Enzyme-Catalyzed Organic Synthesis: Practical Routes to Aza Sugars and Their Analogs for Use as Glycoprocessing Inhibitors

GARY C. LOOK,[1a] CHRISTOPHER H. FOTSCH,[1b] AND CHI-HUEY WONG*

Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037

Received July 22, 1992

The glycosidases and glycosyltransferases are instrumental in the processing of various oligosaccharide-containing glycoproteins and glycolipids.[2] The profound impact of these enzymes on the life processes has made them desirable targets for inhibition.[3] Glycoprocessing inhibitors have been used to treat diabetes and other metabolic disorders[3] and have been implicated in the blocking of microbial infection[4,5] and metastasis.[5c] Some representative examples of glycosidase and glycosyltransferase inhibitors are presented in Figure 1.[6,7]

An important family of inhibitors, primarily for the N-linked oligosaccharide processing enzymes, are the aza sugar compounds. The formation and degradation processes of the O-linked oligosaccharide glycoconjugates are also of interest for inhibition.[3a,8] Because of the potential value of aza sugars as therapeutic agents, the efficient preparation of these compounds and their analogs has been of interest to organic and medicinal chemists.[9]

The mechanisms for glycosidases and to some extent for glycosyltransferases have been studied.[10] It is generally believed that compounds such as aza sugars bind to the enzyme active site by charge–charge interaction and hydrogen bonding. Through the study of the mechanism of glycosyl transfer and glycoside hydrolysis and an understanding of the mode of inhibition of natural products, potent synthetic inhibitors which are transition-state mimics have been developed (as discussed later).

This Account will discuss the recent chemoenzymatic routes developed by our group and several others that have been applied to or have the potential to be applied to the preparation of aza sugars and their derivatives as glycoprocessing inhibitors. The use of enzymatic catalysts in organic synthesis is an emerging field of chemistry that holds great potential due to the high regio-, stereo-, and chemoselectivity of enzymes, the mild reaction conditions, and the ability to conduct reactions with a minimum number of protection steps.[11,12]

Background

One of the most popular approaches to the enzymatic preparation of carbohydrate analogs is to use aldolases since more than one stereogenic center is generated in the enzymatic process.[12] Another feature of aldolases is that a wide variety of aldehydes can be used as acceptors, but often only the natural ketone donor is accepted as a good substrate.[13] For example, fructose-1,6-diphosphate aldolase (FDP aldolase, EC 4.1.2.13)

Gary C. Look received his B.S. in chemistry in 1985 from the University of California at Berkeley and his Ph.D. in chemistry with Professor Larry E. Overman in 1990 from the University of California at Irvine. Since 1991 he has been an American Cancer Society Postdoctoral Fellow in Professor Wong's laboratories at The Scripps Research Institute. His research interests are in the design and synthesis of biologically active compounds through the use of synthetic and bioorganic chemistry methodologies.

Christopher H. Fotsch received his B.A. in chemistry at Macalester College in Saint Paul, MN, and his Ph.D. in chemistry with Professor A. Richard Chamberlin in 1992 from the University of California at Irvine. In that year, he began research with Professor Wong at The Scripps Research Institute as a National Institutes of Health Postdoctoral Fellow. His research interests include using enzymes to synthesize non-natural products that can be used to probe interactions in biopolymers.

Chi-Huey Wong received his B.S. and M.S. degrees from National Taiwan University and his Ph.D. in chemistry with George M. Whitesides in 1982 from MIT. He was a member of the faculty at Texas A&M University for six years, and since 1989 he has been the Ernest W. Hahn Professor of Chemistry at the Scripps Research Institute. His research interests are in the area of bioorganic and synthetic chemistry, especially the development of enzymatic catalysts for asymmetric synthesis and rational design of enzyme inhibitors.

(1) (a) American Cancer Society Postdoctoral Fellow. (b) National Institutes of Health Postdoctoral Fellow.

(2) (a) Schwarz, R. T.; Datema, R. *Trends Biochem. Sci.* **1984**, 9, 32. (b) Leloir, L. F. *Science* **1971**, *172*, 1299. (c) Elbein, A. D. *Trends Biotechnol.* **1991**, *9*, 346.

(3) (a) Hodgson, J. *Bio/Technology* **1991**, *9*, 609. (b) Truscheit, E.; Frommer, W.; Junge, B.; Müller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 744. (c) Elbein, A. D. *Annu. Rev. Biochem.* **1987**, *56*, 497. (d) Müller, L. In *Biotechnology*; Rehm, H.-J., Reed, G., Eds.; VCH: Weinheim, 1985; Vol. 4, pp 1-37.

(4) Schwarz, P. M.; Elbein, A. D. *J. Biol. Chem.* **1985**, *26*, 14452.

(5) (a) Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; De Goede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedena, F.; Ploegh, H. L. *Nature* **1987**, *330*, 74. (b) Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9229. (c) Humphries, M. J.; Matsumoto, K.; White, S. L.; Olden, K. *Cancer Res.* **1986**, *46*, 5215.

(6) (a) Nakayama, T.; Amachi, T.; Murao, S.; Sukai, T.; Shin, T.; Kenny, P. T. M.; Iwashita, T.; Zagarshi, M.; Komura, H.; Nomoto, K. *J. Chem. Soc., Chem. Commun.* **1991**, 919. (b) Baguley, B. C.; Römmele, G.; Gruner, J.; Wehrli, W. *Eur. J. Biochem.* **1979**, *97*, 345. (c) Aoyagi, T.; Yamamoto, T.; Kojiri, K.; Morishima, H.; Nagai, M.; Hamada, M.; Takechi, T.; Umezawa, H. *J. Antibiot.* **1989**, *42*, 883. (d) Dorling, P. G.; Huxtable, C. R.; Colegate, S. M. *Biochem. J.* **1980**, *191*, 649. (e) Kayakiri, H.; Nakamura, K.; Takase, S.; Setoi, H.; Uchida, I.; Terano, H.; Hashimoto, M.; Tada, T.; Koda, S. *Chem. Pharm. Bull.* **1991**, *39*, 2807. (f) Saul, R.; Moylneux, R. J.; Elbein, A. D. *Arch. Biochem. Biophys.* **1984**, *230*, 668. (g) King, I. A.; Tabiowo, A. *Biochem. J.* **1981**, *198*, 331. (h) Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishima, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1974**, 27963. (i) Tropea, J. E.; Kaushal, C. P.; Pastuszak, I.; Mitchell, M.; Aoyagi, T.; Molyneux, R. J.; Elbein, A. D. *Biochemistry* **1990**, *29*, 10062. (j) Schwenden, J.; Borgmann, C.; Legler, G.; Bause, E. *Arch. Biochem. Biophys.* **1986**, *248*, 335. (k) Evans, S. V.; Fellows, L. E.; Shing, T. K. M.; Fleet, G. W. J. *Phytochemistry* **1985**, *24*, 1953. (l) Koga, D.; Isogai, A.; Sakuda, S.; Matsumoto, S.; Suzuki, A.; Kimura, S.; Ide, A. *Agric. Biol. Chem.* **1987**, *51*, 471. (m) Isono, K.; Suzuki, S. *Heterocycles* **1979**, *13*, 333.

(7) (a) Müller, L. In *Novel Microbial Products for Medicine and Agriculture*; Demain, A. L., Somkuti, G. A., Hunter-Cevera, J. C., Rossmoro, H. W., Eds.; Society for Industrial Microbiology: 1989; pp 109-116. (b) Tyms, A. S.; Berrie, E. M.; Ryder, T. A. Nash, R. J.; Hegarty, M. P.; Taylor, D. L.; Mobberley, M. A.; Davis, J. M.; Bell, E. A.; Jeffries, D. J.; Taylor-Robinson, D.; Fellows, L. E. *Lancet* **1987**, *2*, 1025. (c) Robinson, K. M.; Begovic, M. E.; Rhinehart, B. L.; Heineke, E. W.; Ducep, J.-B.; Kastner, P. R.; Marshall, F. N.; Danzin, C. *Diabetes* **1991**, *40*, 825.

