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**SYNTHESIS OF AN ARGININE MIMIC FOR  
AN ANTIFUNGAL OCTAPEPTIDE LIBRARY**

This thesis is presented in partial fulfilment of the requirements for the  
degree of Master of Science in Chemistry at Massey University,  
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**Hao Sun**

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## ABSTRACT

A family of bioactive antifungal octapeptides has been established<sup>1-3</sup>. The template for this library is based on peptides composed of two predefined amino acids, three arginines, and three unknown components. The arginine contains a guanidino group (pKa =12.48) which is readily protonated under physiological conditions, and hence can bind to fungal cell walls which possess a negatively charged surface. Other components of this peptide are used to kill the fungi by inhibition of their sodium transport system<sup>1-5</sup>.

To date this story has survived on the fact that D-amino acids work best. However both D and L arginine derivatives are quite expensive. In collaboration with the research group in the University of Otago, it was planned to develop an effective and economic method to produce a series of compounds to replace arginine. These mimics were designed to be artificial amino acids as  $\text{NH}_2\text{---N}^*(\text{R}')\text{---COOR}$  where the side chain R' carries a guanidino group. Thus the chiral carbon is now replaced by an amide group hence no stereocenter, no enantiomers but the guanidino group will retain a high pKa similar to that of the natural arginine. For the synthetic strategy, the target molecule was divided into two parts, one the amino acid backbone, and the other the guanidine containing side chain. Each segment was built up separately and finally combined together.

The backbone of the arginine mimic must possess two amino groups. One is at the N-terminal for the next step in peptide synthesis, the other one (N\*) is used to connect to the guanidino containing side chain. This was reacted with selected acetate derivatives (*t*-butylchloroacetate, benzylchloroacetate, methylbromoacetate) that had potential to produce the desired backbone in good yield.

Construction of the side chain (R') to be composed of a di-protected guanidine and a carboxylic group was the most challenging and difficult part of this project. To achieve this, two different approaches were studied. One was to use a primary amino acid  $\text{NH}_2\text{---}(\text{CH}_2)_x\text{---COOH}$  (X=1,2,5) to react with a guanylating reagent to make the unprotected side chain, then two protecting groups were added to the two nitrogen containing groups of the guanidine. The other method was protection of a guanylating reagent (usually carboxamide compounds) then reaction with a primary amino acid to make a di-protected guanidine containing side chain.

In amino acid and peptide chemistry, to avoid self condensation and by-product formation, selectivity of amino and carboxylic groups is very crucial hence application of different types of protecting groups are the basis of peptide construction. Therefore in this project, employment of suitable protecting groups at both N- and C-terminals was incorporated in this study.

Finally, one arginine mimic *N*-[*N'*-((9-fluorenyl)methoxycarbonyl)-2-aminoethyl]-*N*-*t*-butyloxycarbonylmethyl-3-*N',N''*-bis(*t*-butyloxycarbonyl)carbamidinopropanamide was successfully built up. It will be used for the construction of octapeptides for the current antifungal programme. The final product will be sent to University of Otago for bio-activity tests.

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## TABLE OF CONTENTS

|                      |  |           |
|----------------------|--|-----------|
| Abstract             | I  |           |
| Acknowledgements     | III  |           |
| List of Figures      | VII  |           |
| List of Tables       | X  |           |
| List of Abbreviation | XI   |           |
| <br>                 |  |           |
| <b>CHAPTER ONE</b>   | <b>INTRODUCTION</b>  | <b>01</b> |
| <br>                 |  |           |
| 1.1                  | A D-Octapeptide Combinatorial Library                                | 01        |
| 1.2                  | Natural Arginine and the Guanidino Group                             | 03        |
| 1.3                  | Structure of a Designed Arginine Mimic<br>and the Synthetic Strategy | 05        |
| 1.4                  | Strategy for the Synthesis of a Protected<br>Guanidino Group         | 07        |
| 1.5                  | Protection of the N-Terminus in Peptide Chemistry                    | 09        |
| 1.6                  | Protection of C-Terminus in Peptide Chemistry                        | 13        |
| 1.7                  | Coupling Reactions   | 14        |
| <br>                 |  |           |
| <b>CHAPTER TWO</b>   | <b>EQUIPMENT AND MATERIALS</b>                                       | <b>16</b> |
| <br>                 |  |           |
| 2.1                  | Equipment  | 16        |
| 2.2                  | Materials  | 16        |
| <br>                 |  |           |
| <b>CHAPTER THREE</b> | <b>FORMATION OF THE PROTECTED<br/>GUANIDINO GROUP</b>                | <b>17</b> |



