Chapter 4

Exploring Methods to Improve 2-Aminoquinoline Binding Affinity 2: Synthesis and Binding Studies of 6-Substituted-2-Aminoquinolines

4.1 Introduction

A key conclusion drawn following the investigations described in Chapters 2 and 3, was that optimal 2-aminoquinoline ligands should be primary amines. Specifically, in Chapter 2, it was illustrated that *N*-methylation (ligand **8**) resulted in approximately three-fold reduced affinity, while the studies presented in Chapter 3 revealed that this reduction in affinity was considerably less severe for *N*-benzylated ligands, indicating that the larger *N*-substituent made a contact with the SH3 domain. Regardless of this, no improvement in affinity had yet been obtained, and therefore it was clearly necessary to design 2-aminoquinoline derivatives that may be able to make additional contacts with the SH3 domain that retain the primary amine.

Inspection of the protein surface residues that are proximal to the 2-aminoquinoline binding site on the 'right-hand' side according to the ligand binding model (Figure 4-1A) indicates there are both hydrophilic [asparagine 211 (N211), aspartate 212 (D212), histidine 214 (H214)] and hydrophobic [leucine 213 (L213)] residues that may be available for making new contacts with 2-aminoquinoline derivatives with substituents in either the 5-, 6-, or 7-positions of the quinoline ring. As illustrated in Figure 4-1B, when 2-amino-6-methylquinoline **33** is manually docked into the 2-aminoquinoline binding site on the SH3 structure (according to the ligand binding model), the distance between the 6-methyl protons of the ligand and the carbonyl oxygen atom of the N211 side-chain is ca. 6.5Å. Similarly, the distance between the 6-methyl protons of **33** and the oxygen atoms of D212 is ca. 7Å. These two residues (N211 and D212) are of particular interest as they potentially may be able to form hydrogen bonds or salt bridges with appropriate functionality on the 2-aminoquinoline nucleus. However, in the absence of a structure to clearly define the binding orientation of the ligand and any binding induced conformational changes of the protein, 2-aminoquinolines with different functionality (eg. methyl, hydroxymethyl and aminomethyl) were selected as targets, to provide a means of exploring the types of contacts that might be possible. In addition substituents likely to substantially alter the electronic properties of the quinoline ring (eg. halogens, or oxygen) were also sought, to provide new structure activity information about the ligand binding process.

For reasons that will become apparent in the next sections of this chapter, 6-substituted-2-aminoquinolines were selected as targets to begin this process of exploring the protein surface for new contacts. The focus of this chapter is the synthesis and binding studies of these compounds.

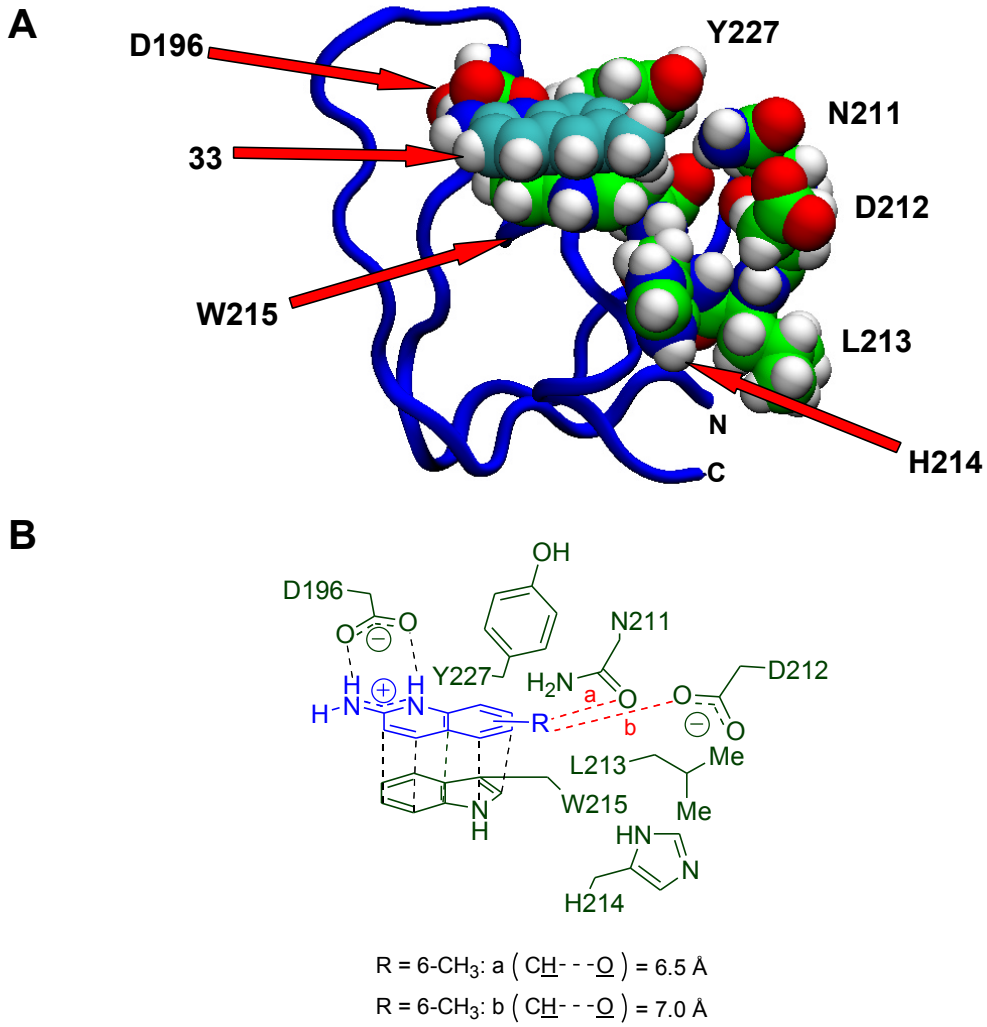


Figure 4-1: Rationale for design of 5, 6, or 7-substituted 2-aminoquinolines. (A) Regions of amino acids proximal to 2-aminoquinoline on the 'right-hand' side of the binding site, according the ligand binding model, represented on the published structure of the Tec SH3 domain. The skeletons for amino acid side-chains of the protein are shown in green, and aqua for the ligand, 2-amino-6-methylquinoline **33**. (B) Cartoon representation of regions of amino acids proximal to 2-aminoquinoline on the 'right-hand' side of the binding site, according to the binding model. Protein amino acids are shown in green, and the ligand is shown in blue.

4.2 Synthesis of simple ring-substituted-2-aminoquinolines

A number of methods for the synthesis of 2-aminoquinolines have been reported. 2-Aminoquinoline itself has been prepared in 52% yield from quinoline by treatment with potassium amide in liquid ammonia in the presence of potassium permanganate.[68] The synthesis of a range of 3-substituted-2-aminoquinolines over two steps from 1-nitro-

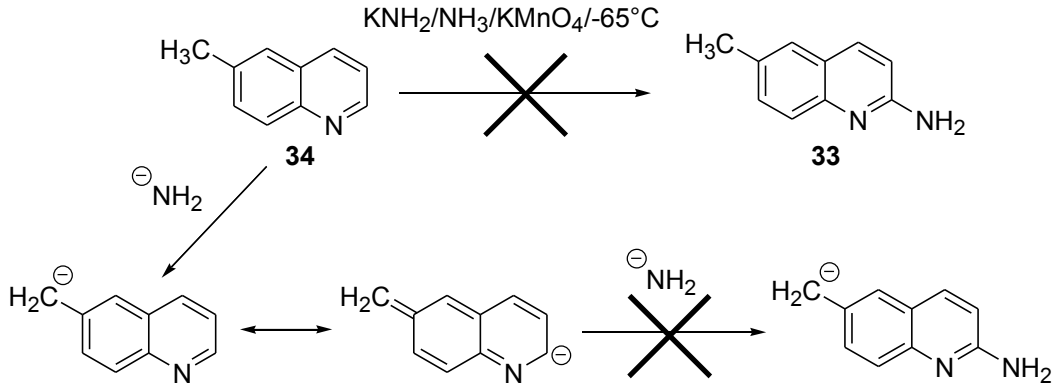
benzaldehyde has also been reported.[69,70] Perhaps the most common approach reported for preparation of 2-aminoquinolines is by nucleophilic aromatic substitution of 2-chloroquinolines with a nitrogenous nucleophile.[71-73] Whilst 2-chloroquinolines are good precursors for introduction of the amino functionality, this method has its own disadvantages. Firstly, the reaction conditions reported for these substitution reactions typically involve high temperatures[72] or pressures[71] which results in lower yields or products that are more difficult to purify. Secondly, the range of 2-chloroquinoline precursors commercially available is limited, and although methods for their synthesis are available, they typically involve several steps and harsh reaction conditions.[74-77] With this in mind, it was decided to start the investigation into methods for preparing 5-, 6-, or 7-substituted-2-aminoquinolines by attempting to adapt the method of Tondys[68] for the synthesis of 2-aminoquinoline from quinoline in liquid ammonia, but instead using the readily available 6-methylquinoline **34** as a precursor for the synthesis of 2-amino-6-methylquinoline **33**.

4.2.1 Synthesis of simple 6-substituted-2-aminoquinolines

The synthesis of 2-amino-6-methylquinoline **33** was attempted by adapting the method of Tondys[68] involving potassium amide as an aminating reagent in liquid ammonia at -65°C in the presence of potassium permanganate (the potassium amide is generated *in situ* by the addition of potassium metal to the liquid ammonia). The suitability of the method for the synthesis of 6-substituted-2-aminoquinolines was tested using 6-methylquinoline **34** as a substrate for the reaction (Scheme 4-1). The main material isolated after attempting this method was the starting material **34**. The most likely reason for the unsuitability of this method with 6-methylquinoline instead of quinoline is that the amide anion acted as a base and deprotonated the methyl group, resulting in a benzylic anion that is delocalised into the quinoline ring, deactivating the 2-postion to nucleophilic attack by amide anion. Specifically, resonance structures can be drawn in which this negative charge is placed on the carbon at the 2-position of the quinoline ring making nucleophilic attack here very unfavourable (Scheme 4-1).

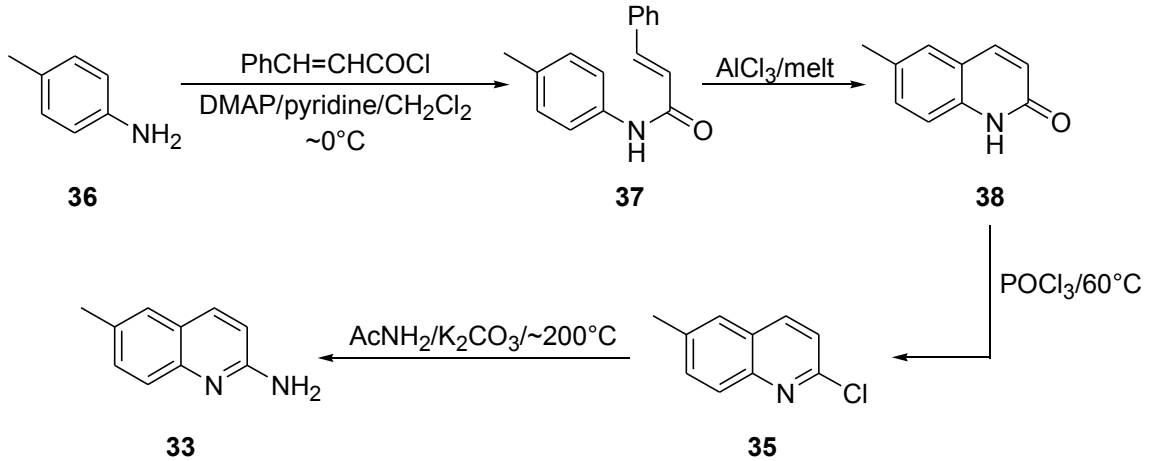
Having established that direct amination of 6-methylquinoline would not work as a method for the synthesis of 6-substituted-2-aminoquinolines, methods involving 2-chloroquinolines as precursors for 2-aminoquinolines were instead selected for investigation. The synthesis of 2-aminoquinolines with a range of simple substituents (methyl, ethyl, chloro, phenyl, etc) at different positions around the quinoline ring has been reported, starting from the corresponding 2-chloroquinolines and treating with a large excess of acetamide near reflux temperature (~200°C) in the presence of potassium carbonate.[72] The reported yields were

respectable (58-69%) and the reaction times short (0.5-6 hours). However, one disadvantage highlighted was the formation of a small amount (~10%) of the corresponding quinolin-2(1*H*)-ones as by-products in the reaction. Although the explicit synthesis of 2-amino-6-methylquinoline **33** was not included as part of this report, there was strong precedence that the method would work for its preparation.



Scheme 4-1: Attempted synthesis of 2-amino-6-methylquinoline **33** from 6-methylquinoline **34** using potassium amide in liquid ammonia. The lower part of the scheme assists with the explanation for the failure of this reaction for the given substrate.

In order to test this method, it was therefore necessary to obtain 2-chloro-6-methylquinoline **35**. This material is not commercially available, however its synthesis has been reported in three steps starting from *p*-toluidine (4-methylaniline) **36**, conversion to cinnamanilide **37** by treatment with cinnamoyl chloride under basic conditions, cyclisation to 6-methylquinolin-2(1*H*)-one **38** using aluminium chloride,[77] and finally treating with phosphoryl chloride[74] to provide 2-chloro-6-methylquinoline **35** (Scheme 4-2).



Scheme 4-2: Method for synthesis of 2-amino-6-methylquinoline **33** in four steps, starting from 4-methylaniline.

The papers[76,77] that described the synthesis of **38** from **37** had prepared **37** by treatment of *p*-toluidine with cinnamoyl chloride using benzene as a solvent. To avoid the use of benzene

for this reaction, an alternative method was sought. (2*E*)-*N*-(4-Methylphenyl)-3-phenylacrylamide **37**, was synthesised in high yield (91%) by treatment of cinnamoyl chloride with *p*-toluidine **36** in dichloromethane in the presence of pyridine and a catalytic amount of DMAP at ca. 0°C (Scheme 4-2). Similar yields were routinely obtained on a large scale (up to 39 g of product). The product isolated was a white powder, judged by ^{1}H NMR spectroscopy to be sufficiently pure to use in the next step without purification. Diagnostic features of the ^{1}H NMR spectrum to assist in the characterisation of this material was the presence of a pair of doublets with coupling constants 15.6 Hz at δ = 6.57 and 7.74 ppm. The signal at δ = 6.57 was assigned to the alkene proton on the carbon adjacent to the carbonyl group (COC<u>H</u>=CH), and the signal at δ = 7.74 assigned to the other alkene proton (COCH=C<u>H</u>) based on resonance arguments. The relative area (integration) for these alkene signals was one third the size of that for a singlet seen at δ 2.32 assigned to the methyl group of the aniline component of the product. Signals seen at 3255, 3181, and 1661, cm^{-1} in the IR spectrum of **37** were consistent with bands expected for an amide.

The aluminium chloride assisted cyclisation step which forms 6-methylquinolin-2(1*H*)-one **38** from **37** (Scheme 4-2) is well documented,[76,77] however it does warrant some further discussion. This is an unusual reaction in that the phenyl anion serves as the leaving group in the ring closure process. The reaction is performed by formation of an 'intimate' mixture of the starting material with three equivalents of aluminium chloride, and melting this mixture with a flame, however it was found that a 'heat-gun' was a more convenient and safer alternative to the use of a flame. A vigorous reaction then takes place briefly, and a dense-black oily material forms. The reaction is then left to stand at ca. 100°C for a further 1 hour. After cooling, the reaction is quenched carefully with ice and water, and an off-white precipitate forms. After collecting and washing the precipitate with both water and dilute acid, and drying the product thoroughly, the quantity of product was consistently approximately 10% in excess of the theoretical yield. ^{1}H NMR spectra of this material in deuteriated chloroform confirmed the product essentially consisted of one organic compound, however, some insoluble impurities were present. The excess was thought to consist of insoluble aluminium containing impurities. Attempts were made to purify the product by recrystallisation from water or filtration through silica gel, however both of these methods were inefficient. It was generally found more effective to use the crude product directly in the next step; treatment with phosphoryl chloride over night at 60°C (Scheme 4-2) to prepare 2-chloro-6-methylquinoline **35.** This product was purified more conveniently by recrystallisation from hexane, to afford pure **35** in 54% overall yield from **37**. A large amount of an insoluble black tar was removed by filtration during this process, consistent with the quinolinone product **38** being in excess of the theoretical yield.

After several attempts at the conversion of **37** to **38**, it was concluded that the optimal scale to perform the reaction on was about 5 g of **37**. When the reaction was attempted on a scale of ~20 g of **37**, a dense gum formed during the quenching process, instead of the powder previously observed. This made the workup more difficult, and less efficient, and the product was of lower quality.

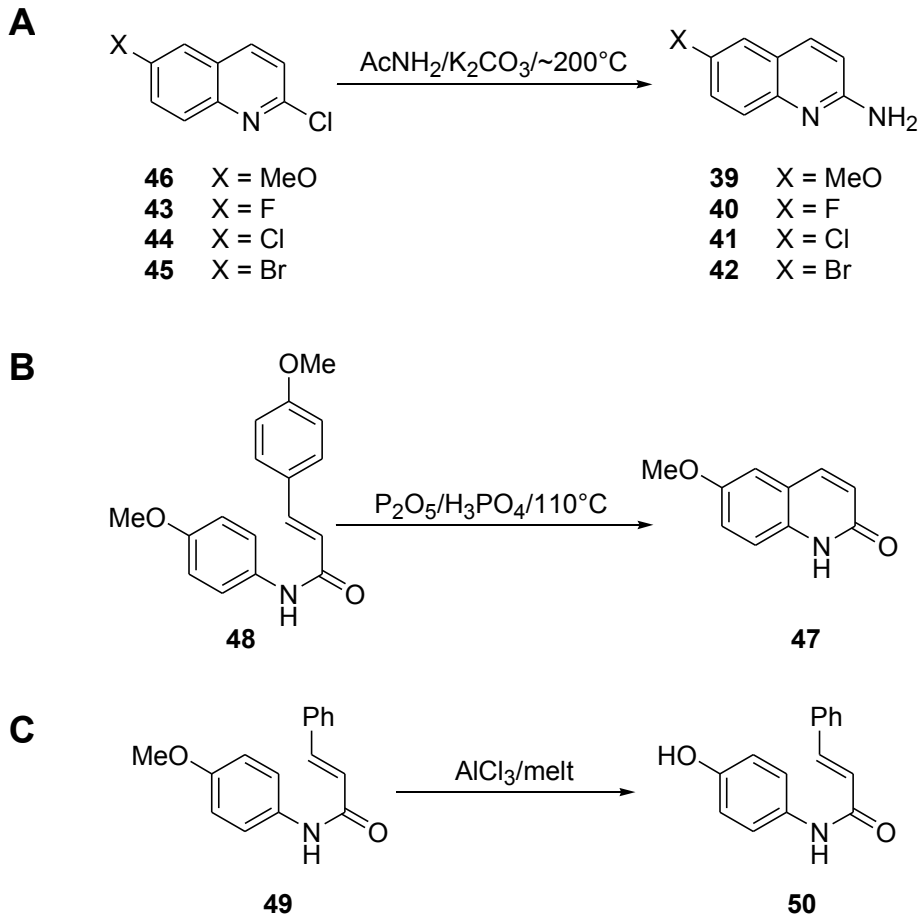
One major feature of the ^{1}H NMR spectrum of **38** used to assist with its characterisation was the change in the coupling constants from ~16 Hz for the pair of alkene protons in the acyclic starting material **37** to ~9 Hz for the pair of protons at the 3 and 4 positions of the quinoline ring of **38**, as would be expected following conversion of the trans double bond in **37** to a cis double bond in **38**. Of this pair of doublets, the most downfield signal (δ 7.78 ppm) was assigned to H4 based on the observation of a ^{3}J coupling from H5 to C4 in the [^{1}H,^{13}C] Heteronuclear Multiple Bond Correlation (HMBC) spectrum of **38**. Thus the signal at δ 6.72 ppm was assigned to H3. In addition, a very downfield broad singlet with variable chemical shift (δ 11-13 ppm) was seen, as expected for a quinolinone type amide proton. The number of signals in the aromatic region was also consistent with the loss of the phenyl group in the reaction. Bands at 3138 and 1662 cm^{-1} were observed in the IR spectrum, again characteristic of an amide. The conversion of **38** to **35** was again accompanied by very large downfield changes in chemical shifts in the ^{1}H NMR spectrum of **35**, specifically for the H3 (6.72 to 7.46 ppm) and H4 (7.78 to 8.26 ppm) protons.

The synthesis of 2-amino-6-methylquinoline **33** from **35** was then tested, using the amination conditions of Kóródi,[72] involving 20 equivalents of acetamide at ca. 200°C in the presence of potassium carbonate (Scheme 4-2). This reaction was performed on a relatively small scale (0.6 g of **35**). Because such a high temperature was required in this reaction, a Woods metal bath on a hot plate was used to heat the vessel. Woods metal consists of 50% bismuth, 25% lead, 12.5% tin and 12.5% cadmium and is well suited to heating vessels at high temperatures. However, due to the hazardous nature of this material, considerable caution needs to be taken for its handling. This is clearly one disadvantage of the method for preparing 2-aminoquinolines. Using this approach, **33** was prepared from **35**. As expected, the ^{1}H NMR spectrum of the crude isolate indicated that a small amount (~10%) of the 6-methylquinolin-2(1*H*)-one **38** by-product was formed, however, following chromatography on silica gel, pure **33** was isolated in 54% yield. The conversion of **35** to **33** was accompanied by very large upfield changes in chemical shifts in the ^{1}H NMR spectrum of **33** compared to **35**, specifically for the H3 (7.46 to 6.72 ppm for **35** and **33** respectively) and H4 (8.26 to 7.81 ppm for **35** and **33** respectively) protons. These assignments were made based on

resonance considerations. A broad singlet at δ 5.04 was also seen, indicative of amino group protons.

This reaction was also tried on starting materials of different qualities. Prior to optimising the synthesis of **35**, the reaction was tried on the crude product isolated from the synthesis of **35** over the three steps, without any purification at all. Although the overall yields obtained for **35** were higher using this approach, and the ^{1}H NMR spectrum of this material suggested the material was essentially pure, the physical form of the product was a brown solid. Use of this material for the amination reaction of Kóródi[72] resulted in a product that was itself a light brown solid, difficult to purify, and the yield was also lower (~40%). It was therefore concluded that high purity of the starting material, particularly freed of coloured impurities, was an essential requirement for success using the method.

Having tested the method of Kóródi[72] for synthesis of 2-aminoquinolines from 2-chloroquinolines, an additional four 2-aminoquinolines (**39-42**) with simple substituents at the 6-position were prepared using the same approach (Scheme 4-3). 2-Chloroquinolines **43-45** for this reaction were also prepared by treatment of the corresponding quinolin-2(1*H*)-ones (obtained from aluminium chloride assisted cyclisation of the appropriate cinnamanilides) with phosphoryl chloride, as set out in Scheme 4-2 for the synthesis of 2-chloro-6-methylquinoline **35**. 2-Chloro-6-methoxyquinoline **46** was similarly prepared by treatment of 6-methoxyquinolin-2(1*H*)-one **47** with phosphoryl chloride. However **47** was prepared by the polyphosphoric acid assisted cyclisation[75] of 3,*N*-bis-(4-methoxy-phenyl)-acrylamide **48** (Scheme 4-3B), as an alternative method for the synthesis of quinolin-2(1*H*)-ones from cinnamanilides. This is because it has been reported that treatment of *N*-4-methoxycinnamanilide **49** with aluminium chloride results in formation of the demethylated cinnamanilide **50** as the only product, as illustrated in Scheme 4-3C. The synthesis of all these additional 6-substituted-2-aminoquinolines and the intermediate compounds was performed by co-workers, and has this been documented.[51]

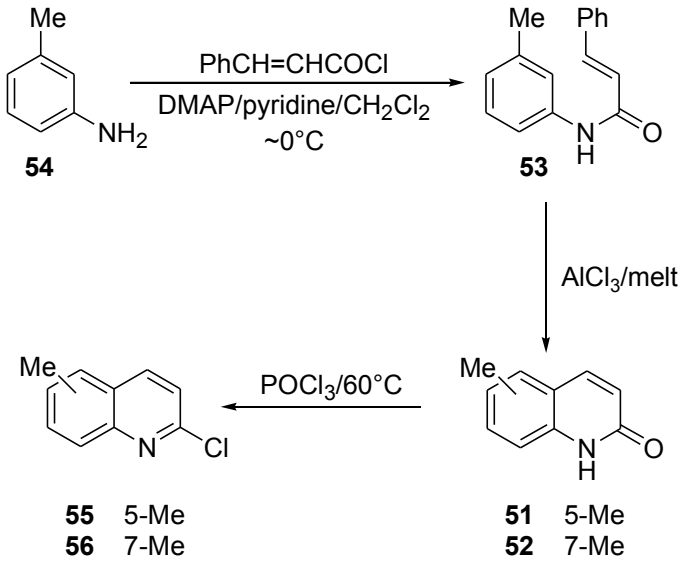


Scheme 4-3: (A) Synthesis of 2-aminoquinolines **39-42** from 2-chloroquinoline precursors. (B) Method for synthesis of 6-methoxyquinolin-2(1*H*)-one **47**. (C) An observation reported in Johnston[76] indicating why aluminium chloride assisted cyclisation is not a suitable method for the synthesis of **47**. The experiments in A and B in this Scheme were performed by co-workers, and have been reported.[51]

4.2.2 Synthesis of simple 5- and 7-substituted-2-aminoquinolines

The aluminium chloride assisted cyclisation of cinnamanilides to quinolin-2(1*H*)-ones has also been reported as a method for the synthesis of 5-methylquinolin-2(1*H*)-one **51** and 7-methylquinolin-2(1*H*)-one **52** starting from (2*E*)-*N*-(3-methylphenyl)-3-phenylacrylamide **53**[76] (Scheme 4-4). Therefore, it was anticipated that this might provide a means for the synthesis of 5- and 7-substituted-2-aminoquinolines, which were also targets. However, as illustrated in Scheme 4-4, these isomers are produced in the same pot from the cyclisation process, and they were reported as a mixture. This was presumably a consequence of the two isomers being inseparable, due to very similar physical properties. Regardless, it was attempted to prepare these materials in essentially the same way as that described for the synthesis of 6-methylquinolin-2(1*H*)-one **38**, but instead, starting from *m*-toluidine (3-methylaniline) **54**

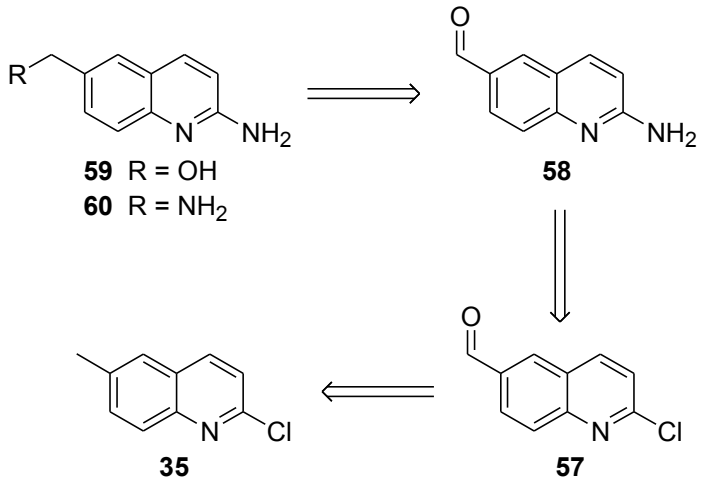
(Scheme 4-4). The ^{1}H NMR spectrum of the crude material isolated following treatment of **53** with aluminium chloride, confirmed the presence of a mixture of quinolin-2(1*H*)ones as products, as evidenced by two distinct sets of doublets at approximately 6.6 and 8.0 ppm with coupling constants of the order 9 Hz representative of the H3 and H4 protons respectively. A broad NH signal at δ 12 ppm was also observed. These features were consistent with those observed for **38**. The ^{1}H NMR spectrum also indicated that the product mixture was approximately a 2:1 ratio, presumably the species in excess being 7-methylquinolin-2(1*H*)-one **52**, a consequence of the formation of 5-methylquinolin-2(1*H*)-one **51** being less favourable due to steric considerations. These two isomers could not be separated by silica gel chromatography. Consequently, the mixture of **52**, and **51** was treated with phosphoryl chloride as per Scheme 4-4 to obtain a mixture of 2-chloro-5-methylquinoline **55** and 2-chloro-7-methylquinoline **56**. The appropriate chemical shift changes in the ^{1}H NMR spectrum of this mixture were observed, consistent with those seen for the formation of 2-chloro-6-methylquinoline **35**. Furthermore, the 2:1 mixture of products was again observed. However, there were still insufficient differences in polarity between the two isomers to allow separation of the compounds using silica gel chromatography. At this stage it was decided to discontinue with the synthesis of the 5- and 7- substituted quinolines, but instead focus on synthesis of the 6-substituted derivatives.



Scheme 4-4: Method for synthesis of 2-chloro-5-methylquinoline **55** and 2-chloro-7-methylquinoline **56** starting from *m*-toluidine.

4.3 Synthesis of 6-substituted-2-aminoquinolines with more complex functionality 1

In order to synthesise 2-aminoquinolines with more diverse functionality, it was envisaged that 2-chloro-6-formylquinoline **57** might be a useful synthetic intermediate from which a range of new compounds may be prepared. For example, conversion of **57** to 2-amino-6-formylquinoline **58** could provide a precursor for reduction or reductive amination to prepare the 6-hydroxymethyl or 6-aminomethyl 2-aminoquinoline derivatives (**59** and **60**) respectively (Scheme 4-5).

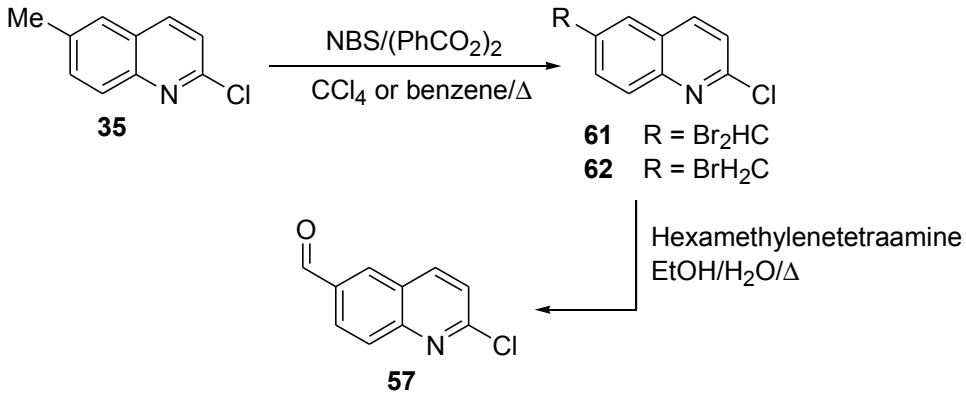


Scheme 4-5: Retrosynthetic plan for synthesis of 2-aminoquinolines **59** and **60** starting from **35**.

4.3.1 Investigation into benzylic oxidation of 2-chloro-6-methylquinoline

In order to test the proposed method for synthesis of 2-aminoquinolines with more diverse functionality as described above, 2-chloro-6-methylquinoline **35** was selected as a substrate for benzylic oxidation. The method of Newman[78] for the synthesis of aryl aldehydes was selected for adaption to **35** (Scheme 4-6). This involves treatment of the aryl-methyl starting material with 2 equivalents of *N*-bromosuccinimide in the presence of a catalytic amount of benzoyl peroxide and heating at reflux in carbon tetrachloride. The reaction was initially tried on a small scale (~0.150 g of recrystallised **35**). After a total reaction time of 4 hours, the cooled solution was filtered to remove precipitated succinimide, and the solvent was removed. The crude material was analysed by ^{1}H NMR spectroscopy, and indicated that an approximately 6:1 mixture of 2-chloro-6-dibromomethylquinoline **61** and 2-chloro-6-bromomethylquinoline **62** was present, as evidenced by signals at δ 6.85 ppm (C<u>H</u>Br_2), and 4.65 (C<u>H_2</u>Br). The presence of both of these products was confirmed by mass spectrometry.

Some other small signals representative of unknown organic materials were also observed, specifically signals in the aromatic region of the spectrum, and a singlet at δ 2.78 ppm suggested that some succinimide also remained in the mixture. This crude material was then treated with ~3 equivalents of hexamethylenetetraamine in 50% aqueous ethanol with heating at reflux for 4 hours, again an adaption of the method of Newman[78] (Scheme 4-6). The crude product isolated after this step was purified by column chromatography over silica gel to furnish pure 2-chloro-6-formylquinoline **57** in 40% overall yield from **35**. The ^{1}H NMR spectrum of **57** featured a singlet at δ 10.19 ppm, as expected for an aldehyde proton, and a strong band was seen at 1697 cm^{-1} in the IR spectrum, characteristic of the carbonyl functionality. The identity of **57** was also confirmed by mass spectrometry and elemental analysis.



Scheme 4-6: Synthesis of 2-chloro-6-formylquinoline **57** from 2-chloro-6-methylquinoline **35**.

Later, this reaction was repeated on a larger scale (4.75 g of recrystallised **35**). Essentially the same procedure was used as described above, however after the first step (treatment with NBS) instead of removing the carbon tetrachloride on a rotary evaporator, it was distilled off and collected for recycling. This was done as carbon tetrachloride is no longer a solvent that can be conveniently purchased at normal prices, since it was listed as a banned chemical as part of the Montreal Protocol. The supply available in our laboratories is limited and hence it is recycled. Following removal of the carbon tetrachloride by distillation, the remaining material was again treated with hexamethylenetetraamine as described above. Following workup, **57** was again isolated as a brown solid in better overall yield (64% from 35). ^{1}H NMR of this material indicated it was essentially pure, but there were additional small signals in the aromatic region, suggesting it was not an analytically pure sample.

An attempt was made to perform the same transformation on a very large scale (~35 g of **35**), however in this case, the starting material **35** had not been recrystallised and freed of coloured impurities accumulated in the steps leading to its synthesis. The reaction mixtures

in each step were dark brown in colour in contrast to previous attempts (pale orange), resulting in products that were brown resins prior to purification attempts. The consequence of this was that the final aldehyde (**57**) obtained over the two steps (radical bromination followed by hexamethylenetetraamine assisted hydrolysis) was isolated in low overall yield (19% mass recovery), and the purity was also very poor. In fact sufficiently pure material for subsequent reactions could not be furnished. As a consequence of this, all future attempts at this transformation were carried out on recrystallised, colour free starting material.

Following the problems encountered above, some additional effort was made investigating this conversion. It was decided to test whether benzene could be used as an alternative solvent for this reaction. Again, on a small scale (~0.1 g **35**) the NBS treatment step of the reaction proceeded essentially as described previously when carbon tetrachloride was used as solvent. This was a useful observation, as all future use of this reaction could be done with a solvent that was less hazardous (although benzene itself is classed as a hazardous chemical), and less expensive than carbon tetrachloride, and the workup was faster, as removal of the solvent by distillation was no longer required.

Another aspect of the previous method that was reviewed was the filtration step for removal of the succinimide by-product. Because such a large amount of succinimide needed to be removed from the reaction, it was thought that this could be a source of product loss; either by crystallising with the succinimide or sticking to it on the filter. In another paper describing radical dibrominations[79] the succinimide was removed by first removing the reaction solvent, redissolving the residue in dichloromethane, and washing out the succinimide with a solution of sodium bicarbonate. This approach was tested and found to be a more efficient means of removing the succinimide from the product, as mass losses were minimal.

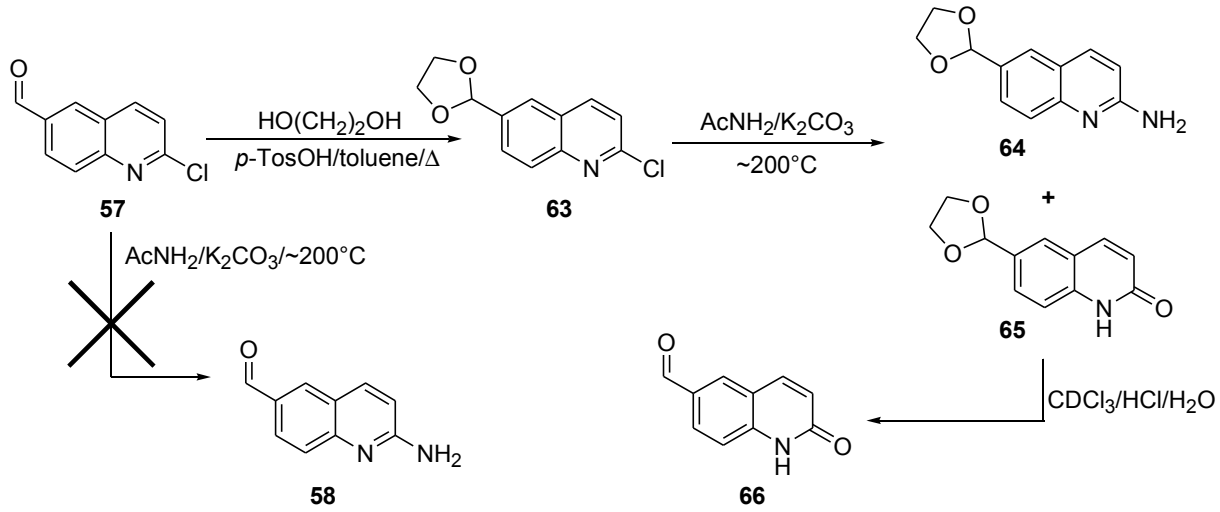
It was decided next to investigate whether purification of the 2-chloro-6-dibromo-methylquinoline **61** and 6-bromomethylquinoline **62** mixture improved the overall efficiency of the reaction. Following the sodium bicarbonate washes of the crude product and removing the solvent, the material was then purified by chromatography over silica gel. On a small scale, essentially pure **61** could be isolated. On a larger scale (3.18 g of starting material **35**) it was possible to remove other unknown organic impurities from the major isolate, however as the difference in R_f between **61** and **62** was small, it was not possible to isolate pure **61**; instead a 6:1 mixture of **61** and **62** was isolated. Some pure **62** (0.234 g, 5%) was also isolated. Fortunately, the inability to isolate pure **61** was of little consequence, as following the subsequent step (hexamethylenetetraamine treatment) pure 2-chloro-6-formylquinoline **57** was isolated as a white solid in 52% overall yield from **35**. At some point during this

reaction and acidic workup, the **62** component of the reaction mixture is most likely converted into a water soluble product, that is not isolated from the organic layer.

In summary, the optimal method for performing the radical dibromination of **35** to **61** was to carry out the reaction on the highest quality starting material possible, particularly freed of coloured impurities, a similar requirement to that of the acetamide amination reaction described in Section 4.2.2. In addition, the reaction is best performed on a maximum of about 3 g scale (of **35**), and losses are minimised by removing the succinimide by-product by washing with a solution of sodium bicarbonate. Partial purification of the product is also beneficial.

4.3.2 Investigation into aldehyde protecting groups

Having established a suitable method for the synthesis of 2-chloro-6-formylquinoline **57**, it was attempted to convert **57** into 2-amino-6-formylquinoline **58** using the amination conditions of Kóródi[72] previously described (Scheme 4-7). Only low recovery of an insoluble material was isolated from this reaction. Perhaps not surprisingly, the aldehyde functionality was not stable under the harsh thermal conditions and/or with the large excess of acetamide used in this reaction. The identity of the material isolated was not determined.



Scheme 4-7: Method for protection of aldehyde **57** by synthesis of dioxolane acetal **63** and subsequent conversion to 2-aminoquinoline **64**, due to observation that **57** was unstable to the amination reaction of Kóródi.[72]

Given the unsuitability of **57** for the above reaction, the incorporation of aldehyde protecting groups was investigated. The first method tried was treatment of **57** with a slight excess of ethylene glycol in the presence of a catalytic amount of *p*-toluenesulfonic acid, and heating at reflux in toluene for 4 hours with a Dean Stark apparatus (Scheme 4-7), a well established method for the protection of aldehydes.[80] The **57** used for the first attempts at this reaction

was the lower purity brown powder described in Section 4.2.2. After cooling, the excess ethylene glycol was removed by washing the toluene solution with sodium hydroxide and water, and then the solvent was removed. The crude material isolated was purified by column chromatography over silica gel and pure 2-chloro-6-(1,3-dioxolan-2-yl)quinoline **63** was furnished in 49% yield. One feature of the ^{1}H NMR spectrum to assist with the characterisation of this material was the loss of the signal at δ 10.19 ppm in **57** representing the aldehyde proton (RC<u>H</u>O), and the appearance of a new signal at δ 5.99 ppm in **63** representing the acetal type proton [RC<u>H</u>(OR^2)$_2$]. In addition a 4-proton multiplet was seen at δ 4.07-4.23 representing the AA'BB' system of the acetal ring protons. The identity of **63** was also confirmed using mass spectrometry and elemental analysis.

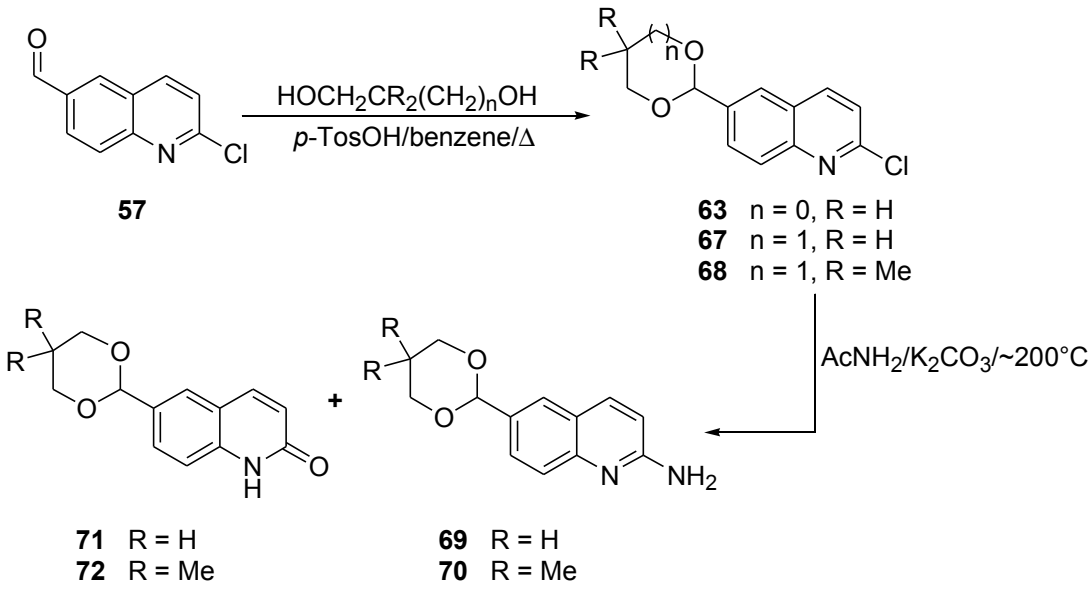
Acetal **63** was stable to the amination conditions of Kóródi[72] and pure 6-(1,3-dioxolan-2-yl)quinolin-2-ylamine **64** was isolated following the usual workup and chromatography system (Scheme 4-7). For solubility reasons, the ^{1}H NMR spectrum of **64** was recorded in d_6-acetone. The characteristic upfield chemical shift changes for conversion of a 2-chloroquinoline to a 2-aminoquinoline for the H3 and H4 ring protons as described for the synthesis of the 2-amino-6-methylquinoline **33** were again observed in the ^{1}H NMR spectrum of **64**, and the identity of the product was also confirmed by mass spectrometry and elemental analysis. A small amount of the 6-(1,3-dioxolan-2-yl)quinolin-2(1*H*)-one **65** by-product was also isolated as evidenced by the characteristic chemical shifts described for the synthesis of 6-methylquinolin-2(1*H*)-one **38** (Section 4.2.2), however some conversion of this acetal to 6-formylquinolin-2(1*H*)-one **66** was also observed by ^{1}H NMR, as evidenced by the existence of an additional set of signals in the aromatic region, and a signal seen at δ 10.02 ppm representative of an aldehyde proton. This observation was most likely a result of traces of hydrochloric acid being present in the deuteriated chloroform, however the equivalent conversion was not seen for **64** due to the different solvents used for acquiring the NMR spectra. Neither of **65** or **66** were isolated in pure a form.

Prior to any attempts to convert **64** into 2-amino-6-formylquinoline **58**, **64** was tested for binding to the Tec SH3 domain, and an unexpected improvement in binding was found (see Section 4.4). Therefore, for the purposes of obtaining additional structure activity information, it was decided to synthesise additional cyclic acetals, by varying the diol used in the acetal formation reaction.

Subsequently, in order to synthesise 2-chloro-6-(1,3-dioxan-2-yl)quinoline **67** (Scheme 4-8), some effort was made to improve the yield from the 49% recovery obtained at the first attempt for the synthesis of **63** (Scheme 4-7). All subsequent experiments were carried out

with the starting aldehyde **57** being of the essentially pure white powder form, instead of the less pure brown powder described above. It was also decided to use benzene as the reaction solvent instead of toluene, because benzene is more easily removed at the end of the reaction due to its lower boiling point. Instead of washing the reaction mixture with sodium hydroxide and water, the benzene was removed first, and the residue was re-dissolved in dichloromethane, and this solution was washed with water and brine. This eliminated the exposure to benzene at the workup stage, but also reduced the mechanical losses during the procedure. When the conversion of ~1.6 g of **57** to **67** was attempted using the modified procedure described above, **67** was isolated as an off-white solid in 78% yield. Furthermore, the ^{1}H NMR spectrum of **67** indicated the material was of sufficient purity to use in subsequent reactions without purification, however a small amount was chromatographed over silica gel to provide an analytical sample. The ^{1}H NMR spectrum of **67** again featured the characteristic loss of the aldehyde signal at δ ~10 ppm and the re-appearance of a signal for the acetal proton at δ ~6 ppm.

The acetal **68** was also prepared in essentially the same way as described above however on a smaller scale (~0.35 g of **57**), and the synthesis of **63** was repeated on a larger scale using the above method (Scheme 4-8), because, as will become apparent in Section 4.5, acetals **63** and **67** were used as precursors for conversion into different functional groups.



Scheme 4-8: Optimised method for synthesis of cyclic acetal derivatives of 2-chloroquinoline (**63, 67** and **68**) and conversion of **67** and **68** to the corresponding 2-aminoquinolines **69** and **70**.

Both 2-chloroquinolines **67** and **68** were converted into 2-aminoquinolines **69** and **70** using the amination reaction of Kóródi[72] and the corresponding quinolin-2-(1*H*)-one by products **71** and **72** were this time isolated and characterised (Scheme 4-8). To ensure that no hydrolysis

of acetals to aldehydes occurred with this set of compounds, the ^{1}H NMR spectra of amines **69** and **70** were recorded in d_6-acetone, and those for the quinolin-2(1*H*)-ones **71** and **72** were recorded in $CDCl_3$ that had been filtered through basic alumina, to remove any residual water and hydrochloric acid that was present in the solvent.

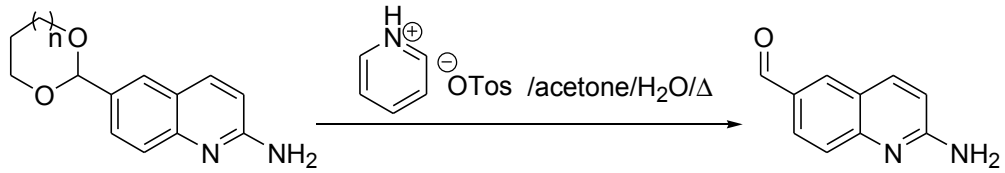
4.3.3 Investigation into methods for de-protection of cyclic acetals

Having established a suitable method for the protection of aldehyde **57**, and having confirmed that the protecting groups were stable to the amination conditions of Kóródi,[72] investigation into methods for regeneration of the aldehyde functionality to form 2-amino-6-formylquinoline **58** was required. As indicated in Scheme 4-5 (Section 4.3.1), it was intended that **58** might be a precursor compound for reduction or reductive amination, necessary to prepare compounds **59** and **60**. Therefore, some effort was made to try and remove the aldehyde protecting groups.

4.3.3.1 Use of pyridinium tosylate as a catalyst for the de-protection of cyclic acetals

The first method tried was treatment of acetal **69** with pyridinium tosylate in wet acetone (Scheme 4-9), an adaption of the method of Sterzycki.[81] This method is presented for the cleavage of dioxolane acetals, however as a more plentiful supply of dioxane acetal **69** was available at the time, the preliminary experiments were carried out with **69**. Using only 0.3 equivalents of pyridinium tosylate and heating at reflux, no reaction was observed as judged by thin layer chromatography. Even after the addition of an excess of pyridinium tosylate and heating at reflux for several more hours, still no reaction could be observed. The reaction was worked up, and the only material isolated was starting material, as indicated by ^{1}H NMR. Hence, it was concluded that the conditions were too mild for a dioxane type acetal, and the reaction was attempted on dioxolane acetal **64**. Dioxolane acetals may be predicted to be more reactive than dioxane acetals, due to the additional ring strain associated with a 5-membered ring, and hence should be more easily hydrolysed. Again, starting the experiment by treatment of **64** with 0.5 equivalents of pyridinium tosylate, no reaction was observed as judged by thin layer chromatography. Therefore, an additional 1 equivalent of pyridinium tosylate was added and the reaction was left at reflux overnight. At this stage, thin layer chromatography suggested that the reaction had proceeded (or commenced to proceed), however it was difficult to judge whether the reaction was complete as the R_f differences between the product and the starting material were very small. Therefore, the reaction was

worked up, and the ^{1}H NMR spectrum indicated that the reaction had proceeded to ~90% completion. The main feature of the ^{1}H NMR spectrum that assisted with the identity of the material was the reappearance of a signal at δ 9.96 ppm indicative of an aldehyde (CHO) type proton. There were clearly two sets of signals, including the characteristic chemical shifts reported for **64**.



64 n = 0
69 n = 1

58

Scheme 4-9: Attempted method for de-protection of acetals **64** and **69** using pyridinium tosylate catalysis.

The above observations provided encouragement for the use of the method for the cleavage of dioxolane acetals. Clearly, the method was not suited to dioxane acetals, and, unlike the results reported[81] an excess of pyridinium tosylate was required for the reaction to proceed. This is most likely because when applied to a 2-aminoquinoline, a substantial proportion of the protons on the pyridyl nitrogen atoms of pyridinium tosylate are transferred to the quinoline nitrogen, as 2-aminoquinoline would be expected to be a stronger base than pyridine. This results in the protons available to participate in the catalysis of the reaction being substantially reduced. Thus, to overcome this deficiency, an excess of pyridinium tosylate is required. However, some problems remained. Firstly, the reaction had not proceeded to completion, and it was difficult to separate the product from the starting material. Secondly, the physical nature of the material also made it difficult to handle. The product was largely insoluble in chloroform and other less polar solvents. The ^{1}H NMR spectrum was run in d_6-DMSO. This also created difficulties when attempting to recover the NMR sample and subsequently purify the product.

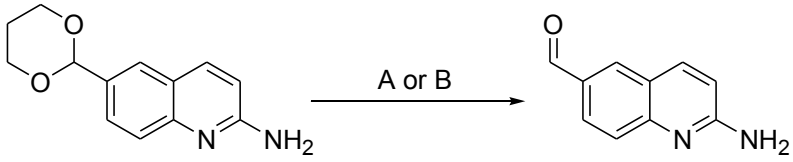
To try to address the indicated problems, the reaction was repeated again using **64**, however this time 2 equivalents of pyridinium tosylate were used from the beginning, and the reaction was allowed to proceed for 12 hours. It was again difficult to monitor the reaction progress using thin layer chromatography. After workup an approximately 1:2 mixture of product and starting material was identified using ^{1}H NMR spectroscopy. Preparative thin layer chromatography was used to try and separate the two materials, however essentially no material (~1 mg) could be recovered from the operation. It appeared as though the material was too polar and could not be effectively stripped off the silica, or due to solubility problems,

the material was not effectively spotted on the plate in the first instance, and that the material was essentially lost throughout these mechanical manipulations.

Based on these above observations, it was concluded that use of pyridinium tosylate applied to the current substrates was an ineffective way to hydrolyse these cyclic acetals. Although there was evidence to indicate that the desired aldehyde was produced using the method, the reaction had proven difficult to be taken to completion, and the outcomes observed over two attempts were inconsistent. It was therefore decided to investigate alternative methods for the de-protection of cyclic acetals.

4.3.3.2 Investigation into de-protection of cyclic acetals using aqueous acids

The hydrolysis of dioxane **69** was next attempted by dissolving it in chloroform with stirring, followed by addition of an equal volume of 1M hydrochloric acid (Scheme 4-10, method A). The mixture was stirred vigorously for 10 minutes. The resulting mixture was added to 10% aqueous sodium hydroxide and extracted with chloroform, however a larger amount of insoluble material remained. A white solid product (in low recovery) was isolated, that was again largely insoluble in $CDCl_3$. However a dilute sample for ^1H NMR analysis was made with the assistance of a few drops of d_6-DMSO. This spectrum indicated that the desired aldehyde **58** was the major material present, however there were a lot of additional signals present in the aromatic region. By a similar approach, **69** was stirred in 80% aqueous acetic acid at 80°C for 3 hours (Scheme 4-10, method B). Again, after workup under basic conditions, a large amount of insoluble material was observed, and only low recovery (~10%) of a largely insoluble material was isolated. The ^1H NMR spectrum of the material suggested that an approximately 2:1 mixture of the desired aldehyde **58** and starting material **69** was present. Given that previous attempts had failed, no effort was made to purify this material.



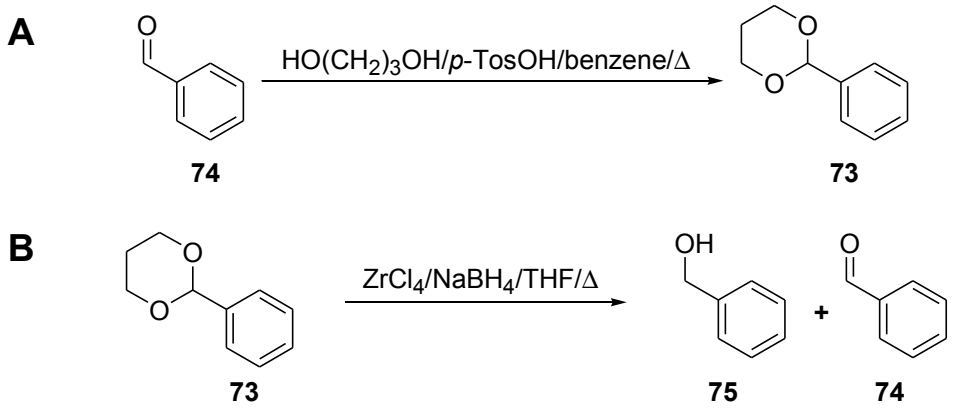
69 **58**

A: 1M aqueous HCl/$CHCl_3$/room temp
B: 80% aqueous AcOH/80°C

Scheme 4-10: Additional methods, involving aqueous acids, tested for de-protection of dioxane **69** to form aldehyde **58**.

4.3.3.3 Use of zirconium tetrachloride/sodium borohydride for the de-protection of cyclic acetals

As part of a final effort to find a suitable method for the hydrolysis of acetal **69** to form aldehyde **58**, it was decided to work with a model compound. At this stage, a substantial amount of **69** (and **64**) had been consumed in exploratory reactions without satisfactory return, hence the need to resort to a model compound for further experiments. As mentioned earlier, because a more plentiful supply of acetal **69** was available than **64**, a dioxane model acetal was sought. Thus, 2-phenyl-1,3-dioxane **73** was prepared by treatment of benzaldehyde **74** with propylene glycol and heating at reflux in benzene in the presence of a catalytic amount of *p*-toluenesulfonic acid (Scheme 4-11A), essentially the same procedure as used for synthesis of the acetals in Scheme 4-8. The chemical shift changes observed on conversion of benzaldehyde to **73** were completely consistent with those seen for the preparation of **67**.



Scheme 4-11: Further investigation into methods for de-protection of dioxane acetals using 2-phenyl-1,3-dioxane **73** as a model system.

A method was found in the literature in which dioxolane and dioxane acetals including **73** were converted back to aldehydes in high yield reductively by treatment with zirconium tetrachloride ($ZrCl_4$) and sodium borohydride ($NaBH_4$) at reflux temperature in THF[82] as illustrated in Scheme 4-11B. This approach was therefore tested with **73** and expected to work well. The stoichiometry used in the reaction was a 1:1:0.5 ratio of **73**:$ZrCl_4$:$NaBH_4$, exactly as reported in the literature. This ratio was required because experiments involving a 1:1:1 ratio of substrate:$ZrCl_4$:$NaBH_4$ were reported to result in formation of benzyl alcohol **75** as the major product, a consequence of the excess hydride reducing the aldehyde to the alcohol as the aldehyde was formed. The material isolated after heating for 1 hour at reflux was approximately a 50:40:10 ratio mixture of **73:75:74**, according to the ^{1}H NMR spectrum (Table 4-1). This indicates that the reaction was indeed proceeding, but not to completion.

Instead, a substantial amount of reduction of the aldehyde to form benzyl alcohol was observed (Scheme 4-11B) (as mentioned was a possibility in Wu[82]). The reaction was therefore repeated with a longer reaction time, however no significant change in the ratio of components in the mixture was observed. Therefore, three more experiments were performed; one in which the reaction time was further increased, and two where the ratio of **73**:$ZrCl_4$:$NaBH_4$ was varied. The results from all these attempts are summarised in Table 4-1 below. These results clearly indicate that benzyl alcohol **75** is the favoured product over benzaldehyde **74**. This investigation would also tend to suggest that when sodium borohydride is used at 0.5 equivalents, the conversion of the acetal to the aldehyde does not go to completion because the aldehyde is further reduced to the alcohol by the sodium borohydride faster than the conversion of the acetal to the aldehyde, and therefore the sodium borohydride becomes a limiting reagent. Additional support for this is gained by considering the effects of using 0.75 equivalents of sodium borohydride, in which an 85:15 ratio of **75**:**73** was obtained, and no benzaldehyde was observed. Changing the ratio of **73**:$ZrCl_4$:$NaBH_4$ to 1:2:0.5 did not significantly impact on the constitution of the recovered material.

The results discussed above did not provide encouragement for the use of zirconium tetrachloride/sodium borohydride as a method for the conversion of cyclic acetals to aldehydes. In fact the results obtained were quite inconsistent with those presented in Wu.[82] The approach was therefore abandoned.

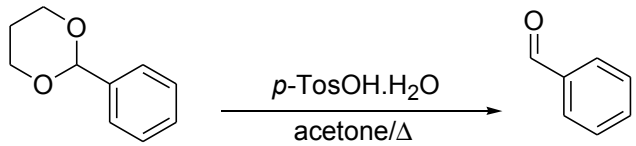
Table 4-1: Summary of results for experiments involving conversion of acetal **73** to benzaldehyde **74** using zirconium tetrachloride/sodium borohydride.[82]

Entry	Ratio of 73:$ZrCl_4$:$NaBH_4$	Reaction Time (hours)	Mass Recovery (%)*	Approximate Ratio of Components in Recovered Material 73:75:74
1	1:1:0.5	1	63	50:40:10
2	1:1:0.5	4	24	60:25:15
3	1:1:0.5	18	28	55:45:5
4	1:1:0.75	3	57	15:85:0
5	1:2:0.5	3	40	55:40:5

* % Recovery based on mass of starting material **73**

4.3.3.4 Use of *p*-toluenesulfonic acid for the de-protection of cyclic acetals

In a last effort to find a method for the conversion of **73** to **74**, it was decided to see if *p*-toluenesulfonic acid could be an alternative to using the methods involving pyridinium tosylate presented in Section 4.3.3.1. Pyridinium tosylate methods had shown some promise when applied to de-protection of **64** to form **58**, but the conditions may be classed as too mild, as the reactions were difficult to get to proceed to completion. This raised the question as to whether *p*-toluenesulfonic acid itself, as opposed to the pyridinium salt form, may provide the additional 'kick' required to push the reaction to completion. Thus, **73** was treated with 0.5 equivalents of *p*-toluenesulfonic acid monohydrate and heated in reflux acetone for 4 hours as illustrated in Scheme 4-12. The isolated material was a 2:1 mixture of the starting material **73** and the product **74**. This provided some promise for the method, but again the reaction did not proceed to completion. The reaction was repeated, however the duration was increased to 14 hours, and 1 equivalent of *p*-toluenesulfonic acid was used. This resulted in a mixture of materials that consisted of approximately 3:1 starting material **73** and the desired product **74**, however these signals in the ^{1}H NMR spectra only compromised a small amount of the mixture. A large amount of signals due to unidentified impurities were also present.



73 **74**

Scheme 4-12: Investigation into deprotection of dioxane acetals using *p*-toluenesulfonic acid catalysed hydrolysis.

4.3.3.5 Summary

After considerable effort was invested in finding a suitable method for the de-protection of cyclic acetals, it was decided to discard the approach. Although there was evidence for formation of the desired product using some of the tested methods, it was difficult to get the conversions to proceed to completion. Furthermore, the product was difficult to handle as it was insoluble in the most convenient solvents. It was also difficult to purify, and the yields obtained were generally low. Therefore **58** was discarded as a target and an alternative approach was sought for the preparation of compounds **59** and **60**, as will be presented in Section 4.7.

4.4 Tec SH3 domain/6-substituted-2-aminoquinolines binding studies 1

All of the 6-substituted 2-aminoquinolines **33**, **39-42**, **64**, **69** and **70** prepared as described in the preceding sections were tested for binding to the Tec SH3 domain using either the Fluorescence Polarisation (FP) peptide competition assays and/or the NMR chemical shift perturbation methods described in previous chapters. In the first instance, where the compound to be tested was adequately soluble in phosphate buffered saline (PBS) (as for **33**, **39-42**, **64**, and **69**) then FP was the preferred method, as it is more efficient (reasons for this were discussed in Chapter 2). The minimum level of solubility required for this method was ~1.5 mM. If a solution of the compound could not be prepared in PBS at a concentration of ~1.5 mM, (as for **70**) it was necessary to use the NMR chemical shift perturbation method, which is compatible with 10% DMSO in the buffer. The DMSO enhances the solubility of the ligand (also discussed in Chapter 2). With this in mind, 2-aminoquinolines **33**, **39-42**, **64** and **69** were tested for binding using FP, however, **70** was tested using NMR. 2-Aminoquinolines **33**, **64** and **69** were tested using both methods. The FP experiments for testing compounds **64** and **69** were performed by Cvetan Stojkoski. These binding studies will now be discussed in detail.

4.4.1 Fluorescence Polarisation peptide competition assays

As determined with the FP peptide displacement assay, 2-aminoquinolines **33**, **39**, **41** and **42**, all competed for binding with **PRP-1** to the Tec SH3 domain with approximately two to three fold improved affinity relative to 2-aminoquinoline **2** (EC_{50} ca. 60-75 μM for **33**, **39**, **41** and **42**, EC_{50} = 160 μM for **2**) (Figure 4-2, Table 4-2). However **40** competed for binding with similar affinity (EC_{50} = 150 μM) to **2** (Table 4-2). 2-Aminoquinolines **64** and **69** were able to compete for binding with **PRP-1** with a further improvement in EC_{50} relative to **33**, **39**, **41** and **42**, and an approximately five to six-fold improvement in affinity relative to **2** (EC_{50} ca. 30 μM for **64** and **69**) (Figure 4-2, Table 4-2).

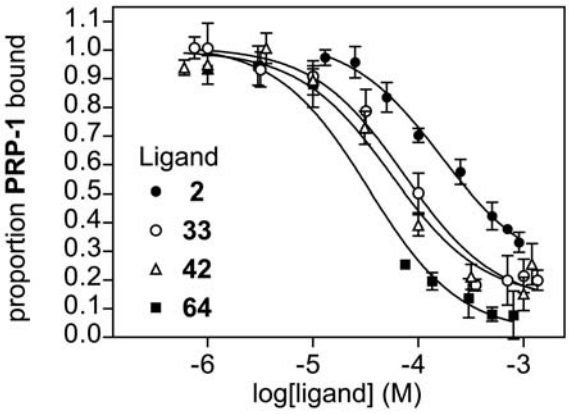
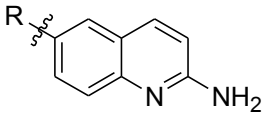
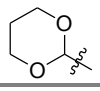


Figure 4-2: 6-Substituted-2-aminoquinolines/Tec SH3 Binding Studies. Overlays of isotherms obtained from independent experiments for competition of fluorescent proline-rich peptide **PRP-1** by **2**, **33**, **42** and **64** from Tec GST-SH3 protein using Fluorescence Polarisation Assay.

Table 4-2: Ligand binding studies of 2-aminoquinolines **33**, **39-42**, **64** and **69** with the Tec SH3 domain, using Fluorescence Polarisation (FP) peptide competition assay.



Ligand	R	EC_{50} (mM)†	Ligand	R	EC_{50} (mM)†
2	H	160 ± 36	**41**	Cl	76 ± 8
33	Me	75 ± 15	**42**	Br	58 ± 15
39	MeO	63 ± 22	**64***		34 ± 5
40	F	150 ± 36	**69***		26 ± 6

† Quoted values are mean ± standard deviation over 3 replicate experiments. * These experiments were performed by Cvetan Stojkoski.

4.4.2 NMR chemical shift perturbation assays

As previously mentioned, compound **70** was not suited to the FP ligand displacement assay for reasons of poor solubility in entirely aqueous conditions. Therefore **70** was tested using the NMR chemical shift perturbation method with a 10% DMSO buffer system. In addition, to complement the results obtained from the FP peptide displacement experiments, **33**, **64** and **69** were also tested using this method.

4.4.2.1 Exchange processes and determination of ligand binding constants

Importantly, for NMR experiments involving **64**, **69** and **70** the ^{1}H (H-N) chemical shifts for the side chain of W215 and the backbones for other residues close to the ligand binding site exhibited intermediate exchange processes, rather than fast exchange processes (on the NMR timescale) at early stages of the titrations. The evidence for this was either complete loss of, or substantial drops in the intensity of the H-N cross peaks at ligand concentrations less than 1 molar equivalent of the protein. Some examples of this are illustrated in Figure 4-3A; two separate regions of overlaid [^{1}H,^{15}N] HSQC spectra of the Tec SH3 domain in the presence of increasing concentrations of **70** are provided. In the case of the signal for the W215ε1 proton (top region of overlaid spectra), at [**70**] = 25 and 50 μM (0.2 and 0.4 molar equivalents of protein respectively), the H-N cross peak disappears completely. When [**70**] = 75 μM, the H-N cross peak reappears, but with reduced intensity. The signal returns to near full intensity when [**70**] = 100 μM, and is at full intensity for the remaining concentrations of **70**. Two more examples are worth noting, as illustrated in the bottom region of overlaid spectra in Figure 4-3A. Firstly, in the case of T192, a very faint signal is observed at [**70**] = 25 μM as illustrated with the red coloured arrow, but the signal returns to near full intensity when [**70**] = 50 μM. Secondly, for H214, the signal at [**70**] = 25 μM is only slightly reduced in intensity, and essentially retains the features of a fast exchange process for the remaining concentrations of **70**. In the case of W216ε1 (top spectra) the chemical shift is characteristic of an entirely fast exchange process.

The consequence of these features of the HSQC spectra was that not all the chemical shift changes observed could be used as monitors for the calculation of equilibrium binding dissociation constants. When there is an intermediate exchange process, some uncertainty exists in the 'true' chemical shifts. Subsequently, if the fast exchange assumption is applied to systems that are in intermediate exchange, errors in the K_d determinations may result. In order to accurately determine the K_d for a system in intermediate exchange, line shape analyses are needed.[83,84] Hence, for ease of comparison, only those residues whose ^{1}H (H-N) cross-peaks exhibited essentially fast exchange processes over the entire range of ligand concentrations were selected as monitors for K_d determinations. Therefore, using the example of **70** (Figure 4-3A) W215ε1 and T192 were excluded from the K_d determination, but W216ε1 and H214 were included. In the case of **33**, there was a small decrease in the intensity of the H-N cross-peak for the side-chain of W215 at 0.33 molar equivalents of **33**, (similar to that observed for H214 in the discussion of **70** binding above – data not shown) however the fast exchange assumption was retained in this case.

2-Amino-6-methylquinoline **33** bound the Tec SH3 domain with equilibrium binding dissociation constant (K_d) of 61 μM (Figure 4-3B, Table 4-3) while **64**, **69** and **70** bound with K_ds of 40, 52 and 22 μM respectively (Figure 4-3B, Table 4-3). These results suggest that **64** and **69** bind with similar affinity, but improved affinity relative to **33**, however **70** binds with a further improvement in affinity relative to **64** and **69**.

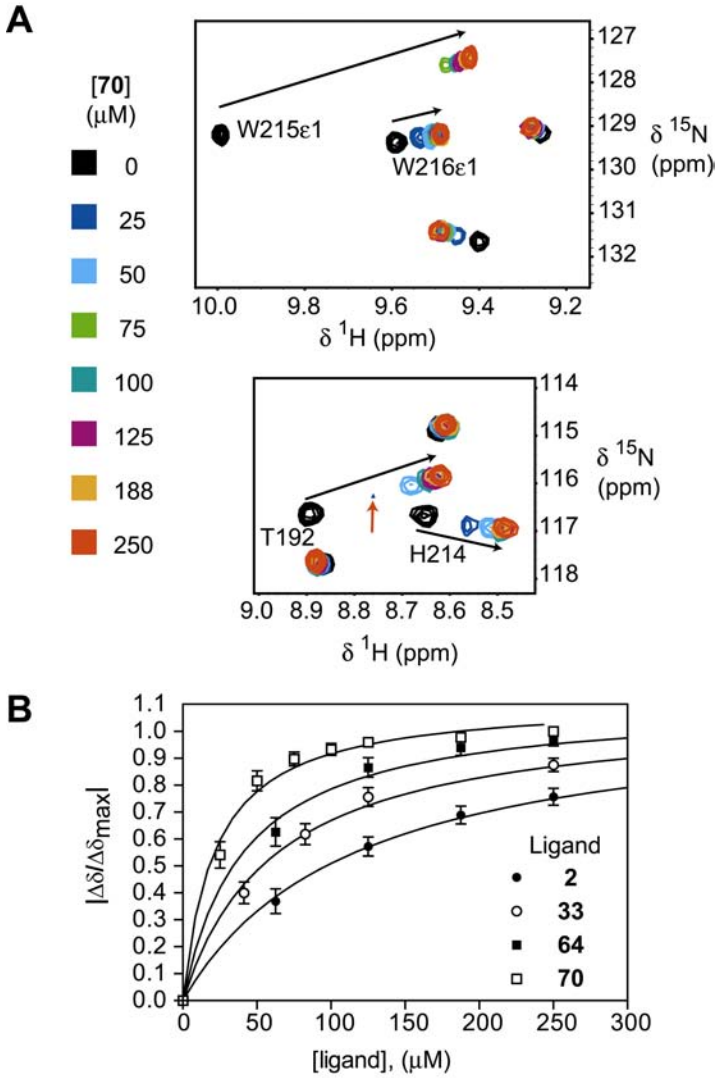
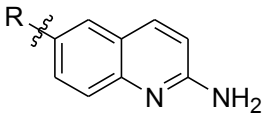
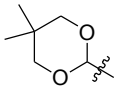


Figure 4-3: Ligand binding studies of 2-aminoquinolines **2**, **33**, **64**, and **70** with the Tec SH3 domain using NMR Spectroscopy. (A) Selected regions of overlaid [^{1}H,^{15}N]-HSQC spectra of uniformly ^{15}N labelled Tec SH3 protein in presence of increasing concentrations of ligand **70**. The labelled cross peaks indicate residues where δ ^{1}H (H-N) was altered by at least 0.1 ppm at saturation binding. These regions show cross peaks that exhibit both intermediate (W215ε1, T192, H214), and fast exchange (W216ε1) processes. (B) Binding isotherms represented by normalised, averaged chemical shift changes for residues involved in binding of ligands **2**, **33**, **64**, and **70**, that exhibited fast exchange processes. (See main text for discussion of the red coloured arrow in the bottom part of A.)

