

# Contents

## Part I     Chemistry of Saccharides

### Vol. 1    Chemical Synthesis of Glycosides and Glycomimetics

List of Contributors .....	LV
Abbreviations Used in Volumes 1 and 2 .....	LXIII
<b>I       Chemical Synthesis of Glycosides.....</b>	<b>1</b>
<b>1      Introduction to Volumes 1 and 2 .....</b>	<b>3</b>
<b>2      Trichloroacetimidates .....</b>	<b>5</b>
<i>Richard R. Schmidt and Karl-Heinz Jung</i>	
Introduction.....	5
Methods.....	6
O-Glycosides.....	7
2.3.1    Synthesis of Oligosaccharides .....	7
β-Glucosides, β-Galactosides, α-Rhamnosides, etc.....	7
Aminosugar Trichloroacetimidates.....	8
β-Mannosides .....	13
2-Deoxyglycosides .....	13
Miscellaneous Compounds .....	14
Complex Oligosaccharides.....	14
2.3.2    Inositol Glycosides.....	36
2.3.3    Glycosylation of Sphingosine Derivatives and Mimics .....	38
2.3.4    Glycosylation of Amino Acids .....	40
2.3.5    Polycyclic and Macroyclic Glycosides .....	42

2.3.6	Glycosides of Phosphoric and Carboxylic Acids .....	44
2.3.7	Solid-Phase Synthesis.....	45
2.4	S-Glycosides .....	49
2.5	N- and P-Glycosides.....	51
2.6	C-Glycosides.....	51
2.7	Conclusion and Outlook .....	53
	References .....	53
<b>3</b>	<b>Iterative Assembly of Glycals and Glycal Derivatives: The Synthesis of Glycosylated Natural Products and Complex Oligosaccharides...</b>	<b>61</b>
	<i>Lawrence J. Williams, Robert M. Garbaccio, and Samuel J. Danishefsky</i>	
3.1	Introduction .....	61
3.2	Cyclamycin 0 .....	64
3.3	Allosamidin .....	66
3.4	KS-502 and Rebeccamycin.....	69
3.5	Extension to Thioethyl Donors .....	74
3.6	Lewis <sup>y</sup> .....	76
3.7	Globo H .....	82
3.8	KH-1 .....	86
3.9	Concluding Remarks .....	90
	Acknowledgments .....	90
	References .....	90
<b>4</b>	<b>Thioglycosides .....</b>	<b>93</b>
	<i>Stefan Oscarson</i>	
4.1	Introduction .....	93
4.2	Synthesis of Thioglycosides .....	94
4.2.1	From Anomeric Acetates.....	94
4.2.2	From Glycosyl Halides.....	95
4.2.3	Protecting Group Manipulations in Thioglycosides .....	96
4.3	Glycosylations with Thioglycoside Donors .....	97
4.3.1	A Two-Step Procedure: Transformation of Thioglycosides into Other Types of Glycosyl Donors.....	97
4.3.2	Direct Activation of Thioglycoside Donors .....	99
	Heavy Metal Salt Promoters .....	99
	Halonium, sulfonium and carbonium type promoters.....	100
	Single-Electron Activation .....	106
	Other Types of Donors With an Anomeric Sulfur .....	108
4.4	Applications of Thioglycosides.....	110
4.4.1	Block Syntheses, Orthogonal Glycosylations .....	110
	Thioglycosides as Acceptors.....	110
	Thioglycosides as Both Donors and Acceptors .....	111
4.4.2	Intramolecular Glycosidations .....	112
4.4.3	Solid Phase Synthesis.....	113
	References .....	113

<b>5</b>	<b>Glycosylation Methods: Use of Phosphites .....</b>	117
	<i>Zhiyuan Zhang and Chi-Huey Wong</i>	
5.1	Introduction.....	117
5.2	Preparation of Glycosyl Phosphites .....	118
5.3	Glycosylation using Glycosyl Phosphites .....	119
5.3.1	Mechanism.....	119
5.3.2	Low Temperature-Dependent Stereoselectivity .....	121
5.3.3	Glycosylation of Sialyl Phosphites .....	122
5.3.4	Glycosylation of C-2-Acylated Glycosyl Phosphites.....	123
5.3.5	Glycosylation with C-2- <i>O</i> -Benzylated Glycosyl Phosphites .....	124
	Glycosylation using Glucosyl Phosphites with a Benzyl Group at C-2 .....	124
	Glycosylation using Galactosyl and Fucosyl Phosphites with a Benzyl Group at C-2.....	125
	Glycosylation using other Glycosyl Phosphites with a Benzyl Group at C-2.....	126
5.3.6	Glycosylation with 2-Deoxy Glycosyl Phosphites .....	127
5.4	Other Applications of Glycosyl Phosphites .....	128
5.4.1	Synthesis of CMP-NeuAc.....	129
5.4.2	Synthesis of GDP-Fucose.....	129
5.4.3	Formation of Glycosyl Phosphonate.....	131
5.4.4	Transformation to other Types of Glycosyl Donor.....	131
	Phosphate .....	131
	Phosphorimidate .....	131
	References.....	132
<b>6</b>	<b>Glycosylation Methods: Use of <i>n</i>-Pentenyl Glycosides .....</b>	135
	<i>Bert Fraser-Reid, G. Anilkumar, Mark R. Gilbert, Subodh Joshi, and Ralf Kraehmer</i>	
6.1	Introduction.....	135
6.2	Fundamental Reactions .....	135
6.3	Determination of Relative Reactivities.....	138
6.4	<i>n</i> -Pentenyl Orthoesters as Glycosyl Donors.....	141
6.5	<i>n</i> -Pentenyl Orthoesters as Latent C2 Esters.....	144
6.6	Protecting Groups .....	146
6.7	Solid-Phase Iterative Couple-Deprotect-Couple Strategy .....	146
	References.....	153
<b>7</b>	<b>Glycosylidene Diazirines .....</b>	155
	<i>Andrea Vasella, Bruno Bernet, Martin Weber, and Wolfgang Wenger</i>	
7.1	Introduction.....	155
7.2	Synthesis of Glycosylidene Diazirines.....	155
7.3	Stability of the Glycosylidene Diazirines.....	158
7.4	Glycosidation by Glycosylidene Diazirines .....	158
7.4.1	General Aspects.....	158

7.4.2	Glycosidation of Strongly Acidic Hydroxy Compounds .....	162
	Glycosidation of Phenols.....	162
	Glycosidation of Fluorinated Alcohols .....	163
7.4.3	Glycosylation of Weakly Acidic Hydroxy Compounds .....	163
	Glycosidation of Monovalent Alcohols.....	163
	Glycosidation of Diols and Triols .....	164
7.5	Synthesis of Spirocyclopropanes .....	168
7.6	Addition to Aldehydes and Ketones .....	170
7.7	Exploratory Use of Diazirines: Formation of Glycosyl Phosphines, Stannanes, <i>N</i> -Sulfonylamines, Esters, Boranes, and Alanes, and of 1,1-Difluorides .....	171
	Acknowledgments .....	174
	References .....	174
<b>8</b>	<b>Glycosylation Methods: Alkylation of Reducing Sugars .....</b>	<b>177</b>
	<i>Jun-ichi Tamura</i>	
8.1	Introduction .....	177
8.2	Anomeric O-Alkylation .....	177
8.2.1	Anomeric O-Alkylation of Ribofuranose with Primary Triflates: Effect of the Protecting Group at O-5 of Ribofuranose .....	178
8.2.2	Anomeric O-Alkylation of Mannofuranose with Primary Triflates: The Crown Ether Effect .....	179
8.2.3	Anomeric O-Alkylation of Gluco- and Galactopyranoses with Primary Triflates: High $\beta$ -Selectivity as a Result of the Reactive Anomeric $\beta$ -Anion.....	180
8.2.4	Anomeric O-Alkylation of Acyl-Protected Nucleophiles with Primary Triflates.....	181
8.2.5	Anomeric O-Alkylation of Mannopyranose with Primary Triflates: Possibility of Intramolecular Complexation of the Nucleophile ....	184
8.2.6	Anomeric O-Alkylation of KDO with Primary Triflates.....	185
8.2.7	Anomeric O-Alkylation of Some Protected Aldoses with Primary Triflate .....	186
8.2.8	Anomeric O-Alkylation of Unprotected Aldoses with Primary Triflate, Bromides, and Cyclic Sulfates .....	187
8.2.9	Anomeric O-Alkylation with Secondary Triflates and Nonaflate...	188
8.3	Glycosylation <i>via</i> the Locked Anomeric Configuration .....	189
8.3.1	Synthesis of Methyl, Allyl, and Benzyl Glycosides <i>via</i> Stannylene Acetals .....	189
8.3.2	Epimerization at C-2 by the Locked Anomeric Configuration Method .....	189
8.3.3	The Locked Anomeric Configuration Method for Rhamnosyl Stannylene Acetal .....	190
8.3.4	The Locked Anomeric Configuration Method for Mannosyl Stannylene Acetal: Isomerization of Acetal [25, 26].....	190
8.3.5	The Locked Anomeric Configuration Method for Stannylene Acetal with the Glucose Configuration [25, 26] .....	191

8.4	Conclusion .....	192
	References .....	193
<b>9</b>	<b>Other Methods of Glycosylation .....</b>	<b>195</b>
	<i>Luigi Panza and Luigi Lay</i>	
	Introduction and Summary .....	195
	Highlights .....	195
9.1	Enol Ethers .....	197
9.1.1	Endo-Enol Ethers .....	198
9.1.2	Exo-Enol Ethers .....	201
9.1.3	Endo-Glycals .....	202
9.1.4	Exo-Glycals .....	204
9.1.5	Vinyl Glycosides .....	206
9.2	1-Hydroxy Sugars .....	209
9.2.1	Acidic Activation .....	210
	Acidic Activation With Additional Reagents .....	211
9.2.2	Dehydrative Glycosylation .....	212
	In the Presence of the Acceptor From the Beginning .....	213
9.2.3	Mitsunobu Glycosylation .....	214
9.2.4	1-O-Silyl Glycosides .....	215
9.3	Esters and Related Derivatives .....	216
9.3.1	Esters .....	216
9.3.2	Sugar Carbonates and Derivatives .....	221
9.3.3	Orthoesters and Oxazolines .....	223
9.3.4	Phosphorus and Sulfur Derivatives .....	229
	References .....	233
<b>10</b>	<b>Polymer-Supported Synthesis of Oligosaccharides .....</b>	<b>239</b>
	<i>Jiri J. Krepinsky and Stephen P. Douglas</i>	
10.1	Introduction .....	239
10.2	General Reflections .....	240
10.3	Polymer Supports .....	246
10.4	One-Phase Systems (Syntheses in Solution) .....	247
	Polyethyleneglycol <sub>ω</sub> -monomethyl ether (MPEG) .....	248
	Linear Polystyrene .....	249
10.4.1	Linkers .....	250
	Succinoyl Diester .....	250
	Dioxyxylyl Diether (DOX) .....	252
10.4.2	Chemistry Investigations .....	254
10.5	Two-Phase Systems (Syntheses on Solid Supports) .....	255
	Controlled Pore Glass .....	256
	Cross-Linked Polystyrene .....	256
	Polyethylene Grafts on Cross-Linked Polystyrene .....	256
10.5.1	Linkers .....	259
	Dialkyl- or Diaryl-Silyl .....	259
	Thioglycoside Linkers .....	259

	Linkers Cleavable by Photolysis .....	260
10.6	Examples of Syntheses .....	260
10.7	Combinatorial Libraries.....	261
10.8	Capping.....	262
10.9	Concluding Remarks .....	262
	References .....	262
<b>11</b>	<b>Glycopeptide Synthesis in Solution and on the Solid Phase .....</b>	<b>267</b>
	<i>Horst Kunz and Michael Schultz</i>	
11.1	Introduction .....	267
11.1.1	Which Protecting Groups are Suitable for Carbohydrates (Table 2)? .....	269
11.1.2	Which Glycosylation Methods are Useful for the Formation of Glycopeptides?.....	271
	Formation of Asparagine N-Glycosides .....	271
	$\alpha$ -Fucosylation.....	271
	Formation of the $\beta$ -Lactosamine Linkage .....	272
	$\alpha$ -Sialylation .....	272
11.1.3	Glycopeptides Containing Particularly Sensitive Linkages.....	272
	Acid Sensitivity .....	273
	Base Sensitivity .....	273
11.2	Synthesis of Glycopeptides in Solution .....	274
11.2.1	<i>O</i> -Glycopeptides.....	274
	Glycopeptides Carrying N-Acetylgalactosamine (Tn-Antigen).....	274
	Glycopeptides Carrying the T-Antigen (Gal-GalNAc) .....	276
	Glycopeptides Carrying the Sialyl T Antigen (NeuAca <sub>2,6</sub> [Gal $\beta$ 1,3]GalNAc) .....	278
	Glycopeptides Carrying <i>O</i> -GlcNAc.....	279
11.2.2	<i>N</i> -Glycopeptides .....	280
	<i>N</i> -Glycopeptides Carrying Natural Saccharide Side-Chains .....	280
	<i>N</i> -Glycopeptides with Lewis-Type Saccharide Side-Chains .....	285
11.3	Glycopeptide Synthesis on the Solid Phase .....	286
11.3.1	<i>O</i> -Glycopeptides.....	287
	Glycopeptides Carrying N-Acetylgalactosamine (Tn-Antigen).....	287
	<i>O</i> -Glycopeptides Carrying the T Antigen (Gal-GalNAc) .....	290
	<i>O</i> -Glycopeptides Carrying the Sialyl Tn Antigen (NeuNAc- $\alpha$ 2,6-GalNAc) .....	291
	<i>O</i> -Glycopeptides Carrying the 2,3-Sialyl T Antigen.....	293
	<i>O</i> -Glycopeptides Carrying <i>O</i> -GlcNAc Side-Chains .....	294
	<i>O</i> -Glycopeptides Carrying O-Linked Fucose .....	295
	<i>O</i> -Glycopeptides Carrying a Sialyl Lewis Antigen Structure.....	296
11.3.2	<i>N</i> -Glycopeptides .....	297
	The Construction of <i>N</i> -Glycopeptide Libraries on the Solid Phase.....	298
	Sequential <i>N</i> -Glycopeptide Synthesis on the Solid Phase with Oligosaccharides from Natural Sources.....	299

