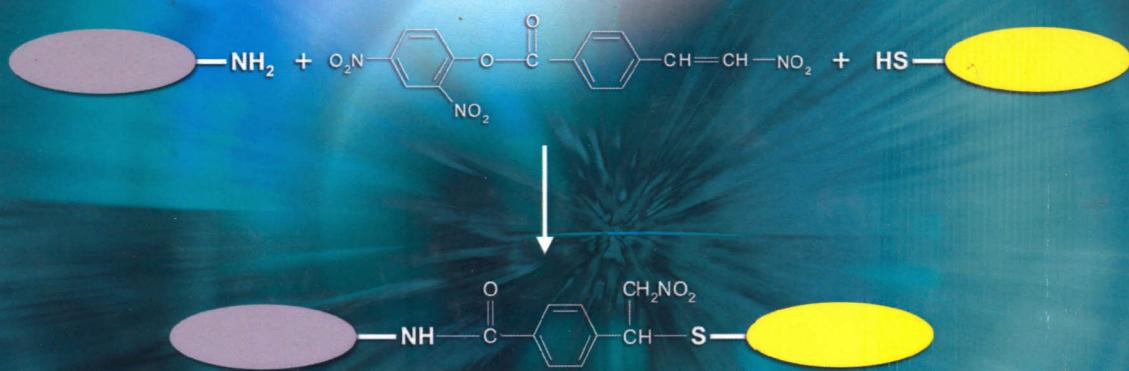


# Chemistry of Protein and Nucleic Acid Cross-Linking and Conjugation



*Second Edition*

Shan S. Wong  
David M. Jameson



CRC Press  
Taylor & Francis Group

# Contents

Preface.....	xv
Authors.....	xvii

<b>Chapter 1</b> Overview of Protein Conjugation.....	1
References .....	6

<b>Chapter 2</b> Review of Protein and Nucleic Acid Chemistry .....	9
---	---

2.1 Introduction .....	9
2.2 Protein Composition .....	10
2.2.1 Amino Acids .....	10
2.2.2 Prosthetic Groups .....	10
2.3 Protein Functional Groups .....	13
2.3.1 Reactive Amino Acid Side Chains.....	13
2.3.1.1 Relationship between Nucleophilicity and Reactivity.....	14
2.3.1.2 Effects of pH.....	15
2.3.1.3 Effects of Microenvironment.....	16
2.3.2 Chemically Introduced Reactive Groups .....	17
2.3.2.1 Reduction of Disulfide Bonds.....	18
2.3.2.2 Interconversion of Functional Groups .....	19
2.3.2.3 Introduction of Carbohydrate Prosthetic Groups .....	25
2.3.2.4 Activation of Carbohydrates by Periodate.....	27
2.4 Nucleic Acid Chemistry .....	27
2.4.1 Photochemical Reactivities of Nucleic Acids.....	27
2.4.2 Chemical Reactivities of Nucleic Acids .....	29
References .....	31

<b>Chapter 3</b> Reagents Targeted to Specific Functional Groups .....	35
--	----

3.1 Introduction .....	35
3.2 Sulfhydryl Reagents .....	35
3.2.1 $\alpha$ -Haloacetyl Compounds.....	35
3.2.2 <i>N</i> -Maleimide Derivatives .....	37
3.2.3 Mercurial Compounds.....	38
3.2.4 Disulfide Reagents.....	38
3.3 Amino Group-Specific Reagents.....	38
3.3.1 Alkylating Agents .....	39
3.3.1.1 $\alpha$ -Haloacetyl Compounds .....	39
3.3.1.2 <i>N</i> -Maleimide Derivatives.....	39
3.3.1.3 Aryl Halides.....	40
3.3.1.4 Aldehydes and Ketones .....	41
3.3.2 Acylating Agents .....	42

