Facile exchange of arsenic between adducts and implications to drug discovery

By Yuxuan Gu

A thesis submitted to McGill University in partial fulfillment of the requirements

for the degree of:

MASTER OF SCIENCE

Department of Chemistry, Faculty of Science

McGill University

Montréal, Québec, Canada

Abstract

Arsenic is a well-known poison that lives a double life as a therapeutic agent. Recently, arsenic trioxide has been used to treat the cancer acute promyelocytic leukemia with high efficacy. Unfortunately, arsenic drug development has been road blocked by a lack of understanding of the mechanisms of interaction. This thesis attempts to elucidate these mechanisms by looking at the reaction kinetics and thermodynamics of various arsenic species, including potent new organic arsenicals such as S-dimethylarsino-glutathione (ZIO-101) and S-dimethylarsino-cysteine (DMAC).

Data shows that rapid thiolate exchange of dimethylarsonim, Me_2As^+, occurs when the two compounds are dissolved in aqueous solution. The equilibrium constants of this interthiol transfer were characterised by the integrals of the diastereotopic methylarsonium resonances of the species in ^1H NMR. Dynamic NMR was used to characterise rapid intra molecular conformational dynamics which lead to the coalescence of diastereotopic methyl resonances. In addition, rapid thiolate exchange was also shown to occur in monomethylarsonium species as well.

The discovery and characterisation of these facile arsenium transfers allows us to think of the arsenicals in a different manner, instead of binding statically to vicinal thiols, arsenic could hop around various thiolates around the cell.

Abstrait

L'Arsenic est un poison bien connu qui mène une double vie en tant qu'agent thérapeutique. Récemment, le trioxide d'arsenic à été utilisé pour le traitement du cancer de la leucémie aiguë promyélocytaire avec une grande efficacité. Malheureusement, le développement des médicaments basé sur l'arsenic connait un ralentissement à cause du manque de compréhension de leurs mécanismes d'interaction. Cette thèse tente d'élucider ces mécanismes en regardant les réactions cinétiques et thermodynamiques de plusieurs espèces de molécules contenant de l'arsenic, incluant de nouveaux composés organique arsenicales comme le S-diméthylarsinoglutathione (ZIO-101) and S-diméthylarsino-cysteine (DMAC).

Les donnés démontrent que l'échange rapide du thiolate de diméthylarsomium, Me2As+, survient lorsque les deux composés sont dissous en solution aqueuse. Les constantes d'équilibres de cet échange interthiole ont étées caractérisé par les intégraux des résonances diastéréotopiques du méthylarsonium des espèces en conformation dynamique intramoléculaire qui ont mené à la coalescence des méthyles diastéréotopiques en résonance des espèces en RMN de 1H. La RMN dynamique à été utilisé pour caractériser les conformations dynamiques intramoléculaires rapides qui ont menées à la coalescence des résonances des méthyles diastéréotopiques. De plus, il à été démontré que

l'échange rapide des thiolate survient aussi dans les espèces de monométhylarsoniums.

La découverte et caractérisation de ces échanges faciles d'espèces d'arseniums nous force à voir ces arsenicales d'un autre œil, au lieu de se lier statiquement aux thiols vicinaux, l'arsenic pourrait sauter entre différent thiolates autour de la cellule.

Acknowledgements

I would like to thank my supervisor Prof D. Scott Bohle, for giving endless support, patience and guidance – even at times when I may be difficult to work with. Dr. Bohle has gone well beyond his role as a supervisor provided me with important life guidance and support. I would like to give special thanks and gratitude to Dr. Inna Perepichka for always being there. Special thanks also goes to ZhiJie Chua who has been my alter ego. My gratitude also goes to Tina Lam for the French translation of the abstract. Great thanks to past and present members of the Bohle research group: Erin Dodd, Kristopher Rosadiuk, Joël Poisson, Andrea Hill, Ivor Wharf, Mirna Veruca, Cheryl Bain, and Laura Brothers.

I would like to thank my parents, Maggie So and my friends for being supportive.

The following staff of the Department of Chemistry, McGill University: Dr Fred Morin, Dr Nadim Saade, Rick Rossi, Weihua Wang, Jean-Philippe Guay and Chantal Marotte own my gratitude for providing their expertise and prompt assistance during the course of my studies.

To end, thanks to McGill University and the supporting grants for funding my doctoral studies throughout.

Contributions of Authors

This dissertation includes content which has been published: Bohle, D. S.; Gu, Y., Facile dimethylarsenic exchange and pyramidal inversion in its cysteine and glutathione adducts. Organic & Biomolecular Chemistry 2013, 11 (16), 2578-2581.

I hereby give copyright clearance for the inclusion of the following papers, of which I am a co-author, in the dissertation of Yuxuan Gu.

Bohle, D. S.; Gu, Y., Facile dimethylarsenic exchange and pyramidal inversion in its cysteine and glutathione adducts. Organic & Biomolecular Chemistry 2013, 11 (16), 2578-2581.

D. Scott Bohle

Professor and CRC Chair in Chemistry

Department of Chemistry

McGill University

801 Sherbrooke St West

Montreal

QC H3A 2K6

Statement of originality and contribution to knowledge

The author performed all work outlined in this thesis, including all the work presented in the paper as specified above, under the supervision of Professor D. Scott Bohle. All work presented in this thesis, with the exception of the introductory literature review, is declared by the author to be original scholarship and distinct contributions to knowledge as is mandatory for doctoral theses.

Table of Contents

Table of Figures

Chapter 1

Thesis Introduction

1.1 The BioInorganic Chemistry of Arsenic

Arsenic is a group 15 metalloid that is abundant in the earth's crust (ca, 1.8 ppm)[1] in the form of various minerals. In order to fully understand the bioinorganic chemistry of arsenic, the periodicity and chemical reactivity of arsenic must be appreciated. As a metalloid, arsenic exhibits a range of class defying properties and has the characteristics of both metal and non-metals. Elemental arsenic is semiconductor, non-ductile and has the capability of forming various allotropes with various crystal structures[2]. Common inorganic forms of

arsenic include arsenic trioxide (As_2O_3) and varies sulfur compounds such as realgar (As_4S_4) and Oripment (As_2S_3).

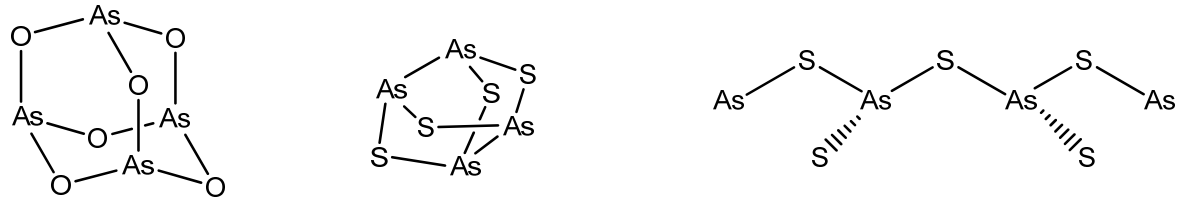


Figure 1: Inorganic forms of arsenic.

In this way arsenic is similar to phosphorus which also forms a variety of sulfur bond compounds.

The chemistry of organic arsenic compounds is just as diverse and varied as its inorganic counterparts. As a pnictogen, arsenic can readily access V, III and -3 oxidation states. Arsenic is closely related to phosphorus in terms of bioinorganic chemistry, and as a result biologically arsenic can occasionally act as a phosphorus analogue. For example enzymes that use phosphate as a substrate recognises arsenate[3]. However there is one key difference between As and P which is that arsenic has filled inner *3d* orbitals and P does not. The *3d* orbital sits between 4s and 4p which makes the 4s orbital less accessible[4]. The consequence when compared with P is that As has a more stable oxidation (III) state at the expense of a less stable (V) state. The increased accessibility of the

As (III) state gives arsenic access a wide range of different chemistry from phosphorus. This is one reason why that As is not as ubiquitous in biology whilst phosphorus is readily used in very inert components such as the phosphate backbone of DNA. In fact many arsenic compounds are toxic to biological species, the point where arsenic trioxide has gained the notoriety as the "*King of poisons*". Despite its toxicity, arsenic compounds may be required by some biological species. This a contrast to the two heavier elements in group 15, antimony and bismuth, which have no known natural biological function[2]. In terms of toxicity, antimony has similar toxicity to arsenic, whilst bismuth has minimal toxicity. Bismuth has been used in over the counter products such as Pepto-Bismol to treat heartburn.

This section will focus on the interactions of arsenic within the human body and highlight recent interests and developments.

1.1.1 Metabolism of arsenic

In order to fully understand arsenic's metabolism by the body, it is necessary to understand that many arsenic species are in rapidly established dynamic equilibria. It is therefore insufficient to just consider one single species, instead an easier way to rationalise the interaction is to consider arsenic as a general system that involves a rapid equilibrium all of the above states. The

dynamic nature of the arsenic species will be discussed in greater detail in section 1.2.3. It could be also argued that the chemistry of arsenic has mostly revolved around the stable forms of arsenic[5], whilst the actual picture is much more distorted as arsenic goes through various unstable and hard to detect species.

Arsenic is ubiquitous in the environment and hence uptake of arsenic by humans is inevitable through food and water. The main form of arsenic that is being absorbed by the body in the inorganic form As (III)[6]. As arsenic is absorbed by the body, it goes through a complicated metabolism pathway that involves a range of oxidation states in addition to methylation on the arsenic. The concern of this section will be focused on the human metabolism of arsenic, however it is important to keep in mind that the metabolism of arsenic between different organisms follows similar patterns[7]. One of the earliest and most referenced studies into arsenic metabolism was done by Challenger in 1947 which introduced the formulation of the Challenger pathway. Despite its age, the Challenger pathway is the framework for which the metabolism of arsenic is rationalised.

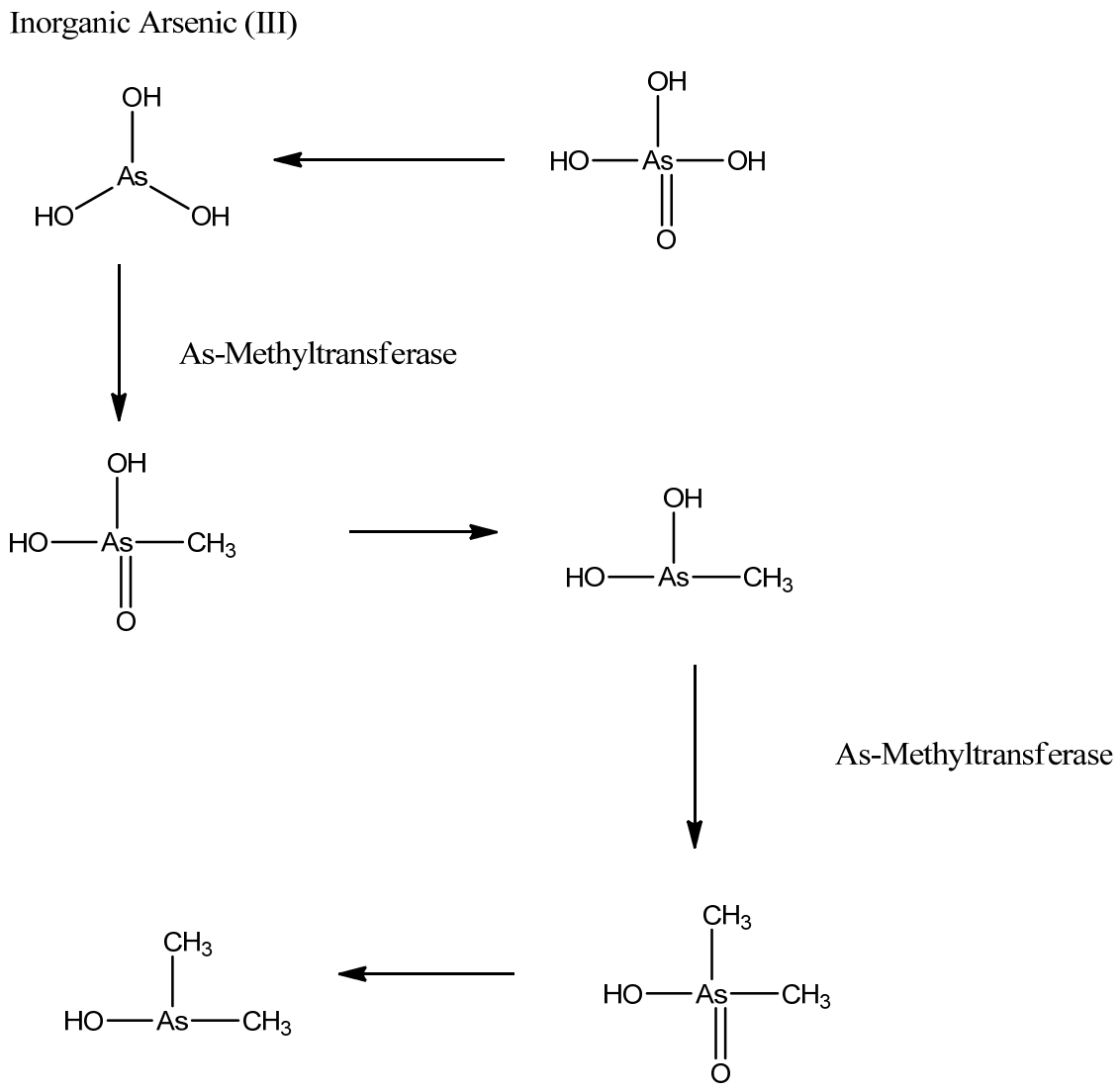


Figure 2: Challenger's proposed pathway of arsenic metabolism

Challenger proposed that arsenic is absorbed as As (V), and is reduced to inorganic As (III) by glutathione (GSH). The arsenite is then methylated and oxidised by As-methyltransferase into mono-methylarsinic acid (V). This is followed by a subsequent reduction to monomethylarsenious acid by GSH. A second methylation then proceeds with the formation of dimethylarsenic acid.

Finally the dimethyl arsenious acid. Both methylated and non-methylated species are arsenite are capable of being removed from the body via the liver into urine. Recent developments[8] in the metabolism of arsenic has shown there are additional processes that allows arsenic to exchange between various states such as thiodimethylarsenopropanoic acid and thiodimethylarsenobutanic acid. There have be additional revisions to the pathway proposed by Naranmandura and Hayakawa[9].