(8) (a) Gary, H. G.; Lyon, N. B. *Adv. Carbohydr. Chem. Biochem.* **1991**, *49*, 239. (b) Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 257.

0001-4842/93/0126-0182$04.00/0

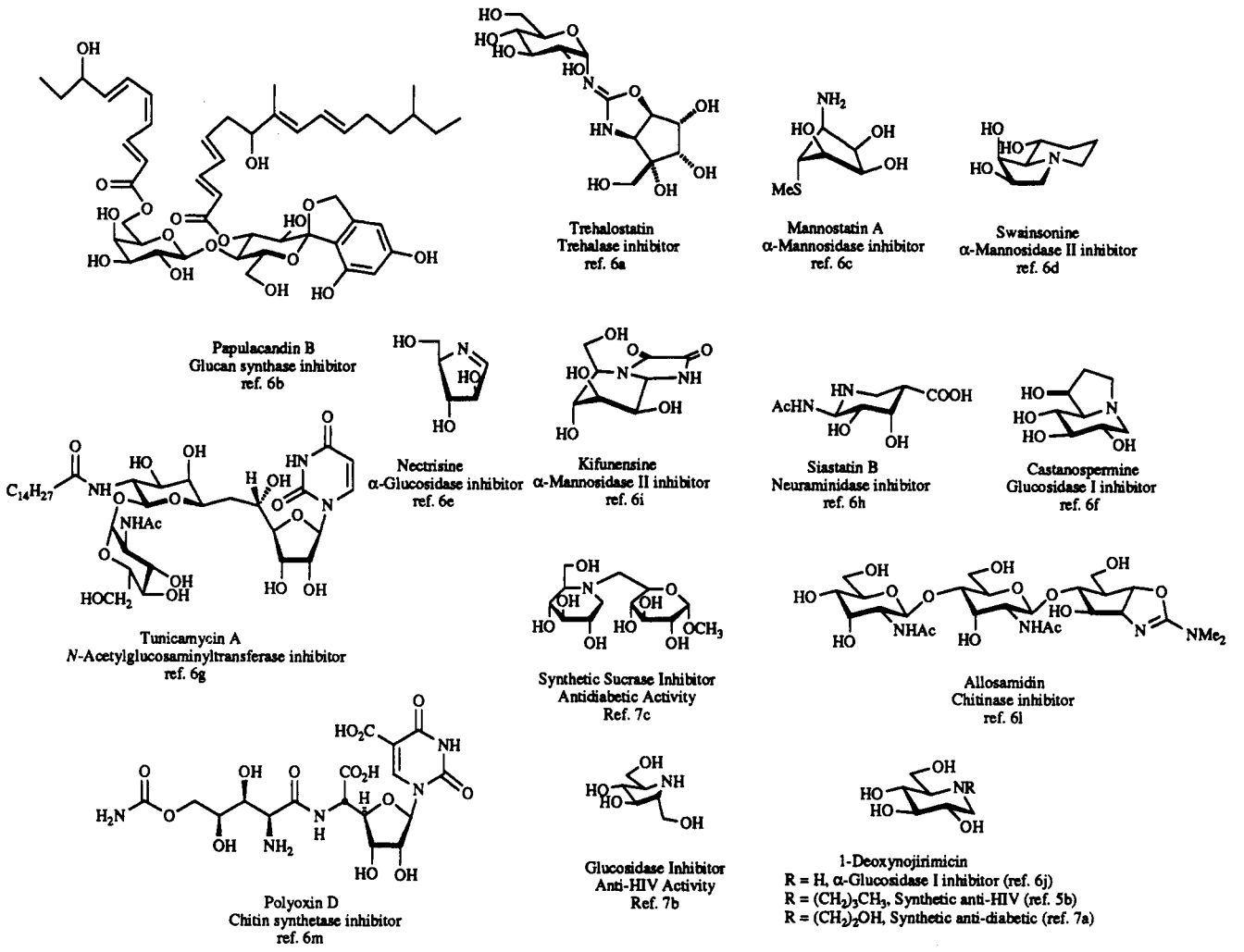


Figure 1. Examples of naturally occurring and synthetic glycosidase and glycosyltransferase inhibitors.

employs D-glyceraldehyde 3-phosphate as the acceptor and dihydroxyacetone phosphate (DHAP) as the donor through the formation of either a Schiff base (Figure 2) or activation with Zn^{2+}.[14] In most cases, the enzyme uses only DHAP or DHA/arsenate as the effective donor,[15] while a wide range of aldehydes react with activated DHAP to generate two new stereocenters with the 3*S*,4*R* configuration. Other DHAP dependent aldolases give stereochemistries complementary to FDP aldolase. Fuculose-1-phosphate aldolase (Fuc-1-PA, EC 4.1.2.17) gives the 3*R*,4*R* configuration,[15-18] rhamnulose-1-phosphate aldolase (Rham-1-PA, EC 4.1.2.19) yields products with the 3*R*,4*S* configuration,[15,17,18] and tagatose-1-phosphate aldolase (Tag-1-PA) provides a 3*S*,4*S* sugar.[19]

In all of the cases above, both enantiomers of aldehydes are acceptable and the stereochemistry of bond formation is dependent on the enzyme and not on the acceptor aldehyde. For example, the FDP aldolase reaction always gives the 3*R*,4*R* stereochemistry

(9) For some representative strategies, see: (a) Fairbanks, A. J.; Carpenter, N. C.; Fleet, G. W. J.; Ramsden, N. G.; De Bello, I. C.; Winchester, B. G.; Al-Daher, S. S.; Nagahashi, G. *Tetrahedron* **1992**, *48*, 3365. (b) Aoyagi, S.; Fujimaki, S.; Yamazaki, N.; Kibayashi, C. *J. Org. Chem.* **1991**, *56*, 815. (c) Paulsen, H.; Hayauchi, Y.; Sinnwell, V. *Chem. Ber.* **1980**, *113*, 2601. (d) Bernotas, R. C.; Pezzone, M. A.; Ganem, B. *Carbohydr. Res.* **1987**, *167*, 305. (e) Kinast, G.; Schedel, M. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 805. (f) Legler, G.; Jülich, E. *Carbohydr. Res.* **1984**, *128*, 614. (g) Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127. (h) See also ref 6c. (i) Fleet, G. W.; Smith, P. W.; Nash, R. J.; Fellows, L. E.; Parekh, R. B.; Rademacher, T. W. *Chem. Lett.* **1986**, 1051. (j) King, S. B.; Ganem, B. *J. Am. Chem. Soc.* **1991**, *113*, 5090. (k) Trost, B. M.; Vranken, D. L. V. *J. Am. Chem. Soc.* **1990**, *112*, 1261. (l) Fleet, G. W. J. *Chem. Ber.* **1989**, 287. (m) Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1991**, *113*, 5863. (n) Winchester B.; Fleet, G. W. J. *Glycobiology*, **1992**, *2*, 199.

(10) (a) For reviews, see: Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319. (b) Sinnott, M. J. *Chem. Rev.* **1990**, *90*, 1171. For recent papers: Strynadka, N. C. J.; James, M. N. G. *J. Mol. Biol.* **1991**, *220*, 401. Withers, S. G.; Warren, R. A.; Street, I. P.; Rupitz, K.; Kempton, J. B.; Aebersold, R. J. *J. Am. Chem. Soc.* **1990**, *112*, 5887. Hardy, L. W.; Poteete, A. R. *Biochemistry* **1991**, *30*, 9457.

(11) Wong, C.-H.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. Jones, J. B. *Tetrahedron* **1986**, *42*, 3341. Yamada, H.; Simizu, S. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695. Ohno, M.; Otsuka, M. *Org. React. (N.Y.)* **1989**, *37*, 1. Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695. Davies, H.-G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in Preparative Organic Chemistry*; Academic Press: New York, 1989. Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114. Schellenberger, V.; Jakubke, H.-D. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1437.

(12) (a) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, E. M. *Tetrahedron* **1989**, *45*, 5365. (b) Drueckhammer, D. G.; Hennen, W. J.; Pederson, R. L.; Barbas, C. F., III; Gautheron, C. M.; Krach, T.; Wong, C.-H. *Synthesis* **1991**, 499.

