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A chemoenzymatic approach to carbohydrate-mediated cell adhesion

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Abstract: Reported here is a chemoenzymatic approach to the synthesis of carbohydrates and carbohydrate mimetics designed for use to study cell adhesion.

Introduction

Carbohydrates on cell surfaces represent an effective class of biomolecules coding for a vast amount of information required in various biological recognition processes such as bacterial and viral infections, cell adhesion in inflammation and metastasis, differentiation, development, regulation and many other intercellular communication and signal transduction processes. The pace of development of carbohydrate-based pharmaceuticals has, however, been slower than that of other classes of biomolecules. Part of the reason is due to the difficult problem associated with the synthesis of carbohydrates on large scales for therapeutic evaluation. The requirement of multiprotection and deprotection steps in conventional carbohydrate synthesis makes it difficult for large-scale processes. Enzymes are able to contribute to the resolution of this issue, and have been increasingly considered as a useful class of catalysts for organic synthesis (1). Numerous novel monosaccharides can now be prepared based on aldolase-catalyzed reactions (2). In addition, bioactive oligosaccharides and their conjugates are now accessible in large quantities based on glycosyltransferase reactions coupled with the regeneration of sugar nucleotides (3).

Synthesis of novel monosaccharides and mimetics based on aldolases

Aldolases catalyze aldol addition reactions. Of the more than 20 aldolases known, approximately half have been exploited for synthesis (2), and the most useful application of these enzymes is in carbohydrate synthesis. Due to the high degree of flexibility in accepting the acceptor component in most aldolase-catalyzed addition reactions, many common and uncommon sugars can be synthesized. The four dihydroxyacetone phosphate-dependent aldolases have been cloned and overexpressed (4,5), and are commercially available for synthesis. Combined with the Sharpless osmium-catalyzed asymmetric dihydroxylation for the preparation of optically pure hydroxy aldehydes, these enzymes are useful for the synthesis of enantiomeric ketoses (6). The aldolases have also been used in the synthesis of pyrrolidines, piperidines (7) and deoxythiosugars (5). The pyruvate-dependent sialic acid aldolasehas been used in the synthesis of enantiomeric high-carbon keto acids (8). Further study of enzymatic aldol reactions lead to the development of new sequential aldol reactions with three and four substrates (e.g., with the use of 2-deoxyribose-5-phosphate aldolase and fructose-1,6-diphosphate aldolase or sialic acid aldolase) reacting in one pot (9, 10). In the former case, the reaction stops when the product forms a stable cyclic hemiacetal. In the latter case, the first aldolase reaction provides a product which is a substrate for the second aldolase. With the increasing understanding of the specificity of various

aldolases, these two sequential aldolase reaction processes are expected to find use in the synthesis of various uncommon monosaccharides. When mercaptoaldehydes are used as substrates, thiosugars are produced (5). When nitroaldehydes are used as substrates, the products undergo a non-enzymatic intromolecular nitroaldol reaction to give nitrocyclitols which may be a useful source of aminocyclitols (11). When phosphonate-containing aldehydes are used as substrates the products spontaneously undergo a Horner-Wadsworth-Emmons olefination to give another type of cyclitols (12). These cyclitols are useful synthons and are building blocks for the synthesis of glycosidase inhibitors.

Synthesis of oligosaccharides and glycopeptides: sialyl Lewis X and derivatives

Due to the advances in recombinant DNA technology, glycosyltransferases are now available in large quantities for the synthesis of oligosaccharides. Coupled with the regeneration of sugar nucleotides (Fig. 1), these enzymes have been developed for the large-scale synthesis of oligosaccharides (3). The cofactor regeneration scheme not only reduces the cost of sugar nucleotides, but also lessens the problem of product inhibition caused by the leaving nucleoside phosphate. This enzymatic strategy of oligosaccharide synthesis has been applied to the kilogram-scale synthesis of the oligosaccharide sialyl Lewis x (13) which is in clinical trials as a new anti-inflammatory agent for the treatment of reperfusion injury. When a glycosyltransferase is not available it may be replaced with a glycosidase for the formation of the glycosidic bond. An example is the synthesis of sialyl Lewis a (14), in which the 1,3-linked *N*-acetyllactosamine was prepared via regioselective acetylation of galactal with an engineered subtilisin in high concentrations of DMF followed by the β-galactosidase reaction and azidonitration. The product then undergoes further enzymatic glycosylation using sialyl and fucosyl transferases to yield the desired product. There are eight sugar nucleotides commonly found in mammalian systems as substrates for glycosyltransferases, and methods for the regeneration of each sugar nucleotides have been developed (15). This sugar nucleotide regeneration strategy has been used in the synthesis of many oligosaccharides, including the recent synthesis of hyaluronic acid with molecular weight of ~500,000 (16).