It has been suggested that reason for the methylation is to reduce the toxicity of arsenic. However, recently this has be disputed as methylated forms of arsenic are shown to be just as toxic as its inorganic counterparts[10].

1.1.2 Arsenic as a poison

Arsenic has an infamous history of being the "King of Poisons", and in famous deaths such as Napoleon and the horse Phar Lap. The LD_{50} for arsenic trioxide is 35 mg per kg and a fatal dose of could be as low as 100 to 200 mg. The effects of arsenic poisoning appear from half an hour of ingestion. The immediate symptoms include abdominal pain, vomiting diarrhea and salvation and death. There are also long term effects to arsenic poison that include rashes in the form of "*arsenicosis*", cancer and damage to the cardiovascular system.

Figure 3: Photo of arsenic induced arsenicosis, retrieved 2013[11]

The exact mechanism of the arsenic interaction is not well established, but it is believed that different species of arsenic are responsible for a range of toxic responses. The initial symptoms have been proposed to be the result of arsenite (III) binding to viscinal thiols of active enzymes. The binding of arsenic may either cause conformational change in the enzyme or block active sites, hence inhibiting enzyme activity. Although has been widely regarded as the mechanism

of action, there has been little direct evidence to substantiate this[12]. Arsenate (V) have been proposed be toxic in another manner, they are transported into cells via phosphate transport pathways. They then to mimic phosphates and are then included into ATP and DNA[7]. As arsenic can do additional chemistry, this may cause mutations in the cell or cellular damage.

Arsenic's poisonous attributes is a large issue today as arsenic is found in ground water. This is a result of the leeching of arsenic from the soil into ground water. This is a major problem for in many countries, but most particularly in developing economies where there is little recourse but to these wells which brings this toxic ground water up and causes health problems in the local population.

1.1.3 Treatment of arsenic poisoning

Dimercaprol, also known as British anti-Lewisite (BAL), is used medically as a treatment against arsenic poisoning. BAL has a capability of binding very strongly to arsenic via a bi-dentate bond.

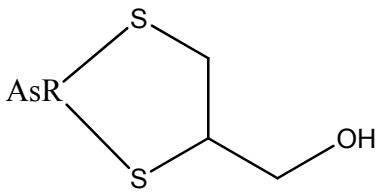


Figure 4: Structure of British Anti-Lewisite (BAL)

It has been proposed that BAL competes with metabolic enzymes for arsenic binding and hence reduces arsenic's toxicity. The chelation would result in subsequent removal of the bound compound from the body via urine.

1.1.4 Medicinal applications of arsenic

It has been proposed by Ehrlich that arsenic based drugs would get converted to "RAsO" and would interact with metallothionein. The exact mechanism of interaction is not known as little direct evidence was found to support this theory. The underlying pattern is that many enzymes are inhibited by arsenic, especially those containing thiol (SH) groups.

More recently arsenic trioxide (As_2O_3) was discovered to be effective against the cancer Acute Promeylocytic Leumkemia (APL). APL is a rare form of myeloid leukemia that affects an estimated 1500 new patients each year. The discovery of the drug initially came from traditional Chinese therapy followed by major research developments in the US, leading to FDA approval in 2000 under tradename of Trisenox. In the clinical trial for Trisenox, it was reported that 77% of the 582 patients treated were alive after 3 years, compared with just 50% for non-arsenic treatment. More recently, Ziopharm oncology has developed Darinaparsin, an oxidation state III organoarsenical bound to glutathione for the treatment of APL.

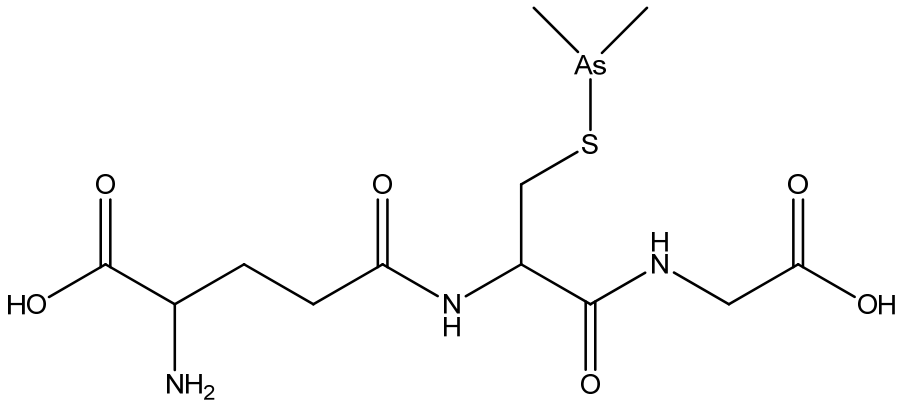


Figure 5: Structure of Darinparsin

Currently the drug is in Phase III trials and has been shown to be more effective that arsenic trioxide with reduced side effects. This is an interesting drug because it is possible metabolite of arsenic trioxide (see section 1.1.1, the metabolism of arsenic) as Me_2AsOH forms an equilibrium with GSH to form Darinparsin in solution. In addition there are two methyls bound to the arsenic already, preventing it from forming more than 1 bond to thiols in the As(III) ground state.

The mechanism for arsenic's activity in the treatment of APL is not fully understood. Luckily APL is a well-studied cancer and work has been done on the oncoprotein PML-RARα. PML-RARα a fusion protein that of Promyelocytic Leukemia protein PML, and retinoic acid receptor RARα. Zhang et al[13] proposed in 2010 that arsenic trioxide interacts the directly the two zinc fingers in PML by replacing the zinc in the zinc finger. They showed using EXAFS that arsenic (III) can interact directly with the sulfurs on the cysteine in the zinc finger.

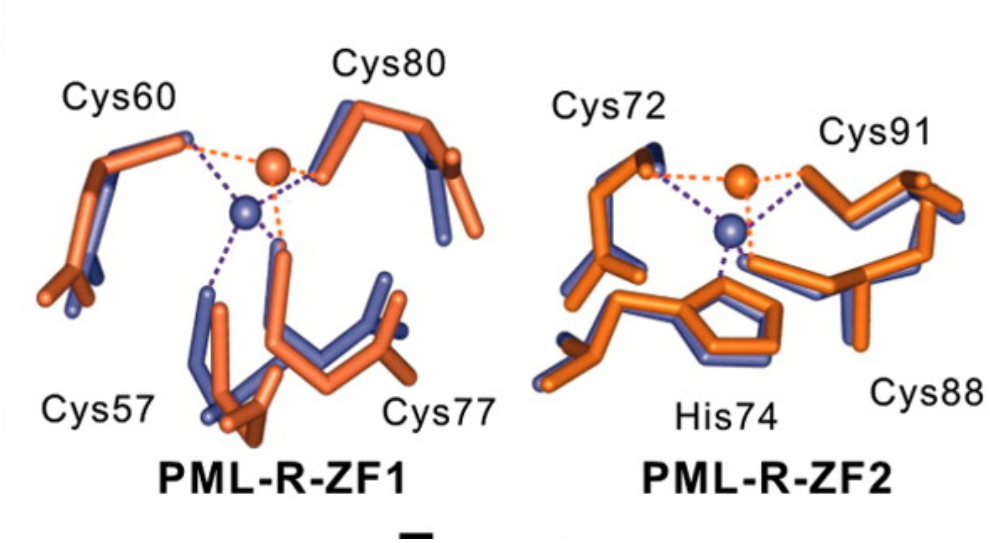


Figure 6: Binding of zinc and arsenic to the PML-R-ZF1 and PML-R-ZF2 Purple: interaction of zinc Orange: Predicted interaction of arsenic (Diagram from Science[13])

They proposed that the coordination of the arsenic causes conformation changes in the protein. These changes would lead to aggregation of this protein and trigger SUMOlyation and eventual cell death. The exact mechanism in which the arsenic displaces the zinc is relatively unknown and not much additional work has been done on the arsenic interaction in the zinc finger domain. In addition this proposal does not apply for dimethylated arsenic species such as Darinaparsin as the methyl groups take space in arsenic's co-ordination sphere.

Another proposal for the mechanism of arsenic[14] has been proposed by involves the ability of arsenic to bind to thiols on PML proteins. Once bound, arsenic would form intermolecular disulfide bridges that result in the

multimerization of PML into a mesh. It is unclear if arsenic has specificity for the thiols in PML.

1.2 Chemistry of Arsenic compounds

The previous section focused on the biological interactions of arsenic with key one underlying theme: the binding of arsenic to thiols is the key interaction involved in its mechanism of action. This interaction has been profusely by biologists. Despite the frequent use of this concept to explain arsenic's poisonous attributes, the chemistry of this interaction is not well understood. This section will outline the approaches taken in chemistry to resolve the kinetics and thermodynamics of the arsenic thiol interaction.

1.2.1 Redox of arsenic by GSH

Cullen et al.[15] first discovered the ability of thiols to reduce $Me_2AsOOH(V)$ to form Me_2AsGSH (III).

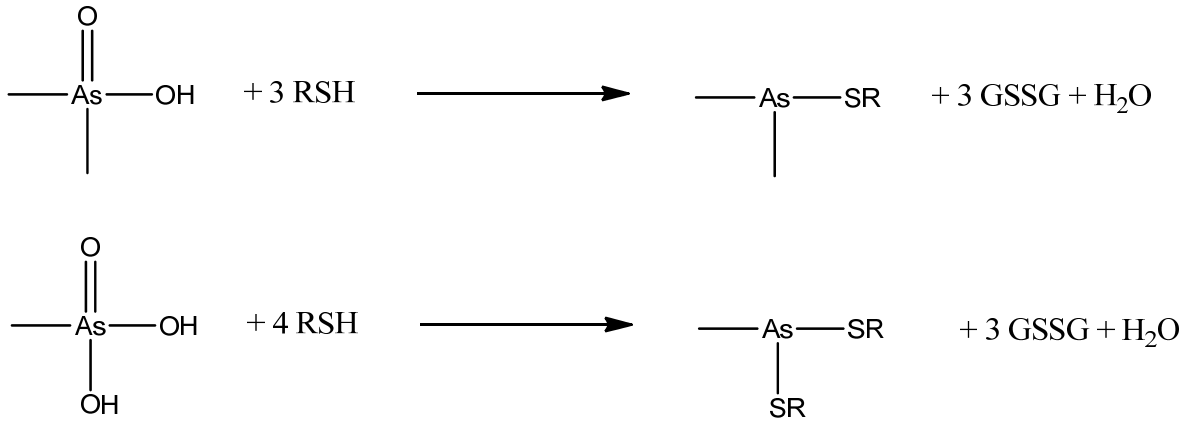


Figure 7: Reduction of dimethylarsinic acid and monomethylarsinic acid by thiols.

They used a range of thiols such as GSH, Cys and mercaptoethanol which had a range of pKas. They concluded that the thiols did not have to be deprotonated before this reduction took place as they could perform this reaction in a range of pH below that of the pKa of the thiol.

Delnomdedieu et al[16] built upon this work using modern NMR spectroscopy. They also noticed at as the pH increased, there is the possibility of the formation of free $Me2As^+$. This is because even though there was no direct peak for that of free $Me2As^+$, the noticed the chemical shift of the methyl peaks would shift slightly as the pH was increased. They proposed change in the chemical shift was caused by the involvement of an increased amount of $Me2As^+$ as solution was basified. Based on this they proposed that the reduction occurred before binding to the thiol. They also noted changes in the spectrum at high pH;

the two diastereoteopic methyl peaks would become a single peak. However no kinetic measurements were done on the system.

Carter et al[17] showed the reduction of arsenate to form $As(GS)_3$

$$3H^+ + AsO_4^{3-} + 5GSH \rightleftharpoons As(SG)_3 + GS\text{-}SG + 4H_2O$$

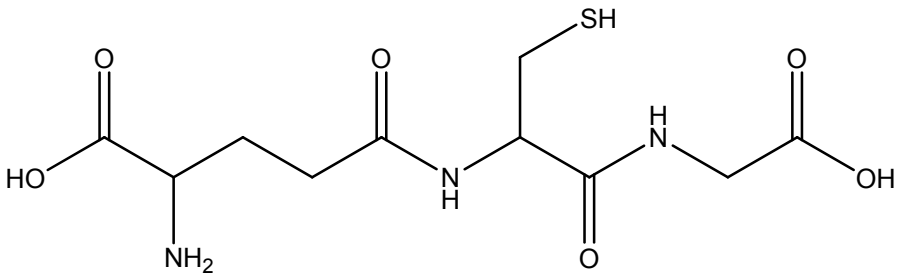
This reaction was found to happen readily at pH 7 and has been fully characterised by ^1H and ^{13}C NMR. They proposed that like for the methylated systems, the arsenate reduction occurred before the binding to GS as a when used a ratio of 1:2 or less, the formation of $As(SG)_3$ was not observed.

Arsenic (III) species are formed in the presence of a reducing environments. However, in non-reducing aqueous environments, arsenic (III) species are unstable. This has been noted by Cullen et al as they found the Me_2AsCys and Me2AsGS were unstable in solution and rapidly decomposed to their parent oxidation state V acid along with the production of the disulfide.

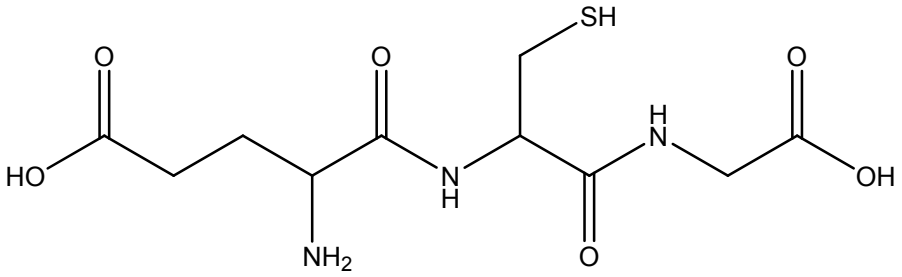
$$2Me_2AsGS + 2H_2O \xrightarrow{Air} 2Me_2AsOOH + GSSG$$

As a result, they degassed all their solvents prior to use and noted this improved their yields when compared to that of Zingaro et al[18] who performed similar synthesis under aerobic conditions. However, the exact mechanism of this degradation was not fully understood. More recently Zhao et al[19] examined the

degradation of the similar system $As(GS)_3$ using HPLC-ESI-MS. They found that an isomer of α-GSH that was the result of the cleavage of GSSG.



glutathione



alpha - glutathione

Figure 8: Structures of glutathione and alpha-glutathione.

