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3-hydroxykynurenine in the human lens

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A thesis submitted in partial fulfillment of the requirements for the award of the degree

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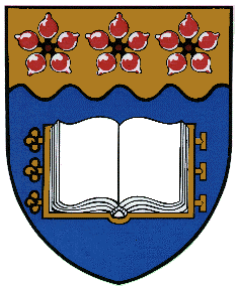

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Certification

I, Anastasia Korlimbinis, declare that this thesis, submitted in partial fulfillment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Anastasia Korlimbinis

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Table of Contents

Certification ..... **ii**

Acknowledgements ..... **iii**

Publications ..... **iv**

Abbreviations ..... **ix**

List of Figures ..... **xi**

List of Schemes ..... **xix**

List of Tables ..... **xx**

Abstract ..... **xxi**

Chapter 1 ..... **1**

Introduction **1**
1.1 General Introduction **1**
1.2 The Human Eye **2**
1.3 The Lens **3**
1.4 Components of the Lens **5**
1.4.1 Proteins in the Lens 7
1.4.2 Antioxidants 8
1.4.3 UV Filters 9
1.5 Aging of the Human Lens **12**
1.6 Cataract **18**
1.7 Age-Related Nuclear (ARN) Cataract **20**
1.8 3OHKyn **23**
1.9 Aims of the Project **26**

Chapter 2 ..... **27**

Synthesis and Characterisation of 3OHKyn Amino Acid Adducts **27**
2.1 Introduction **27**
2.2 Materials and Methods **29**
2.2.1 Materials 29
2.2.2 Synthesis and Purification of 3OHKyn Modified Amino Acids 29
2.2.3 High Performance Liquid Chromatography (HPLC) for Purification of the 3OHKyn Amino Acid Adducts 30
2.2.4 Formation of the 3OHKyn Amino Acid Adducts at pH 7.2 31

2.2.5 Acid Hydrolysis of 3OHKyn Amino Acid Adducts 31
2.2.6 Stability of 3OHKyn Amino Acid Adducts at pH 4.0 31
2.2.7 Stability of 3OHKyn Amino Acid Adducts at pH 7.2 31
2.2.8 Incubation of 3OHKyn-Cys in the Presence of Excess N-α-*t*-Boc-His and N-α-*t*-Boc-Lys 32
2.2.9 HPLC for Acid Hydrolysis and Stability Studies 32
2.2.10 Mass Spectrometry 32
2.2.11 Tandem Mass Spectrometry (MS/MS) 33
2.2.12 High Resolution Mass Spectrometry 33
2.2.13 NMR Spectroscopy 33
2.2.14 Fluorescence and UV-visible Spectroscopy 34
2.3 Results 35
2.3.1 Synthesis of the 3OHKyn Amino Acid Adducts 35
2.3.2 Formation of the 3OHKyn Amino Acid Adducts at pH 7.2 40
2.3.3 Mass Spectrometric Characterisation of the 3OHKyn Amino Acid Adducts 42
2.3.4 NMR Characterisation of 3OHKyn Amino Acid Adducts 50
2.3.5 UV-visible and Fluorescence Characterisation of the 3OHKyn Amino Acid Adducts 52
2.3.6 Stability of 3OHKyn Amino Acid Adducts 60
2.3.7 Acid Hydrolysis of 3OHKyn Amino Acid Adducts 66
2.3.8 Incubation of 3OHKyn-Cys in the Presence of Excess N-α-*t*-Boc-His and N-α-*t*-Boc-Lys 67
2.4 Discussion 75

Chapter 3 83

Lens Proteins Modified with 3OHKyn 83
3.1 Introduction 83
3.2 Materials and Methods 85
3.2.1 Materials 85
3.2.2 Preparation of Calf Lens Protein (CLP)[196] 85
3.2.3 Incubation of CLP with 3OHKyn at pH 7.2 for 48 Hours[103] 85
3.2.4 Incubation of CLP with 3OHKyn at pH 9.5 86
3.2.5 Acid Hydrolysis of CLP and CLP Modified with 3OHKyn 86
3.2.6 Preparation of Human Lens Protein 86
3.2.7 Acid Hydrolysis of Human Lens Protein 86
3.2.8 HPLC 86
3.2.9 Quantification of Protein-Bound 3OHKyn 87
3.2.10 Incubation of CLP with 3OHKyn at pH 7.2 for 24 Days 88
3.2.11 Measurement of Protein Sulfhydryl (PSH) Levels in 3OHKyn-Modified Protein 88
3.2.12 Incubation of α-Crystallin with 3OHKyn at pH 7.2 for 48 Hours 88
3.2.13 HPLC to Purify Modified α-Crystallin 88
3.2.14 Trypsin Digestion 89
3.2.15 HPLC of Trypsin Digests 89
3.2.16 Measurement of Oxygen Levels 89
3.2.17 Hydrogen Peroxide (H_2O_2) Assay 89

3.2.18 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) 89
3.2.19 Mass Spectrometry 90
3.2.20 Tandem Mass Spectrometry (MS/MS) 90
3.2.21 Fluorescence and UV-visible Spectroscopy 90
3.2.22 Statistical Analysis 90
3.3 Results 91
3.3.1 CLP Modified with 3OHKyn 91
3.3.2 Fluorescence and UV-visible Spectroscopy of CLP Modified with 3OHKyn 93
3.3.3 Acid Hydrolysis of Lens Protein 96
3.3.4 Acid Hydrolysis of Human Lens Proteins 98
3.3.5 Determination of 3OHKyn Bound to Proteins 101
3.3.6 CLP Modified with 3OHKyn for 24 Days 136
3.3.7 Identification of CLP Peptides Modified with 3OHKyn 139
3.3.8 Bovine α-Crystallin Modified with 3OHKyn 148
3.3.9 Tryptic Digestion of Modified αA-Crystallin 154
3.3.10 Acid Hydrolysis of Modified αA-Crystallin 160
3.3.11 Tryptic Digestion of Modified αB-Crystallin 161
3.4 Discussion 168