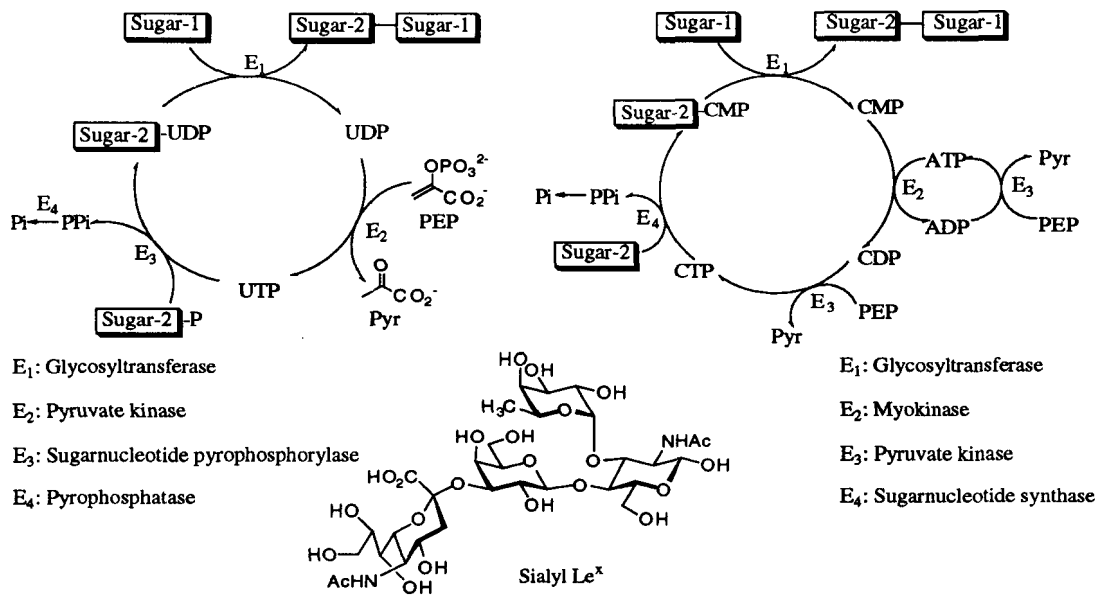


Fig. 1 Regeneration of sugar nucleotides in glycosyltransferase-catalyzed synthesis of oligosaccharides, including the synthesis of sialyl Lewis X.

Glycosyltransferases have also been used in the solid-phase and solution-phase synthesis of glycopeptides (18). In the latter case, glycopeptides were prepared non-enzymatically and coupled with another peptide fragment in aqueous solution via protease-catalyzed aminolysis. Several thermostable subtilisin variants in which the active-site Ser is changed to Cys have been developed *via* site directed mutagenesis for efficient aminolysis reactions (19). A mechanistic investigation of a thermostable thiosubtilisin variant was conducted and an energy diagram was constructed to explain the high ratio of aminolysis to hydrolysis reaction (19).

