Tetrahedron Vol. 45, No. 17, pp. 5365 to 5422, 1989
Printed in Great Britain.

0040–4020/89 $3.00+.00
Pergamon Press plc

TETRAHEDRON REPORT NUMBER 259

ENZYME-CATALYZED SYNTHESIS OF CARBOHYDRATES

Eric J. Toone, Ethan S. Simon, Mark D. Bednarski
and George M. Whitesides*

Department of Chemistry, Harvard University, Cambridge, MA 02138 USA

(*Received in USA* 14 *June* 1989)

CONTENTS

1. INTRODUCTION.

Carbohydrates are one of the most important classes of compounds in biology.[1-6] In cells, for example, carbohydrates function as structural components, to regulate viscosity, for energy storage, and as key components of cell surfaces. Their fundamental role in molecular biology, coupled with the synthetic challenges they pose, has made carbohydrates a target for synthetic chemists since the work of Emil Fischer. Classical techniques for the synthesis of carbohydrates are extensively developed.[7-15] The difficulties in these techniques center on selective protection and deprotection. These difficulties, coupled with those of isolating and purifying carbohydrates and of analyzing their structures, have made this area of chemistry a demanding one. This review examines the potential of enzymes as catalysts for the synthesis of carbohydrates. The major advantage of enzyme-catalyzed synthesis is the potential for effecting regioselective reactions using unprotected carbohydrates under mild conditions in aqueous solutions. Enzymatic methods certainly offer the most plausible routes for the modification of oligosaccharides on proteins and on cell surfaces. The major uncertainty in the potential of enzymes as catalysts in carbohydrate synthesis involves the availability of the range of enzymes required to accomplish major tasks in this area, and the stability and breadth in substrate acceptability characterizing these enzymes. The rapidly developing methodology of genetic engineering holds great promise for making important enzymes readily available, and may eventually make it possible to modify the selectivity of these enzymes.

Current interest in carbohydrate chemistry ranges from fundamental biology to applied biotechnology. Glycoproteins and glycolipids play important roles in intra- and intercellular communication.[2-4,16] They serve as binding sites for antibodies, enzymes, hormones, toxins, bacteria, drugs, and viruses.[17-24] Cell-surface glycoconjugates function as cellular labels during differentiation and development. Oligosaccharides are important for sorting newly synthesized proteins between cellular compartments and influencing growth through intercellular contact.[25-31] The presence of modified glycoconjugates is associated with several disease states, including a variety of malignancies.[14,32-35] Glycosylation of proteins may increase solubility, alter uptake and residency time *in vivo*,[36,37] and decrease antigenicity.[38] Modification of the carbohydrate groups on therapeutic glycoproteins is also an area of interest as companies attempt to differentiate products such as tissue plasminogen activator (tPA) from similar products of their competitors. Other areas of practical interest concern biodegradable polymers, site-specific and controlled drug delivery, agents for modifying viscosity, immunomodulation, and non-nutritive fat substitutes and sweeteners.

Problems of Classical Synthesis.

The synthesis and modification of carbohydrates is a difficult problem in classical synthetic chemistry. In particular, four features of carbohydrate chemistry limit the application of existing synthetic methods for the practical (ie. multi-gram) preparation of this class of molecules:

1) Glycosyl activation. The development of good, general chemical methods for the construction of glycosidic linkages is still an area of ongoing concern. Few general techniques provide high yields of a single anomer. This issue has been discussed elsewhere.[7,12]

2) Selectivity. High regio- and stereoselectivity are required for the construction of mono- and oligosaccharides. The presence of multiple functional groups (such as hydroxyl or amino groups) of similar reactivity makes this issue non-trivial. To ensure regio- and stereoselectivity, classical organic synthesis uses sequential protection and deprotection steps. Such an approach often results in complex synthetic schemes.

3) Complexity. The diversity in linkages in carbohydrates exceeds that found in other areas of biological chemistry, including peptide chemistry. Even simple biologically important structures often contain several distinct monosaccharide units joined by specific linkages, and are frequently linked to proteins or lipids.

4) Aqueous media. Although many carbohydrates are only soluble in water, a large fraction of the reactions in the armamentarium of organic chemists is incompatible with aqueous systems. This incompatibility has posed problems for organic chemists who are reluctant to manipulate compounds in aqueous media, and has tended to require extensive, if reversible, modification of the carbohydrate groups simply to achieve solubility in non-aqueous solvents.

The application of enzymes as catalysts in organic synthesis has succeeded in solving synthetic problems of similar complexity, notably the production of chiral synthons.[39-42] The use of enzymatic synthesis is now being extended to the synthesis and modification of carbohydrates.[43-44] This review evaluates the current level of application of enzymes to the synthesis of both monomeric and oligomeric carbohydrate structures, and the potential for further developments in the field.

Structure and Scope of Review.

The first section of this review describes advances in the enzyme-catalyzed construction of natural and unnatural monosaccharides. The primary emphasis in this section is on the construction of carbon-carbon bonds by enzyme-catalyzed aldol reactions. The following sections outline methods for enzyme-catalyzed formation of glycosyl bonds

by Leloir and non-Leloir pathways. These sections include descriptions of methods for the synthesis of oligosaccharides and, in less detail, of polysaccharides. The area of enzyme-catalyzed formation of glycosyl linkages is developing rapidly; a number of successful syntheses of oligosaccharides and polysaccharides have recently been reported. The final section illustrates unsolved problems and targets in the enzyme-catalyzed synthesis of carbohydrates. Although the success of enzymology in providing practical catalysts for the synthesis of carbohydrates will build on advances in biochemistry, a discussion of the fundamental biology of carbohydrates is beyond the scope of this review.

Enzymes as Catalysts; Auxiliary Technology.

A perception remains in the community of synthetic organic chemists that most enzymes are highly specific for their natural substrates, and that this substrate specificity precludes their general use in synthesis. The use of enzymes is further hampered by concerns regarding their cost, stability, and requirements for expensive cofactors. Previous developments in applied enzymology have successfully addressed many of these problems.

Many enzymes accept a wide range of substrates. Although the rates of reaction of unnatural substrates may be slower than that of the natural substrate, the substrate specificity of many enzymes is broad enough to allow a wide range of compounds to be utilized as substrates at an acceptable rate. Among enzymes showing broad substrate specificity are the esterases and lipases,[45] chymotrypsin,[46] lactate dehydrogenase,[47,48] acylase I,[49] and rabbit muscle fructose 1,6-diphosphate aldolase.[50,51] Many enzymes are sufficiently inexpensive that they can be used in large quantities, in order to achieve an acceptable rate of reaction with even a slowly-reacting substrate. The development of techniques such as enzyme immobilization,[52-54] and containment in hollow fiber reactors[55] and with membranes (membrane enclosed enzymatic catalysis, MEEC)[56] allows the recovery and reuse of enzymes, thereby reducing their cost as catalysts. Immobilization can also enhance the stability of enzymes.[52] The deactivation of enzymes on oxidation by O_2 can often be avoided through the use of inert atmospheres and thiol-containing reducing agents.[57-59]

Several groups of enzymes require added cofactors. In many cases, these cofactors are too expensive to use stoichiometrically, and this limitation initially hampered the use of enzymes in synthesis. This problem has now been solved for ATP, the nicotinamide cofactors, and certain others, through the development of regenerating systems, which allows the use of catalytic amounts of cofactor. This field has been reviewed.[60,61]

2. MONOSACCHARIDES.

Two types of enzyme-catalyzed reactions have been used in preparing monosaccharides: carbon-carbon bond forming reactions and reactions that alter functional groups. Aldolases are a class of enzymes that catalyze the stereospecific construction of carbon-carbon bonds in monosaccharides.[62,63] The first part of this section discusses the demonstrated use of aldolases (particularly the most well-explored aldolase, fructose 1,6-diphosphate aldolase from rabbit muscle) in synthesis. The second part describes other classes of enzymes that alter functional groups of monosaccharides. Throughout this review, sugars are of the D-configuration unless otherwise noted.

Aldolases

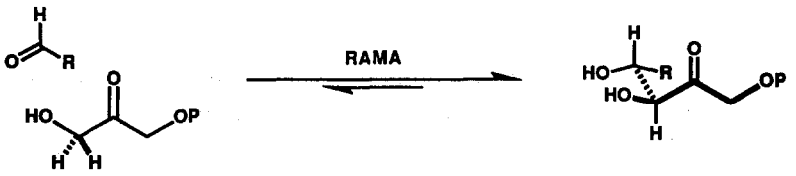
Aldolases were first recognized near the beginning of this century as an ubiquitous class of enzymes that catalyze the interconversion of hexoses and their three-carbon components. Their activity was first described by Meyerhof and coworkers, and was originally named zymohexase.[64] It is now known that aldolases operate on a much wider range of substrates than hexoses, and a variety of enzymes has been described that add a one-, two- or three-carbon fragment stereospecifically to an aldehyde via an aldol condensation.

All organisms possess aldolase enzymes, and two distinct groups have been recognized.[65] Type I aldolases, found predominantly in higher plants and animals, require no metal cofactor, and catalyze the aldol condensation through a Schiff base intermediate. These enzymes are inactivated by borohydride in the presence of substrate, and are unaffected by EDTA. Class II aldolases are found primarily in bacteria and fungi, and are Zn^{2+} dependent enzymes. These enzymes are not affected by borohydride, but are inactivated by EDTA.

To date, the aldolases have been the most useful enzymes for the formation of monosaccharides, although only a small number of the known aldolases have been exploited synthetically. The most useful of these enzymes are described below.

Fructose 1,6-diphosphate aldolase.

Fructose 1,6-diphosphate aldolase from rabbit muscle (FDP aldolase, E.C. 4.1.2.13, also commonly known as rabbit muscle aldolase, RAMA) catalyzes the equilibrium condensation of dihydroxyacetone phosphate (DHAP) with D-glyceraldehyde-3-phosphate (G-3-P) to form D-fructose 1,6-diphosphate (FDP).[50] The equilibrium constant for this reaction is $K = 10^4\ M^{-1}$ in favor of the formation of FDP (Scheme 1, R = $CHOHCH_2OP_i$). The stereospecificity of the reaction is absolute; the configuration of the vicinal diols at C-3 and C-4 is always D-*threo*. There is significant discrimination (~20:1) between the antipodes of the natural substrate, G-3-P; a few examples with unnatural



Scheme 1.

substrates also demonstrate some limited diastereoselectivity.[50] In the context of organic synthesis, however, RAMA does not exhibit useful diasteroselectivity with respect to C-5.

Substrate Specificity.

RAMA accepts a wide range of aldehydes in place of its natural substrate, G-3-P, allowing the synthesis of carbohydrates such as nitrogen-containing sugars,[51] deoxysugars,[66-68] fluorosugars,[68] and eight- and nine-carbon sugars.[69] More than 75 aldehydes have been identified as substrates based on enzymatic assay. Table 1 lists aldol adducts which have been isolated and characterized.[50,51,66-93] In general, unhindered aliphatic, α-heteroatom-substituted, and differentially protected alkoxy aldehydes are substrates; severely hindered aliphatic aldehydes such as pivaldehyde do not react with RAMA, nor do α,β-unsaturated aldehydes or compounds that can eliminate to form α,β-unsaturated aldehydes. Aromatic aldehydes are either poor substrates or are unreactive. A rate enhancement has been observed for substrates phosphorylated at the terminal hydroxyl relative to the non-phosphorylated species.[50] This effect has been noted for trioses (glyceraldehyde), tetroses (erythrose), pentoses and hexoses, and the rate enhancement on phosphorylation is on the order of 2- to 10-fold. The effect has been attributed to an interaction of phosphate with some positively charged group at the active site.

The requirement for the nucleophilic component (DHAP) is much more stringent than for the electrophilic component. Several DHAP analogues have been tested as substrates for RAMA (Table 2); so far investigations have demonstrated that only 1,3-dihydroxy-2-butanone-3-phosphate and 1,4-dihydroxy-3-butanone-1-phosphonate are substrates.[48]

Preparation of DHAP.

DHAP itself may be generated by three procedures: (1) *in situ* from fructose 1,6-diphosphate with the enzyme triosephosphate isomerase (E.C. 5.3.1.1),[66] (2) from the dimer of dihydroxyacetone by chemical phosphorylation with $POCl_3$,[83] (3) or from dihydroxyacetone by enzymatic phosphorylation using ATP and glycerol kinase (E.C. 2.7.1.30), with *in situ* regeneration of the ATP using phosphoenolpyruvate (PEP) or acetyl phophate as the ultimate phosphate donor.[94] The *in situ* generation of DHAP from FDP is the most convenient of the above methods, but in some instances, particularly when the

Table 1. Products Isolated From RAMA-Catalyzed Condensation of Electrophile with DHAP

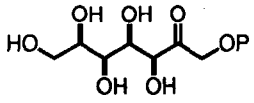
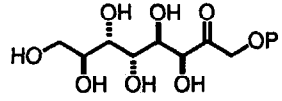
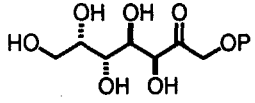
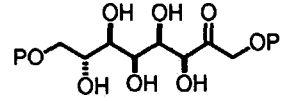
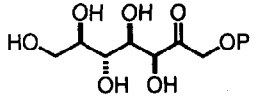
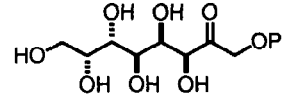
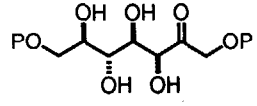
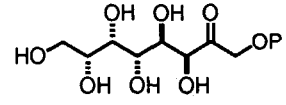
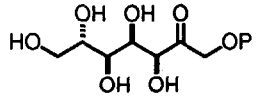
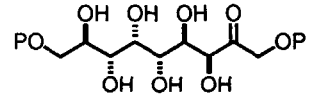
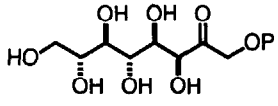
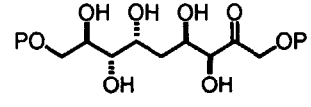
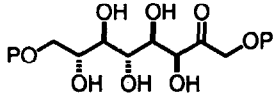
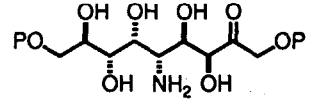
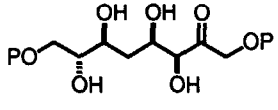
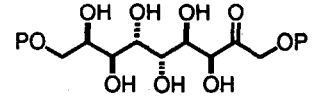
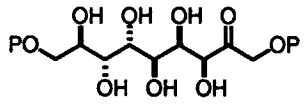
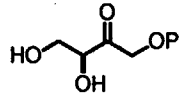
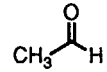
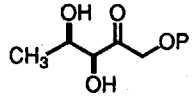
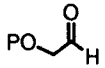
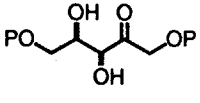
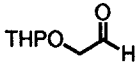
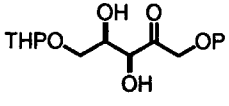
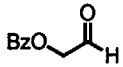
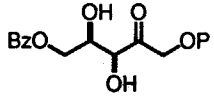
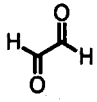
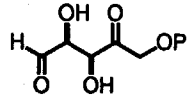
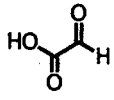
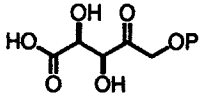
Electrophile	Product	Reference	Electrophile	Product	Reference
D-Threose		88	L-Arabinose		70
L-Threose		72	D-Arabinose-5-P		69,75, 76,80
D-Erythrose		82	D-Lyxose		70
D-Erythrose-4-P		89,91	D-Xylose		70
L-Erythrose		79	D-Glucose-6-P		69
D-Ribose		70,74	2-Deoxy-D-Glucose-6-P		69
D-Ribose-5-P		69,75,76,80	D-Glucosamine-6-P		69
2-Deoxy-Ribose-5-P		69	D-Galactose-6-P		69

Table 1 (con't).

Electrophile	Product	Reference
D-Mannose-6-P		69
		92
		77
		72
		50
		50
		83
		83

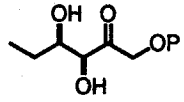
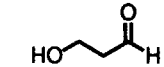
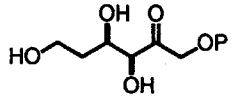
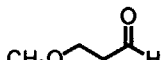
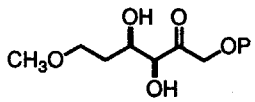
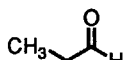
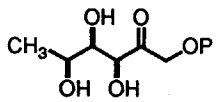
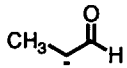
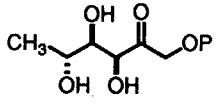
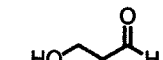
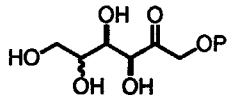
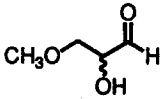
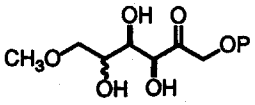
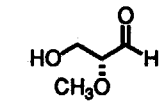
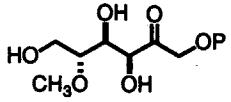
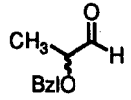
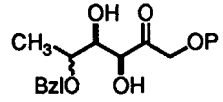
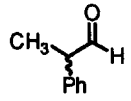
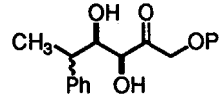
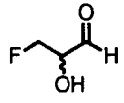
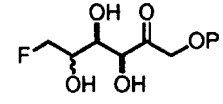
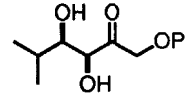
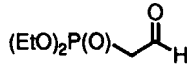
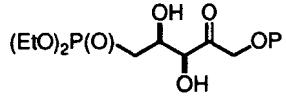
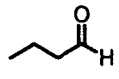
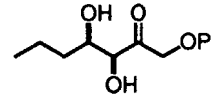
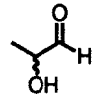
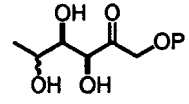
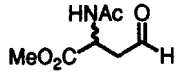
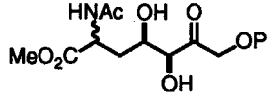
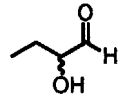
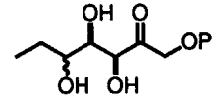
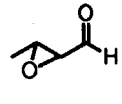
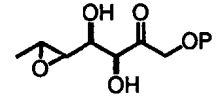
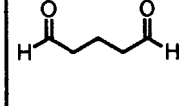
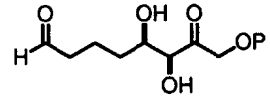
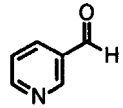
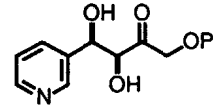
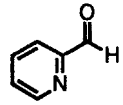
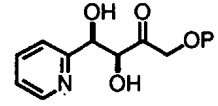
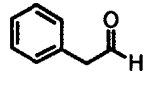
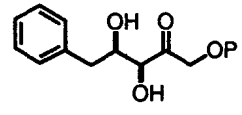
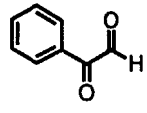
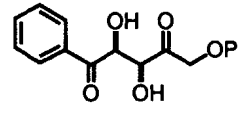
Electrophile	Product	Reference
		90,50
		68
		90
		67,68
		67
		66,68, 86
		68
		50

Table 1 (con't).

Electrophile	Product	Reference
		50
		50
		68
		83
		83
		50,83
		65,85,86
		84

Electrophile	Product	Reference
		67
		83
		83
		83
		83
		83
		83

reaction does not go to completion, the presence of excess FDP complicates the isolation

Table 2. Relative Rates of DHAP Analogues R_1COR_2 with G-3-P in RAMA-Catalyzed Aldol Condensations.[a]

R_1	R_2	V_{rel}
$HOCH_2$	CH_2OP_i	100
$HOCH_2$	$CH(CH_3)OP_i$	10
$HOCH_2$	$CH_2CH_2PO_3H_2$	10
$HOCH_2$	CH_2SO_3H	< 0.1
$HOCH_2$	CH_2OH	< 0.1
H_3C	CH_2OP_i	< 0.1
N_3CH_2	CH_2OP_i	< 0.1
$AcNHCH_2$	CH_2OP_i	< 0.1
$HO(CH_3)CH$	CH_2OP_i	< 0.1
$ClCH_2$	CH_2OP_i	0
$BrCH_2$	CH_2OP_i	0
ICH_2	CH_2OP_i	0

[a] Relative velocities were measured in 0.2 M triethanolamine buffer (pH 7.0, 25 °C) containing 50 mM substrate.

of products. The use of pure preparations of DHAP (rather than an equilibrium mixture of DHAP and G-3-P derived from FDP) also favors the formation of products. The synthetic route based on chemical phosphorylation of the dihydroxyacetone dimer gives the highest purity DHAP (95%), although the required eight steps give the lowest overall chemical yield (34%). Enzymatic phosphorylation of dihydroxyacetone gives DHAP of slightly lower purity (87%), but the procedure requires only one step, and gives a satisfactory chemical yield (83%).