Table 4-3: Ligand Binding Studies of 2-aminoquinolines **33**, **64**, **69** and **70** with Tec SH3 Domain, using NMR Chemical Shift Perturbation Experiments.



Ligand	R	K_d (mM)*	Ligand	R	K_d (mM)*
2	H	125 ± 24	**69**		52 ± 16
33	Me	61 ± 6	**70**		22 ± 5
64		40 ± 8			

*Quoted values are mean ± standard deviation over residues where ^{1}H (H-N) chemical shift changes of protein exceeded 0.1 ppm at or near saturation binding of ligand.

4.4.2.3 Chemical shift mapping of ligand binding events

The amino acid residues whose ^{1}H (H-N) chemical shifts were altered by at least 0.1 ppm (including those that were in intermediate exchange as discussed in Section 4.4.2.1) at or near saturation binding of ligands **64**, **69** and **70** were mapped onto the SH3 fold (see for **70** in Figure 4-4). This revealed that essentially the same residues were involved in binding of these ligands, as those involved in the binding of 2-aminoquinoline **2**, with a few additional changes in chemical shift observed. [Compare left (**2**) and right (**70**) portions of Figure 4-4] The majority of these additional chemical shift changes were observed at residues adjacent to the 'right-hand' side of the ligand binding site, according to the binding model, consistent with new interactions being made with one or more of the residues illustrated in Figure 4-1.

4.4.3 Interpretation of SAR information

All of the presented structure activity information for the 6-substituted-2-aminoquinolines suggests that a new lipophilic contact is made between the substituents on the quinoline ring and the protein. Where a simple lipophilic group is placed in the 6-position (eg. Me, MeO, Cl, and Br as in **33**, **39**, **41**, and **42**), a two to three-fold improvement in affinity is obtained, whereas if a smaller lipophilic group is present (eg. F or H as for **40** and **2**), this contact is not made (Table 4-4). These results also suggest that electronegative substituents at the 6-position have little or no effect on the ligand binding process according to the ligand binding model (Figure 4-1). When more complex and larger (cyclic-acetal) groups are placed at the 6-position of the quinoline ring (**64**, **69** and **70**) a further improvement in affinity is obtained (Table 4-4), and this interaction is optimal in the case of **70**, that has extra methyl groups that

may make additional contacts with the protein surface. 2-Aminoquinoline derivative **70** was the highest affinity Tec SH3 domain ligand identified from these studies (K_d = 22 μM) and has six-fold improved affinity relative to unsubstituted 2-aminoquinoline **2**. But in the absence of a 3D structure of the **70**/SH3 complex, the precise mechanism that mediates the improvement in affinity obtained for ligands **64**, **69** and **70** remains unclear. Of the amino acid residues that are close to where the 6-position substituent sits, according to the ligand binding model, leucine 213 (L213) (Figure 4-1) is the only bulky lipophilic side-chain present. It would seem that L213 is therefore the most likely residue to mediate a lipophilic interaction with bulky lipophilic functionality on the ligand. However, on inspection of Figure 4-1, L213 appears to be too far from where the 6-position substituent sits (according to the ligand binding model). Therefore, a conformational change in this region may occur, that puts the L213 side-chain in place to make the lipophilic contact with the ligand. This highlights the need for a 3D structure of this complex, to provide confirmation of the mechanism of binding of these ligands. However, ligand **70** remains unsuitable for ligand/protein structure determination using NMR methods as slow exchange ligands are required to allow adequate time to transfer magnetisation from the ligand to the protein to perform Nuclear Overhauser experiments for structure calculations.

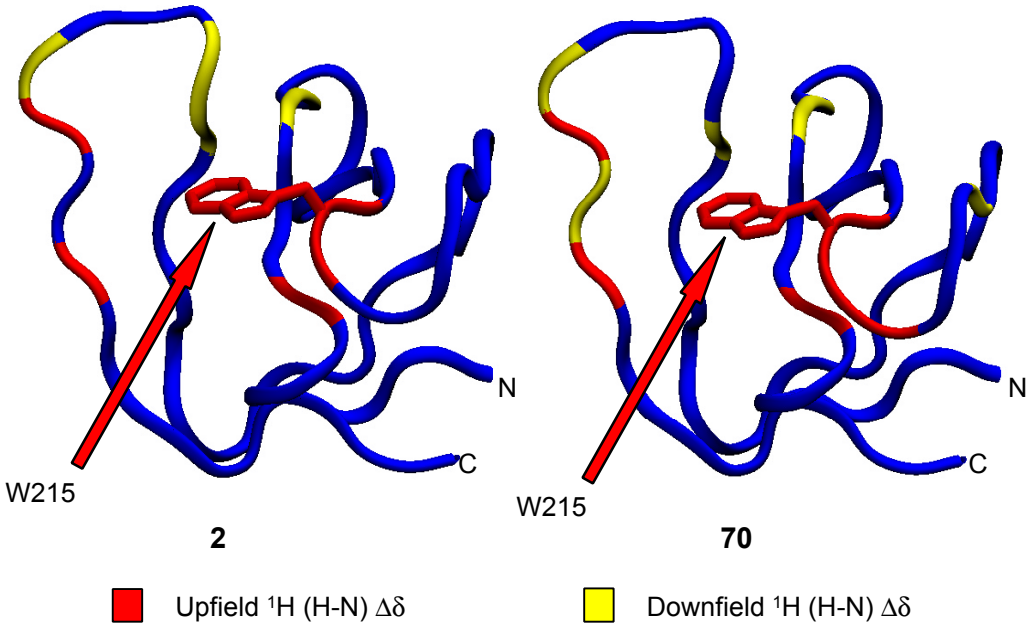


Figure 4-4: Chemical shift mapping of backbone or side-chain (H-N) resonance where δ ^{1}H (H-N) was altered by at least 0.1 ppm at saturation binding of **2** (left) and **70** (right).

4.4.4 Investigation into stability of acetals during ligand binding experiments

Although the highest affinity ligands (**64**, **69** and **70**) identified in these studies provide useful SAR information and are good starting points for developing ligands with improved potency, the acetal functionality is not 'drug like' in character. Specifically, acetals hydrolyse to carbonyl compounds under acidic aqueous conditions. To determine whether a substantial amount of hydrolysis of these acetals was occurring during the protein binding experiments, the rate of hydrolysis of **64** to form aldehyde **58** was investigated using NMR spectroscopy. A dilute sample of **64** was made in a 10% DMSO/phosphate buffer system identical to the conditions used for the NMR K_d determinations. Similarly a sample that mimicked the conditions of the FP assay system was also prepared. ^{1}H NMR experiments were recorded over a time course of several hours and the relative percentage of **58** present was calculated by comparing the relative integrations of the ^{1}H signals representing the acetal and the aldehyde protons. In the case of the 10% DMSO sample, only 3-4% of **58** was present after 4.5 hours and about 5% of **58** was present after 8 hours (see Figure 4-5). Given that a typical series of experiments for determining the K_d of a ligand by NMR is of the order of 8 hours, this amount of hydrolysis is not expected to have a significant impact on the accuracy of the K_d determinations for **64**, **69** and **70**. In the case of the sample that mimicked the FP conditions, approximately 7% of **58** was present after 4 hours, and after 10 hours this had risen to 9% (Figure 4-5). It is likely that the slightly higher amount of **58** present at the earlier times in this case was a result of the heating required to assist dissolving **58** in the aqueous

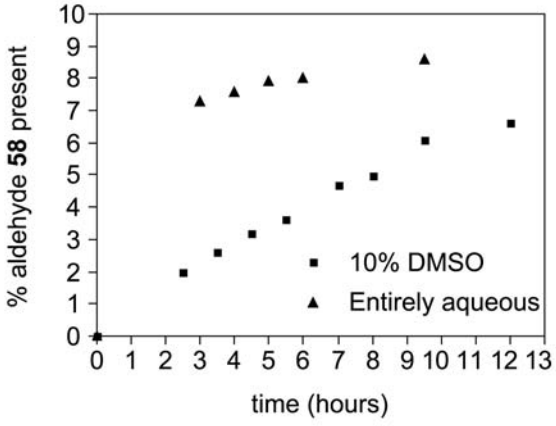


Figure 4-5: The rates of hydrolysis of **64** to **58** under aqueous conditions, as determined by ^{1}H NMR Spectroscopy. The series represented by the square was derived from a sample of **64** (1 mg) dissolved in 0.5 mL of 10 mM Na_2HPO_4 with 10% v/v d_6-DMSO and 10% v/v D_2O pH 6.5. The series represented by a triangle was derived from a sample of **64** (1 mg) dissolved in 0.5 mL of 10 mM Na_2HPO_4, with 10% v/v D_2O (heating of sample required to dissolve ligand).

medium. A typical FP experiment takes of the order of 3 hours between commencing to dissolve a ligand and recording measurements. Therefore this level of aldehyde present in the sample is again unlikely to substantially influence the accuracy of the EC_{50} values determined. Furthermore, it is expected that the rates of acetal hydrolysis for ligands **69** and **70** would be even lower due to the increased stability associated with dioxane acetals relative to dioxolane acetals, hence even less influence of **58** is expected in determining the binding constants for these ligands.

4.4.5 Summary

A series of 6-substituted-2-aminoquinolines with both simple (**33**, **40-42** and **39**) and bulkier (**64**, **69**, and **70**) lipophilic groups were tested for binding to the Tec SH3 domain. With the exception of **40**, all these ligands bound the SH3 domain with an improvement in affinity. The ligands with simple substituents bound with approximately two to three-fold improved affinity relative to 2-aminoquinoline **2**, and the ligands with the bulkier hydrophobic groups bound with a further improvement in affinity. In the case of ligand **70**, a six-fold improvement relative to **2** was observed. These results provide useful structure activity information and suggest that a new lipophilic contact with the protein is made. However, the highest affinity ligands (**64**, **69**, and **70**) are not 'drug-like' due to their aqueous instability. In addition these compounds were originally intended as intermediates towards synthesis of ligands more hydrophilic groups such as compounds **59** and **60**, but were unable to fulfil this role. Furthermore, compounds **64**, **69**, and **70** remain in fast to intermediate exchange on the NMR timescale, and hence are still unsuitable for structure determination using NMR spectroscopy, as this would require a slow exchange ligand.

Therefore, this prompted investigation into the synthesis of ligands with alternative functionality, with improved drug-like character. Clearly ligands with bulky hydrophobic character are desirable, however ligands with hydrophilic groups still require investigation. The major goal of improving affinity remains.

4.5 Synthesis of 6-substituted-2-aminoquinolines with more complex functionality 2: Uncovering the limitations of the Kóródi method

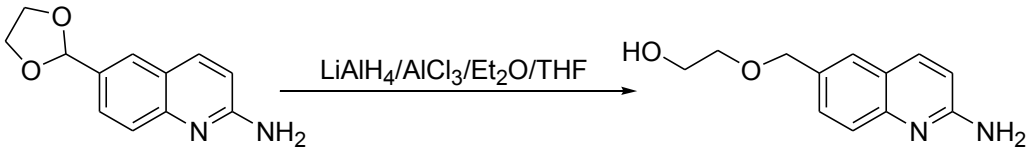
As just discussed, ligands with hydrophilic groups such as hydroxyl or amino groups were still required, as a means of continuing to explore the protein surface for new contacts. As part of the original 5-, 6-, or 7-substituted-2-aminoquinoline design process (Figure 4-1),

compounds such as **59** and **60** (Scheme 4-5) were sought. The synthesis of these compounds was further delayed however, because it has been reported that cyclic acetals can be converted to acyclic alcohols by reduction with lithium aluminium hydride in the presence of aluminium chloride.[80] Given that cyclic acetal derivatives of 2-aminoquinoline had been studied as SH3 domain ligands in Section 4.4, it was envisaged that conversion of these acetals into acyclic alcohols, be useful in synthetic strategies for the synthesis of 2-aminoquinolines with hydrophilic functional groups. Hence some time was invested into testing the approach, and investigating the best way to these alcohols into synthetic strategies for the synthesis of 2-aminoquinolines with more diverse functionality. The utility of the amination method of Kóródi with a few different 2-chloroquinoline derivatives was also a significant feature of this investigation, and thus, some limitations of this method will also be presented.

4.5.1 Cyclic acetals as precursors for acyclic alcohols

4.5.1.1 A preliminary investigation

The first part of this new investigation was to test the reaction for conversion of cyclic acetals to acyclic alcohols. In the first case, this was attempted using dioxolane **64** as a substrate, as illustrated in Scheme 4-13 below. The stoichiometry used the first time was retained as reported.[80] Also, the reported method used ether as the solvent. However, it was found that acetal **64** was poorly soluble in ether, hence the compound was dissolved in THF for addition to the prepared mixture of lithium aluminium hydride/aluminium chloride/ether. Following workup, ^{1}H NMR spectroscopy revealed that the isolated material consisted of mainly the desired product **76**, in conjunction with a small amount (~10%) of aldehyde **58**. The yield however was low (22% assuming pure **76**). A feature of the ^{1}H NMR spectrum that provided evidence for the presence of **76** included a large upfield change in chemical shift from δ 5.8 to 4.6 ppm in combination with a doubling in the relative area, for the conversion of the acetal proton [$\underline{H}C(OR)_2$] to two methylene type protons ($OC\underline{H}_2$). In addition, the complex multiplet seen at δ 3.90-4.13 ppm for the ethylene protons in **64** was changed to two 2H signals characteristic of an AA'BB' system ($OC\underline{H}_2C\underline{H}_2O$) at ~ δ 3.55 and 3.67 ppm. A second set of signals in the aromatic region of the ^{1}H NMR spectrum, in conjunction with a singlet at δ 10 ppm provided evidence for the formation of **58** in the reaction. The formation of **58** as a by-product in the reaction was most likely a result of the acetal reduction not having reached completion when the reaction was worked up. Given that the workup involved aqueous conditions, the acetal hydrolysed back to the aldehyde on exposure to aqueous, mildly acidic conditions.



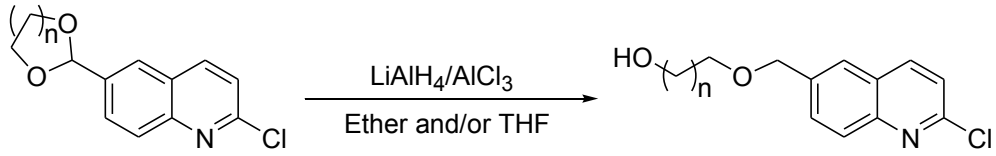
64 **76**

Scheme 4-13: Preliminary investigation into the synthesis alcohol **76** from **64**.

Although the synthesis described above was far from optimal, it did provide some encouragement for use of the method to prepare the desired alcohols. Prior to investing any additional time trying to improve the conditions for applicability to the systems of interest, the crude product of **76** isolated was tested for binding to the Tec SH3 domain using the NMR Chemical Shift Perturbation method, and found to bind with a K_d of ca. 40 μM (see Section 4.8). This indicates that these compounds bind with similar affinity to the best ligands identified so far, those being the cyclic acetal 2-aminoquinoline derivatives **64**, **69** and **70**. This preliminary binding data therefore did provide encouragement for this approach to preparing ligands with hydrophilic functionality. This approach offered some additional appeal, as it potentially provided a means for obtaining lipophilic groups in conjunction with hydrophilic groups. Furthermore, the reaction could be applied to both dioxolane or dioxane acetals to yield either ethanol or propanol derivatives respectively. This provided potential for extending the carbon skeleton, and providing a possible linkage point for additional functionality. A thorough investigation into the use of this method, and subsequent conversions is therefore the focus of the next sections.

4.5.1.2 Investigation into optimising the reaction

Due to the low yield obtained when performing the acetal reduction on the 2-aminoquinoline derivative as illustrated in Scheme 4-13, it was decided to instead test the method on the 2-chloroquinoline acetal derivatives **63** and **67**. The 2-chloroquinolines are considerably less polar compounds than the 2-aminoquinolines, and given that this acetal reduction results in formation of an alcohol, when carried out on a 2-aminoquinoline derivative, the resulting



63 n = 1
67 n = 2

77 n = 1
78 n = 2

Scheme 4-14: Conversion of acetals **63** and **67** to alcohols **77** and **78** respectively.

product is a 2-aminoquinoline/alcohol derivative, resulting in a highly polar compound, that may be difficult to purify. However, a 2-chloroquinoline/alcohol derivative is considerably less polar than the latter, and should be a material that is more convenient to work with.

The conversion of **63** to **77** was attempted as illustrated in Scheme 4-14. Again, the stoichiometry as reported[80] was used involving approximately 2 equivalents of aluminium chloride and 0.5 equivalents of lithium aluminium hydride. The substrate was again added in THF instead of ether, for reasons of poor solubility of **63** in ether alone. After aqueous acidic workup, a product was isolated that consisted of approximately 4:1 of the desired product **77** and aldehyde **57** in quite good yield (~80% assuming pure **77** as product). The changes in chemical shift observed for the formation of **77** from **63** were consistent with those observed for the conversion of **64** to **76**, as discussed in Section 4.5.1, and the characteristic resonance at δ = 10 ppm again provided evidence for the formation of aldehyde **57**. Signals seen in the aromatic region were also consistent with those seen in the spectrum of **57** prepared in Section 4.3.1. This observation again suggested that the reaction had not proceeded to completion, and that the aldehyde had most likely formed during the workup when the unreacted acetal was exposed to aqueous acidic conditions. Thin layer chromatography (TLC) was used to monitor the reaction progress, and the developed plates were indicative of the formation of **57** during the 'mini-workup' prior to spotting the TLC plates. The intensities of the spots representing the desired product **77** and aldehyde **57** were however misleading, making this reaction difficult to follow by TLC.

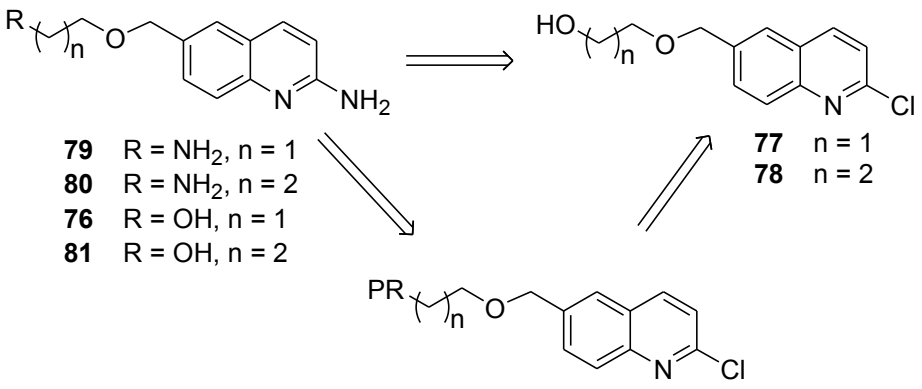
These preliminary investigations had so far not lead to an ideal procedure for the acetal to alcohol conversion. Although the yield was considerably improved when the method was applied to the 2-chloroquinoline instead of the 2-aminoquioline derivative, the underlying problem was that the reaction did not proceed to completion. To address this problem, the next reaction was carried out in THF alone instead of the ether/THF mixtures previously used. Furthermore, in contrast to the previous attempts that were performed at room temperature, this time, the reaction was heated at reflux for 8 hours, to try to improve the conversion to product. For reasons of supply, this experiment was carried out on acetal **67**. Following acidic aqueous workup, the 4:1 mixture of product **78** and aldehyde **57** was again observed. This indicated that temperature was not the limiting factor in assisting this reaction to proceed to completion. Thus it was concluded that the next experiment should involve some changes in the stoichiometry of the reagents. Thus, the reaction was next performed with 1.0 equivalents of $LiAlH_4$ instead of the 0.5 equivalents previously used. This approach was successful; this time a 19:1 ratio of alcohol **78** to aldehyde **57** was observed. Thus future

experiments involved an excess of $LiAlH_4$ (~1.2 equiv), and NMR spectra of the isolated products indicated that no **57** was formed at all.

Following the above investigations, both acetals **63** and **67** could be routinely converted to alcohols **77** and **78** respectively in good yields (~80%) on relatively large scales (~1 g of starting material). The crude products isolated were also of sufficient purity to be used in subsequent reactions without any purification as judged by ^1H NMR. However, to assist in characterisation, analytical samples were easily prepared following chromatography over silica gel.

4.5.2 Acyclic alcohols as precursors for synthesis of new 2-aminoquinolines with diverse functionality

Having established a suitable method for the synthesis of alcohols **77** and **78**, it was then time to investigate what types of compounds could be prepared from these precursors. At this stage, it was assumed that the conversion of 2-chloroquinolines to 2-aminoquinolines using the method of Kóródi[72] would be suited to any 2-chloroquinolines, provided that suitable protecting groups for additional functionality were in place. Therefore, one of the first approaches to test was the conversion of either of the alcohols **77** or **78** into protected amino derivatives for conversion into 2-aminoquinolines using the method of Kóródi, and subsequent de-protection to give di-amino derivatives **79** or **80** as illustrated in Scheme 4-15. Similarly, an improved method for the preparation of alcohol derivatives of 2-aminoquinolines **76** and **81**, would also be investigated. As illustrated in Scheme 4-15, one approach may be to test the conversion of 2-chloroquinolines **77** or **78** to 2-aminoquinolines **76** and **81** using



Scheme 4-15: Retrosynthetic plan for use of alcohols **77** and **78** as precursors of 2-aminoquinolines **79**, **80**, **76**, and **81**.

the method of Kóródi without the use of protecting groups. If necessary, incorporation of protecting groups for the hydroxyl group would also be investigated. The outcomes of these investigations are the focus of this section.

4.5.2.1 Adding new functionality to 2-chloroquinolines

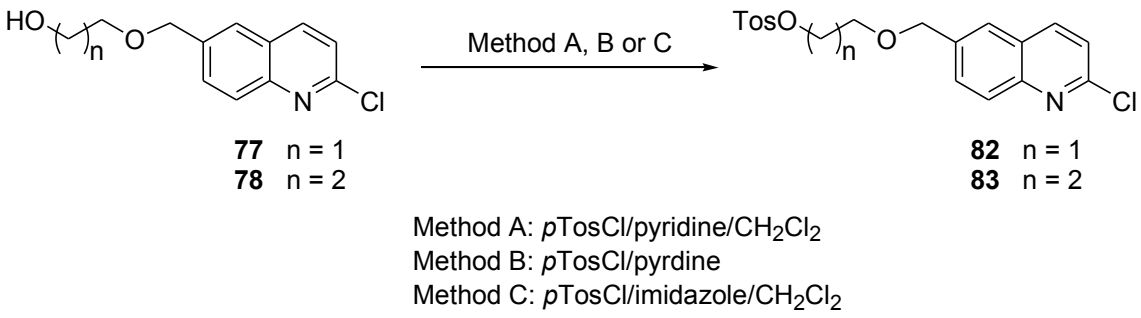
Investigation into conversion of alcohols 77 and 78 into tosylates

The first investigative step towards synthesis of diamines **79** or **80** was the conversion of alcohols **77** or **78** into tosylates **82** or **83**. These tosylates were envisaged to be useful in substitution chemistry with nitrogenous nucleophiles that might lead to 2-chloroquinolines potentially suited to conversion into 2-aminoquinolines using the method of Kóródi (see later).

The synthesis of tosylate **82** was tested by treatment of alcohol **77** with *p*-toluenesulfonyl chloride in the presence of an excess of pyridine in dichloromethane at room temperature (Scheme 4-16, method A). After a few hours, thin layer chromatography suggested that the reaction was proceeding, although substantial starting material was still observed. Thus additional *p*-toluenesulfonyl chloride was added such that a total of ~ 2 equivalents was present, and the reaction was allowed to proceed for an additional 60 hours, before workup. The isolated material was an approximately 3:1 mixture of the desired tosylate **82** and starting alcohol **77** as indicated in the ^{1}H NMR spectrum. One feature of the ^{1}H NMR spectrum to provide evidence for the formation of **82** was the substantial downfield shift of one portion of the AA'BB' multiplet from 3.80-3.83 ppm in **77** ($CH_2C\underline{H}_2OH$) to 4.21-4.24 ppm in **82** ($CH_2C\underline{H}_2OTos$), as would be expected by replacement of a C-OH bond with a C-OSO_2 bond. In addition, a signal with relative area indicative of 3H relative to the 2H signal just described was observed, suggesting the presence of a methyl group. However, there was also evidence for *p*-toluenesulfonyl chloride or *p*-toluenesulfonic acid remaining in the mixture. 116 Milligrams of material was recovered from the reaction when 109 mg of **77** was invested, and given that the mixture was not pure, this is indicative of a poor yield.

The next approach taken in an attempt to improve the yield and conversion of the above reaction was to carrying out the reaction in neat pyridine instead of using dichloromethane as solvent (Scheme 4-16, method B). Hence the pyridine was in large excess. Using this approach, involving again approximately 1.5 equivalents in total of *p*-toluenesulfonyl chloride, and allowing the reaction to proceed for a total of ca. 48 hours, the isolated product consisted of 60:40 **82** and **77**, and again low recovery (73 mg from 123 mg invested) was observed. It is likely that extra losses resulted from the acid washes used for removing the pyridine from

the reaction mixture, due to protonation and subsequent loss of the quinoline product to the aqueous layer. Thus, the use of pyridine as a base for this reaction was discontinued.



77 n = 1
78 n = 2

82 n = 1
83 n = 2

Method A: *p*TosCl/pyridine/CH_2Cl_2
Method B: *p*TosCl/pyrdine
Method C: *p*TosCl/imidazole/CH_2Cl_2

Scheme 4-16: Investigation into synthesis of tosylates **82** and **83**.

In a final attempt to find a better method to prepare the desired tosylates, the experiment was repeated one more time. Again for reasons of supply, the reaction was this time tried with alcohol **78** instead of **77**. The reaction conditions were changed back to use of dichloromethane as a solvent, but imidazole was instead used as the base (Scheme 4-16, method C). Imidazole offers the advantage of being more easily removed by washing with water, instead of requiring washing with acid, as in the case of pyridine. The reaction was again allowed to proceed for around 60 hours, and even after a total of ca. 2 equivalents of *p*-toluenesulfonyl chloride and a total of 5 equivalents of imidazole were added, only a mixture of starting material **78**, imidazole, and *p*-toluenesulfonic acid (or *p*-toluenesulfonyl chloride) was isolated after workup, as judged by ^{1}H NMR spectroscopy. There was no convincing evidence for the formation of the desired tosylate.

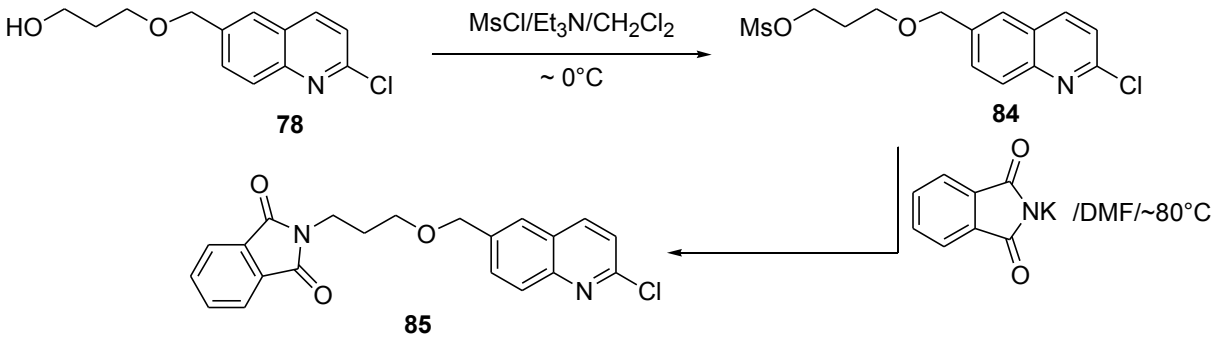
The results described above did not provide encouragement for the use of imidazole/ dichloromethane as an alternative to pyridine/dichloromethane or neat pyridine as the optimal conditions for the synthesis of the desired tosylates. In fact, for reasons that are unclear, the use of tosylates as precursors for substitution chemistry was not looking promising at all. The underlying problem was getting the reaction to proceed to completion, and devising conditions that minimised the losses of product in the workup.

Activation of alcohols for substitution chemistry via mesylates

In order to overcome the problems encountered above for the preparation tosylates, it was decided to try to synthesise mesylates instead. This method was tested with instant success, as illustrated in Scheme 4-17. Mesylate **84** was prepared in 91% yield following treatment of **78** with methanesufonyl chloride in the presence of triethylamine in dichloromethane at ca. 0°C. Also, given that triethylamine can be removed by evaporation, and excess methanesulfonyl chloride can be removed by washing with water, the losses in yield were minimal during the workup relative to those encountered for synthesis of the equivalent

tosylates. Features of the ^{1}H NMR spectrum of **84** that provided evidence for its formation included a similar downfield shift of a triplet at seen at δ 3.78 ppm for the ethylene protons in **78** ($CH_2C\underline{H}_2OH$) to a triplet at δ 4.41 ppm in **84** ($CH_2C\underline{H}_2OMs$), consistent with the change in chemical shifts seen in the tosylates described above. In addition, a singlet with relative area three was observed at δ 3.0 ppm, as expected for the methyl group of a sulfonate ester.

Having established that mesylates were much more conveniently prepared than tosylates in the present case, it was then time to test the use of mesylate **84** in substitution chemistry. A classic method for the synthesis of amines using substitution reactions is by treatment of an



Scheme 4-17: Synthesis of mesylate **84** and its conversion to phthalimido derivative **85**.

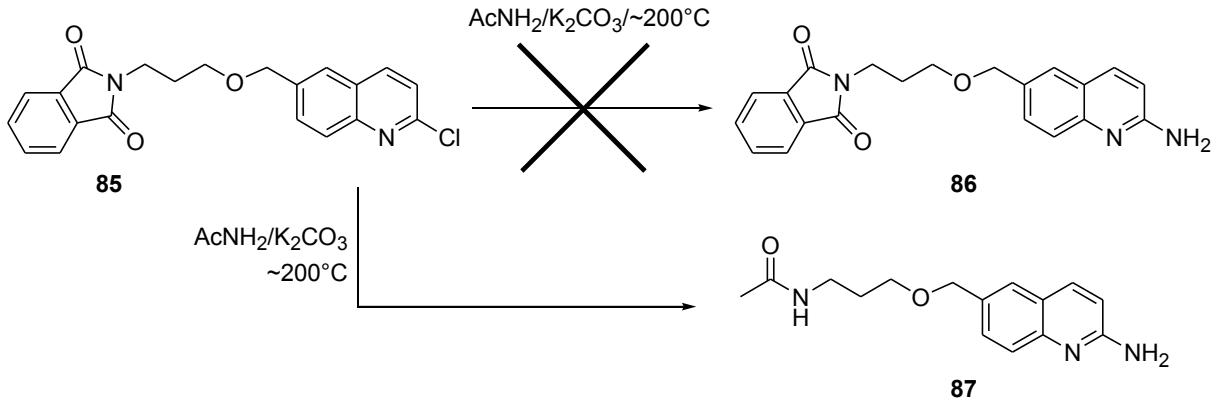
alkyl tosylate or halide with potassium phthalimide. The resulting imido derivative can then be hydrolysed to an amine. Hence, **84** prepared above (without any purification) was treated with potassium phthalimide in DMF overnight at ca. 80°C, as illustrated in Scheme 4-17. This resulted in the formation of phthalimide derivative **85** in high yield (89%). Features of the ^{1}H NMR spectrum of **85** that provided evidence for its structure included an upfield shift of the triplet at δ 4.40 ppm for the methylene protons adjacent to the mesylate functionality ($CH_2C\underline{H}_2OMs$) in **84** to a triplet at δ 3.87 ppm in **85** for the methylene protons adjacent to the imido functionality ($CH_2C\underline{H}_2N$). In addition, the singlet seen at δ 3.0 ppm for the methyl group of **84** was not observed in the spectrum of **85**. Additional signals with total relative area of four were also seen in the aromatic region as expected for a phthalimide system. Characteristic stretches in the IR spectrum were also observed at 1770 and 1719 cm^{-1} consistent with asymmetric and symmetric vibrations for the imido carbonyl groups. The identity of **85** was also confirmed by mass spectrometry, and following recrystallisation of a portion from methanol, an analytical sample was also obtained.

4.5.2.2 Investigation into compatibility of the amination method of Kóródi with a range of 2-chloroquinoline derivatives

A position had then been reached where the suitability of phthalimide derivative **85** (prepared above), to the amination conditions of Kóródi could be tested. This, in addition to testing the suitability of alcohols **77** and **78** is the emphasis of the next section.

Testing the method of Kóródi with 2-chloroquinoline 85

Having finally established a suitable method to generate 2-chloroquinoline derivative **85** that had a protected amine in place, testing its suitability for conversion into 2-aminoquinoline **86** (Scheme 4-18) using the method of Kóródi was required. When **85** was treated with acetamide at ca. 200°C, the phthalimido derivative **86** could not be identified as a component of the isolated product mixture. There was evidence to suggest that the reaction had not proceeded as planned prior to the major workup; when a 'mini-workup' was performed leading up to thin layer chromatography for analysis of reaction progress, the majority of the hot reaction contents appeared to dissolve in water, suggesting that the generated product was water soluble. This observation was not made during the workups of other compounds used in similar reactions. For instance in the preparation of amines such as **33** or **64**, large precipitates were observed on addition of the reaction contents to water, and these precipitates were then readily dissolved in organic solvents. The subsequent thin layer chromatograms were also inconsistent with those observed for the other amines, with the resulting products having lower R_f values than typically observed, suggesting that a more polar product had been formed.



Scheme 4-18: Investigation into the suitability of phthalimide **85** as a substrate for the amination method of Kóródi.

Following workup of the reaction mixture, the isolated material was analysed by ^{1}H NMR spectroscopy, and this suggested that a major component of the mixture was acetamide. However, there was also evidence for a 2-aminoquinoline present in the mixture, provided by

the presence of a doublet at δ ~ 6.7 ppm with *J* = ~ 9.0 Hz, consistent with the chemical shifts observed for H3 of the quinoline ring in the other 2-aminoquinolines. All the alkyl signals were in place as expected, and there was evidence for the presence of a phthalimido group in the mixture, however, the relative areas of these signals were not as expected for a phthalimido adduct. Due to the low purity of the product, it was not possible to be convinced of the structure of the product prior to additional purification and other analytical techniques. The required $M^{+\cdot}$ for **86** was not observed in the electron impact mass spectrum.

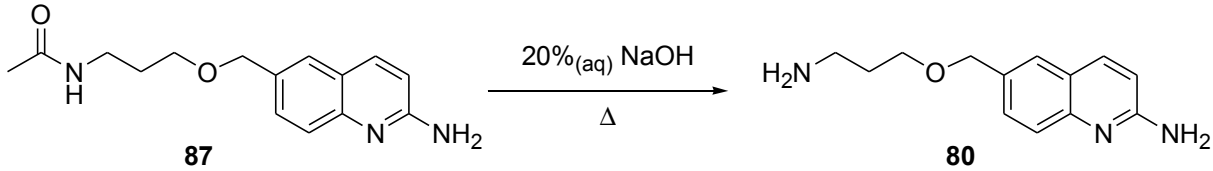
Some thought was then required to devise a plan for purification of the product. Given the apparent high water solubility of the material, and the high degree of polarity of the product as judged by TLC (R_f ~ 0 in 9:1 dichloromethane/ethanol), removal of the excess acetamide by washing with water was not considered a sensible option. Hence, the first attempt to purify the material was to use cation exchange chromatography, with a strongly acidic Amberlite resin [IR-120(H)]. The sample was loaded onto the column and washed with 1:1 water/methanol as eluant to remove the excess acetamide. The product was then eluted from the column by changing the eluant to 1% ammonia in 1:1 water/methanol. Once the solvent was removed, ^{1}H NMR of the isolated material indicated that the ion exchange procedure was effective for removal of the acetamide, but the resulting material had significant insoluble impurities, and the purity remained inadequate for effectively characterising the product.

The resulting material was filtered to remove insoluble impurities, and the filtrate was subsequently purified using preparative silica gel thin layer chromatography, from which an essentially pure product could be isolated, as judged by ^{1}H NMR spectroscopy. The identity of the material was then determined, and found to be **87** as illustrated in Scheme 4-18. Thus the final yield could be determined and was low (10%).

There was a lot of evidence to assist in assigning the structure of **87** as the major product formed in the reaction. Firstly, in the purified material, no signals were observed representative of the phthalimide functionality, indicating that this group was clearly lost during the reaction. However there was the appearance of an additional NH as well as the NH_2 signal, that was suggestive of the acetamide ($N\underline{H}COCH_3$) functionality. This was accompanied by a three proton singlet seen at δ 1.86 ppm for the CH_3 group of the acetamide functionality. The ^{13}C NMR spectrum contained the correct number of carbons for the assigned structure, including a signal at δ ~170 ppm characteristic of a carbonyl (CO) carbon. The identity of the material was further supported by low and high resolution mass spectrometry techniques.

Although compound **87** was not the expected product, it was feasible to imagine that such a product may be formed in a reaction involving a large excess of acetamide, and high temperatures. These conditions were clearly able to effect the substitution of the phthalimido group with another nucleophile. In addition, the observation that the reaction product was soluble in water can be better rationalised for **87** than for **86**, which has a large hydrophobic group attached.

Given the low yield and difficult workup associated with this reaction no additional time was spent investigating ways of improving this reaction, or preparing similar substrates that may work better. A small portion of **87** was put aside for a ligand binding experiment (refer to Section 4.8.1). The remaining material was hydrolysed under basic conditions to generate the target amine **80**, as illustrated in Scheme 4-19.



87 **80**

Scheme 4-19: Hydrolysis of amino-amide derivative **87** to diamine **80**.

It should be highlighted however that, due to the low yield obtained for the preparation of **87**, this hydrolysis was only performed on a small scale (11 mg of **87**). The desired amine **80** was formed in the reaction, but as the scale was so small, only ~ 2 mg of **80** could be isolated, and thus a thorough characterisation of the material was not possible. The main evidence for confirmation of the structure was the disappearance of the singlet at δ 1.86 ppm for the acetamido methyl group (CH_3CO). Solid conclusions could not be made from inspection of the ^{13}C NMR spectrum of the compound due to low signal to noise. The desired $M^{+\cdot}$ was however observed in the electron impact mass spectrum.

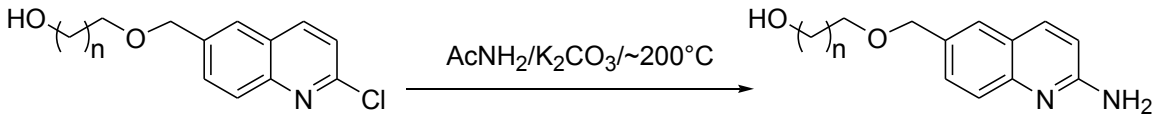
The resulting material was then all invested in a ligand binding experiment (refer to Section 4.8.2). After the ligand binding assay, the material was recovered from a DMSO solution by evaporation under reduced pressure. An attempt was made to confirm the structure of **80** by obtaining a high resolution mass spectrum of the residue after evaporation, however the required mass was not observed. Thus, some doubts still remain about the identity of this material.

Unfortunately the results from the synthetic investigation, and the subsequent ligand binding studies (presented later) remain unsatisfactory. Although compound **87** was not the desired product from the reaction in which it was formed, it remains a potentially useful intermediate

for obtaining target amine **80**. However, because the yield of **87** was low and the reaction was performed on a relatively small scale in this case, overall, this approach was not very effective for the synthesis of target 2-aminoquinoline **80**. Furthermore, in the case of **80** some uncertainty remains about the accuracy of the results for the ligand binding studies, a consequence of only a small amount of material being available for the experiment. This is because there are technical difficulties associated with accurately weighing out a compound when only ~ 1-2 mg is available, particularly if the compound is not in the form of a dry powder. If there is uncertainty about the concentration of ligand solutions prepared for binding studies, then there must be subsequent uncertainty about the accuracy of the binding constants derived. Additionally, as mentioned above, some uncertainty remains about the actual identity of the final amine **80** (also a consequence of the low yields in the synthesis). With this in mind, some effort was made, albeit presently still unsuccessful, to seek improved methods for the synthesis of amines **79** and **80**, and these are presented in Sections 4.6 and 4.7.

Testing the method of Kóródi with 2-chloroquinolines 77 and 78

Having established in the previous section that a 2-chloroquinoline like **85** was poorly suited to conversion to the 2-aminoquinoline using the method of Kóródi, the next part of the investigation was to test the suitability of 2-chloroquinolines such as **77** or **78**, that contain unprotected hydroxyl groups, for conversion to 2-aminoquinolines **76** and **81** under the same conditions. The method was therefore tested using **77** as the starting material, as illustrated in Scheme 4-20. There were early indications during the reaction that it was not proceeding as smoothly as for previous substrates such as the synthesis of **64** or **33**, in that when the reaction was followed by thin layer chromatography, a substantial amount of insoluble material was observed during the 'mini-workup'. The thin layer chromatograms themselves did suggest that the reaction was proceeding, however, there were more spots observed than usual suggesting the formation of additional by-products, and the intensity of the spots both by ultra violet visualisation, and potassium permanganate staining suggested that less material was present on the plate.



77 n = 1
78 n = 2

76 n = 1
81 n = 2

Scheme 4-20: Synthesis of 2-aminoquinolines **76** and **81** directly from 2-chloroquinoline precursors **77** and **78** respectively, using the method of Kóródi.

It was envisaged that the target product **76** would be quite water soluble due to the combination of the amino and hydroxy functionality on the one molecule. Therefore, when the reaction was worked up, the procedure was altered slightly in an attempt to maximise the yield. Instead of resuspending the cooled reaction mixture into water and extracting into chloroform (as was done for the other compounds) the reaction mixture was resuspended in saturated brine, and extracted with 3:1 chloroform/isopropanol. The ^{1}H NMR spectrum of the crude product indicated that a large amount of acetamide was present in the mixture. In fact, without increasing the 'gain' of the NMR spectrum, the only detectable signals in this spectrum were from the protons in acetamide; a large singlet at δ 1.85 ppm (CH_3), and two broad singlets at δ 6.19 and 6.80 ppm (each 1 x NH). This would suggest that composition of the sample was > 95% acetamide. When the gain of the spectrum was increased, small signals could be seen that suggested the expected product **76** was present, as evidenced by a doublet also at ~ δ 6.80 ppm, consistent with the signal expected for the H3 proton of the quinoline ring. In addition the expected signals were also seen in the aliphatic region of the spectrum. However more signals were also observed in the aromatic region of the spectrum suggesting the presence of one or more by-products.

The crude material was purified by preparative thin layer chromatography with silica gel plates, and eluting twice in 3:1 dichloromethane/ethanol. Following this, essentially pure **76** was isolated in very low yield (3%), and the identity was confirmed using mass spectrometry.

The low yield for **76** (3%) is consistent with the observation that the crude material consisted mainly of acetamide. The likely reason for such a large recovery of acetamide from the reaction is that the high salt concentration from the brine workup forced a lot more acetamide into the organic layer. The fact that the yield of **76** was so low may be either that a lot of the product was lost during the workup to the aqueous layer, or more likely, that there was a lot of degradation of the product or the starting material during the reaction. This would seem consistent with the large amount of insoluble material observed during the workup. Regardless, if the method was to be of any use, then a more effective method for the removal of the acetamide from the reaction would be necessary.

It was decided that another attempt at the reaction was warranted, to see if by altering the workup, an improvement in yield was possible. The next experiment was performed using **78** as the starting material, for reasons of supply. Exactly the same procedure as described for the reaction above was used (Scheme 4-20). However, this time, an attempt was made to remove the excess acetamide by sublimation under reduced pressure. While this was effective for removal of perhaps the majority of the excess acetamide, a considerable

quantity of acetamide remained according to the ^{1}H NMR spectrum of the remaining material. One limitation of this approach, especially for removing the last portions of acetamide, is that prolonged heating at the required temperatures could contribute to degradation of the product.

Another different feature of this workup was the avoidance of water during the process. Following the sublimation, instead of washing, the resulting material was resuspended in methanol, and filtered to remove insoluble impurities. The remaining residue was then purified using column chromatography with silica gel and eluting with 3:1 dichloromethane/ethanol. The main material isolated was an approximately 7:3 mixture of **81** and acetamide. A sample of **81**, free of acetamide was finally obtained after preparative thin layer chromatography, however the final yield remained very low (3%). The characteristic chemical shift at δ ~ 6.8 ppm for H3 of the quinoline ring was observed, providing evidence for the structure of **81**.

The synthesis of **81** was repeated one last time using the method described above. To work it up this time, the product was again partitioned between brine and 3:1 chloroform/isopropanol. However, the resulting residue was then purified by ion exchange chromatography using strongly acidic Amberlite [IR-120(H)], in a similar method described for the purification of **87** in Section 4.5.3.2. This approach was effective for the removal of excess acetamide, but the resulting sample of **81** was still isolated in an impure form, and low yield (<10%).

The results presented above indicate that if the method of Kóródi was to be applicable to the hydroxy derivatives illustrated in this case, then protection of the hydroxy functionality was required. However, the use of protecting groups needs to be assessed on a case by case basis and may be difficult to predict; in the case of the aldehyde protected as dioxolane or dioxane acetals, the method was satisfactory (see Section 4.3). However, in the case of an amine protected as a phthalimide, the method was unsatisfactory. The use of protecting groups for the hydroxy functionality at this point had not yet been investigated.

4.5.2.3 Investigation into methods for protection of aliphatic alcohol derivatives of 2-chloroquinoline

Having established in the previous section that the desired amines **76** and **81** could not be prepared satisfactorily directly from the 2-chloroquinolines **77** and **78** respectively using the

method of Kóródi, an investigation into incorporation of protecting groups for the hydroxyl functionality was next required.

Attempts to protect alcohols 77 and 78 as benzyl ethers

It was envisaged that a suitable protecting group for these compounds might be a benzyl ether. However, on consideration of the structure of **77** or **78**, or its derivatives, it can be seen that **77** or **78** are in fact benzyl ethers themselves. Therefore, if the hydroxyl functionality of **77** or **78** was protected as a benzyl ether, a selectivity problem may be encountered when trying to remove the protecting group by catalytic hydrogenolysis, the standard method for removal of benzyl ethers, as illustrated in Figure 4-6. Therefore, to allow for this possible selectivity problem, it was decided to try to protect the alcohols as 4-methoxybenzyl ethers, as these may be removed by alternative methods such as treatment with cerium (IV) ammonium nitrate[85] or DDQ.[86] The attempted preparation of these 4-methoxybenzyl ethers is the focus of this next section.

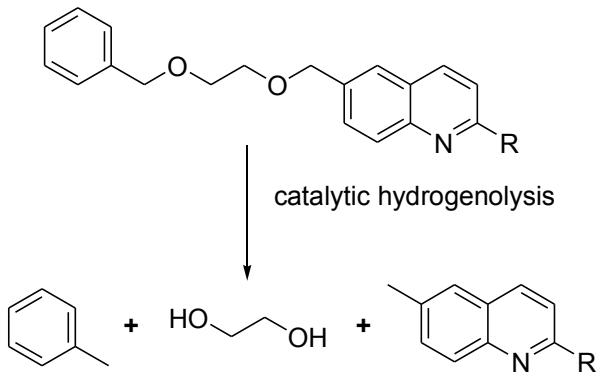
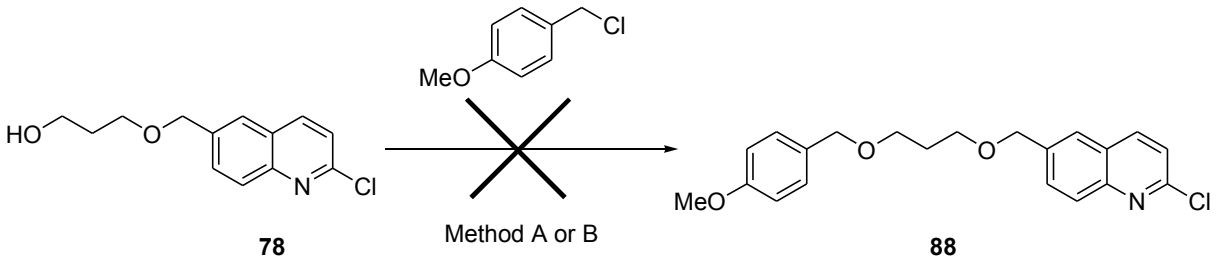


Figure 4-6: Thought experiment: catalytic hydrogenolysis as a de-benzylation method may result in non-selective double de-benzylation when applied to the quinoline derivative illustrated in the above example.

The first investigation into protection of alcohols as 4-methoxybenzyl ethers was made using alcohol **78**. The method used was to try and preform the alkoxide of **78** by treatment with sodium hydride in THF under a nitrogen atmosphere. Then 4-methoxybenzyl chloride was added and the mixture stirred at room temperature for 36 hours, as illustrated in Scheme 4-21 method A. After workup, no evidence for formation of the desired product **88** was seen, as judged by ^{1}H NMR spectroscopy. Only a mixture of both starting materials (**78** and 4-methoxybenzyl chloride), and a small amount of an unknown by-product(s) was obtained. This suggested that either the reaction conditions were not suitable for the desired substitution to occur, or that the alkoxide of **78** was not formed efficiently.



Method A: NaH/THF/room temp
Method B: NaH/DMSO/THF/room temp

Scheme 4-21: Investigation into protection of alcohol **78** as 4-methoxybenzyl ether **88**.

In an attempt to overcome this, the next time the reaction was tested, a solution of sodium hydride was prepared in DMSO instead of THF (Scheme 4-21 method B). Sodium hydride is insoluble in THF, and therefore acid base reactions involving sodium hydride/THF take place as suspensions. Alkoxides formed in such a system may not always be soluble in THF. However, sodium hydride is soluble in DMSO with gentle heating, with the dimsyl sodium salt being formed, which subsequently acts as a base, as illustrated in Figure 4-7. In addition, by using a more polar solvent such as DMSO, this significantly enhances the solubility and nucleophilicity of the alkoxides formed.

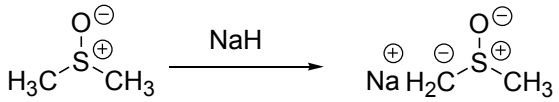


Figure 4-7: Formation of dimsyl sodium by treatment of DMSO with sodium hydride.

Evidence for formation of the dimsyl anion was observed as the solution turned a pale green colour. This solution was stirred for around 30 minutes before alcohol **78** dissolved in THF was added drop-wise to the solution over 10 minutes. At this stage, a darker green solution was observed that shortly after became a very dark, almost black solution. Despite this discouraging observation, 4-methoxybenzyl chloride dissolved in THF was added after an additional 30 minutes of stirring this dark solution, and the resulting solution was stirred overnight. Following workup, again none of the desired product **88** was isolated. Only a mixture of unreacted 4-methoxybenzyl chloride, and an unidentified product was isolated, as indicated in the ^{1}H NMR spectrum. The signals of the unidentified material were all seen at chemical shifts approximately consistent with the starting material **78**, however the heights of these signals were all considerably smaller than the signals for the 4-methoxybenzyl chloride. Furthermore, these signals were also broadened.

Given that a large amount of 4-methoxybenzyl chloride was recovered unreacted from the reaction mixture, and the large colour changes observed in the solution prior to the addition of the 4-methoxybenzyl chloride, it may be concluded that the 4-methoxybenzyl chloride did not participate in the reaction at all, and that all the chemistry had taken place prior to its addition. Thus a possible explanation for the observations is that a polymer was formed, resulting from a nucleophilic aromatic substitution of chlorine for oxygen at the 2-position of the quinoline ring, as illustrated in Figure 4-8, with the alkoxide anion playing the role of the nucleophile. However, no additional data is available to support to this conclusion.

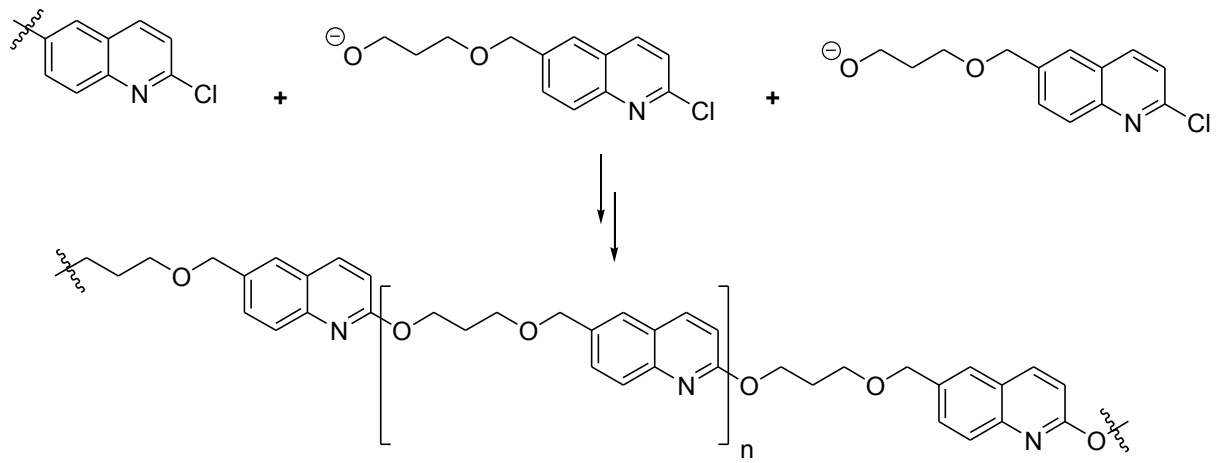
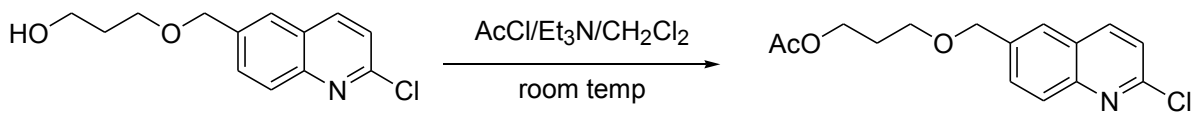


Figure 4-8: Thought experiment: proposed explanation for the failure of the reaction intended to add a 4-methoxybenzyl group to alcohol **78**. The proposed polymerisation most likely occurred prior to the addition of the 4-methoxybenzyl chloride.

Protection of alcohol 78 as an acetate ester

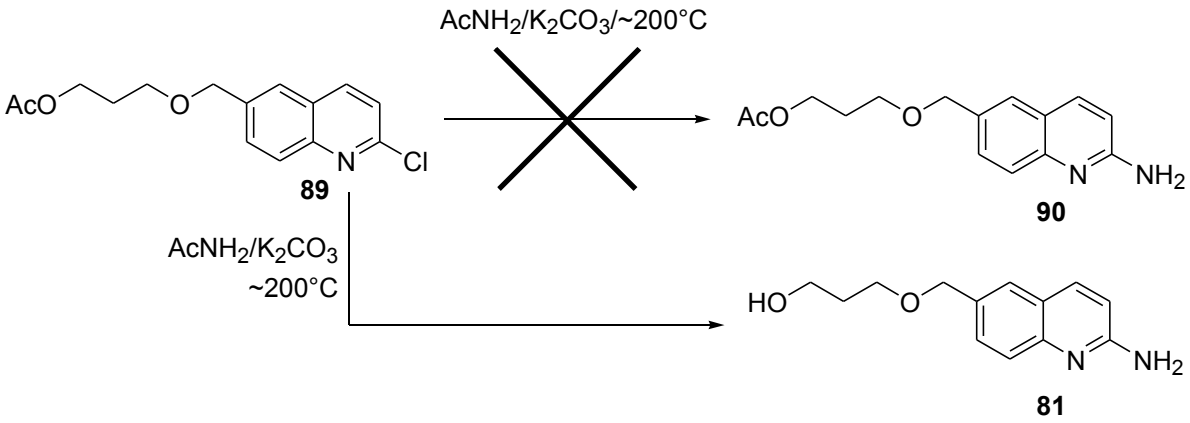
The investigation into methods for protection of alcohols **77** and **78** was so far not encouraging. The conditions in the first instance (Scheme 4-21, method A) were either too mild for the reaction to proceed as required, or in the second case (Scheme 4-21, method B) the conditions were too vigorous and the desired product was not formed. Therefore, it was next decided to try to protect alcohol **78** as an ester, because it was anticipated that this group should be more readily installed onto the molecule, under mild conditions. However, it was also envisaged that the ester may not be a protecting group that was stable to the harsh amination conditions of Kóródi.



78 **89**

Scheme 4-22: Synthesis of ester **89** from alcohol **78**.

Alcohol **78** was readily converted into ester **89** in 91% yield by treatment with acetyl chloride in the presence of triethylamine in dichloromethane as illustrated in Scheme 4-22. One feature of the ^{1}H NMR spectrum that provided evidence for the formation of **89** was a large downfield shift for the protons at δ ~ 3.8 ppm (C$\underline{H}_2$OH) in **78** to 4.17 ppm (C$\underline{H}_2$OAc) in **89**. In addition a singlet was observed at δ 1.97 ppm in **89** for the methyl protons of the acetate group that was not observed in the spectrum of **78**. The correct number of signals was observed in the ^{13}C NMR of spectrum of **89** to support the addition of the acetate group, and a signal was observed at δ 171.6 ppm that is characteristic of a carbonyl (CO) carbon. Given that this material formed the basis of a quick experiment to test the suitability of esters as protecting groups for the amination reaction, no additional data was collected for **89**.



Scheme 4-23: Investigation into the suitability of ester **89** as a substrate for the amination method of Kóródi.

Ester **89** was promptly tested for suitability in the amination reaction of Kóródi, as illustrated in Scheme 4-23. The reaction was worked up in the normal way for substrates (partitioned between water and chloroform) that are well suited to the reaction. The ^{1}H NMR spectrum of the isolated material indicated that multiple products were present. There was evidence to suggest that a 2-aminoquinoline(s) was present in the mixture, based on the observation that several signals were present at δ ~ 6.8 ppm, consistent with the expected chemical shift for the H3 proton of the quinoline ring. However, the singlet for the methyl protons at δ ~ 1.97 in **89** was not observed in the spectrum for the isolated material. This was suggestive that the ester group had not withstood the reaction conditions, and that **81** was the likely major component of the mixture, instead of **90**. The recovery from this experiment was very low however (18 mg isolated from 90 mg of starting material), and given that the material was a mixture of multiple products, the conclusion drawn from this experiment was that there was no benefit to be gained from protection of **78** as an ester.

4.5.3 Summary

Starting from cyclic acetals **63** and **67** a thorough investigation was made into effective conversion of these into acyclic alcohols **77** and **78** respectively. A method was then established for the conversion of **78** into phthalimide **85**. All these 2-chloroquinolines **77, 78** and **85** were found to be poor substrates for the amination method of Kóródi, as the yields were very poor, and the products were difficult to purify. In addition, for the case of **85** the target product was not formed. Some effort was made to protect alcohol **78** as a 4-methoxybenzyl ether, but a suitable method could not be developed for the incorporation of the protecting group. Alcohol **78** was easily protected as an acetate ester, however this protecting group did not survive the harsh amination reaction.

Thus it can be concluded that the method of Kóródi is not a method for the preparation of 2-aminoquinolines from 2-chloroquinolines that is applicable to all 2-chloroquinoline derivatives. The method is clearly only suited to 2-chloroquinolines that contain simple, non-polar functional groups.

4.6 Synthesis of 6-substituted-2-aminoquinolines with more complex functionality 3: Investigation into alternative amination methods

Following the thorough investigation into the method of Kóródi for the preparation of 2-aminoquinolines from a range of 2-chloroquinolines, and the discovery that the method was not suitable for the preparation of amines such as **76**, **81** and **86**, it was decided that developing new methods for preparing 2-aminoquinolines from 2-chloroquinolines was required.

One feature of the method of Kóródi that might appear unusual to an organic chemist is the use of acetamide as a nucleophile. Amides are generally not considered as nucleophiles due to the strong resonance delocalising effect of the carbonyl group, as illustrated in Figure 4-9 below. This probably explains why such a high temperature (~200°C) is required for the reaction of Kóródi to proceed. Thus, if acetamide is sufficiently nucleophilic to undergo a nucleophilic aromatic substitution reaction with 2-chloroquinoline at 200°C, then it was envisaged that an amine that is not resonance stabilised (eg. an aliphatic amine) should be suited to perform a similar reaction with a 2-chloroquinoline at reduced temperatures. Indeed, several methods have been reported where nucleophilic aromatic substitutions using

nucleophiles such as benzylamine or other primary amines have been performed on systems with chlorides *ortho* to a pyridine type nitrogen, including a 2-chloroquinoline derivative.[87-91] The effectiveness of these methods was varied however; in some cases high temperatures and long reaction times were still involved and the yields were not outstanding (<50%),[88,89] but methods involving shorter reaction times under milder conditions with good yields have also been reported.[87,90,91] Also, a method involving coupling of aliphatic amines to 2-bromopyridines using palladium-catalysis on a potassium fluoride-alumina surface has also been reported resulting in the formation of a range of 2-aminopyridines in good to excellent yields.[92] Methods have also been reported where benzylamino derivatives of pyridine were converted to primary amines by catalytic hydrogenolysis.[87,88,93] Although a step involving catalytic hydrogenation may not be the most desirable method for systems such as **76** or **81** (for reasons that were discussed in Section 4.5.4.1), being able to effectively regenerate the primary amine from a benzyl protected amine would be an essential requirement, if any of the methods outlined above were to be useful for the synthesis of 2-aminoquinolines. This is because primary amines are the focus of the current ligand binding studies.

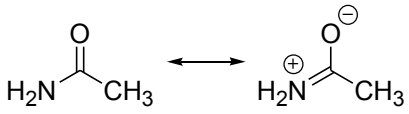


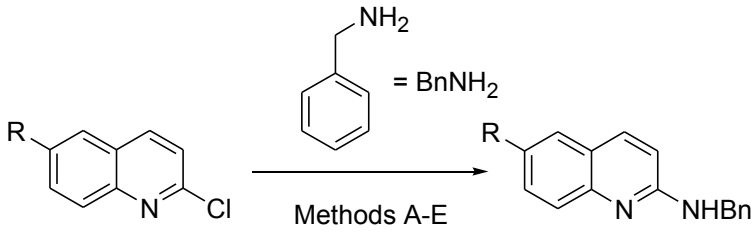
Figure 4-9: Resonance stabilisation of acetamide, accounting for its reduced nucleophilicity and basicity.

4.6.1 Investigation into conversion of simple 2-chloroquinolines into 2-(benzylamino)quinolines using benzylamines as nucleophiles

4.6.1.1 Preliminary investigation

The planned strategy of using benzylamines as nucleophiles to undergo nucleophilic aromatic substitutions with 2-chloroquinolines was tested using 2-chloro-6-methylquinoline **35** and benzlyamine. Several methods were tested until a satisfactory procedure was identified (Scheme 4-24). Initially, the reaction was tried using only a slight excess of benzylamine and heating at reflux in toluene in the presence of potassium carbonate (Scheme 4-24, Method A), but no reaction was observed using this method, according to thin layer chromatographic analysis. Thus, the next approach taken was to use a large excess of neat benzylamine and heat at reflux in the presence of potassium carbonate (Scheme 4-24, Method B). This method proved effective, with **91** isolated in 69% yield following chromatography. One feature of the ^{1}H NMR spectrum that provided evidence for formation

of **91** included an upfield shift of the H3 proton of the quinoline ring from δ 7.35 ppm in **35** to δ 6.60 ppm in **91**. This change in chemical shift is consistent with those observed for the formation of 2-aminoquinolines from 2-chloroquinolines, however, in this case, the resulting chemical shift for H3 is slightly more upfield than those observed for the primary amines. Additional evidence for the structure of **91** from the ^{1}H NMR spectrum included a doublet at δ 4.71 ppm with *J* = 5.6 Hz, for the benzylic protons that couple to the amino proton (NHC$\underline{H}_2$Ph), and the addition of a 5 proton multiplet at δ ~ 7.2-7.5 ppm for the phenyl group of the benzyl amino functionality. The identity of **91** was confirmed by mass spectrometry.



35 R = Me
11 R = H

91 R = Me
24 R = H

Method A: $BnNH_2$ (1.1 equiv)/K_2CO_3/Toluene/Δ
Method B: neat $BnNH_2$/K_2CO_3/Δ
Method C: neat $BnNH_2$/60°C*/66 hours
Method D: neat $BnNH_2$/120°C*/38 hours
Method E: neat $BnNH_2$/120°C*/66 hours
Method F: neat $BnNH_2$/140/°C*/30 hours

Methods A-E: **35**, Method F: **11**.
*Oil bath temp

Scheme 4-24: Investigation into benzylamination of 2-chloroquinolines using **35** and **11**, as a test system. Methods D and E were performed by co-worker Daniel Fritz (unpublished).

As it stands, the results described above provided encouragement for the use of the method. However, given that the boiling point of benzylamine is 184-185°C and the above reaction involved reflux temperature, then the above does not offer substantially milder conditions than the amination method of Kóródi. Furthermore, when performed on a small scale, the Woods metal bath was still required to bring the benzylamine to reflux, and it would be preferable to develop an alternative amination method that avoids the need for this. Hence additional experiments were performed in an attempt to identify milder conditions to effect the same transformation.

The next approach was to stir **35** in neat benzylamine at 60°C for 66 hours under a nitrogen atmosphere (Scheme 4-24, Method C). This method was adapted from a report involving treatment of a 2-chloroquinoline derivative with 1,3-diaminopropane that resulted in formation of the 2-alkyaminoquinoline derivative in 77% yield.[91] When the same conditions were

applied here, **91** was isolated in only 19% yield following chromatography, however an 80% recovery of the starting material **35** was obtained from the chromatographic procedure. This indicates that the method was inefficient. Hence in the next experiment, the same reaction was heated at 120°C for 38 hours, and this resulted in formation of **91** in 74% yield accompanied by 25% recovery of **35** (Scheme 4-24, Method D). When the reaction was allowed to proceed at 120°C for 66 hours, **91** was isolated in 93% yield (Scheme 4-24, Method E). One last experiment was performed, this time using the 2-chloroquinoline **11** instead of **35**, in order to conserve the supply of **35** (Scheme 4-24, Method F). In this case, the reaction time was 30 hours at ca. 130°C, and the product **24** was isolated in 97% yield following chromatography. The ^{1}H NMR spectrum and the melting point of this material was identical with those obtained for **24** synthesised by reductive amination in Chapter 3.

The results from Scheme 4-24 (Methods D-F) were an improvement on the first successful experiment (Scheme 4-24, Method B), with the conditions used for Method F representing the most efficient method developed at this stage, although the yields were excellent in each case. One disadvantage that remained however was the long reaction times required (30 hours at best). Even so, at this stage this was not considered a large problem. What was of greater importance was to pursue other aspects of the investigation such as developing it as a method for preparing primary amines such as **76** or **81** in good yields, something that was not possible so far.

4.6.1.2 Modification of approach for convenient de-protection

The next important part of the investigation required, was to find a method for conversion of the 2-(benzylamino)quinolines into primary amines, as a step towards possible application of the new strategy to the synthesis of amines such as **76** or **81**. As discussed earlier in Section 4.6, successful de-protection of 2-(benzylamino)pyridine derivatives by catalytic hydrogenolysis to give primary amines has been reported.[87,88,93] However it was also mentioned in Section 4.5.2.3 with relevance to protection of alcohols as benzyl ethers, that if applied to a compound such as **76** or **81**, catalytic hydrogenolysis might lead to non-selective double de-benzylation as illustrated in Figure 4-10 (now using a benzylamine derivative as an example). Therefore, using a similar logic to that discussed in Section 4.5.2.3 for the protection of alcohols, it was envisaged that it might be preferable to synthesise 2-(4-methoxybenzylamino)quinolines that can be de-benzylated by different methods.

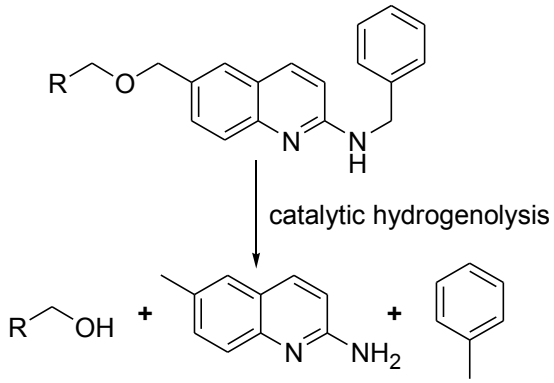
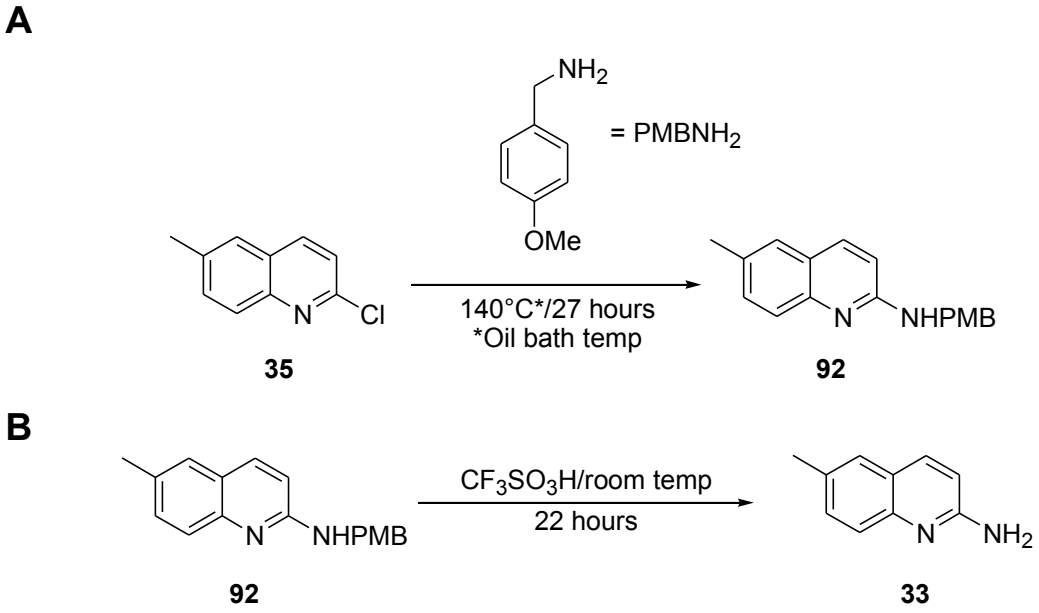


Figure 4-10: Thought experiment: catalytic hydrogenolysis as a de-benzylation method may result in non-selective double de-benzylation when applied to the 2-aminoquinoline derivative illustrated in the above example.