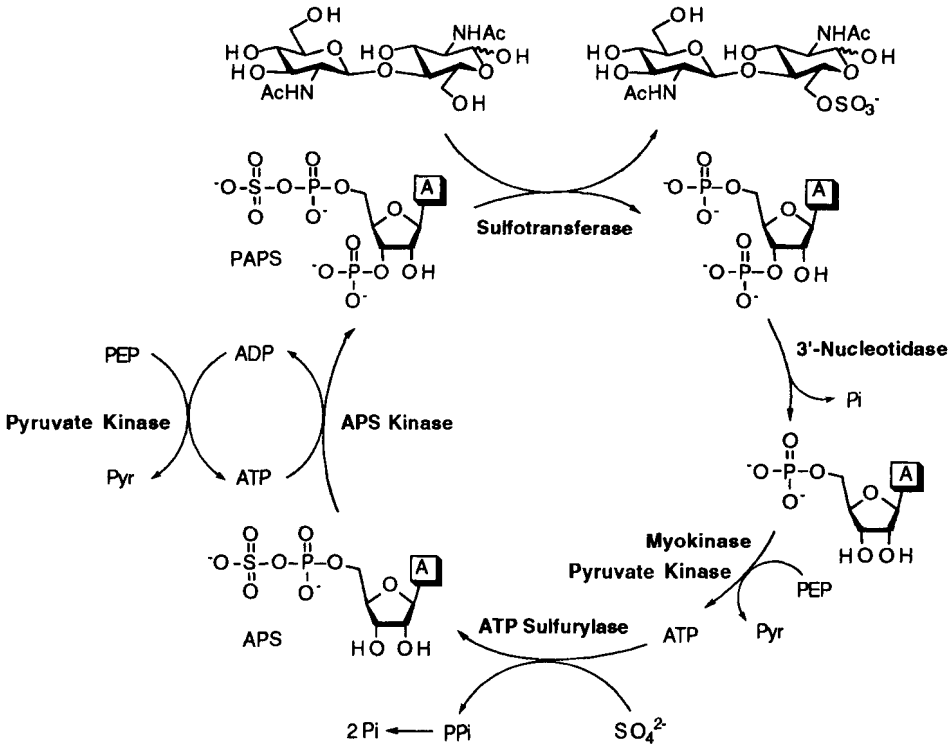
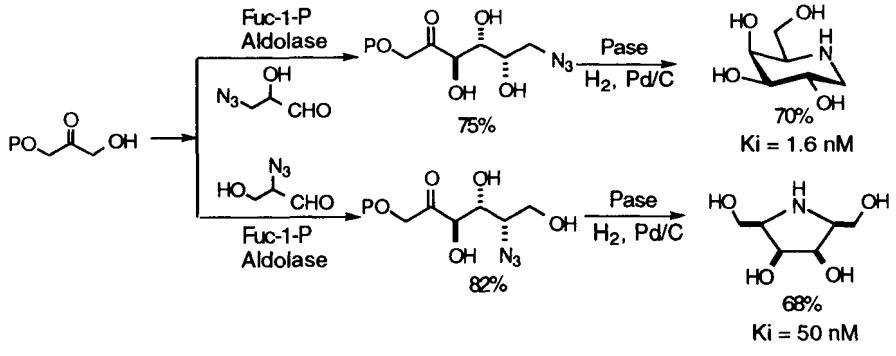


Fig. 2 Regeneration of PAPS in enzyme-catalyzed sulfation of oligosaccharides.
A stands for adenine.

In another effort directed toward the development of new methods for the synthesis of complex carbohydrates, a method for the introduction of a sulfate group to an oligosaccharide has been developed using a recombinant sulfotransferase coupled with regeneration of the sulfation cofactor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) (Fig. 2) (20). This method is generally useful for the enzymatic synthesis of oligosaccharide sulfates such as heparin and sulfated sialyl Lewis X as inhibitors of P- and L-selectins.



