ENZYMES AS CATALYSTS IN CARBOHYDRATE SYNTHESIS

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INTRODUCTION

The field of carbohydrate chemistry is perhaps one of the most thoroughly investigated of all disciplines of chemistry. Thousands of investigators, from the time of Emil Fischer, have probed the structure, function and reactivity of mono-, oligo-, and polysaccharides. Nevertheless, carbohydrate chemistry and biology are currently undergoing a major renaissance. In addition to their well known roles as energy storage vehicles and structural components of cells, carbohydrates are now recognized as vital components in biological recognition phenomena.[1-3] The involvement of carbohydrates has been implicated in many biological processes, including cell-cell recognition in growth and differentiation, clearance of aging cells from circulation, and cell sorting and targeting. Cell-surface carbohydrates are specific attachment sites for certain pathogenic viruses, bacteria, and parasites, and for several soluble bacterial toxins.[4-7] With the realization of these new roles has come a renewed demand for improved synthetic routes to carbohydrates.

Synthetic methodologies for the preparation of monosaccharides, oligosaccharides and polysaccharides have been pursued by legions of chemists for over a century.[8-11] Although impressive progress has been made, carbohydrates continue to be an extremely challenging group of compounds to prepare. Recently, enzymes have been applied to the field of carbohydrate synthesis.[12] Enzymes have been utilized as catalysts in other areas of organic synthesis for many years.[13-15] Many of the

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advantages of enzyme-based synthesis are especially relevant to the preparation of carbohydrates:

i) Compatibility with aqueous media. Carbohydrates are soluble primarily in aqueous solution. Many of the reactions available to the organic chemist, however, are incompatible with water. As a result, carbohydrates must be modified to solublize them in organic media. Enzymes operate in aqueous solution, at or near room temperature, and at neutral pH. Complex protection/deprotection schemes can therefore be avoided.

ii) Specificity. The single most attractive feature of enzymes as catalysts in synthesis is their specificity. This specificity is manifested in three major ways. First, enzymes display absolute chemo-, or reaction, specificity. Carbohydrates frequently possess a variety of sensitive functionalities, which may be susceptible to undesirable modification during manipulation with conventional reagents. Second, enzymes in many cases display high regioselectivity. Since carbohydrates are composed of a number of functionalities of similar reactivity, the ability to operate selectively on a single functional group is highly desirable. This characteristic is an especially important issue in oligosaccharide syntheses, where, for example, a branched-chain carbohydrate may contain more than one identical monosaccharide unit. Finally, enzymes display great stereoselectivity. Carbohydrates contain much stereochemical information in the form of multiple stereogenic centers. This chirality must be correctly installed.

Probably no group of compounds is better suited to enzyme-based synthesis than the carbohydrates. We have developed synthetic methodologies for the synthesis of mono-, oligo- and polysaccharides. This review presents some of the more recent work on the use of aldolases and transketolase for monosaccharide synthesis, and of the enzymes of the Leloir pathway for oligosaccharide synthesis.

MONOSACCHARIDE SYNTHESIS

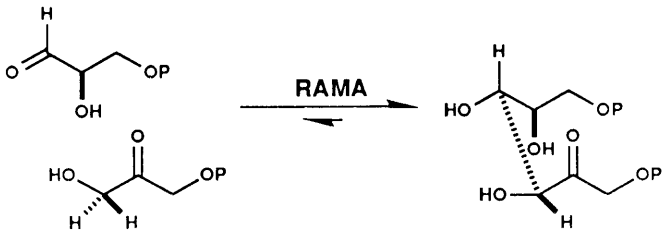
1. Aldolases

A. FDP Aldolases. The "Inversion Strategy"

The aldolases (E.C. 4.1.2.x) are an ubiquitous group of enzymes that catalyze the interconversion of higher aldoses and ketoses and their respective components. Two groups of aldolases are found in Nature.[16] Type 1 aldolases, present in mammalian and higher plant systems, utilize a lysine amino functionality to catalyze aldol condensation *via* a Schiff base

intermediate. Type 2 aldolases, isolated primarily from prokaryotic sources, are Zn^{2+}-dependent enzymes.

Since the early 1950's, aldolases have been utilized as catalysts for the synthesis of monosaccharides.[12] The best studied of the aldolases is a fructose-1,6-diphosphate (FDP) aldolase from rabbit muscle (E.C. 4.1.2.13, frequently referred to as RAMA).[17] *In vivo*, this enzyme catalyzes the interconversion of FDP and dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (Scheme 1).



