Chapter 1

Enzymes as Catalysts in Carbohydrate Synthesis

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The discovery of the myriad roles of carbohydrates in biological recognition phenomena has led to an increased demand for practical routes to gram-scale quantities of unnatural carbohydrate structures. We have investigated the applicability of enzymes as catalysts for mono-oligo-, and polysaccharide synthesis. Here, we review our progress in the area, in particular the use of aldolases for monosaccharide synthesis, the synthesis of the activated sugar nucleosides required for Leloir-pathway glycosidic bond formation, and the use of both Leloir- and non-Leloir-pathway enzymes for the synthesis of carbohydrate polymers.

The past two decades have seen explosive growth in the field of carbohydrate chemistry. The synthesis and structural analysis of natural and unnatural carbohydrate structures has reached levels impossible only a few years ago. To a large extent, growth in carbohydrate chemistry has been driven by advances in carbohydrate biology, or glycobiology. Until recently, carbohydrates were generally regarded solely as energy storage vehicles and structural units in cells. These notions have now been challenged: it is clear that carbohydrates govern a wide range of biological recognition phenomena (*1-3*). In addition to their well-known recognition roles as blood group antigens, it is now known that carbohydrates act as binding sites for a wide range of bacteria, viruses, hormones and soluble toxins (*4-7*). Cell-surface carbohydrates also play key roles in intercellular communication events that control growth and differentiation as well as organogenesis (*8*). In addition to these now well-defined roles, research is continually establishing new roles for carbohydrates in other systems, including, for instance, as binding sites for cell-adhesion molecules (CAMs) (*9*) and tumor necrosis factor (*10*).

With these new-found roles for carbohydrates has come an increased demand for practical synthetic routes to this class of compounds. An enormous number of carbohydrate and carbohydrate-like structures exist that are of biomedical interest (Figure 1). Despite efforts towards general methodologies, carbohydrates remain one of the most challenging groups of compounds to prepare (*11-14*). In the past, enzymes have been

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utilized as catalysts for the synthesis of a range of non-carbohydrate compounds (*15-17*). More recently, enzymes have been applied to carbohydrate synthesis (*18*). Briefly, enzymes offer two major advantages over classical methodologies for the synthesis of carbohydrates:

i) *Compatibility with aqueous media.* Many of the reactions commonly utilized by organic chemists are incompatible with water. Water, however, is the most practical medium for synthetic manipulations of unprotected, hydrophilic compounds such as carbohydrates. Enzymes generally operate in aqueous solution, at or near neutral pH, at or near room temperature. The use of enzymes as catalysts for carbohydrate synthesis therefore avoids the necessity for protection/deprotection schemes.

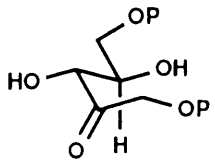
ii) *Specificity.* The specificity of enzymes is manifested in three major ways, all of which are vital to successful carbohydrate syntheses. First, enzyme-catalyzed reactions demonstrate absolute *chemospecificity*. A second important feature of enzyme-catalyzed reactions is high *regiospecificity*. Since carbohydrates generally contain a number of hydroxyl groups of approximately equal reactivity, the ability to selectively manipulate a single hydroxyl residue is clearly important. Third, enzymes display exquisite *stereospecificity*. Carbohydrates are chirotopic species, and usually possess multiple stereogenic centers. This stereochemical information must be correctly installed in any successful synthetic methodology.

It is clear, then, that perhaps no other group of compounds is as well suited to enzyme-based synthesis as is the carbohydrates. During the past several years, we have developed a range of enzyme-based syntheses for mono-, oligo-, and polysaccharides. This paper reviews some of our efforts on the use of aldolases and transketolase for monosaccharide synthesis, as well as the use of Leloir and non-Leloir pathway enzymes for glycosidic bond formation.

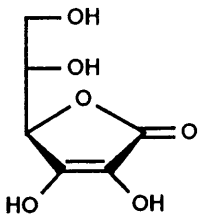
Monosaccharide Synthesis Using Aldolases

FDP Aldolase. The most extensively utilized class of enzymes for monosaccharide synthesis are the aldolases (E.C. sub-class 4.1.2.). This ubiquitous group of enzymes catalyzes reversible aldol reactions *in vivo*. Two major groups of aldolases exist: type I aldolases, found primarily in higher plants and animals, catalyze aldol condensations by means of a Schiff base formed between an enzyme lysine e-amino group and the nucleophilic carbonyl group; type II aldolases, found primarily in microorganisms, utilize a divalent zinc to activate the nucleophilic component (*19*). Approximately 25 aldolases have been identified to date (*18*).

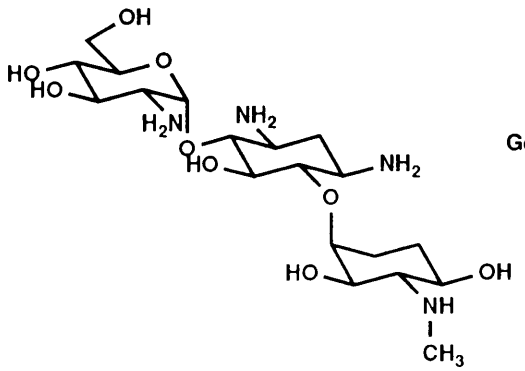
Aldolases have been studied as catalysts for monosaccharide synthesis for nearly 40 years. The best studied member of the group is a fructose-1,6-diphosphate (FDP) aldolase from rabbit muscle (RAMA, E.C. 4.1.2.13) (*20*). *In vivo*, this enzyme catalyzes the reversible condensation of D-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (DHAP) to generate FDP (Scheme 1). In the synthetic direction, the enzyme catalyzes the formation of two new stereogenic centers with absolute stereospecificity: the stereochemistry of the new vicinal diol is always D-*threo*. RAMA will accept a wide range of aldehydes as electrophiles in the aldol condensation. Studies have demonstrated that virtually any aldehyde except aldehydes sterically hindered at the α-position, α,β-unsaturated



Ribulose-1,5-P_2

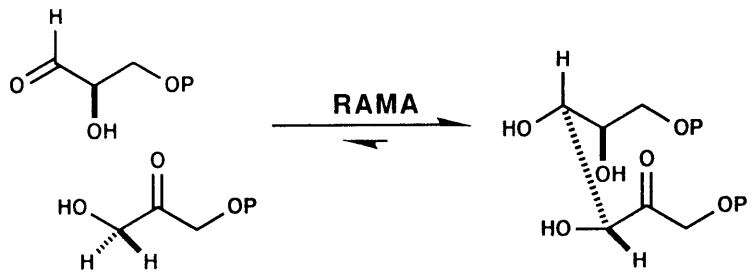


Ascorbic Acid



Gentamycin A

Figure 1. Carbohydrate Structures of Biomedical Interest.