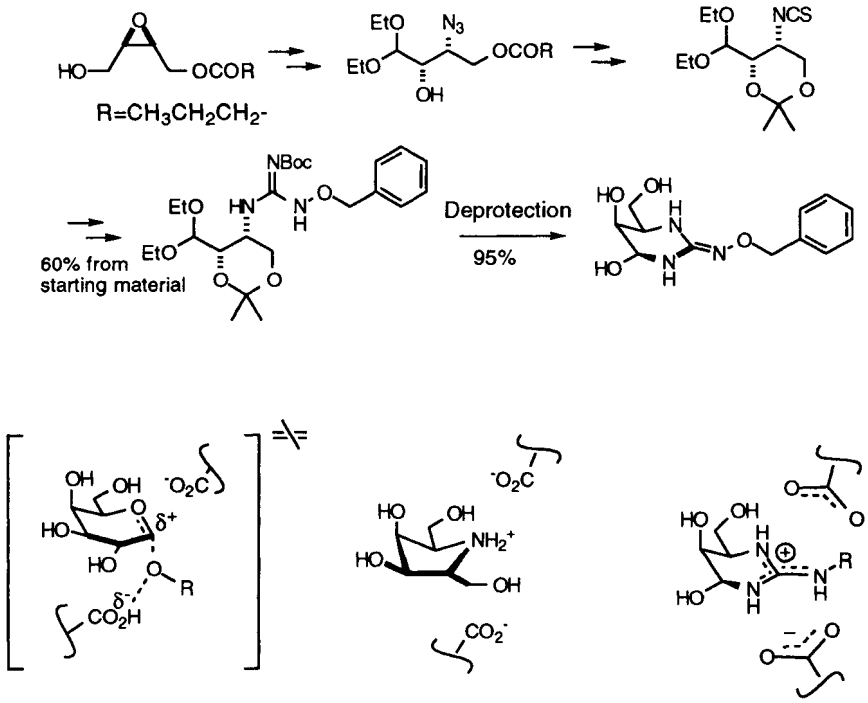


Fig. 3 Synthesis and proposed modes of action of inhibitors of α-galactosidase. The neutral form of the inhibitors is bound to the enzyme, followed by a proton transfer to form a tight complex. Fuc-1-P, fuculose-1-phosphate; Pase, phosphatase.

Synthesis of carbohydrate mimetics: sialyl Lewis X mimetics and inhibitors of glycosidases and glycosyltransferases

One of the major problems in the development of carbohydrate-based pharmaceuticals is, in addition to the difficulty in synthesis, that carbohydrates are unstable and orally inactive. This problem requires a new approach to the development of inhibitors of glycosidases and glycosyltransferases. In addition, many interesting oligosaccharide ligands potentially useful as inhibitors of carbohydrate binding proteins such as lectins may only be useful as injectable drugs for acute symptoms, not as orally active forms for the treatment of chronic diseases. Development of carbohydrate mimetics is therefore of great interest. With regard to the inhibition of glycosidases and glycosyltransferases, various cyclic imitols have proven to be effective and aldolases have been shown to be useful for the synthesis of pyrrolidines and piperidines and their homoanalogs (Fig. 3) (2). Cyclic guanidino-sugars recently developed are also effective glycosidase inhibitors (21). The starting material used in the synthesis of cyclic-guanidino-sugars was prepared from a lipase-catalyzed hydrolysis of a meso-epoxy diol (Fig. 3) (22). Both homopyrrolidines and piperidines can also be used as building blocks in the synthesis of sequence-specific inhibitors of glycosidases and glycosyltransferases (23). A representative example is the synthesis of a designed aza-trisaccharide as α-1,3-fucosyltransferase inhibitor in which an acceptor moiety is incorporated. The inhibitory activity of this aza-saccharide increases in the presence of GDP, indicating the formation of a complex which mimics the transition state of the glycosyl transfer reaction (Fig.4).

