adapted to afford **101**.



100 **101** **102**

Reagents and Conditions: (**i**)[246] 1 eq. *n*-BuLi, ether, -30-(-40) °C, 1 h, then 1 eq. $PhSO_2Cl$, 30 min, 90%; (**ii**) 2.2 eq. *n*-BuLi, THF, -30-(-40) °C, 0.75 h, then 2.5 eq. $PhSO_2Cl$, 30 min, 99%.

Scheme 2.15: *Attempted synthesis of sulfide* ***102***

The synthesis of **101** from diphenyl sulfone proceeded in almost quantitative yield. However, all attempts at the selective reduction of its sulfone group failed. Because of the susceptibility of aryl chlorides to reduction by the reagents used most commonly to reduce sulfone groups, for example DIBAL,[247] it was not possible to use them in this instance. Of the methods attempted, the reflux of sulfone **101** with samarium iodide in THF-DMPU and THF-HMPA,[248] and with elemental sulfur in ethanol,[249] left the compound unchanged. The reduction of the sulfone with DIBAL was attempted; however, an inseparable mixture of products resulted, probably from unselective de-oxygenations and dechlorinations effected by this reductant.

Following the failure to synthesise sulfide **102**, the sulfide **99** was prepared using the procedure outlined in Scheme 2.14. This worked well except for the final Sandmeyer reaction, the yield of which could not be increased above 35%. Sulfide **99** was then converted to the desired borinic acid **84** in a yield of 67%, using a method similar to that shown for the preparation of **83** in Scheme 2.13. The lithium-halogen exchange reaction proceeded very quickly and, at -70 °C, was found to be complete within 5 minutes.

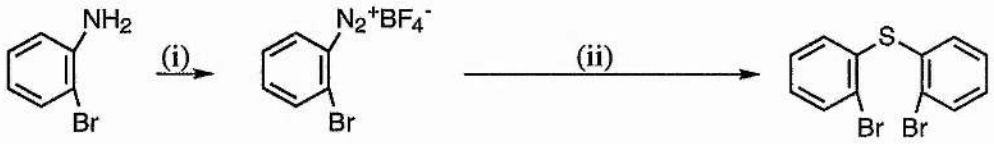
For reasons discussed later, greater quantities of both borinic acid **84** and sulfide **99** were required than could satisfactorily be made using the known low-yielding synthesis of **99** (23% over three steps). Because of both this, and the fact that the synthesis is probably intolerant of additional substitution on the aromatic ring, four further methods of preparing **99** were investigated. These are outlined below:

(1) Arylation of arene thiolate ions

Aryl iodides are known to react with the thiophenoxide ion in liquid ammonia under irradiation to form diaryl sulfides in high yield.[250] However, under the reaction conditions, several 1,2-dihalobenzenes afforded disubstitution products,[251] and hence this method was ruled out.

(2) Reaction of arenediazonium tetrafluoroborates with excess arenethiolate

This procedure has been used to give high yields of diaryl sulfides.[252] Disappointingly though, the reaction between diazonium salt **103**,[253] and 2-bromothiophenol gave an extremely poor yield (8%) of **99** and was not pursued further (Scheme 2.16).



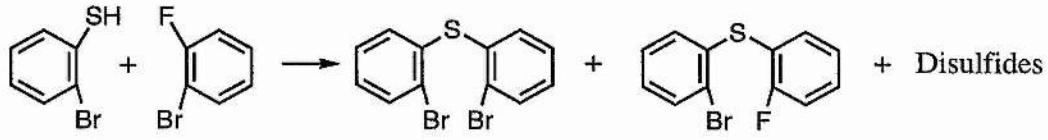
103 **99**

Reagents and Conditions: **(i)** $NaNO_2$, $HCl_{(aq.)}$, $NaBF_4$, 0-5 °C; **(ii)** 2-bromothiophenol, $NaHCO_3$, DMF, 90 °C, 30 min, 8% (over two steps).

Scheme 2.16: *Preparation of sulfide **99** from diazonium salt **103***

(3) Nucleophilic substitution of unactivated haloarenes with thiolate ions

In the nucleophilic aromatic substitution of chloride by thiolate shown in the first step of Scheme 2.14, the displacement is activated by the presence of the nitro group in the *ortho* position. It is also known that unactivated aryl halides can be displaced by the thiolate ion in polar solvents such as DMF and DMSO at high temperatures.[254] As the rate determining step in nucleophilic aromatic substitutions is almost always the attack of the nucleophile, strongly electron-withdrawing substituents such as fluorine or NO_2 are the "best" leaving groups. With these considerations in mind, 2-bromothiophenol and 1-bromo-2-fluorobenzene were reacted using conditions modified from those used to prepare **97** (Scheme 2.17, overleaf).



99 **104**

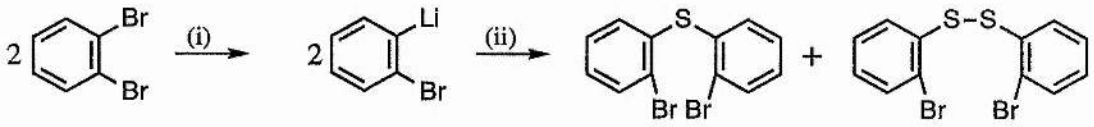
Reagents and Conditions: $NaHCO_3$, DMF, 153 °C, 10 h.

Scheme 2.17: *The reaction of 2-bromothiophenol and 1-bromo-2-fluorobenzene*

Analysis of the products from this reaction by mass spectrometry showed that **99** had been formed. Despite this, it proved impossible to separate it from by-products of similar polarity such as 2-bromo-2'-fluorodiphenyl sulfide (**104**) and other material thought to be disulfides. Attempting to use this mixture to prepare borinic acid **84** was unsuccessful and this method of preparation was not pursued further.

(4) Reaction of 2-bromophenyllithium with sulfur dichloride

A synthesis of 2-bromophenyllithium (**105**) in > 95% yield by the lithiation of 1,2-dibromobenzene with *n*-BuLi has been reported by the group of Tamborski (Scheme 2.18).[255,256] In order to prevent lithium halide elimination and thus formation of benzyne, this reaction must be performed in a mixture of THF and ether and cooled to below -110 °C. At this temperature, 2-bromophenyllithium is stable for at least 2 h, but it is extremely temperature sensitive; if warmed to -90 °C, almost all of it decomposes to give at least 17 products.[255] However, at -110 °C, it has been reacted with a number of electrophiles including several chlorosilanes to give silylated products in good yields. We hoped that it would be possible to react 2 equivalents of **105** with sulfur dichloride to afford **99** in one step (Scheme 2.18). Although, only one equivalent of **105** had been found to react with dichlorodimethylsilane (Me_2SiCl_2),[255] this had been attributed to the steric bulk of the methyl groups and it was hoped that the reaction between **105** and sulfur dichloride would occur with a stoichiometry of 2:1.



105 **99** **106**

Reagents and Conditions: (**i**) *n*-BuLi, THF-ether, -110 °C, 1 h; (**ii**) SCl_2, -110→25 °C, 16 h, 37% (of **99**) and 15% (of **106**).

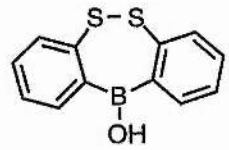
Scheme 2.18: *Expedient synthesis of sulfide **99***

This experiment proved very successful. Whilst the optimised yield of sulfide **99** using these conditions was a modest 37%, this was found to be the best procedure and was used repeatedly to synthesise **99**. Although the temperature of the reaction was maintained between -120 °C and -110 °C (with the use of a slurry of liquid nitrogen and pentane), there are two possible reasons for the relatively poor yield. Firstly, even with the slow addition of sulfur dichloride, it was difficult to prevent the temperature from rising several degrees (although it was possible to prevent it from rising above -110 °C). Secondly, the electrophile is known to disproportionate to sulfur monochloride and chlorine.

$$2SCl_2 \longrightarrow S_2Cl_2 + Cl_2$$

Even although the sulfur dichloride was distilled *immediately* before its use (after the *n*-BuLi had been added to the solution of dibromobenzene), its disproportionation caused partly by the need to add it very slowly to **105** led to the isolation of the disulfide **106** in around 15% yield. It proved impossible to obtain a pure sample of this disulfide; its identification was confirmed by the use of mass spectrometry. Considering the rather unusual reaction conditions, the incorporation of around 70% of the sulfur dichloride into **99** and **106** was pleasing. Despite its unintended synthesis, two attempts were made to convert **106** to borinic acid **107** using the same conditions that had been used to prepare borinic acid **84** from sulfide **99**. These did not work because of the formation of many products during the attempted dilithiation. The failure of the lithiation step was almost

certainly caused by the electrophilicity of the disulfide bond; it was probably attacked by either the *n*-BuLi itself or by monolithiated **106**.



107

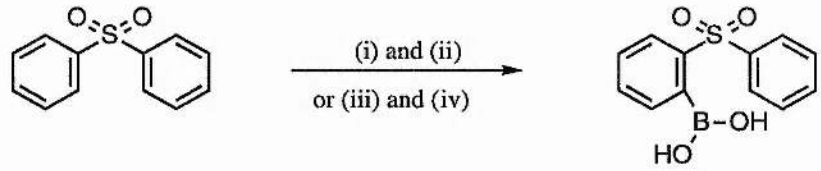
2.6.4 Attempted Synthesis of Borinic Acid 82

As borinic acid **83** was being prepared, attempts were being made to synthesise the cyclic borinic acid that contained a sulfone group (**82**). We envisaged that this would be a relatively straightforward process because, as the phenylsulfonyl group is an extremely good *ortho* director, the intermediate 2,2'-dilithiodiphenyl sulfone can be easily prepared. Curiously, this dilithiated species is reported to be made by the treatment of diphenyl sulfone with three or four equivalents of *n*-BuLi.[257] The necessity for one or two extra equivalents of *n*-BuLi was attributed to the formation of a stable complex between the sulfone group and the butyl lithium molecule.[257]

The lithiation of diphenyl sulfone was achieved by treating a solution of it, cooled to -30 °C in ether, with *n*-BuLi, to give a characteristically bright red solution,[258] which decolourised upon quenching with D_2O. The use of a moderately low temperature is essential as the dilithiated species was found to decompose at temperatures above -20 °C. The effect of different numbers of equivalents of *n*-BuLi on the degree of lithiation was investigated by comparing of the ^1H NMR spectra of the D_2O-quenched material: the *ortho* protons of diphenyl sulfone stand distinct (*ca.* 0.2 ppm downfield) from the *meta* and *para* protons. This ratio (which is 4:6 in diphenyl sulfone) is lower in deuterated material. Treatment with one equivalent of *n*-BuLi led to the incorporation of one deuterium atom. However, in contrast to the reported procedure, we found that four equivalents of *n*-BuLi were not required to lithiate diphenyl sulfone twice; only two equivalents were needed.

Indeed, the use of three and four equivalents led to the incorporation of three deuterium atoms into diphenyl sulfone.

After establishing exactly how to synthesise 2,2'-dilithiodiphenyl sulfone, attention was turned to reacting it with boron-containing electrophiles. Because of problems experienced with an impure batch of starting material, it was decided to synthesise the diphenyl sulfone-derived boronic acid (**108**) by reaction of monolithiated diphenyl sulfone with a trialkyl borate before attempting the synthesis of the more challenging borinic acid (**82**). After a number of experiments, in which the temperature of the reaction was varied, and two electrophiles (tri*iso*propyl borate and trimethyl borate) were used, the highest yield obtained was 32% of **108** (Scheme 2.19). If the reaction was allowed to warm above -30 °C before quenching, no products were isolated. Changing the solvent to THF was not considered as 2,2'-dilithiodiphenyl sulfone, in which the main interest lay, had been found to be insoluble in it. Despite its insolubility, however, it was discovered much later that reactions involving dilithiated diphenyl sulfone worked better in THF. Through the use of this solvent and by quenching with saturated aqueous ammonium chloride, the yield of **108** was increased, to a still moderate (58%).



108

Reagents and Conditions: **(i)** *n*-BuLi, ether, -30 °C, 10 min, then →-70 °C; **(ii)** $B(OPr^i)_3$, -70→-30 °C, 40 min, then H_2O and $HCl_{(aq.)}$, 32%; **(iii)** *n*-BuLi (1 eq.), THF, -30 °C, 30 min, then →-70 °C; **(ii)** $B(OPr^i)_3$, -70→-30 °C, 30 min, then $NH_4Cl_{(aq.)}$, 58%

Scheme 2.19: *Preparations of boronic acid **108***

Despite the unsatisfactory preparation of **108**, we decided to attempt the synthesis of the desired borinic acid **82**. This proved to be another unsuccessful

endeavour. The only product initially isolated was the boronic acid **108**. We thought that the use of a more powerful electrophile might enable the attack of the second lithiated carbon atom on the boron atom (Fig. 2.12). Unfortunately, when a solution of boron trichloride in heptane was added to a solution of 2,2-dilithiodiphenyl sulfone, cooled to -70 °C, total decomposition resulted. The inability to form the second carbon-boron bond was thought perhaps to result from the formation of a chelate ring from the interaction between the lithium and sulfonyl oxygen atoms (Fig. 2.12). In an attempt to enhance the

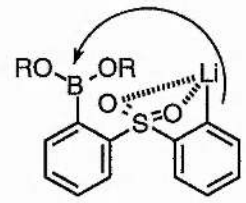
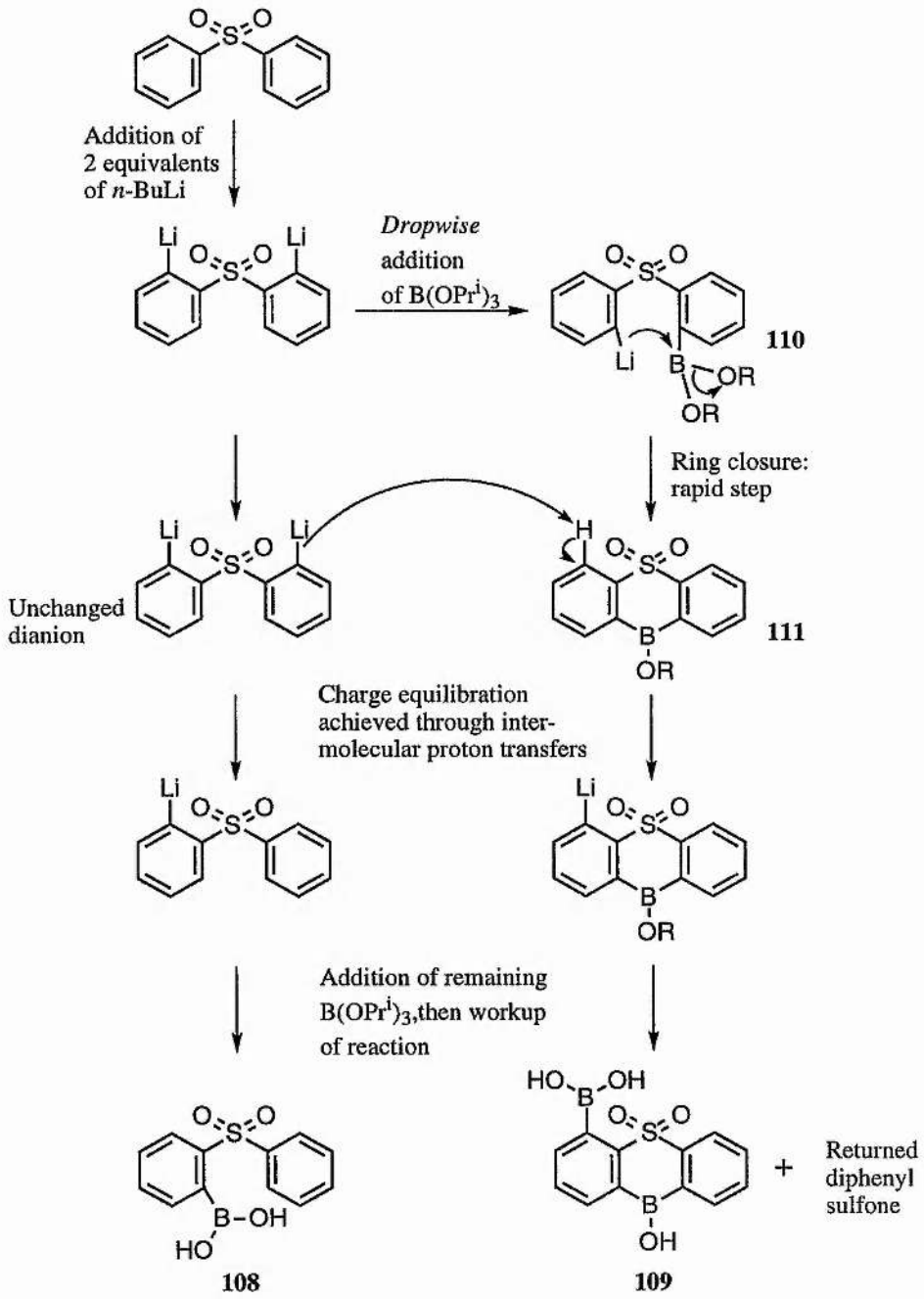


Figure 2.12: *Prevention of synthesis of 82 by the formation of a stable chelate ring*

reactivity of this lithiated intermediate, the reaction was performed in the presence of TMEDA. This experiment was analysed by TLC by extracting small aliquots from the reaction vessel and quenching them with dilute hydrochloric acid. TLC indicated the presence of a previously undetected compound. However, TLC examination of the organic solution resulting from the treatment of the reaction with dilute hydrochloric acid showed no sign of this product. Only when the reaction solution was added to ice-cold dilute hydrochloric acid was the isolation of a small quantity of it made possible. Subsequent analysis showed it not to be the desired borinic acid (**82**). Instead, it was thought to be a small quantity (7%) of the diboronated product **109**. The assignment of this structure was made on the basis of its ^{1}H, ^{13}C and ^{11}B NMR spectra, and by the presence of an *m/z* ratio of 289 Da in its mass spectrum that corresponds to $[M + H]^{+}$.

109

The isolation of this product was perplexing. In addition to **109**, the boronic acid **108** was also formed in 40% yield. Furthermore, greater than 50% of the diphenyl sulfone used in the reaction was recovered intact. This led to the hypothesis that intermolecular proton transfers between intermediates might be operating (Scheme 2.20).



Scheme 2.20: *Possible mechanism for the formation of diboronated product **109***

We thought that, as the borate is added dropwise, it reacts with the 2,2'-dilithiodiphenyl sulfone species to afford the boronate **110**. The rapid ring closure of this intermediate, caused by the intramolecular attack of the remaining carbanion on the boron atom, could generate a charge imbalance between borinate **111** and the remaining 2,2'-dilithiodiphenyl sulfone. Equilibration of charge could be achieved by an intermolecular proton transfer. This would explain the isolation of **108** and **109**, as well as unchanged starting material. The reason for the formation of different quantities of **108** and **109** probably arises because of the lower stability of **109**, indicated by the difficulty in its isolation.

Experiments were undertaken to circumvent this problem, if it was indeed occurring, in two different ways. In one, the dilithiated diphenyl sulfone was added to a chilled solution of tri*iso*propyl borate; in a second, *n*-BuLi was added to a chilled solution of both the borate solution and diphenyl. Nevertheless, in neither case was the desired borinic acid isolated.

Because trialkyl borates appeared to be too unreactive to allow the formation of the desired cyclic structure, and boron trichloride appeared to be too reactive, the use of boron-containing reagents of intermediate electrophilicity was considered (Fig. 2.13).

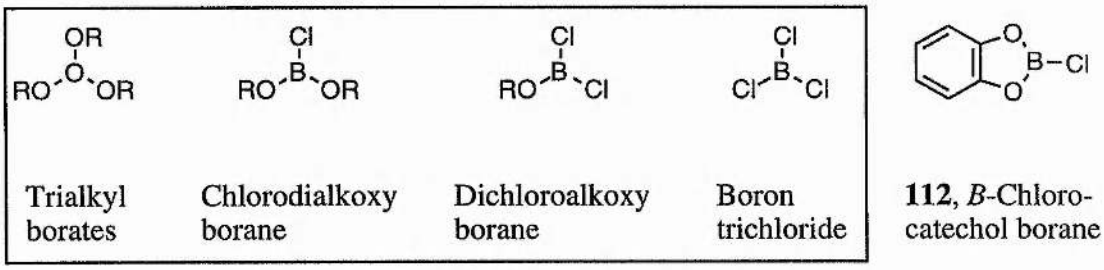
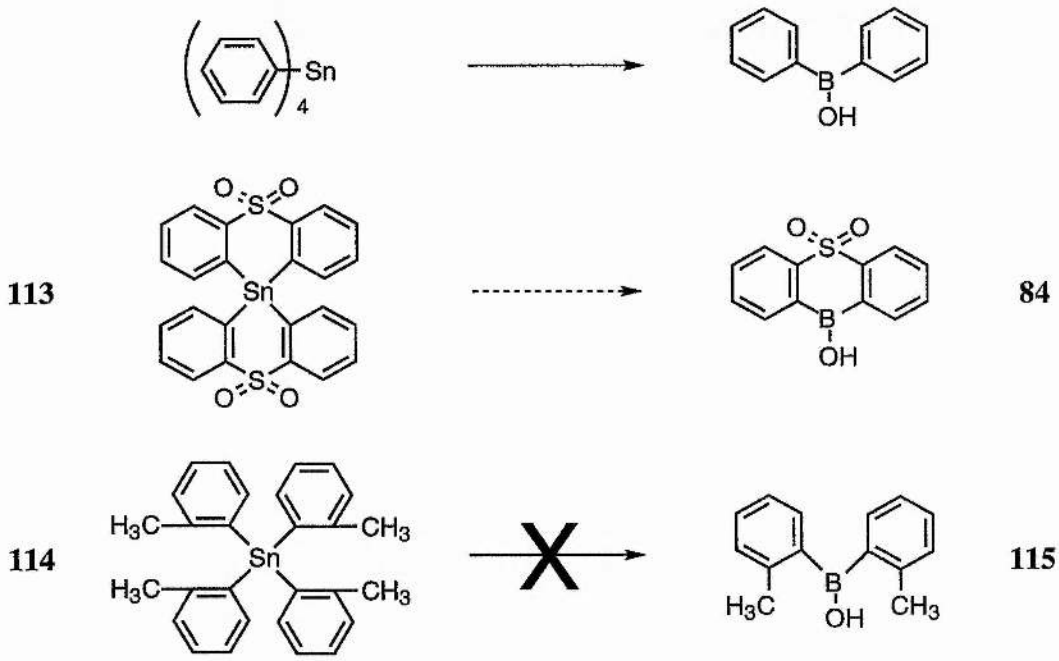


Figure 2.13: *Different boron-containing electrophiles*

The only commercially available dichloroalkoxyborane or chlorodialkoxyborane is *B*-2-chlorocatecholborane (**112**). Addition of this to a solution of 2,2'-dilithiodiphenyl sulfone, cooled to -70 °C, again resulted in total decomposition. Rather than undertake a

study of the syntheses and reactivities of different chlorodialkoxy boranes and dialkoxychloro boranes, we decided to investigate alternative ways of making the target borinic acid.

Diphenylborinic acid has been prepared in 42% yield from tetraphenyl tin by its treatment with diborane and hydrolysis of the resultant borane (Scheme 2.21).[259] We envisaged, therefore, that the target borinic acid **84** could be prepared by similar treatment of the known organotin compound (**113**).[260] However, this was not pursued for two reasons. Firstly, the yield of **113** (from 2,2'-dilithiodiphenyl sulfone and stannic chloride) was only 15%.[260] Secondly, it had not been possible to prepare borinic acid **115** from the related tetraaryl tin compound (**114**), implying that the *ortho* substituent had prevented the transmetallation reaction from occurring.



Reagents and Conditions: B_2H_6, THF, 65 °C, then water.

Scheme 2.21: *Preparation of borinic acids from tetraaryl tin compounds*

The next approach that we pursued was the attempted oxidation of the sulfur atom in the borinic acid synthesised previously (**84**). An advantage of this approach was that it might have enabled the sulfoxide-containing borinic acid **85** to be prepared. It had proved impossible to lithiate diphenyl sulfoxide in the manner attempted with diphenyl sulfone. This is known to result from the attack of the sulfoxide group by the organolithium.[261]

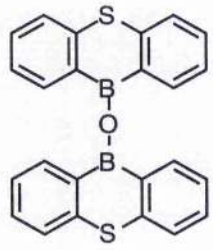
Whilst aware of the susceptibility of boron-containing compounds towards oxidation, the use of sodium periodate in an oxidative cleavage reaction in the presence of a boronate,[262] prompted us to use this well-known sulfide oxidant,[263] in the attempted oxidation of the sulfide functionality in borinic acid **84**. No oxidation was observed in either aqueous methanol or aqueous acetone. This may have resulted from the very poor insolubility of **84** in these solutions. To try to overcome this insolubility problem, the reaction was performed in the presence of Aliquot 336 under phase-transfer conditions,[264] using dichloromethane and water as solvents. These conditions resulted in the slight decomposition of **84**, but otherwise failed to alter the starting material; consequently other oxidants were tried. The use of sodium hypochlorite, using phase-transfer catalysis also failed to affect **84** in any way.[264] Furthermore, the use of Oxone®,[265] and sulfuryl chloride,[266] did not affect the starting material. Attempted oxidation with *m*CPBA gave an inseparable mixture containing many products, probably as a result, at least in part, of the oxidation of the boron atom. Finally, the oxidant dimethyldioxirane was prepared (**116**, Scheme 2.22),[267,268] and its ability to oxidise the sulfide functionality of borinic acid **84** was investigated.

116

Reagents and conditions: H_2O, $NaHCO_3$, pH ~ 7.4, 5→25 °C, *ca*, 5%

Scheme 2.22: *Preparation of dimethyldioxirane,* ***116***

However, although the dimethyldioxirane was shown to be capable of oxidising diphenyl sulfide, no oxidation of compound **84** was observed when treated with it. In an attempt to understand the inability to oxidise the sulfur atom in borinic acid **84**, it was crystallised and X-ray diffraction data obtained (see Fig. 2.14).



117

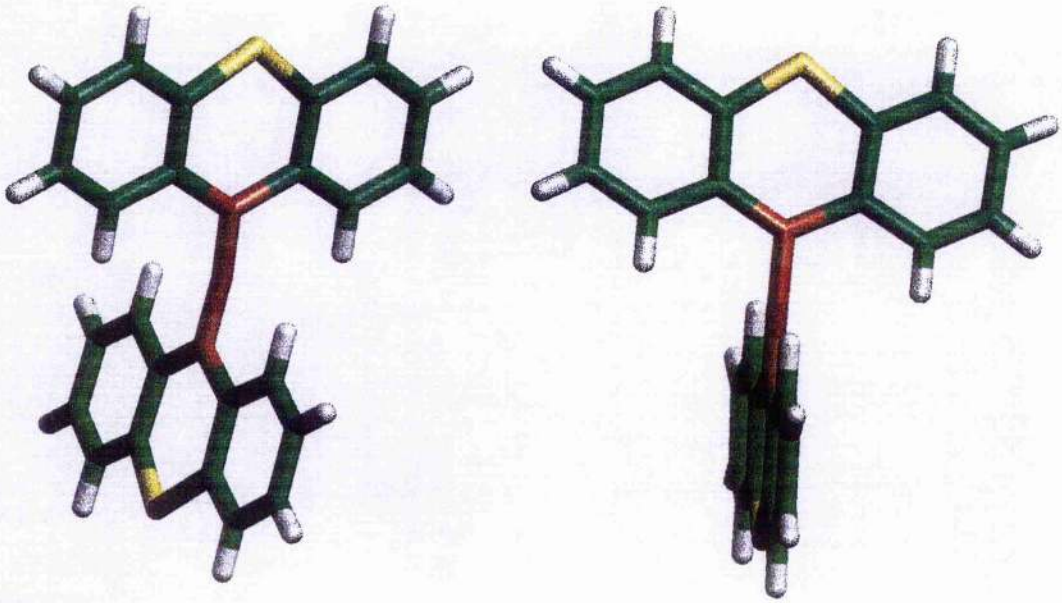


Figure 2.14: *Two representations of 117 generated from crystallographic data*

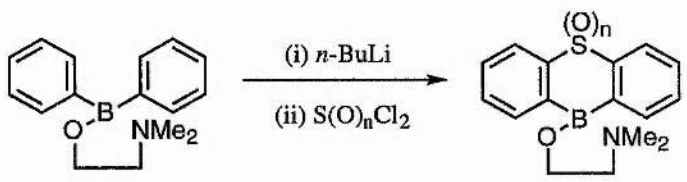
Two aspects of its structure were immediately clear from the data obtained:

- Firstly, the compound thought to have been borinic acid **84** had in fact been isolated as the borinic anhydride (**117**). This was in agreement with both the micro-analytical data and the IR spectrum which did not contain an absorption corresponding to BO-H. The ^{1}H NMR spectrum of **117**, however, does contain a singlet (δ = 9.33 ppm) that corresponds to the acidic proton of the

borinic acid. Presumably borinic acid **84** is formed through the hydrolysis of borinic anhydride **117** by the traces of water present in deuterated DMSO. It proved to be impossible to obtain a ^{1}H NMR spectrum of the anhydride itself.

- Secondly, it had been thought that the failure to oxidise the sulfur atom might have been caused by its forming an intramolecular Lewis acid-Lewis base complex with the boron atom. This, however, would have resulted in the puckering of the central ring; examination of the structure reveals it to be almost totally flat (see Fig. 2.14). It may well be that this ring is aromatic as has been proposed for the central ring of the analogous oxygen-containing borinic acid **83**.[230] The involvement of lone pairs of the sulfur atom in aromatic bonding may explain, in part, the resistance of the sulfur atom to oxidation.

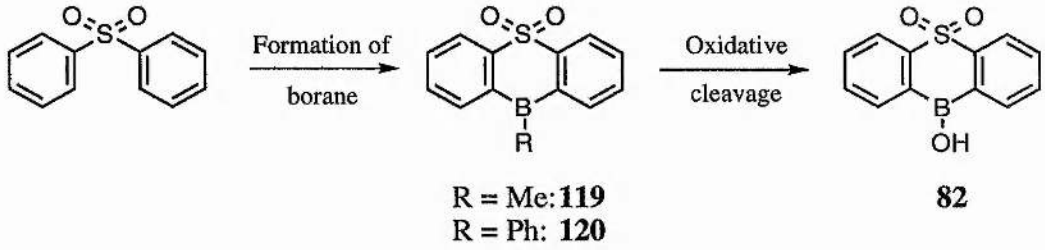
Such was the interest in the synthesis of borinic acid **82**, we decided to investigate other methods of preparation. As one of the problems in the initial approach had been effecting ring closure through the use of boron-containing electrophiles, an alternative cyclisation strategy was envisaged. We thought that it might be possible to 2,2'-dilithiate the *N*,*N*-dimethylethanolamine ester of diphenyl borinic acid (**118**),[269] before treatment with either thionyl chloride or sulfuryl chloride. This would achieve ring closure with the use of sulfur-containing electrophiles (Scheme 2.23). It was thought that the pendant *N*, *N*-dimethyl(ethyl) group might act as an *ortho* director. Unfortunately, this seemed not to be the case as quenching, with D_2O, a solution of **118** in THF that had been treated with two equivalents of *n*-BuLi did not show any sign of deuterium incorporation.



