

## Contents

### List of Contributors XVII

<b>1</b>	<b>Protection Reactions</b>	<b>1</b>
<i>Vorminna V. Sureshbabu and Narasimhamurthy Narendra</i>		
1.1	General Considerations	1
1.2	$\alpha$ -Amino Protection ( $N^{\alpha}$ Protection)	4
1.2.1	Non-Urethanes	4
1.2.1.1	Acyl Type	4
1.2.1.1.1	Monoacyl Groups	5
1.2.1.1.2	Groups Cleavable via Lactam Formation	6
1.2.1.1.3	Diacyl Groups	7
1.2.1.2	Phosphine-Type Groups	10
1.2.1.3	Sulfonyl-Type Groups	10
1.2.1.4	Alkyl-Type Groups	11
1.2.1.4.1	Triphenylmethyl (Trityl or Trt) Group	11
1.2.1.4.2	Benzhydryl Groups	12
1.2.1.4.3	<i>N,N</i> -Bis-Benzyl Protection	12
1.2.1.4.4	Vinyl Groups	12
1.2.1.5	Sulfanyl-Type Groups	13
1.2.2	Urethanes (Carbamates or Alkyloxycarbonyl Groups)	14
1.2.2.1	Formation of the Urethane Bond	16
1.2.2.2	Urethanes Derived from Primary Alcohols	16
1.2.2.2.1	Benzyoxy carbonyl (Cbz or Z) Group	16
1.2.2.2.2	Urethanes Cleaved by $\beta$ -Elimination	19
1.2.2.2.3	Urethanes Cleaved via Michael-Type Addition	24
1.2.2.2.4	Allyloxycarbonyl (Aloc) Group	25
1.2.2.3	Urethane Groups Derived from Secondary Alcohols	25
1.2.2.4	Urethanes Derived from Tertiary Alcohols	25
1.2.2.4.1	<i>tert</i> -Butoxycarbonyl (Boc) Group	25
1.2.2.4.2	Boc Analogs	28
1.2.2.5	Other Aspects of Urethane Protectors	29

1.2.2.5.1	Formation of Dipeptide Impurities during the Introduction of Urethanes and Protocols to Overcome It	29
1.2.2.5.2	Introduction of Urethanes via Transprotection	30
1.2.2.5.3	Protection of the Nitrogen of $\alpha$ -Amino Acid <i>N</i> -Carboxy Anhydrides (NCAs)	31
1.2.2.5.4	$N^{\alpha},N^{\alpha}$ -bis-Protected Amino Acids	32
1.2.3	Other $N^{\alpha}$ -Protecting Groups	32
1.2.3.1	$\alpha$ -Azido Acids as $\alpha$ -Amino Acid Precursors	33
1.2.3.2	One-Pot $N^{\alpha}$ Protection and $C^{\alpha}$ Activation	33
1.2.3.3	Effect of $N^{\alpha}$ -Protecting Groups in the Synthesis of NMAs	33
1.3	Carboxy Protection	34
1.3.1	Methyl and Ethyl Esters	35
1.3.1.1	Substituted Methyl and Ethyl Esters	36
1.3.2	Benzyl Ester	36
1.3.2.1	Cleavage	36
1.3.3	Substituted Benzyl Esters	38
1.3.4	<i>tert</i> -Butyl Ester	38
1.3.5	Other Acid-Labile Esters	39
1.3.6	Temporary $\alpha$ -Carboxy Protection	39
1.3.7	$\alpha$ -Carboxy Protectors as Precursors to Useful Amino Acid Derivatives: Formation of Acid Hydrazides	41
1.4	Side-Chain Protection	41
1.4.1	$\omega$ -Amino Group of Diamino Acids	41
1.4.2	Guanidino Group of Arg	43
1.4.2.1	Protection Through Protonation	43
1.4.2.2	Nitration	44
1.4.2.3	Arg Precursors	45
1.4.3	Imidazole Group of His	45
1.4.4	Indole Group of Trp	48
1.4.5	$\omega$ -Amido Group of Asn and Gln	49
1.4.6	$\beta$ -Thiol Group of Cys	50
1.4.6.1	Common Side-Reactions with S-Protected Cys Derivatives	51
1.4.6.1.1	Racemization	51
1.4.6.1.2	$\beta$ -Elimination	51
1.4.6.1.3	Oxidation	51
1.4.6.2	Synthesis of Peptides Using Cystine as "Self-Protected" Cys	51
1.4.7	Thioether Group of Met	53
1.4.8	Hydroxy Group of Ser, Thr, and the Phenolic Group of Tyr	54
1.4.9	$\omega$ -Carboxy Group of Asp and Glu	55
1.4.9.1	Aspartimide Formation	55
1.5	Photocleavable Protections	57
1.6	Conclusions	58
1.7	Experimental Procedures	59
1.7.1	Protection Reactions	59
1.7.1.1	General Procedure for the Preparation of Tfa-Arg-OH	59