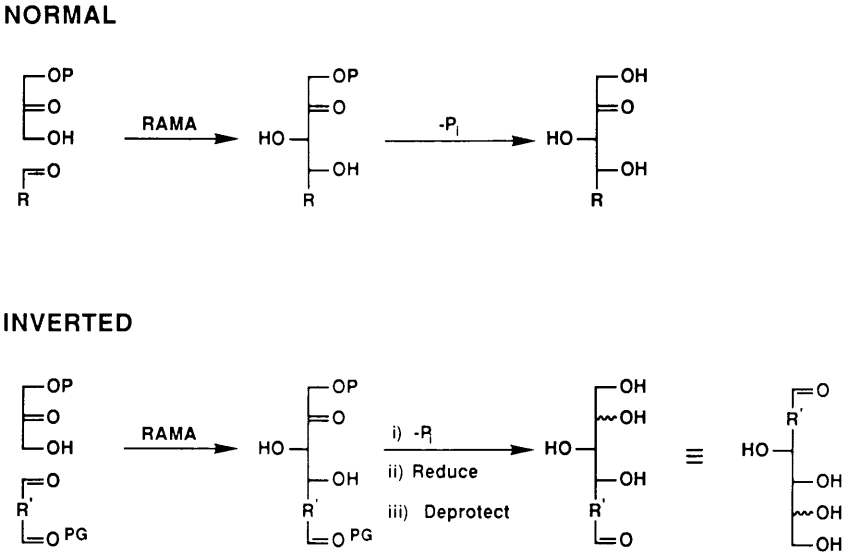
Scheme 1. The RAMA-Catalyzed Aldol Condensation

The enzyme displays absolute stereospecificity for the two new stereogenic centers formed during the reaction: the vicinal diol formed during the condensation is always D-*threo*. RAMA will accept a wide range of aldehydes as the electrophilic component of the reaction, although the enzyme appears to be absolute in its requirement for DHAP as the nucleophile.[12,17] A number of useful monosaccharides have been prepared using RAMA. More recently, a bacterial FDP-aldolase has been cloned and overproduced. In initial studies, the bacterial enzyme is reported to have similar substrate specificity to RAMA.[18]

Although clearly useful as a catalyst in monosaccharide synthesis, RAMA suffers a number of serious drawbacks. Most notably, RAMA produces only ketoses, while a number of important naturally occurring structures are aldose sugars. Furthermore, only a single diastereomeric product is available. Much of the recent work on monosaccharide synthesis in our group has focussed on strategies to overcome these limitations.

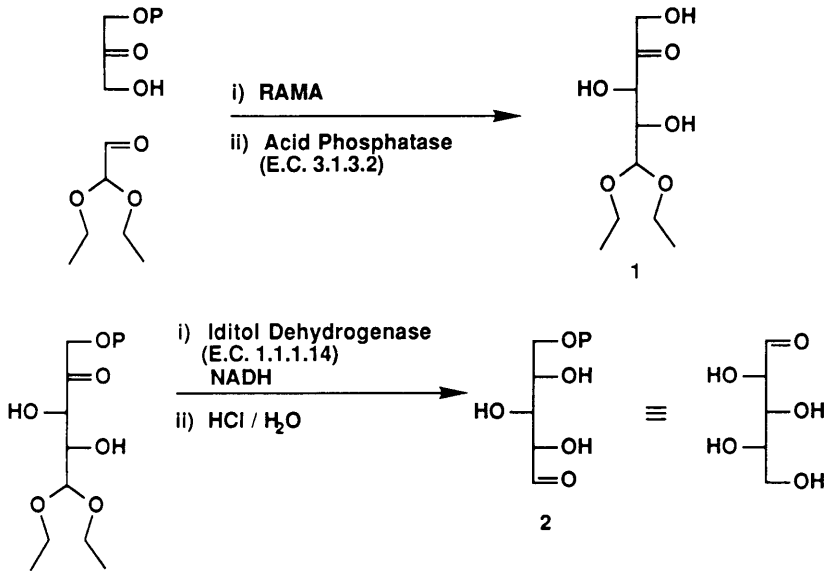
One potential route to aldose sugars is the so-called "inversion strategy" (Scheme 2).[19] The idea behind this methodology is to utilize a monoprotected dialdehyde as the electrophile in an enzyme-catalyzed aldol condensation. Monoprotected dialdehydes of this nature are conveniently prepared using a vinyl group as a masked aldehyde functionality. Ozonolysis is then used to release the desired carbonyl.

Following aldolase-catalyzed condensation, the ketone functionality is reduced stereospecifically, and the protected aldehyde is exposed to yield a new aldose sugar. This methodology accomplishes two goals: the generation of aldose sugars, and the placement of the vicinal diol formed during the enzyme-catalyzed aldol condensation in a position other than C3/C4. We have demonstrated the utility of this methodology through the synthesis of two monosaccharides, L-xylose (**2**, Scheme 3) and 2-deoxy-D-*arabino*-hexose (**5**, Scheme 4).[19]

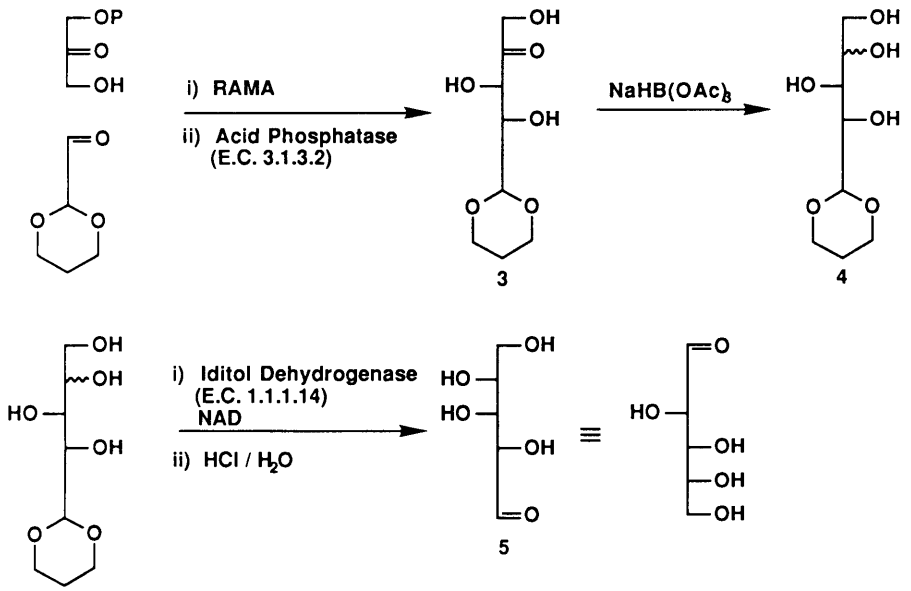


Scheme 2. The Inversion Strategy.