118

Scheme 2.23: *Alternative strategy to synthesise borinic acids 82 and 85 using protected diphenyl borinic acid*

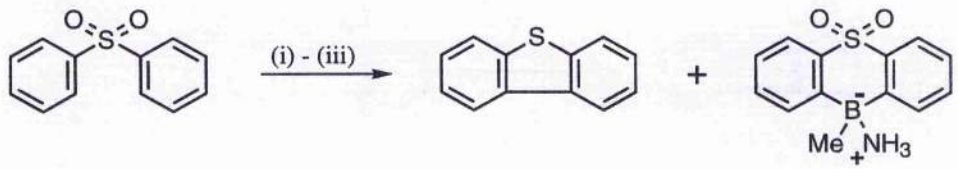
One of the causes of the failure to prepare borinic acid **82** by oxidising the sulfide in **84/117** is thought to have been the susceptibility of the boron atom to oxidation. This had precluded the successful use of oxidants such as *m*CPBA and alkaline hydrogen peroxide. It was thought that if this ability of boron to be oxidised could be exploited, it might be possible to prepare **82**. By the formation of a cyclic borane derived from diphenyl sulfone, followed by the selective oxidative cleavage of the alkyl or aryl group not derived from diphenyl sulfone, we hoped to realise this goal (Scheme 2.24).



Scheme 2.24: *Potential synthesis of target 82 by an oxidative cleavage reaction on a diphenyl sulfone-derived borane*

In principle, "R" in Scheme 2.24 could be any group. However, it was decided to try to make borane **119**, in which R = Me, first. The use of a methyl-containing electrophile was expected to minimise any steric hindrance during the cyclisation reaction. To try to prepare **119**, 2,2'-dilithiodiphenyl sulfone was treated with the commercially available reagent di*iso*propoxymethylborane, followed by the addition of saturated aqueous ammonium chloride (Scheme 2.25). This reaction was performed in THF, from which the dianion precipitated. Rather than being disadvantageous, the poor solubility of the dianion is thought to have been to the benefit of its reaction with the di*iso*propoxymethylborane. Because the majority of the dianion is not in solution, it is not free to react with itself (the reason thought to lie behind its reported instability at temperatures greater than -30 °C).[258] As the temperature of the solution in THF was increased, however, more of the dianion gradually dissolved. Thus, because the dissolution of the dianion is a slow process, it is always more probable that it will react with the di*iso*propoxymethylborane than with itself.

Following the workup of the reaction described above, two products were isolated. However, neither of these was the expected compound (**119**).



121 **122**

Reagents and Conditions: (**i**) *n*-BuLi, THF, -30 °C, 30 min; (**ii**) $(Pr^iO)_2BMe$, -30 °C, 1 h, then →25 °C, 16 h; (**iii**) $NH_4Cl_{(aq.)}$, 7% (of **121**) and 52% (of **122**).

Scheme 2.25: *The isolation of **122**, the complex of borane **119** and ammonia*

The isolation of a small quantity (7%) of dibenzothiophene (**121**) is not understood at all, in contrast to that of the ammonia adduct of the desired borane (**122**). It is thought to result from the interaction between **119** and ammonia generated in solution during the quenching of lithium *iso*propoxide and *n*-BuLi. The highly novel structure proposed for **122** was confirmed by X-ray crystallography. From the structure elucidated, it was determined that one of the hydrogen atoms of the ammonia molecule is hydrogen bonded to an oxygen atom of the sulfone group (Fig. 2.15).

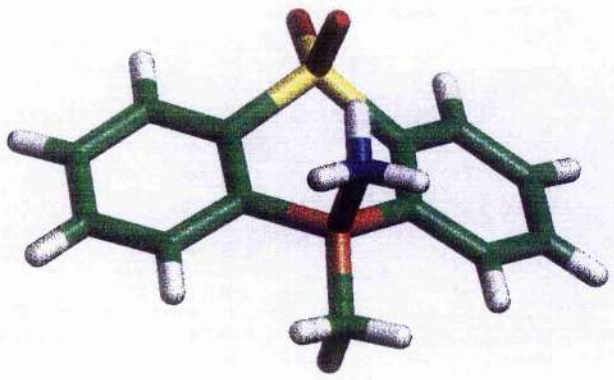
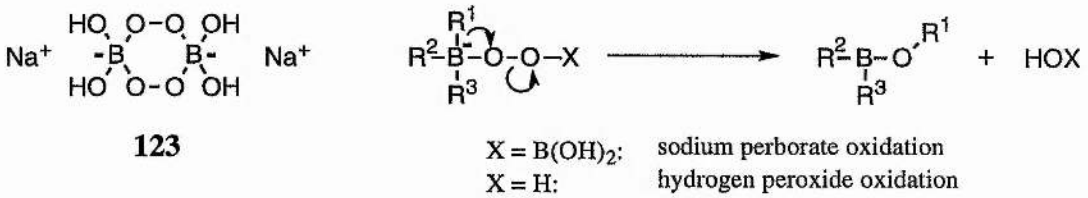


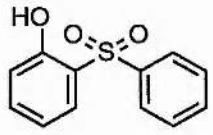
Figure 2.15: *Representation of **122** generated from crystallographic data*

Following the synthesis of **122**, attempts were made to cleave oxidatively its B-Me bond. In the oxidative workup procedure that commonly follows the hydroboration of unsaturated carbon-carbon bonds, alkaline hydrogen peroxide is normally used. However, because the exact concentration of aqueous hydrogen peroxide is not normally known, it was decided to investigate the use of an alternative oxidant, sodium perborate (**123**).[270] The mechanisms for both sodium perborate and peroxide oxidations are thought to be the same (Scheme 2.26). At this point, it was not at all clear how the presence of the ammonia ligand would affect the oxidation of **122**.

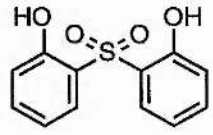


Scheme 2.26: *The mechanism of the oxidative cleavage of boranes by sodium perborate (**123**) and hydrogen peroxide*

Treatment of a solution of **122** in THF with an aqueous solution containing one equivalent of sodium perborate gave none of the desired product (**82**) when stirred at room temperature overnight. It had been intended not to use more than one equivalent of oxidant to prevent over-oxidation of the boron atom. The use of alkaline hydrogen peroxide was attempted but this too had no effect. When the solution was heated to 50 °C for 30 min, an inseparable mixture of products was obtained. We decided, therefore, to investigate the effect of excess alkaline hydrogen peroxide. After heating at 50 °C for 2 h, phenol **124** (13% yield) and diphenol **125** (31% yield) were isolated. Diphenol **125** must have

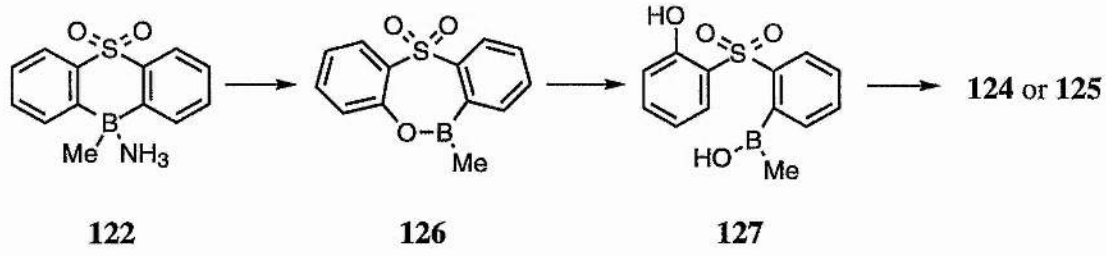


124



125

resulted from the migration of the phenyl groups in preference to the methyl group. It is known that the ability of a group to migrate to an electron-deficient oxygen atom is determined by its ability to stabilise the partial positive charge developed in the transition state during such a migration.[271] Although, the methyl group is known to have poor migratory aptitude, it had been thought that it would migrate in preference to the electron-deficient phenyl sulfonyl groups. This, however, appears not to be the case. The generation of **124** and **125** is thought to result from the series of reactions shown in Scheme 2.27. Migration of one phenyl group would give the cyclic borinate **126** which probably hydrolyses to give the intermediate borinic acid **127**. The major product **125** is formed from another cycle of oxidation-rearrangement and hydrolysis. The minor product **124** is probably formed by the side-reaction of non-oxidative hydrolytic cleavage of the bond connecting the remaining phenyl group to the boron atom. As only a small quantity (20 mg) of **124** was produced from **122**, its identity was confirmed by comparison with an "authentic" sample that was synthesised independently (from the oxidation of boronic acid **108** with sodium perborate).



122 **126** **127**

Scheme 2.27: *Probable intermediates in the syntheses of 124 and 125*

The failure of yet another attempted synthesis of borinic acid **82** was disappointing; nevertheless it was thought that the parent borane (**119**) might behave differently from the ammonia adduct **122** under the oxidising conditions that had generated **124** and **125**. The synthesis of **119** was therefore attempted. When the reaction in which it was synthesised was quenched with aqueous citric acid, rather than ammonium chloride

solution, the desired product was thought to have been isolated on the basis of its ^{1}H NMR spectrum. However the resultant oil, in contrast to crystalline white solid **122**, decomposed shortly after its synthesis.

The surprising isolation of **122** prompted two attempts to make similar complexes for two reasons: both to try to develop novel HIV-1 PR inhibitors and out of curiosity. We envisaged that, if instead of quenching the reaction with aqueous ammonium chloride (see Scheme 2.25), the reaction was treated with hydroxyammonium chloride solution or hydrazine hydrochloride solution, it would be possible to synthesise the compounds **128** and **129** respectively.

X = OH: **128**
X = NH_2: **129**

Although it proved impossible to synthesise the hydrazine derivative (**129**), the hydroxylamine adduct **128** was prepared in 38% yield, and X-ray diffraction data obtained in order to corroborate its structure (Fig. 2.16).

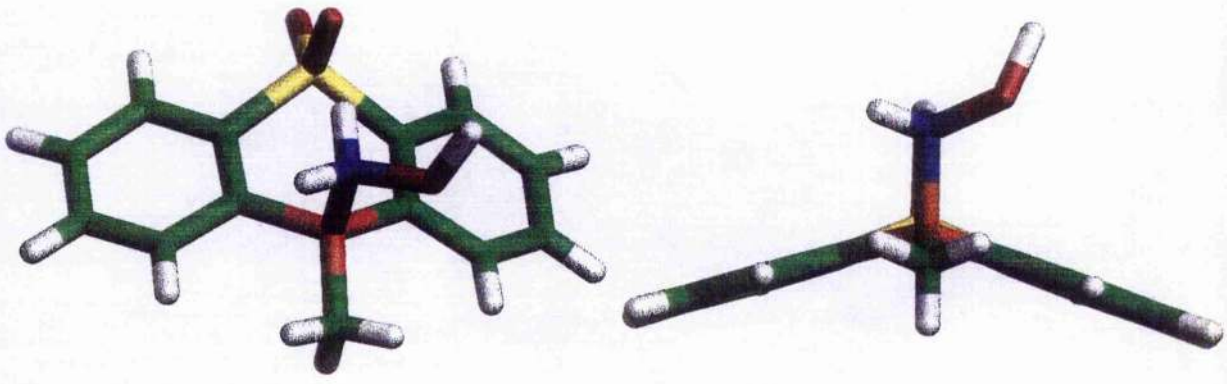


Figure 2.16: *Two representations of 128 generated from crystallographic data*

It was expected that the hydroxyl group might hydrogen bond to the sulfonyl oxygen atoms in **128**. However, it was determined that such a non-bonding interaction is only made to one of the these oxygen atoms (Fig. 2.17). As can be seen from the representation below, it was again found that the amine itself interacts with both the oxygen atoms of the sulfone.

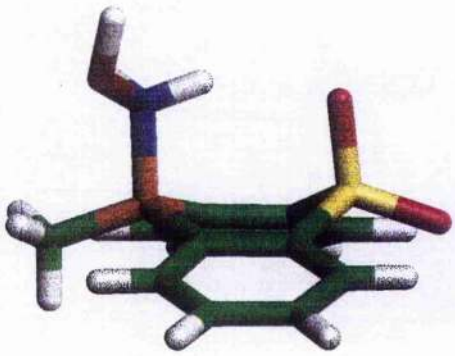
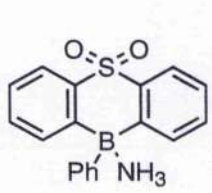


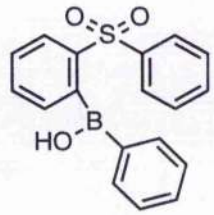
Figure 2.17: *A further representations of 128 generated from crystallographic data*

While it had proved possible to synthesise **128**, it was decided not to submit the small quantities obtained to an attempted oxidative cleavage reaction. Instead we thought that the phenyl borane (**120**), on account of the superior migratory aptitude of the phenyl group over the methyl group, would allow the generation of the desired borinic acid **82** by applying the oxidative cleavage protocol. Additionally, it was of great interest to discover whether phenyl borane **120** would form similar Lewis acid-Lewis base complexes with amines to those formed by methyl borane **119**. The same conditions used to prepare **122** were employed to try to synthesise **120**, except for the use of di*iso*propoxyphenylborane in place of di*iso*propoxymethylborane. In addition to the synthesis of a small quantity of dibenzothiophene (**121**), a white crystalline solid was obtained. The mass spectrum of this product contained an *m/z* ratio of 322 Da thought to correspond to the $[M + H]^+$ ion. Additionally, the mass spectrum indicated the presence of boron. [Mass spectrometry indicates the presence of a boron atom if two adjacent peaks are found in the ratio 4:1. These arise from the presence of both isotopes of boron: ^{11}B (80% abundant) and ^{10}B (20% abundant)]. The IR spectrum did not contain a great deal of information other

information other than an absorption at 3479 cm^{-1}. These data correspond to the ammonia adduct of **120** having been formed (**130**). However, the ^{1}H and ^{13}C NMR spectral data did not corroborate this structure nor did they assist in the elucidation of the correct one; they only contained chemical shifts that corresponded to aromatic proton and carbon atoms, plus an additional broad singlet in the ^{1}H spectrum. In order to establish the correct structure, X-ray diffraction data were obtained for these crystals. The structure deduced was not **130** but the borinic acid **131** (Fig. 2.18), formed from the incomplete reaction between di*iso*propoxy-methylborane and 2,2'-dilithiodiphenyl sulfone.



130



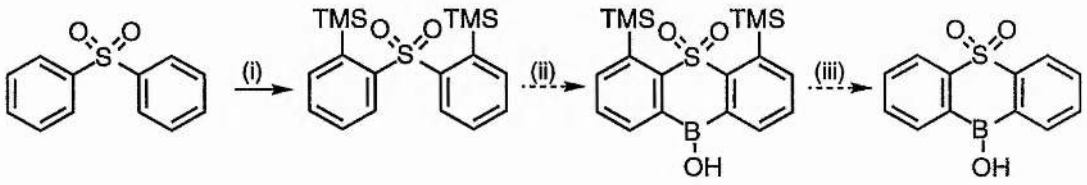
131

Figure 2.18: *A representation of **131** generated from crystallographic data*

This was disappointing as the phenyl borane **120** had been considered to be an excellent candidate for conversion to borinic acid **82**. The reason thought to be behind the failure to synthesise **120** was a steric one. An attempt was made, therefore, to prepare an

analogue of **119** in which the methyl group was replaced by an allyl group. The reagent di*iso*propoxyallylborane is not commercially available but the preparation of analogous dimethoxyallylborane from allylmagnesium bromide and trimethyl borate has been described.[272,273] However, the attempted preparation and reaction of this reagent with 2,2'-dilithiodiphenyl sulfone did not yield any products that contained the allyl group (as determined by ^{1}H NMR spectroscopy) and so the investigation into the oxidation of diphenyl sulfone-derived boranes was not pursued further.

One final attempt to synthesise borinic acid **82** was then undertaken. Of the problems encountered with the lithiation strategy initially attempted, one was thought to be that of intermolecular proton transfer reactions (see Scheme 2.20). It was thought that if such reactions could be prevented, it might prove possible to make **82**. In an attempt to achieve this, we decided to mask two of the four acidic hydrogen atoms *ortho* to the sulfone group in diphenyl sulfone. This was achieved by the reaction of 2,2'-dilithiodiphenyl sulfone with two equivalents of TMS chloride to form sulfone **132** (Scheme 2.28). It was envisaged that by treatment of this compound with another two equivalents of *n*-BuLi, followed by one equivalent of a trialkyl borate, borinic acid **133** could be synthesised. Simple TBAF-deprotection would then afford **82**.

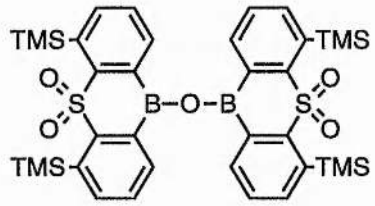


132 **133** **82**

Reagents and Conditions: **(i)** *n*-BuLi, THF, -30 °C, 1 h, then →-50 °C then TMS-Cl, -50→25 °C, 16 h, 81%; **(ii)** *n*-BuLi, THF, -30 °C, 30 min, then →-70 °C then $B(OR)_3$, -70→-30 °C, 30 min; **(iii)** TBAF, THF, 25 °C.

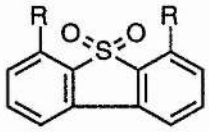
Scheme 2.28: *Alternative proposed synthesis of borinic acid 82*

Although, the required *bis*(trimethylsilyl)diphenyl sulfone **132** was readily obtained, when it was treated with either tri-*n*-butyl borate or boron trifluoride etherate, the predicted product (**133**) was not isolated. In both cases, the same product, whose identity could not be elucidated, was obtained. Both the ^{1}H and ^{13}C NMR spectra provided evidence for a symmetrical product in which C-6 and C-6' no longer bore hydrogen atoms. In the ^{1}H spectrum, there are three multiplets which correspond to six aromatic hydrogen atoms when compared to the singlet that represents two equivalent TMS groups. The ^{13}C spectrum contains, in addition to the TMS singlet, six resonances, three of which appeared to correspond to quaternary aromatic carbon atoms. On the basis of this information we postulated that this product might be the borinic anhydride **134**. However, as the same



134

product was isolated from the reaction of 2,2'-*bis*(trimethylsilyl)-6,6'-dilithiodiphenyl sulfone with di*iso*propoxyphenylborane, and the product did not contain boron (according to ^{11}B NMR spectroscopy), this structure had to be dismissed. Despite its structure not being known, the product was treated with TBAF, and was found to afford exclusively dibenzothiophene-5,5-dioxide **136**. We concluded, therefore, that the unknown product thought at first to be **134** was in fact 4,6-*bis*(trimethylsilyl)-dibenzothiophene-5,5-dioxide (**135**).



135, R = TMS
136, R = H

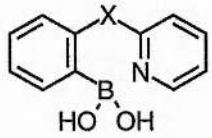
The mechanism by which **135** is formed from **132** when treated with *n*-BuLi is not known. The failure of this approach led us to abandon the attempted synthesis of borinic acid **82**.

As has already been described, compound **82** could not be synthesised directly from diphenyl sulfone by the initial lithiation strategy (see Schemes 2.19 and 2.20). This may be because either the intermediate borinate formed during this reaction, or the borinic acid **82** that results from its hydrolysis, is so electrophilic that it is attacked by water, when the reaction is quenched, thereby causing the formation of boronic acid **108**. Similar electronic effects may have prevented the synthesis of the diphenyl sulfone-derived phenyl borane (**120**); as already discussed, however, this may arise more as a result of the steric bulk of the phenyl group than because of the stability of the product, or the intermediate borinate.

The most unusual compounds to have been synthesised from all the above reactions are the ammonia (**122**) and hydroxylamine (**128**) adducts of the target diphenyl sulfone-derived methylborane (**120**). Why exactly ammonia complexes only to borane **120** is not understood as it is undoubtedly present during the quenching of many of the reactions discussed above.

2.7 Design of Acyclic Borinic and Boronic Acids

A final set of target molecules, whose syntheses again we hoped would be easier than all of those previously described, were designed. The rationale for this came initially from an idea to synthesise a single compound that would resemble, as closely as possible, borinic acid **82**. As has been demonstrated (by the syntheses of compounds **122** and **128**), the co-ordinative bond formed between nitrogen- and boron-containing compounds can be very strong. On the basis of these considerations, compound **137** was designed.

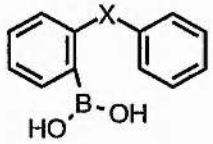


X = SO_2: **137**
X = S: **138**
X = O: **139**

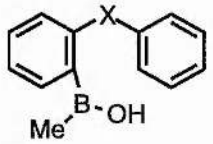
Although we hoped that the sulfone present in boronic acid **137** could be generated from the oxidation of the sulfur atom in the analogous sulfide-containing boronic acid **138**, were this not to prove possible **138** itself would still be a valid target compound. The oxygen-containing analogue (**139**) was also thought to be worthy of examination.

In addition to these pyridine-containing boronic acids, it was decided to try to synthesise the following two final sets of compounds:

- the sulfur- and oxygen-containing analogues (**140** and **141**) of the previously synthesised sulfone-containing boronic acid (**108**).
- three closely related borinic acids (**142** - **144**), differing only from the boronic acids **108**, **140** and **141** in the replacement of one of their hydroxyl groups with a methyl group.



X = SO_2: **108**
X = S: **140**
X = O: **141**



X = SO_2: **142**
X = S: **143**
X = O: **144**

It was intended that any inhibition of HIV-1 PR exhibited by boronic acids **140** and **141** could be compared directly with the cyclic borinic acids **83** and **84** (the compound obtained when borinic anhydride **117** is dissolved in aqueous solution) synthesised earlier. The purpose in making borinic acids **142-144** was to assess whether the borinic acid functionality might be better than the boronic acid group at binding to the catalytic aspartic acids even if the cyclic borinic acids **83** and **84** proved to be inactive. It was hoped that these more flexible acyclic analogues might be active on account of being able to adapt their conformations to complement the active site. The small methyl group was not expected to sabotage the interaction of the borinic acid group contained within these molecules with the active site. This premise was based on the earlier successful

development of inhibitors of the phosphonamidate methyl ester class by Camp and others within the Gani group at St Andrews (see page 29).[119,206]

2.8 Syntheses of Acyclic Borinic and Boronic Acids

2.8.1 Attempted Syntheses of Pyridyl-containing Boronic Acids

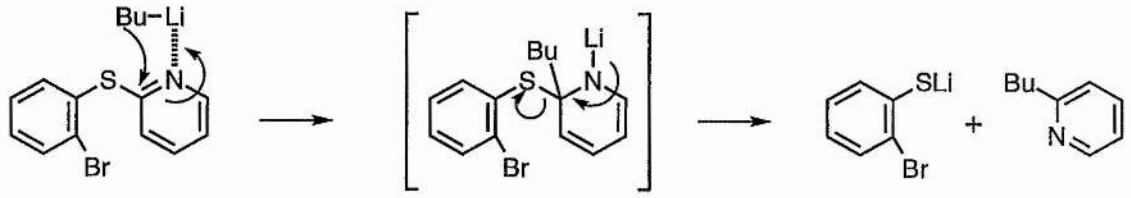
We intended to synthesise sulfide **137**, the precursor to borinic acid **138**, by treating sulfide **145** with *n*-BuLi, followed by reaction with a trialkyl borate [Scheme 2.29 (**ii**)].

145 **138**

Reagents and Conditions: (**i**) $NaHCO_3$, DMF, 130 °C, 2 h, 54%; (**ii**) *n*-BuLi, THF, -70 °C, then $B(OR)_3$, -70→25 °C.

Scheme 2.29: *Attempted synthesis of sulfide **138** by a lithiation strategy*

The reaction between 2-bromothiophenol and 2-bromopyridine under the same conditions used to generate sulfide **97** gave the desired sulfide **145** in 54% yield. However, its attempted lithiation and subsequent reaction with tri-*n*-butyl borate resulted in the total decomposition of the starting material, as determined by TLC. The failure of the lithiation may have resulted partially from a Ziegler-type alkylation of the pyridine ring (Fig. 2.19).[274] Consequently, an alternative method of synthesising **138** was sought.



145

Figure 2.19: *Possible Ziegler-type reaction between* n-*BuLi and sulfide* ***145***

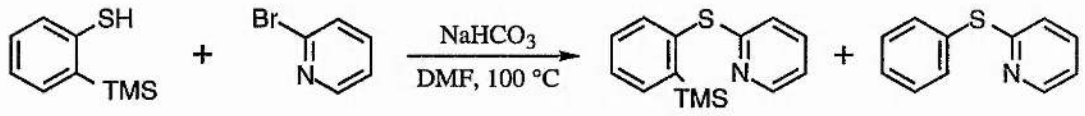
The *ipso* borodesilylation strategy developed independently by Kaufmann,[275] and Snieckus et al.,[276] seemed an appropriate strategy to adopt. This procedure exploits the well-known electronic effect of the TMS group on electrophilic aromatic substitution reactions.

Aryl silicon bonds are thought to be cleaved by electrophilic reagents in a mechanism similar to that which operates in normal electrophilic aromatic substitutions. However, for most electrophiles, substitution of the silyl group is faster than for the replacement of a hydrogen atom (the so-called "*ipso* effect"). This is thought to result from preferential stabilisation by the silicon atom of the β-cation contained within one of the resonance forms of the intermediate addition complex (Fig. 2.20).[277]

Figure 2.20: ipso *Substitution of trimethylsilylbenzene*

We hoped that *ipso* borodesilylation of the sulfide **147** would afford the desired boronic acid **138**. However, an acceptable synthesis of **147** was uncovered only after a number of attempts. At first, it was thought that it could be prepared from the reaction between 2-bromopyridine and 2-(trimethylsilyl)thiophenol (**146**), using the same

conditions previously employed to make sulfides **97** and **145**. However, the main product isolated under these conditions was the sulfide **148** (62%), which did not contain the TMS group. The desired sulfide **147** was formed in a yield of 12% (Scheme 2.30).

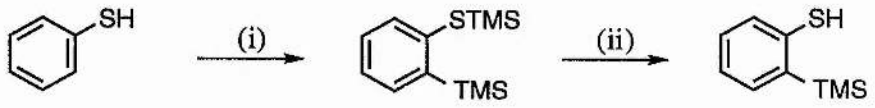


146 **147** (12%) **148** (62%)

Scheme 2.30: *Initial synthesis of sulfide **147***

It was thought that the cause of the cleavage of the carbon-silicon bond was the presence of aqueous base during the reaction. The reaction conditions were, therefore, changed. The sodium salt of thiol **146** was pre-formed by treating **146** with sodium hydride, before the addition of 2-bromopyridine. By heating this mixture under reflux in THF (65 °C), small quantities of the desired sulfide **147** were formed. However, the reaction proceeded extremely slowly in this solvent. When exchanged for the higher-boiling solvent, 1,4-dioxane (bp 99 °C), although the yield was modest even after heating under reflux for 48 h, a sufficient quantity was obtained to permit the borodesilylation reaction to be studied.

One of the reasons for attempting to optimise the yield of **147** was to minimise the number of times that the high-yielding but odorous synthesis of thiol **146** had to be prepared. The procedure,[278] involved the dilithiation of thiophenol, followed by treatment with two equivalents of TMS chloride. The intermediate silylsulfide (**149**) was then heated under reflux in methanol to afford the desired thiol in 94% overall yield (Scheme 2.31).



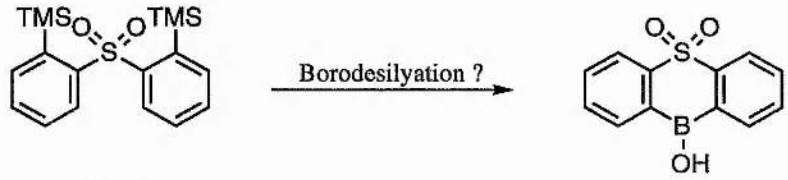
149 **146**

Reagents and Conditions: **(i)** *n*-BuLi, TMEDA, cyclohexane, 25 °C, 24 h, then TMS-Cl, 25 °C, 16 h; **(ii)** MeOH, 65 °C, 9.5 h, 94% (over 2 steps).

Scheme 2.31: *Synthesis of 2-(trimethylsilyl)thiophenol* ***146***

With an adequate quantity of **147** secured, it was discovered that the desired borodesilylation reaction could not be performed. The reaction in which a solution of **147** in dichloromethane was treated with a solution of boron tribromide, also in dichloromethane, was repeated a number of times. Although the starting material was consumed, none of the resultant mixture of products could be identified and the borodesilylation route to boronic acid **138** was not pursued further.

Considering that the reason for the failure of the borodesilylation route to deliver **138** was not understood, it was perhaps rather ambitious to apply the same reaction to another attempted synthesis of borinic acid **82** from sulfide **132** (Fig. 2.21).



132 **82**

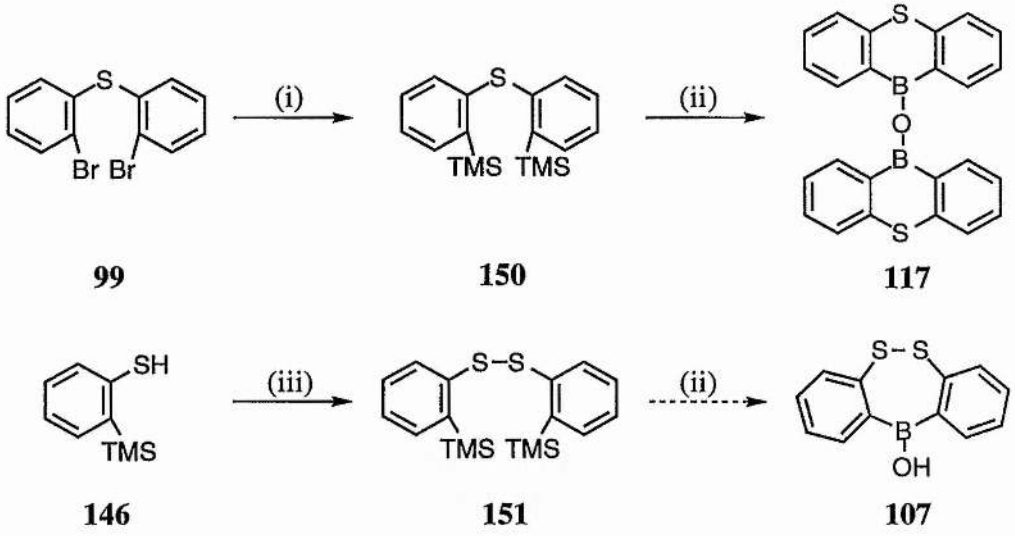
Figure 2.21: *A borodesilylation strategy to borinic acid 82*

Additionally, as the *ipso* borodesilylation reaction is simply a directed electrophilic aromatic substitution, it might be expected to be retarded by the presence of the adjacent electron-withdrawing sulfone group. However, as other *ipso* desilylation reactions have proceeded in the presence of electron-withdrawing groups (*e.g.* CO_2H),[279] the attempted diborodesilylation of **132** appeared to be worthwhile. However, the two main products

isolated following the reaction between **132** and boron tribromide did not contain boron (as determined by mass spectrometry) and were discarded.

