carbanions, carbenes, and nitrenes.

Concluding Remarks

This Account has highlighted ways in which the inclusion of organic molecules within zeolites can alter the basic photochemical and photophysical properties of guest molecules. One might predict that in the coming years photochemistry will be a tool that will make a significant contribution toward a better understanding of the physical characteristics of zeolites—location, aggregation, mobility, and diffusion of guests within zeolites. The host–guest complex strategy allows one to control the size of the reaction cavity. Relative sizes of the guest and the free space around it are important factors to be considered when choosing a host system. With the proper choice of a guest and a host, one can control the relative sizes of the reaction cavity and the free space around the guest, and thus one can have a handle on the photobehavior of the included guest. In this context, zeolites offer several advantages over organic hosts: (a) zeolites with a number of sizes and shapes are commercially available, and therefore one has a choice;[36] (b) zeolites do not absorb light in the region where most organic molecules do; (c) generally, they do not undergo reaction with the guest molecules under nonforcing conditions; and (d) the internal properties of zeolites can also be fine tuned by variation of the silicon to aluminum ratio, by co-inclusion of solvents[37] or other adsorbents, and by the variation of cations. This mix of advantageous properties will prove valuable in future studies and applications.

Outstanding technical assistance provided by D. Sanderson, J. Lockhart, P. Hollins, and A. Pittman and in-depth discussions and a continuous supply of zeolites provided by Dr. D. R. Corbin are gratefully acknowledged. Discussions with Professors N. J. Turro and G. S. Hammond over a period of time have been very valuable. It is a pleasure to thank Professors C. Dybowski, A. Maki, J. Scheffer, C. V. Kumar, and Dr. L. Johnston, whose contributions have been vital to the program.

(36) Zeolites X, Y, L, M-5, Ω-5, and silicalite are available from Molecular Sieve Department (UOP) of Union Carbide, and pentasils (ZSM-5) can be obtained from Conteka B. V., Sweden. Pentasils may also be prepared by the literature procedure: Rollman, L. S.; Volyocsik, E. K. In *Inorganic Syntheses*; Wiley: New York, 1983; pp 61–68. Zeolite-β is prepared by following the literature procedure. Zeolites were activated by heating them in an aerated furnace at 500 °C for about 12 h.

(37) Ramamurthy, V.; Corbin, D. R.; Turro, N. J.; Zhang, Z.; Garcia-Garibay, M. A. *J. Org. Chem.* **1991**, *56*, 255.

Nucleoside Phosphate Sugars: Syntheses on Practical Scales for Use as Reagents in the Enzymatic Preparation of Oligosaccharides and Glycoconjugates

JÜRGEN E. HEIDLAS, KEVIN W. WILLIAMS, and GEORGE M. WHITESIDES*

Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

Received October 25, 1991

For many years, the carbohydrates present in living organisms have been identified primarily with passive roles: compounds used for energy storage, as structural units of cells, and for solubilizing and sorting components of glycoproteins. Recent advances in glycobiology and carbohydrate biochemistry have caused a fundamental reexamination of the importance of carbohydrates in biology. It is now evident that the oligosaccharide residues of glycoconjugates (glycoproteins, glycolipids, and glycophospholipids) serve as recognition sites for a variety of important intra- and intermolecular communication events.[1-3] These structures function, inter alia, as specific binding sites for various bacteria, viruses, and soluble toxins.[3-7] Cell-surface glycoconjugates help to regulate the growth and differentiation of cells, and they play a role in organogenesis.[8] The cell-surface lectins of mammalian cell adhesion (LEC-CAMs or selectins)[9-13] may be crucial mediators

Jürgen E. Heidlas studied food chemistry at the Julius-Maximilians-University, Würzburg, Germany. He received his Ph.D. from the Department of Food and Biotechnology of the Technical University of Berlin (with Roland Tressl) in 1990, working on the enzymatic formation of chiral hydroxy compounds. During 1990–1991, he was a postdoctoral fellow at Harvard University with Professor G. M. Whitesides. His major scientific interest is the application of enzymes to biotechnological processes.