Chapter 4 178

Does 3OHKyn Crosslink Lens Proteins? 178
4.1 Introduction 178
4.2 Materials and Methods 180
4.2.1 Materials 180
4.2.2 Incubations with 3OHKyn-*t*-Boc-His and 3OHKyn-*t*-Boc-Lys 180
4.2.3 Incubation of CLP Modified by 3OHKyn at pH 7.2 181
4.2.4 SDS-PAGE 182
4.2.5 HPLC 182
4.2.6 Mass Spectrometry 182
4.2.7 Tandem Mass Spectrometry (MS/MS) 182
4.2.8 Fluorescence and UV-visible Spectroscopy 182
4.2.9 NMR Spectroscopy 182
4.3 Results 183
4.3.1 Incubations with the 3OHKyn-*t*-Boc-His Adduct 183
4.3.2 Incubations with the 3OHKyn-*t*-Boc-Lys Adduct 201
4.3.3 Incubation of 3OHKyn-Modified CLP (pH 7.2) 210
4.3.4 Incubation of 3OHKyn-Modified CLP (pH 9.5) 221
4.4 Discussion 233

Chapter 5 239

Isolation of Novel Compounds from Human Cataract Lenses 239
5.1 Introduction 239
5.2 Materials and Methods 242
5.2.1 Materials 242

5.2.2 Preparation of Cataract Lens Protein 242
5.2.3 Hydrolysis of Cataract Lens Protein 242
5.2.4 First Stage of HPLC Purification 242
5.2.5 Second Stage of HPLC Purification 243
5.2.5.1 HPLC Gradient for P1 243
5.2.5.2 HPLC Gradient for P2, P3, P4 and P5 243
5.2.5.3 HPLC Gradient for P6 (second HPLC purification) 243
5.2.5.4 HPLC Gradient for P6 (third HPLC purification) 244
5.2.6 Mass Spectrometry 244
5.2.7 Tandem Mass Spectrometry (MS/MS) 244
5.2.8 High Resolution Mass Spectrometry 244
5.2.9 NMR Spectroscopy 244
5.2.10 UV-visible Spectroscopy 244
5.3 Results 245
5.3.1 Analysis of P1 245
5.3.2 Analysis of P2 250
5.3.3 Analysis of P3 255
5.3.4 Analysis of P4 259
5.3.5 Analysis of P5 268
5.3.6 Analysis of P6 272
5.4 Discussion 274

Chapter 6 280

Conclusions and Future Directions 280

References 283

Appendix 1 296

Abbreviations

ACN	acetonitrile
AGE	advanced glycation end
AHBDG	4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-β-D-diglucoside
AHBG	4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-β-D-glucoside
ARN	age-related nuclear
Boc	butyloxycarbonyl
CLP	calf lens protein
Cys	cysteine
Da	dalton
DCl	deuterium chloride
D_2O	deuterium oxide
DTND	5,5'-dithio-bis(2-nitrobenzoic acid)
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
Em	emission
ESI-MS	electrospray ionisation mass spectrometry
Ex	excitation
Guanidine HCl	guanidine hydrochloride
GSH	glutathione (reduced)
His	histidine
H_2O_2	hydrogen peroxide
HRP	horseradish peroxidase
Kyn	kynurenine
Kyn-GSH	kynurenine-glutathione
Lys	lysine
Met	methionine
M_{ox}	methionine sulfoxide
MS/MS	tandem mass spectrometry
Msr	methionine sulfoxide reductase

MW	molecular weight
NanoESI-MS	nanoelectrospray ionisation mass spectrometry
Na_2CO_3	sodium carbonate
$NaHCO_3$	sodium bicarbonate
NaN_3	sodium azide
NMR	nuclear magnetic resonance
3OHKyn	3-hydroxykynurenine
3OHKyn-GSH	3-hydroxykynurenine-glutathione
3OHKynG	3-hydroxykynurenine *O*-β-D-glucoside
3OHKynG-GSH	3-hydroxykynurenine *O*-β-D-glucoside-glutathione
OPD	*o*-phenylenediamine
PDA	photodiode array
PMSF	phenylmethylsulfonyl fluoride
PSH	protein sulfhydryl
PTM	post-translational modification
RP-HPLC	reversed phase high performance liquid chromatography
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SH	sulfhydryl
TFA	trifluoroacetic acid
Tris-HCl	tris(hydroxymethyl)aminomethane hydrochloride
Trp	tryptophan
W_{ox}	oxidised tryptophan