(13) (a) Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3199. (b) Durrwachter, J. R.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175. (c) Bednarski, M. D.; Simon, E. S.; Bischofberger, N.; Fessner, W.-D.; Kim, M. J.; Lee, W.; Saito, T.; Waldemann, H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *11*, 627. (d) Liu, K. K.-C.; Pederson, R. L.; Wong, C.-H. *J. Chem. Soc., Perkin Trans.* **1991**, 2669.

(14) Schiff base enzymes: Horecker, B. L.; Tsolas, O.; Lai, C. Y. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic: New York, 1972; Vol 7, p 213. Midelfort, C. F.; Gupta, R. K.; Rose, I. A. *Biochemistry* **1976**, *15*, 2178. X-ray structure: Sygusch, J.; Beaudry, D.; Allaire, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7846. Zn^{2+} enzyme from *Escherichia coli*: Kadonaga, J. T.; Knowles, J. R. *Biochemistry* **1983**, *22*, 130.

(15) Drueckhammer, D. G.; Durrwachter, J. R.; Pederson, R. L.; Crans, D. C.; Daniels, L.; Wong, C.-H. *J. Org. Chem.* **1989**, *54*, 70.

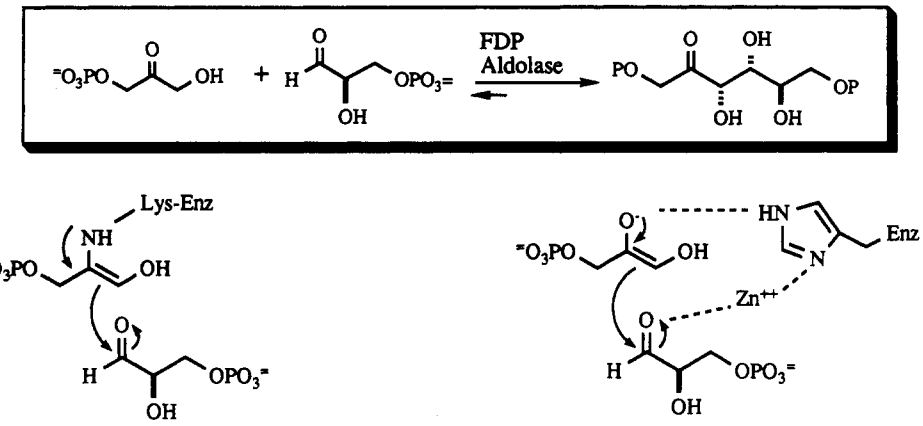


Figure 2. Reactions of FDP aldolase from rabbit muscle (left) and *E. coli* (right). In general, the Zn^{2+} enzyme is more stable.

regardless of the stereochemistry of the α-hydroxy aldehyde. However, the (*R*)-α-hydroxy aldehyde is accepted by the enzyme at a faster rate than the *S* enantiomer.[12a,19,20] Rham-1-PA reacts faster with the (*S*)-α-hydroxy aldehydes that allow for Cram–Felkin addition of the DHAP. FDP aldolase also proceeds with the Cram-type selectivity. Fuc-1-PA on the other hand, reacts faster with the (*S*)-α-hydroxy aldehyde that undergoes anti-Cram attack by the DHAP. Since the rates of reactivity are different for epimeric α-hydroxy aldehydes, only one product from racemic α-hydroxy aldehydes is obtained when the enzymatic reaction is run under kinetic control.[12a,19,20]

Racemic β-hydroxy aldehydes can also be used in the aldolase reaction to give predominantly one isomer. FDP aldolase catalyzed additions to β-hydroxy aldehydes yield ketoses which preferentially form a pyranose. Under thermodynamic control, the all equatorial product is favored when 2 equiv of racemic aldehyde is reacted with 1 equiv of DHAP.[12a,19,20]

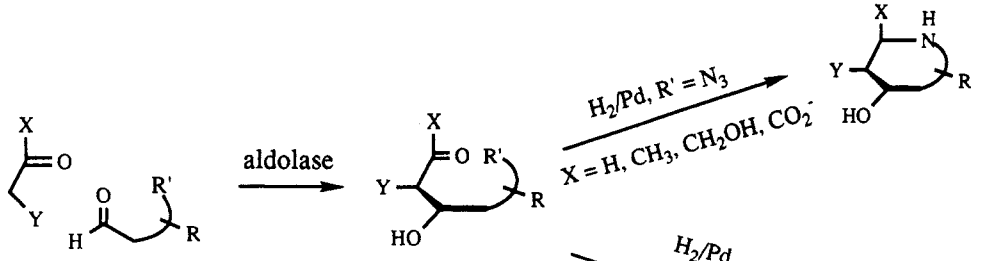
In addition to DHAP dependent aldolases, 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4),[21] sialic acid aldolase (EC 4.1.3.3),[12,22,23] KDO aldolase,[23c] KDO-8 phosphate synthase,[24] DAHP synthetase,[25] 3-hexulose phosphate synthase,[26] 2-keto-3-deoxy-6-phosphonogluconate aldolase,[27] and 2-keto-4-hydroxyglutarate aldolase[28] have been used in the generation of carbohydrates. Several aldolase genes have been cloned and overexpressed.[16,18,21,25,29] DERA is able to accept a large number of aldehydes as acceptors, and unlike the DHAP dependent aldolases, DERA shows some flexibility for the donor component. The natural donor, acetaldehyde, adds to an acceptor aldehyde to give the 3*S* configuration, again, irrespective of what substituents are on the acceptor. Reaction with propionaldehyde as the donor generated two new stereocenters having the 2*R* and 3*S* configurations.[21] Both (*R*)- and (*S*)-α-hydroxy aldehydes were substrates in these reactions. However, *R* isomers were much better substrates for DERA (following the Cram–Felkin mode of attack), so selective condensation of one enantiomer from a racemic mixture could be performed using this enzyme.

Sialic acid aldolase and KDO aldolase use pyruvate as the donor and, like the other aldolases, are flexible in the aldehydes they accept. The selectivity of addition, on the other hand, is not as strict as for the other aldolases. Sialic acid aldolase catalyzed additions to simple α-hydroxy aldehydes normally give the *S* configuration at the newly generated stereogenic center. However, with D-arabinose a mixture of KDO and 4-epi-KDO was generated,[22b] and with larger aldehyde acceptors, such as L-mannose and L-6-deoxymannose,[23a] and L-*N*-acetylmannosamine,[23b] the newly formed stereocenter had the *R* configuration only. This unprecedented dependence on aldehyde stereochemistry has been exploited for the preparation of complementary enantiomers of high-carbon sugars.[23b] The KDO aldolase reactions always give a new stereocenter with the *R* configuration.[23c]

Aza Sugar Construction

A direct extension of the aldolase strategy is the synthesis of nitrogen-containing sugars and analogs. In principle, an aldehyde acceptor species containing a suitable amine synthon could be converted to the corresponding azido keto sugar by aldolase action. The azido keto sugar subsequently could be reduced to form