Kevin W. Williams received his B.A. in chemistry at Lincoln University in Pennsylvania. He received his Ph.D. from the University of Pittsburgh, Department of Chemistry (with Julius Rebek, Jr.), in 1990. During 1991–1992 he was an NSF postdoctoral fellow at Harvard University with G. M. Whitesides. His major scientific interest is in the area of medicinal chemistry with an emphasis on rational drug design.

George M. Whitesides received his B.A. from Harvard University and his Ph.D. (with J. D. Roberts) from the California Institute of Technology in 1964. He worked at MIT for almost 20 years and is now a member of the Department of Chemistry at Harvard University. His research interests include materials science, surface chemistry, rational drug design, carbohydrate biochemistry, and molecular recognition.

(1) Sharon, N. *Complex Carbohydrates*; Addison-Wesley: Reading, MA, 1975.

(2) Horowitz, M. I., Pigman, W., Eds. *The Glycoconjugates*; Academic: New York, 1977, 1978; Vols. I and II.

(3) Horowitz, M. I., Ed. *The Glycoconjugates*; Academic: New York, 1982; Vols. III and IV.

(4) Schauer, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 131–234.

(5) Liener, I. E., Goldstein, I. J., Eds. *The Lectins: Properties, Functions and Applications in Biology and Medicine*; Academic: New York, 1986.

(6) Paulson, J. C. In *The Receptors*; Conn, P. M., Ed.; Academic: New York, 1985; Vol. 2, pp 131–219.

(7) Sairam, M. R. In *The Receptors*; Conn, P. M., Ed.; Academic: New York, 1985; Vol. 2, pp 307–340.

(8) Rademacher, T. W.; Parekh, R. B.; Dewek, R. A. *Annu. Rev. Biochem.* **1988**, *57*, 785–838.

(9) Bevilacqua, M. P.; Stenglein, S.; Gimbrone, M. A., Jr.; Seed, B. *Science* **1989**, *243*, 1160–1165.

(10) Brandley, B. K.; Swieder, S. J.; Robbins, P. W. *Cell* **1990**, *63*, 861–863.

(11) Larsen, E.; Palabrica, T.; Sajer, S.; Gilbert, G. E.; Wagner, D. D.; Furie, B. C.; Furie, B. *Cell* **1990**, *63*, 467–474.

(12) Moore, K. L.; Varki, A.; McEver, R. P. *Cell Biol.* **1991**, *112*, 491–500.

0001-4842/92/0125-0307$03.00/0

in inflammation. Bacterial endotoxin (LPS)[14,15] is an amphiphilic lipopolysaccharide, located in the outer membrane of Gram-negative bacteria, that is active in induction of the tumor necrosis factor[16,17] and responsible for many of the toxic effects these microorganisms have toward mammals.[18] One segment of LPS, the so-called lipid A region (a disaccharide, β-GlcN-1,6-GlcN acylated with fatty acids and phosphorylated), causes many of the physiological effects common to the bacterial endotoxin.[14]

Current interest in carbohydrate structures, and the increased demand for practical synthetic approaches to these compounds, is not restricted to fundamental biology. Carbohydrate-based inhibitors may provide the basis for therapies for diseases involving carbohydrates,[19] and many oligosaccharides and their derivatives now have pharmaceutical relevance. For example, heparin—a heterogeneous glycosaminoglycan polymer consisting of repeating units of sulfated and nonsulfated hexosamines and alduronic acids (mainly L-iduronic acid)—has widespread usage in medical therapy as an anticoagulant and antilipemic agent.[20,21] The biological activity of heparin preparations correlates well with the composition of the polymer.[22] Hyaluronic acid—a polymer based on repeating units of β-GlcUA-1,3-β-GlcNAc-1,4—has become important for its ability to increase the viscosity of biological fluids in surgery. The biotechnological community is also interested in oligo- and polysaccharide derivatives for their potential applications as biodegradable polymers, agents for modifying viscosity, and nonnutritive fat substitutes.