List of Figures

Figure 1.1 Schematic diagram of the cross section of the human eye.[10] 2
Figure 1.2 Cross-section of the human lens (adapted from J. Harding).[14] 4
Figure 1.3 Structure of GSH. 9
Figure 1.4 Structures of the AGE products found in human lenses.[105,106,130,131] 16
Figure 1.5 The Pirie classification of nuclear cataracts[161] using slit lamp photographs.[164] The colour of the nucleus increases with increasing severity of nuclear cataract. 20
Figure 1.6 Structures of non-disulfide bond crosslink compounds identified in cataract lens proteins. 22
Figure 1.7 Structure of Phenoxazone. 24
Figure 2.1 Structures of the 3OHKyn amino acid adducts. 28
Figure 2.2 HPLC trace of 3OHKyn and L-Cys. 3OHKyn was incubated with L-Cys at pH 9.5, 37^0C for 48 hours (UV detection monitored at 360 nm). The peak at 15.6 min is unreacted 3OHKyn, and the peak at 18.9 min is the 3OHKyn amino acid adduct, 3OHKyn-Cys. 37
Figure 2.3 HPLC trace of 3OHKyn and N-α-*t*-Boc-L-His. 3OHKyn was incubated with N-α-*t*-Boc-L-His at pH 9.5, 37^0C for 48 hours (UV detection monitored at 360 nm). The peak at 14.8 min is unreacted 3OHKyn, the peak at 18.3 min contained N-α-*t*-Boc-L-His, the peak at 19.8 min is unknown. The peak at 22.6 min is 3OHKyn-yellow, the peak at 25.0 min is xanthommatin, and the peak at 26.2 min is the 3OHKyn amino acid adduct, 3OHKyn-*t*-Boc-His. 38
Figure 2.4 HPLC trace of 3OHKyn and N-α-*t*-Boc-L-Lys. 3OHKyn was incubated with N-α-*t*-Boc-L-Lys at pH 9.5 and 37^0C for 48 hours (UV detection monitored at 360 nm). The peak at 14.8 min is unreacted 3OHKyn, the peak at 18.7 min contained N-α-*t*-Boc-L-Lys, the peak at 20.1 min is unknown. The peak at 22.5 min is 3OHKyn-yellow, the peak at 25.0 min is xanthommatin, and the peak at 26.8 min is the 3OHKyn amino acid adduct, 3OHKyn-*t*-Boc-Lys. 39
Figure 2.5 Formation of 3OHKyn amino acid adducts over a 5 day incubation. 3OHKyn was incubated with a 25-fold molar excess of N-α-*t*-Boc-L-His, N-α-*t*-Boc-L-Lys, or Cys at pH 7.2, 37^0C. 41
Figure 2.6 MS/MS spectrum of the protonated molecular ion of 3OHKyn-Cys *m/z* 329. 44
Figure 2.7 MS/MS spectrum of the protonated molecular ion of 3OHKyn-*t*-Boc-His *m/z* 463. 46
Figure 2.8 MS/MS spectrum of the protonated molecular ion of 3OHKyn-*t*-Boc-Lys *m/z* 454. 48
Figure 2.9 UV-visible spectra of 3OHKyn-Cys at various pH values; *A*, pH 2.1; *B*, pH 5.5; *C*, pH 7.2; *D*, pH 9.5. 54
Figure 2.10 3-D Fluorescence spectra of 3OHKyn-Cys at various pH values; *A*, pH 2.1; *B*, pH 5.5; *C*, pH 7.2; *D*, pH 9.5. 55
Figure 2.11 UV-visible spectra of 3OHKyn-*t*-Boc-His at various pH values; *A*, pH 2.1; *B*, pH 5.5; *C*, pH 7.2; *D*, pH 9.5. 56
Figure 2.12 3-D Fluorescence spectra of 3OHKyn-*t*-Boc-His at various pH values; *A*, pH 2.1; *B*, pH 5.5; *C*, pH 7.2; *D*, pH 9.5. 57
Figure 2.13 UV-visible spectra of 3OHKyn-*t*-Boc-Lys at various pH values; *A*, pH 2.1; *B*, pH 5.5; *C*, pH 7.2; *D*, pH 9.5. 58

Figure 2.14 3-D Fluorescence spectra of 3OHKyn-*t*-Boc-Lys at various pH values; *A*, pH 2.1; *B*, pH 5.5; *C*, pH 7.2; *D*, pH 9.5. 59
Figure 2.15 Stability of the 3OHKyn amino acid adducts and 3OHKyn at pH 7.2 in the absence (■) and presence (◆) of oxygen. *A*, 3OHKyn; *B*, 3OHKyn-Cys; *C*, 3OHKyn-*t*-Boc-His; *D*, 3OHKyn-*t*-Boc-Lys. 63
Figure 2.16 Stability of the 3OHKyn amino acid adducts and 3OHKyn at pH 4.0 in the absence (■) and presence (◆) of oxygen. *A*, 3OHKyn; *B*, 3OHKyn-Cys; *C*, 3OHKyn-*t*-Boc-His; *D*, 3OHKyn-*t*-Boc-Lys. 64
Figure 2.17 Formation of breakdown products from stability study of 3OHKyn amino acid adducts at pH 7.2 in the absence of oxygen. *A*, 3OHKyn-Cys; *B*, 3OHKyn-*t*-Boc-His; *C*, 3OHKyn-*t*-Boc-Lys. 65
Figure 2.18 3OHKyn-Cys was incubated with excess N-α-*t*-Boc-His and N-α-*t*-Boc-Lys at pH 7.2 for a total of 48 hours. Shown is the 48 hour time sample (aliquot). The peak at 28.4 min is unreacted 3OHKyn-Cys. The peak at 30.6 min contained unreacted N-α-*t*-Boc-His, and the peak at 32.4 min contained unreacted N-α-*t*-Boc-Lys. The peak at 33.5 min is 3OHKyn-yellow, the peak at 35.1 min is 3OHKyn-*t*-Boc-His and the peak at 36.0 min is 3OHKyn-*t*-Boc-Lys. The peak at 39.5 min is unknown (U39.5). 69
Figure 2.19 The rate of loss of 3OHKyn-Cys in relation to the rate of formation of 3OHKyn-yellow, 3OHKyn-*t*-Boc-His, 3OHKyn-*t*-Boc-Lys and unknown compound eluting at 39.5 min (U39.5) on the HPLC chromatogram (Figure 2.18). 70
Figure 2.20 Mass spectra of U39.5. *A*, ESI mass spectrum; *B*, MS/MS spectrum of *m/z* 664 ion. 71
Figure 2.21 Proposed structures of molecular ion *m/z* 664, and fragment ions *m/z* 608, 564 and 409. 72
Figure 2.22 Mass spectra of aliquot from autoxidation of 3OHKyn at pH 7.2. *A*, ESI mass spectrum of aliquot after 3 hours of incubation; *B*, MS/MS spectrum of ion *m/z* 409. 73
Figure 2.23 UV-visible spectrum of U39.5. 74
Figure 2.24 Structures of the common fragment ions for all three 3OHKyn amino acid adducts. 76
Figure 3.1 Photograph of the CLP modified with 3OHKyn at pH 7.2 on the left; and CLP modified with 3OHKyn at pH 9.5 on the right. CLP was incubated with 3OHKyn at 37^0C for 48 hours. As can be seen the protein modified at pH 7.2 was pink and the protein modified at pH 9.5 was brown. 91
Figure 3.2 SDS-PAGE of proteins. Lane 1: Marker; Lane 2: CLP; Lane 3: CLP modified with 3OHKyn at pH 7.2; Lane 4: CLP modified with 3OHKyn at pH 9.5. *A*, Non-reducing conditions; *B*, Reducing conditions. 92
Figure 3.3 CLP modified with 3OHKyn at pH 7.2 for 48 hours. *A*, UV-visible spectrum, (protein concentration: 10 mg/mL in 6 M guanidine HCl); *B*, 3-D fluorescence spectrum, (protein concentration: 2 mg/mL in 6 M guanidine HCl). 94
Figure 3.4 CLP modified with 3OHKyn at pH 9.5 for 48 hours. *A*, UV-visible spectrum, (protein concentration: 1 mg/mL in 6 M guanidine HCl); *B*, 3-D fluorescence spectrum, (protein concentration: 2 mg/mL in 6 M guanidine HCl). 95
Figure 3.5 HPLC chromatograms of acid hydrolysed lens protein samples. *A*, CLP; *B*, CLP modified by 3OHKyn at pH 7.2 for 48 hours; *C*, CLP modified by 3OHKyn at pH 9.5 for 48 hours. 97