- 1.7.1.2 General Procedure for the Preparation of  $N^{\alpha}$ -Phthaloyl Amino Acids using *N*-(Ethoxycarbonyl)phthalimide 59
- 1.7.1.3 General Procedure for the Preparation of  $N^{\alpha}$ -Trt-Amino Acids 59
- 1.7.1.4 General Procedure for the Preparation of  $N^{\alpha}$ -Ns-Amino Acids 60
- 1.7.1.5 General Procedure for the Preparation of  $N^{\alpha}$ -Z-Amino Acids 61
- 1.7.1.5.1 Method A: Using Z-Cl 61
- 1.7.1.5.2 Method B: Using Z-OSu 62
- 1.7.1.6 General Procedures for the Preparation of  $N^{\alpha}$ -Fmoc-Amino Acids 62
- 1.7.1.6.1 Method A: Using Fmoc-OSu 62
- 1.7.1.6.2 Method B: Using Fmoc-Cl and *N,O*-bis-TMS-Amino Acids 62
- 1.7.1.6.3 Method C: Using Fmoc-Cl in the Presence of Zinc Dust 63
- 1.7.1.6.4 Method D: Using Fmoc-N<sub>3</sub> 63
- 1.7.1.7 General Procedure for the Preparation of  $N^{\alpha}$ -Nsc-Amino Acids 64
- 1.7.1.8 General Procedure for the Preparation of  $N^{\alpha}$ -Bsmoc-Amino Acids 64
- 1.7.1.9 General Procedure for the Preparation of  $N^{\alpha}$ -Aloc-Amino Acids 65
- 1.7.1.10 General Procedures for the Preparation of  $N^{\alpha}$ -Boc-Amino Acids 65
- 1.7.1.10.1 Method A: Using (Boc)<sub>2</sub>O 65
- 1.7.1.10.2 Method B: Using Boc-ON 65
- 1.7.1.10.3 Method C: Using Boc-N<sub>3</sub> 66
- 1.7.1.11 General Procedure for the Preparation of *N,N'*-di-Boc-Amino Acids 66
- 1.7.1.12 General Procedure for the Preparation of  $N^{\alpha}$ -Bpoc-Amino Acids 67
- 1.7.1.13 General Procedures for the Preparation of Amino Acid Methyl Esters 68
- 1.7.1.13.1 Preparation of Amino Acid Methyl Ester Hydrochloride Salts 68
- 1.7.1.13.2 Isolation of Amino Acid Methyl Esters: Deprotonation of the Hydrochloride Salt Using Zinc Dust 69
- 1.7.1.13.3 Glutamic Acid  $\alpha$ -Methyl,  $\gamma$ -*tert*-Butyl Diester Using Diazomethane 69
- 1.7.1.13.4 Z-Glu-OMe via Methanolysis of Cyclic Anhydride 69
- 1.7.1.14 General Procedure for the Preparation of Amino Acid Ethyl Esters 69
- 1.7.1.15 General Procedure for the Preparation of Amino Acid Benzyl Ester *p*-Toluenesulfonate Salts 70
- 1.7.1.15.1 Preparation of Amino Acid Benzyl Ester *p*-Toluenesulfonate Salts Under Microwave Irradiation 70
- 1.7.1.16 General Procedure for the Preparation of *tert*-Butyl Esters of  $N^{\alpha}$ -Unprotected Amino Acids Using Isobutene 71
- 1.7.1.16.1 Preparation of Z-Phe-O*t*Bu by the Silver Salt Method 71
- 1.7.1.17 General Procedure for Concomitant Protection and Activation of Amino Acids Using Pentafluorophenyl Carbonate 80