|                      |   |           |
|----------------------|---|-----------|
| 4.4                  | Experimental  | 52        |
| <b>CHAPTER FIVE</b>  | <b>BACKBONE SYNTHESIS</b>   | <b>54</b> |
| 5.1                  | Introduction  | 54        |
| 5.2                  | Synthesis and Discussion  | 56        |
| 5.3                  | Conclusion  | 58        |
| 5.4                  | Experimental  | 58        |
| <b>CHAPTER SIX</b>   | <b>COUPLING OF THE GUANIDINE<br/>CONTAINING SIDE CHAIN TO<br/>THE AMINO ACID BACKBONE</b> | <b>60</b> |
| 6.1                  | Introduction  | 60        |
| 6.2                  | Discussion  | 61        |
| 6.3                  | Conclusion  | 62        |
| 6.4                  | Experimental  | 62        |
| <b>CHAPTER SEVEN</b> | <b>SUMMARY AND CONCLUSION</b>   | <b>64</b> |
| <b>CHAPTER EIGHT</b> | <b>CONCLUSION</b>   | <b>67</b> |
| <b>REFERENCES</b>    |   | <b>69</b> |

## LIST OF FIGURES

|           |   |    |
|-----------|---|----|
| Figure 1  | Designed D-octapeptide and the combinatorial library  | 02 |
| Figure 2  | Structure of natural L and D arginine   | 03 |
| Figure 3  | Resonance structures of guanidine   | 04 |
| Figure 4  | Structure of the target arginine mimic in this project  | 05 |
| Figure 5  | Synthetic strategy of arginine mimics   | 06 |
| Figure 6  | Structure and molecular weight of glycine,<br>$\beta$ -alanine and 6-aminohexanoic acid                     | 06 |
| Figure 7  | Structure of a typical guanylation reagent and the<br>normal process of formation of a guanidino group      | 07 |
| Figure 8  | Four possibilities of di-peptide formation using<br>unprotected amino acids                                 | 10 |
| Figure 9  | Design of a protected arginine mimic  | 11 |
| Figure 10 | Mechanisms of cleavage of Fmoc and Boc groups   | 12 |
| Figure 11 | The structures of DCC and HBTU  | 15 |
| Figure 12 | The three routes for the formation of substituted<br>guanidines   | 17 |
| Figure 13 | The mechanism of Mitsunobu reaction to achieve<br>substituted guanidine formation                           | 19 |
| Figure 14 | Synthetic strategy for making the di-Boc guanidino<br>acid from the guanidine hydrochloride                 | 20 |
| Figure 15 | Formation of 1H-pyrazole-1-carboxamidine<br>hydrochloride   | 24 |
| Figure 16 | Reaction of 1H-pyrazole-1-carboxamidine<br>hydrochloride with a primary amino acid                          | 24 |
| Figure 17 | Self-condensation of 1H-pyrazole-1-carboxamidine<br>and of <i>N</i> -alkyldiguanidine                       | 26 |
| Figure 18 | Formation of guanidino acids from reaction<br>of aminoiminomethanesulfonic acid with primary<br>amino acids | 27 |
| Figure 19 | Structure of unprotected guanidino acids  | 32 |
| Figure 20 | Structure of di- <i>t</i> -butyl dicarbonate and the  |    |

