3.3.1 Zinc Fingers

Recent advances[35](#_ENREF_4_4),[36](#_ENREF_4_5) into therapeutic arsenic trioxide chemistry has led to the identification of the zinc finger contained within the PML-RARα as a possible site of interaction. PML-RARα contains two zinc fingers, PML-R-ZF1 is a 4 cysteine zinc finger and **PML-R-ZF2** contains 3 cysteine[37](#_ENREF_4_6) and 1 histidine. It is proposed that arsenic interacts directly with cysteine residues and it is this interaction which leads to the eventual death of the cell. Although the group showed there was interaction between the protein and arsenic, the mechanism and origin of the specificity of this interaction is unclear. Even more unclear is how arsenic could displace the zinc from a four co-ordinated site.

The key part of the zinc finger is a four co-ordinate zinc which holds the finger together by providing structural support[38](#_ENREF_4_7). The “finger” aspect is provided 16 generic residues in the form of a loop[39](#_ENREF_4_8). The zinc could be co-ordinated to either the sulfur on cysteine or the nitrogen on histidine molecules. As one of the zinc fingers in the PML-RARα protein is a 4 cysteine zinc finger. After looking at existing zing finger analogues[40](#_ENREF_4_9),[39](#_ENREF_4_8) and searching the Cambridge crystal database for solved crystal structures of zinc fingers[41](#_ENREF_4_10),[42](#_ENREF_4_11), we propose the follow molecules as models for zinc fingers:



**Figure 19: Structures of proposed zinc finger models**

Compounds that contained tetrahedral zinc molecules bound to four sulfurs are preferred as they are better models for zinc fingers. Zinc 1,2 ethane ditholate (**Zn1b**) is a charged species. We thought this would allow for reactions with charged arsenic species.

*O*,*O*’-dialkyldithiophosphate zinc(II) (**Zn2a**) is a neutral species which contains a phosphorus linking the sulfurs together. Although phosphates are not present in zinc fingers, having such a group allows us the study the system using the phosphorus NMR. This is a major advantage as the phosphorus is very close to the sulphurs and will be a major indicator of interactions that occur.

Experimental procedure for Zn1b

  
Figure 20: Preparation of Zn1b

*Preparation of 1,2 ethanedithiol sodium salt*[*43*](#_ENREF_4_12): Dry tetrahydrofuran (200 ml, previously distilled from sodium and stored inside glove box) was added to finely divided sodium (5.0, 220 mmol). Ethanedithiol was added (10 ml, 95.5 mmols) and the solution was headed under reflux for 5 days until no pieces of sodium are seen. The white precipitate was filtered using Schlenk apparatus and dried under vacuum. The resulting white solid had a mass of 13.44g (92.8 mmols, 97% yield). The salt had very low solubility in all solvents and hence a NMR was not possible.

*Preparation of (Et­4N)2 [Zn(S2C2H4)2]:*The original preparation called for the use of tetraethyl ammonium chloride. This was used in the initial run of the experiment. The yield (c. 10%, purified but decomposed shortly after) of the first attempt was extremely low due to the water sensitive nature of the product. As a result the experiment was modified to use tetraethyl ammonium iodide which is less hydroscopic than tetraethyl ammonium chloride. Zinc chloride (0.7699g, 5.66 mmols) and tetraethylammonium iodide (3.200g, 12.4 mmols) was stirred in a solution of dry acetonitrile (50ml), forming a c solution. Ethanedithiol sodium salt (1.56g, 11.3 mmols). The white solid did not dissolve. The cloudy solution was left stirring for 1 day. The solution was filtered and the filtrate was concentrated to 10ml. Ether was added and the solution was cooled overnight. The solid crystals were filtered. Mass of final product was 0.7062 (1.42 mmols, 25% yield). 1H NMR (400 MHz, DMSO) δ 3.35 (s, 33H), 3.21 (d, J = 7.3 Hz, 16H), 2.50 (s, 13H), 1.24 – 1.08 (m, 24H).

In the 1H NMR there is a peak at 3.21 ppm which can be assigned to be the -CH2 and the 1.16 peak is the -CH3 on the ethyl. The 3.35 peak could be assigned to be the CH2 peaks on the ethanedithiol. The integrals suggest there are more (S2C2H4) than the formula suggests. This is most likely due to remaining sodium ethanedithiol salt which is difficult to purify.

Experimental procedure for Zn2a:

**Figure 21: Reaction scheme for the preparation of Zn2a**

Preparation of the zinc salt: Potassium diethyl dithiophosphate (0.5029g, 2.22 mmols) and zinc chloride (0.2045g, 1.11 mmols) was dissolved in acetonitrile (20ml). After 30 minutes, the colorless solution turned cloudy with a white precipitate. Stirring was continued for 5 hours. The solution was subsequently filtered, and the filtrate was concentrated yielding the product as a colorless solid. Product was recrystallized into a colorless solid with 32% yield. The low yield is possibly attributed to an incomplete recrystallization. 1H NMR (200 MHz, CD3OD) δ 4.14 (dq, J = 9.5, 7.1 Hz, 2H), 1.32 (td, J = 7.1, 0.5 Hz, 3H). 31P NMR (81 MHz, CD3OD) δ 105.69 (s).

This reaction gave a much cleaner product than ethanedithiol and the purification was much easier. The yield is low, but this most likely due to product precipitating out of solution and getting filtered with the NaCl. This is verified by NMR the filtrand which was similar NMR peaks to the final product. Although this is not optimal in terms of being a model for zinc fingers, the zinc is still co-ordinated to 4 sulfurs and thus still serving as a chemical model.