Scheme 1. The RAMA-Catalyzed Aldol Condensation.

aldehydes, or those that can readily eliminate to α,β-unsaturated aldehydes, is accepted as a substrate. The demand for DHAP as nucleophile seems to be absolute (*18*). We have prepared a number of useful monosaccharides using RAMA, including 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphonate, an inhibitor of the shikimate pathway (DAHP, Scheme 2) (*21*) and Furaneol®, a flavoring component (*22*).

Despite the demonstrated utility of RAMA in carbohydrate synthesis, an inability to install stereochemistries other than D-*threo* at the C3/C4 vicinal diol limits the range of applications of the enzyme. We have pursued strategies designed to overcome this limitation. The "inversion strategy" (Scheme 3) makes use of a monoprotected dialdehyde as the electrophile during a RAMA-catalyzed condensation (*23*). The resulting ketose can then be reduced, and the protected aldehyde functionality unmasked to generate a new aldose sugar.

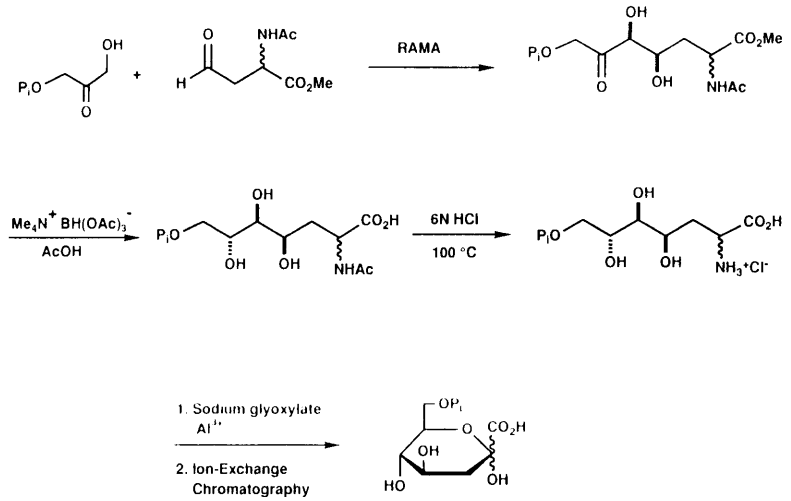
The stereospecific reduction of the ketone functionality is clearly of paramount importance to the inversion strategy. We have used iditol dehydrogenase (also known as sorbitol dehydrogenase or polyol dehydrogenase, E.C. 1.1.1.14) from *Candida utilis* or sheep liver to selectively generate *either* diastereomer of the new polyol using a single oxido-reductase (*23*). Reduction of the ketose with iditol dehydrogenase generates the 2R polyol exclusively (Scheme 4). Alternatively, chemical reduction of the ketone followed by stereospecific *oxidation* of the unwanted enantiomer with iditol dehydrogenase generates the 2S diastereomer (Scheme 5). The ketose resulting from the unwanted enantiomer can then be recycled.

Fuculose 1-Phosphate Aldolase. Another potential route to different stereochemistries at C3 and C4 is *via* different aldolases. Of the four possible diastereomers that can result from an aldol condensation between DHAP and an electrophile, aldolases have been identified in Nature that stereoselectively generate three (Scheme 6).

We have recently overexpressed a fuculose 1-phosphate (Fuc-1-P) aldolase from *E. coli* and have begun to evaluate its utility as a catalyst for carbohydrate synthesis (*24*). We have expressed the enzyme using a *tac* promoter, to a level of 6 X 10^3 units per liter (one unit of enzyme catalyzes the conversion of 1 μmol of substrates to products per minute under optimal conditions of temperature and pH). The enzyme is easily purified to crystallinity using ion-exchange chromatography. Fuc-1-P aldolase has a usefully broad substrate specificity, at least with regard to the electrophilic component: the enzyme appears to accept over 40 compounds in kinetic assays. Investigations on the substrate specificity with regard to the nucleophilic component remain to be completed. Fuc-1-P aldolase has been used in a preparative-scale (10 μmol) synthesis of D-ribulose (Scheme 7) (*24*).

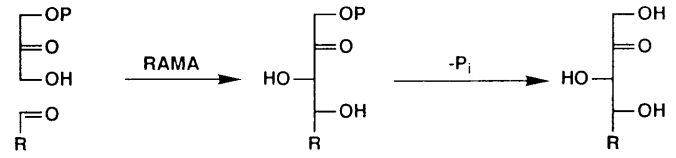
Synthesis of DHAP. Both RAMA and Fuc-1-P aldolase require DHAP as the nucleophilic component. Although DHAP is commercially available, it is too expensive for synthetic use, and must be synthesized for preparative-scale enzymatic reactions. DHAP has been prepared *via* three major routes: enzymatically from FDP using a combination of RAMA and triose isomerase (TIM, E.C. 5.3.1.1) (*25*), chemically, by phosphorylation of dihydroxyacetone dimer (*26*), and enzymatically by phosphorylation of dihydroxyacetone catalyzed by glycerokinase (Scheme 8) (*25*).

The preparation of DHAP by phosphorylation of dihydroxyacetone with glycerokinase is most effective for large-scale (mole) syntheses of

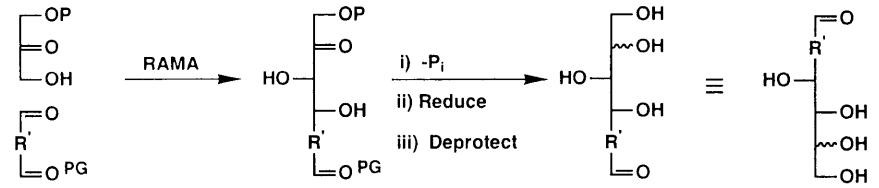


Scheme 2. Synthesis of DAHP.