Both arsenate and vanadate form kinetically labile esters with a variety of alcohols in aqueous solution. This phenomenon has been used to avoid the use of phosphates as substrates for a variety of enzymes, including phosphoketolase,[95] G-3-P dehydrogenase,[96] glucose-6-phosphate dehydrogenase,[97] and RAMA.[98] A transient substrate-arsenate/vanadate ester probably forms, followed by an enzyme-catalyzed reaction. This methodology avoids the often difficult preparation of phosphate substrates. This strategy has been used synthetically in a RAMA-catalyzed system, using a mixture of dihydroxyacetone and inorganic arsenate.[68] The toxicity of arsenate limits the attractiveness of this method. Arsenate could not be replaced with inorganic vanadate.[68]

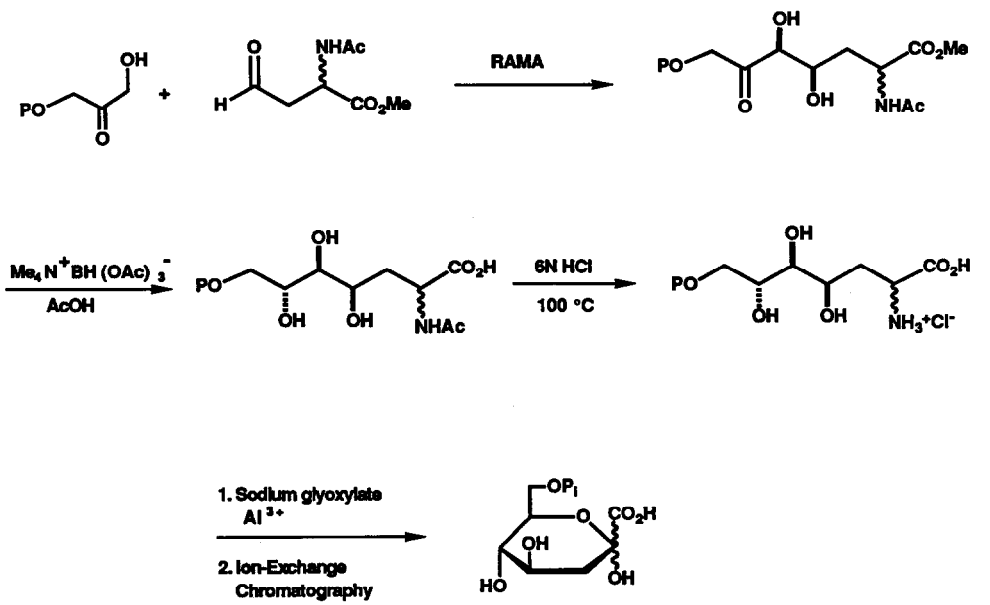
The phosphate group of aldol adducts facilitates their purification by ion-exchange chromatography or by precipitation as the barium or silver salts. Either enzymatic (using acid or alkaline phosphatase, E.C. 3.1.3.2 and 3.1.3.1 respectively) or chemical methods allow cleavage of this group.[50]

Enzyme characteristics.

Several characteristics of RAMA make this enzyme useful for forming carbon-carbon bonds. Commercial preparations of the enzyme are inexpensive ($0.04/U) and have a reasonable specific activity (60 U/mg of protein). RAMA requires no metal ions or cofactors, is stable in the presence of oxygen and added organic cosolvents, and is not inhibited to a significant extent by the natural product, FDP. The enzyme has been used both in soluble and immobilized forms, as well as enclosed within a dialysis membrane.[50]

Examples of the Use of RAMA in Synthesis.

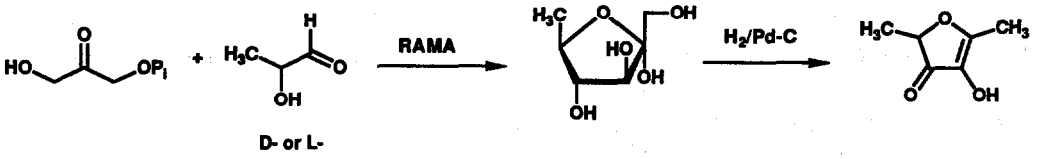
Many biologically-active monosaccharides contain vicinal diols having the stereochemistry generated by RAMA. For example, 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) is an important intermediate in the biosynthesis of aromatic amino acids in plants (the shikimate pathway). This compound has been produced in a combined chemical and enzymatic synthesis from racemic *N*-acetyl-aspartate β-semialdehyde and dihydroxyacetone phosphate (Scheme 2).[84] The four-step synthesis proceeds in an overall



Scheme 2.

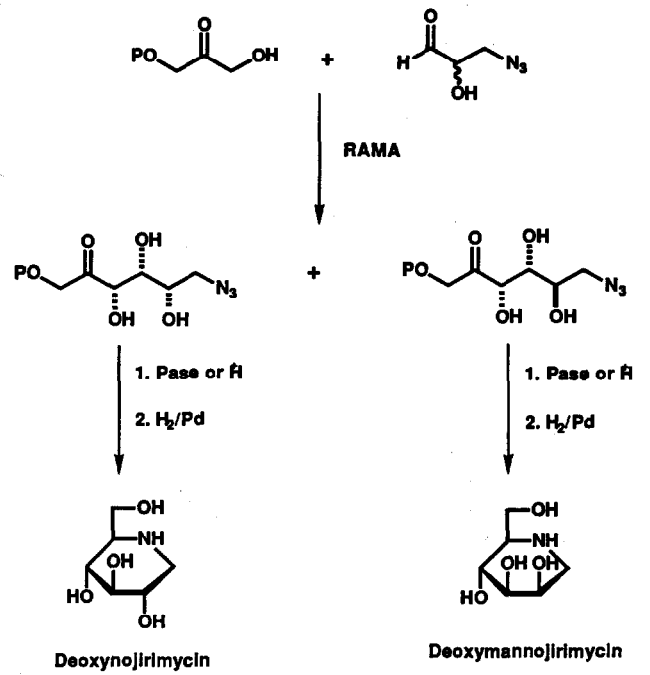
yield of 13% (37% for the enzymatic step). The key step generates the required, enantiomerically pure, *threo* stereochemistry by using RAMA as a catalyst. In view of the broad range of substrates tolerated by RAMA, this method should be applicable to the production of analogues of DAHP.

RAMA has been used to prepare other useful compounds on a preparative scale. RAMA-catalyzed addition of DHAP to lactaldehyde generated 6-deoxyfructose. This compound was then converted chemically to Furaneol,[99] a caramel flavor component (Scheme 3).[67] RAMA has also been utilized by two groups to prepare nitrogen-containing



Scheme 3.

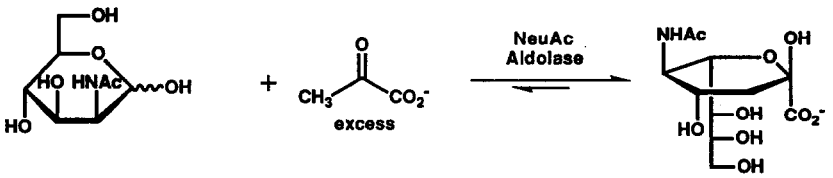
sugar analogues (Scheme 4).[100,101] The products of the enzyme-catalyzed reaction were converted to deoxynojirimycin and deoxymannojirimycin, both potent glycosidase inhibitors.[102]



Scheme 4.

***N*-Acetylneuraminic acid aldolase.**

N-Acetylneuraminic acid aldolase (NeuAc aldolase, E.C. 4.1.3.3) catalyzes the reversible aldol condensation of pyruvate and *N*-acetylmannosamine (ManNAc) to form *N*-acetylneuraminic acid (NeuAc, *N*-acetyl-5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid).[103-108] *In vivo* the enzyme has a catabolic function and the equilibrium for this reaction is near unity (Scheme 5), although the presence of excess pyruvate shifts this equilibrium to favor the aldol product. NeuAc and other derivatives of neuraminic acid are termed sialic acids. These compounds, found at the termini of



Scheme 5.

mammalian glycoconjugates, play an important role in biochemical recognition.[109-112] The production of analogues of NeuAc is of interest to synthetic and medicinal chemists. The synthesis of NeuAc itself is only of moderate interest because, although many chemical syntheses of NeuAc exist (see leading references in ref. 108), most commercial suppliers isolate NeuAc from natural sources (eg. from cow's milk in the Snow Brand process). The enzymatic approach has not been fully explored but it may be a practical alternative to the chemical synthesis of certain sialic acids, and a good route to isotopically labeled materials.

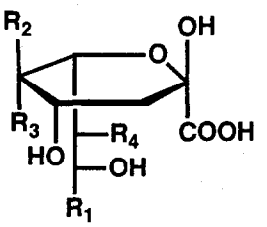
Laboratory-scale reactions catalyzed by NeuAc aldolase routinely produce multi-gram quantities of pure NeuAc.[56,108,113-116] Although the specificity for pyruvate appears to be absolute, enzymatic assays suggest that NeuAc aldolase accepts a range of substrates in place of ManNAc.[104,105,113-121,142] Several groups have taken advantage of this observation to synthesize and isolate derivatives of NeuAc (Table 3). General observations from these syntheses and from assay results suggest that NeuAc aldolase will be quite useful in synthesis: substitution at C-2 and C-6 in ManNAc is readily tolerated, and the enzyme exhibits only a slight preference for defined stereochemistry at other centers. One report describes the isolation of a mixture of derivatives of KDO and 4-epi-KDO from NeuAc aldolase-catalyzed condensation of Ara and pyruvate (see Scheme 6 for the structure of KDO).[142]

In addition to its acceptance of unnatural substrates, several other characteristics make NeuAc aldolase a useful catalyst for synthesis. The cloning of the enzyme[123] has reduced its cost. Techniques of genetic engineering offer the potential to produce in large quantity new proteins with improved stability or with altered substrate specificity. Although the optimal pH for activity of NeuAc aldolase is near pH 7.5 at 37 °C, the enzyme is active between pH 7 and 9.[108] The protein is stable in the presence of oxygen, and does

not require added cofactors.[108] One drawback is that an excess of pyruvate (the less expensive reagent) must be used in synthetic reactions to shift the equilibrium towards the formation of product; approximately seven equivalents of pyruvate are needed to attain ~90% conversion of ManNAc to NeuAc at equilibrium. It may be possible to avoid the need for an excess of pyruvate by coupling the synthesis of NeuAc to a more thermodynamically favored process.

An advantage of the enzymatic route compared with chemical routes is that purification of NeuAc or its derivatives may be avoided. For example, an unpurified solution of NeuAc generated enzymatically from an unpurified solution of ManNAc was used in the enzymatic synthesis of cytidine 5'-monophospho-NeuAc (CMP-NeuAc, see Scheme 17). The unpurified preparation of ManNAc was derived from base-catalyzed epimerization of the much less expensive starting material, GlcNAc.[108]

Table 3. Sialic acids synthesized by condensation of pyruvate with analogues of ManNAc in the presence of NeuAc aldolase. Other products have been reported based on enzymatic assay but not characterized.



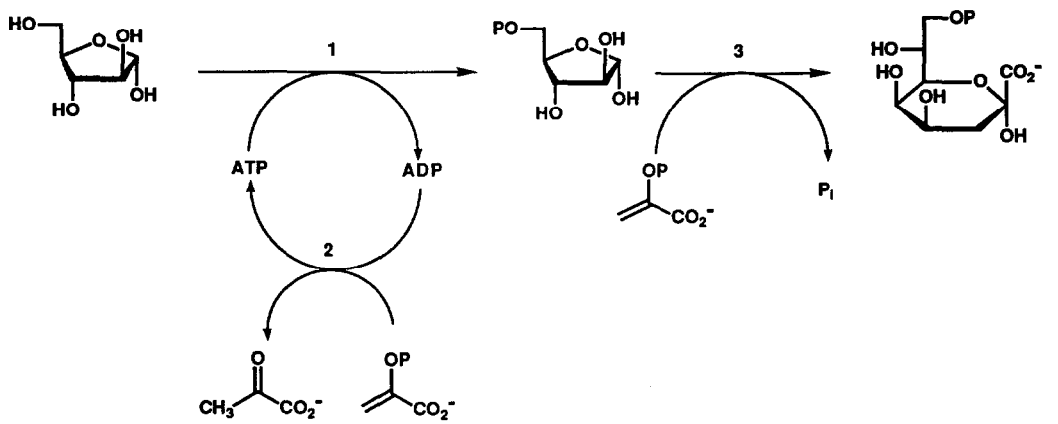
R_1	R_2	R_3	R_4	Reference
CH_2N_3	NHAc	H	OH	121
CH_2OCH_3	NHAc	H	OH	119
CH_2OAc	NHAc	H	OH	115, 119, 120
$CH_2OCOCHOHCH_3$	NHAc	H	OH	119
CH_2OAc	$NHCOCH_2OH$	H	OH	119
CH_2OH	H	H	OH	119, 122, 142
CH_2OH	OH	H	OH	119, 122, 142
CH_2OH	N_3	H	OH	142
CH_2OH	$NHCOCH_2OH$	H	OH	119
CH_2OH	$NHCOCH_2OCOCH_3$	H	OH	119
H	OH	H	OH	119, 122, 142
H	H	OH	OH	142 [a]
CH_2OH	H	OH	OH	142 [a]
CH_2OH	NHAc	H	OCH_3	119
CH_2OH	OH	H	H	142

[a] Characterized as the peracetylated methyl ester; diastereomeric purity not proven.

KDO Synthetase.

KDO synthetase (E.C. 4.1.2.16) synthesizes KDO-8-P (3-deoxy-D-*manno*-2-octulosonate-8-phosphate) from arabinose 5-phosphate (Ara-5-P) and PEP.[124] KDO synthetase is not commercially available but has been isolated from *E. coli* and used in the synthesis of KDO-8-P (Scheme 6, 63% from Ara-5-P, 38 mmol).[125] KDO-8-P and its biologically activated form, CMP-KDO, are key intermediates in the synthesis of the lipopolysaccharide (LPS) region of Gram-negative bacteria. Inhibitors of LPS biosynthesis are targets in the design of antimicrobial pharmaceuticals.[126-128] Although the substrate specificity of the enzyme has not been examined, enzyme-catalyzed syntheses may also allow the preparation of analogues of KDO-8-P.

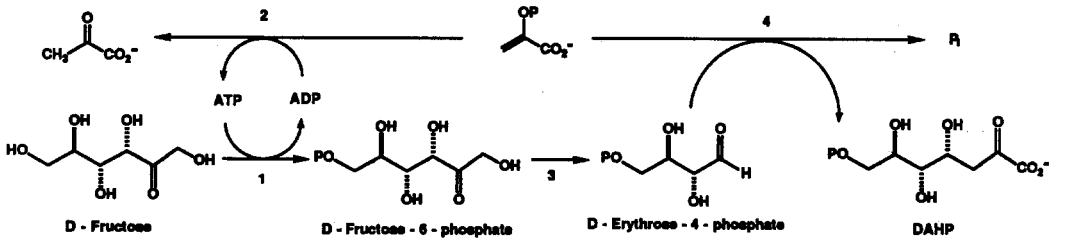
In the synthesis of KDO-8-P outlined in Scheme 6, arabinose 5-phosphate was generated from arabinose by hexokinase-catalyzed phosphorylation of arabinose. The fact that arabinose is not a natural substrate for hexokinase was not a limiting factor because large quantities of inexpensive hexokinase could be used, confined within a dialysis membrane (MEEC).[56]



Scheme 6. Synthesis of KDO-8-P. 1 = hexokinase; 2 = pyruvate kinase; 3 = KDO synthetase.

DAHP Synthetase.

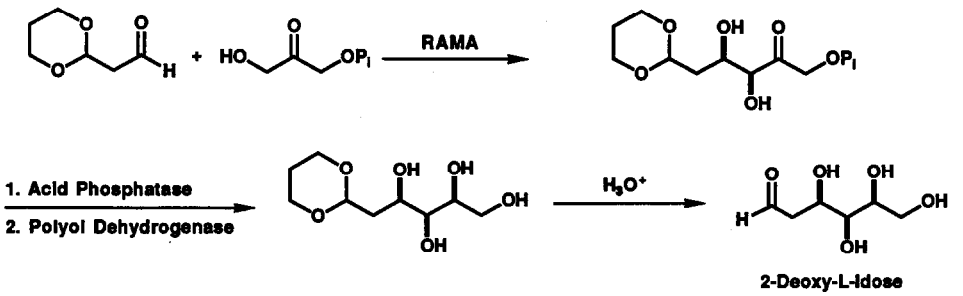
DAHP synthetase (E.C. 4.1.2.15) produces DAHP (3-deoxy-D-*arabino*-heptulsonic acid 7-phosphate) from D-erythrose 4-phosphate. The enzyme was used by Frost to synthesize DAHP (Scheme 7) as an intermediate in the chemical synthesis of its phosphonate analogue, 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphonate (DAH phosphonate), a potential inhibitor of the shikimate pathway.[129]



Scheme 7. 1 = hexokinase; 2 = pyruvate kinase; 3 = transketolase; 4 = DAHP synthetase.

Alternative Stereochemical Configurations.

A potential limitation with the use of aldolases for the synthesis of monosaccharides is that the stereochemistry at C-3 and C-4 is fixed. In the case of RAMA this stereochemistry is always D-*threo*. There are, however, methods of installing this stereochemistry at other centers. One technique, referred to as inversion, makes use of a mono-protected dialdehyde.[130] This technique is shown in Scheme 8 for the synthesis of L-xylose. After the RAMA-catalyzed aldol reaction, the resulting ketone is reduced stereospecifically with polyol dehydrogenase (E.C. 1.1.1.14). The remaining aldehyde is then deprotected, to yield a new aldose. The stereochemistry at C-3 and C-4 is now controlled by the stereochemistry of the aldehyde substrate.



Scheme 8.

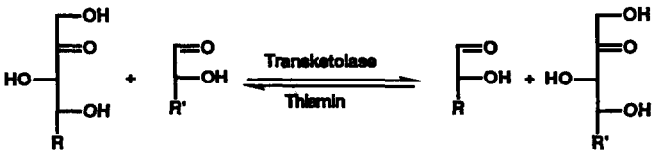
A second strategy to control stereochemistry is to use an aldolase other than RAMA. A large number of aldolases have been isolated and characterized.[50,62,63,65,113,131-150] These enzymes and the reactions they catalyze are shown in Table 4. Several stereochemical series are available through the use of the appropriate enzyme. Limited explorations of substrate specificity have been made in certain cases, and their use in synthetic organic chemistry has now been reported.[151] Work in this field has been hindered by an inavailability of the enzymes. Modern techniques in molecular biology should, however, alleviate this problem during the next several years. A bacterial FDP-aldolase has now been cloned and overexpressed in *E. coli*.[151] Many of the enzymes shown

in Table 4 are from microbiological sources, which will facilitate straightforward cloning and overexpression.

Transketolase/Transaldolase.

Transketolase (E.C. 2.2.1.1, D-sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate glycoaldehyde-transferase) has been used to extend stereospecifically the chain of a variety of aldoses by two carbon units.

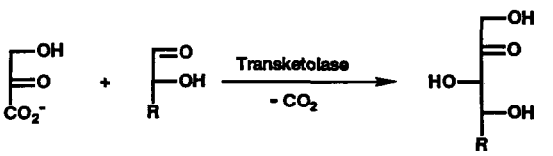
In the pentose pathway, the enzyme catalyzes the reversible transfer of a hydroxyketo group from a ketose phosphate to an aldose phosphate.[152,153] The cofactor thiamin pyrophosphate (TPP) is associated with the enzyme and activates the ketone (Scheme 9). Most donors of the -$COCH_2OH$ group (xylulose 5-phosphate, sedoheptulose 7-phosphate, fructose 6-phosphate, L-erythrose) have a *threo* arrangement of hydroxyl



Scheme 9.

groups at C-3 and C-4; hydroxypyruvate is an exception.[154] A range of aldehydes (such as D-glyceraldehyde 3-phosphate, D-ribose 5-phosphate, D-erythrose 4-phosphate, glycoaldehyde) are known as acceptors. Transketolase has been used in synthesis with its natural substrates (see leading references in ref. 155) and has been used to prepare ^{14}C-labeled intermediates of the pentose pathway.[156]

Although the substrate specificity of transketolase has not been thoroughly explored, it appears to be a promising catalyst for use in synthesis. Hydroxypyruvate can replace the ketose, providing a reactive hydroxyketo group after spontaneous decarboxylation of hydroxypyruvate; TPP is not required. The hydroxyketo group is transferred to the acceptor aldehyde in an irreversible reaction (Scheme 10). This method has allowed the synthesis of the synthesis of a number of monosaccharides on scales of ~ 2 - 5 mmol with yields ranging from 24 - 85% (Table 5).[155,157] Transketolase is commercially available and has been immobilized.[158]



Scheme 10.

Table 4. Aldolases Identified to Date and the Reactions They Catalyze *In Vivo*.