3.4	Reagents Directed toward Carboxyl Groups .....	43
3.4.1	Diazoacetate Esters and Diazoacetamides.....	43
3.4.2	Carbodiimides .....	44
3.5	Tyrosine Selective Reagents .....	44
3.5.1	Acyling Agents .....	44
3.5.2	Electrophilic Reagents .....	45
3.6	Arginine-Specific Reagents.....	45
3.7	Histidine-Selective Reagents.....	46
3.8	Methionine-Alkylyating Reagents .....	47
3.9	Tryptophan-Specific Reagents.....	47
3.10	Serine-Modifying Reagents.....	48
	References .....	48
<b>Chapter 4</b>	<b>How to Design and Choose Cross-Linking Reagents .....</b>	<b>53</b>
4.1	Introduction .....	53
4.2	Use of Nucleophilic Reactions .....	55
4.2.1	The Basic Reaction.....	55
4.2.1.1	Electrophilicity of the Substrate .....	55
4.2.1.2	Leaving Group Reactivity.....	56
4.2.2	Alkylation.....	56
4.2.3	Acylation .....	58
4.3	Use of Electrophilic Reactions .....	60
4.4	Incorporating Group-Directed Reagents.....	61
4.4.1	Disulfide Reagents.....	62
4.4.2	Mercurial Reagents .....	62
4.4.3	Reductive Alkylation.....	62
4.4.4	Vicinal Dicarbonyl Reagents.....	63
4.5	Incorporating Photoactivatable Nonspecific Groups.....	63
4.6	Changing the Water Solubility of Cross-Linkers .....	65
4.7	Incorporating Special Characteristics in the Bridge Spacer .....	66
4.7.1	Incorporation of Cleavable Bonds.....	66
4.7.1.1	Disulfide Bond .....	66
4.7.1.2	Mercurial Group .....	66
4.7.1.3	Vicinal Glycol Bond .....	66
4.7.1.4	Azo Linkage .....	66
4.7.1.5	Sulfone Linkage.....	69
4.7.1.6	Selenoethylene Group .....	69
4.7.1.7	Ester Bond .....	69
4.7.1.8	Thioester Bond .....	69
4.7.1.9	Maleylamide Linkage .....	69
4.7.1.10	Acetals, Ketals, and Ortho Esters.....	69
4.7.2	Incorporating Molecular Distance Rulers.....	70
4.7.3	Incorporating Reporter Groups .....	72
4.7.3.1	UV-VIS Absorption Chromophores.....	72
4.7.3.2	Infrared-Absorbing Chromophores .....	73
4.7.3.3	Fluorescent Probes.....	73
4.7.3.4	Spin Labels .....	74
4.7.3.5	Radioactive and Nonradioactive Isotopes.....	75
	References .....	76

