3.1 Mechanism elucidation of dimethyl arsenic exchange

In Chapter 2 we showed that facile dimethylarsenic exchange occurred in the dimethylarsenic adducts of cysteine and glutathione. The aim of this chapter is to attempt to elucidate the mechanism behind the exchange. Several proposed mechanisms have been outlined in Figure 14.



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

Another possibility is an intermolecular attack from the nitrogen from another Me2AsCys into the arsenic, thus forming pyramidal intermediate.

This nucleophilic attack could also be intramolecular, causing the formation of a 5 membered ring. If this was the case, chemical substitution of electron withdrawing or donation substituents on the nitrogen would cause changes in the rate of reaction. Section 3.1.3 of this chapter deals with the attempts at synthesizing the chemically substituted species dimethylarseno-penicillamine and dimethylarseno-N-acetyl cysteine.

Dimethyl arseno species get demethylated in the body as outlined in section 1.1.1. The monomethylated species is not only biologically relevant, but might interact in a similar way to the demethylated species. In section 3.2.1, monomethylated species was synthesized and its interaction with cysteine in solution was investigated.

3.1.3 Synthetic analogues to dimethylarseno cysteine

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 cysteinewhere 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.* Me2AsI was prepared using the Burrows method[32](#_ENREF_4_1). Potassium iodide, 15g, and 5g of Me2AsOOH 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, CDCl3) 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. 1H NMR (400 MHz, d2o) 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 Me2AsCys required for DNMR experimentation. Purity is especially important because contaminants of other thiol containing products are expected to significantly alter the kinetics.