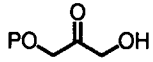
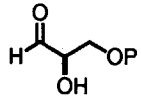
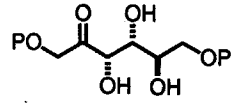
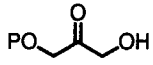
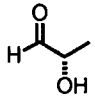
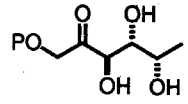
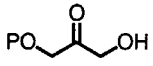
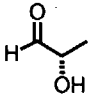
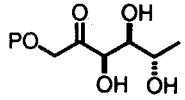
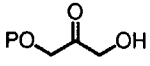
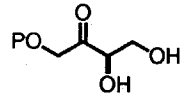
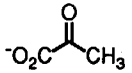
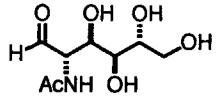
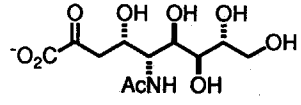
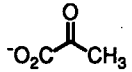
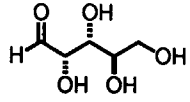
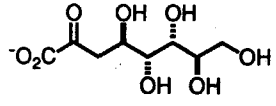
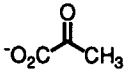
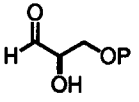
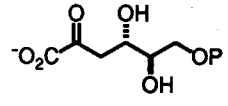
Nucleophile	Electrophile	Enzyme	Product	Reference
		Fructose 1,6-diphosphate aldolase (RAMA, E.C. 4.1.2.7)		65,131,132
		Fuculose 1-phosphate aldolase (E.C. 4.1.2.17)		133
		Rhamnulose 1-phosphate aldolase (E.C. 4.1.2.19)		134
		Erythulose 1-phosphate aldolase (E.C. 4.1.2.2)		135
		Neuraminic acid aldolase (E.C. 4.1.3.3)		108,136,138
		2-Keto-3-deoxyoctanoate aldolase (E.C. 4.1.2.23)		137
		2-Keto-3-deoxy-6-phosphogluconate aldolase (E.C. 4.1.2.14)		138

Table 4 (con't).

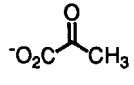
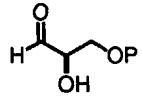
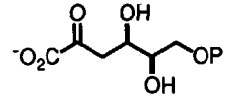
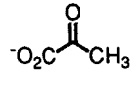
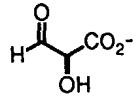
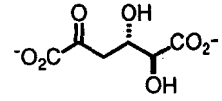
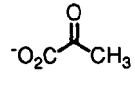
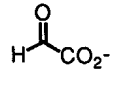
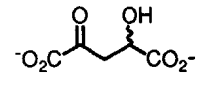
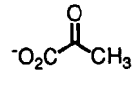
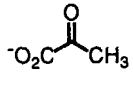
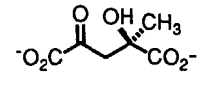
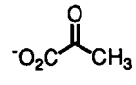
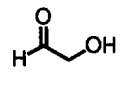
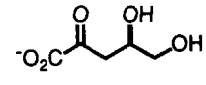
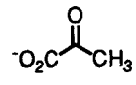
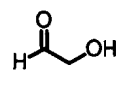
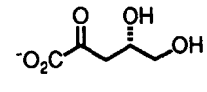
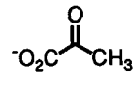
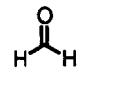
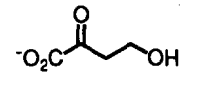
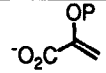
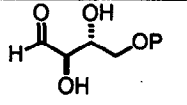
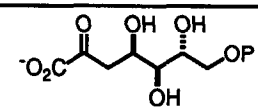
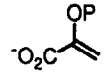
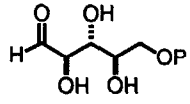
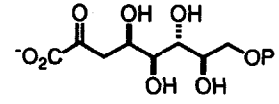
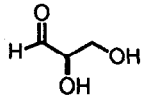
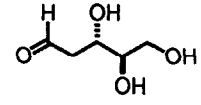
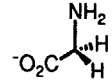
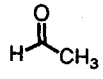
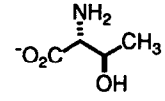
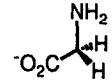
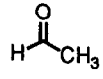
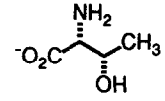
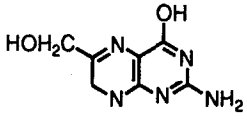
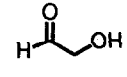
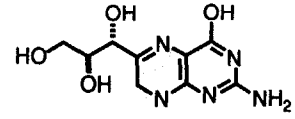
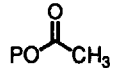
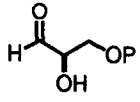
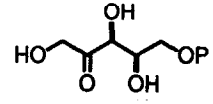
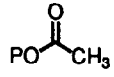
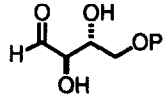
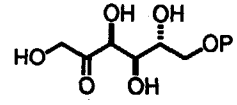
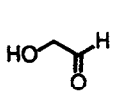
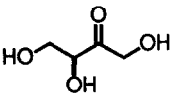
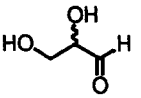
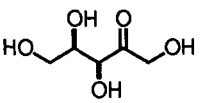
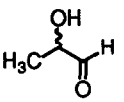
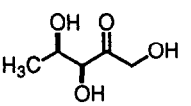
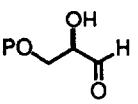
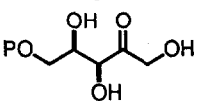
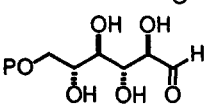
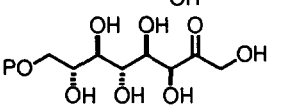
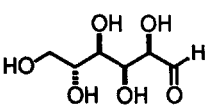
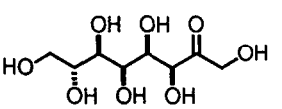
Nucleophile	Electrophile	Enzyme	Product	Reference
		2-Keto-3-deoxy-6-phosphogalactonate aldolase (E.C. 4.1.2.21)		138
		2-Keto-3-deoxy-D-glucarate aldolase (E.C. 4.1.2.20)		138
		2-Keto-4-hydroxyglutarate aldolase (E.C. 4.1.2.31)		138
		4-Methyl-4-hydroxy-2-ketoglutarate aldolase		138
		2-Keto-3-deoxy-L-arabinoate aldolase (E.C. 4.1.2.18)		138
		2-Keto-3-deoxy-D-pentanoate aldolase (E.C. 4.1.2.28)		139
		Hydroxybutyrate aldolase (E.C. 4.2.1.1)		140

Table 4 (con't).

Nucleophile	Electrophile	Enzyme	Product	Reference
		Phosphono-2-keto-3-deoxyheptanoate aldolase (E.C. 4.2.1.15)		141
		Phosphono-2-keto-3-deoxyoctanoate aldolase (E.C. 4.2.1.16)		138
		Deoxyribose aldolase (E.C. 4.1.2.4)		143
		Threonine aldolase (E.C. 4.1.2.5)		144[a]
		Serine hydroxymethytransferase (Allothreonine aldolase, E.C. 4.1.2.6)		145
		Dihydroneopterin aldolase (E.C. 4.1.2.25)		146[a]
		Phosphoketolase/TPP (E.C. 4.1.2.9)		147
		Fructose-6-phosphate phospho-ketolase/TPP (E.C. 4.1.2.22)		148

[a] Equilibrium favors reactants, or equilibrium constant not reported.

Table 5. Products synthesized using transketolase according to Scheme 10.

Substrate	Product	Reference
		155
		155
		155
		157
		156
		156

Enzymes Useful in the Synthetic Manipulation of Monosaccharides that are not Aldolases.

Other enzymes in addition to aldolases are useful catalysts for the construction and manipulation of monosaccharides.

Kinases. Phosphorylated sugars are important as chemically-activated intermediates in many biochemical pathways. As such, these compounds are often intermediates or targets in synthetic procedures. In biochemical systems, the kinases catalyze the transfer of phosphate groups to and from nucleoside triphosphates and sugars. The following examples illustrate the use of kinases in the synthesis of phosphorylated monosaccharides.

Much of the utility of the kinases in organic synthesis is based on the observation that these enzymes often accept unnatural substrates. Hexokinase (E.C. 2.7.1.1), for

example, accepts other sugars in place of glucose.[159] This breadth of specificity allows the multi-gram synthesis of Ara-5-P, the precursor to KDO-8-P (Scheme 6 above).[125] Acetate kinase (E.C. 2.7.2.1) and pyruvate kinase (E.C. 2.7.1.40) are key components of cofactor recycling systems.[61] Pyruvate kinase also accepts other nucleoside diphosphates in addition to adenosine 5′-diphosphate (ADP) at synthetically useful rates, allowing, for example, the generation of cytidine 5′-triphosphate (CTP) (Scheme 13),[160,161] uridine 5′-triphosphate (UTP),[161] and ribavirin 5′-triphosphate[162] from the corresponding monophosphate and PEP, using adenylate kinase (E.C. 2.7.4.3). The nucleoside monophosphate kinases (E.C. 2.7.4.4) and nucleoside diphosphate kinases (E.C. 2.7.4.6), although expensive and relatively unstable,[62] have been used to generate nucleoside triphosphates.[161,163,164] Glycerol kinase (E.C. 2.7.1.30) is useful for the production of DHAP for use in reactions catalyzed by aldolase as well as for the synthesis of a number of unnatural phosphorylated analogues of glycerol.[94,165]

Oxidoreductases. The class of oxidoreductase enzymes is generally less important in the synthesis of carbohydrates than for the installation of critical stereocenters in the production of chiral building blocks, such as R- or S-butene oxide.[166] The utility of only a few enzymes in this class has been explored in the context of carbohydrate synthesis. Polyol dehydrogenase (E.C. 1.1.1.14) is relatively non-specific and may be widely useful in synthesis for the stereospecifc oxidation and reduction of ketones.[167-169] Galactose oxidase (E.C. 1.1.3.9) suffers from severe product inhibition and has not yet proved to be a useful catalyst for synthesis.[170] There are, however, several strategies to overcome inhibition of enzyme-catalyzed reactions.[171] Oxidoreductases have also been used to install required stereochemistries in monosaccharide precursors.[178]

Isomerases/Epimerases. Glucose isomerase is used to produce millions of killograms of high fructose corn syrup (HFCS) annually,[173] but has been used only to a limited extent in organic synthesis. The enzyme has been used to catalyze the isomerization of products from condensations catalyzed by aldolase,[70] and to produce analogues of fructose from analogues of glucose in the synthesis of disaccharides.[174,175] UDP-Galactose epimerase catalyzes the epimerization of UDP-glucose to UDP-galactose, and is of use in the synthesis of *N*-acetyllactosamine (see Scheme 16).[176]

Hydrolytic enzymes. The enzymatic synthesis of carbohydrates generally avoids the need for protection and deprotection chemistry. Nevertheless, enzyme-catalyzed methods for the selective hydrolysis and transesterification of carbohydrates exist.[177,179-181]

3. Oligosaccharides.

Introduction.

Oligosaccharides and polysaccharides are important classes of biologically occurring structures.[1-5] Oligosaccharides containing fewer than ~20 sugars are the subject of this section, and polysaccharides of the following section.

In mammalian systems, eight monosaccharides activated in the form of nucleoside mono- and diphosphate sugars are the building blocks for most oligosaccharides: UDP-Glc, UDP-GlcUA, UDP-GlcNAc, UDP-Gal, UDP-GalNAc, GDP-Man, GDP-Fuc, and CMP-NeuAc.[2,184] These are the intermediates of the Leloir pathway. A much larger number of sugars (eg. xylose, arabinose) and oligosaccharides are present in microorganisms, plants and insects: we will not discuss these substances, although biosyntheses involving them usually follow the same principles as those in mammalian biochemistry. Other monosaccharides are found in mammalian systems but these sugars (for example iduronic acid and *N*- and *O*-sulfated or acylated sugars), found mainly in polymers, do not occur as nucleoside phosphates, but result from postpolymerization modification of other sugars.[5] *In vivo*, oligosaccharides are usually covalently attached to lipids or proteins during and after biosynthesis.

The three major classes of glycolipids are glycosphingolipids, glycoglycerolipids, and the dolichol-bound intermediates formed during the synthesis of *N*-linked glycoproteins. Of these, glycosphingolipids predominate in mammalian systems.[1]

There are two important classes of glycoproteins, differing from each other in their attachment to the peptide chain: *O*-linked glycoproteins usually contain an *O*-glycosidic linkage between GalNAc and serine (Ser) or threonine (Thr); *N*-linked glycoproteins contain *N*-glycosidic linkages between GlcNAc and the amide moiety of asparagine (Asn).

The basic biosynthetic routes to these two classes of glycoproteins differ, although the initial formation of the bond between protein and sugar is cotranslational in each. The synthesis of *O*-linked glycoproteins involves the stepwise addition of activated sugars (usually starting with GalNAc) directly to a protein, to a serine or threonine residue (Scheme 11). The synthesis of *N*-linked glycoproteins is more involved.[183,184]

Protein-Ser/Thr → Protein-Ser/Thr-GalNAc → → *O*-linked glycoproteins

Scheme 11.

First, an oligosaccharide of composition DolPP(GlcNAc)$_2$Man$_9$Glc$_3$ is built in a stepwise manner onto dolichol phosphate (DolP), a polyisoprenoid lipid. This entire oligosaccharide is transferred to Asn, at a site characterized by the sequence Asn-X-Ser(Thr), where X is not Pro or Asp. Removal (trimming) of the glucose residues and most of the mannose by several glycosidases leaves the inner core exposed (peptide-Asn-

GlcNAc-GlcNAc-(Man)3). Elaboration of this core by transfer of sugars from nucleoside mono- and diphosphate sugars provides mature glycoproteins (Scheme 12).[185] A similar synthetic strategy involving the assembly of a lipid-bound intermediate is also important in the biosynthesis of bacterial cell walls.[2]

Dol-P → DolPP-GlcNAc → DolPP-GlcNAcGlcNAc(Man)9(Glc)3 →

Protein-Asn-GlcNAcGlcNAc(Man)9(Glc)3 → Protein-Asn-GlcNAcGlcNAc(Man)3

→ → *N*-linked glycoproteins

Scheme 12.

There are two groups of enzymes used for synthesizing oligosaccharides *in vivo*, and both use prior activation of the sugar monomer by phosphorylation. The largest group, the enzymes of the Leloir pathway, transfer sugars activated as sugar nucleoside phosphates to the growing oligosaccharide chain.[2,186] Non-Leloir pathway enzymes transfer carbohydrate units activated as sugar phosphates, but not as sugar nucleoside phosphates.[2]

There are two strategies available for enzyme-catalyzed *in vitro* synthesis of oligosaccharides. The first uses the glycosyl transferases (EC 2.4.), which are used *in vivo* to elaborate oligo- and polysaccharides. The second strategy uses the glycosidases, or glycosyl hydrolases (EC 3.2.). *In vivo*, these enzymes have a catabolic function, and cleave glycosidic linkages to form mono- or oligosaccharides. They can be used synthetically to form glycosidic linkages, however, in the presence of a suitable nucleophile other than water.

Glycosyl Transferases.

Leloir Pathway Glycosyl Transferases.

Biochemical Synthesis.

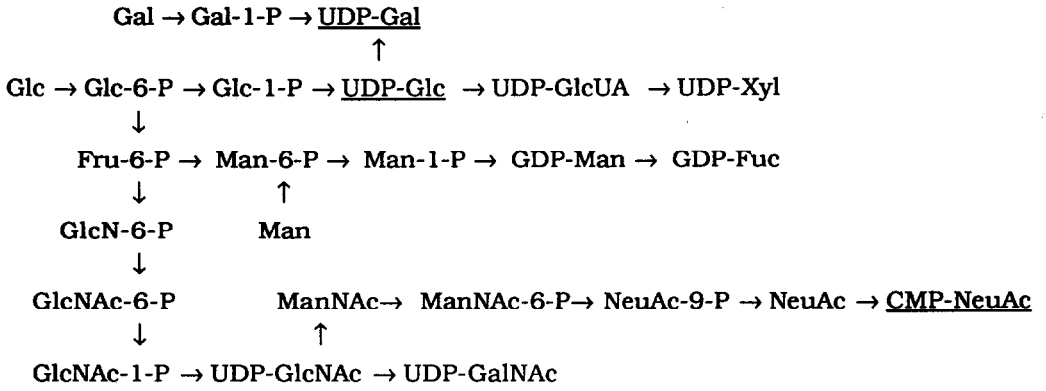
Three fundamental steps constitute the Leloir pathway: activation, transfer, and modification.[2-4,187] These steps represent biological solutions to the problems also faced by chemists: chemical activation of sugars, stereospecific and regioselective formation of glycosidic linkages, and elaboration of products.

In the first steps of the Leloir pathway for all sugars except NeuAc and its derivatives, a sugar (glucose, galactose or mannose) is transformed into a sugar-1-phosphate (sugar-1-P) by a kinase (E.C. 2.7.). This sugar-1-P reacts with a nucleoside triphosphate (NTP) in an enzyme-catalyzed reaction (scheme 13) and forms a chemically activated nucleoside diphosphate sugar, UDP-Glc, UDP-GlcNAc, UDP-Gal, or GDP-Man (Scheme 14, from ref. 2, p. 129). These enzymes are known as pyrophosphorylases, or nucleoside transferases (E.C. 2.7.)

$$\text{sugar-1-P} + \text{NTP} \rightarrow \text{NDP-sugar} + PP_i$$

Scheme 13.

Subsequent transformations of these key nucleoside diphosphate sugars lead to the remaining activated sugars (Scheme 14). In some cases, more than one route may lead to the same sugar nucleotide. For example, UDP-Glc epimerase (E.C. 5.1.3.2) converts UDP-Glc into UDP-Gal. UDP-Gal is also synthesized directly from galactose.



Scheme 14. Biosynthesis of nucleoside phosphate sugars used in the Leloir pathway in mammalian systems. Underlined nucleoside phosphate sugars have been prepared on a greater than 1-g scale in cell-free enzyme-catalyzed reactions.

The activation of sialic acid (NeuAc) is an exception: a nucleoside monophosphate sugar forms directly from NeuAc (Scheme 15).

$$\text{NeuAc} + \text{CTP} \rightarrow \text{CMP-NeuAc} + PP_i$$

Scheme 15.

A second class of enzymes (the glycosyltransferses) catalyze the addition of the activated sugars in a stepwise fashion to a protein or lipid, or to the non-reducing end of a growing oligosaccharide. For glycoproteins, these steps occur in the rough endoplasmic reticulum and in the Golgi apparatus. A large number of glycosyl transferases appear to be necessary: each NDP-sugar requires a distinct class of glycosyltransferases and each of the more than 100 glycosyltransferases identified to date appears to catalyze the formation of a unique glycosidic linkage. Exact details concerning the specificity of the glycosyltransferases are not known. It is not clear, for example, what sequence of carbohydrates is recognized by most of these enzymes.[188]

Modification of *N*-linked glycoproteins occurs in two steps: specific glycosidases catalyze the trimming of sugar residues, then the residual oligosaccharide is elaborated by several glycosyl transferases. The synthesis of inhibitors (eg. nojirimycin, castanospermine, swainsonine) of these glycosidases is a goal of several synthetic programs; tunicamycin represents a useful example of an inhibitor of glycosylation.[189]

Chemists have begun to apply the enzymes of the Leloir pathway to the synthesis of oligosaccharides. Two requirements are essential to the success of this approach: the availability of the sugar nucleoside phosphates at practical costs and the availability of the glycosyltransferases. The first issue is being resolved for the most common NDP~sugars (including those important in mammalian biosynthesis). Only a small number of glycosyltransferases are, however, presently available, and access to these enzymes is the limiting factor in this type of synthesis.

NDP~Sugars. Availability and Preparation.

The fact that only a small number of the known NDP~sugars[190] are used in mammalian biochemistry simplifies the problems faced by the organic chemist. Methods based on chemical synthesis[191] or on fermentation[192] exist for the preparation of most of these compounds, although a chemical preparation of CMP-NeuAc remains to be reported.[44] All of the common NDP~sugars are available from commercial sources, but most are expensive. This high cost has made large scale syntheses of oligosaccharides using glycosyl transferases impractical.

Enzymatic methods of preparing the NDP~sugars can have several advantages relative to chemical methods. For example, the NDP~sugar can be generated *in situ*, making it possible to drive thermodynamically unfavorable equilibria (such as the epimerization of UDP-Glc to UDP-Gal).[193] Purification steps may be eliminated altogether because the byproducts of enzyme-catalyzed methods often do not interfere with further enzymatic steps. Enzyme-catalyzed syntheses have produced UDP-Glc,[193] UDP-Gal,[193] and CMP-NeuAc[108] on > 1-g scale. All of the other important NDP~sugars have been made enzymatically on an analytical scale.[190]

Characteristics of Glycosyltransferases.

Availability and Stability. Most glycosyl transferases are difficult to isolate, especially from mammalian sources, because the proteins are present in low concentrations, and are membrane-bound.[188,194] From the perspective of synthesis, rigorously purified preparations of enzymes are not necessary, although the activity of certain enzymes, such as the phosphatases, must be avoided.