1.7.2	Deprotection Reactions	81
1.7.2.1	Removal of the Phth Group by Hydrazinolysis	81
1.7.2.2	Removal of the Nps Group	81
1.7.2.3	Removal the Z-group	82
1.7.2.3.1	Protocol A: Employing CH	82
1.7.2.3.2	Protocol B: Employing Silylhydride	82
1.7.2.3.3	Protocol C: Through CTH using 1,4-Cyclohexadieneas Hydrogen Donor	83
1.7.2.4	Cleavage of the Fmoc Group	83
1.7.2.4.1	Method A: Using TAEA [67]	83
1.7.2.4.2	Method B: Using DEA: Simultaneous Removal of the Fmoc Group and 9-Fluorenylmethyl Ester	83
1.7.2.5	Cleavage of the Boc Group	84
1.7.2.5.1	Protocol A: Removal of the Boc group with TFA in the Presence of Scavengers	84
1.7.2.5.2	Protocol B: Cleavage of Boc Group with TMS/Phenol	84
1.7.2.6	Transprotection of $N^{\alpha}$ -Protecting Groups: Fmoc-Met-OH to Boc-Met-OH	84
1.7.2.7	Selective Methyl Ester Hydrolysis in the Presence of the $N^{\alpha}$ -Fmoc Group	84
1.7.2.8	Cleavage of <i>tert</i> -Butyl Ester Using $BF_3 \cdot Et_2O$	84
1.7.2.9	Selective Cleavage of Phenacyl Ester in the Presence of the $N^{\alpha}$ -Nosyl Group	85
1.7.2.10	Removal of the Trt Group (Iodolysis)	85
1.7.2.11	Deprotection of the Pbf Group from Z-Arg(Pbf)-OH	85
1.7.2.12	Removal of the Phenoc Group through Photolysis	85
1.7.2.13	Conversion of the DCHA Salt of $N^{\alpha}$ -Protected Amino Acids into Free Acids	85
	References	86

**Part One Amino Acid-Based Peptidomimetics** 99

2	<b>Huisgen Cycloaddition in Peptidomimetic Chemistry</b>	101
	<i>Daniel Sejer Pedersen and Andrew David Abell</i>	
2.1	Introduction	101
2.2	Huisgen [2 + 3] Cycloaddition Between Azides and Acetylenes	102
2.3	Mechanistic Consideration for the Cu-Huisgen and Ru-Huisgen Cycloadditions	103
2.4	Building Blocks for the Synthesis of Triazole-Modified Peptidomimetics	106
2.5	Cyclic Triazole Peptidomimetics	109
2.6	Acylic Triazole Peptidomimetics	113
2.7	Useful Experimental Procedures	121