(16) Ozaki, A.; Toone, E. J.; von der Osten, C. H.; Sinskey, A. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 4970.
(17) (a) Kajimoto, T.; Chen, L.; Liu, K. K.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6678. (b) Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280.
(18) Fessner, W.-D.; Sinerius, G.; Schneider, A.; Dreyer, M.; Schultz, G. E.; Badia, J.; Aquilar, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 555.
(19) Fessner, W.-D.; Eyrisch, O. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 56.
(20) (a) Durrwachter, J. R.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175. (b) Liu, K. K.-C.; Pederson, R. L.; Wong, C.-H. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2669.
(21) Chen, L.; Dumas, D. P.; Wong, C.-H. *J. Am. Chem. Soc.*, in press.
(22) (a) Augé, C.; Gautheron, C. *Adv. Carbohydr. Chem.* **1991**, *49*, 175. (b) Augé, C.; David, S.; Gautheron, C.; Malleron, A.; Cavaye, B. *New J. Chem.* **1988**, *12*, 733. Augé, C.; Gautheron, C.; David, S.; Malleron, A.; Cavaye, B.; Bouxom, B. *Tetrahedron* **1990**, *46*, 201. (c) Kim, M.-J.; Hennen, W. J.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 6481.
(23) (a) Gautheron-Le Narvor, C. M.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 7816. (b) Lin, C.-H.; Sugai, T.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 10138. (c) Sugai, T.; Shen, G. J.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 413. (d) Halcomb, R. L.; Wong, C.-H. Unpublished material.
(24) Bednarski, M. D.; Crans, D. C.; DiCosimo, R.; Simon, E. S.; Stein, P. D.; Whitesides, G. M.; Schneider, M. J. *Tetrahedron Lett.* **1988**, *29*, 427.
(25) Reimer, L. M.; Conley, D. L.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1986**, *108*, 8010.
(26) Beisswenger, R.; Snatzke, G.; Thiem, J.; Kula, M.-R. *Tetrahedron Lett.* **1991**, *32*, 3156.
(27) Allen, S. T.; Heintzelman, G. R.; Toone, E. J. *J. Org. Chem.* **1992**, *57*, 426.
(28) Floyd, N. C.; Liebster, M. H.; Turner, N. J. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1085.
(29) Overproduction of *E. coli* FDP aldolase: (a) von der Osten, C. H.; Sinskey, A. J.; Barbas, C. F., III; Pederson, R. L.; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1989**, *111*, 3924. (b) Alefounder, P. R.; Baldwin, S. A.; Perham, R. N.; Short, N. J. *Biochem. J.* **1989**, *257*, 529.



Enzyme	X	Y
2-Deoxyribose 5-P-Aldolase	-H	H, CH_3, $ClCH_2$
	$-CH_3$, CH_2F	CH_3
DHAP-dependent Aldolase	$-CH_2OPO_3^=$	OH
Pyruvate Aldolase	$-CO_2^-$	CH_3

Figure 3. Aza sugars from aldolase reactions and Pd-mediated reductive aminations.

a transient imine, which would be further reduced to the aza sugar (Figure 3). The in situ reductive amination of azido keto sugars to aza sugars was demonstrated earlier by Card and Hitz[30] while the Pd-mediated reductive amination of amino sugars was pioneered by Paulsen and co-workers.[31] The chemoenzymatic strategy was first reported for the preparation of a mixture of 1-deoxymannonojirimycin and 1-deoxynojirimycin[32,33] starting with racemic 3-azido-2-hydroxypropanal. With the use of different aldolases and enantiomerically pure azido aldehydes, a variety of aza sugars are now readily accessible.[20,21,29a] The phosphate group in the product not only facilitates product recovery but also serves as an activating group in the reductive amination to give a dideoxy aza sugar.[17]

Preparation of Substrates. The efficient preparation of DHAP is required for DHAP-dependent aldolase reactions. Three efficient methods have been reported: the first method involves the chemical phosphorylation of ethyl dihydroxyacetone dimer,[34] the second method employs glycerol kinase to phosphorylate dihydroxyacetone,[13a] and the third method uses rabbit muscle aldolase to convert fructose diphosphate to glycerol 3-phosphate and then triosephosphate isomerase to convert G-3-P to DHAP.[13] The most effective method perhaps is the one based on dibenzyl *N,N*-diethylphosphoramidite.[34b] Another strategy is the in situ formation of an arsenate ester equivalent of DHAP.[15] While enzymatic reactions using arsenate are slower, the method can be readily performed on large scales using relatively inexpensive starting materials. Studies on the acceptor specificity[35,36] indicate that the enzymes only accommodate limited substitution at C-1 with a marked decrease in reactivity.

Since the DHAP-dependent enzymes are quite flexible for the acceptor, a wide variety of azido aldehydes have been prepared and examined for use in aza sugar synthesis. Azido aldehydes in high enantiomeric purity can be prepared by kinetic resolution based on *Pseudomonas* lipases.[29a,37-39] A detailed study on the use of various amine synthons as substrates for FDP aldolase indicates that the best amine synthon substrates are those that contain the azido group.[40] This may be due in part to the increased water solubility of azides and their general affinity due to size and charge for the active site of the aldolases.

Sugar Synthesis. Figure 4 presents aza sugars that have been reported based on certain aldolase reactions and Pd/C-mediated reductive amination. Figure 5 depicts enantiocomplementary routes used for the preparation of a series of sugars and aza sugars. By the proper choice of enzymes, both the D and L sugars can be readily accessible through this strategy.[17,23,41]

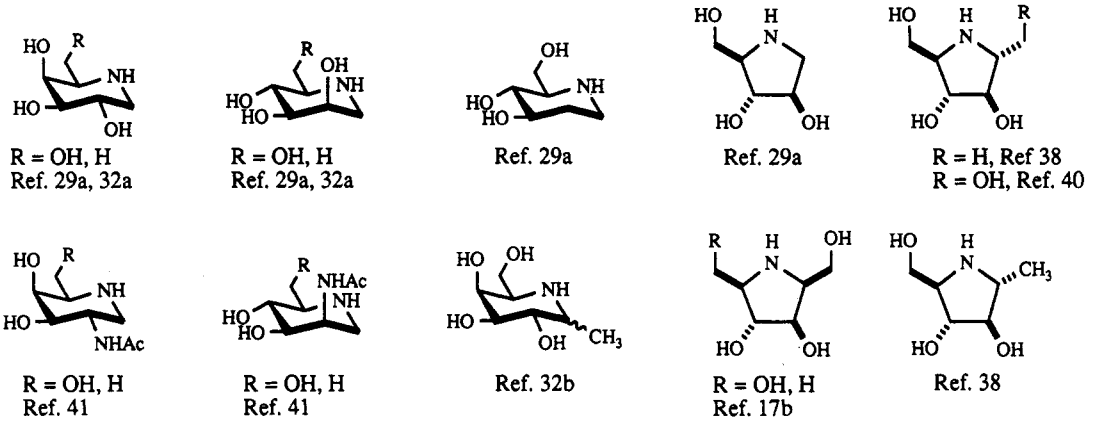
Stereoselective Imine Reduction. A key step in the synthesis of aza sugars is the heterogeneous palladium-mediated reduction of the transient imine which arises from the reduction of the azido group. In the six-membered-ring case, the stereoselectivity of this reduction step is usually quite high with the delivery of hydrogen occurring from the face opposite the axial hydroxyl groups or from the face that affords the least torsional strain upon rehybridization of the six-membered ring.[17,41] When these two effects are in conflict, the hydroxyl directing influence usually takes precedence.

Reductive amination of the FDP aldolase product from γ-azido-β-hydroxybutyraldehyde in neutral aqueous solution gave a six-membered amino ketose instead of a seven-membered aza sugar.[13a] We are currently exploring the use of homogeneous catalysts for the imine reduction with hopes of yielding hydroxyl-directed products[42] to expand the scope of aza sugar formation.

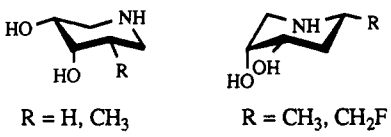
Modification of Aza Sugars with Lipases and Proteases. The naturally-occurring aza sugar castano-

(30) Carel, P. J.; Hitz, W. D. *J. Org. Chem.* **1985**, *50*, 891.
(31) Paulsen, H.; Sangster, I.; Heyns, K. *Chem. Ber.* **1967**, *100*, 802.
(32) (a) Zeiger, T.; Straub, A.; Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 716. (b) Straub, A.; Effenberger, F.; Fischer, P. *J. Org. Chem.* **1990**, *55*, 3926.
(33) Pederson, R. L.; Kim, M.-J.; Wong, C.-H. *Tetrahedron Lett.* **1988**, *29*, 4645.
(34) (a) Effenberger, F.; Straub, A. *Tetrahedron Lett.* **1987**, *28*, 1641. (b) Pederson, R. L.; Esker, J.; Wong, C.-H. *Tetrahedron* **1991**, *47*, 2643.
(35) Bischofberger, N.; Waldmann, H.; Saito, T.; Simon, E. S.; Lees, W.; Bednarski, M. D.; Whitesides, G. M. *J. Org. Chem.* **1988**, *53*, 3457.
(36) Stribling, D. *Biochem. J.* **1974**, *141*, 724.
(37) Pederson, R. L.; Liu, K. K.-C.; Rutan, J. F.; Chen, L.; Wong, C.-H. *J. Org. Chem.* **1990**, *55*, 4899.
(38) Wang, Y.-F.; Dumas, D. P.; Wong, C.-H. *Tetrahedron Lett.* **1993**, *34*, 403.
(39) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656.
(40) Hung, R. R.; Straub, J. A.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 3849.
(41) (a) Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, J. A., Jr.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187. (b) Lees, W. J.; Whitesides, G. M. *Bioorg. Chem.* **1992**, *20*, 173.
(42) Stork, G.; Kahne, D. *J. Am. Chem. Soc.* **1983**, *105*, 1072.