Glycosyltransferases are reported to be unstable,[193] although a few enzymes have been immobilized.[195,196] Only a small number of glycosyltransferases are available from commercial sources at this time, and these preparations are expensive, although many others have been isolated and characterized.[188,194] Genetic engineering (ie. cloning or modification of enzymes) will have a large impact in this area. Already several glycosyl

transferases have been cloned, including glucosyl-, fucosyl-, and sialyltransferases.[197-202] Recent advances in cloning techniques should speed the cloning of other glycosyl transferases.[201,202]

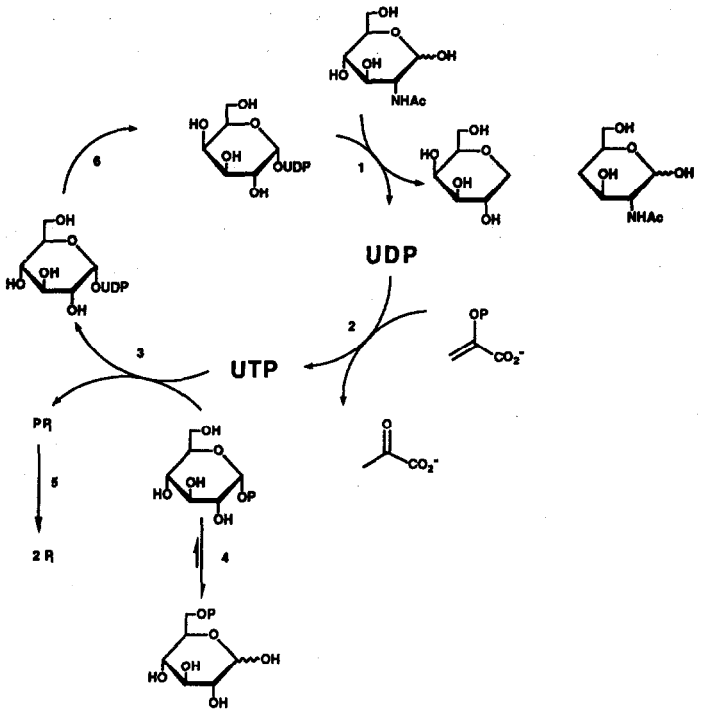
Specificity. Glycosyltransferases form regio- and stereospecific linkages between the activated donor (a nucleoside diphosphate sugar) and an acceptor (a mono- or oligosaccharide). The point of interest to synthetic chemists is the range of acceptors and donors that can be used in synthesis. Research in the biochemical community in understanding how the fidelity of oligosaccharide construction is maintained[172] and in designing inhibitors of glycoprotein synthesis[203] has shown that the specificity of the glycosyltransferases is not absolute.

UDP-Gal transferase (lactose synthetase) is the best-studied glycosyl transferase in terms of specificity for the acceptor sugar. Several groups have demonstrated that this enzyme transfers UDP-Gal to a wide range of acceptor substrates.[204-208,217] The broad substrate specificity of the enzyme has allowed the preparation and isolation of well-characterized oligosaccharides (see Table 6) Other glycosyltransferases, although less well-studied than UDP-Gal transferase, also appear to accept a range of acceptor substrates.[209,210]

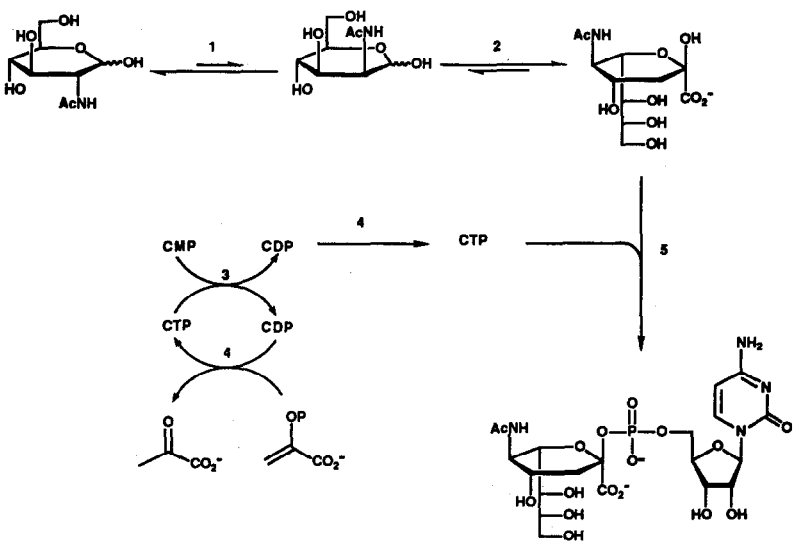
Although the glycosyltransferases are considered to be specific for the NDP~sugars, recent observations suggest that this specificity is not absolute. Several groups, for example, have transferred analogues of UDP-Gal,[211] GDP-Man[212,213] and CMP-NeuAc[164,214] to acceptor sugars. It is important to realize, however, that not all of the enzymes have been purified to homogeneity, so it is unclear whether this apparently loose specificity for donor and acceptor is real, or whether it represents activity of a mixture of several enzymes.[194] As more enzymes become available, it should be possible to evaluate better the substrate specificity of the glycosyl transferases and their potential use in the synthesis of natural and unnatural oligosaccharides.

Examples And Potential Uses.

Enzyme-catalyzed syntheses, particularly using the galactosyl- and sialyltransferases, have produced a number of oligosaccharides (Table 6).[18,114,204,215-226] Techniques of detecting, isolating, and characterizing reaction products exist, but the amount of product that can be formed is limited by the availability of the glycosyltransferases. For example, enzyme-catalyzed preparations of ~10 g of *N*-acetyl lactosamine[193] (Scheme 16) and ~3 g of CMP-NeuAc[108] (Scheme 17) were both straightforward. The addition of NeuAc to N-acetyllactosamine, however, produced only mg quantities of the trisaccharide, sialyl *N*-acetyllactosamine (Scheme 18), because of limited availability of the required sialyltransferase.[18,108,195] The techniques of solid phase synthesis, well demonstrated in the field of peptide synthesis, have been used in the synthesis of carbohydrates.[218-220]



Scheme 16. Synthesis of *N*-acetyllactosamine. The six-enzyme system generates UDP-Glc and UDP-Gal *in situ*. 1 = galactosyl transferase; 2 = pyruvate kinase; 3 = UDP-glucose pyrophosphorylase; 4 = phosphoglucomutase; 5 = inorganic pyrophosphatase; 6 = UDP-galactose epimerase.



Scheme 17. Synthesis of CMP-NeuAc from GlcNAc, CMP. PEP, and pyruvate. 1) NaOH; 2) NeuAc aldolase, pyruvate; 3) adenylate kinase; 4) pyruvate kinase; 5) CMP-NeuAc synthetase.

Table 6. Examples of oligosaccharides synthesized using glycosyltransferases.

Acceptor	Product	Scale[a]	Reference
Galactosyl transferase (Donor: UDP-Gal)			
Glc-OH	β-Gal-(1→4)-Glc-OH	C	215
GlcNAc-OH	β-Gal-(1→4)-GlcNAc-OH	A	176,[b] 215, 216
GlcNAc-agarose	β-Gal-(1→4)-GlcNAc-agarose	C	215
GlcNAc-hexanolamine	β-Gal-(1→4)-GlcNAc-hexanolamine	C	215
β-GlcNAc-1→4)-Gal-OH	β-Gal-(1→4)-β-GlcNAc-(1→4)-Gal-OH	C	215
β-GlcNAc-(1→6)-Gal-OH	β-Gal-(1→4)-β-GlcNAc-(1→6)-Gal-OH	C	114,217
β-GlcNAc-(1→3)-Gal-OH	β-Gal-(1→4)-β-GlcNAc-(1→3)-Gal-OH	C	114,217
β-Glc-$OCH_2C_6H_4(NO_2)$-CONH-polymer	β-Gal-(1→4)-β-Glc-$OCH_2C_6H_4(NO_2)$CONH-polymer	D	218-220[c]
β-Glc-(1→4)-β-Glc-OCH_2-$C_6H_4(NO_2)$-CONH-polymer	β-Gal-(1→4)-β-Glc-(1→4)-β-Glc-OCH_2-$C_6H_4(NO_2)$-CONH-polymer	D	218-220[c]
β-Glc-(1→4)-β-Glc-OCH_2-NH-L-Phe-CO-polymer	β-Gal-(1→4)-β-Glc-(1→4)-β-Glc-OCH_2-NH-L-Phe-CO-polymer	D	218-220[c]
β-GlcNAc-(1→3) \ β-Gal-(1→4)-β-Glc-OCH_3 / β-GlcNAc-(1→6)	β-Gal-(1→4)-β-GlcNAc-(1→3) \ β-Gal-(1→4)-β-Glc-OCH_3 / β-Gal-(1→4)-β-GlcNAc-(1→6)	C	114,221
β-GlcNAc-(1→3) \ β-Gal-(1→4)-β-Glc-OCH_3 / β-GlcNAc-(1→6)	β-GlcNAc-(1→3) \ β-Gal-(1→4)-β-Glc-OCH_3 / β-Gal-(1→4)-β-GlcNAc-(1→6)	C	114, 221
α-L-Fuc-(1→6)-β-GlcNAc-$O(CH_2)_8CO_2CH_3$	α-L-Fuc-(1→6) \ β-GlcNAc-$O(CH_2)_8CO_2CH_3$ / β-Gal-(1→4)	E	204

α-NeuAc-OMe-(2→ 6)- β-GlcNAc-O(CH$_2$)$_8$CO$_2$CH$_3$	α-NeuAc-(2→ 6) \ β-GlcNAc-O(CH$_2$)$_8$CO$_2$CH$_3$ / β-Gal-(1→ 4)	E	204

Sialyltransferases
(Donor: CMP-NeuAc)

2,6-Sialyltransferases

β-Gal-OCH$_3$	α-NeuAc-(2→ 6)-β-Gal-OCH$_3$	D	18
β-Gal-(1→ 4)-β-Glc-OCH$_3$	α-NeuAc-(2→ 6)-β-Gal-(1→ 4)-β-Glc-OCH$_3$	D	18
β-Gal-(1→ 4)-β-GlcNAc-OH	α-NeuAc-(2→ 6)-β-Gal-(1→ 4)-β-GlcNAc-OH	C	18,108,114,195
β-Gal-(1→ 4)-β-GlcNAc-OCH$_3$	α-NeuAc-(2→ 6)-β-Gal-(1→ 4)-β-GlcNAc-OCH$_3$	C	18
α-NeuAc(-2→ 3)-β-Gal-(1→ 3)-α-GlcNAc-OPh	α-NeuAc-(2→ 3) \ β-Gal-(1→ 3)-α-GlcNAc-OPh / α-NeuAc-(2→ 6)	C	222
β-Gal-(1→ 4)-β-GlcNAc-(1→ 3)-β-Gal(1→ 4)-Glc	α-NeuAc-(2→ 6)β-Gal-(1→ 4)-β-GlcNAc-(1→ 3)- β-Gal-(1→ 4)-Glc	C	18

2,3-Sialyltransferases

β-Gal-(1→ 4)-β-Glc-OCH$_3$	α-NeuAc-(2→ 3)-β-Gal-(1→ 4)-β-Glc-OCH$_3$	D	18
β-Gal-(1→ 4) -β-GlcNAc-OCH$_3$	α-NeuAc-(2→ 3)-β-Gal-(1→ 4)-β-GlcNAc-OCH$_3$	D	18
β-Gal-(1→ 3)-β-GlcNAc-OCH$_3$	α-NeuAc-(2→ 3)-β-Gal-(1→ 3)-β-GlcNAc-OCH$_3$	D	18
β-Gal-(1→ 3)-α-GlcNAc-OPh	α-NeuAc-(2→ 3)-β-Gal-(1→ 3)-α-GlcNAc-OPh		222
β-Gal-(1→ 3)-β-GlcNAc-(1→ 3)-β-Gal-(1→4)-Glc	α-NeuAc-(2→ 3)-β-Gal-(1→ 3)-β-GlcNAc-(1→ 3)- β-Gal-(1→ 4)-Glc	D	18
β-Gal-(1→ 3)-β-GlcNAc-O(CH$_2$)$_5$-COOCH$_3$	α-NeuAc-(2→ 3)-β-Gal-(1→ 3)-β-GlcNAc-*O*-(CH$_2$)$_5$-COOCH$_3$	D	18
β-Gal-(1→ 3)-β-GlcNAc-(1→ 3)-β-Gal-*O*-(CH$_2$)$_5$-COOCH$_3$	α-NeuAc-(2→ 3)-β-Gal-(1→ 3)-β-GlcNAc-β-Gal-*O*-(CH$_2$)$_5$-COOCH$_3$	D	209
β-Gal-(1→ 3)-β-GlcNAc-(1→ 6)-β-Gal-*O*-(CH$_2$)$_5$-COOCH$_3$	α-NeuAc-(2→ 3)-β-Gal-(1→ 3)-β-GlcNAc-(1→ 6)-β-Gal-*O*-(CH$_2$)$_5$-COOCH$_3$	D	209

Fucosyltransferases
(Donor: GDP-Fuc)

β-Gal-OCH$_2$CH$_3$	α-L-Fuc-(1→ 2)-β-Gal-OCH$_2$CH$_3$	C	223

β-Gal-(1→4)-β-GlcNAc	α-L-Fuc-(1→ 2)-β-Gal-(1→ 4)-β-GlcNAc	C	223
β-Gal-hexanolamine	α-L-Fuc-(1→ 2)-β-Gal-hexanolamine	C	223
β-Gal-(1→4)-β-GlcNAc-hexanolamine	α-L-Fuc-(1→ 2)-β-Gal-(1→ 4)-β-GlcNAc-hexanolamine	C	223
α-NeuAc-(2→ 3)-β-Gal-(1→ 3)-β-GlcNAc-(1→ 3)-β-Gal-*O*-$(CH_2)_5$-$COOCH_3$	α-NeuAc-(2→ 3)-β-Gal-(1→ 3) \ β-GlcNAc-(1→3)-β-Gal-*O*-$(CH_2)_5$-CO_2CH_3 / α-L-Fuc-(1→ 4)	D	209
α-NeuAc-(2→ 3)-β-Gal-(1→ 3)-β-GlcNAc-(1→ 6)-β-Gal-*O*-$(CH_2)_5$-$COOCH_3$	α-NeuAc-(2→ 3)-β-Gal-(1→ 3) \ β-GlcNAc-(1→ 6)-β-Gal-*O*-$(CH_2)_5$-CO_2CH_3 / α-L-Fuc-(1→ 4)	D	209

Glucosyltransferase
(*Donor: UDP-Glc*)

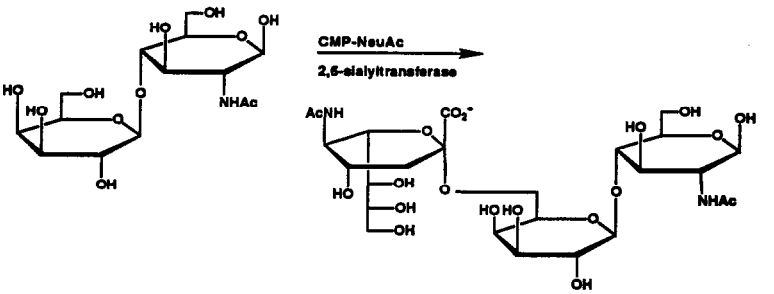
R-C_6H_4-COOH	β-Glc-OCO-C_6H_4-R		
R = H, 4-OH, 4-OMe, 3,4-diOH, 4-OH-3-OMe, 3,4-diOMe, 3,4,5-triOH, 4-OH-3,5-diOMe		C/D	224

***N*-Acetylglucosaminyltransferase**
(Donor: UDP-GlcNAc)

β-GlcNAc-(1→ 2)-α-Man-(1→6)-β-Man-$O(CH_2)_8CO_2CH_3$	β-GlcNAc-(1→ 2) \ α-Man-(1→ 6)-β-Man-$O(CH_2)_8CO_2CH_3$ / β-GlcNAc-(1→ 6)	D	225, 226

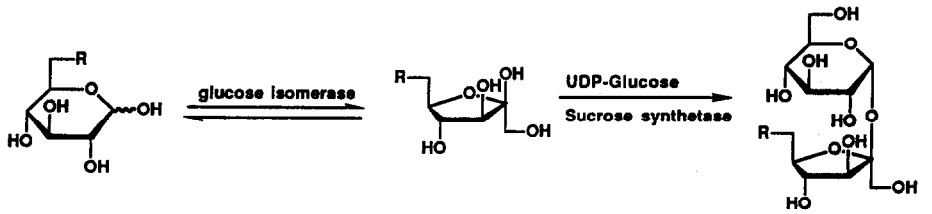
---

[a] Amount of isolated product: A: >1g; B: 100mg to 1 g; C: 10 mg to 100 mg; D: <10 mg; E: product not isolated or no yield reported.
[b] A coupled recycling system was used.
[c] A subsequent step released the product from the polymer .



Scheme 18. Synthesis of sialyl *N*-acetyllactosamine using 2,6-sialyltransferase.

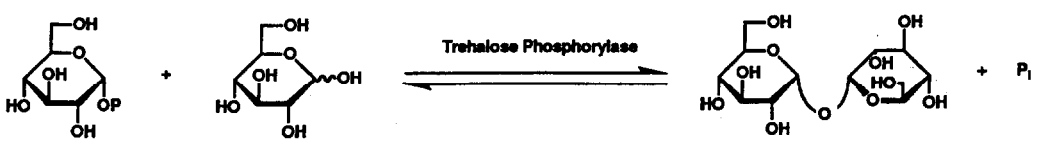
The synthesis of fluorinated carbohydrate analogues is an area of interest in both chemistry and biochemistry.[227] Card and Hitz have used sucrose synthetase to transfer glucose from UDP-glucose to 1-deoxy-1-fluorofructose, to generate 1′-deoxy-1′-fluorosucrose (Scheme 19).[174,175]



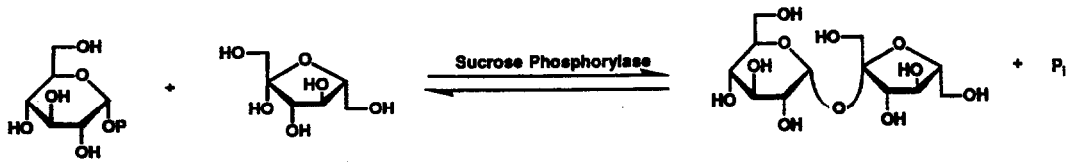
Scheme 19. Synthesis of 1′-deoxy-1′-fluorosucrose using sucrose synthetase.

Non-Leloir Glycosyltransferases.

Oligosaccharides have also been prepared by non-Leloir routes. Two particularly important examples are sucrose and trehalose (a major storage carbohydrate in plants, fungi and insects[1]). Both compounds have been successfully synthesized in cell-free enzymatic systems using routes that reverse the routes followed for their catabolism *in vivo* (Schemes 20a and 20b).[228] Other examples of this class are dextran sucrase and sucrose phosphorylase. *In vivo*, these forms of synthesis are primarily used for the preparation of carbohydrate polymers, such as the microbial levans and dextrans.[229,230] Several enzymes of this type have been used synthetically.



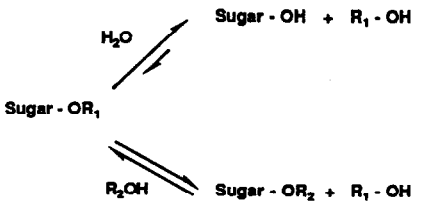
Scheme 20a. Synthesis of trehalose.



Scheme 20b. Synthesis of sucrose.

Glycosidases

The glycosidases cleave glycosidic bonds in nature (Scheme 21). Two groups of glycosidases exist; the exoglycosidases, which cleave terminal glycosidic units, and the endoglycosidases, which cleave both terminal and non-terminal glycosidic linkages in oligo- or polysaccharide chains.



Scheme 21.

In general, the glycosidases show high specificity for both the glycosyl moiety and the glycosidic linkage, but little if any specificity for the aglycon component. For example disaccharides, alkyl-, aryl-, thioalkyl-, azido- and fluoroglycosides have all been used as substrates for β-galactosidase from *E. coli*.[231]

The mechanism of hydrolysis is presumed to proceed in the same fashion as the acid-catalyzed cleavage of a glycosidic bond, via a carbocation intermediate. Hydrolysis of glycosidic linkages leads to net retention of configuration at the anomeric center. This observation suggests one of two possible mechanisms: i) a double displacement mechanism, with an enzyme-glycoside covalent intermediate, or ii) the binding and stabilization of a carbocation intermediate in such a fashion that only one face is accessible to an attacking nucleophile. The existence and nature of a covalent intermediate is still the subject of debate.[232,233]

It has long been recognized that the intermediate in the hydrolytic pathway shown in Scheme 21 can be intercepted by a second nucleophile. Thus, reaction of a carbohydrate in the presence of a nucleophile other than water can result in the formation of a new glycosidic bond: the glycohydrolase can serve as a transglycosidase. Huber and coworkers demonstrated that during the hydrolysis of lactose (β–Gal-(1→4)-Glc) by β-galactosidase, approximately 50% of the lactose molecules were converted to allolactose (β–Gal-(1→6)-Glc), either by trapping of the intermediate by the newly formed glucose, or

by an internal transfer reaction of undefined nature.[234] Bourquelot's group has reported the preparation of over 40 crystalline glycosides by reactions of this type.[235,236]

Glycosidases can be used synthetically in two modes. The first utilizes a glycosidase, the corresponding free monosaccharide, and a nucleophile. This procedure has been referred to as "direct glycosylation", or "reverse hydrolysis" (Scheme 21).[237] Since the equilibrium constant for this reaction lies strongly in favor of hydrolysis, however, high concentrations of both the monosaccharide and alcohol must be used. Yields in these reactions are generally low.