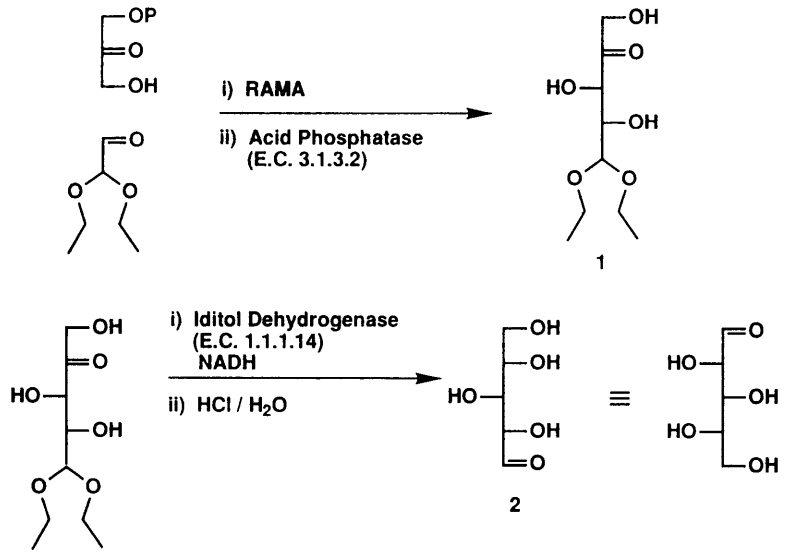
NORMAL



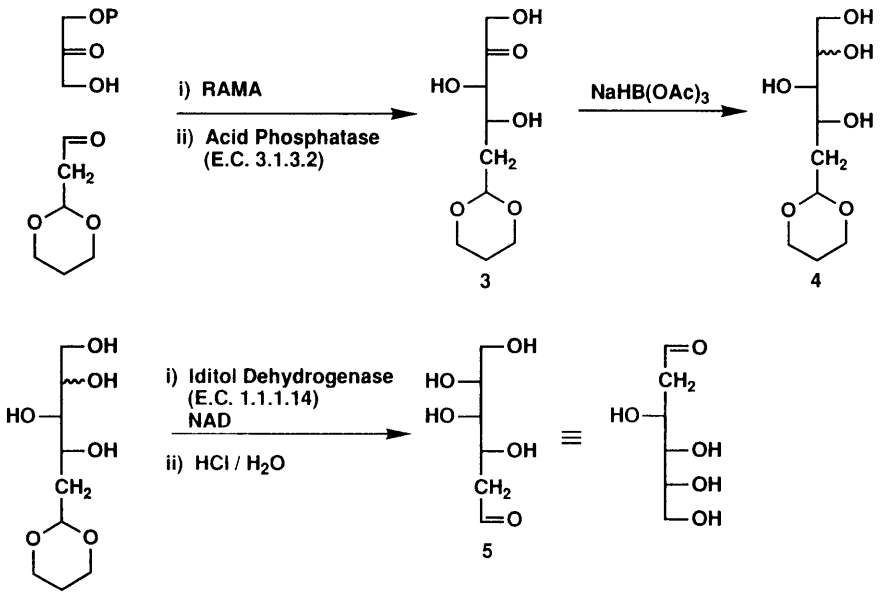
INVERTED



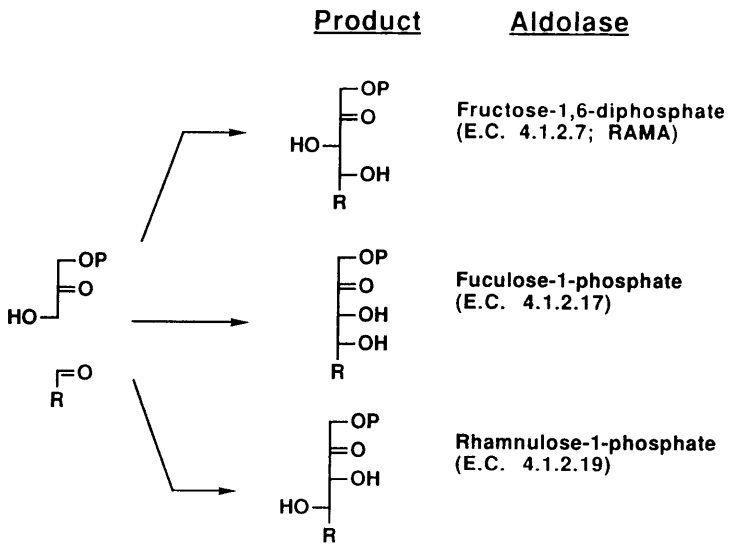
Scheme 3. The Inversion Strategy. P = phosphate; PG = protecting group.



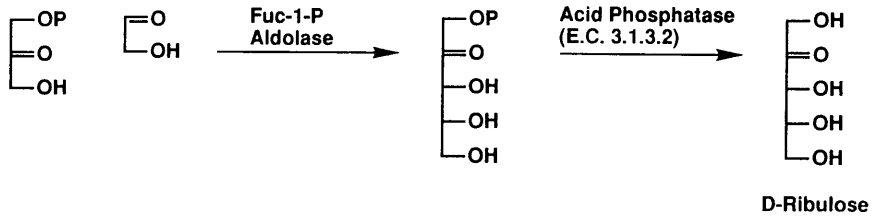
Scheme 4. Synthesis of L-Xylose.



Scheme 5. Synthesis of 2-Deoxy-D-*arabino*-hexose.



Scheme 6. Identified DHAP Aldolases.



Scheme 7. Synthesis of D-Ribulose.

DHAP (*27*). This strategy, however, requires an effective regeneration scheme for ATP. We have utilized two regeneration schemes for ATP, one based on pyruvate kinase (E.C. 2.7.1.40), and one based on acetyl kinase (E.C. 2.7.2.1). Both phosphate donors, acetyl phosphate and phosphoenolpyruvate (PEP) must be prepared: both have been prepared chemically on a mole scale (*27*).

Recently, we have developed a method for preparing PEP *in situ* from commercially available 3-phosphoglyceric acid (PGA), using phosphoglyceromutase and enolase (Scheme 9) (*28*).

Other Aldolases. In addition to the DHAP aldolases, we have conducted preliminary investigations of two other aldolases. KDO synthase (E.C. 4.1.2.16) catalyzes the formation of 2-keto-3-deoxy-L-*arabino*-octulosonic acid 8-phosphate (KDO-8-P) from arabinose 5-phosphate and PEP (Scheme 10) (*29*). KDO is an integral component of Gram-negative bacterial cell walls, and derivatives of KDO are of interest as inhibitors of cell wall formation (*30, 31*).

N-Acetylneuraminic acid (NeuAc) aldolase (E.C. 4.1.3.3) catalyzes the formation of NeuAc from *N*-acetylmannosamine and pyruvate (Scheme 11). The sialic acids are key cell-surface determinants of mammalian glycoconjugates. We and others have prepared *N*-acetylneuraminic acid (*32*) *via* a NeuAc aldolase-catalyzed condensation between *N*-acetylmannosamine and pyruvate. A number of derivatives of sialic acid have been prepared using derivatives of mannose (*33-35*).

Transketolase. Another group of enzymes that catalyze the stereospecific formation and cleavage of carbohydrates *in vivo* are the transketolases and transaldolases. Transketolase (E.C. 2.2.1.1) is a thiamin pyrophosphate (TPP) dependent enzyme that catalyzes the transfer of a hydroxyketo group from a ketose phosphate to an aldose phosphate in the pentose pathway (Scheme 12) (*36*).

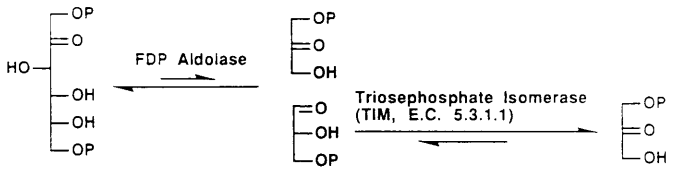
The action of transketolase generates vicinal diols having the same stereochemistry as the products of RAMA-catalyzed condensation. The enzyme, however, has two significant advantages over RAMA: the reaction does not require DHAP, and the products are not phosphorylated. The ketose functionality can be replaced by hydroxy pyruvate, which provides a hydroxyketo equivalent after decarboxylation. No other hydroxy acid has yet been found that is accepted by transketolase. Although the enzyme is absolute in its requirement for the R configuration of the hydroxy functionality at C2 of the aldehyde, there seem to be no other stereochemical requirements. Transketolase accepts a range of aldoses as substrates, and should be a useful enzyme for carbohydrate synthesis (Table 1) (*37*).