<b>Chapter 5</b>	<b>Homobifunctional Cross-Linking Reagents.....</b>	<b>81</b>
5.1	Introduction .....	81
5.2	Amino Group–Directed Cross-Linkers .....	82
5.2.1	Bisimidoesters (Bisimides).....	82
5.2.2	Bis-Succinimidyl Derivatives ( <i>N</i> -Hydroxysuccinimidyl Esters, NHS Esters).....	85
5.2.3	Bifunctional Aryl Halides .....	86
5.2.4	DiIsocyanates and DiIsothiocyanates .....	87
5.2.5	Bifunctional Sulfonyl Halides .....	87
5.2.6	Bis-Nitrophenyl Esters .....	88
5.2.7	Bifunctional Acylazides .....	88
5.2.8	Dicarbonyl Compounds.....	88
5.2.9	Other Amino Group–Reacting Cross-Linking Reagents.....	91
5.3	Sulfhydryl Group–Directed Cross-Linkers.....	93
5.3.1	Mercurial Reagents .....	95
5.3.2	Disulfide-Forming Reagents .....	95
5.3.3	Bismaleimides .....	97
5.3.4	Bis-Haloacetyl Derivatives.....	98
5.3.5	Di-Alkyl Halides .....	98
5.3.6	Chloro- <i>s</i> -Triazines .....	99
5.3.7	Aziridines (Ethyleneimines) .....	99
5.3.8	Bis-Epoxides (Bisoxiranes) .....	99
5.3.9	Sulfone Derivatives .....	100
5.4	Carboxyl Group–Directed Cross-Linking Agents .....	101
5.5	Phenolate and Imidazolyl Group–Directed Cross-Linking Reagents.....	102
5.6	Arginine Residue–Directed Cross-Linkers.....	102
5.7	Methionine Residue Cross-Linking Agent.....	103
5.8	Carbohydrate Moiety–Specific Reagents .....	103
5.9	Nondiscriminatory Photoactivatable Cross-Linkers .....	104
5.10	Noncovalent Homobifunctional Cross-Linking Reagents.....	104
5.11	Nucleic Acid Cross-Linking Reagents .....	105
5.11.1	Metal Compounds .....	105
5.11.2	Azinomycin Bis-Epoxides.....	140
5.11.3	Bis-Pyrrolobenzodiazepines .....	141
5.11.4	Bis-Cyclopropylpyrroloindole (CPI)-Based Reagents.....	143
5.11.5	Bis-Cyclopropanebenz[e]indoline (CBI)-Based Reagents .....	145
5.11.6	Diaziridinyl Benzoquinones.....	146
5.11.7	Mitomycin C Dimers.....	147
5.11.8	Bis-Chloroethylamine Derivatives .....	147
5.11.9	Bis-Carbamate Derivatives .....	158
5.11.10	Pyrrolizidine Alkaloids (PAs) .....	160
5.11.11	Bis-Catechol Derivatives.....	161
5.11.12	Quinone Methides .....	162
5.11.13	Nitrosourea Derivatives.....	164
	References .....	165
<b>Chapter 6</b>	<b>Heterobifunctional Cross-Linkers.....</b>	<b>191</b>
6.1	Introduction .....	191
6.2	Group-Selective Heterobifunctional Reagents for Protein Cross-Linking .....	191

6.2.1	Amino- and Sulfhydryl-Group–Directed Cross-Linkers .....	191
6.2.2	Cross-Linkers Directed toward Carboxyl and Either Sulfhydryl or Amino Groups .....	199
6.2.3	Carbonyl- and Amino- or Sulfhydryl-Group–Directed Cross-Linkers .....	200
6.2.4	Miscellaneous Heterobifunctional Cross-Linkers with Undefined Specificity .....	200
6.3	Protein-Photosensitive Heterobifunctional Cross-Linking Reagents.....	202
6.3.1	Amino Group–Anchored Photosensitive Reagents.....	203
6.3.2	Sulfhydryl Group–Anchored Photoactivatable Reagents .....	204
6.3.3	Guanidinyl Group–Anchored Photoactivatable Reagents.....	205
6.3.4	Carboxyl-, Carboxamide-, and Carbonyl-Group–Anchored Photoactivatable Reagents.....	205
6.3.5	Photoaffinity-Labeling Reagents.....	205
6.4	Noncovalent Immunoglobulin Cross-Linking System.....	206
6.5	Heterobifunctional Nucleic Acid Cross-Linking Reagents .....	208
	References .....	225
<b>Chapter 7</b>	<b>Multifunctional Cross-Linking Reagents.....</b>	<b>239</b>
7.1	Introduction .....	239
7.2	Trifunctional Cross-Linkers .....	239
7.3	Tetrafunctional Cross-Linkers.....	256
7.4	Multifunctional Cross-Linkers .....	257
7.5	Noncovalent Cross-Linkers .....	258
7.5.1	Avidin and Streptavidin.....	258
7.5.2	Lectins.....	259
7.5.3	Multifunctional Antibodies .....	260
	References .....	261
<b>Chapter 8</b>	<b>Monofunctional and Zero-Length Cross-Linking Reagents .....</b>	<b>265</b>
8.1	Introduction .....	265
8.2	Monofunctional Cross-Linking Reagents .....	266
8.2.1	Imidoesters .....	266
8.2.2	Formaldehyde .....	266
8.2.3	Chloroformates .....	268
8.2.4	Mercuric Ion .....	269
8.2.5	Functional Group–Modifying Reagents .....	269
8.3	Zero-Length Cross-Linking Reagents.....	270
8.3.1	Carboxyl Group–Activating Reagents .....	270
8.3.1.1	Carbodiimides .....	270
8.3.1.2	Isoxazolium Compounds .....	276
8.3.1.3	Ethylchloroformate .....	276
8.3.1.4	Carbodiimidazole .....	277
8.3.1.5	<i>N</i> -Alkoxy carbonyl-2-Alkoxy-1,2-Dihydroquinolines .....	278
8.3.1.6	Diethylpyrocarbonate .....	279
8.3.2	Reagents for Disulfide Formation .....	279
8.3.3	Oxidation Cross-Linking Reagents .....	280
8.3.4	Carbohydrate Activation Reagents .....	281
8.3.5	Enzymes as Zero-Length Cross-Linkers .....	282