The second route utilizes a preformed glycoside, which is hydrolyzed by an appropriate glycosidase. The intermediate is then trapped by a second nucleophile (other than water) to yield a new glycoside (Scheme 21). Several species have been used as the donor glycoside, including substituted aryl glycosides, glycosylfluorides and disaccharides. This method has been referred to as "transglycosylation." In early works the transglycosylation ability of a glycosidase was sometimes referred to as a separate activity. Transglycosylation gives higher yields, and is generally the method of choice.

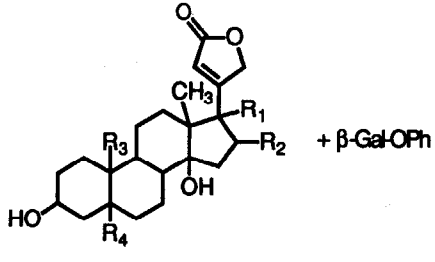
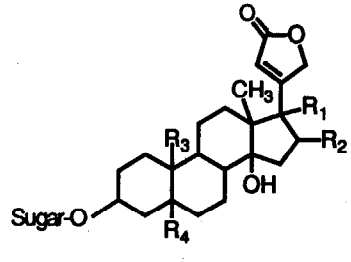
The primary advantage of these modes of synthesis compared with the use of the glycosyl transferases discussed above is that activated sugar nucleosides are not required. Several examples of synthesis using glycosidases have now been reported (Table 7).[238-260] The major drawbacks of these syntheses are low yields, and the formation of mixtures of products.

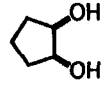
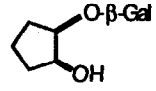
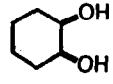
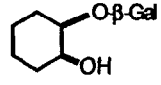
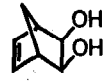
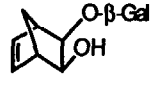
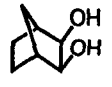
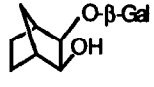
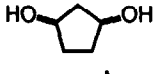
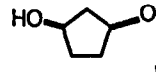
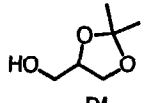
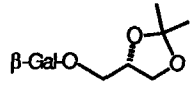
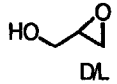
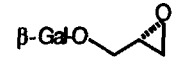
In the formation of oligosaccharides, the regiospecificity of the transfer is clearly of great importance. Nilsson has demonstrated that the nature of the glycosyl acceptor affects the regiospecificity of the transfer.[247] Thus, the addition of α-Gal-*O*-*p*-NO_2Ph to α-Gal-*O*-Me gives predominantly the 1→6 linked product, while the same addition to β-Gal-*O*-Me gives predominantly the 1→3 linked product.

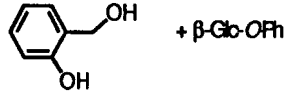
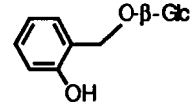
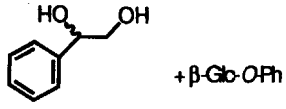
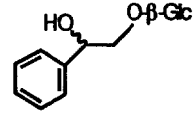
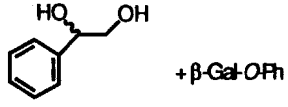
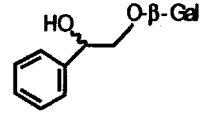
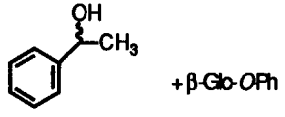
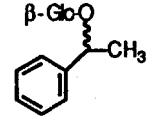
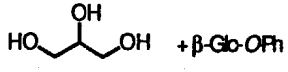
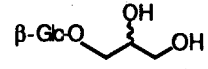
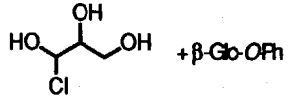
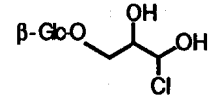
More recently, several groups have begun to investigate the diastereoselectivity of glycosidase-catalyzed transfers. This effort has included the addition of sugars to racemic and prochiral substrates (refs 255-259, Table 7). The addition of α-Gal-*O*-*p*-NO_2Ph to both racemic glycidol and isopropylidene glycerol catalyzed by the β-galactosidase from *E. coli* is reported to proceed in ~40% diastereomeric excess.[257] The transfer of an α-Glc unit from maltose to a racemic mixture of trans-1,2-cyclohexanediol was reported to give 100% diastereomeric excess, using a crude enzyme preparation from *Aspergillus oryzae*. Enzymes from other sources gave lower selectivities.[258] Gais and coworkers found diastereomeric excesses of 50-96% resulting from the transfer of β-Gal from either lactose or β-Gal-*O*-Ph to a series of meso diols, catalyzed by β-galactosidase from both *E. coli* and *Aspergillus oryzae*.[255] These investigators also noted a dependence of diastereomeric excess on enzyme source, reaction conditions (addition of acetone cosolvent) and the nature of the donating species. Much work remains to be done on the stereospecificity of glycosidase-catalyzed glycosyl transfer. A dependence of diastereomeric excess on the nature of the donating glycoside could have important mechanistic implications.

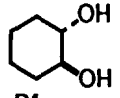
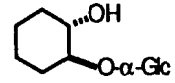
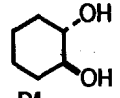
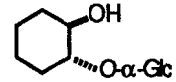
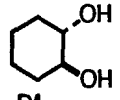
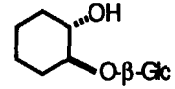
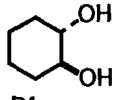
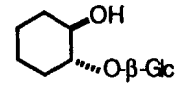
Table 7. Examples of Oligosaccharides synthesized using glycosidases.[a]

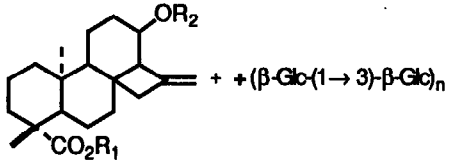
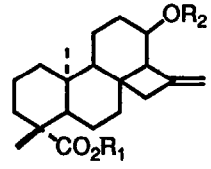
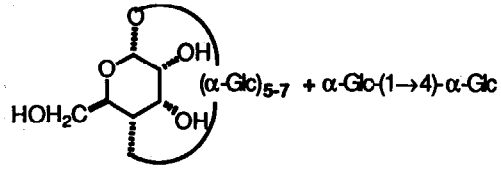
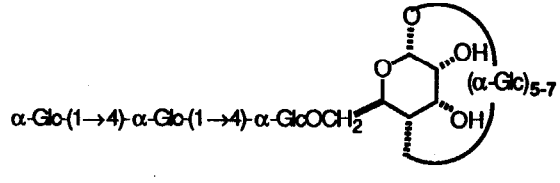
Substrate	Product	Scale[b]	Reference
α-Galactosidase			
Raffinose + allyl alcohol	α-Gal-*O*-$CH_2CH{=}CH_2$	A	249
α-Gal-*O*-*p*-$PhNO_2$ + α-Gal-*O*-$CH_2CH{=}CH_2$	α-Gal-(1→3)-α-Gal-*O*-$CH_2CH{=}CH_2$	B	249
α-Gal-*O*-Me + α-Gal-*O*-*p*-NO_2Ph	α-Gal-(1→3)-α-Gal-*O*-Me	B	247
	α-Gal-(1→6)-α-Gal-*O*-Me		
β-Gal-*O*-Me + α-Gal-*O*-*p*-NO_2Ph	α-Gal-(1→3)-β-Gal-*O*-Me	B	247
	α-Gal-(1→6)-β-Gal-*O*-Me		
α-Gal-*O*-*p*-NO_2Ph + α-Gal-*O*-*p*-NO_2Ph	α-Gal-(1→2)-β-Gal-*O*-*p*-NO_2Ph	C	247
	α-Gal-(1→3)-β-Gal-*O*-*p*-NO_2Ph		
β-Gal-*O*-*p*-NO_2Ph + α-Gal-*O*-*o*-NO_2Ph	α-Gal-(1→2)-β-Gal-*O*-*p*-NO_2Ph	C	247
	α-Gal-(1→3)-β-Gal-*O*-*p*-NO_2Ph		
β-Galactosidase			
$CH_3(CH_2)_3OH$ + β-Glc-OPh	$CH_3(CH_2)_3O$-β-Glc-OH	C	260
$CH_3(CH_2)_7OH$ + β-Glc-OPh	$CH_3(CH_2)_7O$-β-Glc-OPh	C	260
$[CH_3(CH_2)_3]_2CHOH$	$[CH_3(CH_2)_3]_2CHO$-β-Glc-OPh	C	260
$(CH_3)_2COH$ + β-Glc-OPh	$(CH_3)_2CO$-β-Glc-OH	C	260
$C_6H_{11}OH$ + β-Glc-OPh	$C_6H_{11}O$-β-Glc-OH	C	260
$HO(CH_2)_2OH$ + β-Glc-OPh	$HO(CH_2)_2O$-β-Glc-OH	C	260
$(CH_3)_2CH(CH_2)_3OH$ + β-Glc-OPh	$(CH_3)_2CH(CH_2)_3O$-β-Glc-OH	C	260
$(CH_3)_2C(OH)CH(CH_3)OH$ + β-Glc-OPh	$(CH_3)_2C(OH)CH(CH_3)O$-β-Glc-OH	C	260
$PhCH_2CH_2OH$ + β-Glc-OPh	$PhCH_2CH_2O$-β-Glc-OPh	C	260
Gal-(1→4)-β-Glc + GlcNAc	β-Gal-(1→6)-GalNAc	B[c]	240
Lactose + allyl alcohol	β-Gal-*O*-$CH_2CH{=}CH_2$	A	249
	β-Gal-(1→3)-β-Gal-*O*-$CH_2CH{=}CH_2$	B	
	β-Gal-(1→6)-β-Gal-*O*-$CH_2CH{=}CH_2$	B	

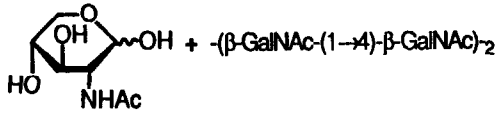
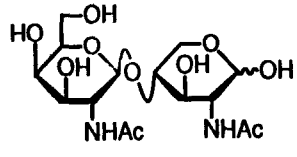
Substrate	Product	Scale[b]	Reference
Lactose + $HOCH_2Ph$	β-Gal-*O*-CH_2Ph	A	249
	β-Gal-(1→3)-β-Gal-*O*-CH_2Ph	B	
	β-Gal-(1→6)-β-Gal-*O*-CH_2Ph	B	
Lactose + $HOCH_2CH_2Si(Me)_3$	β-Gal-*O*-$CH_2CH_2Si(CH_3)_3$	B	249
	β-Gal-(1→3)-β-Gal-*O*-$CH_2CH_2Si(Me)_3$	E	
			
1. $R_1 = R_4 = H$; $R_2 = OH$, $R_3 = CH_3$ (Gitoxigenin)	1. Sugar = β-Gal	B	248
2. $R_1 = R_2 = R_4 = H$; $R_3 = CH_3$ (Digitoxigenin)	2. Sugar = β-Gal	B	248
3. $R_1 = R_2 = O$, $R_3 = CH_3$, $R_4 = H$ (16β,17β-Epoxy-17α-digitoxigenin)	3. Sugar = β-Gal	B	248
4. $R_1 = R_2 = H$; $R_3 = CHO$ $R_4 = OH$ (Strophanthidin)	4. Sugar = β-Gal	B	248
α-Gal-*O*-Me + β-Gal-*O*-*o*-NO_2Ph	β-Gal-(1→6)-α-Gal-*O*-CH_3	C	247
β-Gal-*O*-Me + β-Gal-*O*-*o*-NO_2Ph	β-Gal-(1→6)-β-Gal-*O*-CH_3	C	247
	β-Gal-(1→3)-β-Gal-*O*-CH_3		
β-Gal-*O*-Ph + CH_3OH	β-Gal-*O*-CH_3	B	252

Substrate	Product	Scale[b]	Reference
β-Gal-*O*-Ph + $HO(CH_2)_3CH_3$	β-Gal-*O*-$(CH_2)_3CH_3$	B	252
β-Gal-*O*-Ph + $HOCH_2(CH_3)_3$	β-Gal-$OCH_2(CH_3)_3$	B	252
β-Gal-*O*-Ph + C_6H_{11}-OH	β-Gal-*O*-cyclohexyl	B	252
β-Glc-*O*-Ph + CH_3OH	β-Glc-*O*-CH_3	B	252
β-Glc-*O*-Ph + C_6H_{11}-OH	β-Glc-*O*-cyclohexyl	B	252
β-Gal-*O*-*p*-NO_2Ph + sucrose	β-Gal-(1→ 6)-α-Glc-(1→ 2)-α-Fru	E	253
β-Gal-(1→ 4)-Glc + sucrose	β-Gal-(1→ 6)-α-Glc-(1→ 2)-α-Fru	E	253
	+ β-Gal-*O*-Ph or β-Gal-(1→ 4)-Glc	 89% de	E	255
	+ β-Gal-*O*-Ph or β-Gal-(1→ 4)-Glc	 90% de	E	255
	+ β-Gal-*O*-Ph or β-Gal-(1→ 4)-Glc	 75% de	E	255
	+ β-Gal-*O*-Ph or β-Gal-(1→ 4)-Glc	 63% de	E	255
	+ β-Gal-*O*-Ph or β-Gal-(1→ 4)-Glc	 50% de	E	255
 D/L	+ β-Gal-(1→ 4)-Glc	 100% de[e]	A	256
 D/L	+ β-Gal-(1→ 4)-Glc or β-Gal-*O*-*o*-NO_2Ph	 40% de	A	257

Substrate	Product	Scale[b]	Reference
		B	258
		B[c]	258
		B	258
		B[c]	258
		B	310
			
		E	254
α-Mannosidase			
Man	isomers of α-Man-Man	B	239
α-Man-*O*-Me + α-Man-*O*-*p*-NO_2Ph	α-Man-(1→2)-α-Man-*O*-Me α-Man-(1→6)-α-Man-*O*-Me	B	247

Substrate	Product	Scale[b]	Reference
α-Man-*O*-*p*-NO_2Ph + α-Man-*O*-*p*-NO_2Ph	α-Man-(1→ 2)-α-Man-*O*-*p*-NO_2Ph α-Man-(1→ 6)-α-Man-*O*-*p*-NO_2Ph	B	247
α-Glucosidase			
Glc + Fru	α-Glc-(1→ 1)-Fru	D	241
Glc + Fru	α-Glc-(1→ 4)-Fru	D[c]	241
 + α-Glc-(1→ 4)-Glc	 100%de	C[e]	260
 + α-Glc-(1→ 4)-Glc		E[e]	259
 + α-Glc-(1→ 4)-Glc	 100%de	A[e]	259
β-Glucosidase			
Glc	β-Glc-(1→ 4)-Glc	C	251
	β-Glc-(1→ 6)-Glc	C	
β-Glc-(1→ 4)-Glc	β-Glc-(1→ 4)-β-Glc-(1→ 4)-Glc	C	251
 + α-Glc-(1→ 4)-Glc		E[e]	259

Substrate	Product	Scale[b]	Reference
			
R_1 = β-Glc R_2 = β-Glc-(1→ 2)-β-Glc	1. R_1 = β-Glc-(1→ 3)-β-Glc; R_2 = β-Glc-(1→ 2)-β-Glc 2. R_1 = β-Glc; R_2 = β-Glc-(1→ 3)-β-Glc-(1→ 2)-β-Glc 3. R_1 = β-Glc-(1→ 3)-β-Glc-(1→ 3)-β-Glc; R_2 = β-Glc-(1→ 2)-β-Glc	B B B	250 250 250
Amylase			
1-deoxynorjirimycin + α-cyclodextrin	α-Glc-(1→ 4)-1-deoxynojirimicin	A	242
		E[c]	259
Amylosucrase			
α-Glc-1-F + Fru	amylopolysaccharides	D	243
Cyclodextrin Glycosyltransferase			
α-Glc-1-F	α-cyclodextrin	B	244[c]

Substrate	Product	Scale[b]	Reference
	β-cyclodextrin	B	
	maltooligomers	B	
Starch + 2-Deoxyglucose	α-Glc-(1→ 4)-2-deoxyglucose	E	245
starch + α-Glc-(1→ 2)-Glc	α-Glc-(1→ 4)-α-Glc-(1→ 2)-Glc	B	246
Lysozyme			
		E[e]	259

a Sugars are of D configuration unless noted.
b Amount of product isolated:

A : > 1 g
B: 100 mg to 1 g
C: 10 mg to 100 mg
D: <10 mg
E: product not isolated or no yield reported

c Immobilized enzyme used.
d de not reported

4. Polysaccharides.

Polysaccharides play important roles as storage, structural, and protective components in biological systems.[1,2,5] From a commercial standpoint, polysaccharides are the most widely used group of carbohydrates. Polysaccharides are important both as commodity chemicals in the food,[261,262] textile,[263] and petroleum[263] industries, and as specialty chemicals, primarily in the medical field.[264] Some of the more important polysaccharides from the point of view of human health are heparin, hyaluronic acid, and chondroitin sulfate (Figure 1). Modification of these and other carbohydrate polymers may lead to important new materials.

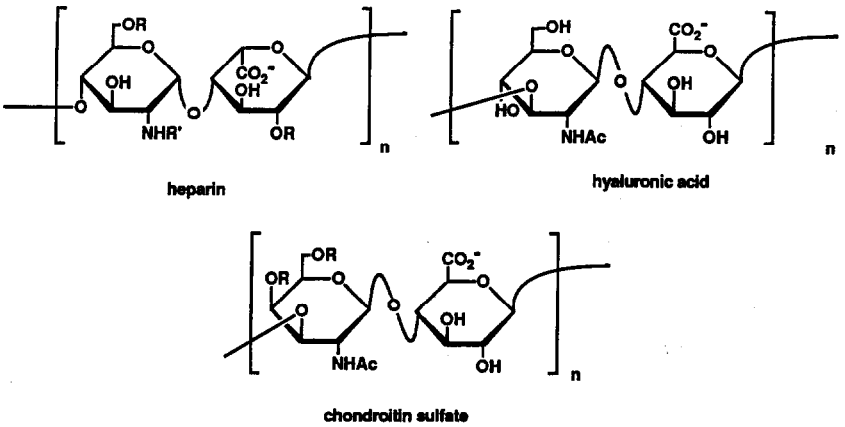


Figure 1. Representative repeating units of important glycosylaminoglycan polysaccharides. The full structures are heterogeneous. For heparin , R = $-SO_3^{2-}$ or -H and R' = -Ac or -H; for chondroitin sulfate, R = $-SO_3^{2-}$ or -H.

Synthesis.