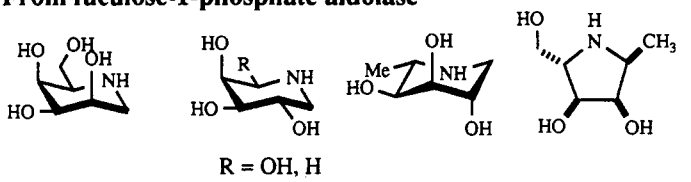
From fructose-1,6-diphosphate aldolase



From 2-deoxyribose-5-phosphate aldolase[21a]



From fuculose-1-phosphate aldolase[38,41]



From sialic acid aldolase[23d]

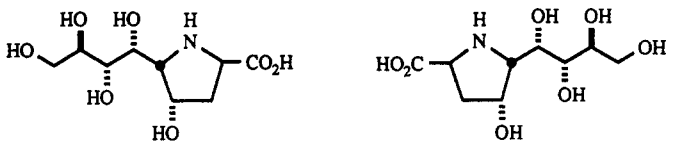


Figure 4. Aza sugars prepared by aldolase reactions.

spermine has been intensely studied since it shows antineoplastic activity[43] and is potentially useful for the treatment for AIDS.[5b] Recently it was found that some O-acetylated derivatives of castanospermine are much more potent than castanospermine itself.[44] Preparation of such compounds is not easily done by standard synthetic methods; however, this difficulty was overcome by employing subtilisin and lipases to provide several different O-acetylated derivatives.[45] The subtilisin-catalyzed acylation occurs at the OH group equivalent to the primary hydroxyl group of glucose. Another regioselective acylation catalyzed by lipase CV followed by subtilisin-catalyzed hydrolysis gave 7-butanoylcastanospermine. Of the many acylation reagents used in enzymatic acylation, the most effective ones are enol esters[22c,46] which gave ketones or aldehydes as leaving groups, making the process irreversible. The best organic solvent for sugar transformation appears to be dimethylformamide (DMF). Although enzymes are generally very unstable in DMF, the technique of site-directed mutagenesis can be employed to reengineer subtilisin to become stable and active in DMF.[47]

Enzyme-Mediated Oligosaccharide Synthesis. Glycosidases and glycosyltransferases catalyze the formation of glycoside linkages between saccharides with high regio- and stereoselectivity without the need for harsh conditions and multiple protecting groups. These enzymes have been studied extensively for the synthesis of oligosaccharides.[12,48] Some work has been performed chemically[49a] or enzymatically[49b] to incorporate aza sugars into oligosaccharides which have potential value as glycosidase and glycosyltransferase inhibitors.[49] The glycosyltransferase activity of cyclodextrin glycosyltransferase (EC 2.4.1.19) has been utilized in combination with glucoamylase (EC 3.2.1.3) to prepare an aza disaccharide in 20–30% yield from either α-cyclodextrin or soluble starch (poly-α-1,4-glucose) and various 1-deoxynojirimycin *N*-derivatives.[50] In a limited study 1-deoxy-nojirimycin and 1,6-dideoxynojirimycin were shown not to be acceptors for the β-galactosidase-catalyzed coupling reaction.[51]

Glycosyltransferases are known to exhibit higher regioselectivity than the glycosidases and have been shown to accommodate a wide variety of acceptor substrates. Thus, these enzymes hold great potential

(43) For a leading reference, see: Humphries, M. J.; Matsumoto, K.; White, S. L.; Molyneux, R. J.; Olden, K. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1752.

(44) Sunkara, P. S.; Taylor, D. L.; Kang, M. S.; Bowlin, T. L.; Liu, P. S.; Tymes, A. S.; Sjoedsma, A. *Lancet* **1989**, 1206.

(45) Margolin, A. L.; Delinck, D. L.; Whalon, M. R. *J. Am. Chem. Soc.* **1990**, *112*, 2849.

(46) Sweers, H. W.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 6421.

(47) Zhong, Z.; Liu, J. L.-C.; Dinterman, L. M.; Finkelman, M. A. J.; Mueller, T. W.; Rollence, M. L.; Whitlow, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 683.

(48) (a) Ichikawa, Y.; Look, G. C.; Wong, C.-H. *Anal. Biochem.* **1992**, *202*, 215. (b) Nilsson, K. G. I. *Trends Biotechnol.* **1988**, *6*, 256. (c) Coté, G. L.; Tao, B. Y. *Glycoconjugate J.* **1990**, *7*, 145.

(49) (a) Liotta, L. J.; Bernotas, R. C.; Wilson, D. B.; Ganem, B. *J. Am. Chem. Soc.* **1989**, *111*, 783. Furui, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1992**, *229*, C1. (b) Wong, C.-H. *TIBTECH* **1992**, *10*, 337.

(50) (a) Ezure, Y. *Agric. Biol. Chem.* **1985**, *49*, 2159. (b) Ezure, Y.; Maruo, S.; Ojima, N.; Konno, K.; Yamada, N.; Sugaiyama, M. *Agric. Biol. Chem.* **1989**, *53*, 61.

(51) Wong, C.-H.; Ichikawa, Y.; Krach, T.; Gautheron-Le Narvor, C.; Dumas, D. P.; Look, G. C. *J. Am. Chem. Soc.* **1991**, *113*, 8137.