The stereospecific reduction of the ketone functionality generated in the RAMA adduct is another vital component of the inversion strategy. We have found iditol dehydrogenase (E.C. 1.1.1.14), from *Candida utilis*, also known as sorbitol or polyol dehydrogenase) to be a useful catalyst for this reduction. Together, Schemes 3 and 4 demonstrate the accessibility of ***either*** diastereomer using a single dehydrogenase. Iditol dehydrogenase-catalyzed reduction of adduct **1** produces exclusively the 2R polyol **1** (Scheme 3). The 2S configuration is available using a two-step procedure: the ketone is first reduced *non-specifically* with a hydride reducing agent, and the undesired 2R enantiomer is oxidized enzymatically to the original ketone (Scheme 4). The ketone can then be recycled, to produce a new diastereomeric mixture of polyols.



Scheme 3. Synthesis of L-xylose

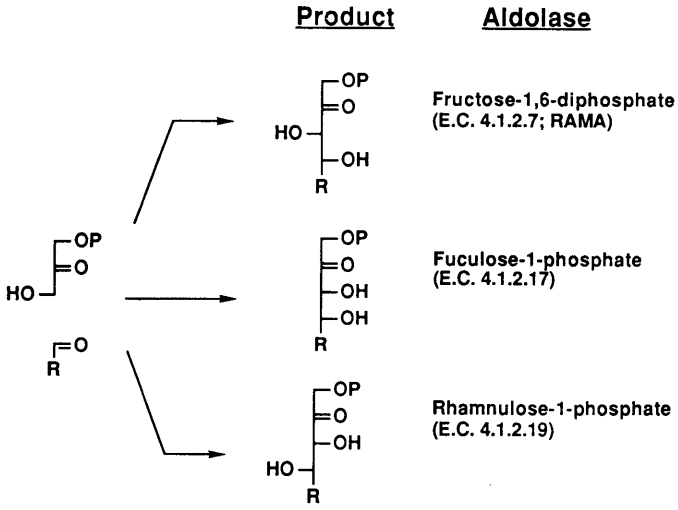


Scheme 4. Synthesis of 2-deoxy-D-*arabino*-hexose.

B. Fuculose-1-Phosphate Aldolase

Another potential route to other stereochemistries at C3 and C4 relies on the availability of other aldolases. Approximately 25 aldolases have been identified to date from various sources.[12] Of the four possible diastereomeric products resulting from an aldolase-catalyzed condensation of DHAP with an aldehyde, enzymes have been identified which generate three stereochemically distinct products (Scheme 5). We have begun investigations on the utility of bacterial aldolases that generate products analogous to but stereochemically distinct from those generated by FDP-aldolases. We have now begun an evaluation of a bacterial fuculose-1-phosphate (fuc-1-P) aldolase as a catalyst for monosaccharide synthesis.[20]

Unlike FDP aldolases, which are synthesized by most organisms under all growth conditions, fuc-1-P aldolase is an inducible enzyme; it is expressed only when cells are grown on fucose as a carbon source.



Scheme 5. Identified DHAP Aldolases.

Even under these conditions, the amount of enzyme produced is small, on the order of <1 unit of enzyme per gram of wet cells (a unit of enzyme catalyzes the conversion of 1 μmol of the substrate to products per minute, under optimal conditions of temperature and pH).[21] In order to

produce the thousands of units of enzyme we required for carbohydrate synthesis, overproduction of the enzyme through recombinant technology seemed the only feasible route. We have overexpressed fuc-1-P aldolase from *E. coli*, using a *tac* promoter. Approximately 300 units of fuc-1-P aldolase are obtained per gram of wet cells. The enzyme has been purified to crystallinity (Table 1), although crude preparations can also be used synthetically. Fuculose-1-phosphate aldolase is a Type 2 aldolase, and is zinc-dependent. In initial substrate specificity studies, the enzyme shows broad substrate specificity with regard to the aldehyde component (Table 2).

Table 1. Cloned Fuc-1-P Aldolase.