Before completing the study of the borodesilylation reaction, curiosity prompted two final attempts at successfully applying it in two other syntheses. We reasoned that it should be possible to synthesise borinic anhydride **117** and borinic acid **107** (perhaps isolable also as its anhydride) through diborodesilylation reactions performed on sulfide **150** and disulfide **151** respectively (Scheme 2.32). The electron-donating properties of the sulfide or disulfide groups were expected to provide an additional driving force for substitution at the positions occupied by the TMS groups.

Sulfide **150** was synthesised from sulfide **99** by sequential treatment with two equivalents each of *n*-BuLi and TMS chloride. Disulfide **151** was synthesised by sodium perborate oxidation,[280] of the previously prepared thiol **146**. However, whilst the addition of aluminium chloride enabled the synthesis of **117** in 48% yield, application of this and other methods only caused the decomposition of the second starting material, **151**.



99 **150** **117**

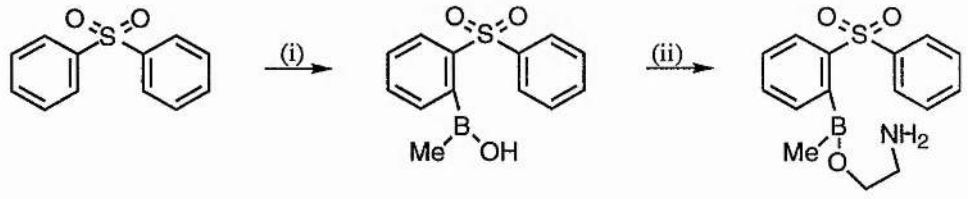
146 **151** **107**

Reagents and Conditions: **(i)** *n*-BuLi, THF, -70 °C, 30 min, then TMS-Cl, -70→25 °C, 1 h, 61%; **(ii)** $AlCl_3$, CH_2Cl_2, -70 °C, then BBr_3, -70→25 °C, 16 h, 47% (of **117**); **(iii)** $NaBO_3.4H_2O$, Aliquot 336, MeOH, 25 °C, 1 h, 73%

Scheme 2.32: *Application of* ipso *diborodesilylation to the synthesis of borinic anhydride **117**, and to the attempted synthesis of borinic acid **107***

2.8.2 Syntheses of Borinic and Borinic acids Derived from Diphenyl Sulfone, Diphenyl Sulfide and Diphenyl Ether

Finally, attention was turned to the syntheses of the two additional sets of compounds described on page 102. Of these, boronic acid **108** had already been synthesised. The related borinic acid (**142**) was prepared from 2-lithiodiphenyl sulfone and di*iso*propoxymethylborane, but was found to be unstable (Scheme 2.33). It was therefore stabilised by the formation of its ethanolamine ester (**152**).[204] Rather frustratingly, this protection could not performed on the crude product, as an intractable viscous yellow oil was formed. Instead the derivatisation was carried out on **142** (following its purification by column chromatography). Although the ethanolamine ester **152** was obtained this way, the yield was disappointingly low (36%).



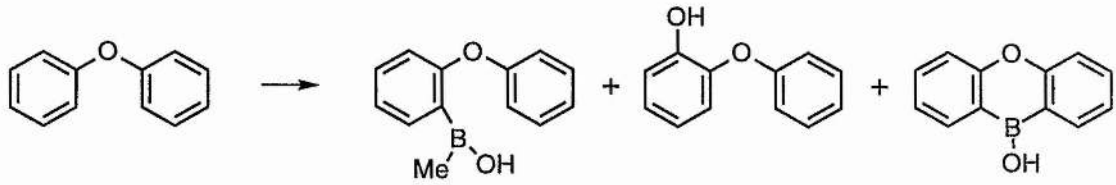
142 **152**

Reagents and Conditions: **(i)** *n*-BuLi, THF, -30 °C, 30 min, then $B(OPr^i)_2Me$, -30 °C, 1h, →25 °C, 1 h; **(ii)** ethanolamine, ether, 0 °C, 36% (over two steps).

Scheme 2.33: *Preparation of borinic acid **142** and its ethanolamine ester **152***

Of the remaining four compounds, it was expected that the two based on the diphenyl ether skeleton (**141** and **144**) could be synthesised directly from diphenyl ether like the cyclic borinic acid **84**. At the time, unfortunately, it proved impossible to obtain a quality solution of *s*-BuLi. Therefore, the lithiation of diphenyl ether was attempted using *t*-BuLi. However, addition of di*iso*propoxymethylborane to the resulting solution,

afforded, after quenching the reaction, a mixture of the products **147**, **153** and **83** (Scheme 2.34).



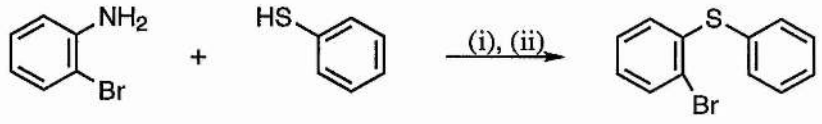
144 (7%) **153** (16%) **83** (33%)

Reagents and Conditions: *t*-BuLi, THF, -70→25 °C, 30 min, then, $B(OPr^i)_2Me$, -50 °C, 2 h, then →25 °C, 1h.

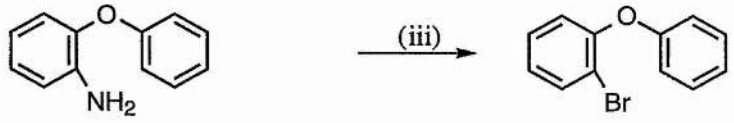
Scheme 2.34: *Products obtained from the first attempted preparation of borinic acid **144***

In addition to these products, 40% of starting material was recovered intact. Although it is not known how the 2-phenoxyphenol (**153**) was produced, it would seem that the only way in which the borinic acid **83** could have been generated is by the dilithiation of diphenyl ether and the reaction of the 2,2'-dilithiodiphenyl ether with tri*iso*propyl borate. This could be a contaminant in the di*iso*propoxymethylborane.

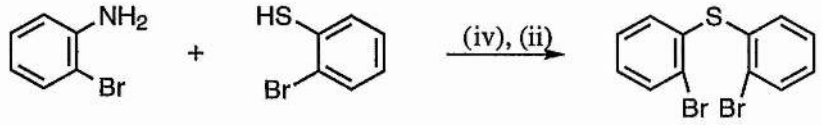
Because of this failure, it was decided to synthesise the 2-lithiodiphenyl sulfide and 2-lithiodiphenyl ether required to make **140** & **143** and **141** & **144**, respectively, by lithiation of the appropriate bromo-compounds **154** and **155**. The syntheses of these are shown in Scheme 2.35 overleaf. The preparation of the known sulfide **154**,[281] was achieved using the method of Hilbert and Johnson.[282] Out of curiosity, another preparation of **99** was attempted by a modification of this procedure. Although this was successful, only a low yield of 32% was achieved. The 2-bromodiphenyl ether **155** was prepared from 2-aminodiphenyl ether according to a known procedure.[283]



154 (48%)



155 (73%)

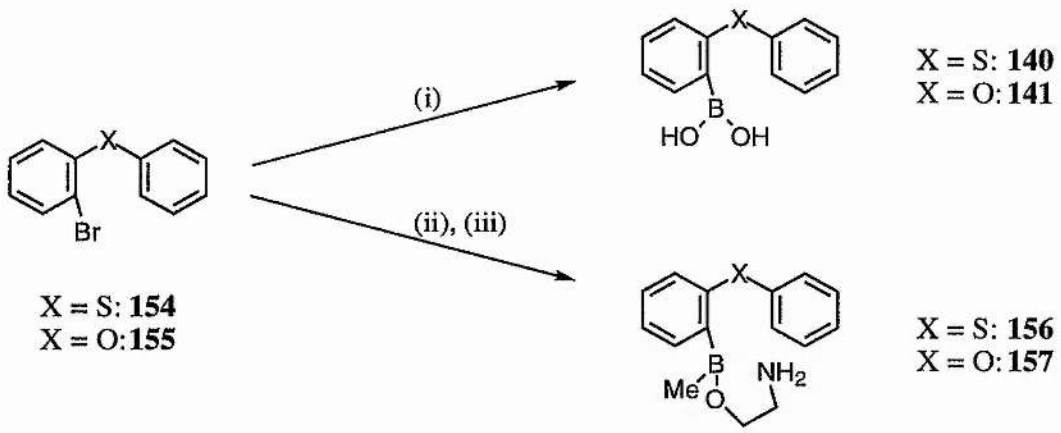


99 (32%)

Reagents and Conditions: (**i**) $H_2SO_{4(aq.)}$, $NaNO_2$, 0-3 °C, then NaOAc; (**ii**) NaOH, Cu, H_2O, 0-5 °C, then →90 °C, 1h.; (**iii**) 48% HBr, $NaNO_2$, 0-5 °C, then CuBr, 48% HBr, 100 °C, 30 min; (**iv**) $HCl_{(aq.)}$, $NaNO_2$, 0-2 °C, then NaOAc.

Scheme 2.35: *Preparation of **154**, **155** and **99** from aromatic amines*

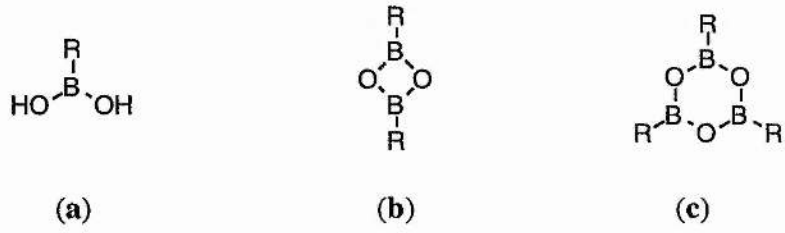
Aryl bromides **154** and **155** were then used to synthesise both the boronic acids **140** and **141** and the borinic acids **143** and **144**. Although like **142**, these borinic acids were found to be unstable, it proved possible to prepare their corresponding ethanolamine esters (**156** and **157**) directly from the crude mixtures containing **143** and **144**. The preparation of **140**, **141**, **156** and **157** are shown in Scheme 2.36.



Reagents and Conditions: (**i**) *n*-BuLi, THF, -70 °C, 30 min, then $B(OPr^i)_3$, -70 °C, 30 min, -70→25 °C, 1 h, 80% (of **140**), 84% (of **141**); (**ii**) *n*-BuLi, THF, -70 °C, 30 min, then $B(OPr^i)_2Me$, -70 °C, 30 min, -70→25 °C, 1 h; (**iii**) ethanolamine, 2 d, 59% (of **156** from **154**), 82% (of **157** from **155**).

Scheme 2.36: *Preparation of boronic acids **140, 141** and protected borinic acids **156** and **157***

Although all three ethanolamine esters (**152**, **156** and **157**) are highly stable crystalline solids, and proved relatively straightforward to characterise, the same cannot be said about the analogous series of boronic acids (**108**, **140** and **141**). Boronic acids are recognised as being notoriously difficult to characterise because of the ease with which they fully or partially dehydrate to oligomeric anhydrides (Fig. 2.22).[284]



(a) **(b)** **(c)**

Figure 2.22: *A boronic acid in the form of the free acid (a) and as a dehydrated dimeric (b) or a dehydrated trimeric (c) anhydride*

Although the boronic acids **140** and **141** both gave, in addition to ^{1}H, ^{13}C and ^{11}B NMR spectral data consistent with the structures of the free acids, *m/z* ratios corresponding to $[M + H]^+$ ions, great difficulty was found in obtaining the elemental analyses for carbon and hydrogen that would corroborate these structures. As **140** and **141** were highly crystalline, X-ray diffraction data were obtained for both to determine if they had been isolated as dehydrated oligomers. However, both were found to be present, at least in the crystals studied, as the free acids (Fig. 2.23).

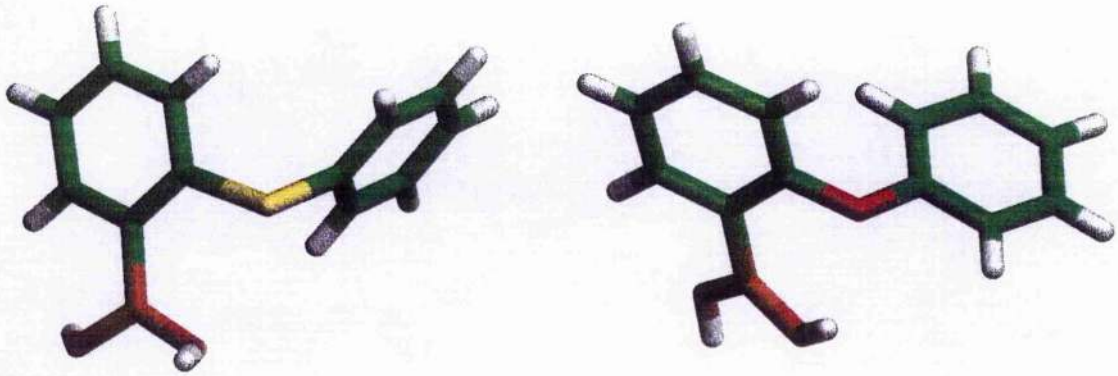


Figure 2.23: *Representations of **140** (left) and **141** (right) generated from X-ray crystallographic data*

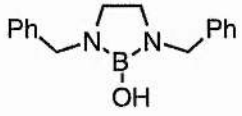
Paradoxically, although boronic acid **108** exhibited NMR spectra consistent with and gave the C and H analyses appropriate for the structure of the free acid, it proved impossible to observe an *m/z* ratio that corresponded to the M^+ or $[M + H]^+$ for the free acid.

2.9 Testing of Boron-Containing Compounds and Conclusion

All of the boron-containing compounds described above (**83**, **108**, **109**, **117**, **118**, **122**, **128**, **131**, **140**, **141**, **152**, **156** and **157**) with the exception of **118** (the *N,N*-dimethylethanolamine ester of diphenylborinic acid), were tested against HIV-1 PR in the *in vitro* assay described in Chapter 3. In addition the commercially available compounds phenylboronic acid and diphenylborinic acid (as its ethanolamine ester) were tested. [Ethanolamine was not found to inhibit the enzyme and the ethanolamine ester of diphenyl borinic acid was found to hydrolyse within five minutes of its addition to the assay buffer. Thus this and the other ethanolamine esters (**152**, **156** and **157**) were all tested without deprotection].

Unexpectedly, none of the above compounds were found to be inhibitors of the enzyme (when tested at concentrations of up to 200 μM; see also Appendix 2). This was obviously a great disappointment. Although it had been feared that the groups designed to interact with the enzyme might be too close to one another, it could not have been anticipated that no activity would be detected, especially as, by the time the synthetic work was complete, a variety of boron-containing compounds had been prepared. Indeed, the boron atoms in compounds **122** and **128** were not expected to play any role in binding.

It may be, therefore, that the failure of all these compounds to inhibit HIV-1 PR is a consequence of the closeness of the phenyl group(s) to the boron atom. However, as it had not been possible to synthesise the more desirable targets (see pages 64 - 68), it had proved necessary to design different targets in order to prepare any borinic acids at all. Other potential targets, such as **158**, in which the inclusion of a group designed to interact with the flap isoleucine residues has been sacrificed for synthetic reasons, have since been considered, but lack of time did not permit their study.



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CHAPTER THREE

EXPERIMENTAL

Experimental

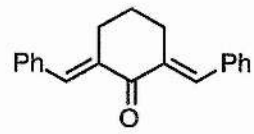
General procedures

^1H and ^{13}C NMR spectra were recorded on Bruker AM 300 (^1H, 300 MHz), Varian Gemini 500 (^1H, 500.3 MHz), Varian Gemini 300 (^1H, 300 MHz; ^{13}C, 75.4 MHz) or Varian Gemini 200 (^1H, 200 MHz; ^{13}C, 50.3 MHz) spectrometers. Chemical shifts are described in ppm downfield from tetramethylsilane and are reported as follows: position, (δ_H or δ_C) [relative integral, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet and br = broad), coupling constant (*J* /Hz, if of practical importance) and assignment (numbered according to the IUPAC nomenclature for the compound)]. ^1H spectra were referenced internally on $CHCl_3$ (δ 7.27 ppm) or $(CH_3)_2SO$ (δ 2.47 ppm). ^{13}C NMR spectra were referenced internally to the central resonances of either C^2HCl_3 (δ 77.0 ppm) or $(CH_3)_2SO$ (δ 39.7 ppm). ^{11}B NMR spectra were recorded on a Bruker AM 300 spectrometer at 96.3 MHz and are described in ppm downfield from boron trifluoride etherate, which was referenced as an external standard in a co-axial cell. Infra-red spectra were recorded on a Perkin-Elmer 1710 FT-IR spectrometer. The samples were prepared as Nujol mulls or as thin films between sodium chloride discs. The frequencies (υ) as absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Microanalyses were determined in the microanalytical laboratory at the University of St Andrews. Mass spectra and accurate mass (HRMS) measurements were recorded in St Andrews on a VG 70-250 SE or a Kratos MS-50, by the E.P.S.R.C. service at Swansea on a VG AZB-E, or at GlaxoWellcome Medicines Research Centre, Stevenage, on a Hewlett-Packard Engine (Thermospray), a VG MicroMass Platform (ES), or a Finnigan 4600 (CI). Major fragments are given as percentages of the base peak intensity (100%). Multiple signals arising through the presence of isotopes of chlorine or bromine are all given; however, the base peak intensity given refers to the major peak. Melting points were determined on either a Reichert hot stage (< 230 °C) or an Electrothermal (>230 °C) apparatus and are uncorrected.

Reagents were used without purification unless otherwise stated. Quantities of reagents were calculated from the manufacturers' stated purities. Experiments were conducted at room temperature (20-25 °C) unless otherwise stated. All reactions that employed organometallic regents or other moisture sensitive reagents were performed in dry solvent under an atmosphere of dry nitrogen or argon in oven-dried and/or flame-dried glassware. Solutions in organic solvents were dried over anhydrous magnesium sulfate and concentrated or evaporated under reduced pressure on a Büchi rotary evaporator unless otherwise stated. Flash column chromatography was performed according to the method of Still *et al.*,[285] with Fluka Kieselgel (220-400 mesh). Analytical TLC was carried out on 0.25 mm precoated silica gel plates (Whatman PE SIL G/UV) and compounds were visualised by UV fluorescence, ethanolic phosphomolybdic acid, iodine vapour or aqueous potassium permanganate.

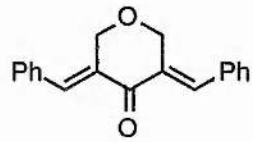
The solvents used were either distilled or of analar quality and petrol refers to the portion of petroleum ether that boils between 40 and 60 °C. Solvents were dried according to literature procedures: ethanol and methanol were dried over magnesium turnings; cyclohexane, dichloromethane, DMF, pyridine and triethylamine were distilled over calcium hydride; THF and diethylether (referred to as ether) and 1,4-dioxane were dried over sodium and benzophenone. Organolithium solutions were titrated according to the method of Kofron and Baclawski.[286] All other chemicals were of analytical grade or were recrystallised or distilled before use.

2,6-*bis*(Phenylmethylene)-cyclohexanone, 58



To a stirred solution of NaOH (15 g, 375 mmol) in aqueous ethanol (50% v/v; 120 cm^3), cooled to 0 °C, was added dropwise over 30 min a solution of cyclohexanone (9.81 g, 100 mmol) and benzaldehyde (21.22 g, 200 mmol) in ethanol (50 cm^3). The solution was stirred for a further 16 h and the resultant yellow solid filtered and washed with water (10 x 100 cm^3). Recrystallisation from acetone gave yellow crystals (18.65 g, 68%), mp 116-118 °C (lit.,[208] 118-119 °C) (Found: C, 87.4; H, 6.7. Calc. for $C_{20}H_{18}O$: C, 87.55; H, 6.6%) (HRMS: found $[M + H]^+$, 275.1436. Calc. for $C_{20}H_{19}O$: 275.1436); υ_{max}(Nujol)/cm^{-1} 1662 (CO) and 1606, 1576 and 1569 (C=C); δ_H(200 MHz; C^2HCl_3) 1.80 (2 H, m, 2 x 4-H), 2.95 (4 H, m, 2 x 3-H and 2 x 5-H), 7.30-7.60 (10 H, m, Ar-H) and 7.84 (2 H, s, 2 x C=CH); δ_C(50.3 MHz; C^2HCl_3) 22.97 (C-4), 28.42 (C-3 and C-5), 128.33, 128.55 and 130.33 (Ar-CH), 135.91 and 136.13 (Ar-C quaternary, C-2 and C-6), 136.88 (C=*C*H) and 190.33 (CO); *m/z* (CI) 275 (100%, $[M + H]^+$).

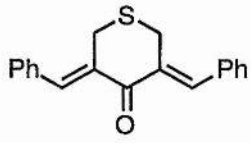
Tetrahydro-3,5-*bis*(phenylmethylene)-4*H*-pyran-4-one, 59



A solution of sodium hydroxide (1 g, 25 mmol) in aqueous ethanol (50% v/v; 20 cm^3) was prepared and cooled to 0 °C. Benzaldehyde (1.06 g, 10 mmol) was dissolved in the minimum volume of ethanol (5 cm^3) and added to a solution of tetrahydro-4*H*-pyran-4-one (500 mg, 5 mmol) in ethanol (5 cm^3). Half of this mixture was added dropwise to the alkaline solution with vigorous stirring and the reaction mixture was left for a further 15 min at 25 °C. The remainder was then added and the resultant mixture was left to stir for a further 1 h. The yellow precipitate was filtered then washed with water (100 cm^3) to remove excess alkali.

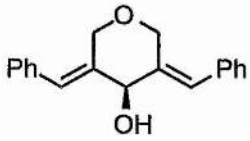
Recrystallisation from ethanol-acetone gave yellow plates (897 mg, 65%), mp 181.5-182.5 °C (lit.,[207] 185 °C) (Found: C, 82.35; H, 5.95. Calc. for $C_{19}H_{16}O_2$: C, 82.6; H, 5.85%) (HRMS: found $[M + H]^+$, 277.1229. Calc. for $C_{19}H_{17}O_2$: 277.1228); υ_{max}(Nujol)/cm^{-1} 1668 (CO) and 1612 and 1582 (C=C); δ_H(200 MHz; C^2HCl_3) 4.95 (4 H, s, 2 x 2-H and 2 x 6-H), 7.30-7.60 (10 H, m, Ar-H) and 7.85 (2 H, s, 2 x C=CH); δ_C(50.3 MHz; C^2HCl_3) 68.58 (C-2 and C-6), 128.65, 129.36 and 130.43 (Ar-CH) 133.02 and 134.67 (Ar-C quaternary, C-3 and C-5), 136.44 (C=*C*H) and 185.50 (CO); *m/z* (CI) 277 (100%, $[M + H]^+$).

Tetrahydro-3,5-*bis*(phenylmethylene)-4*H*-thiopyran-4-one, 60



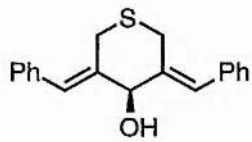
This was prepared in an manner identical to *pyranone* **59** by using sodium hydroxide (1 g, 25 mmol), benzaldehyde (1.06 g, 10 mmol) and tetrahydrothio-4*H*-pyran-4-one (580 mg, 5 mmol). This afforded a yellow solid which was recrystallised from acetone to give yellow crystals (900 mg, 62%), mp 150-151 °C (lit.,[207] 149-151 °C) (HRMS: found M^+, 292.0922. Calc. for $C_{19}H_{16}SO$: 292.0922); υ_{max}(Nujol)/cm^{-1} 1660 (CO) and 1600, 1577 and 1569 (C=C); δ_H(200 MHz; C^2HCl_3) 3.95 (4 H, s, 2 x 2-H and 2 x 6-H), 7.20-7.55 (10 H, m, Ar-H) and 7.80 (2 H, s, 2 x C=CH); δ_C(50.3 MHz; C^2HCl_3) 30.13 (C-2 and C-6), 128.63, 129.00 and 130.08 (Ar-CH), 133.90 (C-3 and C-5), 135.14 (Ar-C quaternary), 136.92 (C=*C*H) and 189.00 (CO); *m/z* (CI) 293 (100%, $[M + H]^+$).

Tetrahydro-3,5-*bis*(phenylmethylene)-4*H*-pyran-4-ol, 62



To a suspension of *pyranone* **59** (168 mg, 610 μmol) in ethanol (3 cm^3) was added sodium borohydride (25 mg, 660 μmol) in three portions over 15 min. The reaction was followed to completion (*ca.* 1 h) both by colour change (yellow to colourless solution) and by TLC. Water (5 cm^3) was added to the solution and the resultant white precipitate filtered off and washed with more water (10 cm^3). Recrystallisation from acetone-water afforded colourless needles (156 mg, 92%) which were prone to decolourisation at room temperature and were stored at 0 °C in the dark. Mp 115 °C (decomp.) (Found: C, 82.1; H, 6.8; M^+, 278.1307. $C_{19}H_{18}O_2$ requires C, 82.0; H, 6.5%; M^+, 278.1307); υ_{max}(Nujol)/cm^{-1} 3300 (OH), 1599 and 1575 (C=C) and 1166 (ether); δ_H(200 MHz; C^2HCl_3) 2.48 (1 H, d, *J* 6.2, OH), 4.55 (2 H, d, *J* 13.6, 1 x 2-H and 1 x 6-H), 4.93 (1 H, d, *J* 6.2, 4-H), 6.68 (2 H, s, 2 x C=CH) and 7.15-7.40 (10 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 66.70 (C-2 and C-6), 75.72 (C-4), 125.50 (C=*C*H), 127.19, 128.35 and 128.80 (Ar-CH) and 136.08 and 138.66 (Ar-C quaternary, C-3 and C-5); *m/z* (EI) 278 (3%, M^+), 262 (2, $[M + H - OH]^+$), 248 (4, $[M - CH_2O]^+$) and 91 (100).

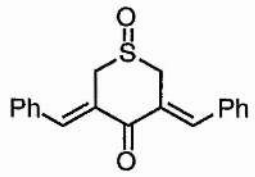
Tetrahydro-3,5-*bis*(phenylmethylene)-4*H*-thiopyran-4-ol, 63



This was prepared in an manner identical to *pyranol* **62** by using *thiopyranone* **60** (140 mg, 480 μmol) and sodium borohydride (20 mg, 480 μmol). This afforded a white solid which was recrystallised from acetone-water to give colourless needles (136 mg, 94%) which were prone to decolourisation at room temperature and were stored at 0 °C in the dark, mp 117-119 °C (Found: C, 77.55; H, 6.3; M^+, 308.0875. $C_{19}H_{16}O_2S$ requires

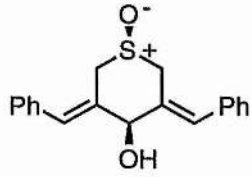
C, 77.5; H, 6.15%; M^+, 308.0879); υ_{max}(Nujol)/cm^{-1} 3367 (OH) and 1598 and 1576 (C=C); δ_H(200 MHz; C^2HCl_3) 2.0 (1 H, br s, OH), 3.54 (2 H, d, *J* 13.6, 1 x 2-H and 1 x 6-H), 3.86 (2 H, d, *J* 13.6, 1 x 2-H and 1 x 6-H), 4.79 (1 H, s, 4-H), 6.63 (2 H, s, C=CH) and 7.25-7.40 (10 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 26.23 (C-2 and C-6), 80.58 (C-4), 124.87 (C=*C*H), 127.11, 128.36 and 128.94 (Ar-CH) and 136.23 and 140.23 (Ar-C quaternary, C-3 and C-5); *m/z* (EI) 294 (8%, M^+), 278 (15, $[M + H - OH]^+$), 187 (85) and 91 (100).

Tetrahydro-3,5-*bis*(phenylmethylene)-4*H*-thiopyran-4-one-1-oxide, 64

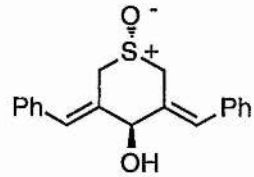


To a stirred solution of *thiopyranone* **60** (309 mg, 1 mmol) in dichloromethane (20 cm^3) was added dropwise *m*CPBA (225 mg, 1 mmol) in dichloromethane (8 cm^3). The resultant solution was stirred for 2 h, diluted with dichloromethane (50 cm^3) and extracted with aqueous sodium hydrogen-carbonate (5% w/v; 3 x 25 cm^3). The organic phase was dried and evaporated to leave a solid which was recrystallised from methanol to give bright yellow crystals (240 mg, 77%), mp 155-159 °C (lit.,[210] 155-160 °C) (Found: C, 74.1; H, 5.4; M^+, 308.0875. Calc. for $C_{19}H_{16}O_2S$: C, 74.0; H, 5.25%; M^+, 308.0879); υ_{max}(Nujol)/cm^{-1} 1662 (CO), 1597 and 1570 (C=C) and 1049 (SO); δ_H(200 MHz; C^2HCl_3) 4.22 (4 H, s, 2 x 2-H and 2 x 6-H), 7.40 (10 H, s, Ar-H) and 8.05 (2 H, 2 x C=CH); δ_C(50.3 MHz; C^2HCl_3) 50.30 (C-2 and C-6), 126.27 (C-3 and C-5), 127.86, 129.67 and 129.75 (Ar-CH), 134.11 (Ar-C quaternary), 144.40 (C=*C*H) and 187.61 (CO); *m/z* (EI) 308 (18%, M^+), 292 (33, $[M - O]^+$), 260 (42, $[M - SO]^+$) and 115 (100).