The α -GSH was identified comparing the fragmentation patters of degradation products of $As(GS)_3$ compared to that of standard GSH. They noted that α-GSH is not normally formed from the degradation of GSSG in the absence of arsenic. The mechanism and reason for the formation of this isomer is not known.

1.2.2 Interaction of arsenic with thiols

One of the well-studied systems in terms of thermodynamics is that of $As(OH)_3$ and its interaction with Glutathione to form $As(GSH)_3$. Rey et al[20] used potentiometric and spectroscopic data to show that the formation constant, log K, of $As(GS)_3$ to be 32. They noted that pH played important role in this interaction

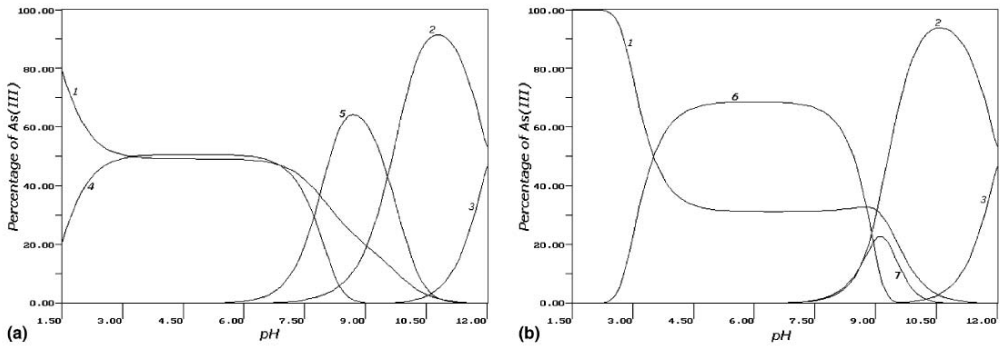


Fig. 2. Species distribution curves for the systems: (a) As–Cys (1) $As(OH)_3$; (2) $As(OH)_2O^-$; (3) $As(OH)O_2^{2-}$; (4) $[As(HCys)_3]$; (5) $As(Cys)(OH)_2]^-$; and (b) As–GSH (1) $As(OH)_3$; (2) $As(OH)_2O^-$; (3) $As(OH)O_2^{2-}$; (6) $[As(HGS)_3]^{3-}$; (7) $[As(GS)(OH)_2]^{2-}$. In both cases the total ligand concentration is 15 mM and the total As(III) concentration is 5 mM.

Figure 9: Effect of pH on the speciation of arsenic (Figure from Rey et al paper[21])

Further research has been done by Wilcox[12] et al. who used colormetric and calorimetric studies to quantify the formation constants of arsenothiolates.

Table 3. Thermodynamic Properties for the Formation of As(III)−Thiolate Complexes

	$As(OH)_3$	MMA
GSH	1:3 $\beta_3 = 1.8 \times 10^6$ $\Delta G = -8.8$ kcal/mol $\Delta H = -38.7$ kcal/mol $\Delta S = -96$ cal/(mol·K)	1:2 $\beta_2 = 1.3 \times 10^7$ $\Delta G = -10.1$ kcal/mol $\Delta H = -17.8$ kcal/mol $\Delta S = -25$ cal/(mol·K)
DMSA (5)[a]	1:2 $\beta_2 = 8.3 \times 10^8$ $\Delta G = -12.7$ kcal/mol $\Delta H = -27.3$ kcal/mol $\Delta S = -47$ cal/(mol·K)	1:1 $K = 1.0 \times 10^7$ $\Delta G = -9.9$ kcal/mol $\Delta H = -13.2$ kcal/mol $\Delta S = -9$ cal/(mol·K)
DHLA (6)[a]	2:3 $\beta_{2:3} = 4 \times 10^{18}$ [b] $\Delta G = -25$ kcal/mol $\Delta H = -43$ kcal/mol[c] $\Delta S = -59$ cal/(mol·K)	1:1 $K = 1.1 \times 10^7$ $\Delta G = -10.0$ kcal/mol $\Delta H = -17.0$ kcal/mol $\Delta S = -20$ cal/(mol·K)
DTT (7)[a]	1:1 $K = 9.5 \times 10^5$ $\Delta G = -8.5$ kcal/mol $\Delta H = -13.7$ kcal/mol $\Delta S = -17$ cal/(mol·K)	1:1 $K = 8.2 \times 10^5$ $\Delta G = -8.4$ kcal/mol $\Delta H = -15.9$ kcal/mol $\Delta S = -24$ cal/(mol·K)

Figure 10: Thermodynamic for the formation of As(III)-Thiolate complexes (from Wilcox et al. 2008[22])

They found that for $As(OH)_3$ the binding of glutathione has a stability constant β_3 =2 x10^6. In addition, they found a co-operative effect in the binding of thiols and the formation constant after each binding in increased. For example ΔH_3 was found to be -33.1 kcal /mol compared to ΔH_1 of -2.5 kcal/mol. They also looked at monomethylarsenite and its equilvalent binding to GSH and found that the binding of GSH to a monomethylated species gave comparable numbers to that of the non-methylated species. Gailer[23] offered an alternative view and proposed after passing both methylated and non-methylated compounds through size exclusion chromatography, the methylated versions were found to be more stable.

Finally they worked out the enthalpy of thiolate displacement to be -2.8 kcal/mol. They also found that there is a large unfavourable ΔS term involved in the binding as a result of the loss of conformational degrees of freedom. As a result they proposed that viscinal thiols that are conformationally constrained will have a higher affinity for arsenic III. This argument extends to conformationally unconstrained Cys residues found in zinc fingers would by this theory have a lower affinity for As (III).

Zingaro et al used the ability of thiols to displace the hydroxyl group in numerous synthesis of new dimethyl arsenious derivatives[24].

1.2.3 Lability of the Arsenic Thiol bond

It was found experimentally that as $As(SG)_3$ was passed through a size exclusion chromatography (SEC)[23], an increase of temperature of the column would cause retention shifts of the arsenic peaks towards small-molecular-mass regions. This is indicative of labile arsenic sulfur bonds which could allow the GS to break away from the molecule.

Arsenic III compounds have the ability are known have labile bonds. The is shown by the ability of meso-2,3-dimercaptosuccinic acid to displace GSH from the complex from $As(GS)_3$[25]

Arsenic systems have been studied by theoretical chemistry by Orthaber et al[26] using DFT calculations with B3lYP/6-31G basis set. They were interested in the interaction of H_2S with Arsenic (V) species

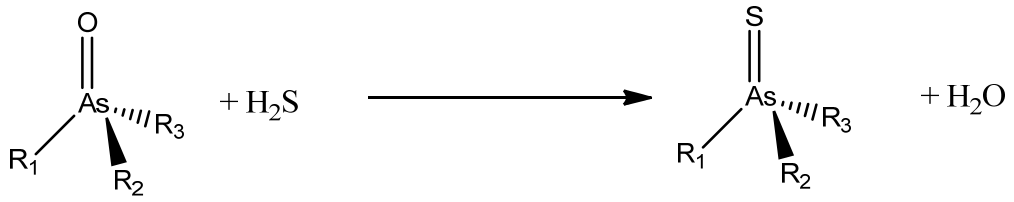


Figure 11: Interaction of H_2S with arsenic V species

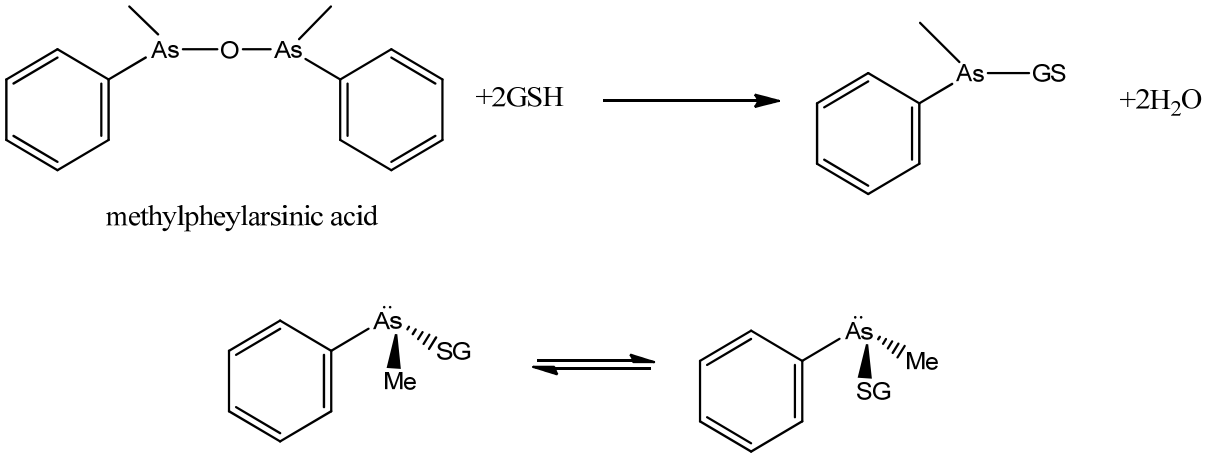
They found that the formation constants is very favorable with ΔG of up to 51 kJ/mol for dimethylarsinoylethanol. Interesting they found that the pentavalent state, the –OH and –SH could interconvert between axial and equatorial positions similar to that of the Berry pseudorotation. They found that the relative intermediate of the interconversion is 6.7 kJ/mol.

One analogous system for the lability of arsenic is that of Mercury. The mercury thiolate bond has been shown to be labile and capable of rapidly breaking and reforming[27].

1.2.4 Co-ordination and geometry of arsenic compounds

The co-ordination chemistry of arsenic is both rich and diverse. As previously discussed in chapter 1.1, arsenic is a metalloid and can both accept and donate lone pairs. As it result it can accept co-ordination and act as a metal, in addition to donating its lone pair and acting as a ligand.

Edmonds et al[28] found that chiral arsenite species are capable rapidly racemizing. The studied this using the chiral arsenite, methylphenylarsinic acid and reacted it with (L)-glutathione to form two diastereomers. The then tried to separate the diastereomers using HPLC



They tried to resolve the diastereomers using NMR spectroscopy with the aid of lanthanide shift reagents and COSY 2D techniques. They were surprised to find that the species have racemized during the purification process (with heating was less than 40°C). The concluded that no pyramidal inversion of the arsenic was seen but could not give a definitive mechanism for this inversion.

1.2.5 Mechanism of arsenic bond lablity

The mechanism under which arsenic can perform its bond lability is not fully understood. Zampella et al[29] tried to calculate the binding of structures of arsenite systems using DFT calculations in order to understand how arsenite

interacts with thiols of proteins. They modelled the arsenic binding systems with $As(CH_3S)_3$ and determined that there are two major forms of binding: the endo and exo isomers.

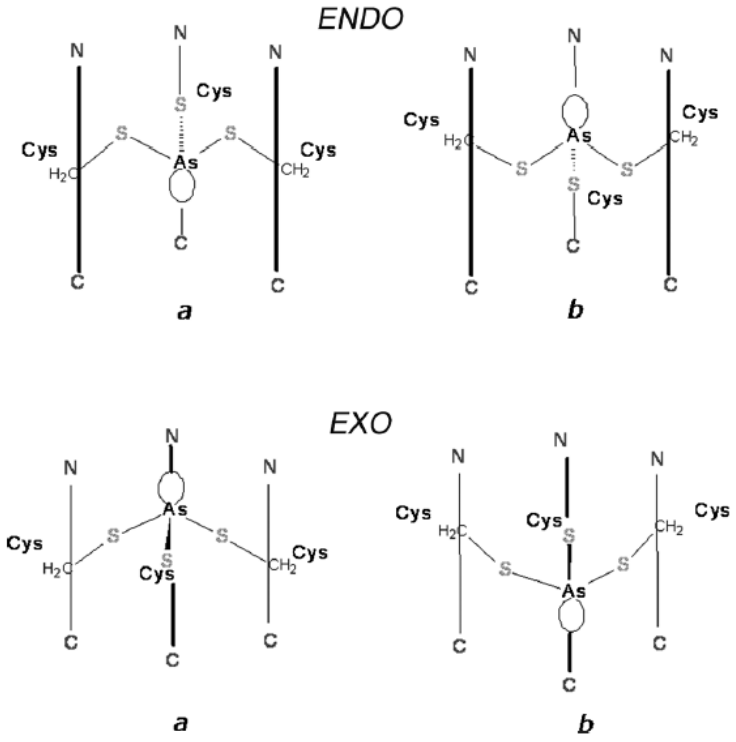


Figure 12: endo and exo binding forms of $As(CH_3S)_3$ (Figure from Zampella et al[29])

They concluded that the calculated lowest energy forms were similar to that found in protein systems. This meant that the mode of binding was strongly influenced by the steric bulk of the substituents. They also determined that the direction of the lone pair was a crucial factor influencing the selectivity binding for particular metal ions.

1.3 Conclusions

The mechanism of arsenic based drugs is currently not well understood and poses a road-block to arsenic based drug development. It is hypothesised that whilst arsenic is often thought react in an analogous fashion phosphorus, the kinetics and thermodynamics of pnicogen bonds formed by these two species far from analogous. The Challenger pathway shows that arsenic can be prone to both oxidation and methylation, a sharp contrast to phosphorus bonds which are stable enough to be used in genetic material. In addition to having different reactivity than phosphorus, arsenic seems to have bond lability, where arsenic appears to move between different thiol containing compounds. The mechanism of this interaction has not been thoroughly characterised, the thermodynamics of arsenic thiol bonds remains unknown. This project aims at using modern kinetic techniques to study the kinetics and thermodynamics of arsenic compounds. This is a pivotal step in the greater picture of understanding of arsenic drug interaction and the transportation of arsenic within the body.

References

1. Norman, N. C., *Chemistry of Arsenic, Antimony and Bismuth*. Springer: 1998.

2. Sun, H.; Editor, *Biological Chemistry Of Arsenic, Antimony And Bismuth*. John Wiley & Sons Ltd.: 2011; p 383 pp.

3. Tawfik, D. S.; Viola, R. E., Arsenate replacing phosphate: alternative life chemistries and ion promiscuity. *Biochemistry* **2011,** *50*, 1128-34.