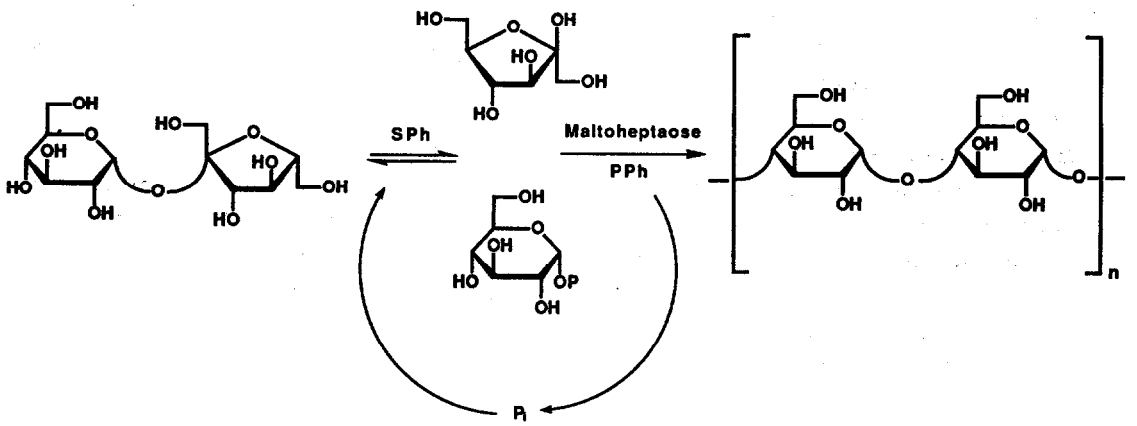
Biosynthesis and Isolation. The biosynthesis of polysaccharides is achieved in one of two ways. The first uses the Leloir pathway, described above for oligosaccharides. Extensive post-polymerization modification can, however, strongly influence the structure and properties of carbohydrate biopolymers, particularly in eukaryotic systems. Modifications include acetylation, deacetylation, sulfation, epimerization, oxidation, reduction, and methylation. The system of enzymes and cofactors needed to accomplish many of these transformations are now developed for synthetic applications: one important exception is the system, based on 3'-phosphoadenosine 5'-phosphosulfate (PAPS), that is involved in essentially all sulfation reactions. Although *in vitro* sulfation of polymeric systems with cell-

free enzymes has been accomplished on an analytical scale,[265] preparative-scale polymer sulfation remains an important unsolved problem.[61] The second route utilizes non-Leloir pathways, and is used in the biosynthesis of several important classes of polymers, including the dextrans and levans.[266] In this synthetic route, sugar monomers are activated as the sugar-1-phosphates before incorporation into the growing chain.[1,2,5,267]

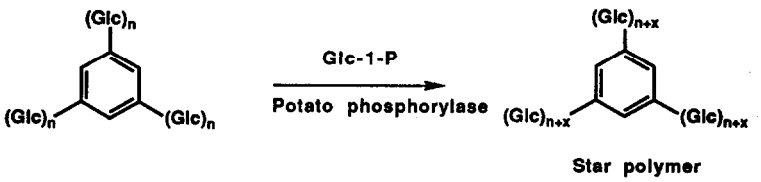
Common methods for the production of polysaccharides by isolation from biological sources or by fermentation suffer several drawbacks. Isolation does not allow control of either polymer molecular weight or the extent of branching. Isolation of polysaccharides also creates an inconvenient dependence on natural sources. Separation of the desired polymer from a variety of extra- and intracellular (when cells must be lysed) products is difficult. Shear degradation of the polymer may also occur during lysis.

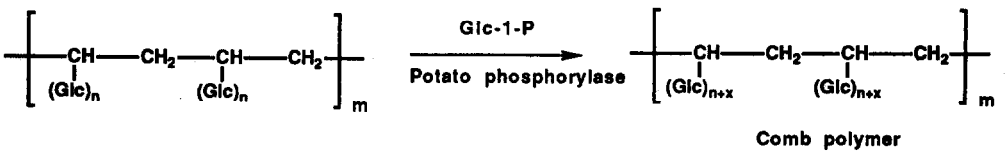
Cell-free Enzymatic Synthesis. The synthesis of modified carbohydrate polymers might provide materials with more desirable physical and biological properties than their natural counterparts. One approach to controlling the characteristics of polymers is to control the genes encoding the enzymes responsible for their production, to regulate the activity of these enzymes, or to influence *in vivo* synthesis in some other way.[268,269] Another approach is to use cell-free systems of enzymes. Potato phosphorylase, which uses Glc-1-P to synthesize amylose, has been used synthetically *in vitro*. Pfannemüller and co-workers have used this enzyme to prepare maltose oligomers.[270] Whitesides and co-workers extended this method by preparing Glc-1-P *in situ* from sucrose, using sucrose phosphorylase (E.C. 2.4.1.7) (Scheme 22).[271] Pfannemüller and colleagues have also produced an extensive family of linear, star- and comb- shaped polymers carrying amylose chains using potato phosphorylase (E.C. 2.4.1.1) (Scheme 23).[272,273] Other important cell-free enzymatic systems include those used in the commercial preparation of cyclodextrins from starch.[274]

In spite of the progress that has been made, several difficulties limit the use of cell-free enzymes for the synthesis of polysaccharides. The major problem is the complexity of many polysaccharide-synthesizing systems. The large number of enzymes involved in synthesis (and in post-polymerization modification, when it occurs), the need for primers, and, in the case of glycoproteins, the involvement of protein- or dolichol-bound intermediates all contribute to this problem. Isolation, purification, and stabilization of the required enzymes is often difficult. Many enzymes lose activity when they are no longer membrane-associated. Like the glycosyl transferases, the concentration of many of these enzymes in cells is low. This problem is especially significant with enzymes isolated from eukaryotic sources. It is unlikely that cell-free enzymatic synthesis will provide better routes to most natural polysaccharides than do fermentation and isolation. The use of genetic engineering, both using classical genetics and recombinant DNA technology, is now being used to prepare modified carbohydrate polymers.[275]



Scheme 22. SPh = sucrose phosphorylase; PPh = potato phosphorylase





Scheme 23.

Several Leloir-pathway systems have been isolated, and hyaluronic acid,[276-278] chitin,[279] and chitosan[280] have all been synthesized on small (μmol) scale using cell-free enzyme preparations. To date, the focus of this work has been the elucidation of biochemical pathways, and only natural polymers have been synthesized. The use of these systems to generate novel polymers is an area of current interest.

5. Prospects, Targets, and Unsolved Problems

Assessment. Enzymes will be a useful class of catalysts in the synthesis of monosaccharides, activated monosaccharides, oligosaccharides and polysaccharides. The aldolases presently appear to be the most useful group of catalysts for the preparation of monosaccharides; other enzymes (kinases, lipases, oxidoreductases) catalyze specific functional group transformations of monosaccharides but are less general in applicability than the aldolases.

Enzymes of the Leloir pathway have already been used successfully to prepare certain oligosaccharides by formation of glycosidic linkages. The eight nucleoside phosphate sugars required to synthesize the large majority of mammalian oligosaccharides will soon be available by practical synthetic routes. Difficulties in obtaining the required glycosyl transferases currently limit the scope and scale of syntheses in this area.

The use of cell-free enzymes for the synthesis of polysaccharides is just beginning to be explored. The lack of the necessary glycosyl transferases, and the requirement, in some systems, for enzymes for post-polymerization modification, are both unresolved problems. Methods for producing or modifying selected polysaccharides having high value may prove practical; these methods will draw upon results in the area of oligosaccharide synthesis.

This section sketches a few of the specific areas in which enzyme-based carbohydrate synthesis has potential. While this list of potential targets for synthesis is by no means complete, it illustrates the range of targets to which this synthetic methodology might be applied.

Research Chemicals and Biochemicals; Synthetic Intermediates.

Biochemicals. Compounds such as nucleoside phosphate sugars, glycoproteins, glycolipids (eg. gangliosides) are important as materials for studies of biochemical systems and as intermediates for the synthesis of other biochemicals. Modified, fluorescent-labelled, or radioactively-tagged biochemical intermediates are also useful as inhibitors or tracers. The stereochemical demands posed by the synthesis of these water-soluble materials challenge the methods of organic synthesis. Enzyme-catalyzed reactions provide routes to many simple biochemical intermediates and may be the method of choice for the synthesis of more complex structures. These reactions are particularly useful in syntheses requiring a high degree of stereoselectivity.

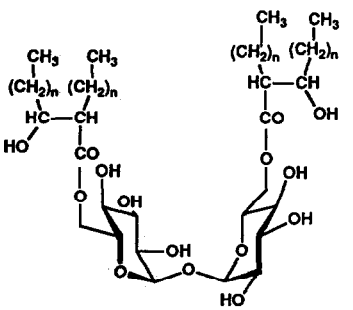
Chemicals. Naturally-occurring sugars are already widely used in organic synthesis, primarily as sources of chiral centers. The capacity of aldolases to generate large numbers of rare and unnatural sugars and of sugar-like molecules offers a route to many new chiral synthons.[281,282]

Immunomodulators.

Glycolipids and Glycoproteins. The identification and preparation of compounds that enhance the response of the immune system is a key target of biomedical research.[149,283,284] Bacterial sources have provided a large number of glycoconjugates that function as immunomodulators.[285] Two of the most interesting classes of such compounds are muramyl dipeptide (MDP) and trehalose diesters, or cord factor (Figure 2).

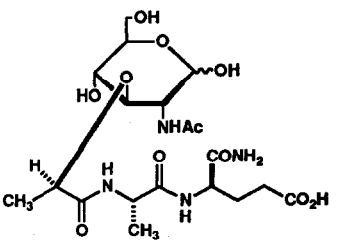
Immunomodulators are of great value as adjuvants in vaccine therapy. Muramyl dipeptide has been used in this capacity since the beginning of the century. Originally used as a whole cell preparation (Freund's adjuvant), MDP was shown in 1974 to be the minimum active structure from mycobacterial cells.[286] Certain vaccine and anti-viral therapies use MDP to activate macrophages, increase levels of antibodies against specific antigens, and induce a delayed hypersensitivity reaction.[287] MDP also stimulates non-specific resistance to bacterial infection[288] and to oncogenic animal viruses.[289] Unfortunately, native MDP displays a variety of deleterious effects *in vivo*, including pyrogenicity, thrombocytolysis, and sensitization to endotoxin.[290] As a result, the synthesis of analogues of MDP is of interest in efforts to maximize the value of this class of biologically active compounds. To date, these studies have focussed primarily on the modification of the peptide moiety.[290] Improved methods for the synthesis of both unnatural monosaccharides and glycoproteins may allow the preparation of a variety of new compounds with variation in the sugar moiety.

Trehalose diesters show immunostimulant, antibacterial, antiparasitic, and antitumor activity (Figure 2).[290] The preparation of modified trehalose derivatives has concentrated on alterations of the acyl side chains, in part due to the synthetic difficulty in modifying the trehalose moiety.[290] The development of techniques based on enzymes for the formation of the unusual glycosidic bond of trehalose should allow for the preparation of new analogues of trehalose, based on the preparation of novel disaccharide cores.



Trehalose Diester

n = 30 - 90



Muramyl Dipeptide

Figure 2.

Bacterial Endotoxin/Lipid A.[292,293] The outer membrane of Gram-negative organisms contains significant amounts of an amphiphilic lipopolysaccharide (LPS, or bacterial endotoxin). This biopolymer is responsible for many of the toxic effects due to Gram-negative bacteria infection, including fever, hypotension, coagulation abnormalities and death in laboratory animals.[294] LPS consists of three segments: lipid A, an acylated disaccharide of β-GlcN-(1→6)-GlcN; a core polysaccharide region; and an antigenic outer polysaccharide (Figures 3 and 4). These components show considerable species

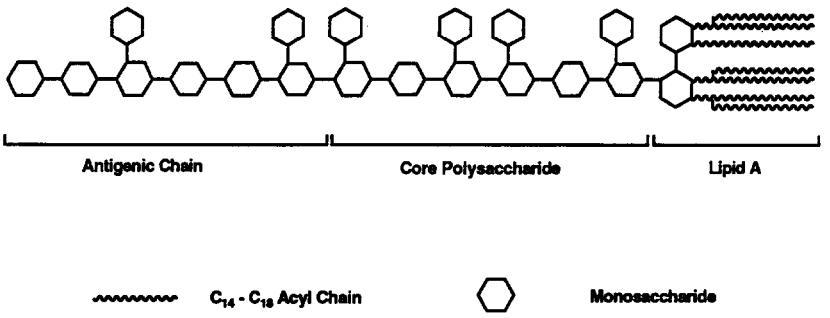
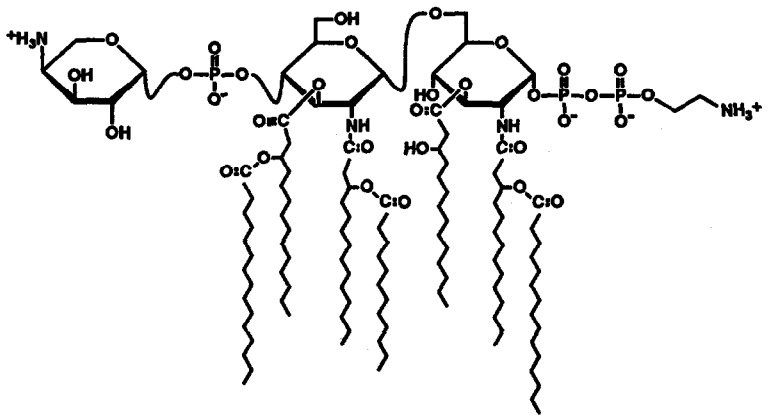


Figure 3.

variation. Lipid A alone causes many of the physiological effects of bacterial endotoxin.[295] It is a potent stimulator of tumor necrosis factor,[296] and also stimulates the formation of antibodies, which cross-react with a wide range of other Gram-negative bacteria.[297] Lipid A stimulates the immune system, causing the differentiation of B-lymphocytes and the release of secondary mediators by macrophages, such as prostaglandins.[294] The synthesis of modified endotoxin structures is clearly an area of great interest in the search for non-protein immunomodulators and adjuvants. To date, the study of modified bacterial LPS has relied on the isolation of incomplete endotoxin structures, often from mutant strains,[298] or by total chemical synthesis.[299] Enzymes should prove useful in the synthesis of components and analogues of LPS.

Polysaccharides. A number of polysaccharides (for example dextran, heparin, hyaluronic acid, Figure 1, Section 4) have well defined places in medicinal chemistry, and a number of others have shown biological activity.[300,301] Heparin is a highly heterogeneous structure, with a number of apparently distinct biological activities due to different sequences. The pentasaccharide shown in Figure 5 has been shown to be a minimum sequence required for binding to antithrombin III.[153,302]

While cell-free synthesis of polysaccharides is not presently feasible on a large scale, the preparation of useful quantities of oligosaccharides may well be possible. These oligosaccharide sequences would be valuable in defining relationships between biological activity and structure, and might constitute useful drug candidates by themselves.



1

α-Gal-(1→6) Hep-(1→7) P-P-NEt KDO-P-NEt

α-GlcNAc-(1→2)-α-Glc-(1→2)-α-Gal-(1→3)-α-Glc-(1→3)-β-Hep-(1→3)-β-Hep-(1→5)-KDO→KDO→LipidA 2

β-Glc-(1→4)

[→4)-β-GlcUA-(1→3)-α-FucNAc-(1→3)-α-GlcNAc-(1→6)-Gal-β-(1→] 3

Figure 4. Representative units of bacterial endotoxin. 1 = Lipid A from *Salmonella minnesota*. 2 = Core polysaccharide of *Salmonella*. 3 = Repeating *O*-antigenic polysaccharide of *E. coli* O32 (P: phosphate; Hep: heptose).

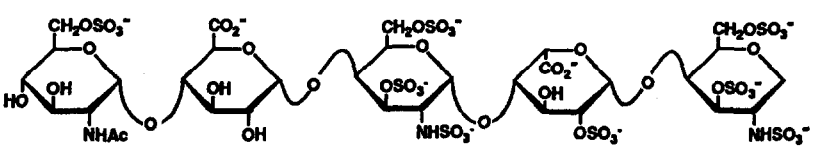


Figure 5.

Drug delivery.

Carbohydrates have already proved useful in three areas of drug delivery: in solubilization of hydrophobic drugs, as biodegradable polymers for controlled drug release, and for specific organ targeting.[291,303] Improved enzymatic methods for synthesis of carbohydrates and derivatives will provide more flexible methods for preparing drug-carbohydrate conjugates, and for generating new carbohydrates.

Solubilization of large, hydrophobic molecules, such as steroids, through glycosylation has long been recognized as one mechanism of detoxification *in vivo*.[38] This same principle is used to solubilize drugs that might otherwise have to be used as suspensions. *In vivo*, glycosidases remove the glycosyl unit, unmasking the drug. Increased availability of glycosyl transferases should, in combination with techniques for preparing nucleoside mono- and diphosphate sugars, be especially helpful in synthesizing drug-sugar conjugates.

Polymeric drug delivery systems have been used clinically.[304] The primary advantage of polysaccharides as delivery vehicles is the biocompatibility of both the initial polymer and its degradation products. Furthermore, an effective mechanism for decomposition, the glycosidase enzyme system, exists *in vivo*. The hydrophilic surfaces presented to serum by polysaccharides may be less thrombogenic than synthetic polymers.

It may be possible to exploit the fact that carbohydrates are often important in biological recognition to design strategies for specific organ targeting. For example, the use of mannosylated proteins in conjunction with muramyl dipeptide (*vide supra*) allows specific delivery of MDP to macrophages. The glycoprotein conjugate binds to the mannose receptors on the surface of macrophage cells, and is subsequently endocytosed.

Glycosidase and Glycosyl Transferase Inhibitors.

A range of analogues and derivatives of carbohydrates are proving interesting in studying the biosynthesis and modification of oligosaccharides: deoxynojirimycin, swainsonine, and castanospermine inhibit trimming of the *N*-linked oligosaccharides of glycoproteins; tunicamycin and streptovirudin also inhibit glycosylation in the Leloir pathway;[305,306] acarbose inhibits amylase.[307] These inhibitor systems have attracted substantial interest for two reasons. First, they provide a way of exploring cell-surface oligosaccharide chemistry, a topic of central interest in differentiation and development, as well as other areas. Second, most are relatively easily understood as transition state analogues, and there is a good chance that the design of other, new sugar analogues to inhibit other glycosidase and glycosyl transferases can be accomplished.

The syntheses of these types of structures are not straightforward using classical synthetic methods. Enzymatic methods have already proved very useful in syntheses of deoxynojirimycin and related materials, and will probably prove widely applicable to other preparations in this series.

Glycoprotein Remodeling.

A number of the proteins of interest as human pharmaceuticals (tissue plasminogen activator, juvenile human growth hormone, CD4) are glycoproteins. There is substantial interest in developing synthetic methods that will permit modification of oligosaccharide structures on these glycoproteins by removing and adding sugar units ("remodeling") and in making new types of protein-oligosaccharide conjugates.[308,309] The motivation for these efforts is the hope that modification of the sugar components of naturally-occurring or unnatural glycoproteins might increase serum lifetime, increase solubility, decrease antigenicity, and promote uptake by target cells and tissues.

Enzymes are plausible catalysts for manipulating the oligosaccharide content and structure of glycoproteins. The delicacy and polyfunctional character of proteins, and the requirement for high selectivity in their modification, indicate that classical synthetic methods will be of limited use. The major problems in the widespread use of enzymes in glycoprotein remodeling and generation are that many of the glycosyl transferases that are plausible candidates for this area are not available, and the uncertainty in whether glycosyl transferases that act during synthesis *in vivo* (probably on unfolded or partially folded protein) will be active at the surface of a completely folded protein.

Biocompatible Materials.

The carbohydrate portions of glycoproteins and glycolipids are a major constituent of the exterior surface of mammalian cells. A number of functions have been suggested for these oligosaccharide moieties, ranging from highly structure-specific roles in cellular recognition to rather general roles in cellular protection. At least two general mechanisms have been suggested for the hypothesized activities in the latter category, both based on analogy with phenomena known to be important in colloid chemistry. First, the presence of NeuAc as the terminal constituent of many cell-surface oligosaccharides gives the cell some aspect of a negatively charged particle, and may thus prevent too-intimate cell-cell contact. Second, the presence of highly solvated, conformationally mobile oligosaccharide chains on the cell surface may provide steric protection for the cell surfaces.

The recognition that oligosaccharides on cell surfaces play an important role in their interaction with cells and other constituents of biological systems has lead to the suggestion that the ability to prepare surface-immobilized sugars and oligosaccharides might lead to new strategies for controlling biocompatibility. A variety of mono- and oligosaccharides, from glucose to heparin, have been attached to surfaces using classical chemical coupling methods. The enzyme-based synthetic methods discussed here should also be applicable to certain types of synthetic reactions at interfaces (using of course, soluble enzymes). There is, however, an important uncertainty concerning the character of steric interactions

between the enzyme and the surface: is a potential substrate for an enzyme-catalyzed transformation accessible to the enzyme if it is immobilized close to a surface?

Other Opportunities.

The pace of development of carbohydrate-derived pharmaceutical agents has, in general, been slower than that of more conventional classes of materials. The difficulties in synthesis and analysis of carbohydrates have undoubtedly contributed to this slow pace, but at least three areas of biology and medicinal chemistry have redirected attention to carbohydrates. First, interfering with the assembly of bacterial cell walls remains one of the most successful strategies for the development of antimicrobials. As bacterial resistance to penams and cephams becomes more widespread, there is increasing interest in interfering with the biosynthesis of the characteristic carbohydrate components of the cell wall, especially KDO, heptulose, lipid A and related materials. Interest in cell-wall constituents is also heightened by their relevance to vaccines and as leads toward non-protein immunomodulating compounds. Second, cell-surface carbohydrates are central to differentiation and development, and may be relevant to abnormal states of differentiation, such as those characterizing some malignancies. Third, the broad interest in diagnostics has finally begun to generate interest in carbohydrates as markers of human health. In addition, there are a number of other possible applications of carbohydrates, for example as dietary constituents, in antivirals, as components of liposomes, which warrant attention. Enzymatic methods of synthesis, by rendering carbohydrates more accessible, will contribute to investigations of all of these areas.

