

# Natural Products Applied in Reverse Chemical Proteomics

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## Thesis Abstract

Natural products are a rich source of structurally diverse and biologically active small molecules. They constitute a useful class of compounds as leads in rational drug design and development. However, drug discovery faces a major bottleneck due to the lack of knowledge about the active compounds' cellular targets and mode of action. For this thesis, several natural products with interesting biological activity have been applied as ligands in a technique we refer to as reverse chemical proteomics. This method rapidly generates protein-ligand pairs, which will be useful for the rational design of new and more potent therapeutics, identification of druggable targets as well as for understanding the underlying biochemical pathways of these active ligands. This thesis is divided into four main chapters. Chapter 1 introduces the techniques behind reverse chemical proteomics. Chapter 2 describes the bioassay-guided fractionation of eleven marine sponges, the isolation and characterisation of two new and seven known bromotyrosines of the highly antibacterial active extract from the marine sponge *Pseudoceratina purpurea*, as well as isolation of bromotyrosines from the opisthobranch *Tylodina corticalis*, which was collected while feeding on *P. purpurea*. In Chapter 3, the chemical derivatisation of natural products is presented alongside the synthesis and characterisation of novel, linkers and reagents required for performing reverse chemical proteomics. Chapter 4 describes the application of T7 phage display utilising the immunosuppressant natural product FK506 as a model affinity probe. Consecutively, protein binding partners for biotinylated artesunate, daptomycin and manzamine are isolated from various T7 phage-displayed human cDNA libraries. An experimental chapter and concluding remarks follow thereafter.

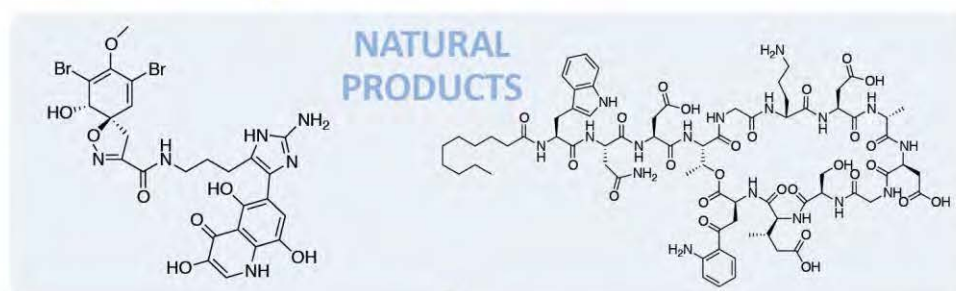


# Graphical Abstract

## Chapter 2

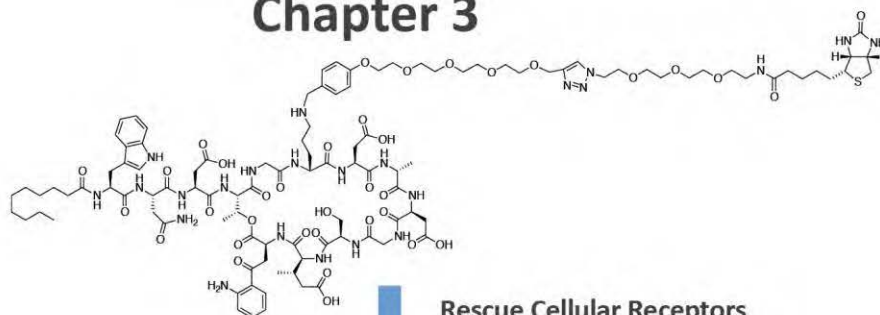


Isolate Marine  
Natural Products



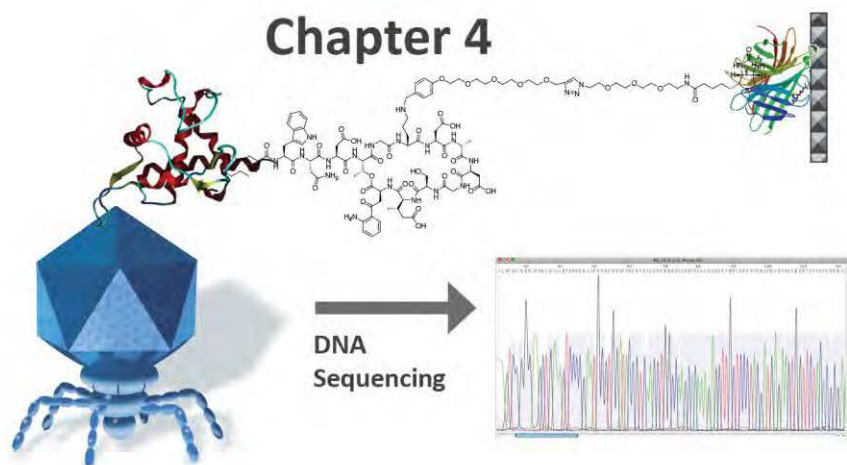
Derivatised  
Natural Products

## Chapter 3



Rescue Cellular Receptors  
for the Natural Products from  
T7 Phage-Displayed cDNA library