8.3.5.1	Transglutaminase.....	282
8.3.5.2	Tyrosinase .....	282
8.3.5.3	Peroxidases .....	283
8.3.5.4	Xanthine Oxidase and Others.....	283
8.3.6	Radiation as Zero-Length Cross-Linker .....	284
8.3.7	Miscellaneous Reagents .....	285
8.3.7.1	Tetranitromethane.....	285
8.3.7.2	Potassium Nitrosyl Disulfonate .....	286
8.3.7.3	Bisulfite.....	286
	References .....	286

**Chapter 9 General Approaches for Chemical Cross-Linking.....** 297

9.1	Introduction .....	297
9.2	Classification of Cross-Linking Procedures.....	297
9.2.1	One-Step Cross-Linking Reactions.....	297
9.2.2	Two-Step Cross-Linking Reactions .....	298
9.2.3	Three-Step Cross-Linking Reactions.....	300
9.2.4	Multistep Cross-Linking Reactions .....	300
9.3	General Conditions for Cross-Linking.....	303
9.3.1	Choice of Reaction Medium.....	303
9.3.2	Choice of Reaction Temperature and Time.....	303
9.3.3	Choice of Reactant Concentrations .....	304
9.4	Cross-Linking Protocols for Commonly Used Reagents .....	304
9.4.1	Examples for Zero-Length Cross-Linker.....	304
9.4.1.1	Cross-Linking a Peptide and a Protein Using EDC .....	304
9.4.1.2	Cross-Linking of Porcine Luteinizing Hormone with EDC to Study a and b Subunit Interactions.....	305
9.4.2	Examples for Homobifunctional Reagents.....	305
9.4.2.1	Bis-Imidoesters .....	305
9.4.2.2	Bis- <i>N</i> -Hydroxysuccinimide (NHS) Esters.....	305
9.4.2.3	Bis-Maleimido Reagents.....	306
9.4.2.4	Bis- $\alpha$ -Haloacetyl Reagents .....	307
9.4.3	Examples for Heterobifunctional Reagents.....	307
9.4.3.1	Conjugation of Human Serum Albumin (HSA) and Monoclonal Antibody (mAb) with SPDP .....	308
9.4.3.2	Cross-Linking of Demineralized Bone Matrix (DBM) and Monoclonal Antibody with Sulfo-SMCC .....	308
9.4.4	Examples for Heterobifunctional Photosensitive Reagents.....	309
9.4.4.1	Cross-Linking of Proteins with the Photoreagent <i>N</i> -(4-Azido-2,3,5,6-Tetrafluorobenzyl)-3- Maleimidylpropionamide (TFPAM-3).....	309
9.4.4.2	Cross-Linking UvsY Hexamer Protein Complex with the Photo-Reagent Ruthenium(II) Tris-Bipyridyl Dichloride ( $\text{Ru}(\text{II})\text{bpy}_3\text{Cl}_2$ ) .....	310
9.5	Cross-Linking Protocols Based on Biological Systems .....	310
9.5.1	Soluble Macromolecules .....	310
9.5.1.1	Cross-Linking Nonassociated Proteins .....	310
9.5.1.2	Cross-Linking Multisubunit Complexes.....	311
9.5.2	Membrane-Bound Proteins .....	311
9.5.3	Nucleic Acids and Nucleic Acid–Protein Complexes .....	312