11.4	Conclusion .....	300
	References.....	300
<b>12</b>	<b>Glycolipid Synthesis.....</b>	<b>305</b>
	<i>Hideharu Ishida</i>	
12.1	Introduction.....	305
12.2	Synthesis of Ganglio-Series Gangliosides .....	305
12.2.1	Retrosynthetic Analysis of Ganglioside GD1a .....	305
12.2.2	Preparation of Sialylgalactose Donor as Building Block .....	306
12.2.3	Construction of Oligosaccharide .....	308
12.2.4	Transformation of Oligosaccharide into Glycolipid .....	310
12.3	Synthesis of Polysialo Ganglio-Series Gangliosides.....	311
12.3.1	Retrosynthetic Analysis of GQ1b .....	313
12.3.2	Preparation of Building Block.....	313
12.3.3	Construction of Oligosaccharide .....	314
12.4	Conclusion .....	315
	References.....	316
<b>13</b>	<b>Stereoselective Synthesis of <math>\beta</math>-Mannosides.....</b>	<b>319</b>
	<i>Vince Pozsgay</i>	
13.1	Introduction.....	319
13.2	Chemical Methods.....	320
13.2.1	Glycosylation with Mannosyl Donors .....	320
	Mannosylation using Insoluble Promoters .....	320
	The Sulfonate Approach.....	322
	Intramolecular Mannosylation .....	324
	Other Mannosyl Donor-Based Methods.....	327
13.2.2	Epimerization of $\beta$ -Glucopyranosides at C-2 .....	329
	The Oxidation–Reduction Approach .....	329
	Direct Inversion.....	329
13.2.3	The 2-Ulosyl Donor Method .....	331
13.2.4	Anomeric <i>O</i> -alkylation .....	332
	Alkylation of 1- <i>O</i> -Metal Complexes .....	332
	The Stannylene Acetal Method .....	332
13.2.5	Miscellaneous Methods.....	333
	Radical Inversion of the Anomeric Chirality of $\alpha$ -D-	
	Mannopyranosides.....	333
	Reductive Cleavage of Cyclic Orthoesters .....	334
	<i>De novo</i> Syntheses .....	334
13.2.6	2-Acetamido-2-deoxy- $\beta$ -D-mannopyranosides .....	335
13.2.7	Aryl $\beta$ -D-mannopyranosides .....	336
13.2.8	1-Thio- $\beta$ -D-mannopyranosides .....	336
13.2.9	$\beta$ -D-Mannopyranosylamines .....	337
13.3	Enzymatic Synthesis .....	337
13.4	Conclusions .....	338
	References.....	338

<b>14</b>	<b>Special Problems in Glycosylation Reactions: Sialidations.....</b>	<b>345</b>
	<i>Makoto Kiso, Hideharu Ishida and Hiromi Ito</i>	
14.1	Introduction .....	345
14.2	Sialidation by the Koenigs-Knorr Method.....	345
14.3	Sialidation Using an Auxiliary Group at C-3 .....	347
14.4	Sialidation Using 2-Thioglycosides, Xanthates, or Phosphites of Sialic Acids in Acetonitrile .....	349
14.4.1	Thioglycosides .....	349
14.4.2	Xanthates and Phosphites .....	356
14.4.3	Reaction Mechanism .....	359
14.5	Further Solutions to the Problem .....	359
14.5.1	Combination of C-3 Auxiliary and Sterically less Hindered Sugar Acceptors .....	359
14.5.2	Combination of C-3 Auxiliary and Specific Activation of the Anomeric Center C-2.....	360
14.5.3	Thioglycoside of <i>N,N</i> -Diacetylneuraminic Acid and Combination with C-3 Auxiliary.....	363
	References .....	364
<b>15</b>	<b>Special Problems in Glycosylation Reactions: 2-Deoxy Sugars .....</b>	<b>367</b>
	<i>Alain Veyrières</i>	
15.1	Introduction .....	367
15.2	Electrophilic Additions to Glycals: Mechanistic Aspects and Applications to the Synthesis of 2-Deoxyglycosides.....	368
15.2.1	Protonation of Glycals .....	369
15.2.2	Enzyme-Catalyzed Additions to Glycals.....	370
15.2.3	Halogenation of Glycals .....	370
15.2.4	Bromo- and Iodoalkoxylation of Glycals.....	372
15.2.5	Epoxidation of Glycals.....	377
15.2.6	Addition of Sulfur Based Electrophiles to Glycals .....	379
15.2.7	Addition of Selenium Based Electrophiles to Glycals .....	382
15.3	The Cycloaddition Way to Glycosyl Transfer .....	384
15.4	Fluoroglycosylation of Glycals.....	385
15.5	Glycosyl Donors with a C-2 Heteroatom.....	386
15.5.1	2-Bromo-2-deoxyglycosyl bromides.....	386
15.5.2	2-Deoxy-2-(thiophenyl)-glycosyl fluorides .....	387
15.5.3	2,6-Anhydro-2-Thio-Glycosyl Donors .....	388
15.5.4	1,2-Di- <i>O</i> -Acetyl- $\beta$ -Hexopyranoses and <i>N</i> -Formylglucosamine Derivatives.....	392
15.6	2-Deoxyglycosyl Donors .....	393
15.6.1	2-Deoxy-Hexopyranoses .....	394
15.6.2	Tert-Butyldimethylsilyl 2-Deoxyglycosides .....	394
15.6.3	1- <i>O</i> -Acyl- and Acetimidyl-2-Deoxy-Hexopyranoses.....	394
15.6.4	2-Deoxyglycosyl Bromides and Fluorides .....	395
15.6.5	<i>S</i> -(2-Deoxyglycosyl)phosphorodithioates .....	396

15.6.6	2-Deoxyglycosyl Phosphates, Phosphoramidites and Phosphites . . . . .	397
15.6.7	2-Deoxy Thioglycosides . . . . .	398
15.6.8	2-Deoxyglycosyl Sulfoxides . . . . .	399
15.7	Other Approaches to 2-Deoxyglycosides . . . . .	400
15.7.1	Cyclization of Acyclic Sugars . . . . .	401
15.7.2	Use of Alkoxy-Substituted Anomeric Radicals . . . . .	402
	References . . . . .	403
<b>16</b>	<b>Orthogonal Strategy in Oligosaccharide Synthesis . . . . .</b>	<b>407</b>
	<i>Osamu Kanie</i>	
16.1	Introduction . . . . .	407
16.2	Analysis of the Strategic Aspects of Oligosaccharide Synthesis . . . . .	408
16.2.1	General Aspects . . . . .	408
16.2.2	The Pursuit of Efficiency in Oligosaccharide Synthesis . . . . .	408
16.3	The Introduction of the Orthogonal Glycosylation Strategy . . . . .	410
16.3.1	Limitation of Current Concepts . . . . .	410
16.3.2	The Orthogonal Coupling Concept . . . . .	412
16.3.3	What is Orthogonality Anyway? . . . . .	413
16.3.4	Orthogonal Glycosylation and Solid-Phase Oligosaccharide Synthesis . . . . .	414
16.4	The Orthogonal Glycosylation Strategy . . . . .	414
16.4.1	Orthogonal Chain Elongation of Homo-Oligosaccharides: Synthesis of Chito-Oligosaccharides [19] . . . . .	414
16.4.2	Orthogonal Coupling for Hetero-Oligomer Synthesis [22] . . . . .	418
16.4.3	Application to Polymer-Supported Synthesis [26] . . . . .	420
16.5	Conclusions and Prospects . . . . .	421
	Acknowledgments . . . . .	424
	References . . . . .	424
<b>17</b>	<b>Protecting Groups: Effects on Reactivity, Glycosylation Stereoselectivity, and Coupling Efficiency . . . . .</b>	<b>427</b>
	<i>Luke G. Green and Steven V. Ley</i>	
17.1	Introduction . . . . .	427
17.2	Glycosidic Mechanism . . . . .	428
17.3	Electronic and Torsional Effects . . . . .	430
17.4	Influence of Protecting Group on Donor Reactivity . . . . .	431
17.5	Stereoselectivity . . . . .	436
17.5.1	Neighboring-Group Participation . . . . .	436
17.5.2	Reactivity Control . . . . .	437
17.6	Influence of the Protecting Group on the Acceptor . . . . .	441
17.7	Steric Effects on Glycosylation . . . . .	443
17.8	Conclusions . . . . .	444
	Acknowledgments . . . . .	445
	References . . . . .	446

<b>18</b>	<b>Intramolecular Glycosidation Reactions .....</b>	<b>449</b>
	<i>Jacob Madsen and Mikael Bols</i>	
18.1	Introduction .....	449
18.2	Reactions in which the Tether Participates in the Reaction.....	450
18.2.1	Tethering to the Glycosyl Donor .....	450
	Carbon Tethers .....	450
	Silicon Tethers .....	454
18.2.2	Tethering to the Leaving Group .....	459
18.3	Reactions in which the Tether does not Participate in the Reaction	459
18.4	Conclusion.....	464
	References .....	465
<b>19</b>	<b>Classics In Total Synthesis of Oligosaccharides and Glycoconjugates.....</b>	<b>467</b>
	<i>Jean-Maurice Mallet and Pierre Sinay</i>	
19.1	Introduction .....	467
19.2	Syntheses of Nod factors .....	467
19.2.1	Introduction .....	467
19.2.2	The K. C. Nicolaou Synthesis (1992) [3].....	468
19.2.3	The J.-M. Beau Synthesis (1994) [12] .....	471
19.2.4	The T. Ogawa Synthesis (1994) [16] .....	475
19.2.5	The Y. Z. Hui Synthesis (1992) [18] .....	477
19.2.6	Conclusion.....	480
19.3	Synthesis of the Antithrombin-Binding Pentasaccharide Sequence in Heparin (1984) [19, 20].....	480
19.3.1	Introduction .....	480
19.3.2	An Overview of the Synthesis of the Protected Pentasaccharide 73 .....	481
19.3.3	Synthesis of the Disaccharidic Bromide Donor 68 .....	483
19.3.4	Synthesis of the Disaccharidic Acceptor 69.....	484
19.3.5	Synthesis of the Protected Pentasaccharide 73.....	484
19.3.6	Synthesis of the Active Site of Heparin .....	485
19.4	Total Synthesis of VIM-2 Ganglioside [31] .....	485
19.4.1	Introduction .....	485
19.4.2	The Total Synthesis of VIM-2—a General Strategy .....	486
19.4.3	Preparation of the Key Protected Octasaccharide 87.....	487
19.5	Epilogue .....	490
	References .....	491
<b>II</b>	<b>Synthesis of Oligosaccharide Mimics .....</b>	<b>493</b>
<b>20</b>	<b>Synthesis of C-Oligosaccharides .....</b>	<b>495</b>
	<i>Troels Skrydstrup, Boris Vauzeilles, and Jean-Marie Beau</i>	
20.1	Introduction .....	495
20.2	The Anionic Approach.....	496
20.2.1	C5-Alkynyl Anions .....	496

20.2.2	C1-Glycal Carbanions .....	500
20.2.3	Anomeric Samarium Species .....	502
20.2.4	C-Branched Carbanions .....	506
20.2.5	C6-Phosphoranes .....	508
20.3	The Radical Approach .....	511
20.3.1	Intermolecular Anomeric Radical Addition .....	511
20.3.3	Intramolecular Anomeric Radical Addition .....	513
20.4	The Partial <i>de Novo</i> Approach .....	518
20.5	The Cycloaddition and Rearrangement Approach .....	527
	References .....	528
<b>21</b>	<b>Synthesis of Oligosaccharide Mimics: S-Analogs .....</b>	<b>531</b>
	<i>Jon K. Fairweather and Hugues Driguez</i>	
21.1	Introduction .....	531
21.2	General Synthesis .....	532
21.2.1	Preparation of Thioglycoses .....	532
	1-Thioglycoses .....	532
	2-, 3-, 4-, 5-, or 6-Thioglycoses .....	532
	Selective S-Deprotection of Thioglycoses .....	533
	Glycosylation Methods .....	534
21.3	Establishment of 1,6-Thio Linkages .....	534
21.3.1	6-Thiodisaccharides .....	534
21.3.2	6-Thiooligosaccharides .....	538
21.3.3	Branched Thiocyclodextrins .....	538
21.4	Establishment of 1,4-Thio Linkages .....	541
21.4.1	1,4-Thiodisaccharides .....	541
	General Approaches .....	541
	S <sub>N</sub> 2-Displacement on Triflates .....	541
21.4.2	1,4-Thiooligosaccharides .....	546
	Conventional Approaches .....	546
	Chemoenzymatic Approaches .....	548
	Michael Addition to Unsaturated Acceptors .....	549
	Solid-Support Synthesis .....	550
21.5	Establishment of 1,3-Thio Linkages .....	551
21.5.1	1,3-Thiodisaccharides .....	551
	Conventional Methods .....	551
	Cyclic Sulfamide and Aziridine .....	551
21.5.2	1,3-Thiooligosaccharides .....	552
21.6	Establishment of 1,2-Thio Linkages .....	553
21.6.1	1,2-Thiodisaccharides .....	553
	Conventional Methods .....	554
	Other Approaches .....	555
21.7	Establishment of 1,1-Thio Linkages .....	557
21.8	Establishment of Mixed Thio linkages .....	558
21.9	Thiooligosaccharides and Proteins .....	558
21.9.1	The Conformation of Thiooligosaccharides in Solution .....	558

21.9.2	Enzyme–Substrate Interactions .....	560
	$\alpha$ -Glucan-Active Enzymes.....	560
	$\beta$ -Glucan-Active Enzymes.....	561
21.9.3	Lectin–Ligand Interactions .....	562
21.10	Conclusion .....	562
	Acknowledgments .....	562
	References .....	562
<b>22</b>	<b>Saccharide–Peptide Hybrids.....</b>	<b>565</b>
	<i>Hans Peter Wessel</i>	
22.1	Introduction .....	565
22.2	Carbohydrate Amino Acids .....	566
22.2.1	Natural Carbohydrate Amino Acids.....	566
22.2.2	Synthetic Carbohydrate Amino Acids .....	567
22.3	Amide-Linked Carbohydrate Polymers.....	572
22.4	Amide-Linked Carbohydrate Oligomers.....	574
22.4.1	Solution Synthesis .....	574
22.4.2	Solid-Phase Synthesis.....	578
22.4.3	Biological Activity.....	579
22.4.4	Conformational Properties .....	582
	References .....	583
	<b>Index .....</b>	<b>I 1</b>

