

Contents

Preface to the second edition *XIII*

Preface to the first edition *XV*

1	Introduction and Background	1
	References	4
2	Fundamental Chemical and Structural Principles	5
2.1	Definitions and Main Conformational Features of the Peptide Bond	5
2.2	Building Blocks, Classification, and Nomenclature	7
2.3	Analysis of the Covalent Structure of Peptides and Proteins	11
2.3.1	Separation and Purification	13
2.3.1.1	Separation Principles	13
2.3.1.2	Purification Techniques	17
2.3.1.3	Stability Problems	19
2.3.1.4	Evaluation of Homogeneity	20
2.3.2	Primary Structure Determination	20
2.3.2.1	End Group Analysis	21
2.3.2.2	Cleavage of Disulfide Bonds	24
2.3.2.3	Analysis of Amino Acid Composition	24
2.3.2.4	Selective Methods of Cleaving Peptide Bonds	26
2.3.2.5	N-Terminal Sequence Analysis (Edman Degradation)	28
2.3.2.6	C-Terminal Sequence Analysis	30
2.3.2.7	Mass Spectrometry	31
2.3.2.8	Peptide Ladder Sequencing	32
2.3.2.9	Assignment of Disulfide Bonds and Peptide Fragment Ordering	33
2.3.2.10	Location of Post-Translational Modifications and Bound Cofactors	34
2.4	Three-Dimensional Structure	36
2.4.1	Secondary Structure	36
2.4.1.1	Helices	37
2.4.1.2	β -Sheets	39
2.4.1.3	Turns	40
2.4.1.4	Amphiphilic Structures	42

2.4.2	Tertiary Structure	44
2.4.2.1	Structure Prediction	48
2.5	Methods of Structural Analysis	49
2.5.1	Circular Dichroism	49
2.5.2	Infrared Spectroscopy	51
2.5.3	NMR Spectroscopy	52
2.5.4	X-Ray Crystallography	54
2.5.5	UV Fluorescence Spectroscopy	55
2.6	Review Questions	56
	References	57
3	Biology of Peptides	63
3.1	Historical Aspects and Biological Functions	63
3.2	Biosynthesis	75
3.2.1	Ribosomal Peptide Synthesis	75
3.2.2	Post-Translational Modification	79
3.2.2.1	Enzymatic Cleavage of Peptide Bonds	79
3.2.2.2	Hydroxylation	80
3.2.2.3	Carboxylation	81
3.2.2.4	Glycosylation	81
3.2.2.5	Amidation	86
3.2.2.6	Phosphorylation	87
3.2.2.7	Lipidation	88
3.2.2.8	Pyroglutamyl Formation	90
3.2.2.9	Sulfation	91
3.2.2.10	Further Post-Translational Modifications	92
3.2.3	Nonribosomal Peptide Synthesis	94
3.3	Selected Biologically Active Peptides	96
3.3.1	Gastroenteropancreatic Peptide Families	96
3.3.1.1	The Gastrin Family	97
3.3.1.2	Secretin Family	98
3.3.1.3	The Insulin Superfamily	101
3.3.1.4	The Somatostatin Family	104
3.3.1.5	The Tachykinin Family	105
3.3.1.6	The Neuropeptide Y family	106
3.3.1.7	The Ghrelin Family	108
3.3.1.8	The EGF Family	109
3.3.2	Hypothalamic Liberins and Statins	110
3.3.2.1	Thyroliberin	112
3.3.2.2	Gonadoliberin	113
3.3.2.3	Corticoliberin	113
3.3.2.4	Growth Hormone-Releasing Hormone	114
3.3.3	Pituitary Hormones	115
3.3.3.1	Growth Hormone	115
3.3.3.2	Corticotropin	115

