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***N*-Linked Glycopeptide Mimetics as  
Tools in Kinetic, Mechanistic and  
Structural Studies of Peptide  
*N*:Glycanase F**

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A thesis  
presented in partial fulfilment  
of the requirements for the degree  
of  
**Doctor of Philosophy in Biochemistry**  
at  
**Massey University**  
by  
**Dirk Henning Lenz**

---



**Massey University**

**Palmerston North  
New Zealand  
2003**



### CERTIFICATE OF REGULATORY COMPLIANCE

This is to certify that the research carried out in the Doctoral Thesis entitled "N-linked Glycopeptide Mimetics as Tools in Kinetic, Mechanistic and Structural Studies of Peptide N:Glycanase F" in the Institute of Molecular BioSciences at Massey University, New Zealand, and at IRL, Lower Hutt (with the approval of the Head of Institute of IMBS):

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**Date:** 22 March 2004

**Supervisor's Name:** Gillian E. Norris

**Signature:**

**Date:** 22 March 2004



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This is to state that the research carried out for my Doctoral thesis entitled "N-linked Glycopeptide Mimetics as Tools in Kinetic, Mechanistic and Structural Studies of Peptide N:Glycanase F" under the auspices of IMBS at IRL with the approval of the Head of Institute and in the Institute of Molecular BioSciences at Massey University, Palmerston North, New Zealand is all my own work. This is also to certify that thesis material has not been used for any other degree.

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**Supervisor's Name:** Gillian E. Norris

**Signature:**



**Date:** 22 March 2004

# ABSTRACT

PNGases (Peptide- $N^4$ -( $N$ -acetyl- $\beta$ -glucosaminyl)asparagine amidases (E.C. 3.5.1.52) cleave the carbohydrate chains from the asparagine side chains of glycoproteins. They are widely used to deglycosylate  $N$ -linked glycoproteins and glycopeptides for analytical purposes. PNGase F from *Flavobacterium meningosepticum* is the best characterised of this class of enzymes but little is known so far about the biological significance or the catalytic mechanism of these intriguing enzymes.

The substrate binding and cleavage mechanism of PNGase F has now been investigated.

The first part of this work describes the synthesis of various novel  $N$ -linked glycopeptide mimetics which were then used in kinetic investigations with PNGase F. To facilitate kinetic studies at low substrate concentrations, a discontinuous HPLC based assay using a fluorescently labelled ovalbumin glycopeptide had to be developed. These experiments led to a better understanding of the structural requirements for substrate binding which will aid the future development of potent PNGase F inhibitors.

In the second part of the thesis, a virtual  $N$ -linked glycopeptide from human lactoferrin was modelled into the active site region of PNGase F using molecular modelling techniques. This model has resulted in the proposal of a mechanism for catalysis that predicts an important role for Arginine 248, a residue that had previously not been considered part of the catalytic machinery. The model also provides a basis for explaining the substrate specificity of the enzyme. The mechanism is supported by kinetic studies with targeted PNGase F mutants. As a result of this study, new PNGase F mutants have been designed to test the current findings.

## *To my Family*

To be consistent [...]: no penicillin, no lightning rods, no eyeglasses, no DDT, no radar and so on. We live technologically, with man as the master of nature, man as the engineer, and let anyone who raises his voice against it stop using bridges not built by nature. To be consistent, they would have to reject any kind of operation; that would mean people dying every time they had appendicitis. What an outlook! No electric-light bulbs, no engines, no atomic energy, no adding machines, no anesthetics-back to jungle!

From "Homo Faber", A Report by Max Frisch (1959)

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Posthumous **André Citroën, Pierre Boulanger, André Lefèbvre, Paul Mages and Flaminio Bertoni** the names of which are inseparably connected with a pinnacle of automobile engineering, “la déesse (DS)” (the goddess) [1].

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# TABLE OF CONTENTS

Table of Contents		i
List of Abbreviations		iv
<b>Chapter 1</b>	<b>Introduction</b>	<b>1</b>
1.1	Peptide <i>N</i> :Glycanases	2
1.2	<i>In vivo</i> Functions of PNGases	3
1.3	PNGases: Sources and Properties	13
1.4	Assays for the Determination of PNGase Activity	18
1.5	PNGase A from Almonds ( <i>Prunus amygdalus</i> )	20
1.6	PNGase F from <i>Flavobacterium meningosepticum</i>	22
1.7	Concepts of Inhibitors Towards PNGases	25
1.7.1	<i>N</i> -Linked Glycopeptide Mimetics	26
1.7.2	<i>N</i> -Glycosyl Phosphoramidates	30
1.8	Aims of the Thesis	33
<b>Chapter 2</b>	<b>Development of a New Assay for PNGases</b>	<b>35</b>
2.1	Introduction	36
2.2	Labelling with Fluorescamine	36
2.3	Labelling with Fluoresceine Isothiocyanate	38
<b>Chapter 3</b>	<b>Synthesis of <i>N</i>-linked Glycopeptides and Glycopeptide Mimetics</b>	<b>41</b>
3.1	Introduction	42