Background

Two groups of glycosyltransferases—the Leloir pathway enzymes and those of non-Leloir pathways—are involved biosynthetically in the attachment of monosaccharide units to oligo- and polysaccharide chains. Both classes of enzymes use activated monosaccharides as substrates. The larger group of enzymes—the Leloir pathway glycosyltransferases—transfer monosaccharides activated as sugar nucleoside phosphates to the end of the growing oligosaccharide chain (eq 1) (XDP = nucleoside diphosphate; *N*-acetylneuraminic acid activated as a monophosphate, CMP-Neu-5-Ac).[1,23]

$$\text{sugar} + \text{acceptor} \rightarrow \text{sugar–acceptor} \qquad (1)$$

Non-Leloir pathway glycosyltransferases utilize monosaccharide units activated as sugar phosphates. Although we have used non-Leloir pathway enzymes in the syntheses of sucrose and trehalose,[24] the practical importance of this class of enzymes is primarily in the preparation of microbial polysaccharides, such as levans and dextrans,[25,26] and we will not discuss them further.

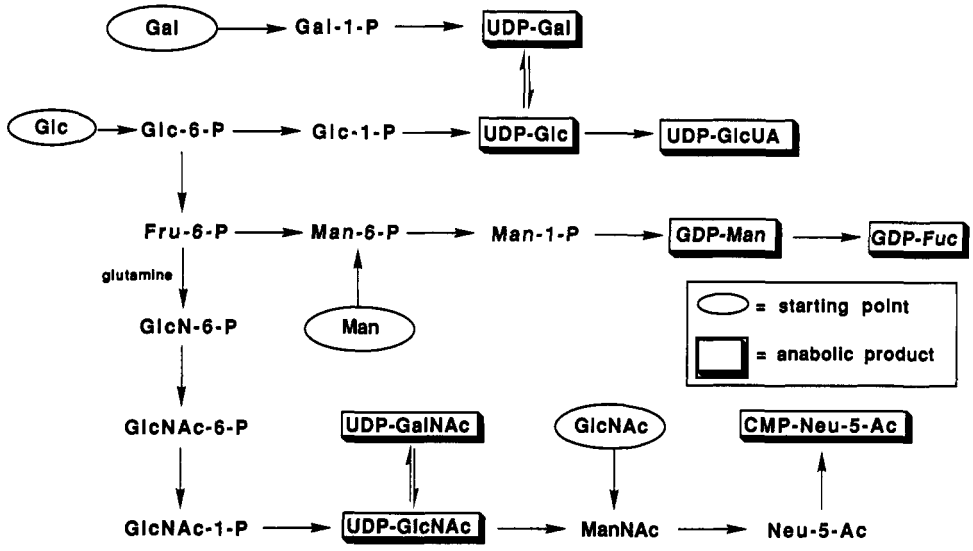
In mammalian systems, the Leloir pathway glycosyltransferases play the central role in the biosynthesis of glycosidic bonds. The mammalian system for biosynthesis of oligosaccharides requires a total of only eight activated monosaccharides as substrates in vivo: uridine 5′-diphosphoglucose (UDP-Glc), uridine 5′-diphosphoglucuronic acid (UDP-GlcUA), uridine 5′-diphospho-*N*-acetylglucosamine (UDP-GlcNAc), uridine 5′-diphosphogalactose (UDP-Gal), uridine 5′-diphospho-*N*-acetylgalactosamine (UDP-GalNAc), guanosine 5′-diphosphomannose (GDP-Man), guanosine 5′-diphosphofucose (GDP-Fuc), and cytidine 5′-monophospho-*N*-acetylneuraminic acid (CMP-Neu-5-Ac).[1,27] Other monosaccharide units occur in mammalian oligo- and polysaccharides (for example iduronic acid and N- and O-sulfated or acetylated sugars), but these sugars do not exist as activated nucleoside phosphates and are formed by a postpolymerization modification.[28] In vivo, the oligosaccharide moieties are usually attached to lipids or proteins during their biosyntheses by glycosyltransferases.[29]