Synthesis of Oligo- and Polysaccharides Using Glycosyltransferases

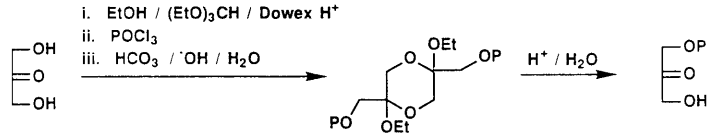
One of the greatest single problems in the field of carbohydrate synthesis is the development of reliable methods for the formation of glycosidic bonds. Despite the development of numerous protocols for the synthesis of oligosaccharides, the available coupling reactions often give mixtures of anomers, occur with low yields, and lack generality (*38*). The formation of glycosidic linkages is another area of carbohydrate chemistry where enzymes are now beginning to have an impact.

The glycosyltransferases transfer activated monosaccharides to nascent oligo- or polysaccharide chains. There are two basic motifs by

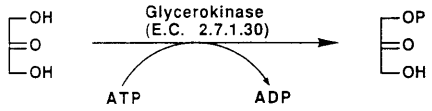
1. From FDP.



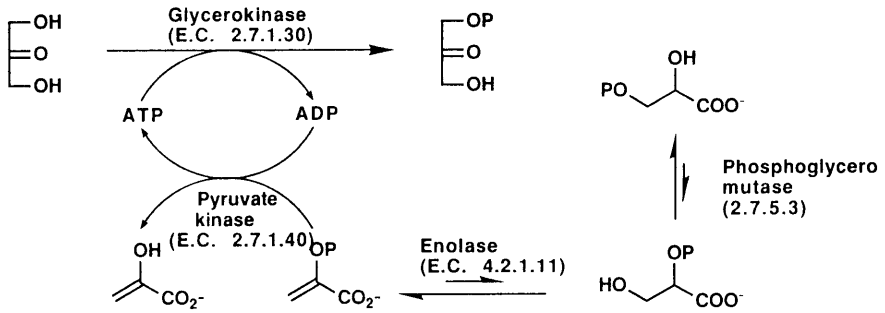
2. Chemical Synthesis from Dihydroxyacetone.



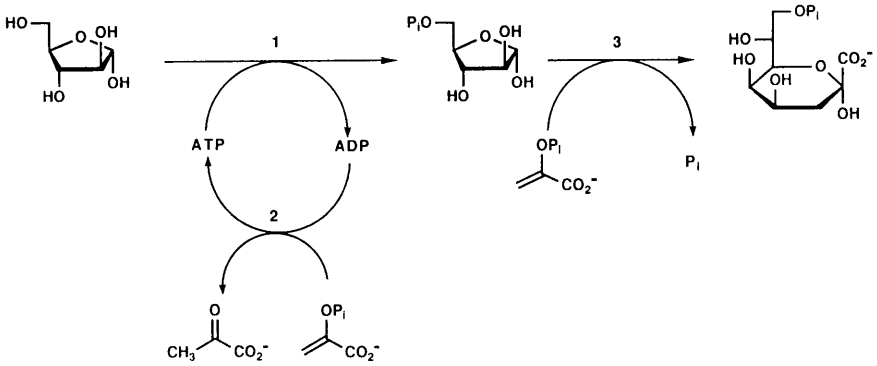
3. Enzymatic Synthesis Using Glycerokinase



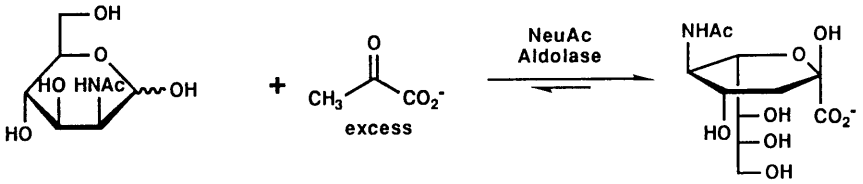
Scheme 8. Synthesis of DHAP.



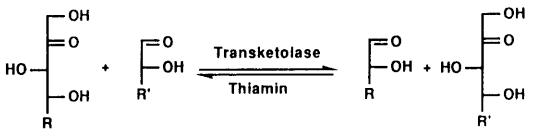
Scheme 9. Enzymatic Synthesis of DHAP.



Scheme 10. Synthesis of KDO-8-P.

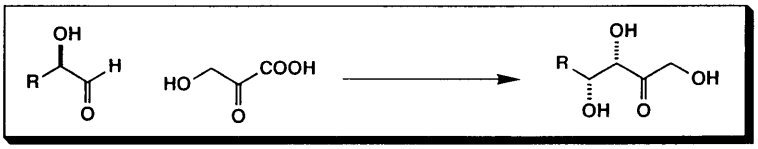


Scheme 11. Synthesis of NeuAc.

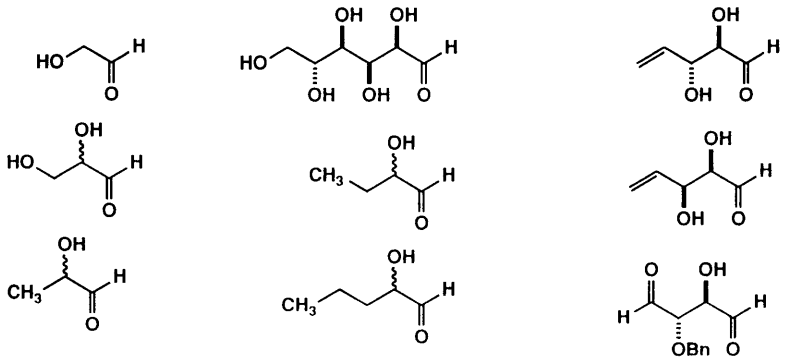


Scheme 12. Transketolase-Catalyzed Ketose Transfer.

Table 1. Substrate Specificity of Transketolase



Aldehyde Substrates:



Scheme 13. Synthetic Routes to Activated Monosaccharides.

which monosaccharides are activated *in vivo*. The majority of glycosyltransferases utilize sugars activated as the nucleoside phosphate derivatives. The enzymes that catalyze the formation and transfer of nucleoside phosphate sugars are collectively referred to as the enzymes of the Leloir pathway, after the Argentinian biochemist who elucidated this biosynthetic route. A second group of glycosyltransferases use monosaccharides activated as the sugar-1-phosphate. Examples of this class of enzymes are sucrose phosphorylase and amylose phosphorylase. We have conducted investigations over the past several years on both groups of enzymes as catalysts for glycosidic bond formation.