The 2-(4-methoxybenzlyamino)-6-methylquinoline derivative **92** was synthesised by co-worker Daniel Fritz by treatment of **35** with 4-methoxybenzylamine at 140°C for 27 hours as illustrated in Scheme 4-25A: these conditions closely matched the optimised method developed in Scheme 4-24 Method F, and the desired product **92** was formed in 94% yield. Having established this, an investigation into methods for de-protection of **92** was then required. Some experiments were attempted by Daniel Fritz involving adaption of known methods for de-protection of 4-methoxybenzyl ethers; using either DDQ in dichloromethane and water,[86] or cerium (IV) ammonium nitrate in acetonitrile and water.[85] Only starting material was returned following workup for the experiment involving DDQ. In the case of the



Scheme 4-25: Investigation into synthesis and de-benzylation of 2-(4-methoxybenzylamino)-quinolines. (A) Synthesis of 2-(4-methoxybenzylamino)-6-methylquinoline **92**. (B) De-protection of **92** using trifluoromethanesulfonic acid. The work presented in this Scheme was performed by a co-worker, Daniel Fritz (unpublished).

cerium (IV) ammonium nitrate treatment, a mixture of several products was formed, as judged by ^{1}H NMR analysis. There was some evidence that the desired product **33** was present, however, no effort was made to isolate the product, or to pursue this method any further.

The above observations indicated that the methods for de-protection of 4-methoxybenzyl ethers were not suited to de-protection of 4-methoxybenzyl amines, at least for the substrate of interest. But soon after this, it was brought to our attention that 4-methoxybenzylamino compounds could be converted into primary amines by treatment with either trifluoromethanesulfonic acid, or trifluoroacetic acid.[94] Hence the conversion of **92** to **33** was attempted, again by co-worker Daniel Fritz, by treatment with trifluoromethanesulfonic acid as illustrated in Scheme 4-25B, and using this approach, the desired product **33** was formed in high yield (95%). The ^{1}H NMR spectrum obtained for the product isolated from the reaction was identical to that for **33** prepared by the method of Kóródi in Section 4.2.2.

4.6.2 Investigation into conversion of more complex 2-chloroquinolines to 2-(4-methoxybenzylamino)quinolines, and their subsequent de-benzylations

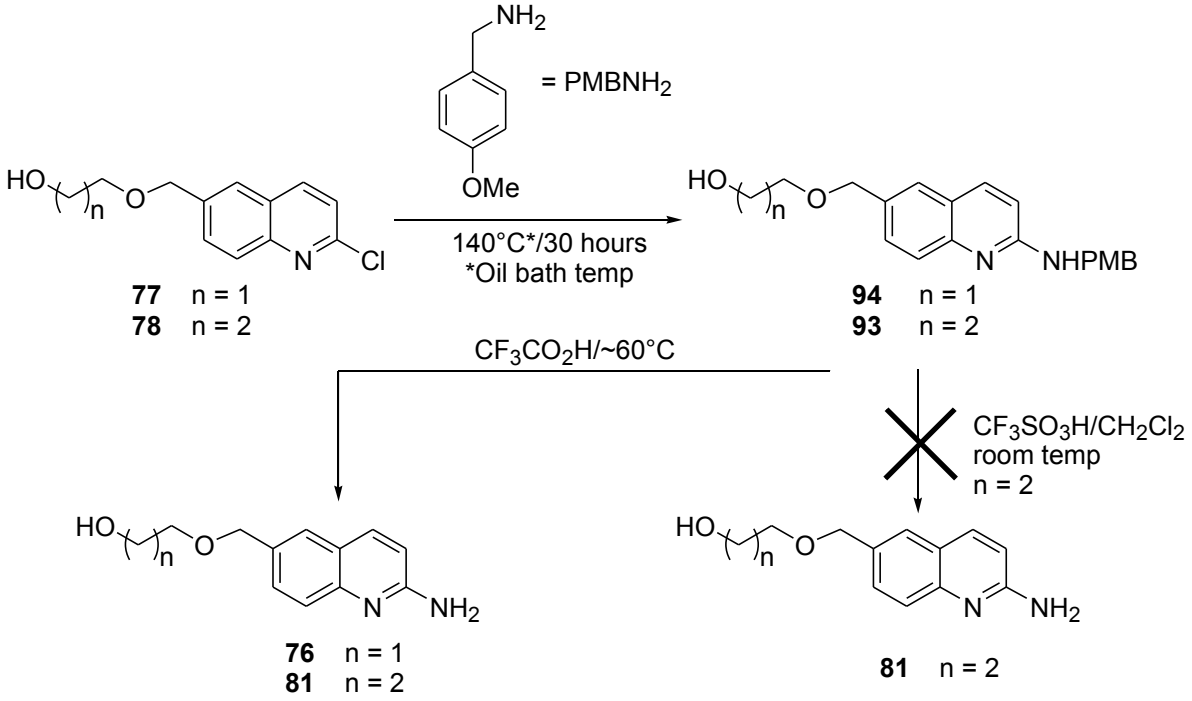
Having established a method for conversion of simple 2-chloroquinolines into 2-(4-methoxybenzylamino)quinolines and their subsequent de-benzylation into primary amines, as presented in the previous section, it was then time to try to apply the method to 2-chloroquinolines with more complex functionality such as **77**, **78** or **85**.

4.6.2.1 Investigation into suitability of aliphatic alcohol derivatives of 2-chloroquinoline

Because it was not possible to place a robust protecting group such as a benzyl ether onto the nucleus of **78** as discussed in Section 4.5.2.3, it was decided to try the conversion of **78** into 4-methoxybenzylamino derivative **93** in the absence of protecting groups as illustrated in Scheme 4-26. Pleasingly, this method was effective, and **93** was formed in 91% yield following treatment of **78** with 4-methoxybenzylamine at ca. 140°C for 30 hours. Features of the ^{1}H NMR spectrum of **93** that provided evidence for its formation included the characteristic chemical shifts seen at δ 6.65 ppm with J = 9.0 Hz, for the H3 proton of the quinoline ring, a doublet at δ 4.62 ppm with J = 5.4 Hz, for the benzylic protons (NHC$\underline{H}_2$Ar), and the two multiplets at δ 6.85-6.90 and δ 7.30-7.35 for the AA'XX' system of the 4-

methoxybenzyl motif. All the chemical shifts for the aliphatic portion of the molecule were essentially unchanged in **93** relative to **78**, suggesting that portion of the molecule was unaltered during the reaction. Furthermore, the identity of **93** was confirmed by mass spectrometry.

Having established that amination of **78** with 4-methoxybenzylamine to produce **93** in high yield was possible, it was then time to test its suitability for de-benzylation using trifluoromethanesulfonic acid, as illustrated in Scheme 4-26. Instead of treating **93** with neat trifluoromethanesulfonic acid, as was the case for the de-benzylation of **92** in Section 4.6.1.2 (Scheme 4-25B), **93** was dissolved in dichloromethane and 10 equivalents of trifluoromethanesulfonic acid was added to the stirred solution. A deep red colour change



Scheme 4-26: Investigation into synthesis and subsequent de-benzylation of 2-(4-methoxybenzylamino)quinoline derivatives, starting from 2-chloroquinolines **77** and **78**.

was then observed. Thin layer chromatography was used to try and monitor the progress of the reaction, and this indicated that the starting material had been consumed, however a clear spot with the expected R_f for the product (compared to **81** prepared in Section 4.5.2.2) could not be observed by either ultraviolet visualisation, or by staining the plate with potassium permanganate/potassium carbonate solution. Following workup of the reaction and ^{1}H NMR analysis of the isolated material, it was difficult to conclude whether the reaction had worked or not; many signals were observed with approximate chemical shifts that were representative of those expected for **81**, however, large broad signals that had co-incidental

chemical shifts with some of the key expected resonances were also observed. Therefore the conclusion made at this point was that the reaction had not worked.

The de-benzylation of **93** to **81** was instead attempted by treatment with neat trifluoroacetic acid as illustrated in Scheme 4-26, as this was suggested as another method that may be applicable for the de-benzylation of 4-methoxybenzyl amino derivatives.[94] When **93** was stirred in trifluoroacetic acid at room temperature, no reaction could be observed by thin layer chromatography. But when the vessel was heated at ca. 60°C, the reaction did proceed as evidenced by the disappearance of the spot for the starting material and the appearance of a spot with R_f that was identical to **81** prepared in Section 4.5.2.2. Thin layer chromatography indicated that the reaction was complete after 1 hour.

Some discussion of the workup method for the above reaction is now required. The first time the reaction was attempted, the cooled reaction mixture was cautiously added to a cooled solution of 10% sodium hydroxide and a precipitate was formed. The resulting mixture was extracted with a 3:1 mixture of chloroform/isopropanol. The organic extracts were dried and the solvent was removed to afford a 'gluey' residue. The ^{1}H NMR spectrum of this material featured large broad signals at regions of the spectrum where the resonances were expected for the product **81**, and hence it was difficult confirm whether the reaction had worked. Thus, the resulting residue was purified by column chromatography with silica gel, and from this, a material was isolated that essentially resembled **81** that was prepared by the other method. However, the chemical shifts for the H3 and H4 protons of the quinoline ring were both shifted slightly downfield by 0.07 and 0.09 ppm respectively. A clear resonance with good signal to noise was observed in the ^{19}F NMR spectrum of the material, indicating that there was a fluorine containing impurity (most likely sodium trifluoroacetate) in the sample. For reasons that are unclear, it is possible that this impurity was responsible for the differences in some of the chemical shifts observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of the isolated material was essentially identical to that obtained for **81** obtained previously, and the identity of the material was confirmed by mass spectrometry. Assuming that the material was pure, the yield of **81** was 82% following the de-benzylation reaction.

Some revision of the workup method was therefore required, in order to minimise the quantity of sodium trifluoroacetate that remained in the product following the trifluoroacetic acid de-benzylation method. So when the reaction was repeated, instead of quenching the trifluoroacetic acid by addition to sodium hydroxide, the excess trifluoroacetic acid was cautiously removed under reduced pressure on the rotary evaporator. The residue was then further dried under high vacuum for 1 hour to remove any residual trifluoroacetic acid. The

resulting residue was then partitioned between sodium hydroxide and 3:1 chloroform/isopropanol and worked up in the usual manner. The subsequent sticky residue was then purified by silica gel chromatography twice to yield **81** that was analytically pure in 59% yield. In addition the chemical shifts of the signals for the H3 and H4 protons in the ^{1}H NMR spectrum of the product were in better agreement with those for **81** prepared using the other method. A small signal was observed in the ^{19}F NMR spectrum for this product, but on this occasion, the signal to noise was considerably lower after only a few transients (in contrast to the first attempt when considerably better signal to noise was observed after only a few transients). Some ^{19}F NMR experiments were also performed in which an insert with a known concentration of α,α,α-trifluortoluene was placed in the NMR tube. From this, the sodium trifluoroacetate content of the powder form of **81** was estimated to be < 0.02% w/w. The decreased yield obtained for the analytically pure product this time is also consistent with the previous sample containing a substantial amount of impurity, and indicates that the alternative workup method was effective for obtaining the pure product. Hence all future syntheses using this method were worked up this way.

By using the same approach as described above, **76** was also prepared in two steps from **77** as illustrated in Scheme 4-26, in 68% overall yield from **77** (98% and 69% for 4-methyoxybenzyl amination and de-benzlyation respectively). The characteristic changes in chemical shifts on going from **77** to **94** and then to **76** were all consistent with those observed for the synthesis of **81** from **78** and the ^{1}H NMR spectrum of **76** prepared here was also identical to the spectrum of the product that had been prepared using the other method. The identities of **94** and **76** were also confirmed using mass spectrometry.

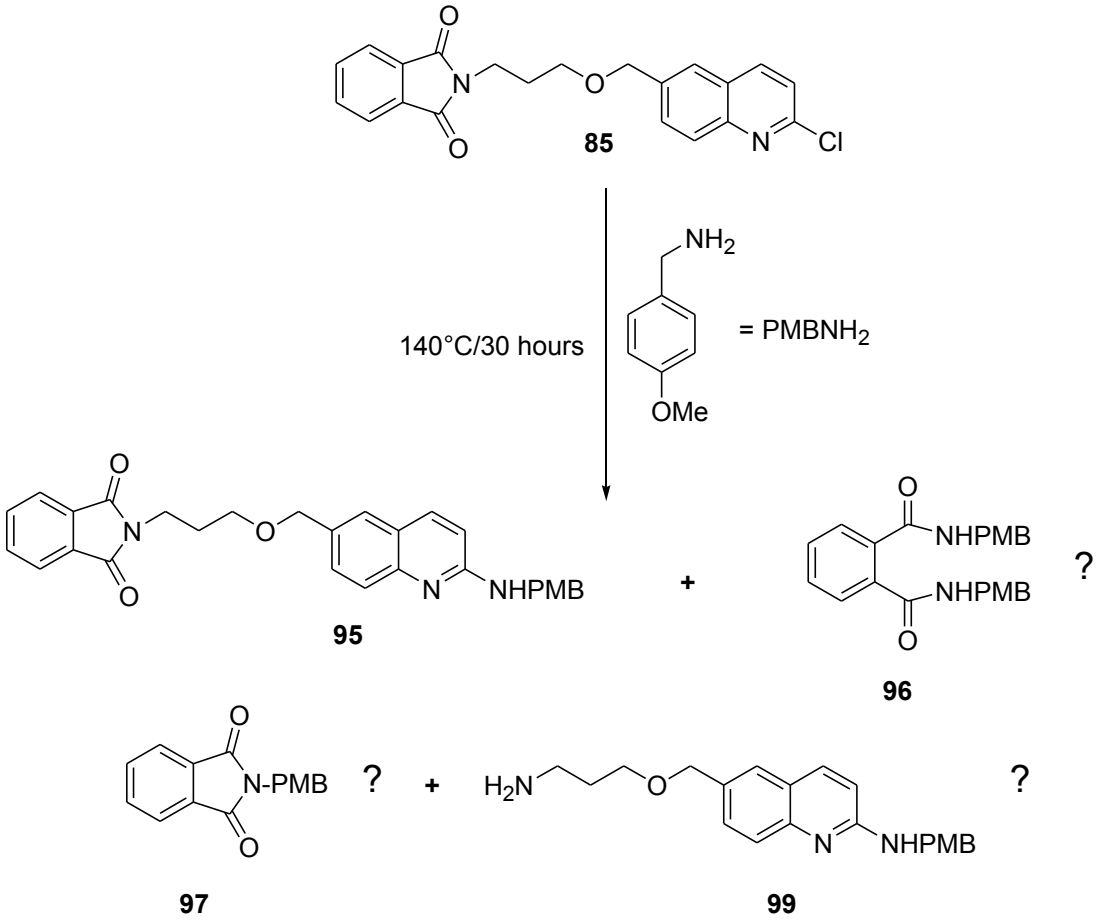
Hence, a much more satisfying method was developed for the conversion of 2-chloroquinolines with polar functional groups such as **77** and **78** into 2-aminoquinolines **76** and **81** respectively. The yields were greatly improved (68% and 59% overall yields in two steps from **77** and **78** respectively) compared to the very low yields (2% for both **76** and **81** in one step) obtained using the amination method of Kóródi. The desired products could be prepared and purified more conveniently, and sufficient material was obtained so that the products could be characterised more thoroughly. Furthermore, protection of the hydroxyl groups on the starting materials **77** and **78** was not necessary.

4.6.2.2 Investigation into suitability of the phthalimido derivative of 2-chloroquinoline

Having successfully developed an alternative amination method that was compatible with 2-chloroquinolines **77** and **78** as described above, an investigation into the suitability of the method with the phthalimido 2-chloroquinoline derivative **85** was required. This was another substrate that was poorly suited to the method of Kóródi. Hence, 0.100 g of **85** was treated with 4-methoxybenzylamine at ca. 140°C for 30 hours as illustrated in Scheme 4-27. Thin layer chromatography at this stage indicated that all of the starting material had been consumed, however, there were at least two spots present in the product mixture. Prior to ^{1}H NMR analysis of the crude residue (following removal of the excess 4-methoxybenzyl amine under reduced pressure) an attempt was made to purify the material using chromatography with silica gel. However, it was not possible to get to effective separation of the two components of the mixture. Two materials were isolated, one major and one minor.

The major isolate (0.126 g) was the higher R_f material, and the ^{1}H NMR spectrum of this did not exhibit many features that were indicative of the expected product **95**. This spectrum contained two large signals at δ ~ 3.8 ppm consistent with the expected chemical shifts for the methyl protons of the methoxy group ($C\underline{H}_3OPh$) (as observed in other compounds such as **93** and **94**), suggesting that two products containing 4-methoxybenzyl groups were present. A doublet was also observed at δ 4.4 ppm that is consistent with the chemical shifts observed for benzylic protons coupling to an NH proton, as seen in the spectra for other derivatives **93** or **94**. In addition, major signals were observed in the aromatic region at δ ~ 7.4 and 7.6 ppm (characteristic of the phthalimide motif), and at δ ~ 6.9 and 7.2 ppm (characteristic of the AA'XX' system of the 4-methoxybenzyl motif). The expected signals for all the aliphatic protons of **86** (ie $ArCH_2O(C\underline{H}_2)_3R$) were however not observed. It was difficult to use the relative integrations of the observed signals to draw solid conclusions about the identity of the material, due to additional signals from impurities existing at similar chemical shifts for some of the signals. Therefore, electron impact mass spectrometry was used to try and determine the structure of the possible product(s) in this mixture, and a peak was observed at *m/z* 404 that reflects the possible existence of **96** illustrated in Scheme 4-27 below. However, the peak at *m/z* 404 was only observed at an ionisation temperature of 95°C; when the temperature was raised beyond this, the peak was no longer seen. However, another peak at *m/z* 267 was also observed at ionisation temperatures of 95°C and higher that reflects the possible existence of structure **97** (Scheme 4-27). The intensity of this signal was greater than that at *m/z* 404 at both temperatures, suggesting that this is a stable species formed as part of the fragmentation process of **96**.

When the minor material isolated (0.023 g) was analysed by ^{1}H NMR spectroscopy, good evidence was observed for the formation of the expected product **95**. This included the characteristic doublets seen at δ 6.61 and 7.73 ppm with *J* = 8.8 Hz for the H3 and H4 protons of the quinoline ring respectively. In addition, signals for the aliphatic protons were all present in the expected regions of the spectrum. However the presence of several extra signals (from impurities) at the same chemical shifts of many from **95** made it difficult to assign all of the signals of **95**. Electron impact mass spectrometry was used to confirm the presence of **95** in this minor isolate.



Scheme 4-27: Attempted synthesis of 2-(4-methoxybenzylamino)quinoline derivative **95**, and the structures of other possible products formed in the reaction.

Yet again, this particular 2-chloroquinoline had not behaved as expected in this reaction. In this case, the large excess of 4-methoxybenzylamine used in the reaction probably resulted in attack from the amine at the imido motif to subsequently cleave the phthalimide group and form by-product **96**, and reveal the primary amine from the starting material **85** or the product **95** as illustrated in Figure 4-11 below. It is possible that the reactions proceeded in two different orders also as illustrated; the 4-methoxybenzylamine may attack at the phthalimido motif prior to substitution of the chlorine to form **98**, followed by subsequent amination of the quinoline to form **99**, or the conversion to the aminoquinoline may have occurred first forming

95 followed by attack at the imido motif resulting in formation **99**. This process can be rationalised by considering that a known method for de-protection of phthalimides to generate primary amines involves hydrazinolysis. In the current case, a similar process has occurred using 4-methoxybenzylamine instead of hydrazine. Unfortunately, this explanation is not completely supported by the mass spectra because the resulting primary aliphatic amines (**98** or **99**) were not observed. It is possible that **98** and **99** were formed in the reaction, but were not isolated after column chromatography. Due to the high polarity of aliphatic primary amines, these compounds are frequently difficult to chromatograph using silica gel. Given that an additional spot was observed close to the baseline by thin layer chromatography, it is possible that this accounts for the lost material. Unfortunately this was not realised until after the silica gel from the column had been discarded, and therefore no attempt was made to retrieve this material from the column. In addition, when the column

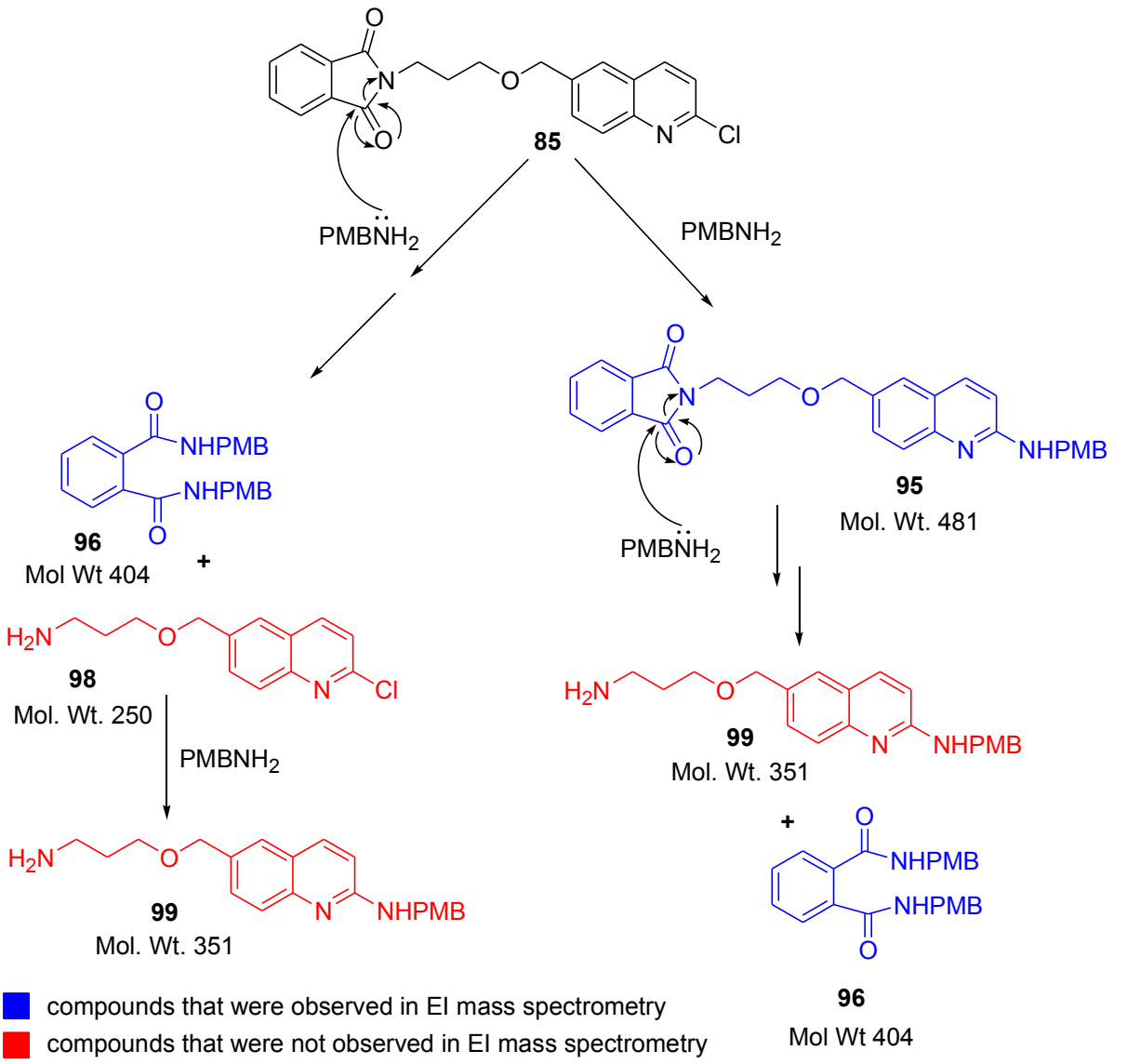


Figure 4-11: Thought experiment: proposed explanation for failure to isolate the desired product **95** following treatment of 4-methoxybenzylamine with phthalimide **85**.

was first performed, it was thought that this large spot observed at the base line was most likely to be residual 4-methoxybenzylammonium chloride, produced after 'mop-up' of the hydrochloric acid formed in the nucleophilic aromatic substitution reaction.

Hence, it is difficult to draw solid conclusions about the effectiveness of this method with 2-chloroquinoline **85**. Clearly only a small amount of the expected product **95** (<20%) was isolated, and the other major material isolated did not appear to contain many structural features of the starting material or the expected product as judged by ^{1}H NMR and mass spectrometry. Therefore, a large portion of the starting material (~50-80%) remains un-accounted for. Therefore, this experiment perhaps warrants another investigation, to see if di-amine **99** can be isolated using a modified chromatographic method.

4.6.3 Summary

The investigations made in this section resulted in the identification of an alternative and much more effective method for the amination of 2-chloroquinolines **77** and **78**, that were poorly suited to the method of Kóródi. Although the approach requires an additional step (benzyl amination followed by de-protection), the yields were much improved, and the materials were easier to work with. However, whether the method was genuinely effective when tried with 2-chloroquinoline **85** is unclear. However, it was clear that a substantial side-reaction(s) occurred.

Therefore, the method has some appeal, but is perhaps not optimised. One drawback of the approach is that it still requires a large excess of the amine, relatively high temperatures and long reaction times to drive the reaction to completion, and these conditions remain unsuited to certain substrates. Therefore, it would certainly be worthwhile investigating alternative, milder conditions for this type of coupling such as the use of palladium catalysis.[92] Given the ease of de-protection of the 2-(4-methoxybenzylamino)quinoline derivatives with trifluoroacetic acid (or less mild trifluoromethanesulfonic acid), identifying a milder method for the amine coupling step will potentially lead to a powerful method for the preparation of 2-aminoquinolines with diverse functionality. In addition, the method for the synthesis of 2-(benzylamino)quinolines from 2-chloroquinolines described here is much improved to that described in Chapter 3, where reductive alkylation of 2-aminoquinoline was used. The yields are excellent, and the formation of the undesired by products is eliminated.

4.7 Synthesis of 6-substituted-2-aminoquinolines with more complex functionality 4: Towards convergent synthesis

In a broader context, one drawback of the general approach for the synthesis of 2-aminoquinolines as presented in both Sections 4.5 and 4.6 is that it is very linear. There are too many steps involved in reaching the final targets. This results in lower overall yields, and generally a less efficient method for preparing a range of 2-aminoquinolines for SH3 domain ligand binding studies. In addition, when many exploratory reactions are tried that result in low yields, or failed reactions, the supply of starting material(s) soon diminishes.

For example, as illustrated in Figure 4-12A, the current approach involves three steps starting from the 4-methylaniline, to reach 2-chloro-6-methylquinoline **35** which may be classed as the 'key intermediate'. Depending on the functionality that is required, this key intermediate will be subject to additional steps to obtain the 'amination precursor' compound. Assuming the 'amination precursor' compound is suited to the available amination methods, one or two steps are then required for conversion to the 'protected derivative', and depending on the nature of the protecting groups in place, an additional one or more steps may be required to obtain the 'target' compound. Furthermore, although some of the 'protected derivatives' can also be tested for ligand binding activity, all this hard work with multi-step syntheses essentially results in the preparation of just one ligand! In the case of the attempted synthesis of di-amine **80** where phthalimide **85** was intended as the 'amination precursor' compound, six steps were required [radical di-bromination, hydrolysis to form aldehyde (Scheme 4-6), acetal formation (Scheme 4-8), acetal reduction (Scheme 4-14), alcohol activation, and phthalimide substitution (Scheme 4-17)] to undergo the transformation from the 'key intermediate' to the 'amination precursor'. Then, given that the amination reaction worked poorly with **85** resulting in very low yields of the 'protected derivative', the supply of **85** soon diminished following exploratory reactions. In order to perform additional exploratory reactions, then the multi-step synthesis of the 'amination precursor' would require repeating.

With this in mind, it was decided to begin investigation into a more efficient 'convergent' synthetic approach, for example as illustrated in Figure 4-12B. The idea was to develop a system in which the 'key intermediate' compound was structurally, much further progressed towards the 'target' compound. For example, to synthesise 6-substituted-2-aminoquinolines such as **80**, the 'key intermediate' should be a quinoline derivative that already has the

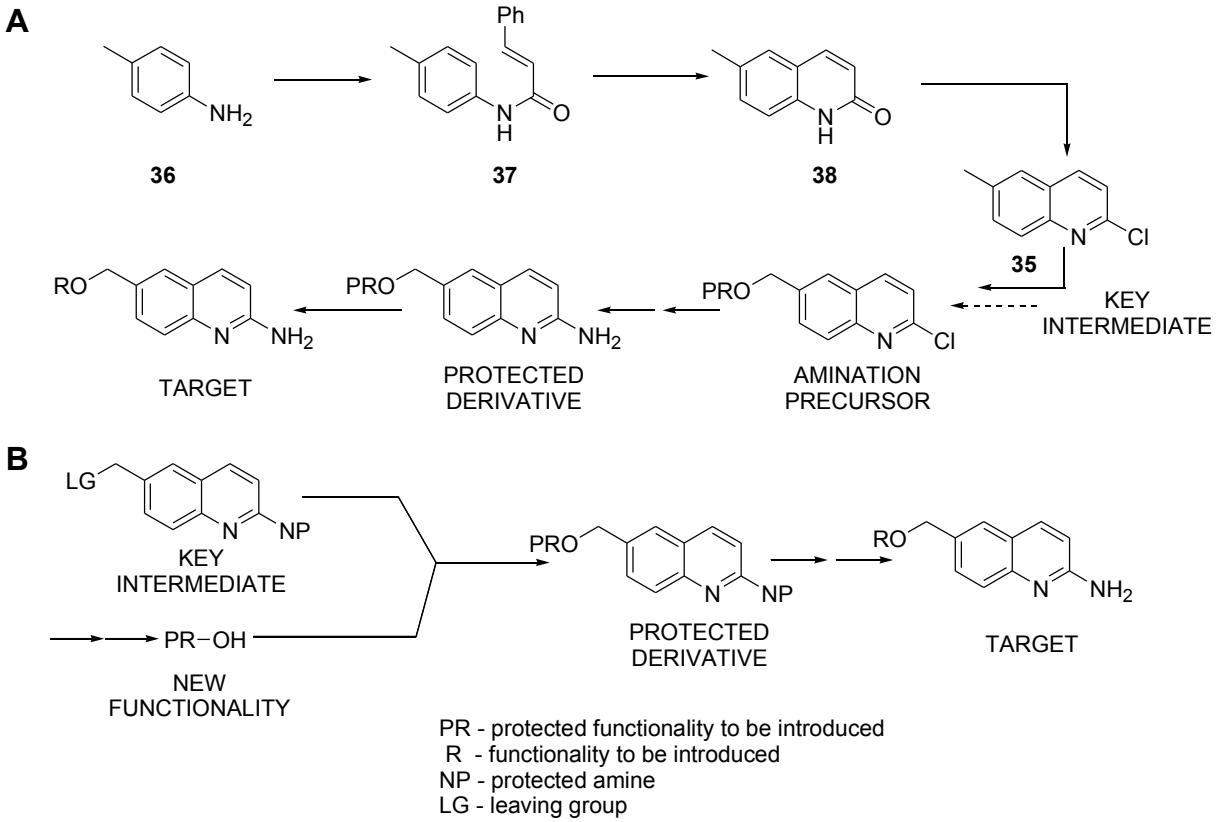


Figure 4-12: Thought experiment: comparison of 'linear' versus 'convergent' synthetic approaches for synthesis of 2-aminoquinolines. (A) 'Linear' approach that has so far been the focus of the investigation, highlighting the many steps involved in reaching the target molecule. (B) Proposed 'convergent' approach to be investigated as an alternative method, that potentially will result in a more efficient method with fewer steps required to reach the target molecule.

2-amino-functionality in place, but in a protected form. In addition, this intermediate should have a leaving group in place at the 6-benzylic position, so that the intermediate can be involved in substitution chemistry. Then, by an independent pathway, compounds with 'new functionality' (with appropriate protecting groups if necessary) can be synthesised. An essential feature of this compound is that it can play the role of a nucleophile (eg. an alcohol derivative, as illustrated in Figure 4-12B). The 'new functionality' and 'key intermediate' compounds are then joined together by way of a nucleophilic substitution reaction. The resulting compound, the 'protected derivative' is then de-protected in one or two steps to provide the 'target' compound.

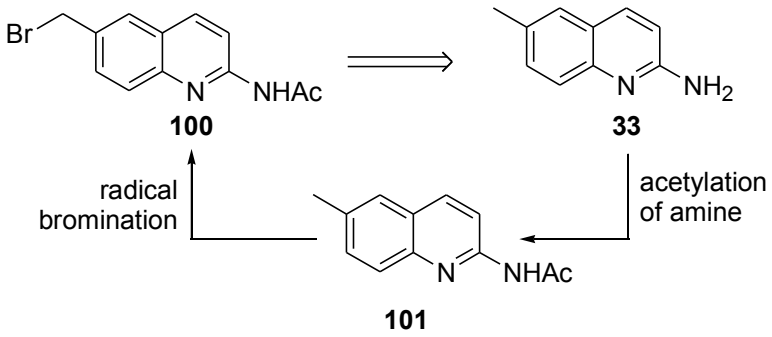
There would be several advantages of such a system. Firstly, the number of steps that the 'key intermediate' has to be involved in is very much reduced, resulting in better overall yields, and potentially allowing several compounds to be synthesised with the same (or a lesser) amount of material than would otherwise be required to synthesise one just compound using a linear synthetic approach. The 'new functionality' compounds can be synthesised on a large scale using readily available, and cheap starting materials, and thus a

range of diverse functionality can be investigated, and potentially a large range of new ligands could be synthesised quite quickly, to provide much needed new SAR information.

The work presented in this section is an investigation into the development of a 'convergent' synthetic strategy for the synthesis of 6-substituted-2-aminoquinolines.

4.7.1 Synthesis of a 'key intermediate' for use in convergent synthetic strategy

As illustrated in Figure 4-12B, the 'key intermediate' compound for use in a convergent synthetic strategy should be a 6-substituted-2-aminoquinoline derivative in which the amino functionality is in a protected form, and the substituent at the 6-position should have a suitable leaving group in place for involvement in substitution chemistry. Hence, a simple example of a compound that fits this criterion is *N*-[6-(bromomethyl)quinolin-2-yl]acetamide **100** (Scheme 4-28) with the 2-amino functionality protected as an acetamide, and the bromomethyl functionality at the 6-position being suited to substitution chemistry. It was anticipated **100** would be prepared in two steps starting from **33** by acetylation of the amino group to form **101**, followed by radical bromination to form **100**, as illustrated in Scheme 4-28.



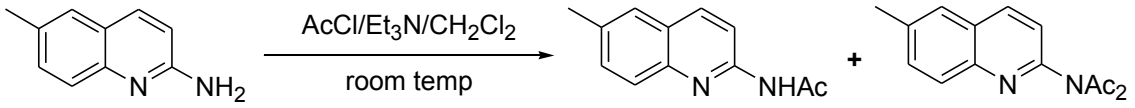
Scheme 4-28: Proposed method for synthesis of 'key intermediate' compound **100** for use in convergent synthetic strategy.

4.7.1.1 Synthesis of *N*-(6-methylquinolin-2-yl)acetamide

The first step towards synthesis of 'key intermediate' compound **100** was to develop a suitable method for the synthesis of radical bromination precursor **101**. Two approaches are presented.

Synthesis of *N*-(6-methylquinolin-2-yl)acetamide 101 by acetylation of 2-amino-6-methylquinoline 33

The first method tried was treatment of 2-amino-6-methylquinoline **33** with an excess of acetyl chloride in the presence of triethylamine in dichloromethane, as illustrated in Scheme 4-29.



33 **101** **102**

Scheme 4-29: Synthesis of acetamide derivatives **101** and **102** from **33**.

The acetylation reaction produced the mono-acetyl and di-acetyl products **101** and **102** respectively as illustrated in Scheme 4-29. These products could be separated using column chromatography on silica gel, and **101** and **102** were isolated in 24% and 50% yield respectively. Although this observation is consistent with that made when the acetylated derivative of 2-aminoquinoline was prepared using similar conditions, as described in Chapter 2, this result was not expected from the point of view that the mono-acetyl derivative **101** would not normally be predicted to be sufficiently nucleophilic (due to resonance considerations) to undergo the second acetylation to produce the di-acetylated product **102**. On the other hand, it may be predicted that the NH proton of **101** is significantly more acidic than that of other acetamides. This is because the resultant negative charge obtained after deprotonation of **101** may be delocalised onto the quinoline ring nitrogen atom, in addition to the oxygen atom of the acetamido carbonyl group. If this was the case, then deprotonation of the NH of **101** with triethylamine may be possible, thereby enhancing the nucleophilicity of the actetamido nitrogen atom, thus leading to the second acylation. This may also explain why a substantially higher yield of the di-acetyl product **102** was observed than the mono-acetyl product **101** (50% vs 24%).

One feature of the ^{1}H NMR spectrum of **101** that provided evidence for its formation included a very large downfield change in chemical shift for the H3 proton of the quinoline ring from δ = 6.72 ppm in **33** to δ = 8.39 ppm in **101**. In addition the broadening of the signal at δ 8.39 ppm in **101** was observed, as was the case for the synthesis of the acetamide derivative of 2-aminoquinoline (**9**) in Chapter 2. A singlet at δ 2.17 ppm for the methyl protons of the acetyl motif ($C\underline{H}_3CO$) was also observed, and a band was seen at 1661 cm^{-1} for the carbonyl functionality in the IR spectrum. The identity of **101** was confirmed by mass spectrometry and elemental analysis.

Features of the ^{1}H NMR spectrum of **102** that provided evidence for its formation included a doubling of the height of the signal at δ 2.32 ppm for the methyl protons of the acetyl motif (2 x $C\underline{H}_3CO$), relative to the signal for the methyl group attached to the quinoline ring ($ArC\underline{H}_3$) at δ 2.54 ppm, suggesting that two acetyl groups had been added to the molecule. In addition, there

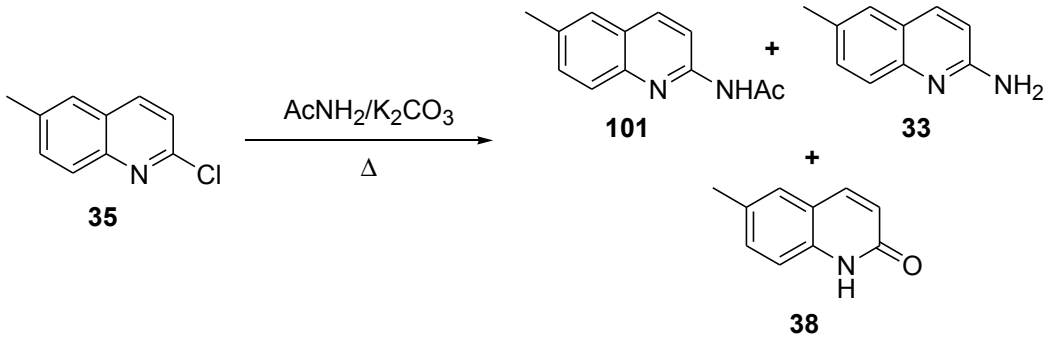
was an upfield shift of the H3 proton in **102** relative to the mono-acetyl derivative **101**, from δ 8.39 ppm in **101** to δ 7.27 ppm in **102**. In contrast to **101**, the broadening of the signal for H3 was not observed. There was a signal at δ 173.3 ppm in the carbon spectrum of **102** that was indicative of a large quaternary signal relative to other quaternary signals, suggestive of two carbonyl carbons (CO). In addition, there were two bands seen at 1723 cm^{-1} and 1701 cm^{-1} in the IR spectrum indicative of asymmetric and symmetric carbonyl stretches respectively. The identity of **102** was confirmed by mass spectrometry.

Based on the results discussed above, and those from the synthesis of the 2-aminoquinoline derivatives presented in Chapter 2, it can be concluded that acetylation of 2-aminoquinolines by treatment with acetyl chloride using the conditions as illustrated in Scheme 4-29, is not an effective method. The problem is, controlling the reaction to produce only the mono acetyl derivative without the accompanying doubly acetylated product. Furthermore, in order to control the reaction, it seems that it is not simply a matter of ensuring there is no excess of acetyl chloride. For the synthesis of **101** and **102** as presented in Scheme 4-29, when just one equivalent of acetyl chloride had been added, thin layer chromatography indicated that three compounds (starting material **33** and the two products **101** and **102**) were present in the reaction mixture. This suggests that all the acetyl chloride had been consumed, and that the mono-acetyl compound **101** had preferentially reacted with the acetyl chloride (forming more **102**) instead of the remaining starting material **33**. This observation provides some evidence for the enhanced acidity of the NH proton of **101**, proposed above. Hence, additional acetyl chloride was added, and the remaining starting material was promptly consumed, however, the consequence of this was that at the end of the reaction, a mixture of mono- and di-acetyl compounds was isolated. The major product was the di-acetyl compound **102**. In the case of the synthesis of the 2-aminoquinoline derivatives as presented in Chapter 2, an excess of acetyl chloride was present at the beginning of the reaction, and again the mixture of mono and di-acetylated products was obtained. But on that occasion, the ratio of mono- to di-acetylated product was in favour of the mono-acetylated derivative. It is unclear why the mono-acetyl compound was the major product in this case. It was very clear however, that some effort would have been necessary to investigate the optimal method for acetylation of 2-amino-6-methylquinoline **33** using this approach, in order to avoid or minimise the quantity of the di-acetylated product formed.

Synthesis of *N*-(6-methylquinolin-2-yl)acetamide 101 by acetamidation of 2-chloro-6-methylquinoline 35

It has also been reported that 2-chloroquinolines may be converted to 2-acetamidoquinolines in one step, by treatment with a large excess of acetamide and potassium carbonate, and heating at reflux overnight.[73] These conditions are similar to the amination method of Kóródi[72] however the Kóródi method involves ca. 20 equivalents of acetamide, 5 equivalents of

potassium carbonate, relatively short reaction times (~1-3 hours) at temperatures ca. 200°C and results in formation of 2-aminoquinolines. The alternative conditions as presented in Watanabe[73] involve ca. 80 equivalents of acetamide, 5 equivalents of potassium carbonate and heating at reflux temperature for 14 hours, resulting in formation 2-acetamidoquinolines. Given that the boiling point of acetamide is 221°C,[95] the only significant differences between these two sets of conditions is the reaction time and the number of equivalents of acetamide used. Hence, it was decided to investigate the use of the Watanabe method as an alternative method for the synthesis of **101** in one step from **35**. This would potentially provide a method for the synthesis of **101** that avoids the selectivity problem associated with acetylation of the 2-aminoquinoline precursor, and it has the added advantage of eliminating one step.



Scheme 4-30: Investigation into the synthesis of **101** in one step from **35** according to the method of Watanabe.[73]

Thus, the approach was tested as illustrated in Scheme 4-30, on a fairly small scale (0.25 g of **35**). Thin layer chromatography was used to monitor the reaction progress and this suggested that after only a short time (~ 2 hours) the starting material had been consumed, however the R_f value of the major product observed at this stage was consistent with a 2-aminoquinoline instead of a 2-acetamidoquinoline. This was a peculiar observation in that it would seem more likely that the amination of 2-chloroquinoline using acetamide would proceed via the 2-acetamidoquinoline followed by subsequent hydrolysis, and therefore after a relatively short reaction time, the expected product would be the 2-acetamidoquinoline. On the other hand, this observation is consistent with the Kóródi method in which the 2-aminoquinoline is formed in a relatively short reaction time, and that no acetamido derivative is observed during the reaction as judged by thin layer chromatography. Hence, the reaction was allowed to proceed overnight as specified in the Watanabe method. After this additional time, thin layer chromatography indicated that the acetamido derivative had indeed formed. Following workup, ^{1}H NMR of the crude material confirmed the main product from the reaction was **101**, as evidenced by the presence of all the signals observed in the purified sample of **101** prepared using the other method (Scheme 4-29). In particular, the broadened doublet at δ 8.39 ppm was observed again, a very diagnostic feature. However, a small

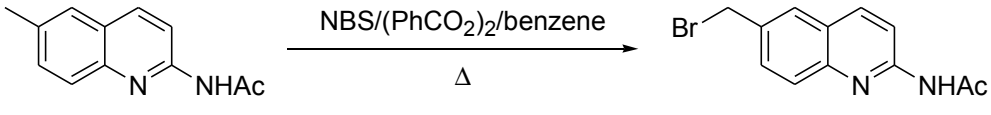
amount of **33** and **38** were also both observed in the ^{1}H NMR spectrum, as evidenced by the diagnostic doublets seen for both at δ ~ 6.7 ppm for the H3 protons of the quinoline ring, amongst other signals in the aromatic region, consistent with those seen for **33** and **38** when prepared by other methods. By comparing the relative areas of the signals from the by-products and the major product, the amount of **33** and **38** present in the sample was estimated at 15% in total. Following chromatography, pure **101** was isolated in 68% yield, consistent with the reported yield for the synthesis of 2-acetamidoquinoline.

This reaction behaved similarly to the amination method of Kóródi from the point of view that the yields and the quality of the isolated product were optimal if the starting material was recrystallised and freed of coloured impurities. Yields of around 40% or less were obtained when the starting material contained coloured impurities.

In order to obtain a larger supply of **101** for exploratory reactions, the reaction was repeated on a large scale (7.54 g of recrystallised **35**). On this scale, a considerably large amount of acetamide (200 g) was required. Subsequently, to avoid blockage of the condenser with sublimed acetamide whilst heating at reflux, a wide diameter air condenser was used instead of a standard water condenser. The success of this reaction when performed on such a large scale was quite different to that obtained on a smaller scale. Only a 39% yield of the desired product **101** was isolated after chromatography. However, on this occasion, 31% recovery of the quinolin-2(1*H*)-one **38** was also isolated, but none of the 2-aminoquinoline product **33** was observed. The reasons for this are unclear, however, it is possible that the acetamide used in the reaction was not as dry as supplies used for other reactions resulting in more of the competing reaction that forms the quinolin-2(1*H*)-one. In spite of this lower yield, an adequate supply of **101** was obtained in order to proceed with the current investigation.

4.7.1.2 Synthesis of *N*-[6-(bromomethyl)quinolin-2-yl]acetamide

Having established a suitable method for the preparation of *N*-(6-methyl-quinolin-2-yl)-acetamide **101**, this was tested as a suitable substrate for radical bromination by treatment with *N*-bromosuccinimide and a catalytic amount of benzoyl peroxide with heating at reflux in benzene, as illustrated in Scheme 4-31. These conditions were essentially the same as those used for the radical di-bromination of 2-chloro-6-methylquinoline **35** (Scheme 4-6) however in the present case only 1.1 instead of 2 equivalents of *N*-bromosuccinimide were used.



101 **100**

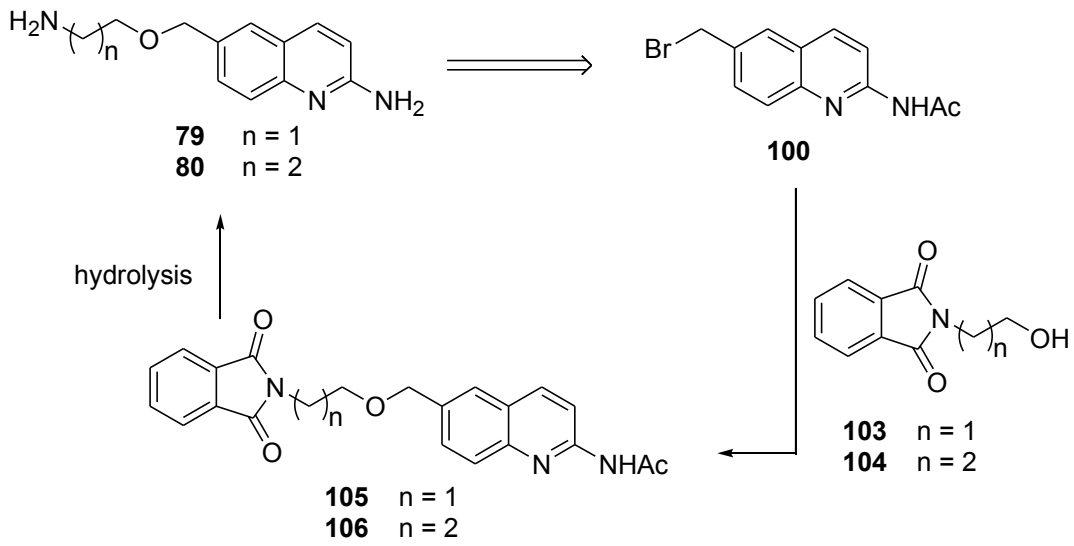
Scheme 4-31: Synthesis of bromomethylquinoline derivative **100** by radical bromination of **101**.

The reaction was heated at reflux for 5 hours and ^{1}H NMR was used to monitor the reaction progress. This indicated that a substantial amount of the desired mono-bromomethyl **100** product had formed, as evidenced by a signal at δ 4.65 ppm for the methylene (C$\underline{H}_2$Br) protons, consistent with the chemical shift observed for the mono-bromo derivative of 2-chloroquinoline **62** (Scheme 4-6). A small amount (~8%) of the corresponding di-bromo compound was also present as evidenced by a signal at δ 6.8 ppm, again consistent with the chemical shift observed for the di-bromomethyl derivative of 2-chloroquinoline **61** (Scheme 4-6). In addition, some unreacted starting material **101** was also present.

This mixture was purified by silica gel chromatography from which pure **100** was isolated in 56% yield. The identity of **100** was confirmed by mass spectrometry and elemental analysis. The di-brominated material was not isolated from the column and hence no additional data is provided for this compound. The yields obtained for this reaction were typically ~50% after several attempts. However, consistent with radical bromination reactions performed in Section 4.3.1, the yields were optimal and the product was higher quality when the starting materials used for this reaction were of the highest purity possible.

4.7.2 Testing suitability of 'key intermediate' for use in convergent synthetic strategy 1: Attempted coupling with primary alcohols

Given that in Sections 4.5 and 4.6, so much effort was made to develop satisfactory methods for the synthesis of di-amines **79** and **80** without success, identification of an alternative method for their synthesis remained a priority. Hence, it was envisaged that the planned convergent synthetic strategy might be an effective method to achieve this goal. Specifically, as presented in Scheme 4-32, by coupling the phthalimido-alcohol derivatives **103** or **104** to **100** to form protected derivatives **105** or **106**, following a one-pot hydrolysis, the di-amines **79** and **80** might be prepared in two simple steps. This approach is further simplified by the commercial availability of alcohols **103** and **104.**



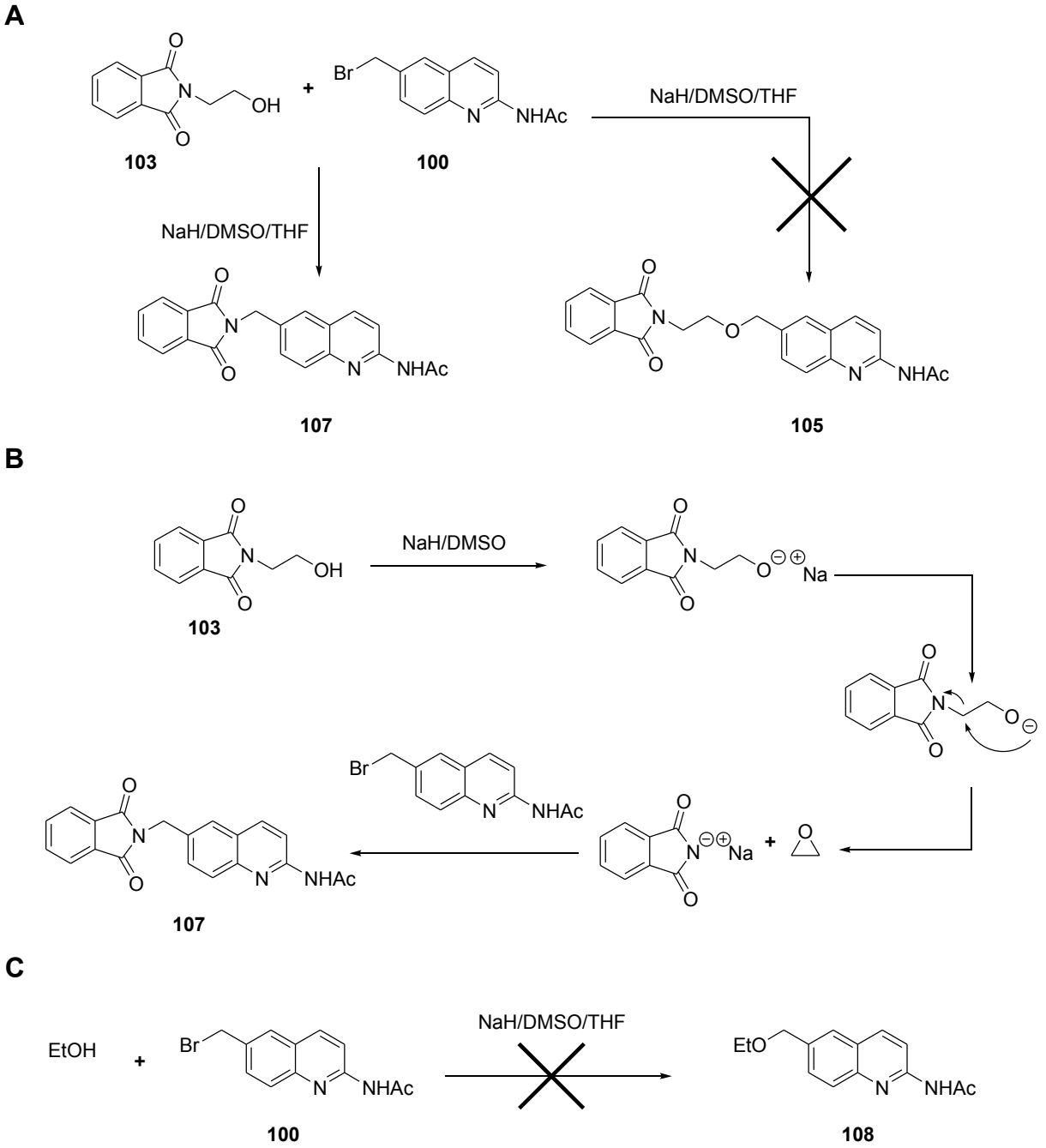
Scheme 4-32: Planned method for synthesis of diamines **79** and **80** using convergent synthetic strategy, in 2 steps from **100**.

4.7.2.1 Testing the coupling reaction through substitution via alkoxide formation

Attempted coupling of 100 with alcohol 103

The coupling of the phthalimido-alcohol **103** with bromomethyl derivative **100** was first tested as illustrated in Scheme 4-33A. It was decided that it might be preferable to pre-form the alkoxide of **103** in DMSO, and then add this drop-wise to a stirred solution of **100** in THF, in order to effect the alkylation of the alkoxide preferentially to the possible de-protonation of the amide proton of **100**. The resulting solution was stirred overnight at room temperature under an inert atmosphere. After this time, thin layer chromatography indicated that three major materials were present, however, two of their R_f values were very similar to the R_f values of both the starting materials **100** and **103**. Following workup, ^{1}H NMR analysis of the resulting material indicated a mixture of products was present, however, there was little evidence to suggest that the desired product **105** was part of the mixture. The material was purified by column chromatography on silica gel, and three materials were isolated. ^{1}H NMR analysis of the major product was suggestive of the product **107** as illustrated in Scheme 4-33A below. The evidence for this was the presence of a 2-proton singlet at δ 5.00 ppm, suggestive of displacement of the bromine atom of **100** (δ = 4.65 ppm for Ar$C\underline{H}_2$Br). In addition a 3-proton singlet was observed at δ = 2.23 ppm for the methyl group protons (CH_3CO), suggesting the presence of the intact amide functionality. Clear signals were also observed in the aromatic region of the spectrum for the NH, H3, and H4 protons of the quinoline ring (consistent with the starting material **100**), in conjunction with two multiplets at

δ 7.69-7.74 and 7.80-7.89 respectively characteristic of the phthalimido motif (overlapping with the signals for the H5, H7 and H8 protons of the quinoline ring respectively). However, no additional signals were observed in the aliphatic region, in particular the expected signals for the methylene protons of the desired product **105** (OC$\underline{H}_2$C$\underline{H}_2$N). The identity of **107** was indeed confirmed by mass spectrometry.



Scheme 4-33: Investigation into coupling of primary alcohols with bromomethylquinoline derivative **100**. (A) Attempted synthesis of phthalimide derivative **105** from **100**. (B) Explanation for the formation of phthalimide derivative **107** as the major product from the reaction in (A) instead of the desired product **105.** (C) Investigation into coupling conditions with model alcohol, ethanol.

The formation of product **107** instead of the desired product **105** indicated that a nucleophilic substitution reaction had taken place with the phthalimide anion as the nucleophile instead of the alkoxide. The reason for the formation of this product is likely to be the result of an unexpected intra-molecular epoxidisation of the alkoxide anion formed, resulting in the leaving of the phthalimide anion as illustrated in Scheme 4-33B; the phthalimide anion then plays the role of the nucleophile in the substitution reaction.

The identity of the other two materials that were isolated in low mass recovery (< 10%) and low purity were not determined, however there was evidence to suggest that unreacted **100** was a component of one of the materials.

Attempted coupling of 100 with model alcohol

To gain more information about the reactivity of **100** towards nucleophilic substitution reactions, and the types of conditions that might be compatible with **100**, an attempt was made to alkylate a model alcohol, ethanol, by alkoxide formation (Scheme 4-33C). Specifically, this experiment was intended to provide information about the suitability of the acetamide functionality under the strongly basic conditions used in these experiments. Thus, in this case the ethoxide was pre-formed in a DMSO solution, and then **100** was added to this solution. The reaction was allowed to proceed overnight, prior to workup. Thin layer chromatography after this time indicated that all of the starting material had been consumed, however, only a spot with low UV activity at the base line of the thin layer chromatography plate was observed. ^{1}H NMR analysis of the isolated material suggested that degradation of the starting material to form an unknown by-product had occurred, as evidenced by the presence of large broad signals in the aromatic region of the spectra, and several additional signals in the region of ~2.0-2.5 ppm. No signals indicative of the expected product **108** were observed in the spectrum. No further attempt was made to identify the isolated material.

The results from both of the investigations presented above suggest that the current method was not suitable for achieving the desired coupling reaction; there was now evidence from two different angles highlighting the problems associated with the approach. Firstly, trying to get the substitution reaction to proceed via generation of an alkoxide with the alcohol **103** resulted in an intra-molecular reaction to generate an unintended nucleophilic species (Scheme 4-33B). Secondly, even with a simple alcohol where no intra-molecular reaction is possible, the desired product still does not form (Scheme 4-33C), suggesting that side reactions, possibly involving the acetamido motif of **100** are occurring. It may therefore be

concluded that the acetamido group is not a suitable protecting group for the amino functionality in this case.

4.7.2.2 Testing the coupling reaction through silver oxide catalysis

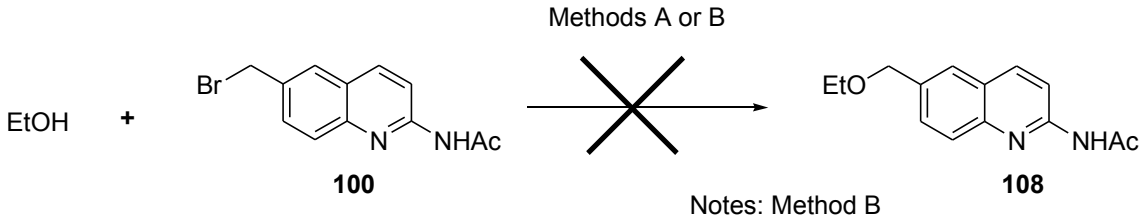
Given that the experiments described in Section 4.7.2.1 for the alkylation of alcohols by alkoxide formation had both failed (Scheme 4-33), an alternative approach to achieve the same coupling was sought. It was also envisaged that the undesired intra-molecular expoxidisation reaction illustrated in Scheme 4-33B should be eliminated if a method for the coupling reaction could be developed that proceeded under neutral conditions. Hence, it was predicted that the coupling reaction might proceed using silver(I) oxide catalysis. (Silver(I) oxide catalyses substitution of alkyl halides by promotion of halide dissociation through precipitation of the silver halide complex.)

Several methods have been reported for the alkylation of alcohols with benzyl bromides using silver(I) oxide.[96-99] Typical conditions for the reaction involve one or more equivalents of silver(I) oxide and an excess of benzyl bromide, and common solvents for the reaction are THF[99] or DMF,[96] however methods involving non-polar solvents such as dichloromethane have also been reported.[97,98]

With this in mind, it was decided to first trial the method using the model alcohol ethanol, and its alkylation with **100**, as illustrated in Scheme 4-34A. In the first instance, dichloromethane was selected as the solvent, and the reaction was allowed to proceed at room temperature for 24 hours (Scheme 4-34A, Method A), however, only the starting material **100** was returned after workup of this reaction. Next, three very small scale (~5-10 mg of **100**) experiments were performed using THF as solvent with varied stoichiometric ratios of ethanol:**100**: silver(I) oxide as illustrated in Scheme 4-34A (Methods B1-3), but again no reaction was observed after 24 hours, as judged by thin layer chromatography. The results from these investigations (Scheme 4-34A) were suggestive that the conditions selected were too mild for the reaction to take place. Either an increase in temperature was necessary, or a more polar solvent was required. Indeed, all the reactions tested so far were inhomogeneous, with the silver(I) oxide remaining as a suspension in the solution.

Thus, for the next experiment, a more polar solvent, DMF, was selected for the reaction. For the purposes of saving time and the starting material **100**, it was decided to perform the

A

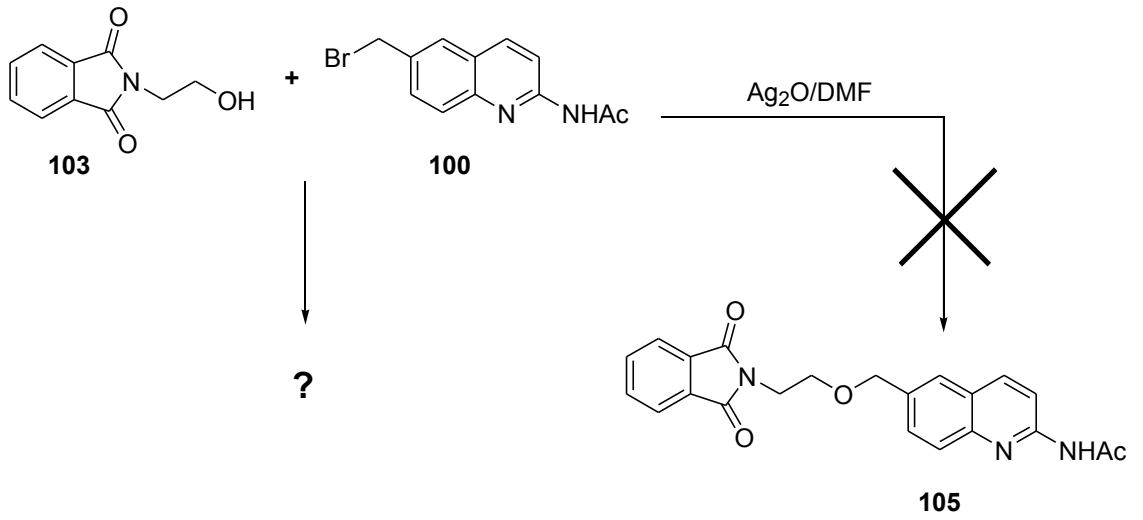


Notes: Method B

Method	Ratio EtOH/**100**/Ag_2O
B1	1:1:1
B2	1:2:1
B3	1:2:2

Method A: Ag_2O/CH_2Cl_2
Method B: Ag_2O/THF

B

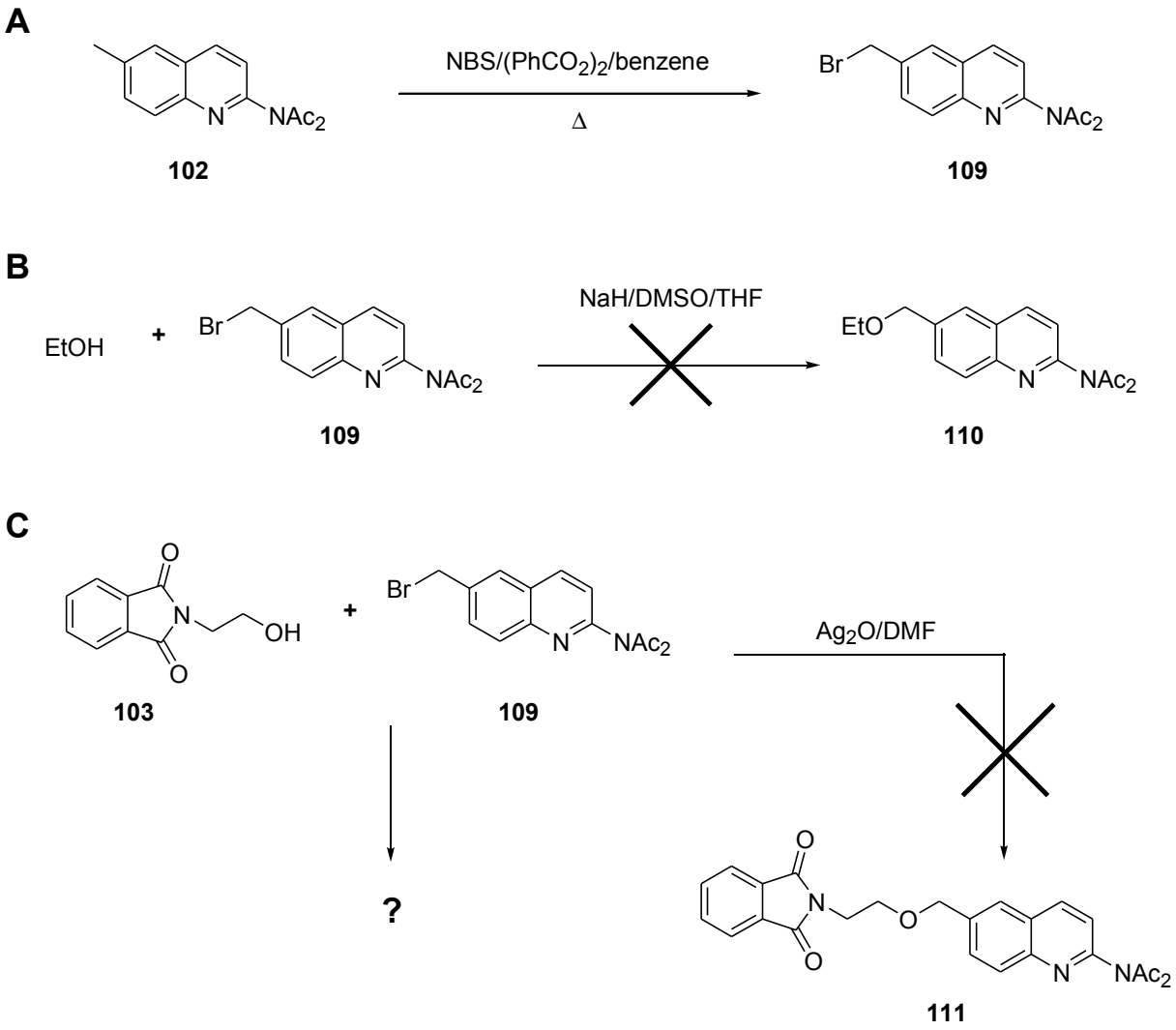


Scheme 4-34: Investigation into alkylation of alcohols with **100** using silver(I) oxide catalysis. (A) Attempted alkylation of ethanol with **100**. (B) Attempted alkylation of **103** with **100**.

reaction using the phthalimido ethanol derivative **103,** instead of the model alcohol ethanol, as illustrated in Scheme 4-34B. In this case, a 1:1 ratio of **103** and **100** was used, and 2 equivalents of silver(I) oxide were used, and the reaction was stirred in DMF for 19 hours at room temperature. After workup, ^{1}H NMR analysis indicated a mixture of several compounds was present, however there was good evidence to suggest that the alcohol **103** was largely unreacted, as there was a large signal at δ 3.90 ppm for the methylene protons ($OC\underline{H}_2C\underline{H}_2N$) consistent with the chemical shift for **103**. Signals were also observed in the aromatic region of the spectrum at δ ~ 7.7-7.9 ppm indicative of the phthalimide motif. However, large, broad signals were coincident with this, and other regions of the aromatic part of the spectrum. Several other signals were also observed in the aliphatic region of the spectrum, suggesting that substantial side reactions had occurred. No additional data was obtained for this material. This suggests that use of the highly polar solvent DMF, led to a range of side reactions, and the conditions were too severe for obtaining the desired product.

4.7.2.3 Brief investigation into 'key intermediate' with alternative protecting group for amino functionality

The investigations presented in Sections 4.7.2.1 and 4.7.2.2 had both suggested that one of the possible problems associated with these methods was that the acetamido motif of 'key intermediate' **100** was not a robust enough protecting group for the desired reactions. Under both strongly basic conditions, and neutral conditions, the reaction had not gone to plan. Therefore, to assess whether this was a consequence of the acetamide group, and specifically the potentially acidic amide proton ($N\underline{H}COCH_3$) causing complications in the reaction, the bromomethyl derivative of the doubly acetylated compound **102** (Scheme 4-29) was prepared with the aim of investigating its suitability for the desired coupling reactions.



Scheme 4-35: Investigation into use of doubly acetylated quinoline derivative **109** as 'key intermediate' in convergent synthetic strategy. (A) Radical bromination of **102** to form **109**. (B) Attempted synthesis of ethoxy derivative **110** using basic conditions. (C) Attempted synthesis of phthalimido derivative **111** using silver(I) oxide catalysis.

