SYNTHESIS OF AN ARGININE MIMIC FOR AN ANTIFUNGAL OCTAPEPTIDE LIBRARY

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ABSTRACT

A family of bioactive antifungal octapeptides has been established[1-3]. 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[1-5].

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 NH_2~~N*(R')~~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 NH_2-$(CH_2)_x$-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 carboxamidine 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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LIST OF ABBREVIATIONS

Arg	arginine
Boc	*t*-butyloxycarbonyl
$(Boc)_2O$	di-*t*-butyl dicarbonate
Cbz	benzyloxycarbonyl
$(Cbz)_2O$	dibenzyl dicarbonate
Cbz-OSu	benzyloxycarbonylsuccinimide
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIEA	diisopropylethylamine
Fmoc	9-fluorenylmethyloxycarbonyl
Fmoc-OSu	*N*-(9-fluorenylmethoxycarbonyloxy)succinimide
HBTU	O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate
ortho-Br-Cbz	ortho-bromobenzyloxycarbonyl
ortho-Cl-Cbz	ortho-chlorobenzyloxycarbonyl
PNA	peptide nucleic acid
PPh_3	triphenylphosphine
SPPS	solid phase peptide synthesis
TFA	trifluoroacetic acid