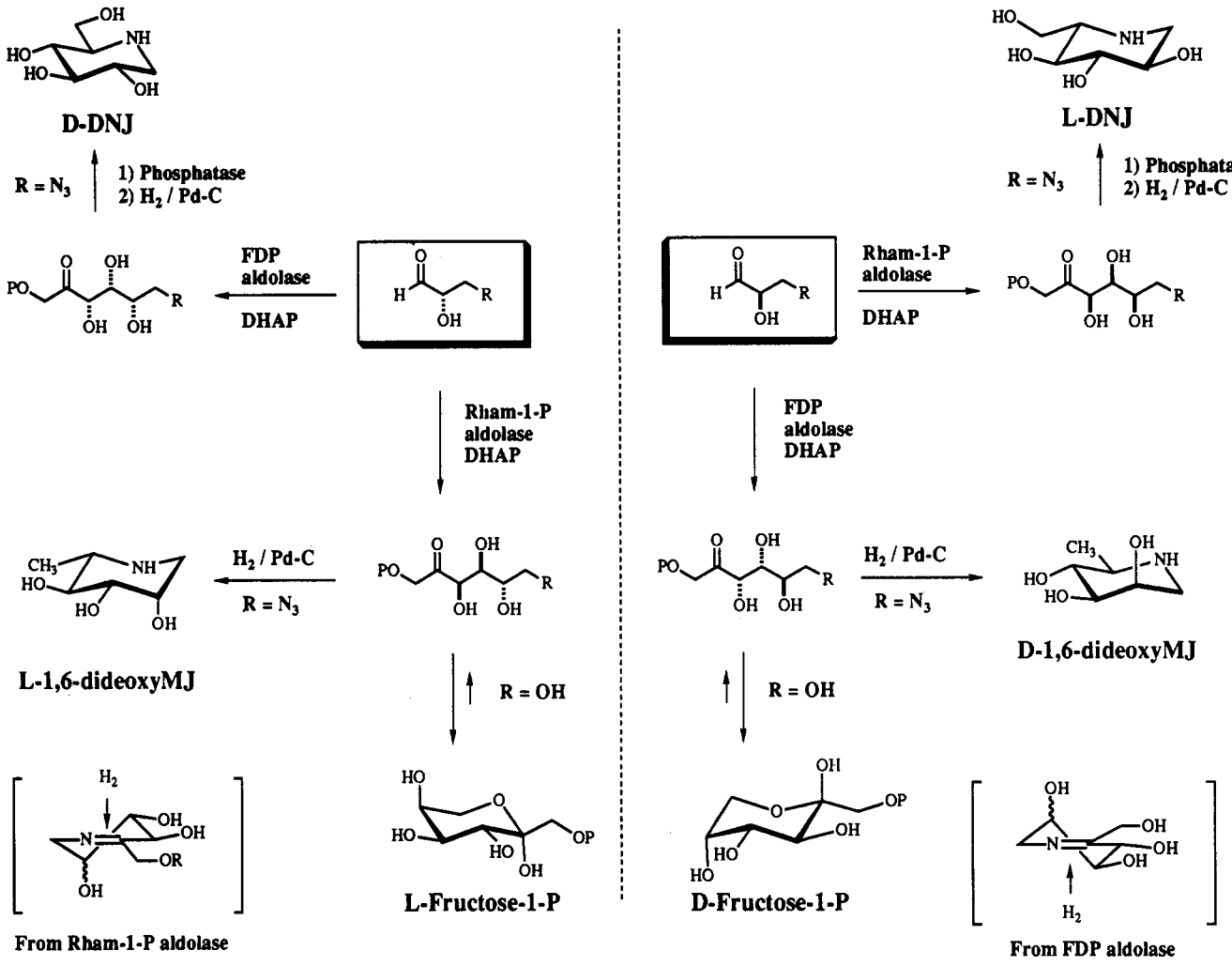


Figure 5. Enantiocomplementary nature of FDP aldolase and Rham-1-PA.

for the in vitro formation of oligosaccharides. The major problems involved with the use of glycosyltransferases are that the requisite sugar nucleotides are expensive for large scale synthesis and the released nucleoside phosphates are frequently inhibitors of the coupling reaction. These difficulties have been circumvented by the in situ regeneration of the sugar nucleotide during the course of the enzymatic reaction.[48a,52,53]

Regeneration systems for the eight sugar nucleotide substrates (UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, UDP-GluA, GDP-Man, GDP-Fuc, CMP-NeuAc) employed by mammalian glycosyltransferases have been developed.[52,53] While the glycosyltransferase systems have yet to be extensively applied for the coupling of aza sugars, galactosyltransferase [EC 2.4.1.22] has been used to couple galactose to 1-deoxynojirimycin, affording galactosyl β-1,4-deoxynojirimycin as the sole disaccharide product in 20% yield (Figure 6).[54] The 1,6-dideoxy derivative of nojirimycin has also been used as a substrate, although the relative rate of product formation (versus the natural acceptor glucose) is low. The aza disaccharide is an inhibitor of a recombinant α-1,3-fucosyltransferase (IC_{50} = 8 mM).[55] The galactosyl 5-thioglucose analog prepared through the galactosyltransferase reaction, however, is a good substrate for α-1,3-fucosyltransferase.[55]

Inhibition Studies. With the number of aza sugar inhibitors prepared by these enzymatic methods, it was interesting to compare the features of the inhibitors with their inhibition constants (K_i). In theory, one might expect that the best inhibitors would have features similar to those of the glycosyl cation and match the electronic and steric requirements necessary to bind tightly in the enzyme active site.[56] For example, deoxynojirimycin analogs[41] mimic the charge and the positioning of the hydroxyl groups on the glycosyl cation. At physiological pH, when these inhibitors bind to the active site of a glucosidase, they interact with the base residues normally involved in the hydrolysis of a glucoside[56] (see A, in Figure 7). The hydroxyl groups on the ring interact favorably with the hydrophilic region in the active site of the enzyme, and as expected, when hydroxyl groups are removed from the aza sugar, binding diminishes. The positioning of these hydroxyl groups is also an important binding factor since it has been shown that the glucosidase inhibitor deoxynojir-

(52) Wong, C.-H.; Ichikawa, Y.; Kajimoto, T.; Liu, K. K.-C.; Shen, G.-J.; Liu, C.-H.; Wang, Y.-F.; Wang, R.; Look, G. C. *Pure Appl. Chem.*, in press.

(53) (a) Wong, C.-H.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 5416. (b) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, S.; Paulson, J.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.

(54) Gautheron-Le Narvor, C.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, 1130.

(55) Wong, C.-H.; Dumas, D. P.; Ichikawa, Y.; Koseki, K.; Danishefsky, S. J.; Weston, B. W.; Lowe, J. B. *J. Am. Chem. Soc.* **1992**, *114*, 7321.

(56) Dale, M. P.; Ensley, H. E.; Kein, K.; Sastry, K. A. R.; Byers, L. *Biochemistry* **1985**, *24*, 3503.

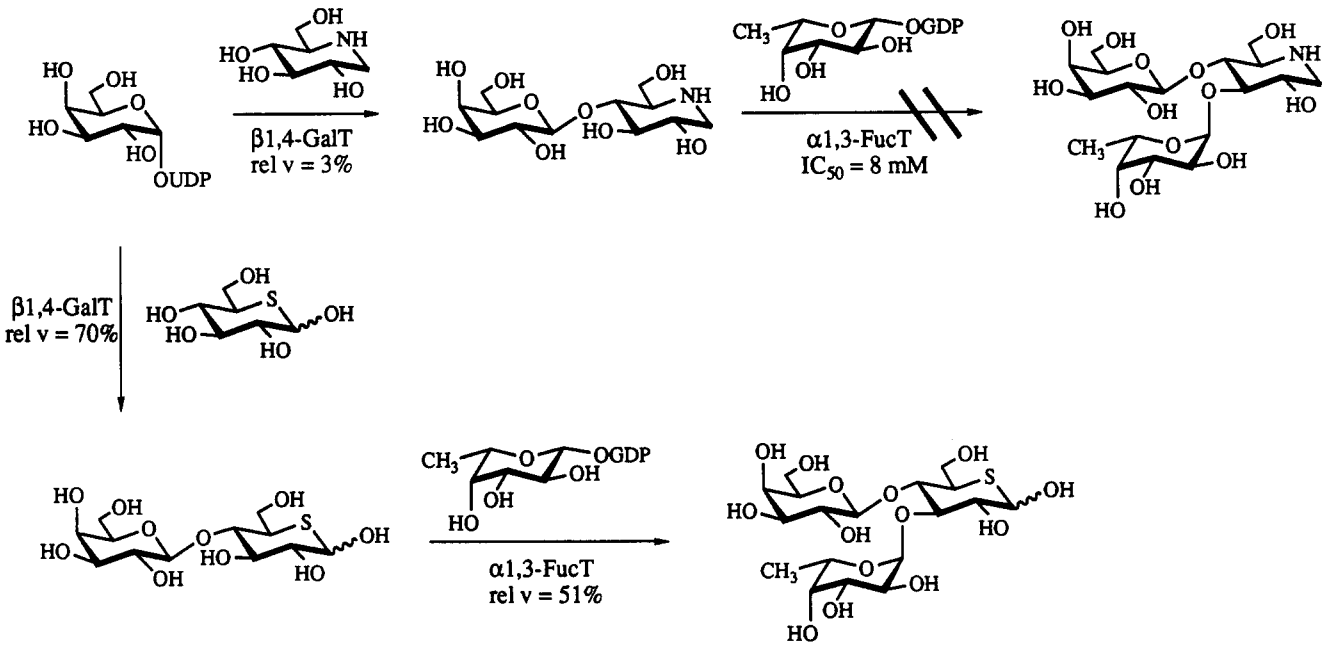


Figure 6. Glycosyltransferase-catalyzed incorporation of thio sugar and aza sugar into oligosaccharides.