The bromomethyl derivative **109** was synthesised by treatment of **102** with *N*-bromosuccinimide and a catalytic amount of benzoyl peroxide and heating at reflux in benzene as illustrated in Scheme 4-35A (as described for the synthesis of **100** in Scheme 4-31). The ^{1}H NMR spectrum of the crude product suggested that during the radical bromination reaction, some hydrolysis of the di-acetylamino motif had occurred to form the mono-acetyl material, as evidenced by the presence of two sets of signals in the spectrum, and additional resonances in the aromatic region consistent with those of **100**. Specifically, the diagnostic broad doublet at δ ~ 8.4 ppm for the H3 proton of the quinoline ring was observed for the minor component (**100**) of the mixture. The reasons for this hydrolysis are unclear. The main feature of the ^{1}H NMR spectrum of this crude product to provide evidence for the formation of the expected product **109** was the large downfield change in chemical shift for the benzylic protons (Ar$C\underline{H}_3$) from 2.54 ppm in **102** to 4.66 ppm (Ar$C\underline{H}_2$Br) in the product **109**. Following chromatography, pure **109** was isolated in relatively low yield (31%), and the identity of the product was confirmed by mass spectrometry.

Two experiments that were essentially the same as those tried with mono-acetyl derivative **100** were attempted using **109** as illustrated in Scheme 4-35; namely the attempted coupling of **109** with ethanol under basic conditions (Scheme 4-35B) and the attempted coupling of **109** with phthalimido derivative **103** using silver(I) oxide catalysis (Scheme 4-35B). Both of these investigations resulted in similar outcomes to those observed for the equivalent experiments using the mono-acetyl derivative **100**, as described in Sections 4.7.2.1 and 4.7.2.2. No evidence for formation of the desired products (**110** or **111**) was seen. These results suggest that the presence of the amide proton was not causing the side reactions, but some other features of the substrate were not suited to the reaction conditions being tested.

At this point, after several attempts to couple alcohols with the bromomethyl-acetamidoquinoline derivatives **100** and **109** without success, it was decided to discard the current approach, and investigate other methods for the synthesis of di-amines **79** and **80**.

4.7.3 Testing suitability of 'key intermediate' for use in convergent synthetic strategy 2: Substitution reactions under milder conditions

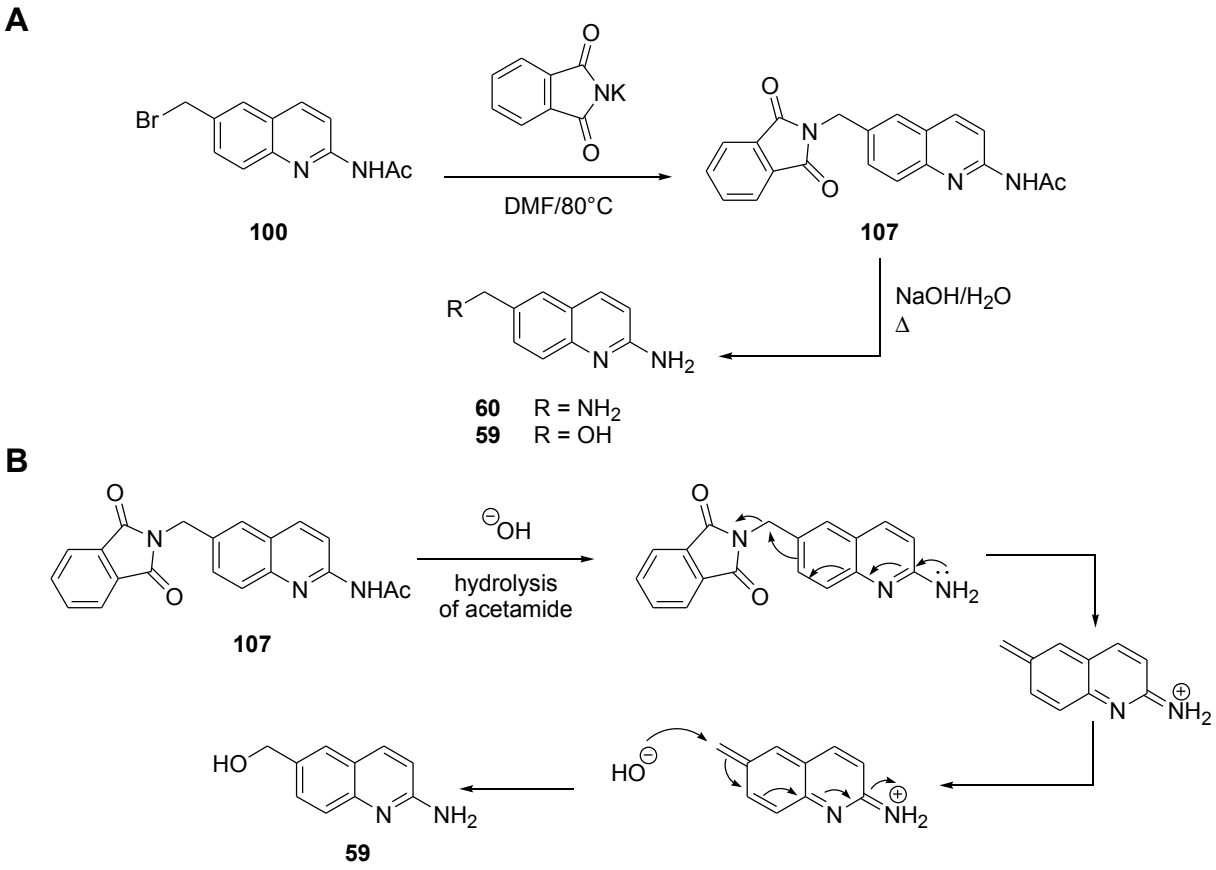
Given that several unsuccessful attempts had been made to effect substitution reactions using alcohols as nucleophiles with the proposed 'key intermediate' **100** as part of a convergent synthetic strategy, it was decided to investigate whether substitution reactions would occur with **100**, using simple, weakly to non-basic nucleophiles, for example the

phthalimide anion. Indeed, there was already evidence to suggest that substitution reactions with **100** were possible with non-basic nucleophiles, given that in Section 4.7.2.1, where the coupling of phthalimido-ethanol derivative **103** was attempted under strongly basic conditions, the *N*-benzylated product **107** was isolated instead of the intended *O*-benzylated product (Scheme 4-33). In this case the *N*-benzylated product was most likely the result of an intra-molecular epoxidisation reaction that took place leading to the formation of sodium phthalimide that subsequently played the role of nucleophile in the substitution reaction. Thus, substitution of **100** with weakly to non-basic nucleophiles, including phthalimide and others is the subject of this next section, and as will be presented, several encouraging consequences resulted from these studies.

4.7.3.1 Substitution with 'key intermediate' and phthalimide: A simple synthesis of 6-aminomethylquinolin-2-ylamine

The coupling of phthalimide with **100** was tested by treatment of **100** with potassium phthalimide and stirring in DMF at ca. 80°C overnight as illustrated in Scheme 4-36A. The reaction proceeded as expected, and the desired product **107** was isolated in good yield of 81% following workup and purification. Features of the ^{1}H NMR spectrum of **107** that provided evidence for its formation included a downfield change in chemical shift from 4.65 ppm in **100** for the benzylic protons (ArC$\underline{H_2}$Br) to 4.98 ppm in **107** (ArC$\underline{H_2}$N), and the characteristic signals for the phthalimido motif at δ 7.69-7.72 and δ 7.82-7.86 ppm were also observed in the aromatic region. Three signals were seen at 1768, 1717 and 1706 cm^{-1} in the infrared spectrum: the signals at 1768 and 1717 cm^{-1} are consistent with asymmetric and symmetric stretches respectively for an imide, whilst that at 1706 cm^{-1} is consistent with the acetamide carbonyl group. The identity of **107** was confirmed by mass spectrometry and elemental analysis. In addition, the ^{1}H and ^{13}C NMR spectra for **107** synthesised here were in good agreement with those obtained for the unexpected synthesis of **107**, presented in Section 4.7.2.1, providing additional evidence for its formation in that case.

This was an encouraging result from two perspectives. Firstly it confirmed that substitution reactions with **100** and non-basic nucleophiles were indeed possible, but in addition, it also potentially provided a simple method for the synthesis of the 6-aminomethyl-2-aminoquinoline derivative **60**, obtainable following hydrolysis of the substitution product **107**. This compound had been a long-term target, sought during the early 6-substituted-2-aminoquinoline design process, but up to this point, a suitable method for its synthesis had not been identified. Thus, the substitution product, **107** was treated with 20% w/v aqueous



Scheme 4-36: (A) Method for synthesis of 2-aminoquinoline **60** in two steps starting from **100**. (B) Proposed explanation for formation of by-product **59** in the hydrolysis step (A).

sodium hydroxide and heated at reflux for 4 hours, as illustrated in Scheme 4-36A. Following workup, ^{1}H NMR analysis of the isolated material indicated that the expected product **60** was present as the major component of the sample. The evidence for this was the loss of the signals in the aromatic region for the phthalimido motif, in conjunction with an upfield change in chemical shift from δ 4.98 for the benzylic protons (C$\underline{H}_2$NR) in **107** to δ 4.50 ppm in **60**. In addition, the signal at δ 2.20 ppm for the methyl protons of the acetyl motif in **107** was no longer observed. A large upfield change in chemical shift from 8.30 ppm for the H3 proton of the quinoline ring in **107**, to the characteristic chemical shift at δ ~ 6.80 ppm in **60** was also observed. The identity of **60** was confirmed by mass spectrometry.

There was however a minor impurity present in the sample of **60**, determined to be the 6-hydroxymethyl-2-aminoquinoline derivative **59**. The evidence for this included a small singlet at δ 4.69 ppm suggestive of another material containing benzylic protons attached to an electronegative element. In addition, small signals were observed in the aromatic region of the spectrum of the ^{1}H NMR spectrum, particularly an additional set of signals having chemical shifts consistent with the H3 and H4 protons of the quinoline ring. This was suggestive of another 2-aminoquinoline being present in the sample. Mass spectrometry was

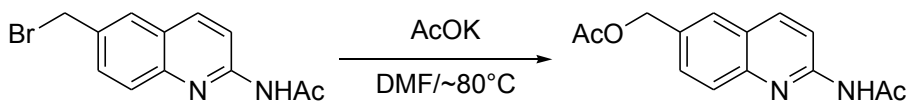
used to confirm that this by-product was indeed the hydroxymethyl derivative **59**, and comparison of the relative integration ratios from the ^{1}H NMR spectrum indicated that the level of **59** present in the mixture was 5% or less. Thus, no attempt was made to remove this impurity from the sample, and the ligand binding experiments presented in Section 4.8 were performed on the unpurified material.

Although the precise reasons for the formation of the by-product **59** above are unclear, it is possible that the resonance electron donating effect of the amino group at the 2-position (resulting from hydrolysis of the acetamide) can result in displacement of the phthalimide functionality to form an activated alkene intermediate, as illustrated in Scheme 4-36B. Subsequent attack by hydroxide in a 'Michael' sense results in formation of the hydroxymethyl compound. This explanation at a glance may seem more realistic than direct substitution of the phthalimide by hydroxide in an 'S_N2' sense, however, given that two other experiments in this report have resulted in S_N2 reactions with phthalimide as a leaving group, the S_N2 mechanism can not be eliminated.

4.7.3.2 Substitution with 'key intermediate' and acetate: A simple synthesis of 6-hydroxymethylquinolin-2-ylamine, and potential utility in a modified convergent synthetic strategy

Substitution of 100 with acetate

Having established that **100** could be coupled with phthalimide as presented above, it was then decided to investigate whether a similar reaction would occur using acetate as the nucleophile. Hence, in a similar manner to the method used for the phthalimide coupling, **100** was treated overnight with potassium acetate in DMF with heating at ca. 80°C as illustrated in Scheme 4-37. Again, this experiment proceeded as expected, with the desired product **112** isolated in high yield (87%) after workup.



100 **112**

Scheme 4-37: Substitution of **100** to form di-acetyl derivative **112**.

The main feature of the ^{1}H NMR spectrum of **112** to provide evidence for its structure was a large downfield change in the chemical shift of the benzylic protons in **100** from δ 4.65 ppm (ArC$\underline{H}_2$Br) to δ 5.26 (ArC$\underline{H}_2$OH) ppm in **112**, and the appearance of an additional signal at δ

2.15 ppm for the methyl protons of the added acetyl functionality ($C\underline{H}_3CO_2$). Two stretches were seen at 1689 and 1699 cm^{-1} in infrared spectrum of **112** indicative of the presence of two carbonyl groups. The identity of **112** was also confirmed by mass spectrometry.

Thought experiment: selective de-protection of 112 to form new 'key intermediate'

The above result provided additional encouragement for the use of weakly to non-basic nucleophiles in substitution reactions with **100**, but it was also envisaged that the coupling product **112** could be a useful precursor for other target compounds. For example, in a similar manner to the use of the phthalimide derivative **107**, simple hydrolysis of **112** should provide a convenient synthesis of 6-hydroxymethyl-2-aminoquinoline derivative **59**, another target that had been sought for sometime, but a suitable method for its synthesis had not yet been developed. In addition, it was predicted that under mild hydrolysis conditions, **112** might also be a substrate suited to selective de-protection of the *O*-Ac group to form the 6-hydroxymethyl-2-acetamidoquinoline derivative **113** (Scheme 4-38) that has the *N*-Ac motif intact. It was further envisaged that this selectively de-acetylated product **113** could be a useful intermediate as part of a 'modified' convergent synthetic strategy. Specifically, **113** would replace **100** as the 'key intermediate' whereby **113** would instead play the role of the nucleophile in substitution reactions, with derivatives containing 'new functionality' instead playing the role of electrophile, as illustrated in Figure 4-13. This will be further discussed below.

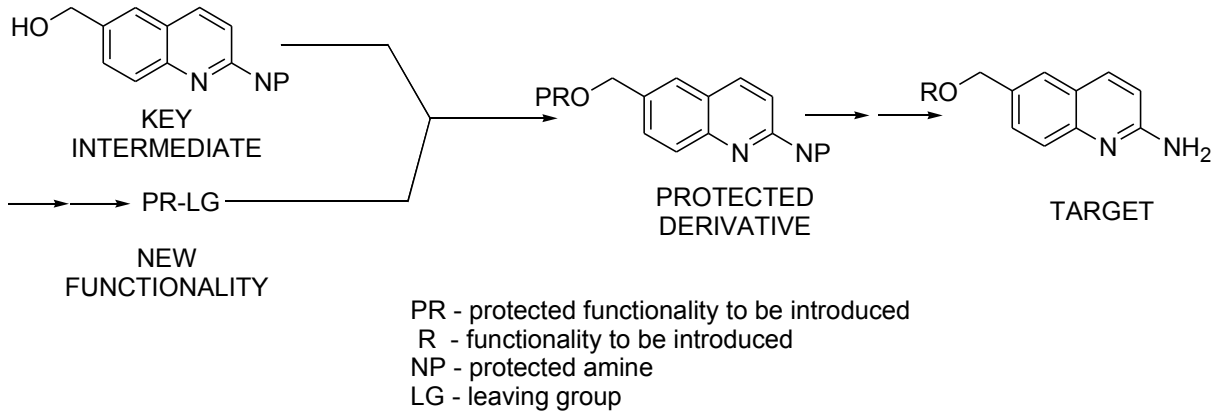
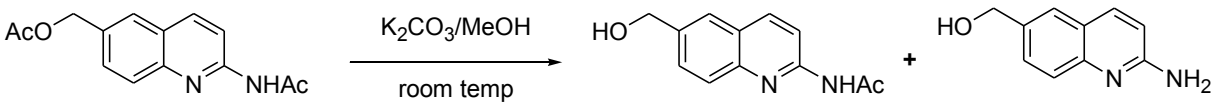


Figure 4-13: Thought experiment; a modification of the approach for convergent synthetic strategy, where the nucleophilic and electrophilic components of the plan are reversed relative to the original plan illustrated in Figure 4-12. It was envisaged that **113** prepared in Scheme 4-38 below might play the role of 'key intermediate' using this approach.

One pot synthesis of acetamide 113 and amine 59

Thus, as illustrated in Scheme 4-38, **112** was treated with 0.5 equivalents of potassium carbonate and stirred in methanol at room temperature for 2 hours. After this time, thin layer chromatography indicated that the starting material had been consumed, however an

additional spot on the plate suggested that two products were present in the mixture. Following workup and chromatography, the desired singly de-acetylated product **113** was isolated in good yield (72%), but in addition, a small amount (20%) of the doubly de-acetylated product **59** was also isolated. Although the formation of **59** was not expected from this reaction, this approach was pleasing in that the desired compound **113** was the major product, but **59**, a long sought target for ligand binding studies was also formed in the reaction and conveniently separated.



112 **113** **59**

Scheme 4-38: One pot synthesis of amide **113** and amine **59**.

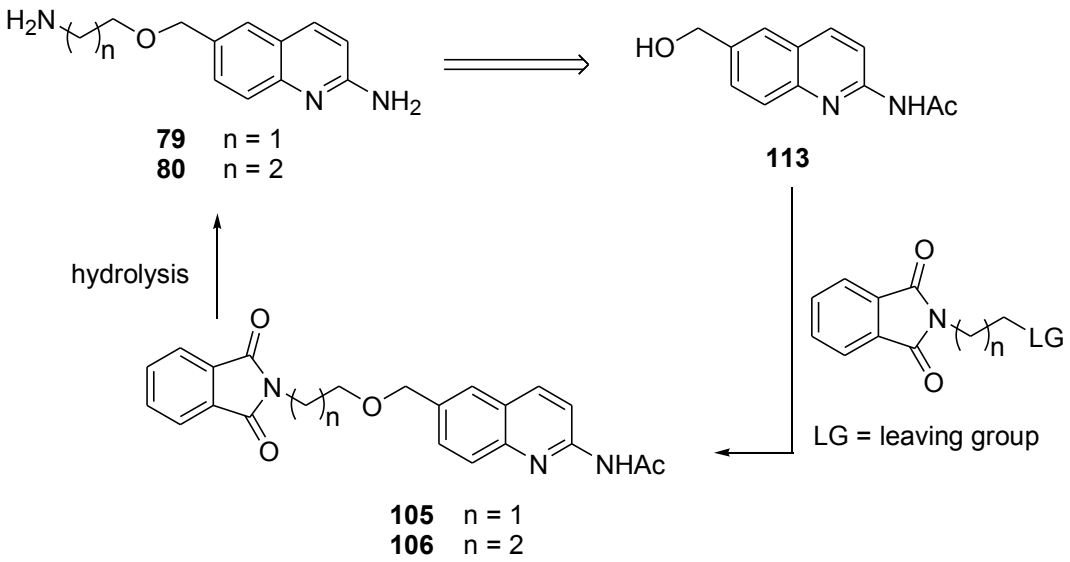
Features of the ^{1}H NMR spectrum that provided evidence for the formation of **113** included a loss of the signal observed at δ 2.15 ppm for the methyl protons for the acetate motif ($C\underline{H}_3CO_2$) in **112** accompanied by an upfield change in chemical shift for the benzylic protons from δ 5.26 ppm (a singlet, $ArC\underline{H}_2OAc$) in **112** to 4.79 ppm (a doublet, $ArC\underline{H}_2OH$) in **113**. In addition, a triplet was observed at δ 4.40 ppm suggestive of the OH proton that was coupled to the benzylic protons. Only one band at 1674 cm^{-1} characteristic of a carbonyl group was observed in the infrared spectrum, unlike the starting material **112** in which two carbonyl bands were observed. The identity of **113** was confirmed by mass spectrometry.

Features of the ^{1}H NMR spectrum of **59** that provided evidence for its structure included the loss of both of the methyl group signals from the acetyl motifs that were found at δ 2.15 and 2.23 ppm in **59**, and this was accompanied by a change in chemical shift for the benzylic protons from 5.26 ppm ($ArC\underline{H}_2OAc$) in **112** to 4.55 ppm ($ArC\underline{H}_2OH$) in **59**. Again, the signal for the H3 proton of the quinoline in **59** had returned to δ 6.77 ppm, the characteristic shift for all the 2-aminoquinolines presented in this report. The identity of **59** was confirmed by mass spectrometry.

Investigation into use of 113 as an alternative 'key intermediate' in the convergent synthetic strategy: another attempt at the synthesis of diamine 79

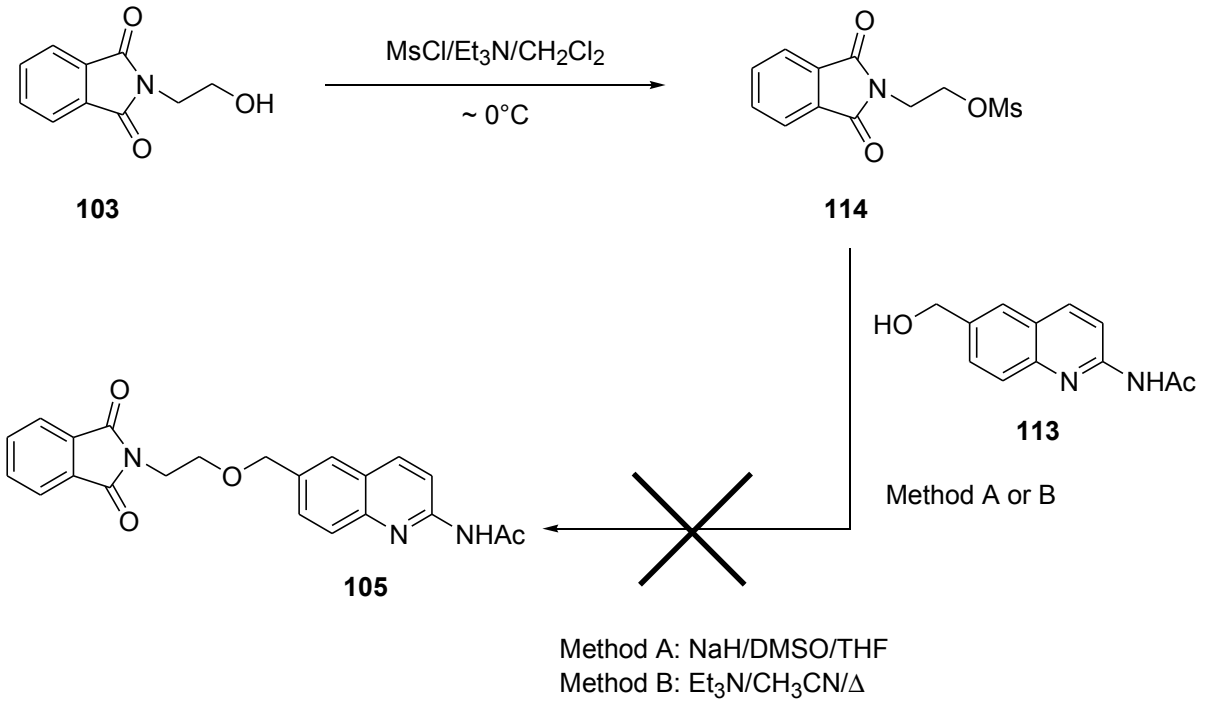
As discussed above, it was proposed that the 6-hydroxymethyl-2-acetamidoquinoline derivative **113** could be used in a modified 'convergent' synthetic strategy, as illustrated in Figure 4-13. The difference between this approach and that presented in Figure 4-12B is that the 'key intermediate' in the present context plays the role of nucleophile whereas previously, the 'key intermediate' was the electrophilic species. The utility of a convergent synthetic

strategy has been demonstrated for the synthesis of 2-aminoquinolines **59** and **60** as described above, but the approach so far has proven ineffective for the synthesis of long term targets, diamines **79** and **80**. Thus, the preparation of **79** was once again investigated, using this modified approach as illustrated in Scheme 4-39.



Scheme 4-39: Planned method for synthesis of di-amines **79** and **80** using modified convergent synthetic strategy, in 2 steps from **113**.

In this modified strategy, by reversing the role of the 'key intermediate', there must also be a subsequent role reversal for the 'new functionality' component of the system. Thus in the current context, the electrophilic component now needs to be the compound with 'new functionality'. Hence, for the synthesis of **79** by this approach, the alcohol **103** needed to be replaced by a derivative that had a leaving group in place. This was achieved simply by treatment of alcohol **103** with methanesulfonyl chloride in the presence of triethylamine in dichloromethane as illustrated in Scheme 4-40. This reaction proceeded as expected and the desired product **114** was isolated after workup in 80% yield. Features of the ^{1}H NMR spectrum of **114** that provided evidence for its formation included a large downfield change in chemical shift for the methylene protons adjacent to the hydroxy group ($CH_2C\underline{H}_2OH$) from δ 3.90 ppm in **103** to δ 4.48 ppm in **114** ($CH_2C\underline{H}_2OMs$), and the appearance of a three proton singlet at δ 3.01 ppm, as expected for the methyl group protons of a sulfonate ester ($C\underline{H}_3SO_3$). These chemical shifts were consistent with those observed for the mesylate prepared in Section 4.5.2.1. The mesylate was used in subsequent experiments without additional purification.



Scheme 4-40: Investigation into synthesis of 'protected derivative' **105** from 'key intermediate' **113**, by conversion of alcohol **103** into mesylate **114**, followed by the attempted coupling of **113** with mesylate **114** by using two different approaches both under basic conditions.

The coupling of **113** with **114** was promptly tested using two different approaches. The first method (Scheme 4-40, Method A) involved formation of a solution of dimsyl sodium by gentle heating of sodium hydride in DMSO, before addition of **113** dissolved in THF. Later, mesylate **114** was added to the mixture also dissolved in THF. The reaction was stirred at room temperature for 2 hours prior to workup, and ^{1}H NMR analysis indicated that both of the starting materials **113** with **114** were intact after the attempted reaction. Thin layer chromatography had been used to try to monitor the reaction progress, and this had suggested that the mesylate **114** had been consumed, however, this was clearly not the case. This observation was in contrast to attempts to couple alcohols to **100** in Section 4.7.2.1 using strongly basic conditions, from which complex mixtures of several products were isolated, indicating undesired side reactions had occurred. In the current experiment, no reaction had taken place at all. The reasons for this are unclear, but it is possible that the alkoxide of **113** was not formed throughout the procedure, due to the reagents not being sufficiently dry. Alternatively, due to the very small scale of the experiment (10 mg of **113** requiring only ~ 1.5 mg of sodium hydride) the portion of the material weighed out of the sodium hydride container was inactive (ie. was in fact sodium hydroxide). This is potentially a problem when such small amounts of sodium hydride must be weighed out. However, this explanation is partly inconsistent with the results obtained from reactions using the same supply of reagents (dry DMSO, dry THF, and sodium hydride) as those used in Section 4.7.2.1 where the intra-molecular epoxidisation reaction was apparent (Scheme 4-33A and

B), given that this epoxidisation process relies on the generation of an alkoxide anion. It is also possible that the vessel required heating for the reaction to proceed. Whether this method will genuinely be effective or not awaits further investigation; experiments need to be tried on a larger scale so that more workable quantities of sodium hydride are used, and the solvents, particularly the DMSO used for the reaction, may need to be dried more thoroughly. In addition, alternative conditions need to be tested, for example using a single polar solvent well suited to S_N2 chemistry like DMF. The use of different bases could also be investigated.

An additional experiment for the same coupling was attempted using an alternative method (Scheme 4-40, Method B). In this case, the mesylate **114** and **113** were simply heated at reflux in acetonitrile in the presence of triethylamine, and in this case, no reaction could be observed after 5 hours as judged by thin layer chromatography. Given that triethylamine is a much weaker base than sodium hydride (and in fact the pK_a of triethylamine is such that the equilibrium of alkoxide formation of alcohol **113** using triethylamine as a base lies very much in favour of the neutral alcohol), for a substitution reaction to take place, it must do so via the neutral form of the alcohol, followed by removal of the proton by the base after the substitution has occurred. It was therefore predicted that this was unlikely to occur. Nevertheless, given that mesylates are very good leaving groups, the experiment was still attempted. However, clearly these conditions were too mild for the reaction to take place.

At this point, no additional experiments for developing a suitable coupling method were performed. Although the current approach can't yet be ruled out as an option, the experiments using strongly basic conditions for the attempted coupling of alcohols to **100** (in which a large amount side reactions had occurred) suggest that the current approach may also be ineffective. One conclusion that may be drawn is that an acetamide is not a robust enough protecting group for these reactions. The strongly basic conditions result in undesirable side reactions that might involve the acetamide, and the expected products don't form. Thus, the convergent synthetic strategy may require further modification. Specifically, different amino protecting groups should be investigated. It is possible that the 4-methoxylbenzyl amination strategy for synthesis of 2-aminoquinolines described in Section 4.6 may find additional use in the convergent synthetic strategy.

Thought experiment: coupling via trichloroacetimidate intermediates

An alternative approach that has since been considered for coupling reactions involving **113** that could be used for the synthesis of 'protected derivatives' **105** and **106** is the use of trichloroacetimidates as coupling partners instead of mesylates. The use of trichloroacetimidates is a well known approach for activation of alcohols towards nucleophilic

attack with partner alcohols to form ethers, particularly as a method for the protection of the hydroxyl group in carbohydrate chemistry.[100] Trichloroacetimidates in conjunction with a catalyst, typically trifluoromethanesulfonic acid, react with alcohols to form ethers in non polar solvents such as dichloromethane.[100] Methods involving even milder catalysts such as trimethylsilyl triflate have also been reported.[101] An appealing feature of this approach is that the conditions are relatively mild, and therefore tend to be well suited to systems with functional groups that are otherwise not stable to strongly acidic or basic conditions.

Recently, a method for the coupling of the trichloroacetimidate of *N*-hydroxymethylphthalimide to several alcohols including benzyl alcohol, using trimethylsilyl triflate catalysis was reported.[101] The trichloroacetimidate was easily prepared in high yield by treatment of the alcohol with trichloroacetonitrile in dichloromethane in the presence of a mild base. Therefore, it was envisaged that similar methods might be suited to the current system. Indeed, preliminary experiments performed by a co-worker have indicated that the trichloroacetimidate of alcohol **103** can be prepared easily. Hence, an outline for the use of trichloroacetimidate chemistry as a possible means for the synthesis of 'protected derivatives' **105** and **106** is provided in Figure 4-14. Due to time restraints however, the coupling step still awaits investigation. Given that a large amount of preliminary work has been done in establishing possible substrates for coupling reactions that may lead to the synthesis of new 2-aminoquinolines **79** and **80** and others, the use of the trichloroacetimidate approach warrants further investigation.

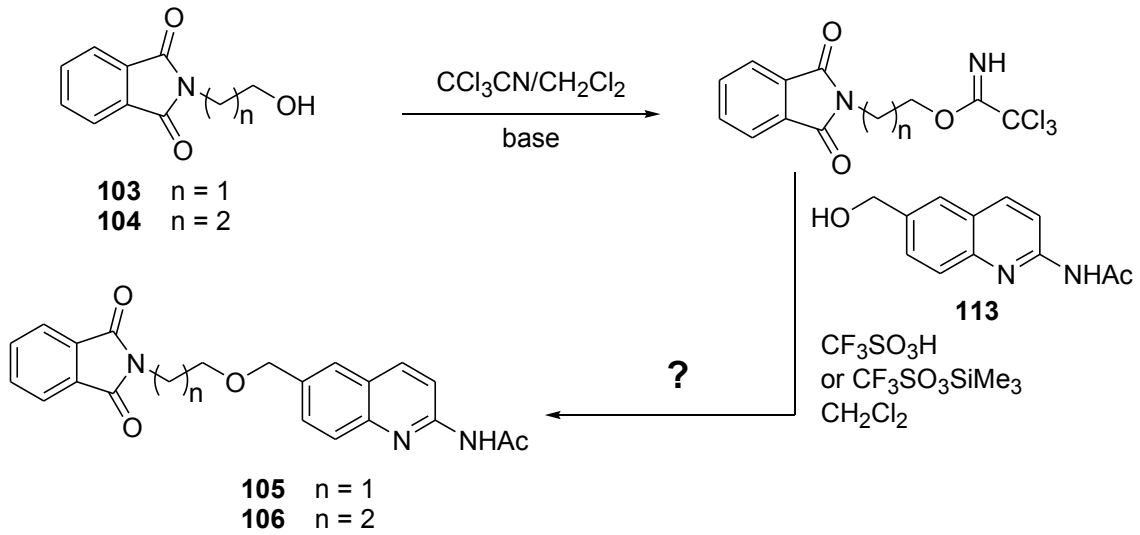


Figure 4-14: Thought experiment: proposed method for synthesis of protected derivatives **105** and **106** by coupling of **113** with trichloroacetimidate derivatives generated from alcohols **103** and **104**.

4.7.3.3 Substitution with original 'key intermediate' and phenoxide: A brief yet promising investigation

So far, the most useful results obtained from substitution experiments involving the originally planned 'key intermediate' **100** were those involving the weakly to non-basic nucleophiles phthalimide and acetate, and this led to a simple synthesis of 2-aminoquinolines **59** and **60**. However, until a method is identified that makes use of the alternative 'key intermediate' **113**, potentially leading to a convenient method for the synthesis of many new 2-aminoquinolines, the successful substitution reactions using **100** are of limited value. Furthermore, the investigations presented in Sections 4.5-7 have been quite thorough and focussed, with the aim of developing convenient methods for the synthesis of a relatively small set of ligands, and they have not yielded a large return, considering the amount of time invested.

Currently, the highest affinity Tec SH3 domain ligand that has been identified is the acetal derivative **70** that has a K_d of 22 μM (Section 4.4). Structure activity information also suggests that the main feature of this compound that contributes to the improved affinity is the large, bulky hydrophobic motif attached to the quinoline ring. However, a problem associated with **70** as a candidate for drug development is the aqueous instability of the acetal functionality (Section 4.4.4). Therefore, it was decided that some focus should be returned to developing new ligands that contain the desirable features of acetal **70** (ie bulky hydrophobic groups) but have the non-desirable characteristics (ie the aqueous instability) removed.

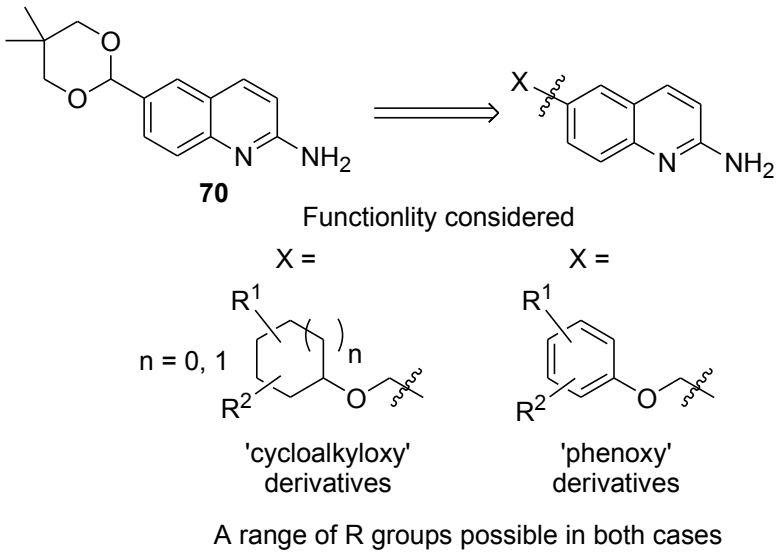
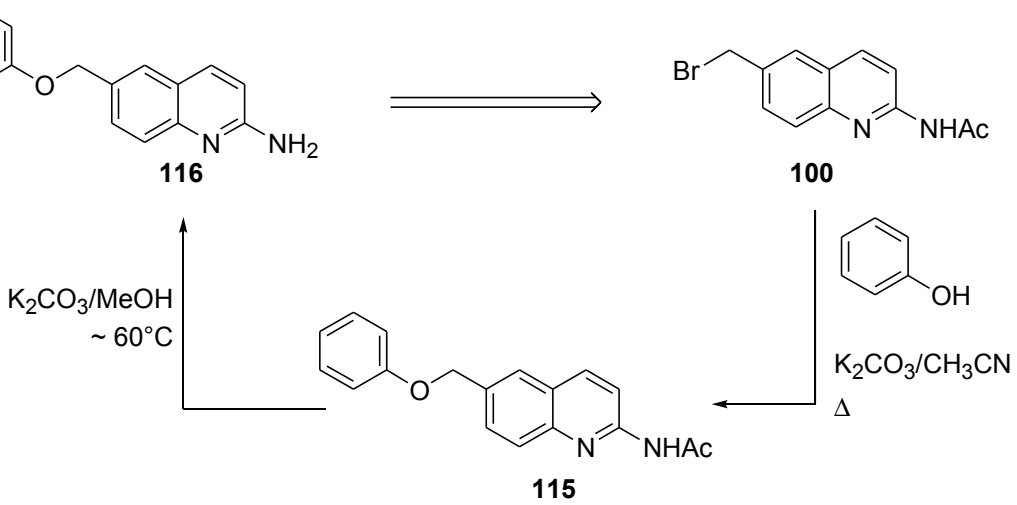


Figure 4-15: Thought experiment: some ideas considered for development of 2-aminoquinolines that may bind to the Tec SH3 domain with similar affinity to highest affinity ligand **70**, but have more drug like characteristics.

When the original convergent synthetic strategy was first planned using **100** as the 'key intermediate' it was anticipated that substitution reactions could also be performed using nucleophiles such as cyclohexanol or cyclopentanol derivatives that would lead to ligands with 'cycloalkyloxy' groups that could mimic the shape of the acetal motif in **70** and be more 'drug-like' as illustrated in Figure 4-15. However, given the difficulty encountered with trying to do substitution reactions using aliphatic alcohols as nucleophiles, synthesis of such derivatives was never tested.

However, based on the fact that the best nucleophiles for substitution reactions with **100** have been shown to be weakly to non-basic species, it was subsequently predicted that phenoxides, generated from phenols could also serve as nucleophiles in substitution reactions with **100**. The phenolic proton is substantially more acidic (and the phenoxide anion is therefore much less basic) than the proton of the aliphatic alcohol, due to the resonance stabilisation of the phenoxide, provided by the benzene ring. Indeed phenoxides can be generated with weak bases such as potassium carbonate. Therefore it was envisaged that 'phenoxy' derivatives of 2-aminoquinolines could serve as simple derivatives that are easy to synthesise, and that could mimic the acetal motif as illustrated in Figure 4-15. Although the shape of the 'phenoxy' motif will differ to that of the dioxane motif of **70** due to the planar benzene ring (compared to the chair conformation of the dioxanes), the potential ease of synthesis of such compounds warrants investigation into their binding activity.



Scheme 4-41: Investigation into coupling of phenol to **100** under mild conditions to give amide **115**, and subsequent hydrolysis to afford the 6-phenoxymethyl-2-aminoquinoline derivative **116**.

Hence, the coupling of phenol to **100** was tested, by simply treating **100** with phenol in the presence of an excess of potassium carbonate, and heating at reflux for 5 hours in acetonitrile, as illustrated in Scheme 4-41. Following workup, ^{1}H NMR analysis of the

material isolated suggested that the reaction had proceeded, and that **115** had formed as expected, as evidenced by a large downfield change in chemical shift for the benzylic protons from δ 4.65 ppm (ArC$\underline{H}_2$Br) in **100** to δ 5.26 ppm (ArC$\underline{H}_2$OPh) in **115**. In addition, a characteristic set of signals at δ 6.93, 7.04-7.05, and 7.27-7.30 was observed for the protons on the phenyl ring of the phenoxy motif, assigned to H4' (1H), H2'/H6' (2H), and H3'/H5' (2H) respectively based on a resonance argument. This material was purified by silica gel chromatography, leading to isolation of analytically pure **115** in 70% yield. The identity of **115** was also confirmed by mass spectrometry.

In Section 4.7.3.2, during the attempted selective de-acetylation of di-acetyl derivative **112**, some de-acetylation of the acetamide also occurred when the reaction was performed at room temperature, when 0.5 equivalents of potassium carbonate were used. Given this observation, it was envisaged that the hydrolysis of 2-acetamidoquinolines to form 2-aminoquinolines should proceed quickly and conveniently using a larger amount of potassium carbonate, and at higher temperature. Thus, for the conversion of 2-acetamidoquinoline derivative **115** to the corresponding 2-aminoquinoline **116**, amide **115** was treated with a slight excess of potassium carbonate in methanol at ca. 60°C for 1 hour (Scheme 4-41), at which point thin layer chromatography indicated that all of the starting material had been consumed, and a product with an R_f characteristic of a 2-aminoquinoline was present. After cooling, a precipitate formed in the reaction vessel, and this was collected by filtration. Additional material was also isolated following a standard workup. ^{1}H NMR analysis indicated that the precipitate was the expected product **116** in a highly pure form: the same diagnostic features were observed for this de-acetylation reaction compared to others presented in this report. ^{1}H NMR spectroscopy confirmed that the additional material isolated was extra product: hence the overall yield for the reaction was 84%. The identity of **116** was confirmed by elemental analysis and mass spectrometry.

Overall, the results of the current investigation were very encouraging: a simple method for the coupling of phenols to 'key intermediate' **100** was identified that gave good yields, and the conditions were sufficiently mild to not cause side reactions as observed in previous investigations involving strongly basic conditions. Furthermore, the approach for the hydrolysis of the acetamide also proved to be an efficient, high yielding, mild method, that did not require the use of strong bases such as sodium hydroxide, typically used for hydrolysis of an amide. Hence this method may well find additional use for future syntheses by this approach.

4.7.4 Summary

Good progress has been made towards the development of a convergent synthetic strategy in this section. Simple and effective procedures have been outlined for the synthesis of compound **100**, proposed as a 'key intermediate' in the strategy. The utility of this key intermediate was subsequently tested in a range of substitution reactions. This led to a simple method for the synthesis of 2-aminoquinolines **59** and **60** following substitution with weakly to non-basic nucleophiles. However, substitution reactions under strongly basic conditions that were intended to lead to the synthesis of 2-aminoquinolines **79** and **80** were unsuccessful. A modified 'key intermediate' **113** was also prepared from **100**, and tested for its utility in a convergent synthetic strategy. However the level of testing of **113** has not been thorough enough at this stage, to draw solid conclusions about its usefulness. It is possible that the *N*-acetyl motif is not a suitable protecting group for the types of reactions required to prepare diamines **79** and **80**, and that other protecting groups for the amino group may need investigating.

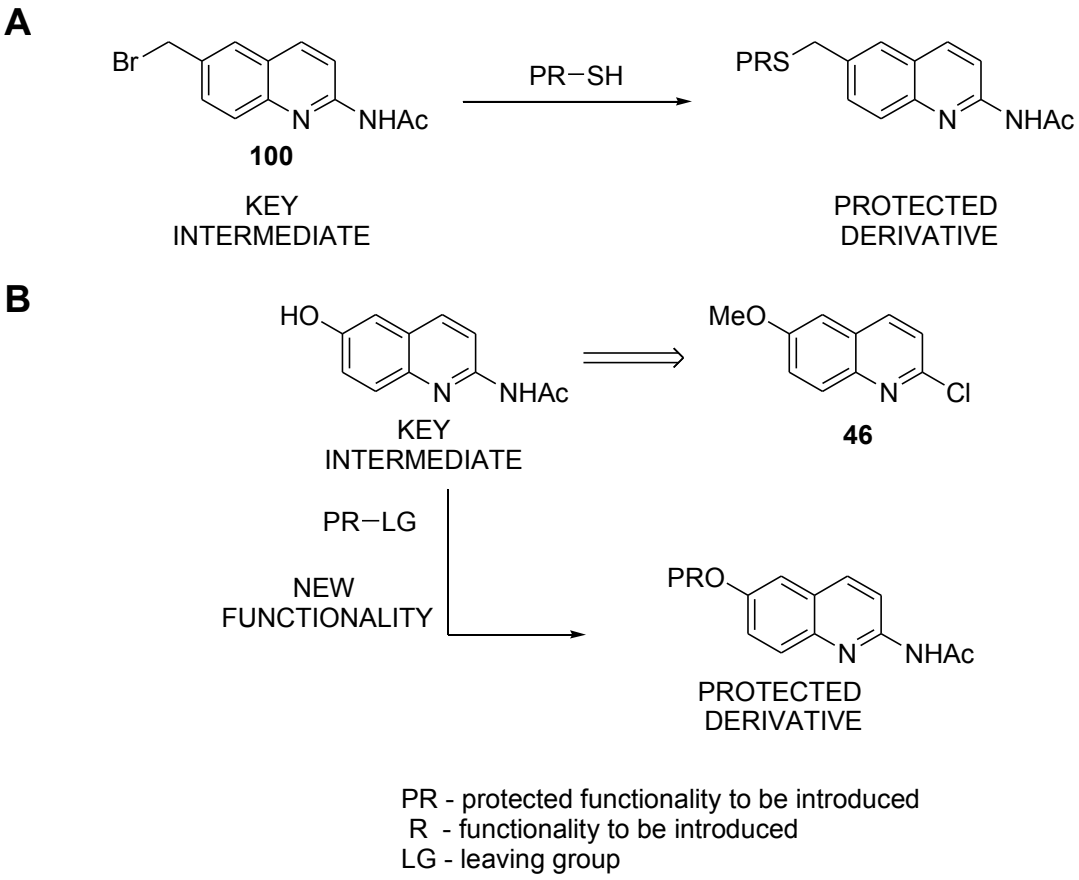


Figure 4-16: Thought experiment: modified approaches for the convergent synthetic strategy, involving alternative 'linkages' that could be investigated as part of future work. (A) Use of initially planned 'key intermediate' and coupling to thiols instead of alcohols previously tested. (B) Use of a hydroxy-2-aminoquinoline derivative and linkage to new functionality via the hydroxyl group.

However, further alternative strategies have been considered that may result in the preparation of compounds similar to **79** and **80**, but with slightly modified frameworks. For example, the use of aliphatic thiols as nucleophiles in substitution reactions with **100** instead of alcohols, resulting in the synthesis of thio-ethers instead of ethers as illustrated in Figure 4-16A, is one approach that could be investigated. Thiolates generated from thiols have advantages such as enhanced nucleophilicity and reduced basicity over alkoxides, and thus substitution reactions involving thiols with **100** may proceed under milder conditions. Also, in Section 4.4.1 it was shown that oxygen directly attached to the quinoline ring in the 6-position is tolerated for ligand binding (ligand **39**). Thus, linkages to additional functionality via this phenolic oxygen (Figure 4-16B) could also be investigated, and should similarly allow coupling reactions to proceed under mild conditions.

The utility of **100** in a convergent synthetic strategy for the synthesis of 'phenoxymethyl' derivatives of 2-aminoquinoline has also been demonstrated; substitution reactions with phenols under weakly basic conditions were possible, and high yielding. As will be presented in the next Section (4.8) the ligand binding studies for 2-aminoquinoline **116** indicated that **116** binds to the Tec SH3 domain with affinity that is comparable to the best ligand, **70**. Therefore, the synthesis of 'phenoxy' derivatives certainly warrants additional investigation, especially given that such a large range of phenols are commercially available. The addition of extra functionality (at one or more position, as illustrated in Figure 4-15) on the phenyl ring could result in extra contacts with the protein surface, leading to further improvements in affinity. Furthermore, coupling of phenol derivatives that have hydrophilic groups (eg aliphatic amines or alcohols) in place should also be feasible using the mild conditions established here without the need for protecting groups, considering that when a weak base was used for the attempted coupling of alcohol **113** to mesylate **114**, no reaction was observed. Therefore, it would seem that this approach may have endless possibilities, and will surely lead to the synthesis of a variety of ligands quickly that can be used to explore the protein surface at the ligand binding site.

In conclusion, the investigations presented in this section clearly demonstrate that convergent synthetic strategies will be greatly beneficial for the synthesis of a range of 2-aminoquinolines. With a little more exploratory work, it is likely that several suitable 'key intermediates' could be established, each tailored for the incorporation of different types of functionality onto the 2-aminoquinoline scaffold.

4.8 Tec SH3 domain/6-substituted-2-aminoquinolines binding studies 2

The work presented in the previous sections of this chapter resulted in the preparation of seven new 2-aminoquinolines (**59**, **60**, **76**, **80**, **81**, **87**, and **116**) to be tested for binding to the Tec SH3 domain. As mentioned in Section 4.5.1.1, the crude sample of **76**, isolated after it was first synthesised, was tested by the NMR chemical shift perturbation method. However, after establishing an improved method for the synthesis of **76**, and the related compound **81**, both of these compounds were also tested for binding using the Fluorescence Polarisation (FP) peptide displacement assay. In addition, compounds, **59**, **60** and **87** were selected for testing using FP. All of these compounds (**59**, **60**, **76**, **81** and **87**) were predicted to be sufficiently water soluble to be suited to the FP method, or in the case of **87**, there was already evidence for its solubility in water (as discussed in Section 4.5.2.2). Furthermore, compounds **59**, **60**, **76**, and **81** were predicted to have enhanced water solubility over several other ligands tested using the FP method (see Table 4-2 in Section 4.4.1) due to the presence of hydrophilic groups (OH or NH_2) on their structures. In the case of **80**, only a very small amount of this material was available (as discussed in Section 4.5.2.2). In order to promptly determine whether **80** bound the SH3 domain in slow exchange on the NMR timescale (for the purpose of deciding whether it was suited to structure determination when bound to the SH3 domain by NMR methods), which was considered a priority at the time, the NMR method was selected for the testing of this compound. 2-Aminoquinoline **116** that contains the large hydrophobic phenyl group was predicted to be poorly soluble in buffer alone, and therefore the NMR chemical shift perturbation method, that uses a 10% DMSO/buffer system was selected for the testing of this compound.

In addition, it was also decided to test at least one of the 2-(4-methoxybenzylamino)-quinolines (**93** and **94**) that were intermediates in the synthesis of 2-aminoquinolines **76** and **81** (Section 4.6.2). Whilst there was already evidence for substitution on the amino group with benzyl substituents leading to a small reduction in affinity (Chapter 3), there was also evidence for the phenyl group of the benzyl substituent making a contact with the SH3 domain. Given that studies presented in Sections 4.4.1 and 4.4.2 indicated that substitution at the 6-position of the quinoline ring with large hydrophobic groups also resulted in formation of a new contact with the SH3 domain, it was of interest to investigate the influence of 2-aminoquinolines with substituents at both the 6-position and the amino group, and whether multiple contacts with different parts of the protein surface were possible. This potentially could lead to a ligand with much improved affinity. Therefore, 2-(4-methoxybenzyl-amino)quinoline derivative **93** was selected for ligand binding studies, and the NMR chemical

shift perturbation method was chosen for this compound, as it was anticipated that this compound would have low solubility in buffer alone.

The results from these ligand binding studies (FP and NMR), and a subsequent discussion of the SAR information they provide is the focus of this next section.

4.8.1 Fluorescence Polarisation peptide competition assays

4.8.1.1 Testing of compounds 59, 60, 76, and 81

As discussed above, 2-aminoquinolines **59**, **60**, **76**, **81** and **87** were all selected for testing for binding to the Tec SH3 domain using the FP peptide displacement assay. Of these, **76** and **81** both competed with the proline rich peptide **PRP-1** for binding to the Tec SH3 domain with

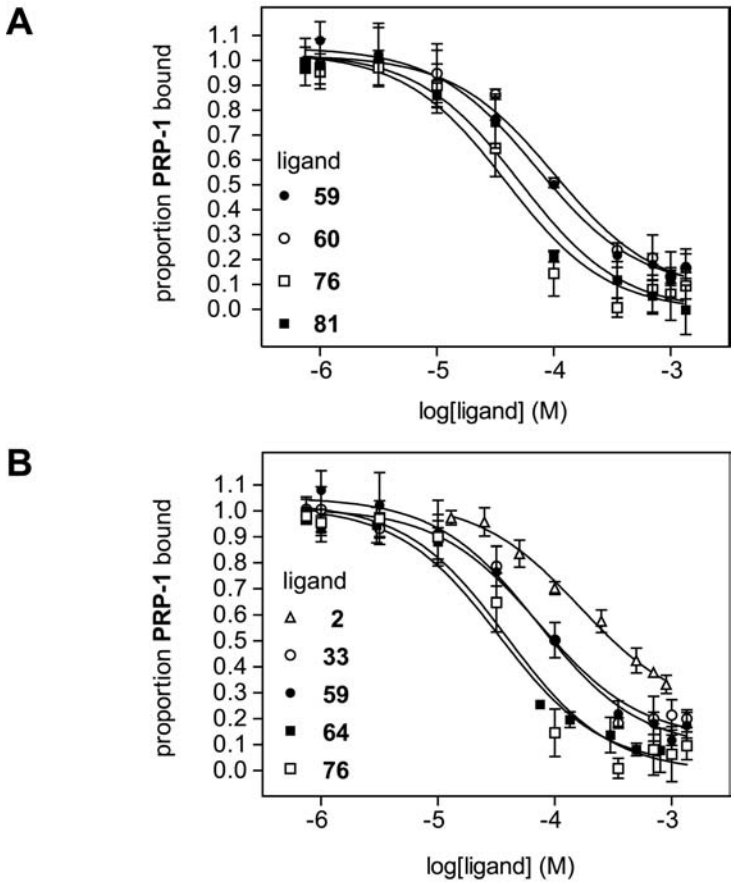
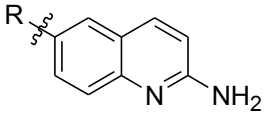


Figure 4-17: Binding Studies of 6-substituted-2-aminoquinolines with Tec SH3 domain using Fluorescence Polarisation (FP) Peptide Displacement Assays. (A) Overlays of isotherms obtained from independent experiments for competition of fluorescent proline-rich peptide **PRP-1** by ligands **59**, **60**, **76** and **81** from Tec GST-SH3 protein. (B) Comparison of isotherms for experiments with ligands **59** and **76** from (A) and isotherms obtained from independent experiments for ligands **2**, **33** and **64** in previous sections.

approximately three to four-fold improved affinity relative to 2-aminoquinoline **2** (EC_{50}s ca. 40-50 μM for **76** and **81**, EC_{50} = 160 μM for **2**) (Figure 4-17A, Table 4-4). 2-Aminoquinolines **59** and **60** also competed for binding to the SH3 domain with an improvement in affinity relative to **2**, however the extent of this improvement was smaller than in the case of **76** and **81** (EC_{50}s 71 and 102 μM for **59** and **60** respectively, EC_{50} = 160 μM for **2**) (Figure 4-17A, Table 4-4).

Table 4-4: Ligand Binding Studies of 2-aminoquinolines **59**, **60**, **76**, **81** and **87** with the Tec SH3 Domain, and comparison with results for ligands **2**, **33** and **64** from previous studies, as determined by Fluorescence Polarisation (FP) peptide competition studies.



Ligand	R	EC_{50} (mM)†	Ligand	R	EC_{50} (mM)†
76	$HO(CH_2)_2OCH_2$	40 ± 8	**64**		34 ± 5
81	$HO(CH_2)_3OCH_2$	51 ± 4	**33**	Me	75 ± 15
59	$HOCH_2$	71 ± 9	**2**	H	160 ± 36
60	H_2NCH_2	102 ± 17	**87*♠**	$AcHN(CH_2)_3OCH_2$	88 ± 13

† Quoted values are mean ± standard deviation over 3 replicate experiments. * The discussion of this ligand is in Section 4.8.1.2 ♠ This experiment was performed by Cvetan Stojkoski.

4.8.1.2 Testing of compound 87: a description of important considerations in non-linear regression analysis

The FP assay for the testing of compound **87** was carried out by Cvetan Stojkoski. The results from these experiments are discussed separately in this section, because the data was analysed in a slightly different manner to that typically used for the testing of other compounds using the FP method. Before the ligand binding data for **87** can be presented however, some discussion of the EC_{50} determination process is required.

Determining EC_{50} values: Planning experiments, and data-analysis

In order to obtain an EC_{50} value for a ligand as presented here, about ten different ligand concentrations are selected in the range ~ 0.5 to 1300 μM. Ligand concentrations must be selected such that a sufficiently well defined binding isotherm can be obtained, and the key

requirement for this is that an adequate number of data points are present at or near the beginning and end points of the series. A well defined isotherm should ideally have a 'sigmoidal shape', with an inflection point at the halfway point of the sigmoidal curve. The ligand concentration corresponding to this inflection point is theoretically the EC_{50} value for the ligand.

For example, consider the isotherms presented in Figure 4-17B in Section 4.8.1.1. In the case of the lower isotherms for the two highest affinity ligands **64** and **76**, the curve begins to flatten out around the last few data points. This stage corresponds to approximately 0.1 (or 10%) of proportion of **PRP-1** bound (Y-axis), and may be considered as being at or near saturation binding of the ligand to the SH3 domain. This is a well defined isotherm. In the case of the intermediate affinity ligands presented in Figure 4-17B (**33** and **59**), a well defined isotherm is again observed, however in this case, the curve flattens out at with a higher (~0.2) corresponding proportion **PRP-1** bound value. In order to reach the same level observed for the lower isotherms, higher ligand concentrations would be required. In the case of the lowest affinity ligand **2** presented in Figure 4-17B, a substantially lower degree of 'saturation' binding is observed, based on the observation that the curve has barely begun to 'flatten' out. Ideally, the isotherm for **2** could be better defined, with additional data points added at the beginning and end points of the series. This becomes more apparent when comparing the isotherm for **2** with isotherms obtained for other ligands that are better defined.

When calculating EC_{50} values using the non-linear regression curve-fit software Prism[55] the quality of the fit, and subsequently the accuracy of the binding constants should be assessed by inspection of some key parameters in the output table. A sample of some of the key parameters is given in Table 4-5 below. When examining the quality of the data produced, it is important to check the values given for 'BOTTOM' and 'TOP', as illustrated in Table 4.5. When Prism fits curves to data with its default settings, it attempts to judge what the BOTTOM and TOP values should be, based on the data provided in the data set. TOP refers to the fitted value corresponding to the expected Y-coordinate at the beginning of the isotherm. In the context of the present examples, 'TOP' should have a value of approximately one. Similarly, BOTTOM refers to the fitted value corresponding to expected Y-coordinate at the end of the isotherm. In the present examples, bottom should be approximately zero. However, it is reasonable to assume that in practice, BOTTOM values of zero will not always be obtained. In the context of a peptide competition assay with a small molecule, there will always be a small amount of peptide binding, resulting in some detectable background signal. Thus, in practice, a BOTTOM value based at ca. 0.1 proportion **PRP-1** bound (10%)

therefore seems reasonable. So, as illustrated in Table 4-5, which includes data for ligands **2**, **59** and **64**, the fitted TOP and BOTTOM values all fit quite well with the expected values. Another feature of the Prism output tables that should be inspected is the regression 'goodness of fit coefficient', R^2. Generally, when the R^2 value is ≥ 0.95, the goodness of fit is classed as satisfactory, but ideally the R^2 value should be as close to 1 as possible. If $R^2 \leq 0.9$, then there is likely to be some uncertainty associated with the calculated EC_{50} values. The combination of the R^2 values, and the realistic TOP and BOTTOM values as presented in Table 4-5 provides good grounds to accept the given EC_{50} values are meaningful.

Table 4-5: Sample Prism output, highlighting some key parameters that should be inspected following EC_{50} determinations

	64	**59**	**2**
Best Fit Values			
BOTTOM	0.01339	0.08344	0.2180
TOP	1.019	1.053	1.041
log[EC_{50}]	-4.471	-4.144	-3.784
EC_{50}	3.3780e-005	7.1750e-005	0.0001645
Goodness of Fit			
R^2	0.9838	0.9946	0.9941

EC_{50} determination for 87: an example of a poor fit, and how to resolve the problem

Now, consider the isotherm for the EC_{50} determination for ligand **87** illustrated below in Figure 4-18. In this case, the range of concentrations that were selected for the binding experiments did not produce an isotherm that is adequately defined, mainly at the end points of the series. At least two or three additional data points corresponding to concentrations closer to 1000 μM would be required to produce an isotherm that begins to flatten out like those in Figure 4-17. In Figure 4-18, the isotherm in black corresponds to the fit produced by Prism with the default settings; note that this curve has no inflection point. Thus, when Prism attempted to fit the curve in this case, the BOTTOM value that it calculated (based on the fitted curve shape) was –1.041, and the corresponding EC_{50} value was approximately 500 μM (Table 4.6). Whilst the given curve shape is certainly indicative of a lower affinity ligand, compared to those presented in Section 4.8.1.1 for example, the given BOTTOM value of ca. –1 indicates that it has not fitted the curve correctly, because as discussed above, a BOTTOM value should be in theory ~ zero. However, Prism also caters for this situation. If the BOTTOM value that it fits is inappropriate, then the value can be altered as desired by the user. Hence, a bottom value of 0.15 was selected, and the non-linear regression was performed again, and the consequences of this can be seen in Figure 4-18 (the red

isotherm), and Table 4-6. As illustrated in Figure 4-18, the red isotherm has a considerably different shape compared to that obtained previously (black), and appears to commence 'flattening' out towards the end, more like the isotherms in Figure 4-17. Also, as presented in Table 4-6, the EC_{50} value is substantially altered as a consequence of this manipulation (~90 μM vs 500 μM), and as can be seen, the goodness of fit is now reduced as reflected in the R^2 value (0.9472 vs 0.9976).

The manipulation described above proved to be useful in this situation because it was able to provide an approximation of the EC_{50} value for **87** as 88 ± 13 μM (Table 4-4) using data obtained from a poorly designed experiment. When this experiment was performed, it was (inappropriately) assumed that the ligand would be higher affinity than any others that had been tested so far, (due to the presence of the amide functionality and its potential for involvement in hydrogen bonding) and thus lower concentrations of ligand would be adequate to obtain a sufficiently defined binding isotherm. Thus a maximum ligand concentration of 316 μM (log[**87**] = 3.5) was used. The ligand clearly did not bind with the anticipated affinity, and thus the results obtained required re-evaluation. Whilst the adjustment made to the BOTTOM value was able to improve the data such that an approximation of the EC_{50} could be calculated, a better quality EC_{50} value will only be obtained by repetition of the experiment, and the inclusion of additional data points. Due to lack of supply of **87**, this has not been done.

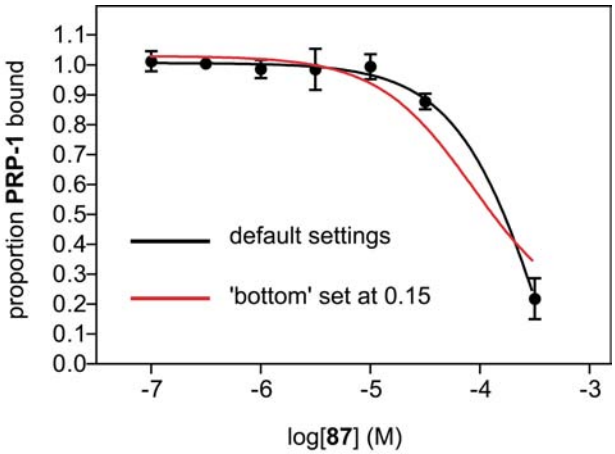


Figure 4-18: EC_{50} isotherms for FP peptide competition assay of compound **87**. The isotherm in black was that obtained using the default Prism settings, whilst that in red was obtained when the BOTTOM value was held constant at proportion **PRP-1** bound of 0.15.

Table 4-6: Sample Prism output following non-linear regression analysis for FP peptide competition assay with compound **87**. Descriptions of the two columns are provided in the text. This analysis was based on a single data set, generated from the average of three replicate sets.

	Default Settings	BOTTOM set constant
Best Fit Values		
BOTTOM	-1.041	0.15
TOP	1.006	1.029
log[EC_{50}]	-3.297	-4.062
EC_{50}	0.0005052	8.67E-05
Goodness of Fit		
R^2	0.9976	0.9472

4.8.2 NMR chemical shift perturbation assays

4.8.2.1 Testing of compounds 76, 80, 93, and 116

As discussed in Section 4.8, compounds **76**, **80**, **93** and **116** were selected for testing for SH3 domain binding using the NMR chemical shift perturbation method. In a similar fashion to the observations made for ligands **64**, **69** and **70** in Section 4.4.2.1, the [^{1}H,^{15}N] HSQC NMR spectra for the present ligand binding experiments indicated that the binding of all the ligands was again in intermediate exchange on the NMR timescale. The evidence for this was, again, either complete loss of, or substantial drops in the intensity of the H-N cross peaks for some of the residues involved in ligand binding, at some of the ligand concentrations (see for **93** in Figure 4-19). In the case of ligands **76**, **80** and **116**, all the signals returned to full intensity at or near ligand concentrations of 1 molar equivalent of the protein (data not shown). However, as illustrated in Figure 4-19, in the case of ligand **93**, for several of the residues involved in binding, the signals did not return to full intensity, even at ligand concentrations corresponding to near saturation binding (see for Y227 in Figure 4-19A). Furthermore, in the case of the tryptophan side-chain H-N (W215ε1) signal, complete loss of the signal was observed for all ligand concentrations (Figure 4-19B) (the implications of this particular example will be discussed later). However as illustrated in Figure 4-19C, in other cases, the signals did return to full intensity at the highest ligand concentration (S230), whilst for D196, the signal was characteristic of fast exchange for all ligand concentrations. Consistent with the method described in Section 4.4.2.1, to facilitate the data analysis, only amino-acid residues that exhibited entirely fast exchange characteristics were selected for use as monitors for calculation of equilibrium binding dissociation constants (K_d).

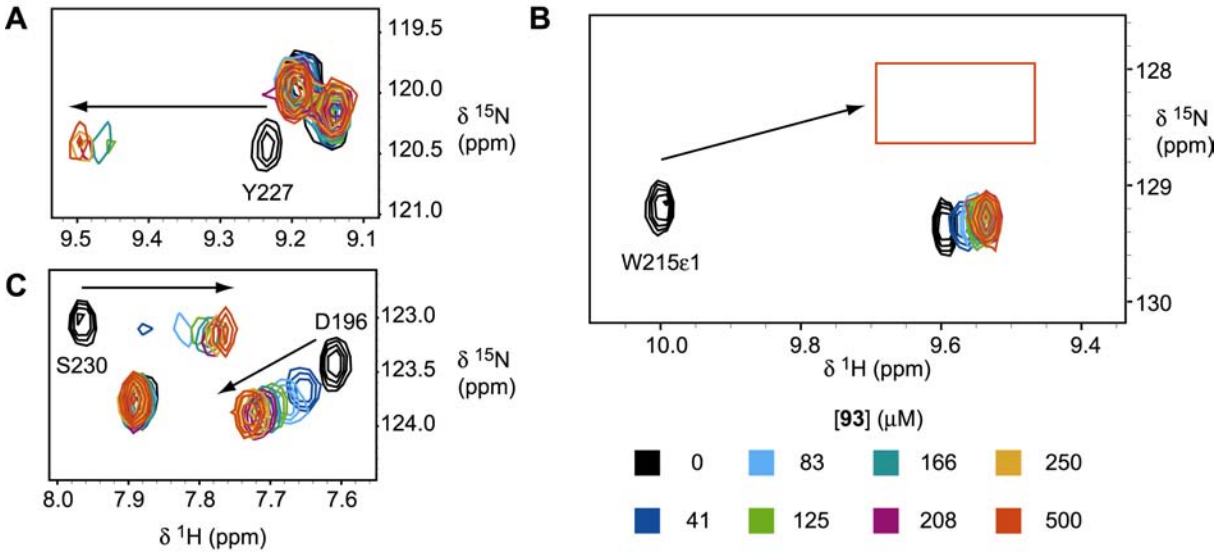


Figure 4-19: [^{1}H,^{15}N] HSQC spectra overlays for binding experiments involving intermediate exchange ligand **93**. (A) Region of spectrum demonstrating complete loss of signal for residue Y227 at low ligand concentration, followed by substantial loss of signal at higher ligand concentrations. (B) Region of spectrum demonstrating the complete loss of the signal for the W215ε1 side-chain (H-N) resonance for all ligand concentrations. The red box signifies the chemical shift region where the signal typically returns at higher ligand concentrations as observed with other intermediate exchange ligands (eg. compare with Figure 4-3A in Section 4.4.2, for ligand **70**). (C) Region of spectrum demonstrating substantial loss of signal at low ligand concentration followed by return of the signal to near full intensity at high concentration in the case of residue S230, while for D196 essentially fast exchange character is observed for all ligand concentrations.

2-Aminoquinoline derivative **116** bound the SH3 domain with an approximately three to four-fold improvement in affinity relative to **2** (K_ds 32 μM and 125 μM for **116** and **2** respectively) (Figure 4-20, Table 4-7), whilst **76** bound with slightly lower affinity (K_d 38 μM, Figure 4-20, Table 4-7). In the case of **80**, the affinity (K_d ca. 225 μM) was slightly lower than **2**, however, there is some doubt as to the accuracy of this result. Because only a very small amount of this ligand was available (~1 mg), there was some uncertainty about the amount weighed out for preparation of the stock solution. Hence there is uncertainty associated with actual concentration of the solution, and therefore the calculated K_d. Ligand **93** bound the SH3 domain with K_d of 81 μM (Table 4-7) an approximately one to two-fold improvement relative to **2**, but approximately two-fold reduced affinity relative to structurally related **76**.

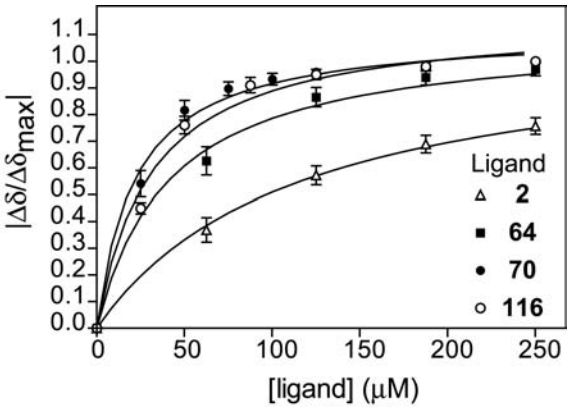
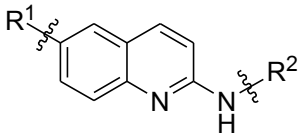
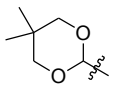
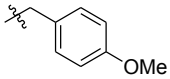


Figure 4-20: Binding studies of 6-substituted-2-aminoquinolines to the Tec SH3 domain using NMR Spectroscopy. Overlays of isotherms obtained from independent experiments, represented by the mean of the normalised chemical shift changes for amino acid residues that exhibited fast exchange processes, involved in binding of ligand **116** and for comparison **2**, **64** and **70**.

Table 4-7: Equilibrium binding dissociation constants (K_d) of 2-aminoquinolines **76**, **80**, **93**, and **116** as determined in the present NMR studies, and comparison of these with K_ds of selected ligands from previous studies.