***cis*- and *trans*-Tetrahydro-3,5-*bis*(phenylmethylene)-4*H*-thiopyran-4-ol-1-oxides, 65 and 66**



65



66

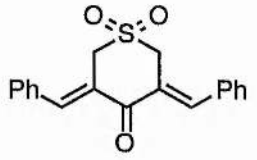
To a rapidly stirred solution of *thiopyran-4-one-1-oxide* **64** (280 mg, 900 μmol) in ethanol (5 cm^3) was added in four portions over 10 min sodium borohydride (37 mg, 1 mmol). The suspension was stirred for a further 20 min. Water (20 cm^3) was added and the white precipitate was filtered then washed with more water (100 cm^3). Purification of the crude diastereomeric mixture by flash column chromatography (eluant dichloromethane-ethanol 50:1) afforded *alcohols* **65** (92 mg, 32.9%) and **66** (161 mg, 57.6%), both as white solids.

Less polar **65**, mp 173-175 °C (Found: C, 73.35; H, 5.8; M^+, 310.1024. $C_{19}H_{18}O_2S$ requires C, 73.5; H, 5.85%; M^+, 310.1028); υ_{max}(Nujol)/cm^{-1} 3358 (OH), 1074 (SO) and 1001 (CO); δ_H(300 MHz; C^2HCl_3) 2.02 (1 H, d, *J* 2.0, OH), 3.72 (2 H, d, *J* 13.5, 1 x 2-H and 1 x 6-H), 4.11 (2 H, d, *J* 13.5, 1 x 2-H and 1 x 6-H), 4.95 (1 H, br s, 4-H), 7.12 (2 H, s, 2 x C=CH), 7.26-7.39 (10 H, m, Ar-H); δ_C[50.3 MHz; $(C^2H_3)_2SO$] 46.04 (C-2 and C-6), 78.77 (C-4), 127.72, 128.61, 128.82 and 130.80 (Ar-CH and C=*C*H) and 133.88 and 135.77 (Ar-C quaternary, C-3 and C-5); *m/z* (CI) 311 (48%, $[M + H]^+$), 293 (100, $[M + H - H_2O]^+$) and 243 (83).

More polar **66**, mp 168-169 °C (Found: C, 73.2; H, 5.7; M^+, 310.1026. $C_{19}H_{18}O_2S$ requires C, 73.5; H, 5.85%; M^+, 310.1028); υ_{max}(Nujol)/cm^{-1} 3367 (OH), 1076 (SO) and 1035 (CO); δ_H(300 MHz; C^2HCl_3) 2.19 (1 H, d, *J* 2.2, OH), 4.01 (2 H, dd, *J* 11.6 and 1.2, 1 x 2-H and 1 x 6-H), 4.30 (2 H, dd, *J* 11.6 and 1.3, 1 x 2-H and 1 x 6-H), 4.82 (1 H, m, 4-H), 6.92 (2 H, s, 2 x C=CH) and 7.30-7.43 (10 H, m, Ar-H); δ_C[50.3 MHz; $(C^2H_3)_2SO$] 49.52 (C-2 and C-6), 74.70 (C-4), 127.48, 127.90, 128.66, 128.81 (Ar-CH and C=*C*H) and

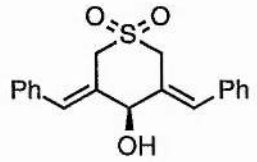
134.34 and 136.11 (Ar-C quaternary, C-3 and C-5); *m/z* (EI) 310 (68%, M^+) and 293 (100, $[M - OH]^+$).

Tetrahydro-3,5-*bis*(phenylmethylene)-4*H*-thiopyran-4-one-1,1-dioxide, 67



To a stirred solution of *thiopyranone* **60** (900 mg, 3 mmol) in glacial acetic acid (9 cm^3), cooled to 0 °C, was added dropwise aqueous hydrogen peroxide (30% v/v; 8 cm^3). The solution became turbid and was stirred at room temperature for a further 72 h. Water (20 cm^3) was added and the mixture was extracted with dichloromethane (3 x 50 cm^3). The combined organic extracts were then dried and evaporated to leave a crude solid. Purification by column chromatography (eluant petrol-ethyl acetate 1:1) followed by recrystallisation from glacial acetic acid gave bright yellow needles (590 mg, 59%), mp 199-200 °C (lit.,[210] 198-200 °C) (Found: C, 70.55; H, 4.95; M^+, 324.0818. Calc. for $C_{19}H_{16}O_3S$: C, 70.35; H, 4.95%; M^+, 324.0820); υ_{max}(Nujol)/cm^{-1} 1664 (CO), 1598 and 1566 (C=C) and 1333, 1316 and 1129 (SO_2); δ_H(200 MHz; C^2HCl_3) 4.47 (4 H, s, 2 x 2-H and 2 x 6-H), 7.35-7.52 (10 H, m, Ar-CH) and 8.02 (2 H, s, 2 x C=CH); δ_C(50.3 MHz; C^2HCl_3) 53.11 (C-2 and C-6), 126.59 (C-3 and C-5), 129.02, 129.61 and 130.10 (Ar-CH), 133.47 (Ar-C quaternary), 144.31 (C=*C*H) and 186.27 (CO); *m/z* (EI) 324 (16%, M^+), 259 (68, $[M - SO_2 - H]^+$) and 115 (100).

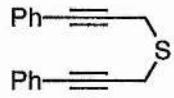
Tetrahydro-3,5-*bis*(phenylmethylene)-4*H*-thiopyran-4-ol-1,1-dioxide, 68



This was prepared in an manner identical to **65** and **66** above by using *thiopyran-4-one-1,1-dioxide* **67** (300 mg, 930 μmol) and sodium borohydride (32 mg, 1 mmol) to afford a pure white solid (270 mg, 89%), mp 160-162 °C (Found: C, 70.5; H, 5.7; M^+, 326.0970.

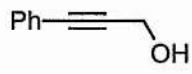
$C_{19}H_{18}O_3S$ requires C, 69.9; H, 5.55%; M^+, 326.0977); υ_{max}(Nujol)/cm^{-1} 3492, 3457 and 3369 (OH) and 1320 and 1145 (SO_2); δ_H(300 MHz; C^2HCl_3) 2.15 (1 H, d, *J* 2.0, OH), 4.22 (4 H, m, 2 x 2-H and 2 x 6-H), 5.00 (1 H, d, *J* 2.0, 4-H), 7.02 (2 H, s, 2 x C=CH) and 7.31-7.40 (10 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 50.87 (C-2 and C-6), 79.04 (C-4), 128.21, 128.68, 128.86 and 129.72 (Ar-CH and C=*C*H) and 133.24 and 134.43 (Ar-C quaternary, C-3 and C-5); *m/z* (EI) 326 (15%, M^+), 262 (59, $[M - SO_2]^+$) and 116 (100).

1,1'-(Thiodi-1-propyne-3,1-diyl)*bis*benzene, 75



To a stirred solution of *bromide* **78** (1.95 g, 10 mmol) in methanol (3 cm^3), under an atmosphere of argon, was added sodium sulfide nonahydrate (1.44 g, 6 mmol) in methanol (10 cm^3). The solution was then stirred for 16 h under argon, diluted with water (50 cm^3) and extracted with ether (3 x 50 cm^3). The combined organic extracts were dried and concentrated to afford an oil that was purified by flash column chromatography (eluant petrol) to give a pale yellow oil (1.30 g, 99%); υ_{max}(thin film)/cm^{-1} 2189 (C≡C); δ_H(200 MHz; C^2HCl_3) 3.74 (4 H, s, 2 x CH_2), 7.30-7.33 (6 H, m, *meta* Ar-H and *para* Ar-H) and 7.44-7.49 (4 H, m, *ortho* Ar-H); δ_C[50.3 MHz; $(C^2H_3)_2SO$] 19.90 (CH_2), 83.07 and 86.13 (*C*≡*C*), 122.61 (Ar-C quaternary) and 128.84, 128.94 and 131.78 (Ar-CH); *m/z* (CI) 263 (100%, $[M + H]^+$), 229 (13, $[M + H - SH_2]^+$) and 115 (100).

3-Phenyl-2-propyn-1-ol, 77



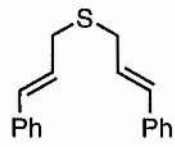
To a stirred solution of phenyl acetylene (5 cm^3; 5.38 g, 52.2 mmol) in THF (150 cm^3), cooled to -40 °C, was added dropwise *n*-BuLi (2.5 mol dm^{-3} in hexanes; 22 cm^3;

55 mmol). The solution was allowed to warm to -10 °C over 1 h before paraformaldehyde (1.8 g, 60 mmol) was added in one portion and the solution allowed to warm further to room temperature over 3 h. Water (50 cm^3) was added, the layers separated and the aqueous layer extracted further with ether (3 x 60 cm^3). The combined organic extracts were dried and evaporated and the resultant yellow oil purified by flash column chromatography (eluant petrol-ethyl acetate 5:1) to afford *alcohol* **77** as a clear pale yellow oil (6.2 g, 90%); υ_{max}(thin film)/cm^{-1} 3338 (OH) and 2237 (C≡C); δ_H(200 MHz; C^2HCl_3) 2.10 (1 H, br s, OH), 4.51 (2 H, br s, 1-H), 7.30-7.34 (3 H, m, *meta* Ar-H and *para* Ar-H) and 7.43-7.48 (2 H, m, *ortho* Ar-H); δ_C(50.3 MHz; C^2HCl_3) 51.54 (C-1), 85.63 and 87.17 (C-2 and C-3), 122.48 (Ar-C quaternary) and 128.26, 128.44 and 131.63 (Ar-CH); *m/z* (EI) 132 (74%, M^+), 131 (100, $[M - H]^+$), 115 (32, $[M - OH]^+$) and 102 (42, $[M - CH_2O]^+$).

(3-Bromo-1-propynyl)benzene, 78

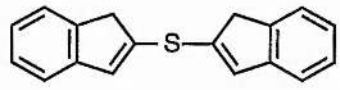
To a stirred solution of *alcohol* **77** (4.0 g, 30.3 mmol) and pyridine (450 mm^3) in ether (5 cm^3), cooled to 0 °C, was added dropwise phosphorus tribromide (4 g, 38.4 mmol). The mixture was then heated to 50 °C for 2 h, cooled to room temperature and poured onto aqueous sodium hydrogen-carbonate (10% w/v; 200 cm^3). After extraction with ether (5 x 50 cm^3), the combined organic extracts were washed with saturated brine (2 x 50 cm^3), dried and evaporated to leave an oil which was purified by Kügelrohr distillation (oven temperature 90 °C; 0.2 mm Hg) (lit.,[222] 92 °C; 2 mm Hg) to give a clear oil (3.83 g, 65%); υ_{max}(thin film)/cm^{-1} 2220 (C≡C); δ_H(200 MHz; C^2HCl_3) 4.17 (2 H, s, 3-H), 7.30-7.34 (3 H, m, *meta* Ar-H and *para* Ar-H) and 7.43-7.47 (2 H, m, *ortho* Ar-H); δ_C(50.3 MHz; C^2HCl_3) 15.28 (C-3), 84.19 and 86.70 (C-1 and C-2), 122.10 (Ar-C quaternary) and 128.30, 128.83 and 131.84 (Ar-CH); *m/z* (EI) 194 and 196 (8%, M^+), 115 (90, $[M - Br]^+$) and 105 (100).

1,1'-(Thiodi-1-propene-3,1-diyl)*bis*benzene, 79



To a stirred solution of cinnamyl chloride (10 cm^3; 10.74 g, 70.4 mmol) in methanol (50 cm^3), maintained under an atmosphere of argon was added a solution of sodium sulfide nonahydrate (9.4 g, 39 mmol) in methanol (150 cm^3). The solution was then stirred for 1 h under argon, diluted with water (150 cm^3) and extracted with ether (4 x 50 cm^3). The combined organic extracts were combined, washed with brine and dried to afford an oil. Purification by flash column chromatography (eluant petrol) gave a cloudy oil which solidified on standing (7.63 g, 77%), mp 29-35 °C (from heptane) (lit.,[225] 33-34 °C); υ_{max}(Nujol)/cm^{-1} 1596 and 1576 (C=C); δ_H(200 MHz; C^2HCl_3) 3.36 (4 H, d, *J* 7.2, 2 x CH_2), 6.24 (2 H, dt, *J* 15.8 and 7.2, CH_2-C*H*=CH), 6.50 (2 H, d, *J* 15.8, CH_2-CH=C*H*) and 7.28-7.46 (10 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 33.08 (CH_2), 125.86, 126.22, 127.50 and 128.53 and 132.39 (Ar-CH and C=*C*H) and 136.66 (Ar-C quaternary); *m/z* (CI) 383 (100%), 267 (22, $[M + H]^+$), 233 (8, $[M + H - SH_2]^+$) and 117 {44, $[C_6H_5$-CH=CH$(CH_2)]^+$}.

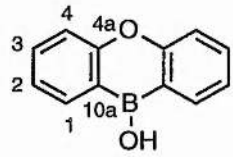
2,2'-Thio*bis*-1*H*-indene, 80



A solution of 2-indanone (1.5 g, 11.35 mmol) and Lawesson's reagent (2.25 g, 6.7 mmol) in toluene (20 cm^3) was heated under reflux for 2.5 h. The solution was then stirred at room temperature for a further 12 h, before the solvent was removed under reduced pressure. The resultant brown residue was purified by flash column chromatography (eluant petrol-ethyl acetate 20:1) to afford *sulfide* **80** as a white odorous solid (739 mg, 67%), mp 95-97 °C (lit.,[227] 96 °C); υ_{max}(Nujol)/cm^{-1} 1605 (C=C); δ_H(200 MHz;

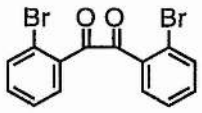
C^2HCl_3) 3.61 (4 H s, CH_2), 6.92 (2 H, s, C=CH) and 7.15-7.42 (8 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 42.51 (CH_2), 120.31, 123.40, 124.68 and 126.64 (Ar-CH), 133.08 (C=*C*H) and 139.82, 143.37 and 144.30 (Ar-C quaternary and *C*=CH); *m/z* (CI) 263 (100%, $[M + H]^+$) and 229 (8, $[M + H - SH_2]^+$).

10-Hydroxy-10*H*-phenoxaborin, 83

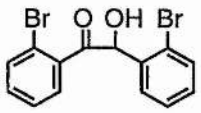


To a stirred solution of diphenyl ether (710 mg, 4.19 mmol) in THF (30 cm^3) was added dropwise *s*-BuLi (0.44 mol dm^{-3} in hexanes; 20 cm^3; 8.8 mmol). The solution was then heated under reflux for 1 h. To this boiling solution was added dropwise tri-*n*-butyl borate (1.13 cm^3; 964 mg, 4.19 mmol) and the solution heated under reflux for a further 2 h. The solution was cooled to room temperature, cautiously treated with hydrochloric acid (2 mol dm^{-3}; 20 cm^3) then extracted with ether (3 x 50 cm^3). The combined organic extracts were dried and evaporated to leave a white solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 10:1) to give *borinic acid* **83** as a white solid (500 mg, 61%), mp 274.5-275.5 °C (lit.,[230] 285 °C) (Found: C, 73.75; H, 4.8; M^+, 196.0705. Calc. for $C_{12}H_9BO_2$: C, 73.55; H, 4.65%; M^+, 196.0696); υ_{max}(Nujol)/cm^{-1} 3295 (OH), 1346 (BO) and 1144 (ether); δ_H[200 MHz; $(C^2H_3)_2SO$] 7.26-7.33 (2 H, m, 2 x 2-H), 7.44 (2 H, d, *J* 8.3, 2 x 4-H), 7.65-7.72 (2 H, m, 2 x 3-H), 8.18 (2 H, d, *J* 7.3, 2 x 1-H) and 9.92 (1 H, s, OH); δ_C[50.3 MHz; $(C^2H_3)_2SO$] 117.22, 122.30, 132.03 and 133.36 (Ar-CH), 120.38 (br, Ar-CB) and 160.82 (Ar-CO); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 37.46; *m/z* (CI) 196 (100%, M^+).

***bis*(2-Bromophenyl)-ethanedione, 94 and 1,2-*bis*(2-bromophenyl)-2-hydroxyethanone, 89**



94



89

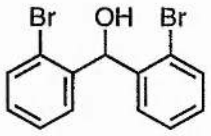
A solution of 2-bromobenzaldehyde (5.55 g, 30 mmol), 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (400 mg, 1.6 mmol) and triethylamine (900 mg, 9 mmol) in ethanol (12 cm^3) was heated under reflux for 12 h and then allowed to cool. The mixture was poured onto ice, acidified to pH 1 with hydrochloric acid (2 mol dm^{-3}), then extracted with dichloromethane (100 cm^3). The organic phase was washed with water (2 x 30 cm^3), dried and then evaporated to leave a crude solid. Purification by flash column chromatography (eluant petrol-ethyl acetate 5:1) gave *benzil* **94** as a bright yellow solid (200 mg, 3.5%) and *benzoin* **89** as a white solid (3.35 g, 60%).

Less polar **94**, mp 127-128 °C (from ethanol) (lit.,[237] 127-128 °C); υ_{max}(Nujol)/cm^{-1} 1678 (CO); δ_H(200 MHz; C^2HCl_3) 7.47-7.55 (4 H, m, 2 x 4-H and 2 x 5-H), 7.69-7.74 (2 H, m, 2 x 3-H) and 7.99-8.04 (2 H, m, 2 x 6-H); δ_C(50.3 MHz; C^2HCl_3) 123.20 (Ar-CBr)), 127.62, 133.33, 134.41 and 134.58 (Ar-CH), 134.10 (Ar-C quaternary) and 191.13 (CO); *m/z* (CI) 367, 369 and 371 (100%, $[M + H]^+$), 339, 341 and 343 (63, $[M + H - CO]^+$), 323, 325 and 327 (10, $[M + H - CO_2]^+$), 261 and 263 (21, $[M + H - Br - CO]^+$), 209 (5, $[M + H - 2\ Br]^+$) and 181 (17, $[M + H - CO - 2\ Br]^+$).

More polar **89**, mp 77-78 °C (from ethanol) (Found: C, 45.7; H, 2.6. $C_{14}H_{10}Br_2O_2$ requires C, 45.45; H, 2.7%) (HRMS: found $[M + H]^+$, 368.9138. $C_{14}H_{10}Br_2O_2$ requires 368.9126); υ_{max}(Nujol)/cm^{-1} 3484 (OH) and 1699 (CO); δ_H(200 MHz; C^2HCl_3) 4.46 (1 H, d, *J* 5.2, OH), 6.35 (1 H, d, *J* 5.2, 2-H) and 7.08-7.57 (8 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 77.20 (C-2), 119.67 and 124.16

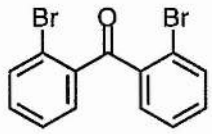
(Ar-CBr), 126.94, 127.92, 128.85, 129.23, 130.20, 132.21, 133.11 and 133.57 (Ar-CH), 136.12 and 137.30 (Ar-C quaternary) and 201.45 (CO); *m/z* (CI) 368, 370 and 372 (3%, M^+), 351, 353 and 355 (100, $[M - OH]^+$), 323, 325 and 327 (85, $[M - CO_2H]^+$), 273 and 275 (14, $[M - Br - OH]^+$), 211 (32, $[M + H - 2\ Br]^+$) and 195 (22, $[M + H - OH - 2\ Br]^+$).

2-Bromo-α-(2-bromophenyl)-α–benzene methanol, 91



To a solution of *benzophenone* **92** (340 mg, 1 mmol) in ethanol (30 cm^3), cooled to 0 °C, was added sodium borohydride (38 mg, 1 mmol). The solution was stirred for 1 h at 0 °C. Water (30 cm^3) was added and the solution was concentrated under reduced pressure to *ca.* 30 cm^3. More water (30 cm^3) was added and the solution was extracted with dichloromethane (3 x 30 cm^3). The combined organic extracts were dried, evaporated and purified, firstly by flash column chromatography (eluant petrol-ethyl acetate 10:1) and then by Kügelrohr distillation (oven temp. 160 °C; 0.7 mm Hg). This gave the desired *benzhydrol* **91** as a clear oil that solidified on standing (314 mg, 92%), mp 69-71.5 °C (HRMS: found M^+, 339.9095. $C_{13}H_{10}Br_2O$ requires 339.9098); υ_{max}(Nujol)/cm^{-1} 3270 (OH); δ_H(200 MHz; C^2HCl_3) 2.64 (1 H, d, *J* 4.1, OH), 6.42 [1 H, d, *J* 4.1, C*H*(OH)], 7.15-7.34 (6 H, m, 2 x 4-H, 2 x 5-H and 2 x 6-H) and 7.59 (2 H, d, *J* 7.4, 2 x 3-H); δ_C(50.3 MHz; C^2HCl_3) 74.16 [CH(OH)], 123.82 (Ar-CBr), 127.57, 128.62, 129.37 and 132.91 (Ar-CH) and 140.83 (Ar-C quaternary); *m/z* (CI) 340, 342 and 344 (13%, M^+) and 323, 325 and 327 (100, $[M - OH]^+$).

***bis*(2-Bromophenyl)methanone, 92**



Method 1: *via* 2-Bromo-α-(2-bromophenyl)-α–hydroxy benzene acetic acid, 90

Method 1.1: Preparation of 90 by the method of Lee *et al.*[233]

To a solution of NaOH (770 mg, 19.2 mmol) and potassium bromate (700 mg, 4 mmol) in water (3 cm^3) was added *benzoin* **89** (1.5 g, 4 mmol). The mixture was then heated under reflux for 20 h. The resultant oil was treated with excess sulfuric acid (4 mol dm^{-3}; 30 cm^3) which caused the precipitation of a sticky yellow solid. Dichloromethane (50 cm^3) was added, the layers were separated and the aqueous layer was extracted further with dichloromethane (2 x 20 cm^3). The combined organic extracts were washed with brine (2 x 40 cm^3), dried and evaporated to leave a pale yellow solid (1.54 g, quant. recovery) which was used in method 1.3 below without any further purification; υ_{max}(Nujol)/cm^{-1} 3432 (OH), 2900 (acid OH) and 1706 (CO); δ_H(200 MHz; C^2HCl_3) 7.18-7.38 (6 H, m, Ar-H) and 7.60-7.70 (2 H, m, Ar-3-H and Ar-3'-H); δ_C(50.3 MHz; C^2HCl_3) 83.73 [*C*(OH)(CO_2H)], 122.69 (Ar-CBr), 127.30, 130.10, 130.43 and 135.37 (Ar-CH), 137.70 (Ar-C quaternary) and 175.12 (CO_2H).

Method 1.2: Preparation of 90 by the method of Bickelhaupt *et al.*[237]

A mixture of *benzil* **94** (1.00 g, 2.72 mmol) and KOH (560 mg, 9.8 mmol) in aqueous ethanol (55 v/v; 2.55 cm^3) was heated under reflux for 15 min. After the mixture had

cooled to room temperature it was diluted with water (50 cm^3), acidified with hydrochloric acid (2 mol dm^{-3}; 20 cm^3) and then extracted with dichloromethane (3 x 40 cm^3). The combined organic extracts were washed with brine (2 x 30 cm^3), dried and evaporated to leave a cream solid (1.07 g, quant. recovery) which was used in method 1.3 below without any further purification. All physical properties were identical to those described in method 1.1 above.

Method 1.3: Preparation of 92 by the method of Bickelhaupt *et al.*[237]

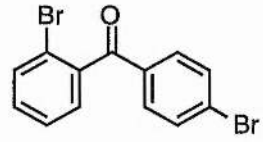
To a vigorously stirred solution of *benzilic acid* **90** (500 mg, 1.3 mmol) in glacial acetic acid (2 cm^3), heated to 50-60 °C, was added sodium bismuthate (450 mg, 1.64 mmol). The resultant suspension was heated at 50-60 °C for a further 5 h. Addition of phosphoric acid (85%, 15 mol dm^{-3}; 270 mm^3) and water (400 mm^3) caused the precipitation of a white solid which was left to stand for 16 h. Ether (50 cm^3) was added and the organic solution washed with water (2 x 20 cm^3). The aqueous extracts were repeatedly extracted with ether (8 x 20 cm^3) and all the combined organic extracts washed with NaOH (1 mol dm^{-3}; 30 cm^3) and water (30 cm^3). The organic solution was dried and evaporated to leave *benzophenone* **92** as white crystals (360 mg, 82%), mp 84-85 °C (from ethanol) (lit.,[237] 84.5-85.5 °C); υ_{max}(Nujol)/cm^{-1} 1678 (CO); δ_H(200 MHz; C^2HCl_3) 7.35-7.50 (6 H, m, 2 x 3-H, 2 x 4-H and 2 x 5-H) and 7.63-7.68 (2 H, m, 2 x 6-H); δ_C(50.3 MHz; C^2HCl_3) 121.15 (Ar-CBr), 127.29, 131.29, 132.52 and 134.06 (Ar-CH), 139.25 (Ar-C quaternary) and 195.39 (CO); *m/z* (CI) 339, 341 and 343 (100%, $[M + H]^+$), 259 and 261 (35, $[M + H - HBr]^+$) and 181 (76, $[M + H - 2\ Br]^+$).

Method 2

A solution of 2-bromobenzaldehyde (1.33 g, 60 mmol), 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (1.10 g, 4.0 mmol) and triethylamine (1.8 g, 18 mmol) in ethanol (12 cm^3) was heated under reflux for 12 h. The solution was cooled to room

temperature, poured onto ice, acidified to pH 1 with hydrochloric acid (2 mol dm^{-3}), extracted with dichloromethane (4 x 50 cm^3) and the combined organic extracts dried and evaporated. Purification of the resultant oil by flash column chromatography (eluant petrol-ethyl acetate 5:1) gave a mixture of *benzil* **94** and *benzoin* **89** (6.7 g). To this mixture was added a solution of potassium bromate (3.2 g, 18 mmol) and NaOH (3.5 g, 87.2 mmol) in water (13 cm^3) and the resultant suspension heated under reflux for 18 h. After acidification with hydrochloric acid (12 mol dm^{-3}; 30 cm^3), the resultant slurry was extracted with dichloromethane (4 x 50 cm^3) and the combined organic extracts washed with saturated brine (2 x 50 cm^3), dried and evaporated to afford an oil. Purification by flash column chromatography (eluant petrol-ethyl acetate 30:1) gave the desired *benzophenone* **92** as a white solid (3.51 g, 60%). All physical properties were identical to those described in method 1.3 above.

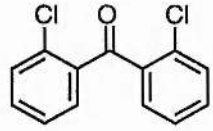
(2-Bromophenyl)(4'-bromophenyl)methanone, 93



To a stirred mixture of bromobenzene (20 cm^3; 29.82 g, 190 mmol) and 2-bromobenzoyl chloride (2.23 cm^3; 3.73 g, 17.0 mmol) was added anhydrous aluminium chloride (2.54 g, 18.7 mmol) in four portions over 10 min. The mixture was then heated to 100 °C for 30 min. Excess bromobenzene was distilled off to leave a brown residue which was purified by flash column chromatography (eluant petrol-ethyl acetate 20:1) to afford a pale yellow oil. Further purification by Kügelrohr distillation (oven temperature 160 °C; 0.5 mm Hg) gave a clear oil which solidified on standing (1.74 g, 30%), mp 48-50 °C (lit.,[235] 62 °C and lit.,[236] 51-52 °C) (Found: C, 46.25; H, 2.2. $C_{13}H_8Br_2O$ requires C, 45.90; H, 2.35%) (HRMS: found $[M + H]^+$, 338.9029. $C_{14}H_{10}Br_2O_2$ requires 338.9020); υ_{max}(Nujol)/cm^{-1} 1674 (CO); δ_H(200 MHz; C^2HCl_3) 7.32-7.50 (3 H, m, Ar-H) and 7.58-7.12 (5 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 119.42 and 129.136

(Ar-CBr), 127.33, 128.93, 131.40, 131.57, 131.99, 133.24 (Ar-CH), 134.85 and 140.07 (Ar-C quaternary) and 194.88 (CO); *m/z* (CI) 339, 341 and 343 (100%, $[M + H]^+$), 258 and 260 (15, $[M - HBr]^+$), 180 (11, $[M - 2\ Br]^+$) and 152 (9, $[M - 2\ Br - CO]^+$).

bis(2-Chlorophenyl)methanone, 95

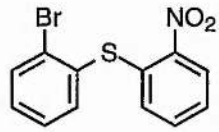


A solution of 2-chlorobenzaldehyde (13.34 g, 95 mmol), 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (1.5 g, 5.95 mmol) and triethylamine (3 g, 30 mmol) in ethanol (40 cm^3) was heated under reflux for 5 h. The solution was allowed to cool to room temperature, poured onto ice and acidified to pH 1 with hydrochloric acid (2 mol dm^{-3}). The solution was then extracted with dichloromethane (4 x 50 cm^3) and the combined organic extracts were combined, dried and evaporated to leave impure 1,2-*bis*(2-chlorophenyl)-2-hydroxyethanone as a soft yellow solid (14.64 g), which was used in the next step with no purification; υ_{max}(Nujol)/cm^{-1} 3480 (OH) and 1698 (CO); δ_H(200 MHz; C^2HCl_3) 4.43 (1 H, br s, OH), 6.35 (1 H, s, 2-H) and 7.15-7.59 (8 H, m, Ar-H); *m/z* (CI) 281, 283 and 285 (17%, $[M + H]^+$), 265 (95) and 237 (100).

A mixture of the crude 1,2-*bis*(2-chlorophenyl)-2-hydroxyethanone (13.64 g), water (38 cm^3), NaOH (9.61 g, 240 mmol) and potassium bromate (8.8 g, 49.45 mmol) was heated under reflux for 5 h. The heat source was removed and hydrochloric acid (12 mol dm^{-3}; 25 cm^3) was added which caused the mixture to boil again. Once cool, the mixture was diluted with water (50 cm^3) and the solution was extracted with dichloromethane (6 x 50 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow oil which was purified by flash column chromatography (eluant petrol-ethyl acetate 10:1) to afford *benzophenone* **95** (7.46 g, 62.6%) as white solid, mp 43-46 °C (from ethanol) (lit.,[240] 45-46 °C); υ_{max}(Nujol)/cm^{-1} 1668 (CO);

δ_H(200 MHz; C^2HCl_3) 7.30-7.55 (8 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 126.80, 130.61, 130.878 and 132.45 (Ar-CH), 132.59 (Ar-CCl), 137.90 [Ar-C(CO)] and 194.26 (CO); *m/z* (CI) 251, 253 and 255 (100%, $[M + H]^+$), 215 and 217 (10, $[M + H - HCl]^+$) and 139 and 141 (10, $[M + H - C_6H_5Cl]^+$).