Figure 3.6 HPLC chromatograms of human lens sample (76 year old male) hydrolysed with HCl and antioxidants. *A*, Lens sample spiked with 3OHKyn amino acid adducts prior to hydrolysis, in order to observe the elution time of the standards; *B*, Hydrolysed human lens sample (unspiked). 99
Figure 3.7 MS/MS spectra of 3OHKyn-Cys. *A*, Authentic 3OHKyn-Cys; *B*, HPLC peak isolated from an aged human lens (76 year old male); *m/z* 329 is the molecular ion. 100
Figure 3.8 Synthesis of standard 3OHKyn-GSH. *A*, HPLC chromatogram; *B*, MS/MS spectrum of ion *m/z* 515. 103
Figure 3.9 Incubation of 3OHKyn-Cys with excess GSH at pH 9.5. Recovery of 3OHKyn-Cys and the yield of 3OHKyn-GSH..... 104
Figure 3.10 Incubation of 3OHKyn-*t*-Boc-His with excess GSH at pH 9.5. Recovery of 3OHKyn-*t*-Boc-His and the yield of 3OHKyn-GSH. 105
Figure 3.11 Incubation of 3OHKyn-*t*-Boc-Lys with excess GSH at pH 9.5. Recovery of 3OHKyn-*t*-Boc-Lys and the yield of 3OHKyn-GSH..... 105
Figure 3.12 Incubation of Kyn-Cys with excess GSH at pH 9.5. Recovery of Kyn-Cys and the yield of Kyn-GSH. 106
Figure 3.13 Incubation of Kyn-*t*-Boc-His with excess GSH at pH 9.5. Recovery of Kyn-*t*-Boc-His and yield of Kyn-GSH. 107
Figure 3.14 Incubation of Kyn-*t*-Boc-Lys and excess GSH at pH 9.5. Recovery of Kyn-*t*-Boc-Lys and yield of Kyn-GSH. 108
Figure 3.15 Yield of 3OHKyn-GSH from CLP that had been modified with 3OHKyn. Modified protein was incubated with excess GSH at pH 9.5 for 4 hours. *A*, HPLC chromatogram of filtrate; *B*, MS/MS spectrum of ion *m/z* 515 in the 30 min peak (Figure 3.15A)..... 109
Figure 3.16 HPLC chromatogram of the ethanol extract from a human normal lens cortex (81 years old)..... 111
Figure 3.17 HPLC chromatograms of human lens protein incubated with excess GSH at pH 9.5 for 4 hours. *A*, Normal 79 year old nucleus; *B*, Normal 79 year old cortex. 113
Figure 3.18 MS/MS spectra of the three kynurenine derived UV filter GSH adducts isolated from a normal human lens nucleus. *A*, Molecular ion *m/z* 677; *B*, Molecular ion *m/z* 499; *C*, Molecular ion *m/z* 515..... 114
Figure 3.19 Protein – bound 3OHKyn. The concentration of 3OHKyn bound to the nuclear proteins of normal human lenses as a function of age. 115
Figure 3.20 Free 3OHKyn. The concentration of free 3OHKyn in the nucleus. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses. 117
Figure 3.21 Free 3OHKyn. The concentration of free 3OHKyn in the cortex. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses..... 118
Figure 3.22 Plot of the concentration of free 3OHKyn in the nucleus versus the concentration of bound 3OHKyn in the nucleus of normal aged lenses..... 119
Figure 3.23 Protein – bound 3OHKynG. The concentration of bound 3OHKynG in the nucleus. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses..... 121
Figure 3.24 Protein – bound 3OHKynG. The concentration of bound 3OHKynG in the cortex. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses..... 122
Figure 3.25 Free 3OHKynG. The concentration of free 3OHKynG in the nucleus. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses. 124