9.6	Conditions for Cleavage of Cross-Linked Complexes.....	313
9.6.1	Disulfide Linkages .....	313
9.6.2	Glycol Bonds .....	313
9.6.3	Azo Bonds .....	313
9.6.4	Sulfone Linkages.....	313
9.6.5	Ester and Thioester Bonds .....	314
9.6.6	Acetals, Ketals, and Orthoesters.....	314
9.7	Reaction Complications.....	314
9.7.1	General Considerations .....	314
9.7.2	Immunogenicity .....	315
9.7.3	Stability .....	315
	References .....	316
<b>Chapter 10</b>	<b>Analysis of Cross-Linked Products.....</b>	<b>321</b>
10.1	Introduction .....	321
10.2	Techniques.....	321
10.2.1	Size-Exclusion Chromatography .....	321
10.2.2	Electrophoresis .....	323
10.2.3	Light Scattering .....	323
10.2.4	Mass Spectrometry.....	325
	References .....	326
<b>Chapter 11</b>	<b>Applications of Chemical Cross-Linking to the Study of Biological Macromolecules .....</b>	<b>327</b>
11.1	Introduction .....	327
11.2	Determination of Tertiary Structures of Proteins .....	328
11.2.1	Molecular Distances of Cross-Linking Reagents.....	328
11.2.2	Examples of Interresidue Distance Measurements .....	329
11.2.3	Examples of Applications to 3D Protein Structure Determination.....	330
11.3	Determination of Quaternary Structures of Proteins .....	331
11.3.1	Nearest Neighbor Analysis.....	332
11.3.2	Examples of Determination of Geometric Arrangements of Subunits within a Multiprotein Complex.....	332
11.3.2.1	Subunit Arrangement in Hexameric Protein Oligomers .....	332
11.3.2.2	Three-Dimensional Arrangement of F <sub>1</sub> -Adenosine Triphosphatase Subunits .....	332
11.3.2.3	Three-Dimensional Structure of the RNA Polymerase II-TFIIF Complex .....	333
11.3.2.4	Three-Dimensional Structure of the Ribosome .....	334
11.3.2.5	Organization of Contractile Protein Systems .....	336
11.4	Determination of Protein–Protein Interactions .....	338
11.4.1	Examples of Determinations of Protein–Protein Interactions of Soluble Proteins .....	338
11.4.2	Examples of Protein–Protein Interactions of Membrane-Bound Proteins: Ligand–Receptor Interactions .....	339
11.4.2.1	Interactions between Membrane-Bound Proteins .....	340
11.4.2.2	Interactions between Membrane-Bound Proteins and Soluble Proteins .....	340
11.5	Detection of Protein Conformational Changes .....	341