3.3.3.3	Melanotropin	117
3.3.4	Neurohypophyseal Hormones	118
3.3.4.1	Oxytocin	118
3.3.4.2	Vasopressin	119
3.3.5	Parathyroid Hormone and Calcitonin/Calcitonin Gene-Related Peptide Family	120
3.3.5.1	Parathyroid Hormone	120
3.3.5.2	Parathyroid Hormone-Related Peptides	121
3.3.5.3	The Calcitonin/Calcitonin Gene-Related Peptide Family	121
3.3.6	The Blood Pressure Regulating Peptide Families	123
3.3.6.1	Angiotensin-Kinin System	123
3.3.6.2	Endothelins and Endothelin-Like Peptides	125
3.3.6.3	Cardiac Peptide Hormones	127
3.3.7	Neuropeptides	128
3.3.7.1	Endorphins	131
3.3.7.2	Dynorphins	136
3.3.7.3	Hypocretins (Orexins)	136
3.3.7.4	Dermorphins	137
3.3.7.5	Deltorphins	138
3.3.7.6	Nociceptin/Orphanin and Nocistatin	138
3.3.7.7	Exorphins	139
3.3.7.8	The Adipokinetic Hormone/Red Pigment-Concentrating Hormone Family	140
3.3.7.9	Endomorphins	141
3.3.7.10	The Allatostatin Families	141
3.3.7.11	Neuromedins	142
3.3.7.12	Additional Neuroactive Peptides	143
3.3.8	Peptide Antibiotics	146
3.3.8.1	Nonribosomally Synthesized Peptide Antibiotics	147
3.3.8.2	Ribosomally Synthesized Peptide Antibiotics	152
3.3.9	Peptide Toxins	156
3.4	Review Questions	162
	References	163
4	Peptide Synthesis	175
4.1	Principles and Objectives	175
4.1.1	Main Targets of Peptide Synthesis	175
4.1.2	Basic Principles of Peptide Bond Formation	178
4.2	Protection of Functional Groups	181
4.2.1	N ^α -Amino Protection	182
4.2.1.1	Alkoxy carbonyl-Type (Urethane-Type) Protecting Groups	183
4.2.1.2	Carboxamide-Type Protecting Groups	192
4.2.1.3	Sulfonamide and Sulfenamide-Type Protecting Groups	192
4.2.1.4	Alkyl-Type Protecting Groups	192
4.2.2	C ^α -Carboxy Protection	193

4.2.2.1	Esters	194
4.2.2.2	Amides and Hydrazides	199
4.2.3	C-Terminal and Backbone N ^α -carboxamide Protection	199
4.2.4	Side-Chain Protection	201
4.2.4.1	Guanidino Protection	202
4.2.4.2	ω-Amino Protection	204
4.2.4.3	ω-Carboxy Protection	205
4.2.4.4	Thiol Protection	208
4.2.4.5	Imidazole Protection	211
4.2.4.6	Hydroxy Protection	214
4.2.4.7	Thioether Protection	216
4.2.4.8	Indole Protection	217
4.2.4.9	ω-Amide Protection	218
4.2.5	Enzyme-Labile Protecting Groups	220
4.2.5.1	Enzyme-labile N ^α -amino Protection	221
4.2.5.2	Enzyme-labile C ^α -carboxy Protection and Enzyme-labile Linker Moieties	223
4.2.6	Protecting Group Compatibility	223
4.3	Peptide Bond Formation	224
4.3.1	Acyl Azides	225
4.3.2	Anhydrides	226
4.3.2.1	Mixed Anhydrides	227
4.3.2.2	Symmetrical Anhydrides	229
4.3.2.3	N-Carboxy Anhydrides	229
4.3.3	Carbodiimides	231
4.3.4	Active Esters	235
4.3.5	Acyl Halides	237
4.3.6	Phosphonium Reagents	239
4.3.7	Guanidinium/Uronium Reagents	240
4.3.8	Immonium Type Coupling Reagents	242
4.3.9	Further Special Methods for Peptide Synthesis	243
4.4	Racemization During Synthesis	246
4.4.1	Direct Enolization	246
4.4.2	5(4H)-Oxazolone Mechanism	247
4.4.3	Racemization Tests: Stereochemical Product Analysis	249
4.5	Solid-Phase Peptide Synthesis (SPPS)	251
4.5.1	Solid Supports and Linker Systems	253
4.5.2	Safety-Catch Linkers	262
4.5.3	Protection Schemes	265
4.5.3.1	Boc/Bzl-protecting Groups Scheme (Merrifield Tactics)	265
4.5.3.2	Fmoc/tBu-Protecting Groups Scheme (Sheppard Tactics)	267
4.5.3.3	Three- and More-Dimensional Orthogonality	268
4.5.4	Chain Elongation	269
4.5.4.1	Coupling Methods	269
4.5.4.2	Undesired Problems During Elongation	269