Figure 3.26 Free 3OHKynG. The concentration of free 3OHKynG in the cortex. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses. 125
Figure 3.27 Plot of the concentration of free vs bound 3OHKynG. *A*, Nucleus; *i*, Normal lenses; *ii*, Dark cataract; *iii*, Light cataract lenses; *B*, Cortex; *i*, Normal lenses; *ii*, Dark cataract; *iii*, Light cataract lenses. 127
Figure 3.28 Protein – bound Kyn. The concentration of bound Kyn in the nucleus. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses. 129
Figure 3.29 Protein – bound Kyn. The concentration of bound Kyn in the cortex. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses. 130
Figure 3.30 Free Kyn. The concentration of free Kyn in the nucleus. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses. 132
Figure 3.31 Free Kyn. The concentration of free Kyn in the cortex. *A*, Normal lenses; *B*, Dark cataract lenses; *C*, Light cataract lenses. 133
Figure 3.32 Plot of the concentration of free vs bound Kyn. *A*, Nucleus; *i*, Normal lenses; *ii*, Dark cataract; *iii*, Light cataract lenses; *B*, Cortex; *i*, Normal lenses; *ii*, Dark cataract; *iii*, Light cataract lenses. 135
Figure 3.33 Photograph of CLP modified with 3OHKyn at pH 7.2 under low oxygen. Aliquots were taken at 0, 3, 6, 9, 12, 15, 18, 21 and 24 days. 137
Figure 3.34 Formation of 3OHKyn amino acid adducts in CLP over time. CLP was incubated with 3OHKyn at 37^0C for a total of 24 days. 138
Figure 3.35 The content of PSH in CLP modified with 3OHKyn at 37^0C for 24 days. 138
Figure 3.36 HPLC chromatograms following trypsin digestion of CLP modified with 3OHKyn for 12 days. Arrowed peaks were collected for mass spectral analysis. *A*, UV trace; *B*, Fluorescence trace. 139
Figure 3.37 ESI mass spectrum of the peak eluting at 45.1 min in Figure 3.36. 140
Figure 3.38 ESI mass spectrum of the peak eluting at 50.5 min (Figure 3.36). 143
Figure 3.39 MS/MS spectrum of ion $(M+3H)^{3+}$ *m/z* 398.3. The sequence confirms that the peptide is αB T6-7 (DRFSVNLDVK). 145
Figure 3.40 MS/MS spectrum of ion $(M+2H)^{2+}$ *m/z* 519.4. The sequence confirms that the peptide is αA T3 (TLGPFYPSR). 146
Figure 3.41 MS/MS spectrum of ion $(M+2H)^{2+}$ *m/z* 594.4. The sequence confirms that the peptide is βB1 T14 (WDTWSSSYR). 146
Figure 3.42 α-Crystallin was modified with 3OHKyn at 37 ^{0}C for 48 hours. *A*, HPLC chromatogram of the initial aliquot of the reaction mixture at time zero; *B*, HPLC chromatogram of the reaction mixture after incubation for 48 hours. 150
Figure 3.43 Transformed mass spectra. *A*, Native bovine αB-crystallin; *B*, Modified bovine αB-crystallin. 151
Figure 3.44 Transformed mass spectra. *A*, Native bovine αA-crystallin; *B*, Modified bovine αA-crystallin. 152
Figure 3.45 Photograph of modified α-crystallins. Bovine α-crystallin was incubated with 3OHKyn at 37^0C for 48 hours under low oxygen tension. Native α-crystallin is white in colour. As shown, the modified αA- and αB-crystallins are pink in colour, and were purified by HPLC. 153
Figure 3.46 HPLC chromatogram of tryptic digest products of αA-crystallin following modification with 3OHKyn. Arrow indicates the peak that contained a peptide with an oxidised amino acid residue. 155

Figure 3.47 MS/MS spectrum of an oxidised αA-crystallin peptide, residues 1-11, showing oxidation at Met1. The sequence for this peptide is shown above the spectrum. 156
Figure 3.48 nanoESI-MS spectrum of tryptically digested 3OHKyn-modified αA-crystallin. 158
Figure 3.49 MS/MS spectrum of an oxidised αA-crystallin peptide, residues 120-145, showing oxidation at Met138. 159
Figure 3.50 HPLC chromatogram of acid hydrolysed 3OHKyn-modifed αA-crystallin. 160
Figure 3.51 HPLC chromatogram of tryptic digest products of αB-crystallin after modification with 3OHKyn. Arrows indicate peaks that contained peptides with oxidatively modified amino acid residues. 162
Figure 3.52 MS/MS spectrum of an oxidised αB-crystallin peptide, residues 1-11, showing oxidation at Met1. 163
Figure 3.53 MS/MS spectrum of an oxidised αB-crystallin peptide, residues 1-11, showing oxidation at Met1 and Trp9. 164
Figure 3.54 MS/MS spectrum of an oxidised αB-crystallin peptide, residues 57-69, showing oxidation at Met68. 166
Figure 3.55 MS/MS spectrum of an oxidised αB-crystallin peptide, residues 57-69, showing oxidation at Met68 and Trp60. 167
Figure 4.1 HPLC separation of 3OHKyn-*t*-Boc-His incubated at pH 7.2. X is 3OHKyn- 185
t-Boc-His. Peak 3 is an impurity stable to oxidation. *A*, Initial reaction mixture; *B*, Reaction mixture after 12 days of incubation. Arrowed peaks were collected for mass spectrometry. Absorbance monitored at 360 nm; *C*, Absorbance monitored at 440 nm. 185
Figure 4.2 ESI mass spectra of peaks eluting from HPLC chromatogram in Figure 4.1B. 186
Figure 4.3 Rate of formation of unknown products in peaks 1, 2, 3, 4, and 5 from Figure 4.1B. The inset shows the rate of loss of 3OHKyn-*t*-Boc-His, in relation to the formation of products. Peak 3 is an impurity from the synthesis of 3OHKyn-*t*-Boc-His, which is stable to oxidation. 187
Figure 4.4 HPLC of the acid digest of 3OHKyn-*t*-Boc-His following incubation for 12 days. HPLC chromatograms of, *A*, Absorbance monitored at 360 nm. Peaks 1, 2, 3, and 4 were collected for mass spectral analysis. *B*, Absorbance monitored at 440 nm. 189
Figure 4.5 ESI mass spectra of peaks eluting from the HPLC chromatogram in Figure 4.4A. 190
Figure 4.6 Incubation of 3OHKyn-*t*-Boc-His with a 20-fold molar excess of N-α-*t*-Boc-His for 12 days. HPLC chromatograms of, *A*, Initial reaction mixture; *B*, Reaction mixture after 12 days of incubation. Peak 1 eluted at 32.5 min, peak 2 at 33.5 min, peak 3 at 34.9 min, peak 4 at 39.7 min and peak 5 at 40.6 min (identical to Figure 4.1B). 192
Figure 4.7 HPLC of the acid digest of 3OHKyn-*t*-Boc-His with a 20-fold molar excess of N-α-*t*-Boc-His following incubation for 12 days. HPLC chromatograms of, *A*, Absorbance monitored at 360 nm. Peak 1 eluted at 25.0 min, peak 2 at 28.6 min,