11.6	Determination of Nucleic Acid Interaction and Nucleic Acid–Protein Interaction .....	344
11.7	Effects of Cross-Linking on Structural Stability and Biological Activity .....	345
11.7.1	Increased Structural Stability and Activity .....	345
11.7.2	Conformation Lock .....	345
	References .....	346
<b>Chapter 12</b>	<b>Applications of Chemical Conjugation in the Preparation of Immunoconjugates and Immunogens .....</b>	<b>353</b>
12.1	Introduction .....	353
12.2	Preparation of Immunoconjugates .....	353
12.2.1	Components of Enzyme Immunoconjugates .....	354
12.2.1.1	Enzymes.....	354
12.2.1.2	Antibodies and Their Fragments .....	356
12.2.2	Introduction of Thiol Groups into Immunoglobulins .....	356
12.2.3	Preparation of Horseradish Peroxidase Immunoconjugates .....	358
12.2.3.1	Conjugation with Amino- and Thiol-Directed Cross-Linkers.....	358
12.2.3.2	Conjugation through Disulfide Formation .....	358
12.2.3.3	Conjugation with Glutaraldehyde .....	359
12.2.3.4	Conjugation Using Periodate Oxidation .....	360
12.2.3.5	Zero-Length Conjugation <i>In Vacuo</i> .....	360
12.2.3.6	Conjugation with Miscellaneous Cross-Linkers.....	360
12.2.4	Preparation of Alkaline Phosphatase Immunoconjugates .....	361
12.2.4.1	Conjugation with Amino- and Thiol-Directed Reagents .....	361
12.2.4.2	Conjugation with Glutaraldehyde .....	361
12.2.4.3	Conjugation with Periodate Oxidation .....	362
12.2.4.4	Zero-Length Conjugation .....	362
12.2.5	Preparation of $\alpha$ -D-Galactosidase Immunoconjugates.....	362
12.2.5.1	Conjugation with Amino- and Thiol-Directed Reagents.....	362
12.2.5.2	Conjugation with Thiol Group–Directed Dimaleimides.....	362
12.2.5.3	Conjugation with Phenolate and Thiol Group–Directed Reagent.....	363
12.2.5.4	Conjugation with Glutaraldehyde .....	364
12.2.6	Preparation of Glucose-6-Phosphate Dehydrogenase Immunoconjugates .....	364
12.2.7	Preparation of Glucose Oxidase Immunoconjugates .....	364
12.2.7.1	Coupling with <i>N</i> -Ethoxycarbonyl-2-Ethoxy-1,2-Dihydroquinoline.....	364
12.2.7.2	Coupling with Amino- and Thiol-Directed Reagents .....	364
12.2.7.3	Coupling with Other Cross-Linkers .....	364
12.2.8	Preparation of Other Enzyme Immunoconjugates.....	364
12.2.9	Preparation of Nonenzyme Protein Immunoconjugates.....	365
12.2.10	Coupling Enzymes to Proteins Other than Antibodies .....	365
12.2.10.1	Examples of Conjugations of Enzymes and Biotin-Binding Proteins .....	365
12.2.10.2	Examples of Conjugation of Enzymes and Other Proteins .....	365
12.2.10.3	Examples of Conjugation of Enzymes and Antigens.....	366