|           |   |    |
|-----------|---|----|
|           | mechanism of its amine protecting reaction  | 34 |
| Figure 21 | Preparation of di-Boc-protected guanidino acid  | 35 |
| Figure 22 | Three reagents for the introduction of the Cbz group  | 36 |
| Figure 23 | Preparation of di-Cbz protected guanidino acids<br>using benzyloxycarbonylsuccinimide   | 37 |
| Figure 24 | Di-tosyl protected arginine mimic   | 38 |
| Figure 25 | Attempted di-protection of a guanidino group<br>with <i>p</i> -toluenesulfonyl chloride   | 39 |
| Figure 26 | Angles of the guanidino group for the unprotected<br>and mono protected guanidino acids   | 41 |
| Figure 27 | Angles of the guanidino group for thiourea and<br>S-methylisothiourea and their di-Boc products   | 43 |
| Figure 28 | Basic mechanism for the reaction of<br><i>N,N'</i> -bis- <i>t</i> -Butoxycarbonylthiourea with amines<br>catalyzed by HgCl <sub>2</sub>   | 45 |
| Figure 29 | Formation of di-protected guanidine acids from<br>S-methylisothiourea   | 46 |
| Figure 30 | Protonation of pyrazole   | 49 |
| Figure 31 | Formation of 1H-pyrazole-1-[ <i>N,N''</i> -<br>-bis( <i>t</i> -Butoxycarbonyl)]carboxamidine  | 49 |
| Figure 32 | Production of guanidines from 1H-Pyrazole-1-<br>[ <i>N,N''</i> -bis( <i>t</i> -butoxycarbonyl)]carboxamidine  | 50 |
| Figure 33 | Designed backbone structure   | 54 |
| Figure 34 | Basic process of solid phase peptide synthesis  | 55 |
| Figure 35 | Mechanism for removal of the 9-<br>fluoromethyloxycarbonyl group (Fmoc)   | 56 |
| Figure 36 | Reaction scheme for the construction of the<br>protected backbone amino acid  | 57 |
| Figure 37 | Structure and reaction scheme for the<br>coupling reagents DCC and HBTU   | 61 |
| Figure 38 | The arginine mimic <i>N</i> -[ <i>N'</i> -((9-fluorenyl)methoxycarbonyl)-<br>2-aminoethyl]- <i>N-t</i> -butyloxycarbonylmethyl-<br>3- <i>N',N''</i> -bis( <i>t</i> -butyloxycarbonyl)carbamidinopropanamide<br>produced in this project | 64 |

|           |   |    |
|-----------|---|----|
| Figure 39 | Designed arginine mimic in which benzyl ester is used as C-terminal block group   | 65 |
| Figure 40 | Mechanism for the removal of the benzyl group on the C-terminal by hydrogenolysis | 65 |

## LIST OF TABLES

|         |   |    |
|---------|---|----|
| Table 1 | Experimental results for the reaction of<br>1H-Pyrazole-1-carboxamide<br>hydrochloride with three primary amino acids   | 25 |
| Table 2 | Experimental results for the reaction of<br>aminoiminomethanesulfonic acid with three<br>primary amino acids  | 27 |
| Table 3 | Experimental results for the formation of di-Boc guanidino<br>acids from the reaction of the <i>N,N'</i> -bis<br>( <i>t</i> -butyloxycarbonyl)- <i>S</i> -methylisothiourea with<br>three primary amino acids | 47 |
| Table 4 | Basicity of common amines   | 60 |

## LIST OF ABBREVIATIONS

|                      |  |
|----------------------|--|
| Arg                  | arginine   |
| Boc                  | <i>t</i> -butyloxycarbonyl   |
| (Boc) <sub>2</sub> O | di- <i>t</i> -butyl dicarbonate  |
| Cbz                  | benzyloxycarbonyl  |
| (Cbz) <sub>2</sub> O | dibenzyl dicarbonate   |
| Cbz-OSu              | benzyloxycarbonylsuccinimide   |
| DCC                  | dicyclohexylcarbodiimide   |
| DCM                  | dichloromethane  |
| DEAD                 | diethyl azodicarboxylate   |
| DIEA                 | diisopropylethylamine  |
| Fmoc                 | 9-fluorenylmethyloxycarbonyl   |
| Fmoc-OSu             | <i>N</i> -(9-fluorenylmethoxycarbonyloxy)succinimide                             |
| HBTU                 | O-Benzotriazol-1-yl- <i>N,N,N',N'</i> -tetramethyluronium<br>hexafluorophosphate |
| ortho-Br-Cbz         | ortho-bromobenzyloxycarbonyl   |
| ortho-Cl-Cbz         | ortho-chlorobenzyloxycarbonyl  |
| PNA                  | peptide nucleic acid   |
| PPh <sub>3</sub>     | triphenylphosphine   |
| SPPS                 | solid phase peptide synthesis  |
| TFA                  | trifluoroacetic acid   |