Although this compound looks plausible, many five and six coordinate zinc complexes are known, the behaviour of the complex in solution is not. To begin with, in addition to being a strong chelating ligand, the dithiophosphate ligand can bridge between zinc centers and thus form either dimeric or polymeric structures[44](#_ENREF_4_13). If this process is fast compared to the NMR timescale, the spectroscopic result would be a single peak with an average chemical shift.

The initial attempt to co-ordinate arsenic with the zinc complex was by the use of triphenylarsine which is an excellent Lewis base. Triphenylarsine was added to a solution of **Zn2a** in CDCl3 and possible interactions were observed via NMR. 1H NMR (200 MHz, CDCl3) δ 7.32 – 7.30 (m, 2H), 4.33 – 4.14 (m, 2H), 1.50 – 1.17 (m, 3H).  31P NMR (81 MHz, CDCl3) δ 97.68 (s). The 1H NMR spectrum indicates a multiplet at 7.3 ppm which corresponds to the hydrogens on triphenyl arsine. The ethyl peaks are present and in the correct integration. The resolution of the peaks seems reduced and hence we can no longer distinctly see the doublet of quartets and triplet of doublets seen in the pure sample of **Zn2a**. While this might be due to the shimming of the sample, both changes could also be due to signal averaging and rapid exchange. There is only one phosphorus signal suggesting there is only one averaged species present. Overall, the NMR does not suggest a strong interaction between the triphenylarsine and the complex. (However, given the interaction seen later on with Me2AsCys and the fast exchange which results in a single peak for the phosphorus, this experiment should be looked at again).

The second attempt to co-ordinate arsenic to the zinc complex involved the use of Me2AsCys. Me2AsCys was added to a solution of **Zn2a** in Chloroform. The following stacked spectra shows the –CH3group of Zn2a on top and the NMR of the mixture after the addition of Me2AsCys.

  
**Figure 22: Stacked 1H NMR of Zn2a (top) and the same solution following the addition of Me2AsCys**

The most important peak is the broad peak at 1.85 ppm which indicates an exchange process is happening. This peak is from the methyls on Me2AsCys. The phosphorus NMR, indicated a single peak at 98.0 ppm. This is quite surprising as interaction was seen in the 1H|HH NMR spectrum and one would expect multiple peaks in the phosphorus spectra if the diethyl dithiophosphate ligand was being displaced by Me2AsCys. It was decided free ligand and zinc complex interaction should be looked at in the form of a NMR titration.

NMR titration experimental: 0.011g of **Zn2a** was dissolved in 1ml of CD3OD. 0.4 ml of this solution was placed into the NMR tube. A solution of potassium diethyl-phosphoro dithiolate was made with 0.033g of the compound in 1.5 ml of CD3OD. 1ml of this solution was taken then in injected 0.05 ml aliquots to the NMR tube. The titration indicated that instead of having separate peaks for bound ligand and free ligand, exchange was occurring and hence only a single peak was observed. The following plot shows the chemical shift of the phosphorus signal after addition free ligand.

**Figure 23: Titration of ligand against zinc salt**

Note for the free ligand: 31P NMR (81 MHz, CD3OD) δ 111.99 (s).

The data could be fit to an exponential decay curve with the following formula:

y = A1\*exp(-x/t1) + y0 with y0 = 112.24509, A1 = -6.24402, t1 = 2.07497 with R2= 0.9992.

The good fit to the exponential decay equation indicates that there is one dominating exchange reaction that is happening. In addition to this, the curve does not match that of a simple average of the shift of the free and bound ligand, indicating that there is indeed an interaction and a third species as opposed to simple averaging of the peaks.

Due to the slow timescale of NMR, a fluorescent zinc finger model composed of zinc complex to 8-mercaptoquinoline is proposed. This could be synthesized using the following scheme.



**Figure 24: Synthesis of 8-mercapto quinoline zinc salt.**

Starting material 8-aminoquinoline had a melting point of 82°C. 1H NMR (400 MHz, dmso) δ 8.72 (dd, J = 4.1, 1.6 Hz, 1H), 8.17 (dd, J = 8.3, 1.6 Hz, 1H), 7.68 – 6.54 (m, 4H), 5.90 (s, 2H).

Preparation of **Zn3a**. 8-aminoquinoline (Light green powder, 82°C melting point) (0.3460g) was placed in a round bottom flask. HBr (48%, from Gleason Group, light yellow in color) (1.7ml) was added. The solid turned to deep orange and dissolved upon addition of water (11 ml) forming a transparent orange solution which was cooled by the addition of ice. A solution of 0.2088 g of sodium nitrite in 2 ml of water was added to the cooled mixture. There is little sign of a visible reaction. The solution was then added to a warm solution of thiourea (0.187g in 5 ml of water). Gas evolution started upon warming to 40°C. The product was recrystallized using ethanol and ether. A fraction of the product was not soluble (0.053g) and this is most likely the disulfide. The recrystallized product had a mass of (0.049g). 1H NMR (400 MHz, dmso) δ 9.39 – 8.61 (m, 1H), 8.45 (d, J = 8.2 Hz, 1H), 8.03 – 7.21 (m, 4H).

**Fluorescence data:**

8-aminoquinoline sodium salt was dissolved in ethanol. The UV spectra indicated two peaks, one at 243 nm and 321 nm. Using this as a starting point, emission spectrum shows a maxima at 450 nm. The excitation spectrum showed 2 major excitation peaks at 270nm and 365nm. (see next page)

**Figure 25: Excitation spectra of 8-aminoquinoline in ethanol (450 emission)**

**Figure 26: Emission spectra with 365 nm excitation**

Given that the ligand is fluorescent, the next step is to synthesize the complex by reacting the 8-amino quinolone with ZnCl2.