2.7.1	Monitoring Huisgen Cycloadditions and Characterizing Triazoles	121
2.7.2	General Procedure for the Synthesis of 1,4-Triazoles Using Cu-Huisgen Cycloaddition	122
2.7.3	General Procedure for the Synthesis of 1,5-Triazoles Using Ru-Huisgen Cycloaddition	123
	References	124
<b>3</b>	<b>Recent Advances in <math>\beta</math>-Strand Mimetics</b>	<b>129</b>
	<i>Wendy A. Loughlin and David P. Fairlie</i>	
3.1	Introduction	129
3.1.1	$\beta$ -Strands	129
3.1.2	$\beta$ -Sheets	130
3.1.3	Differences in Strand/Sheet/Turn/Helix Recognition	130
3.1.4	Towards $\beta$ -Strand Mimetics	131
3.2	Macrocyclic Peptidomimetics	133
3.3	Acyclic Compounds	135
3.4	Aliphatic and Aromatic Carbocycles	136
3.5	Ligands Containing One Ring with One Heteroatom (N)	137
3.6	Ligands Containing One or Multiple Rings with One Heteroatom (O, S)	138
3.7	Ligands Containing One Ring with Two Heteroatoms (N,N)	139
3.8	Ligands Containing One Ring with Two Heteroatoms (N,S) or Three Heteroatoms (N,N,S or N,N,N)	140
3.9	Ligands Containing Two Rings with One Heteroatom (N or O)	140
3.10	Ligands Containing Two Rings with Two or Three Heteroatoms (N,N or N,S or N,N,N)	141
3.11	Conclusions	142
	References	143
<b>Part Two</b>	<b>Medicinal Chemistry of Amino Acids</b>	<b>149</b>
<b>4</b>	<b>Medicinal Chemistry of <math>\alpha</math>-Amino Acids</b>	<b>151</b>
	<i>Lennart Bunch and Povl Krogsgaard-Larsen</i>	
4.1	Introduction	151
4.2	Glutamic Acid	151
4.3	Conformational Restriction	153
4.3.1	Synthesis – General Considerations	154
4.3.2	Case Study: Synthesis of DCAN	155
4.3.3	Case Study: Synthesis of LY354740	157
4.3.4	Case Study: Synthesis of ABHD-V and ABHD-VI	158
4.4	Bioisosterism	159
4.4.1	Case Study: Design and Synthesis of AMPA	160
4.4.2	Case Study: Design and Synthesis of Thioibotenic Acid	161
4.5	Structure–Activity Studies	162

4.5.1	Case Study: AMPA Analogs	162
4.5.2	Case Study: 4-Substituted Glu analogs	163
4.6	Conclusions	168
	References	169
<b>5</b>	<b>Medicinal Chemistry of Alicyclic <math>\beta</math>-Amino Acids</b>	175
	<i>Nils Griebeinow</i>	
5.1	Introduction	175
5.2	Five-Membered Alicyclic $\beta$ -Amino Acids	175
5.3	Six-Membered Alicyclic $\beta$ -Amino Acids	183
	References	186
<b>6</b>	<b>Medicinal Chemistry of <math>\alpha</math>-Hydroxy-<math>\beta</math>-Amino Acids</b>	189
	<i>Zyta Ziora, Mariusz Skwarczynski, and Yoshiaki Kiso</i>	
6.1	Introduction	189
6.2	$\alpha$ -Hydroxy- $\beta$ -Amino Acids	189
6.2.1	$\alpha$ -Hydroxy- $\beta$ -Amino Acids Occurring in Natural Products	189
6.2.2	Synthesis of $\alpha$ -Hydroxy- $\beta$ -Amino Acids	191
6.2.2.1	Isoserine	191
6.2.2.2	Isothreonine	193
6.2.2.3	Phenylisoserine	197
6.2.2.4	Norstatines	197
6.2.2.5	3-Amino-2-Hydroxydecanoic Acid and its Analogs	204
6.2.2.6	Synthetic Demands	205
6.3	Antibacterial Agents	205
6.4	Inhibitors of Aminopeptidases	207
6.5	Aspartyl Proteases Inhibitors	211
6.5.1	Renin Inhibitors	212
6.5.2	HIV-1 Protease Inhibitors	216
6.5.3	HTLV-I Inhibitors	220
6.5.4	Plasmeprin II Inhibitors	222
6.5.5	BACE-1 Inhibitors	224
6.6	Paclitaxel and its Derivatives	228
	References	234
<b>7</b>	<b>Peptide Drugs</b>	247
	<i>Chiara Falciani, Alessandro Pini, and Luisa Bracci</i>	
7.1	Lights and Shades of Peptide and Protein Drugs	247
7.2	Peptide Drugs Available on the Market	249
7.2.1	Natriuretic Peptide (Nesiritide)	249
7.2.2	Oxytocin	249
7.2.3	Vasopressin	250
7.2.4	Desmopressin	251
7.2.5	Blood Coagulation Inhibitors	251
7.2.5.1	Bivalirudin	251