2-[(2'-Nitrophenyl)thio]bromobenzene, 97

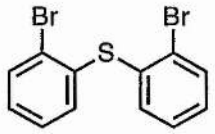


To a solution of 2-bromothiophenol (12.4 cm^3; 19.5 g, 100 mmol) and 2-chloronitrobenzene (12.0 cm^3; 16.1 g, 100 mmol) in DMF (125 cm^3) was added sodium hydrogen-carbonate (10.1 g, 120 mmol). The suspension was heated at 70 °C for 4 h. The sodium chloride that precipitated was filtered off and washed with chloroform (2 x 50 cm^3). The combined organic extracts were concentrated and the resultant yellow oil dissolved in ethyl acetate (600 cm^3) and washed with water (2 x 200 cm^3). The organic solution was then dried and evaporated to leave a bright yellow solid (29.80 g, 96%) which was used without further purification, mp 114-115 °C (from ethanol) (lit.,[287] 116-117 °C) υ_{max}(Nujol)/cm^{-1} 1515 and 1338 (NO_2); δ_H(200 MHz; C^2HCl_3) 6.77 (1 H dd, *J* 6.6 and 1.4, Ar-H), 7.23-7.49 (4 H, m, Ar-H), 7.70-7.81 (2 H, m, Ar-H) and 8.28 (1 H, dd *J* 8.2 and 1.6, 3'-H); δ_C(50.3 MHz; C^2HCl_3) 125.30, 125.87, 127.85, 128.83, 131.69, 133.63, 134.24 and 137.98 (Ar-CH), 130.99, 132.18 and 137.24 (Ar-C-1, Ar-C-2 and Ar-C-1') and 144.98 (Ar-CNO_2); *m/z* (CI) 310 and 312 (100%, $[M + H]^+$), 230 (18, $[M + H - HBr]^+$), 214 (20, $[M + H - HBr - O]^+$) and 184 (6, $[M + H - HBr - NO_2]^+$).

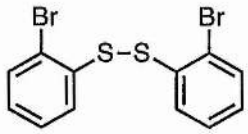
2-[(2'-Bromophenyl)thio]benzeneamine, 98

To a solution of $SnCl_2.2H_2O$ (100 g, 452 mmol) in hydrochloric acid (12 mol dm^{-3}; 150 cm^3) was added *sulfide* **97** (14 g, 45.2 mmol) followed by ethanol (150 cm^3). The suspension was heated at 70-80 °C for 4 h and then cooled to room temperature. The resultant solution was extracted with toluene (5 x 300 cm^3) and the combined organic extracts dried and evaporated to leave a red solid (12.80 g). The acidic aqueous layer was neutralised with aqueous sodium hydroxide (10 mol dm^{-3}) and the precipitated tin salts filtered off over celite. The salts were washed with chloroform (4 x 500 cm^3) and the combined organic extracts dried and evaporated to leave a white solid (5.17 g). Purification of both organic solids by flash column chromatography (eluant petrol-ethyl acetate 7:1 - 2:1) gave the desired product as a white solid (8.77 g, 70%), mp 67-68 °C (from ethanol) (lit.,[287] 62-63 °C); υ_{max}(Nujol)/cm^{-1} 3463 and 3365 (NH_2); δ_H(200 MHz; C^2HCl_3) 4.32 (2 H, br s, NH_2), 6.62 (1 H, dd, *J* 7.8 and 1.7, Ar-H), 6.77-6.85 (2 H, m, Ar-H), 6.94-7.02 (1 H, m, Ar-H), 7.07-7.15 (1 H, m, Ar-H), 7.27-7.35 (1 H, m Ar-H) and 7.46-7.56 (2 H, m, Ar-H); δ_C[50.3 MHz; $(C^2H_3)_2SO$] 110.50, 120.16 and 138.09 (Ar-C-2, Ar-C-1' and Ar-C-2'), 115.44, 117.30, 126.16, 126.78, 128.39, 132.07, 133.00 and 137.63 (Ar-CH) and 151.07 (Ar-CNH_2); *m/z* (CI) 280 and 282 (90%, $[M + H]^+$), 279 and 281 (100, M^+) and 199 (45, $[M - HBr]^+$).

1,1'-Thio*bis*(2-bromobenzene), 99 and *bis*(2-bromophenyl)disulfide, 106



99



106

Method 1: Method of Wiley *et al.*[241]

To stirred sulfuric acid (18 mol dm^{-3}; 8.5 cm^3) was added in 5 portions sodium nitrite (1.23 g, 17.5 mmol) and the mixture stirred for 30 min. A solution of *amine* **98** (4.5 g, 16 mmol) in glacial acetic acid (29 cm^3) was added dropwise to the diazotization solution at such a rate that the temperature was maintained at 16-20 °C. The resultant brown suspension was stirred for an additional 30 min at 16-20 °C then poured slowly onto a suspension of cuprous bromide (2.92 g, 20 mmol) in 45% HBr in glacial acetic acid (12 cm^3) and rinsed with additional 45% HBr in glacial acetic acid (2 x 8 cm^3). The mixture was partially neutralised by the extremely cautious addition of aqueous ammonia (35%, 18 mol dm^{-3}; 29 cm^3), then diluted with water (50 cm^3) and the resultant solution extracted with chloroform (5 x 50 cm^3). The combined organic extracts were washed with aqueous ammonia (3.2%, 1.8 mol dm^{-3}; 3 x 30 cm^3) and water (30 cm^3) then dried and evaporated to leave a crude red oil. Purification by flash column chromatography (eluant petrol-ethyl acetate 100:0.5) afforded *sulfide* **99** as a white solid (1.95 g, 35%), mp 71.5-72.5 °C (from pentane) (lit.,[241] 67.5-68 °C); δ_H(200 MHz; C^2HCl_3) 7.11-7.29 (6 H, m, 2 x 4-H, 2 x 5-H and 2 x 6-H) and 7.64 (2 H, d, *J* 7.7, 2 x 3-H); δ_C(50.3 MHz; C^2HCl_3) 125.77 (Ar-CBr), 128.14, 128.76, 132.38 and 133.47 (Ar-CH) and 135.60 (Ar-CS); *m/z* (CI) 360, 362 and 364 (15%, $[M + NH_4]^+$), 342, 344 and 346 (20, M^+) and 184 (100, $[M - 2Br]^+$).

Method 2

To a vigorously stirred suspension of 2-bromoaniline (14.14 g, 82.07 mmol) in hydrochloric acid (9 mol dm^{-3}; 40 cm^3), cooled to 0 °C, was added dropwise over 30 min a solution of sodium nitrite (6 g, 85 mmol) in water (7.5 cm^3). The resultant cloudy solution was treated with ice-cold water (5 cm^3) and the solution decanted from the sediment into another ice-cold beaker. A solution of sodium fluoroborate (11 g, 100 mmol) in ice-cold water (25 cm^3) was added and the cream-coloured precipitate left to stand for 20 min at 0 °C before it was filtered through a chilled (5 °C) filtration apparatus and washed with ice-cold water (2 x 5 cm^3). Drying under reduced pressure in a desiccator over phosphorus pentoxide for 3 days afforded crude 2-bromobenzene diazonium fluoroborate **103** as a white solid (16 g, 72%) which was used in the next step with no further purification, mp 132 °C (decomp.) [lit.,[253] 156 °C (decomp.)]; υ_{max}(Nujol)/cm^{-1} 2291 (N≡N); δ_H(200 MHz; C^2HCl_3) 7.96-8.04 and 8.13-8.23 (2 H, 2 x m, 4-H and 5-H), 8.33 (1 H, d, *J* 8.3, 3-H) and 8.87 (1 H, d, *J* 8.2, 6-H); δ_C(50.3 MHz; C^2HCl_3) 118.75 (Ar-CN≡N), 124.63 (Ar-CBr) and 130.49, 135.15, 135.35 and 141.95 (Ar-CH); *m/z* (CI) 175 and 177 (10%, $[M + H]^+$), 174 and 176 (100, M^+) and 73 (40).

To a stirred solution of 2-bromothiophenol (1 cm^3; 1.573 g, 8.32 mmol) and sodium hydrogen-carbonate (2 g, 16 mmol) in DMF (20 cm^3) was added a solution of *diazonium salt* **103** (4.32 g, 16 mmol) in DMF (20 cm^3). The mixture was then heated at 90 °C for 30 min, cooled to room temperature and poured onto saturated aqueous brine (50 cm^3). After extraction of the solution with ethyl acetate (4 x 50 cm^3), the combined organic extracts were washed with water (3 x 80 cm^3), dried and evaporated to leave an oil. Purification by flash column chromatography (eluant petrol-ethyl acetate 200:1) gave *sulfide* **99** as a white solid (250 mg, 8.7%). Physical properties were identical to those described in method 1 above.

Method 3

A solution of 1,2-dibromobenzene (14.15 g, 60 mmol) in a mixture of ether (45 cm^3) and THF (270 cm^3) was cooled with a liquid nitrogen-pentane slurry such that the temperature could be maintained between -110 and -120 °C. *n*-BuLi (1.6 mol dm^{-3} in hexanes; 37.5 cm^3; 60 mmol) was added dropwise at a rate that at which the temperature could be maintained below -110 °C (45 min). Freshly distilled sulfur dichloride (2 cm^3; 30 mmol) was then added dropwise over 30 min. The mixture was stirred at -110 °C for a further 60 min then allowed to warm to room temperature over 16 h. Saturated aqueous ammonium chloride (100 cm^3) was added, the layers were separated and the aqueous layer extracted further with ether (4 x 100 cm^3). The combined organic extracts were dried and concentrated to give a yellow oil which. Purification by flash column chromatography (eluant petrol-ethyl acetate 100:0.5) afforded less polar impure *disulfide* **106** as a white solid (1.7 g, 15%) and more polar *sulfide* **99** as a clear oil which crystallised on standing (3.8 g, 37%).

Less polar **106** was found to be contaminated with **99**, even after several attempts at purification: δ_H(200 MHz; C^2HCl_3) 7.24-7.44 (6 H, m, 2 x 4-H, 2 x 5-H and 2 x 6-H) and 7.69 (2 H, d, *J* 7.0, 2 x 3-H); δ_C(50.3 MHz; C^2HCl_3) 123.47 (Ar-CBr), 127.08, 129.35 and 130.94 (Ar-CH) and 132.54 (Ar-CS); *m/z* (CI) 431, 433 and 435 (17%, $[M + H + C_4H_8]^+$), 375, 377 and 379 (77, $[M + H]^+$) and 59 (100).

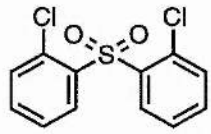
More polar **99** had physical properties identical to those described in method 1 above.

Method 4

To stirred molten 2-bromoaniline (4.76 g, 87.65 mmol) was added hydrochloric acid (12 mol dm^{-3}; 7.5 cm^3; 90 mmol) in water (50 cm^3). The mixture was heated to 60 °C to effect complete dissolution of the amine. The solution was then cooled to and maintained at

0-2 °C and vigorously stirred whilst a solution of sodium nitrite (2.36 g, 33.2 mmol) in water (6 cm^3) was added dropwise. After confirmation of the presence of excess nitrous acid (starch-iodide paper), the solution was neutralised to pH 4 with sodium acetate (3.31 g, 40 mmol). The resultant clear yellow solution was transferred to a dropping funnel and added dropwise to a stirred mixture of 2-bromothiophenol (5.39 g, 27.65 mmol), NaOH (3.4 g, 85 mmol) and copper powder (2.1 g, 33 mmol) in water (20 cm^3), cooled to 0-5 °C. After the addition of the *diazonium chloride* was complete, the solution was heated to 90 °C for 1 h. The solution was then cooled to room temperature, extracted with ether (3 x 50 cm^3) and the combined organic extracts washed with brine (2 x 50 cm^3), dried and evaporated to leave a jet black oil. Purification by flash column chromatography (eluant petrol-ethyl acetate 200:1) afforded a mixture of less polar impure *disulfide* **106** as a white solid (1.8 g) and more polar *sulfide* **99** as a white solid (3.08 g, 32.4%). These solids had physical properties identical to those described in methods 1 and 2 above.

1,1'-Sulfonyl *bis*(2-chlorobenzene), 101



To a stirred solution of diphenyl sulfone (2.25 g, 10 mmol) in THF (100 cm^3), cooled to -30 °C, was added dropwise *n*-BuLi (1.6 mol dm^{-3} in hexanes; 13.70 cm^3; 22 mmol). The red solution was stirred for 45 min at -30 °C and then benzene sulfonyl chloride (4.41 g, 25 mmol) in THF (50 cm^3) was added dropwise. After a further 30 min, aqueous NaOH (2 mol dm^{-3}; 20 cm^3) and then water (30 cm^3) were added. The layers were separated and the aqueous layer was extracted further with chloroform (3 x 70 cm^3). The combined organic extracts were dried and evaporated to leave the product as a white solid (2.86 g, 99%), mp 185-185.5 °C (from ethanol) (lit.,[245] 183 °C) (Found: C, 50.05; H, 2.7. Calc. for $C_{12}H_8Cl_2O_2S$ requires C, 50.2; H, 2.8%); υ_{max}(Nujol)/cm^{-1} 1327 and 1158 (SO_2);

δ_H(200 MHz; C^2HCl_3) 7.38-7.61 (6 H, m, 2 x 3-H, 2 x 4-H and 2 x 5-H) and 8.42-8.48 (2 H, m, 2 x 6-H); δ_C(50.3 MHz; C^2HCl_3) 126.89, 131.61, 132.76 and 134.85 (Ar-CH), 132.41 (Ar-CBr) and 137.34 (Ar-CSO_2); *m/z* (thermospray) 304, 306 and 308 (100%, $[M+ NH_4]^+$).

2-(Benzenesulfonyl)-phenyl-boronic acid, 108 and (10-Hydroxy-10*H*-phenothiaborin-4-yl)-boronic acid 5,5-dioxide, 109

108

109

Method 1

To a stirred solution of diphenyl sulfone (450 mg, 2 mmol) and TMEDA (660 mm^3; 4.4 mmol) in ether (20 cm^3), cooled to -30 °C, was added dropwise *n*-BuLi (1.6 mol dm^{-3} in hexanes; 2.8 cm^3; 4.4 mmol). The resultant yellow solution was stirred for 10 min and then cooled to -70 °C. Tri*iso*propyl borate (425 mm^3; 2.2 mmol) was added dropwise and the solution stirred for a further 30 min then poured onto hydrochloric acid (2 mol dm^{-3}; 20 cm^3). The layers were separated and the aqueous phase was extracted further with ether (3 x 30 cm^3). The combined organic extracts were dried and evaporated to leave a crude solid which was purified by flash column chromatography (eluant chloroform-methanol; 20:1 - 12:1) to afford **108** (210 mg, 40%) and **109** (40 mg, 7%), both as white solids.

Less polar **108**, mp *ca.* 110-160 °C (Found: C, 55.3; H, 4.0. $C_{12}H_{11}BO_4S$ requires C, 55.2; H, 3.85%); υ_{max}(Nujol)/cm^{-1} 3456 and 3337 (OH), 1340 (br, BO and SO_2) and 1157 (SO_2); δ_H[300 MHz; $(C^2H_3)_2SO$] 7.46-7.67 (6 H, m, Ar-H), 7.83 (1 H, d, *J* 7.5, Ar-H), 8.05 (2 H, dd, *J* 7.5 and 1.2, Ar-H) and 8.88 [2 H, br s, $B(OH)_2$]; δ_C[75.4 MHz;

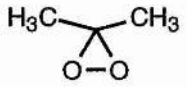
$(C^2H_3)_2SO$] 128.24, 128.39, 129.31, 129.83, 132.63, 132.91 and 133.87 (Ar-CH), 140.33 (br, Ar-CB) and 142.19 and 142.98 (Ar-CSO_2); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 30.54; *m/z* (ES^+) 733 (13%, $[3M - 3H_2O + H]^+$), 489 (100, $[2M - 2H_2O + H]^+$) and 245 (28, $[M - H_2O + H]^+$).

More polar **109**, mp 192-194 °C; υ_{max}(Nujol)/cm^{-1} 3223 (OH), 1309 (br, BO and SO_2), 1163 (SO_2); δ_H[200 MHz; $(C^2H_3)_2SO$] 7.42-8.09 (7 H, m, Ar-H), 8.21 [2 H, s, $B(OH)_2$] and 9.34 [1 H, s, B(OH)]; δ_C[75.4 MHz; $(C^2H_3)_2SO$] 127.11, 128.93, 131.25, 132.17, 132.57, 133.43 and 136.33 (Ar-CH), 135.30 and 140.10 (br, Ar-CB) and 143.46 and 145.14 (Ar-CSO_2); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 21.04 and 28.52; *m/z* (ES^+) 289 (100%, $[M + H]^+$) and 271 (28, $[M + H - H_2O]^+$).

Method 2

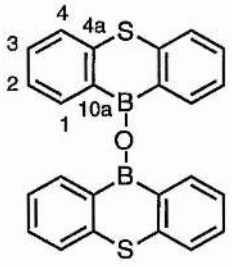
This preparation was similar in manner to method 1 above, but only 1 equivalent of *n*-BuLi was used: the reaction employed diphenyl sulfone (2.26 g, 10 mmol) in THF (100 cm^3), *n*-BuLi (2.4 mol dm^{-3} in hexanes; 4.6 cm^3; 11 mmol), tri*iso*propyl borate (2.6 cm^3; 11 mmol) and saturated aqueous ammonium chloride (30 cm^3). Additionally, after the addition of the tri*iso*propyl borate, the solution was warmed to 30 °C and maintained at this temperature for 30 min before it was quenched by addition of the saturated aqueous ammonium chloride. Purification by flash column chromatography (eluant petrol-ethyl acetate 1:1) gave *boronic acid* **108** (1.53 g, 58%) as a white solid which had physical properties identical to those described in method 1 above.

Dimethyldioxirane 116



A 2000 cm^3 round-bottomed flask equipped with a stillhead, solids addition funnel and thermometer was connected to a two-necked receiving flask, which was cooled to -70 °C. The reaction flask was charged with a mixture of water (127 cm^3), acetone (96 cm^3), and sodium hydrogen-carbonate (29 g, 35 mmol) and cooled to 0-5 °C. With vigorous stirring, Oxone® ($2KHSO_5 \bullet KHSO_4 \bullet K_2HSO_4$) (60 g, 98 mmol) was added in five portions over 15 min. 3 min after the final addition, a moderate vacuum (80-100 mm Hg) was applied, and the cooling flask removed from the reaction flask. The dimethyldioxirane/acetone solution distilled (*ca.* 50 cm^3) and collected in the cooled receiving flask. An approximate measure of the concentration (*ca.* 0.1 mol dm^{-3}) was obtained by oxidising diphenyl sulfide to its sulfoxide with an aliquot of the dimethyldioxirane solution, monitoring the reaction by TLC.

10,10'-Oxy*bis*(10*H*-phenothiaborin), 117



Method 1

To a stirred solution of *sulfide* **99** (1.03 mg, 3 mmol) in THF (50 cm^3), cooled to -70 °C, was added dropwise *n*-BuLi (1.6 mol dm^{-3} in hexanes; 3.75 cm^3, 6 mmol). The yellow solution was then stirred at -70 °C for a further 5 min. Tri-*n*-butyl borate (825 mm^3; 690 mg, 3 mmol) added dropwise and the solution was stirred for a further 30 min at -70 °C before it was allowed to warm to room temperature over 30 min. Saturated

aqueous ammonium chloride (20 cm^3) was added, the layers were separated and the aqueous layer was extracted further with ether (3 x 30 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow solid. Purification of the crude solid by flash column chromatography (eluant petrol-ethyl acetate 7:1) gave pure *borinic anhydride* **117** as a white solid (425 mg, 67%), mp 301-303 °C (from ethyl acetate-cyclohexane) (Found: C, 71.25; H, 4.0; M^+, 406.0822. $C_{24}H_{16}B_2OS_2$ requires C, 71.0; H, 3.95%; M^+, 406.0829); υ_{max}(Nujol)/cm^{-1} 1378 (BO); δ_H[200 MHz; $(C^2H_3)_2SO$] 7.38-7.44 (2 H, m, 2 x 2-H), 7.60-7.62 (4 H, m, 2 x 3-H and 2 x 4-H), 7.36 (2 H, d, *J* 7.4, 2 x 1-H) and 9.93 (1 H, s, OH, caused by *in situ* hydrolysis of the borinic anhydride); δ_C[50.3 MHz; $(C^2H_3)_2SO$] 124.08, 125.20, 131.43 and 134.00 (Ar-CH), 128.57 (br, Ar-CB) and 143.20 (Ar-CS); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 30.39; *m/z* (CI) 424 (10%, $[M + NH_4]^+$), 406 (5, M^+) and 212 {100, $([M + H_2O]/2)^+$}.

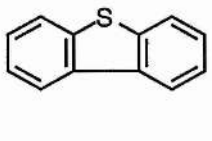
Method 2

To *sulfide* **150** (330 mg, 1 mmol) was added anhydrous aluminium chloride (66 mg, 500 μmol). Dichloromethane (20 cm^3) was added and the solution cooled to -70 °C. Boron tribromide (1 mol dm^{-3} in dichloromethane; 1 cm^3; 1 mmol) was added dropwise and the solution was allowed to warm to room temperature over 16 h. Saturated aqueous ammonium chloride (20 cm^3) was then added, the layers were separated and the aqueous phase was extracted further with ether (3 x 30 cm^3). The combined organic extracts were dried and evaporated to afford a crude solid. Purification by flash column chromatography (eluant petrol-ethyl acetate 7:1) gave pure *borinic anhydride* **117** (95 mg, 47%) which had physical properties identical to those described in method 1 above.

2-(Dimethylamino)ethyl diphenyl borinate, 118

2-aminoethyl diphenyl borinate (500 mg, 2.2 mmol) was suspended in a mixture of ether (30 cm^3) and hydrochloric acid (1 mol dm^{-3}; 30 cm^3). The suspension was vigorously stirred for 30 min. The layers were separated and the aqueous layer was extracted further with ether (3 x 30 cm^3). The combined organic fractions were then dried and concentrated to *ca.* 10 cm^3. A mixture of *N,N*-dimethylethanolamine (3 cm^3) and methanol (3 cm^3) was then added. The resultant suspension was left to stir for 1 h, during which time a white solid precipitated. The solid was filtered and washed with water (3 x 10 cm^3) to afford *dimethylethanolamine ester* **118** (370 mg, 66%), mp 161-164 °C (from ethyl acetate-cyclohexane) (lit.,[269] 165-166 °C); υ_{max}(Nujol)/cm^{-1} 1072 (C-O); δ_H[300 MHz; $(C^2H_3)_2SO$] 2.52 (6 H, s, 2 x CH_3), 2.93 [2 H, br s, $CH_2N(CH_3)_2$], 4.11 (2 H, br s, CH_2OB), 7.08-7.24 (6 H, m, *meta* and *para* Ar-H) and 7.66-7.70 (4 H, m, *ortho* Ar-H); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 46.80 [$N(CH_3)_2$], 60.19 and 60.34 (CH_2CH_2), 125.82 and 127.10 (*meta* Ar-CH and *para* Ar-CH), 132.63 (*ortho* Ar-CH) and Ar-CB (not detected); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 9.09; *m/z* (CI) 254 (100%, $[M + H]^+$), 176 (28, $[M + H - C_6H_6]^+$) and 90 (19).

Dibenzothiophene, 121 and (10-Methyl-10*H*-phenothiaborin-5,5-dioxide)-ammonia (1:1), 122



121

122

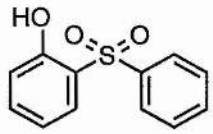
To a stirred solution of diphenyl sulfone (1.09 g, 5 mmol) in THF (50 cm^3), cooled to -30 °C, was added dropwise *n*-BuLi (2.5 mol dm^{-3} in hexanes; 4 cm^3; 10 mmol). The resultant yellow suspension was then stirred for 30 min. Di*iso*propoxymethylborane (950 mm^3; 720 mg, 5 mmol) was added dropwise and the suspension stirred for a further 1 h at -30 °C before it was allowed to warm to room temperature over 16 h. Saturated aqueous ammonium chloride (30 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 50 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow solid. Purification by flash column chromatography (eluant petrol-ethyl acetate 3:1) gave less polar dibenzothiophene **121** (70 mg, 7%) and more polar *ammonia adduct* **122** (670 mg, 52%) both as a white solids.

Less polar dibenzothiophene **121**, mp 97-99 °C (lit.,[288] 97-100 °C); δ_H[200 MHz; $(C^2H_3)_2SO$] 7.52-7.56 (4 H, m, Ar-H), 8.03-8.08 (2 H, m, Ar-H) and 8.37-8.42 (2 H, m, Ar-H); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 122.30, 123.30, 125.00 and 127.35 (Ar-CH) and 135.28 and 138.79 (Ar-C quaternary); *m/z* (CI) 185 (100%, $[M + H]^+$).

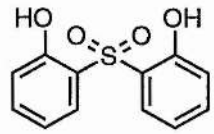
More polar *ammonia adduct* **122**, mp 255 °C (decomp.) (from ethyl acetate-cyclohexane) (Found: C, 60.15; H, 5.55; N, 5.35. $C_{13}H_{14}BNO_2S$ requires C, 60.25; H, 5.45; N, 5.4%) (HRMS: found $[M + H]^+$, 260.0913. $C_{13}H_{15}BNO_2S$ requires 260.0917); υ_{max}(Nujol)/cm^{-1} 3283, 3214 and 3160 (NH_3) and 1302 and 1159 (SO_2); δ_H[500.3 MHz;

$(C^2H_3)_2SO$] 0.26 (3 H, s, CH_3), 5.53 (3 H, NH_3), 7.47-7.50 (2 H, m, 2 x 3-H), 7.58-7.62 (2 H, m, 2 x 2-H), 7.88 (2 H, d, *J* 7.0, 2 x 1-H) and 7.86 (2 H, d, *J* 8.0, 2 x 4-H); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 10.51 (br, CH_3), 122.46 (C-4), 126.08 (C-3), 131.34 (C-2), 132.03 (C-1), 142.62 (Ar-CSO_2) and 155.47 (br, Ar-CB); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] -12.29; *m/z* (CI) 260 (55%, $[M + H]^+$), 243 (100, $[M + H - NH_3]^+$) and 227 (5).

2-(Phenylsulfonyl)phenol, 124 and 2,2'-Sulfonyl*bis*phenol, 125



124



125

Method 1

To a stirred solution of *ammonia adduct* **122** (168 mg, 646 μmol) in THF (5 cm^3) was added dropwise aqueous sodium hydroxide (3 mol dm^{-3}; 750 mm^3; 2.25 mmol). Aqueous H_2O_2 (30% v/v; 750 mm^3) was added dropwise and the mixture was heated to 50 °C for 2 h. The solution was then cooled to room temperature and neutralised to pH 7 with aqueous citric acid (7.5% w/v). The layers were separated and the aqueous phase was extracted further with ethyl acetate (3 x 20 cm^3). The combined organic extracts were dried and evaporated to leave a white solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 2:1) to give less polar *phenol* **124** (20 mg, 13%) and more polar bis*phenol* **125** (50 mg, 31%), both as white solids.

Less polar **124**, mp 98.5-99 °C (lit.,[289] 98-99 °C); υ_{max}(Nujol)/cm^{-1} 3360 (OH), 1305 and 1146 (SO_2) and 1090 (CO); δ_H[200 MHz; $(C^2H_3)_2SO$] 6.92 (1 H, d, *J* 8.1, Ar-H), 7.00-7.07 (1 H, m, Ar-H), 7.47-7.69 (5 H, m, Ar-H), 7.89-7.96 (2 H, m, Ar-H) and 10.80 (1 H, br s, OH); δ_C(50.3 MHz; C^2HCl_3) 119.14, 120.78, 126.79, 129.18, 129.44,

133.70 and 136.14 (Ar-CH), 123.46 and 141.60 (Ar-CSO_2) and 155.88 [Ar-C(OH)]; *m/z* (CI) 235 (100%, $[M + H]^+$) and 219 (50, $[Ph_2SO_2]^+$).

More polar **125**, mp 189.5-191 °C (lit.,[290] 189.5-191 °C) (HRMS: found M^+, 250.0305. Calc. for $C_{12}H_{10}BOS$: 250.0300); υ_{max}(Nujol)/cm^{-1} 3356 (OH), 1310 and 1144 (SO_2) and 1209 (CO); δ_H[200 MHz; $(C^2H_3)_2SO$] 6.85 (2 H, dd, *J* 8.2 and 1.0, 2 x 6-H), 6.96-7.04 and 7.42-7.51 (2 x 2 H, 2 x m, 2 x 4-H and 2 x 5-H), 7.91 (2 H, dd, *J* 8.0 and 1.7, 2 x 6-H); δ_C[50.3 MHz; $(C^2H_3)_2SO$] 117.35, 119.13, 130 and 135.64 (Ar-CH), 126.76 (Ar-C-2) and 155.87 (Ar-C-1); *m/z* (EI) 250 (52%, M^+), 157 (45, $[M - C_6H_5O]^+$) and 94 (100, $[C_6H_5OH]^+$).

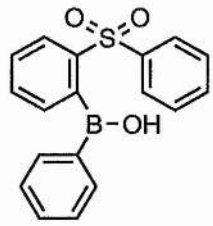
Method 2

To a stirred solution of *boronic acid* **108** (375 mg, 1.44 mmol) in THF (10 cm^3) was added in one portion sodium perborate tetrahydrate (220 mg, 1.44 mmol). The suspension was stirred for 2 h at room temperature then diluted with hydrochloric acid (1 mol dm^{-3}; 20 cm^3) and ether (20 cm^3). The layers were separated and the aqueous phase was extracted further with ether (3 x 20 cm^3). The combined organic extracts were dried and evaporated to leave a white solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 3:1) to give *phenol* **124** (310 mg, 92%) which had physical properties identical to those described in method 1 above.

(10-Methyl-10*H*-phenothiaborin-5,5-dioxide)-hydroxyammonia-N (1:1), 128

This was prepared in a manner identical to the *ammonia adduct* **122** and employed diphenyl sulfone (1.69 g, 7.5 mmol) in THF (70 cm^3), *n*-BuLi (2.5 mol dm^{-3} in hexanes; 6.5 cm^3; 16.25 mmol), di*iso*propoxymethylborane (1.5 cm^3; 8 mmol) and saturated aqueous hydroxyammonium chloride (30 cm^3). Purification of the crude product by flash column chromatography (eluant petrol-ethyl acetate 5:1 - 3:1) gave *hydroxylamine adduct* **128** as a white solid (791 mg, 38%), mp 190-192 °C (from ethyl acetate-cyclohexane) (Found: C, 56.85; H, 5.0; N, 5.0. $C_{13}H_{14}BNO_3S$ requires C, 56.75; H, 5.15; N, 5.1%) (HRMS: found $[M + H]^+$, 276.0855. $C_{13}H_{15}BNO_3S$ requires 276.0866); υ_{max}(Nujol)/cm^{-1} 3424 (OH), 3243, 3213 and 3138 (NH_3) and 1305 and 1111 (SO_2); δ_H[500.3 MHz; $(C^2H_3)_2SO$] 0.17 (3 H, s, CH_3), 7.35-7.38 (2 H, m, 2 x 3-H), 7.45-7.48 (2 H, m, 2 x 2-H), 7.79 (2 H, d, *J* 7.1, 2 x 1-H), 7.86 (2 H, d, *J* 7.1, 2 x 4-H), 8.34 (1 H, t, *J* 2.7, OH) and 8.60 (2 H, d, *J* 2.7, NH_2); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 5.62 (br, CH_3), 122.18 (C-4), 126.28 (C-3), 131.09 (C-2), 133.18 (C-1), 143.02 (Ar-CSO_2) and 151.33 (br, Ar-CB); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] -8.21; *m/z* (CI) 276 (10%, $[M + H]^+$), 243 (100, $[M + H - NH_2OH]^+$) and 219 (23, $[PhSO_2Ph + H]^+$).