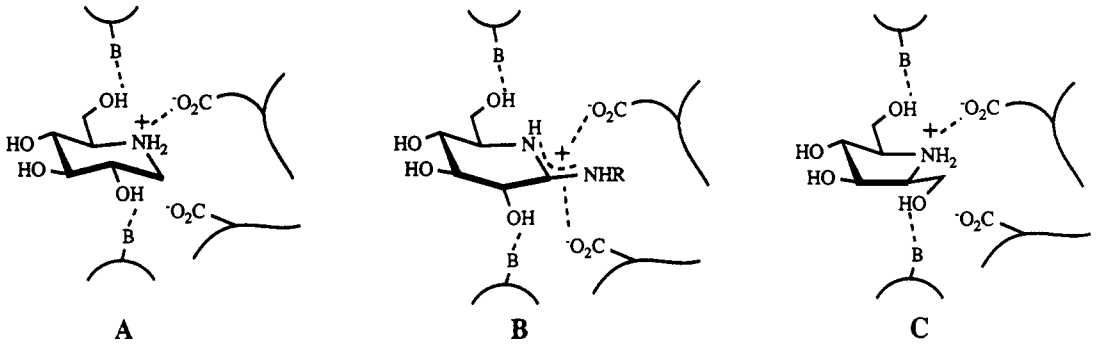


Figure 7. Binding of nitrogen-containing inhibitors in the glycosidase enzyme active site.

imycin does not inhibit β-galactosidase or α-mannosidase, and the mannosidase inhibitor deoxymannonojirimycin is a poor inhibitor of glucosidase.[41a,57]

One common feature of the deoxynojirimycin analogs is that they all exist in a six-membered-ring chair configuration. A class of inhibitors which are more representative of the flattened half-chair found in the glucosyl cation is the amidine inhibitors (see B, Figure 7).[58] This inhibitor is one of the most potent synthetic inhibitors known; amidines are, however, unstable since they can be readily hydrolyzed, and their syntheses are not trivial. To overcome this problem, five-membered-ring pyrrolidine inhibitors which do not possess a labile moiety have been developed.[17,38] These inhibitors have a similar conformation and charge distribution to the half-chair glycosyl cation, and their inhibition constants are comparable with those of the amidine inhibitors (see C, Figure 7).

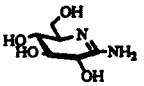
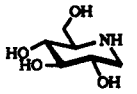
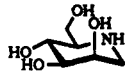
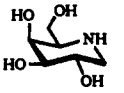
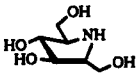
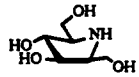
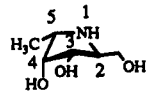
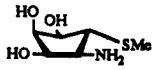
As shown in Table I, a remarkable feature of the flattened half-chair compounds is that they usually inhibit a broad range of glycosidases. The amidine[58] is a potent inhibitor of sweet almond β-glucosidases as one would predict based on the position of the hydroxyl groups. However, jack bean α-mannosidase and β-galactosidase are also inhibited by this compound, which is not expected if inhibition is only a function of hydroxyl group topography. Inhibitors that are in a chair conformation, on the other hand (e.g., deoxynojirimycin,[41] deoxymannonojirimycin,[57] and galactostatin[9d]), are very specific for the enzymes whose natural substrate they mimic.

Broad spectrum inhibition was often observed with five-membered-ring inhibitors.[9g,17b,38] Compound C in Figure 7 inhibits both α- and β-glucosidases;[17b] it also inhibits α-galactosidase, α-mannosidase, and α-L-fucosidase.[59] In another study with fucosidases, several five-membered-ring inhibitors, all with differing hydroxyl group topography, inhibited α-L-fucosidase.[38] Finally, the five-membered-ring inhibitor mannostatin, which inhibits α-mannosidase with a K_i of 48 nM,[6c] has a hydroxyl group topography that does not correspond well at all with mannose. Apparently, it is more important for an inhibitor to mimic the flattened half-chair conformation of the glycosyl cation than to have the correct hydroxyl group topography.

To better understand the structure–inhibition relationship, the free energy of dissociation, ΔG_{diss}, was separated into four components: conformation, positioning (or existence) of the 2-OH group, position (or existence) of the 6-OH group, and the charge on the heteroatom.[17b,41] Using multivariable least-squares

(57) Legler, G.; Jülich, E. *Carbohydr. Res.* **1984**, *128*, 61.
(58) Tong, M. T.; Papandreou, G.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 6137.
(59) Dumas, D. P.; Kajimoto, T.; Liu, K. K.-C.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 33.

Table I. Inhibition Potencies of Various Aza Sugars[a]

	sweet almond β-glucosidase	yeast α-glucosidase	jack bean α-mannosidase	green coffee bean α-galactosidase	bovine liver β-galactosidase	*Aspergillus niger* β-xylosidase	bovine kidney α-L-fucosidase
	8 ± 5[b]		9 ± 1[b]	8 ± 5[b]			
	18[c]	8.7[c]	400[c]	NI[c]			
	5300[d]		68[d]				30 ± 10[e]
	NI[f]	NI[f]	NI[f]	0.0053[f]	NI[f]		
	7.8[g]	3.3[g]	NI	NI		250[g]	
	19[h]	2.8[h]	3100[h]	50[h]			910 ± 20[e]
							1.4[i]
			0.048[j] (rat epididymus)		>100[j]		

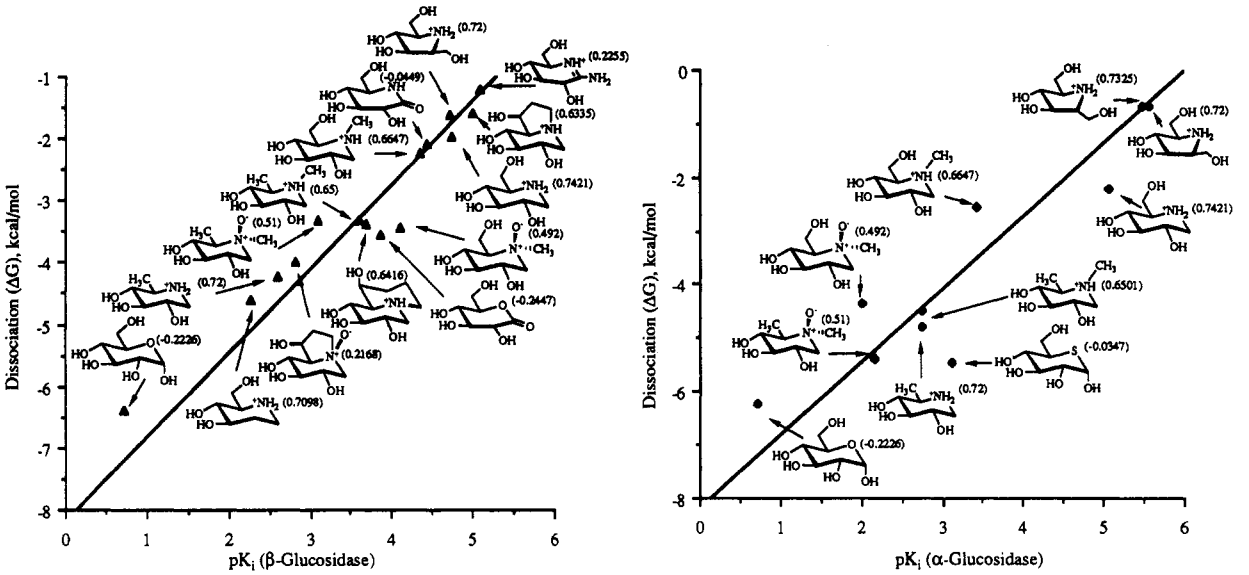
[a] All K_i measured in μM; NI = no inhibition. [b] See ref 58. [c] See ref 41. [d] See ref 57. [e] See ref 59. [f] See ref 9d. [g] See ref 9g. [h] See ref 17b. [i] See ref 38. K_i values for other epimers: 8 μM for 3-epimer, 22 μM for 2,3,5-epimer, 4 μM for 3,5-epimer. [j] See ref 6c.

analysis, parameters were calculated based on constants (F) which best fit the observed data (observed data is the line $\Delta G_{diss} = -RT\ pK_i = 8.181$ where all K_i values are standardized to 1 μM (Figure 8)). According to this equation, the most important contributor to binding is charge since the largest parameter (3.268) value is in front of the charge component. Also, the parameter value for F(conformation) is nearly as large as, if not larger than, the parameter values for the hydroxyls F(2-OH) and F(6-OH). This suggests that if the inhibitor is in a positively charged half-chair conformation, the positions of the hydroxyl groups are less important, which coincides with the observations summarized in Table I.