7.2.5.2	Integrilin (Eptifibatide)	251
7.2.6	Gonadotropin-Releasing Hormone Agonists and Antagonists	251
7.2.6.1	Gonadorelin	251
7.2.6.2	Lupron(Leuprolide)	252
7.2.6.3	Cetorelix	253
7.2.6.4	Degarelix	253
7.2.7	Antihyperglycemics	254
7.2.7.1	Symlin (Pramlintide)	254
7.2.7.2	Exendin-4	254
7.2.7.3	Liraglutide	255
7.2.8	Icatibant	255
7.2.9	Sermorelin	256
7.2.10	Calcitonin	256
7.2.11	Parathyroid Hormone	256
7.2.12	Cyclosporine	257
7.2.13	Fuzeon	257
7.3	Approved Peptides in Oncology	258
7.3.1	Bortezomib	259
7.3.2	Actinomycin D	259
7.3.3	Marimastat	260
7.3.4	Octreotide	260
7.3.5	Vapreotide	261
7.3.6	Octreoscan	262
7.4	Antimicrobial peptides	263
7.4.1	Polymyxin	265
7.4.2	Daptomycin	266
7.4.3	Gramicidin S	267
7.5	Perspectives	267
7.5.1	Branched Peptides as Tumor-Targeting Agents	268
7.5.2	Branched Peptides as Antimicrobials	270
	References	271
<b>8</b>	<b>Oral Bioavailability of Peptide and Peptidomimetic Drugs</b>	<b>277</b>
	<i>Arik Dahan, Yasuhiro Tsume, Jing Sun, Jonathan M. Miller, and Gordon L. Amidon</i>	
8.1	Introduction	277
8.2	Fundamental Considerations of Intestinal Absorption	277
8.3	Barriers Limiting Oral Peptide/Peptidomimetic Drug Bioavailability	279
8.4	Strategies to Improve Oral Bioavailability of Peptide-Based Drugs	280
8.4.1	Chemical Modifications	280
8.4.1.1	Prodrug Approach	280
8.4.1.2	Structural Modifications	281

8.4.2	Formulation Technologies	284
8.4.2.1	Absorption Enhancers	284
8.4.2.2	Coadministration with Protease Inhibitors	285
8.4.2.3	Formulation Vehicles	285
8.4.2.4	Site-Specific Delivery	286
8.5	Conclusions	287
	References	287
<b>9</b>	<b>Asymmetric Synthesis of <math>\beta</math>-Lactams via the Staudinger Reaction</b>	<b>293</b>
	<i>Monika I. Konaklieva and Balbina J. Plotkin</i>	
9.1	Introduction	293
9.2	Staudinger Reaction	293
9.3	Influence of the Geometry of the Imine on Stereoselectivity in the Reaction	294
9.4	Influence of the Polarity of the Solvent on Stereoselectivity of the Reaction	296
9.5	Influence of the Isomerization of the Imine Prior to its Nucleophilic Attack onto the Ketene Stereoselectivity in the Reaction	296
9.6	Influence of the Order of Addition of the Reactants to the Reaction	297
9.7	Influence of Chiral Substituents on the Stereoselectivity of the Reaction	298
9.8	Asymmetric Induction from the Imine Component	298
9.9	Asymmetric Induction from the Ketene Component	305
9.10	Double Asymmetric Cycloinduction	308
9.11	Influence of Catalysts on the Stereoselectivity of the Reaction	309
9.11.1	General Procedure for $\beta$ -Lactams 106 with Proton Sponge	312
9.11.2	General Procedure for the Tandem Nucleophile/Lewis Acid-Promoted Synthesis of $\beta$ -Lactams 110	312
9.11.3	General Procedure for Catalytic Asymmetric Synthesis of <i>Trans</i> - $\beta$ -Lactams 113	314
9.11.4	Example for Kinugasa Reaction with Cu (II) Catalyst	316
9.11.4.1	General Procedure for Catalytic Asymmetric Synthesis of $\beta$ -Lactams 122	316
9.12	Conclusions	316
	References	317
<b>10</b>	<b>Advances in <i>N</i>- and O-Glycopeptide Synthesis – A Tool to Study Glycosylation and Develop New Therapeutics</b>	<b>321</b>
	<i>Ulrika Westerlind and Horst Kunz</i>	
10.1	Introduction	321
10.2	Synthesis of O-Glycopeptides	324
10.2.1	Synthesis of Mucin-Type Glycopeptides	325