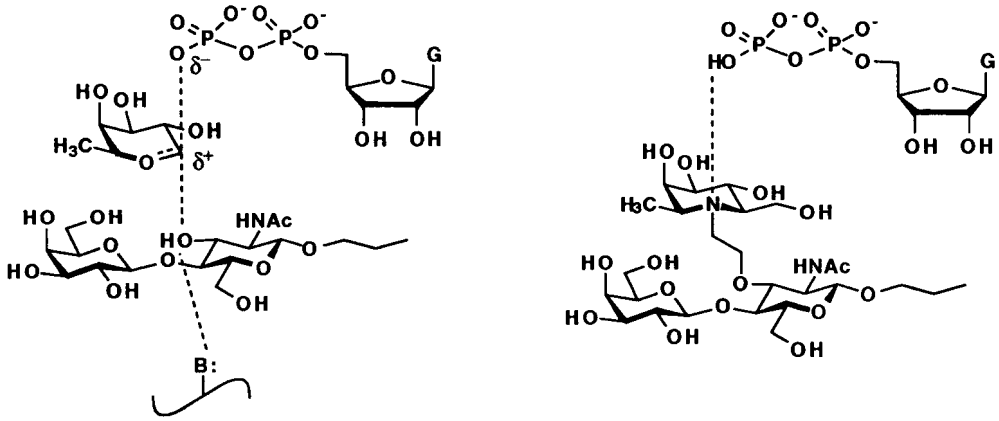


Fig. 4 A proposed mechanism of α1,3-fucosyltransferase reaction (left) and the transition-state analog inhibitor presented as a mixture of an azatrisaccharide containing the acceptor moiety *N*-acetyllactosamine and GDP.