Ligand	R^1	R^2	K_d (mM)*	Ligand	R^1	R^2	K_d (mM)*
116	$PhOCH_2$	H	32 ± 4	**70**		H	22 ± 5
76	$HO(CH_2)_2OCH_2$	H	38 ± 9	**64**		H	40 ± 8
80	$H_2N(CH_2)_3OCH_2$	H	225 ± 18	**2**	H	H	125 ± 24
93	$HO(CH_2)_3OCH_2$		81 ± 10	**24**	H	CH_2Ph	193 ± 15

* Quoted values are mean ± standard deviation over residues where ^{1}H (H-N) chemical shift changes of protein exceeded 0.1 ppm at or near saturation binding of ligand.

4.8.2.2 Chemical shift mapping of ligand binding events

Chemical shift mapping of all residues whose backbone ^{1}H (H-N) (or side-chains as indicated) chemical shifts were altered by at least 0.1 ppm at or near saturation binding, was performed for ligands **116** and **93** as illustrated in Figures 4-21 and 4-22 respectively. In the case of the **116** (Figure 4-21), the mapping was compared with that for ligand **70** from the studies in Section 4.4.2.3, due to the structural similarities between these compounds. This mapping indicates that mostly the same residues are involved for the binding of both of the ligands, especially on the 'left' side of the tryptophan residue (W215) as illustrated in Figure

4-21, although, on the 'right' side of W215, some additional features are worth noting. Specifically, additional residues have been influenced on the binding of **116**, as evidenced by a larger footprint of upfield chemical shift changes. This may be accounted for by the large anisotropic shielding effect that is provided by the π electrons of the phenyl group of ligand **116**, but such a dramatic upfield alteration of chemical shifts would not be expected for the alkyl-type cyclic structure of ligand **70**. In addition, the involvement of one of the H-N protons of the side-chain of asparagine 211 (N211) was also implicated, as evidenced by an upfield change in chemical shift, a change that had not been observed with any other ligands previously tested. This may also be a consequence of anisotropic effects from the phenyl ring.

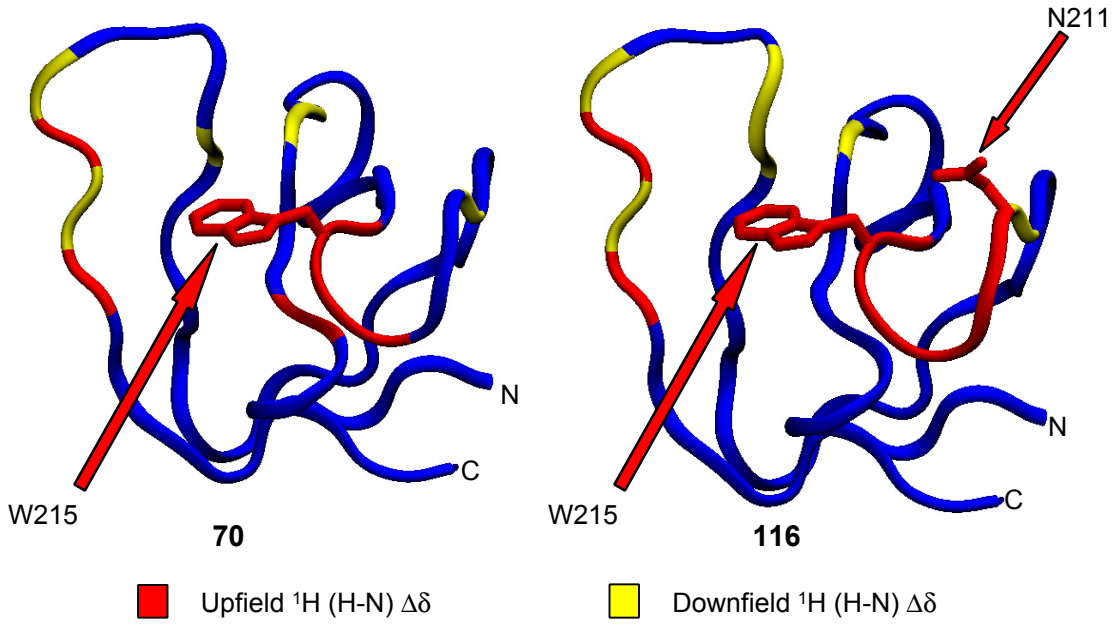


Figure 4-21: Chemical shift mapping of backbone or side-chain resonances where δ ^{1}H (H-N) was altered by at least 0.1 ppm at saturation binding of **70** (left) for comparison to **116** (right).

In the case of **93**, the mapping has been compared with that of the *N*-benzylated derivative of 2-aminoquinoline **24** with no substitution at the 6-position as illustrated in Figure 4-22. Ligand **24** was selected for comparison in this case so that the footprint provided by the benzyl group could be compared with that of the 4-methoxybenzyl group in **93**. Comparison of the regions to the 'left' side of W215 in Figure 4-22 indicates that largely the same residues were involved in binding of the two ligands. However, additional changes in chemical shift are observed on binding of **93**. It is possible that these additional changes are a consequence of the 4-methoxy group attached to the phenyl ring, and/or that the orientation of the rings differs in the two cases, resulting in more chemical shift changes being observed. An additional upfield change in chemical shift was observed for **93** on the 'right' side of the

W215 side-chain, consistent with extra interactions taking place in this region, from the substituent at the 6-position of the quinoline ring.

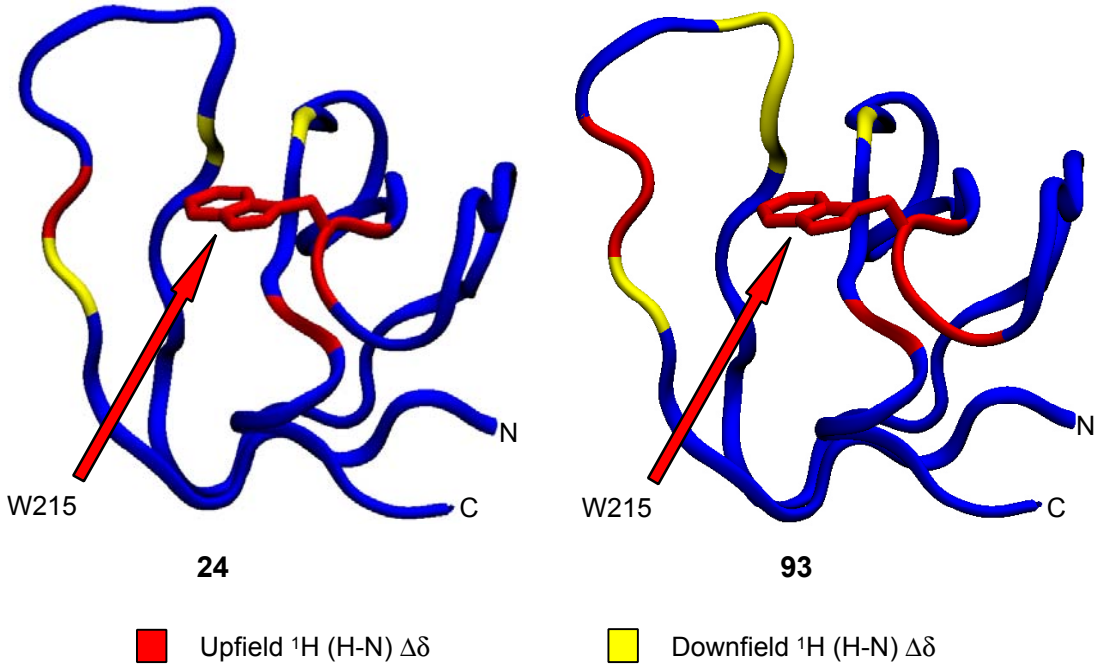


Figure 4-22: Chemical shift mapping of backbone or side-chain resonances where δ ^{1}H (H-N) was altered by at least 0.1 ppm at highest concentration of **24** (left) for comparison to **93** (right).

4.8.3 Interpretation of SAR information

The results obtained from both the FP peptide competition and the NMR chemical shift perturbation assays provide useful structure activity information. In the case of ligands **76** and **81**, the results suggest that whether there is two or three carbon atoms respectively in the side-chain of the substituent at the 6-position of the quinoline ring, has little influence on the binding affinity, as evidenced by the high similarity in the EC_{50} values determined and the characteristic binding isotherms (Figure 4-17A). These results also indicate that there is little difference in the affinities for ligands **76** and **81** and the 'parent' cyclic acetal compounds **64** and **69** (eg. EC_{50} 40 ± 8 μM and 35 ± 5 μM for **76** and **64** respectively: an overlay of the binding isotherms obtained for **76** and **64** is provided in Figure 4-17B to illustrate this point, and see Tables 4-2, 4-3, 4-4, and 4-7). However, these results also suggest that the hydroxyl group on the side-chain is not participating in an 'ideal' hydrogen bond with residues of the protein surface, as a substantially larger improvement in affinity would have been expected if this was the case.[41] On the other hand, given that there are several rotatable bonds on the alkyl substituents of ligands **76** and **81**, it may be argued a hydrogen bond has been formed between the hydroxyl group and a protein surface residue, but this is not reflected in the EC_{50} value, because of an entropic cost associated with formation of the hydrogen bond. Given

that there is little difference in affinity between ligands **76** and **81**, and the parent acetal compounds **64** and **69**, the use of the scaffold offered by the alkyl substituents of **76** and **81** as a basis for the development of new ligands, *perhaps* warrants further investigation. This is because, from the perspective of the higher aqueous stability of ligands **76** and **81**, it may be said that they have more drug-like characteristics than **64** and **69** (an issue that was addressed in Section 4.4.4). On the other hand, ligands **76** and **81** are not drug like from the perspective of the number of rotatable bonds on their structures, and thus by entropy considerations.

Based on the approximation for the EC_{50} value of **87** as 88 μM, as presented in Section 4.8.1.2, it can be concluded that this ligand binds with approximately two-fold improved affinity to **2** (EC_{50} **2** = 160 μM). But compared to ligands **76** and **81** which have similar structure to **87**, but altered functionality at the terminus of the 6-position substituent, (OH for **76** and **81**, NHAc for **87**) **87** binds with approximately two-fold reduced affinity (EC_{50} ca. 50 μM for **76** and **81**). However, due to the uncertainty associated with the EC_{50} value of **87** it is not possible to make solid interpretations about the structure activity information obtained here. It is possible that the NHAc motif of **87** may make less favourable interactions with the protein surface relative to the OH groups of **76** and **81** hence explaining the reduced affinity. It is also possible that the functionality at the terminus of the substituents in all cases has no influence on the binding affinity: a repetition of the ligand binding experiment for **87** would be required to confirm this. Based on the arguments presented above regarding the expected improvements in affinity associated with forming ideal hydrogen bonds, it can also be concluded that no ideal hydrogen bonds have been formed here.

Similarly, the result for **80** also needs careful consideration. As mentioned in Section 4.8.2.1, there was some uncertainty associated with the concentration of the d_6-DMSO stock solution of **80**, resulting in uncertainty in the accuracy of the K_d value, calculated to be ca. 225 μM (Table 4-7). This uncertainty appears justified when the K_d for **80** is compared to either the K_d for **76** (38 μM, Table 4-7), or the EC_{50} values of **76** and **81** (40 and 51 μM respectively, Table 4-4). Based on the observation that there was not a significant difference between the affinities of **76** and **81** as discussed above, it was concluded that the presence of the hydroxyl group did not influence the binding. In the present context, given that the only difference structurally between **81** and **80** is, the replacement of the hydroxyl group at the terminus of the alkyl-substituent of **81** with an amino group in **80**, a calculated K_d of 225 μM for **80** is not consistent with the previous conclusion. Hence, as was the case for the EC_{50} estimate of **87**, no solid SAR interpretations can be made from the K_d calculation for **80**. The

ligand binding experiment would require repetition in order to make better conclusions, but as no more material was available, this repetition has not been done.

In the case of ligands **59** and **60**, the results suggest that there is little influence on affinity from the nature of the substituent (OH vs NH_2) on the methyl group at the 6-position of the quinoline ring. For example, comparison of the data obtained here for **59** with that obtained for 2-amino-6-methylquinoline **33** in Section 4.4.1 suggests that there is no difference in affinity (EC_{50}s for **59** and **33** 71 ± 9 μM and 75 ± 15 μM respectively: overlays of the binding isotherms are provided in Figure 4-17B to illustrate this point, and see Tables 4-2 and 4-4). Given the similarity in the affinities between **59** and **33**, and using a similar argument to that discussed above for ligands **76** and **81** in terms of the expected improvement in affinity, these results also suggest that a hydrogen bond (or salt bridge in the case **60**) has not been formed between either of the ligands **59** and **60** and hydrophilic residues on the protein surface. Furthermore, the entropic considerations described above are less relevant in these cases because ligands **59** and **60** have fewer rotatable bonds. Although the calculated EC_{50} for **60** is somewhat different to that of **59** (102 ± 17 μM vs. 71 ± 9 μM, see Table 4-4), consideration of the standard deviations associated with the values, in conjunction with the high similarity in the binding isotherms (Figure 4-17A), suggests that the difference in the affinities for these two ligands is not significant.

In a broad context, perhaps the most exciting result obtained from the present studies was that the 6-phenoxymethyl-2-aminoquinoline derivative **116** bound the SH3 domain with similar affinity to the dioxane-acetal derivative **70**, the highest affinity SH3 domain ligand identified so far (K_ds 32 and 22 μM for **116** and **70** respectively) (Figure 4-20, Table 4-7). Inspection of the over-laid binding isotherms for **116**, **70** and the dioxolane-acetal **64** (Figure 4-20) indicates that there is not a significant difference in affinity between **116** and **70** as the isotherms are almost coincident. However there is a substantial 'right' shift between the isotherm for **64** and those for **116** and **70** suggesting that **64** binds with lower affinity, as is indicated by its K_d value (40 μM, Table 4-7). The main motivation for synthesising and testing ligand **116** was to investigate whether the phenoxy motif could mimic the acetal motif of ligands **64**, **69** and **70**, previously identified as the highest affinity ligands for the SH3 domain. The results from this experiment indicate that the desired outcome has been obtained. However, ligand **116** has a major advantage over ligands **64**, **69** and **70** in that **116** is substantially more 'drug-like' in character than **64**, **69** and **70**, that hydrolyse to form aldehyde derivative **58** under aqueous conditions (as discussed in Section 4.4.4). This result now paves the way to investigate the synthesis of a range of phenoxy derivatives, with the aim of seeking a further improvement in affinity.

Perhaps of equal interest was the result obtained for the binding experiments with ligand **93**. Comparison of the K_d values of **93** with those obtained for structurally related **76** and **24** and unsubstituted 2-aminoquinoline **2** (K_ds 81, 38, 193 and 125 μM for **93**, **76**, **24** and **2** respectively, Table 4-7), indicates that **93** binds with approximately two-fold reduced affinity relative to **76**, suggesting a reduction in affinity has resulted from substitution on the amino-group. However, an approximately two-fold improvement in affinity is obtained relative to **24**. This indicates that the affinity of **93** lies approximately in between that of **76** and **24**. This result is consistent with the conclusion drawn following the studies presented in Chapter 3, that optimal 2-aminoquinoline ligands should be primary amines. Despite this, it was predicted that a compound such as **93** would make contacts with the protein on both of the respective sides of the ligand binding site, as illustrated in Figure 4-23. But it was unclear whether using a compound such as **93** that is not a primary amine, yet has functionality that may make multiple contacts with the protein, was a sensible approach to identify a tightly binding ligand. These results suggest that this was not the preferable method for identifying a tightly binding ligand.

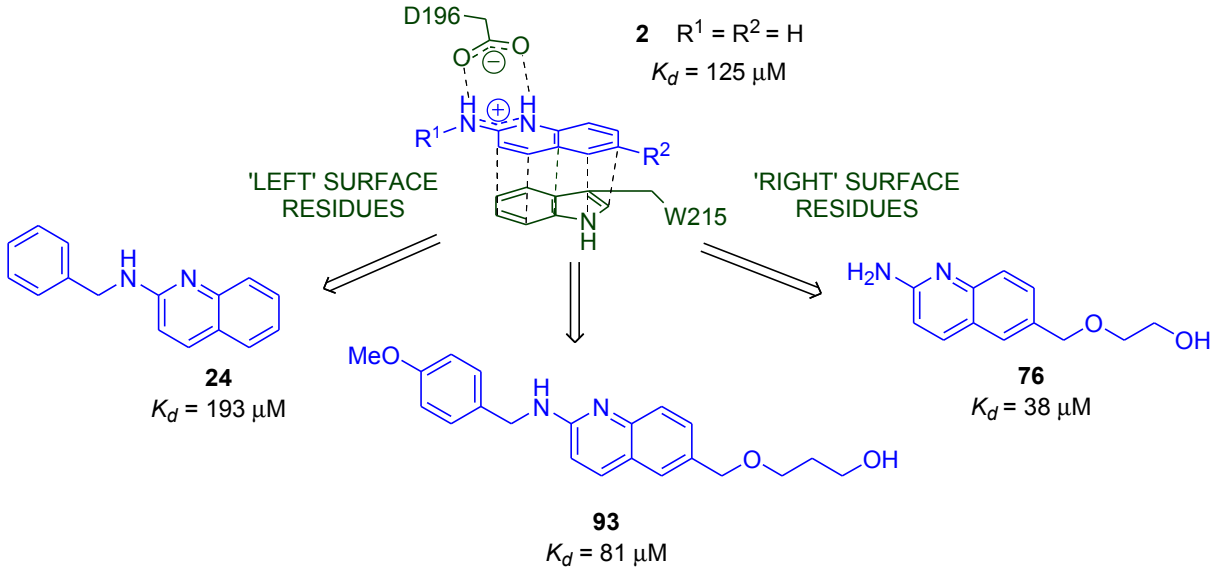


Figure 4-23: Conceptual illustration of Tec SH3/2-aminoquinoline **2** ligand binding model, with 'left' and 'right' regions highlighted, intended to make contacts with new functionality at appropriate positions on the 2-aminoquinoline platform, for example ligand **93**.

Despite the above, a discussion of the features of the [^{1}H,^{15}N] HSQC spectra (Figure 4-19) for the experiments involving ligand **93**, and their implications for interpretation of the SAR information, is still required. These spectra exhibited characteristics of an exchange process not observed so far for any other ligands. Whilst the binding appears to be in intermediate exchange, having some features consistent with the other intermediate exchange ligands (see for **70** in Figure 4-3A, Section 4.4.2.1) there were less residues that exhibited entirely

fast exchange processes in this case, and some residues that never returned to full signal intensity, even in the presence of an excess of ligand (eg Y227, Figure 4-19A). Of particular interest, the signal for the side-chain H-N of tryptophan 215 (W215ε1), a residue that constitutes a major part of the ligand binding site according to the ligand binding model (Figure 4-1) was not observed in the [^{1}H,^{15}N] HSQC spectra collected, at any of the ligand concentrations (Figure 4-19B). In contrast to the other intermediate exchange ligands, this indicates that the exchange process observed for the W215ε1 resonance is not influenced by the concentration of the ligand, and the exchange process for this ligand is more characteristic of slow exchange than fast exchange. Hence it is reasonable to conclude that the 'off-rate' constant or k_{off} for ligand **93** is lower than the k_{off} for any of the other ligands studied so far. Although a slow exchange situation has not been observed here, it is worth noting that the slow exchange condition generally applies to tightly binding ligands, with K_d ~≤ 10^{-7} M.[83] Given that K_d may be also expressed as $K_d \approx k_{off}/k_{on}$, (Appendix 1) then a reasonable description of a tightly binding ligand is one that has a 'small' off rate constant, and a 'large' on rate constant, resulting in a lower K_d. But in the present case for ligand **93**, clearly $K_d >> 10^{-7}$ M, and slow exchange is not observed. Therefore, in order to account for the resultant K_d value, the apparent reduction in the off rate constant k_{off} observed in the NMR spectrum must also be accompanied by a reduced on rate constant, k_{on}.

The reason for this apparent reduction in k_{off} may indeed be a consequence of formation of new contacts with the protein surface. These additional contacts may increase the lifetime of the binding event resulting in a reduced k_{off}. The reduced k_{on} may be a consequence of the substitution of the amino group. As mentioned above, and as concluded from Chapters 2 and 3, substitution on the amino group is not preferable for retaining optimal affinity 2-aminoquinoline ligands. Substitution on the amino group may reduce its ability to efficiently form the salt bridge with the aspartate 196 (D196) residue required for binding, based on the rotamer and thus entropic argument presented in Chapter 2 (Section 2.4.3). Therefore, although the affinity of ligand **93** (K_d = 81 μM) is not indicative of a tightly binding ligand from a thermodynamic point of view, it may be concluded that an additional contact(s) has been made with the protein, as evidenced by the [^{1}H,^{15}N] HSQC spectra. Thus, it may be further concluded that a ligand has been identified that makes multiple contacts with the protein, on both the 'left' and 'right' sides of the ligand binding site, in a 'non-ideal' fashion, as illustrated in Figure 4-23. An 'ideal' ligand that makes the same contacts on both the left and right sides of the ligand binding site, might be better achieved with the 'left' side contacts made via the 3- (or 4-) position of the quinoline ring, rather than via the amino group as illustrated in Figure 4-24, resulting in a complex with an increased life time (or a reduced k_{off}) but without a subsequent reduction in the on-rate constant (or k_{on}).

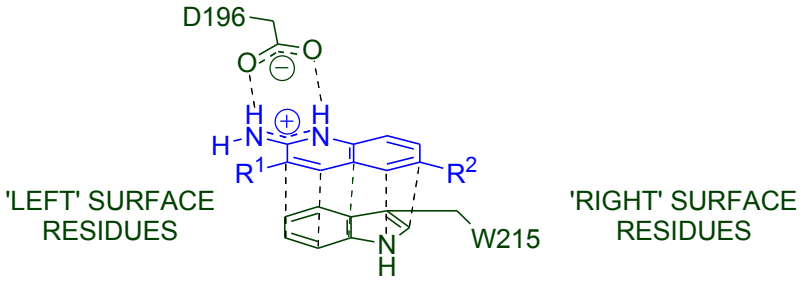


Figure 4-24: Conceptual illustration of Tec SH3/2-aminoquinoline **2** ligand binding model, with 'left' and 'right' regions highlighted, intended to make contacts with new functionality at appropriate positions on the 2-aminoquinoline platform. This diagram highlights a method that may be preferable for making contacts on the 'left' side of the binding site, via substitution at the 3-position of the quinoline ring, in contrast to the example in Figure 4-23, where 'left' side contacts were made via substitution on the amino group.

Furthermore, it would also be of interest to investigate the effect of reduced temperature on the [^{1}H,^{15}N] HSQC NMR spectrum of the Tec SH3 domain in the presence of ligand **93**. Given that the ligand exhibits more characteristics of slow exchange than fast exchange (as discussed above) when the NMR experiments were performed at 25°C, it may be predicted that this exchange process could be driven into slow exchange at reduced temperature. Slow exchange ligands are desirable from the point of view that sufficient time is then available for Nuclear Overhauser Effect transfer from the ligand to the protein, which is essential for determination of structures of ligand-protein complexes. Thus, ligand **93** may offer an advantage over all other ligands so far studied.

4.8.4 Summary

A second series of 2-aminoquinolines (**59**, **60**, **76**, **80**, **81**, **87**, **93**, and **116**) that were described in Sections 4.5 to 4.7 of this chapter were tested for binding to the Tec SH3 domain. As determined by the FP assay, **76** and **81** competed with the proline rich peptide **PRP-1** for binding to the SH3 domain with similar affinity, and an approximately three to four fold improvement relative to 2-aminoquinoline **2** (EC_{50} ca. 45 μM for **76** and **81**, EC_{50} = 160 μM for **2**) (Table 4-4, Figure 4-17). Compounds **59** and **60** also competed with **PRP-1** for binding to the SH3 domain with an improvement in affinity relative to **2**, but this binding was of lower affinity (EC_{50}s 70, 102 and 160 μM for **59**, **60** and **2** respectively) (Table 4-4, Figure 4-17). The binding of compounds **80** and **87** was also demonstrated. There are however, doubts as to the accuracy of the binding constants calculated in these cases, and therefore no solid conclusions can be made about these ligands.

The highest affinity ligand identified from these studies is the phenoxy derivative **116**, which bound the SH3 domain with K_d = 32 μM, an approximately four-fold improvement relative to

2-aminoquinoline **2** (K_d = 125 μM). This ligand binds with similar affinity to the previously identified, highest affinity ligand **70** (K_d **70** = 22 μM), but **116** has advantages over **70** in that it has more 'drug-like' characteristics. Furthermore, due to the ease of synthesis of **116**, it is likely that several more derivatives of **116** could be synthesised promptly, potentially leading to a simple and rapid method for obtaining higher affinity Tec SH3 domain ligands.

Ligand **93** bound the SH3 domain with an approximately one and a half-fold improvement in affinity (K_d = 81 μM) relative to **2** (K_d 125 μM). However, the interpretation of this result is complicated by the intermediate exchange nature of the binding, as evidenced in [^{1}H,^{15}N] HSQC spectra of the SH3 domain in the presence of ligand **93**. It may be concluded that this ligand makes multiple contacts with the protein surface, but the overall stability of the ligand is not optimal due to substitution of the amino group.

4.9 Final Summary: Chapter 4

In this chapter, a thorough investigation into methods for synthesis of 6-substituted-2-aminoquinolines, resulting in the preparation of several new 2-aminoquinolines for ligand binding studies with the Tec SH3 domain, has been presented. These compounds, with a range of functionality at the 6-position, were sought as a means of exploring the protein surface at regions adjacent to the ligand binding site, according to the ligand binding model (Section 4.1), to identify the types of ligand-protein contacts that could be made.

4.9.1 Synthesis of a range of 6-substituted-2-aminoquinolines

The general approach for the synthesis of 2-aminoquinolines was by nucleophilic aromatic substitution reactions, involving treatment of 2-chloroquinoline precursors with nitrogenous nucleophiles. Starting out from 2-chloro-6-methylquinoline **35**, 2-chloroquinolines with a range of functionality at the 6-position (with both hydrophobic and hydrophilic character) were prepared, after benzylic oxidation of the methyl group at the 6-position (Section 4.3.1, 4.3.2, and 4.5.1). Two different approaches for conversion of the 2-chloroquinolines to the 2-aminoquinolines were then used. A literature method[72] using acetamide as the amination reagent at high temperature, was suitable in cases where simple, non-polar functional groups were present at the 6-position (Sections 4.2.1 and 4.3.2). However, this approach was not suitable in cases where hydrophilic/polar functionality (eg a hydroxyl group) was present (Section 4.5.3). For these cases, an alternative, more versatile amination method was established, by where the 2-chloroquinolines were converted into 2-[(4-methoxybenzyl)-amino]quinolines in high yields, by treatment with 4-methoxybenzylamine (Section 4.6.2).

The 2-[(4-methoxybenzyl)amino]quinolines were readily converted into 2-aminoquinolines by treatment with trifluoroacetic acid (Section 4.6.2.1). With additional investigations into optimisation of the 4-methoxylbenzylamination reaction, this method will potentially provide a powerful tool for the synthesis of 2-aminoquinolines with diverse functionality. Still, a general disadvantage of the above syntheses was that they were very 'linear', and the approach was inefficient for production of a large number of ligands.

Alternatively, methods were explored where the 2-amino functionality was incorporated onto the quinoline nucleus in a protected form, prior to benzylic oxidation. Then, new functionality was added at the 6-position. Following de-protection, additional 2-aminoquinolines were prepared. However, the full scope and utility of this, more 'convergent' synthetic approach is yet to be explored (Section 4.7).

4.9.2 Ligand binding studies

With only two exceptions, all of the sixteen 6-substituted-2-aminoquinolines prepared above bound the SH3 domain with an improvement in affinity. In the cases where simple, lipophilic groups were present (eg, Me, Br, MeO as in ligands **33**, **42** and **39** respectively), an approximately two to three-fold improvement in affinity was obtained relative to unsubstituted 2-aminoquinoline **2** (Section 4.4). However, when large lipophilic functional groups were present, up to six-fold improved affinity was obtained. The highest affinity ligand identified was the dioxane-acetal derivative **70** (K_d = 22 μM) (Section 4.4). A disadvantage of this ligand however is that it is not 'drug-like' in character due to its aqueous instability. But, the phenoxymethyl derivative **116** bound the SH3 domain with similar affinity (K_d = 32 μM) to **70** (Section 4.8), indicating that the non 'drug-like' acetal-motif may be replaced with the more drug-like phenoxymethyl group. Given the ease of synthesis of **116**, and the large amount of commercially available phenol precursor starting materials that could be used in the reaction, synthesis of phenoxymethyl derivatives with varied substitution around the phenyl ring warrants further investigation, as a means of identifying higher affinity ligands.

In the cases where the ligands had hydrophilic functionality with the potential to be involved in hydrogen bonding with protein surface residues (eg ligands **59** and **76**), it was concluded that ideal hydrogen bonds were probably not formed, as more substantial improvements in affinity would have been expected (Section 4.8). On the other hand, entropic costs associated with binding of the hydrophilic ligands with many rotatable bonds, could have 'masked' improvements in affinity obtained by formation of hydrogen bonds. Despite this, the major conclusion drawn from all the SAR information obtained was that a lipophilic contact

between the SH3 domain, and the substituent at the 6-position of the ligand, was the driving force for the observed improvements in affinity.

Although the goal of identifying a ligand suited to structure determination of its complex with the SH3 domain by NMR methods was not fulfilled, encouragement for achieving this was gained, as the NMR studies of several of the highest affinity ligands (eg. **70** and **116**) revealed that they were binding in intermediate exchange on the NMR timescale (Sections 4.4.2 and 4.8.2). Furthermore, the 2-(4-methoxybenzylamino)quinoline derivative **93** appeared to be close to slow exchange on the NMR timescale (Sections 4.8.2 and 4.8.3). Slow exchange is an essential feature for achieving a 3D structure of ligand bound to a protein. The reason for this 'near' slow exchange character is likely to be a consequence of a larger contact surface area between the ligand and the protein, due to multiple substituents on the 2-aminoquinoline nucleus. Therefore, higher affinity ligands might be better obtained by substitution at more than one position around the quinoline ring, for example a 3,6-disubstituted-2-aminoquinoline, with the primary amine intact, consistent with conclusions drawn in Chapters 2 and 3. Thus, in a broad sense, the information obtained from the testing of ligand **93** provides major assistance in this project.

4.9.3 5- and 7-substituted-2-aminoquinolines

Finally, as outlined at the commencement of this chapter, 5- and 7-substituted-2-amino-quinoline derivatives were also sought, as part of the process of exploring the protein surface at regions adjacent to the 2-aminoquinoline binding site. The synthesis of 5- and 7-substituted-2-aminoquinolines was commenced using essentially the same approach for the synthesis of simple 6-substituted-2-aminoquinolines, but, the 5- and 7-methylquinolin-2(1*H*)-one mixture obtained was not separable (Section 4.2.2). It was envisaged however, that this mixture could be converted to a 5- and 7-formyl mixture at the 2-chloroquinoline stage in a similar fashion to the method used for the synthesis of 2-chloro-6-formylquinoline **57** (Section 4.3.1), and that the two formyl compounds may be separable. This aspect has since been investigated by a colleague (Rhiannon Jones, unpublished), and the desired conversion and separation was possible. Hence, much of the subsequent chemistry presented in this report has also been applied to the 5- and 7-substituted compounds, leading to the synthesis of 5- and 7-substituted-2-aminoquinolines, for ligand binding studies. These studies have not been reported here.

Chapter 5

Specificity Studies of 2-Aminoquinoline and Derivatives with other SH3 Domains

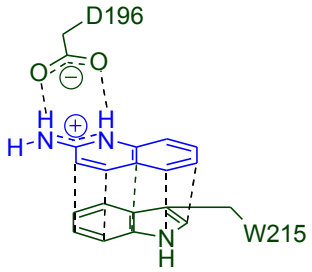
5.1 Introduction

As has now been well established, the Tec SH3 domain/2-aminoquinoline **2** ligand binding model that was presented in Chapter 1, and was further investigated in Chapter 2 (Figure 5-1A) indicates that two key residues in the proline-rich peptide binding site of the Tec SH3 domain are involved in binding of the 2-aminoquinoline scaffold: the side-chains of tryptophan 215 (W215) (involved in π-π stacking), and aspartate 196 (D196) (involved in salt bridge formation). Comparison of the mouse Tec IV SH3 domain amino-acid sequence with sequences from a range of human SH3 domains indicates that both W215 and D196 are highly conserved (Figure 5-1B). These residues both play key roles in the binding of **PRP-1** to the Tec SH3 domain.[13] The tryptophan residue corresponding to W215 is almost completely conserved throughout all SH3 domain sequences (data not shown), consistent with its role as part of the hydrophobic surface that contacts one of the core proline residues within the PxxP ligand sequence. The position corresponding to D196 also makes an important contribution to binding of proline-rich peptides to the SH3 domains through either salt bridges or hydrophilic interactions with residues on the proline-rich peptide. However, as indicated in the sequence alignment (Figure 5-1B), some variety is found at the position corresponding to D196. The other amino acid containing the carboxylate motif glutamate, or the hydrophilic residues threonine, asparagine and glutamine are frequently found in place of aspartate at this position.

To complement the sequence alignments, structure alignments were performed with selected SH3 domains to investigate the relative orientations of the important residues for 2-aminoquinoline **2** binding in Tec. Specifically, the tryptophan side-chain was superimposed for several SH3 domain structures that contain either aspartate or glutamate residues at positions corresponding to position 196 in the Tec sequence, and the relative positions of the aspartate and glutamate side-chains were inspected. An example of this is provided in Figure 5-1C, in which the Tec, Hck and Nck-1(2) SH3 domains were investigated. Hck is a member of the Src family of tyrosine kinase enzymes that has an aspartate at the site of interest, while the second of three SH3 domains from the Nck-1 adaptor protein [Nck-1(2), hereafter referred to as Nck] has a glutamate at the site of interest. The Hck structure was obtained from a published crystal structure, with molecule B selected out of a total of six models A-F[102] (PDB entry 1BU1). (The significance of the selection of molecule B will be further discussed

in Section 5.2.2.) The Nck structure was obtained using the homology modelling software MODELLER,[103,104] performed by a co-worker (unpublished). This inspection indicates that the negatively charged residues are all in essentially the same position, relative to the position of D196 in Tec.

A



B

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mTec          182 IVVAMYDFQATEAHDLRLERGQEYIIL-EKNDLHWWRARDKYGS-----EGYIPSNYV 233
Nck1_2        110 PAYVKFNYMAEREDELSLIKGTKVIVM-EKCSDGWWRG-SYNGQ-----VGWFPSNYV 160
Hck            61 IVVALYDYEAIHHEDLSFQKGDQMVVL-EES-GEWWKARSLATR----KEGYIPSNYV 112
Fyn            86 LFVALYDYEARTEDDLSFHKGEKFQIL-NSSEGDWWEARSLTTG----ETGYIPSNYV 138
Src            88 TFVALYDYESRTETDLSFKKGERLQIV-NNTEGDWWLAHSLSTG----QTGYIPSNYV 140
Crk           136 YVRALFDFNGNDEEDLPFKKGDILRIR-DKPEEQWWNAEDSEGK-----RGMIPVPYV 187
vav           786 TAKARYDFCARDRSELSLKEGDIIKILNKKGQQGWWRG-EIYGR-----VGWFPANYV 827
PLCg          773 TVKALYDYKAKRSDELSFCRGALIHNV-SKEPGGWWKG-DYGTR----IQQYFPSNYV 824
Grb2_C        160 YVQALFDFDPQEDGELGFRRGDFIHVMDNSDPN-WWKG-ACHGQ-----TGMFPRNYV 210
Txk            86 QVKALYDFLPREPCNLALRRAEEYLIL-EKYNPHWWKARDRLGN-----EGLIPSNYV 137
ITSN-1_4     1078 IAQVIASYTATGPEQLTLAPGQLILIR-KKNPGGWWEG-ELQARGKKRQIGWFPANYV 1113
Trip10        544 HCVAIYHFEGSSEGTISMAEGEDLSLMEEDKGDGWTRVRRKEGG-----EGYVPTSYL 596
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C

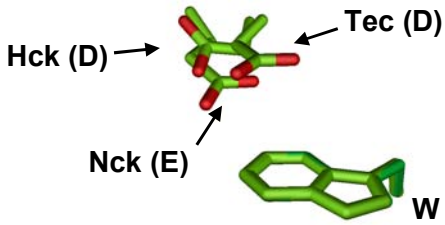


Figure 5-1: (A) Tec SH3 domain/2-aminoquinoline **2** ligand binding model, as presented in earlier sections. (B) Sequence Alignments of mouse Tec (denoted mTec) SH3 Domain with selected human SH3 domains, indicating the degrees of conservation of Tryptophan (W215) and Aspartatic Acid (D196) residues of mTec, both important for binding of 2-aminoquinolines (residues of interest are shown in red, and boxed). Sequence alignment courtesy of Dr. Grant Booker. (C) Structure alignments of Tec, Nck, and Hck SH3 domains: the tryptophan side-chains were superimposed and the relative positions of the side-chains corresponding to position D196 in the Tec sequence are illustrated. The oxygen atoms on the negatively charged residues are coloured in red.

It was therefore of interest to determine whether 2-aminoquinoline or its derivatives could bind to other SH3 domains. Hence a small test set of SH3 domains, including the Hck and Nck proteins described above was selected for specificity studies. In addition, another Src kinase family member Fyn, that also has an aspartate residue at the position equivalent to D196 in Tec, was also chosen for investigation. The results of these studies are the focus of this chapter.

5.2 Specificity of 2-aminoquinoline

To investigate the specificity of 2-aminoquinoline **2** binding with other SH3 domains, the ability of **2** to displace fluorescently labelled proline-rich peptides from GST-SH3 fusion proteins was tested using the Fluorescence Polarisation (FP) ligand-competition assay. The same proline-rich peptide that has been used in all previous FP experiments described in this report, **PRP-1**, also bound the Nck and the Fyn GST-SH3 proteins (data not shown). Hence, **PRP-1** also could be used for FP peptide displacement experiments with both of these SH3 domains. The testing of the Fyn SH3 domain was performed by a co-worker, Cvetan Stojkoski. In the case of the Hck SH3 domain, **PRP-1** did not bind to this GST-SH3 protein, and thus another fluorescent proline-rich peptide, fluorescein-βA-STPRPLPPLPTTR-CO_2H **PRP-2**, was obtained for this experiment.

5.2.1 FP competition assays with 2-aminoquinoline and the Nck, Hck, and Fyn SH3 domains

Ligand **2** was able to compete with **PRP-1** for binding to the Tec and Nck SH3 domains with comparable affinity (EC_{50}s 160 and 150 μM respectively) (Figure 5-2, Table 5-1). It should be noted however, that inspection of the competition binding isotherms as illustrated in Figure 5-2, suggests that the **2** may actually compete with **PRP-1** for binding to the Nck SH3 domain with higher affinity as judged by the small 'left-shift' observed for the Nck isotherm relative to that for Tec. However, considering that a small amount of overlap of the isotherms is evident when taking the error bars for the respective experiments into account, it may be concluded that the difference in the affinities for the Nck and Tec SH3 domains is not significant.

In contrast, the competition by **2** with the proline-rich peptides for binding to the Hck and Fyn SH3 domains was very weak (EC_{50}s > 1000 μM) (Figure 5-2, Table 5-1). Furthermore, in a similar fashion to the data manipulations discussed in Chapter 4 (Section 4.8.1.2) for EC_{50} determinations using poorly defined binding isotherms, a manipulation of the data was also required in order to obtain meaningful estimates of the EC_{50} values in both of these cases.

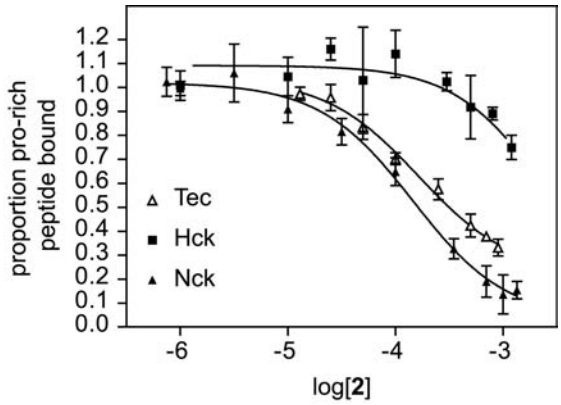


Figure 5-2: Fluorescence Polarisation peptide displacement studies of fluorescent proline rich peptide from either Tec, Nck and Hck GST-SH3 proteins with **2**. The isotherms were obtained from independent experiments using either **PRP-1** (for Tec and Nck) or **PRP-2** for Hck. In the case of Hck, the value of BOTTOM was set constant at 0.15 in the non-linear regression analysis, as is discussed in the main text in this section.

Table 5-1: Specificity Studies Round 1: Compound **2** with **Nck**, **Hck** and **Fyn** SH3 domains

	Tec[†] **EC_{50} (μM)**[*]	**Nck**[†] **EC_{50} (μM)**[*]	**Hck**[‡♦] **EC_{50} (μM)**	**Fyn**[†♦♠] **EC_{50} (μM)**
2	160 ± 35	150 ± 30	> 2000	>1000

[*] Quoted values are mean ± standard deviation over 3 replicate experiments. [†] **PRP-1** displacement, [‡] **PRP-2** displacement. [♦] BOTTOM held constant at 0.15 in the non-linear regression analysis in this case (see main text in this section for discussion). [♠] This experiment was performed by a colleague, Cvetan Stojkoski.

Specifically, for the Hck **2**/**PRP-2** competition assay, the calculated BOTTOM value using the default Prism settings was –1.78, a large over estimate. Conversely, in the case of the Fyn experiment, the calculated BOTTOM value using the default Prism settings was 0.64, a large under estimate. In both of these situations, the BOTTOM values were set constant at 0.15, and the non-linear regressions were recalculated, to provide more meaningful estimates of the EC_{50} values, as indicated in Table 5-1 (also, see for Hck in Figure 5-2). Although these manipulations result in very poor regression coefficient R^2 values, (R^2 = 0.79 and 0.65 for Hck and Fyn respectively) the results are clearly indicative of the binding of **2** to the SH3 domains of Hck and Fyn being very weak.

5.2.2 Discussion of SAR information

On consideration of the data presented in Section 5.2.1 above, the results indicate that in the correct context, glutamate side-chains (at the equivalent position to D196 in Tec) can be tolerated, as in Nck. However in the wrong context, aspartate and tryptophan (as in Hck and Fyn), are not sufficient to facilitate **2** binding.

These results also show that it is not justified to predict whether **2** may bind to a particular SH3 domain based on sequence alone, but that an inspection of the relative positions of the tryptophan and the negatively charged residue on the structure of the SH3 domain, as in Figure 5-1C, may be of assistance. For example, it can be seen from Figure 5-1C, that the relative positions of the negatively charged residues (aspartate or glutamate) are similar, and are consistent with that of the Tec SH3 domain. However, in the case of Hck, the direction of the carboxylate motif of the aspartate side-chain is different relative to the other two, with the oxygen atoms pointing in the opposite direction. Considering that the importance of D196 in Tec has been demonstrated for the binding of **2**, the fact that the oxygen atoms of the aspartate residue point in the opposite direction in Hck might have the consequence that it is less favourable for formation of a salt bridge with **2**, and therefore might explain why **2** binds to the Hck so weakly. However, this comparison is perhaps not ideal, from the point of view that a simple comparison of these 'static' structures, gives no information about the motions of the amino acid side-chains that would be expected under physiological conditions. Specifically, it is worth noting that three of the six published structures of Hck[102] (molecules A, D and F) have the carboxylate motif of the aspartate side-chain pointing in a similar direction to that in the Tec aspartate residue, while the remaining structures (molecules B, C and E) have the carboxylate group in the opposite direction (not shown). This indicates that flexibility at this position is indeed possible. However, a minimised average structure of the Fyn SH3 domain determined by NMR methods (PDB entry 1NYF) has the aspartate residue placed in a similar position to that in the Tec SH3 domain (not shown), yet **2** also binds very weakly to the Fyn SH3 domain. This provides an example where even inspection of an 'average' structure from an ensemble was not sufficient for predicting whether **2** may bind to the protein.

In the case of the Nck glutamate side-chain, although there is one extra carbon atom in this side-chain, the final position and direction of the carboxylate motif is very similar to that of the Tec situation, according to the *'predicted'* structure illustrated in Figure 5-1C. This suggests that the overall positioning of the tryptophan and negatively charged residues, not the size of the negatively charged residue, is important for determining whether 2-aminoquinoline can bind to a particular SH3 domain. However, again solid conclusions cannot be drawn here, because the Nck structure is based on homology modelling, and is not experimentally determined. It is intended that the structure of the Nck SH3 domain will be determined by NMR methods in the future to assess the quality of the predicted structure, and to assist in characterising the Nck SH3 domain/**2** ligand binding event. This may indeed provide additional support for the proposed ligand binding model for the Tec SH3 domain.

5.3 Specificity of 6-substituted-2-aminoquinolines with the Nck SH3 domain

The results described above for the 2-aminoquinoline binding studies with the Hck and Fyn SH3 domains (very weak binding) indicate that even a simple, unsubstituted heterocyclic compound like **2** provides some selectivity between SH3 domains. However, in the case of the Nck SH3 domain, **2** was able to bind with comparable affinity as it does to Tec. Hence, in this situation, the selectivity can be described as poor. However, because **2** may be classed as a 'lead' compound for ligand development, the fact that the 'lead' also binds to the Nck SH3 domain is not necessarily disadvantageous, given that from a therapeutic point of view, the affinity of **2** for both the Tec and the Nck SH3 domains remains very low. Furthermore, the 'lead' ultimately requires optimisation to obtain a higher affinity ligand, by synthesis of derivatives of the 'lead'. The ultimate test of specificity of a ligand comes down to the selectivity of the optimised ligand, not the 'lead'. On the other hand, the fact that **2** also binds to the Nck SH3 domain, suggests that **2** may also be used as a 'lead' for development of high affinity ligands for the Nck SH3 domain.

Therefore, to begin to address these issues, it was of interest to investigate whether some of the 6-substituted-2-aminoquinolines synthesised in Chapter 4, particularly the higher affinity derivatives, provide additional specificity. Thus 2-aminoquinolines **33**, **64** and **69** were selected for binding studies with the Nck SH3 domain, and the results from this investigation is the focus of this section.

5.3.1 FP competition assays with Nck SH3 domain and 2-aminoquinolines 33, 64 and 69

Compounds **33**, **64** and **69** were tested for binding to the Nck SH3 domain again using the FP peptide displacement assay with the GST-SH3 fusion protein. 2-Aminoquinoline **33** was able to compete for binding with **PRP-1** from the Nck SH3 domain with similar affinity as it does for the Tec SH3 domain (EC_{50}s 90 and 75 μM for Nck and Tec respectively) (Figure 5-3, Table 5-2), which also equates to an approximately two-fold improvement relative to **2** for Nck. In contrast, the derivatives with the bulkier cyclic-acetal motifs **64** and **69**, which are two of the higher affinity ligands for the Tec SH3 domain (EC_{50}s 34 and 25 μM respectively), both competed with **PRP-1** for binding to the Nck SH3 domain weakly (EC_{50}s 300 and > 500 μM for **64** and **69** respectively) (Figure 5-3, Table 5-2). This equates to a two to three-fold reduction in affinity relative to **2** for binding to the Nck SH3 domain, but when combined with

the improved affinity of **64** and **69** for the Tec SH3 domain, it can be seen that approximately ten to twenty-fold selectivity between the Tec and Nck SH3 domains was achieved.

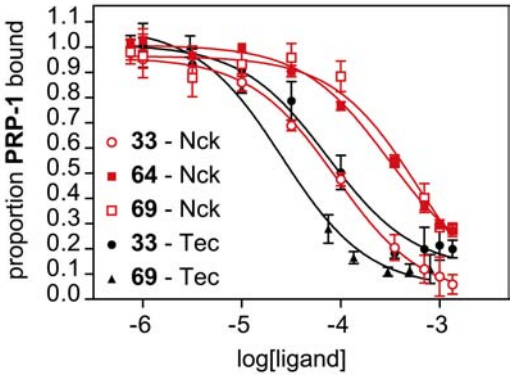


Figure 5-4: Fluorescence Polarisation peptide displacement studies of fluorescent proline rich peptide **PRP-1** from either Tec (black), or Nck (red) GST-SH3 proteins with 6-substituted-2-aminoquinolines, **33**, **64** and **69**. The isotherms were obtained from independent experiments.

Table 5-2: Specificity Studies Round 2: 6-substituted-2-aminoquinolines binding to the Nck SH3 domain, using FP peptide competition assay.

Ligand	Tec EC_{50} (mM)*	Nck EC_{50} (mM)*
2	160 ± 35	150 ± 30
33	75 ± 15	90 ± 12
64	34 ± 5	300 ± 30
69	26 ± 6	> 500

* Quoted values are mean ± standard deviation over 3 replicate experiments.

5.3.2 Discussion of SAR information

Although the SAR information obtained from testing of 6-substituted-2-aminoquinolines with the Tec SH3 domain indicates that a lipophilic contact most likely mediates the improvement in affinities obtained, the precise nature (ie which surface residue(s) of the SH3 domain is involved) of this lipophilic interaction is unclear, in the absence of a 3D structure of the complex. Similarly, following inspection of the equivalent location of the predicted Nck structure, it remains unclear why a small improvement in affinity is obtained for ligand **33** for binding to the Nck SH3 domain (EC_{50}s 150 and 90 μM for **2** and **33** respectively). However, inspection of the Nck SH3 structure does indicate that there is an aspartate residue (D143) on the Nck surface, that sits proximal to where the oxygen atoms of the acetal motifs of ligands **64** and **69** are likely to be placed. Although there is also an aspartate residue in a similar location on the Tec SH3 domain (D212), the positioning of D212 in Tec is further

away from the 2-aminoquinoline binding site. By using a repulsion argument (between oxygen atoms), this may therefore explain the substantially reduced affinity of ligands **64** and **69** for the Nck SH3 domain, compared to Tec.

5.4 Summary: Chapter 5

A brief, yet informative study into the specificity of some 2-aminoquinolines has been presented in this chapter.

A small test set of different SH3 domains, including two of the Src family members, Hck and Fyn, and the second of three SH3 domains from the Nck-1 adaptor protein [Nck-1(2)] was tested for binding to **2** using the FP peptide competition assay with GST-SH3 proteins. Of these three SH3 domains, **2** was able to compete for binding with the proline rich peptide for the Nck SH3 domain with similar affinity as it does to the Tec SH3 domain (EC_{50}s 150 and 160 μM respectively). However only very weak competition of the proline-rich peptide was detected from both the Fyn and Hck SH3 domains (EC_{50}s > 1000 μM) (Section 5.2). Given that at the equivalent positions to W215 and D196 in the Tec amino acid sequence (the two key residues that form the 2-aminoquinoline binding site) there are a combination of W/D, W/D and W/E residues for the Hck, Fyn and Nck SH3 domains respectively, the results described above indicate that it is not possible to predict whether 2-aminoquinoline may bind to a particular SH3 domain, based on sequence alone. Furthermore, inspection of the structures of the SH3 domain at the binding site to view the relative positioning of the two key residues was unable to consistently assist with making predictions.

To determine whether any additional specificity is gained through derivatisation of **2**, three 6-substituted-2-aminoquinolines **33**, **64** and **69** were subsequently tested for proline-rich peptide competition from the Nck SH3 domain (Section 5.3). Approximately ten to twenty-fold selectivity between the Tec and Nck SH3 domains was obtained with ligands **64** and **69**, two of the higher affinity ligands for the Tec SH3 domain (EC_{50}s ca. 30 μM and 300-500 μM for Tec and Nck respectively). These results provide encouragement for the use of 2-aminoquinoline as a scaffold for development of SH3 domain small molecule ligands with engineered specificity. However, given that such a large number of SH3 domains exist in the human genome, a major challenge will now be to identify which SH3 domains are appropriate targets for small molecule ligand development with the 2-aminoquinoline scaffold.

Chapter 6

Conclusions, Future Work and Final Discussion

6.1 Conclusions and Future Work

At the commencement of this PhD research project, several aims were set out. Ultimately, these aims were intended to contribute towards development of high affinity small molecule ligands for the Tec SH3 domain, using the lead compound 2-aminoquinoline **2** as a platform. This would ideally lead to a ligand that would be suited to structure determination of its complex with the SH3 domain using NMR methods. In addition, an examination of the specificity of 2-aminoquinoline, and any derivatives that were developed was also planned. In this chapter, each of these aims will again be briefly revisited, with relevance to the outcomes obtained. Then, as appropriate, future work related to the different sections will be proposed. A final discussion will then follow.

6.1.1 Aim 1: Additional characterisation of the 2-amino-quinoline/SH3 domain binding event (Chapter 2)

An additional series of simple heterocyclic compounds with structural similarities to 2-aminoquinoline were either synthesised, or obtained from colleagues or commercial sources, and were tested for binding to the Tec SH3 domain. This led to an improved understanding of the mechanism of binding of **2** to the Tec SH3 domain and confirmed that **2** was the highest affinity 'lead' compound available for development of ligands with improved affinity. A key result from this section was the discovery that substitution on the amino nitrogen atom of 2-aminoquinoline with a methyl group (ligand **8**) resulted in an approximately three-fold reduction in affinity. Additionally in this section, a relationship between pH and affinity of **2** for the SH3 domain was demonstrated, and hence further confirmed the importance of protonation of **2** in the ligand binding event.

6.1.2 Aim 2: Development of 2-aminoquinoline derivatives with improved affinity for the Tec SH3 domain (Chapters 3 and 4)

Two approaches were pursued as a means of investigating methods for the development of 2-aminoquinolines with improved affinity.

6.1.2.1 Synthesis and binding studies of *N*-benzylated-2-aminoquinoline derivatives (Chapter 3)

As was presented in Chapter 3, a brief investigation was made into the synthesis and binding of *N*-benzylated-2-aminoquinolines, as a possible approach for the development of 2-aminoquinoline ligands with improved affinity. Although evidence was already obtained based on the work described in Chapter 2 that *N*-methylation of 2-aminoquinoline (ligand **8**) led to an approximately three-fold reduction in affinity, it was predicted that substitution with bulkier hydrophobic groups (such as a benzyl group) at the amino nitrogen atom, might result in the formation of additional contacts with the SH3 domain, and hence an improvement in affinity may result.

Therefore, a small series of *N*-benzylated-2-aminoquinolines was synthesised. All of these compounds bound the SH3 domain with improved affinity relative to the *N*-methylated derivative **8**, and in the best case, an approximately two-fold improvement was obtained. Furthermore, this improvement in affinity almost brought back the affinity to where it was prior to substitution on the amino group (K_ds = 177 and 125 μM for *N*-benzyl derivative **26** and **2** respectively) (Figure 6-1A). This indicates that a new contact was made between the aryl rings of the *N*-benzyl substituents, that was able to offset the entropic cost associated with the *N*-methyl derivative on ligand binding. Whilst these results provided new SAR information, they did not lead to the development of ligands with improved affinity. Hence, it was further concluded based on these results that optimal 2-aminoquinoline ligands should be primary amines.

Proposed future work

Based on the conclusions discussed above, as part of future work, it would be of interest to synthesise ligands with bulky hydrophobic substituents, positioned so they may be able to make a similar contact(s) to that obtained with ligand **24**, but instead placing the substituents on the quinoline ring instead of the amino group. Using this approach, an improvement in affinity relative to **2** should be obtained. As illustrated in Figure 6-1B, this may be achieved by substitution at the 3-position of the quinoline ring: it may be necessary to synthesise

derivatives with varied 'linkers' in order to obtain the optimal affinity. Currently, some progress has been made towards the synthesis of 3-substituted-2-aminoquinolines by a co-worker, Rhiannon Jones.

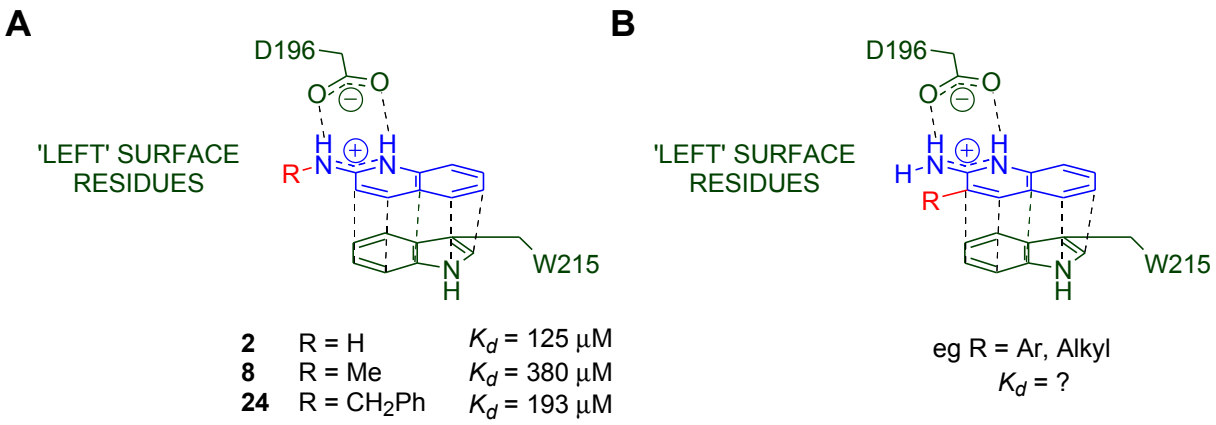


Figure 6-1: (A) Summary of key results relating to the *N*-substituted-2-aminoquinolines. (B) Proposed alternative approach for forming the same or similar contact made with ligand **24**, but instead by substitution at the 3-position of the quinoline ring.

6.1.2.2 Synthesis and binding studies of 6-substituted-2-aminoquinoline derivatives (Chapter 4)

Following the results described above, it was clear that an effort towards synthesis of ring-substituted-2-aminoquinolines, with the primary amino group intact, was necessary. Hence, as presented in Chapter 4, it was envisaged that 2-aminoquinoline derivatives with substituents at the 5-, 6-, or 7-position of the quinoline ring would be placed well to potentially make hydrogen bonds with hydrophilic amino-acid residues in that region of the protein surface. However, derivatives with a range of functionality, not just with hydrophilic groups were sought, as a means of exploring the protein surface in this region, to determine the types of contacts with the protein that may be possible.

Thus, an investigation into methodology for the synthesis of 5-, 6- or 7-substituted-2-aminoquinolines was pursued. However, the synthesis of 6-substituted-2-aminoquinolines soon became the focus of this investigation, as these were easier to prepare. As part of this synthetic investigation, different strategies for the synthesis of 2-aminoquinolines were explored, and the advantages and limitations of different approaches were uncovered. In addition, good progress was made towards the development of a 'convergent' synthetic strategy. From this work, a number of 6-substituted-2-aminoquinolines were prepared with a range of functionality, including compounds with both hydrophobic and hydrophilic functional groups. These syntheses resulted in the discovery of 2-aminoquinolines with up to six-fold

improved affinity relative to **2**. In fact, only two 6-substituted-2-aminoquinolines were prepared that did not bind with higher affinity than **2**, indicating that the 6-position is well placed for making new protein contacts. In contrast to what was initially anticipated, the 2-aminoquinolines that had large, hydrophobic functionality placed at the 6-position of the quinoline ring were the highest affinity (eg ligand **70** K_d = 22 μM), whilst the ligands that had the highest probability of participation in ideal hydrogen bonding were lower affinity. This suggests that a lipophilic contact was made between the SH3 domain and the hydrophobic functionality of the ligands. On the other hand, ligands like **76** and **81**, that had several rotatable bonds in conjunction with hydroxyl groups (that may participate in hydrogen bonding) did not bind with substantially lower affinity than the bulky hydrophobic ligands (eg ligand **76** K_d = 38 μM). In these cases, it is possible that hydrogen bonds may have been formed, but significant improvements in affinity were not observed, due to entropic costs associated with forming the hydrogen bonds.

As part of a new method that was developed for the synthesis of 6-substituted-2-aminoquinolines, 2-[(4-methoxybenzyl)amino]quinoline intermediates were prepared. Because it had been demonstrated that *N*-benzylated-2-aminoquinolines were able to make a lipophilic contact with the SH3 domain, it was of interest to investigate whether such a ligand that also had a substituent at the 6-position may bind with improved affinity, relative to the related 6-substituted primary amines (eg **76**), due to the potential formation of multiple contacts with the SH3 domain. Thus, one 6-substituted-2-[(4-methyoxybenzyl)-amino]quinoline intermediate (**93**) was tested for binding to the Tec SH3 domain. Although the affinity of this ligand was not higher than **76**, features of the [^{1}H,^{15}N] HSQC NMR spectra used for the ligand binding assay provided some evidence for the formation of multiple contacts with the SH3 domain. Hence, this provides encouragement for the idea that ligands with multiple substituents around the 2-aminoquinoline nucleus may lead to higher affinity ligands than have been identified so far.

Proposed future work

Following the extensive synthetic investigations presented in Chapter 4, with particular regard to the synthesis of 2-aminoquinolines using the 'convergent' synthetic strategy, some additional work is required to expand the diversity of 2-aminoquinoline derivatives that can be prepared efficiently. Then, it should be possible for a range of new 6-substituted-2-aminoquinolines to be synthesised for ligand binding assays, leading to optimal 6-substituted ligands. As part of this optimisation process, it would be of interest to prepare ligands with substituents at the 6-position with hydrophilic groups (that may be able to form hydrogen bonds) but with less rotatable bonds. This approach may have an increased likelihood of

leading to potent ligands, as the entropic costs associated with hydrogen bond formation would be reduced. Having identified the optimal functionality at the 6-position, in conjunction with identifying a suitable method for the synthesis of 3-substituted-2-aminoquinolines (that may also lead to improved affinity), it would then potentially be of interest to synthesise 2-aminoquinoline derivatives that have substituents at both the 3- and 6-positions of the quinoline ring. This could lead to a ligand that makes multiple contacts, with both the 'left' and 'right' surface residues, as illustrated in Figure 6-2. Given that there is evidence for formation of multiple ligand-protein contacts with the disubstituted ligand **93** (as discussed above), but no significant improvements in affinity resulted (probably due to a loss of affinity arising from *N*-substitution), synthesis of 3,6-disubstituted-2-aminoquinolines without *N*-substitution may well turn out to be a preferable method for making multiple contacts with the SH3 domain. This may subsequently result in a synergistic gain in affinity.

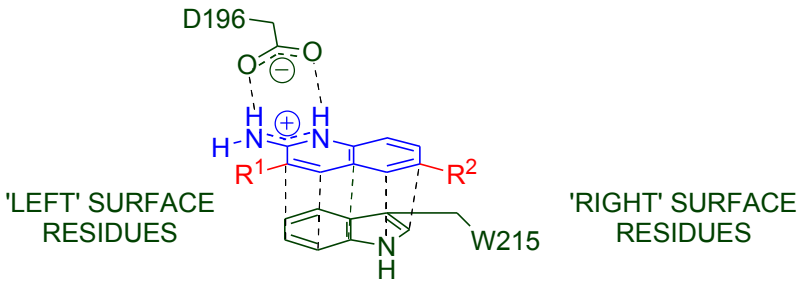


Figure 6-2: Conceptual illustration of Tec SH3/2-aminoquinoline **2** ligand binding model, with 'left' and 'right' regions highlighted, intended to make contacts with new functionality at appropriate positions on the 2-aminoquinoline platform, potentially leading to synergistic improvements in affinity.

6.1.3 Aim 3: Identification of a ligand suited to structure determination of its complex with the SH3 domain by NMR methods (Chapter 4)

The feature of ligand/protein binding that is essential for structure determination by NMR methods is that the exchange rate of the binding must be slow on the NMR timescale. Several of the compounds that were synthesised as part of the investigations presented in Chapter 4 bound the SH3 domain in intermediate exchange on the NMR timescale (eg ligands **70**, **116** and **93**). This suggests that the exchange rate for ligand binding in these cases, lies in-between the rate characteristic of fast and slow exchange, and is a step in the right direction, towards identifying a slow exchange ligand. Furthermore, in the case of intermediate exchange ligand **93**, it was concluded that the exchange rate for this ligand probably lies even closer to that characteristic of slow exchange, than the rates for the other intermediate exchange ligands (described above).

However, slow exchange binding was not observed for any of the ligands, and therefore a 3D structure of a 2-aminoquinoline ligand bound to the SH3 domain was not obtained.

Proposed future work

For the intermediate exchange ligands identified (eg **93** or **116**), it would be of interest to investigate the influence of reduced temperature on the observed exchange process in the NMR spectrum. Reduced temperature should result in a further decrease of the off rate constant k_{off}, which would thus result in an increased lifetime of the complex. This may subsequently lead to the slow exchange observation on the NMR timescale and hence, provide conditions suited to recording of NOE experiments, required for structure determination. On a similar note, recording of spectra using a spectrometer with higher field strength may likewise change the exchange process that is observed during the experiments for the intermediate exchange ligands, because the exchange process observed also has a dependence on field strength.

If either of the above approaches did not result in the slow exchange process being observed, the combination of both reduced temperature and increased field strength may also be worth investigating. On the other hand, given that such substantial progress has been presented in Chapter 4 towards methods for the synthesis of a diverse range of 6-substituted-2-aminoquinolines, and that the synthesis of 3,6-disubstituted-2-aminoquinolines has also been proposed, a new ligand may well soon be identified, that demonstrates the slow exchange process at room temperature, and without the need for a higher field magnet.

Another approach that may be considered for obtaining new structural information about the 2-aminoquinoline/SH3 ligand binding event with some of the current ligands, is the use of the 'Transferred NOE' NMR experiment. This experiment provides information about the conformation of ligands bound to proteins, by way of intra-molecular Nuclear Overhauser Effects,[105] and is better suited to ligands that bind in fast exchange on the NMR timescale. However, this approach would probably only be of use when applied to a ligand that has several rotatable bonds, and adopts a specific conformation on binding to the SH3 domain. Little information would be obtained when applied to a simple heterocyclic compound like **2**, as no conformational changes occur to **2** on ligand binding. Furthermore, this approach is still unlikely to provide significant information about the structure of the SH3 domain on ligand binding. The 3D structure of the complex, obtained from measurement of inter-molecular Nuclear Overhauser Effects, remains as the preferred approach for defining the ligand binding mode.

6.1.4 Aim 4: Investigation into specificity of 2-aminoquinoline and derivatives with other SH3 domains (Chapter 5)

A small test set of SH3 domains [Nck-1(2), Fyn, and Hck] was selected for specificity studies. Of these proteins, **2** was able to compete for binding with the proline-rich peptide for the Nck SH3 domain with similar affinity to Tec, however, **2** could only compete very weakly with the peptides in the case of the Fyn and Hck SH3 domains. This indicates that some specificity is possible, even without any substitution on the ligand. On the other hand, this reveals that **2** may also be used as a lead for the development of ligands for the Nck SH3 domain. However, ligands **64** and **69** (two of the higher affinity ligands for the Tec SH3 domain) competed for binding with the proline-rich peptide with approximately ten to twenty-fold reduced affinity to Tec. This provides encouragement for the use of **2** as a scaffold for the development of high affinity ligands for SH3 domains, with engineered specificity.

Proposed future work

Considerably more work is required from several perspectives. The main challenge now is to identify which SH3 domains may be targets for ligand development with **2**. This would be a desirable future undertaking: in order that 2-aminoquinolines may become useful from a medicinal point of view, a suitable, more 'clinically relevant' SH3 domain target is required. However, the difficulty associated with this is that there are hundreds of SH3 domains encoded in the human genome, and it would be very inefficient and costly to test every single one using an experimental approach. Hence a high throughput, computational approach for shortlisting possible targets would certainly be beneficial. Development of such a method is currently being addressed by colleagues Iain Murchland and Cvetan Stojkoski.

Then, development of high affinity, highly specific 2-aminoquinolines for the different targets on the shortlist will become important. To facilitate this aspect, a large selection of synthetic tools for the preparation of a diverse range of 2-aminoquinolines will be necessary.

6.2 Final Discussion

The SH3 domains are small protein-protein interaction domains that bind to proline-rich peptide sequences, mediating a range of important biological processes. For example, SH3 domains often feature as regulatory domains in enzymes like the protein tyrosine kinases such as the Src or Tec families. Alternatively, they often feature in adaptor proteins that are involved in signalling cascades that control cell growth, for example Grb-2. Since, de-

regulated signalling pathways form the basis of many diseases such as cancer, immunodeficiency and osteoporosis,[15] the SH3 domains have been common targets for the development of potential therapeutics, able to alter the normal function of the domain. However no examples of entirely non-peptide ligands for the SH3 domains have previously been reported. In our research group, we have identified 2-aminoquinoline **2**, a simple heterocyclic compound, as a moderate affinity ligand for the Tec SH3 domain. Small molecule ligands are preferable to peptides for utility as therapeutics for reasons such as improved cell permeability, oral delivery, stability, and cost effectiveness. Thus the identification of **2** as an SH3 ligand is potentially of high value, towards development of medicines targeted to the domain. As a step towards this, the main aim of this PhD project was to develop high affinity small molecule ligands for the Tec SH3 domain (a model system) using the 'lead' ligand **2** as a platform. The outcomes of the research presented in this thesis, have, and will continue to make a substantial contribution to the development of 2-aminoquinolines as high affinity SH3 domain ligands.

Using the Tec model system, ligands with up to six-fold improved affinity relative to **2**, were synthesised. Furthermore, a large amount of time was invested into the development of different methods for the synthesis of 6-substituted-2-aminoquinolines with a range of functionality, and it may be fair to say that much of the 'fruit' obtainable from this effort is yet to be harvested. It seems inevitable that higher affinity ligands will be developed in a relatively short time frame. In addition, the proposed synthesis of 3,6-disubstituted-2-aminoquinolines will also potentially lead to the identification of higher affinity ligands.

Although the goal of identifying a ligand suited to structure determination by NMR methods was not fulfilled, some of the higher affinity ligands tested by the NMR assay method demonstrated characteristics of 'slower' exchange on the NMR timescale (relative to the fast exchange processes observed for all the other ligands studied prior to this), a feature necessary for obtaining a structure. Thus, it is likely that as ligands with further 'jumps' in improvement (of affinity) are discovered, they will bring the slow exchange property with them. Having solved the 3D structure of the complex of a 2-aminoquinoline ligand with the SH3 domain, future derivatives should be easier to design.

Therefore, the achievements described above provide a good foundation for the development of 2-aminoquinolines as potential therapeutic agents. However, a new phase of this research, involving a thorough investigation into the specificity of the 2-aminoquinolines is now becoming of greater importance. In order for the developments made here to have more useful applications, discovery of alternative SH3 domain targets that have clear roles in

disease states is necessary. This is currently being addressed by colleagues, using a computational approach. Given that the preliminary studies described here revealed that good selectivity of the higher affinity 2-aminoquinolines for the Tec SH3 domain exists, then, should the identification of a more clinically appealing SH3 target take place, engineering specificity for the new target can be approached with confidence.