4. Levason, W.; Reid, G., 3.6 - Arsenic, Antimony, and Bismuth. In *Comprehensive Coordination Chemistry II*, Editors-in-Chief: , J. A. M.; Meyer, T. J., Eds. Pergamon: Oxford, 2003; pp 465-544.

5. Raab, A.; Hansen, H.; Feldmann, J., Labile Arsenic Compounds in Biological Matrices, or Possible Problems Finding the Metal Species Present in Cells. *Special Publication-Royal Society of Chemistry* **2005,** *301*, 72.

6. Jomova, K.; Jenisova, Z.; Feszterova, M.; Baros, S.; Liska, J.; Hudecova, D.; Rhodes, C. J.; Valko, M., Arsenic: toxicity, oxidative stress and human disease. *Journal of applied toxicology : JAT* **2011,** *31* (2), 95-107.

7. Cullen, W. R., *Is Arsenic an Aphrodisiac?* The Royal Society of Chemistry: 2008; p P001-P412.

8. Thomas, D. J.; Li, J.; Waters, S. B.; Xing, W.; Adair, B. M.; Drobna, Z.; Devesa, V.; Styblo, M., Arsenic (+3 oxidation state) methyltransferase and the

methylation of arsenicals. *Experimental biology and medicine (Maywood, N.J.)* **2007,** *232*, 3-13.

9. Rehman, K.; Naranmandura, H., Arsenic metabolism and thioarsenicals. *Metallomics : integrated biometal science* **2012,** *4* (9), 881-92.

10. Petrick, J. S.; Ayala-Fierro, F.; Cullen, W. R.; Carter, D. E.; Vasken Aposhian, H., Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. *Toxicol. Appl. Pharmacol.* **2000,** *163* (2), 203-207.

11. Arsenic Contamination of Groundwater in South and East Asian Countries. http://go.worldbank.org/WVH5RSX920 (accessed Jan. 2013).

12. Spuches, A. M.; Kruszyna, H. G.; Rich, A. M.; Wilcox, D. E., Thermodynamics of the As(III)-thiol interaction: arsenite and monomethylarsenite complexes with glutathione, dihydrolipoic acid, and other thiol ligands. *Inorg. Chem.* **2005,** *44*, 2964-72.

13. Zhang, X. W.; Yan, X. J.; Zhou, Z. R.; Yang, F. F.; Wu, Z. Y.; Sun, H. B.; Liang, W. X.; Song, A. X.; Lallemand-Breitenbach, V.; Jeanne, M.; others, Arsenic trioxide controls the fate of the PML-RARα oncoprotein by directly binding PML. *Science* **2010,** *328* (5975), 240-3.

14. Lallemand-Breitenbach, V.; Zhu, J.; Chen, Z.; de Thé, H., Curing APL through PML/RARA degradation by As2O3. *Trends in molecular medicine* **2012,** *18*, 36-42.

15. Cullen, W.; McBride, B.; Reglinski, J., The reaction of methylarsenicals with thiols: some biological implications. *J. Inorg. Biochem.* **1984,** *21*, 179-193.

16. Delnomdedieu, M.; Basti, M. M.; Otvos, J. D.; Thomas, D. J., Reduction and binding of arsenate and dimethylarsinate by glutathione: a magnetic resonance study. *Chem. Biol. Interact.* **1994,** *90*, 139-55.

17. Scott, N.; Hatlelid, K. M.; MacKenzie, N. E.; Carter, D. E., Reactions of arsenic(III) and arsenic(V) species with glutathione. *Chem. Res. Toxicol.* **1993,** *6*, 102-6.

18. Chen, G. C.; Zingaro, R. A.; Thompson, C. R., 6-thio and-seleno-[beta]–glucose esters of dimethylarsinous acid. *Carbohydr. Res.* **1975,** *39* (1), 61-66.

19. Zhao, F.; Chen, Y.; Qiao, B.; Wang, J.; Na, P., Analysis of two new degradation products of arsenic triglutathione in aqueous solution. *Frontiers of Chemical Science and Engineering* **2012,** *6*, 292-300.

20. Rey, N.; Howarth, O., Equilibrium characterization of the As (III)-cysteine and the As (III)-glutathione systems in aqueous solution. *J. Inorg. Biochem.* **2004,** *98*, 1151-9.

21. Rey, N. A.; Howarth, O. W., Equilibrium characterization of the As (III)-cysteine and the As (III)-glutathione systems in aqueous solution. *J. Inorg. Biochem.* **2004,** *98* (6), 1151-9.

22. Wilcox, D. E., Isothermal titration calorimetry of metal ions binding to proteins: An overview of recent studies. *Inorg. Chim. Acta* **2008,** *361*, 857-867.

23. Percy, A. J.; Gailer, J., Methylated trivalent arsenic-glutathione complexes are more stable than their arsenite analog. *Bioinorganic chemistry and applications* **2008,** *2008*, 539082.

24. Banks, C. H.; Daniel, J. R.; Zingaro, R. A., Biomolecules Bearing the S- or SeAsMe2 Function : Amino Acid and Steroid Derivatives. *J. Med. Chem.* **1979,** *22*, 572-575.

25. Delnomdedieu, M.; Basti, M. M.; Otvos, J. D.; Thomas, D. J., Transfer of arsenite from glutathione to dithiols: A model of interaction. *Chem. Res. Toxicol.* **1993,** *6*, 598-602.

26. Orthaber, A.; Sax, A. F.; Francesconi, K. a., Oxygen versus sulfur: Structure and reactivity of substituted arsine oxides and arsine sulfides. *J. Comput. Chem.* **2012,** *33*, 112-7.

27. Carson, G.; Dean, P., The Metal NMR Spectra of Thiolate. *Inorg. Chim. Acta* **1982,** *66*, 157-161.

28. Edmonds, J. S.; Nakayama, T.; Kondo, T.; Morita, M., Diastereoisomerism of thiol complexes of arsenic acids and pseudoasymmetry of arsenic: a 1H and 13C NMR study. *Magnetic resonance in chemistry : MRC* **2006,** *44*, 151-62.

29. Zampella, G.; Neupane, K. P.; De Gioia, L.; Pecoraro, V. L., The importance of stereochemically active lone pairs for influencing Pb(II) and As(III) protein binding. *Chemistry (Weinheim an der Bergstrasse, Germany)* **2012,** *18*, 2040-50.

Chapter 2:

2.1 Kinetics and thermodynamics of rapid Me_2As exchange by thiols.

The research from this project is used in the following paper published on 05 March 2013 in the journal Organic & Biomolecular Chemistry.

Organic & Biomolecular Chemistry

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Facile dimethylarsenic exchange and pyramidal inversion in its cysteine and glutathione adducts†

D. Scott Bohle* and Yuxuan Gu

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 2578

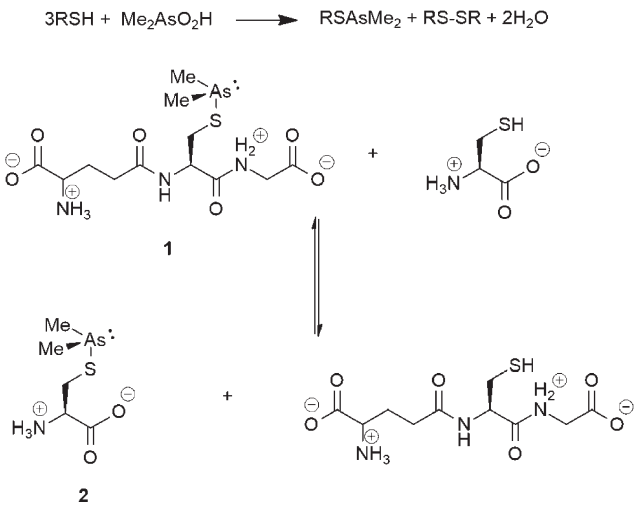
Received 5th February 2013,
Accepted 4th March 2013

DOI: 10.1039/c3ob40268a

www.rsc.org/obc

Rapid thiolate exchange of dimethylarsonium, Me_2As^+, is observed between two different thiolate species in solution. NMR is used to characterize the equilibrium constants for interthiol transfer as well the rapid intra molecular conformational dynamics which leads to the coalescence of diastereotopic methyl resonances. These rapid exchange kinetics have important consequences of arsenic's toxicity and pharmacology.

Regardless of its oxidation state or its substitution arsenic and its compounds are to varying degrees universally toxic.[1] Surprisingly though As_4O_6, arsenous oxide, is the FDA approved therapy for acute promyelocytic leukemia (APL).[2] As with many metals and metalloids our current understanding of arsenic detoxification centers on its methyl derivatives, their transport, and localization.[3] In addition to its methylation arsenic binding and transport frequently involves thiols and thiol containing proteins, which reflects arsenic's strong thiophilicity.[4] For example, arsenic(III) compounds form very strong bonds to glutathione, GSH, Scheme 1, and the tris glutathione adduct, $As(SG)_3$, has a high formation constant of log K_f = 32.0.[5] Nevertheless, thiolate exchange even from this tris-thiol adduct by either *meso*-2,3-dimercaptosuccinic acid or British Anti-Lewisite is well established.[6] The mechanisms for these facile exchange reactions have not been examined in any detail, but recent studies for As(III) species suggested that their Lewis basicity is a factor in their lability.[7] However the kinetics of arsenic-thiol exchange remains poorly characterised. In the course of characterising darinaparsin, dimethyl-arsino-glutathione, **1**, and dimethyl-arsino-cysteine, **2**,[8] as part of a program to understand the formers anticancer role in APL,[9] we have discovered examples of rapid thiolate exchange. Herein we report: (1) Equilibrium constants for the rapid exchange of dimethylarsenium groups between cysteine and glutathione; (2) Dynamic NMR studies for the self-exchange of the methyl sites in these dimethylarsenic thiolate adducts; and (3) The dependence of these self-exchange reactions on pH, concentration, and thiol. Taken together these studies lay a foundation for understanding biochemical arsenic lability and transport.



Scheme 1 Synthesis and equilibrium of **1** and **2**. In H_2O at 25 °C.

In aqueous solution GSH rapidly exchanges Me_2As^+ with **2** to give $GSAsMe_2$, **1**. ESI mass spectroscopy reveals the presence of the two dimethylarsenio derivatives in solution, with the peaks at 225.889 and 411.991 *m*/*z* corresponding to **1** and **2**. When handled under nitrogen atmosphere both **1** and **2** are stable for several days at room temperature and during the time course of the NMR experiments described here. In the presence of oxygen there is the slow evolution of cacodylic acid and the corresponding disulfide. In the ^{1}H NMR spectra of these solutions at room temperature and high field, 500 MHz, Fig. 1, there are total of 4 peaks between 1–2 ppm which correspond to the diastereotopic, non-equivalent methyl resonances of compounds **1** and **2**. Warming this mixture leads to reversible coalescence of first the methyls resonances of the cysteine

Department of Chemistry, McGill University, 801 Sherbrooke St. W. Montreal, H3A 2K6 Canada. E-mail: scott.bohle@mcgill.ca; Fax: +1-514-398-3797; Tel: +1-514-398-7409

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c3ob40268a

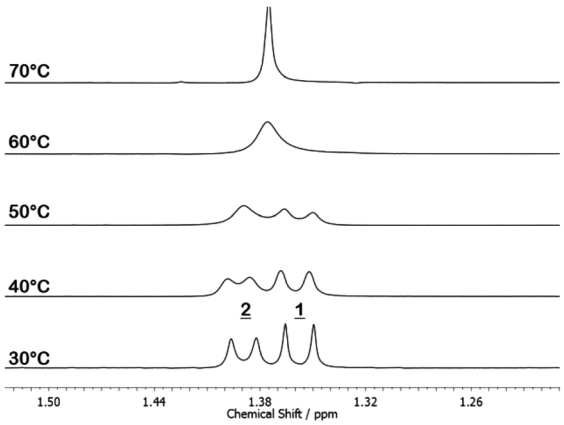


Fig. 1 Variable temperature ^{1}H NMR spectra for the diastereotopic methyl resonances on **1** and **2**. The observed coalescence of the inequivalent methyls is caused by dynamic inter and intramolecular exchange.

derivative, and then at higher temperature the glutathione, and finally all four methyls. Up until 50 °C the ratio of **1** : **2** remains constant. Subsequent titration and integration of related samples gives the equilibrium constant (K_{eq}) for Scheme 1 to be 0.6 and favoring the glutathione derivative. This corresponds to a relatively small free energy difference (ΔG) of 1.4 kJ mol^{-1} between the two species and suggests similar arsenic–sulfur bond energies. Raising the pH to between 5.5 and 7.0 also results in similar spectroscopic changes as shown in Fig. 1. More basic conditions promote methyl site exchange. Attempts to also measure the kinetics of these reactions have been hampered by the lack of useful UV-vis chromophores, in **1** and **2**, and that the reaction occurs in the mixing time (ESI†), of a typical NMR experiment. We conclude that this coalescence is due facile methyl exchange, and to test this facile exchange we opted to perform the study of **2** in isolation.

Individually the ^{1}H and ^{13}C NMR spectra of **1** and **2** are markedly temperature, pH, and concentration sensitive. For example in Fig. 2 the solution spectra for a 5 mM solution of **2** in 0.1 M phosphate buffer at pD = 4.5 exhibited a reversible

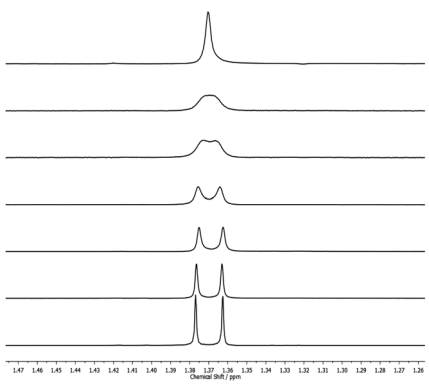
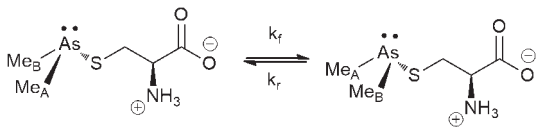


Fig. 2 Variable temperature ^{1}H NMR for the diastereotopic methyl signals in **2** in 0.1 M phosphate buffer at pD = 4.5. Bottom trace is at 10 °C, followed by 20, 30, 40, 45, 50, 55, and 70 °C, at top.