Acknowledgements. This work was supported by the National Institutes of Health, Grant GM 30367. ESS acknowledges a fellowship from DuPont. A number of individuals provided very helpful comments concerning this review, especially Ole Hindsgual (Alberta), Bryan Jones (Toronto), James Paulson (UCLA), Subramaniam Sabesan (DuPont), and Chi-Huey Wong (Texas A&M).

Abbreviations

AcP	acetyl phosphate
AMP	adenosine 5′-monophosphate
ADP	adenosine 5′-diphosphate
Ara-5-P	arabinose 5′-phosphate
Asn	asparagine
ATP	adenosine 5′-triphosphate
CMP	cytidine 5′-monophosphate
CMP-NeuAc	cytidine 5′-monophospho-*N*-acetylneuraminic acid
CDP	cytidine 5′-diphosphate
CTP	cytidine 5′-triphosphate
DAHP	3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate
DHAP	dihydroxyacetone phosphate
Dol	dolichol
FDP	fructose 1, 6-diphosphate
Gal	galactose
GalNAc	*N*-acetylgalactosamine
Glc	glucose
GlcNAc	*N*-acetylglucosamine
G-3-P	glyceraldehyde-3-phosphate
GMP	guanosine 5′-monophosphate
GDP	guanosine 5′-diphosphate
GDP-Fuc	guanosine 5′-diphosphofucose
GDP-Man	guanosine 5′-diphosphomannosamine
GTP	guanosine 5′-triphosphate
Hep	heptulose
KDO-8-P	3-deoxy-D-manno-2-octulosonic acid 8-phosphate
LPS	lipopolysaccharide
Man	mannose
ManNAc	*N*-acetylmannosamine
MDP	muramyl dipeptide
MEEC	membrane-enclosed catalysis
NeuAc	*N*-acetylneuraminic acid
NDP	nucleoside diphosphate
NTP	nucleoside triphosphate
P	phosphate
P_i	inorganic phosphate
PEP	phosphoenolpyruvate
PP_i	inorganic pyrophosphate
Pro	proline
RAMA	rabbit muscle FDP aldolase
Ser	serine
Thr	threonine
UMP	uridine 5′-monophosphate
UDP	uridine 5′-diphosphate
UDP-Gal	uridine 5′-diphosphogalactose
UDP-GalNAc	uridine 5′-diphospho-*N*-acetylgalactosamine
UDP-Glc	uridine 5′-diphosphoglucose
UDP-GlcNAc	uridine 5′-diphospho-*N*-acetylglucosamine
UDP-GlcUA	uridine 5′-diphosphoglucuronic acid
UDP-Xyl	uridine 5′-diphosphoxylose
UTP	uridine 5′-triphosphate

References

1. Kennedy, J. F.; White, C. A. *Bioactive Carbohydrates*; Ellis Horwood Ltd.: West Sussex, 1983.
2. Sharon, N. *Complex Carbohydrates*; Addison-Wesley: Reading, MA, 1975.
3. Horowitz, M. I.; Pigman, W., Eds. *The Glycoconjugates, Vol I - II*; Academic: New York, 1977-1978.
4. Horowitz, M. I., Ed. *The Glycoconjugates, Vol III - IV*; Academic: New York, 1982.
5. Aspinall, G. O., Ed. *The Polysaccharides, Vol I - III*; Academic: New York, 1982-1985.
6. Metzler, D. E. *Biochemistry: The Chemical Reactions of Living Cells*; Academic: New York, 1977.
7. Paulsen, H. *Chem. Soc. Rev.* **1984**, *13*, 15.
8. Zamojski, A.; Grynkiewicz, G. In *The Total Synthesis of Natural Products*; ApSimon, J., Ed.; Wiley-Interscience: New York, 1984; Vol. 6, p 141.
9. McGarvey, G. J.; Kimura, M.; Oh, T.; Williams, J. M. *J. Carbohydr. Chem.* **1982**, *40*, 1.
10. Zamojski, A.; Banaszek, A.; Grynkiewicz, G. *Adv. Carbohydr. Chem. Biochem.* **1984**, *3*, 125.
11. Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212.
12. Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 294.
13. Paulsen, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155.
14. Lemieux, R. U. *Chem. Soc. Rev.* **1978**, *7*, 423.
15. Ogawa, T.; Yamamoto, H.; Nukada, H.; Kitajima, T.; Sugimoto, M. *Pure Appl. Chem.* **1984**, *56*, 779.
16. For a collection of recent papers on glycoproteins and glycolipids, see: *Carbohyd. Res.* **1987**, *164*.
17. Schauer, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 131.
18. Sabesan, S.; Paulson, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 2068.
19. *The Lectins: Properties, Functions and Applications in Biology and Medicine*; Liener, I. E.; Sharon, N.; Goldstein, I. J., Eds.; Academic: New York, 1986.
20. Feizi, T. *Nature* **1985**, *314*, 53.
21. Paulson, J. E. In *The Receptors*; Cohn, P. M., Ed.; Academic: New York, 1985; Vol. 2, p 131.
22. Sairam, M. R. In *The Receptors*; Cohn, P. M., Ed.; Academic: New York, 1985; Vol. 2, p 307
23. Kanfer, J. N.; Hakomori, S. *Handbook of Lipid Research*; Plenum: New York, 1983; Vol. 3.
24. Montreuil, J. In *Comprehensive Biochemistry*; Neuberger, A.; Van Deenen, L. L. M., Eds.; Elsevier: Amsterdam, 1982; Vol. 19B part II, p 1.
25. Hakomori, S. *Ann. Rev. Biochem.* **1981**, *50*, 733.
26. Feizi, T.; Childs, R. A. *Biochem. J.* **1987**, *245*, 1.
27. Rademacher, T. W.; Parekh, R. B.; Dwek, R. A. *Ann. Rev. Biochem.* **1988**, *57*, 785.
28. Sariola, H.; Aufderheide, E.; Bernhard, H.; Henke-Fahle, S.; Dippold, W.; Ekblom, P. *Cell* **1988**, *54*, 235.
29. Nojiri, H.; Kitagawa, S.; Nakamura, M.; Kirito, K.; Enomoto, Y.; Saito, M. *J. Biol. Chem.* **1988**, *263*, 7443.
30. Seyfried, T. N. *Developmental Biology* **1987**, *123*, 286.
31. Usuki, S.; Lyu, S.-C.; Sweeley, C. C. *J. Biol. Chem.* **1988**, *263*, 6847.
32. Hakomori, S. *Cancer Res.* **1985**, *45*, 2405.
33. Kornfeld, R.; Kornfeld, S. In *The Biochemistry of Glycoproteins and Proteoglycans*; Lenmarz, W. J., Ed.; Plenum: New York, 1980; p 1.
34. Neufeld, E. F.; Ashwell, G. In *The Biochemistry of Glycoproteins and Proteoglycans*; Lenmarz, W. J., Ed. Plenum: New York, 1980; p 266.
35. Roseman, S. In *Cell Membranes: Biochemistry, Cell Biology and Pathology*; Weissmann, G.; Claiborne, R. Eds.; Hospital Practice: New York, 1975; p 55.
36. Ashwell, G.; Harford, J. *Ann. Rev. Biochem.* **1982**, *51*, 531.
37. Berger, E. G.; Greber, U. F.; Mosbach, K. *FEBS Lett.* **1986**, *203*, 64.
38. See: *Enzymatic Bases of Detoxication*; Jakoby, W. B. Ed.; Academic: New York, 1980; Vol. II,.
39. Jones, J. B. *Tetrahedron* **1986**, *42*, 3351.
40. *Enzymes in Organic Synthesis (Ciba Foundation Symposium 111)*; Porter, R.; Clark, S., Eds.; Pitman: London, 1985.
41. Akiyama, A.; Bednarski, M. D.; Kim, M.-J.; Simon, E. S.; Waldmann, H.; Whitesides, G. M. *CHEMTECH* **1988**, 627.
42. Whitesides, G. M.; Wong, C.-H. *Aldrichchem. Acta* **1983** *16*, 27.
43. Nilsson, K. G. I. *TIBTECH* **1988**, *6*, 256.
44. Shibaev, V. N. *Prog. Khim. Uglevodov* **1985**, 149 (Abstract: CA: **108**:112841e).
45. *Lipases*; Borgström, B.; Brockman, H. L., Eds.; Elsevier: Amsterdam, 1984.
46. Jones, J. B.; Beck, J. F. in *Techniques in Chemistry*; Jones, J. B.; Sih, C. J.; Perlman, D., Eds.; Wiley-Interscience: New York, 1976; p 107.
47. Kim, M.-J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 2959.
48. Simon, E. S.; Plante, R.; Whitesides, G. M. *Appl. Biochem. Biotechnol.* **1989**, in press.
49 Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.*, in press.
50. Bednarski, M. D.; Simon, E. S.; Bischofberger, N.; Fessner, W.-D.; Kim, M.-J.; Lees, W.; Saito, T.; Waldmann, H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 627.
51. Durrwachter, J. R.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175.
52. For a collection of papers see: *Methods Enzymol.* **1976**, *XLIV*.
53. Chibata, I. *Immobilized Enzymes - Research and Development*; Halsted: New York, 1978.
54. For a collection of papers see: *Methods Enzymol.* **1987**, *136*.
55. Lee, E. K. In *Encyclopedia of Physical Science and Technology*; Academic: New York, 1987; Vol. 8, p 20.

56. Bednarski, M. D.; Chenault, H. K.; Simon, E. S.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 1283.
57. Jocelyn, P. C. *Methods Enzymol.* **1987**, *143*, 246.
58. Trotta, P. P.; Pinkus, L. M.; Meister, A. *J. Biol. Chem.* **1974**, *249*, 1915.
59. Costa, M.; Pecci, L.; Pensa, B.; Cannella, C. *Biochem. Biophys. Res. Commun.* **1977**, *78*, 596.
60. Chenault, H. K.; Whitesides, G. M. *Appl. Biochem. Biotech.* **1987**, *14*, 147.
61. Chenault, H. K.; Simon, E. S.; Whitesides, G. M. In *Biotechnol. and Genetic Eng. Rev.*; Russell, G. E., Ed.; Intercept Ltd.: Wimborne, Dorset, 1988; Vol. 6, Chapter 6.
62. Whitesides, G, M.; Wong, C.-H. *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 617.
63. Wong, C.-H. In *Enzymes as Catalysts in Organic Synthesis*; Schneider, M. P., Ed.; Reidel: Dordrecht, 1986, p. 199.
64. Meyerhof, O.; Lohmann, K. *Biochem. Z.* **1934**, *271*, 89.
65. Horecker, L.; Tsolas, O.; Lai, C. Y. In *The Enzymes*, Boyer, P. D. Ed.; Academic: New York, 1972; Vol. VII, p 213.
66. Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3199.
67. Wong, C.-H.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3493.
68. Durrwachter, J. R.; Drueckhammer, D. G.; Nozaki, K.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 7812.
69. Bednarski, M. D.; Waldmann, H. J.; Whitesides, G. M. *Tetrahedron Lett.* **1986**, *27*, 5807.
70. Jones, J. K. N.; Sephton, H. H. *Can. J. Chem.* **1960**, *38*, 753.
71. Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1952**, 4047.
72. Jones, J. K. N.; Matheson, N. K. *Can. J. Chem.* **1959**, *37*, 1754.
73. Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1953**, 342.
74. Haustveit, G. *Carbohydr. Res.* **1976**, *47*, 164.
75. Franke, F. P.; Kapuscinski, M.; MacLeod, J. K.; Williams, J. F. *Carbohydr. Res.* **1984**, *125*, 177.
76. Mehler, A. H.; Cusic, M. E., Jr. *Science* **1967**, *155*, 1101.
77. Gorin, P. A. J.; Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1953**, 2140.
78. Gorin, P. A. J.; Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1955**, 3843.
79. Jones, J. K. N.; Kelly, R. B. *Can. J. Chem.* **1956**, *34*, 95.
80. Kapuscinski, M.; Franke, F. P.; Flanigan, I.; MacLeod, J. K.; Williams, J. F. *Carbohydr. Res.* **1985**, *140*, 65.
81. Bally, C.; Leuthardt, F. *Helv. Chim. Acta* **1970**, *53*, 732.
82. Horecker, B. L.; Smyrniotis, P. Z. *J. Am. Chem. Soc.* **1952**, *74*, 2123.
83. Effenberger, F.; Straub, A. *Tetrahedron Lett.* **1987**, *28*, 1641.
84. Turner, N. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 624.
85. Huang, P. C.; Miller, O. N. *J. Biol. Chem.* **1958**, *330*, 805.
86. Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1952**, 4052.
87. Byrne, W. L.; Lardy, H. A. *Biochim. Biophys. Acta* **1954**, *14*, 495.
88. Gorin, P. A. J.; Jones, J. K. N. *J. Chem. Soc.* **1953**, 1537.
89. Lardy, H. A.; Wiebelhaus, V. D.; Mann, K. M. *J. Biol. Chem.* **1950**, *187*, 325.
90. Lehninger, A. L.; Sice, J. *J. Am. Chem. Soc.* **1955**, *77*, 5343.
91. Ballou, C. E.; Fischer, H. O. L.; MacDonald, D. L. *J. Am. Chem. Soc.* **1955**, *77*, 5967.
92. Charalampous, F. C. *J. Biol. Chem.* **1954**, *211*, 249.
93. Gorin, P. A. J.; Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1953**, 2140.
94. Crans, D. C.; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 7019.
95. Goldberg, M.; Fessenden, J. M.; Racker, E. *Methods Enzymol.* **1966**, *9*, 515.
96. DeMaster, E. G.; Mitchell, R. A. *Biochem.* **1973** *12*, 3616.
97. Nour-Eldeen, A. F.; Craig, M. M.; Gresser, M. J. *J. Biol. Chem.* **1985**, *260*, 6836.
98. Lagunas, R.; Sols, A. *FEBS. Lett.* **1968**, *1*, 32.
99. Furaneol is a registered trademark of Firmenich Inc. Geneva, Switzerland.
100. Pederson, R. L.; Kim, M.-J.; Wong, C.-H. *Tetrahedron Lett.* **1988**, *29*, 4645.
101. Ziegler, T.; Straub, A.; Effenberger, F. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 716.
102. Truscheit, E.; Frommer, W.; Junge, B.; Muller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 744.
103. Comb, D. G.; Roseman, S. *J. Biol. Chem.* **1960**, *235*, 2529.
104. Brunetti, P.; Jourdian, G. W.; Roseman, S. *J. Biol. Chem.* **1962**, *237*, 2447.
105. DeVries, G. H.; Binkley, S. B. *Arch. Biochem. Biophys.* **1972**, *151*, 234.
106. Uchida, Y.; Tsukada, Y.; Sugimori, T. *Biochem.*. **1984**, *96*, 507.
107. Uchida, Y.; Tsukada, Y.; Sugimori, T. *Agric. Biol. Chem.* **1985**, *49*, 181.
108. Simon. E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159.
109. Schauer, R. *TIBS* **1985**, *10*, 357.
110. Corfield, A. P.; Schauer, R. In *Sialic Acids*; Schauer, R. Ed.; Springer-Verlag: New York, 1982; p 195.
111. McGuire, E. J. In *Biological Roles of Sialic Acid*; Rosenberg, A.; Schengrund, C.-L., Eds.; Plenum: New York, 1976; Chapter 4.
112. Gottschalk, A. *The Chemistry and Biology of Sialic Acids and Related Substances*; Cambridge University: Cambridge, England, 1960.
113. Augé, C.; David, S.; Gautheron, C. *Tetrahedron Lett.* **1984**, *25*, 4663.
114. David, S.; Augé, C. *Pure Appl. Chem.* **1987**, *59*, 1501.

115. Kim, M.-J.; Hennen, W. J.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 6481.
116. Suttajit, M.; Urban, C.; McLean, R. L. *J. Biol. Chem.* **1971**, *246*, 810.
117. Schauer, R.; Wember, M.; Wirtz-Peitz, F.; Do Amaral, C. F. *Hoppe-Seylers Z. Physiol. Chem.* **1971**, 352, 1073.
118. Faillard, H.; Do Amaral, C. F.; Blohm, M. *Hoppe-Seyler's Z. Physiol. Chem.* **1969**, *350*, 798.
119. Augé, C.; David, S.; Gautheron, C.; Malleron, A.; Cavaye, B. *New. J. Chem.*. **1988**, *12*, 733.
120. Augé, C.; David, S.; Gautheron, C.; Veyrieres, A. *Tetrahedron Lett.* **1985**, *26*, 2439.
121. Brossmer, R.; Rose, U.; Kasper, D.; Smith, T. L.; Grasmuk, H.; Unger, F. M. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 1282.
122. Augé, C.; Gautheron, C. *J. Chem. Soc. Chem. Commun.* **1987**, 859.
123. Ota, Y.; Shimosaka, M.; Murata, K.; Tsudaka, Y.; Kimura, A. *Appl. Microbiol. Biotech.* **1986**, *24*, 386.
124. Ray, P. H. *J. Bacteriol.* **1980**, *141*, 635.
125. Bednarski, M. D.; Crans, D. C.; DiCosmio, R.; Simon. E. S.; Stein, P. D.; Whitesides, G. M.; Schneider, M. *Tetrahedron Lett.* **1988**, *29*, 427.
126. Hammond, S. M.; Claesson, A.; Jansson, A. M.; Larsson, L.-G.; Pring, B. G.; Town, C. M.; Ekström, B. *Nature* **1987**, *327*, 730.
127. Luthman, K.; Orbe, M.; Waglund, T.; Claesson, A. *J. Org. Chem.* **1987**, *52*, 3777.
128. Danishefsky, S. J.; DeNinno, M. P. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 15.
129. Reimer, L. M.; Conley, D. L.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1986**, *108*, 8010.
130. Borysenko, C.; Straub, J.; Spaltenstein, A.; Whitesides, G. M., unpublished results.
131. Serianni, A. S.; Cadman, E.; Pierce, J.; Hayes, M. L.; Barker, R. *Methods Enzymol.* **1982**, *89*, 83.
132. Rose, I. A. *J. Am. Chem. Soc.* **1958**, *80*, 5835.
133. Ghalambor, M. A.; Heath, E. C. *J. Biol. Chem.* **1962**, *237*, 2427.
134. Chiu, T. H.; Feingold, D. S. *Biochem.* **1969**, *8*, 98.
135. Charalampous, G. C. *Methods Enzymol.* **1962**, *5*, 283.
136. Comb, D. G.; Roseman, S. *J. Biol. Chem.* **1960**, *235*, 2529.
137. Ghalambor, M. A.; Heath, F. C. *J. Biol. Chem.* **1966**, *241*, 3222.
138. Wood, W. A.; In *The Enzymes*; Boyer, P. D. Ed.; Academic: New York, 1970; Vol. VII, p 281.
139. Dahms, A. S. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 1433.
140. Hift, H.; Mahler, H. R. *J. Biol. Chem.* **1952**, *198*, 901.
141. Weissbach, A.; Hurwitz, J. *J. Biol. Chem.* **1959**, *234*, 705.
142. Augé, C.; Bouxom, B.; Cavaye, B.; Gautheron, C. *Tetrahedron Lett.* **1989**, *30*, 2217.
143. Hoffee, P.; Rosen, O. M.; Horecker, B. L. *Methods Enzymol.* **1966**, *9*, 545.
144. Malkin, L. I.; Greenberg, D. M. *Biochim. Biophys. Acta* **1964**, *85*, 117.
145. Greenberg, D. M. *Methods Enzymol.* **1962**, *5*, 931.
146. Mathis, J. B.; Brown, G. M. *J. Biol. Chem.* **1970**, *245*, 3015.
147. Heath, E. C.; Hurwitz, J.; Horecker, B. L.; Ginsberg, A. *J. Biol. Chem.* **1958**, *231*, 1009.
148. Racker, E. *Methods Enzymol.* **1962**, *5*, 276.
149. Fauci, A. S.; Rosenberg, S. A.; Sherwin, S. A.; Dinarello, C. A. Longo, D. L.; Lane, H. C. *Ann. Intern. Med.* **1987**, *106*, 421.
150. Whistler, R. L.; Bushway, A. A.; Singh, P. P. *Adv. Carbohydr. Chem. Biochem.* **1975**, *32*, 235.
151. von der Osten, C. H.; Sinskey, A. J.; Barbas, C. F.; Pederson, R. L.; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.*, **1989**, *111*, 3924.
152. Racker, E. In *The Enzymes*; Boyer, P. D.; Lardy, H.; Myrbäch, K., Eds.; Academic: New York; 1961; Vol. 5, p 397.
153. Thunberg, L.; Backstrom, G.; Lindahl, U. *Carbohydr. Res.* **1982**, *100*, 393.
154. Kochetov, G. A. *Methods Enzymol.* **1982**, *90*, 209.
155. Bolte, J.; Demuynck, C.; Samaki, H. *Tetrahedron Lett.* **1987**, *28*, 5525.
156. Arora, K. K.; MacLeoad, J. K.; Williams, J. F. *J. Labelled Compd. Radiopharm.* **1987**, *24*, 205.
157. Mocali, A.; Aldinucci, D.; Paoletti, F. *Carbohydr. Res.* **1985**, *143*, 288.
158. Kochetov, G. A.; Solov'eva, O. N. *Biokhimiya* (Moscow) **1977**, *42*, 1872 (Abstract: CA 87:196453p).
159. Dixon, M.; Webb, E. C. *Enzymes*; Academic: New York, 1979; p 258..
160. Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron Lett.* **1988**, *29*, 1123.
161. Simon, E. S.; Grabowski, S.; Whitesides, G. M. unpublished results.
162. Kim, M.-J.; Whitesides, G. M. *Appl. Biochem. Biotech.* **1987**, *16*, 95.
163. Seip, J. E,.; Fager, S. K.; Grosz, R.; Gavagan, J. E.; DiCosmio, R.; Anton, D. L. *Enzyme Microb. Technol.* **1989**, submitted for publication.
164. Augé, C.; Gautheron, C. *Tetrahedron Lett.* **1988**, *29*, 789.
165. Crans, D. C.; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 7008.
166. Chenault, H. K.; Kim, M.-J.; Akiyama, A.; Miyazawa, T.; Simon, E.; Whitesides, G. M. *J. Org. Chem.* **1987**, *52*, 2608.
167. Dhawale, M. R.; Szarek, W. A.; Hay, G. W.; Kropinski, A. M. B. *Carbohydr. Res.* **1986**, *155*, 262.
168. Chambers, R. P.; Walle, E. M.; Baricos, W. H.; Cohen, W. In *Enzyme Engineering*; Pye, E. K. ; Weetall, H. H., Eds.; Plenum: New York, 1978; Vol. 3, p 263.
169. Maret, W.; Auld, D. S. *Biochemistry* **1988**, 27, 1622.
170. Root, L. R.; Durrwachter, J. R.; Wong, C.-H. *J. Am. Chem. Soc.* **1985**, *107*, 2997.
171. Lee, L. G.; Whitesides, G. M. *J. Org. Chem.* **1986**, *51*, 25.