## Part I      Chemistry of Saccharides

### Vol. 2      Enzymatic Synthesis of Glycosides and Carbohydrate-Receptor Interaction

<b>III</b>	<b>Enzymatic Synthesis of Glycosides .....</b>	<b>587</b>
<b>23</b>	<b>On the Origin of Oligosaccharide Species—Glycosyltransferases in Action .....</b>	<b>589</b>
	<i>Dirk H. van den Eijnden</i>	
23.1	Introduction .....	589
23.2	Protein N-Glycosylation: Pre-assembly of Oligosaccharide-PP-Dolichol and en bloc Transfer .....	591
23.3	Trimming of the Polypeptide-Bound Oligosaccharide.....	592
23.4	Folding and Quality Control.....	593
23.5	Committed Steps in the Formation of Complex-Type Oligosaccharide Chains and Branching.....	594
23.6	Topology of the Reaction Catalyzed by a Typical GlcNAcT.....	596
23.7	Elongation and Termination Reactions in the <i>trans</i> -Golgi.....	596
23.8	Activity with Branched Substrates .....	598

23.9	Branch Specificity.....	600
23.10	Essential Requirements for Activity with LacNAc .....	601
23.11	Further Terminal Reactions in Complex-Type Oligosaccharide Synthesis .....	602
23.12	Specific Modifications of Polylactosaminoglycans.....	603
23.13	The Invariable Core of N-linked Oligosaccharide Chains, and Site- and Protein-Specific Processing .....	606
23.14	Comparison of the Synthesis of Type 1 (Gal(β1-3)GlcNAcβ-R) and Type 2 (Gal(β1-4)GlcNAcβ-R) Chains .....	607
23.15	The LacdiNAc Pathway of Complex-Type Oligosaccharide Synthesis .....	607
23.16	Protein <i>O</i> -Glycosylation .....	608
23.17	Glycosyltransferase Families .....	608
23.18	Sialyltransferase Family .....	610
23.19	α2-Fucosyltransferase Family .....	611
23.20	α3/4-Fucosyltransferase Family .....	612
23.21	α3-Galactosyl/ <i>N</i> -Acetylgalactosaminyltransferase (Histo-Blood Group ABO) Family .....	613
23.22	β6- <i>N</i> -Acetylglucosaminyltransferase Family.....	613
23.23	Polypeptide <i>N</i> -Acetylgalactosaminyltransferase Family .....	614
23.24	β4- <i>N</i> -Acetylgalactosaminyltransferase Family .....	615
23.25	β4-Galactosyltransferase Family .....	615
23.26	β3-Galactosyltransferase Family .....	617
23.27	β3-Glucuronyltransferase Family .....	617
23.28	Glycosyltransferases Standing Alone .....	617
23.29	Concluding Remarks .....	618
	References.....	618
24	<b>Synthesis of Sugar Nucleotides .....</b>	625
	<i>Reinhold Öhrlein</i>	
24.1	Introduction.....	625
24.2	Synthesis of Sugar Nucleotides .....	626
24.2.1	Chemical Synthesis .....	626
	UDP-Activated Donors .....	626
	CMP-Activated Sugars.....	629
	GDP-Activated Donors .....	632
	Comments.....	634
24.2.2	Chemo-Enzymatic Synthesis.....	635
	Uridine Diphosphate-Activated Donor Sugars .....	635
	CMP-Activated Sugars.....	637
	GDP-activated sugars .....	639
	Comments.....	640
24.3	In situ Generation of Sugar Nucleotides .....	641
	Comments.....	641
24.4	Outlook .....	644
	References.....	644

<b>25</b>	<b>Enzymatic Glycosylations with Glycosyltransferases . . . . .</b>	<b>647</b>
	<i>Ossi Renkonen</i>	
25.1	Introduction . . . . .	647
25.2	<i>In vitro</i> Synthesis of the Core Region of <i>O</i> -Glycans . . . . .	648
25.2.1	Initialization of O-Glycan Biosynthesis . . . . .	648
25.2.2	Synthesis of Core 1 . . . . .	648
25.2.3	Synthesis of Core 2 . . . . .	649
25.2.4	Synthesis of Core 3 and Core 4 . . . . .	650
25.2.5	<i>In vitro</i> Extension of Core 1 Glycans . . . . .	650
25.2.6	<i>In vitro</i> Extension of Core 2 Glycans . . . . .	651
25.2.7	Extension of Core 3 and Core 4 Glycans . . . . .	651
25.3	Enzymatic <i>in vitro</i> Synthesis of Polylactosamine Backbones . . . . .	651
25.3.1	Enzymatic Synthesis of the Primary Chains of Blood Group i-Type . . . . .	652
25.3.2	Distal Branching of i-Type Polylactosamine Backbones . . . . .	653
25.3.3	Central Branching of i-Type Polylactosamine Backbones . . . . .	654
25.3.4	$\beta$ 4-Galactosylation in Polylactosamine Backbones . . . . .	656
25.4	$\alpha$ 3-Sialylation of <i>N</i> -Acetyllactosaminoglycans at the Terminal Gal . . . . .	656
25.5	$\alpha$ 3-Fucosylation of Lactosamine Saccharides . . . . .	657
	References . . . . .	659
<b>26</b>	<b>Recycling of Sugar Nucleotides in Enzymatic Glycosylation . . . . .</b>	<b>663</b>
	<i>Kathryn M. Koeller and Chi-Huey Wong</i>	
26.1	Introduction . . . . .	663
26.2	Glycosyltransferases of the Leloir Pathway and their Sugar Nucleotide Substrates . . . . .	663
26.3	Design of Regeneration Systems . . . . .	665
26.4	Practical Regeneration Systems . . . . .	666
26.4.1	UDP-Galactose . . . . .	666
26.4.2	Other UDP-Sugars . . . . .	669
26.4.3	CMP-NeuAc . . . . .	671
26.4.4	GDP-Sugars . . . . .	676
26.4.5	Other Carbohydrate-Based Regeneration Systems . . . . .	680
26.5	Conclusion . . . . .	682
	References . . . . .	683
<b>27</b>	<b>Enzymatic Glycosylations with Non-Natural Donors and Acceptors . . . . .</b>	<b>685</b>
	<i>Xiangping Qian, Keiko Sujino, and Monica M. Palecic</i>	
27.1	Introduction . . . . .	685
27.2	Enzymatic Glycosylations . . . . .	686
27.2.1	Galactosylations . . . . .	686
	$\beta$ 1,4-Galactosyltransferase . . . . .	686
	$\alpha$ 1,3-Galactosyltransferase . . . . .	688

27.2.2	Fucosylations .....	690
	Human Milk $\alpha$ 1,3/4-Fucosyltransferase.....	690
	FucT III and VI .....	692
	FucT V.....	692
27.2.3	Sialylations.....	692
	$\alpha$ 2,3-Sialyltransferase and $\alpha$ 2,6-Sialyltransferase.....	692
27.2.4	N-Acetylglucosaminylation .....	696
	N-Acetylglucosaminyltransferase I, II, and III.....	696
	N-Acetylglucosaminyltransferase V .....	698
27.3	Summary.....	700
	Acknowledgments .....	700
	References.....	700
<b>28</b>	<b>Solid-Phase Synthesis with Glycosyltransferases .....</b>	<b>705</b>
	<i>Claudine Augé, Christine Le Narvor, and André Lubineau</i>	
28.1	Introduction.....	705
28.2	General Aspects.....	705
28.3	Enzymatic Synthesis on Insoluble Supports.....	707
28.3.1	Enzymatic Synthesis of Oligosaccharides .....	707
	Use of an Amino-Functionalized Water-Compatible Polyacrylamide Gel .....	707
	Use of a Sepharose Matrix .....	708
	Use of Controlled-Pore Glass .....	711
28.3.2	Enzymatic Synthesis of Glycopeptides .....	712
	Use of Controlled-Pore Glass .....	712
	Use of Polyethylene Glycol Polyacrylamide (PEGA).....	715
28.4	Enzymatic Synthesis of Oligosaccharides and Glycoconjugates on Soluble Supports .....	715
28.4.1	Enzymatic Synthesis of Oligosaccharides .....	715
	Use of Water-Soluble Amino-Substituted Poly(vinyl alcohol) ....	715
	Use of Water-Soluble Glycopolymer Synthesized by Polymerization .....	717
28.4.2	Enzymatic Synthesis of Glycolipids on Water-Soluble Polyacrylamide–Poly( <i>N</i> -acryloxysuccinimide) (PAN) .....	718
	References.....	722
<b>29</b>	<b>Glycosidase-Catalysed Oligosaccharide Synthesis .....</b>	<b>723</b>
	<i>David J. Vocadlo and Stephen G. Withers</i>	
29.1	Introduction.....	723
29.2	Background on Glycosidases .....	723
29.3	Basic Mechanisms .....	724
29.4	Synthesis by the ‘Thermodynamic’ Approach .....	724
29.5	The Kinetic Approach.....	728
29.6	Recent Developments and New Directions .....	732
	References.....	838

<b>30</b>	<b>Production of Heterologous Oligosaccharides by Recombinant Bacteria (Recombinant Oligosaccharides) .....</b>	845
	<i>Roberto A. Geremia and Eric Samain</i>	
30.1	Introduction .....	845
30.2	Concept and Methodology of Heterologous ('Recombinant') Oligosaccharide Production in <i>E. coli</i> .....	847
30.2.1	Biosynthesis of Nod Factors .....	847
30.2.2	Expression Systems and Cloning Strategy .....	849
30.2.3	High Cell-Density Cultivation .....	851
30.2.4	Purification of Recombinant Oligosaccharides .....	852
30.3	Examples of Recombinant Oligosaccharides .....	852
30.3.1	Production of Chitin Oligosaccharides in <i>E. coli</i> Expressing NodC .....	852
30.3.2	Production of Nod Factor Precursors .....	853
30.3.3	Production of Derivatives of <i>N</i> -Acetyllactosamine .....	855
30.4	Conclusions and Future Perspectives .....	856
30.4.1	Production of Labeled Chitin Oligosaccharides to Study Their Interactions with Proteins .....	856
30.4.2	Improvement of Oligosaccharide Production, and Metabolic Engineering .....	858
30.4.3	Production of More Complex Oligosaccharides .....	858
	Acknowledgments .....	859
	References .....	859
<b>IV</b>	<b>Carbohydrate–Protein Interactions .....</b>	861
<b>31</b>	<b>Protein–Carbohydrate Interaction: Fundamental Considerations ..</b>	863
	<i>Nikki F. Burkhalter, Sarah M. Dimick, and Eric J. Toone</i>	
31.1	Introduction .....	863
31.2	Association in Aqueous Solution .....	864
31.2.1	Gas Phase Non Covalent Interactions .....	864
	Dipole–Dipole Interactions .....	864
	Dipole–Induced Dipole .....	866
	Dispersive Interactions .....	867
	Specific Forces: Hydrogen Bonding and $n\text{-}\sigma$ Bonding .....	868
31.2.2	The Effect of Water on Intermolecular Interactions .....	869
	Coulombic Stabilization .....	870
	Hydrogen Bonding .....	871
	Dispersive Interactions .....	872
31.2.3	'Hydrophobic' Interactions .....	872
31.3	The Evaluation of Protein–Carbohydrate Binding .....	876
31.3.1	Precipitin Assays .....	877
31.3.2	Enzyme-Linked Lectin Assay (ELLA) .....	878
31.3.3	Isothermal Titration Microcalorimetry .....	878
31.4	The Interpretation of Calorimetric Data .....	882

31.4.1	Solvation/Desolvation .....	882
	Solvation Entropy .....	883
	Translational/Rotational Entropy.....	884
31.4.2	Other Contributions to Thermodynamics of Association .....	885
	Proton Transfer .....	885
	Salt Effects/Binding Site Reorganization .....	885
31.4.3	van't Hoff versus Calorimetric Enthalpies .....	886
31.5	The Thermodynamics of Protein–Carbohydrate Interaction .....	887
31.6	The Role of Multivalency in Protein–Carbohydrate Interaction...	901
31.6.1	Phenomenology .....	901
31.6.2	The Energetic Consequence of Ligand Linkage .....	905
	Enthalpic Contributions to $\Delta G_i$ .....	906
	Entropic Contributions to $\Delta G_i$ .....	907
31.6.3	A Molecular Basis for the Cluster Glycoside Effect .....	910
	Acknowledgments .....	911
	References.....	911
32	<b>Structural Analysis of Oligosaccharides: FAB-MS, ES-MS and MALDI-MS .....</b>	915
	<i>Anne Dell, Howard R. Morris, Richard Easton, Stuart Haslam, Maria Panico, Mark Sutton-Smith, Andrew J. Reason, and Kay-Hooi Khoo</i>	
32.1	Introduction.....	915
32.2	Fast Atom Bombardment-Mass Spectrometry (FAB-MS) .....	915
32.3	Matrix Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF-MS) .....	917
32.4	Electrospray-Mass Spectrometry (ES-MS).....	918
32.5	Appearance of Mass Spectra Obtained in FAB-MS, MALDI-MS and ES-MS Experiments .....	919
32.6	Assignment of Mass Values .....	921
32.7	Derivatisation.....	921
32.8	Fragmentation Pathways .....	922
32.9	Protocols for MS Analysis.....	924
32.9.1	Protocol 1—Sample Loading for FAB-MS Analysis.....	924
32.9.2	Protocol 2—Sample Loading for NanoES-MS and MS-MS Analysis on the Q-TOF .....	925
32.9.3	Protocol 3—Sample Loading for LC-ES-MS and LC-ES-MS-MS on the Q-TOF.....	925
32.9.4	Protocol 4—Sample Loading for MALDI-MS Analysis .....	925
32.10	Applications of FAB-MS, MALDI-MS and ES-MS in Glycobiology.....	926
32.10.1	Case Study 1—Molecular Weight Profiling of Polysaccharides by MALDI-MS .....	926
32.10.2	Case Study 2—Analysis of Glycoproteins by LC-ES-MS and FAB-MS .....	927