Scheme 2 Methyl site exchange in **2**.

coalescence of the two methyl resonances. Formally this corresponds to the two site exchange shown in Scheme 2, which corresponds to an effective inversion of the arsenic stereochemistry. Although this mechanism may not be the same as interthiol exchange of Me_2As^+, both reactions suggest a markedly unexpected lability for the otherwise thermodynamically stable Me_2As–S bond.

The ^{1}H NMR spectra measurements of **2** were performed on a 5 mM phosphate buffered solution at pH = 4.6 between the temperatures of 270 K to 335 K. The rate constant $(d[\mathbf{2}])/dt = -k_f[\mathbf{2}]$ was calculated using the chemical shift difference between the methyl peaks using the Sandstrom's equation[10] $k = \{\pi/\sqrt{2}\}[\sqrt{(\delta\nu^2 - \delta\nu_\varepsilon{}^2)}]$. Peak width was also used to independently calculate the rate; both methods yielded the same activation parameters.

The data give good linear Arrhenius fits for all data above 10 °C with only slight deviations being found for the temperatures just above the freezing point of water. The activation energy (E_a) is 14 kJ mol^{-1}, indicating that very little energy is required to cause the coalescence. In terms of the activation parameters from the Eyring equation, $\Delta G^\ddagger$ = 73 kJ mol^{-1}, $\Delta H^\ddagger$ = 11 kJ mol^{-1} and $\Delta S^\ddagger$ = −190 J mol^{-1} K^{-1}. The relatively small $\Delta G^\ddagger$ suggests that As–S bond dissociation is an unlikely mechanism as the bond enthalpy of the As–S bond is around 380 kJ mol^{-1}.[11] In addition, the $\Delta S^\ddagger$ is negative indicating a markedly more ordered transition state, this suggests that there might be an associative mechanism for this exchange.

The zwitterionic ionization of the amine and carboxylic acid groups in **1** and **2** play an important role in the mechanism of the exchange. Exchange kinetics as a function of pH and substrate concentration are shown in Fig. 3. The slowest methyl site exchange kinetics correspond to a singly protonated species. This is in accord with a prior potentiometric titration result for **1** which was suggested to be particularly labile at pH ≥ 7.[5] While under basic conditions there may be significant formal dissociation of Me_2As^+ through an associative nucleophilic hydrolysis, for the pH used in these studies there is no substantial build-up of side products or other indications of competing side reactions.

The concentration dependence for the methyl site exchange, as reflected in its coalescence temperature, is shown in Fig. 3b. The marginal decrease in coalescence temperature with increased concentration suggest that in addition to a rapid intramolecular mechanism there is second intermolecular, bimolecular, pathway. This second pathway is consistent with the **1**/**2** exchange results shown in Fig. 1. However, the rate and thus contribution, of this second pathway makes to methyl site exchange is minor compared to basal unimolecular rate of site exchange. There are several mechanisms for methyl

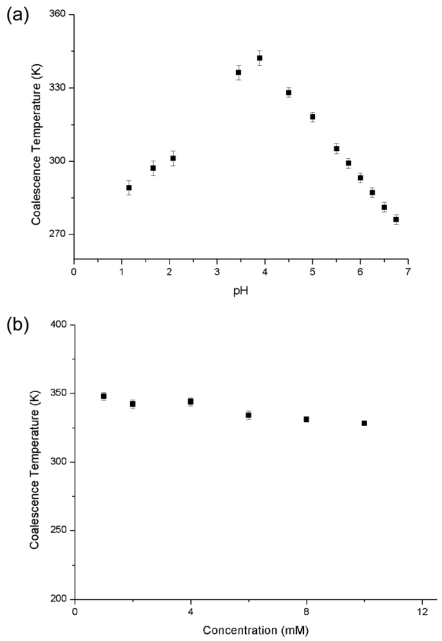
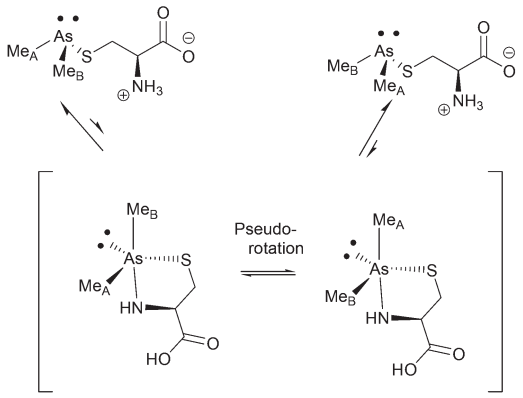


Fig. 3 Dependence of coalescence temperature upon (a) the pH of a 5 mM solution of **2** and (b) concentration of **2** in 5 mM phosphate buffer.



Scheme 3 Proposed fluxionality in **2**.

site exchange, with most obvious, a formal inversion of the arsenic geometry, being unlikely. Experimentally, arsenic(III) pyramidal inversion through a trigonal planar transition state has a high barrier, 176 kJ mol^{-1} for PhEtMeAs.[12] Theoretical calculations[13] also suggest these transition states should be in excess of 150 kJ mol^{-1}, which is much higher than our experimentally determined barrier of 80 kJ mol^{-1}. Surprisingly facile racemisation at arsenic of the diastereomeric methylphenylarsinic acid adduct with glutathione was observed by Edmonds *et al.*, and interpreted in terms of an unexpected and unaccountably low inversion barrier.[14]

To account for the rapid methyl site exchange in **1** and **2** we note that As(III) species are of course ambiphilic being potent nucleophiles and ligands as well being as metalloids with latent Lewis acidity. It is this latter character which would allow for an associative chelation of the amine to the arsenic to give a net five coordinate intermediate with four substituents and a stereochemically active lone pair, Scheme 3. For this geometry Berry pseudorotation barriers are expected to be low, and their action will lead to rapid methyl site exchange. This mechanism is in accord with the negative entropy of activation and the rate enhancement at higher pH. The increase in rate at lower pH may be due to a separate acid catalyzed exchange, but the generally low of solubility of these species limits a more extensive study under these conditions.

In conclusion, we have shown that the As–S bond is kinetically labile and can be interact with other thiols in aqueous solutions. In addition to being more stable to oxidation, **1** is 1.4 kJ mol^{-1} more stable than **2** in aqueous solutions. This type of facile thiol exchange has important implications for the activity of methylarsenic species in cells and proteins. Also given the enhanced electrophilicity of the AsO_2 moiety in arsenous oxide suggests that its exchange rates may be considerably faster than those reported here. In what may be a helpful analogy, the facile dimethylarsenium transfer reactions discovered here have many parallels with the *trans*-nitrosylation chemistry of the nitrosylated thiolates, RSNO, which have been more extensively studied.[15]

Support from NSERC, FQRNT, and CRC is gratefully acknowledged.

Notes and references

1 W. R. Cullen, *Is Arsenic an Aphrodisiac?*, The Royal Society of Chemistry, 2008, pp. P001–P412.
2 G. Q. Chen, X. G. Shi, W. Tang, S. M. Xiong, J. Zhu, X. Cai, Z. G. Han, J. H. Ni, G. Y. Shi and P. M. Jia, *et al.*, *Blood*, 1997, **89**, 3345–3345.
3 D. J. Thomas, J. Li, S. B. Waters, W. Xing, B. M. Adair, Z. Drobna, V. Devesa and M. Styblo, *Exp. Bio. Med.*, 2007, **232**, 3–13.
4 (*a*) V. Lallemand-Breitenbach, J. Zhu, Z. Chen and H. de Thé, *Trend Mol. Med.*, 2012, **18**, 36–42; (*b*) A. M. Spuches, *J. Am. Chem.*, 2008, **2**, 8148–8149; (*c*) A. M. Spuches, H. G. Kruszyna, A. M. Rich and D. E. Wilcox, *Inorg. Chem.*, 2005, **44**, 2964–2972.
5 N. Rey and O. Howarth, *J. Inorg. Biochem.*, 2004, **98**, 1151–1159.
6 (*a*) M. Delnomdedieu, M. M. Basti, J. D. Otvos and D. J. Thomas, *Chem. Res. Toxicol.*, 1993, **6**, 598–602; (*b*) K. Dill, E. R. Adams, R. J. O'Connor, S. Chong and E. L. McGown, *Arch. Biochem. Biophys.*, 1987, **257**, 293–301; (*c*) D. E. Wilcox, *Inorg. Chim. Acta*, 2008, **361**, 857–867.
7 M. Delnomdedieu, M. M. Basti, J. D. Otvos and D. J. Thomas, *Chem.-Biol. Interact.*, 1994, **90**, 139–155.
8 W. R. Cullen, B. C. McBride and J. Reglinski, *J. Inorg. Biochem.*, 1984, **21**, 179–193.
9 K. K. Mann, B. Wallner, I. S. Lossos and W. H. Miller, *Expert Opin. Invest. Drugs*, 2009, **18**, 1727–1734.
10 J. Sandström, *J. Mol. Struct.*, 1983, **102**, 417–420.

11 Y. R. Luo, *Comprehensive Handbook of Chemical Bond Energies*, CRC Press, Boca Raton, 2007, p. 488.
12 G. H. Senkler Jr. and K. Mislow, *J. Am. Chem. Soc.*, 1972, **94**, 291.
13 (*a*) M. Rekhis, O. Ouamerali, L. Joubert, V. Tognetti and C. Adamo, *J. Mol. Struct. (THEOCHEM)*, 2008, **863**, 79–83; (*b*) X. Xu and D. G. Truhlar, *J. Chem. Theor. Comput.*, 2011, **7**, 2766–2779.
14 (*a*) J. S. Edmonds, T. Nakayama, T. Kondo and M. Morita, *Magn. Reson. Chem.*, 2006, **44**, 151–162; (*b*) K. Nakamiya, T. Nakayama, J. S. Edmonds and M. Morita, *Appl. Organomet. Chem.*, 2006, **20**, 580–584.
15 (*a*) M. Digicaylioglu and S. A. Lipton, *Nature*, 2001, **412**, 641–647; (*b*) G. E. Hardingham and S. A. Lipton, *Antioxid. Redox Signaling*, 2011, **14**, 1421–1424.

2.2 Supplementary material

The following supplementary material details the methodology and calculations used in the chapter 2.1.

Prepration of S-(dimethylarseno)cysteine (Me_2AsCys). The synthetic preparation was adapted from the Cullen method[15], with the main change being nitrogen gas is used instead of carbon dioxide to produce an inert atmosphere. Cacodylic acid (0.445g) of and 0.929g of L-Cystine was mixed in distilled water under nitrogen for 16 hours. The precipitate was filtered dried under reduced pressure without heating. ^{1}H NMR (500 MHz, D_2O) δ 3.99 – 3.91 (m, 1H), 3.24 – 3.13 (m, 1H), 1.37 (s, 1H), 1.36 (s, 1H).

Preparation of Me_2AsCys Solution. 0.0115g of Me_2AsCys was dissolved in 1 ml of Phosphate buffer at pH 4.2 (10% potassium phosphate buffer was prepared by the dissolution of potassium phosphate into water and subsequent adjustment of pH using NaOH. The solution was deuterated by drying the mixture and re-hydrating it with D2O). This solution was diluted to form a desired concentration of 6.40 mM by a 1/5 dilution using 200ul of the solution and 800 ul of buffer. To ensure the temperature is changing constantly and as expected, a plot of the

solvent chemical shift against temperature. This indicates minimal changes to the pH or random errors in the temperature readings, as the R^2 is 0.999. This however does not rule out the possibility of systematic errors to the temperature readings. This could be remedied by using a temperature reference.

NMR data was acquired with a Varian 500 MHz instrument. Temperature calibration was done using standard samples of ethylene glycol and methanol[30]. All NMR acquisitions were made with 16 scans, transform size of 32k and shims were done with a gradient shimming on each measurement. Data was processed using Mnova 6.1.1 FID processing software.

To determine the rate constant of the reaction, the separation of the methyl peaks were used to calculate the rate constant using the following equation[31]:

$$k = \frac{\pi}{\sqrt{2}}\sqrt{\delta v^2 - \delta v_e^2}$$

This equation is applicable in this situation as δv and k are much larger than the bandwidth in absence of exchange (0.6 Hz). As it is not possible to lower the sample below 0 degrees due to the freezing point of water, δv was estimated using the lowest experimentally obtained separation of 7.8 Hz (solution of 1mM sample at 10 °C).

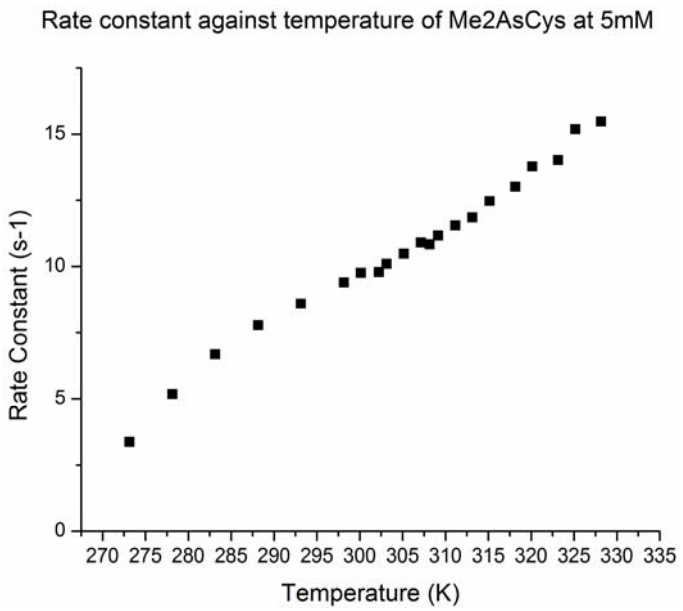


Figure 13: Plot of rate constant against temperature of a 5mM solution of Me_2AsCys

We notice an almost linear build up of the rate constant.