Leloir Pathway Glycosyltransferases. The Preparation of Activated Monosaccharides. In mammalian biochemistry, eight monosaccharides are commonly encountered: uridine 5'-diphosphoglucose (UDP-Glc), uridine 5'-diphosphogalactose (UDP-Gal), uridine 5'-diphosphoglucuronic acid (UDP-GlcUA), uridine 5'-diphospho-*N*-acetylglucosamine (UDP-GlcNAc), uridine 5'-diphospho-*N*-acetylgalactose (UDP-GalNAc), guanidine 5'-diphosphomannose (GDP-Man), guanidine 5'-diphosphofucose (GDP-Fuc), and cytidine 5'-monophospho-*N*-acetylneuraminic acid (CMP-NeuAc). A substantial portion of our research in the area of Leloir-pathway synthesis has focussed on the development of practical synthetic routes to multigram quantities of the nucleoside phosphate sugars required to transfer these eight sugars. The biosynthetic routes by which these sugars are synthesized are shown in Scheme 13. Our progress in this area is also shown schematically. We discuss below our recent efforts towards the syntheses of UDP-GlcUA, and CMP-NeuAc. We have previously reported multigram preparations of UDP-Glc and UDP-Gal (*39*).

Synthesis of Nucleoside Triphosphates (XTPs). *In vivo*, the nucleoside diphosphate sugars are synthesized from the sugar-1-phosphate and the appropriate nucleoside triphosphates (Scheme 14).

$$\text{Sugar-1-P} + \text{XTP} \rightarrow \text{XDP-Sugar}$$

Scheme 14. Biosynthesis of Nucleoside Diphosphate Sugars.

An exception is the synthesis of CMPNeuAc, which proceeds directly from neuraminic acid (Scheme 15).

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

Scheme 15. Biosynthesis of CMPNeuAc.

Clearly, good synthetic routes to all of the required XTPs is a prerequisite to successful preparation of activated nucleoside phosphate sugars. Although literature methods exist for the preparation of all XTPs from the corresponding XMP, few methods for the convenient preparation of gram quantities exist. We therefore undertook an examination of the various potential routes, both enzymatic and chemical, to determine the optimal preparation of each XTP. Scheme 16 outlines the most effective enzymatic route to the nucleoside triphosphates (*40*).

Adenylate kinase, which *in vivo* catalyzes the equilibrium between adenosine mono-, di- and triphosphates, has been used extensively in the production of ATP (*27, 41*). Although the enzyme has a broad substrate specificity for nucleoside di- and triphosphates, the specificity for monophosphates is much more restrictive. Nonetheless, the specificity is

broad enough to permit a multigram synthesis of CTP (*42*). Guanylate kinase offers the most effective route to GTP, while UTP is best prepared by chemical deamination of CTP (*40*). In all cases the ultimate phosphate donor is PEP, which can be generated from PGA (*vide supra*). All of the nucleoside monophosphates are commercially available at low cost.

UDP-GlcUA. Glucuronic acid occurs *in vivo* as a conjugate for xenobiotic removal (*43*). The uronic acids (glucuronic acid and L-iduronic acid) also occur in the glycoamineglycan polysaccharides, previously referred to as the mucopolysaccharides. Important members of the glycosamineglycans include hyaluronic acid, chondroitin, and heparin. Iduronic acid is not transferred as a monosaccharide: it is generated by epimerization of glucuronic acid in an intact polymer.

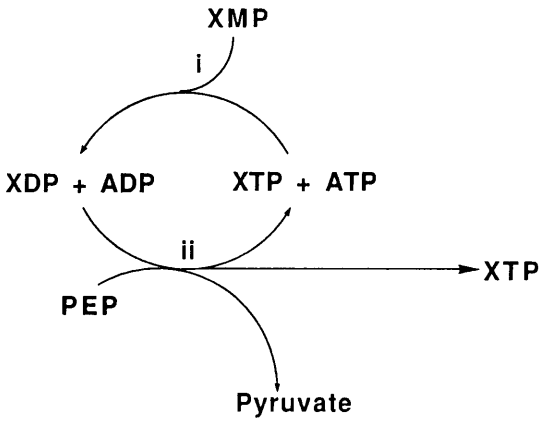
UDP-GlcUA is synthesized *in vivo* by oxidation of UDP-Glc. The nicotinamide dependent UDP-Glc dehydrogenase (E.C. 1.1.1.22, from bovine liver) is commercially available at $20/U. In our hands, the enzyme was unstable, and unsuitable for the preparation of gram-scale quantities of UDP-GlcUA. UDP-Glc dehydrogenase can be isolated from whole bovine liver, according to literature methods (*44*). This isolation yields approximately 450 U of enzyme from 2.5 kg of frozen liver. We were able to prepare UDP-GlcUA from UDP-Glc on a 1-gram scale using this preparation (Scheme 17). The overall yield was 76% (91% mass recovery, 84% pure by enzymatic assay) (*45*). The nicotinamide cofactor was regenerated using pyruvate with L-lactate dehydrogenase.

We were able to synthesize the simplest member of the glycosamineglycans, hyaluronic acid, using a cell-free system of enzymes isolated from *Streptococcus zooepidemicus* (Scheme 18) (*46*). UDP-GlcUA was generated *in situ* from UDP-Glc using commercially available UDP-Glc dehydrogenase. Although the current enzyme preparation is not suitable for large-scale production of hyaluronic acid, the cell-free enzyme system offers the possibility of incorporating unnatural carbohydrates into the polymer. This could potentially lead to the synthesis of polymers with more desirable properties than the naturally occurring material.

CMP-NeuAc. Derivatives of neuraminic acid frequently terminate mammalian glycoconjugates. Activated NeuAc is therefore an especially important target for enzyme-based synthesis. NeuAc is synthesized *in vivo* by an NeuAc aldolase-catalyzed condensation of *N*-acetylmannosamine and pyruvate (Scheme 11 above). NeuAc is then coupled directly to CTP by CMP-NeuAc synthase (E.C. 2.7.7.43). We have recently published a multigram preparation of CMP-NeuAc which makes use of both NeuAc aldolase and CMP-NeuAc synthase (Scheme 19) (*47*). NeuAc aldolase has been cloned and overexpressed, and is commercially available. The final enzyme required, CMP-NeuAc synthase, was isolated from calf brain.

Non-Leloir Pathway Glycosyltransferases. Glycosidic linkages have also been formed using glycosyl transferases which utilize sugar-1-phosphates as activated monosaccharides. Both sucrose phosphorylase and trehalose phosphorylase have been utilized *in vitro* to synthesize disaccharides (Scheme 20) (*48*). Synthetic methodologies based on isolated enzymes as catalysts may allow the preparation of unnatural analogues of these two important sugars.