Despite the absence of a clinically appealing SH3 target for the 2-aminoquinoline ligands currently, a high affinity 2-aminoquinoline ligand for the Tec SH3 domain might still find use as a probe for elucidation of a more clearly defined biological function of the Tec Kinase enzyme.

Chapter 7

Experimental

7.1 Chemistry General

All solvents were distilled, dried and stored according to standard procedures.[106] Melting points were determined using a Kofler hot-stage apparatus equipped with a Reichart microscope, and values are uncorrected. Infrared spectra were recorded on an ATI Mattson Genesis FTIR spectrometer. Unless otherwise stated, the samples were prepared as either nujol mulls or as liquid films between sodium chloride plates. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Gemini-2000 spectrometer (^{1}H: 200.13 MHz, ^{13}C: 50.32 or ^{1}H: 300.13 MHz, ^{13}C: 74.47 MHz), or a Varian INOVA 600 spectrometer (^{1}H: 599.842 MHz, ^{13}C: 150.842 MHz). The Varian INOVA 600 spectrometer was fitted with a $^{1}H\{^{13}C/^{15}N\}$ inverse triple resonance PFG probe with z-axis gradients. Spectra were recorded as solutions in $CDCl_3$ [tetramethylsilane (δ_H = 0.0) or $CDCl_3$ (δ_C = 77.7) as internal standards], d_6-acetone [δ_H = 2.05, δ_C = 29.9 as internal standards], or d_6-DMSO [δ_H = 2.50, δ_C = 39.5 as internal standards]. Chemical shift values are given on the δ scale quoted in parts per million, followed by the integration, multiplicity, coupling constant *J* and assignment. The following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet; br, broad. ^{13}C signals for new compounds were assigned from Heteronuclear Multiple Quantum Coherence (HMQC), and Heteronuclear Multiple Bond Correlation (HMBC) experiments. Flash chromatography was performed using Scharlau Silica Gel 60, 230-400 mesh. Thin layer chromatography (TLC) was performed on aluminium backed silica gel 60 plates (Merck), and plates were visualised under UV light (254 nm) or by staining with a $KMnO_4/K_2CO_3$ solution. Preparative thin layer chromatography was performed on Merck Silica Gel 60 F254 pre-coated, 20 x 20 cm glass backed plates, 0.25 mm layer thickness. Electron impact (EI) mass spectra were recorded using a ZAB 2HF mass spectrometer. High resolution mass spectrometry was performed at Monash University (Victoria, Australia) or the University of Tasmania, (Tasmania, Australia). Elemental analyses were performed at the University of Otago, (Dunedin, New Zealand).

Notes regarding notation used for assignments of NMR spectra

The following system has been used for assignments: HX/HY refers to a signal that has been unambiguously assigned to HX and HY. H[X or Y] refers to a signal that may be assigned to either HX or HY (ie an ambiguous assignment). In this case, the data set will include two signals that are assigned this fashion. In some instances, there may be up to three ambiguous assignments in a data set. In addition, combinations of both notations

described here are sometimes used. For example (3H, m, HA / 2 x H[X or Y or Z]), indicates that HA and two of the HX, HY or HZ protons give rise to the multiplet.

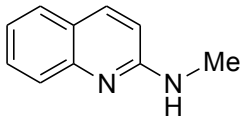
7.2 Sources of ligands not included in the experimental chapter

Compounds **2** and **19-21** were all obtained from commercial sources. Compound **1**[107] was synthesised by a co-worker, Anna Hardie, according to a literature procedure. Compounds **22** and **23** were synthesised and kindly donated by Lisa Kaminskas. Compounds **39-42** were synthesised by co-workers and this has been documented.[51]

7.3 Synthesis of compounds presented in Chapter 2

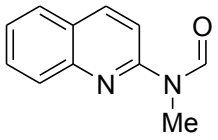
(*N*-Methyl)quinolin-2-yl-amine 8/(*N*-methyl-*N*-quinolin-2-yl)formamide 12[56] **(Scheme 2-1)**
A mixture of 2-chloroquinoline (0.205 g, 1.25 mmol), diethanolamine (0.325 g, 3.10 mmol) and *N*-methylformamide (5.05 g, 85.6 mmol) was heated at ca. 130°C for 12 hours. After cooling, most of the excess *N*-methylformamide was removed under reduced pressure and then water (5 mL) was added to the remaining mixture. This mixture was extracted with dichloromethane (3 x 10 mL) and the combined organic extracts were washed with brine (30 mL), dried (Na_2SO_4) then the solvent was removed. The residue (0.114 g) was partially purified by filtration through silica gel using 19:1 dichloromethane/ethyl acetate as eluant to afford a material (0.034 g) that consisted of at least 2 products as judged by ^{1}H NMR spectroscopy. This mixture was rechromatographed on silica gel using 3:2 hexane/ethyl acetate as eluant to afford the title compound **8** (0.0021 g, 1%) (R_f 0.14) as a white solid, mp 68-71°C (lit.[108] 69-71°C). In addition the by-product **12** (0.0066 g, 3%) (R_f 0.48) was also isolated as a white solid, mp 63-69°C.

8



IR (nujol) υ/cm^{-1}: 3241 (NH), 1622, 1588, 1549. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.09 (3H, d, *J* = 4.8 Hz, CH_3), 4.77 (1H, br s, NH), 6.64 (1H, d, *J* = 9.0 Hz, H3), 7.21 (1H, ddd, *J* = 1.0, 6.9, 7.7 Hz, H6), 7.53 (1H, ddd, *J* = 1.3, 6.9, 8.6 Hz, H7), 7.58 (1H, dd, *J* = 1.3, 7.7 Hz, H5), 7.70 (1H, br d, *J* = 8.6 Hz, H8), 7.81 (1H, d, *J* = 9.0 Hz, H4).

12

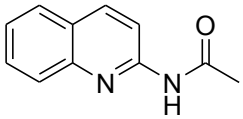


IR ($CDCl_3$) υ/cm^{-1}: 1679 (CO), 1620, 1601, 1504. ^1H NMR (200 MHz, $CDCl_3$) δ: 3.49 (3H, s, CH_3), 7.25 (1H, d, *J* = 8.9 Hz, H3), 7.49 (1H, ddd, *J* = 1.2, 6.9, 8.1 Hz, H6), 7.71 (1H, ddd, *J* = 1.4, 6.9, 8.4 Hz, H7), 7.80 (1H, dd, *J* = 1.4, 8.1 Hz, H5), 7.95 (1H, br d, *J* = 8.4 Hz, H8), 8.19 (1H, d, *J* = 8.9 Hz, H4), 9.52 (1H, s, CHO). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 28.9 (CH_3), 111.5 (C3), 125.6 (C[6 or 8]), 126.0 (C4a), 127.4 (C[6 or 8]), 128.4 (C5), 130.5 (C7), 138.9 (C4), 146.9 (C8a), 152.6 (C2), 162.5 (CHO). *m/z* (EI): 186 ($M^{+\cdot}$,10%), 158 ($M^{+\cdot}$ – CO + H, 90), 157 ($M^{+\cdot}$ – HCO, 90), 130 (35), 129 (100), 128 (50).

***N*-(Quinolin-2-yl)acetamide 9/*N*-acetyl-*N*-(quinolin-2-yl)acetamide 13 (Scheme 2-2)**

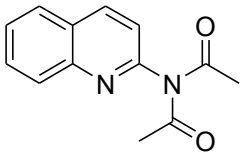
To a stirred solution of 2-aminoquinoline **2** (0.207 g, 1.44 mmol) in dichloromethane (3 mL) under a nitrogen atmosphere at room temperature was added triethyamine (0.315 g, 3.13 mmol). Stirring was continued for 10 minutes before the drop-wise addition of acetyl chloride (0.235 g, 3.00 mmol) in dichloromethane (1 mL) over 5 minutes. Stirring was continued for 3 hours, and the mixture was diluted with dichloromethane to a total volume of 20 mL, and washed with brine (2 x 20 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed. The residue (0.316 g) was chromatographed over silica gel (9:1 dichloromethane/ethyl acetate) to provide the title compound **9** (0.124 g, 46%) (R_f 0.2) as a white solid, mp 128-130°C (lit[73] 129-130°C). In addition the by-product **13** (0.108 g, 33%) (R_f 0.39) was also isolated. A portion was recrystallised from water to afford an analytically pure sample as white needles, mp 98-100°C.

9



IR: (nujol) υ/cm^{-1}: 3236 (NH), 1674 (CO), 1600. ^1H NMR (200 MHz, $CDCl_3$) δ: 2.20 (3H, s, CH_3), 7.44 (1H, ddd, *J* = 1,2, 6.8, 8.1 Hz, H[6 or 7]), 7.64 (1H, ddd, *J* = 1.6, 6.8, 8.4 Hz, H[6 or 7]), 7.75-7.84 (2H, m, H5/H8), 8.17 (1H, d, *J* = 9.0 Hz, H4), 8.42 (1H, br d, *J* = 9.0 Hz, H3), 9.11 (1H, br s, NH).

13



$C_{13}H_{12}N_2O_2$ requires C 68.41, H 5.30, N 12.27%; Found C 68.18, H 5.21, N 12.28%. IR (nujol) υ/cm^{-1}: 1730 (CO asym), 1698 (CO), 1591, 1571. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.38 (6H, s, 2 x CH_3), 7.33 (1H, d, *J* = 8.6 Hz, H3), 7.64 (1H, ddd, *J* = 1.4, 7.0, 8.2 Hz, H6), 7.78 (1H, ddd, *J* = 1.6, 7.0, 8.5 Hz, H7), 7.91 (1H, dd, *J* = 1.6, 8.2 Hz, H5), 8.08 (1H, br d, *J* = 8.5 Hz, H8), 8.31 (1H, d, *J* = 8.6 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 26.7 (2 x CH_3), 121.3 (C3), 127.5 (C[6 or 8]), 127.6 (C4a), 127.7 (C[6 or 8]), 129.2 (C5), 130.3 (C7), 139.0 (C4), 147.4 (C8a), 151.9 (C2), 172.6 (2 x CO). *m/z* (EI): 228 ($M^{+\cdot}$,10%), 186 ($M^{+\cdot}$ – CH_2=C=O, 38), 171 ($M^{+\cdot}$ – CH_2=C=O – CH_3, 100), 144 ($M^{+\cdot}$ – CH_2=C=O – CH_3CO + H, 56), 128 (46).

1-(Cyclohex-1-enyl)pyrrolidine 15[57] (Scheme 2-3)

A mixture of cyclohexanone (10.4 g, 0.106 mol), and pyrrolidine (15.1 g, 0.212 mol) was heated at reflux in benzene (30 mL) with a Dean Stark apparatus attached for 5 hours. After cooling, the excess benzene was removed under reduced pressure, and the remaining liquid was distilled, to afford the title compound **15** (12.42 g, 77%) as a colourless liquid, bp 75-80°C/0.02 mm (lit.[57] 104-106°C/12 mm). IR (neat) υ/cm^{-1}: 1641 (C=C). This material was used in the next step without additional characterisation.

3-(2-Oxocyclohexyl)propionitrile 16[57]

A mixture of the pyrrolidine derivative **15** (11.0 g, 0.072 mol) and acrylonitrile (5.0 g, 0.094 mol) was heated at reflux in dry 1,4-dioxane (40 mL) under an inert atmosphere for 12 hours. After cooling, water (4.3 mL) was added, and heating at reflux was continued for a further 1.5 hours. After cooling, the excess 1,4-dioxane was removed under reduced pressure, and the residue was dissolved in ether (40 mL). The ether solution was washed with a hydrochloric acid solution (5%, 3 x 40 mL), brine (40 mL), dried (Na_2SO_4), and the solvent was removed. The residue was then distilled to afford the title compound **16** (7.0 g, 64%) as a colourless liquid, bp 108-112°C/0.06 mm (lit.[57] 141-145°C/10 mm). IR (neat) υ/cm^{-1}: 2245 (CN), 1708 (CO), 1450. This material was used in the next step without additional characterisation.

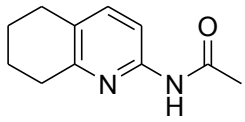
3-[(2-Hydroxyimino)cyclohexyl]propionitrile 17[58]

A solution of di(hydroxylammonium) sulfate (2.96 g, 0.036 mol) in water (13 mL) was stirred before the addition of 3-(2-oxocyclohexyl)propionitrile **16** (5.00 g, 0.033 mol) in ether (13 mL) at ca. 10°C. Then, sodium hydroxide (1.64 g, 0.041 mol) in water (8 mL) was added at such a rate as to maintain the temperature below 20°C. The resulting mixture was stirred at room

temperature for 19 hours. The organic layer was then separated and the aqueous layer was extracted with additional ether (3 x 15 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed to afford the crude product **17** (2.88 g, 53%) as a clear viscous oil, that later solidified. IR (nujol) υ/cm^{-1}: 3272 (OH), 2241 (CN), 1642 (C=N–O). This material was used for the subsequent step without purification, or additional characterisation.

_N_-(5,6,7,8-Tetrahydroquinolin-2-yl)acetamide 18[58]

Acetic anhydride (1.20 g, 11.8 mmol) was added to crude 3-[(2-hydroxyimino)cyclohexyl]-propionitrile **17** (1.30 g, 7.82 mmol) (prepared above) with stirring and cooling to ca. 5°C. Then acetyl chloride (1.23 g, 15.7 mmol) was added portion-wise over 15 minutes with stirring, and the resulting mixture was heated at reflux for 4.5 hours. After cooling, sodium hydroxide (1.62 g, 40.5 mmol) in water (4 mL) was added, and the mixture was heated at reflux for a further 3 hours. After cooling, the mixture was extracted with dichloromethane (4 x 20 mL), and the combined organic extracts were washed with brine (100 mL), dried (Na_2SO_4) and the solvent was removed to afford a black tar (0.995 g). ^{1}H NMR analysis of the material suggested it consisted mostly of the title compound **18** in an impure form. Approximately half of the material (0.470 g) was chromatographed over silica gel using ethyl acetate as eluant to afford purified **18** (0.112 g, 8%) (R_f 0.33) as an off white solid, mp 108-125°C.



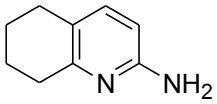
IR: (nujol) υ/cm^{-1}: 3236 (NH), 1660 (CO), 1594, 1538. ^{1}H NMR (200 MHz, $CDCl_3$)* δ: 1.71-1.91 (4H, m, H6/H7), 2.14 (3H, s, CH_3), 2.67-2.77 (4H, m, H5/H8), 7.35 (1H, d, J = 8.3 Hz, H4), 7.88 (1H, br d, J = 8.3 Hz, H3), 8.20 (1H, br s, NH). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 23.4 (CH_3), 23.6 (C[6 or 7]), 25.2 (C[6 or 7]), 28.7 (C[5 or 8]), 32.7 (C[5 or 8]), 112.1 (C3), 128.9 (C4a), 139.8 (C4), 149.3 (C8a), 155.7 (C2), 169.2 (CO). *m/z* (EI): 190 ($M^{+\cdot}$, 80%), 148 ($M^{+\cdot}$ – CH_2=C=O, 100).

* Data consistent with that presented in literature.[58]

2-Amino-5,6,7,8-tetrahydroquinoline 10[58]

A mixture of *N*-(5,6,7,8-tetrahydroquinolin-2-yl)acetamide **18** (0.065 g, 0.34 mmol) and sodium hydroxide (20%, 5 mL) was heated at reflux for 16 hours. After cooling, the resulting mixture was extracted with ether (3 x 3 mL), and the combined organic extracts were washed

with brine (5 mL), dried (Na_2SO_4) and the solvent was removed to afford the title compound **10** (0.025 g, 49%) as a pale yellow solid, mp 87-90°C (lit.[58] 87-90°C).



IR: (nujol) υ/cm^{-1}: 3450 (NH), 3148 (NH), 1643 (CO), 1605, 1570. ^1H NMR (200 MHz, $CDCl_3$)* δ: 1.71-1.98 (4H, m, H6/H7), 2.61 (2H, t, *J* = 5.9 Hz, H[5 or 8]), 2.72 (2H, t, *J* = 6.1 Hz, H[5 or 8]), 4.23 (2H, br s, NH_2), 6.30 (1H, d, *J* = 8.3 Hz, H3), 7.12 (1H, d, *J* = 8.3 Hz, H4).
* Data consistent with that presented in literature.[58]

7.4 Synthesis of compounds presented in Chapter 3

2-(Benzylamino)quinoline 24 (first attempt, using method of Manescalchi[65]) (Scheme 3-2)

A mixture of 2-aminoquinoline **2** (0.120 g, 0.832 mmol), sodium triacetoxyborohydride (0.176 g, 0.832 mmol), potassium hydroxide (0.0470 g, 0.832 mmol) and acetic acid (0.0250 g, 0.416 mmol) was stirred in methanol (3 mL) under a nitrogen atmosphere until a homogeneous mixture was obtained. The temperature was then lowered to ca. 5°C. Benzaldehyde (0.0940 g, 0.886 mmol) in methanol (0.5 mL) was then added drop-wise over 30 minutes, and the resulting solution was maintained at ca. 5°C for 6 hours. The vessel was then allowed to reach room temperature and stirring was continued for 24 hours, at which point thin layer chromatography suggested that no product had formed. The temperature of the vessel was then raised to ca. 40°C and stirring was continued for a further 24 hours. After cooling, the solvent was removed under reduced pressure, the residue was resuspended in sodium hydroxide (10%, 6 mL), and the resulting solution was extracted with ether (3 x 6 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent was removed to afford the crude residue (0.101 g). ^1H NMR analysis of this material revealed a mixture of 2-aminoquinoline **2** and benzaldehyde was present.

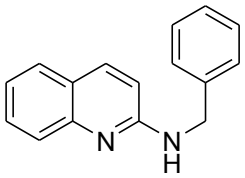
General procedure for reductive alkylation of 2-aminoquinoline according to the method of Mattson[66] (Scheme 3-3)

2-Aminoquinoline **2** (1 equiv.) and the aryl aldehyde (1 equiv.) were stirred with titanium(IV) isopropoxide (1.3-2 equiv) at room temperature with a calcium chloride drying tube attached. If the mixture was too viscous for effective stirring, THF (~0.5 mL/mmol **2**) was added. After ~2 hours, the mixture was diluted with ethanol (~0.5 mL/mmol **2**) and sodium cyanoborohydride (0.7 equiv) was added and the resulting mixture was stirred for a further

24 hours at room temperature. The solvent was removed and the residue was taken up into ethyl acetate and filtered through celite to remove insoluble impurities. The solvent was removed and the residue was chromatographed on silica gel to afford the pure amine and the by-products. However, a characterisation is only provided for the by-products **30** and **31**, isolated from the synthesis of 2-aminoquinoline derivative **26**.

2-(Benzylamino)quinoline 24

2-Aminoquinoline **2**, (0.100 g, 0.694 mmol), benzaldehyde (0.0750 g, 0.694 mmol) and titanium(IV) isopropoxide (0.385 g, 1.35 mmol) were stirred as described above for 12 hours, before dilution with ethanol (1 mL) and the addition of sodium cyanoborohydride (0.0295 g, 0.469 mmol). The resulting solution was stirred for an additional 24 hours. Following workup and chromatography using 7:3 hexane/ethyl acetate as eluant, the pure title compound **24** (0.0740 g, 46%) (R_f 0.42) was isolated as a white solid, mp 95-100°C (lit.[109] 96-97°C).

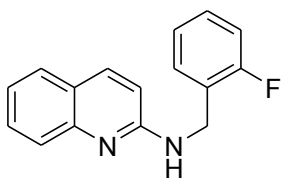


IR (nujol) υ/cm^{-1}: 3266 (NH), 1623, 1571, 1508, 1494, 1429. ^{1}H NMR (200 MHz, $CDCl_3$)* δ: 4.73 (2H, d, *J* = 5.4 Hz, CH_2), 5.00 (1H, br s, NH), 6.63 (1H, d, *J* = 8.9 Hz, H3), 7.18-7.45 (6H, m, H2'-6'/H6), 7.50-7.62 (2H, m, H5/H7), 7.71 (1H, br d, *J* = 8.1 Hz, H8), 7.82 (1H, d, *J* = 8.9 Hz, H4). *m/z* (EI): 235 ($M^{+\cdot}$ + H, 50%), 234 ($M^{+\cdot}$, 100), 233 ($M^{+\cdot}$ – H, 50), 157 ($M^{+\cdot}$ – C_6H_5, 9), 129 (50), 106 (100).

* Data consistent with that presented in literature.[109]

2-[(2-Fluorobenzyl)amino]quinoline 25

2-Aminoquinoline **2**, (0.250 g, 1.74 mmol), 2-fluorobenzaldehyde (0.215 g, 1.74 mmol) and titanium(IV) isopropoxide (0.621 g, 2.18 mmol) were stirred as described above for 2 hours, before dilution with ethanol (1.7 mL) and the addition of sodium cyanoborohydride (0.0730 g, 1.16 mmol). The resulting solution was stirred for an additional 24 hours. Following workup and chromatography using 3:1 hexane/ethyl acetate as eluant, the pure title compound **25** (0.101 g, 23%) (R_f 0.48) was isolated as a pale yellow solid, mp. 96-101°C.



HRMS (EI): $C_{16}H_{13}FN_2$ requires 252.1063; Found 252.1070. IR (nujol) υ/cm^{-1}: 3278 (NH), 1621, 1587, 1570, 1523, 1508, 1486. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 4.81 (2H, d, *J* = 6.0 Hz, CH_2), 5.06 (1H, br s, NH), 6.64 (1H, d, *J* = 9.0 Hz, H3), 7.04-7.09 (2H, m, H3'/H5'), 7.21 (1H,

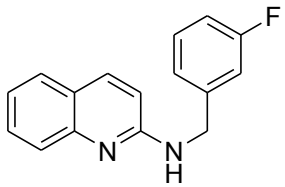
ddd, *J* = 1.2, 6.9, 7.9 Hz, H6), 7.21-7.26 (1H, m, H4'), 7.47-7.50 (1H, m, H6'), 7.53 (1H, ddd, *J* = 1.5, 6.9, 8.5 Hz, H7), 7.58 (1H, dd, *J* = 1.5, 7.9 Hz, H5), 7.71 (1H, br d, *J* = 8.5 Hz, H8), 7.82 (1H, d, *J* = 9.0 Hz, H4). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 40.2 (d, $^3J_{CF}$ = 4.4 Hz, CH_2), 112.0 (C3), 116.0 (d, $^2J_{CF}$ = 21.3 Hz, C3'), 122.9 (C6), 124.3 (C4a), 124.8 (d, $^4J_{CF}$ = 3.3 Hz, C5'), 127.1 (C8), 127.1 (d, $^2J_{CF}$ = 14.7 Hz, C1'), 128.1 (C5), 129.6 (d, $^3J_{CF}$ = 8.3 Hz, C4'), 130.2 (C7), 130.9 (d, $^3J_{CF}$ = 4.4 Hz, C6'), 138.1 (C4), 148.7 (C8a), 157.2 (C2), 161.8 (d, $^1J_{CF}$ = 244.2 Hz, C2'). *m/z* (EI): 253 ($M^{+\cdot}$ + H, 10%), 252 ($M^{+\cdot}$, 55), 251 ($M^{+\cdot}$ – H, 10), 157 ($M^{+\cdot}$ – C_6H_4F, 10), 144 ($M^{+\cdot}$ – $C_6H_4FCH_2$ + H, 15), 129 ($M^{+\cdot}$ – $C_6H_4FCH_2NH$ + H, 70), 124 (100).

A portion of **25** was dissolved in ether, and dry hydrogen chloride gas was bubbled through the ethereal solution, and a precipitate formed. The precipitate was collected, washed with additional ether, and dried to obtain the hydrochloride salt of **25**, mp 190-212°C.
Anal. ($C_{16}H_{13}FN_2$.HCl) requires C 66.55, H 4.89, N 9.70 %; Found C 66.09, H 5.12, N 9.50 %.

2-[(3-Fluorobenzyl)amino]quinoline 26

2-Aminoquinoline **2**, (0.250 g, 1.74 mmol), 3-fluorobenzaldehyde (0.215 g, 1.74 mmol) and titanium(IV) isopropoxide (0.621 g, 2.18 mmol) were stirred as described above for 2 hours, before dilution with ethanol (1.7 mL) and the addition of sodium cyanoborohydride (0.0730 g, 1.16 mmol). The resulting solution was stirred for an additional 24 hours. Following workup and chromatography using 3:1 hexane/ethyl acetate as eluant, the pure title compound **26** (0.136 g, 31%) (R_f 0.38) was isolated as a pale yellow solid, mp. 59-64°C. In addition, the by-product 2-(3-flourophenyl)imidazo[1,2-*a*]quinolin-1-ylamine **30** (0.0495 g, 10%) (R_f 0.20) was also furnished, mp 145-160°C. An unknown by-product, with partial structure **31** (0.015 g) (R_f 0.11) was also isolated, mp 140-152°C.

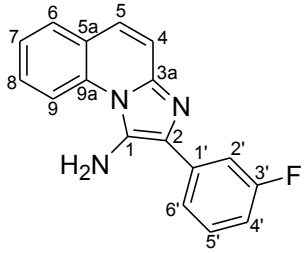
26



Anal. ($C_{16}H_{13}N_2F$) requires C 76.17, H 5.19, N 11.10 %; Found C 76.14, H 5.25, N 10.94 %. IR (nujol) υ/cm^{-1}: 3283 (NH), 1616, 1591, 1571, 1509. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 4.74 (2H, d, *J* = 5.4 Hz, CH_2), 5.15 (1H, br s, NH), 6.63 (1H, d, *J* = 8.7 Hz, H3), 6.96 (1H, dt, $^4J_{2',4'}$ = 2.5, $^3J_{HF}$ = $^3J_{4',5'}$ = 8.2 Hz, H4'), 7.13 (1H, dt, $^4J_{2',4'}$ = $^4J_{2',6'}$ = 2.5 Hz, $^3J_{HF}$ = 9.8 Hz, H2'), 7.18-7.19 (1H, m, H6'), 7.24 (1H, m, H6), 7.30 (1H, dt, $^4J_{HF}$ = 5.9 Hz, $^3J_{4',5'}$ = $^3J_{5',6'}$ = 8.2 Hz, H5'), 7.55 (1H, ddd, *J* = 1.7, 6.8, 8.4 Hz, H7), 7.60 (1H, dd, *J* = 1.7, 8.0 Hz, H5), 7.71 (1H, br d, *J* = 8.4 Hz, H8), 7.83 (1H, d, *J* = 8.7 Hz, H4). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 45.9 (d, $^4J_{CF}$ = 1.7

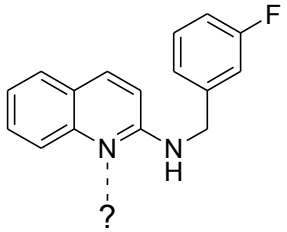
Hz, CH_2), 112.0 (C3), 114.8 (d, $^2J_{CF}$ = 20.7 Hz, C4'), 115.2 (d, $^2J_{CF}$ = 21.3 Hz, C2'), 123.1 (C6), 123.8 (d, $^4J_{CF}$ = 2.7 Hz, C6'), 124.3 (C4a), 126.9 (C8), 128.1 (C5), 130.4 (C7), 130.8 (d, $^3J_{CF}$ = 8.3 Hz, C5'), 138.3 (C4), 142.8 (d, $^3J_{CF}$ = 7.0 Hz, C1'), 148.4 (C8a), 157.1 (C2), 163.7 (d, $^1J_{CF}$ = 244.8 Hz, C3'). *m/z* (EI): 253 ($M^{+\cdot}$ + H, 10%), 252 ($M^{+\cdot}$, 58), 251 ($M^{+\cdot}$ – H, 20), 157 ($M^{+\cdot}$ – C_6H_4F, 12), 144 ($M^{+\cdot}$ – $C_6H_4FCH_2$ + H, 10), 129 ($M^{+\cdot}$ – $C_6H_4FCH_2NH$ + H, 100).

30



HRMS (EI): $C_{17}H_{12}FN_3$ requires 277.1015; Found 277.1015. IR (nujol) υ/cm^{-1}: 3367 (NH), 3294 (NH), 1614, 1583, 1544, 1491. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 3.85 (2H, br s, NH_2), 7.00 (1H, tdd, $^3J_{HF}$ = $^3J_{4',5'}$ = 8.4 Hz, $^4J_{4',6'}$ = 1.3 Hz, $^4J_{2',4'}$ = 2.6 Hz, H4'), 7.34 (1H, d, *J* = 9.3 Hz, H4), 7.40-7.43 (2H, m, H5'/H7), 7.41 (1H, d, *J* = 9.1 Hz, H5), 7.54 (1H, ddd, *J* = 1.6, 7.2, 8.5 Hz, H8), 7.58 (1H, ddd, $^4J_{2',6'}$ = 1.3 Hz, $^4J_{2',4'}$ = 2.6 Hz, $^3J_{HF}$ = 10.2 Hz, H2'), 7.61 (1H, dt, $^4J_{2',6'}$ = $^4J_{4',6'}$ = 1.3 Hz, $^3J_{5',6'}$ = 7.7 Hz, H6'), 7.73 (1H, dd, *J* = 1.6, 7.8 Hz, H6), 9.10 (1H, d, *J* = 8.5 Hz, H9). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 113.8 (d, $^2J_{CF}$ = 21.3 Hz, C4'), 114.0 (d, $^2J_{CF}$ = 21.9 Hz, C2'), 116.2 (C9), 117.4 (C5), 122.6 (d, $^4J_{CF}$ = 2.7 Hz, C6'), 124.5 (C7), 124.7 (C5a), 125.4 (C4), 127.6 (C8), 128.8 (C6), 129.1 (C1), 130.3 (d, $^3J_{CF}$ = 8.7 Hz, C5'), 130.6 (d, $^4J_{CF}$ = 2.7 Hz, C2), 134.6 (C9a), 136.6 (d, $^3J_{CF}$ = 8.3 Hz, C1'), 139.8 (C3a), 163.2 (d, $^1J_{CF}$ = 244.4 Hz, C3'). *m/z* (EI): 278 ($M^{+\cdot}$ + H, 20%), 277 ($M^{+\cdot}$, 100), 249 (5), 129 (74), 128 (42).

31

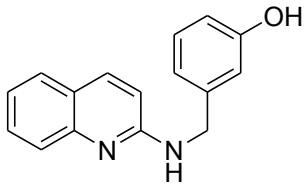


IR (nujol) υ/cm^{-1}: 3315 (NH), 1630, 1580, 1542. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 4.72 (2H, d, *J* = 6.0 Hz, CH_2), 6.82 (1H, d, *J* = 9.3 Hz, H3), 7.04 (1H, dt, $^4J_{2',4'}$ = 2.5 Hz, $^3J_{HF}$ = $^3J_{4',5'}$ = 8.2 Hz, H4'), 7.07 (1H, dt, $^4J_{2',4'}$ = $^4J_{2',6'}$ = 2.5 Hz, $^3J_{HF}$ = 9.4 Hz, H2'), 7.16-7.18 (1H, m, H6'), 7.37 (1H, dt, $^4J_{HF}$ = 5.8 Hz, $^3J_{4',5'}$ = $^3J_{5',6'}$ = 8.2 Hz, H5'), 7.43 (1H, ddd, *J* = 1.0, 7.0, 8.0 Hz, H6), 7.52 (1H, br s, NH), 7.67 (1H, dd, *J* = 1.6, 8.0 Hz, H5), 7.78 (1H, ddd, *J* = 1.6, 7.0, 8.6 Hz, H7), 8.04 (1H, d, *J* = 9.3 Hz, H4), 8.50 (1H, br d, *J* = 8.6 Hz, H8). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 47.4 (d, $^4J_{CF}$ = 1.7 Hz, CH_2), 109.8 (C3), 114.5 (d, $^2J_{CF}$ = 21.8 Hz, C2'), 116.0 (d, $^2J_{CF}$ = 21.3

Hz, C4'), 122.9 (d, $^4J_{CF}$ = 3.2 Hz, C6'), 123.0 (C8), 123.5 (C4a), 125.3 (C6), 129.4 (C5), 131.7 (d, $^3J_{CF}$ = 8.3 Hz, C5'), 133.4 (C7), 139.0 (d, $^3J_{CF}$ = 6.6 Hz, C1'), 142.6 (C8a), 143.0 (C4), 156.0 (C2), 163.9 (d, $^1J_{CF}$ = 246.5 Hz, C3'). *m/z* (EI): 278 (20%), 277 (85), 253 (18), 252 (65), 251 (20), 157 (10), 129 (100), 128 (50). *m/z* (ESI): 605 (30%), 604 (10), 315 (20), 314 (100), 313 (25).

2-[(3-Hydroxybenzyl)amino]quinoline 27

2-Aminoquinoline **2**, (0.250 g, 1.74 mmol), 3-hydroxybenzaldehyde (0.212 g, 1.74 mmol) and titanium(IV) isopropoxide (0.621 g, 2.18 mmol) were stirred as described above for 1 hour, before dilution with ethanol (1.7 mL) and the addition of sodium cyanoborohydride (0.0730 g, 1.16 mmol). The resulting solution was stirred for an additional 24 hours. Following workup and chromatography using 4:1 dichloromethane/ethyl acetate as eluant, the partially purified product (0.0220 g) was obtained as a white solid (R_f 0.23). This material was subsequently repurified using preparative thin layer chromatography on silica gel plates, developing twice with 6:3:1 hexane/ethyl acetate/methanol as eluant, to afford the pure title compound **27** (0.0139 g, 3%) (R_f 0.59) as an off white solid, mp 155-158°C.

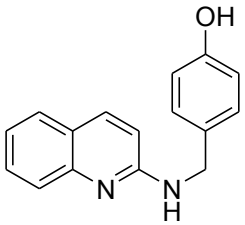


HRMS (EI): $C_{16}H_{14}N_2O$ requires 250.1106; Found 250.1102. IR (nujol) υ/cm^{-1}: 3352 (NH), 1616, 1608, 1586, 1572. ^{1}H NMR (300 MHz, d_6-acetone/$CDCl_3$) δ: 4.07 (1H, br s, OH), 4.61 (2H, s, CH_2), 5.60 (1H, br s, NH), 6.65 (1H, d, *J* = 9.2 Hz, H3), 6.74 (1H, dd, *J* = 1.8, 7.8 Hz, H[4' or 6']), 6.83 (1H, br d, *J* = 7.8 Hz, H[4' or 6']), 6.88 (1H, br s, H2'), 7.12 (1H, t, *J* = 7.8 Hz, H5'), 7.17 (1H, ddd, *J* = 1.2, 6.8, 8.0 Hz, H6), 7.47 (1H, ddd, *J* = 1.6, 6.8, 8.5 Hz, H7), 7.54 (1H, dd, *J* = 1.6, 8.1 Hz, H5), 7.64 (1H, d, *J* = 8.5 Hz, H8), 7.72 (1H, d, *J* = 9.2 Hz, H4). ^{13}C NMR (75 MHz, d_6-acetone) δ: 45.2 (CH_2), 113.6 (C3), 114.5 (C[2' or 4']), 115.4 (C[2' or 4']), 119.5 (C6'), 122.3 (C6), 124.4 (C4a), 127.1 (C8), 128.2 (C5), 129.8 (C5'), 130.1 (C7), 137.3 (C4), 143.0 (C1'), 143.0 (C1'), 149.2 (C8a), 157.9 (C2), 158.4 (C3'). *m/z* (EI): 252 (30%), 251 ($M^{+\cdot}$ + H, 80), 250 ($M^{+\cdot}$, 100), 249 ($M^{+\cdot}$ – H, 30), 157 ($M^{+\cdot}$ – HOC_6H_4, 18), 144 ($M^{+\cdot}$ – $HOC_6H_4CH_2NH$ + H, 144).

2-[(4-Hydroxybenzyl)amino]quinoline 28

2-Aminoquinoline **2**, (0.300 g, 2.08 mmol), 4-hydroxybenzaldehyde (0.254 g, 2.08 mmol) and titanium(IV) isopropoxide (1.18 g, 4.16 mmol) were stirred with THF (1 mL) as described above for 3 hours, before dilution with ethanol (1 mL) and the addition of sodium cyanoborohydride (0.0880 g, 1.40 mmol). The resulting solution was stirred for 24 hours. The

solvent was removed, and the residue was dried for a further 1 hour under high vacuum. The residue was chromatographed using 4:1 dichloromethane/ethyl acetate as eluant to afford the title compound **28** (0.106 g, 20%) (R_f 0.50) as an off white solid, mp. 138-140°C.

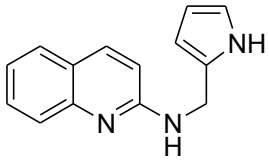


HRMS (EI): $C_{16}H_{14}N_2O$ requires 250.1106; Found 250.1101. IR (nujol) υ/cm^{-1}: 3410 (NH), 1626, 1610, 1572, 1531, 1517. ^{1}H NMR (300 MHz, d_6-acetone) δ: 4.64 (2H, d, *J* = 5.4 Hz, CH_2), 6.55 (1H, br s, NH), 6.76-6.81 (2H, m, AA' portion of AA'XX', H3'/H5'), 6.85 (1H, d, *J* = 9.0 Hz, H3), 7.16 (1H, ddd, *J* = 1.2, 6.9, 8.0 Hz, H6), 7.25-7.29 (2H, m, XX' portion of AA'XX', H2'/H6'), 7.48 (1H, ddd, *J* = 1.6, 6.9, 8.5 Hz, H7), 7.60 (1H, br d, *J* = 8.5 Hz, H8), 7.61 (1H, d, *J* = 8.0 Hz, H5), 7.85 (1H, d, *J* = 9.0 Hz, H4), 8.25 (1H, br s, OH). ^{13}C NMR (75 MHz, d_6-acetone) δ: 45.0 (CH_2), 113.6 (C3), 115.9 (C3'/C5'), 122.1 (C6), 124.3 (C4a), 126.9 (C8), 128.2 (C5), 129.8 (C7), 130.0 (C2'/C6'), 131.9 (C1'), 137.3 (C4), 149.1 (C8a), 157.2 (C[2 or 4']), 157.8 (C[2 or 4']). *m/z* (EI): 251 ($M^{+\cdot}$ + H, 16%), 250 ($M^{+\cdot}$, 95), 251 ($M^{+\cdot}$ – H, 22), 157 ($M^{+\cdot}$ – HOC_6H_4, 5), 144 ($M^{+\cdot}$ – $HOC_6H_4CH_2NH$ + H, 100).

A portion of **28** was dissolved in THF, and dry hydrogen chloride gas was bubbled through the THF solution, and a precipitate formed. The precipitate was collected, washed with ether, and dried to obtain the hydrochloride salt of **28**, mp. 230-250°C.
Anal. ($C_{16}H_{14}N_2O$.HCl) requires C 67.02, H 5.27, N 9.77 %; Found C 66.44, H 5.56, N 9.47 %.

2-[(1*H*-Pyrrol-2-ylmethyl)amino]quinoline 29

2-Aminoquinoline **2**, (0.250 g, 1.74 mmol), 2-formyl-1*H*-pyrrole (0.165 g, 1.74 mmol) and titanium(IV) isopropoxide (0.621 g, 2.18 mmol) were stirred as described above for 2 hours, before dilution with ethanol (1.7 mL) and the addition of sodium cyanoborohydride (0.0730 g, 1.16 mmol). The resulting solution was stirred for an additional 24 hours. Following workup and chromatography using 3:2 hexane/ethyl acetate as eluant, the pure title compound **29** (0.109, 28%) was isolated as an off white solid, mp 113-116°C.



Anal. ($C_{14}H_{13}N_3$) requires C 75.31, H 5.87, N 18.82 %; Found C 75.18, H 6.13, N 18.84 %. IR (nujol) υ/cm^{-1}: 3395 (NH), 3373 (NH), 1621, 1531. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 4.63 (2H, d,

J = 6.0 Hz, CH_2), 5.16 (1H, br s, NH), 6.08-6.10 (2H, m, H3'/H4'), 6.60 (1H, d, *J* = 8.7 Hz, H3), 6.68 (1H, br d, *J* = 1.2 Hz, H5'), 7.24-7.27 (1H, m, H6), 7.57-7.60 (2H, m, H5/H7), 7.77 (2H, d, *J* = 8.7 Hz, H4/H8). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 39.0 (CH_2), 106.7 (C3'), 108.1 (C4'), 113.5 (C3), 118.3 (C5'), 123.1 (C6), 124.2 (C4a), 126.7 (C8), 128.2 (C5), 130.4 (C7), 132.1 (C2'), 138.1 (C4), 148.1 (C8a), 157.6 (C2). *m/z* (EI): 224 ($M^{+\cdot}$ + H, 14%), 223 ($M^{+\cdot}$, 70), 144 ($M^{+\cdot} - C_4H_5NCH_2NH$ + H, 100).

7.5 Synthesis of compounds presented in Chapter 4

7.5.1 Synthesis of compounds presented in Sections 4.2 and 4.3

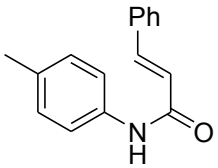
Attempted synthesis of 2-amino-6-methylquinoline 33 from 6-methylquinoline 34 (Scheme 4-1)[68]

Potassium metal (0.205 g, 5.25 mmol) was carefully added to liquid ammonia (35 mL) at ca. -65°C with stirring, and the resulting solution was stirred for 10 minutes, under a nitrogen atmosphere, at which point 6-methylquinoline **34** (0.300 g, 2.10 mmol) was added drop-wise over 5 minutes. Stirring was continued for an additional 10 minutes before the portion-wise addition of potassium permanganate (2.30 g, 14.5 mmol) over 5 minutes. The resulting mixture was stirred at ca. -65°C for 7 hours, before quenching carefully by the portion-wise addition of saturated ammonium chloride. The ammonia was evaporated, and the resulting solution was extracted with ethyl acetate, from which the starting material **34** (0.205 g, 68%) was recovered as a pale brown liquid, as judged by ^1H NMR.

(2*E*)-*N*-(4-Methylphenyl)-3-phenylacrylamide 37 (Scheme 4-2)

To a stirred mixture of pyridine (14.3 g, 0.18 mol) and DMAP (2.2 g, 0.018 mol) in dichloromethane (40 mL) under a nitrogen atmosphere at ca. 0°C was added cinnamoyl chloride (30.0 g, 0.18 mol) in dichloromethane (120 mL) drop-wise over 1 hour during which a dense white precipitate was formed. Then a solution of *p*-toluidine (19.3 g, 0.18 mol) in dichloromethane (90 mL) was added drop-wise over 30 minutes and the mixture began to return to homogeneity. Stirring was continued for a further 30 minutes. The resultant solution was diluted with dichloromethane (100 mL) prior to washing with hydrochloric acid (5%, 3 x 300 mL), 10% sodium hydroxide solution (10%, 2 x 300 mL), water (1 x 300 mL), and drying (Na_2SO_4). The solvent was removed, and the resulting white powder was dried under high vacuum to afford the title compound **37** (38.8 g, 91%) in high purity. This material was used in the next step without purification. To assist in characterisation, a small portion was purified

by chromatography on silica gel using 199:1 dichloromethane/ethyl acetate as eluant (R_f 0.35) to afford white crystals, mp 165-166°C (lit.[110] 162°C).

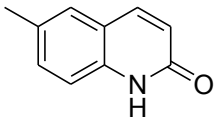


IR (Nujol) υ/cm^{-1}: 3255 (NH), 3181 (NH), 1661 (CO), 1622, 1598, 1538, 1514. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.32 (3H, s, CH_3), 6.57 (1H, d, *J* = 15.6 Hz, COCH=CH), 7.14 (2H, AA' portion of AA'XX', H3/H5), 7.35-7.37 (3H, m, ArH), 7.50-7.57 (5H, m, 4xArH/1xNH), 7.74 (1H, d, *J* = 15.6 Hz, COCH=CH).

6-Methylquinolin-2(1*H*)-one 38[77] (Scheme 4-2)

An intimate mixture of (2*E*)-*N*-(4-Methylphenyl)-3-phenylacrylamide **37** (17.1 g, 0.072 mol) and aluminium chloride (28.7 g, 0.215 mol) was heated rapidly to melting,* and then maintained at ca. 100°C for 1 hour. After cooling, water and ice were cautiously added while mixing with a spatula. The precipitate that formed was collected by filtration, washed with water and hydrochloric acid (5%), and dried. The resulting material (12.9 g†) was used for the next step without further purification. However, to assist in characterisation, a small portion was recrystallised from water, to obtain the title compound **38** as white needles, mp 230-237 °C (lit.[77] 237 °C).

*To avoid the formation of a dense gum during the workup, it was preferable to divide the intimate mixture into three separate vessels to perform the reaction. † Theoretical yield 11.5 g.

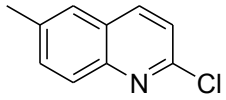


IR (Nujol) υ/cm^{-1}: 3138 (NH), 1662 (CO), 1567, 1503, 1426. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.41 (3H, s, CH_3), 6.72 (1H, d, *J* = 9.3 Hz, H3), 7.33-7.40 (3H, m, H5/H7/H8), 7.78 (1H, d, *J* = 9.3 Hz, H4), 12.67 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.5 (CH_3), 116.8 (C8), 120.7 (C4a), 121.7 (C3), 128.0 (C5 or C7), 132.8 (C5 or C7), 133.1 (C6), 137.1 (C8a), 141.6 (C4), 165.1 (C2).

2-Chloro-6-methylquinoline 35 (Scheme 4-2)

The crude 6-methylquinolin-2(1*H*)-one **38** formed above, (12.75 g) was stirred in phosphoryl chloride (70 mL) at 60°C overnight. Most of the $POCl_3$ was removed by vacuum distillation then the residual $POCl_3$ was quenched by the careful addition of crushed ice. The resulting precipitate was collected by filtration, and washed with water, then dried. The crude material

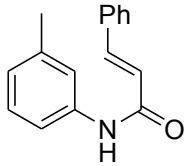
was recrystallised from hexane to afford pure 2-chloro-6-methylquinoline **35** (6.83g, 54% from **37**) as white needles, mp. 113-115°C (lit.[74] 115.2-115.5°C).



IR (Nujol) υ/cm^{-1}: 1586, 1564, 1498. ^{1}H NMR (200 MHz, d_6-acetone) δ: 2.52 (3H, s, CH_3), 7.46 (1H, d, *J* = 8.6 Hz, H3), 7.65 (1H, dd, *J* = 1.8, 8.6 Hz, H7), 7.75 (1H, br s, H5), 7.83 (1H, d, *J* = 8.6 Hz, H8), 8.26 (1H, d, *J* = 8.6 Hz, H4). ^{13}C NMR (50 MHz, d_6-acetone) δ: 21.4 (CH_3), 123.1 (C3), 127.6 (C5), 128.0 (C4a), 128.9 (C8), 133.6 (C7), 138.0 (C6), 139.6 (C4), 147.4 (C8a), 150.2 (C2).

(2*E*)-*N*-(3-Methylphenyl)-3-phenylacrylamide 53 (Scheme 4-4)

The same procedure described above for the synthesis of **37** was used with DMAP (0.37 g, 3.00 mmol) and pyridine (2.37 g, 30.0 mmol) in 8 mL of dichloromethane, and addition of cinnammoyl chloride (5.00 g, 30 mmol) in 20 mL of dichloromethane, followed by addition of *m*-toluidine (3.22 g, 30 mmol) in 15 mL of dichloromethane. Following workup, the title compound **53** (6.05 g, 85%) was isolated as an off white solid, mp 100-112°C (lit.[110] 114°C). This material was used in the next step without further purification.

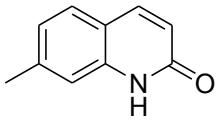


IR (Nujol) υ/cm^{-1}: 3264 (NH), 3209 (NH), 3137 (NH), 1658 (CO), 1614, 1555. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.26 (3H, s, CH_3), 6.68 (1H, d, *J* = 15.6 Hz, COC<u>H</u>=CH), 6.90 (1H, d, *J* = 7.8 Hz, H4), 7.17 (1H, t, *J* = 7.8 Hz, H5), 7.23-7.33 (3H, m, ArH), 7.39-7.42 (2H, m, ArH), 7.47 (1H, d, *J* = 7.8 Hz, H6), 7.51 (1H, br s, H2), 7.73 (1H, d, *J* = 15.6 Hz, COCH=C<u>H</u>), 8.31 (1H, br s, NH).

5-Methylquinolin-2(1*H*)-one 51 / 7-methylquinolin-2(1*H*)-one 52 (Scheme 4-4)[76]

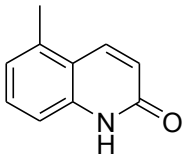
An intimate mixture of **53** prepared above (5.00 g, 21.0 mmol) and aluminium chloride (8.43 g, 63.2 mmol) was heated rapidly to melting and then treated at ca. 100°C for 1 hour. The resulting mixture was carefully quenched by addition of ice and water whilst mixing with a spatula, and a dense gum formed. This gum was extracted with ethyl acetate (3 x 80 mL) and the combined organic extracts were washed with water (2 x 200 mL) and brine (1 x 200 mL) and dried (Na_2SO_4) and then the solvent was removed to afford the ca. 2:1 mixture of **52** and **51** (2.63 g, 79%) as a pale brown solid.

52



^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.45 (3H, s, CH_3), 6.67 (1H, d, *J* = 9.4 Hz, H3), 7.01-7.06 (1H, m, H6), 7.34 (1H, br s, H8), 7.44 (1H, d, *J* = 8.2 Hz, H5), 7.78 (1H, d, *J* = 9.4 Hz, H4), 12.50 (1H, br s, NH).

51

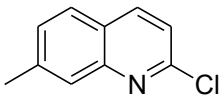


^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.57 (3H, s, CH_3), 6.75 (1H, d, *J* = 9.7 Hz, H3), 7.01-7.06 (1H, m, H[6 or 7 or 8]), 7.26-7.43 (2H, m, 2 x H[6 or 7 or 8]), 8.03 (1H, d, *J* = 9.7 Hz, H4), 12.50 (1H, br s, NH).

2-Chloro-5-methylquinoline 55 / 2-chloro-7-methylquinoline 56 (Scheme 4-4)[76]

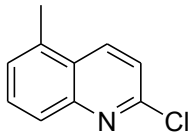
The 2:1 mixture of **51** and **52** prepared above was stirred in phosphoryl chloride (16 mL) at ca. 60°C overnight. Most of the phosphoryl chloride was removed by vacuum distillation, then the residual phosphoryl chloride was carefully quenched by the addition of crushed ice. The resulting mixture was extracted with ether (3 x 60 mL) and the combined organic extracts were washed with water (2 x 200 mL) and brine (1 x 200 mL) and dried (Na_2SO_4) then the solvent was removed to afford the ca. 2:1 mixture of **56** and **55** (2.58 g, 88%) as a brown solid.

56



^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.57 (3H, s, CH_3), 7.32 (1H, d, *J* = 8.5 Hz, H3), 7.40 (1H, dd, *J* = 1.8, 8.4 Hz, H6), 7.71 (1H, d, *J* = 8.4 Hz, H5), 7.80 (1H, br s, H8), 8.05 (1H, d, *J* = 8.5 Hz, H4).

55

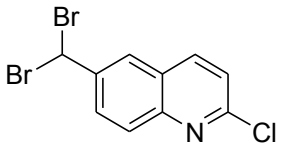


^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.68 (3H, s, CH_3), 7.37-7.43 (2H, m, H3/H[6 or 8]), 7.58-7.66 (1H, m, H5), 7.88 (1H, d, *J* = 8.4 Hz, H[6 or 8]), 8.28 (1H, d, *J* = 8.8 Hz, H4).

2-Chloro-6-formylquinoline 57 (Scheme 4-6)

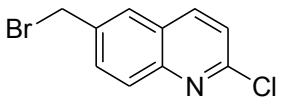
A mixture of recrystallised 2-chloro-6-methylquinoline **35** (3.18 g, 17.9 mmol), *N*-bromosuccinimide (6.37 g, 35.8 mmol), and benzoyl peroxide (0.43 g, 1.79 mmol) was heated at reflux in benzene (20 mL) for 4 hours. After cooling, the benzene was removed under reduced pressure, the residue was dissolved in dichloromethane (120 mL), and washed with 10% sodium bicarbonate (3 x 120 mL). The organic layer was dried (Na_2SO_4) then the solvent was removed. The crude product (5.75 g) was chromatographed over silica gel using 4:1 dichloromethane/hexane as solvent to afford 3.85 g of a white solid that consisted of approximately a 6:1 mixture of 2-chloro-6-(dibromomethyl)quinoline **61** (R_f 0.58) and 6-bromomethyl-2-chloroquinoline **62** (R_f 0.48) as judged by ^{1}H NMR. In addition, pure 6-bromomethyl-2-chloroquinoline **62** (0.23 g, 5%) was also isolated.

61



HRMS (ESI): $C_{10}H_7{}^{79}Br_2{}^{35}ClN^+$ ($M+H^+$) requires 333.8634; Found 333.8627. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.85 (1H, s, $CHBr_2$), 7.49 (1H, d, *J* = 8.6 Hz, H3), 7.94 (1H, d, *J* = 1.9 Hz, H5), 8.08 (1H, dd, *J* = 1.9, 9.0 Hz, H7), 8.11 (1H, d, *J* = 9.0 Hz, H8), 8.16 (1H, d, *J* = 8.6 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 40.5 ($CHBr_2$), 124.1 (C3), 125.0 (C5), 126.5 (C4a), 130.2 (C8), 130.4 (C7), 138.8 (C4), 140.9 (C6), 148.7 (C8a), 152.8 (C2). *m/z* (EI): 338, ($M^{+\cdot}$ [$^{81}Br_2{}^{37}Cl$] – H, 15%), 337 ($M^{+\cdot}$ [$^{81}Br_2{}^{35}Cl$], 2), 336 ($M^{+\cdot}$ [$^{81}Br_2{}^{35}Cl$] - H, 15), 335 ($M^{+\cdot}$ [$^{81}Br^{79}Br^{35}Cl$], 4), 334 ($M^{+\cdot}$ [$^{81}Br^{79}Br^{35}Cl$] - H, 20), 333 ($M^{+\cdot}$ [$^{79}Br_2{}^{35}Cl$], 1), 258 ($M^{+\cdot}$ - Br , 41), 256 ($M^{+\cdot}$ - Br, 100), 254 ($M^{+\cdot}$ - Br, 80).

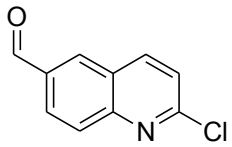
62



mp 140-150°C. HRMS (ESI): $C_{10}H_8{}^{79}Br^{35}ClN^+$ ($M+H^+$) requires 255.9529; Found 255.9522. IR (nujol) υ/cm^{-1}: 1625, 1586, 1499. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.65 (2H, s, CH_2Br), 7.4

(1H, d, *J* = 8.4 Hz, H3), 7.76 (1H, dd, *J* = 1.8, 8.7 Hz, H7), 7.81 (1H, d, *J* = 1.8 Hz, H5), 8.01 (1H, d, *J* = 8.7 Hz, H8), 8.07 (1H, d, *J* = 8.4 Hz, H4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 33.3 (CH_2Br), 123.6 (C3), 127.3 (C4a), 128 (C5), 130 (C8), 132.2 (C7), 137.2 (C6), 139.4 (C4), 148.1 (C8a), 152 (C2). *m/z* (EI): 259 ($M^{+\cdot}$ [$^{81}Br^{37}Cl$], 20%), 258 ($M^{+\cdot}$ [$^{81}Br^{35}Cl$] - H, 80), 257 ($M^{+\cdot}$ [$^{81}Br^{35}Cl$], 22), 256 ($M^{+\cdot}$ [$^{81}Br^{35}Cl$] – H, 93), 255 ($M^{+\cdot}$ [$^{79}Br^{35}Cl$], 14), 254 ($M^{+\cdot}$ [$^{79}Br^{35}Cl$] - H , 23), 178 ($M^{+\cdot}$ - Br, 32), 176 ($M^{+\cdot}$ - Br, 100).

The 6:1 mixture of **61** and **62** prepared above (3.85 g), hexamethylenetetraamine (4.42 g, 0.0316 mol) was heated at reflux in 50% aqueous ethanol (20 mL) for 1 hour at which point thin layer chromatography indicated that all of the starting material had been consumed. After cooling, the mixture was diluted with water (10 mL) and concentrated hydrochloric acid (2.1 mL) was carefully added over 5 mins, and the mixture was brought to reflux for a further 30 minutes. After cooling, the mixture was added to brine (40 mL), and extracted with dichloromethane (4 x 80 mL). The combined organic extracts were then washed with brine (250 mL), dried (Na_2SO_4) then the solvent was removed to provide 2-chloro-6-formylquinoline **57** as a white solid (1.77 g, 52% from **35**). This material was used for further synthesis without purification. However a small amount was chromatographed over silica gel using 49:1 dichloromethane/ethyl acetate as solvent to obtain an analytical sample (mp 165-167°C).

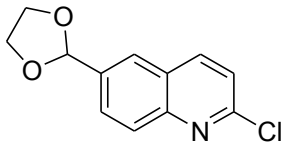


Anal. ($C_{10}H_6ClNO$) requires C 62.68, H 3.16, N 7.31%; Found C 62.95, H 3.19, N 7.15%. IR (nujol) υ/cm^{-1}: 1697, 1624, 1583. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.5 (1H, d, *J* = 8.7 Hz, H3), 8.13 (1H, d, *J* = 8.7 Hz, H8), 8.22 (1H, dd, *J* = 1.8, 8.7 Hz, H7), 8.27 (1H, d, *J* = 8.7 Hz, H4), 8.35 (1H, d, *J* = 1.8 Hz, H5), 10.19 (1H, s, CHO). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 124.4 (C3), 127 (C4a), 128.9 (C8), 130.6 (C7), 133.3 (C5), 135.3 (C6), 140.6 (C4), 151.3 (C8a), 154.4 (C2), 191.7 (CHO). *m/z* (EI): 193 ($M^{+\cdot}$ [^{37}Cl], 32%), 192 ($M^{+\cdot}$ [^{37}Cl] – H, 45), 191 ($M^{+\cdot}$ [^{35}Cl], 100), 190 ($M^{+\cdot}$ [^{35}Cl] – H, 100), 164 ($M^{+\cdot}$ [^{37}Cl] – H – CO, 45), 162 ($M^{+\cdot}$ [^{35}Cl] – H – CO, 45), 127 (63).

2-Chloro-6-(1,3-dioxolan-2-yl)quinoline 63 (Scheme 4-7)

A mixture of 2-chloro-6-formylquinoline **57** (256 mg, 1.33 mmol), ethylene glycol (91.0 mg, 1.47 mmol), and *p*-toluenesulfonic acid monohydrate (6.00 mg, 0.0300 mmol), was heated at reflux in toluene (3 mL) with a Dean Stark apparatus attached for 4 hours. After cooling, the toluene solution was washed with a 10% sodium hydroxide solution (2 x 6 mL), water (2 x 6 mL), and brine (6 mL), dried (Na_2SO_4) then the solvent was removed. The residue was

chromatographed on silica gel using 49:1 dichloromethane/ethyl acetate as eluant to afford the analytically pure title compound **63** (153 mg, 49%) (R_f 0.29) as a white powder, mp 99-102°C.



Anal. ($C_{12}H_{10}ClNO_2$) requires C 61.16, H 4.28, N 5.94%; Found C 61.02, H 4.39, N 5.91%. IR (nujol) υ/cm^{-1}: 1587, 1497. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.07-4.23 (4H, m, H4'/H5'), 5.99 (1H, s, H2'), 7.41 (1H, d, *J* = 8.4 Hz, H3), 7.85 (1H, dd, *J* = 1.7, 8.7 Hz, H7), 7.94 (1H, d, *J* = 1.7 Hz, H5), 8.05 (1H, d, *J* = 8.7 Hz, H8), 8.13 (1H, d, *J* = 8.4 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 66.2 (C4'/C5'), 103.8 (C2'), 123.4 (C3), 126.2 (C5), 127.0 (C4a), 129.4 (C8), 129.6 (C7), 137.6 (C6), 139.8 (C4), 148.9 (C8a), 151.9 (C2). *m/z* (EI): 237 ($M^{+\cdot}$ [^{37}Cl], 26%), 236 ($M^{+\cdot}$ [^{37}Cl] - H, 34), 235 ($M^{+\cdot}$ [^{35}Cl], 68), 234 ($M^{+\cdot}$ [^{35}Cl] - H, 67), 192 (37), 190 (100).

General procedure for acetal formation, optimised method (Scheme 4-8)

2-Chloro-6-formylquinoline **57**, the diol (1.1 - 2 equiv.) and *p*-toluenesulfonic acid monohydrate (0.05 - 0.1 equiv.) were heated at reflux in benzene (~4 mL/mmol aldehyde) using a Dean Stark apparatus until thin layer chromatography indicated that all the starting material had been consumed. After cooling, the benzene was removed under reduced pressure, and the residue was taken up into dichloromethane and washed twice with water and once with brine, dried (Na_2SO_4) then the solvent was removed to afford a product that was sufficiently pure for subsequent use. In the case of **67** and **68**, small amounts were purified using silica gel chromatography to obtain analytically pure samples.

2-Chloro-6-(1,3-dioxolan-2-yl)quinoline 63

2-Chloro-6-formylquinoline **57** (1.25 g, 6.56 mmol) was treated with ethylene glycol (0.814 g, 13 mmol) and *p*-toluenesulfonic acid monohydrate (0.062 g, 0.33 mmol) in 25 mL of benzene for 4.5 hours as described above. After workup the title compound **63** was isolated as a white solid (1.20 g, 77%).

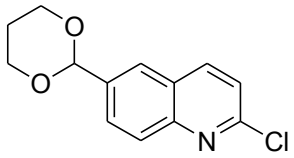
^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.08-4.21 (4H, m, H4'/H5'), 5.98 (1H, s, H2'), 7.41 (1H, d, *J* = 8.6 Hz, H3), 7.85 (1H, dd, *J* = 1.8, 8.7 Hz, H7), 7.94 (1H, d, *J* = 1.8 Hz, H5), 8.05 (1H, d, *J* = 8.7 Hz, H8), 8.12 (1H, d, *J* = 8.6 Hz, H4).

[The complete characterisation for **63** is provided above from its first synthesis, Scheme 4-7.]

2-Chloro-6-(1,3-dioxan-2-yl)quinoline 67

2-Chloro-6-formylquinoline **57** (1.65 g, 8.65 mmol) was treated with 1,3-propanediol (0.724 g, 9.52 mmol) and *p*-toluenesulfonic acid monohydrate (0.083 g, 0.43 mmol) in 25 mL of

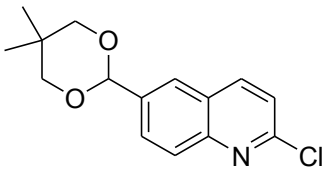
benzene for 5 hours as described above. After workup the title compound was isolated as a white solid (1.68 g, 78%). An analytical sample was obtained after chromatography with 24:1 dichloromethane/ethyl acetate as solvent, mp 111-113°C.



Anal. ($C_{13}H_{12}ClNO_2$) requires C 62.53, H 4.84, N 5.61%; Found C 62.53, H 4.89, N 5.53%. IR (nujol) υ/cm^{-1}: 1582, 1568, 1502. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.51 (1H, dtt, *J* = 1.4, 2.6, 13.6 Hz, H5'$_{eq}$), 2.27 (1H, dtt, *J* = 5.0, 12.4, 13.6 Hz, H5'$_{ax}$), 4.02-4.11 (2H, m, H4'/H6'), 4.30-4.36 (2H, m, H4'/H6'), 5.67 (1H, s, H2'), 7.39 (1H, d, *J* = 8.6 Hz, H3), 7.86 (1H, dd, *J* = 1.7, 8.7 Hz, H7), 7.97 (1H, d, *J* = 1.7 Hz, H5), 8.03 (1H, d, *J* = 8.7 Hz, H8), 8.11 (1H, d, *J* = 8.6 Hz, H4). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 26.4 (C5'), 68.2 (C4'/C6'), 101.4 (C2'), 123.3 (C3), 125.6 (C5), 127.1 (C4a), 129.2 (C8), 129.5 (C7), 138.2 (C6), 140.1 (C4), 148.5 (C8a), 151.7 (C2). *m/z* (EI): 251 ($M^{+\cdot}$ [^{37}Cl], 30%), 250 ($M^{+\cdot}$ [^{37}Cl] - H, 30), 249 ($M^{+\cdot}$ [^{35}Cl], 85), 248 ($M^{+\cdot}$ [^{35}Cl] – H, 50), 192 (50), 190 (100).

2-Chloro-6-(5,5-dimethyl-1,3-dioxan-2-yl)quinoline 68

2-Chloro-6-formylquinoline **57** (0.350 g, 1.83 mmol) was treated with 2,2-dimethyl-1,3-propanediol (0.209 g, 2.0 mmol) and *p*-toluenesulfonic acid monohydrate (0.017 g, 0.09 mmol) in 5 mL of benzene for 4 hours as described above. After workup the title compound **68** was isolated as a white solid (0.395 g, 78%). An analytical sample was obtained after chromatography with dichloromethane as eluant, mp 135-140°C.



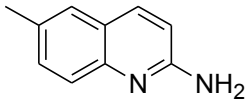
Anal. ($C_{13}H_{12}ClNO_2$) requires C 64.87, H 5.81, N 5.04%; Found C 64.77, H 6.08, N 4.91%. IR (nujol) υ/cm^{-1}: 1584, 1501. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.83 (3H, s, CH_3), 1.31 (3H, s, CH_3), 3.71 (2H, d, *J* = 11.0 Hz, H4'/H6'), 3.82 (2H, d, *J* = 11.0 Hz, H4'/H6'), 5.55 (1H, s, H2'), 7.38 (1H, d, *J* = 8.6 Hz, H3), 7.88 (1H, d, *J* = 1.8, 8.7 Hz, H7), 7.97 (1H. br s, H5), 8.03 (1H, d, *J* = 8.7 Hz, H8), 8.12 (1H, d, *J* = 8.6 Hz, H4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.5 (CH_3), 23.7 (CH_3), 31.0 (C5'), 78.4 (C4'/C6'), 101.6 (C2'), 123.2 (C3), 125.8 (C5), 127.1 (C4a), 129.3 (C8), 129.5 (C7), 138.0 (C6), 140.0 (C4), 148.6 (C8a), 151.7 (C2). *m/z* (EI): 279 ($M^{+\cdot}$ [^{37}Cl], 10%), 278 ($M^{+\cdot}$ [^{37}Cl] - H, 10), 277 ($M^{+\cdot}$ [^{35}Cl], 30), 276 ($M^{+\cdot}$ [^{35}Cl] – H, 12), 192 (50), 190 (100).

General procedure for amination of 2-chloroquinolines[72] (Schemes 4-2, 4-7 and 4-8)

The 2-chloroquinoline (1 equiv.) was treated with acetamide (20 equiv.) and potassium carbonate (5 equiv.) at ~200°C until thin layer chromatography (9:1 dichloromethane/ethanol) indicated the reaction was complete (1-2 hours). After cooling, water was added to the residue, and the aqueous layer was extracted three times with chloroform. The combined organic extracts were washed with brine, dried (Na_2SO_4) then the solvent was removed. The residues were chromatographed over silica gel using 9:1 dichloromethane/ethanol as eluant to afford the pure 2-aminoquinolines. In the case of amines **64**, **69** and **70**, the accompanying quinolin-2(1*H*)-ones were also isolated.

2-Amino-6-methylquinoline 33 (Scheme 4-2)

2-Chloro-6-methylquinoline **35** (0.600 g, 3.38 mmol) was treated with acetamide (3.99 g, 67.6 mmol) and potassium carbonate (2.33 g, 16.9 mmol) as described above. Following workup and chromatography, the title compound **33** (0.288 g, 54%) was isolated as a white fluffy solid, mp 137-145°C. (lit. mp[111] 147-149°C).

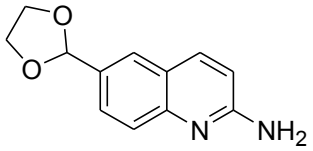


IR (nujol) υ/cm^{-1}: 3453, 3149, 1625, 1610, 1563. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (3H, s, CH_3), 5.04 (2H, br s, NH_2), 6.72 (1H, d, *J* = 9.0 Hz, H3), 7.4 (2H, m, H5/H7), 7.57 (1H, d, *J* = 9.0 Hz, H8), 7.81 (1H, d, *J* = 9.0 Hz, H4). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.8 (CH_3), 112.3 (C3), 124.3 (C4a), 126.5 (C8), 127.3 (C5), 132.4 (C7), 132.8 (C6), 138.2 (C4), 146.6 (C8a), 157.1 (C2). *m/z* (EI): 158 ($M^{+\cdot}$, 100%), 157 ($M^{+\cdot}$ – H, 50), 130 (20).

6-(1,3-Dioxolan-2-yl)quinolin-2-ylamine 64 (Scheme 4-7)

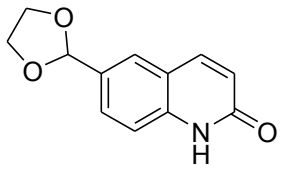
2-Chloro-6-(1,3-dioxolan-2-yl)quinoline **63** (0.275 g, 1.17 mmol) was treated with acetamide (1.38 g, 23.4 mmol) and potassium carbonate (0.806 g, 5.83 mmol) as described above. Following workup and chromatography, the title compound **64** (0.114 g, 46%) (R_f 0.20) was isolated as a pale yellow solid (mp 156-162°C). In addition a yellow solid (0.035 g) (R_f 0.51) was isolated and ^{1}H NMR in $CDCl_3$ revealed this material consisted of approximately a 4:1 mixture of 6-(1,3-dioxolan-2-yl)quinolin-2(1*H*)-one **65** and 6-formylquinolin-2(1*H*)-one **66**.

64



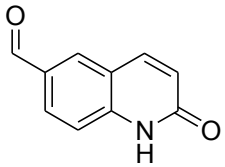
Anal. ($C_{12}H_{12}N_2O_2$) requires C 66.65, H 5.59, N 12.96%; Found C 66.61, H 5.64, N 12.76%. IR (nujol) υ/cm^{-1}: 3419, 3124, 1654, 1621, 1610, 1566. ^{1}H NMR (300 MHz, d_6-acetone) δ: 3.94-4.16 (4H, m, H4'/H5'), 5.80 (1H, s, H2'), 6.00 (2H, br s, NH_2), 6.86 (1H, d, *J* = 9.0 Hz, H3), 7.52 (1H, d, *J* = 8.4 Hz, H8), 7.57 (1H, dd, *J* = 1.7, 8.4 Hz, H7), 7.70 (1H, d, *J* = 1.7 Hz, H5), 7.92 (1H, d, *J* = 9.0 Hz, H4). ^{13}C NMR (75 MHz, d_6-acetone) δ: 66.0 (C4'/C5'), 104.6 (C2'), 113.2 (C3), 123.5 (C4a), 126.5 (C8), 126.8 (C5), 128.4 (C7), 132.9 (C6), 138.4 (C4), 149.4 (C8a), 159.4 (C2). *m/z* (EI): 216 ($M^{+\cdot}$, 40%), 215 ($M^{+\cdot}$ - H, 20), 171 (38), 44 (100).