Crude cell extracts:

E. coli JM105 (FDP-Aldolase)	**6.6 U/mg**
E. coli A03 (No FDP-Aldolase)	**1.7 U/mg**

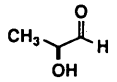
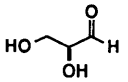
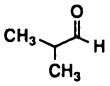
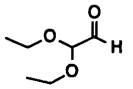
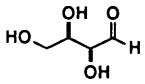
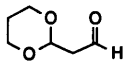
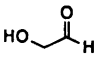
Purification

	Protein Yield (mg)	**Activity (U)**	**Specific Activity (U/mg)**	(%)
Cell-Free Extracts	1063	5.8×10^3	5.5	100
$(NH_4)_2SO_4$ (40-60%)	755	5.4×10^3	7.1	93
DEAE-Sepharose	393	3.9×10^3	9.9	67

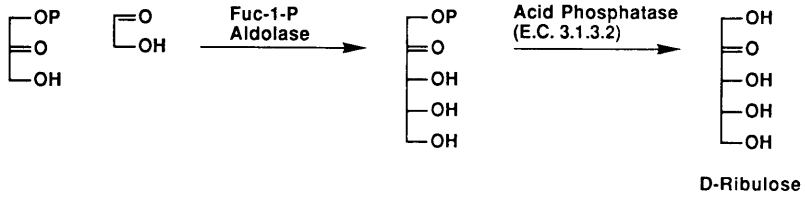
We have now extended the list of aldehydes examined for activity to approximately 40, and the enzyme appears to be a useful catalyst for the preparation of monosaccharides. Investigations on the specificity of the enzyme for the nucleophilic component remain to be completed.
The enzyme has also been used to prepare D-ribulose, on a 10 mmol scale (Scheme 6). The product pentose was identical to authentic commercial material.

Synthetic routes to monosaccharides utilizing all of the aldolases discussed here require DHAP. DHAP is commercially available, although at $120/mmol DHAP clearly must be prepared for synthetic purposes. There are three methods generally used for the preparation of DHAP for use in aldolase-catalyzed syntheses: from fructose-1,6-diphosphate (FDP), by chemical synthesis from dihydroxyacetone, and by enzymatic synthesis from dihydroxyacetone using glycerol kinase (Schemes 7 and 8). The

Table 2. Substrate Specificity of Fuc-1-P Aldolase

Electrophile	Relative Rate	Electrophile	Relative Rate
	100		56
	83		44
	~70[a]		22
	65		59

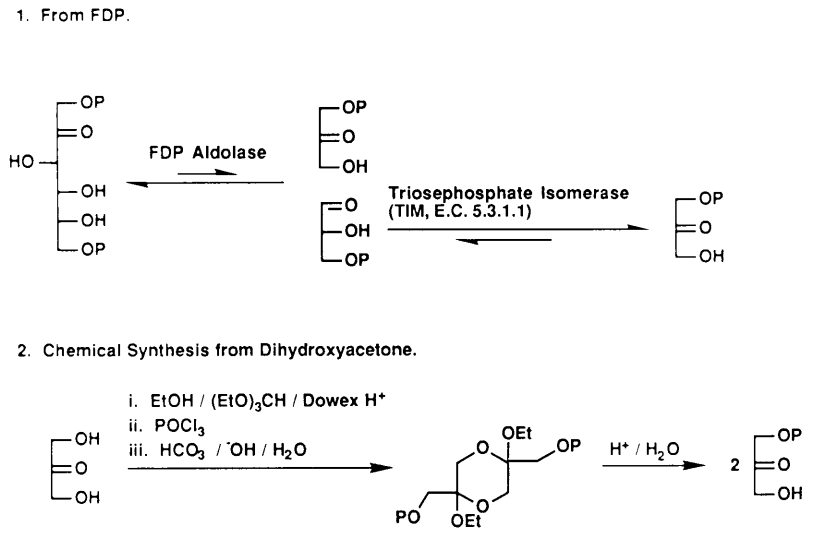
[a] Substrate insoluble; approximate rate.



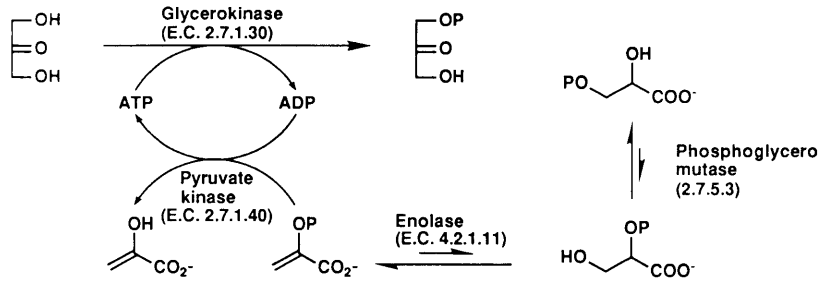
Scheme 6. Synthesis of D-Ribulose.

relative merits of these three methodologies have been discussed elsewhere.[12] Briefly, synthesis from fructose-1,6-diphosphate has the advantages of low cost and simplicity. The major disadvantage is the presence of large amounts of FDP during purification of products. Furthermore, this method is obviously only applicable to syntheses using FDP-aldolases. Chemical synthesis of DHAP provides high purity material, but the synthesis is technically awkward for amounts greater than 10

mmol. This method originally published[22] has recently been improved.[23] The major enhancements are the use of ion exchange resin as the acid in the formation of dihydroxyacetone cyclic dimer ethyl ketal, and the use of triethylorthoformate to drive this reaction (Scheme 7).



SCHEME 7. Synthesis of DHAP.



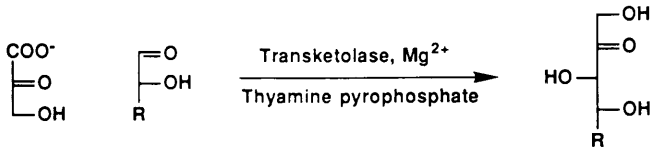
Scheme 8. Enzymatic Synthesis of DHAP.