A larger number of activated sugar phosphonucleosides are found in plants, microorganisms, and insects (e.g., GDP-xylose, UDP-arabinose) than in mammals. These compounds will not be covered in this review, although the biosynthetic principles are similar to those of the mammalian systems.

The intense research activity in carbohydrate biology and glycobiology has challenged carbohydrate synthetic chemists to provide specific classes of biologically important carbohydrate structures. In response, numerous sophisticated chemical strategies have been developed for the stereocontrolled synthesis of oligosaccharides and glycoconjugates.[30-40] Nevertheless, one of the most difficult steps in "classical" chemical syntheses of oligosaccharides has remained the stereospecific formation of the glycosidic linkage. This Account will outline work that has established the Leloir pathway in vitro as an attractive alternative to nonbiological synthetic methods for the stereo- and regioselective formation of glycosidic bonds.

(13) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Science* **1990**, *250*, 1130–1132.
(14) Homma, J. Y., Kanegasaki, S., Lüderitz, O., Shiba, T., Westphal, O., Eds. *Bacterial Endotoxins: Chemical, Biological and Clinical Aspects*; Verlag Chemie: Weinheim, 1984.
(15) Morrison, D. C.; Ryan, J. L. *Annu. Rev. Med.* **1987**, *38*, 417–432.
(16) Smith, C. A.; Davis, T.; Anderson, T.; Solam, L.; Beckman, M. P.; Jerzy, R.; Dower, S. K.; Cosman, D.; Goodwin, R. G. *Science* **1990**, *248*, 1019–1023.
(17) Beutler, B.; Cerami, A. *Annu. Rev. Biochem.* **1988**, *57*, 505–518.
(18) Lüderitz, O.; Freudenberg, M. A.; Galanos, C.; Lehmann, V.; Rietschel, E. T.; Shaw, D. H. *Curr. Top. Membr. Transp.* **1982**, *17*, 79–151.
(19) Rao, B. N. N.; Moreland, M.; Brandley, B. K. *Med. Chem. Res.* **1991**, *1*, 1–8.
(20) Erlich, J.; Stivala, S. S. *J. Pharm. Sci.* **1973**, *62*, 517–522.
(21) Jaues, L. B. *Pharmacol. Rev.* **1980**, *31*, 99–166.
(22) Casu, B. *Adv. Carbohydr. Chem. Biochem.* **1985**, *43*, 51–134.
(23) Leloir, L. F. *Science* **1971**, *172*, 1299–1303.
(24) Haynie, S. L.; Whitesides, G. M. *Appl. Biochem. Biotechnol.* **1990**, *23*, 155–170.
(25) Deonder, R. *Methods Enzymol.* **1966**, *8*, 500–505.
(26) Sutherland, I. W. *Biotechnology of Microbial Exopolysaccharides*; Cambridge University: Cambridge, 1990.
(27) Kornfeld, R.; Kornfeld, S. *Annu. Rev. Biochem.* **1985**, *54*, 631–664.
(28) Aspinell, G. O., Ed. *The Polysaccharides*; Academic: New York, 1982–1985; Vols. I–III.
(29) Beyer, T. A.; Sadler, J. E.; Rearick, J. J.; Paulson, J. C.; Hill, R. L. *Adv. Enzymol.* **1981**, *52*, 23–173.
(30) Lopez, J. C.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1991**, 159–161.
(31) Klaffke, W.; Thiem, J. *Nachr. Chem. Tech. Lab.* **1991**, *39*, 290–298.
(32) Waldmann, H. *Nachr. Chem., Tech. Lab.* **1991**, *39*, 675–682.
(33) Paulson, H. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 823–839.
(34) Friesen, R. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6656–6660.
(35) Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583–5586.
(36) Nicolaou, K. C.; Caufield, T.; Kataoka, H.; Kumazara, T. *J. Am. Chem. Soc.* **1988**, *110*, 7910–7912.
(37) Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294–308.
(38) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235.
(39) Ogawa, T.; Yamamoto, T.; Nukada, T.; Kitajima, T.; Sugimoto, M. *Pure Appl. Chem.* **1984**, *56*, 779–795.
(40) Lemieux, R. U.; Hendricks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.