172. For example, see: Joziasse, D. H.; Schiphosrt, W. E. C. M.; Van den Eijnden, D. H.; Van Kuik, J. A.; Van Halbeek, H.; Vliegenthart, J. F. G. *J. Biol. Chem.* **1987**, *262*, 2025.
173. *Commercial Biotechnology: An International Analysis*; OTA-BA-218; US Congress Office of Technology Assessment: Washington, 1984; p 199.
174. Card, P. J.; Hitz, W. D.; Ripp, K. G. *J. Am. Chem. Soc.* **1986**, *108*, 158.
175. Card, P. J.; Hitz, W. D. *J. Am. Chem. Soc.* **1984**, *106*, 5348.
176. Wong, C.-H.; Haynie, S. H.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 5416.
177. Klibanov, A. M. *CHEMTECH* **1986**, 354.
178. Fronza, G.; Fuganti, C.; Grasselli, P.; Sewrvi, S. *Tetrahedron Lett.* **1986**, *27*, 4363.
179. Kloosterman, M.; Mosmuller, E. W. J.; Schoemaker, H. E.; Meijer, E. M. *Tetrahedron Lett.* **1987**, *28*, 2989.
180. Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 6421.
181. Riva, S.; Chopineau, J.; Kieboom, A. P. G.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 584.
182. Berger, E. G.; Buddecke, E.; Kamerling, J. P.; Kobata, A.; Paulson, J. C.; Vliegenthart, J. F. G. *Experientia* **1982**, *38*, 1129.
183. Hubbard, S. C. *Ann. Rev. Biochem.* **1981**, *50*, 555.
184. Kornfeld, R.; Kornfeld, S. *Ann. Rev. Biochem.* **1985**, *54*, 631.
185. Kornfeld, S. In *The Glycoconjugates*; Horowitz, M. I., Ed.; Academic: New York, 1982, Vol. III, p 3.
186. Leloir, L. F. *Science* **1971**, *172*, 1299.
187. Schachter, H.; Roseman, S. In *The Biochemistry of Glycoproteins and Proteoglycans*; Lennarz, W. J. Ed.; Plenum: New York, 1980, p. 85.
188. Sadler, J. E.; Beyer, T. A.; Oppenheimer, C. L.; Paulson, J. C.; Prieels, J.-P.; Rearick, J. I.; Hill, R. L. *Methods Enzymol.* **1982**, *83*, 458.
189. Fellows, L. E. *Chem. Brit.* **1987**, 842.
190. Ginsburg, V. In *Advances in Enzymology*; Nord, F. F., Ed.; Wiley Interscience: New York, 1964; Vol. 26, p 35.
191. Kochetkov, N. K.; Shibaev, V. N. In *Adv. Carbohydr. Chem. Biochem.*; Tipson, R. S.; Horton, D. Eds; Academic: New York, 1973; Vol 28, 307.
192. Tochikura, T.; Kawai, H.,; Kawaguchi, K.; Mugibayhashi, Y.; Ogata, K. *Ferment. Technol. Today, Proc. IVth Int. Ferment. Symp.*, **1972**, 463.
193. Wong, C.-H.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 5416.
194. Beyer, T. A.; Sadler, J. E.; Rearick, J. I.; Paulson, J. C.; Hill, R. L. In *Advances in Enzymol.*; Meister, A., Ed.; Wiley Interscience: New York, 1981; Vol 52, p 23.
195. Thiem, J.; Treder, W. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 1096.
196. Demers, A. G.; Wong, S. S. *J. Appl. Biochem.* **1985**, *7*, 122.
197. Creeger, E. S.; Rothfield. L. I. *Methods Enzymol.* **1982**, *83*, 326.
198. Creeger, E. S.; Rothfield. L. I. *J. Biol. Chem.* **1979**, *254*, 804.
199. Weinstein, J.; Lee, E. U.; McEntee, K.; Lai, P-H.; Paulson, J. C. *J. Biol. Chem.*, **1987**, *262*, 17735.
200. Paulson, J. C.; Weinstein, J.; Ujita, E. L.; Riggs, K. J.; Lai, P.-L *Biochem. Soc. Trans.* **1987**, *15*, 618.
201. Ernst, L. K.; Rajan, V. P.; Larsen, R. D.; Ruff, M. M.; Lowe, J. B. *J. Biol. Chem.* **1989**, *264*, 3436.
202. Rajan, V. P.; Larsen, R. D.; Ajmera, S.; Ernst, L. K.; Lowe, J. B. *J. Biol. Chem.* **1989**, *264*, in press.
203. Schwarz, R. T.; Datema, R. *Adv. Carbohydr. Chem. Biochem.*, **1982**, *40*, 287.
204. Palcic, M. M.; Srivastava, O. P.; Hindsgaul, O. *Carbohydr. Res.* **1987**, *159*, 315.
205. Berliner, L. J.; Robinson, R. D. *Biochem.* **1982**, *21*, 6340.
206. Andree, P.; Berliner, L. J. *Biochim. Biophys. Acta*, **1978**, *544*, 489.
207. Lambright, D. G.; Lee, T. K.; Wong, S. S. *Biochem.* **1985**, *24*, 910.
208. Beyer, T. A.; Sadler, J. E.; Rearick, J. I.; Paulson, J. C.; Hill, R. L. *Adv. Enzymol.* **1981**, *52*, 23.
209. Palcic, M. M.; Venot, A. P.; Ratcliffe, R. M.; Hindsgaul, O. *Carbohydr. Res* **1989**, *189*, in press.
210. Srivastava, O. P.; Hindsgaul, O.; Shoreibah, M.; Pierce, M. *Carbohydr. Res.* **1988**, *179*, 137.
211. Morin, M. J.; Porter, C. W.; Petrie, C. R.; Korytnyk, W.; Bernacki, R. J. *Biochem. Pharm.* **1983**, *32*, 553.
212. McDowell, W.; Grier, T. J.; Rasmussen, J. R.; Schwarz, R. T. *Biochem. J.* **1987**, *248*, 523.
213. Shibaev, V. N. *Pure Appl. Chem.* **1978**, *50*, 1421.
214. Gross, H. J.; Bünsch, A.; Paulson, J. C.; Brossmer, R. *Eur. J. Biochem.* **1987**, *168*, 595.
215. Nunez, H. A.; Barker, R. *Biochemistry* **1980**, *19*, 489.
216. Deya, E.; Amaya, M.; Igarashi, S.; Ahiko, K. *Yuki. Nyugyo Kenkyu. Hokoku* **1986**, *25* (Abstract: CA **106**: 115628k).
217. Augé, C.; David, S.; Mathieu, C.; Gautheron, C. *Tetrahedron Lett.* **1984**, *25*, 1467.
218. Zehavi, U; Herchman, M. *Carbohydr. Res* **1984**, *128*, 160.
219. Zehavi, U; Sadeh, S.; Herchman, M. *Carbohydr. Res* **1983**, *124*, 23.
220. Zehavi, U; Herchman, M. *Carbohydr. Res* **1984**, *133*, 339.
221. Augé, C.; Mathieu, C.; Mérienne, C. *Carbohyd. Res.* **1986**, *151*, 147.
222. Kloosterman, M., Ph.D. thesis, Leiden.
223. Rosevear, P. R.; Nunez, H. A.; Barker, R. *Biochemistry* **1982**, *21*, 1421.
224. Weisesmann, S.; Denzel, K.; Schilling, G.; Gross, G. G. *Bioorganic Chem.* **1988**, *16*, 29.
225. Hindsgaul, O.; Tahir, S. H.; Srivastava, O. P. *Carbohydr. Res* **1988**, *173*, 263.
226. Tahir, S. H.; Hindsgaul, O. *Can. J. Chem.* **1986**, *64*, 1771.
227. Penglis, A. A. E. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 195.
228. Haynie, S. L., Whitesides, G. M. *Appl. Biotech. Biochem.* **1989**, submitted for publication.

229. Dedonder, R. *Methods in Enzymol.* **1966**, *VIII*, 500.
230. Walker, G. J. In *International Review of Chemistry of Carbohydrates II*; Manners, D. J. Ed.; University Park: Baltimore, 1978; p 75.
231. Wallenfels, K.; Weil, R. In *The Enzymes*, 3rd Ed.; Boyer, P. D. Ed.; Academic Press: New York, 1972; Vol. VII, p 618.
232. Post, C. B.; Karplus, M. *J. Am. Chem. Soc.* **1986**, *108*, 1317.
233. Withers, S. G.; Street, I. P. *J. Am. Chem. Soc.* **1988**, *110*, 8551.
234. Huber, R. E.; Kurz, G.; Wallenfels, K. *Biochem.* **1976**, *15*, 1994.
235. Bourquelot, E. *Ann. Chim.* **1913**, *29*, 145.
236. Bourquelot, E. *J. Pharm. Chem.* **1914**, *10*, 361.
237. Veibel, S. *Enzymologia* **1936**, *1*, 124.
238. Johansson, E.; Hedbys, L.; Larsson, P.-O.; Mosbach, K.; Gunnarsson, A.; Svensson, S. *Biotechnol. Lett.* **1986**, *8*, 421.
239. Hedbys, L.; Larsson, P.-O.; Mosbach, K.; Svensson, S. *Biochem. Biophys. Res. Commun.* **1984**, *123*, 8.
240. Larsson, P.-O.; Hedbys, L.; Svensson, S.; Mosbach, K. *Methods Enzymol.* **1987**, *136*, 230.
241. Fujimoto, H.; Ajisaka, K. *Biotechnol. Lett.* **1988**, *10*, 107.
242. Ezure, Y. *Agric. Biol. Chem.* **1985**, *49*, 2159.
243. Okada, G.; Hehre, E. J. *Carbohydr. Res.* **1973**, *26*, 240.
244. Treder, W.; Thiem, J.; Schlingmann, M. *Tetrahedron Lett.* **1986**, *27*, 5605.
245. Kitahata, S.; Okada, S.; Fukui, T. *Agric. Biol. Chem.* **1978**, *42*, 2369.
246. Chiba, S.; Okada; S. Kitahata, S.; Shimomura, T. *Agric. Biol. Chem.* **1975**, *39*, 2353.
247. Nilsson, K. G. I. *Carbohydr. Res.* **1987**, *167*, 95.
248. Ooi, Y.; Hashimoto, T.; Mitsuo, N.; Satoh, T. *Tetrahedron Lett.* **1984**, *25*, 2241.
249. Nilsson, K. G. I. *Carbohydr. Res.* **1988**, *180*, 53.
250. Kusama, S.; Kusakabe, I.; Nakamura, Y.; Eda, S.; Murakami, K. *Agric. Biol. Chem.* **1986**, *50*, 2445.
251. Tanaka, T.; Oi, S. *Agric. Biol. Chem.* **1985**, *49*, 1267.
252. Ooi, Y.; Hasimoto, T.; Mitsuo, N.; Satoh, T. *Chem. Pharm. Bull.* **1985**, *33*, 1808.
253. Suyama, K.; Adachi, S.; Toba, T.; Sohma, T.; Hwang, C.-J.; Itoh, T. *Agric. Biol. Chem.* **1986**, *50*, 2069.
254. van Eikeren, P.; White, W. A.; Chipman, D. M. *J. Org. Chem.* **1973**, *38*, 1831.
255. Gais, H.-J.; Zeissler, A.; Maidonis, P. *Tetrahedron Letters* **1988**, *29*, 5743.
256. Boos, W. *Methods Enzymol.* **1982**, *89*, 59.
257. Björkling, F.; Godtfredsen, S. E. *Tetrahedron* **1988**, *44*, 2957.
258. Itano, K.; Yamasaki, K.; Kihara, C.; Tanaka, O. *Carbohydr. Res.* **1980**, *87*, 27.
259. Ooi, Y.; Mitsuo, N.; Satoh, T. *Chem. Pharm. Bull.* **1985**, *33*, 5547.
260. Mitsuo, N.; Takeichi, H.; Satoh, T.; *Chem. Pharm. Bull.* **1984**, *32*, 1183.
261. *Polysaccharides in Food*; Blanshard, J. M. V.; Mitchell, J. R. Eds.; Butterworth: London, 1979.
262. Birch, G. G.; Parker, K. J. *Sugar: Science and Technology*; Applied Science: London, 1979.
263. Sandford, P. A.; Baird, J. In *The Polysaccharides*; Aspinall, G. O. Ed.; Academic: New York, 1983; Vol. 2, p 412.
264. *Polymers in Medicine*; Chiellini, E.; Giusti, P., Eds.; Plenum: New York, 1983.
265. Greiling, H.; Momburg, M.; Stuhlsatz, H. W. *Scand. J. Clin. Lab. Invest.* **1972**, *29* Suppl 123, 37.
266. Monsan, P.; Paul, F.; Auriol, D.; Lopez, A. *Methods Enzymol.* **1987**, *136*, 239.
267. Shibaev, V. N. Adv. *Carbohydr. Chem. Biochem.* **1986**, *44*, 277.
268. Botstein, D.; Davis, R. W. In *Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression*. Strathern, J. N; Jones, E. W.; Broach, J. R. Cold Spring Harbor Laboratory, 1981; p 607.
269. Carlson, D. M. *Pure Appl. Chem.* **1987**, *59*, 1489.
270. Pfannemüller, B. *Staerke* **1968**, *11*, 341.
271. Waldmann, H.; Gygax, D.; Bednarski, M. D.; Shangraw, W. R.; Whitesides, G. M. *Carbohydr. Res.* **1986**, *157*, c4.
272. Ziegast, G.; Pfannemüller, B. *Carbohydr. Res.* **1987**, *160*, 185, and references therein.
273. Ziegast, G.; Pfannemüller, B. *Makromol. Chem., Rapid Commun.* **1984**, *5*, 373.
274. Horikoshi, K. *Proc. Biochem.* **1979** *14*, 26.
275. Jamas, S.; Rha, C. K.; Sinskey, A. *J. Biotechnol. Bioeng.* **1986**, *28*, 769.
276. Stoolmiller, A. C.; Dorfman, A. *J. Biol. Chem.* **1969**, *244*, 236.
277. Simon, E. S.; Toone, E. J.; Ostroff, G.; Bednarski, M. D.; Whitesides, G. M. *Methods Enzymol.* **1989**, *138(2)*, in press.
278. Toone, E. J.; Ostroff, G.; Whitesides, G. M. **1989**, unpublished results.
279. Gooday, G. W.; Trinci, A. P. J. In *The Eukaryotic Microbial Cell*; Gooday, G. W.; Lloyd, D.; Trinci, A. P. J., Eds.; Cambridge University: Cambridge, England, 1980; p 207.
280. Davis, L. L.; Bartnicki-Garcia, S. *Biochemistry* **1984**, *23*, 1065.
281. Hanessian, S. *The Total Synthesis of Natural Products. The "Chiron" Approach*; Pergamon: New York; 1983.
282. Fleet, G. *Chem. Brit.* **1989**, *25*, 287.
283. St. Georgiev, V. *Ann. Rep. Med. Chem.* **1987**, *22*, 127.
284. Fenichel, R. L.; Chirigos, M. A. *Immune Modulation Agents and their Mechanisms*; Marcel Decker: New York, 1984.
285. Stewart-Tull, D. E. S. *Prog. Drug. Res.* **1988**, *32*, 305.

286. Ellouz, F.; Adam, A.; Ciorbaru, R.; Lederer, E. *Biochem. Biophys. Res. Comm.* **1974**, *59*, 1317.
287. Adam, A.; Lederer, E. *Medicinal Res. Rev.* **1984**, *4*, 111.
288. Chedid, L.; Parant, M.; Parant, F.; Lefrancier, P.; Choay, J.; Lederer, E. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 2089.
289. Acevedo, H. F.; Raikow, R. B.; Acevedo, H. O.; Delgado, T. F.; Pardo, M. *Antimicrob. Agents Chemother.* **1985**, *28*, 589.
290. Lederer, E. *J. Med. Chem.* **1980**, *23*, 819.
291. Murray, G. J. *Methods Enzymol.* **1987**, *149*, 25.
292. For a collection of articles on bacterial endotoxin see: *Bacterial Endotoxin: Chemical, Biological and Clinical Aspects*; Homma, J. Y.; Kanegasaki, S.; Lüderitz, O.; Shiba, T.; Westphal, O. Eds.; Verlag Chemie: Weinheim, 1984.
293. Morrison, D. C.; Ryan, J. L. *Ann. Rev. Med.* **1987**, *38*, 417.
294. Luderitz, O.; Freudenberg, M. A.; Galanos, C.; Lehmann, V.; Rietschel, E. T.; Shaw, D. H. *Curr. Top. Membr. Transp.* **1982**, *17*, 79.
295. Galanos, C.; Luderitz, O.; Rietschel, E. T.; Westphal, O. *Int. Rev. Biochem.* **1977**, *14*, 213.
296. Beutler, B.; Cerami, A. *Ann. Rev. Biochem.* **1988**, *57*, 505.
297. Galanos, C.; Luderitz, O.; Westphal, O. *Eur. J. Biochem.* **1971**, *24*, 116.
298. Luderitz, O.; Galanos, C.; Lehmann, V.; Mayer, H.; Rietschel, E. T.; Weckesser, J. *Naturwissenchaften* **1978**, *65*, 578.
299. Galanos, C.; Lehmann, V.; Luderitz, O.; Rietschel, E. T.; Westphal, O.; Brade, H.; Brade, L.; Freudenberg, M. A.; Hansen-Hagge, T.; Luderitz, T.; Mckenzie, G.; Schade, U.; Strittmatter, W.; Tanamoto, K.-I.; Zahringer, U.; Imoto, M.; Yoshimura, H.; Yamamoto, M.; Shimamoto, T.; Kusumoto, S.; Shiba, T. *Eur. J. Biochem.* **1984**, *140*, 221.
300. Fransson, L.-A. *TIBS* **1987**, *12*, 406.
301. Casu, B. *Adv. Carbohydr. Chem. Biochem.* **1985**, *43*, 51.
302. Lindahl, U.; Thunberg, T.; Backstrom, G.; Riesenfeld, J.; Nordling, K.; Bjork, I. *J. Biol. Chem.* **1984**, *259*, 12368.
303. Bodor, N.; Kaminski, J. J. *Ann. Rep. Med. Chem.* **1987**, *22*, 303.
304. Kim, S. W.; Petersen, R. V.; Feijen, J. In *Drug Design*; Ariëns, E. J., Ed.; Academic: New York, 1980; Vol. X, Chapter 6.
305. Elbein, A. D. *Ann. Rev. Biochem.* **1987**, *56*, 497.
306. Schwarz, R. T.; Datema, R. *TIBS* **1984**, *9* 32.
307. Truscheit, E., Frommer, W.; Junge, B.; Müller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 744.
308. Conradt, H. S.; Egge, H.; Peter-Katalinic, J.; Reiser, W.; Siklosi, T.; Schaper, K. *J. Biol. Chem.*, **1987**, *262*, 14600.
309. Little, S. P.; Bang, N. U.; Harms, C. S.; Marks, C. A.; Mattler, L. E. *Biochem.* **1984**, *23*, 6191.
310. Abe, J.-I.; Mizowaki, N.; Hizukuri, S.; Koizumi, K.; Utamura, T. *Carbohydr. Res.* **1986**, *154*, 81.