The enzymatic synthesis of DHAP, on the other hand, is capable of generating product on a mole scale.[24] Glycerol kinase (E.C. 2.7.1.30) transfers a phosphate from ATP to commercially available

dihydroxyacetone. ATP must be regenerated, and regeneration schemes for ATP in syntheses of DHAP have been reported using both acetyl phosphate with acetyl kinase, and phosphoenolpyruvate (PEP) with pyruvate kinase.[24] These schemes still require synthesis of the ultimate phosphate donors, acetyl phosphate or PEP. More recently, we have developed a method for the generation of PEP *in situ* from commercially available D-3-phosphoglycerate.[25] This synthesis of PEP can be used to regenerate ATP in syntheses of DHAP (Scheme 8). The net result is a one-pot synthesis of DHAP using only commercially available starting materials. The quantity of each of the four enzymes used represents approximately $10 per enzyme, while D-3-PGA is commercially available for $400/mole. This methodology represents an extremely attractive synthetic approach to large quantities of DHAP.

2. Transketolase

In addition to aldolases, the other major group of enzymes used *in vivo* to generate and degrade monosaccharides are the transaldolases and transketolases.[26] One of these enzymes, transketolase (E.C. 2.2.1.1), is a potentially useful catalyst for the synthesis of carbohydrates. The enzyme catalyzes the transfer of a two-carbon unit from hydroxypyruvate to a variety of aldehydes (Scheme 9).

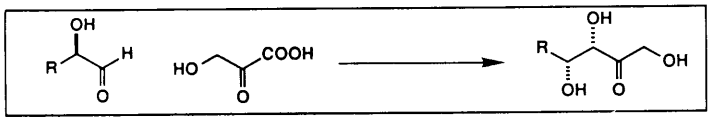


SCHEME 9. Transketolase.

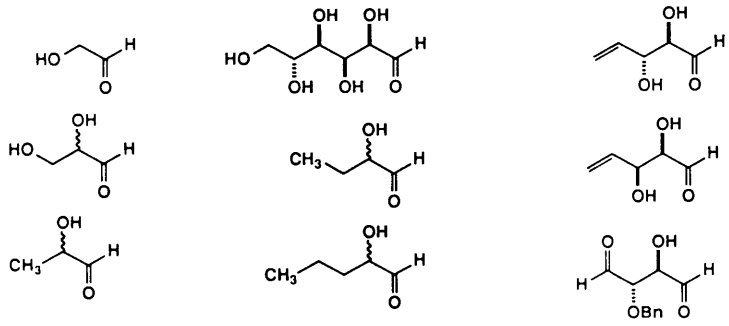
Transketolase is a thyamine pyrophosphate dependant enzyme, and also requires divalent zinc as a cofactor. Transketolase from baker's yeast is commercially available for $1.5/unit. Transketolase can also be readily isolated from spinach; approximately 150 units per kilogram of spinach can be recovered.[28]

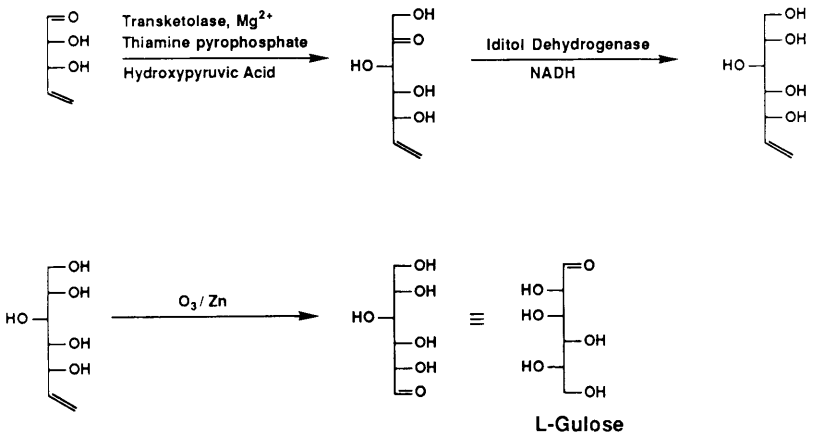
Transketolase has received scant attention in the past.[29,30] We are now investigating the enzyme in more detail. Although transketolase generates a vicinal diol with the same configuration as those produced by FDP-aldolases, there are two significant advantages to transketolase-catalyzed syntheses: the enzyme does not require DHAP, and the products are not phosphorylated. Substrate specificity studies to date have shown the enzyme will accept a wide range of aldehydes as substrates (Table 3).[31]

TABLE 3. Substrate Specificity of Transketolase.



Aldehyde Substrates:





SCHEME 10. Transketolase-Based Synthesis of L-Gulose.

The enzyme seems to be absolute in its requirement for a 2R hydroxyl group in the aldehyde; mixtures of aldehydes epimeric at C2 can be resolved using transketolase. The enzyme appears to have no preference

for configuration beyond C2. To date, no keto acid has been found which is accepted by the enzyme in place of hydroxypyruvate. Transketolase has been used for a synthesis of L-gulose (Scheme 10).[32] It seems certain that transketolase will be a useful enzyme for the synthesis of monosaccharides.

