

Table of contents

Table of contents.....	ix
1. Summary	1
2. Zusammenfassung	7
3. Introduction	13
3.1 Gene expression	13
3.2 Natural modified nucleotides.....	14
3.3 tRNA	15
3.4 Modifications at position 37.....	18
3.4.1 Structure and distribution in organisms	18
3.4.2 Function	20
3.4.2.1 <i>Structural role</i>	20
3.4.2.2 <i>Codon-anticodon interaction</i>	22
3.4.2.3 <i>Frameshift prevention and translocation</i>	23
3.4.2.4 <i>Aminoacylation</i>	25
3.4.2.5 <i>Diseases</i>	26
3.5 Summary	27
4. Aims of the Project.....	29
5. Synthesis of modified tRNA nucleosides	33
5.1 The t⁶A carbamoyl family	33
5.2 Methylated adenosine modifications.....	35
5.3 N⁶-Acetyladenosine.....	37
5.4 Synthesis of modifications.....	38
5.4.1 Synthesis of t ⁶ A	38
5.4.2 Synthesis of isotope-labeled t ⁶ A	39
5.4.3 Synthesis of g ⁶ A.....	39
5.4.4 Synthesis of m ⁶ A and m ⁶ ₂ A	40
5.4.5 Synthesis of m ⁶ t ⁶ A	41
5.4.6 Synthesis of isotope-labeled m ⁶ t ⁶ A	44
5.4.7 Synthesis of Am and m ¹ A.....	46
5.4.8 Synthesis of ac ⁶ A	47
5.5 Overview.....	47
5.6 Building blocks for RNA synthesis	48
6. Quantification method	51
6.1 Extraction and purification of tRNA	52
6.1.1 Extraction of tRNA.....	52
6.1.2 Purification of tRNA.....	52
6.2 Enzymatic hydrolysis of tRNA	54

6.3	HPLC-ESI-MS.....	55
6.4	Stock solutions	57
6.5	Calibration curves	58
6.6	Accuracy of quantification.....	60
7.	Results tRNA modifications	63
7.1	Differences between <i>E. coli</i>, mammalian tissue, and cell lines	63
7.2	Strategy.....	65
7.3	Porcine tissue	67
7.4	Cancer cell lines	79
7.5	Phylogenetic analysis.....	81
7.6	Pathogenic bacteria	88
7.7	Stress response	89
8.	Modified nucleosides in DNA	95
8.1	5-Hydroxymethylcytosine	95
8.2	Quantification of hmC by HPLC-ESI-MS	96
8.3	Distribution of hmC in mammalian tissue	99
8.4	hmC as a putative intermediate in the demethylation process?.....	106
8.5	hmC in cancer cell lines	110
9.	Outlook.....	113
10.	Experimental Section	115
10.1	General chemical materials and methods	115
10.2	Tissue samples, bacterial strains, and cell culture.....	116
10.3	Biochemical materials	117
10.3.1	Equipment.....	117
10.3.2	Bacterial strains and cell lines.....	118
10.4	Biochemical methods.....	119
10.4.1	Bacterial strains and growth conditions.....	119
10.4.2	tRNA purification	120
10.4.2.1	<i>tRNA extraction.....</i>	120
10.4.2.2	<i>tRNA purification</i>	121
10.4.3	DNA isolation from tissue samples and cancer cell lines	122
10.4.4	Enzymatic digestion of tRNA	122
10.4.5	Enzymatic digestion of DNA	123
10.4.6	HPLC-ESI-MS.....	124
10.4.6.1	<i>Mass filter</i>	124
10.4.6.2	<i>Calibration curves.....</i>	125
10.4.7	Separation of mitochondria and cytosol.....	126
10.4.8	<i>In vitro</i> translation assay	127
10.4.9	Immunohistochemistry	127
10.5	Phylogenetic analysis.....	128
10.6	Syntheses	129

10.6.1	Synthesis of t ⁶ A	129
10.6.2	Synthesis of ¹³ C ₄ , ¹⁵ N-t ⁶ A	138
10.6.3	Synthesis of g ⁶ A.....	140
10.6.4	Synthesis of m ⁶ A, d ₃ -m ⁶ A, m ⁶ ₂ A, and d ₃ -m ⁶ ₂ A	142
10.6.5	Synthesis of m ⁶ t ⁶ A	148
10.6.6	Synthesis of d ₃ -m ⁶ t ⁶ A	155
10.6.7	Synthesis of Am, d ₃ -Am, and d ₃ -m ¹ A.....	161
10.6.8	Synthesis of ac ⁶ A.....	164
10.6.9	Synthesis towards incorporation of t ⁶ A into RNA	166
10.6.10	Synthesis towards incorporation of m ⁶ t ⁶ A into RNA.....	171
11.	Abbreviations.....	177
12.	References	181