A number of polysaccharides can also be prepared using non-Leloir glycosyl transferases. Maltooligomers could be polymerized by potato phosphorylase (PPh, E.C. 2.4.1.1). The activated glucose 1-phosphate in this scheme was generated *in situ* from sucrose and inorganic phosphate by the action of sucrose phosphorylase (SPh, E.C. 2.4.1.7, Scheme 21) (*49*).



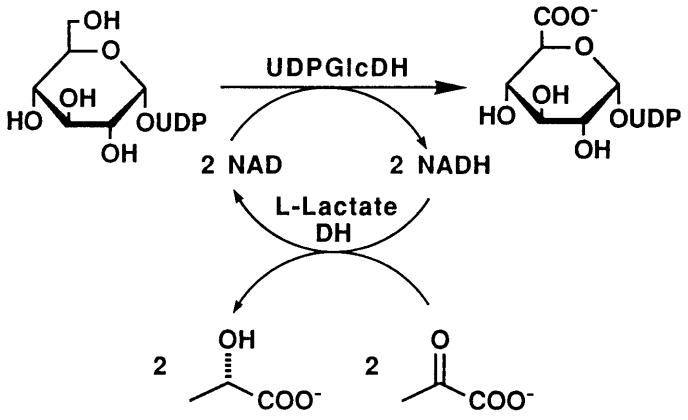
i) **Adenylate Kinase**
(E.C. 2.7.4.3, X = A, C, U)

Guanylate Kinase
(E.C. 2.7.4.8, X = G)

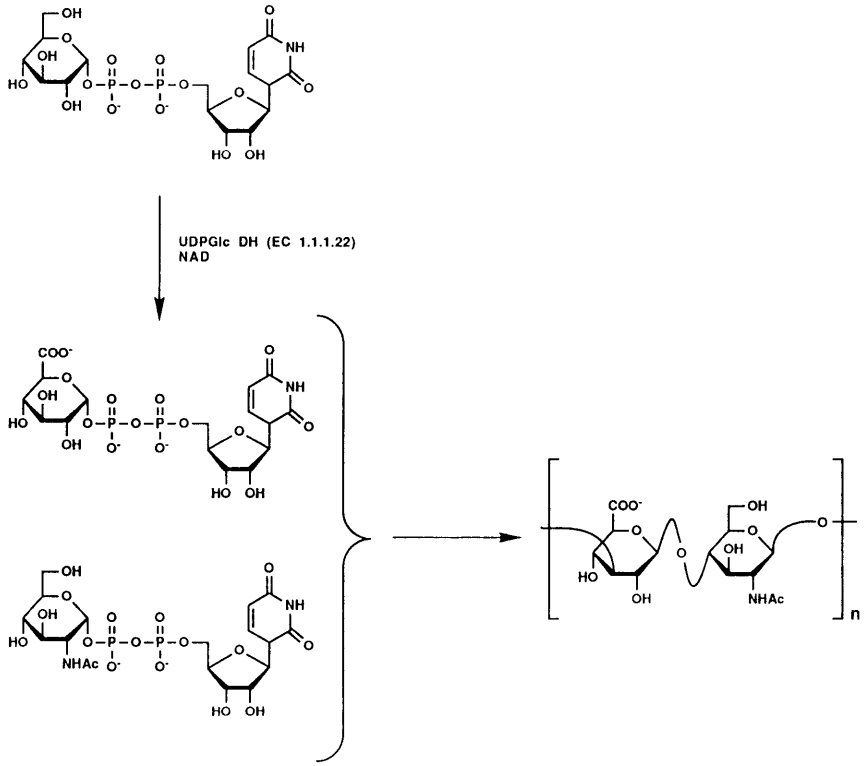
Nucleoside Monophosphate Kinase
(E.C. 2.7.4.4, X = U)

ii) **Pyruvate Kinase**
(E.C. 2.7.1.40)

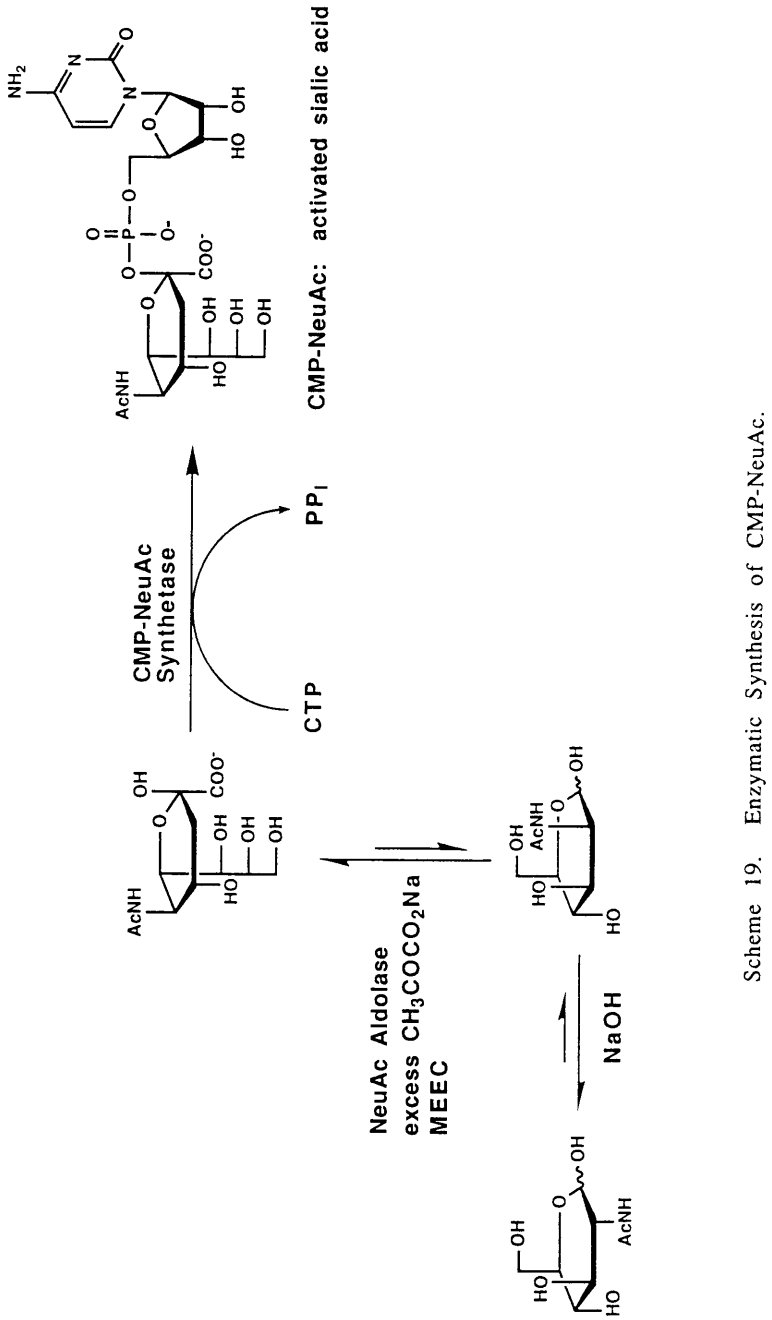
Scheme 16. Enzymatic Syntheses of Nucleoside Triphosphates.