GLYCOSIDIC BOND FORMATION. THE PREPARATION OF ACTIVATED MONOSACCHARIDES

1. Introduction

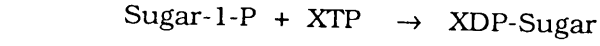
Another area of carbohydrate chemistry where enzymes are now finding extensive use is in the formation of glycosidic linkages. Although great progress has been made in the development of chemical methodologies for glycosidic bond formation, few general methods exist to generate high yields of a single anomeric product.[10,32] Furthermore, the exquisite specificity required in the synthesis of many important glycoconjugates makes chemical synthesis formidable.

Nature uses two basic motifs to activate monosaccharides for transfer to nascent chains.[1] In several systems, glycosyl phosphates are used as activated sugars to form glycosidic linkages. Sucrose phosphorylase and trehalose phosphorylase are examples of enzymes of this type: both have been used synthetically.[33,34] More commonly, monosaccharides are activated as nucleoside phosphate sugars. In mammalian biochemistry, eight activated monosaccharides are commonly encountered: uridine-5'-diphosphoglucose (UDPGlc), uridine-5'-diphosphoglucuronic acid (UDPGlcUA), uridine-5'-diphosphogalactose (UDPGal), uridine-5'-diphospho-N-acetylglucoamine (UDPGlcNAc), uridine-5'-diphospho-N-acetylgalactosamine (UDPGalNAc), guanidine-5'-diphosphomannose (GDPMan), guanidine-5'-diphosphofucose (GDPFuc), and cytidine-5'-monophospho-N-acetylneuraminic acid (CMPNeuAc). Monosaccharides are transferred from these activated sugars to nascent oligosaccharide chains by the glycosyl transferases. Collectively, the enzymes that form the nucleoside phosphate sugars and transfer them to growing carbohydrate chains are referred to as the enzymes of the Leloir pathway (named for the Argentinean biochemist who first elucidated this biosynthetic route). We have been active for several years in the study of glycosidic bond formation using Leloir pathway enzymes. Most recently, we have been engaged in elucidating practical routes to large quantities (>1g) of the important nucleoside phosphate sugars.

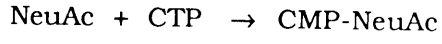
2. Synthesis of Nucleoside Triphosphates

The synthesis of most of the nucleoside diphosphate sugars is accomplished *in vivo* by coupling a sugar-1-phosphate with a nucleoside

triphosphate (Scheme 11). An exception is CMPNeuAc, in which case N-acetylneuraminic acid is coupled directly with CTP (Scheme 12).

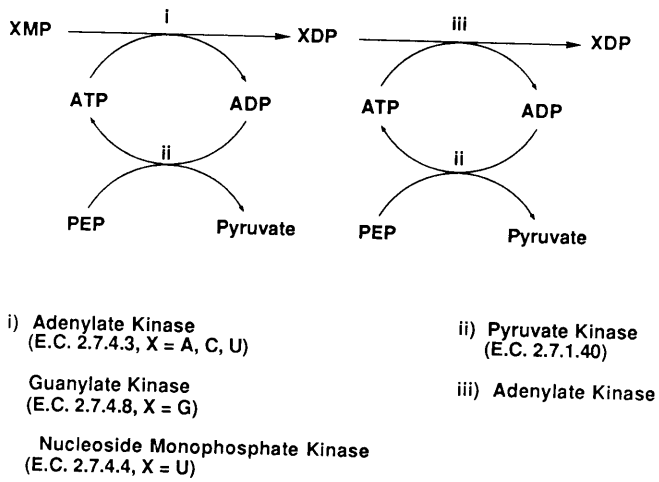


Scheme 11. Biosynthesis of Nucleoside Diphosphate Sugars.



Scheme 12. Biosynthesis of CMPNeuAc.

Clearly, a source of nucleoside triphosphates (XTP's) is vital for enzymatic syntheses of nucleoside phosphate sugars. We have compared chemical and enzymatic methodologies for the preparation of XTP's.[35] Although numerous chemical methodologies are available in the literature, we have



Scheme 13. Enzymatic Synthesis of Nucleoside Triphosphates.

found that enzymatic methods for the preparation of XTP's are operationally much simpler, especially for large scale production. Scheme 13 outlines the most effective enzymatic route to each of the nucleoside triphosphates from the corresponding monophosphate.
All of the nucleoside monophosphates are available from yeast RNA digests at low cost. Adenylate kinase (E.C. 2.7.4.3), which catalyzes the

equilibrium between adenosine mono-, di- and triphosphate, has been used extensively for the production of ATP.[24,36] Although adenylate kinase will accept all of the nucleoside diphosphates as substrates, its substrate specificity for monophosphates is much more restrictive. Nonetheless, cytidine monophosphate can be phosphorylated at a synthetically useful rate using adenylate kinase.[37] The monophosphates of both uridine and guanosine are not accepted by adenylate kinase. GTP is best prepared using guanylate kinase, while UTP can be prepared by chemical deamination of CTP.[35] In all cases, the ultimate phosphate donor is PEP, which can be prepared from 3-PGA.