[2-(Phenylsulfonyl)phenyl](phenyl) borinic acid, 131



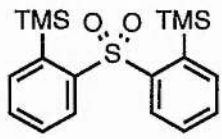
Method 1

To a stirred solution of diphenyl sulfone (960 mg, 4.25 mmol) in THF (50 cm^3), cooled to -30 °C, was added dropwise *n*-BuLi (2.5 mol dm^{-3} in hexanes; 3.6 cm^3; 9 mmol). The resultant suspension was stirred for a further 15 min. Di*iso*propoxyphenylborane (1 cm^3; 877 mg, 4.25 mmol) was added dropwise and the suspension was allowed to warm to room temperature over 3 h. Saturated aqueous ammonium chloride (30 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 30 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 3:1) to give *borinic acid* **131** as a white solid (510 mg, 37%), mp 142.5-145.5 °C (from ethyl acetate-cyclohexane) (Found: C, 67.4; H, 4.85; M^+, 322.0831. $C_{18}H_{15}BO_3S$ requires C, 67.1; H, 4.7%; M^+, 322.0835); υ_{max}(Nujol)/cm^{-1} 3479 (OH) and 1345 and 1153 (SO_2); δ_H[300 MHz; $(C^2H_3)_2SO$] 7.26-7.31 (2 H, m, Ar-H), 7.37-7.67 (9 H, m, Ar-H), 7.73-7.77 (2 H, m, Ar-H), 7.90 (1 H, dd, *J* 7.1 and 0.7, Ar-H) and 10.07 (1 H, s, OH); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 127.67, 127.88, 128.23, 129.28, 129.62, 130.70, 131.24, 132.76, 133.85 and 134.77 (Ar-CH), 138.61 and 142.79 (br, Ar-CB) and 141.21 and 143.26 (Ar-CSO_2); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 44.78; *m/z* (CI) 322 (10%, M^+) and 305 (100, $[M - OH]^+$).

Method 2

This was prepared in a manner identical to method 1 above, but only 1 equivalent of *n*-BuLi solution was used; the reaction employed diphenyl sulfone (1.69 g, 7.5 mmol) in THF (60 cm^3), *n*-BuLi (2.5 mol dm^{-3} in hexanes; 6.5 cm^3; 16.25 mmol), di*iso*propoxyphenylborane (1.8 cm^3; 1.58 g, 7.65 mmol) and saturated aqueous ammonium chloride (30 cm^3). Purification by flash column chromatography (eluant petrol-ethyl acetate 3:1) gave *borinic acid* **131** (1.45 g, 60%) as a white solid which had physical properties identical to those described in method 1 above.

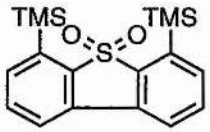
***bis*[2-(Trimethylsilyl)phenyl]sulfone, 132**



To a stirred solution of diphenyl sulfone (11.25 g, 50 mmol) in THF (600 cm^3), cooled to -40 °C, was added dropwise *n*-BuLi (2.1 mol dm^{-3} in hexanes; 50 cm^3; 105 mmol). The resultant suspension was then stirred at a temperature between -30 and -50 °C for 1 h. Chlorotrimethylsilane (14.5 cm^3; 110 mmol) was added dropwise and the solution was allowed to warm to room temperature over 16 h. Saturated aqueous ammonium chloride (100 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 100 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 30:1 - 20:1) to give *sulfone* **132** as a white solid (14.62 g, 81%), mp 138.5-139.5 °C (from ethyl acetate-cyclohexane) (Found: C, 59.9; H, 7.1. $C_{18}H_{26}O_2SSi_2$ requires C, 59.6; H, 7.25%) (HRMS: found $[M + NH_4]^+$, 380.1536. $C_{18}H_{30}NO_2SSi_2$ requires 380.1536); υ_{max}(Nujol)/cm^{-1} 1423 and 1114 (Si-Ph), 1310 and 1160 (SO_2) and 1260, 844 and 752 [Si-$(CH_3)_3$]; δ_H(200 MHz; C^2HCl_3) 0.40 [18 H, s, 2 x Si$(CH_3)_3$], 7.34-7.57 (6 H, m, 2 x 3-H,

2 x 4-H and 2 x 5-H) and 7.79-7.86 (2 H, m, 2 x 6-H); δ_C(50.3 MHz; C^2HCl_3) 1.14 [$Si(CH_3)_3$], 128.96, 129.11, 131.65 and 136.74 (Ar-CH), 140.08 (Ar-CSi) and 146.80 (Ar-CSO_2); *m/z* (CI) 380 (100, $[M + NH_4]^+$) and 347 (42, $[M + NH_4 - NH_3 - CH_4]^+$).

4,6-*bis*(Trimethylsilyl)-dibenzothiophene-5,5-dioxide, 135



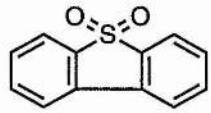
Method 1

To a stirred solution of *sulfone* **132** (1.45 g, 4 mmol) in THF (100 cm^3), cooled to -40 °C, was added dropwise *n*-BuLi (2.1 mol dm^{-3} in hexanes; 4 cm^3; 8.4 mmol). The resultant red solution was allowed to warm to -30 °C over 30 min before it was cooled to -70 °C. Tri-*n*-butyl borate (1.2 cm^3; 4.4 mmol) was then added dropwise and the solution was allowed to warm to room temperature over 16 h. Saturated aqueous ammonium chloride (50 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 50 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 30:1) to give pure *sulfone* **135** as a white solid (430 mg, 30%), mp 139-139.5 °C (from ethyl acetate-cyclohexane) (Found: C, 59.7; H, 7.0. $C_{18}H_{24}O_2SSi_2$ requires C, 59.95; H, 6.7%) (HRMS: found $[M + H]^+$, 361.1106. $C_{18}H_{25}O_2SSi_2$ requires 361.1114); υ_{max}(Nujol)/cm^{-1} 1160 (SO_2) and 1254, 844 and 765 [Si-$(CH_3)_3$]; δ_H(200 MHz; C^2HCl_3) 0.54 [18 H, s, 2 x $Si(CH_3)_3$], 7.47-7.55 (2 H, m, Ar-H), 7.64 (2 H, dd, *J* 7.4 and 1.2, Ar-H) and 7.72 (2 H, dd, *J* 7.5 and 1.3, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 0.20 [$Si(CH_3)_3$], 121.56, 132.23 and 136.44 (Ar-CH) and 131.13, 137.13 and 142.65 (Ar-C quaternary); *m/z* (CI) 361 (33%, $[M + H]^+$) and 345 (100, $[M + H - CH_3]^+$).

Method 2

This preparation was identical in manner to method 1 above, but di*iso*propoxyphenylborane (950 mm^3; 833 mg, 4 mmol) was used as the electrophile. Pure *sulfone* **135** was isolated as a white solid (600 mg, 42%) which had physical properties identical to those described in method 1 above.

Dibenzothiophene-5,5-dioxide, 136



Method 1

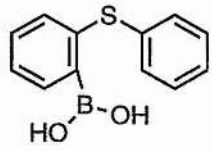
A stirred solution of *sulfone* **135** (76 mg, 210 μmol) in THF (10 cm^3) was treated with TBAF (1 mol dm^{-3} in THF; 1 cm^3; 1 mmol). After 2 h, saturated aqueous brine (10 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (2 x 10 cm^3). The combined organic extracts were dried and evaporated to leave crude dibenzothiophene-5,5-dioxide **136** (53 mg, quant. recovery). Pure **136** was prepared by method 2 below.

Method 2

To a stirred solution of *sulfone* **132** (1.45 g, 4 mmol) in THF (80 cm^3), cooled to -40 °C, was added dropwise *n*-BuLi (2.3 mol dm^{-3} in hexanes; 3.55 cm^3; 8.2 mmol). The resultant red solution was allowed to warm to -30 °C over a further 30 min. Boron trifluoride etherate (540 mm^3; 605 mg, 4.2 mmol) was added dropwise and the solution was allowed to warm additionally over 3 h to room temperature. TBAF (1 mol dm^{-3} in THF; 8.2 cm^3; 8.2 mmol) was added and the solution stirred for 16 h. Saturated aqueous brine (30 cm^3) was added, the layers were separated and the aqueous phase was extracted

further with ether (3 x 30 cm^3). The combined organic extracts were dried and evaporated to leave a crude solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 3:1) to give pure dibenzothiophene-5,5-dioxide **132** as a white solid (300 mg, 31%), mp 230-232 °C (lit.,[291] 233-237 °C); υ_{max}(Nujol)/cm^{-1} 1166 (SO_2); δ_H[200 MHz; $(C^2H_3)_2SO$] 7.64-7.72 (2 H, m, Ar-H), 7.80-7.88 (2 H, m, Ar-H), 8.01 (2 H, d, *J* 7.1, Ar-H) and 8.24 (2 H, d, *J* 7.7, Ar-H); δ_C[50.3 MHz; $(C^2H_3)_2SO$] 122.25, 122.99, 131.22 and 134.84 (Ar-CH) and 131.07 and 137.16 (Ar-C quaternary); *m/z* (CI) 217 (100%, $[M + H]^+$) and 186 (35).

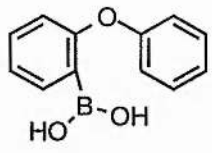
2-Phenylthiobenzene boronic acid, 140



To a stirred solution of 2-(bromophenylthio)benzene **154** (3.1 g, 11.7 mmol) in THF (100 cm^3), cooled to -70 °C, was added dropwise *n*-BuLi (2.4 mol dm^{-3} in hexanes; 6.0 cm^3; 14.4 mmol). The resultant solution was stirred for 30 min. Tri*iso*propyl borate (3.4 cm^3; 2.77 g, 14.4 mmol) was added dropwise and the solution was stirred for a further 30 min at -70 °C before it was allowed to warm to room temperature over 1 h. Saturated aqueous ammonium chloride (50 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 50 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow solid which was purified by flash column chromatography (eluant hexane-ethyl acetate 4:1) to give *boronic acid* **140** as a white solid (2.16 g, 80%), mp 163-165 °C (from ethyl acetate-cyclohexane) (Found: C, 62.95; H, 4.6; M^+, 230.0581. $C_{12}H_{11}BO_2S$ requires C, 62.64; H, 4.82%; M^+, 230.0573); υ_{max}(Nujol)/cm^{-1} 3304 (OH) and 1085 (BO); δ_H[300 MHz; $(C^2H_3)_2SO$] 7.14-7.17 (1 H, m, Ar-H), 7.22-7.35 (6 H, m, Ar-H), 7.44-7.47 (1 H, m, Ar-H) and 8.15 (2 H, br s, OH); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 126.90, 127.06, 129.57, 129.77, 130.60, 131.95 and 133.32 (Ar-CH), 136.97 and 137.52 (Ar-CS) and

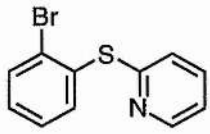
141.64 (br, Ar-CB); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 30.50; *m/z* (CI) 231 (100%, $[M + H]^+$), 213 {6, $[(2M - 2H_2O + H)/2]^+$} and 187 (14, $[Ph_2S + H]^+$).

2-Phenyloxybenzene boronic acid, 141



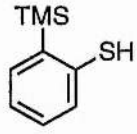
This was prepared in a manner identical to the *boronic acid* **140** and employed 2-bromophenyloxybenzene **155** (1.69 g, 6.78 mmol) in THF (80 cm^3), *n*-BuLi (2.4 mol dm^{-3} in hexanes; 3.35 cm^3; 8.4 mmol), tri*iso*propyl borate (2.0 cm^3; 1.6 g; 8.4 mmol) and saturated aqueous ammonium chloride (40 cm^3). After the layers were separated and the aqueous phase extracted further with ether (3 x 50 cm^3), the combined organic extracts were dried and evaporated to leave a pale yellow solid. Purification by flash column chromatography (eluant hexane-ethyl acetate 5:1 - 2:1) afforded *boronic acid* **141** as a white solid (1.29 g, 84%), mp 128-131 °C (lit.,[230] 114 °C) (ethyl acetate-cyclohexane) (Found: C, 67.65; H, 5.15. Calc. for $C_{12}H_{11}BO_3$: C, 67.35; H, 5.2%) (HRMS: found $[M + H]^+$, 215.0875. Calc. for $C_{12}H_{12}BO_3$: 215.0880); υ_{max}(Nujol)/cm^{-1} 3502 and 3328 (OH), 1072 (BO) and 1229 (ether); δ_H[300 MHz; $(C^2H_3)_2SO$] 6.74-6.78 (1 H, m, Ar-H), 6.93-6.98 (2 H, m, Ar-H), 7.04-7.12 (2 H, m, Ar-H), 7.30-7.36 (3 H, m, Ar-H), 7.58 (1 H, dd, *J* 7.4 and 1.7, Ar-H) and 7.88 (2 H, br s, OH); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 118.32, 119.12, 123.48, 123.54, 130.31, 131.52 and 135.62 (Ar-CH), 127.38 (br, Ar-CB) and 157.81 and 160.48 (Ar-CO); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 30.12; *m/z* (CI) 215 (100%, $[M + H]^+$), 197 {19, $[(2M - 2H_2O + H)/2]^+$} and 171 (12, $[Ph_2O + H]^+$).

2-(2'-Bromophenylthio)pyridine, 145



A mixture of 2-bromopyridine (1.63 g, 10 mmol), 2-bromophenol (1.42 cm^3; 1.95 g, 10 mmol) and sodium hydrogen-carbonate (1 g, 12 mmol) in DMF (20 cm^3) was heated at 130 °C for 2 h, after which time evolution of carbon dioxide had ceased. The solvent was distilled off under reduced pressure and the residue was mixed with ethyl acetate (60 cm^3) and water (20 cm^3). The layers were separated and the organic phase was washed further with water (2 x 20 cm^3), dried and evaporated. The resultant oil was purified by flash column chromatography (eluant petrol-ethyl acetate 20:1 - 3:1) to afford *sulfide* **145** as a clear oil (1.43 g, 54%) (Found: C, 49.7; H, 3.25; N, 5.2. $C_{11}H_8BrNS$ requires C, 49.65; H, 3.05; N, 5.25%) (HRMS: found $[M + H]^+$, 265.9648. $C_{11}H_9BrNS$ requires 265.9639); δ_H(200 MHz; C^2HCl_3) 6.92 (1 H, d, *J* 8.1, Ar-H), 7.10-7.07 (1 H, m, Ar-H), 7.20-7.39 (2 H, m, Ar-H), 7.46-7.54 (1 H, m, Ar-H), 7.62-7.73 (2 H, m, Ar-H) and 8.45 (1 H, d, *J* 4.0, 6-H); δ_C(50.3 MHz; C^2HCl_3) 119.08 (Ar-CBr), 129.41 and 132.80 (Ar-C-2 and Ar-C-1'), 149 (Ar-C-6) and 120.35, 122.03, 128.23, 130.36, 133.75, 136.40 and 136.80 (other Ar-CH); *m/z* (CI) 267 and 269 (15%, $[M + H]^+$), 266 and 268 (100, M^+) and 186 (15%, $[M - HBr]^+$).

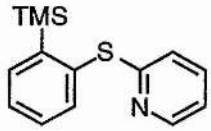
2-(Trimethylsilyl)thiophenol, 146



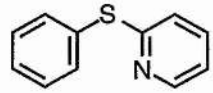
To a solution of TMEDA (16 cm^3; 12.2 g, 105 mmol) and *n*-BuLi (2.2 mol dm^{-3} in hexanes; 50 cm^3; 110 mmol) in cyclohexane (120 cm^3), cooled to 0 °C, was added dropwise a solution of thiophenol (5.3 cm^3; 5.51 g, 50 mmol) in cyclohexane (10 cm^3). The solution was then stirred at room temperature for 24 h. To the resultant off-white

suspension was added chlorotrimethylsilane (16.2 cm^3; 13.58 g, 125 mmol) and the mixture stirred for a further 16 h to afford a lilac-coloured suspension. Water was added (40 cm^3), the organic solvent was removed under reduced pressure. The residue was mixed with ether (350 cm^3), washed with hydrochloric acid (5% v/v; 3 x 130 cm^3) and water (2 x 70 cm^3) then dried and evaporated to leave the crude intermediate *silylsulfide* **149** as a pungent oily yellow liquid. The crude liquid was dissolved in methanol (100 cm^3) and then heated under reflux for 9.5 h. The solution was evaporated and the resultant oil purified by Kügelrohr distillation (oven temperature 130 °C; 1 mm Hg) (lit.,[278] 55 °C, 0.1 mm Hg) to afford a clear pungent oil (8.58 g, 94%); υ_{max}(thin film)/cm^{-1} 2569 (SH), 1422 and 1126 (Si-Ph) and 1251, 841 and 744 [Si-$(CH_3)_3$]; δ_H(200 MHz; C^2HCl_3) 0.45 [9 H, s, $Si(CH_3)_3$], 3.56 (1 H, s, SH), 7.19-7.37 (3 H, m, 4-H, 5-H and 6-H) and 7.47-7.52 (1 H, m, 3-H); δ_C(50.3 MHz; C^2HCl_3) -0.45 [$Si(CH_3)_3$], 125.19, 129.61, 131.48 and 135.08 (Ar-CH), 137.14 (Ar-CS) and 139.53 (Ar-CSi); *m/z* (EI) 182 (20%, M^+), 167 (100, $[M - CH_3]^+$) and 151 (95, $[M - CH_3 - CH_4]^+$).

2-[2'-(Trimethylsilyl)phenylthio]pyridine, 147 and 2-(Phenylthio)pyridine, 148



147



148

Method 1

A stirred suspension of 2-bromopyridine (1.61 g, 10 mmol), *thiophenol* **146** (1.82 g, 10 mmol) and sodium hydrogen-carbonate (1 g, 12 mmol) in DMF (20 cm^3) was heated at 100 °C for 1 h. The solvent was evaporated and the residue mixed with ethyl acetate (50 cm^3) and water (20 cm^3). The layers were separated and the organic phase was washed with more water (4 x 50 cm^3). The organic phase was dried and evaporated to

afford an oil which was purified by flash column chromatography (eluant petrol-ethyl acetate 12:1) to give less polar **147** (310 mg, 12%) and more polar **148** (1.15 g, 62%), both as clear oils.

Less polar **147** (Found: C, 64.85; H, 6.85; N, 5.35; M^+, 259.0857. $C_{14}H_{17}NSSi$ requires C, 64.8; H, 6.6; N, 5.4%; M^+, 259.0851); υ_{max}(thin film)/cm^{-1} 1417 and 1122 (Si-Ph) and 1249, 841 and 756 [Si-$(CH_3)_3$]; δ_H(200 MHz; C^2HCl_3) 0.30 [9 H, s, $Si(CH_3)_3$], 6.64 (1 H, d, *J* 7.8, Ar-H), 6.92-6.98 (1 H, m, Ar-H), 7.35-7.46 (3 H, m, Ar-H), 7.58-7.67 (2 H, m, Ar-H) and 8.41 (1 H, d, *J* 4.0, 6-H); δ_C(50.3 MHz; C^2HCl_3) -0.07 [$Si(CH_3)_3$], 119.32, 120.48, 128.60, 130.35, 135.85, 136.52, 137.34 and 149.28 (Ar-CH), 136.66 and 146.69 (Ar-C-1' and Ar-C-2') and 163.08 (Ar-C-1); *m/z* (EI) 259 (1%, M^+), 244 (35, $[M - CH_3]^+$) and 186 {100, $[M - Si(CH_3)_3]^+$}.

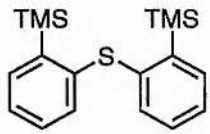
More polar **148**; δ_H(200 MHz; C^2HCl_3) 6.87 (1 H, dd, *J* 8.1 and 1.1, Ar-H), 6.94-6.98 (1 H, m, Ar-H), 7.39-7.48 (4 H, m, Ar-H), 7.57-7.62 (2 H, m, Ar-H) and 8.42 (1 H, d, *J* 3.9, 6-H); δ_C(50.3 MHz; C^2HCl_3) 119.77, 121.19, 128.99, 129.53, 134.83, 136.60 and 149.45 (Ar-CH), 130.89 (Ar-C-1') and 161.41 (Ar-C-1); *m/z* (EI) 186 (100%, M^+).

Method 2

To a stirred suspension of sodium hydride (60% dispersion in mineral oil; 684 mg, 17.1 mmol) in 1,4-dioxane (35 cm^3) was added a solution of *thiophenol* **146** (2.835 g, 15.55 mmol) in 1,4-dioxane (35 cm^3). After 20 min, evolution of hydrogen had ceased and a solution of 2-bromopyridine (2.39 g, 15 mmol) in 1,4-dioxane (35 cm^3) was added dropwise and the mixture heated under reflux for 48 h. The mixture was cooled to 0 °C and saturated aqueous ammonium chloride (50 cm^3) was added. The layers were separated and the aqueous phase was extracted further with ether (3 x 100 cm^3). The combined organic extracts were dried and evaporated to leave an oil which was purified by

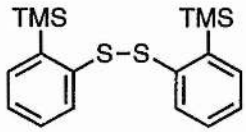
flash column chromatography (eluant petrol-ethyl acetate 12:1) to give pure *sulfide* **147** a clear oil (2.01 g, 52%), which had physical properties identical to those described in method 1 above.

1,1'-Thio*bis*[2-(trimethylsilyl)benzene], 150



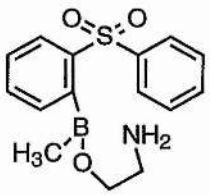
To a stirred solution of *sulfide* **99** (1.38 g, 4 mmol) in THF (50 cm^3), cooled to -70 °C, was added dropwise *n*-BuLi (2.5 mol dm^{-3} in hexanes; 3.6 cm^3; 9 mmol) and the resultant solution stirred for 30 min. Chlorotrimethylsilane (1 mol dm^{-3} in THF; 9 cm^3; 9 mmol) was added dropwise and the solution allowed to warm to 25 °C over 1 h. Saturated aqueous ammonium chloride (20 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 50 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow oil which was purified first by flash column chromatography (eluant petrol) and then by Kügelrohr distillation (oven temperature 140 °C; 0.4 mm Hg) to give *sulfide* **150** as a clear oil which solidified on standing (800 mg, 61%), mp 45-45.5 °C (Found: C, 65.55; H, 8.15; M^+, 330.1301. $C_{18}H_{26}SSi_2$ requires C, 65.4; H, 7.95%; M^+, 330.1294); υ_{max}(Nujol)/cm^{-1} 1422 and 1110 (Si-Ph) and 1249, 840 and 746 [Si-$(CH_3)_3$]; δ_H(200 MHz; C^2HCl_3) 0.41 [18 H, s, 2 x $Si(CH_3)_3$], 7.04-7.09 (2 H, m, Ar-H), 7.21-7.26 (4 H, m, Ar-H) and 7.52-7.56 (2 H, m, 2 x 6-H); δ_C(50.3 MHz; C^2HCl_3) -0.10 [$Si(CH_3)_3$], 125.98, 130.01, 131.74 and 134.96 (Ar-CH), 141.86 (Ar-CS) and 143.51 (Ar-CSi); *m/z* (CI) 330 (55%, M^+), 315 (100, $[M - CH_3]^+$) and 285 (26).

***bis*[2-(Trimethylsilyl)phenyl)disulfide, 151**



A stirred solution of *thiophenol* **146** (1.82 g, 10 mmol) in methanol (25 cm^3) was treated with a solution of sodium perborate tetrahydrate (3.07, 20 mmol) in water (10 cm^3) at 25 °C. Aliquot 336 (*ca.* 100 mg) was added and the solution stirred for 1 h. The solution was extracted with pentane (5 x 20 cm^3) and the combined organic extracts were dried and evaporated to leave a pale yellow oil. Purification first by flash column chromatography (eluant petrol) and then by Kügelrohr distillation (oven temperature 160 °C; 0.3 mm Hg) afforded *disulfide* **151** as a yellow oil (1.32 g, 73%) (Found: C, 59.55; H, 7.5. $C_{18}H_{26}S_2Si_2$ requires C, 59.6; H, 7.25%) (HRMS: found $[M + H]^+$, 363.1102. $C_{18}H_{27}S_2Si_2$ requires 363.1093); υ_{max}(Nujol)/cm^{-1} 1420, 1125 and 1103 (Si-Ph) and 1250, 840 and 748 [Si-$(CH_3)_3$]; δ_H(200 MHz; C^2HCl_3) 0.41 [18 H, s, 2 x $Si(CH_3)_3$], 7.20-7.37 (4 H, m, Ar-H), 7.46-7.57 (2 H, m, Ar-H) and 7.61-7.66 (2 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 0.2 [$Si(CH_3)_3$], 126.55, 128.83, 130.00 and 134.69 (Ar-CH) and 140.15 and 143.59 (Ar-C quaternary); *m/z* (CI) 363 (100, $[M + H]^+$), 347 (60, $[M + H - CH_3]^+$) 317 (15, $[M + H - CH_4 - 2CH_3]^+$), 275 {20, $[M + H - Si(CH_3)_4]^+$}, 259 {35, $[M + H - CH_4 - Si(CH_3)_4]^+$} and 167 {31, $[M + H - (CH_3)_3Si\text{-}C_6H_4SCH_3]^+$}.

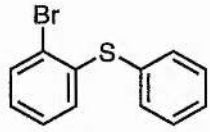
2-Aminoethyl [2-(phenylsulfonyl)phenyl](methyl) borinate, 152



To a stirred solution of diphenyl sulfone (1.688 g, 7.5 mmol) in THF (60 cm^3), cooled to -30 °C, was added dropwise *n*-BuLi (2.5 mol dm^{-3} in hexanes; 3.25 cm^3; 8.12 mmol). The resultant suspension was stirred for 30 min. Di*iso*propoxymethylborane (1.5 cm^3; 1.14 g, 8 mmol) was then added dropwise and the suspension was stirred for an additional 1 h at -30 °C before it was allowed to warm to room temperature over 1 h. Saturated aqueous ammonium chloride (30 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 50 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow oil which was immediately purified by flash column chromatography (eluant petrol-ethyl acetate 3:1) to give *borinic* acid **142** as a clear oil (1.65 g, 85%). Because of the instability of the *borinic acid*, it was immediately dissolved in ether (10 cm^3), cooled to 0 °C and treated with ethanolamine (2 cm^3). This caused the precipitation of a white solid and a viscous yellow oil. The solid was filtered off and washed with water (200 cm^3) and recrystallised from ethyl acetate-cyclohexane to give *ethanolamine ester* **152** as white needles (810 mg, 36%), mp 154-158 °C (Found: C, 59.7; H, 5.85; N, 4.55. $C_{15}H_{18}BNO_3S$ requires C, 59.4; H, 6.0; N, 4.6%) (HRMS: found $[M + H]^+$, 260.0913. $C_{13}H_{15}BNO_2S$ requires 260.0917); υ_{max}(Nujol)/cm^{-1} 3320 (NH_2), 1321, 1150 and 1137 (SO_2) and 1086 (CO); δ_H[300 MHz; $(C^2H_3)_2SO$; 80 °C] -0.24 (3 H, s, CH_3), 2.80 (2 H, t, *J* 6.3, CH_2NH_2), 3.64 (2 H, t, *J* 6.3, CH_2OB), 5.63 (2 H, br s, NH_2), 7.23-7.28 (1 H, m, Ar-H), 7.39-7.44 (1 H, m, Ar-H), 7.51-7.68 (4 H, m, Ar-H), 7.77-7.81 (2 H, m, Ar-H) and 7.89-7.97 (1 H, m, Ar-H); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 8.50 (br, CH_3), 41.62 (CH_2NH_2), 61.95 (CH_2OB), 126.25, 127.27, 129.35, 129.80, 131.83, 133.04 and 134.74 (Ar-CH), 143.15 and 143.37 [Ar-(CSO_2)] and 154.64 (br, Ar-CB); δ_B[96.3 MHz;

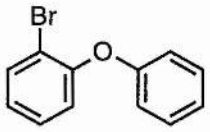
$(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 6.09; *m/z* (CI) 546 {37%, [2M + H - $NH_2(CH_2)_2OH$]$^+$}, 344 (100), 304 (70, [M + H]$^+$), 243 {12, [M + H - $NH_2(CH_2)_2OH$ - H_2O]$^+$} and 102 (45).

2-(Bromophenylthio)benzene, 154



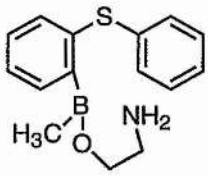
To stirred molten 2 bromoaniline (6.88 g, 40 mmol) was added sulfuric acid (18 mol dm^{-3}; 5 g, 50 mmol) in water (75 cm^3). The mixture was heated to 60 °C to effect complete dissolution of the amine. The solution was then cooled to 0 °C, vigorously stirred and treated with ice (25 g) then dropwise with a solution of sodium nitrite (3 g, 42.2 mmol) in water (25 cm^3), maintaining the temperature at 0-3 °C. After confirmation of the presence of excess nitrous acid (starch-iodide paper), the bright yellow suspension was neutralised to pH 4 with sodium acetate (2.5 g, 30 mmol). The suspension was transferred to a dropping funnel and added dropwise to a stirred, ice-cold mixture of thiophenol (4.54 g, 40 mmol), NaOH (4.82 g, 120.5 mmol) and copper powder (3 g, 47.14 mmol) in water (25 cm^3). After the addition of the *diazonium salt* was complete, the solution was heated to 90 °C for 1 h. The solution was then cooled to room temperature, extracted with ether (4 x 100 cm^3) and the combined organic extracts washed with brine (50 cm^3), dried and evaporated to leave a brown oil. Purification by flash column chromatography (eluant petrol-ethyl acetate 100:0.5) and then by Kügelrohr distillation (oven temp. 120 °C; 10 mm Hg) afforded *sulfide* **281** as a pale yellow oil (4.98 g, 48%) which did not crystallise upon standing (lit.,[281] mp 34-35 °C); δ_H(200 MHz; C^2HCl_3) 6.93 (1 H, dd, *J* 7.7 and 1.8, Ar-H), 7.00-7.08 (1 H, m, Ar-H), 7.12-7.20 (1 H, m, Ar-H), 7.37- 7.50 (5 H, m, Ar-H) and 7.57 (1 H, dd, *J* 7.8 and 1.4, 3'-H); δ_C(50.3 MHz; C^2HCl_3) 122.96 (Ar-CBr), 127.19, 127.77, 128.44, 129.61, 129.67, 132.97 and 133.48 (Ar-CH) 132.78 (Ar-C-1) and 138.74 (Ar-C1'); *m/z* (CI) 365 and 367 (100%, [M + H]$^+$), 218 (20) and 185 (15, [M + H - HBr]$^+$).