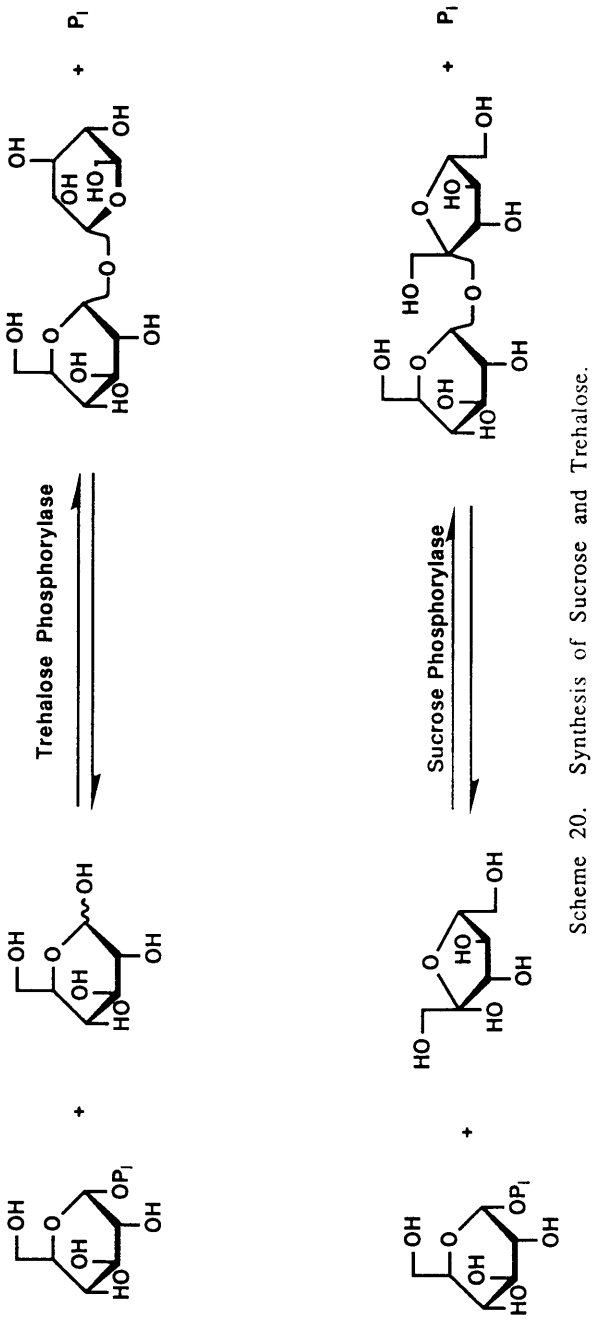
Scheme 17. Enzymatic Synthesis of UDP-GlcUA.



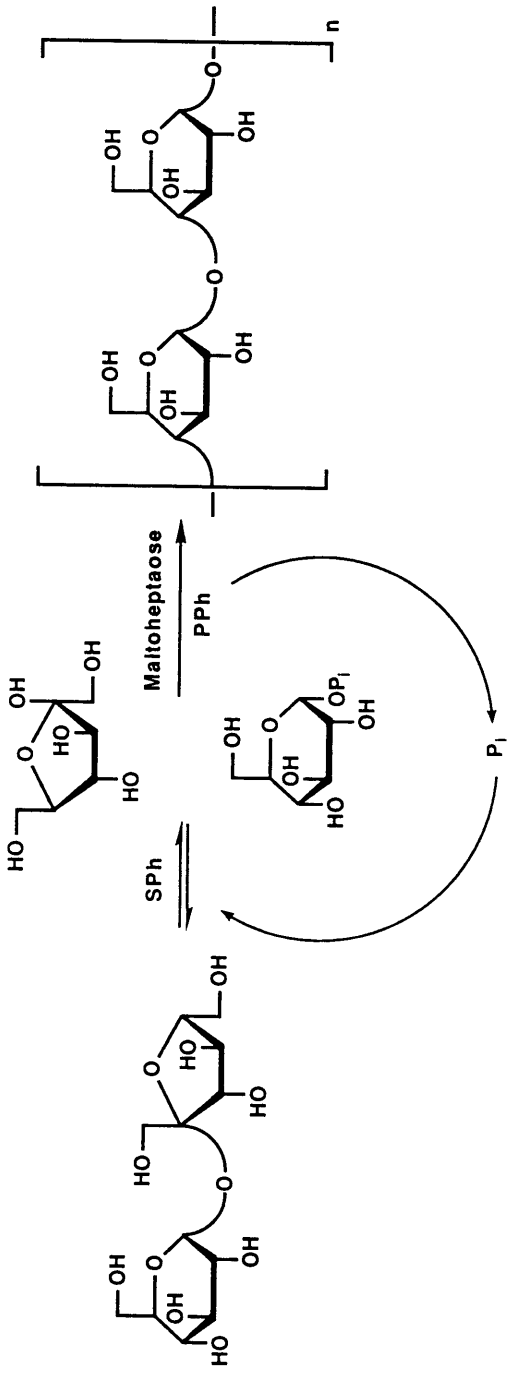
Scheme 18. Cell-Free Synthesis of Hyaluronic Acid.



Scheme 19. Enzymatic Synthesis of CMP-NeuAc.



Scheme 20. Synthesis of Sucrose and Trehalose.



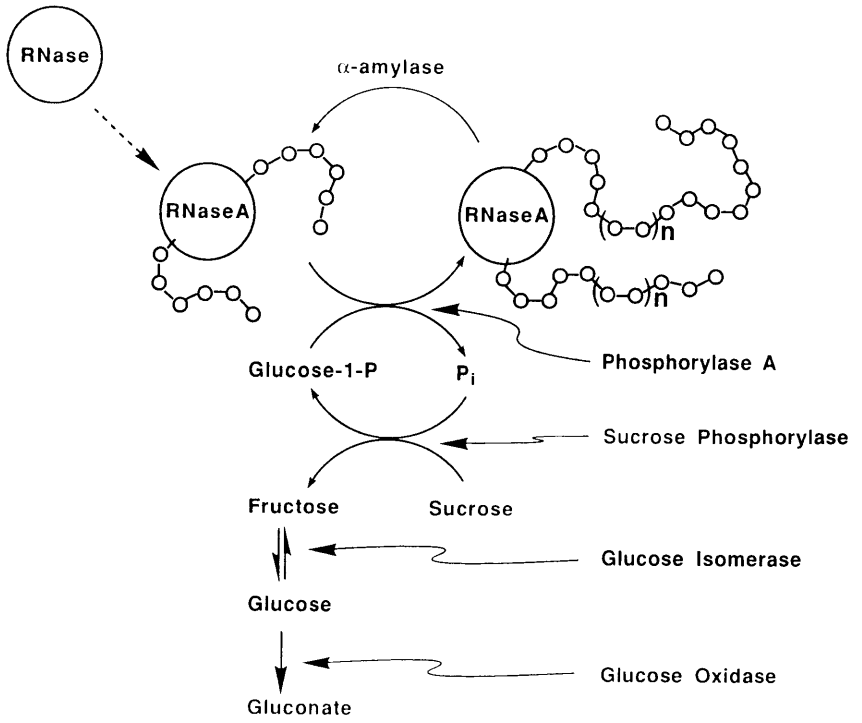
Scheme 21. Synthesis of Maltooligomers.