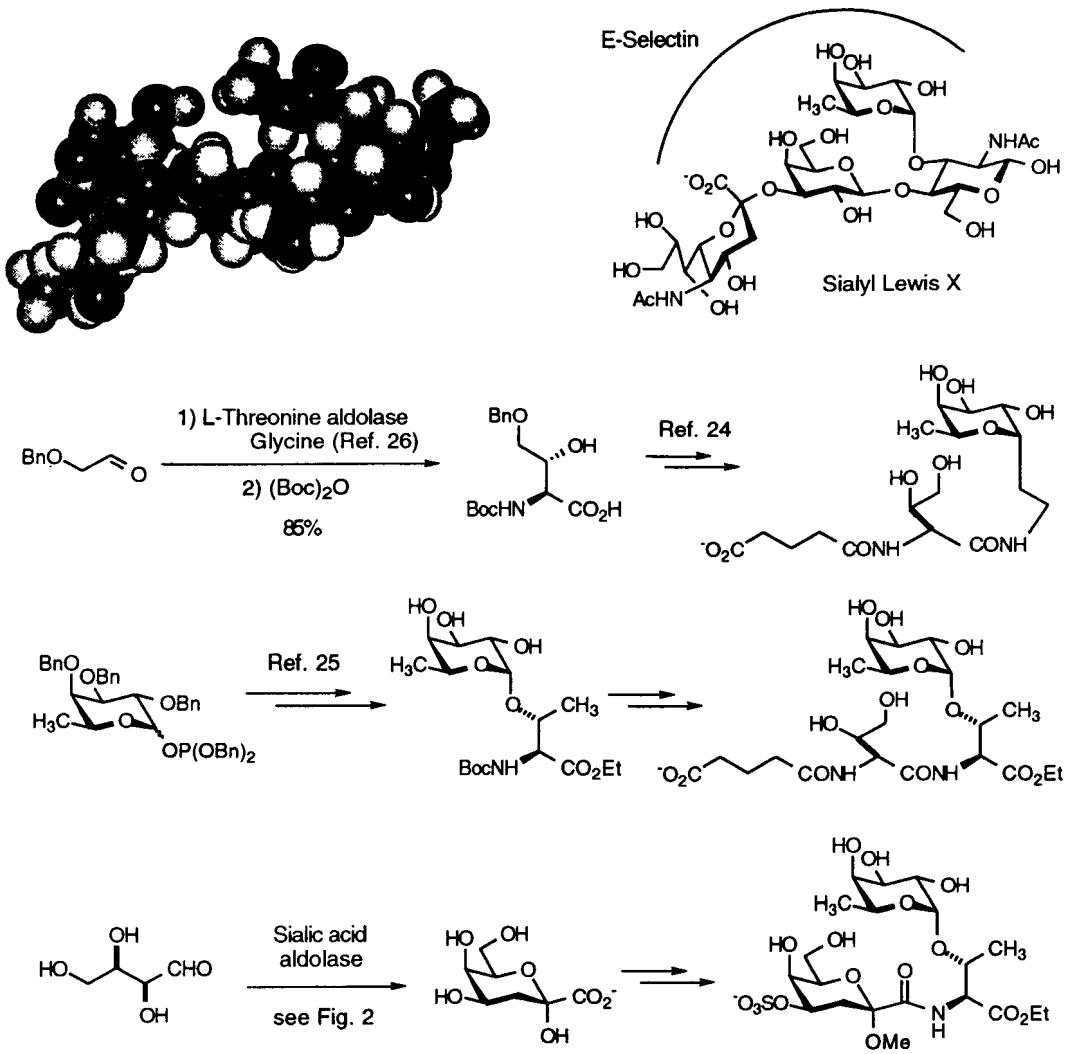


Fig. 5 Chemo-enzymatic synthesis of sialyl Lewis X mimetics. The E-selectin binding site was determined and transformed into mimetics. Top right, the five OH-groups and the CO_2^- group are essential for binding. Top left, the 3D-structure of sialyl Lewis X (see ref. 13).

In our effort toward the development of oligosaccharide mimetics, several sialyl Lewis X mimetics have recently been designed and synthesized, and two of such mimetics have inhibitory activities comparable to that of sialyl Lewis X (Fig. 5) (24,25). A key component used in the synthesis is (2S, 3R)-2-amino-3,4-dihydroxybutanoic acid (L-hydroxythreonine), which can be easily prepared via a threonine aldolase-catalyzed addition reaction (26).

Multivalency

The receptor-ligand interactions on cell surfaces are often multivalent. This mechanism is particularly important in carbohydrate-protein recognition as it will enhance the binding. We have recently prepared a phosphatidyl sialic acid liposome (Fig. 6) which was shown to be approximately 10^4 times more active than sialic acid against human rotavirus. Work is in progress to prepare the phosphatidyl derivative of sialyl Lewis X mimetics to improve the inhibitory activity.

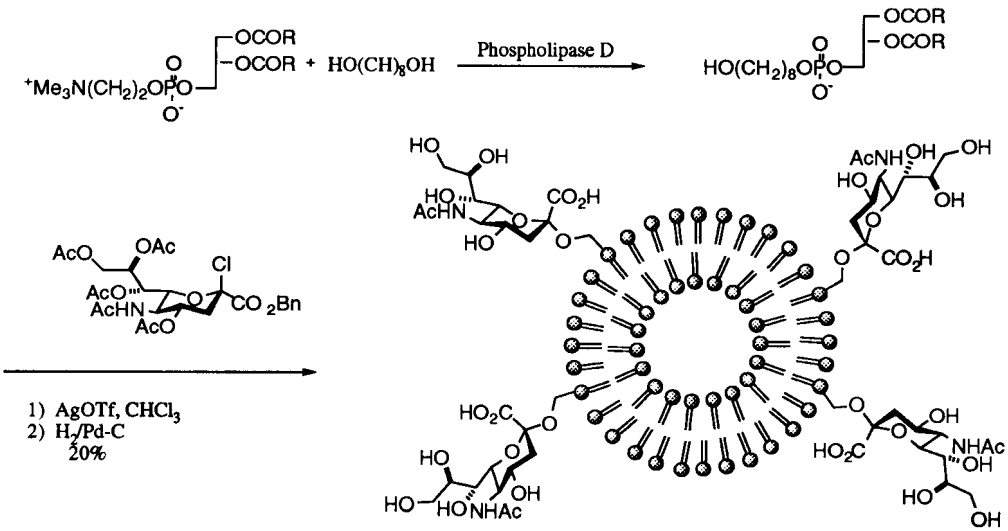


Fig. 6 Synthesis of phosphatidyl sialic acid liposome. The IC_{50} toward human rotavirus is 16 μM compared to 0.14 M for sialic acid.

In summary, the aldolase reaction strategy described above provides a new approach to the synthesis of novel monosaccharide structures and mimetics, and the glycosyltransferase reaction has proven to be useful for the large-scale synthesis of oligosaccharides. One can also alter the specificity and stability of subtilisin to peptide ligases for the synthesis of glycopeptides. As various monosaccharide building blocks and glycosyltransferases become readily available, many carbohydrate recognition problems in biological systems can be studied, and the development of novel carbohydrates and mimetics for use to control the function of carbohydrates involved in certain diseases and metabolic disorders will follow.

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