Scheme I
Biosynthesis of Nucleoside Mono- and Diphosphate Sugars



The practical use of the Leloir pathway in oligosaccharide synthesis has been limited by (i) the availability of the enzymes and (ii) the high costs and difficult syntheses of the nucleoside phosphate sugars required as reactants. Molecular cloning is now increasing the number of available glycosyltransferases,[41-46] and a range of them may soon be available as tools in carbohydrate chemistry. This Account summarizes the current state of the art in preparations of the eight essential nucleoside mono- and diphosphate sugars. Many synthetic preparations of these crucial compounds have been developed on a small scale in research biochemistry; these preparations have been reviewed in 1973[47] and 1982.[48] Only a few of the reported protocols can be scaled up easily. Although both biological and nonbiological procedures are possible for the nucleoside phosphate sugars and their precursors and although we (and others) have used both, we have focused our efforts on enzyme-based strategies. These have the potential to be used either in stand-alone syntheses or in in situ syntheses coupled directly to glycosyltransferases or other enzymes.

We focus on three subjects, all of which are essential for practical enzymatic or enzyme-based syntheses of the nucleoside phosphate sugars. These areas are the following: (i) syntheses of the required nucleoside triphosphates (UTP, GTP, and CTP); (ii) availability of the monosaccharides; and (iii) assembly of these building blocks to afford the nucleoside mono- and diphosphate sugars. The first section summarizes the biosynthetic pathways for the nucleoside phosphate sugars in mammals to demonstrate the potentials and limitations of enzyme-based synthetic routes. Further sections summarize routes to (or sources of) the nucleoside triphosphates and sugars and synthetic routes to the eight nucleoside phosphate sugars and give references to applications of these materials in synthesis.

Biosynthesis of Nucleoside Di- and Monophosphate Sugars

Scheme I summarizes the biosynthetic routes leading to the eight nucleoside phosphate sugars that are the activated substrates for the Leloir pathway glycosyltransferases in mammalian metabolism.[1-3] The first step in the anabolic sequence to UDP-Glc, UDP-GlcNAc, UDP-Gal, and GDP-Man is the synthesis of the sugar α-1-phosphate from the corresponding sugars. These compounds can be synthesized in several multistep sequences. Only D-galactose (Gal) is transformed directly to Gal-α-1-P in a single kinase-catalyzed step (catalyzed by galactokinase EC 2.7.1.6). Glucose 6-phosphate, mannose 6-phosphate, and *N*-acetylglucosamine 6-phosphate, which are synthesized either by a kinase-catalyzed reaction from the corresponding unphosphorylated sugars or by enzymatic isomerizations starting from fructose 6-phosphate, are rearranged to the corresponding sugar α-1-phosphates by appropriate phosphomutases (EC 2.7.5). Specific nucleotide pyrophosphorylases (EC 2.7.7) catalyze the condensation between the sugar α-1-phosphates and the appropriate nucleoside triphosphates (XTPs) and generate the nucleoside diphosphate sugars with the release of inorganic pyrophosphate (eq 2). UDP-GlcUA, UDP-GalNAc,

$$\text{sugar-1-P} + \text{XTP} \rightarrow \text{XDP–sugar} + \text{PP}_i \quad (2)$$