peak 3 at 32.5 min and peak 4 at 34.7 min. *B*, Absorbance monitored at 440 nm. 193
Figure 4.8 Incubation of 3OHKyn-*t*-Boc-His with a 20-fold molar excess of N-α-*t*-Boc-Lys for 12 days. HPLC chromatograms of, *A*, Initial reaction mixture; *B*, Reaction mixture after 12 days of incubation. Peak 1 eluted at 32.3 min, peak 2 at 33.2 min, peak 3 at 34.5 min, peak 4 at 39.9 min and peak 5 at 40.3 min (~identical to Figure 4.1B). 195
Figure 4.9 HPLC of the acid digest of 3OHKyn-*t*-Boc-His with a 20-fold molar excess of N-α-*t*-Boc-Lys following incubation for 12 days. HPLC chromatograms of, *A*, Absorbance monitored at 360 nm. Peak 1 eluted at 25.3 min, peak 2 at 28.7 min, peak 3 at 32.5 min and peak 4 at 34.6 min. *B*, Absorbance monitored at 440 nm. 196
Figure 4.10 MS/MS spectrum of the molecular ion *m/z* 550, eluting as a doublet in the hydrolysed HPLC profiles, involving 3OHKyn-*t*-Boc-His incubations. 197
Figure 4.11 HPLC chromatogram of peak 2 (Figures 4.1B, 4.6B and 4.8B) following deprotection with acid. 198
Figure 4.12 UV-visible spectra. *A*, Peak 2 (Figures 4.1B, 4.6B and 4.8B) (*m/z* 650); *B*, Peak 2' (Figures 4.4A, 4.7A and 4.9A) (*m/z* 550). 199
Figure 4.13 Proton NMR spectrum of peak 2' (Figures 4.4A, 4.7A and 4.9A) (*m/z* 550). 200
Figure 4.14 Incubation of 3OHKyn-*t*-Boc-Lys at pH 7.2. HPLC chromatograms, *A*, Initial reaction mixture; *B*, Reaction mixture after 12 days of incubation. 202
Figure 4.15 HPLC of the acid digest of 3OHKyn-*t*-Boc-Lys following incubation for 12 days. HPLC chromatograms of, *A*, Absorbance monitored at 360 nm; *B*, Absorbance monitored at 440 nm. 203
Figure 4.16 Incubation of 3OHKyn-*t*-Boc-Lys with a 20-fold molar excess of N-α-*t*-Boc-His for 12 days. HPLC chromatograms of, *A*, Initial reaction mixture; *B*, Reaction mixture after 12 days of incubation. 205
Figure 4.17 HPLC of the acid digest of 3OHKyn-*t*-Boc-Lys with a 20-fold molar excess of N-α-*t*-Boc-His following incubation for 12 days. HPLC chromatograms of, *A*, Absorbance monitored at 360 nm. *B*, Absorbance monitored at 440 nm. 206
Figure 4.18 Incubation of 3OHKyn-*t*-Boc-Lys with a 20-fold molar excess of N-α-*t*-Boc-Lys for 12 days. HPLC chromatograms of, *A*, Initial reaction mixture; *B*, Reaction mixture after 12 days of incubation. 208
Figure 4.19 HPLC of the acid digest of 3OHKyn-*t*-Boc-Lys with a 20-fold molar excess of N-α-*t*-Boc-Lys following incubation for 12 days. HPLC chromatograms of, *A*, Absorbance monitored at 360 nm. *B*, Absorbance monitored at 440 nm. 209
Figure 4.20 SDS-PAGE of proteins from incubation of 3OHKyn modified CLP. Lane 1: Marker; Lane 2: Aliquot time = 0 days; Lane 3: Aliquot time = 3 days; Lane 4: Aliquot time = 6 days; Lane 5: Aliquot time = 9 days; Lane 6: Aliquot time = 12 days; Lane 7: Aliquot time = 15 days. *A*, Non-reducing conditions; *B*, Reducing conditions. 211
Figure 4.21 3-D Fluorescence spectra of aliquots from the protein mixture. *A*, Initial aliquot; *B*, Aliquot after 15 days of incubation. 212
Figure 4.22 HPLC chromatogram of the filtrate from the protein aliquot after 1 day of incubation at pH 7.2. 213
Figure 4.23 ESI mass spectra of the peaks eluting in Figure 4.22. *A*, Peak 1 eluting at 30 min; *B*, Peak 2 eluting at 32.4 min; *C*, Peak 3 eluting at 33.7 min. 214

Figure 4.24 HPLC chromatograms of acid hydrolysed proteins. *A*, Initial aliquot; *B*, Aliquot after 15 days of incubation. Arrowed peaks were collected for mass spectrometry. 217
Figure 4.25 ESI mass spectra of peaks eluting in Figure 4.24B. *A*, Peak 2; *B*, Peak 3; *C*, Peak 4; *D*, Peak 5; *E*, Peak 6; *F*, Peak 7 218
Figure 4.26 ESI mass spectra of peaks eluting in Figure 4.24B. *A*, Peak 8; *B*, Peak 9; *C*, Peak 10; *D*, Peak 11; *E*, Peak 12; *F*, Peak 13 219
Figure 4.27 Concentration of 3OHKyn-Cys and 3OHKyn-His in protein samples during incubation of CLP originally modified by 3OHKyn at pH 7.2. 220
Figure 4.28 SDS-PAGE of proteins from incubation of 3OHKyn modified CLP. Lane 1: Marker; Lane 2: Aliquot time = 0 days; Lane 3: Aliquot time = 1 days; Lane 4: Aliquot time = 2 days; Lane 5: Aliquot time = 3 days; Lane 6: Aliquot time = 4 days; Lane 7: Aliquot time = 6 days; Lane 8: Aliquot time = 8 days; Lane 9: Aliquot time = 10 days. *A*, Non-reducing conditions; *B*, Reducing conditions. ... 222
Figure 4.29 3-D Fluorescence spectra of the aliquots from the protein mixture. *A*, Initial aliquot; *B*, Aliquot after 10 days of incubation. 223
Figure 4.30 HPLC chromatogram of the filtrate from the protein aliquot after 1 day of incubation. 224
Figure 4.31 ESI mass spectra of the peaks eluting in Figure 4.30. *A*, Peak 1 eluting at 27 min; *B*, Peak 2 eluting at 28.3 min; *C*, Peak 3 eluting at 29.4 min; *D*, Peak 4 eluting at 29.8 min; *E*, Peak 5 eluting at 31.9 min; *F*, Peak 6 eluting at 40.5 min. 226
Figure 4.32 HPLC chromatograms of acid hydrolysed proteins. *A*, Initial aliquot; *B*, Aliquot after 10 days of incubation. Arrowed peaks were collected for mass spectrometry. 229
Figure 4.33 ESI mass spectra of peaks eluting in Figure 4.32B. *A*, Peak 2; *B*, Peak 3; *C*, Peak 4; *D*, Peak 5. 230
Figure 4.34 ESI mass spectra of peaks eluting in Figure 4.32B. *A*, Peak 6; *B*, Peak 7; *C*, Peak 8; *D*, Peak 9. 231
Figure 4.35 Concentration of 3OHKyn-Cys, 3OHKyn-His and 3OHKyn-Lys in protein samples during incubation of CLP originally modified by 3OHKyn at pH 9.5. .. 232
Figure 4.36 Possible structures of the unknown compound formed from the 3OHKyn-*t*-Boc-His incubations, whereby the imidazole ring is linked twice. 235
Figure 5.1 HPLC chromatograms of acid hydrolysed lens protein samples (~ 1 mg hydrolysed protein was injected in each case). *A*, CLP; *B*, Normal human lens nuclear protein, from a 76 year old lens; *C*, Pooled human cataract lens nuclear protein. The HPLC profiles of all human cataract lens protein hydrolysed were consistent. 241
Figure 5.2 HPLC chromatogram of P1 after a second purification stage using a Phenomenex Synergi Fusion column, and 0.1% (v/v) formic acid HPLC buffers. 245
Figure 5.3 UV-visible spectrum of the peak eluting at 14.7 min in Figure 5.2. 246
Figure 5.4 ESI mass spectrum of the peak eluting at 14.7 min in Figure 5.2. 247
Figure 5.5 MS/MS spectra. MS/MS of ions that were present in the ESI mass spectrum (Figure 5.4). *A*, *m/z* 658; *B*, *m/z* 505; *C*, *m/z* 419; *D*, *m/z* 331; *E*, *m/z* 270; *F*, *m/z* 166. 249