The NMR dynamic exchange was modeled using DNMR3 with Spinworks 3 software[32]. The experimentally determined variables (low temperature limit) of the system at 5°C with a concentration of 1mM was used as the input parameter for the simulation (two spins at 1.3832 and 1.3698 with 0.6 line width). Other parameters include Permutation vector set as 2,1 (mutual exchange of the system), the relaxation time 1 sec (same as acquisition), populations were 0 as this is a case of mutual exchange and maximum iterations = 30. The rate constant RC (1,2) was gradually increased by 1 s^{-1} until the simulated system exhibited coalescence. It was found that a rate constant of 16 s^{-1} gave coalescence for the two peaks.

2.3 References

15. Cullen, W.; McBride, B.; Reglinski, J., The reaction of methylarsenicals with thiols: some biological implications. *J. Inorg. Biochem.* **1984,** *21*, 179-193.

30. Holz, M.; Heil, S. R.; Sacco, A., Temperature-dependent self-diffusion coefficients of water and six selected molecular liquids for calibration in accurate 1H NMR PFG measurements. *PCCP* **2000,** *2* (20), 4740-4742.

31. Sandström, J., *Dynamic Nmr Spectroscopy*. Academic Press: 1982.

32. Spinworks, SpinWorks3 Manual. *Data Processing* **2010,** *7*, 1-117.

Chapter 3: Rapid exchange in related arsenic derivatives.

3.1 Mechanism elucidation of dimethyl arsenic exchange

In Chapter 2 we showed that facile dimethylarsenic exchange occurred between dimethylarsenic cysteine and glutathione adducts. In addition to exchange between different dimethylarsenic adducts, we showed that the diastereotopic methyls of Me_2AsCys also underwent rapid exchange on the NMR timescale. The self-exchange was of particular interest because a good understanding of this was required to understand the intra-system exchange. In this second part of this project, I focused on elucidating the mechanism of the facile dimethylarsenic self-exchange.

There are several possible mechanisms for the observed effect. One possibility is a hydrolysis mechanism where H_2O would hydrolyse the arsenic species thus causing the formation of a Me_2AsOH which no longer contains diastereotopic methyls. Another possibility is an intermolecular attack from the nitrogen from another Me_2AsCys into the arsenic, thus forming pyramidal intermediate. This nucleophilic attack could also be intramolecular, causing the formation of a 5 membered ring.

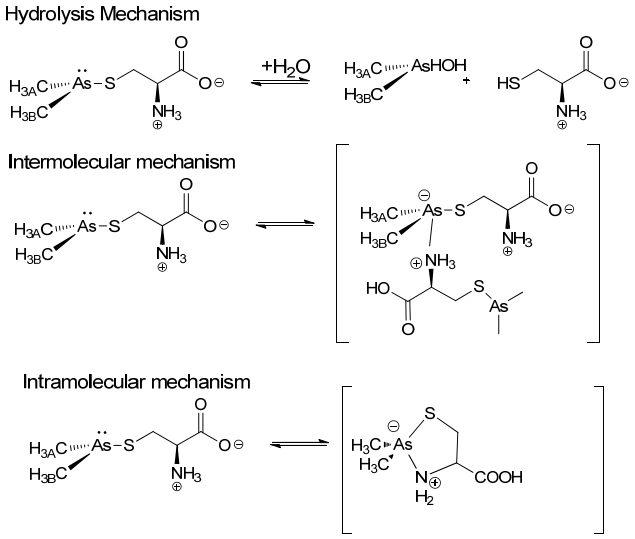


Figure 14: Possible mechanisms for the observed coalescence of the dimethylarsonium peaks on ^1H NMR.

In order to further validate this mechanism, we attempted to use chemical substitution to produce other dimethylarsenic derivatives and compare their kinetics. The first synthesis target is dimethylarseno-penicillamine which contains penicilamine, a cysteine derivative which contains two additional methyls between the thiol and the β-carbon. With additional methyls substituents different

rates of 5 membered ring formation are expected, and thus significantly different DNMR kinetics. Another synthesic target was dimethylarseno-N-acetyl cysteine where the nitrogen is tied up with an acetyl group, thus tying up its lone pairs and preventing it from nucleophillically attacking or chelating to the arsenic.

Preparation of Dimethylarsenoiodide. Me_2AsI was prepared using the Burrows method[33]. Potassium iodide, 15g, and 5g of Me_2AsOOH are dissolved in 45ml of distilled water. Concentrated HCl 5ml is added to make a clear colorless solution. Sulfur dioxide is bubbled for 15 minutes through the solution at which point the solution turned to light yellow. After around 5 minutes of bubbling the solution darkened to an opaque black, followed by the formation of a bottom layer which was clear yellow. The bottom layer was extracted and distilled under reduced pressure of 16 mm at 401K. ^1H NMR (400 MHz, $CDCl_3$) δ 2.01.

Preparation of Dimethylarseno-penicillamine

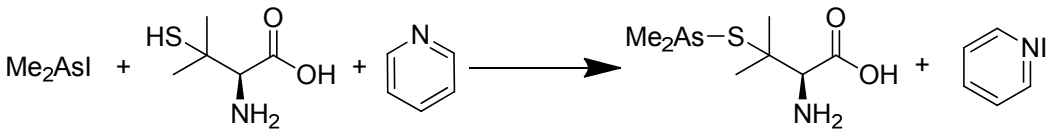


Figure 15: Synthetic scheme for dimethylarseno-penicillamine

Penicillamine, 0.5g, was suspended in dimethoxyethane. 1 ml of Me2AsI was added by syringe causing the full dissolution was penicillamine. 1 ml of pyridine

was added and precipitation immediately occurred. The solution was refluxed for 15 minutes and left to stir for 2 hours. The solution was filtered and the filtrate was dried. Note this compound has an extremely unpleasant smell, Schlenk apparatus and proper fume hood containment methods are required. NMR revealed the filtrand to be pyridinium iodide. ^{1}H NMR (400 MHz, d_2o) δ 3.86 (d, *J* = 2.3 Hz, 1H), 3.60 (s, 1H), 3.36 (s, 1H), 2.02 (d, *J* = 1.1 Hz, 1H), 1.60 (s, 4H), 1.44 (s, 4H), 1.38 (s, 3H), 1.35 – 1.28 (m, 6H). The NMR revealed additional unexpected peaks that could be attributed to cacodylic acid and the disulfide adduct of penicillamine. Attempts to further purify the product proved unsuccessful.

Preparation of dimethylarseno-N-acetyl cysteine

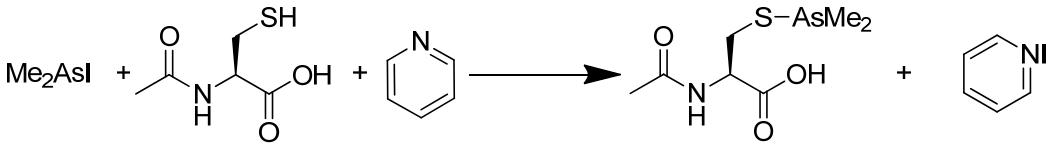


Figure 16: Synthetic scheme for the preparation of dimethylarseno-N-acetyl cysteine

0.5g of NAC was dissolved in dimethoxyethane and 1 ml of Me2AsI was added by syringe. 1 ml of pyridine was added and precipitation immediately occurred. The solution was refluxed for 15 minutes and left to stir for 2 hours. The solution was filtered and the filtrate was dried. NMR revealed the filtrand to

be pyridinium iodide. Unfortunately this reaction mixture is contaminated by Me2AsOOH and N-acethylcysteine disulfide. This was difficult to purify as the product was a thick liquid and could not be recrystallized.

Unfortunately it proved difficult to synthesize the pure derivatives of Me_2AsCys required for DNMR experimentation. Purity is especially important because contaminants of other thiol containing products are expected to significantly alter the kinetics.

3.2.1 Monomethylated derivatives

The kinetics and thermodynamics of monomethyl arsenic (III) derivatives is of immense interest and complements the work done on the dimethyl arsenic system. One particularly interesting property is the ability of monomethyl arsenic derivatives to bind to two thiols, thus allowing it to bind very strongly to vicinal dicysteine residues. The starting material for monomethyl derivatives is monomethyl arsenous acid sodium salt, $(MeAsO)_x$, which was synthesised by literature methods[34].

Preparation of Methylarsenate(V) acid sodium salt. 3g of Arsenic trioxide was dissolved in 10 ml of 10M NaOH. 15 ml of MeI was added, forming a bilayer

solution. The solution mixture was heated to reflux for 16 hours, which resulted in a white precipitate of methylarsenate(V) acid sodium salt in 72% yield.

Preparation of Methyl arsonious acid sodium salt. $MeAsO(ONa)_2$ was dissolved in 50 ml of H_2O. Dissolution of the initial salt was promoted by gradual heating of the solution. Once dissolved the solution is treated with sulfur dioxide which is bubbled through the solution. The solution quickly becomes clear (suggesting acid sensitivity) then light yellow after 2 minutes. After saturating with SO_2, the solution was quickly boiled for 2 minutes then cooled for 15 minutes. Neutralisation with sodium carbonate turned the solution from light yellow to clear. Solution was dried and $(MeAsO)_x$ was extracted with benzene. After removing the benzene in vacuo resulting in a white solid (70% yield).

^1H NMR (500 MHz, $CDCl_3$) δ 1.58 (d, *J* = 6.8 Hz, 5.9%), 1.52 – 1.49 (m, 27%), 1.48 (d, *J* = 2.1 Hz, 58%), 1.44 – 1.42 (m, 8%). This corresponds to the literature reference (Aposhian et al[35]) of ($CDCl_3$): δ 1.58/1.59 (5.0%), 1.50/1.51 (26.8%), 1.48 (60.0%), 1.43 (8.1%). ^1H NMR (400 MHz, D_2O) δ 1.17 (s, 1H). ^1H NMR (500 MHz, C_6D_6) δ 1.21 (s, 1H). ^1H NMR (500 MHz, CD_3OD) δ 1.25 (dd, *J* = 11.1, 7.1 Hz, 1H). ESI of the compound did not reveal any tetramer peaks as NMR later indicated that it possibly hydrolyses.

Monomethyl arsinous acid takes the form of cyclic and linear oligomers, hence resulting in the formula $(MeAsO)_{x,}$ where the exact number of oligomers depends on the concentration of the solution. This is shown in the $CDCl_3$ NMR which contains 4 sets of multiplets in $CDCl_3$.

3.2.2 Examining the nature of Monomethyl arsinous acid

Monomethyl arsinous acid exists in different oligomers and a more detailed study of this mixture is necessary. Marsmann and Wazer[34] proposed the possibility of the species olgimerizing at higher concentrations and temperatures, in particular the preference of the tetrameric form at 48% wt concentrations of arsenosomethane at 120°C (in diphenyl ether). It is interesting to note that the Wazer did not observe a hydrolysis with diphenyl ether.

To validate this possibility of oligiomer, a temperature dependant NMR experiment was performed with the sample in $CDCl_3$. For this experiment 0.0975g of **$(MeAsO)_x$** was dissolved in 1000 ul of $CDCl_3$. The sample was initially cooled down to 273.15K and the temperature was slowly brought up in 10° increments.

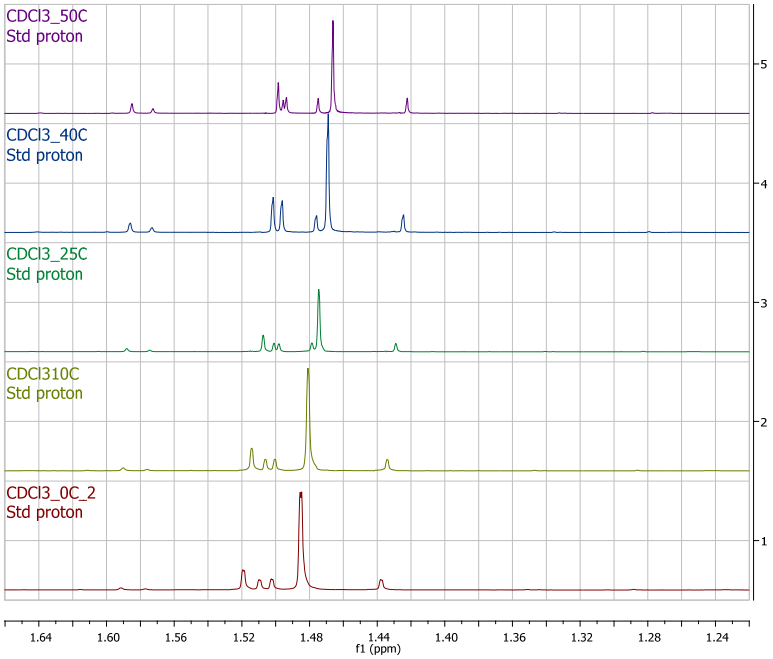


Figure 17: Temperature variation on the sample of (MeAsO)x dissolved in CDCl3, 1) 273.15 K, 2) 283.15, 3)298.15 K, 4)313.15K, 5) 323.15K.

As the temperature increases, we notice a shoulder peak appearing at 1.48 ppm. We also notice in increase in the intensity of the peaks at 1.57 and 1.59. No coalescence of the peaks is observed suggesting that this process is slow on the NMR time scale. The integrals of the peaks stay constant during the heating and the mixture returns to its original composition upon cooling to room temperature. Additional experiments are necessary, in particular the use of different solvents (possibly diphenyl ether, the solvent used by Marsmann et al[34]). Concentration variation, especially that of high concentrations of **$(MeAsO)_x$** may confirm the oligiomerization. In addition it is difficult to heat the solvent to higher temperatures inside the NMR and this experiment could be extended by

heating the compounds separately other solvents. The oligomerization is might not be a reversible process in which cause this would not cause a coalescence of the peaks in the NMR.

3.2.3 Interaction of $MeAs(OH)_2$ with cysteine

Previously in the project we characterised the interaction of dimethyl arsenicals with cysteine. This provided us with a new and expected insight into the lability and kinetics of the As-S bond. With monomethyl derivatives the situation is more complicated as the arsenic can bind with two cysteines.

Preparation of Monomethyl arsenious acid solution. A solution of $(MeAsO)_x$ was prepared by dissolving 0.0245 g of the compound in 1.0 ml of D_2O (buffered with 10% deuterated sodium phosphate). A 231 mM solution of cysteine was prepared by dissolving 0.0277g of cysteine in 2000 ul of the same buffered D_2O. All solutions were deoxygenated by bubbling nitrogen for 10 minutes. For the NMR titration, 500ul of the stock $(MeAsO)_x$ solution placed in a NMR tube. The Cysteine solution was titrated into the NMR tube at 100ul aliquots followed by 30 sec of mixing.