32.10.3	Case Study 3—Characterization of a Novel <i>N</i> -Glycan by FAB-MS and FAB-MS-MS.....	930
32.10.4	Case Study 4—High Sensitivity Sequencing of a Novel Glycopeptide by Q-TOF ES-MS-MS and MALDI-MS.....	933
32.10.5	Case Study 5—FAB-MS Screening of Biological Samples for Glycan Content.....	935
32.10.6	Case Study 6—MS Analysis of Mycobacterial Glycoconjugates ..	942
32.11	Concluding Remarks .....	944
	References .....	945
<b>33</b>	<b>Conformational Analysis in Solution by NMR .....</b>	<b>947</b>
	<i>S. W. Homans</i>	
33.1	Introduction .....	947
33.2	Solution Conformations of Oligosaccharides .....	947
33.2.1	The NMR Technique.....	947
33.2.2	Conformational Parameters in Oligosaccharides .....	948
33.2.3	Conformational Restraints .....	949
33.2.4	$^{13}\text{C}$ Isotopic Enrichment .....	949
33.2.5	Additional Conformational Restraints.....	950
	Exchangeable Protons .....	950
	Heteronuclear Overhauser Effects.....	952
	$^{13}\text{C}$ - $^{13}\text{C}$ Coupling-Constants .....	953
	Dipolar Couplings .....	954
33.3	Experimental Restraints in Conformational Analysis .....	955
33.3.1	Restraining Protocol.....	955
	Biharmonic Restraints.....	955
	Time-Dependent Restraints .....	957
33.3.2	Dynamical Simulated Annealing.....	957
33.4	Analysis of Oligosaccharide Dynamics .....	958
33.4.1	Monte-Carlo Simulations .....	959
33.4.2	Molecular Dynamics Simulations.....	959
33.5	A Case Study on Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc .....	959
33.5.1	Resonance Assignments in Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc .....	960
33.5.2	ROE Connectivities .....	960
33.5.3	'Global Minimum' Conformation of Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc .....	961
33.5.4	Conformational Dynamics of Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc .....	962
33.5.5	Short-range vs Long-range Restraints .....	963
33.6	Conclusions .....	966
	References .....	966
<b>34</b>	<b>Oligosaccharide Conformations by Diffraction Methods .....</b>	<b>969</b>
	<i>Serge Pérez, Catherine Gautier, and Anne Iniberty</i>	
34.1	Introduction .....	969
34.2	General Analysis .....	970
34.3	Crystalline Conformations of Disaccharide Moieties .....	973
34.3.1	The Disaccharides .....	973

34.3.2	The Analogs (S, C, N, . . .) . . . . .	985
34.4	Hydrogen Bonding in Crystalline Oligosaccharides . . . . .	987
34.5	Packing Features . . . . .	988
34.6	Selected Examples . . . . .	990
34.7	Crystalline Conformations of Oligosaccharides Complexed with Lectins . . . . .	992
34.8	Concluding Remarks . . . . .	996
	References . . . . .	998
<b>35</b>	<b>Transfer NOE Experiments for the Study of Carbohydrate–Protein Interactions . . . . .</b>	<b>1003</b>
	<i>Thomas Peters</i>	
35.1	Introduction . . . . .	1003
35.2	The Transfer NOE Experiment . . . . .	1004
35.3	Measurement of trNOEs . . . . .	1006
35.4	Bioactive Conformations of Carbohydrate Ligands From trNOE Experiments . . . . .	1008
35.5	Spin Diffusion may Generate Misleading Distance Constraints . . . . .	1009
35.6	The Conformation of Sialyl Lewis <sup>x</sup> Bound to E-selectin . . . . .	1011
35.7	Interaction of Bacterial Lipopolysaccharide Fragments with Monoclonal Antibodies . . . . .	1016
35.8	Conclusions and Future Directions . . . . .	1019
	References . . . . .	1021
<b>36</b>	<b>Carbohydrate–Protein Interactions: Use of the Laser Photo Chemically Induced Dynamic Nuclear Polarization(CIDNP)-NMR Technique . . . . .</b>	<b>1025</b>
	<i>Hans-Christian Siebert and Johannes F. G. Vliegenthart</i>	
36.1	Introduction . . . . .	1025
36.2	The CIDNP Method . . . . .	1026
36.3	CIDNP-related Molecular Modelling . . . . .	1027
36.4	Applications . . . . .	1027
36.5	Hevein-like Lectins . . . . .	1029
36.6	Galactoside-binding Lectins from Plant and Animal Origin . . . . .	1032
36.7	Sialidase from <i>Clostridium Perfringens</i> (Wild Type and Mutants) . . . . .	1037
36.8	CIDNP Analysis of Glycoproteins . . . . .	1039
36.9	Conclusions . . . . .	1040
	Acknowledgments . . . . .	1041
	References . . . . .	1042
<b>37</b>	<b>Biacore . . . . .</b>	<b>1045</b>
	<i>Wolfgang Jäger</i>	
37.1	Introduction . . . . .	1045
37.1.1	Real-time Analysis by Surface Plasmon Resonance . . . . .	1045
37.1.2	Information in a Sensorgram . . . . .	1047
37.2	Experimental Procedures . . . . .	1048

37.2.1	Immobilization of Biomolecules at the Sensor Surface .....	1048
37.2.2	Surface Regeneration.....	1050
37.2.3	Interaction Analysis and Controls .....	1051
37.2.4	Determination of Kinetic Rate Constants .....	1052
37.2.5	Affinity Determination .....	1053
37.3	Application Areas .....	1054
37.3.1	Selectin Binding to a Glycoprotein Ligand .....	1054
37.3.2	Oligosaccharide Characterization .....	1055
37.3.3	<i>In situ</i> Modification of Immobilized Carbohydrates.....	1056
	References .....	1056
V	<b>Carbohydrate–Carbohydrate Interactions .....</b>	1059
38	<b>Carbohydrate–Carbohydrate Interactions .....</b>	1061
	<i>Dorothe Spillmann and Max M. Burger</i>	
38.1	Introduction .....	1061
38.2	From Structural Components to Cell Recognition .....	1063
38.2.1	Carbohydrate–Carbohydrate Interactions as Part of Structural Components .....	1063
	Extracellular Matrix of Seaweeds—Agarose, Carrageenan and Alginate.....	1063
	Cell Walls.....	1064
	Mammalian Extracellular Matrix Components .....	1066
38.2.2	Carbohydrate–Carbohydrate Interactions as Part of Recognition Keys?.....	1068
	Carbohydrate Interactions in Invertebrates—The Marine Sponge <i>Microciona prolifera</i> as a Model System .....	1069
	Carbohydrate Interactions in Vertebrates—Embryonal and Tumor Cells.....	1071
	Repulsive Carbohydrate–Carbohydrate Interactions.....	1072
38.3	Molecular Aspects of Carbohydrate Interactions.....	1074
38.3.1	Polyvalence to Inforce Weak Interactions .....	1074
38.3.2	Arrangement of Motifs and the Possibility to Control Specificity ..	1075
38.3.3	Molecular Basis of Carbohydrate–Carbohydrate Interactions.....	1076
38.4	Experimental Approaches.....	1078
38.4.1	General Considerations .....	1078
38.4.2	Affinity Interactions .....	1079
	Cell Binding Studies .....	1079
	Aggregation of <i>de novo</i> Complexes .....	1081
	Affinity Chromatography .....	1082
	Distribution between Compartments .....	1082
38.4.3	Microscopy .....	1083
	Electron Microscopy .....	1083
	Atomic Force Microscopy .....	1083
38.4.4	Crystallography.....	1084

38.4.5	Mass Spectrometry .....	1085
38.4.6	Nuclear Magnetic Resonance .....	1085
38.4.7	Molecular Modelling .....	1086
38.4.8	Tools .....	1086
	Synthetic Oligosaccharides .....	1086
	Antibodies against Carbohydrate Motifs .....	1087
	Cells .....	1088
	References.....	1088
<b>VI</b>	<b>Carbohydrate–Nucleic Acid Interactions .....</b>	<b>1093</b>
<b>39</b>	<b>Carbohydrate–Nucleic Acid Interactions .....</b>	<b>1095</b>
	<i>Heinz E. Moser</i>	
39.1	Introduction.....	1095
39.2	Carbohydrates Binding to DNA .....	1096
39.2.1	Ene-Diyne Antibiotics and Antitumor Agents.....	1096
	Esperamicins.....	1096
	Calicheamicins.....	1100
39.2.2	Anthracyclins .....	1106
39.2.3	Pluramycins and Aureolic Acids .....	1111
39.3	Carbohydrates Binding to RNA .....	1112
39.3.1	Aminoglycosides .....	1113
	References.....	1120
	<b>Index .....</b>	<b>I 1</b>

## Part II Biology of Saccharides

### Vol. 3 Biosynthesis and Degradation of Glycoconjugates

	<b>Introduction to Volumes 3 and 4 .....</b>	<b>V</b>
	<b>Abbreviations Used in Volumes 3 and 4 .....</b>	<b>LV</b>
<b>I</b>	<b>Biosynthesis of Glycoconjugates .....</b>	<b>1</b>
<b>1</b>	<b>Metabolism of Sugars and Sugar Nucleotides .....</b>	<b>3</b>
	<i>Hudson H. Freeze</i>	
1.1	Introduction.....	3
1.2	Basic Principles .....	3
1.3	Transporters Deliver Monosaccharides to Cells .....	4
1.4	Intracellular Sources of Sugars .....	5
1.4.1	Salvage .....	5

## XXVI Contents

1.4.2	Activation and Interconversion of Monosaccharides.....	6
	Glycogen.....	6
	Glucose .....	7
	Glucuronic acid.....	8
	Iduronic acid.....	8
	Xylose.....	8
	Mannose .....	8
	Fucose .....	9
	Galactose .....	10
	N-Acetylglucosamine .....	10
	N-Acetylgalactosamine .....	10
	Sialic acids .....	11
1.5	Sugar Nucleotide Transporters.....	11
1.6	Control of Sugar Nucleotide Levels .....	13
1.7	Possible Future Directions .....	13
	References .....	14
<b>2</b>	<b>Nucleotide Sugar Transporters .....</b>	<b>19</b>
	<i>Rita Gerardy-Schahn and Matthias Eckhardt</i>	
2.1	Introduction .....	19
2.2	General Considerations .....	20
2.3	The Requirement for Nucleotide Sugar Transporters and Their Mechanism of Function: A Comprehensive Overview of the Last 20 Years .....	20
2.4	Molecular Cloning of Nucleotide Sugar Transporters .....	22
2.5	The Structure of Nucleotide Sugar Transporters .....	25
2.6	The Subcellular Distribution of Nucleotide Sugar Transporters....	27
2.7	Molecular Defects that Cause Inactive UDP-Galactose and CMP-Sialic Acid Transporters.....	28
2.8	Association Between Defects in Nucleotide Sugar Transporters and Diseases.....	29
2.9	Involvement of Nucleotide Sugar Transporters in the Regulation of Glycosylation .....	29
2.10	Future Perspectives.....	30
	Acknowledgements .....	31
	References .....	32
<b>3</b>	<b>Biosynthesis of Oligosaccharyl Dolichol.....</b>	<b>37</b>
	<i>Sharon S. Krag</i>	
3.1	General Overview .....	37
3.2	Oligosaccharyl Dolichol.....	38
3.3	Key Enzymatic Steps in the Assembly Process .....	39
3.4	Topology of the Assembly Process.....	42
3.5	Utilization of Oligosaccharyl Dolichol .....	42
	Acknowledgment .....	43
	References .....	43

<b>4</b>	<b>Biochemistry and Molecular Biology of the <i>N</i>-Oligosaccharyl-transferase Complex.....</b>	<b>45</b>
	<i>Roland Knauer and Ludwig Lehle</i>	
4.1	Introduction.....	45
4.2	Biochemistry of OST .....	46
4.2.1	Lipid–Saccharide Donor .....	47
4.2.2	Acceptor Specificity of OST .....	48
4.2.3	Catalytic Mechanism of OST .....	49
4.2.4	Regulation of OST Activity .....	51
4.3	Isolation of OST Complexes from Different Sources .....	51
4.4	Molecular Biology of OST .....	52
4.4.1	WBP1/OST48.....	54
4.4.2	SWP1/Ribophorin II .....	54
4.4.3	OST1/Ribophorin I.....	55
4.4.4	OST3/OST6.....	55
4.4.5	OST5 .....	56
4.4.6	OST4 .....	56
4.4.7	OST2/DAD1.....	57
4.4.8	STT3 .....	58
4.5	Structural Organization of the OST Complex .....	59
	Acknowledgments .....	60
	References.....	60
<b>5</b>	<b>Processing Enzymes Involved in the Deglucosylation of <i>N</i>-Linked Oligosaccharides of Glycoproteins: Glucosidases I and II and Endomannosidase .....</b>	<b>65</b>
	<i>Robert G. Spiro</i>	
5.1	Introduction.....	65
5.2	Glucosidase I.....	66
5.3	Glucosidase II.....	68
5.4	Endo- $\alpha$ -mannosidase.....	70
5.5	Concerted Action of Deglucosylation Enzymes.....	72
5.6	Mutants .....	74
5.7	Role of Monoglycosylated <i>N</i> -Linked Oligosaccharides and Glucose Trimming Enzymes in Regulating Quality Control of Glycoproteins .....	75
5.8	Effect of Glucosidase Inhibitors on Viral Proliferation.....	77
	Acknowledgments .....	78
	References.....	78
<b>6</b>	<b><math>\alpha</math>-Mannosidases in Asparagine-linked Oligosaccharide Processing and Catabolism.....</b>	<b>81</b>
	<i>Kelley W. Moremen</i>	
6.1	Overview .....	81
6.2	Introduction.....	82

## XXVIII *Contents*

6.2.1	Roles of <i>N</i> - and <i>O</i> -Linked Glycans and Compartmentalization of Biosynthetic and Catabolic Reactions .....	82
6.2.2	Processing of Asn-Linked Oligosaccharides .....	82
6.2.3	Early Trimming Events: importance for quality control glycoprotein degradation and anteriograde transport.....	85
6.2.4	Glycoprotein Catabolism: multiple routes for glycoprotein breakdown .....	87
6.2.5	Consequences of Genetic Defects in Oligosaccharide Biosynthesis and Catabolism.....	88
6.3	Mannosidases in Glycoprotein Processing and Catabolism .....	89
6.3.1	Classification of Mannosidases.....	89
6.3.2	Class 1 Mannosidases: enzymes of the ER and Golgi.....	93
	ER mannosidase I subfamily.....	93
	Golgi mannosidase I sub-family .....	95
	Fungal secreted mannosidases .....	97
	New genes with unknown functions .....	98
6.3.3	Class 2 Mannosidases: enzymes of the cytosol, ER, Golgi, and Lysosomes .....	98
	Golgi mannosidase II.....	99
	Lysosomal mannosidase.....	101
	Epididymal/sperm mannosidase .....	103
	Heterogeneous cluster of mannosidase homologs among eukarya, eubacteria, and archaea .....	104
6.4	Conclusions and Future Prospects .....	106
	Acknowledgments .....	107
	References .....	107
7	<b>The Role of UDP-Glcylglycoprotein Glucosyltransferase as a Sensor of Glycoprotein Conformations .....</b>	119
	<i>Armando J. Parodi</i>	
7.1	Introduction .....	119
7.2	General Properties.....	120
7.3	GT Recognizes Glycoprotein Conformations .....	121
7.4	The Primary Structure of the UDP-Glcylglycoprotein Glucosyltransferase.....	122
7.5	The Role of Monoglycosylated Oligosaccharides in Glycoprotein Folding.....	123
	Acknowledgments .....	126
	References .....	127
8	<b>Mannosyltransferases .....</b>	129
	<i>Peter Orleans</i>	
8.1	Introduction .....	129
8.2	Occurrence of Covalently-linked Mannose .....	130