65



HRMS (ESI): $C_{12}H_{11}NNaO_3^+$ ($M+Na^+$) requires 240.0637; Found 240.0630. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.01-4.21 (4H, m, H4'/H5'), 5.87 (1H, s, H2'), 6.75 (1H, d, *J* = 9.5 Hz, H3), 7.49 (1H, d, *J* = 8.4 Hz, H8), 7.64 (1H, dd, *J* = 1.8, 8.4 Hz, H7), 7.71 (1H, d, *J* = 1.8 Hz, H5), 7.85 (1H, d, *J* = 9.5 Hz, H4), 12.58 (1H, br s, NH). *m/z* (EI): 217 ($M^{+\cdot}$, 5%), 216 ($M^{+\cdot}$ – H, 5), 172 (100).

66



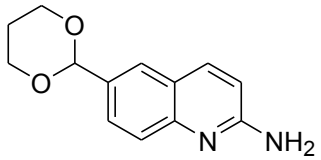
HRMS (ESI): $C_{10}H_7NNaO_2^+$ ($M+Na^+$) requires 196.0374; Found 196.0366. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.80 (1H, d, *J* = 9.6 Hz, H3), 7.63 (1H, d, *J* = 8.6 Hz, H8), 7.92 (1H, d, *J* = 9.6 Hz, H4), 8.03 (1H, dd, *J* = 1.7, 8.6 Hz, H7), 8.09 (1H, d, *J* = 1.7 Hz, H5), 10.02 (1H, s, CHO), 13.00 (1H, br s, NH). *m/z* (EI): 173 ($M^{+\cdot}$, 100%), 172 ($M^{+\cdot}$ - H, 91), 144 ($M^{+\cdot}$ - H – CO, 19).

6-(1,3-Dioxan-2-yl)quinolin-2-ylamine 69 (Scheme 4-8)

2-Chloro-6-(1,3-dioxan-2-yl)quinoline **67** (0.485 g, 1.94 mmol) was treated with acetamide (2.30 g, 38.8 mmol) and potassium carbonate (1.34 g, 9.70 mmol) for 1 hour as described above. Following workup and chromatography, the title compound **69** (0.278 g, 62%) (R_f

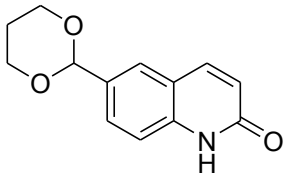
0.21) was isolated as a pale yellow solid (mp 155-170°C). In addition 6-(1,3-dioxan-2-yl)-quinolin-2(1*H*)-one **71** (0.035 g, 8%) (R_f 0.50) was isolated (mp 230-240°C).

69



Anal. ($C_{13}H_{14}N_2O_2$) requires C 67.81, H 6.13, N 12.17%; Found C 67.20, H 6.14, N 11.86%. HRMS (ESI): $C_{13}H_{15}N_2O_2$ ($M+H^+$) requires 231.1134; Found 231.1130. IR (nujol) υ/cm^{-1}: 3441, 3100, 1649, 1624, 1609, 1570. ^{1}H NMR (300 MHz, d_6-acetone) δ: 1.47 (1H, dtt, *J* = 1.4, 2.6, 13.4 Hz, H5'$_{eq}$), 2.07 (1H, dtt, *J* = 5.0, 12.3, 13.4 Hz, H5'$_{ax}$), 3.96-4.05 (2H, m, H4'/H6'), 4.16-4.22 (2H, m, H4'/H6'), 5.59 (1H, s, H2'), 5.88 (2H, br s, NH_2), 6.85 (1H, d, *J* = 8.7 Hz, H3), 7.48 (1H, d, *J* = 8.4 Hz, H8), 7.56 (1H, dd, *J* = 1.9, 8.4 Hz, H7), 7.68 (1H, d, *J* = 1.9 Hz, H5), 7.91 (1H, d, *J* = 8.7 Hz, H4). ^{13}C NMR (75 MHz, d_6-acetone) δ: 26.8 (C5'), 67.8 (C4'/C6'), 102.3 (C2'), 113.1 (C3), 123.5 (C4a), 125.9 (C8), 126.4 (C5), 128.2 (C7), 140.0 (C6), 138.3 (C4), 149.4 (C8a), 159.4 (C2). *m/z* (EI): 230 ($M^{+\cdot}$, 95%), 229 ($M^{+\cdot}$ – H, 35), 171 (100).

71



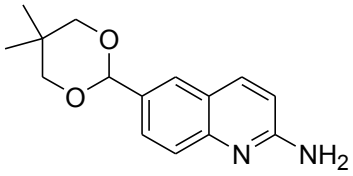
HRMS (ESI): $C_{13}H_{13}NNaO_3^+$ ($M+Na^+$) requires 254.0793; Found 254.0787. IR (nujol) υ/cm^{-1}: 3311, 3150, 1662, 1610, 1568. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.48 (1H, dtt, *J* = 1.4, 2.6, 13.5 Hz, H5'$_{eq}$), 2.25 (1H, dtt, *J* = 5.0, 12.3, 13.5 Hz, H5'$_{ax}$), 3.95-4.09 (2H, m, H4'/H6'), 4.25-4.34 (2H, m, H4'/H6'), 5.57 (1H, s, H2'), 6.71 (1H, d, *J* = 9.3 Hz, H3), 7.39 (1H, d, *J* = 8.6 Hz, H8), 7.63 (1H, dd, *J* = 1.8, 8.6 Hz, H7), 7.72 (1H, d, *J* = 1.8 Hz, H5), 7.81 (1H, d, *J* = 9.3 Hz, H4), 12.06 (1H, br s, NH). ^{13}C NMR (150 MHz, $CDCl_3$/d_6-acetone) δ: 26.3 (C5'), 67.9 (C4'/C6'), 101.4 (C2'), 116.4 (C8), 120.0 (C4a), 122.2 (C3), 126.0 (C5), 129.2 (C7), 134.0 (C6), 139.2 (C8a), 141.6 (C4), 164.8 (C2). *m/z* (EI): 231 ($M^{+\cdot}$, 20%), 230 ($M^{+\cdot}$ – H, 20), 172 (30), 43 (100).

6-(5,5-Dimethyl-1,3-dioxan-2-yl)quinolin-2-ylamine 70

2-Chloro-6-(5,5-dimethyl-1,3-dioxan-2-yl)quinoline **68** (0.180 g, 0.65 mmol) was treated with acetamide (0.765 g, 13 mmol) and potassium carbonate (0.448 g, 3.3 mmol) for 1 hour as described above. Following workup and chromatography, the title compound **70** (0.108 g,

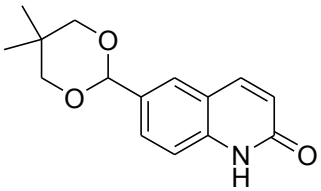
65%) (R_f 0.29) was isolated as a white fluffy solid (mp 192-202°C). In addition 6-(5,5-dimethyl-1,3-dioxan-2-yl)quinolin-2(1*H*)-one **72** (0.012 g, 7%) (R_f 0.58) was isolated as a white solid (mp 242-246°C).

70



Anal. ($C_{15}H_{18}N_2O_2$) requires C 69.74, H 7.02, N 10.84%; Found C 69.70, H 7.14, N 10.92%. HRMS (ESI): $C_{15}H_{19}N_2O_2^+$ ($M+H^+$) requires 259.1447; Found 259.1442. IR (nujol) υ/cm^{-1}: 3452, 3120, 1649, 1623, 1610, 1568. ^{1}H NMR (300 MHz, d_6-acetone) δ: 0.8 (3H, s, CH_3), 1.26 (3H, s, CH_3), 3.67-3.75 (4H, m, H4'/ H6'), 5.49 (1H, s, H2'), 5.98 (2H, br s, NH_2), 6.86 (1H, d, *J* = 8.8 Hz, H3), 7.51 (1H, d, *J* = 8.7 Hz, H8), 7.61 (1H, dd, *J* = 2.0, 8.7 Hz, H7), 7.71 (1H, d, *J* = 2.0 Hz, H5), 7.93 (1H, d, *J* = 8.8 Hz, H4). ^{13}C NMR (50 MHz, d_6-acetone) δ: 22.0 (CH_3), 23.4 (CH_3), 30.8 (C5'), 78.1 (C4'/C6'), 102.5 (C2'), 113.1 (C3), 123.5 (C4a), 126.1 (C8), 126.4 (C5), 128.3 (C7), 133.7 (C6), 138.4 (C4), 149.4 (C8a), 159.4 (C2). *m/z* (EI): 258 ($M^{+\cdot}$,18%), 257 ($M^{+\cdot}$ – H, 8), 171 (95), 41 (100).

72



HRMS (ESI): $C_{15}H_{17}NNaO_3^+$ ($M+Na^+$) requires 282.1106; Found 282.1103. IR (nujol) υ/cm^{-1}: 3435, 1668, 1610, 1568. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.82 (3H, s, CH_3), 1.31 (3H, s, CH_3), 3.69 (2H, d, *J* = 11.0 Hz, H4'/H6'), 3.80 (2H, d, *J* = 11.0 Hz, H4'/H6'), 5.46 (1H, s, H2'), 6.74 (1H, d, *J* = 9.6 Hz, H3), 7.46 (1H, d, *J* = 8.6 Hz, H8), 7.66 (1H, dd, *J* = 1.7, 8.6 Hz, H7), 7.75 (1H, d, *J* = 1.7 Hz, H5), 7.85 (1H, d, *J* = 9.6 Hz, H4), 12.54 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.6 (CH_3), 23.8 (CH_3), 30.9 (C5'), 78.4 (2 x C4'/C6'), 101.7 (C2'), 116.7 (C8), 120.3 (C4a), 122.3 (C3), 126.2 (C5), 129.5 (C7), 134.0 (C6), 139.3 (C8a), 141.8 (C4), 165.2 (C2). *m/z* (EI): 259 ($M^{+\cdot}$, 70%), 258 ($M^{+\cdot}$ – H, 45), 173 (100), 172 (65).

Attempted synthesis of 2-amino-6-formylquinoline 58 from cyclic acetal precursors 64 and 69 using pyridinium tosylate (Scheme 4-9)

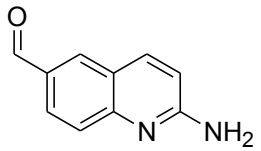
From 69

Acetal **69** (0.035 g, 0.152 mmol) and pyridinium tosylate (0.012 g, 0.0456 mmol) were heated at reflux in acetone (2 mL) with 2 drops of water for 1 hour, when thin layer chromatography suggested that no product had formed. Thus additional pyridinium tosylate (total 0.057 g, 0.228 mmol) was added at 2 hourly intervals with heating at reflux continued for an additional 6 hours in total, at which point, thin layer chromatography again suggested that no product had formed. Thus the solvent was removed, the residue was dissolved in chloroform (4 mL) and washed with 10% sodium bicarbonate (3 x 4 mL), dried (Na_2SO_4) and then the solvent was removed to afford a sticky solid (0.019 g). ^{1}H NMR analysis of the isolated material indicated that it was the starting material **69** (54% recovery).

From 64 (first attempt)

Acetal **64** (0.033 g, 0.152 mmol) and pyridinium tosylate (0.019 g, 0.0760 mmol) were heated at reflux in acetone (3 mL) with 2 drops of water for 1 hour at which point thin layer chromatography indicated that no product had formed. Thus additional pyridinium tosylate (0.038 g, 0.152 mmol) was added and heating at reflux was continued for a further 16 hours. The solvent was removed, and the residue was taken up into chloroform (5 mL) and washed with 10% sodium bicarbonate (3 x 5 mL), dried (Na_2SO_4) and the solvent was removed to afford a yellow 'sticky' solid (0.018 g). ^{1}H NMR analysis of this material indicated it consisted of an approximately 9:1 mixture of the desired aldehyde **58** and the starting material **64**.

58



^{1}H NMR (300 MHz, d_6-DMSO) δ: 6.83 (1H, d, *J* = 9.0 Hz, H3), 7.00 (2H, br s, NH_2), 7.50 (1H, d, *J* = 8.4 Hz, H8), 7.88 (1H, dd, *J* = 1.6, 8.4 Hz, H7), 8.05 (1H, d, *J* = 9.0 Hz, H4), 8.29 (1H, d, *J* = 1.6 Hz, H5), 9.96 (1H, s, CHO). ^{13}C NMR (150 MHz, d_6-DMSO) δ: 113.4 (C3), 121.9 (C4a), 125.8 (C8), 126.8 (C7), 129.5 (C6), 133.4 (C5), 138.1 (C4), 152.1 (C8a), 160.3 (C2), 191.6 (CO). *m/z* (EI): 173 (15%, $M^{+\cdot}$ + H), 172 ($M^{+\cdot}$, 100), 171 ($M^{+\cdot}$ – H, 100), 144 ($M^{+\cdot}$ – H – HCN, 20), 143 (50), 117 (10), 116 ($M^{+\cdot}$ – H – HCN – CO, 40).

From 64 (second attempt)

Acetal **64** (0.045 g, 0.208 mmol) and pyridinium tosylate (0.105 g, 0.416 mmol) were heated at reflux in acetone (5 mL) with 3 drops of water for 12 hours. After cooling, the solvent was removed and the residue was redissolved in chloroform (6 mL) and washed with 10% sodium bicarbonate (3 x 6 mL), and dried (Na_2SO_4), and the solvent was removed, to a afford a light brown solid (0.014 g). ^{1}H NMR analysis indicated that the material consisted of ca. 1:2 mixture of product **58** and **64**. An attempt was made to purify the material using preparative thin layer chromatography, however this resulted in only low recovery (~ 0.001 g) of material.

Attempted synthesis of 2-amino-6-formylquinoline 58 from cyclic acetal 69 with aqueous acids (Scheme 4-10)

Using hydrochloric acid

Acetal **69** (0.026 g, 0.113 mmol) was dissolved in chloroform (2 mL) with stirring, and hydrochloric acid (1M, 2 mL) was added with vigorous stirring. After 10 minutes, thin layer chromatography suggested that all of the staring material had been consumed. The reaction mixture was then added to sodium hydroxide (10%, 5 mL) and a white precipitate formed, that was subsequently extracted with chloroform (3 x 5 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent was removed to afford a white solid (0.006 g). ^{1}H NMR analysis of this material suggested that the desired product had formed, however the purity was low, and a small amount (<10%) of the starting material was present.

Using acetic acid

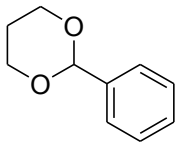
Acetal **69** (0.030 g, 0.136 mmol) was stirred in 80% acetic acid (1 mL) at ca. 80°C for 3 hours. The excess acetic acid was removed under reduced pressure, and sodium hydroxide (10%, 3 mL) was added to the residue. The resulting mixture was extracted with chloroform (3 x 4 mL) and the combined extracts were dried (Na_2SO_4), then the solvent was removed to afford a pale yellow solid (0.003 g). The material was sparingly soluble in d_6-acetone, and ^{1}H NMR analysis indicated it consisted of ca. 2:1 mixture of aldehyde **58** and starting material **69**.

Investigation into de-protection of dioxane acetals using model acetal 2-phenyl-1,3-dioxane 73 (Scheme 4-11)

2-Phenyl-1,3-dioxane 73 (Scheme 4-11A)

A mixture of benzaldehyde **74** (2.50 g, 0.0235 mol), 1,3-propanediol (8.96 g, 0.118 mol) and *p*-toluenesulfonic acid monohydrate (4.48 g, 0.0235 mol) was heated at reflux in benzene for

3 hours with a Dean Stark apparatus attached. After cooling, the benzene was removed under reduced pressure, and the residue was redissolved in ether (100 mL). The ether solution was washed with water (4 x 100 mL) and saturated brine (1 x 100 mL), dried (Na_2SO_4) and then the solvent was removed to afford a crude oil (3.27 g). The crude product was distilled under reduced pressure to afford the pure title compound **73** (1.62 g, 42%) as a colourless oil, bp. 55°C/0.015 mm.



IR (Neat) υ/cm^{-1}: 2965, 2854, 1718, 1602, 1495, 1455, 1379. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.41 (1H, dtt, *J* = 1.5, 2.6, 13.5 Hz, $H5_{eq}$), 2.19 (1H, dtt, *J* = 5.0, 12.3, 13.5 Hz, $H5_{ax}$), 3.89-4.03 (2H, m, H4/H6),* 4.20-4.29 (2H, m, H4/H6),* 5.49 (1H, s, H2),* 7.30-7.50 (5H, m, ArH).
* Data consistent with that presented in literature.[112]

Attempted syntheses of benzaldehyde 74 from 2-phenyl-1,3-dioxane 73 using zirconium tetrachloride/sodium borohydride[82] (Scheme 4-11B)

The following procedure was carried out with stoichiometric ratios and reaction times as presented in Table 4-1 in Chapter 4 (Section 4.3.3.3), using the following quantities of starting acetal **73**: Table entry 1 (0.15 g, 0.91 mmol), entry 2 (0.075 g, 0.46 mmol), entry 3 (0.075 g, 0.46 mmol), entry 4 (0.100 g, 0.61 mmol), entry 5 (0.100 g, 0.61 mmol).

General procedure for attempted conversion of acetal 73 to benzaldehyde 74
To an oven dried 2-necked flask was placed zirconium tetrachloride (1-2 equiv.) and dry THF (~10 mL/mmol zirconium tetrachloride) was added under an inert atmosphere. Sodium borohydride (0.5-0.75 equiv.) was then added and the mixture was stirred at room temperature for 30 minutes. The acetal (1 equiv.) was then added in dry THF (~2 mL/mmol acetal) and the mixture was brought to reflux for the specified time. After cooling, the reaction mixture was added portion-wise to a solution of 10% sodium bicarbonate, and the mixture was stirred until the evolution of gas had ceased. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with saturated brine, dried, then the solvent was removed. The components of the crude material were identified by ^1H NMR analysis of $CDCl_3$ solutions. The identities of compounds **75** and **74** in product mixtures were confirmed by comparison of the chemical shifts obtained with those from authentic samples.

Attempted syntheses of benzaldehyde 74 from 2-phenyl-1,3-dioxane 73 using *p*-toluenesulfonic acid (Scheme 4-12)

First attempt

Acetal **73** (0.054 g, 0.33 mmol) and *p*-toluenesulfonic acid monohydrate (0.031 g, 0.16 mmol) were heated at reflux in acetone (1 mL) for 4 hours. After cooling, the solvent was removed, and the residue was redissolved in dichloromethane (5 mL). The dichloromethane solution was washed with 10% sodium bicarbonate (2 x 5 mL) and water (5 mL), dried (Na_2SO_4) and the solvent was removed. ^{1}H NMR analysis of the recovered material (0.028 g) indicated that a ca. 2:1 mixture of starting material **73** and aldehyde **74** was present. The identity of **74** was confirmed by comparison of the chemical shifts obtained with those from an authentic sample.

Second attempt

Acetal **73** (0.100 g, 0.61 mmol) and *p*-toluenesulfonic acid monohydrate (0.116 g, 0.61 mmol) were heated at reflux in acetone (2 mL) for 14 hours. After cooling, the solvent was removed, and the residue was redissolved in dichloromethane (5 mL). The dichloromethane solution was washed with 10% sodium bicarbonate (2 x 5 mL) and water (5 mL), dried (Na_2SO_4) and the solvent was removed. ^{1}H NMR analysis of the recovered material (0.068 g) indicated that a ca. 3:1 mixture of starting material **73** and aldehyde **74** was present. In addition, substantial impurities were also present, as evidenced by additional signals in the aromatic region of the spectrum. The identity of **74** was confirmed by comparison of the chemical shifts obtained with those from an authentic sample.

7.5.2 Synthesis of compounds presented in Section 4.5

6-[(2-Hydroxyethoxy)methyl]quinolin-2-ylamine 76 from 64 (Scheme 4-13)

To an oven dried flask was placed aluminium chloride (0.037 g, 0.28 mmol) and dry ether (0.5 mL) was added under a nitrogen atmosphere, and the mixture was stirred on ice for 30 minutes before lithium aluminium hydride (0.006 g, 0.16 mmol) was added. Stirring was continued for a further 30 minutes before the addition of acetal **64** (0.029 g, 0.13 mmol) in dry THF (2 mL) over 10 minutes. The resulting mixture was stirred overnight at room temperature. The mixture was then carefully added portion-wise to saturated ammonium chloride (2 mL), and the resulting mixture was extracted with 3:1 chloroform/isopropanol (3 x 1.5 mL), washed with saturated brine (3 mL), dried (Na_2SO_4) then the solvent was removed, to afford a sticky solid (0.005 g). ^{1}H NMR analysis of the isolated material indicated it consisted of a ca. 9:1 mixture of the target alcohol **76** and the aldehyde **58**.

76

^{1}H NMR (300 MHz, d_6-acetone) δ: 3.04 (1H, br s, OH), 3.54-3.57 (2H, m, AA' portion of AA'BB', H1), 3.66-3.69 (2H, m, BB' portion of AA'BB', H2), 4.59 (2H, s, ArC$\underline{H_2}$O), 5.89 (2H, br s, NH_2), 6.84 (1H, d, *J* = 8.7 Hz, H3'), 7.45-7.49 (2H, m, H7'/H8'), 7.59 (1H, br s, H5), 7.88 (1H, d, *J* = 8.7 Hz, H4').

[A more thorough characterisation of this product is provided later (Section 7.5.3) for its synthesis from the 2-(4-methoxybenzylamino)quinoline derivative **94**, Scheme 4-26.]

2-Chloro-6-[(2-hydroxyethoxy)methyl]quinoline 77 (Scheme 4-14, first attempt)

To an oven dried, ice cooled 2 necked flask was placed aluminium chloride (0.840 g, 6.30 mmol), and dry ether (5 mL). The mixture was stirred under nitrogen until a uniform suspension was obtained, and then lithium aluminium hydride (0.057 g, 1.50 mmol) was carefully added portion-wise over 5 minutes. This mixture was stirred for a further 30 minutes, before 2-chloro-6-(1,3-dioxolan-2-yl)quinoline **63** (0.720 g, 3.05 mmol) dissolved in dry THF (7 mL) was added drop-wise over 10 minutes. The resulting mixture was then stirred at room temperature overnight. The reaction mixture was then carefully added portion-wise to ice (ca. 50 g) with stirring in a water bath, before the addition of concentrated sulfuric acid (0.6 mL). The volatiles were then removed under reduced pressure, and the resulting aqueous mixture was extracted with ether (3 x 30 mL). The combined organic extracts were washed with brine (1 x 100 mL), dried (Na_2SO_4) then the solvent was removed to afford a pale brown residue. ^{1}H NMR analysis of this material suggested it consisted of a ca. 4:1 mixture of the desired alcohol **77** and the aldehyde **57**.

77

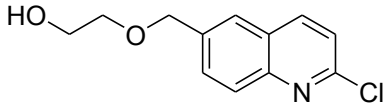
^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.14 (1H, br s, OH), 3.54-3.57 (2H, m, AA' portion of AA'BB', H1), 3.70-3.73 (2H, m, BB' portion of AA'BB', H2), 4.66 (2H, s, $ArCH_2$), 7.19 (1H, d, *J* = 8.4 Hz, H3'), 7.52 (1H, dd, *J* = 1.8, 8.7 Hz, H7'), 7.58 (1H, br s, H5'), 7.80 (1H, d, *J* = 8.7 Hz, H8'), 7.88 (1H, d, *J* = 8.4 Hz, H4').

[A more thorough characterisation of compound **77** is provided below for its synthesis from the optimised method.]

2-Chloro-6-[(2-hydroxyethoxy)methyl]quinoline 77 (Scheme 4-14, optimised method)

To an oven dried, ice cooled 2 necked flask was placed aluminium chloride (0.988 g, 7.41 mmol), and dry THF (6 mL). The mixture was stirred under nitrogen until a uniform suspension was obtained, then lithium aluminium hydride (0.161 g, 4.24 mmol) was carefully added portion-wise over 5 minutes. This mixture was stirred for a further 30 minutes, before 2-chloro-6-(1,3-dioxolan-2-yl)quinoline **63** (0.832 g, 3.53 mmol) dissolved in dry THF (11 mL)

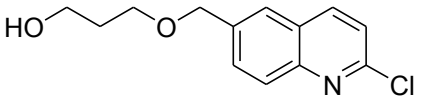
was added drop-wise over 10 minutes. The resulting mixture was then heated at reflux for 4 hours at which point thin layer chromatography (3:1 dichloromethane/ethyl acetate) indicated that all of the starting material had been consumed. After cooling, the reaction mixture was carefully added portion-wise to ice (ca. 100 g) with stirring in a water bath, before the addition of concentrated sulfuric acid (1.4 mL). The THF was then removed under reduced pressure, and the resulting aqueous mixture was extracted with dichloromethane (3 x 80 mL). The combined organic extracts were washed with brine (1 x 250 mL), dried (Na_2SO_4) then the solvent was removed to afford a pale brown solid. This material was chromatographed over silica gel using 3:1 dichloromethane/ethyl acetate as eluant to afford the title compound **77** (0.621 g, 74%) (R_f 0.20) as fluffy white crystals, mp 51-53°C.



Anal. ($C_{12}H_{12}ClNO_2$) requires C 60.64, H 5.09, N 5.89%; Found C 60.41, H 5.07, N 5.86%. IR (Nujol) υ/cm^{-1}: 3332 (OH), 1586, 1565, 1499. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.17 (1H, br s, OH), 3.65-3.68 (2H, m, AA' portion of AA'BB', H1), 3.80-3.83 (2H, m, BB' portion of AA'BB', H2), 4.73 (2H, s, $ArCH_2$), 7.38 (1H, d, *J* = 8.9 Hz, H3'), 7.70 (1H, dd, *J* = 1.5, 8.5 Hz, H7'), 7.77 (1H, br s, H5'), 8.00 (1H, d, *J* = 8.5 Hz, H8'), 8.08 (1H, d, *J* = 8.9 Hz, H4'). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 62.6 (C2), 72.5 (C1), 73.4 ($ArCH_2$), 123.3 (C3'), 126.4 (C5'), 127.3 (C4'a), 129.5 (C8'a), 130.8 (C7'), 137.8 (C6'), 139.4 (C4'), 148.2 (C8'a), 151.4 (C2'). *m/z* (EI): 239 ($M^{+\cdot}$ [^{37}Cl], 8%), 237 ($M^{+\cdot}$ [^{35}Cl], 24), 194 (8), 192 (24), 178 ($M^{+\cdot}$ [^{37}Cl] - $HO(CH_2)_2O$, 40), 176 ($M^{+\cdot}$ [^{35}Cl] - $HO(CH_2)_2O$, 100).

2-Chloro-6-[(3-hydroxypropoxy)methyl]quinoline 78 (Scheme 4-14)

Essentially the same procedure as that described for the preparation of **77** (optimised method) was used with 1.06 g (7.99 mmol) of aluminium chloride in 6 mL of dry THF, 0.173 g (4.56 mmol) of lithium aluminium hydride, and 0.95 g (3.80 mmol) of 2-chloro-6-(1,3-dioxan-2-yl)quinoline **67** dissolved in 12 mL of dry THF and heating at reflux for 5 hours. Following workup and chromatography (3:1 dichloromethane/ethyl acetate), pure **78** (0.735 g, 77%) (R_f 0.20) was isolated as a white fluffy solid, mp 58-60°C.



Anal. ($C_{13}H_{14}ClNO_2$) requires C 62.03, H 5.61, N 5.56%; Found: C 62.08, H 5.53, N 5.64%. IR (Nujol) υ/cm^{-1}: 3307 (OH), 1585, 1562, 1502. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.91 (2H, quin, *J* = 6.0 Hz, H2), 2.21 (1H, br s, OH), 3.72 (2H, t, *J* = 6.0 Hz, H1), 3.81 (2H, t, *J* = 6.0 Hz, H3), 4.69 (2H, s, $ArCH_2$), 7.38 (1H, d, *J* = 8.4 Hz, H3'), 7.69 (1H, dd, *J* = 1.8, 8.4 Hz, H7'), 7.76 (1H, br s, H5'), 8.0 (1H, d, *J* = 8.4 Hz, H8'), 8.08 (1H, d, *J* = 8.4 Hz, H4'). ^{13}C NMR: (150

MHz, $CDCl_3$) δ: 32.9 (C2), 62.2 (C3), 70.2 (C1), 73.3 ($ArCH_2$), 123.2 (C3'), 126.2 (C5'), 127.3 (C4'a), 129.3 (C8'), 130.9 (C7'), 138.0 (C6'), 139.6 (C4'), 148.0 (C8'a), 151.2 (C2'). *m/z* (EI): 253 ($M^{+\cdot}$ [^{37}Cl], 8%), 251 ($M^{+\cdot}$ [^{35}Cl], 22), 194 (30), 192 (100), 178 ($M^{+\cdot}$ [^{37}Cl] - $HO(CH_2)_3O$, 28), 176 ($M^{+\cdot}$ [^{35}Cl] - $HO(CH_2)_3O$, 68).

Synthesis of tosylates of alcohols 77 and 78 (Scheme 4-16)

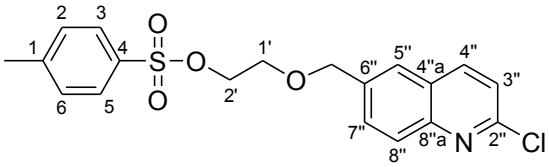
2-Chloro-6-{[2-(toluenesulfonyloxy)ethoxy]methyl}quinoline 82 (Scheme 4-16, Method A)

A mixture of alcohol **77** (0.109 g, 0.457 mmol) and pyridine (0.072 g, 0.915 mmol) was stirred in dichloromethane (2 mL) under a nitrogen atmosphere at room temperature, and recrystallised *p*-toluenesulfonyl chloride (0.087 g, 0.457 mmol) was added. Stirring was continued for 1 hour when thin layer chromatography indicated that some product had formed but substantial starting material was still present. Hence additional *p*-toluenesulfonyl chloride (0.087 g, 0.457 mmol) was added and stirring was continued for a further 66 hours. The mixture was then diluted with dichloromethane (8 mL) and washed with hydrochloric acid (5%, 3 x 10 mL), dried (Na_2SO_4) and the solvent was removed. ^1H NMR of the crude material (0.116 g) indicated that the isolated material consisted of a ca. 3:1 mixture of the desired tosylate **82** and the starting material **77**. In addition, some evidence was seen for the presence of *p*-toluenesulfonic acid or *p*-toluenesulfonyl chloride in the mixture.

82 (Scheme 4-16, Method B)

Alcohol **77** (0.123 g, 0.517 mmol) was stirred in neat pyridine (2 mL) under a nitrogen atmosphere, and *p*-toluenesulfonyl chloride (0.098 g, 0.517 mmol) was added, and the resulting mixture was stirred over night at room temperature. The following day, thin layer chromatography suggested that all of the starting material had not been consumed, so additional portions of *p*-toluenesulfonyl chloride [2 x (0.020 g, 0.103 mmol)] were added at two hourly intervals, and the reaction was left to stir for a further 24 hours. The mixture was then diluted with dichloromethane (15 mL), and washed with hydrochloric acid (5%, 3 x 15 mL) and dried (Na_2SO_4) and the solvent was removed. ^1H NMR of the crude material (0.073 g) indicated that the isolated material consisted of a ca. 1:1 mixture of the desired tosylate **82** and the starting material **77**.

The crude products from both the first and second attempts described above were combined and chromatographed over silica gel using 9:1 dichloromethane/ethyl acetate as eluant to isolate a purified sample of the title compound **82** (0.089 g, 23%) as a dense colourless oil.



^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.40 (3H, s, CH_3), 3.72-3.75 (2H, m, AA' portion of AA'XX', H1''), 4.22-4.24 (2H, m, XX' portion of AA'XX', H2'), 4.66 (2H, s, C$\underline{H}_2$Ar), 7.26-7.30 (2H, m, AA' portion of AA'XX', H2/H6), 7.38 (1H, d, *J* = 9.0 Hz, H3''), 7.60 (1H, dd, *J* = 1.8, 8.4 Hz, H7''), 7.73 (1H, br s, H5''), 7.77-7.94 (2H, m, XX' portion of AA'XX', H3/H5), 7.96 (1H, d, *J* = 8.4 Hz, H8''), 8.07 (1H, d, J = 9.0 Hz, H4''). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.3 (CH_3), 68.7 (C[1' or 2']), 69.9 (C[1' or 2']), 73.3 (Ar$\underline{C}H_2$O), 123.3 (C3''), 126.3 (C5''), 127.3 (C4''a), 128.6 (C3/C5), 129.4 (C8''), 130.5 (C2/C6), 130.6 (C7''), 133.7 (C1), 137.3 (C6''), 139.5 (C4''), 145.5 (C4), 148.2 (C8''a), 151.4 (C2'').

2-Chloro-6-{[3-(toluenesulfonyloxy)propoxy]methyl}quinoline 83 (Scheme 4-16, Method C)

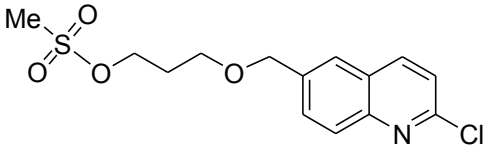
A solution of imidazole (0.025 g, 0.37 mmol), in dichloromethane (1 mL) was stirred at ca. 0°C under a nitrogen atmosphere for 30 minutes before *p*-toluenesulfonyl chloride (0.074 g, 0.39 mmol) was added, and stirring was continued for a further 15 minutes. Alcohol **78** (0.093 g, 0.37 mmol) was then added in dichloromethane (1 mL) over 5 minutes, and stirring was continued for 15 minutes at room temperature, when thin layer chromatography suggested that some product had formed, but substantial starting material was still present. Thus, further imidazole (0.10 g, 1.5 mmol) was added, in addition to two portions of *p*-toluenesulfonyl chloride (2 x [0.037 g, 0.019 mmol]) over two hourly intervals. Stirring was continued for a further 48 hours at room temperature, then the mixture was diluted with dichloromethane (8 mL), and washed with water (3 x 8 mL), dried (Na_2SO_4) and the solvent was removed. ^{1}H NMR analysis of the isolated material (0.174 g) indicated that it consisted of a mixture of starting alcohol **78**, imidazole and either *p*-toluenesulfonyl chloride, or *p*-toluenesulfonic acid.

Synthesis of mesylate 84 and substitution with potassium phthalimide (Scheme 4-17)

2-Chloro-6-{[3-(methanesulfonyloxy)propoxy]methyl}quinoline 84

A mixture of 2-chloro-6-[(3-hydroxypropoxy)methyl]quinoline **78** (0.550 g, 2.20 mmol) and triethylamine (0.265 g, 2.60 mmol) was stirred in dichloromethane (8 mL) under a nitrogen atmosphere at ca. 0°C for 10 minutes before addition of methanesulfonyl chloride (0.302 g, 2.60 mmol). Stirring was continued for 30 minutes when thin layer chromatography revealed that all of the starting material had been consumed. The mixture was diluted with

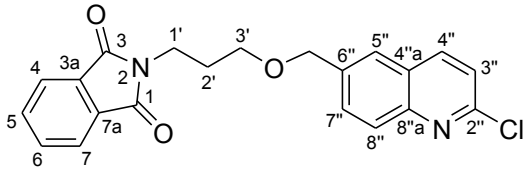
dichloromethane (12 mL) and washed with water (3 x 20 mL), brine (1 x 20 mL), dried (Na_2SO_4) then the solvent was removed. The residue, a dense orange oil was then dried under high vacuum for 1 hour, resulting in the title compound **84** (0.643 g, 89%) that was used for the subsequent step without further purification.



^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.09 (2H, quin, *J* = 6.0 Hz, H2), 3.00 (3H, s, CH_3), 3.67 (2H, t, *J* = 6.0 Hz, H1), 4.41 (2H, t, *J* = 6.0 Hz, H3), 4.68 (2H, s, Ar$C\underline{H}_2$O), 7.38 (1H, d, *J* = 8.9 Hz, H3'), 7.69 (1H, dd, *J* = 1.8, 8.7 Hz, H7'), 7.77 (1H, br s, H5'), 7.98 (1H, d, *J* = 8.7 Hz, H8'), 8.10 (1H, d, *J* = 8.9 Hz, H4'). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 30.1 (C2), 37.8 (CH_3), 66.5 (C3), 67.7 (C1), 73.0 (Ar$\underline{C}H_2$O), 123.1 (C3'), 126.2 (C5'), 127.2 (C4'a), 129.2 (C8'), 130.7 (C7'), 137.7 (C6'), 139.4 (C4'), 147.9 (C8'a), 151.1 (C2').

2-{3-[(2-Chloroquinolin-6-yl)methoxy]propyl}-1*H*-isoindole-1,3(2*H*)-dione 85

2-Chloro-6-{[3-(methanesulfonyloxy)propoxy]methyl}quinoline **84** prepared above (0.643 g, 1.95 mmol) was treated with potassium phthalimide (0.397 g, 2.15 mmol) in DMF (16 mL) with stirring at ca. 80°C for 14 hours. After cooling, the mixture was diluted with ethyl acetate (90 mL) and washed with water (3 x 60 mL), brine (3 x 60 mL), dried (Na_2SO_4) then the solvent was removed to afford the title compound **85** (0.659 g, 89%) as an off-white solid. A portion was recrystallised from methanol to obtain an analytical sample as a white powder, mp 115-117°C.

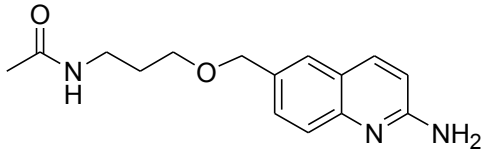


Anal. ($C_{12}H_{17}ClN_2O_3$) requires C 66.23, H 4.50, N 7.36%; Found C 65.93, H 4.39, N 7.37%. IR (Nujol) υ/cm^{-1}: 1770 (CO, asym) 1719 (CO, sym), 1588, 1499. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.06 (2H, quin, *J* = 6.3 Hz, H2'), 3.64 (2H, t, *J* = 6.3 Hz, H3'), 3.87 (2H, t, *J* = 6.3 Hz, H1'), 4.62 (2H, s, Ar$C\underline{H}_2$O), 7.37 (1H, d, *J* = 8.6 Hz, H3''), 7.60-7.65 (3H, m, H5/H6/H7''), 7.70 (1H, br s, H5''), 7.75-7.78 (2H, m, H4/H7), 7.92 (1H, d, *J* = 8.7 Hz, H8''), 8.04 (1H, d, J = 8.6 Hz, H4''). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 29.3 (C2'), 36.4 (C1'), 69.3 (C3'), 73.1 (Ar$C\underline{H}_2$O), 123.0 (C3''), 123.7 (C4/C7), 126.2 (C5''), 127.3 (C4''a), 129.2 (C8''), 130.9 (C7''), 132.9 (C3a/C7a), 134.4 (C5/C6), 138.1 (C6''), 139.5 (C4''), 148.0 (C8''a), 151.1 (C2''), 169.1 (C1/C3). *m/z* (EI): 382 ($M^{+\cdot}$ [^{37}Cl], 2%), 380 ($M^{+\cdot}$ [^{35}Cl], 10), 192 (35), 189 (15), 188 (45), 178 ($M^{+\cdot}$ [^{37}Cl] - $C_8H_4NO_2(CH_2)_3O$, 15), 177 (18), 176 ($M^{+\cdot}$ [^{35}Cl] - $C_8H_4NO_2(CH_2)_3O$, 40), 161 (40), 160 (100).

Attempted synthesis of 80 from 85

6-{[3-(Acetylamino)propoxy]methyl}quinolin-2-ylamine 87 (Scheme 4-18)

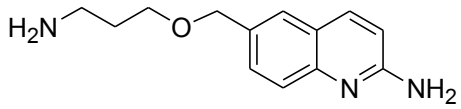
2-{3-[(2-Chloroquinolin-6-yl)methoxy]propyl}-1*H*-isoindole-1,3(2*H*)-dione **85** (0.150 g, 0.394 mmol), was treated with acetamide (0.465 g, 7.88 mmol), and potassium carbonate (0.272 g, 1.97 mmol) at ca. 200 °C for 1 hour. After cooling, the residue was resuspended in saturated brine (5 mL), and this was extracted with 3:1 chloroform/isopropanol (10 x 5 mL). The combined organic extracts were dried (Na_2SO_4) then the solvent was removed to afford 0.342 g of crude material. ^{1}H NMR indicated that a small amount of the title compound **87** was present, however the major component of this material was acetamide. The crude material was partially purified by adsorbing onto strongly acidic Amberlite resin [IR-120(H)], pre-equilibrated in 1:1 methanol/water, and eluting the product with 1% ammonia in 1:1 methanol/water, and then the solvent was removed. The resulting material was resuspended in methanol and filtered, before the filtrate was purified on preparative thin layer chromatography plates using 1:1 dichloromethane/ethanol as eluant (R_f 0.35). The title compound **87** (11 mg, 10%) was isolated as a yellow 'sticky' solid.



HRMS (EI): $C_{15}H_{19}N_3O_2$ ($M^{+\cdot}$) requires 273.1477; Found 273.1475. ^{1}H NMR (600 MHz, d_6-acetone) δ: 1.75 (2H, quin, *J* = 6.6 Hz, H2), 1.80 (3H, s, CH_3), 3.24-3.27 (2H, m, H3), 3.52 (2H, t, *J* = 6.6 Hz, H1), 4.55 (2H, s, Ar$C\underline{H}_2$), 5.83 (2H, br s, NH_2), 6.85 (1H, d, *J* = 9 Hz, H3'), 6.99 (1H, br s, $CH_2N\underline{H}CO$), 7.47 (1H, dd, *J* = 1.8, 8.4 Hz, H7'), 7.5 (1H, d, *J* = 8.4 Hz, H8'), 7.58 (1H, m, H5'), 7.89 (1H, d, *J* = 9 Hz, H4'). ^{13}C NMR (150 MHz, d_6-acetone) δ: 22.5 (CH_3), 30.7 (C2), 37.4 (C3), 68.7 (C1), 73.3 (Ar$\underline{C}H_2$), 113.1(C3'), 123.9 (C4'a), 126.7 (C8'), 127.1 (C5'), 130 (C7'), 133.2 (C6'), 138.1 (C4'), 148.6 (C8'a), 159.1 (C2'), 169.8 (CO). *m/z* (EI): 273 ($M^{+\cdot}$, 15%), 173 (90), 158 ($M^{+\cdot}$ - $CH_3CONH(CH_2)_3O$ + H, 90), 157 ($M^{+\cdot}$ - $CH_3CONH(CH_2)_3O$, 80), 60 (90), 59 (90), 44 (100).

6-[(3-Aminopropoxy)methyl]quinolin-2-ylamine 80 (Scheme 4-19)

6-{[3-(Acetylamino)propoxy]methyl}quinolin-2-ylamine **87** (11 mg, 0.402 mmol) was heated at reflux in 20% aqueous sodium hydroxide (0.5 mL) for 36 hours. After cooling, the water was removed under reduced pressure, and the residue was suspended in 3:1 chloroform/isopropanol (1 mL), filtered, then the solvent was removed to afford the title compound **80** (2.2 mg, 24%) as a 'sticky solid'.



^{1}H NMR (200 MHz, d_6-acetone) δ: 1.3 (2H, br s, $CH_2N\underline{H}_2$), 1.85 (2H, quin, *J* = 6.6 Hz, H2'), 3.22-3.30 (2H, m, H3'), 3.52-3.58 (2H, m, H1'), 4.55 (2H, s, $ArC\underline{H}_2O$), 6.84 (1H, d, *J* = 8.8 Hz, H3), 7.48-7.54 (2H, m, H7/H8), 7.57 (1H, br s, H5), 7.88 (1H, d, *J* = 8.8 Hz, H4). ^{13}C NMR (50 MHz, d_6-acetone) δ: 32.1 (C2'), 48.6 (C3'), 68.9 (C1'), 73.3 ($Ar\underline{C}H_2O$), 113.0 (C3), 124.0 (C4a), 126.8 (C8), 127.0 (C5), 130.0 (C7), 133.3 (C6), 138.0 (C4). *m/z* (EI): 231 ($M^{+\cdot}$, 3%), 174 (20), 173 (100), 158 ($M^{+\cdot}$ - $H_2N(CH_2)_3O$ + H, 15), 157 ($M^{+\cdot}$ - $H_2N(CH_2)_3O$, 50).

Synthesis of amines 76 and 81 using method of Kóródi (Scheme 4-20)

6-[(2-Hydroxyethoxy)methyl]quinolin-2-ylamine 76

A mixture of 2-chloroquinoline **77** (0.122 g, 0.513 mmol), acetamide (0.606 g, 10.3 mmol) and potassium carbonate (0.355 g, 2.57 mmol) was heated at ca. 200°C for 45 minutes, at which point thin layer chromatography indicated that all of the starting material had been consumed. Saturated brine (20 mL) was added to the residue, and the resulting mixture was extracted with 3:1 chloroform/isopropanol (3 x 10 mL), dried (Na_2SO_4) and the solvent was removed. ^{1}H NMR of the residue indicated that it consisted of mostly acetamide, and a small amount of desired product. The residue was purified by preparative thin layer chromatography, over two silica gel plates, using 3:1 dichloromethane/ethanol as eluant with successive developments, to afford the purified product **76** (0.003 g, 3%) as a white solid.

^{1}H NMR (300 MHz, d_6-Acetone) δ: 1.29 (1H, br s, OH), 3.56-3.59 (2H, m, AA' portion of AA'BB', H1), 3.67-3.70 (2H, m, BB' portion of AA'BB', H2), 4.61 (2H, s, $ArC\underline{H}_2O$), 5.95 (2H, br s, NH_2), 6.87 (1H, d, *J* = 8.7 Hz, H3'), 7.48-7.51 (2H, m, H7'/H8'), 7.61 (1H, br s, H5'), 7.91 (1H, d, *J* = 8.7 Hz, H4').

[A more thorough characterisation of this product is provided below (Section 7.5.3) for its synthesis from the 2-(4-methoxybenzylamino)quinoline derivative **94**, Scheme 4-26.]

6-[(3-Hydroxypropoxy)methyl]quinolin-2-ylamine 81 (first attempt)

A mixture of 2-chloroquinoline 78 (0.125 g, 0.497 mmol), acetamide (0.586 g, 9.93 mmol) and potassium carbonate (0.343 g, 2.48 mmol) was heated at ca. 200°C for 45 minutes. After cooling, some of the excess acetamide was removed by sublimation under reduced pressure (oil bath temperature ca. 80°C at 0.02 mm) for 1 hour. The residue was resuspended in methanol (6 mL) and filtered and the solvent was removed. The residue (0.245 g) was chromatographed over silica gel using 3:1 dichloromethane/ethanol as eluant to afford a partially purified sample of the title compound **81** (0.010 g). This sample was purified again

using preparative thin layer chromatography with a silica gel plate and developing twice using 3:1 dichloromethane/ethanol as eluant, to afford essentially pure **81** (0.003 g, 3%) as a while solid.

^{1}H NMR (300 MHz, d_6-acetone) δ: 1.80 (2H, quin, *J* = 6.3 Hz, H2), 3.60 (2H, t, *J* = 6.3 Hz, H[1 or 3]), 3.65 (2H, t, *J* = 6.3 Hz, H[1 or 3]), 4.56 (2H, s, ArC$\underline{H_2}$O), 5.83 (2H, br s, N$\underline{H_2}$), 6.85 (1H, d, *J* = 8.7 Hz, H3'), 7.47 (1H, dd, *J* = 1.8, 8.5 Hz, H7'), 7.51 (1H, d, *J* = 8.5 Hz, H8'), 7.58 (1H, br s, H5') 7.90 (1H, d, *J* = 9.0 H2, H4').
[A more thorough characterisation of this product is provided below (Section 7.5.3) for its synthesis from the 2-(4-methoxybenzylamino)quinoline derivative **93**, Scheme 4-26.]

6-[(3-Hydroxypropoxy)methyl]quinolin-2-ylamine 81 (second attempt)
A mixture of 2-chloroquinoline 78 (0.115 g, 0.477 mmol), acetamide (0.564 g, 9.53 mmol) and potassium carbonate (0.340 g, 2.38 mmol) was heated at ca. 200°C for 45 minutes. After cooling, water was added (10 mL), and the aqueous mixture was extracted with 3:1 chloroform/isopropanol (8 x 10 mL). The combined organic layers were washed with saturated brine (80 mL) dried (Na_2SO_4) and the solvent was removed. The residue was loaded onto a strongly acidic ion exchange column [Amberlite IR-120(H)] that had been pre-equilibrated with 1:1 methanol/water. Additional 1:1 methanol/water was eluted through the column (ca. 50 mL), before eluting retained analytes with a solution of 1% ammonia in 1:1 methanol/water (ca. 50 mL). The solvent was removed and ^{1}H NMR analysis of the isolated material (0.010 g) indicated that it consisted largely of the desired amine **81**, accompanied by some impurities that gave rise to small signals in both the aromatic and aliphatic regions of the spectrum. Yield, 9% (assuming pure **81**).

The 200 MHz, ^{1}H NMR spectrum of the product in d_6-acetone was consistent with those obtained for **81** described above (first attempt) and below (Section 7.5.3) for its synthesis from the 2-(4-methoxybenzylamino)quinoline derivative 93, Scheme 4-26.)

Investigation into protection of alcohol 78 as a 4-methoxybenzyl ether (Scheme 4-21)

2-Chloro-6-({3-[(4-methoxybenzyl)oxy]propoxy}methyl)quinoline 88 (Method A)
Sodium hydride [as 80% mineral oil dispersion] (0.021 g, 0.685 mmol) was placed into an oven dried 2-necked round bottom flask, and dry THF (4 mL) was added under a nitrogen atmosphere. The mixture was stirred on an ice bath for 10 minutes before the drop-wise addition of alcohol **78** (0.15 g, 0.596 mmol) dissolved in dry THF (2.5 mL) over 10 minutes. The ice bath was removed and the resulting mixture was stirred at room temperature for a

further 30 minutes before 4-methoxybenzyl chloride (0.103 g, 0.656 mmol) was added in dry THF (0.5 mL) over 5 minutes. The resulting mixture was stirred at room temperature for 36 hours, then the mixture was carefully added to saturated ammonium chloride (10 mL). The THF was then removed under reduced pressure, and the remaining aqueous solution was extracted with dichloromethane (3 x 8 mL). The combined organic extracts were washed with brine (20 mL) dried (Na_2SO_4) and the solvent was removed. ^{1}H NMR analysis of the resulting material (0.188 g) indicated that it consisted of mostly 4-methoxybenzyl chloride, accompanied by starting material **78** and unidentified impurities. There was no evidence for the formation of the desired ether **88**.

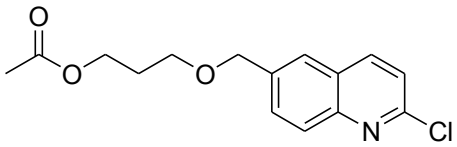
88 (Method B)

Sodium hydride [as 80% mineral oil dispersion] (0.018 g, 0.596 mmol) was placed into an oven dried 2-necked round bottom flask and rinsed twice with hexane under a nitrogen atmosphere. Dry DMSO (0.5 mL) was then added and the solution was stirred with gentle heating, for 30 minutes, during which the solution became a pale green colour. Alcohol **78** (0.10 g, 0.397 mmol) dissolved in dry THF (1 mL) was then added over 10 minutes during which the solution became a darker green colour, promptly followed by an almost black colour. 4-Methoxybenzyl chloride (0.065 g, 0.417 mmol) dissolved in dry THF (1 mL) was then added over 5 minutes, and the resulting mixture was stirred at room temperature for 16 hours. The mixture was then carefully added to saturated ammonium chloride (2 mL) and the resulting mixture was then extracted with ether (3 x 2 mL), the combined organic extracts were washed with saturated brine (6 mL), dried (Na_2SO_4) and the solvent was removed. ^{1}H NMR analysis of the resulting material (0.093 g) indicated that it consisted of mostly 4-methoxybenzyl chloride, accompanied by impurities that gave rise to small broadened signals at chemical shifts approximately equal to those of the starting alcohol **78**. No further attempt was made to characterise the material.

6-{[3-(Acetyloxy)propoxy]methyl}-2-chloroquinoline 89 (Scheme 4-22)

A mixture of alcohol **78** (0.100 g, 0.397 mmol) and triethylamine (0.044 g, 0.0437 mmol) was stirred in dichloromethane (2 mL) under a nitrogen atmosphere at room temperature for 15 minutes when acetyl chloride (0.034 g, 0.437 mmol) in dichloromethane (0.5 mL) was added. Stirring was continued for 1 hour when thin layer chromatography suggested that some product had formed, but substantial starting material remained. Hence further acetyl chloride (0.045 g, 0.573 mmol) in dichloromethane (0.5 mL) was added. After an additional 15 minutes of stirring, a precipitate formed, and thin layer chromatography indicated that all of the starting material had been consumed. The resulting mixture was diluted with extra dichloromethane (8 mL) and washed with water (3 x 7 mL), dried (Na_2SO_4) and the solvent

was removed, to afford the pure ester **89** (0.107 g, 91%) in high yield, as judged by 1H NMR analysis. The material was used in the next step without purification.



^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.93 (2H, quin, *J* = 6.3 Hz, H2), 1.97 (3H, s, CH_3), 3.57 (2H, t, *J* = 6.3 Hz, H1), 4.17 (2H, t, *J* = 6.3 Hz, H3), 4.61 (2H, s, $ArCH_2$), 7.31 (1H, d, *J* = 8.4 Hz, H3'), 7.63 (1H, dd, *J* = 1.8, 8.5 Hz, H7'), 7.70 (1H, br s, H5'), 7.92 (1H, d, *J* = 8.5 Hz, H8'), 8.02 (1H, d, *J* = 8.4 Hz, H4'). ^{13}C NMR: (75 MHz, $CDCl_3$) δ: 21.5 (CH_3), 29.6 (C2), 62.1 (C[1 or 3]), 67.6 (C[1 or 3]), 72.9 ($ArCH_2$), 123.0 (C3'), 126.1 (C5'), 127.2 (C4'a), 129.2 (C8'), 130.6 (C7'), 138.0 (C6'), 139.3 (C4'), 148.0 (C8'a), 151.1 (C2'), 171.6 (CO).

6-{[3-(Acetyloxy)propoxy]methyl}quinolin-2-ylamine 90 (Scheme 4-23)

A mixture of the acetylated 2-chloroquinoline **89** prepared above (0.090 g, 0.306 mmol), acetamide (0.362 g, 6.16 mmol) and potassium carbonate (0.211 g, 1.53 mmol) was heated at ca. 200°C for 1 hour. After cooling, water (8 mL) was added to the residue and this mixture was extracted with chloroform (3 x 8 mL). The combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4) and the solvent was removed. ^{1}H NMR analysis of the resulting material (0.018 g) indicated that the main product was the de-acetylated 2-aminoquinoline **81**. However multiple by-products were also present.

7.5.3 Synthesis of compounds presented in Section 4.6

Investigation into amination of simple 2-chloroquinolines with benzylamine (Scheme 4-24)

Attempted synthesis of 2-(benzylamino)-6-methylquinoline 91 (Scheme 4-24, method A)

A mixture of 2-chloro-6-methylquinoline **35** (0.10 g, 0.563 mmol), benzylamine (0.066 g, 0.619 mmol) and potassium carbonate (0.39 g, 2.80 mmol) was heated at reflux in toluene (2 mL) for 4 hours, at which point no reaction was observed, as judged by thin layer chromatography.

2-(Benzylamino)-6-methylquinoline 91 (Scheme 4-24, Method B)

A mixture of 2-chloro-6-methylquinoline **35** (0.10 g, 0.563 mmol), benzylamine (2 mL) and potassium carbonate (0.39 g, 2.80 mmol) was heated at reflux for 4 hours, at which point thin layer chromatography suggested that substantial product had formed. The heating was

ceased, and the reaction was allowed to cool. The resulting mixture was purified by chromatography on silica gel using 17:3 dichloromethane/ethyl acetate as eluant, from which the desired product **91** (0.096 g, 69%) (R_f 0.36) was isolated as yellow/orange solid. [A full characterisation of **91** is provided below.]

General procedure for benzylamination of simple 2-chloroquinolines (Scheme 4-24, Methods C-F)

The 2-chloroquinoline (1 equiv.) and benzylamine (ca. 25 equiv.) were heated in an oil bath at the specified oil temperature with stirring under a nitrogen atmosphere, for the specified time. After cooling, the excess benzylamine was removed under reduced pressure using a Kugelrohr apparatus, and the residue was purified by column chromatography on silica gel using 17:3 dichloromethane/ethyl acetate as eluant, to afford the pure 2-(benzylamino)quinoline derivatives. In the case of Methods C and D some of the starting 2-chloroquinoline was also recovered.

2-(Benzylamino)-6-methylquinoline 91 (Method C)

2-Chloro-6-methylquinoline **35** (0.10 g, 0.563 mmol) was treated with benzylamine (1.5 mL) at 60°C for 66 hours as described above. Following chromatography the title compound **91** (0.026 g, 19%) was isolated, in addition to recovered starting material **35** (0.082 g, 80%).

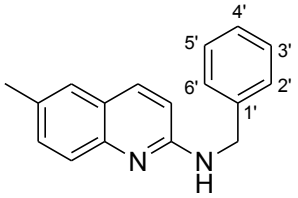
91 (Method D)

2-Chloro-6-methylquinoline **35** (0.10 g, 0.563 mmol) was treated with benzylamine (1.5 mL) at 120°C for 38 hours as described above. Following chromatography the title compound **91** (0.103 g, 74%) was isolated in good yield, in addition to recovered starting material **35** (0.025 g, 25%).

91 (Method E)

2-Chloro-6-methylquinoline **35** (0.070 g, 0.394 mmol) was treated with benzylamine (1.1 mL) at 120°C for 66 hours as described above. Following chromatography the title compound **91** (0.093 g, 95%) was isolated in excellent yield.

91



mp 45-49°C. HRMS (ESI): $C_{17}H_{17}N_2^+$ ($M+H^+$) requires 249.1386; Found 249.1387. IR (Nujol) υ/cm^{-1}: 3418 (NH), 3251 (NH), 1660, 1614, 1504. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.43 (3H, s, CH_3), 4.68 (2H, d, *J* = 5.7 Hz, C$\underline{H}_2$NH), 5.10 (1H, br s, NH), 6.57 (1H, d, *J* = 8.7 Hz, H3), 7.23-7.40 (7H, m, H2'-H6'/H5/H7), 7.61 (1H, d, *J* = 8.7 Hz, H8), 7.72 (1H, d, *J* = 8.7 Hz, H4). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 21.1 (CH_3), 46.0 (CH_2), 111.0 (C3), 123.3 (C3), 123.3 (C4a), 125.4 (C1'), 126.7 (C8), 127.3 (C5), 127.7 (C[2'/6' or 3'/5']), 128.6 (C[2'/6' or 3'/5']), 131.8 (C7), 131.8 (C6), 137.3 (C4), 139.2 (C1'), 145.4 (C8a), 156.1 (C2). *m/z* (EI): 249 ($M^{+\cdot}$ + H, 30%), 248 ($M^{+\cdot}$, 100), 247 ($M^{+\cdot}$ – H, 30), 171 ($M^{+\cdot}$ – C_6H_5, 6), 158 (15), 143 (34), 106 (55).

2-(Benzylamino)quinoline 24 (Method F)

2-Chloroquinoline **11** (0.230 g, 1.4 mmol) was treated with benzylamine (3.8 mL) at 140°C for 30 hours as described above. Following chromatography the title compound **24** (0.318 g, 97%) was isolated as a white waxy solid in excellent yield, mp 97-100°C (lit.[109] 96-97°C).

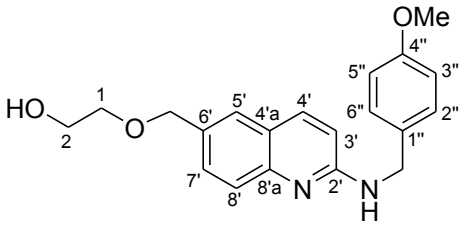
IR (Nujol) υ/cm^{-1}: 3265 (NH), 1622, 1570, 1508. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 4.68 (2H, d, *J* = 5.4 Hz, C$\underline{H}_2$NH), 5.21 (1H, br s, NH), 6.60 (1H, d, *J* = 9.0 Hz, H3), 7.17-7.42 (6H, m, H2'-6'/H6), 7.48-7.59 (2H, m, H5/H7), 7.71 (1H, d, *J* = 8.2 Hz, H8), 7.78 (1H, d, *J* = 9.0 Hz, H4). [Additional data for **24** is included in Section 7.4, for its synthesis by reductive amination from 2-aminoquinoline (Scheme 3-3)].

Investigation into amination and de-benzylation of more complex 2-chloroquinolines with 4-methoxybenzylamine

76 and 81 (Scheme 4-26)

6-[(2-Hydroxyethoxy)methyl]-2-[(4-methoxybenzyl)amino]quinoline 94

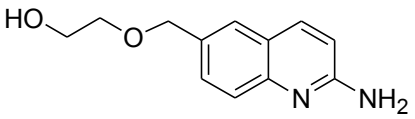
2-Chloro-6-[(2-hydroxyethoxy)methyl]quinoline **77** (0.248 g, 1.04 mmol) was stirred in 4-methoxybenzylamine (3 mL, 3.15 g, 23.0 mmol) at ca. 140°C for 30 hours. After cooling, the excess 4-methoxybenzylamine was removed under reduced pressure using a Kugelrohr apparatus, and the residue was chromatographed over silica gel using 9:1 dichloromethane/ethanol as eluant to afford the title compound **94** (0.347 g, 98%) as a pale orange oil (R_f 0.61) that eventually solidified, mp 85-93°C.



HRMS (ESI-TOF): $C_{20}H_{23}N_2O_3^+$ ($M+H^+$) requires 339.1703; Found 339.1703. IR (Nujol) υ/cm^{-1}: 3519 (NH/OH), 3311 (NH/OH), 1614, 1576, 1537, 1513. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.46 (1H, br s. OH), 3.62-3.65 (2H, m, AA' portion of AA'BB', H1), 3.77-3.80 (2H, m, BB' portion of AA'BB', H2), 3.80 (3H, s, CH_3), 4.62 (2H, d, *J* = 4.5 Hz, $C\underline{H}_2NH$), 4.65 (2H, s, $ArC\underline{H}_2O$), 5.65 (1H, br s, NH), 6.62 (1H, d, *J* = 9.0 Hz, H3'), 6.85-6.90 (2H, m, AA' portion of AA'XX', H3''/H5''), 7.30-7.34 (2H, m, XX' portion of AA'XX', H2''/H6''), 7.51-7.55 (2H, m, H5'/H7'), 7.72 (1H, d, *J* = 8.1 Hz, H8'), 7.81 (1H, d, *J* = 9.0 Hz, H4'). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.1 ($Ar\underline{C}H_2NH$), 55.9 (CH_3), 62.5 (C2), 72.1 (C1), 73.8 ($ArC\underline{H}_2O$), 112.0 (C3'), 114.8 (C3''/C5''), 123.6 (C4'a), 126.3 (C8'), 127.2 (C5'), 129.6 (C2''/C6''), 130.6 (C7'), 131.6 (C1''), 132.7 (C6'), 138.6 (C4'), 147.2 (C8'a), 157.2 (C2'), 159.7 (C4''). ***m/z*** (ESI): 339 ([$M+H^+$], 12%). MS/MS (339): 307 (25), 231 (100), 121 (55).

6-[(2-Hydroxyethoxy)methyl]quinolin-2-ylamine 76

6-[(2-Hydroxyethoxy)methyl]-2-[(4-methoxybenzyl)amino]quinoline **94** (0.200 g, 0.591 mmol) was stirred in TFA (3 mL) at ca. 60°C for 1 hour when thin layer chromatography indicated that all of the starting material had been consumed. After cooling, the excess TFA was removed under reduced pressure, and the residue was dried under high vacuum for an additional 1 hour. The residue was resuspended in 10% aqueous sodium hydroxide (10%, 15 mL), and extracted with 3:1 chloroform/isopropanol (4 x 15 mL). The combined organic layers were washed with a 1:1 mixture of 10% aqueous sodium hydroxide/saturated brine (1 x 60 mL), dried (Na_2SO_4) then the solvent was removed. The residue was then chromatographed over silica gel using 9:1 dichloromethane/methanol as eluant to afford the title compound **76** (0.089 g, 69%) (R_f 0.17) as an off white solid. A portion was re-chromatographed as described above to afford a white solid, mp 120-130°C.

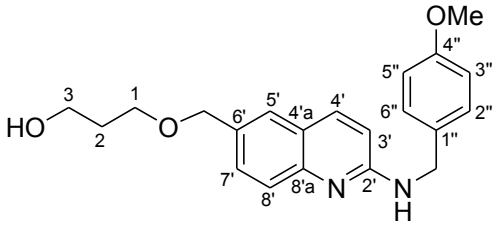


Anal. ($C_{12}H_{14}N_2O_2$) requires C 66.04, H 6.47, N 12.84%; Found C 64.88, H 6.37, N 12.55%. HRMS (ESI): $C_{12}H_{15}N_2O_2^+$ ($M+H^+$) requires 219.1128; Found 219.1127. IR (Nujol) υ/cm^{-1}: 3430 (OH/NH), 3311 (OH/NH), 3205 (OH/NH), 1643, 1625, 1611, 1570, 1507, 1494. ^1H NMR (300 MHz, d_6-acetone) δ: 2.90 (1H, br s, OH), 3.55-3.59 (2H, m, AA' portion of AA'BB', H1), 3.67-3.70 (2H, m, BB' portion of AA'BB', H2), 4.61 (2H, s, $ArC\underline{H}_2O$), 6.85 (1H, d, *J* = 8.9 Hz, H3'), 7.47-7.53 (2H, m, H7'/H8'), 7.60 (1H, br s, H5), 7.89 (1H, d, *J* = 8.9 Hz, H4').

^{13}C NMR (75 MHz, d_6-acetone) δ: 62.1 (C2), 72.8 (C1), 73.5 (Ar<u>C</u>H_2O), 113.2 (C3'), 123.9 (C4'a), 126.7 (C8'), 127.2 (C5'), 130.1 (C7'), 133.2 (C6'), 138.1 (C4'), 148.7 (C8'a), 159.2 (C2'). *m/z* (EI): 218 ($M^{+\cdot}$, 25%), 217 ($M^{+\cdot}$ - H, 4), 174 (15), 173 (40), 172 (30), 158 ($M^{+\cdot}$ - $HO(CH_2)_2O$ + H, 18), 157 ($M^{+\cdot}$ - $HO(CH_2)_2O$, 100).

6-[(3-Hydroxypropoxy)methyl]-2-[(4-methoxybenzyl)amino]quinoline 93

2-Chloro-6-[(3-hydroxypropoxy)methyl]quinoline **78** (0.114 g, 0.453 mmol) was stirred in 4-methoxybenzylamine (1.5 mL, 1.58 g, 11.5 mmol) at ca. 140°C for 30 hours. After cooling, the excess 4-methoxybenzylamine was removed under reduced pressure using a Kugelrohr apparatus, and the residue was chromatographed over silica gel using 9:1 dichloromethane/ethanol as eluant to afford the title compound **93** (0.145 g, 91%) as a pale orange oil (R_f 0.27) that eventually solidified, mp 85-90°C.

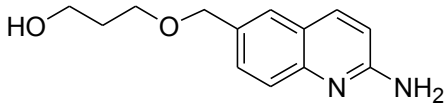


Anal. ($C_{21}H_{24}N_2O_3$) requires C 71.57, H 6.86, N 7.95%; Found C 69.74, H 7.14, N 7.71%. HRMS (ESI-TOF): $C_{21}H_{25}N_2O_3^+$ ($M+H^+$) requires 353.1860; Found 353.1866. IR (Nujol) υ/cm^{-1}: 3418 (NH/OH), 3296 (OH/NH), 1615, 1580, 1536, 1511. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.88 (2H, quin, *J* = 5.8 Hz, H2), 3.50 (1H, br s, OH), 3.69 (2H, t, *J* = 5.8 Hz, H1), 3.78-3.81 (5H, m, H3/CH_3), 4.60 (2H, s, ArC<u>H</u>$_2$O), 4.63 (2H, d, *J* = 5.3 Hz, C<u>H</u>$_2$NH), 5.82 (1H, br s, NH), 6.65 (1H, d, *J* = 9.0 Hz, H3'), 6.85-6.90 (2H, m, AA' portion of AA'XX', H3''/H5''), 7.30-7-35 (2H, m, XX' portion of AA'XX', H2''/H6''), 7.51-7.54 (2H, m, H5'/H7'), 7.71 (1H, d, *J* = 8.4 Hz, H8'), 7.82 (1H, d, *J* = 9.0 H2, H4'). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.8 (C2), 46.0 (<u>C</u>H_2NH), 55.9 (CH_3), 62.2 (C3), 69.6 (C1), 73.7 (Ar<u>C</u>H_2O), 112.1 (C3'), 114.7 (C3''/C5''), 123.7 (C4'a), 126.8 (C8'), 127.0 (C5'), 129.7 (C2''/C6''), 130.2 (C7'), 131.8 (C1''), 132.5 (C6'), 138.1 (C4'), 148.0 (C8'a), 157.4 (C2'), 159.6 (C4''). *m/z* (ESI): 353 ([$M+H^+$], 12%). MS/MS (353): 321 (25), 245 (100), 157 (10), 121 (52).

6-[(3-Hydroxypropoxy)methyl]quinolin-2-ylamine 81

6-[(3-Hydroxypropoxy)methyl]-2-[(4-methoxybenzyl)amino]quinoline **93** (0.100 g, 0.284 mmol) was stirred in TFA (1.5 mL) at ca. 60°C for 1 hour when thin layer chromatography indicated that all of the starting material had been consumed. The reaction was worked up using the same procedure described for the synthesis of **76** above. Following successive chromatography of the crude product over silica gel using 9:1 dichloromethane/methanol as

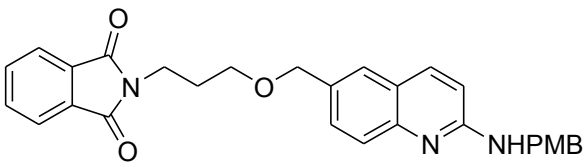
eluant, the title compound **81** (0.039 g, 59%) (R_f 0.13) was isolated as a white solid, mp 118-128°C.