3.2	Synthesis of <i>C</i> -Glycopeptides	42
3.2.1	Preparation of the <i>C</i> -Glycosyl Building Blocks	43
3.2.2	Preparation of the Peptide Building Blocks	52
3.2.3	Coupling of Carbohydrate and Peptide Fragments	53
3.3	Synthesis of $\alpha$ -Linked <i>N</i> -Glycopeptides and their $\beta$ -Linked Analogues	56
3.4	Preparation of an <i>N</i> -Linked Glycopeptide with a Modified Core-Region	62
<b>Chapter 4</b>	<b>Kinetic Investigations using PNGase F</b>	<b>66</b>
4.1	Introduction	67
4.2	Fluorescently Labelled Ovalbumin-Glycopeptide	68
4.3	Synthetic <i>N</i> -Glycopeptides	70
4.4	Inhibition Trials with <i>N</i> -Linked Glycopeptide Mimetics	76
4.4.1	<i>C</i> -Glycopeptides	76
4.4.2	$\alpha$ -Linked <i>N</i> -Glycopeptides	83
4.5	Non-Specific Inhibition	87
4.5.1	Chitobiose, Specific or Non-Specific Inhibition?	88
4.5.2	PEG	92
4.5.3	Variation of PNGase F Activity in Different Buffer Systems	93
<b>Chapter 5</b>	<b>Kinetic Investigations using Two PNGase F Mutants</b>	<b>96</b>
5.1	Introduction	97
5.2	H 193 A	99
5.3	R 248 A	101
<b>Chapter 6</b>	<b>Molecular Modelling Studies</b>	<b>104</b>

6.1	Introduction	105
6.2	Structural Investigations into Non-Specific Inhibition	105
6.3	Structural Investigations into Substrate Binding	108
<b>Chapter 7</b>	<b>Summary</b>	<b>116</b>
<b>Chapter 8</b>	<b>Materials and Methods</b>	<b>119</b>
8.1	General Methods, Materials and Suppliers	120
8.2	General Procedures	122
8.3	Synthesis of the Glycoside Building Blocks	125
8.4	Peptide Synthesis	153
8.5	Synthesis of <i>N</i> -Linked Glycopeptides and Glycopeptide Mimetics	161
8.5.1	<i>C</i> -Glycopeptides	161
8.5.2	<i>N</i> -Glycopeptides	166
8.6	Synthesis of FITC-Labelled Ovalbumin Glycopeptide Substrate	173
<b>Chapter 9</b>	<b>Literature</b>	<b>175</b>
<b>Appendices</b>		<b>193</b>
Appendix I	Experimental Data Sheets	194
Appendix II	Reproduction of a Paper Published in <i>Tetrahedron Letters</i> Journal	208

# LIST OF ABBREVIATIONS

<b>Boc</b>	<i>t</i> -butyl oxycarbonyl
<b>BTP</b>	(1,3-bis[tris(hydroxymethyl)-methylamino]propane)
<b>CNBr</b>	cyanogen bromide
<b>DBU</b>	1,8-diazabicyclo[5.4.0]undec-7-ene
<b>DCC</b>	dicyclohexylcarbodiimide
<b>DDQ</b>	2,3-dichloro-5,6-dicyano- <i>p</i> -benzoquinone
<b>DMF</b>	dimethylformamide
<b>DMSO</b>	dimethylsulfoxide
<b>DTPA</b>	diethylenetriamine pentaacetate
<b>EA</b>	ethylacetate
<b>eq.</b>	equivalent
<b>ER</b>	endoplasmatic reticulum
<b>ES-MS</b>	Electrospray Mass Spectrometry
<b>Eu</b>	europium
<b>FITC</b>	fluoresceine isothiocyanate
<b>Fmoc</b>	9-fluorenyl methoxycarbonyl
<b>FPLC</b>	Fast Performance Liquid Chromatography
<b>Fuc</b>	fucose
<b>Glc</b>	glucose
<b>GlcNAc</b>	<i>N</i> -acetyl glucosamine
<b>HBTU</b>	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
<b>HOBt</b>	1-hydroxybenzotriazole
<b>HPLC</b>	High Pressure/Performance Liquid Chromatography
<b>Man</b>	mannose
<b>NMR</b>	Nuclear Magnetic Resonance
<b>PE</b>	petroleum ether
<b>Phth</b>	phthalimido
<b>PMB</b>	<i>p</i> -methoxybenzyl
<b>PTSA</b>	<i>p</i> -toluenesulphonic acid
<b>r.t.</b>	room temperature
<b>RP-HPLC</b>	Reverse Phase-HPLC

<b>sat.</b>	saturated
<b>SDS</b>	sodium dodecyl sulfate
<b>SDS/PAGE</b>	sodium dodecyl sulfate/Polyacrylamide Gel Electrophoresis
<b>SPPS</b>	Solid Phase Peptide Synthesis
<b>TBDMS</b>	<i>tert</i> -butyl dimethylsilyl
<b>TFA</b>	trifluoroacetic acid
<b>TLC</b>	Thin Layer Chromatography
<b>TMSCN</b>	trimethylsilyl cyanide
<b>TMSOTf</b>	trimethylsilyl trifluoromethanesulfonate
<b>Tol</b>	toluene