8.2.1	Eukaryotic Secretory Glycoproteins .....	130
8.2.2	Glycophospholipids.....	130
8.2.3	Eubacterial and Archaeal Mannose-containing Molecules.....	130
8.2.4	C-linked Mannose .....	130
8.3	Biochemistry of Mannosyl Transfer.....	131
8.3.1	Many Linkages, Two Donors .....	131
8.3.2	Donor Specificity .....	131
8.3.3	Acceptor Specificity.....	132
8.3.4	Structural Features of Man-T .....	132
8.4	Man-T Families and the Pathways They Participate in .....	133
8.4.1	Man-Ts of the ER [1–5].....	134
	Alg1p.....	134
	Alg2p/Alg11p.....	134
	Dpm1p .....	135
	Alg3p.....	135
	Alg9p/PIG-Bp family.....	135
	Pmt1p family.....	136
8.4.2	Golgi Man-Ts and Fungal Mannan Synthesis.....	136
	Och1p family.....	137
	Mnn9p family.....	137
	Mnn10p/Mnn11p family .....	138
	Mnn1p family.....	138
	Ktr1p family .....	138
8.4.3	“Missing” Eukaryotic Man-T .....	138
8.4.4	Eubacterial and Archaeal Man-T .....	139
8.5	Coordinating Man Transfer with the Cell Cycle and Morphogenesis .....	139
8.6	Concluding Remarks .....	140
	Acknowledgments .....	140
	References.....	140
 9	 <b>Branching of N-Glycans: N-Acetylglucosaminyltransferases .....</b>	145
	<i>Harry Schachter</i>	
9.1	Introduction.....	145
9.2	Processing of N-Glycans within the Endomembrane Assembly Line .....	146
9.3	General Properties of the N-Acetylglucosaminyltransferases.....	148
9.3.1	Domain Structure.....	148
9.3.2	Targeting to the Golgi Apparatus.....	150
9.4	UDP-GlcNAc:Man $\alpha$ 1-3R [GlcNAc to Man $\alpha$ 1-3] $\beta$ -1,2-N-Acetylglucosaminyltransferase I (GnT I, EC 2.4.1.101) .....	150
9.5	UDP-GlcNAc:Man $\alpha$ 1-6R [GlcNAc to Man $\alpha$ 1-6] $\beta$ -1,2-N-Acetylglucosaminyltransferase II (GnT II, E.C. 2.4.1.143).....	152
9.6	The Role of GnT I and II in Mammalian Development .....	153

## XXX Contents

9.7	UDP-GlcNAc:R <sub>1</sub> -Manα1-6[GlcNAcβ1-2Manα1-3]Manβ1-4R <sub>2</sub> [GlcNAc to Manβ1-4] β-1,4-N-Acetylglucosaminyltransferase III (GnT III, E.C. 2.4.1.144).....	155
9.7.1	Overexpression of GnT III Activity.....	156
9.7.2	GnT III Activity and Cancer.....	157
9.8	UDP-GlcNAc:R <sub>1</sub> Manα1-3R <sub>2</sub> [GlcNAc to Manα1-3] β-1,4-N-Acetylglucosaminyltransferase IV (GnT IV, E.C. 2.4.1.145).....	157
9.9	UDP-GlcNAc:R <sub>1</sub> Manα1-6R <sub>2</sub> [GlcNAc to Manα1-6] β-1,6-N-Acetylglucosaminyltransferase V (GnT V, E.C. 2.4.1.155) .....	158
9.9.1	GnT V Activity and Cancer.....	159
9.10	UDP-GlcNAc:R <sub>1</sub> (R <sub>2</sub> )Manα1-6R <sub>3</sub> [GlcNAc to Manα1-6] β-1,4-N1-Acetylglucosaminyltransferase VI (GnT VI) .....	161
9.11	GnT VII and GnT VIII .....	161
	References .....	162
<b>10</b>	<b>The Galactosyltransferases .....</b>	<b>175</b>
	<i>Nancy L. Shaper, Martin Charron, Neng-Wen Lo, Jane R. Scocca, and Joel H. Shaper</i>	
10.1	Introduction .....	175
10.2	Using the Databanks to Obtain Information on the Galactosyltransferases.....	177
10.2.1	Nomenclature.....	177
10.3	The Dual Role of β4-Galactosyltransferase-I (β4Galt-I) in Oligosaccharide and Lactose Biosynthesis: The Early Days .....	178
10.3.1	β4Galt-I: Isolation and Characterization of cDNA Clones .....	181
10.3.2	The Murine β4Galt-I Gene: Genomic Organization and Structure of the 5'-End.....	181
10.3.3	β4Galt-I and Lactose Biosynthesis .....	182
10.3.4	β4Galt-I and the Vertebrate β4Galt Gene Family.....	182
10.3.5	Evolution of the β4-Galactosyltransferase Gene Family .....	184
10.4	The Vertebrate β3Galactosyltransferase (β3GalT) Gene Family ..	185
10.4.1	General Characteristics of the β3-Galactosyltransferase Gene Family Members .....	186
10.4.2	β3GalT-IV: UDP-galactose:GM1 β3-galactosyltransferase (GM1 Synthase; GalT-3) .....	187
10.4.3	Other Vertebrate β-Galactosyltransferase Activities.....	187
10.4.4	UDP-Galactose:Ceramide β-Galactosyltransferase (CGalT: EC 2.4.1.45).....	187
10.5	The Vertebrate α3-Galactosyltransferase Gene Family .....	188
10.5.1	α3-Galactosyltransferase (α3GalT: UDP-Gal:Galβ4GlcNAcα3- Galactosyltransferase; EC 2.4.1.87).....	188
10.5.2	The Blood Group B α3-Galactosyltransferase (EC 2.4.1.37).....	190
10.5.3	The Forssman Glycolipid Synthetase (EC 2.4.1.88).....	191
10.5.4	Evolution of the α3GalT Gene Family .....	191

10.6	A UDP-Gal:Gal $\beta$ 3GalNAc $\alpha$ 4Galactosyltransferase Activity .....	192
	Acknowledgments .....	192
	References.....	192
<b>11</b>	<b>Fucosyltransferases.....</b>	<b>197</b>
	<i>Ernesto T. A. Marques, Jr.</i>	
11.1	Introduction.....	197
11.2	General Characteristics .....	198
11.2.1	Nomenclature .....	198
11.2.2	Gene Structure .....	199
11.2.3	Sequence Peptide Motifs.....	199
11.2.4	Specificity .....	199
11.2.5	Protein Structure and Topology.....	200
11.2.6	Enzymatic Reaction Mechanism .....	201
11.2.7	Inhibitors.....	203
11.3	Specific Fucosyltransferases .....	203
11.3.1	GDP-Fucose: Fuco $\alpha$ 1(Fuco $\alpha$ 1,2Fuc) $\alpha$ 2-fucosyltransferase.....	204
11.3.2	GDP-Fucose: Gal $\beta$ 1(Fuco $\alpha$ 1,2Gal) $\alpha$ 2-fucosyltransferase .....	204
11.3.3	GDP-Fucose: Gal $\beta$ 1,4/3GlcNAc(Fuco $\alpha$ 1,3/4GlcNAc) $\alpha$ 3/4-fucosyltransferases .....	204
	Blood group Lewis: FucT III, V and VI .....	204
	Myeloid enzyme: FucT IV.....	205
	Leukocyte enzyme: FucT VII .....	206
	Neuronal enzyme: FucT IX .....	206
11.3.4	GDP-Fucose: Gal $\beta$ 1,3GlcNAc(Fuco $\alpha$ 1,3GlcNAc) bacterial ( <i>Helicobacter pylori</i> ) $\alpha$ 3-fucosyltransferase .....	207
11.3.5	GDP-Fucose: GlcNAc-N(Fuco $\alpha$ 1,6GlcNAc) $\alpha$ 6fucosyltransferases ..	207
11.3.6	GDP-Fucose: O-Ser(Fuco $\alpha$ 1-O-Ser)GlcNAc polypeptide fucosyltransferases .....	207
11.3.7	Unconventional Types of Fucosylation: Fuc $\beta$ 1-P-Ser and cytoplasmic Fuco $\alpha$ 1,2-Gal $\beta$ 1,3-GlcNAc-Pro ( <i>Dictyostelium discoideum</i> ) .....	207
	Fuc $\beta$ 1-P-Ser .....	207
	Fuca $\alpha$ 1,2-Gal $\beta$ 1,3-GlcNAc-Pro .....	208
	Acknowledgments .....	208
	References.....	208
<b>12</b>	<b>Sialyltransferases.....</b>	<b>213</b>
	<i>Joseph T. Y. Lau and Sherry A. Wuensch</i>	
12.1	Introduction.....	213
12.2	General Features of Sialyltransferases .....	213
12.3	Cloning and Identification Strategies for Sialyltransferases .....	215
12.4	Sialyltransferase Classification and Nomenclature.....	216
12.5	The $\alpha$ 2,3-ST Family .....	216
12.6	The $\alpha$ 2,6-ST Family .....	217
12.7	The $\alpha$ 2,8-ST Family .....	218

## XXXII Contents

12.8	Regulation and Functionality of Sialyltransferases.....	219
	References .....	221
<b>13</b>	<b>Biochemistry of Sialic Acid Diversity.....</b>	<b>227</b>
	<i>Roland Schauer</i>	
13.1	Introduction .....	227
13.2	Occurrence and Biosynthesis .....	227
13.3	General Biological Functions .....	229
13.4	<i>N</i> -Glycolylneuraminic Acid .....	231
13.5	<i>O</i> -Acetylated Sialic Acids .....	234
13.6	<i>O</i> -Methylated and <i>O</i> -Sulfated Sialic Acids .....	238
	Acknowledgments .....	239
	References .....	239
<b>14</b>	<b>Carbohydrate Sulfotransferases.....</b>	<b>245</b>
	<i>Steven D. Rosen, Annette Bistrup, and Stefan Hemmerich</i>	
14.1	Introduction .....	245
14.2	Basic Features of Sulfotransferase Reactions .....	245
14.3	Tyrosine Sulfation .....	246
14.4	Diversity of Carbohydrate Sulfation.....	246
14.5	Biochemical Demonstration of Carbohydrate Sulfotransferases ...	249
14.6	Molecular Cloning of Carbohydrate Sulfotransferases.....	250
14.7	Primary Structures of Carbohydrate Sulfotransferases .....	252
	Acknowledgments .....	256
	References .....	256
<b>15</b>	<b>Novel Variant Pathways in Complex-type Oligosaccharide Synthesis .....</b>	<b>261</b>
	<i>Dirk H. van den Eijnden</i>	
15.1	Introduction .....	261
15.2	The lacNAc Pathway of Complex-type Oligosaccharide Synthesis	261
15.3	Occurrence and Biology of lacdiNAc-based Complex-type Oligosaccharides .....	262
15.4	Biosynthesis of lacdiNAc Backbone Units.....	263
15.5	The lacdiNAc Pathway of Complex-type Oligosaccharide Synthesis .....	264
15.6	Other Shared Properties of $\beta$ 4-GalT and $\beta$ 4-GalNAcT .....	266
15.7	Cloning of a snail UDP-GlcNAc:GlcNAc $\beta$ -R $\beta$ 4- <i>N</i> -acetylglucosaminyltransferase .....	266
15.8	The Chitobio Pathway of Complex-type Oligosaccharide Synthesis .....	267
15.9	Competition Between Pathways.....	267
15.10	Concluding Remarks .....	269
	References .....	269

<b>16</b>	<b>Control of Mucin-Type <i>O</i>-Glycosylation: <i>O</i>-Glycan Occupancy is Directed by Substrate Specificities of Polypeptide GalNAc-Transferases.....</b>	273
	<i>Helle Hassan, Eric P. Bennett, Ulla Mandel, Michael A. Hollingsworth, and Henrik Clausen</i>	
16.1	Introduction.....	273
16.2	The Mammalian UDP-GalNAc: Polypeptide GalNAc-Transferase Gene Family .....	274
16.3	The GalNAc-Transferase Gene Family is Evolutionarily Old .....	276
16.4	The Kinetic Properties of GalNAc-Transferase Isoforms are Different.....	278
16.4.1	Lessons from <i>in vivo</i> Analysis of GalNAc-transferase Substrate Specificities.....	279
16.4.2	Lessons from <i>in vitro</i> Analysis of the Acceptor Substrate Specificities of GalNAc-transferase Isoforms .....	280
	Isoforms may have distinct acceptor substrate specificities.....	281
	Isoforms may have overlapping substrate specificities.....	283
	Isoforms may act in different order on substrates with multiple acceptor sites.....	283
	Isoforms may require prior (GalNAc) glycosylation .....	283
16.5	Expression of the GalNAc-Transferase Genes are Differentially Regulated .....	285
16.6	Predictive Value of <i>in vitro</i> <i>O</i> -glycosylation? .....	288
16.7	Conclusions and Future Perspectives.....	288
	References.....	289
<b>17</b>	<b>Glycosyltransferase Inhibitors.....</b>	293
	<i>Xiangping Qian and Monica M. Palcic</i>	
17.1	Introduction.....	293
17.2	Inhibitors of Glycosyltransferases .....	296
17.2.1	Inhibitors of Galactosyltransferases .....	296
	Inhibitors of $\beta$ 1,4-galactosyltransferase .....	296
	Inhibitors of $\alpha$ 1,3-galactosyltransferase .....	297
17.2.2	Inhibitors of Fucosyltransferases .....	298
	Inhibitors of $\alpha$ 1,2-fucosyltransferases .....	300
	Inhibitors of $\alpha$ 1,3-fucosyltransferases .....	300
17.2.3	Inhibitors of Sialyltransferases .....	301
	Inhibitors of $\alpha$ 2,6-sialyltransferase .....	302
	Inhibitors of $\alpha$ 2,3-sialyltransferase .....	304
17.2.4	Inhibitors of <i>N</i> -Acetylglucosaminyltransferases .....	305
17.2.5	Inhibitors of Human Blood Group A and B Glycosyltransferases .....	306
17.3	Summary.....	309
	Acknowledgments .....	309
	References.....	309