2-Bromophenyl)oxybenzene, 155



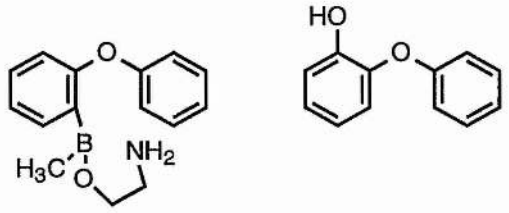
To a stirred mixture of 2-phenoxyaniline (5.15 g, 27.5 mmol) and aqueous HBr (48%; 10 cm^3), cooled to 0-5 °C, was added dropwise a solution of sodium nitrite (1.95 g, 27.5 mmol) in water (5 cm^3). After confirmation of the presence of excess nitrous acid (starch-iodide paper), the solution was added to a boiling solution of aqueous HBr (48%; 4 cm^3) that contained cuprous bromide (4.4 g, 30.14 mmol) and heated under reflux for 30 min. The resultant black solution was extracted with ether (5 x 50 cm^3) and the combined green organic extracts were dried and evaporated to leave a black solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 100:1) to afford *ether* **155** as a white solid (4.82 g, 73%), mp 42.5-44.5 °C (lit.,[283] 43.5-44.5 °C); υ_{max}(Nujol)/cm^{-1} 1236 (ether); δ_H(200 MHz; C^2HCl_3) 6.96-7.41 (8 H, m, Ar-H) and 7.62-7.69 (1 H, m, 3'-H); δ_C(50.3 MHz; C^2HCl_3) 114.87 (Ar-CBr), 118.07, 120.56, 123.35, 124.94, 128.62, 129.75 and 133.78 (Ar-CH) and 153.61 and 156.81 (Ar-CO); *m/z* (CI) 249 and 251 (100%, $[M + H]^+$) and 169 (8, $[M + H - HBr]^+$).

2-Aminoethyl [2-(phenylthio)phenyl](methyl) borinate, 156



To a stirred solution of 2-(bromophenylthio)benzene **154** (1.40 g, 5.3 mmol) in THF (50 cm^3), cooled to -70 °C, was added dropwise *n*-BuLi (2.5 mol dm^{-3} in hexanes; 2.3 cm^3; 5.75 mmol). The resultant solution was stirred for 30 min. Di*iso*propoxymethylborane (1 cm^3; 0.76 g, 5.3 mmol) was added dropwise and the solution stirred for a further 30 min at -70 °C before it was allowed to warm to room temperature over 1 h. Saturated aqueous ammonium chloride (30 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 30 cm^3). The combined organic extracts were dried and concentrated to *ca.* 30 cm^3 and ethanolamine (1 cm^3) was added. The mixture was left for 2 days during which time the volume of the solution reduced to *ca.* 5 cm^3. The resultant pale yellow crystals were recrystallised twice from ethyl acetate-cyclohexane to give pure *ethanolamine ester* **156** as pale yellow crystals (843 mg, 59%), mp 150.5-151.5 °C (Found: C, 66.6; H, 7.0; N, 5.2. $C_{15}H_{18}BNOS$ requires C, 66.45; H, 6.7; N, 5.15%) (HRMS: found $[M + H]^+$, 272.1271. $C_{15}H_{19}BNOS$ requires 272.1280); υ_{max}(Nujol)/cm^{-1} 3301 (NH_2) and 1071 (CO); δ_H[300 MHz; $(C^2H_3)_2SO$; 60 °C] -0.15 (3 H, s, CH_3), 2.77 (2 H, apparent quintet, CH_2NH_2), 3.68 (2 H, t, *J* 6.3, CH_2OB), 5.58 (2 H, br s, NH_2), 6.99-7.30 (8 H, m, Ar-H) and 7.58 (1 H, d, *J* 7.2, Ar-H); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 7.87 (br, CH_3), 41.84 (CH_2NH_2), 62.14 (CH_2OB), 126.11, 126.38, 126.63, 129.28, 130.34, 131.89 and 132.61 (Ar-CH), 137.21 and 138.40 (Ar-CS) and 155.88 (br, Ar-CB); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 6.24; *m/z* (CI) 272 (18%, $[M + H]^+$), 229 (100, $[M + H - CH_2{=}CHNH_2]^+$) and 227 (13, $[Ph_2S + H]^+$).

2-Aminoethyl [2-(phenyloxy)phenyl](methyl) borinate, 157 and 2-Phenoxyphenol, 153



157 **153**

Method 1

To a stirred solution of diphenyl ether (850 mg, 5 mmol) in THF (50 cm^3), cooled to -70 °C, was added dropwise *t*-BuLi (1.5 mol dm^{-3} in pentanes; 7 cm^3; 10.5 mmol). The resultant solution was allowed to warm to room temperature over 30 min, then cooled to -50 °C. Di*iso*propoxymethylborane (920 mm^3; 720 mg, 5 mmol) was added dropwise and the solution stirred for a further 2 h. Saturated aqueous ammonium chloride (30 cm^3) was added, the layers were separated and the aqueous phase was extracted further with ether (3 x 50 cm^3). The combined organic extracts were dried and evaporated to leave a pale yellow solid which was purified by flash column chromatography (eluant petrol-ethyl acetate 15:1) to give returned start material (340 mg, 40%), *borinic acid* **144** (70 mg, 7%), 2-phenoxyphenol **153** (150 mg, 16%) and *borinic acid* **83** (330 mg, 33%).

Borinic acid **144**, an oil, had an R_F identical to intermediate **144** prepared as part of the synthesis of *ethanolamine ester* **157** below; δ_H[300 MHz; $(C^2H_3)_2SO$] 0.6 (3 H, s, CH_3), 6.86-6.94 (3 H, m, Ar-H), 7.05-7.22 (2 H, m, Ar-H), 7.32-7.48 (3 H, m, Ar-H), 7.71 (1 H, d, *J* 7.3, Ar-H) and 9.70 (1 H, s, OH).

2-Phenoxyphenol **153**, mp 105-106 °C (from acetone) (lit.,[292] 104.5-106 °C) (HRMS: found M^+, 186.0688. Calc. for $C_{12}H_{10}O_2$: 186.0681); υ_{max}(Nujol)/cm^{-1} 3412 (OH) and 1240 (ether); δ_H[300 MHz; $(C^2H_3)_2SO$] 6.77-6.84 (3 H, m, Ar-H), 6.92-7.05 (4 H, m,

Ar-H), 7.26-7.31 (2 H, m, Ar-H) and 9.49 (1 H, s, OH); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 116.70, 117.90, 120.24, 122.44, 122.56, 126.05 and 130.15 (Ar-CH) and 143.06, 150.06 and 158.64 (Ar-C quaternary); *m/z* (CI) 243 (33%, $[M + C_4H_8 + H]^+$) and 187 (100, $[M + H]^+$).

Borinic acid **83** had physical properties identical to those described earlier.

Method 2

Ethanolamine ester **157** was better prepared in a manner similar to *ethanolamine ester* **156** and employed 2-bromophenyloxybenzene **155** (2.41 g, 10 mmol) in THF (80 cm^3), *n*-BuLi (2.5 mol dm^{-3} in hexanes; 4.5 cm^3; 11.25 mmol), di*iso*propoxymethylborane (2.15 cm^3; 1.63 g, 11.3 mmol) and saturated aqueous ammonium chloride (40 cm^3). After the layers were separated, the aqueous phase was extracted further with ether (3 x 50 cm^3). The combined organic extracts containing crude borinic acid **144** were dried and concentrated to *ca.* 50 cm^3 and a mixture of ethanolamine (3 cm^3) and methanol (3 cm^3) was added. The mixture was left for 2 days during which time the volume of the solution reduced to *ca.* 5 cm^3. The resultant large white crystals precipitated were filtered off, washed with water (200 cm^3) and dried under reduced pressure in a desiccator over phosphorus pentoxide for 3 days to give pure *ethanolamine ester* **157** (2.13 g, 82%), mp 180.5-181.5 °C (Found: C, 70.8; H, 7.3; N, 5.45. $C_{15}H_{18}BNO_2$ requires C, 70.6; H, 7.1; N, 5.5%) (HRMS: found $[M + H]^+$, 256.1513. $C_{15}H_{19}BNO_2$ requires 256.1509]; υ_{max}(Nujol)/cm^{-1} 3332 (NH_2), 1226 (ether) and 1064 (CO); δ_H[300 MHz; $(C^2H_3)_2SO$; 60 °C] -0.24 (3 H, s, CH_3), 2.75 (2 H, apparent quintet, CH_2NH_2), 3.65 (2 H, t, *J* 6.3, CH_2OB), 5.43 (2 H, br s, NH_2), 6.56 (1 H, dd, *J* 1.0 and 8.0, Ar-H), 6.88-7.04 (5 H, m, Ar-H), 7.27-7.33 (2 H, m, Ar-H) and 7.45 (1 H, dd, *J* 2.0 and 7.2, Ar-H); δ_C[75.4 MHz; $(C^2H_3)_2SO$] 7.74 (br, CH_3), 41.46 (CH_2NH_2), 62.33 (CH_2OB), 117.10, 118.98, 122.55, 122.66, 126.74, 129.93 and 133.33 (Ar-CH), 143.76 (br,

Ar-CB) and 158.24 and 159.54 (Ar-CO); δ_B[96.3 MHz; $(CH_3)_2CO$-$(C^2H_3)_2CO$, 3:1] 5.89; *m/z* (ES+) 295 (15%, $[M + K]^+$), 276 (10, $[M + Na]^+$) and 255 (100, M^+).

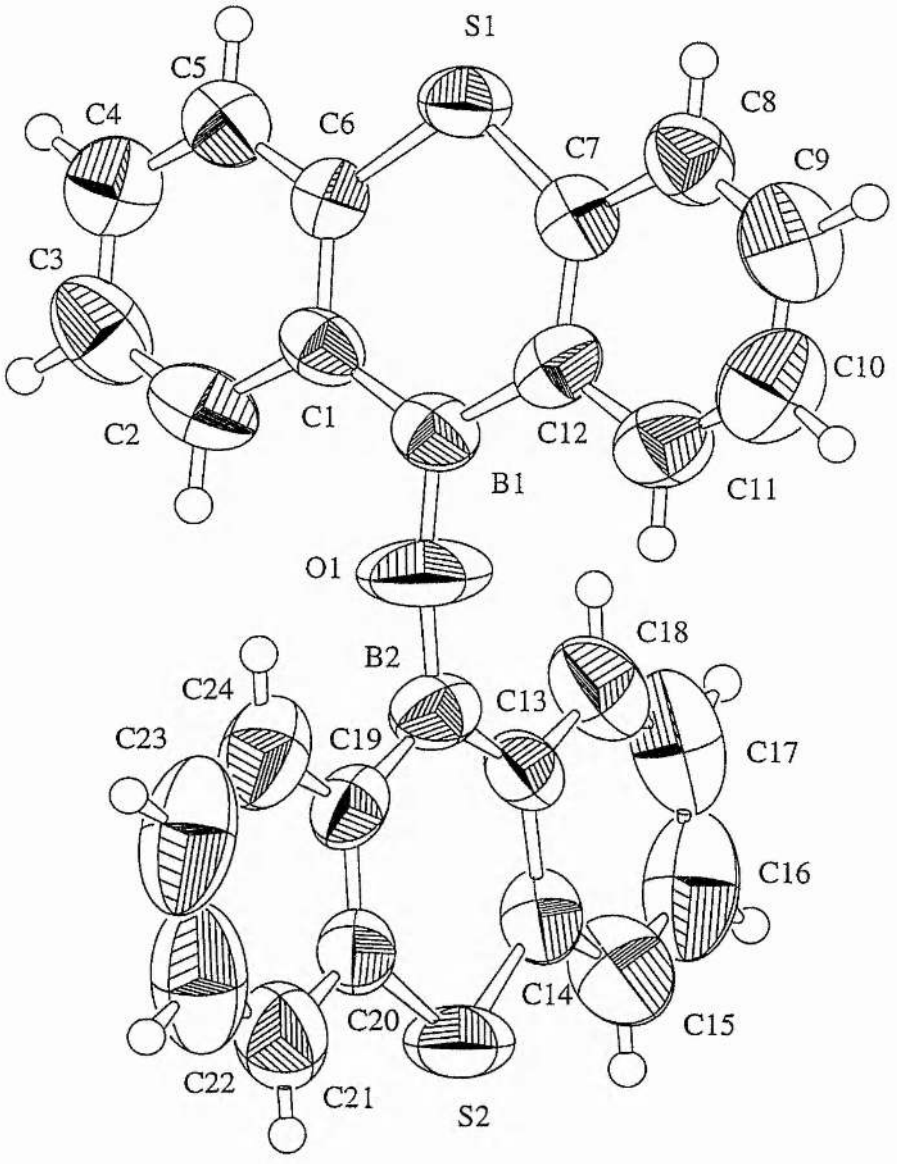
HIV-1 PR *in vitro* assay

The assay was based upon the hydrolytic cleavage of the decapeptide Lys-Ala-Arg-Val-Nle*(NO_2)Phe-Glu-Ala-Nle-Gly-NH_2 which leads to a reduction in the absorbence at 300 nm. The peptide was stored as a 10 mg cm^{-3} solution in water and 2-5 mm^3 of this solution was added to 1 cm^3 of assay buffer (100 mmol dm^{-3} NaOAc, 200 mmol dm^{-3} NaCl, 1 mmol dm^{-3} dithiothreitol at pH 5.6). The compounds to be tested were made up as 10 mmol dm^{-3} stock solutions in methanol and were used at concentrations over the range 1-100 μmol dm^{-3}. Methanol was added to a final concentration of 2% in the assay mixture and the solution was allowed to thermally equilibrate to 37 °C before the reaction was initiated by the addition of 15 mm^3 of enzyme solution. (The enzyme was stored as a 993 mmol dm^{-3} solution in 10 mmol dm^{-3} NaOAc containing 0.05% 2-mercaptoethanol, 1 mmol dm^{-3} EDTA, 20% glycerol and 5% ethylene glycol).

APPENDIX 1

CRYSTALLOGRAPHIC DATA

Crystallographic Data for Compound 117



$C_{24}H_{16}B_2OS_2$, M = 406.13, orthorhombic, space group $Pbca$ (#61), a = 13.44(1), b = 23.36(1), c = 12.71(1) Å, V = 3990(4) Å^3, Z = 8, D_C = 1.352 g cm^{-3}, T = 293 K. 2958 unique reflections were collected on a Rigaku AFC7S diffractometer employing Mo-Kα radiation (λ = 0.71069 Å) of which 1197 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 5.0%, $R_W(F)$ = 3.6% for 262 variables. The structures of **117** and also **122**, **128**, **131**, **140** and **141** were solved using SIR92 and were refined using TEXSAN.[293]

Table A1.1: Bond Lengths (Å) for 117

atom	atom	distance	atom	atom	distance
S(1)	C(6)	1.714(7)	S(1)	C(7)	1.745(8)
S(2)	C(14)	1.735(8)	S(2)	C(20)	1.737(8)
O(1)	B(1)	1.353(9)	O(1)	B(2)	1.337(9)
C(1)	C(2)	1.41(1)	C(1)	C(6)	1.419(9)
C(1)	B(1)	1.50(1)	C(2)	C(3)	1.37(1)
C(3)	C(4)	1.378(10)	C(4)	C(5)	1.367(10)
C(5)	C(6)	1.378(9)	C(7)	C(8)	1.374(9)
C(7)	C(12)	1.431(9)	C(8)	C(9)	1.40(1)
C(9)	C(10)	1.38(1)	C(10)	C(11)	1.33(1)
C(11)	C(12)	1.42(1)	C(12)	B(1)	1.52(1)
C(13)	C(14)	1.422(9)	C(13)	C(18)	1.40(1)
C(13)	B(2)	1.53(1)	C(14)	C(15)	1.36(1)
C(15)	C(16)	1.35(1)	C(16)	C(17)	1.39(1)
C(17)	C(18)	1.35(1)	C(19)	C(20)	1.392(9)
C(19)	C(24)	1.38(1)	C(19)	B(2)	1.54(1)
C(20)	C(21)	1.39(1)	C(21)	C(22)	1.33(1)
C(22)	C(23)	1.37(2)	C(23)	C(24)	1.36(2)
C(2)	H(6)	0.95	C(3)	H(9)	0.95
C(4)	H(5)	0.95	C(5)	H(2)	0.95
C(8)	H(1)	0.95	C(9)	H(7)	0.95
C(10)	H(15)	0.95	C(11)	H(4)	0.95
C(15)	H(3)	0.95	C(16)	H(8)	0.95
C(17)	H(16)	0.95	C(18)	H(14)	0.95
C(21)	H(13)	0.95	C(22)	H(11)	0.95
C(23)	H(10)	0.95	C(24)	H(12)	0.95

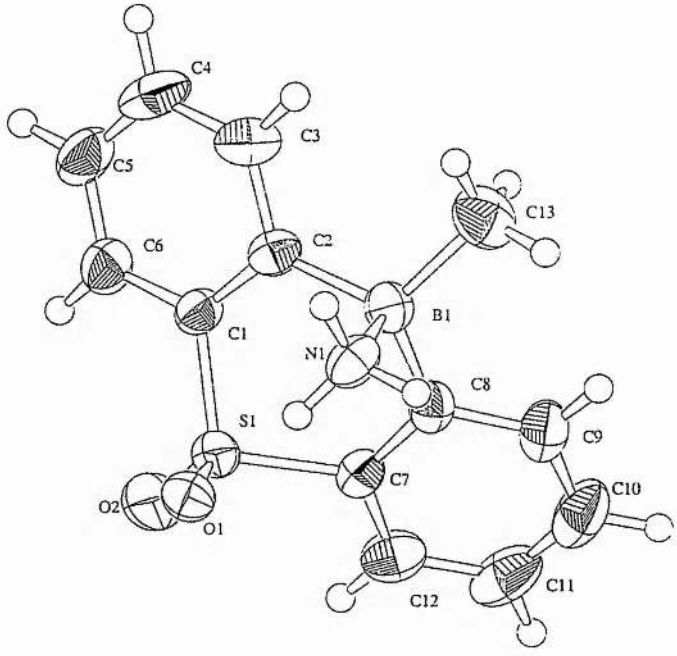
Table A1.2: Bond Angles (°) for 117

atom	atom	atom	distance	atom	atom	atom	distance
C(6)	S(1)	C(7)	107.1(4)	C(14)	S(2)	C(20)	107.0(5)
B(1)	O(1)	B(2)	169.2(9)	C(2)	C(1)	C(6)	115.7(8)
C(2)	C(1)	B(1)	123.4(8)	C(6)	C(1)	B(1)	120.9(8)
C(1)	C(2)	C(3)	123.7(8)	C(2)	C(3)	C(4)	118.9(9)

C(3)	C(4)	C(5)	119.7(9)	C(4)	C(5)	C(6)	122.3(8)
S(1)	C(6)	C(1)	124.9(7)	S(1)	C(6)	C(5)	115.4(7)
C(1)	C(6)	C(5)	119.7(7)	S(1)	C(7)	C(8)	116.1(7)
S(1)	C(7)	C(12)	122.7(7)	C(8)	C(7)	C(12)	121.2(8)
C(7)	C(8)	C(9)	120.9(8)	C(8)	C(9)	C(10)	117.9(10)
C(9)	C(10)	C(11)	121(1)	C(10)	C(11)	C(12)	123.2(9)
C(7)	C(12)	C(11)	114.8(8)	C(7)	C(12)	B(1)	121.5(8)
C(11)	C(12)	B(1)	123.7(8)	C(14)	C(13)	C(18)	114.7(8)
C(14)	C(13)	B(2)	121.1(8)	C(18)	C(13)	B(2)	124.1(9)
S(2)	C(14)	C(13)	124.0(7)	S(2)	C(14)	C(15)	114.5(8)
C(13)	C(14)	C(15)	121.5(8)	C(14)	C(15)	C(16)	121(1)
C(15)	C(16)	C(17)	119(1)	C(16)	C(17)	C(18)	119(1)
C(13)	C(18)	C(17)	123(1)	C(20)	C(19)	C(24)	117.0(9)
C(20)	C(19)	B(2)	121.6(9)	C(24)	C(19)	B(2)	121.4(10)
S(2)	C(20)	C(19)	124.4(8)	S(2)	C(20)	C(21)	115.1(9)
C(19)	C(20)	C(21)	120.5(9)	C(20)	C(21)	C(22)	119(1)
C(21)	C(22)	C(23)	122(1)	C(22)	C(23)	C(24)	118(1)
C(19)	C(24)	C(23)	122(1)	O(1)	B(1)	C(1)	118.9(8)
O(1)	B(1)	C(12)	118.3(8)	C(1)	B(1)	C(12)	122.9(7)
O(1)	B(2)	C(13)	120.0(9)	O(1)	B(2)	C(19)	118.3(9)
C(13)	B(2)	C(19)	121.7(8)				

C(1)	C(2)	H(6)	118.2	C(3)	C(2)	H(6)	118.2
C(2)	C(3)	H(9)	120.6	C(4)	C(3)	H(9)	120.6
C(3	C(4)	H(5)	120.1	C(5)	C(4)	H(5)	120.1
C(4)	C(5)	H(2)	118.9	C(6)	C(5)	H(2)	118.9
C(7)	C(8)	H(1)	119.5	C(9)	C(8)	H(1)	119.5
C(8)	C(9)	H(7)	121.0	C(10)	C(9)	H(7)	121.0
C(9)	C(10)	H(15)	119.1	C(11)	C(10)	H(15)	119.1
C(10)	C(11)	H(4)	118.4	C(12)	C(11)	H(4)	118.4
C(14)	C(15)	H(3)	119.4	C(16)	C(15)	H(3)	119.3
C(15)	C(16)	H(8)	120.1	C(17)	C(16)	H(8)	120.0
C(16)	C(17)	H(16)	120.4	C(18)	C(17)	H(16)	120.4
C(13)	C(18)	H(14)	118.3	C(17)	C(18)	H(14)	118.3
C(20)	C(21)	H(13)	120.2	C(22)	C(21)	H(13)	120.1
C(21)	C(22)	H(11)	119.0	C(23)	C(22)	H(11)	118.9
C(22)	C(23)	H(10)	120.9	C(24)	C(23)	H(10)	120.9
C(19)	C(24)	H(12)	118.8	C(23)	C(24)	H(12)	118.7

Crystallographic Data for Compound 122



$C_{13}H_{14}BNO_2S$, M = 259.13, monoclinic, space group $P2_1/c$ (#14), a = 10.672(6), b = 8.915(4), c = 13.695(5) Å, β = 99.77(4) °, V = 1284(1) Å^3, Z = 4, D_C = 1.340 g cm^{-3}, T = 293 K. 2432 unique reflections were collected on a Rigaku AFC7S diffractometer employing Mo-Kα radiation (λ = 0.71069 Å) of which 1683 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 3.7%, $R_W(F)$ = 2.8% for 163 variables.

Table A1.3: Bond Lengths (Å) for 122

atom	atom	distance	atom	atom	distance
S(1)	O(1)	1.460(2)	S(1)	O(2)	1.449(2)
S(1)	C(1)	1.762(3)	S(1)	C(7)	1.757(2)
N(1)	B(1)	1.642(3)	C(1)	C(2)	1.390(3)
C(1)	C(6)	1.387(3)	C(2)	C(3)	1.400(3)
C(2)	B(1)	1.640(4)	C(3)	C(4)	1.385(4)
C(4)	C(5)	1.371(4)	C(5)	C(6)	1.375(4)
C(7)	C(8)	1.393(3)	C(7)	C(12)	1.392(3)
C(8)	C(9)	1.397(3)	C(8)	B(1)	1.628(4)
C(9)	C(10)	1.379(4)	C(10)	C(11)	1.372(4)
C(11)	C(12)	1.382(4)	C(13)	B(1)	1.607(4)

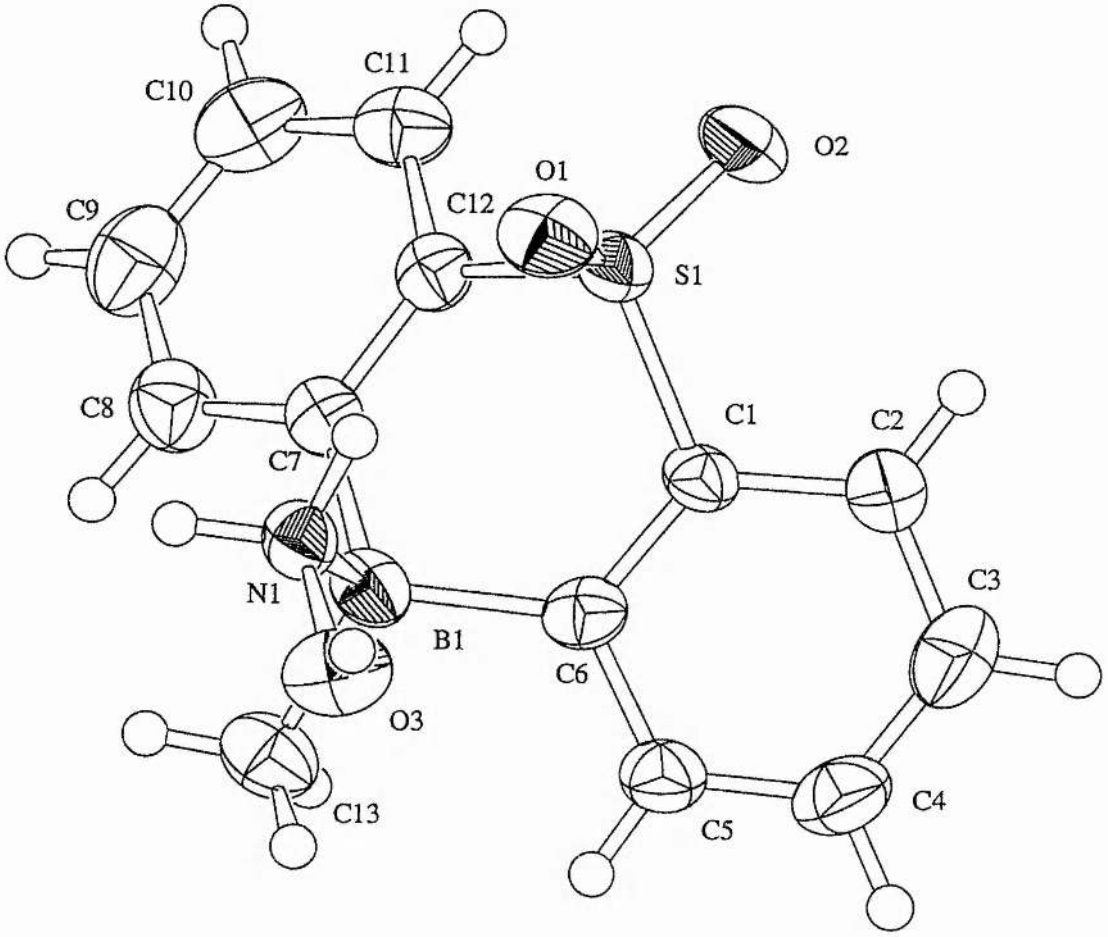
N(1)	H(12)	0.95	N(1)	H(13)	0.95
N(1)	H(14)	0.95	C(3)	H(3)	0.95
C(4)	H(4)	0.95	C(5)	H(2)	0.95
C(6)	H(1)	0.95	C(9)	H(7)	0.95
C(10)	H(6)	0.95	C(11)	H(8)	0.95
C(12)	H(5)	0.95	C(13)	H(9)	0.95
C(13)	H(10)	0.95	C(13)	H(11)	0.95

Table A1.4: Bond Angles (°) for 122

atom	atom	atom	distance	atom	atom	atom	distance
O(1)	S(1)	O(2)	115.7(1)	O(1)	S(1)	C(1)	107.3(1)
O(1)	S(1)	C(7)	107.3(1)	O(2)	S(1)	C(1)	110.9(1)
O(2)	S(1)	C(7)	110.4(1)	C(1)	S(1)	C(7)	104.5(1)
S(1)	C(1)	C(2)	116.8(2)	S(1)	C(1)	C(6)	118.2(2)
C(2)	C(1)	C(6)	124.9(2)	C(1)	C(2)	C(3)	114.0(2)
C(1)	C(2)	B(1)	122.8(2)	C(3)	C(2)	B(1)	123.1(2)
C(2)	C(3)	C(4)	122.4(3)	C(3)	C(4)	C(5)	120.8(3)
C(4)	C(5)	C(6)	119.5(3)	C(1)	C(6)	C(5)	118.3(3)
S(1)	C(7)	C(8)	117.0(2)	S(1)	C(7)	C(12)	117.6(2)
C(8)	C(8)	C(12)	125.5(2)	C(7)	C(8)	C(9)	114.0(2)
C(7)	C(9)	B(1)	123.3(2)	C(9)	C(8)	B(1)	122.7(2)
C(8)	C(11)	C(10)	122.4(3)	C(9)	C(10)	C(11)	121.1(3)
C(10)	C(1)	C(12)	119.8(3)	C(7)	C(12)	C(11)	117.4(3)
N(1)	B(1)	C(2)	105.9(2)	N(1)	B(1)	C(8)	106.9(2)
N(1)	B(1)	C(13)	107.1(2)	C(2)	B(1)	C(8)	110.6(2)
C(2)	B(1)	C(13)	112.7(2)	C(8)	B(1)	C(13)	113.1(2)
B(1)	N(1)	H(12)	109.4	B(1)	N(1)	H(13)	109.3
B(1)	N(1)	H(14)	109.3	H(12)	N(1)	H(13)	109.6
H(12)	N(1)	H(14)	109.6	H(13)	N(1)	H(14)	109.5
C(2)	C(3)	H(3)	118.8	C(4)	C(3)	H(3)	118.8
C(3)	C(4)	H(4)	119.6	C(5)	C(4)	H(4)	119.6
C(4)	C(5)	H(2)	120.3	C(6)	C(5)	H(2)	120.2
C(1)	C(6)	H(1)	120.9	C(5)	C(6)	H(1)	120.8
C(8)	C(9)	H(7)	118.8	C(10)	C(9)	H(7)	118.8
C(9)	C(10)	H(6)	119.5	C(11)	C(10)	H(6)	119.4
C(10)	C(11)	H(8)	120.2	C(12)	C(11)	H(8)	120.0

C(7)	C(12)	H(5)	121.2	C(11)	C(12)	H(5)	121.3
B(1)	C(13)	H(9)	109.2	B(1)	C(13)	H(10)	109.3
B(1)	C(13)	H(11)	109.3	H(9)	C(13)	H(10)	109.6
H(9)	C(13)	H(11)	109.7	H(10)	C(13)	H(11)	109.8

Crystallographic Data for Compound 128



$C_{13}H_{14}BNO_3S$, M = 275.13, monoclinic, space group C2/c (#15), a = 23.615(4), b = 8.665(5), c = 13.643(5) Å, β = 108.09(2) °, V = 2653(1) Å^3, Z = 8, D_C = 1.377 g cm^{-3}, T = 293 K. 2509 unique reflections were collected on a Rigaku AFC7S diffractometer employing Mo-Kα radiation (λ = 0.71069 Å) of which 1941 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 7.5%, $R_W(F)$ = 8.4% for 177 variables.