10.2.1.1	Synthesis of Tumor-Associated Glycopeptides and Glycopeptide Vaccines	325
10.2.1.1.1	Synthesis of Tn, T, Sialyl-Tn, and Sialyl-T Glycosylated Amino Acid Building Blocks	325
10.2.1.1.2	Synthesis of Tn, T, Sialyl-Tn, and Sialyl-T Glycopeptides and Vaccines	329
10.2.1.2	Synthesis of Glycopeptide Recognition Domain of P-Selectin Glycoprotein Ligand-1	331
10.2.1.2.1	Synthesis of a Core 2 sLe <sup>x</sup> Amino Acid Building Block Including a sLe <sup>x</sup> Mimic	332
10.2.1.2.2	Synthesis of Unsulfated and Sulfated Core 2 sLe <sup>x</sup> and Core 2 sLe <sup>x</sup> Mimic PSGL-1 Glycopeptides	334
10.2.1.2.3	Chemoenzymatic Synthesis of Unsulfated and Sulfated sLe <sup>x</sup> PSGL-1 Glycopeptide	336
10.2.2	Synthesis of Other Types of O-Glycopeptides	339
10.2.2.1	Synthesis of Fmoc-GlcNAc-Ser/Thr Amino Acids	340
10.2.2.2	Synthesis of Estrogen Receptor Peptides for Conformational Analysis	340
10.3	Synthesis of N-Glycopeptides	342
10.3.1	Synthesis of RNase C Glycoprotein	343
10.3.2	Synthesis of Erythropoietin N-Glycopeptide Fragment 1–28	346
10.3.2.1	Synthesis of Biantennary Dodecasaccharide	346
10.3.2.2	Synthesis of N-Glycopeptide Fragment 1–28	348
10.3.3	Chemoenzymatic Synthesis of a HIV GP120 V3 Domain N-Glycopeptide	350
10.3.3.1	Synthesis of the Oxazoline Tetrasaccharide Donor	350
10.3.3.2	Synthesis of Fmoc-GlcNAc-Asn Amino Acid Building Block	351
10.3.3.3	Synthesis of V3 Cyclic GlcNAc Peptide and Endo A Coupling with Man <sub>3</sub> GlcNAc Oxazoline Donor	352
	References	353
<b>11</b>	<b>Recent Developments in Neoglycopeptide Synthesis</b>	<b>359</b>
	<i>Margaret A. Brimble, Nicole Miller, and Geoffrey M. Williams</i>	
11.1	Introduction	359
11.2	Neoglycoside and Neoglycopeptide Synthesis	361
11.2.1	S-Glycosides	361
11.2.2	N-Glycosides	362
11.2.3	O-Glycosides	364
11.2.4	C-Glycosides	365
11.2.5	C=N Linkage	365
11.3	Protein Side-Chain Modifications	366
11.3.1	Modifications of Cysteine Side-Chains	366
11.3.2	Modifications of Lysine Side-Chains	369
11.3.3	Other Side-Chain Modifications	370
11.4	Cu(I)-Catalyzed Azide–Alkyne “Click” Cycloaddition	372

11.4.1	General Aspects of Cu(I)-Catalyzed Azide–Alkyne cycloaddition	372
11.4.2	Neoglycoside and Neoglycopeptide Synthesis via CuAAC	373
11.4.3	CuAAC and Neoglycoproteins	376
11.5	Cross-Metathesis	378
11.6	Application of Neoglycopeptides as Synthetic Vaccines	380
11.7	Enzymatic, Molecular, and Cell Biological Techniques	384
11.7.1.1	Enzymatic Glycoprotein Synthesis	385
11.7.2	Molecular and Cell Biological Techniques	385
	References	386