<b>18</b>	<b>Biosynthesis of the <i>O</i>-Glycan Chains of Mucins and Mucin Type Glycoproteins .....</b>	<b>313</b>
	<i>Inka Brockhausen</i>	
18.1	Summary.....	313
18.2	Introduction .....	313
18.3	Structures of <i>O</i> -Glycans.....	314
18.4	Functions of Mucin Type <i>O</i> -Glycans.....	314
18.5	Primary <i>O</i> -Glycosylation.....	315
18.6	Synthesis of <i>O</i> -Glycan Core 1.....	315
18.7	Synthesis of <i>O</i> -Glycan Core 2.....	317
18.8	Synthesis of <i>O</i> -Glycan Core 3.....	319
18.9	Synthesis of <i>O</i> -Glycan Core 4.....	319
18.10	Synthesis of <i>O</i> -Glycan Cores 5–8 .....	319
18.11	Elongation and Branching Reactions.....	320
18.12	Synthesis of Terminal Structures.....	321
	Acknowledgments .....	324
	References .....	324
<b>19</b>	<b>Glycosyltransferases in Glycosphingolipid Biosynthesis .....</b>	<b>329</b>
	<i>Subhash Basu, Kamal Das, and Manju Basu</i>	
19.1	Introduction .....	329
19.2	Fucosyltransferases in Glycolipid Biosynthesis .....	329
19.3	Galactosyltransferases in Glycolipid Biosynthesis .....	332
19.4	<i>N</i> -Acetylgalactosaminyltransferases in Glycolipid Biosynthesis....	334
19.5	<i>N</i> -Acetylglucosaminyltransferases in Glycolipid Biosynthesis....	336
19.6	Sialyltransferases in Glycolipid Biosynthesis .....	337
19.7	Glucuronyltransferases in Glycolipid Biosynthesis .....	340
	Acknowledgments .....	342
	References .....	342
<b>20</b>	<b>Biosynthesis of Glycogen .....</b>	<b>349</b>
	<i>Peter J. Roach</i>	
20.1	Summary.....	349
20.2	Introduction .....	350
20.3	Glycogenin and the Initiation of Glycogen Synthesis .....	351
20.3.1	History.....	351
20.3.2	Properties .....	351
20.3.3	Reaction Mechanism .....	352
20.3.4	Domain Structure .....	352
20.3.5	Function .....	354
20.4	Glycogen Synthase and the Bulk Synthesis of Glycogen .....	354
20.4.1	Properties .....	354
20.4.2	Structure of Glycogen Synthase.....	355
20.4.3	Branching Enzyme .....	356
20.5	Intermediates in the Biosynthesis of Glycogen .....	357

20.6	Conclusion .....	358
	Acknowledgments .....	359
	References.....	359
<b>21</b>	<b>Biosynthesis of Hyaluronan .....</b>	<b>363</b>
	<i>Paraskevi Heldin and Torvard C. Laurent</i>	
21.1	Introduction.....	363
21.2	Site of Biosynthesis .....	364
21.3	Biosynthetic Precursors.....	364
21.4	Hyaluronan Synthases .....	365
21.4.1	Microbial Enzymes .....	365
21.4.2	Vertebrate Synthases.....	366
21.5	Mechanism of Synthesis .....	367
21.5.1	Chain Elongation .....	368
21.5.2	Translocation .....	369
21.5.3	Shedding .....	369
21.6	Regulation of HA Synthesis.....	370
21.7	Concluding Remarks .....	371
	Acknowledgments .....	372
	References.....	372
<b>22</b>	<b>Biosynthesis of Chondroitin Sulfate and Dermatan Sulfate</b>	
	<b>Proteoglycans .....</b>	<b>375</b>
	<i>Geetha Sugumaran and Barbara M. Vertel</i>	
22.1	Introduction.....	375
22.2	Proteoglycan Structure .....	379
22.2.1	Proteoglycans and Their Core Proteins .....	379
22.2.2	What Initiates GAG Chain Addition? .....	381
22.2.3	The Linkage Region .....	381
22.2.4	CS and DS Chains.....	382
22.3	Biosynthesis of CS and DS Proteoglycans .....	383
22.3.1	Biosynthesis of the Core Protein .....	383
22.3.2	Origin of Sugar and Sulfate Precursors .....	384
22.3.3	Addition of the Linkage Oligosaccharides .....	385
	Xylosylation.....	385
	Galactosylation .....	386
	Addition of GlcA and completion of the common tetrasaccharide linkage region .....	387
	Initiation of CS/DS chains by addition of the first GalNAc .....	388
22.3.4	Formation of the CS/DS Chains.....	388
	Addition of the repeating disaccharides.....	388
	Epimerization of GlcA to IdoA to form DS.....	389
	Sulfation of GalNAc.....	390
	Sulfation of uronic acid.....	391

## XXXVI Contents

22.4	Concluding Remarks/Perspectives .....	391
	Acknowledgments .....	392
	References .....	392
23	<b>Biosynthesis of Heparin and Heparan Sulfate Proteoglycans .....</b>	395
	<i>Lena Kjellén and Ulf Lindahl</i>	
23.1	Introduction .....	395
23.2	The Proteoglycans: Structure, Location and Functions .....	396
23.3	Biosynthesis of the Polysaccharide Backbone .....	396
23.4	Outline of Polymer-Modification Reactions .....	397
23.4.1	The N-Deacetylase/N-Sulfotransferases.....	399
23.4.2	The C5-Epimerase .....	399
23.4.3	The 2-O-Sulfotransferase .....	399
23.4.4	The 6-O-Sulfotransferases .....	400
23.4.5	The 3-O Sulfotransferases .....	400
23.5	The Products, Heparin and Heparan Sulfate .....	400
23.6	Interactions with Proteins .....	401
23.7	Regulation of HS Biosynthesis.....	402
	References .....	403
24	<b>Biosynthesis of Proteoglycans with Keratan Sulfates .....</b>	407
	<i>James L. Funderburgh</i>	
24.1	Introduction: Keratan Sulfate Renaissance.....	407
24.2	Keratan Sulfate Structure and Distribution .....	407
24.2.1	Corneal KS .....	408
24.2.2	Non-corneal KSI .....	409
24.2.3	KSII.....	409
24.2.4	KSIII.....	410
24.3	Keratan Sulfate Proteoglycans .....	410
24.3.1	SLRPs.....	410
24.3.2	Aggrecan.....	411
24.3.3	Cell-Associated KS .....	411
24.3.4	Brain .....	412
24.4	Enzymatic Reactions of KS Biosynthesis.....	412
24.5	Metabolic Control of KS Synthesis .....	413
	Acknowledgments .....	414
	References .....	414
25	<b>The Biosynthesis of GPI Anchors.....</b>	417
	<i>Yasu S. Morita, Alvaro Acosta-Serrano, and Paul T. Englund</i>	
25.1	Introduction .....	417
25.2	Structure of GPI Anchors .....	417
25.2.1	Glycan Core Modifications .....	417

25.2.2	Variations in Anchor Lipid Structure .....	419
25.3	GPI Precursor Synthesis .....	419
25.3.1	GlcNAc-PI Synthesis .....	420
25.3.2	GlcNAc-PI Deacetylation .....	421
25.3.3	Inositol Acylation .....	421
25.3.4	GPI Mannosylation .....	422
25.3.5	Transfer of EtN-P .....	423
25.3.6	Lipid Remodeling .....	423
25.3.7	Addition of Carbohydrate Side Chains .....	424
25.3.8	Topology of Biosynthetic Pathways .....	424
25.4	Attachment of the GPI Precursor to a Protein .....	425
25.4.1	Basic Features .....	425
25.4.2	Protein Machinery for GPI Addition .....	426
25.4.3	Signal Sequence for GPI Addition .....	426
25.5	Evolution of GPI Biosynthesis .....	426
25.6	Future Studies .....	427
	Acknowledgments .....	427
	References .....	427
 26	 <b><i>Escherichia coli</i> Lipid A: A Potent Activator of Innate Immunity</b> .....	435
	<i>Teresa A. Garrett and Christian R. H. Raetz</i>	
26.1	Introduction .....	435
26.2	Structure of Lipopolysaccharide .....	435
26.3	Lipid A Biosynthesis in <i>E. coli</i> .....	437
26.3.1	Acylation of UDP-GlcNAc .....	439
26.3.2	Disaccharide Formation .....	440
26.3.3	Phosphorylation by the Lipid A 4' Kinase .....	440
26.3.4	Kdo Addition and the Late Acyltransferases .....	441
26.3.5	Other Acyltransferases .....	442
26.3.6	Transport of Lipid A and the Role of MsbA .....	442
26.4	Lipid A Activation of Signal Transduction in Animal Cells .....	444
26.5	Summary .....	447
	Acknowledgments .....	447
	References .....	447
 II	 <b>Glycosidases</b> .....	453
 27	 <b>Lysosomal Degradation of Glycolipids</b> .....	455
	<i>Thomas Kolter and Konrad Sandhoff</i>	
27.1	Summary .....	455
27.2	Introduction .....	455
27.3	Mechanisms of Lysosomal Glycolipid Degradation .....	456
27.3.1	Glycosidases .....	456

## XXXVIII *Contents*

27.3.2	Topology of Endocytosis and Lysosomal Glycolipid Degradation	457
27.3.3	Sphingolipid Activator Proteins.....	458
	The GM2-activator and its role in lysosomal digestion .....	459
	SAP-A to SAP-D .....	460
27.3.4	Lateral Pressure.....	460
27.3.5	Lipid Composition .....	461
27.3.6	Membrane Curvature .....	462
27.4	Degradation of Selected Lipids .....	462
27.4.1	Ganglioside GM2 .....	462
27.4.2	Lactosylceramide .....	464
27.4.3	Glucosylceramide.....	464
27.4.4	Ceramide.....	465
27.4.5	Sphingomyelin .....	465
27.4.6	Sulfatide .....	465
27.4.7	Galactosylceramide.....	466
27.5	Pathobiochemistry.....	466
27.5.1	Animal Models for Sphingolipidoses .....	467
27.5.2	Therapy .....	469
27.6	Future Directions.....	470
	References .....	470
 28	 <b>Lysosomal Degradation of Glycoproteins .....</b>	473
	<i>Nathan N. Aronson, Jr.</i>	
28.1	Summary.....	473
28.2	Introduction .....	473
28.3	Roles of Lysosomes .....	474
28.4	Lysosomal Degradation of <i>N</i> -Linked Glycoproteins.....	475
28.4.1	General Features .....	475
28.4.2	Carbohydrate Digestion .....	476
28.4.3	Protein and Linkage Hydrolysis .....	476
28.5	Formation of Thyroid Hormone via Lysosomal Degradation of Thyroglobulin .....	477
28.5.1	Synthesis of Thyroid Hormone .....	477
28.5.2	Carbohydrate Degradation.....	478
28.5.3	Proteolysis .....	479
28.6	Degradation of Free Polymannose-Type Oligosaccharides Derived from <i>N</i> -Linked Glycoproteins During Biosynthesis .....	479
	References .....	481
 29	 <b>Sialidases.....</b>	485
	<i>Garry Taylor, Susan Crennell, Carl Thompson, and Marina Chuenkova</i>	
29.1	Abstract.....	485
29.2	Introduction .....	485

29.3	Influenza Virus Neuraminidase.....	486
29.4	Paramyxovirus Hemagglutinin-Neuraminidase (HN) .....	487
29.5	Non-Viral Sialidases .....	487
29.6	Small Sialidases .....	490
29.7	Large Sialidases.....	491
29.8	T. cruzi Trans-Sialidase (TS) .....	491
29.9	Conclusion .....	493
	Acknowledgments .....	494
	References.....	494
<b>30</b>	<b>Microbial Glycosidases.....</b>	<b>497</b>
	<i>Kenji Yamamoto, Su-Chen Li, and Yu-Teh Li</i>	
30.1	Exo-Glycosidases .....	497
30.1.1	$\alpha$ -Glucosidase .....	497
30.1.2	$\beta$ -Glucosidase .....	498
30.1.3	$\alpha$ -Galactosidase .....	498
30.1.4	$\beta$ -Galactosidase .....	499
30.1.5	$\alpha$ -Mannosidase.....	500
30.1.6	$\beta$ -Mannosidase.....	500
30.1.7	$\beta$ -N-Acetylhexosaminidase .....	501
30.1.8	$\alpha$ -N-Acetylgalactosaminidase.....	501
30.1.9	$\alpha$ -L-Fucosidase .....	502
30.1.10	$\beta$ -D-Fucosidase.....	503
30.1.11	Sialidase.....	503
30.1.12	KDNase.....	504
30.1.13	$\alpha$ -L-Rhamnosidase .....	504
30.1.14	$\beta$ -Xylosidase .....	505
30.2	Endo-Glycosidases.....	505
30.2.1	Endo- $\beta$ -N-acetylglucosaminidase.....	505
30.2.2	Peptide-N-glycanase F .....	506
30.2.3	Endo- $\alpha$ -N-acetylgalactosaminidase.....	506
30.2.4	Endo- $\beta$ -galactosidase .....	507
30.2.5	Endoglycoceramidase.....	507
	References.....	508
<b>31</b>	<b>Glycoprotein Processing Inhibitors.....</b>	<b>513</b>
	<i>Magid Osser and Alan D. Elbein</i>	
31.1	Introduction.....	513
31.2	Structural Classification .....	515
31.3	Distribution of Glycosidase Inhibitors in the Plant Kingdom.....	515
31.4	Isolation and Structural Determination .....	516
31.5	Glycosidase Inhibitory Activity .....	517
31.6	Structure-Activity Relationships .....	518
31.7	N-Linked Oligosaccharide Processing.....	519
31.8	Inhibitors of N-Linked Oligosaccharide Processing.....	522

31.8.1	Glucosidase Inhibitors.....	522
31.8.2	Mannosidase Inhibitors .....	525
31.9	Summary and Future Prospects.....	528
	References .....	529