Table A1.5: Bond Lengths (Å) for 128

atom	atom	distance	atom	atom	distance
S(1)	O(1)	1.463(4)	S(1)	O(2	1.448(4)
S(1)	C(1)	1.770(5)	S(1)	C(12)	1.751(5)
O(3)	N(1)	1.436(6)	N(1)	B(1)	1.630(7)
C(1)	C(2)	1.409(7)	C(1)	C(6)	1.381(7)
C(2)	C(3)	1.387(8)	C(3)	C(4)	1.368(9)
C(4)	C(5)	1.368(8)	C(5)	C(6)	1.402(7)
C(6)	B(1)	1.648(8)	C(7)	C(8)	1.396(7)
C(7)	C(12)	1.398(7)	C(7)	B(1)	1.622(8)
C(8)	C(9)	1.393(9)	C(9)	C(10)	1.363(9)
C(10)	C(11)	1.385(8)	C(11)	C(12)	1.392(8)
C(13)	B(1)	1.585(8)			
O(3)	H(14)	1.12(9)	N(1)	H(12)	0.95
N(1)	H(13)	0.95	C(2)	H(1)	0.95
C(3)	H(2)	0.95	C(4)	H(3)	0.95
C(5)	H(4)	0.95	C(8)	H(5)	0.95
C(9)	H(6)	0.95	C(10)	H(7)	0.95
C(11)	H(8)	0.95	C(13)	H(9)	0.95
C(13)	H(10)	0.95	C(13)	H(11)	0.95

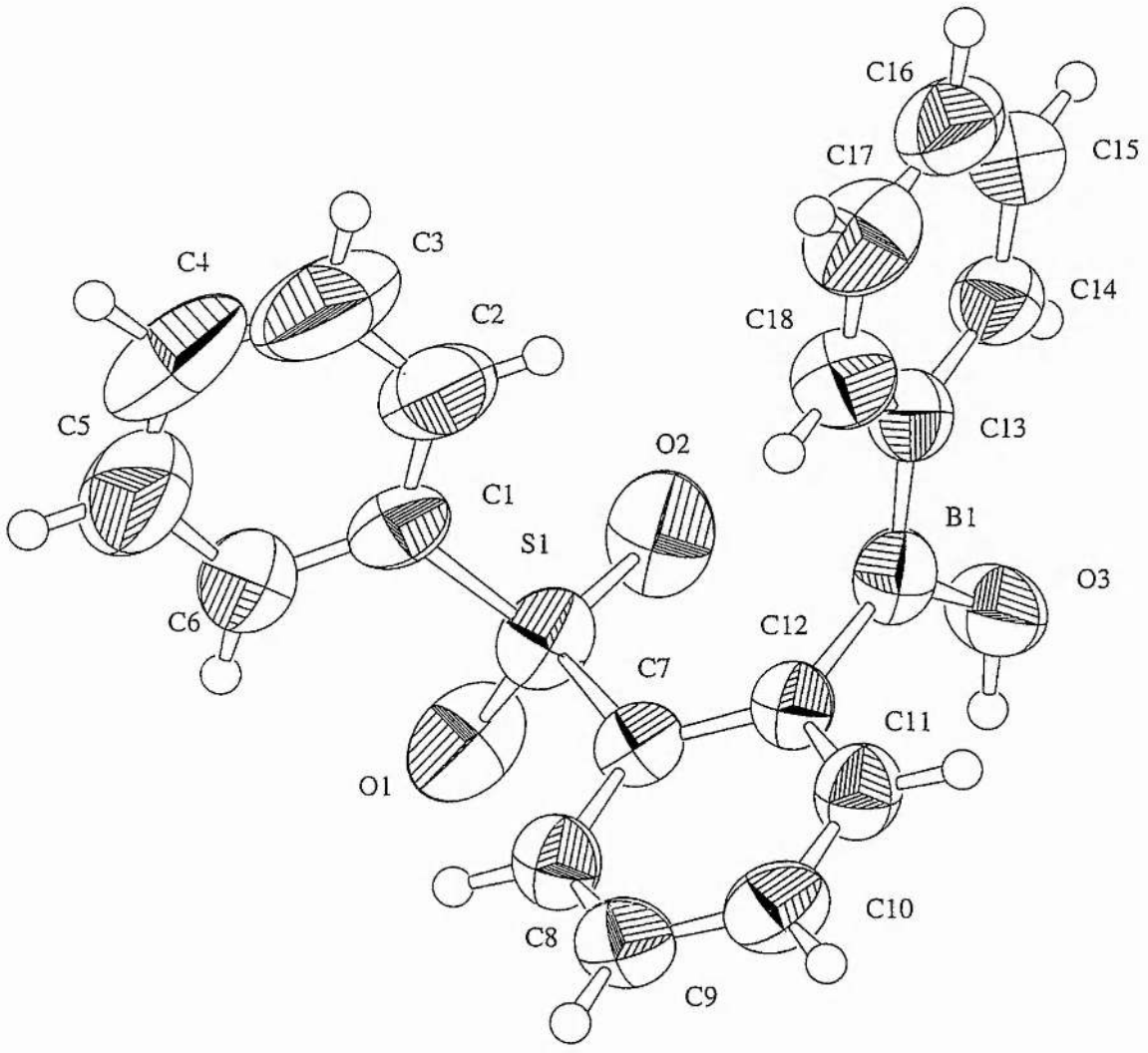
Table A1.6: Bond Angles (°) for 128

atom	atom	atom	distance	atom	atom	atom	distance
O(1)	S(1)	O(2)	115.0(2)	O(1)	S(1)	C(1)	107.1(2)
O(1)	S(1)	C(12)	107.8(2)	O(2)	S(1)	C(1)	110.5(2)
O(2)	S(1)	C(12)	110.8(3)	C(1)	S(1)	C(12)	105.1(3)
O(3)	N(1)	B(1)	112.9(4)	S(1)	C(1)	C(2)	116.1(4)
S(1)	C(1)	C(6)	117.5(4)	C(2)	C(1)	C(6)	126.1)
C(1)	C(2)	C(3)	115.5(5)	C(2)	C(3)	C(4)	120.6(6)
C(3)	C(4)	C(5)	121.6(6)	C(4)	C(5)	C(6)	121.9(6)
C(1)	C(6)	C(5)	114.2(5)	C(1)	C(6)	B(1)	123.4(5)
C(5)	C(6)	B(1)	122.3(5)	C(8)	C(7)	C(12)	114.6(5)
C(8)	C(7)	B(1)	121.8(5)	C(12)	C(7)	B(1)	123.6(5)
C(7)	C(8)	C(9)	122.0(6)	C(8)	C(9)	C(10)	120.8(6)

C(9)	C(10)	C(11)	120.2(6)	C(10)	C(11)	C(12)	117.6(6)
S(1)	C(12)	C(7)	117.5(4)	S(1)	C(12)	C(11)	117.5(4)
C(7)	C(12)	C(11)	124.8(5)	N(1)	B(1)	C(6)	105.3(4)
N(1)	B(1)	C(7)	103.1(4)	N(1)	B(1)	C(13)	107.8(4)
C(6)	B(1)	C(7)	110.9(4)	C(6)	B(1)	C(13)	114.2(5)
C(7)	B(1)	C(13)	114.3(5)				

N(1)	O(3)	H(14)	125(4)	O(3)	N(1)	H(12)	108.5
O(3)	N(1)	H(13)	108.8	B(1)	N(1)	H(12)	108.5
B(1)	N(1)	H(13)	108.6	H(12)	N(1)	H(13)	109.5
C(1)	C(2)	H(1)	122.2	C(3)	C(2)	H(1)	122.3
C(2)	C(3)	H(2)	120.0	C(4)	C(3)	H(2)	119.4
C(3)	C(4)	H(3)	119.6	C(5)	C(4)	H(3)	118.9
C(4)	C(5)	H(4)	119.3	C(6)	C(5)	H(4)	118.9
C(7)	C(8)	H(5)	119.1	C(9)	C(8)	H(5)	118.9
C(8)	C(9)	H(6)	120.1	C(10)	C(9)	H(6)	119.1
C(9)	C(10)	H(7)	120.0	C(11)	C(10)	H(7)	119.8
C(10)	C(11)	H(8)	121.2	C(12)	C(11)	H(8)	121.2
B(1)	C(13)	H(9)	109.5	B(1)	C(13)	H(10)	109.1
B(1)	C(13)	H(11)	109.2	H(9)	C(13)	H(10)	109.8
H(9)	C(13)	H(11)	109.7	H(10)	C(13)	H(11)	109.4

Crystallographic Data for Compound 131



$C_{18}H_{15}BO_3S$, M = 322.18, monoclinic, space group C2/c (#15), a = 24.055(8), b = 7.957(4), c = 18.218(9) Å, β = 104.33(3) °V = 3378(2) Å^3, Z = 8, D_C = 1.267 g cm^{-3}, T = 293 K. 3203 unique reflections were collected on a Rigaku AFC7S diffractometer employing Mo-Kα radiation (λ = 0.71069 Å) of which 1442 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 4.5%, $R_W(F)$ = 3.5% for 208 variables.

Table A1.7: Bond Lengths (Å) for 131

atom	atom	distance	atom	atom	distance
S(1)	O(1)	1.434(3)	S(1)	O(2)	1.444(3)
S(1)	C(1)	1.776(4)	S(1)	C(7)	1.765(4)
O(3)	B(1)	1.363(5)	C(1)	C(2)	1.348(6)
C(1)	C(6)	1.376(6)	C(2)	C(3)	1.374(7)
C(3)	C(4)	1.355(9)	C(4)	C(5)	1.354(9)
C(5)	C(6)	1.389(7)	C(7)	C(8)	1.388(5)
C(7)	C(12)	1.387(5)	C(8)	C(9)	1.367(5)
C(9)	C(10)	1.378(6)	C(10)	C(11)	1.377(5)
C(11)	C(12)	1.400(5)	C(12)	B(1)	1.579(6)
C(13)	C(14)	1.395(5)	C(13)	C(18)	1.387(6)
C(13)	B(1)	1.543(6)	C(14)	C(15)	1.375(6)
C(15)	C(16)	1.367(7)	C(16)	C(17)	1.379(7)
C(17)	C(18)	1.372(6)			
O(3)	H(15)	0.96	C(2)	H(1)	0.95
C(3)	H(2)	0.94	C(4)	H(3)	0.95
C(5)	H(4)	0.96	C(6)	H(5)	0.96
C(8)	H(6)	0.95	C(9)	H(7)	0.95
C(10)	H(8)	0.95	C(11)	H(9)	0.95
C(14)	H(10)	0.94	C(15)	H(11)	0.95
C(16)	H(12)	0.95	C(17)	H(13)	0.95
C(18)	H(14)	0.95			

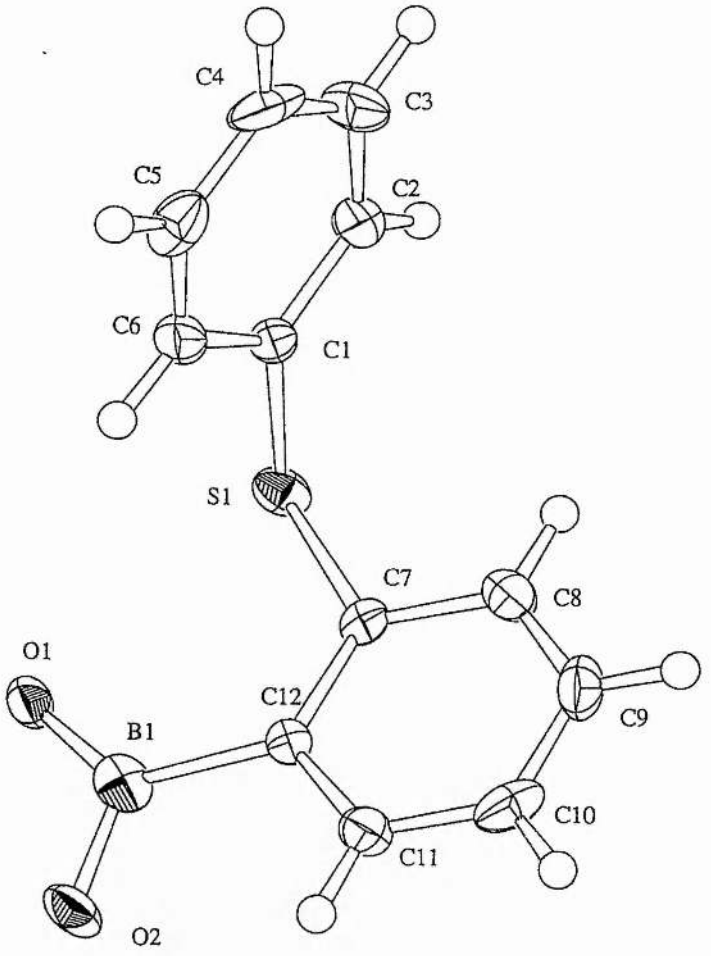
Table A1.8: Bond Angles (°) for 131

atom	atom	atom	distance	atom	atom	atom	distance
O(1)	S(1)	O(2)	120.0(2)	O(1)	S(1)	C(1)	106.9(2)
O(1)	S(1)	C(7)	109.1(2)	O(2)	S(1)	C(1)	107.0(2)
O(2)	S(1)	C(7)	106.7(2)	C(1)	S(1)	C(7)	106.4(2)
S(1)	C(1)	C(2)	119.7(4)	S(1)	C(1)	C(6)	118.2(4)
C(2)	C(1)	C(6)	122.1(4)	C(1)	C(2)	C(3)	119.9(6)
C(2)	C(3)	C(4)	119.1(7)	C(3)	C(4)	C(5)	121.3(7)
C(4)	C(5)	C(6)	120.5(7)	C(1)	C(6)	C(5)	117.1(5)
S(1)	C(7)	C(8)	118.2(3)	S(1)	C(7)	C(12)	117.9(3)

C(8)	C(7)	C(12)	123.9(4)	C(7)	C(8)	C(9)	119.3(4)
C(8)	C(9)	C(10)	119.1(4)	C(9)	C(10)	C(11)	120.8(4)
C(10)	C(11)	C(12)	122.3(4)	C(7)	C(12)	C(11)	114.6(4)
C(7)	C(12)	B(1)	126.1(4)	C(11)	C(12)	B(1)	119.2(4)
C(14)	C(13)	C(18)	116.2(4)	C(14)	C(13)	B(1)	121.2(4)
C(18)	C(13)	B(1)	122.5(4)	C(13)	C(14)	C(15)	121.9(5)
C(14)	C(15)	C(16)	120.4(5)	C(15)	C(16)	C(17)	119.1(5)
C(16)	C(17)	C(18)	120.2(5)	C(13)	C(18)	C(17)	122.1(5)
O(3)	B(1)	C(12)	120.5(4)	O(3)	B(1)	C(13)	116.7(4)
C(12)	B(1)	C(13)	121.6(4)				

B(1)	O(3)	H(15)	117.5	C(1)	C(2)	H(1)	120.2
C(3)	C(2)	H(1)	119.9	C(2)	C(3)	H(2)	121.8
C(4)	C(3)	H(2)	119.1	C(3)	C(4)	H(3)	121.4
C(5)	C(4)	H(3)	117.3	C(4)	C(5)	H(4)	121.0
C(6)	C(5)	H(4)	118.4	C(1)	C(6)	H(5)	121.0
C(5)	C(6)	H(5)	121.9	C(7)	C(8)	H(6)	120.6
C(9)	C(8)	H(6)	120.2	C(8)	C(9)	H(7)	120.4
C(10)	C(9)	H(7)	120.5	C(9)	C(10)	H(8)	119.5
C(11)	C(10)	H(8)	119.8	C(10)	C(11)	H(9)	118.8
C(12)	C(11)	H(9)	118.9	C(13)	C(14)	H(10)	119.5
C(15)	C(14)	H(10)	118.6	C(14)	C(15)	H(11)	120.0
C(16)	C(15)	H(11)	119.6	C(15)	C(16)	H(12)	120.4
C(17)	C(16)	H(12)	120.5	C(16)	C(17)	H(13)	119.9
C(18)	C(17)	H(13)	119.8	C(13)	C(18)	H(14)	119.1
C(17)	C(18)	H(14)	118.7				

Crystallographic Data for Compound 140



$C_{12}H_{11}BO_2S$, M = 230.09, monoclinic, space group $P2_1/c$ (#14), a = 5.129(6), b = 19.777(7), c = 11.337(6) Å, β = 98.74(7) °V = 1136(1) Å^3, Z = 4, D_C = 1.345 g cm^{-3}, T = 293 K. 1738 unique reflections were collected on a Rigaku AFC7S diffractometer employing Mo-Kα radiation (λ = 0.71069 Å) of which 1234 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 11.9%, $R_W(F)$ = 15.2% for 144 variables.

Table A1.9: Bond Lengths (Å) for 140

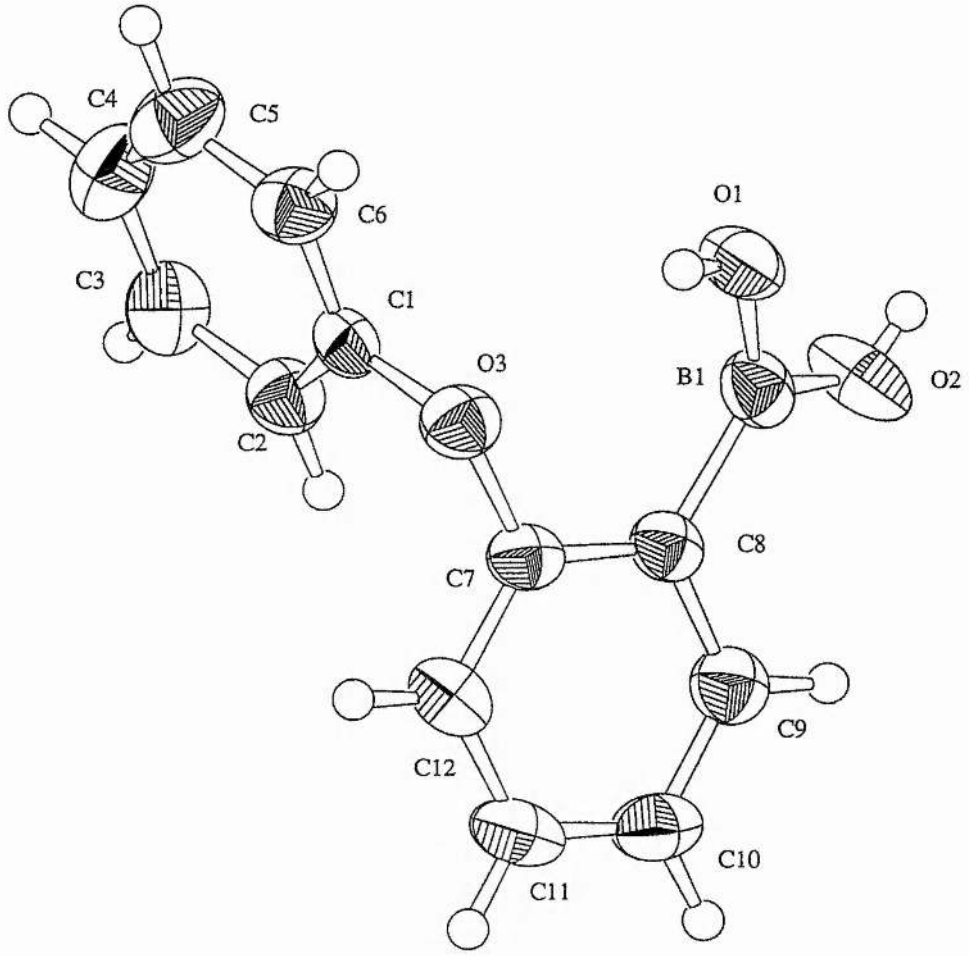
atom	atom	distance	atom	atom	distance
S(1)	C(1)	1.77(1)	S(1)	C(7)	1.80(1)
O(1)	B(1)	1.35(2)	O(2)	B(1)	1.35(2)
C(1)	C(2)	1.36(2)	C(1)	C(6)	1.38(2)
C(2)	C(3)	1.35(2)	C(3)	C(4)	1.36(2)

C(4)	C(5)	1.36(2)	C(5)	C(6)	1.37(2)
C(7)	C(8)	1.39(2)	C(7)	C(12)	1.38(2)
C(8)	C(9)	1.36(2)	C(9)	C(10)	1.36(2)
C(10)	C(11)	1.38(2)	C(11)	C(12)	1.38(2)
C(12)	B(1)	1.60(2)			
C(2)	H(1)	0.95	C(3)	H(2)	0.95
C(4)	H(3)	0.95	C(5)	H(4)	0.95
C(6)	H(5)	0.96	C(8)	H(6)	0.95
C(9)	H(7)	0.94	C(10)	H(8)	0.95
C(11)	H(9)	0.95			

Table A1.10: Bond Angles (°) for 140

atom	atom	atom	distance	atom	atom	atom	distance
C(1)	S(1)	C(7)	99.7(6)	S(1)	C(1)	C(2)	120(1)
S(1)	C(1)	C(6)	121(1)	C(2)	C(1)	C(6)	117(1)
C(1)	C(2)	C(3)	121(1)	C(2)	C(3)	C(4)	120(1)
C(3)	C(4)	C(5)	119(1)	C(4)	C(5)	C(6)	119(1)
C(1)	C(6)	C(5)	120(1)	S(1)	C(7)	C(8)	116(1)
S(1)	C(7)	C(12)	122(1)	C(8)	C(7)	C(12)	121(1)
C(7)	C(8)	C(9)	120(1)	C(8)	C(9)	C(10)	119(1)
C(9)	C(10)	C(11)	119(1)	C(10)	C(11)	C(12)	122(1)
C(7)	C(12)	C(11)	116(1)	C(7)	C(12)	B(1)	126(1)
C(11)	C(12)	B(1)	117(1)	O(1)	B(1)	O(2)	118(1)
O(1)	B(1)	C(12)	124(1)	O(2)	B(1)	C(12)	116(1)
C(1)	C(2)	H(1)	119.0	C(3)	C(2)	H(1)	119.4
C(2)	C(3)	H(2)	119.8	C(4)	C(3)	H(2)	120.1
C(3)	C(4)	H(3)	120.1	C(5)	C(4)	H(3)	120.0
C(4)	C(5)	H(4)	120.4	C(6)	C(5)	H(4)	119.7
C(1)	C(6)	H(5)	119.5	C(5)	C(6)	H(5)	119.9
C(7)	C(8)	H(6)	119.7	C(9)	C(8)	H(6)	120.0
C(8)	C(9)	H(7)	120.4	C(10)	C(9)	H(7)	119.8
C(9)	C(10)	H(8)	120.4	C(11)	C(10)	H(8)	119.9
C(10)	C(11)	H(9)	119.2	C(12)	C(11)	H(9)	118.2

Crystallographic Data for Compound 141



$C_{12}H_{11}BO_3$, M = 214.03, monoclinic, space group $P2_1/c$ (#14), a = 5.33(1), b = 7.639(7), c = 26.44(1) Å, β = 90.4(1) °V = 1077(2) Å^3, Z = 4, D_C = 1.319 g cm^{-3}, T = 293 K. 1652 unique reflections were collected on a Rigaku AFC7S diffractometer employing Mo-Kα radiation (λ = 0.71069 Å) of which 1127 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 3.2%, $R_W(F)$ = 2.5% for 154 variables.

Table A1.11: Bond Lengths (Å) for 141

atom	atom	distance	atom	atom	distance
O(1)	B(1)	1.367(4)	O(2)	B(1)	1.355(4)
O(3)	C(1)	1.385(2)	O(3)	C(7)	1.402(3)
C(1)	C(2)	1.386(3)	C(1)	C(6)	1.379(3)
C(2)	C(3)	1.382(3)	C(3)	C(4)	1.376(4)

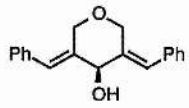
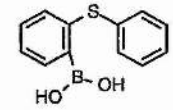
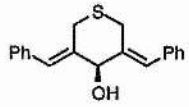
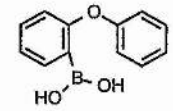
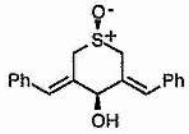
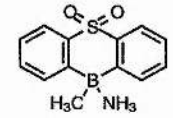
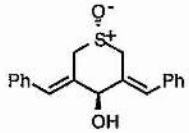
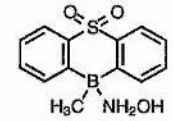
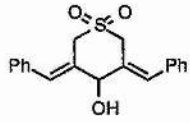
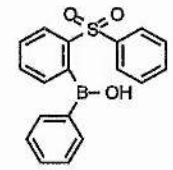
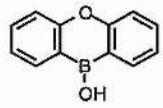
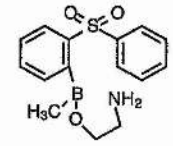
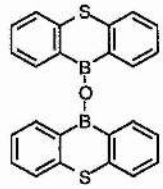
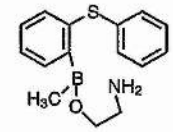
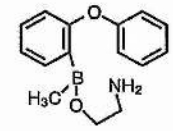
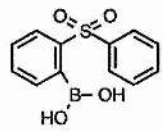
C(4)	C(5)	1.381(4)	C(5)	C(6)	1.370(3)
C(7)	C(8)	1.384(3)	C(7)	C(12)	1.379(3)
C(8)	C(9)	1.396(3)	C(8)	B(1)	1.567(3)
C(9)	C(10)	1.382(3)	C(10)	C(11)	1.382(3)
C(11)	C(12)	1.381(3)			
O(1)	H(10)	0.79(2)	O(2)	H(11)	0.85(2)
C(2)	H(1)	0.95	C(3)	H(2)	0.95
C(4)	H(3)	0.95	C(5)	H(4)	0.95
C(6)	H(5)	0.95	C(9)	H(6)	0.95
C(10)	H(7)	0.95	C(11)	H(8)	0.95
C(12)	H(9)	0.95			

Table A1.12: Bond Angles (°) for 141

atom	atom	atom	distance	atom	atom	atom	distance
C(1)	O(3)	C(7)	118.6(2)	O(3)	C(1)	C(2)	123.6(2)
O(3)	C(1)	C(6)	115.3(2)	C(2)	C(1)	C(6)	121.1(2)
C(1)	C(2)	C(3)	118.3(2)	C(2)	C(3)	C(4)	121.1(2)
C(3)	C(4)	C(5)	119.4(2)	C(4)	C(5)	C(6)	120.6(2)
C(1)	C(6)	C(5)	119.5(2)	O(3)	C(7)	C(8)	118.8(2)
O(3)	C(7)	C(12)	117.8(2)	C(8)	C(7)	C(12)	123.3(2)
C(7)	C(8)	C(9)	116.1(2)	C(7)	C(8)	B(1)	123.2(2)
C(9)	C(8)	B(1)	120.7(2)	C(8)	C(9)	C(10)	122.1(2)
C(9)	C(10)	C(11)	119.5(2)	C(10)	C(11)	C(12)	120.1(2)
C(7)	C(12)	C(11)	118.8(2)	O(1)	B(1)	O(2)	118.3(2)
O(1)	B(1)	C(8)	123.3(2)	O(2)	B(1)	C(8)	118.4(2)
B(1)	O(1)	H(10)	118(2)	B(1)	O(2)	H(11)	115(1)
C(1)	C(2)	H(1)	120.9	C(3)	C(2)	H(1)	120.8
C(2)	C(3)	H(2)	119.5	C(4)	C(3)	H(2)	119.4
C(3)	C(4)	H(3)	120.3	C(5)	C(4)	H(3)	120.3
C(4)	C(5)	H(4)	119.7	C(6)	C(5)	H(4)	119.8
C(1)	C(6)	H(5)	120.2	C(5)	C(6)	H(5)	120.2
C(8)	C(9)	H(6)	119.0	C(10)	C(9)	H(6)	118.9
C(9)	C(10)	H(7)	120.3	C(11)	C(10)	H(7)	120.2
C(10)	C(11)	H(8)	119.9	C(12)	C(11)	H(8)	120.0
C(7)	C(12)	H(9)	120.6	C(11)	C(12)	H(9)	120.6

APPENDIX 2

SUMMARY OF *IN VITRO* ASSAYS

Compound		Assay Result	Compound		Assay Result
62		Insoluble*	**140**		Inactive‡
63		IC_{50} = 40 μM†	**141**		Inactive‡
65		Insoluble*	**122**		Inactive‡
66		Insoluble*	**128**		Inactive‡
68		IC_{50} = 80 μM†	**131**		Inactive‡
83		Inactive‡	**152**		Inactive‡
117		Inactive‡	**156**		Inactive‡
109		Inactive‡	**157**		Inactive‡
108		Inactive‡			

Key to symbols (*, † and ‡) overleaf

Key to symbols (*, † and ‡)

* Insoluble compounds are those which precipitate from the buffer solution at concentrations below which inhibition is not observed. Meaningful analyses could, therefore, not be achieved.

† IC_{50} values were determined for those compounds that were found to inhibit HIV-1 PR (see Chapter 3). Different quantities of inhibitor (corresponding to 0 - 100 μM) were added to a buffered solution of a peptide substrate which absorbed strongly at 300 nm. After thermal equilibration, HIV-1 PR was added and the initial rates calculated. This was achieved by monitoring the rate of the decrease in absorbance at 300 nm. Performing this procedure in duplicate over a range of concentrations allowed graphs of initial rates v concentrations of inhibitor to be plotted. From these IC_{50} values were derived.

‡ Those compounds indicated as being inactive against the enzyme were tested at concentrations of up to 200 μM. However, no decrease in the initial rate of cleavage of the substrate was observed.

CHAPTER FOUR

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