4.5.4.3	Difficult Sequences	271
4.5.4.4	Chemical Strategies for SPPS Methodological Improvements	273
4.5.4.5	On-Resin Monitoring	273
4.5.5	Automation of the Process	274
4.5.6	Peptide Cleavage from the Resin	275
4.5.6.1	Acidolytic Methods	275
4.5.6.2	Side Reactions	276
4.5.6.3	Advantages and Disadvantages of the Boc/Bzl and Fmoc/tBu Schemes	277
4.5.7	Examples of Syntheses by Linear SPPS	277
4.5.8	Special Methods of Polymer-supported Synthesis	278
4.5.9	Microwave-Enhanced Peptide Synthesis	280
4.6	Biochemical Synthesis	281
4.6.1	Recombinant DNA Techniques	281
4.6.1.1	Principles of DNA Technology	282
4.6.1.2	Examples of Synthesis by Genetic Engineering	285
4.6.1.3	Cell-free Translation Systems	288
4.6.1.4	Proteins Containing Non-Proteinogenic Amino Acids – The Expansion of the Genetic Code	290
4.6.2	Enzymatic Peptide Synthesis	291
4.6.2.1	Approaches to Enzymatic Synthesis	291
4.6.2.2	Manipulations to Suppress Competitive Reactions	294
4.6.2.3	Substrate Mimetic Approach	295
4.6.3	Further Selected Biochemical Methods	297
4.6.3.1	Non-ribosomal Peptide Synthesis	297
4.6.3.2	Peptide Bond Formation by LF-Transferase	297
4.6.3.3	Antibody-catalyzed Peptide Bond Formation	297
4.7	Review Questions	300
	References	301
5	Synthesis Concepts for Peptides and Proteins	317
5.1	Strategy and Tactics	317
5.1.1	Linear or Stepwise Synthesis	317
5.1.2	Convergent Synthesis	320
5.1.3	Tactical Considerations	321
5.1.3.1	Selected Protecting Group Schemes	321
5.1.3.2	Preferred Coupling Procedures	324
5.2	Solution Phase Synthesis (SPS)	325
5.2.1	Convergent Synthesis Using Maximally Protected Segments	325
5.2.2	Convergent Synthesis Using Minimally Protected Segments	327
5.2.2.1	Chemical Approaches	327
5.2.2.2	Enzymatic Approaches	329
5.3	Solution Phase/Solid Phase-Hybrid Approaches	332
5.3.1	Solid Phase Synthesis of Protected Segments	332
5.3.2	SPS/SPPS-Hybrid Condensation of Lipophilic Segments	333

5.3.3	Phase Change Synthesis	335
5.3.4	SPS/SPPS-Hybrid Approach to Protein and Large Scale Peptide Synthesis	335
5.4	Optimized Strategies on a Polymeric Support	337
5.4.1	Standard SPPS	337
5.4.2	Convergent Solid-Phase Peptide Synthesis	339
5.4.3	Handle Approaches	341
5.4.3.1	Positively Charged Handles	341
5.4.3.2	Liquid Phase Method	342
5.4.3.3	Excluded Protecting Group Method	343
5.5	Chemical Ligation Strategies	343
5.5.1	Native Chemical Ligation	344
5.5.1.1	Facile Peptide Thioester Synthesis	346
5.5.1.2	Extended Native Chemical Ligation	346
5.5.1.3	Kinetically Controlled Ligation	348
5.5.1.4	Solid Phase Chemical Ligation	350
5.5.1.5	Alternative Approaches to Native Chemical Ligation	350
5.5.2	Expressed Protein Ligation (Intein-mediated Protein Ligation)	351
5.5.3	Prior Capture-mediated Ligation	353
5.5.3.1	Template-mediated Ligation	353
5.5.4	Non-native Chemoselective Ligation	355
5.5.4.1	Thioester- and Thioether-forming Ligations	355
5.5.4.2	Hydrazone- and Oxime-forming Ligations	356
5.5.5	Alternative Ligation Approaches	356
5.5.5.1	Staudinger Ligation	357
5.5.5.2	Ketoacid-hydroxylamine Amide Ligations	357
5.5.5.3	Expressed enzymatic ligation	358
5.5.5.4	Sortase-mediated Ligation	359
5.6	Review Questions	359
	References	360
6	Synthesis of Special Peptides and Peptide Conjugates	365
6.1	Cyclopeptides	365
6.1.1	Backbone Cyclization (Head-to-Tail Cyclization)	371
6.1.2	Side Chain-to-Head and Tail-to-Side Chain Cyclizations	380
6.1.3	Side Chain-to-Side Chain Cyclizations	380
6.2	Cystine Peptides	381
6.3	Glycopeptides	386
6.4	Phosphopeptides	395
6.5	Lipopeptides	398
6.6	Sulfated Peptides	402
6.7	Review Questions	403
	References	403
7	Peptide and Protein Design, Pseudopeptides, and Peptidomimetics	411
7.1	Peptide Design	413