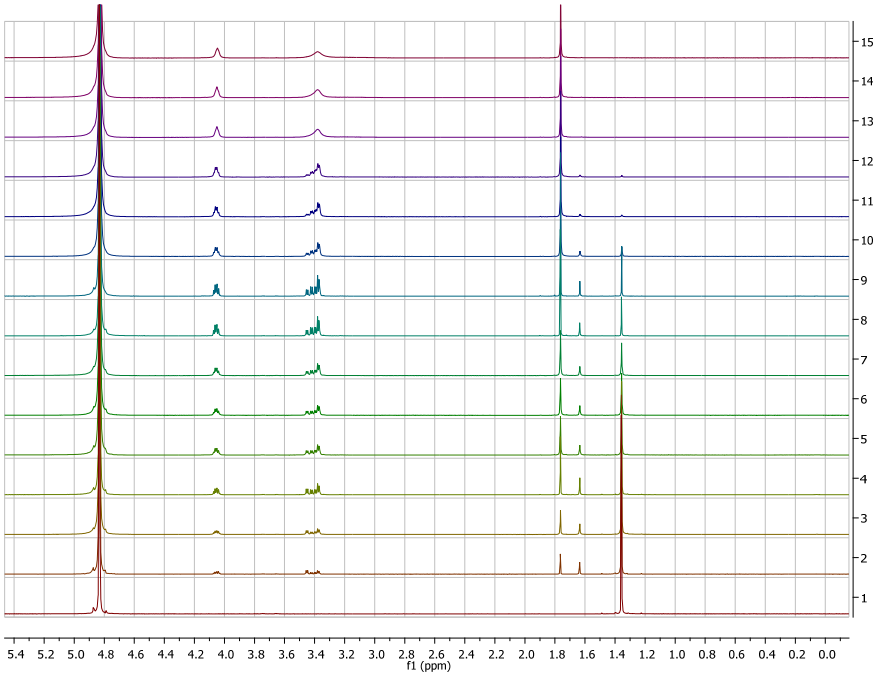
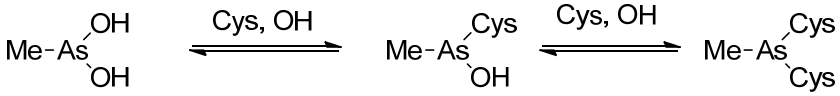


Figure 18: Titration of Cysteine into a solution of (MeAsO)x in increments of 50ul. Top: No cysteine injected, Bottom 2.8 equivalents of cysteine injected.

The peaks corresponds to the following species, 1.76 ppm peak corresponds to $MeAs(Cys)_2$, 1.64 ppm peak to MeAs(OH)(Cys) and 1.358 ppm peak to $MeAs(OH)_2$. The system undergoes the following equilibrium:



From the integrals on the NMR spectra of the system under during the titrations it is possible to work out the concentration of each species after each addition.

As the concentration of the species change due to the dilution caused by the titration, it is easier to look the species in terms of molar ratios:

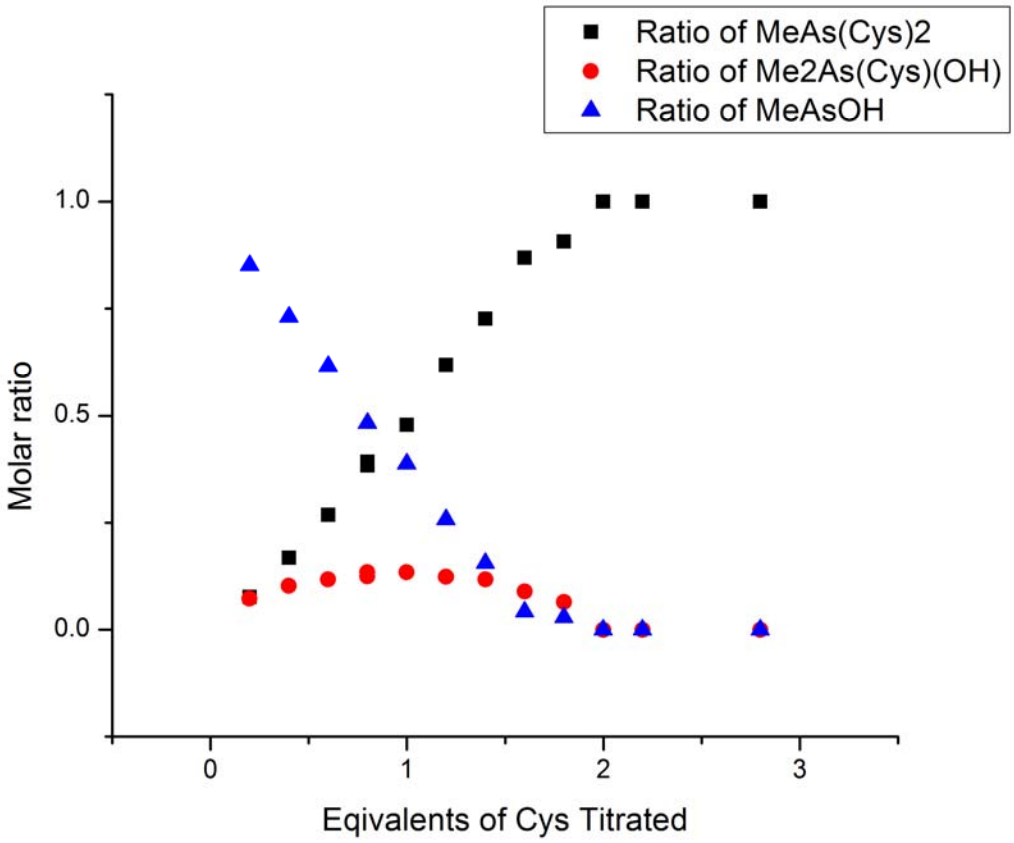


Figure 19: NMR titration of Cysteine against $MeAs(OH)_2$ in D_2O at pH 4.5

We clearly see as more cysteine is titrated into the solution, cysteine would displace the hydroxyl group forming $Me_2As(Cys)_2$ and $Me_2As(Cys)(OH)$. It is interesting to see that the concentration of $Me_2As(Cys)(OH)$ remains relatively constant whilst the preferred product $Me_2As(Cys)_2$ increases over time until it becomes the dominant species.

3.2.4 Temperature sensitivity of the methyl peak

As arsenic has a lone pair, the arsenic the species MeAs(OH)(Cys) is chiral and forms an overall diastereomer with the chiral α Carbon in the cysteine.

This should result in the presence of two peaks for this species as opposed to the singlet that we observe. This suggests there might be dynamic exchanges interactions occurring that is causing the signal to average out. In addition, if the cysteines are labile like in the Me2AsCys case, we might also observe the coalescence of all the methyl peaks.

A preliminary NMR experiment was done with a system with 65 mM of $(MeAsO)_x$ and 77mM of Cysteine at 25°C and 40°C.

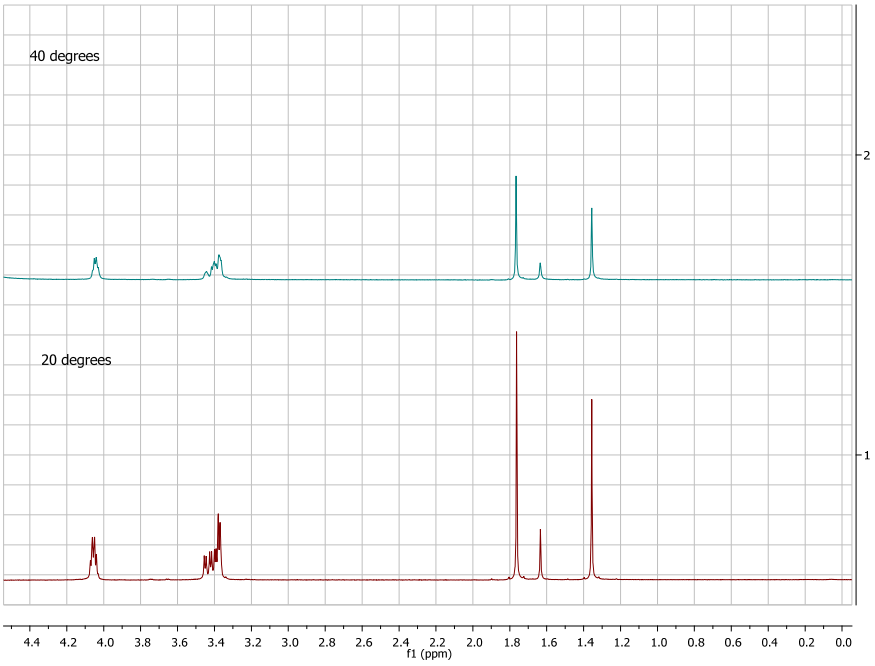


Figure 20: Temperature variation on a solution of $MeAs(OH)_2$ and Cys. Top: 313K, bottom 293K

The results indicate that there is minimal change to the peaks of the arsenic bound methyls and the coalescence of these peaks was not observed.

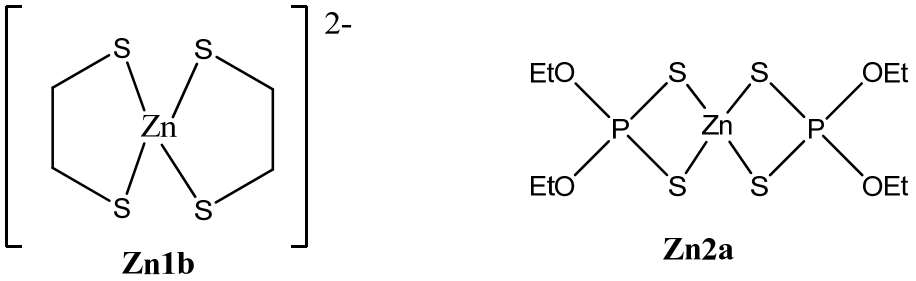
Further experiments into lower temperatures are necessary observe if the peaks would split due chirality.

3.3.1 Zinc Fingers

Recent advances[36,37] 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[38] 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[39]. The “finger” aspect is provided 16 generic residues in the form of a loop[40]. 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[41,40] and searching the Cambridge crystal database

for solved crystal structures of zinc fingers[42,43], we propose the follow molecules as models for zinc fingers:



Zn1b **Zn2a**

Figure 21: 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

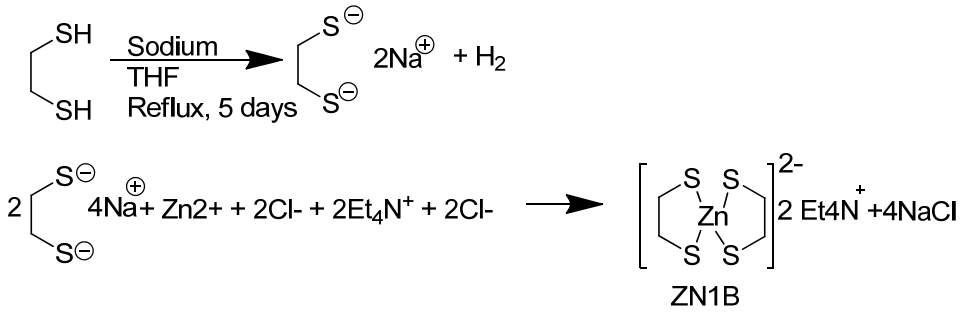


Figure 22: Preparation of Zn1b

Preparation of 1,2 ethanedithiol sodium salt[44]: 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 $(Et_4N)_2$ $[Zn(S_2C_2H_4)_2]$: 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 0.70 of solid crystals were collected with a 25% yield. ^{1}H 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 ^{1}H NMR there is a peak at 3.21 ppm which can be assigned to be the -CH_2 and the 1.16 peak is the -CH_3 on the ethyl. The 3.35 peak could be assigned to be the CH_2 peaks on the ethanedithiol. The integrals suggest there are more ($S_2C_2H_4$) than the formula suggests. This is most likely due to remaining sodium ethanedithiol salt which is difficult to purify.

*Experimental procedure for **Zn2a**:*

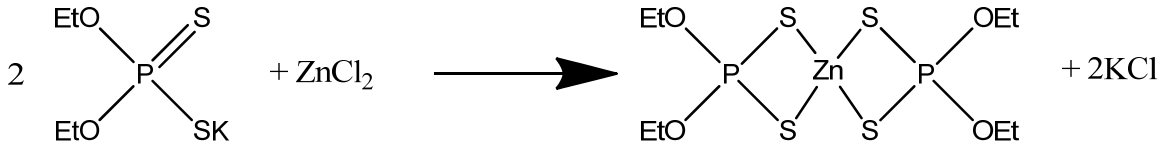


Figure 23: Reaction scheme for the preparation of Zn2a

Potassium diethyl dithiophosphate 0.50g and zinc chloride, 0.20g, was dissolved in 20ml of acetonitrile. 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. ^{1}H NMR (200 MHz, CD_3OD) δ 4.14 (dq, J = 9.5, 7.1 Hz, 2H), 1.32 (td, J = 7.1, 0.5 Hz, 3H). ^{31}P NMR (81 MHz, CD_3OD) 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[45]. 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 $CDCl_3$ and possible interactions were observed via

NMR. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.32 – 7.30 (m, 2H), 4.33 – 4.14 (m, 2H), 1.50 – 1.17 (m, 3H). ^{31}P NMR (81 MHz, $CDCl_3$) δ 97.68 (s). The ^{1}H 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 Me_2AsCys 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 Me_2AsCys. Me_2AsCys was added to a solution of **Zn2a** in Chloroform. The following stacked spectra shows the $–CH_3$ group of Zn2a on top and the NMR of the mixture after the addition of Me_2AsCys.