## Chapter 4





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## Abbreviations

|                             |                                                               |
|-----------------------------|---------------------------------------------------------------|
| aa                          | amino acids                                                   |
| amu                         | atomic mass unit                                              |
| ATP                         | adenosine triphosphate                                        |
| AUC                         | area under curve                                              |
| BES                         | <i>N,N</i> -Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid    |
| Boc                         | <i>tert</i> -butoxycarbonyl                                   |
| bp                          | base pairs                                                    |
| br                          | broad (IR, NMR)                                               |
| BuOH                        | 1-butanol                                                     |
| CDCl <sub>3</sub>           | deuterated chloroform                                         |
| CHCl <sub>3</sub>           | chloroform                                                    |
| cDNA                        | complementary DNA                                             |
| CDS                         | coding sequence                                               |
| COSY                        | correlated spectroscopy                                       |
| d                           | doublet (NMR)                                                 |
| DCC                         | 1,3-dicyclohexylcarbodiimide                                  |
| DCM                         | dichloromethane                                               |
| DHA                         | dihydroartemisinin                                            |
| DMAP                        | 4-dimethylaminopyridine                                       |
| DMSO                        | dimethylsulfoxide                                             |
| DMSO- <i>d</i> <sub>6</sub> | deuterated dimethylsulfoxide                                  |
| DNA                         | deoxyribonucleic acid                                         |
| dNTP                        | deoxyribonucleotide triphosphate                              |
| DSC                         | <i>N,N'</i> -disuccinimidyl carbonate                         |
| dsDNA                       | double-stranded DNA                                           |
| <i>E. coli</i>              | <i>Escherichia coli</i>                                       |
| EDC                         | 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride |
| EDTA                        | ethylenediaminetetraacetic acid                               |
| ESI                         | electrospray ionisation                                       |
| FDA                         | US Food and Drug Administration                               |
| FKBP                        | FK506 binding protein                                         |
| FTIR                        | Fourier transform infrared                                    |
| gp                          | gene product                                                  |
| Grubbs' catalyst            | benzylidene-bis(tricyclohexylphosphine) dichlororuthenium     |

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|                        |                                                                                                       |
|------------------------|-------------------------------------------------------------------------------------------------------|
| HMBC                   | heteronuclear multiple bond coherence                                                                 |
| HOBt                   | hydroxybenzotriazole                                                                                  |
| HPLC                   | high performance liquid chromatography                                                                |
| HRESI                  | high resolution electrospray ionisation (mass spectrometry)                                           |
| HSQC                   | heteronuclear single quantum coherence                                                                |
| Hünig's base           | <i>N,N</i> -diisopropylethylamine                                                                     |
| IL                     | interleukin                                                                                           |
| IPTG                   | isopropyl- $\beta$ -D-thiogalactopyranoside                                                           |
| IR                     | infrared                                                                                              |
| kb                     | kilobase pairs                                                                                        |
| LB                     | Luria Media                                                                                           |
| LC-MS                  | liquid chromatography - mass spectrometry                                                             |
| LR-MS                  | low resolution - mass spectrometry                                                                    |
| m                      | multiplet (NMR), medium (IR)                                                                          |
| MCS                    | multiple cloning site                                                                                 |
| MeCN                   | acetonitrile                                                                                          |
| MeOH                   | methanol                                                                                              |
| MOA                    | mode of action                                                                                        |
| mRNA                   | messenger RNA                                                                                         |
| MS                     | mass spectrometry                                                                                     |
| MTT                    | Thiazolyl Blue Tetrazolium Bromide [or (3-(4,5 Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] |
| NaBH(OAc) <sub>3</sub> | sodium(triacetoxy)borohydride                                                                         |
| NHS                    | <i>N</i> -hydroxysuccinimide                                                                          |
| NMR                    | nuclear magnetic resonance                                                                            |
| ODn                    | optical density at 'n' nanometres                                                                     |
| PBS                    | phosphate buffered saline                                                                             |
| PCR                    | polymerase chain reaction                                                                             |
| PE                     | petroleum ether                                                                                       |
| PEG                    | poly(ethylene glycol)                                                                                 |
| PEG-n                  | poly(ethylene glycol) with average molecular weight of 'n'                                            |
| <i>P. aeruginosa</i>   | <i>Pseudomonas aeruginosa</i>                                                                         |
| PWB                    | phage wash buffer (PBS + 0.05% Tween-20)                                                              |
| q                      | quartet (NMR)                                                                                         |
| RNA                    | ribonucleic acid                                                                                      |
| RP-HPLC                | reverse phase – high performance liquid chromatography                                                |

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|------------------------|-----------------------------------------------|
| rpm                    | revolutions per minute                        |
| RPS19                  | ribosomal protein S19                         |
| rRNA                   | ribosomal RNA                                 |
| s                      | singlet (NMR), sharp (IR)                     |
| SAR                    | structure-activity relationship               |
| <i>S. aureus</i>       | <i>Staphylococcus aureus</i>                  |
| SCUBA                  | self contained underwater breathing apparatus |
| SDS                    | sodium dodecyl sulfate                        |
| sp.                    | species                                       |
| spp                    | species (pl.)                                 |
| ssDNA                  | single-stranded DNA                           |
| t                      | triplet (NMR)                                 |
| Taq                    | <i>Thermus aquaticus</i>                      |
| TEA                    | triethylamine                                 |
| TEG                    | tetra(ethylene glycol)                        |
| TFA                    | trifluoroacetic acid                          |
| THF                    | tetrahydrofuran                               |
| TLC                    | thin layer chromatography                     |
| TNF                    | tumour necrosis factor                        |
| TOCSY                  | totally correlated spectroscopy               |
| Tris                   | tris(hydroxymethyl)aminomethane               |
| UV                     | ultraviolet                                   |
| UV-Vis                 | ultraviolet-visible                           |
| WHO                    | World Health Organisation                     |
| w                      | weak (IR, NMR)                                |
| $\lambda_{\text{max}}$ | wavelength of maximal UV absorption           |
| $\nu_{\text{max}}$     | frequency of maximal IR absorption            |



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## Declaration

I certify that the work in this thesis entitled “Natural Products Applied in Reverse Chemical Proteomics” has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis was approved by Macquarie University Biosafety Review Committee, reference number: 5201001557 NLRD on the 8.12.2010.

Michael Gotsbacher

(SN 41130758)

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