Figure 5.6 HPLC chromatogram of P2 after a second purification stage using a Phenomenex Synergi Fusion column, and 0.1% (v/v) formic acid HPLC buffers. 250
Figure 5.7 UV-visible spectrum of the peak eluting at 27.8 min in Figure 5.6. 251
Figure 5.8 ESI mass spectrum of the sharp peak eluting at 27.8 min in Figure 5.6.....252
Figure 5.9 MS/MS spectra. MS/MS of ions that were present in the ESI mass spectrum (Figure 5.8). *A*, *m/z* 806; *B*, *m/z* 713; *C*, *m/z* 615; *D*, *m/z* 508; *E*, *m/z* 420. 254
Figure 5.10 HPLC chromatogram of P3 after a second purification stage. The single peak eluting at 26.6 min was collected for analysis by UV-visible and mass spectrometry.....255
Figure 5.11 UV-visible spectrum of the peak eluting at 26.6 min in Figure 5.10. 256
Figure 5.12 ESI mass spectrum of the peak eluting at 26.6 min in Figure 5.10. 257
Figure 5.13 MS/MS spectra. MS/MS of ions that were present in the ESI mass spectrum (Figure 5.12). *A*, *m/z* 665; *B*, *m/z* 641; *C*, *m/z* 279. 258
Figure 5.14 HPLC chromatogram of P4 after a second purification stage. The single peak eluting at 31.1 min was collected for analysis by UV-visible and mass spectrometry.....259
Figure 5.15 UV-visible spectrum of the peak eluting at 31.1 min in Figure 5.14. 260
Figure 5.16 ESI mass spectrum of the peak eluting at 31.1 min in Figure 5.14. 261
Figure 5.17 MS/MS spectra. MS/MS of ions that were present in the ESI mass spectrum (Figure 5.16). *A*, *m/z* 790; *B*, *m/z* 670; *C*, *m/z* 404; *D*, *m/z* 387; *E*, *m/z* 353; *F*, *m/z* 284; *G*, *m/z* 192.....264
Figure 5.18 Proton NMR spectrum of the peak eluting at 31.1 min in Figure 5.14. ...266
Figure 5.19 gCOSY spectrum of the peak eluting at 31.1 min in Figure 5.14. *A*, Entire gCOSY spectrum; *B*, Aromatic region of the gCOSY spectrum.....267
Figure 5.20 HPLC chromatogram of P5 after a second purification stage. The single peak eluting at 34.5 min was collected for analysis by UV-visible and mass spectrometry.....268
Figure 5.21 UV-visible spectrum of the peak eluting at 34.5 min in Figure 5.20. 269
Figure 5.22 ESI mass spectrum of the peak eluting at 34.5 min in Figure 5.20. 270
Figure 5.23 MS/MS spectra. MS/MS of ions that were present in the ESI mass spectrum (Figure 5.22). *A*, *m/z* 993; *B*, *m/z* 790; *C*, *m/z* 697; *D*, *m/z* 607.....271
Figure 5.24 Purification of P6. *A*, HPLC chromatogram of second purification (see Section 5.2.5.3 for details); *B*, HPLC chromatogram of third purification (see Section 5.2.5.4 for details); *C*, ESI mass spectrum of the peaks eluting as a doublet at 28 min in Figure 5.24B. 273

List of Schemes

Scheme 1.1 Tryptophan metabolism: The Kynurenine pathway. Glu refers to glucose (β-linked), and GSH refers to glutathione. 11
Scheme 1.2 Formation of protein UV filter adducts. 13
Scheme 1.3 Deamidation of *A*, Glutamine; *B*, Asparagine. 14
Scheme 1.4 Mechanism of formation of H_2O_2, via 3OHKyn autoxidation.[181] 23
Scheme 1.5 Formation of pigments in arthropods from oxidation of 3OHKyn.[184] 25
Scheme 2.1 Synthesis of 3OHKyn amino acid adducts. 3OHKyn is deaminated at pH 9.5, and the intermediate compound is susceptible to nucleophilic attack via a Michael addition. Amino acid side chains of Cys, His or Lys were covalently attached to the 3OHKyn amino acid side chain. 35
Scheme 2.2 Mechanism for the formation of breakdown products of 3OHKyn-Cys at pH 7.2. 79
Scheme 2.3 Mechanism of formation of U39.5, *m/z* 664. 81
Scheme 3.1 Decomposition of UV filter amino acid adducts, and formation of UV filter GSH adducts. 3OHKyn or Kyn amino acid adducts together with excess GSH were incubated at pH 9.5 for 4 hours. 102
Scheme 4.1 Possible scheme for formation of 3OHKyn amino acid crosslinks. 179
Scheme 4.2 Route of formation of xanthurenic acid from autoxidation of protein-bound 3OHKyn. 236