**Part Three Amino Acids in Combinatorial Synthesis 393**

<b>12</b>	<b>Combinatorial/Library Peptide Synthesis</b>	395
	<i>Michal Lebl</i>	
12.1	Introduction	395
12.2	High-Throughput Synthesis of Peptides	396
12.2.1	Parallel Peptide Synthesis	396
12.2.2	Directed Sorting	400
12.3	Synthesis of Peptide Arrays	402
12.4	Peptide Libraries	406
12.4.1	Synthesis of Peptide Mixtures	406
12.4.2	Synthesis of Peptides on a Mixture of Particles	409
12.4.2.1	Determination of the Structure of a Peptide on an Individual Bead	416
12.4.3	Solution-Based Screening of OBOC Libraries	418
12.5	Future of Peptide Libraries	421
12.6	Synthetic Protocols	421
12.6.1	Pin Synthesis	421
12.6.2	SPOT Synthesis	422
12.6.3	Synthesis in Tea-Bags	422
12.6.4	Synthesis on Cotton	423
12.6.4.1	Modification of the Cotton Carrier	423
12.6.5	Split-and-Mix Synthesis of OBOC Noncleavable Libraries	424
12.6.6	Preparation of Dual-Layer Beads	425
12.6.7	Preparation of Library of Libraries	426
12.6.8	Preparation of OBOC Libraries for Testing in Solution	426
12.6.8.1	Synthesis of Multicleavable Linker	426
12.6.8.2	Synthesis of the Library	428
12.6.8.3	Quality Control of the Doubly Releasable Library	428
12.6.8.4	Two-Stage Release Assay in 96-Well Microassay Plates	429
12.6.9	Synthesis of the Positional Scanning Library	430

12.6.10	Synthesis of the Dual Defined Iterative Hexapeptide Library	430
12.6.11	Acylation Monitoring by Bromophenol Blue	431
	References	432
<b>13</b>	<b>Phage-Displayed Combinatorial Peptides</b>	451
	<i>Renhua Huang, Kritika Pershad, Małgorzata Kokoszka, and Brian K. Kay</i>	
13.1	Introduction	451
13.1.1	Types of Phage Vectors	452
13.1.2	Generation of Combinatorial Peptide Libraries	455
13.1.3	Identifying Peptide Ligands to Protein Targets	458
13.1.4	Mapping Protein–Protein Interactions	461
13.1.5	Identifying Peptide Ligands Binding to Cell Surfaces	463
13.1.6	Mapping Protease Specificity	464
13.1.7	Identifying Peptide Ligands to the Surfaces of Inert Materials	464
13.2	Conclusions	465
	References	466
<b>14</b>	<b>Designing New Proteins</b>	473
	<i>Michael I. Sadowski and James T. MacDonald</i>	
14.1	Introduction	473
14.1.1	Why Design New Proteins?	473
14.1.2	How New is “New?”	474
14.2	Protein Design Methods	475
14.2.1	Computational Design	476
14.2.1.1	Computational Enzyme Design	477
14.2.1.2	Results of Computational Design Experiments	478
14.2.2	Directed Evolution Methods	480
14.2.2.1	Randomization Strategies	480
14.2.2.2	Expression Systems and Assays	481
14.2.3	Design of Protein Interfaces	482
14.3	Protocol for Protein Design	484
14.4	Conclusions	486
	References	487
<b>15</b>	<b>Amino Acid-Based Dendrimers</b>	491
	<i>Zhengshuang Shi, Chunhui Zhou, Zhigang Liu, Filbert Totsingan, and Neville R. Kallenbach</i>	
15.1	Introduction	491
15.2	Peptide Dendrimer Synthesis: Divergent and Convergent Approaches	491
15.2.1	Synthesis of the First Peptide Dendrimers: Polylysine Dendrimers	493
15.2.2	Glutamic/Aspartic Acid, Proline, and Arginine Dendrimers	494

15.2.3	Synthesis of MAPs	497
15.2.4	Synthesis of Peptide Dendrimers Grafted on PAMAM and other Peptide Dendrimers	500
15.3	Applications of Peptide Dendrimers	502
15.3.1	Initial Efforts on MAPs	502
15.3.2	Peptide Dendrimers as Antimicrobial Agents	502
15.3.3	Peptide Dendrimers as Protein/Enzyme Mimics	504
15.3.4	Peptide Dendrimers as Ion Sensors and MRI Contrast Agents	505
15.3.5	Peptide Dendrimers as DNA/RNA Delivery Vectors	507
15.3.6	Other Application of Peptide Dendrimers	512
15.4	Conclusions	513
	References	514
	<b>Index</b>	519