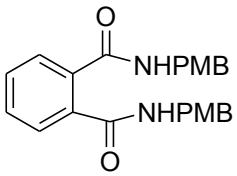
Anal. ($C_{13}H_{16}N_2O_2$) requires C 67.22, H 6.94, N 12.06%; Found C 67.27, H 7.07, N 12.06%. IR (Nujol) υ/cm^{-1}: 3428 (OH/NH), 3311 (OH/NH), 3185 (OH/NH), 1640, 1624, 1610, 1567, 1507, 1483. ^{1}H NMR (300 MHz, d_6-acetone) δ: 1.80 (2H, quin, *J* = 6.3 Hz, H2), 2.95 (1H, br s, OH), 3.60 (2H, t, *J* = 6.3 Hz, H[1 or 3]), 3.65 (2H, t, *J* = 6.3 Hz, H[1 or 3]), 4.56 (2H, s, ArC$\underline{H}_2$O), 5.86 (2H, br s, N$\underline{H}_2$), 6.85 (1H, d, *J* = 9.0 Hz, H3'), 7.47 (1H, dd, *J* = 1.8, 8.6 Hz, H7'), 7.51 (1H, d, *J* = 8.6 Hz, H8'), 7.58 (1H, br s, H5') 7.89 (1H, d, *J* = 9.0 H2, H4'). ^{13}C NMR (75 MHz, d_6-acetone) δ: 33.9 (C2), 59.9 (C3), 68.3 (C1), 73.3 (Ar$\underline{C}$H$_2$O), 113.4 (C3'), 123.7 (C4'a), 125.6 (C8'), 127.1 (C5'), 130.3 (C7'), 133.7 (C6'), 138.8 (C4'), 147.5 (C8'a), 159.0 (C2'). *m/z* (EI): 232 ($M^{+\cdot}$, 50%), 174 (6), 173 (55), 158 ($M^{+\cdot}$ - HO(CH$_2$)$_3$O + H, 40), 157 ($M^{+\cdot}$ - HO(CH$_2$)$_3$O, 100).

Attempted synthesis of 2-{3-[(2-{4-Methoxybenzylamino}quinolin-6-yl)methoxy]-propyl}-1*H*-isoindole-1,3(2*H*)-dione 95 (Scheme 4-27)

The phthalimido 2-chloroquinoline derivative **85** (0.101 g, 0.263 mmol) was stirred with 4-methoxybenzylamine (1 mL) at ca. 140°C for 30 hours under a nitrogen atmosphere. After cooling, the excess 4-methoxybenzylamine was removed under reduced pressure using a Kugelrohr apparatus. The residue was chromatographed on silica gel using 9:1 dichloromethane/ethanol as eluant to afford two materials, one major (0.126 g, R_f 0.56), and one minor (0.023 g, R_f 0.56). The identity of the major material isolated was unclear, as judged by ^{1}H NMR analysis. However the EI mass spectrum suggested the product **96** was present at ionisation temperature 95°C or below. The ^{1}H NMR spectrum of the minor isolate suggested that the desired product **95** was present as a component of a complex mixture, and this was confirmed by the EI mass spectrum.



95
Mol. Wt. 481



96
Mol. Wt. 404

95

m/z (EI): 481 ($M^{+\cdot}$, 5%), 351 (8), 267 (60), 121 (95), 86 (100).

96

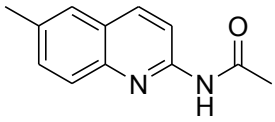
m/z (EI): 404 ($M^{+\cdot}$, 15%), 267 ($M^{+\cdot}$ – $MeOC_6H_4CH_2NH_2$, 50), 121 (100).

7.5.4 Synthesis of compounds presented in Section 4.7

_N_-(6-Methylquinolin-2-yl)acetamide 101 from acetylation of 2-amino-6-methyl-quinoline 33 (Scheme 4-29)

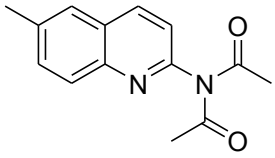
2-Amino-6-methylquinoline **33** (0.150 g, 0.95 mmol) and triethylamine (0.100 g, 0.95 mmol) was stirred in dichloromethane (3 mL) under a nitrogen atmosphere at room temperature for 10 minutes before the drop-wise addition of acetyl chloride (0.074 g, 0.95 mmol) over 10 minutes. At this stage, thin layer chromatography indicated that two products had formed, but not all of the starting material had been consumed. Thus additional triethylamine (0.100 g, 0.95 mmol) and acetyl chloride (0.074 g, 0.95 mmol) was added and thin layer chromatography indicated that all of the starting material had been consumed. The mixture was diluted with additional dichloromethane (7 mL) and washed with water (3 x 10 mL), dried (Na_2SO_4) and the solvent was removed to afford a crude residue (0.210 g). This residue was purified by chromatography on silica gel using 9:1 dichloromethane/ethyl acetate as eluant to afford the title compound **101** (0.045 g, 24%) (R_f 0.15) as white crystals, (mp 181-184°C), in conjunction with the di-acetylated derivative *N*-acetyl-*N*-(6-methyl-quinolin-2-yl)acetamide **102** (0.114 g, 50%) (R_f 0.43) as a white solid, (mp 113-116°C).

101



Anal. ($C_{12}H_{12}N_2O$) requires C 71.98, H 6.04, N 13.99%; Found C 72.12, H 6.08, N 14.00%. IR (Nujol) υ/cm^{-1}: 3240 (NH), 1686, 1661 (CO), 1597, 1577, 1536, 1490. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.17 (3H, s, CH_3CO), 2.49 (3H, s, CH_3Ar), 7.48 (1H, dd, *J* = 1.8, 8.4 Hz, H7), 7.53 (1H, br s, H5), 7.72 (1H, d, *J* = 8.4 Hz, H8), 8.08 (1H, d, *J* = 8.9 Hz, H4), 8.39 (1H, br d, *J* = 8.9 Hz, H3), 9.56 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.0, ($ArCH_3$), 25.4, (CH_3CO), 115.2, (C3), 126.9 (C4a), 127.2 (C5/C8), 132.9 (C7), 135.7 (C6), 138.8 (C4), 145.2 (C8a), 151.3 (C2), 170.1 (CO). *m/z* (EI): 200 ($M^{+\cdot}$, 35%), 159 (10), 158 ($M^{+\cdot}$ – CH_2=C=O, 100), 157 ($M^{+\cdot}$ - CH_3CO, 30).

102



HRMS (EI): $C_{14}H_{14}N_2O_2$ requires 242.1055; Found 242.1055. IR (Nujol) υ/cm^{-1}: 1723 (CO, asym), 1701 (CO, sym), 1597, 1570, 1500. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.32 (6H, s, 2 x CH_3CO), 2.54 (3H, s, $C\underline{H}_3Ar$), 7.27 (1H, d, *J* = 8.6 Hz, H3), 7.58 (1H, dd, *J* = 2.1, 8.7 Hz, H7), 7.63 (1H, br s, H5), 7.95 (1H, d, *J* = 8.7 Hz, H8), 8.18 (1H, d, *J* = 8.4 Hz, H4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.1 ($\underline{C}H_3Ar$), 27.2 (2 x $\underline{C}H_3CO$), 121.8 (C3), 127.0 (C[5 or 8]), 128.2 (C4a), 129.4 (C[5 or 8]), 133.1 (C7), 138.4 (C6), 138.8 (C4), 146.5 (C8a), 151.7 (C2), 173.2 (2 x CO). *m/z* (EI): 242 ($M^{+\cdot}$, 12%), 200 ($M^{+\cdot}$ – CH_2=C=O, 45), 185 (100), 158 (40), 142 (32).

***N*-(6-Methylquinolin-2-yl)acetamide 101 by acetamidation from 2-chloro-6-methylquinoline 35 (Scheme 4-30)**

A mixture of 2-chloro-6-methylquinoline **35** (7.54 g, 0.0424 mol), acetamide (200 g, 3.4 mol), and potassium carbonate (29.33 g, 0.212 mol) was heated at reflux for 14 hours. After cooling, the reaction mixture was added to water (500 mL), and this was extracted with dichloromethane (3 x 300 mL). The combined organic extracts were washed with water (1 x 1000 mL), dried (Na_2SO_4), then the solvent was removed to afford an orange solid (6.3 g). This residue was purified by filtration through silica gel using 17:3 dichloromethane/ethyl acetate as eluant to afford the title compound **101** (3.301 g, 39%) (R_f 0.17) as white crystals. The eluant was then changed to 9:1 dichloromethane/ethanol, and 6-methylquinolin-2(1*H*)-one **38** (2.215 g, 31%) was also isolated as an orange/brown solid.

101

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.23 (3H, s, CH_3CO), 2.51 (3H, s, CH_3Ar), 7.50 (1H, dd, *J* = 2.2, 8.7 Hz, H7), 7.55 (1H, br s, H5), 7.72 (1H, d, *J* = 8.7 Hz, H8), 8.10 (1H, d, *J* = 8.9 Hz, H4), 8.38 (1H, br d, *J* = 8.9 Hz, H3), 8.85 (1H, br s, NH).

[A thorough characterisation of **101** is provided above for its synthesis from 2-amino-6-methylquinoline **33**, Scheme 4-29.]

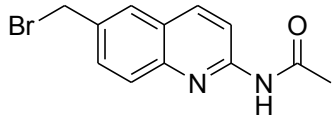
38

^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.41 (3H, s, CH_3), 6.70 (1H, d, *J* = 9.5 Hz, H3), 7.31-7.37 (3H, m, H5/H7/H8), 7.76 (1H, d, *J* = 9.5 Hz, H4), 12.49 (1H, br s, NH).

[Additional data for this compound is included for its explicit synthesis from (2*E*)-*N*-(4-methylphenyl)-3-phenylacrylamide **37**, Scheme 4-2.]

***N*-[6-(Bromomethyl)quinolin-2-yl]acetamide 100 (Scheme 4-31)**

A mixture of *N*-(6-methylquinolin-2-yl)acetamide **101** (1.50 g, 7.5 mmol), *N*-bromosuccinimide (1.47 g, 8.24 mmol), and benzoyl peroxide (0.18 g, 0.75 mmol) was heated at reflux in benzene (10 mL) for 5 hours. After cooling, the benzene was removed under reduced pressure and the residue was dissolved in dichloromethane (50 mL). The dichloromethane solution was then washed with 10% $NaHCO_3$ (3 x 50 mL), dried (Na_2SO_4), then the solvent was removed to afford 1.96 g of a light brown solid. Following chromatography over silica gel using 17:3 dichloromethane/ethyl acetate as eluant, the title compound **100** (1.175 g, 56%) (R_f 0.26) was afforded as a pale yellow fluffy solid, mp 185-187°C.



Anal. ($C_{12}H_{11}BrN_2O$) requires C 51.63, H 3.97, N 10.04%; Found C 51.88, H 4.08, N 9.81%. IR (Nujol) υ/cm^{-1}: 3190, (NH), 1673 (CO), 1602, 1580, 1540, 1494. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.25 (3H, s, CH_3), 4.65 (2H, s, CH_2), 7.69 (1H, dd, *J* = 2.1, 8.7 Hz, H7), 7.78-7.81 (2H, m, H5/H8), 8.14 (1H, d, *J* = 9.0 Hz, H4), 8.43 (1H, br d, *J* = 9.0 Hz, H3), 8.66 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.6 (CH_3), 33.6 (CH_2), 115.4 (C3), 126.4 (C5), 127.4 (C4a), 128.3 (C8), 132.5 (C7), 135.9 (C6), 140.5 (C4), 144.9 (C8a), 152.0 (C2), 170.5 (CO). *m/z* (EI): 280 ($M^{+\cdot}$ [^{81}Br], 6%), 278 ($M^{+\cdot}$ [^{79}Br], 6), 200 ($M^{+\cdot}$ – Br + H, 12), 199 ($M^{+\cdot}$ – Br, 78), 158 ($M^{+\cdot}$ – Br – CH_2=C=O + H, 15), 157 ($M^{+\cdot}$ – Br – CH_2=C=O, 100).

Investigation into substitution with 100 and aliphatic alcohols

Attempted synthesis of *N*-{6-[2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethoxy-methyl]-quinolin-2-yl}acetamide 105 under strongly basic conditions (Scheme 4-33A)

Sodium hydride [as 80% mineral oil suspension] (0.011 g, 0.34 mmol) was placed into an oven dried flask, and dry DMSO (1 mL) was added. The resulting solution was stirred under a nitrogen atmosphere with gentle heating for 30 minutes, during which the solution became a pale green colour. 2-(2-Hydroxyethyl)-1*H*-isoindole-1,3(2*H*)-dione **103** (0.062 g, 0.32 mmol) in dry DMSO (0.5 mL) was then added drop-wise over 10 minutes, and the resulting mixture was stirred at room temperature for 30 minutes. In a separate vessel, the bromomethyl compound **100** (0.090 g, 0.32 mmol) was dissolved in dry THF (2.5 mL) with stirring under a nitrogen atmosphere at room temperature, and then the DMSO solution was added drop-wise via a syringe into the THF solution over 45 minutes. The resulting mixture was stirred at room temperature for 16 hours. The reaction mixture was then carefully added to saturated ammonium chloride (10 mL) and extracted with ethyl acetate (3 x 10 mL). The combined

organic extracts were washed with water (3 x 30 mL) and brine (30 mL) dried (Na_2SO_4) then the solvent was removed. Following chromatography on silica gel, compound **107** (0.021 g, 21%) (R_f 0.48) was isolated as the major product. A small amount of starting material **100** (~0.005 g) in an impure form was also isolated following the chromatographic procedure.

107

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.23 (3H, s, CH_3), 5.00 (CH_2), 7.69-7.74 (4H, m, H5'/H6' / 2 x H[5 or 7 or 8]), 7.80-7.78 (3H, m, H4'/H7' / 1 x H[5 or 7 or 8]), 8.14 (1H, d, *J* = 9.0 Hz, H4), 8.39 (1H, br d, *J* = 9.0 Hz, H3), 8.84 (1H, br s, NH). *m/z* (EI): 345 ($M^{+\cdot}$, 20%), 303 ($M^{+\cdot}$ – CH_2=C=O, 25), 160 (100).

[A complete characterisation of **107** is provided below for its explicit synthesis from **100** and potassium phthalimide, Scheme 4-36.]

Attempted synthesis of *N*-[6-(ethoxymethyl)quinolin-2-yl]acetamide 108 under strongly basic conditions (Scheme 4-33C)

Sodium hydride [as 80% mineral oil suspension] (0.0023 g, 0.10 mmol) was placed into an oven dried flask, and dry DMSO (1 mL) was added. The resulting solution was stirred under a nitrogen atmosphere with gentle heating for 30 minutes, during which the solution became a pale green colour. Ethanol (0.0045 g, 0.10 mmol) in dry THF (0.5 mL) was then added drop-wise over 5 minutes, and the resulting mixture was stirred at room temperature for 30 minutes. Bromomethyl compound **100** (0.025 g, 0.09 mmol) in dry THF (1.5 mL) was then added over 10 minutes, and the resulting mixture was stirred at room temperature for 16 hours. Thin layer chromatography after this time indicated that all of the starting material had been consumed. The mixture was quenched with saturated ammonium chloride (2 mL) and all volatiles were removed under reduced pressure. The residue was resuspended in dichloromethane (2 mL) and filtered through celite, and the solvent was removed to afford the crude residue (0.011 g). ^{1}H NMR analysis of the material did not provide any evidence for formation of the expected product **108**, but suggested that an undesired side reaction(s) had occurred. The identity of the product(s) was not determined.

Attempted synthesis of 108 using silver(I) oxide catalysis (Scheme 4-34A, Method A)

A mixture of bromomethyl derivative **100** (0.044 g, 0.16 mmol), ethanol (0.0080 g, 0.17 mmol), and silver(I) oxide (0.037 g, 0.16 mmol) was stirred in dichloromethane (4 mL) in a vessel that was protected from light, under a nitrogen atmosphere for 22 hours, at which point no reaction was observed, as judged by thin layer chromatography.

108 (Scheme 4-34A, Methods B1-3)

General procedure

The following procedure was carried out according to the stoichiometric ratios as presented in Scheme 4-34A, Methods B1-3.

A mixture of bromomethyl derivative **100** (1 or 2 equiv.), ethanol (1 equiv.), and silver(I) oxide (1 or 2 equiv.) was stirred in THF (~1 mL) in a vessel that was protected from light, under a nitrogen atmosphere for 12 hours, at which point no reaction was observed, as judged by thin layer chromatography.

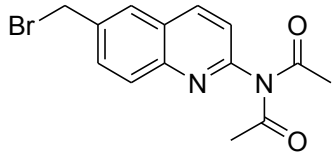
Attempted synthesis of 105 using silver(I) oxide catalysis (Scheme 4-34B)

A mixture of bromomethyl derivative **100** (0.022 g, 0.078 mmol), alcohol **103** (0.015 g, 0.078 mmol), and silver(I) oxide (0.037 g, 0.16 mmol) was stirred in DMF (1.5 mL) in a vessel that was protected from light, under a nitrogen atmosphere for 12 hours. The mixture was then diluted with ethyl acetate (5 mL) and washed with water (4 x 5 mL) and brine (2 x 5 mL), dried (Na_2SO_4) and the solvent was removed to afford a sticky residue (0.013 g). ^{1}H NMR analysis of the material did not provide any evidence for formation of the expected product **105**, but suggested that an undesired side reaction(s) had occurred. The identity of the product(s) was not determined. In addition, several signals were observed consistent with the unreacted alcohol **103**.

Investigation into use of doubly acetylated derivative in substitution chemistry

***N*-Acetyl-*N*-(6-bromomethyl-quinolin-2-yl)acetamide 109 (Scheme 4-35A)**

A mixture of *N*-acetyl-*N*-(6-methyl-quinolin-2-yl)acetamide **102** prepared above (0.099 g, 0.41 mmol), *N*-bromosuccinimide (0.091 g, 0.51 mmol) and benzoyl peroxide (0.010 g, 0.041 mmol) was heated at reflux in benzene (3 mL) for 12 hours. After cooling, the solvent was removed, and the residue was dissolved in dichloromethane (15 mL). The dichloromethane solution was washed with a 10% sodium bicarbonate solution (3 x 15 mL), and dried (Na_2SO_4) and then the solvent was removed. The residue was purified by chromatography on silica gel using 9:1 dichloromethane/ethyl acetate as eluant to afford the title compound **109** (0.040 g, 31%) as a white solid, mp 122-130°C.



^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.35 (6H, s, 2 x CH_3), 4.67 (2H, s, CH_2), 7.34 (1H, d, *J* = 8.5 Hz, H3), 7.80 (1H, dd, *J* = 1.6, 8.8 Hz, H7), 7.89 (1H, d, *J* = 1.6 Hz, H5), 8.06 (1H, d, *J* = 8.8 Hz, H8), 8.27 (1H, d, *J* = 8.5 Hz, H4). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.3 (2 x $\underline{C}H_3CO$), 33.2 (CH_2), 122.6 (C3), 128.0 (C[5 or 8]), 128.1 (C4a), 130.7 (C[5 or 8]), 132.0 (C7), 138.1 (C6), 139.5 (C4), 147.8 (C8a), 153.1 (C2), 173.2 (2 x CO). *m/z* (EI): 322 ($M^{+\cdot}$ [^{81}Br], 5%), 320 ($M^{+\cdot}$ [^{79}Br], 5%), 280 ($M^{+\cdot}$ [^{81}Br] – CH_2=C=O, 25), ($M^{+\cdot}$ [^{79}Br] – CH_2=C=O, 20), 265 (30), 263 (30), 241 ($M^{+\cdot}$ – Br, 10), 199 ($M^{+\cdot}$ – CH_2=C=O – Br, 50), 157 (100).

Attempted synthesis of *N*-acetyl-*N*-[6-(ethoxymethyl)quinolin-2-yl]acetamide 110 under strongly basic conditions (Scheme 3-35B)

Essentially the same procedure as that described above for the attempted coupling of ethanol with the mono-acetylated bromomethyl derivative **100** (Scheme 4-33C) was used with sodium hydride [as 80% mineral oil suspension] (0.0010 g, 0.034 mmol) in dry DMSO (1 mL), addition of ethanol (0.0015 g, 0.034 mmol) in dry THF (0.3 mL), and addition of the di-acetylated bromomethyl derivative **109** (0.010 g, 0.031 mmol) in dry THF (0.9 mL), and stirring overnight at room temperature. Following workup, ^{1}H NMR analysis of the crude residue (0.0015 g) did not provide evidence for the expected product, but instead suggested that an undesired side reaction had occurred. The identity of the material(s) was not determined.

Attempted synthesis of *N*-acetyl-*N*-{6-[2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxymethyl]quinolin-2-yl}acetamide 111 using silver(I) oxide catalysis (Scheme 3-35C)

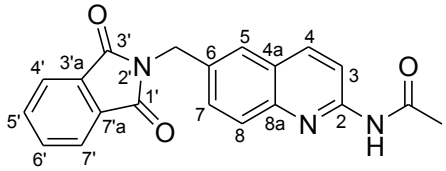
Essentially the same procedure as that described above for the attempted coupling of the mono-acetylated bromomethyl derivative **100** with alcohol **103** (Scheme 4-34B) was used with di-acetylated bromomethyl derivative **109** (0.010 g, 0.031 mmol), **103** (0.0060 g, 0.031 mmol) and silver (I) oxide (0.015 g, 0.62 mmol) with stirring in DMF (1mL). Following workup, ^{1}H NMR analysis of the isolated material (0.017 g) did not provide any evidence for formation of the expected product **111**, but suggested that an undesired side reaction(s) had occurred. The identity of the product(s) was not determined. In addition, several signals were observed consistent with the unreacted alcohol **103**.

Investigation into substitution with 100 and weakly to non-basic nucleophiles

Substitution with potassium phthalimide

***N*-{6-[(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]quinolin-2-yl}acetamide 107 Scheme 4-36A)**

A mixture of *N*-[6-(bromomethyl)quinolin-2-yl]acetamide **100** (0.342 g, 1.22 mmol) and potassium phthalimide (0.238 g, 1.29 mmol) was stirred in DMF (8 mL) at ca. 80°C for 14 hours. After cooling the mixture was diluted with ethyl acetate (60 mL) and washed with water (4 x 60 mL) and brine (2 x 60 mL). During the last three washes a precipitate formed in the organic layer, and this 'sticky' material was collected by filtration. The filtrate was then dried (Na_2SO_4) and the solvent was removed to afford the title compound **107** (0.224 g, 51%) as a pale yellow powder. The glassware used during the workup process was rinsed thoroughly with dichloromethane, and the resulting extract was evaporated under reduced pressure, to afford additional 'sticky' material. The additional products isolated were combined and filtered through silica gel using 17:3 dichloromethane/ethyl acetate as eluant to afford an additional sample of analytically pure **107** (0.123 g, 29%) (R_f 0.13) as a white powder, mp 231-235°C. (Total yield of **107**, 0.347 g, 82%).



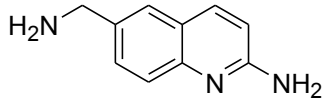
Anal. ($C_{20}H_{15}N_3O_3$) requires C 69.56, H 4.38, N 12.17%; Found C 69.64, H 4.39, N 12.17%. IR (Nujol) υ/cm^{-1}: 3395 (NH), 3334 (NH), 1768 (CO, asym), 1717 (CO, sym), 1706, 1603, 1581, 1493. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.20 (3H, s, CH_3), 4.98 (2H, s, CH_2), 7.69-7.72 (4H, m, H5'/H6' / 2 x H[5 or 7 or 8]), 7.82-7.86 (3H, m, H4'/H7' / 1 x H[5 or 7 or 8]), 8.12 (1H, d, *J* = 8.9 Hz, H4), 8.38 (1H, br d, *J* = 8.9 Hz, H3), 8.73 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.5 (CH_3), 42.0 (CH_2), 115.2 (C3), 124.1 (C4'/C7'), 126.7 (C4a), 128.0 (C5), 128.3 (C8), 131.4 (C7), 132.7 (C3a'/C7a'), 133.9 (C6), 134.8 (C5'/C6'), 139.5 (C4), 146.4 (C8a), 151.9 (C2), 168.7 (C1'/C3'), 169.9 (N<u>C</u>OCH_3). *m/z* (EI): 345 ($M^{+\cdot}$, 85%), 303 ($M^{+\cdot}$ – CH_2=C=O, 100), 157 ($M^{+\cdot}$ – CH_2=C=O – $C_8H_4NO_2$, 15).

6-Aminomethylquinolin-2-ylamine 60 (Scheme 4-36B)

N-{6-[(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]quinolin-2-yl}acetamide **107** (0.216 g, 0.625 mmol) was heated at reflux in 20% aqueous sodium hydroxide (10 mL) for 4 hours. After cooling, this solution was extracted with 3:1 chloroform/isopropanol (5 x 15 mL), the combined organic extracts were dried (Na_2SO_4), then the solvent was removed to afford

0.056 g of a pale yellow solid. ^{1}H NMR analysis of this material indicated it consisted of the title compound **60** accompanied by a small amount (< 5%) of **59**. This material was used in SH3 ligand binding studies without further purification. The presence of **59** was confirmed by mass spectrometry.

60



Yield (assuming pure **60**): 52%

HRMS (EI): $C_{10}H_{11}N_3$ requires 173.0953; Found 173.0949. IR (Nujol) υ/cm^{-1}: 3306 (NH), 3134 (NH), 1646, 1625, 1610, 1590, 1566, 1494. ^{1}H NMR (300 MHz, d_6-acetone) δ: 1.29 (2H, br s, $CH_2N\underline{H}_2$), 4.50 (2H, s, CH_2), 5.81 (2H, br s, $ArN\underline{H}_2$), 6.82 (1H, d, *J* = 8.7 Hz, H3), 7.48 (2H, br s, H7/H8), 7.57 (1H, br s, H5), 7.85 (1H, d, *J* = 8.7 Hz, H4). ^{13}C NMR (75 MHz, d_6-acetone) δ: 55.5 (CH_2), 112.8 (C3), 124.1 (C4a), 126.5 (C5/C8), 130.5 (C7), 135.4 (C6), 137.9 (C4), 148.2 (C8a), 158.8 (C2). *m/z* (ESI): 174 ([M+H]$^+$, 100%). *m/z* (EI): 173 ($M^{+\cdot}$, 70%), 172 ($M^{+\cdot}$ - H, 50), 157 ($M^{+\cdot}$ - NH_2, 32), 145 ($M^{+\cdot}$ – H – HCN, 35), 43 (100).

59

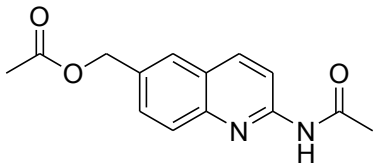
HRMS (EI): $C_{10}H_{10}N_2O$ requires 174.0793; Found 174.0791. *m/z* (ESI): 175 ([M+H]$^+$, 10%). *m/z* (EI): 174 ($M^{+\cdot}$, 10%).

[A more thorough characterisation of **59** is included for its explicit synthesis from **112** below, (Scheme 4-38).]

Substitution with potassium acetate

***N*-{[6-(Acetyloxy)methyl]quinolin-2-yl}acetamide 112 (Scheme 4-37)**

A mixture of *N*-[6-(bromomethyl)quinolin-2-yl]acetamide **100** (0.350 g, 1.25 mmol) and potassium acetate (0.246 g, 2.50 mmol) was stirred in DMF (8 mL) at ca. 80°C for 14 hours. After cooling, the mixture was diluted with ethyl acetate (60 mL) and washed with water (3 x 60 mL) and brine (3 x 60 mL). The resulting ethyl acetate solution was dried (Na_2SO_4), then the solvent was removed to afford the title compound **112** (0.282 g, 87%) as a yellow solid. This material could be used for further synthesis without any additional purification, however a portion was further purified by silica gel chromatography using 3:1 dichloromethane/ethyl acetate as eluant (R_f 0.25) to afford a white solid, mp 140-145°C.

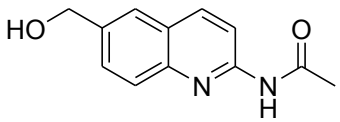


Anal. ($C_{14}H_{14}N_2O_3$) requires C 65.11, H 5.46, N 10.85%; Found C 64.96, H 5.66, N 10.04%. HRMS (EI): $C_{14}H_{14}N_2O_3$ requires 258.1004; Found 258.1004. IR (Nujol) υ/cm^{-1}: 3309 (NH), 1699 (CO), 1689 (CO), 1602, 1580, 1493. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15 (3H, s, CH_3CO_2), 2.23 (3H, s, CH_3CON), 5.26 (2H, s, CH_2), 7.64 (1H, dd, *J* = 1.7, 8.6 Hz, H7), 7.77 (1H, d, *J* = 1.7 Hz, H5), 7.81 (1H, d, *J* = 8.6 Hz, H8), 8.17 (1H, d, *J* = 8.9 Hz, H4), 8.44 (1H, br d, *J* = 8.9 Hz, H3), 8.96 (1H, br s, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.5 ($\underline{C}H_3CO_2$), 25.3 ($\underline{C}H_3CON$), 66.4 (CH_2), 115.5 (C3), 126.5 (C4a), 127.6 (C5), 128.0 (C8), 130.6 (C7), 133.5 (C6), 139.2 (C4), 146.6 (C8a), 152.2 (C2), 170.1 ($CH_3\underline{C}ON$), 171.4 ($CH_3\underline{C}O_2$). *m/z* (EI): 258 ($M^{+\cdot}$, 50%), 216 ($M^{+\cdot}$ – CH_2=C=O, 40), 199 ($M^{+\cdot}$ – CH_3CO_2, 5), 174 ($M^{+\cdot}$ – CH_2=C=O – CH_3CO + H, 38), 157 ($M^{+\cdot}$ – CH_3CO_2 – CH_2=C=O, 100).

_N_-[6-(Hydroxymethyl)quinolin-2-yl]acetamide 113/6-hydroxymethylquinolin-2-ylamine 59 (Scheme 4-38)

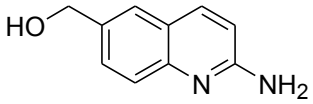
A mixture of *N*-{[6-(acetyloxy)methyl]quinolin-2-yl}acetamide **112** (0.215 g, 0.83 mmol) and potassium carbonate (0.057 g, 0.42 mmol) was stirred in methanol (7 mL) at room temperature for 2 hours. The solvent was removed and the residue was chromatographed over silica gel using 9:1 dichloromethane/methanol as eluant to afford **113** (0.130 g, 72%) (R_f 0.31) as a pale yellow solid, mp 170-174°C. The eluant was changed to 3:1 dichloromethane/methanol and **59** (0.029 g, 20%) (R_f 0.31) was also isolated as a white powder, mp 210-220°C.

113



Anal. ($C_{12}H_{12}N_2O_2$) requires C 66.65, H 5.59, N 12.96%; Found C 66.69, H 5.63, N 12.97%. IR (Nujol) υ/cm^{-1}: 3337 (NH), 1701, 1674 (CO), 1599, 1580, 1493. ^{1}H NMR (300 MHz, d_6-acetone) δ: 2.26 (3H, s, CH_3), 4.40 (1H, t, *J* = 5.6 Hz, OH), 4.79 (2H, d, *J* = 5.6 Hz, CH_2), 7.66 (1H, dd, *J* = 1.8, 8.7 Hz, H7), 7.72 (1H, d, *J* = 8.7 Hz, H8), 7.82 (1H, br s, H5), 8.24 (1H, d, *J* = 8.9 Hz, H4), 8.40 (1H, br d, *J* = 8.9 Hz, H3), 9.66 (1H, br s, NH). ^{13}C NMR (75 MHz, d_6-acetone) δ: 24.6 (CH_3), 64.5 (CH_2), 115.0 (C3), 125.3 (C5), 126.8 (C4a), 128.2 (C8), 129.9 (C7), 138.7 (C6), 140.1 (C4), 147.2 (C8a), 152.5 (C2), 170.2 (CO). *m/z* (EI): 216 ($M^{+\cdot}$, 75%), 174 ($M^{+\cdot}$ – CH_2=C=O, 100), 173 ($M^{+\cdot}$ – CH_2=C=O – H, 38), 157 ($M^{+\cdot}$ – CH_2=C=O – OH, 40).

59

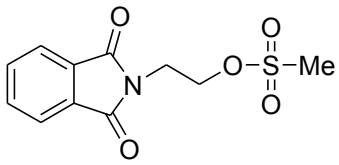


HRMS (EI): $C_{10}H_{10}N_2O$ requires 174.0793; Found 174.0792. IR (Nujol) υ/cm^{-1}: 3458, (NH), 3311 (NH), 1681, 1633, 1626, 1610, 1568, 1491. ^{1}H NMR (300 MHz, d_6-DMSO) δ: 4.55, (2H, s, CH_2), 5.21 (1H, br s, OH), 6.63 (2H, br s, NH_2), 6.77 (1H, d, *J* = 8.9 Hz, H3), 7.41-7.48 (2H, m, H7/H8), 7.56 (1H, br s, H5), 7.92 (1H, d, *J* = 8.9, Hz, H4). ^{13}C NMR (75 MHz, d_6-DMSO) δ: 62.8 (CH_2), 122.1 (C4a), 124.0 (C5), 124.9 (C8), 128.7 (C7), 135.8 (C6), 137.5 (C4), 145.6 (C8a), 158.2 (C2). *m/z* (EI): 175 ($M^{+\cdot}$ + H, 100%), 174 ($M^{+\cdot}$, 55), 173 ($M^{+\cdot}$ - H, 15), 158 ($M^{+\cdot}$ – OH + H, 42), 157 ($M^{+\cdot}$ - OH, 32), 147 ($M^{+\cdot}$ - HCN, 25), 146 ($M^{+\cdot}$ – HCN - H, 42).

Investigation into use of 113 in substitution chemistry

2-{2-(Methanesulfonyloxy)ethyl}-1*H*-isoindole-1,3(2*H*)-dione 114 (Scheme 4-40)

A mixture of alcohol **103** (0.156 g, 0.815 mmol) and triethylamine (0.099 g, 0.979 mmol) was stirred in dichloromethane (3 mL) under a nitrogen atmosphere at ca. 0°C for 10 minutes before addition of methanesulfonyl chloride (0.112 g, 0.979 mmol). Stirring was continued for 30 minutes when thin layer chromatography revealed that all of the starting material had been consumed. The mixture was diluted with dichloromethane (10 mL) and washed with water (3 x 10 mL), brine (1 x 10 mL), dried (Na_2SO_4) then the solvent was removed. The residue, a clear dense oil was then dried under high vacuum for 1 hour, resulting in the title compound **114** (0.176 g, 80%) as a white solid, that was used for the subsequent step without purification.



^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.10 (3H, s, CH_3), 4.04 (2H, t, *J* = 5.6 Hz, H1), 4.48 (2H, t, *J* = 5.6 Hz, H2), 7.72-7.77 (2H, m, H5/H6), 7.83-7.88 (2H, m, H4/H7).

Attempted synthesis of 105 (Scheme 4-40, Method A)

Essentially the same procedure as that described above for the attempted coupling of ethanol with the mono-acetylated bromomethyl derivative **100** (Scheme 4-33C) was used with sodium hydride [as 80% mineral oil suspension] (0.0015 g, 0.050 mmol) in dry DMSO (0.5 mL), addition of alcohol **113** (0.010 g, 0.046 mmol) in dry THF (0.5 mL), and addition of mesylate **114** (0.013 g, 0.049 mmol) in dry THF (1 mL), and stirring at room temperature for

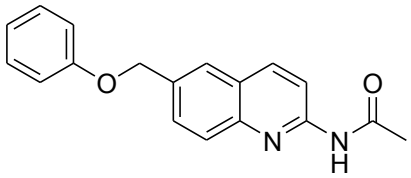
3 hours. The mixture was then added to saturated ammonium chloride (2 mL) and extracted with ethyl acetate (3 x 2 mL). The combined organic extracts were washed with water (3 x 2 mL) and brine (1 x 2 mL), dried (Na_2SO_4) and the solvent was removed. ^{1}H NMR analysis of the resulting material (0.011 g) indicated it consisted of a ca. 1:1 mixture of both starting materials (alcohol **113** and mesylate **114**).

Attempted synthesis of 105 (Scheme 4-40, Method B)

A mixture of alcohol **113** (0.011, 0.053 mmol), mesylate **114** (0.0150 g, 0.053 mmol) and triethylamine (0.0060 g, 0.058 mmol) was heated at reflux in acetonitrile (2 mL) for 5 hours, at which point no reaction was observed, as judged by thin layer chromatography.

***N*-[6-(Phenoxymethyl)quinolin-2-yl]acetamide 115 (Scheme 4-41)**

N-[6-(Bromomethyl)quinolin-2-yl]acetamide **100** (0.180 g, 0.645 mmol), phenol (0.067 g, 0.709 mmol) and potassium carbonate (0.178 g, 1.29 mmol) were stirred in acetonitrile (9 mL) with heating at reflux for 5 hours. After cooling the solvent was removed and the residue was resuspended in ethyl acetate (20 mL), and washed with 5% $NaHCO_3$ (2 x 20 mL), brine (1 x 20 mL), dried (Na_2SO_4) then the solvent was removed. The residue was chromatographed over silica gel using 17:3 dichloromethane/ethyl acetate as eluant to afford the title compound **115** (0.132 g, 70%) (R_f 0.29) as a white solid, mp 148-150°C.

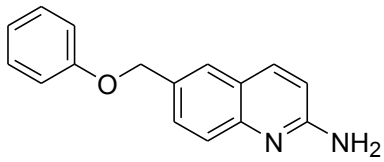


Anal. ($C_{18}H_{16}N_2O_2$) requires C 73.95, H 5.52, N 9.58%; Found C 74.13, H 5.47, N 9.57%. IR (Nujol) υ/cm^{-1}: 3332 (NH), 3246 (NH), 1672 (CO), 1598, 1580, 1537, 1493. ^{1}H NMR (600 MHz, d_6-acetone) δ: 2.27 (3H, s, CH_3), 5.26 (2H, s, CH_2), 6.93 (1H, tt, *J* = 1.1, 7.3 Hz, H4'), 7.04-7.05 (2H, m, H2'/H6'), 7.27-7.30 (2H, m, H3'/H5'), 7.76 (1H, dd, *J* = 1.8, 8.4 Hz, H7), 7.78 (1H, d, *J* = 8.4 Hz, H8), 7.95 (1H, br s, H5), 8.28 (1H, d, *J* = 8.7 Hz, H4), 8.42 (1H, br d, *J* = 8.7 Hz, H3), 9.74 (1H, br s, NH). ^{13}C NMR (150 MHz, d_6-acetone) δ: 24.6 (CH_3), 70.2 (CH_2), 115.3 (C3), 115.8 (C2'/C6'), 121.8 (C4'), 126.8 (C4a), 127.1 (C5), 128.5 (C8), 130.3 (C7), 130.4 (C3'/C5'), 135.1 (C6), 139.0 (C4), 147.3 (C8a), 153.0 (C2), 159.9 (C1'), 170.4 (CO). *m/z* (EI): 292 ($M^{+\cdot}$, 15%), 291 ($M^{+\cdot}$ - H, 3), 249 ($M^{+\cdot}$ – H – CH_2=C=O, 8), 199 ($M^{+\cdot}$ - C_6H_5O, 70), 157 (100).

6-(Phenoxymethyl)quinolin-2-ylamine 116

A mixture of *N*-[6-(phenoxymethyl)quinolin-2-yl]acetamide **115** (0.090 g, 0.308 mmol) and potassium carbonate (0.064 g, 0.462 mmol) was stirred in methanol (5 mL) at ca. 60°C for 2

hours. After cooling, a precipitate formed which was collected by filtration, washed with water and dried, resulting in pure **116** (0.039 g, 51%) as white platelets, mp 190-193°C. The filtrate was then evaporated, and the residue resuspended in chloroform (20 mL). The chloroform solution was washed with water (2 x 20 mL), brine (1 x 20 mL), dried (Na_2SO_4), and the solvent was removed to afford additional **116** (0.026 g, 34%) as an off-white solid. Total yield of **116**: 0.065 g (84%).



Anal. ($C_{16}H_{14}N_2O$) requires C 76.78, H 5.64, N 11.19%; Found C 76.56, H 5.63, N 11.12%. IR (Nujol) υ/cm^{-1}: 3443, (NH), 3306, (NH), 3129 (NH), 1658, 1628, 1596, 1580, 1565, 1484. ^{1}H NMR (300 MHz, d_6-acetone) δ: 5.18 (2H, s, CH_2), 5.88 (2H, br s, NH_2), 6.87 (1H, d, *J* = 8.7 Hz, H3), 6.93 (1H, tt, *J* = 1.2, 7.3 Hz, H4'), 7.02-7.06 (2H, m, H2'/H6'), 7.25-7.32 (2H, m, H3'/H5'), 7.54 (1H, d, *J* = 8.7 Hz, H8), 7.60 (1H, dd, *J* = 1.9, 8.7 Hz, H7), 7.73 (1H, d, *J* = 1.9 Hz, H5), 7.91 (1H, d, *J* = 8.7 Hz, H4). ^{13}C NMR (75 MHz, d_6-acetone) δ: 70.5 (CH_2), 113.2 (C3), 115.8 (C2'/C6'), 121.6 (C4'), 124.0 (C4a), 127.0 (C8), 127.5 (C5), 129.9 (C7), 130.4 (C3'/C5'), 131.5 (C6), 138.0 (C4), 149.1 (C8a), 159.4 (C2), 160.0 (C1'). *m/z* (EI): 250 ($M^{+\cdot}$, 65%), 249 ($M^{+\cdot}$ - H, 10), 171 ($M^{+\cdot}$ – H - C_6H_6, 10), 158 ($M^{+\cdot}$ - C_6H_5O + H, 12), 157 ($M^{+\cdot}$ - C_6H_5O, 100).

7.6 Protein Methods 1: Expression and Purification

7.6.1 General protein methods

The following reagents and methods, are provided in advance of the description of the procedure for protein preparation (Section 7.6.2), and have been included here, as a more detailed reference to some of the techniques used.

7.6.1.1 Common buffers and abbreviations

Common buffers

Milli-Q water at 18.2 mΩ was used for preparation of all buffers, and other biochemical procedures involving aqueous conditions. Hereafter, water refers to Milli-Q water at 18.2 mΩ.

PBS (Phosphate Buffered Saline): disodium hydrogen phosphate (12 mM), sodium chloride (150 mM), dissolved in water, adjusted to pH 7.3, filtered through 0.45 μm nitrocellulose membrane.

TBS (Tris Buffered Saline): tris(hydroxymethyl)aminomethane (Tris) (25 mM), sodium chloride (150 mM) dissolved in water, adjusted to pH ~8.0, filtered through 0.45 μm nitrocellulose membrane.

TTBS: 0.1% v/v Triton-X dissolved in TBS, pH ~8.0, filtered through 0.45 μm nitrocellulose membrane.

Miscellaneous abbreviations

MWCO	molecular weight cut-off
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TEMED	*N*,*N*,*N*',*N*'-tetramethylethylenediamine
Tricine	*N*-[tris(hydroxymethyl)methyl]glycine

7.6.1.2 Purification of Glutathione-*S*-Transferase-SH3 fusion proteins using agarose/glutathione chromatography

Glutathione-*S*-Transferase-SH3 (GST-SH3) fusion proteins were separated from bacterial cell lysis mixtures using an 8 x 2.5 cm id Pharmacia column packed with agarose/glutathione (Zymatrix), attached to a Pharmacia Fast Protein Liquid Chromatography system. The column was equilibrated with at least three column volumes of TBS, followed by at least one column volume of TTBS, at a flow rate of 4 mL/minute. The bacterial extract was then loaded onto the column with the flow rate maintained at 4 mL/minute, and the column was washed with TTBS until all of the lysate had eluted, and then washed extensively with TBS. Bound GST-SH3 fusion protein was eluted by changing the eluant to 10 mM reduced glutathione in TBS pH ~8. An example elution profile is illustrated in Figure 7-1A. The eluted protein was then analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, see below) (Figure 7-1B) prior to the next step. Protein samples were stored at 4°C and with 2% w/v sodium azide added to the solution to final concentration 0.01% w/v.

At the completion of the chromatographic procedure, the column was washed with several volumes of water, and then 20% ethanol. The column was stored in 20% ethanol.

After several uses of one column, the column was regenerated by washing with two column volumes of water, two column volumes of 6M guanidinium hydrochloride, five column volumes of water, two column volumes of 70% ethanol, and a further five column volumes of water, prior to re-equilibration with several column volumes of TBS.

7.6.1.3 Determination of protein concentration using Bradford dye binding assay[113]

10 Microlitres of standard solutions of bovine serum albumin (BSA) with concentrations 0 – 0.8 mg/mL in water, and appropriately diluted samples were pipetted into 96 well flat bottomed clear plates, before the addition of a 3:1 dilution of protein assay concentrate (BIORAD) (in water) to each sample. Absorbance at 600 nm was recorded using an Emax plate reader (Molecular Dynamics). Readings for samples and standards were obtained in duplicate and averages were used for calculation of a standard curve, from which the concentration of the samples was determined.

7.6.1.4 Thrombin digestion

To avoid precipitation of the protein during the digestion, the TBS solution of GST-SH3 obtained above was diluted to a concentration of ~ 1.5 mg/mL. Thrombin (Sigma T 9681, 2.5 units/mg GST-SH3), calcium chloride (to resultant conc. 2.5 mM), and sodium azide (to resultant conc. 0.01% w/v) were added, and the mixture was incubated at room temperature for up to 48 hours, at which point SDS-PAGE was used to confirm the progress of the digestion (Figure 7-1B).

7.6.1.5 Size exclusion chromatography

Size exclusion chromatography was performed using a 60 x 2.5 cm id Pharmacia column packed with Superdex G-75 (Pharmacia) attached to a Pharmacia FPLC system. Prior to loading samples, the column was washed with two column volumes of water and equilibrated overnight in TBS, at a flow rate of 1 mL/minute. GST/SH3 protein mixtures obtained following thrombin digestion were concentrated by ultrafiltration to volumes of ca. 3 mL, and filtered through a 0.45 μm nitrocellulose membrane prior to loading on the column, and the flow rate was maintained at 1 mL/minute. Protein was detected at A_{280} and 5 mL fractions were collected. The appropriate fractions were analysed by SDS-PAGE (Figure 7-2B), prior to pooling, and reconcentration. Protein samples were stored at 4°C and with 2% w/v sodium azide added to the solution to final concentration 0.01% w/v.

An example elution profile is illustrated in Figure 7-2A. At the completion of the chromatographic procedure, the column was washed with two column volumes of water, followed by 20% ethanol. The column was stored in 20% ethanol.

7.6.1.6 PD10 buffer exchange chromatography

PD10 Columns (Pharmacia) were equilibrated in 40 mL of the appropriate buffer prior to loading the sample in a volume of ~ 2 mL. A further 12 mL of buffer was eluted through the column and 12 x 1 mL fractions were collected. The elution was monitored by a Bradford dye binding reaction, and appropriate fractions were pooled, and if necessary, reconcentrated using microsep (Pall Scientific) centrifugal concentrators (3 kDa MWCO for SH3, or 30 kDa MWCO for GST-SH3). Protein samples were stored at 4°C and with 2% w/v sodium azide added to the solution to final concentration 0.01% w/v.

7.6.1.7 SDS-PAGE - sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Gel buffers

3 x gel buffer: 3.0 M tris, 0.3% w/v SDS.

SDS loading buffer: 250 mM tris pH 6.8, 10% w/v SDS, 0.5% w/v bromophenol blue, 50% v/v glycerol, 5% v/v β-mercaptoethanol.

Ingredients for 12.5% acrylamide tris/tricine SDS gels (makes four gels)

Resolving gel: acrylamide (40%, 6.4 mL), glycerol (50%, 5.3 mL), 3 x gel buffer ([3.0 M tris, 0.3% w/v SDS], 8.3 mL), water (5.0 mL), TEMED (35 μL), and ammonium persulfate (10%, 100 μL).

Stacking gel: acrylamide (0.712 mL), 3 x gel buffer (1.95 mL), water (5.20 mL), TEMED (7.5 μL), and ammonium persulfate solution (62.6 μL).

Gels were prepared using a Hoefer gel pouring apparatus.

Electrophoresis protocol

Samples for analysis were diluted 1:1 with a 2 x SDS loading buffer to a final volume of 10 μL and were heated at 100°C for 5 minutes, and given a 'flick' spin prior to loading the gel. Gels were run at 50 milli-amperes using a continuous buffer system (anode: 0.2 M tris pH 8.9,

cathode: 0.1M tris/1% SDS, and 1.0 M tricine). Gels were run until the dye front reached the bottom of the gel.

Completed gels were stained using a Brilliant Blue R stain (1 g Brilliant Blue R in a solution of 3:1:6 methanol/acetic acid/water) with gentle stirring for three hours at 37°C. Gels were then de-stained in a solution of 10:1:9 methanol/acetic acid/water at room temperature for several hours.

7.6.2 Protein preparation methods

7.6.2.1 Bacterial growth media

Luria Broth (LB), per 1 Litre: bacto-tryptone (10 g), yeast extract (5 g), sodium chloride (10 g), dissolved in water, adjusted to pH 7.0 prior to autoclaving.

Minimal A Medium (Min A), per 1 Litre: dipotassium hydrogen phosphate (10.50 g), potassium dihydrogen phosphate (4.50 g), trisodium citrate (1.00 g), ^{15}N-ammonium chloride (0.81 g), dissolved in water, adjusted to pH 7.0 prior to autoclaving. Immediately prior to culture, add solutions (filtered through 0.45 μm nitrocellulose membranes) of magnesium sulfate (1M, 0.8 mL), glucose (20% w/v, 10.0 mL), and thiamine (1% w/v, 0.5 mL).

7.6.2.2 Procedure for preparation of uniformly ^{15}N labelled Tec SH3 protein for NMR spectroscopy

Uniformly ^{15}N labelled Tec SH3 domain was prepared according to developed methods.[52,114] Eschericia coli (E coli) strain BL21-DE3 transfected with the p-Gex 4T-2 expression vector containing regions of cDNA encoding Glutathione-S-Transferase (GST), connected by a thrombin site to amino acids 181-245 of the Mouse Tec IV sequence (representing the SH3 domain) had been previously prepared.[52]

An overnight culture of E coli BL21-DE3 p-GEX 4T-2 in LB medium (+ ampicillin, 0.1 mg/mL) was diluted 1/100 into freshly prepared Min A medium (+ ampicillin, 0.1 mg/mL). This was incubated at 37°C until the medium reached an optical density (at 600 nm) of 0.6, at which point protein expression was induced by the addition of an isopropyl-β-*D*-thiogalactopyranoside (IPTG) solution (100 mM, to resultant conc. 0.2 mM). Incubation of cultures was continued for a further 4 hours, before transferring cultures into 500 mL centrifuge pots, and spinning at 10 000 rpm at 4°C for 10 minutes. The supernatants were discarded, and the pellets were stored at -20°C overnight. The pellets were resuspended in

TTBS, before cell lysis under pressure with addition of an ethanol solution of phenylmethylsulfonyl fluoride (PMSF) (100 mM, to resultant conc. 1 mM). The lysate was then spun at 10 000 rpm at 4°C for 20 minutes, and the supernatant was filtered using a 1.0 μm pre-filter followed by a 0.45 μm nitrocellulose membrane. The filtered sample was chromatographed on an agarose/glutathione affinity column as described above (Figure 7-1A). After chromatography, the concentration of the GST-SH3 fusion protein isolated was estimated using a Bradford Dye binding assay,[113] and the GST-SH3 fusion protein was

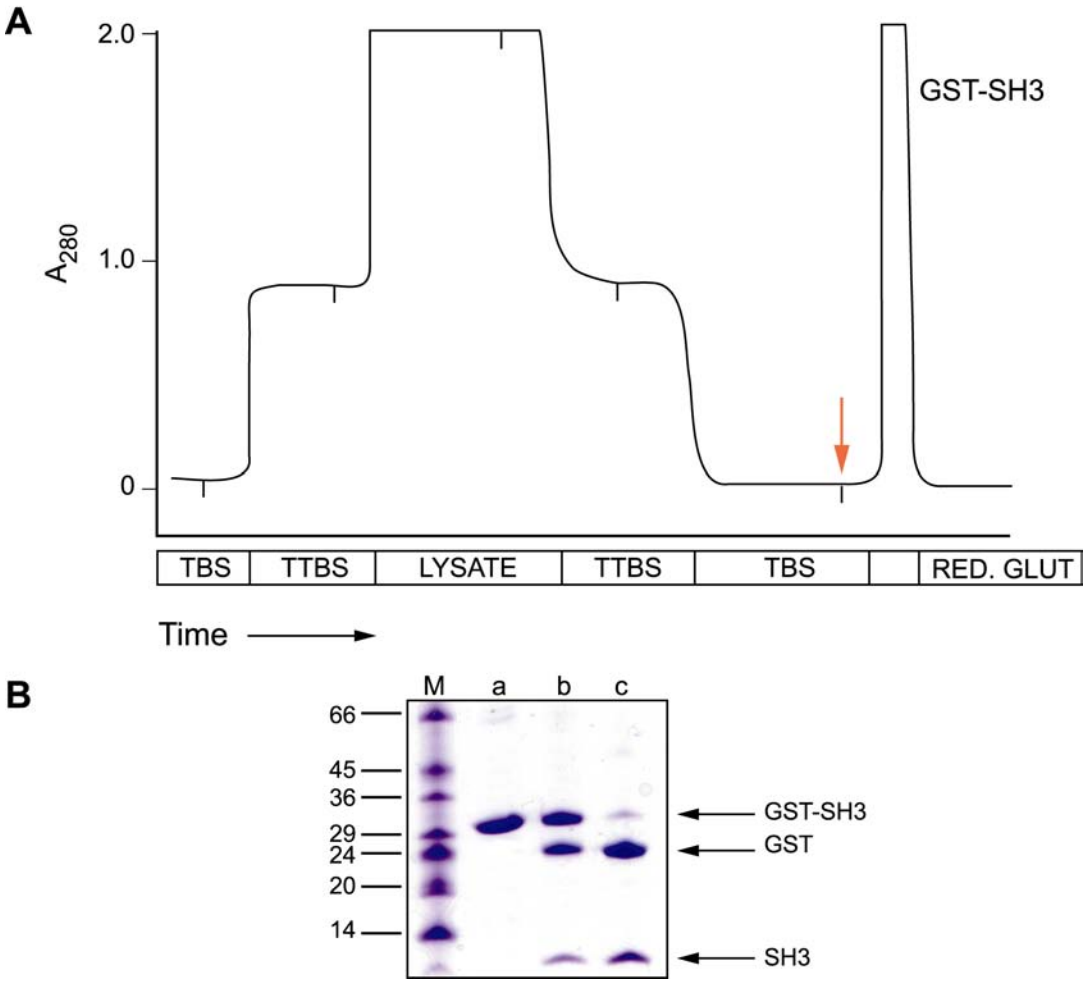


Figure 7-1: Purification and Thrombin digestion of Tec GST-SH3 fusion protein. (A) Fast Protein Liquid Chromatography (FPLC) elution profile for purification of Tec GST-SH3 fusion protein with agarose/glutathione affinity column. The column was equilibrated extensively in TBS followed by TTBS, and the lysate mixture was then loaded, as described in Section 7.6.1.3. Following extensive washing, the eluant was changed to 10 mM reduced glutathione in TBS pH ~8 (denoted with the red arrow) and the purified GST-SH3 protein was eluted. The text in the boxes refers to the reagent responsible for the change in absorbance, and the small marker lines on the trace indicate the points at which the eluants were changed appropriately. The protein was detected at A_{280} with a full scale absorbance of 2. A more detailed description of the agarose/glutathione affinity chromatography protocol is provided in Section 7.6.1.2. (B) SDS-PAGE analysis of protein samples, (a) as eluted from the agarose/glutathione column [as illustrated in (A)], (b) following partial thrombin digestion (12 hours) and (c) following near complete thrombin digestion (36 hours) (as described in Section 7.6.1.3). M refers to the protein markers SDS-7, with the sizes of the reference proteins (in kDa) indicated. A more detailed description of SDS-PAGE analysis protocol is provided in Section 7.6.1.2.

treated with Thrombin as described above (Figure 7-1B). The digested protein solution was concentrated to ca. 3 mL by ultrafiltration using a 3 kDa MWCO membrane. Following size exclusion chromatography on a Superdex G-75 column using TBS as eluant as described above (Figure 7-2B), fractions containing the purified SH3 protein were obtained (Figure 7-2A), and were analysed by SDS-PAGE (Figure 7-2B). The appropriate fractions were combined, and concentrated to ca. 2 mL by ultrafiltration using a 3 kDa MWCO membrane.

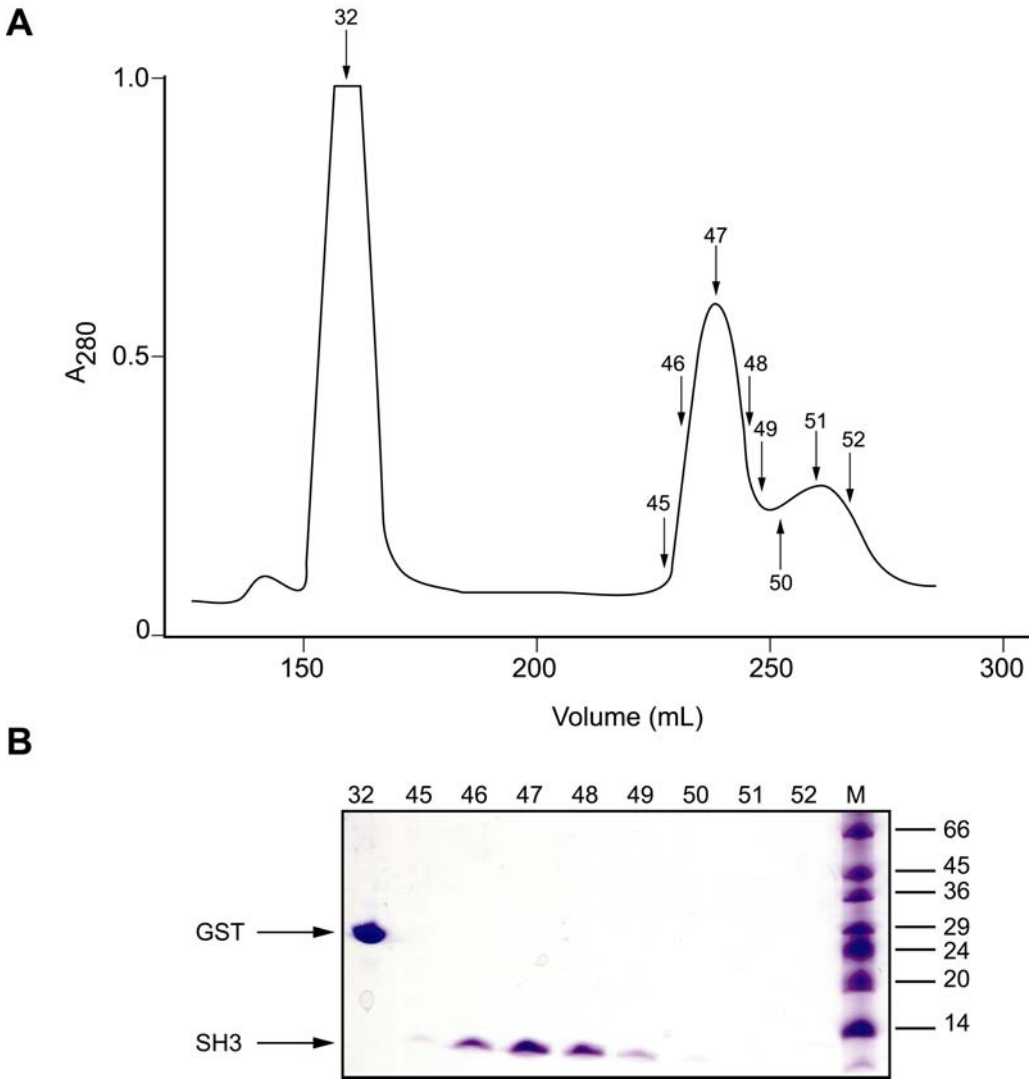


Figure 7-2: Purification of the Tec SH3 protein from the GST/SH3 mixture following thrombin digestion. (A) Fast Protein Liquid Chromatography (FPLC) elution profile for purification of mixture on Superdex G-75 size exclusion chromatography column. The column was equilibrated extensively in TBS, before the concentrated GST/SH3 mixture was loaded. The numbers correspond to the 5 mL fractions that were collected. The protein was detected at A_{280} with a full scale absorbance at 1. A more detailed description of the size-exclusion chromatography protocol is provided in Section 7.6.1.2. (B) SDS-PAGE analysis of protein samples collected from the size-exclusion chromatographic procedure described in (A). The numbers above the gel correspond to the 5 mL fractions that were analysed. M refers to the protein markers SDS-7, with the sizes of the reference proteins (in kDa) indicated on the right. A more detailed description of SDS-PAGE analysis protocol provided in Section 7.6.1.2.

The concentrated sample was exchanged into a solution of disodium hydrogen phosphate (12 mM, pH ~ 6.6) using a PD10 de-salting chromatography column as described above. A Bradford dye binding assay[113] was used to determine the final concentrations of fractions containing the SH3 domain. Originating from a 2 litre culture, typically, a 1 mL fraction was obtained that contained ~ 5-10 mg of the purified SH3 protein (8 kDa), that was used as a stock for preparation of samples for NMR experiments.

A uniformly ^{15}N D196A Tec SH3 sample was prepared by essentially the same procedure to that described above, but instead using E coli strain BL21-DE3 transfected with the p-Gex 4T-2 Tec GST-SH3 D196A mutant construct, prepared by Cvetan Stojkoski.

7.6.2.3 Procedure for preparation of protein samples for FP studies

Preparation of SH3 samples

Samples of the unlabelled SH3 protein were prepared by essentially the same procedure to that described above for the preparation of SH3 samples for NMR spectroscopy, however, the main bacterial culture was grown in LB (instead of Min A). After the size exclusion chromatographic procedure, the appropriate fractions were combined, and concentrated to ca. 2 mL, and exchanged into a solution of PBS (or other buffer as indicated) using a PD10 desalting chromatography column as described above. The appropriate fractions were combined and reconcentrated using a centrifugal concentrator (3 kDa MWCO) to a final concentration of ~40 mg/mL for FP experiments. A Bradford dye binding assay[113] was used to determine the final concentration of the SH3 domain for FP assays.

Preparation of GST-SH3 samples

Samples of the unlabelled GST-SH3 protein were prepared by essentially the same procedure to that described above for the preparation of SH3 samples for NMR spectroscopy, however, the main bacterial culture was grown in LB (instead of Min A). After the agarose/glutathione affinity chromatographic procedure, the concentration of the GST-SH3 fusion protein was estimated using a Bradford Dye binding assay[113] and the protein sample was concentrated to a volume such that [GST-SH3] = ~ 50 mg/mL. (Originating from a 2 litre culture, typically ~ 150 mg total of GST-SH3 protein was obtained.) The concentrated sample was then exchanged into a solution of PBS using a PD10 desalting chromatography column as described above. The appropriate fractions were combined and reconcentrated using a centrifugal concentrator (30 kDa MWCO) to a final concentration of ~40 mg/mL for FP experiments. A Bradford dye binding assay[113] was used to determine the final concentration of the GST-SH3 protein, ready for FP assays.

E coli strains BL21-DE3 transfected with the p-Gex 4T-2 GST-SH3 constructs for the Nck-1(2), and Hck SH3 domains were prepared by Cvetan Stojkoski.

7.7 Protein Methods 2: Ligand Binding Assays

7.7.1 Testing of compounds for binding to the Tec SH3 Domain using NMR Spectroscopy

Samples of the Tec SH3 domain for ligand binding experiments were made at 125 μM in 10 mM Na_2HPO_4, 10% v/v D_2O, 10% v/v d_6-DMSO, 0.01% w/v NaN_3 to a total volume of 600 μL, pH 6.6. NMR spectra were recorded on a Varian INOVA 600 Spectrometer (3 RF channels), using a 5 mm $^1H\{^{13}C/^{15}N\}$ inverse triple resonance PFG probe fitted with z-axis gradients. Sensitivity enhanced [^1H,^{15}N] Heteronuclear Single Quantum Coherence (HSQC)[47] spectra were recorded at 25°C with spectral widths of 8000 and 2000 Hz in F1 and F2 respectively with 64 t_1 increments. The Fourier transformed data resulted in final matrix sizes of 1024 x 512 data points for F1 and F2 respectively.

Stock solutions of compounds to be tested were dissolved in d_6-DMSO at concentrations of ~0.5–1 M. Spectra were first recorded for the protein in the absence of any compounds, before compounds were titrated into the sample at concentrations varying between 0.2 to 1 molar equivalent of the protein in 2 μL of d_6-DMSO. The pH was adjusted by the addition of small amounts of HCl or NaOH if necessary and a new spectrum was recorded. This process was repeated with a range of ligand concentrations until no or only very small changes in protein ^1H (H-N) chemical shifts were observed (typical range of concentrations were 0.5, 1, 2, or 5 molar equivalents of the protein, or greater for the lower affinity ligands). In the case of ligands **24-29**, the above process was repeated until the maximum level of solubility of the ligands was reached (ca. 250 μM). If no changes in chemical shift were observed when 10 molar equivalents of compound were present, then the compound was deemed to not bind to the protein.

Processed NMR spectra were analyzed using SPARKY[115] centred at 4.72 and 120 ppm for F1 and F2 respectively, and chemical shifts for amino acids involved in binding of ligands over the range of concentrations were collected. For all residues whose δ ^1H (H-N) was altered upon ligand binding, $\Delta\delta = \delta_{PL}-\delta_{P0}$ values were calculated where $\delta_{PL} = \delta$ ^1H for the protein in the presence of ligand L at a given [L], and $\delta_{P0} = \delta$ ^1H for the unbound protein. The $\Delta\delta$ values for residues where $|\Delta\delta_{max}| \geq 0.1$ ppm (where $\Delta\delta_{max}$ is the maximum change in

chemical shift observed for a particular residue of the protein), were plotted against ligand concentration from which non-linear regression analyses were performed using GraphPad Prism[55] with a one-site hyperbola binding model and the default settings. The equilibrium binding dissociation constants (K_d) obtained for all the residues were then averaged, to give the overall K_d as the mean ± standard deviation over all residues where $|\Delta\delta_{max}| \geq 0.1$ ppm. For ease of comparison between ligands, the normalised shift $|\Delta\delta/\Delta\delta_{max}|$ was calculated for all residues where $|\Delta\delta_{max}| \geq 0.1$ ppm. The $|\Delta\delta/\Delta\delta_{max}|$ values over the range of ligand concentrations were then averaged, and plotted against ligand concentration, with the error bars representing the standard deviation between the residues.

Chemical shift mapping of residues involved in binding was performed using VMD - Visual Molecular Dynamics.[116]

An example of the data analysis procedure using the NMR chemical shift perturbation method is provided in Appendix 2 of this thesis.

7.7.2 Fluorescence Polarisation (FP) Assays

With the exception of the pH experiments (described below), samples of either SH3 or GST-SH3 proteins used for Fluorescence Polarisation studies were prepared at concentrations of ~ 1 mM in PBS, pH 7.3. Samples of the SH3 protein for the experiments for investigation of the influence of pH on the binding of 2-aminoquinoline to the SH3 domain were performed in either PBS (pH 6.3 and 7.3) or TBS (pH 8.3).

Fluorescence Polarisation[59,60] experiments were carried out with a BMG Laboratories PolarStar Galaxy Plate Reader, using black BMG 96 well plates, pre-blocked with 1% w/v Casein for 2 hours at 37°C. The plate reader was set in polarisation mode with 485 nm and 520 nm excitation and emission filters respectively, and millipolarisation units (mP) were calculated, where P is defined as; $P = (Int_{||} - Int_{\perp})/(Int_{||} + Int_{\perp})$ where $Int_{||}$ = intensity of emission in the plane parallel to excitation (channel 1), and $Int_{\perp}$ = intensity of emission in plane perpendicular to excitation (channel 2).[60] The gain was adjusted for channel 1 and 2 using 100 nM Fluorescein in PBS (or other buffer as indicated) (100 μL), such that an mP value of 35 was obtained.

7.7.2.1 FP peptide binding experiments

Peptide binding experiments were performed in triplicate (5 cycles for each replicate) with Fluorescein-β-A-RRPPPPIPPE-CO_2H (**PRP-1**) maintained at 100 nM, and [SH3] or [GST-SH3] protein varied between 0 and 600 μM, with total well volumes of 100 μL. Millipolarisation units (mP) were calculated as the average of the replicates over 5 cycles, and $\Delta mP = mP_{PT} - mP_T$ was calculated where mP_{PT} is the mP for the protein/**PRP-1** system at a given concentration of protein, and mP_T is the mP for free **PRP-1**. The derived ΔmP values were plotted as a function of protein concentration from which K_d values were determined using GraphPad Prism[55] with a one-site hyperbola binding model and the default settings. The reported values are the K_d ± standard deviation over three replicate experiments.

The peptide binding experiment for investigation on the use of DMSO with the FP assay was performed by Michaela Nicol, of the Biochemistry 3 class 2002, using essentially the same procedure to that described above, however, all reagents were prepared in a 10% DMSO/PBS buffer, pH 7.3.

7.7.2.2 FP peptide competition assays

PRP-1 displacement assays were performed in triplicate (5 cycles for each replicate) with **PRP-1** maintained at 100 nM, and [SH3] or [GST-SH3] maintained at ~50-130 μM. Stock solutions of ligands were prepared in buffer at concentrations of 1.5-10 mM (heating required) prior to dilution. Concentrations of ligands were varied between 0 and 1500 μM, in the presence of the protein/**PRP-1** system (100 μL total). Changes in millipolarisation, $\Delta mP = mP_L - mP_T$ were calculated for each replicate where mP_L is the mP for the protein/**PRP-1**/ligand system at a given ligand concentration, and mP_T is the mP for free **PRP-1**. Proportion of proline-rich peptide bound terms were calculated for each replicate with proportion bound = $\Delta mP_L/\Delta mP_0$, where ΔmP_L = ΔmP for the system at a given ligand concentration, and ΔmP_0 = ΔmP for the system when [ligand] = 0. Proportion bound was then plotted as a function of log[ligand] for each replicate, from which EC_{50} values were determined using GraphPad Prism[55] with a one-site competition binding model and the default settings. If necessary, the EC_{50} values were re-calculated with 'BOTTOM' values held constant at 0.15, as was described in Section 4.8.1.2. The stated EC_{50} values represent the mean ± standard deviation of the calculated EC_{50} values for the three replicate experiments. For ease of comparison between ligands, the proportion bound terms obtained for each replicate were averaged, and plotted against log[ligand] with error bars representing the standard deviation between replicates.

The peptide competition experiments involving the Hck SH3 domain were performed using essentially the same procedure as described above using Fluorescein-βA-STPRPLPPLPTTR-CO_2H (**PRP-2**).

An example of the data analysis procedure using the FP peptide competition method is provided in Appendix 3 of this thesis.