List of Tables

Table 1.1 Major components of the human lens.[9,16-19] 6
Table 2.1 Proposed structures of the major fragment ions of 3OHKyn-Cys observed in the MS/MS spectrum. 45
Table 2.2 Proposed structures of the major fragment ions of 3OHKyn-*t*-Boc-His observed in the MS/MS spectrum. 47
Table 2.3 Proposed structures of the major fragment ions of 3OHKyn-*t*-Boc-Lys observed in the MS/MS spectrum. 49
Table 2.4 Summary of the ^{1}H and ^{13}C NMR spectral assignments for the 3OHKyn amino acid adducts. The atom numbering adopted is shown below. 51
Table 2.5 Recovery of 3OHKyn amino acid adducts after acid hydrolysis for 24 hours at $110^{0}C$ in the presence of antioxidants. 66
Table 2.6 Summary of the 3-D fluorescence intensities for each 3OHKyn amino acid adduct. 78
Table 3.1 Lists all of the doubly charged ions $(M+2H)^{2+}$ in the ESI mass spectrum (Figure 3.37) detected in the peak eluting at 45.1 min on the HPLC chromatogram (Figure 3.36). 141
Table 3.2 Lists all of the triply charged ions $(M+3H)^{3+}$ in the ESI mass spectrum (Figure 3.37) detected in the peak eluting at 45.1 min on the HPLC chromatogram (Figure 3.36). 142
Table 3.3 Lists all of the charged ions in the ESI mass spectrum (Figure 3.38) for the peak eluting at 50.5 min on the HPLC chromatogram (Figure 3.36). 144
Table 4.1 Combination of incubations undertaken with the 3OHKyn amino acid adducts. 181
Table 4.2 List of the 3-D fluorescence intensities for each aliquot from the incubation. 212
Table 4.3 List of the 3-D fluorescence intensities for each aliquot from the incubation. 223
Table 4.4 Expected molecular ions of the 3OHKyn crosslink compounds. 233
Table 5.1 List of characteristic ions, and absorbance maxima for each of the 3OHKyn and Kyn amino acid adducts.[103] 239
Table 5.2 Summary of the ions and fragment ions identified in the peaks, P1, P2, P3, P4, P5 and P6, which were isolated and purified from hydrolysed human cataract lens proteins. 278
Table 5.3 List of modifications in human cataract lens proteins.[104-107,130,212] 279

Abstract

The human lens contains three kynurenine UV filters, 3-hydroxykynurenine *O*-β-D-glucoside (3OHKynG), kynurenine (Kyn) and 3-hydroxykynurenine (3OHKyn), and it absorbs UV light in the 300-400 nm region due to their presence. UV filters may also prevent UV-induced photodamage to the retina and lens. After middle age, the UV filters, 3OHKynG and Kyn become bound progressively to proteins in the centre of our lenses. This feature is, in part, responsible for normal age-dependant human lens colouration.

To provide proof that 3OHKyn is bound to normal human lenses, model studies were undertaken. Cysteine (Cys), histidine (His) and lysine (Lys) residues in lens proteins had been previously shown to bind to UV filters *in vivo*, therefore adducts of these amino acids and 3OHKyn were synthesised and characterised by mass spectrometry, fluorescence, UV-visible and NMR spectroscopy in Chapter 2. The stability properties of each of the 3OHKyn amino acid adducts were also determined, with incubations performed at pH 4.0 and pH 7.2. 3OHKyn-*t*-Boc-His was identified as the most stable of the three adducts. 3OHKyn-*t*-Boc-Lys and 3OHKyn-Cys both decomposed at pH 7.2 forming numerous oxidation products. The stability of each adduct to acid hydrolysis was also examined.

In Chapter 3, calf lens protein was incubated with 3OHKyn, and acid hydrolysis showed that Cys was the primary site of modification when the incubation was undertaken at pH 7.2. However, when the incubation was undertaken at a higher pH (for example, pH 9.5), 3OHKyn readily modified Cys, His and Lys residues. Previously acid hydrolysis of human lens protein had identified Kyn attachment to the proteins. However, acid hydrolysis was not an appropriate method for detecting 3OHKyn attached to human lens proteins because 3OHKynG is also bound to human lens proteins. Therefore, a new assay was developed, and it was found that 3OHKyn does indeed bind to human lens proteins in an age-dependant manner. The assay also provides data for 3OHKynG and Kyn attachment to human lens proteins.

In Chapter 3, α-crystallin was also incubated with 3OHKyn under low oxygen tension, and the findings from this study showed that 3OHKyn modified the Cys residue in αA-crystallin. In addition, oxidation of methionine and tryptophan was observed. Age-related nuclear cataract is associated with colouration, insolubilisation and extensive oxidation of Cys and methionine residues. It appears that 3OHKyn in the lens may promote the oxidation and modifications of proteins, and may contribute to oxidative stress in the human lens.

In Chapter 4, the aim was to examine if 3OHKyn could act as a crosslinker of cataract lens proteins. 3OHKyn is known to readily oxidise and yield highly reactive species. It was therefore proposed that 3OHKyn bound to lens proteins could promote crosslinking, insolubilisation and colouration of lens proteins following formation of oxidised species. 3OHKyn amino acid adducts were incubated with excess amino acids, and the resulting products examined. These compounds may be analogous to those that would form in a cataract lens. In addition, 3OHKyn-modified protein was incubated and the products were examined by SDS-PAGE, fluorescence spectroscopy and mass spectrometry. Results showed that 3OHKyn, under the conditions used, does not crosslink lens protein. Proof of the hypothesis that 3OHKyn crosslinks proteins in the lens requires the isolation of characteristic chemical markers from cataract lens proteins that contain the modified 3OHKyn molecules.

In Chapter 5, the aim was to isolate novel compounds from the hydrolysates of human cataract lens proteins and to determine their chemical properties.

Overall this thesis provides evidence that 3OHKyn plays a role in the post-translational modification of normal human lens proteins, and it also provides preliminary data on the role of 3OHKyn in human cataract.