3. Synthesis of UDPGlcUA

Glucuronic acid is used *in vivo* in mammalian systems as a conjugate for xenobiotic removal.[38] The uronic acids (glucuronic and iduronic acid) are also components of the glycosamineglycans (referred to as mucopolysaccharides in older texts), including heparin, chondroitin, and hyaluronic acid. Iduronic acid (the C5 epimer of glucuronic acid) is generated in vivo from glucuronic acid *after* transfer by a glycosyl transferase.

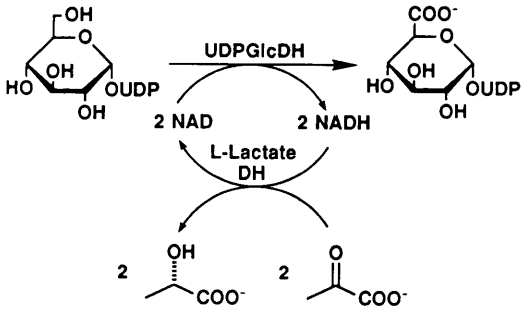
UDPGlcUA is produced from UDPGlc by a nicotinamide-dependent dehydrogenase. UDPGlc dehydrogenase from bovine liver is commercially available. The enzyme is expensive ($10/U), and in our hands was extremely unstable: we found commercial enzyme preparations unsuitable for gram-scale production of UDPGlcUA. UDPGlcUA can also be isolated in straightforward fashion from bovine liver according to literature methods.[39] This preparation furnishes approximately 200U of enzyme per kilogram of liver. We have used crude enzyme (ammonium sulfate fractionation from crude liver homogenate) to prepare UDPGlcUA on a one gram scale (Scheme 14).[40] UDPGlcUA was purified from the crude product mixture by both gel filtration and ion exchange chromatography. The overall yield from UDPGlc was 76% (91% mass recovery, 84% pure by enzymatic analysis).

We have also prepared UDPGlcUA from UDPGlc *via* a platinum catalyzed air oxidation (Scheme 15). This technique suffers two major drawbacks: the catalyst is rapidly deactivated under the reaction conditions, and the rate of oxidation is very slow at pH values <8. This latter concern is significant since UDPGlc is unstable in basic media. It was recognized in previous studies on the oxidation of glucose-1-phosphate to glucuronic acid-1-phosphate that catalyst deactivation was also a serious problem.[41,42] Literature methods exist to prepare catalysts on carbon supports which are resistant to deactivation,[41,42] and this method might be useful under different conditions.

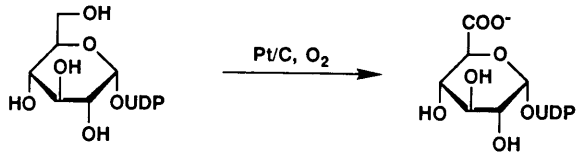
We were unable to generate synthetically useful amounts of UDPGlcUA *via* a platinum-catalyzed oxidation, and at this time oxidation of UDPGlc using crude UDPGlc dehydrogenase from bovine liver is the most useful route to gram-scale quantities of UDPGlcUA.

4. CMPNeuAc

One of the most important sugars in mammalian biochemistry is N-acetylneuraminic acid, which commonly terminates glycoconjugate oligosaccharides. N-Acetylneuraminic acid is synthesized in vivo by an

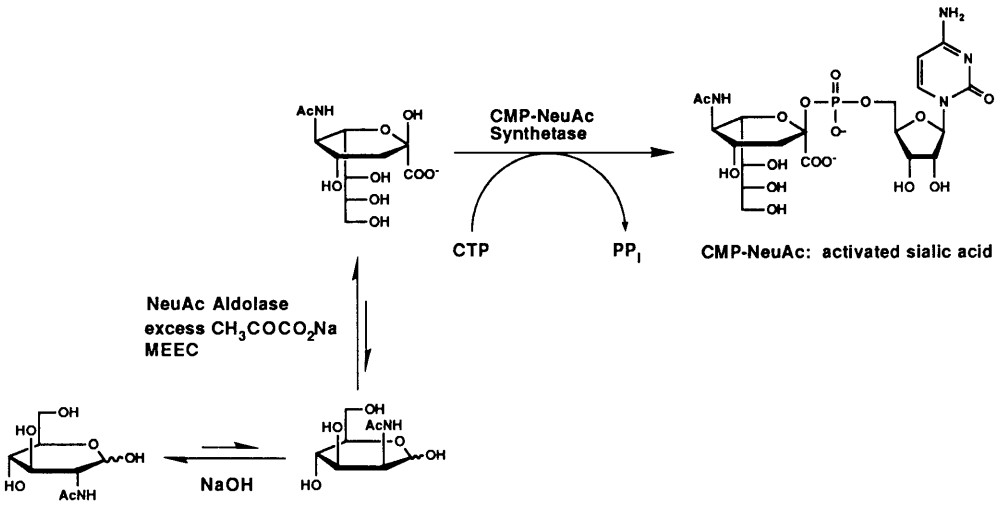


SCHEME 14. Enzymatic Synthesis of UDPGlcUA.



SCHEME 15. Platinum-Catalyzed Oxidation of UDPGlc.

aldolase-catalyzed condensation between N-acetylmannosamine and pyruvate (Scheme 16). CMPNeuAc is synthesized by CMPNeuAc synthetase (E.C. 2.7.7.43), from N-acetylneuraminic acid and CTP. Although preparations of CMPNeuAc have been reported, all are inconvenient for large scale (>250 mg) preparations. A total synthesis of CMPNeuAc from the inexpensive reagents N-acetylglucosamine, CMP, and pyruvate is outlined in Scheme 16.[43]



SCHEME 16. Enzymatic Synthesis of CMPNeuAc.

