

Index

Abbreviations	i
List of figures	iii
List of tables	ix
1 Introduction	1
1.1 Natural products in drug development.....	1
1.2 Non-ribosomal peptide synthetases	3
1.3 Enniatin synthetase	6
1.4 Enniatin.....	7
1.5 Precursor-directed approaches for the biosynthesis of CODPs.....	12
1.6 <i>A. niger</i> as host system for heterologous production of CODPs.....	13
1.7 Semi-synthesis of secondary metabolites derivatives	15
2 Objective	17
3 Materials	18
3.1 Technical equipment.....	18
3.2 Reagents and consumables.....	19
3.3 Microorganisms.....	22
3.4 Media and stock solutions	22
4 Methods	24
4.1 Precursor synthesis	24
4.1.1 Synthesis of 3-chloro- and 3-bromolactate.....	24

4.1.2	Synthesis of 3-fluorolactate	24
4.1.3	Synthesis of 3-iodolactate	25
4.1.4	Synthesis of vinylactate	25
4.1.5	Synthesis of 2-hydroxypentynoic acid.....	25
4.1.6	Synthesis of 2-D-hydroxyvaleric acid	26
4.1.7	Synthesis of 3-azidolactate.....	26
4.1.8	Synthesis of 3-nitrolactate	26
4.2	Cultivation of <i>A. niger</i>	27
4.2.1	Preparation of <i>A. niger</i> conidia	27
4.2.2	Cultivation of <i>A. niger</i> in shaking flasks.....	27
4.3	Purification of enniatin derivatives	28
4.4	Semi-synthesis.....	29
4.4.1	Halogen exchange	29
4.4.2	Substitution of bromine with thiophenolate	29
4.4.3	Cu(I) catalyzed [2+3]-dipolar Huisgen cycloaddition	29
4.4.4	Elimination of HCl and HBr from halogen containing enniatin variants	29
4.4.5	Work up.....	30
4.5	Analytical methods.....	30
4.5.1	High resolution mass spectrometry (HRMS).....	30
4.5.2	Tandem MS analysis of enniatin derivatives.....	31
5	Results and Discussion	33
5.1	Precursor synthesis	33
5.1.1	Synthesis of 3-chlorolactate	34
5.1.2	Synthesis of 3-bromolactate	35

5.1.3	Synthesis of 3-fluorolactate	36
5.1.4	Synthesis of 3-iodolactate	37
5.1.5	Synthesis of 3-nitrolactate	38
5.1.6	Synthesis of 3-azidolactate.....	39
5.1.7	Synthesis of vinylactate	40
5.1.8	Synthesis of 2-hydroxypentynoic acid.....	41
5.1.9	Synthesis of 2-hydroxyvaleric acid	42
5.2	Precursor-directed biosynthesis	43
5.2.1	Isolation of enniatin variants containing 2-hydroxyisovaleric acid-d ₆	45
5.2.2	Isolation of enniatin variants containing 3-chlorolactate	48
5.2.3	Isolation of enniatin variants containing 3-bromolactate	51
5.2.4	Isolation of enniatin variants containing 3-iodolactate	53
5.2.5	Isolation of enniatin variants containing 2-hydroxypentynoic acid.....	55
5.2.6	Isolation of enniatin variants containing 3-azidolactate.....	60
5.2.7	Isolation of enniatin variants containing 3-fluorolactate	63
5.2.8	Isolation of enniatin variants containing 2-hydroxyvaleric acid	65
5.2.9	Strategies of <i>A. niger</i> for the elimination of xenobiotic hydroxy acids.....	67
5.2.10	Summary.....	72
5.2.11	Bioactivity	76
5.3	Semi-synthesis of enniatin derivatives.....	79
5.3.1	Substitution reactions performed with bromine containing enniatin variants.....	80
5.3.2	Elimination of HCl and HBr from enniatin derivatives	82
5.3.3	Cu(I) catalyzed [2+3]-dipolar cyclo-addition	83

6	Prospect	85
6.1.1	Optimization of CODP-biosynthesis in <i>A. niger</i>	85
6.1.2	Advanced semi-synthesis of CODPs	86
6.1.3	Improving lead properties of enniatin	88
7	Appendix.....	I
7.1	Analytical data of 2-hydroxy acid precursor	I
7.1.1	3-Chlorolactate.....	I
7.1.2	3-Bromolactate.....	III
7.1.3	Vinylactate.....	V
7.1.4	3-Azidolactate.....	VII
7.1.5	3-Iodolactate	IX
7.1.6	3-Nitrolactate	XI
7.1.7	2-Hydroxyvaleric acid (Hval).....	XIII
7.1.8	2-Hydroxypentynoic acid.....	XV
7.1.9	3-Fluorolactate	XVII
7.2	Analytical data of enniatin derivatives obtained by biosynthesis	XIX
7.2.1	3-Chlorolactate supplementation	XIX
7.2.2	3-Bromolactate supplementation	XXIV
7.2.3	3-Azidolactate supplementation.....	XXIX
7.2.4	3-Iodolactate supplementation.....	XXXIII
7.2.5	3-Fluorolactate supplementation	XL
7.2.6	2-Hydroxyvaleric acid supplementation.....	XLV
7.2.7	2-Hydroxypentynoic acid supplementation.....	XLVII
7.2.8	¹ H NMR spectra of enniatin derivatives generated by biosynthesis	LX

7.3	Analytical data of the semi-synthesis of enniatin variants	LXX
7.3.1	Cu (I) catalyzed [2+3]-dipolar cyclo-addition	LXX
7.3.2	Substitution (halogen exchange)	LXXIV
7.3.3	Elimination.....	LXXVI
8	References.....	LXXVII
9	Danksagung.....	XCVIII