<b>Index .....</b>	I 1
--------------------	-----

## **Part II    Biology of Saccharides**

### **Vol. 4    Lectins and Saccharides Biology**

<b>III</b>	<b>Lectins .....</b>	533
<b>32</b>	<b>Plant Lectins.....</b>	535
	<i>Marilynn E. Etzler</i>	
32.1	Summary.....	535
32.2	Introduction .....	535
32.3	Carbohydrate Specificity .....	536
32.4	Other Activities.....	539
32.5	Structure .....	540
32.6	Biological Roles .....	543
	Acknowledgments .....	546
	References .....	547
<b>33</b>	<b>Interactions of Oligosaccharides and Glycopeptides with Hepatic Carbohydrate Receptors .....</b>	549
	<i>Yuan C. Lee and Reiko T. Lee</i>	
33.1	Summary.....	549
33.2	Introduction .....	550
33.3	Molecular Characteristics of Hepatic Lectins .....	551
33.4	Cellular Aspects of HL.....	552
33.5	Binding Specificity .....	553
33.6	Photoaffinity Labeling.....	557
33.7	Subunit Organization on Rat Hepatocyte Surface .....	558
33.8	Applications .....	559
	References .....	560
<b>34</b>	<b>P-Type Lectins and Lysosomal Enzyme Trafficking .....</b>	563
	<i>Patricia G. Marron-Terada and Nancy M. Dahms</i>	
34.1	Introduction .....	563
34.2	Intracellular Trafficking of the MPRs .....	564
34.3	Primary Structure and Biosynthesis of the MPRs .....	566

34.3.1	CI-MPR.....	566
34.3.2	CD-MPR.....	567
34.4	Lysosomal Enzyme Recognition by the MPRs .....	569
34.5	Structural Determinants of Man-6-P Recognition.....	571
34.5.1	Expression of Mutant Forms of the MPRs .....	571
34.5.2	Crystal Structure of the CD-MPR .....	572
34.6	Conclusions .....	574
	Acknowledgments .....	574
	References.....	575
<b>35</b>	<b>The Siglec Family of I-Type Lectins.....</b>	<b>579</b>
	<i>Paul R. Crocker and Soerge Kelm</i>	
35.1	Introduction.....	579
35.2	The Immunoglobulin Superfamily and Carbohydrate Recognition .....	579
35.3	Siglecs as a Family of Sialic Acid Binding Proteins .....	580
35.4	Biology of Siglecs .....	581
35.5	Sialic Acids in Cellular Recognition .....	583
35.6	Mode of Carbohydrate Recognition by Siglecs.....	584
35.7	Importance of Multivalent Binding .....	588
35.8	Sialic Acid Recognition by the Immunoglobulin Fold— Evolutionary Considerations .....	588
35.9	Role of <i>cis</i> Interactions in Modulating Adhesion to Other Cells <i>in trans</i> .....	589
35.10	Sialoadhesin as a Macrophage Adhesion Molecule.....	590
35.11	Signalling Versus Adhesion Mediated by Siglecs .....	591
35.12	Conclusions .....	592
	Acknowledgments .....	592
	References.....	592
<b>36</b>	<b>C-Type Lectins and Collectins .....</b>	<b>597</b>
	<i>Russell Wallis</i>	
36.1	Summary .....	597
36.2	Structure and Function of C-Type Animal Lectins.....	598
36.2.1	The Carbohydrate-Recognition Domain.....	599
36.2.2	Ligand Binding .....	600
36.3	Mannose-Binding Protein and Collectins .....	601
36.3.1	Domain Organization .....	601
36.3.2	MBPs as Prototype Collectins .....	603
36.3.3	Ligand Binding by Serum MBP .....	603
36.3.4	MBP and Innate Immunity .....	604
36.3.5	Liver MBP .....	607
36.3.6	Pulmonary Surfactant Proteins .....	608
36.3.7	Conglutinin and CL-43 .....	608
36.4	Conclusions .....	609

Acknowledgments .....	609	
References .....	609	
<b>37</b>	<b>Selectins.....</b>	<b>613</b>
	<i>Roger P. McEver</i>	
37.1	Introduction .....	613
37.2	Structure of Selectins .....	613
37.3	Selectin Ligands .....	614
37.4	Requirements for Selectins to Mediate Tethering and Rolling of Leukocytes under Hydrodynamic Flow .....	619
37.5	Functions of Selectins and their Ligands <i>in vivo</i> .....	621
37.6	Conclusions.....	621
	References .....	622
<b>38</b>	<b>Galectins .....</b>	<b>625</b>
	<i>Douglas N. W. Cooper and Samuel H. Barondes</i>	
38.1	Introduction .....	625
38.2	Galectin Structure .....	626
38.3	Novel Candidate Galectins.....	631
38.4	Unorthodox Subcellular Targeting.....	635
38.5	Regulation of Galectin Expression.....	637
38.6	Galectin Binding Specificity and Identified Ligands.....	639
38.7	Physiological Functions .....	640
38.8	Summary.....	642
	References .....	642
<b>IV</b>	<b>Saccharide Biology.....</b>	<b>649</b>
<b>39</b>	<b>Structures and Functions of Nuclear and Cytoplasmic Glycoproteins</b>	<b>651</b>
	<i>Robert S. Haltiwanger</i>	
39.1	Introduction .....	651
39.2	O-Linked <i>N</i> -Acetylglucosamine (O-GlcNAc).....	652
39.2.1	O-GlcNAc Appears to be a Regulatory Modification much like Phosphorylation .....	653
39.2.2	Modulation of Protein Stability and Function by O-GlcNAc .....	655
39.3	Other Forms of Nuclear and Cytoplasmic Glycosylation.....	658
39.3.1	Unique Cytoplasmic Forms of Glycosylation .....	658
39.3.2	Conventional Forms of Glycosylation in the Nucleus and Cytoplasm .....	660
39.3.3	Nuclear and Cytoplasmic Lectins.....	661
39.4	Conclusions.....	662
	Acknowledgments .....	662
	References .....	662

<b>40</b>	<b>Structure and Functions of Mucins .....</b>	669
	<i>Joyce Taylor-Papadimitriou and Joy M. Burchell</i>	
40.1	Classification of Mucins .....	669
40.2	The Epithelial Mucins .....	670
40.3	Mucin Type O-Glycosylation Pathways .....	670
40.3.1	Initiation of O-Glycosylation .....	671
40.3.2	Chain Extension .....	671
40.3.3	Chain Termination .....	671
40.4	Expression of Epithelial Mucins .....	672
40.5	The Complex Gel-Forming Mucins: Processing and Function .....	672
40.6	Epithelial Membrane Mucins .....	674
40.7	Studies Related to the MUC1 Mucin .....	675
40.7.1	Changes in the Patterns of O-Glycosylation of MUC1 in Breast Cancer .....	675
	Differences in sites of glycosylation .....	675
	Changes in the composition of O-glycans added to MUC1 in Breast Cancer .....	676
	Correlation in changes of Glycosyltransferase activities with changes in O-glycan structure in Breast Cancer .....	676
40.7.2	Changes in Glycosylation of MUC1 in other Cancers .....	677
40.7.3	Effects of MUC1 Expression on the Behavioral Properties of Cancer Cells .....	677
	Effects on cell interactions and tumourogenicity .....	677
40.7.4	MUC1 Expression and Immune Responses .....	678
40.7.5	Active Specific Immunotherapy Based on MUC1 .....	679
	Animal models .....	679
	Clinical studies .....	680
40.8	Comments .....	681
	References .....	681
<b>41</b>	<b>Biological Roles of Hyaluronan .....</b>	685
	<i>Bryan P. Toole</i>	
41.1	Introduction .....	685
41.2	Hyaluronan is a Biopolymer with Unusual Physical Properties .....	685
41.3	Hyaluronan Binds to Several Types of Proteins (Hyaladherins) ...	687
41.3.1	General Properties of Hyaladherins .....	687
41.3.2	Structural Hyaluronan-Binding Proteins .....	688
41.3.3	Hyaluronan Receptors .....	688
41.3.4	Intracellular Hyaluronan-Binding Proteins .....	689
41.3.5	Inter- $\alpha$ -Trypsin Inhibitor .....	689
41.4	Hyaluronan-Dependent Pericellular Matrices Assemble Around Several Cell Types .....	690
41.4.1	Hyaluronan-Dependent Cellular "Coats" .....	690
41.4.2	Assembly of Chondrocyte Pericellular Matrix .....	691

41.4.3	Tethering of Cell Surface Hyaluronan to Hyaluronan Synthase...	691
41.5	Hyaluronan Influences Cell Behavior During Morphogenesis and Tissue Remodeling .....	693
41.5.1	Migratory and Proliferating Cells are Surrounded by Hyaluronan-enriched Matrices.....	693
41.5.2	Hydrated Pericellular Milieux Provide Cellular Pathways .....	693
41.5.3	Receptors Mediate Effects of Hyaluronan .....	693
41.5.4	Hyaluronan-Cell Interactions in Limb Development.....	694
41.5.5	Hyaluronan-Cell Interactions in Other Physiological and Developmental Systems .....	695
41.6	Hyaluronan Plays a Crucial Role in Cancer.....	696
	References .....	696
 <b>42</b>	 <b>Biological Roles of Heparan Sulfate Proteoglycans.....</b>	 <b>701</b>
	<i>Ofer Reizes, Pyong Woo Park, and Merton Bernfield</i>	
42.1	Introduction .....	701
42.2	Heparan Sulfate Biosynthesis .....	701
42.3	Functions of Heparan Sulfate.....	702
42.4	Proteoglycans .....	703
42.5	Intracellular Proteoglycans .....	703
42.5.1	Serglycin and Heparin.....	704
42.6	Cell Surface Heparan Sulfate Proteoglycans.....	705
42.6.1	Syndecans .....	705
42.6.2	Glypicans .....	706
42.7	Part-time Cell Surface Heparan Sulfate Proteoglycans.....	707
42.7.1	Betaglycan .....	707
42.7.2	CD44.....	708
42.8	Functions of Cell Surface Heparan Sulfate Proteoglycans .....	708
42.8.1	Ligand Receptors .....	708
42.8.2	Ligand Coreceptors .....	709
42.8.3	Shed Effectors .....	709
42.9	Extracellular Matrix Heparan Sulfate Proteoglycans and Their Functions .....	710
42.9.1	Perlecan .....	710
42.9.2	Agrin .....	712
42.9.3	Other Extracellular HSPGs .....	713
42.10	Conclusions .....	713
	References .....	713
 <b>43</b>	 <b>Biological Roles of Keratan Sulfate Proteoglycans.....</b>	 <b>717</b>
	<i>Gary W. Conrad</i>	
43.1	Introduction .....	717
43.2	Corneal Transparency .....	718

43.3	Nerve Growth Cone Guidance .....	719
43.4	Cell Adhesion .....	721
43.5	Other Possible Roles of KSPGs .....	722
	Acknowledgment .....	723
	References.....	723
<b>44</b>	<b>Developmental and Aging Changes of Chondroitin/Dermatan Sulfate Proteoglycans .....</b>	<b>729</b>
	<i>J. Michael Sorrell, David A. Carrino, and Arnold I. Caplan</i>	
44.1	Proteoglycans .....	729
44.2	Glycosaminoglycans .....	729
44.3	Core Proteins .....	731
44.3.1	Hyalectans .....	731
44.3.2	Small Leucine-rich Proteoglycans .....	733
44.4	Chondroitin/Dermatan Sulfate Proteoglycans in Development and Aging.....	735
44.4.1	Core Proteins in Development, Aging, and Pathologies.....	735
44.4.2	Chondroitin/Dermatan Sulfate Glycosaminoglycan Chains in Development, Aging, and Pathologies .....	736
44.5	Summary.....	740
	References.....	740
<b>45</b>	<b>Proteoglycans and Hyaluronan in Vascular Disease.....</b>	<b>743</b>
	<i>Thomas N. Wight</i>	
45.1	Introduction.....	743
45.2	Proteoglycans and Hyaluronan.....	744
45.3	Versican (CSPGs).....	745
45.4	Hyaluronan .....	747
45.5	Decorin/Biglycan (DSPGs).....	748
45.6	Perlecan/Syndecans (HSPGs).....	749
45.7	Summary.....	750
	Acknowledgments .....	750
	References.....	750
<b>46</b>	<b>Functions of Glycosyl Phosphatidylinositol.....</b>	<b>757</b>
	<i>Nikola A. Baumann, Anant K. Menon, and David M. Rancour</i>	
46.1	Introduction.....	757
46.2	Parasite Coats: Extreme GPI-Anchoring.....	758
46.3	Yeast GPIs and the Cell Wall .....	758
46.4	Paroxysmal Nocturnal Hemoglobinuria (PNH): Disease and Defects in GPI-Anchoring of Proteins .....	759
46.5	GPIs in the Secretory and Endocytic Pathways.....	760
46.6	Organization of GPI Proteins in the Plasma Membrane .....	762

## XLVI *Contents*

46.7	Association of GPI-Anchored Proteins with Caveolae .....	764
46.8	Detergent Insolubility and Signaling via GPI-Proteins.....	764
46.9	Membrane Release of GPI-Anchored Proteins .....	765
46.10	GPIs as Second Messenger Signaling Molecules .....	766
46.11	Summary.....	767
	Acknowledgments .....	767
	References .....	768
 <b>47</b>	 <b>Glycosphingolipid Microdomains in Signal Transduction, Cancer, and Development.....</b>	 <b>771</b>
	<i>Sen-itiroh Hakomori and Kazuko Handa</i>	
47.1	Clustered GSLs as Functional Units.....	771
47.2	GSL Clusters, Associated with Signal Transducers, are Functional Units Separable from Caveolae .....	772
47.3	Cell Adhesion Coupled with Signal Transduction Initiated by GSL Microdomain: Concept of Glycosignalling Domain (GSD) .....	773
47.4	Role of GSLs in Control of Growth Factor and Hormone Receptors: Possible Relationship with GSL Microdomain.....	774
47.5	Functional Role of Developmentally-Regulated and Tumor-Associated GSLs.....	776
	References .....	778
 <b>48</b>	 <b>The Primary Cell Walls of Higher Plants .....</b>	 <b>783</b>
	<i>Jocelyn K. C. Rose, Malcolm A. O'Neill, Peter Albersheim, and Alan Darvill</i>	
48.1	Introduction (What is a Cell Wall?).....	783
48.2	Purification of Cell Walls and Isolation of Wall Components .....	784
48.3	The Structural Components of the Primary Cell Wall .....	786
48.3.1	Cell Walls and the Diversity of Flowering Plants .....	786
48.3.2	The Structural Components of the Primary Wall .....	786
48.4	Biosynthesis of Wall Components .....	791
48.5	Organization of the Plant Primary Cell Wall.....	793
48.6	Cellulose-Xyloglucan Interactions .....	793
48.7	Interactions Between Pectins and Other Cell Wall Components...	794
48.8	Glycoproteins in the Cell Wall .....	796
48.9	Heterogeneity in the Primary Cell Wall .....	797
48.10	Function and Metabolism of Plant Primary Cell Walls.....	798
48.10.1	Mechanical Support .....	798
48.10.2	Regulation of Cell Expansion.....	798
48.10.3	Morphogenesis and Differentiation .....	800
48.10.4	Plant Cell Wall Oligosaccharides in Defense and Cell Signalling ..	801
48.11	Intercellular Transport and Storage.....	803
48.12	Biotechnology and Future Directions in the Commercial Applications of Plant Primary Cell Walls .....	803