Pfannemüller and coworkers have used similar methodologies to prepare an interesting class of linear, star and comb-shaped polymers (*50, 51*).

This strategy was employed to add amylose polymers to RNAse (Scheme 22) (*49*). It is clear that the carbohydrate component of glycoproteins enhances the stability *in vivo* of circulating proteins, and recombinant proteins modified in this way may be useful therapeutic agents.

Conclusions

To meet the requirements for synthetic routes to a wide range of carbohydrates, we have begun investigating enzyme-based methodologies. The initial results demonstrate conclusively that enzyme technology will play an important role in the future. Although the field is currently limited to some extent by a lack of availability of enzymes, modern molecular biology will almost certainly overcome this problem in the foreseeable future.



Scheme 22. Growth of Amylose Polymers on RNAse.

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Literature Cited

1. Sharon, N. *Complex Carbohydrates*; Addison-Wesley: Reading, MA, 1975.
2. a) *The Glycocongugates, Vol I - II*; Horowitz, M. I.; Pigman, W., Eds., Academic: New York, 1977-1978. b) *The Glycoconjugates, Vol III - IV*; Horowitz, M. I., Ed., Academic: New York, 1982.
3. Collins, P. M. *Carbohydrates*; Chapman and Hall: London, 1987.
4. Schauer, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 131.
5. *The Lectins: Properties, Functions and Applications in Biology and Medicine*; Liener, I. E.; Sharon, N.; Goldstein, I. J., Eds.; Academic: New York, 1986.
6. Paulson, J. C. In *The Receptors*; Cohn, P. M., Ed.; Academic: New York, 1985; Vol. 2, p 131.
7. Sairam, M. R. In *The Receptors*; Cohn, P. M., Ed.; Academic: New York, 1985; Vol. 2, p 307.
8. Rademacher, T. W.; Parekh, R. B.; Dwek, R. A. *Ann. Rev. Biochem.* **1988**, *57*, 785.
9. Rice, G. E.; Bevilacqua, M. P. *Science* **1989**, *246*, 1303.
10. Smith, C. A.; Davis, T.; Anderson, D.; Solam, L.; Beckman, M. P.; Jerzy, R.; Dower, S. K.; Cosman, D.; Goodwin, R. G. *Science* **1990**, *248*, 1019.
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. Wong, C. -H. *Science* **1989**, *244*, 1145.
16. Jones, J. B. *Tetrahedron* **1986**, *42*, 3351.
17. *Enzymes in Organic Synthesis (Ciba Foundation Symposium 111)*; Porter, R.; Clark, S., Eds.; Pitman: London, 1985.
18. Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365.
19. Horecker, L.; Tsolas, O.; Lai, C. Y. In *The Enzymes*, Boyer, P. D., Ed.; Academic: New York, 1972; Vol. 7, p 213.
20. 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.
21. Turner, N. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 624.
22. Wong, C. -H.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3493.
23. Borysenko, C.; Spaltenstein, A.; Straub, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 9275.
24. Ozaki, A.; Toone, E. J.; von der Osten, C. H.; Sinskey, A. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 4970.
25. Bednarski, M. D.; Waldmann, H. J.; Whitesides, G. M. *Tetrahedron Lett.* **1986**, *27*, 5807.
26. Effenberger, F.; Straub, A. *Tetrahedron Lett.* **1987**, *28*, 1641.
27. Crans, D. C.; Kazlauskas, R. J.; Hirschbein, B. L.; Wong, C. -H.; Abril, O.; Whitesides, G. M. *Methods Enzymol.* **1987**, *136*, 263.

28. Simon, E. S.; Grabowski, S.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 8920.
29. Bednarski, M. D.; Crans, D. C.; DiCosimo, R.; Simon. E. S.; Stein, P. D.; Whitesides, G. M.; Schneider, M. *Tetrahedron Lett.* **1988**, *29*, 427.
30. Hammond, S. M.; Claesson, A.; Jansson, A. M.; Larsson, L. -G.; Pring, B. G.; Town, C. M.; Ekström, B. *Nature* **1987**, *327*, 730.
31. Danishefsky, S. J.; DeNinno, M. P. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 15.
32. Bednarski, M. D.; Chenault, H. K.; Simon, E. S.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 1283.
33. David, S.; Augé, C. *Pure Appl. Chem.* **1987**, *59*, 1501.
34. Kim, M. -J.; Hennen, W. J.; Sweers, H. M.; Wong, C. -H. *J. Am. Chem. Soc.* **1988**, *110*, 6481.
35. Augé, C.; Gautheron, C. *J. Chem. Soc. Chem. Commun.* **1987**, 859.
36. Racker, E. In *The Enzymes*; Boyer, P. D.; Lardy, H.; Myrbäch, K., Eds.; Academic: New York; 1961; Vol. 5, p 397.
37. Myles, D.; Kobori, Y.; Whitesides, G. M., unpublished results.
38. Paulsen, H. *Chem. Soc. Rev.* **1984**, *13*, 15.
39. Wong, C. -H.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 5416.
40. Simon, E. S.; Grabowski, S.; Whitesides, G. M. *J. Org. Chem.* **1990**, *55*, 1834.
41. Hirschein, B. ; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 3765.
42. Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron Lett.* **1988**, *29*, 1123.
43. *Enzymatic Bases of Detoxication*; Jakoby, W. B., Ed.; Academic: New York, 1980; Vol. 2.
44. Zalitis, J.; Feingold, D. S. *Arch. Biochem. Biophys.* **1969**, *12*, 457.
45. Toone, E. J.; Simon, E. S.; Whitesides, G. M., unpublished results.
46. Simon, E. S.; Toone, E. J.; Ostroff, G.; Bednarski, M. D.; Whitesides, G. M. *Methods Enzymol.* **1989**, *179*, 275.
47. Simon. E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159.
48. Haynie, S. L., Whitesides, G. M. *Appl. Biochem. Biotech.* **1990**, *23*, 155.
49. Waldmann, H.; Gygax, D.; Bednarski, M. D.; Shangraw, W. R.; Whitesides, G. M. *Carbohydr. Res.* **1986**, *157*, c4.
50. Ziegast, G.; Pfannemüller, B. *Carbohydr. Res.* **1987**, *160*, 185, and references therein.
51. Ziegast, G.; Pfannemüller, B. *Makromol. Chem., Rapid Commun.* **1984**, *5*, 373.

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