7.2	Modified Peptides	418
7.2.1	Side-Chain Modification	418
7.2.2	Backbone Modification	421
7.2.3	Combined Modification (Global Restriction) Approaches	423
7.2.4	Modification by Secondary Structure Mimetics	425
7.2.5	Transition State Inhibitors	427
7.3	Peptidomimetics	428
7.4	Pseudobiopolymers	431
7.4.1	Peptoids	432
7.4.2	Peptide Nucleic Acids (PNA)	434
7.4.3	β -Peptides, Hydrazino Peptides, Aminoxy Peptides, and Oligosulfonamides	435
7.4.4	Oligocarbamates	437
7.4.5	Oligopyrrolinones	438
7.5	Maclopeptides and <i>de novo</i> Design of Peptides and Proteins	439
7.5.1	Protein Design	439
7.5.2	Peptide Dendrimers	444
7.5.3	Peptide Polymers	447
7.6	Review Questions	447
	References	448
8	Combinatorial Peptide Synthesis	457
8.1	Parallel Synthesis	460
8.1.1	Synthesis in Teabags	461
8.1.2	Synthesis on Polyethylene Pins (Multipin Synthesis)	462
8.1.3	Parallel Synthesis of Single Compounds on Cellulose or Polymer Strips	464
8.1.4	Light-Directed, Spatially Addressable Parallel Synthesis	465
8.1.5	Liquid-Phase Synthesis using Soluble Polymeric Support	466
8.2	Synthesis of Mixtures	467
8.2.1	Reagent Mixture Method	468
8.2.2	Split and Combine Method	468
8.2.3	Encoding Methods	470
8.2.4	Peptide Library Deconvolution	474
8.2.5	Dynamic Combinatorial Libraries	476
8.2.6	Biological Methods for the Synthesis of Peptide Libraries	477
8.3	Review Questions	478
	References	479
9	Application of Peptides and Proteins	483
9.1	General Production Strategies	483
9.2	Improvement of the Therapeutic Potential	486
9.2.1	Peptide and Protein Drug Modifications	486
9.2.2	Peptide Drug Delivery Systems	488
9.3	Protein Pharmaceuticals	492
9.3.1	Importance and Sources	492

9.3.2	Endogenous Pharmaceutical Proteins	493
9.3.3	Engineered Protein Pharmaceuticals	493
9.3.3.1	Selected Recombinant Proteins	493
9.3.3.2	Peptide-Based Vaccines	497
9.3.3.3	Monoclonal Antibodies	498
9.3.3.4	Future Perspectives	500
9.4	Peptide Pharmaceuticals	502
9.4.1	Large-Scale Peptide Synthesis	502
9.4.2	Peptide Drugs and Drug Candidates	507
9.4.3	Peptides as Tools in Drug Discovery	515
9.4.4	Peptides Targeted to Functional Sites of Proteins	517
9.4.5	Peptides Used in Target Validation	517
9.4.6	High-throughput Screening (HTS) Using Peptides as Surrogate Ligands	518
9.4.7	Artificial Peptide Analogs in Drug Discovery	521
9.5	Review Questions	522
	References	523
10	Peptides in Proteomics	529
10.1	Genome and Proteome	529
10.2	Separation Methods	530
10.2.1	Depletion Strategies	530
10.2.2	Two-Dimensional Polyacrylamide Gel-Electrophoresis	530
10.2.3	Gel-Free Methods – Two-Dimensional Liquid Chromatography (2D-LC, MudPIT)	531
10.3	Peptide and Protein Analysis in Proteomics	532
10.3.1	Mass Spectrometry	532
10.3.2	Quantitative Proteomics	533
10.3.2.1	Metabolic Stable-Isotope Labelling	533
10.3.2.2	Tagging Methods	533
10.3.2.3	Enzymatic Stable-Isotope Labeling	535
10.4	Activity-Based Proteomics	535
10.4.1	Irreversibly Binding Affinity-Based Probes	536
10.4.2	Reversibly Binding Affinity-Based Probes	539
10.4.2.1	Inhibitor Affinity Chromatography (IAC)	540
10.4.2.2	Labelling Strategies with Reversibly Binding Protein Ligands	541
10.5	Review Questions	543
	References	543
	Glossary	547
	Index	559