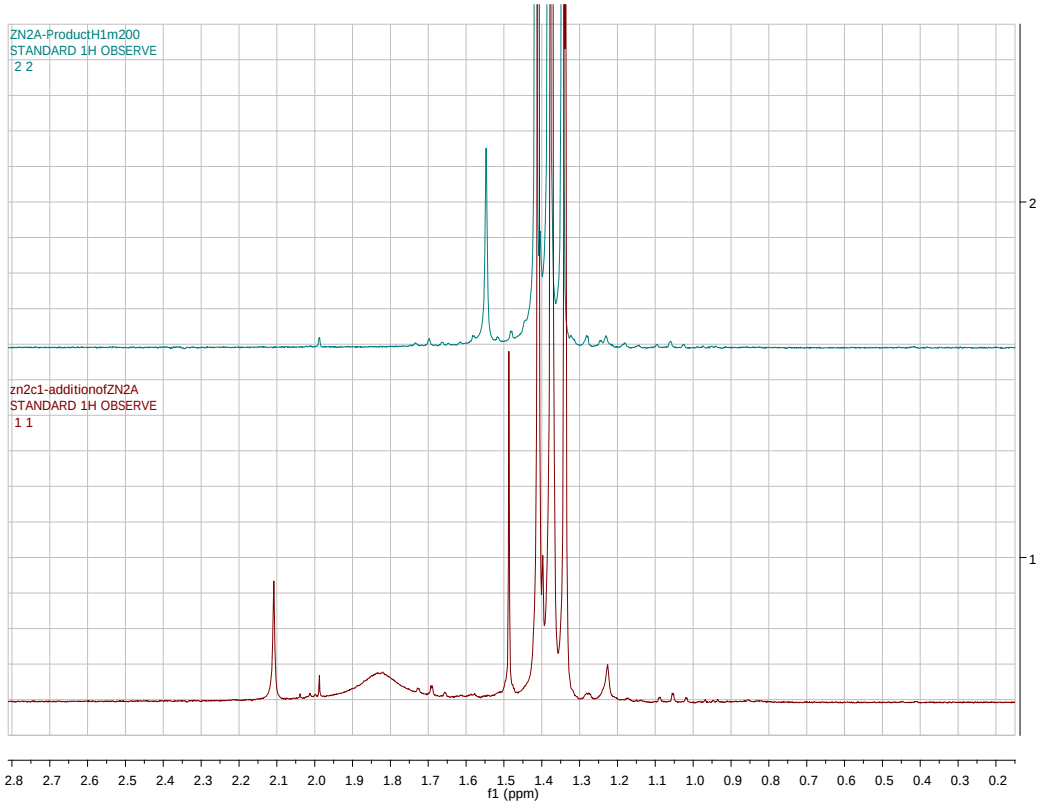


Figure 24: Stacked ^{1}H NMR of Zn2a (top) and the same solution following the addition of Me_2AsCys

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 Me_2AsCys. The phosphorus NMR, indicated a single peak at 98.0 ppm. This is quite surprising as interaction was seen in the ^{1}H NMR spectrum and one would expect multiple peaks in the phosphorus spectra if the diethyl dithiophosphate ligand was being displaced by Me_2AsCys. 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 CD_3OD. 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 CD_3OD. 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 (^{31}P NMR (81 MHz, CD3OD) δ 111.99 (s)).

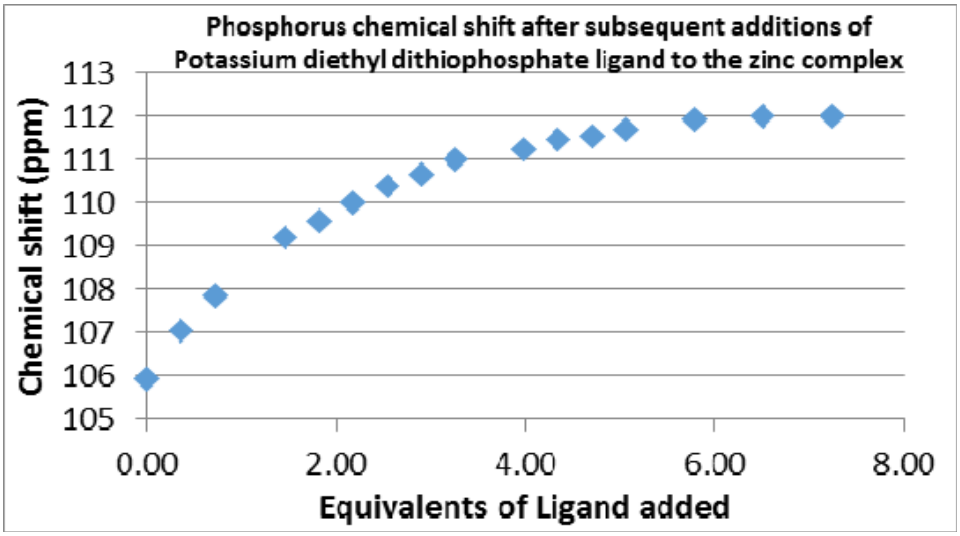


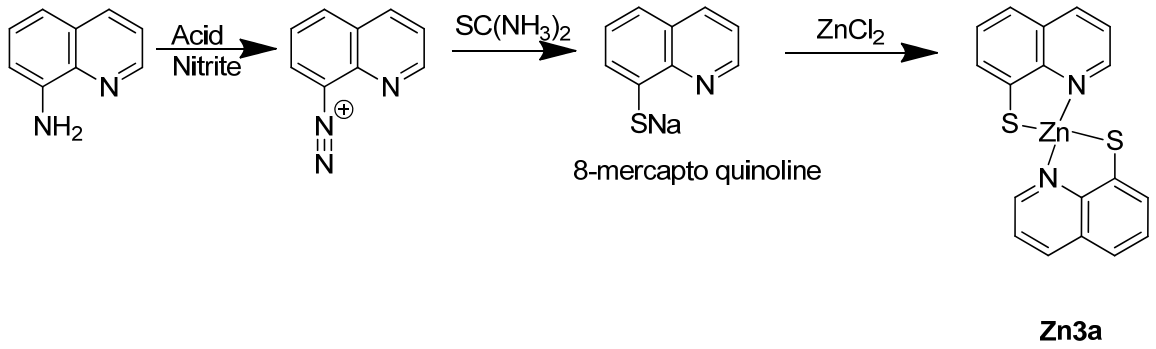
Figure 25: Titration of ligand against zinc salt

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 R^2= 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.



Zn3a

Figure 26: Synthesis of 8-mercapto quinoline zinc salt.

Starting material 8-aminoquinoline had a melting point of 82°C. ^{1}H 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 0.21 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 (0.19g in 5 ml of water). Gas evolution started upon warming to 40°C. The product was recrystallized using ethanol and ether yielding a deep orange crystals with a 89% yield. ^{1}H NMR (400 MHz, DMSO-D6) 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)

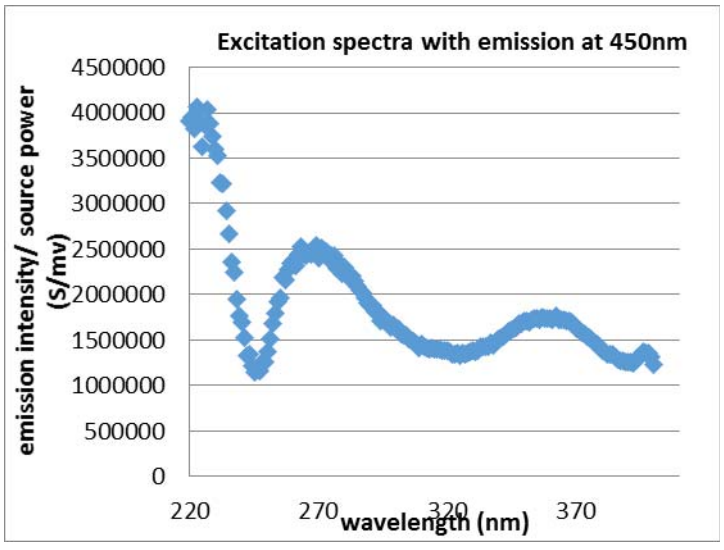


Figure 27: Excitation spectra of 8-aminoquinoline in ethanol (450 emission)

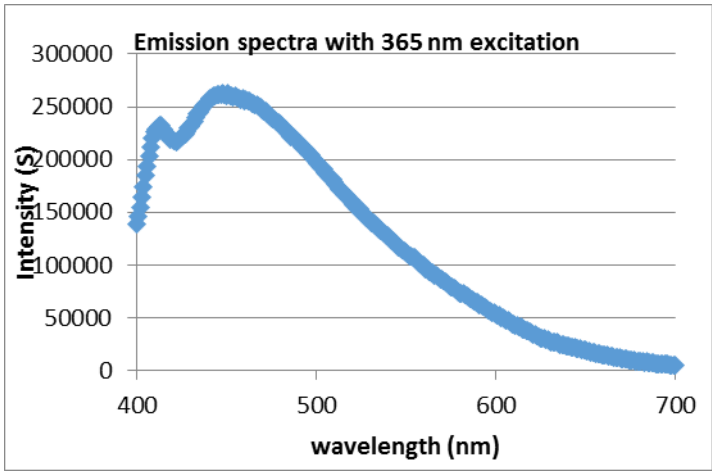


Figure 28: Emission spectra with 365 nm excitation

Given that the ligand is fluorescent, the next step is to synthesize the complex by reacting the 8-amino quinolone with $ZnCl_2$. Unfortunately due to time constraints, this part of could not be completed.

3.3 Conclusions

Throughout the course of this project it was shown that both methylarsenic and dimethylarsenic undergo facile could be exchange between different thiol adducts such as cysteine and glutathione. In the case of dimethylarsenic cysteine, the methyl exchange could be studied using DNMR – elucidating the entropy and enthalpy of the interaction. In addition to transfer between the thiols of cysteines, we also saw dimethylarsenic transfer between cysteine and glutathione groups. Whilst it was not possible to directly model this interaction, it was qualitatively shown that arsenic could move between various thiols. In

addition it was shown that monomethyl arsenic species also have bond lability by titrating cysteine to a solution of monomethylarsenious acid. I have identified the formation of both MeAs(Cys)(OH) and $MeAs(Cys)_2$, and these concentrations would change depending on the amount of cysteine added. Unfortunately it was not possible to explore the kinetics of these species using the DNMR as line shapes did not change with temperature. Further kinetic studies of these systems could be done using stopped flow techniques.

The ability of arsenic to rapidly break and form new bonds has important biological implications. This rapid exchange mechanism gives rise to the possibility that arsenic is transported via a shuttle mechanism where it hops into to various species and is carried around the body. In additional to binding to small molecules such as cysteine and glutathione, arsenic could also bond to viscinal cysteines of large proteins, disrupting its function. Understanding this interaction is key to understanding the mechanism of arsenic based drugs such as ATO and Darinaparsin.

3.4 References

33. Burrows, G. J.; Turner, E. E., A new type of compound containing arsenic. *Journal of the Chemical Society, Transactions* 1920, *117*, 1373-1373.

34. Marsmann, H. C.; Wazer, J. R. V., Methylarsaoxanes. Structural chemistry of cacodyl oxide and arsenosomethane in the liquid state. *JACS* **1970,** *363* (2), 3969-3972.

35. Petrick, J. S.; Jagadish, B.; Mash, E. A.; Aposhian, H. V., Monomethylarsonous Acid (MMA III) and Arsenite: LD 50 in Hamsters and In Vitro Inhibition of Pyruvate Dehydrogenase. *Chem. Res. Toxicol.* **2001,** *14* (6), 651-656.

36. Zhang, X.-W.; Yan, X.-J.; Zhou, Z.-R.; Yang, F.-F.; Wu, Z.-Y.; Sun, H.-B.; Liang, W.-X.; Song, A.-X.; Lallemand-Breitenbach, V.; Jeanne, M.; Zhang, Q.-Y.; Yang, H.-Y.; Huang, Q.-H.; Zhou, G.-B.; Tong, J.-H.; Zhang, Y.; Wu, J.-H.; Hu, H.-Y.; de The, H.; Chen, S.-J.; Chen, Z., Arsenic Trioxide Controls the Fate of the PML-RAR{alpha} Oncoprotein by Directly Binding PML. *Science* **2010,** *328* (5975), 240-243.

37. Borden, K. L.; Lally, J. M.; Martin, S. R.; O'Reilly, N. J.; Solomon, E.; Freemont, P. S., In vivo and in vitro characterization of the B1 and B2 zinc-binding domains from the acute promyelocytic leukemia protooncoprotein PML. *Proceedings of the National Academy of Sciences* **1996,** *93* (4), 1601-1601.

38. Borden, K. L. B.; Boddy, M. N.; Lally, J.; Oreilly, N. J.; Martin, S.; Howe, K.; Solomon, E.; Freemont, P. S., THE SOLUTION STRUCTURE OF THE RING

FINGER DOMAIN FROM THE ACUTE PROMYELOCYTIC LEUKEMIA PROTO-ONCOPROTEIN PML. *Embo J.* **1995,** *14* (7), 1532-1541.

39. Reddy, P. R.; Radhika, M., Synthesis and characterization of mixed ligand complexes of Zn (II) and Co (II) with amino acids: Relevance to zinc binding sites in zinc fingers. *Journal of Chemical Sciences* **2005,** *117* (3), 239-246.

40. Dudev, T.; Lim, C., Modeling Zn2+-cysteinate complexes in proteins. *The Journal of Physical Chemistry B* **2001,** *105* (43), 10709-10714.

41. Barnes, A. M.; Bartle, K. D.; Thibon, V. R. A., A review of zinc dialkyldithiophosphates (ZDDPS): characterisation and role in the lubricating oil. *Tribology International* **2001,** *34* (6), 389-395.

42. Segal, D. J.; Crotty, J. W.; Bhakta, M. S.; Barbas Iii, C. F.; Horton, N. C., Structure of Aart, a designed six-finger zinc finger peptide, bound to DNA. *J. Mol. Biol.* **2006,** *363* (2), 405-421.

43. Pavletich, N. P.; Pabo, C. O., Crystal structure of a five-finger GLI-DNA complex: new perspectives on zinc fingers. *Science* **1993,** *261* (5129), 1701-1701.

44. Rao, C. P.; Dorfman, J. R.; Holm, R. H., Synthesis and structural systematics of ethane-1,2-dithiolato complexes. *Inorganic Chemistry* **1986,** *25* (4), 428-439.

45. Ivanov, A. V.; Antzutkin, O. N.; Larsson, A.-C.; Kritikos, M.; Forsling, W., Polycrystalline and surface O,O'-dialkyldithiophosphate zinc(II) complexes: preparation, 31P CP/MAS NMR and single-crystal X-ray diffraction studies. *Inorganica Chimica Acta* **2001,** *315* (1), 26-35.