N-acetylneuraminic lyase (E.C. 4.1.3.3) is commercially available. The enzyme has been cloned and overexpressed. N-Acetylneuraminic lyase accepts several analogues of N-acetylmannosamine as the electrophile, and has been used in the synthesis of unnatural sialic acids. The final enzyme required for the preparation of CMPNeuAc, CMPNeuAc synthetase (E.C. 2.7.7.43), is isolable from calf brain.

CONCLUSIONS

Enzyme-based carbohydrate synthesis is now clearly established as a useful technology. Its importance will continue to grow, as the demand for unnatural synthetic carbohydrates grows. Although enzyme-based oligosaccharide synthesis is currently limited by the availability of the glyosyl transferases, modern molecular biology will soon provide the necessary enzymes.

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REFERENCES

1. Sharon, N. *Complex Carbohydrates*; Addison-Wesley: Reading, MA, 1975.
2. Horowitz, M. I.; Pigman, W., Eds. *The Glycocongugates, Vol I - II*; Academic: New York, 1977-1978.
3. Horowitz, M. I., Ed. *The Glycoconjugates, Vol III - IV*; Academic: New York, 1982.

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. Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212.
9. Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 294.
10. Paulsen, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155.
11. Lemieux, R. U. *Chem. Soc. Rev.* **1978**, *7*, 423.
12. Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365.
13. Jones, J. B. *Tetrahedron* **1986**, *42*, 3351.
14. *Enzymes in Organic Synthesis (Ciba Foundation Symposium 111)*; Porter, R.; Clark, S., Eds.; Pitman: London, 1985.
15. Akiyama, A.; Bednarski, M. D.; Kim, M.-J.; Simon, E. S.; Waldmann, H.; Whitesides, G. M. *CHEMTECH* **1988**, 627.
16. Horecker, L.; Tsolas, O.; Lai, C. Y. In *The Enzymes*, Boyer, P. D. Ed.; Academic: New York, 1972; Vol. VII, p 213.
17. 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.
18. 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.
19. Borysenko, C.; Spaltenstein, A.; Straub, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 9275.
20. Ozaki, A.; Toone, E. J.; von der Osten, C. H.; Sinskey, A. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, submitted for publication.
21. Ghalamor, M. A.; Heath, E. C. *J. Biol. Chem.* **1962**, *237*, 2427.
22. Effenberger, F.; Straub, A. *Tetrahedron Lett.* **1987**, *28*, 1641.
23. Schmid, W.; Whitesides, G. M. unpublished results.
24. Crans, D. C.; Kazlauskas, R. J.; Hirschbein, B. L.; Wong, C. -H.; Abril, O.; Whitesides, G. M. *Methods Enzymol.* **1987**, *136*, 263.
25. Simon, E. S.; Grabowski, S.; Whtiesides, G. M. *J. Am. Chem. Soc.*, **1989**, *111*, 8920
26. Racker, E. In *The Enzymes*; Boyer, P. D.; Lardy, H.; Myrbäch, K., Eds.; Academic: New York; 1961; Vol. 5, p 397.
27. Villifranca, J. J.; Axelrod, B. *J. Biol. Chem.*, **1971**, *246*, 3126.
28. Bolte, J.; Demuynck, C.; Samaki, H. *Tetrahedron Lett.* **1987**, *28*, 5525.
29. Mocali, A.; Aldinucci, D.; Paoletti, F. *Carbohydr. Res.* **1985**, *143*, 288.
30. Kobori, Y.; Myles, D.; Whitesides, G. M. unpublished results.
31. Myles, D.; Whitesides, G. M. unpublished results.
32 Paulsen, H. *Chem. Soc. Rev.* **1984**, *13*, 15.
33. Haynie, S. L.; Whitesides, G. M. *Appl. Biotech. Biochem.* **1990**, *23*, 155.
34. Waldman, H.; Gygax, D.; Bednarski, M. D.; Shangraw, R.; Whitesides, G. M. *Carbohydr. Res.*, **1986**, *157*, c4.
35. Simon, E. S.; Grabowski, S.; Whitesides, G. M. *J. Org. Chem.* **1990**, submitted.
36. Hirschein, B. :.; Mazenod, F. P.; Whtiesides, G. M. *J. Org. Chem.*, **1982**, *47*, 3765.
37. Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron Lett.*, **1988**, *29*, 1123.
38. *Enzymatic Bases of Detoxication*; Jakoby, W. B. Ed.; Academic: New York, 1980; Vol. II.
39. Zalitis, J.; Feingold, D. S.; *Arch. Biochem. Biophys.* **1969**, *12*, 457.
40. Toone, E. J.; Whitesides, G. M. unpublished results.
41. Van Dam, H. E.; Dieboom, A. P. G.; ban Bekkum, H. *Appl. Catal.*, **1987**, *33*, 367.
42. Van Dam, H. E.; Dieboom, A. P. G.; ban Bekkum, H. *Appl. Catal.*, **1987**, *33*, 373.
43. Simon. E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159.