While the features necessary for a good glucosidase inhibitor seem to be well defined, the characteristics of a good mannosidase inhibitor are still not clear. It has been shown[60] that the lowest energy conformation of the mannosyl cation is the one where three hydroxyls are in a "pseudoaxial" position, and most good inhibitors for mannosidases have hydroxyls which mimic this geometry. However, the importance of charge on the inhibitor is still not clear. For example, kifunensine, which has hydroxyls that overlap with the mannosyl cation and is neutral at physiological pH, is a potent inhibitor of (α)-mannosidase I (IC_{50} = 20 nM)[61] but is a very weak inhibitor of jack bean α-mannosidase.[62] On the other hand, swainsonine, which is protonated and has hydroxyls that correspond to the mannosyl cation, is a potent inhibitor of both (α)-mannosidase II and jack bean α-mannosidase (both IC_{50} ca. 1–2 μM).[63] Interestingly, 6-epi-castanospermine, which is similar to the ground-state conformation of mannose, has virtually no activity toward jack bean α-mannosidases,[64] which exemplifies the importance of the inhibitor mimicking the pseudoaxial conformation of the mannosyl cation. Full understanding of the effects that govern inhibition are also not well defined for other glycosidases, e.g., galactosidase,[9b] sialidase,[65] *N*-acetylglucosaminidase,[9i] *N*-acetylgalactosaminidase,[9i] rhamnosidase,[9a] trehalase,[7d] and chitinase.[6k] Understanding the mechanism of glycosidase reactions will certainly provide useful information for inhibitor design.

Besides inhibiting glycosidases, five-membered-ring aza sugars also inhibit glycosyltransferases. The fucosyl-like five-membered aza sugars inhibit not only α-fucosidase but also α-1,3-fucosyltransferase, in the presence of GDP and an acceptor.[38] This synergistic inhibition of the fucosyltransferase with GDP and the aza sugar may be due to the formation of a complex, where the aza sugar interacts with the acceptor and phosphate on GDP (Figure 9) to mimic the glycosyltransferase reaction.[55] This "synergism" in binding is certainly a very interesting concept and may be useful for the design of inhibitors for glycosyltransferases, which are emerging as interesting targets for inhibition.[66]

(60) Winkler, D. A.; Holan, G. *J. Med. Chem.* **1989**, *32*, 2084.
(61) Elbein, A. D.; Tropca, J. E.; Mitchell, M.; Kaushal, G. P. *J. Biol. Chem.* **1990**, *26*, 15599.
(62) Pan, Y.-T.; Kaushal, G. P.; Papandreou, G.; Ganem, B.; Elbein, A. D. *J. Biol. Chem.* **1992**, *267*, 8313.
(63) Howard, A. S.; Michael, J. P. In *The Alkaloids; Chemistry and Pharmacology*; Brossi, A., Ed.; Academic: New York, 1986; Vol. 28, pp 275–308.
(64) Molyneux, R. J.; Roitman, J. N.; Dunnheim, G.; Szumilo, T.; Elbein, A. D. *Arch. Biochem. Biophys.* **1986**, *251*, 450.
(65) For representative papers, see: Glänzer, B. I.; Györgydeák, Z.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1991**, *74*, 343. Vasella, A.; Wyler, R. *Helv. Chim. Acta* **1991**, *74*, 451. Sastre, A. G.; Cobaleda, C.; Cabezas, J. A.; Villar, E. *Biol. Chem. Hoppe-Seyler* **1991**, *372*, 923. Warner, T. G.; Louie, A.; Potier, M.; Ribeiro, A. *Carbohydr. Res.* **1991**, *215*, 315.



$$\Delta G = \Delta G(\text{conformation}) + \Delta G(2\text{-OH}_{eq}) + \Delta G(6\text{-OH}_{eq}) + \Delta G(\text{charge})$$
$$= 2.450\ F(\text{conformation}) + 2.516\ F(2\text{-OH}) + 1.266\ F(6\text{-OH}) + 3.268\ F(\text{charge}) - 8.181 \text{ for } \beta\text{-glucosidase and}$$
$$= 2.182\ F(\text{conformation}) + 0.6728\ F(2\text{-OH}) + 2.1971\ F(6\text{-OH}) + 4.177\ F(\text{charge}) - 8.181 \text{ for } \alpha\text{-glucosidase}$$

Where:

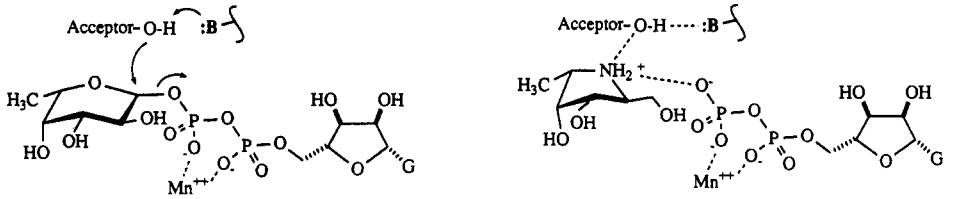
F(conformation) = 1 if the inhibitor is in a half-chair or envelop conformation; 0.2 if the inhibitor is a five-six ring system; 0 if the inhibitor is a chair conformation.

F(2-OH) = 1 for the presence of a 2-OHeq; 0.2 for the 2-CH_2OH group of a 5-membered ring compound; 0 for the absence of a 2-OHeq.

F(6-OH) = 1.2 for castanospermine; 1 if the 6-OH is free of intramolecular H-bonding; 0.5 if the 6-OH has a weak intramolecular H-bond or if the 6-OH has a N-oxide in the vicinity; 0.2 for a bicyclic inhibitor if the -OH corresponds to the 6-OH in glucose and is near an N-oxide or does not overlap well with the corresponding -OH in castanospermine.

F(charge) = partial charge distribution of the ring heteroatom (in parenthesis) calculated by PM3. The values were taken as calculated except for the N-oxide compounds. In those cases, 50% of the calculated values were used for the six-membered ring inhibitors due to possible shielding from the negatively charged oxygen atoms, and in the case of castanospermine N-oxide, the charge was calculated by taking the difference in charge between the nitrogen and oxygen in the N-oxide.

Figure 8. Correlation between dissociation energy and pK_i for α- and β-glucosidase.



Fucosyltransferase reaction Inhibitor-enzyme complex

Figure 9. Inhibition of fucosyltransferase with an aza sugar and GDP.

Conclusion

The combination of chemical and enzymatic methodologies is emerging as a practical route to the synthesis of complex chiral and biologically active molecules, especially mono- and oligosaccharides, and their derivatives. This chemoenzymatic approach to carbohydrates utilizes the flexibility of chemical synthesis and the remarkable regio- and stereoselectivity as well as the relatively mild nature of enzyme-mediated synthesis. The extension of this methodology to the formation of aza sugars and their analogs holds great potential for the design and synthesis of therapeutic agents and mechanistic probes.

We wish to thank our many co-workers, most of whose names are mentioned in the references. This work has been supported by the NIH.

(66) Palcic, M. M.; Roitman, J. N.; Srivastava, O. P.; Hindsgaul, O. *J. Biol. Chem.* **1989**, *264*, 17174. Kim, S. C.; Singh, A. N.; Raushel, F. *J. Biol. Chem.* **1988**, *263*, 10151. Vaghefi, M. M.; Bernacki, R. J.; Dalley, N. K.; Wilson, B. E.; Robins, R. K. *J. Med. Chem.* **1987**, *30*, 1383. Czernecki, S.; Periat, M.-C. *J. Org. Chem.* **1991**, *56*, 6289. Baguley, B. C.; Rommele, G.; Gruner, J.; Wehrli, W. *Eur. J. Biochem.* **1979**, *97*, 345. Noort, D.; van Straten, N. C. R.; Boons, G. J. P. H.; van der Marel, G. A.; Bossuyt, X.; Blanckaert, N.; Mulder, G. J.; van Boom, J. H. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 583.