Acknowledgment .....	804
References.....	804
<b>49      Glycolipids and Bacterial Pathogenesis .....</b>	<b>809</b>
<i>Clifford A. Lingwood</i>	
49.1     Introduction.....	809
49.2     Modulation of Glycolipid Receptor Function .....	810
49.3     Stress Response and Glycolipid Receptors.....	812
49.4     Subcellular Gb <sub>3</sub> Trafficking .....	813
49.5     Model for Lipid Sorting Based on Chain Length.....	815
49.6     Glycosphingolipids and Signal Transduction .....	815
Acknowledgments .....	817
References.....	817
<b>50      Glycobiology of Viruses .....</b>	<b>821</b>
<i>Hildegard Geyer and Rudolf Geyer</i>	
50.1     Summary.....	821
50.2     General Aspects.....	821
50.2.1    Functions of Viral Surface Glycoproteins.....	825
50.2.2    Biosynthesis .....	826
50.2.3    Function of Carbohydrate Substituents.....	826
50.2.4    Oligosaccharide Diversity .....	827
50.3     Examples.....	830
50.3.1    Friend Murine Leukemia Virus Complex.....	830
50.3.2    Marburg Virus (MBGV) .....	832
50.3.3    Hepatitis B Virus (HBV) .....	833
Acknowledgments .....	836
References.....	836
<b>51      The Glycobiology of Influenza Viruses.....</b>	<b>839</b>
<i>Stephen J. Stray and Gillian M. Air</i>	
51.1     Introduction.....	839
51.2     Receptor Binding Proteins: Influenza A HAg and Influenza C HEF .....	840
51.2.1    Structure of Receptor Binding Domain and Mechanism of Sialic Acid Recognition.....	843
51.2.2    HEF Esterase Domain and Mechanism of Cleavage.....	844
51.3     Influenza NAm (types A and B) .....	844
51.3.1    Mechanism of Sialic Acid Cleavage.....	845
51.4     Function of Viral Receptor Destroying Enzymes.....	847
Acknowledgments .....	847
References.....	848

## XLVIII Contents

<b>52</b>	<b>Glycobiology of Aids.....</b>	851
	<i>Ten Feizi</i>	
52.1	Abstract.....	851
52.2	Introduction .....	851
52.3	The Repertoire of <i>N</i> -Glycans on the Envelope Glycoprotein of HIV of Human Immunodeficiency Virus Produced in Different Cell Types.....	853
52.4	Evidence for the Occurrence of <i>O</i> -Glycans on the Envelope Glycoproteins of HIV-1 Produced in Certain Cell Lines.....	854
52.5	Oligosaccharides of gp 120 and gp 41 at <i>N</i> -Glycosylation Sites and Their Possible Influence on Viral Infectivity .....	855
52.6	gp 120 Glycosylation Can Influence Antigenicity and Immunogenicity .....	856
52.7	Saccharides Recognized by Carbohydrate-binding Proteins and Antibodies as Potential Neutralization Epitopes on the Envelope Glycoprotein of HIV-1 .....	857
52.7.1	Lectins and Antibodies with Mannose-related Specificities .....	857
52.7.2	Antibodies to <i>O</i> -Glycan Sequences .....	858
52.7.3	Antibodies to Blood Group A .....	858
52.7.4	Xeno-antibodies to Gal $\alpha$ 1-3Gal Sequence .....	859
52.7.5	Potential Medical Relevance .....	859
52.8	Does Viral Oligosaccharide Display Influence Tissue Tropism? ..	860
52.9	Concluding Remarks .....	862
	Acknowledgment .....	862
	References .....	863
 <b>53</b>	 <b>Glycobiology of Protozoan and Helminthic Parasites.....</b>	867
	<i>Richard D. Cummings, and A. Kwame Nyame</i>	
53.1	Introduction .....	867
53.2	General Classification of Parasites .....	867
53.3	The Major Protozoan Parasites .....	868
53.3.1	Malaria .....	868
53.3.2	Trypanosomiasis.....	873
53.3.3	Leishmaniasis .....	874
53.4	Other Protozoan Parasites.....	878
53.4.1	<i>Entamoeba histolytica</i> .....	878
53.4.2	<i>Acanthamoeba</i> .....	878
53.4.3	<i>Giardia lamblia</i> .....	878
53.4.4	<i>Cryptosporidium parvum</i> .....	878
53.4.5	<i>Sarcocystis</i> spp.....	879
53.4.6	<i>Toxoplasma gondii</i> .....	879
53.4.7	<i>Pneumocystis carinii</i> .....	879
53.5	Helminthic Parasites.....	879
53.6	Carbohydrate-Binding Proteins in Parasitic Helminths .....	883

53.7	Unusual Glycans in Other Helminthic Parasites.....	883
53.8	Future Directions .....	885
	Acknowledgments .....	885
	References.....	886
<b>54</b>	<b>The Involvement of the Oligosaccharide Chains of Glycoproteins in Gamete Interactions at Fertilization .....</b>	<b>895</b>
	<i>Noritaka Hirohashi and William J. Lennarz</i>	
54.1	Introduction.....	895
54.2	Advantages of Marine Invertebrates as an Experimental System ..	895
54.3	Induction of the Acrosome Reaction.....	896
54.3.1	Studies in Sea Urchins .....	896
54.3.2	Studies in Starfish .....	899
54.4	Sperm–Egg Coat Binding .....	899
54.4.1	Studies in Mammals .....	900
54.4.2	Studies in Frog.....	900
54.4.3	Studies in Ascidians.....	902
54.4.4	Studies in Sea Urchins .....	904
54.5	Carbohydrate as a Species-Specific Determinant .....	906
	References.....	907
<b>55</b>	<b>Glycosylation and Development.....</b>	<b>909</b>
	<i>Michèle Aubery and Christian Deraupe</i>	
55.1	Summary .....	909
55.2	Introduction.....	911
55.3	Lectins as Tools to Analyze Changes in Cell-surface Glycoconjugates During Development.....	911
55.4	Cell-adhesion Molecules .....	912
55.4.1	Neural Cell-adhesion Molecule.....	912
55.4.2	The Adhesion Molecule L1.....	914
55.5	Glycosyltransferases .....	914
55.6	Altered Expression of Endogenous Lectins During Development ..	915
55.6.1	Galectins .....	915
55.6.2	Selectins .....	917
55.6.3	Other Endogenous Lectins .....	917
55.7	Conclusion .....	918
	References.....	918
<b>56</b>	<b>Protein Glycosylation and Cancer .....</b>	<b>923</b>
	<i>James W. Dennis and Maria Granovsky</i>	
56.1	Introduction .....	923
56.2	Protein Glycosylation Generates Molecular Diversity.....	923
56.3	Cancer Initiation and Progression.....	926

56.4	Tumor Cell Proliferation .....	927
56.5	Cell Migration .....	930
56.6	Sialylation and Metastasis.....	933
56.7	Endogenous Lectins and Tumor Cell Adhesion .....	934
56.8	Carbohydrate Processing Inhibitors as Anti-Cancer Agents .....	935
56.9	Other Considerations .....	936
	Acknowledgments .....	937
	References .....	937
<b>57</b>	<b>Lysosomal Storage Diseases .....</b>	<b>945</b>
	<i>Nathan N. Aronson, Jr.</i>	
57.1	Summary .....	945
57.2	Introduction .....	946
57.3	Animal Models .....	947
57.4	Mucopolysaccharidoses .....	947
57.5	Cathepsin K Deficiency and Pycnodynostosis .....	949
57.6	Mouse Models for Tay-Sachs and Sandhoff Diseases.....	951
57.7	Impact of Lysosomal Diseases and Their Study .....	953
	References .....	954
<b>58</b>	<b>Genetic Diseases of Glycosylation .....</b>	<b>959</b>
	<i>Tomoya Akama and Michiko N. Fukuda</i>	
58.1	Introduction .....	959
58.2	CDGS.....	959
58.2.1	CDGS Type I.....	959
58.2.2	CDGS Type II.....	961
58.3	HEMPAS.....	963
	References .....	964
<b>59</b>	<b>Glycobiology of <i>Helicobacter pylori</i> and Gastric Disease .....</b>	<b>967</b>
	<i>Karl-Anders Karlsson</i>	
59.1	Introduction .....	967
59.2	The Bacterial Surface and Molecular Mimicry .....	968
59.3	Host Surfaces and <i>H. pylori</i> Recognition of Glycoconjugates: Unique Complexity.....	968
59.3.1	Sialic Acid .....	969
59.3.2	Sulfatide .....	970
59.3.3	Heparan Sulfate .....	970
59.3.4	Fucose-Dependent Binding (H-1 and Lewis b).....	971
59.3.5	Gangliotetraosylceramide .....	971
59.3.6	Lactosylceramide .....	972
59.4	The Meaning of Multiple Binding Specificities .....	972
59.5	Aspects for the Future.....	973
	References .....	973

<b>60</b>	<b>Immunoglobulin G Glycosylation and Galactosyltransferase Changes in Rheumatoid Arthritis .....</b>	977
	<i>John S. Axford</i>	
60.1	Introduction.....	977
60.2	Oligosaccharide Synthesis .....	977
60.3	Galactosyltransferase .....	978
60.4	Immunoglobulin G .....	978
60.5	Rheumatoid Arthritis .....	979
60.6	Quantification of IgG sugars in RAr .....	979
60.7	RAr and Pregnancy.....	980
60.7.1	Galactosylation of IgG .....	980
60.7.2	$\alpha$ 3-Fucosylation of $\alpha$ 1-Acid Glycoprotein .....	981
60.8	Agalactosyl-IgG and Rheumatoid Factor Binding .....	982
60.9	Tissue-specific Galactosyltransferase Abnormalities in an Experimental Model of Rheumatoid Arthritis.....	983
60.10	Glycosylation Homeostasis within RAr Lymphocytes is Abnormal .....	985
60.11	Are the Rheumatoid Arthritis Associated Glycosylation Abnormalities Unique? .....	986
60.12	Sugar Printing Rheumatic Disease is Possible .....	990
60.13	Rapid Profiling of IgG N-Glycans by Fluorophore-coupled Oligosaccharide Electrophoresis has the Potential of Differentiating Rheumatic Diseases.....	992
60.14	In What Way could GTase Enzymatic Control be Abnormal? ....	992
60.15	Conclusion .....	993
	References.....	994
<b>61</b>	<b>Calnexin, Calreticulin and Glycoprotein Folding Within the Endoplasmic Reticulum .....</b>	997
	<i>Michael R. Leach and David B. Williams</i>	
61.1	Structure and Properties of Calnexin and Calreticulin .....	997
61.2	Biological Functions.....	1000
61.3	Mechanism of Action.....	1002
61.4	Functional Relationship Between Calnexin and Calreticulin....	1005
61.5	Relationship with other ER Chaperones and Folding Catalysts ..	1007
	References.....	1008
<b>62</b>	<b>Glycobiology of The Nervous System.....</b>	1013
	<i>Ronald L. Schnaar</i>	
62.1	Introduction.....	1013
62.2	Nervous System Glycoconjugates—Overview .....	1013
62.3	Nervous System Glycolipids.....	1014
62.3.1	Galactosylceramides .....	1014

62.3.2	Gangliosides and Related Anionic Glycosphingolipids .....	1016
62.4	Nervous System Glycoproteins.....	1019
62.4.1	Polysialic Acid.....	1020
62.4.2	The HNK-1 Determinant .....	1020
62.5	Nervous System Glycosaminoglycans .....	1020
62.6	Lectins in the Brain.....	1021
62.6.1	Myelin-Associated Glycoprotein .....	1021
62.6.2	Other Nervous System Lectins .....	1022
62.7	Concluding Remarks .....	1023
	References .....	1023
 <b>63</b>	 <b>Glycobiology of the Immune System .....</b>	 1029
	<i>Elizabeth F. Hounsell</i>	
63.1	Infection and Pathogenesis .....	1029
63.2	Control of the Immune Response .....	1033
63.3	Bacterial and Tumor Antigens, Mucins and Mucin-like Molecules .....	1034
63.4	Immunoglobulins and Pathology .....	1036
	References .....	1038
 <b>64</b>	 <b>Metabolic Engineering Glycosylation: Biotechnology's Challenge to the Glycobiologist in the Next Millennium .....</b>	 1043
	<i>Thomas G. Warner</i>	
64.1	Introduction .....	1043
64.2	Recent Developments in Carbohydrate Biosynthesis.....	1044
64.2.1	Optimizing Sialylation of Recombinant Proteins by Metabolic Engineering Sialic Acid Biosynthesis.....	1044
64.2.2	Optimizing Galactosylation of Recombinant Proteins by Metabolically Engineering Galactose Biosynthesis.....	1049
64.2.3	Mannose Biosynthesis and Mannosylation of Recombinant Proteins .....	1052
64.3	Glycosylation Engineering Alternate Expression Hosts For Recombinant Protein Therapeutic Production .....	1053
64.3.1	Engineering Glycosylation of Recombinant Proteins Expressed in Baculovirus-Insect Cells .....	1053
	Genes needed to supplement glycosylation of recombinant proteins in insect cells .....	1054
	Deleterious genes may need to be deleted or inhibited to enhance recombinant glycoprotein biosynthesis in insect cells.....	1054
64.3.2	Engineering Glycosylation of Recombinant Proteins Expressed in Plants.....	1056
	Genetic addition and supplementation needed to improve plant recombinant protein glycosylation .....	1058
	Inhibition or deletion of plant glycosylation genes .....	1059

64.4	Summary .....	1059
	Acknowledgments .....	1060
	References.....	1060
	<b>Index .....</b>	<b>I 1</b>