12.3	Preparation of Immunogens .....	366
12.3.1	Examples of Conjugation of Hapten to Albumin .....	366
12.3.2	Examples of Conjugation of Hapten to Keyhole Limpet Hemocyanin .....	368
12.3.3	Examples of Hapten Conjugation to Other Carriers .....	368
12.4	Characterization of Conjugation Methods .....	369
	References .....	369
<b>Chapter 13</b>	Application of Chemical Conjugation for the Preparation of Immunotoxins and Other Drug Conjugates for Targeting Therapeutics .....	377
13.1	Introduction .....	377
13.2	Targeting Agents and Toxins .....	377
13.2.1	Choice of Targeting Agents.....	377
13.2.1.1	Antibodies.....	378
13.2.1.2	Other Naturally Occurring Molecules.....	378
13.2.1.3	Synthetic Peptides and Nucleotides.....	380
13.2.1.4	Synthetic Polymers .....	381
13.2.2	Choice of Toxins.....	382
13.3	Preparation of Therapeutic Conjugates .....	383
13.3.1	Choice of Cross-Linking Reagents .....	383
13.3.2	Conjugation through Disulfide Bond .....	384
13.3.2.1	Coupling with <i>N</i> -Succinimidyl-3-(2-Pyridyldithio) propionate .....	384
13.3.2.2	Coupling with Other Disulfide Generating Agents .....	387
13.3.3	Conjugation through Thioether Linkage.....	390
13.3.3.1	Use of Iodoacetyl Compounds.....	390
13.3.3.2	Use of Amino and Thiol Directed Cross-Linkers .....	392
13.3.4	Conjugation with Activated Chlorambucil.....	392
13.3.5	Conjugation with Acid-Labile Cross-Linkers .....	393
13.3.6	Conjugation with Photocleavable Cross-Linkers .....	396
13.3.7	Coupling through Carbohydrate Residues .....	396
13.3.7.1	Use of Intrinsic Carbohydrate Moieties .....	396
13.3.7.2	Use of Polysaccharide Spacers .....	398
13.3.8	Conjugation Using Avidin–Biotin Linkage.....	398
13.3.9	Conjugation Using Enzymes .....	399
13.3.10	Conjugation Using Solid-Phase Procedures .....	399
13.3.11	Conjugation with Glutaraldehyde and Carbodiimides .....	400
	References .....	400
<b>Chapter 14</b>	Application of Chemical Conjugation to Solid-State Chemistry .....	409
14.1	Introduction .....	409
14.2	Functionalities of Matrices .....	409
14.3	Protein Immobilization by Matrix Activation.....	412
14.3.1	Activation of Hydroxyl Groups .....	412
14.3.2	Activation of Carboxyl Groups .....	413
14.3.3	Activation of Acyl Hydrazide.....	413

14.3.4 Activation of Amines .....	414
14.3.4.1 Use of Nitrous Acid .....	414
14.3.4.2 Use of Phosgene and Thiophosgene .....	414
14.3.4.3 Use of Cyanogen Bromide.....	414
14.3.5 Activation of Polyacrylonitrile .....	415
14.4 Cross-Linking Reagents Commonly Used for Immobilization of Biomolecules .....	415
14.4.1 Use of Zero-Length Cross-Linking Reagents .....	415
14.4.2 Use of Mono- and Homobifunctional Cross-Linkers.....	417
14.4.2.1 Glutaraldehyde.....	417
14.4.2.2 Chloroformates and Carbonyldiimidazole .....	417
14.4.2.3 Heterocyclic Halides.....	418
14.4.2.4 Bisoxiranes .....	418
14.4.2.5 Divinylsulfone.....	419
14.4.2.6 Quinones.....	419
14.4.2.7 Transition Metal Ions.....	419
14.4.2.8 Other Homobifunctional Cross-Linkers.....	419
14.4.3 Use of Heterobifunctional Cross-Linkers .....	420
14.4.3.1 Monohalogenacetyl Halide .....	420
14.4.3.2 Epichlorohydrin .....	420
14.4.3.3 Amino- and Thiol-Group-Directed Reagents.....	421
14.5 Immobilization by Cross-Linking through Carbohydrate Chains .....	422
14.6 Examples of Applications of Solid-Phase Immobilization Chemistry .....	422
14.6.1 Affinity Chromatography .....	422
14.6.2 Biosensors.....	425
14.6.3 Microarrays .....	426
14.6.3.1 DNA Microarrays.....	426
14.6.3.2 Protein/Peptide Microarrays.....	431
14.6.3.3 Antibody Microarrays .....	433
14.6.3.4 Carbohydrate Microarrays.....	434
14.6.4 Industrial Applications .....	435
References .....	436
<b>Appendix A:</b> Amino Group-Directed Homobifunctional Cross-Linkers.....	447
<b>Appendix B:</b> Sulfhydryl Group-Directed Homobifunctional Cross-Linkers .....	483
<b>Appendix C:</b> Phenolate- and Imidazolyl-Group-Directed Reagents: Bis diazonium Precursors.....	513
<b>Appendix D:</b> Group Selective Heterobifunctional Cross-Linkers .....	515
<b>Appendix E:</b> Photoactivatable Heterobifunctional Cross-Linking Reagents .....	549
<b>Index.....</b>	587