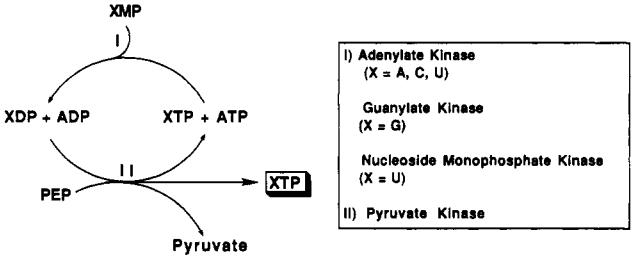
and GDP-Fuc are not formed biosynthetically by coupling of the sugars with the XTPs: UDP-GlcUA and GDP-Fuc are formed by oxidative and reductive steps from UDP-Glc and GDP-Man, respectively. UDP-GalNAc is anabolized by isomerization from UDP-GlcNAc.

The activation of neuraminic acid (Neu-5-Ac) is an exception: the nucleoside monophosphate sugar is formed directly from *N*-acetylneuraminic acid (Neu-5-Ac, eq 3) in a single-step reaction catalyzed by CMP-Neu-5-Ac synthetase (EC 2.7.7.43).

$$\text{Neu-5-Ac} + \text{CTP} \rightarrow \text{CMP-Neu-5-Ac} + \text{PP}_i \quad (3)$$

(41) Joziasse, D. H.; Shaper, N. L.; van den Eijnden, D. H.; van der Spoel, A. C.; Shaper, J. H. *Eur. J. Biochem.* **1990**, *191*, 75–83.
(42) Toghrol, F.; Kimura, T.; Owens, I. S. *Biochemistry* **1990**, *29*, 2349–2356.
(43) Aoki, D.; Appert, H. E.; Johnson, D.; Wong, S. S. *EMBO J.* **1990**, *9*, 3171–3178.
(44) Paulson, J. C.; Colley, K. J. *J. Biol. Chem.* **1989**, *264*, 17615–17618.
(45) Masibay, A. S.; Sasba, P. K. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5733–5737.
(46) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365–5421.
(47) Kochetkov, N. K.; Shibaev, V. N. *Adv. Carbohydr. Chem. Biochem.* **1973**, *28*, 307–399.
(48) Gabriel, O. *Methods Enzymol.* **1982**, *83*, 332–353.

Scheme II
Enzymatic Synthesis of Nucleoside Triphosphates



Synthesis of Nucleoside Triphosphates

All of the nucleoside diphosphate sugars are synthesized in vivo in sequences that ultimately require the corresponding nucleoside triphosphates (XTPs). Practical methods for the preparation of UTP, CTP, GTP are thus a prerequisite for practical syntheses of the nucleoside phosphate sugars. ATP is also required for enzyme-catalyzed phosphorylations of the monosaccharides.

Although small-scale protocols for the synthesis of all XTPs are available in the literature,[49] we have spent substantial effort in developing preparations that can be used on a scale of 1–100 g. A comprehensive comparison of various strategies—both chemical and enzymatic—indicated that the enzymatic approach, summarized in Scheme II, was the most effective and the most practical method to convert all of the XDPs to XTPs.[50,51] Synthesis of the XDPs from the commercially available XMPs is slightly more complicated.

Adenylate kinase (EC 2.7.4.3) catalyzes the equilibration of AMP, ADP, and ATP and has been used extensively in the production of ATP.[52-54] Although this enzyme has a broad substrate specificity for nucleoside di- and triphosphates, its acceptance of monophosphates is more limited. Nonetheless, its activity toward CMP is high enough to enable it to provide multigram quantities of CTP.[55,56] The use of guanylate kinase (EC 2.7.4.8) offers the most effective approach to GTP.[50,57] UTP is best prepared by a chemoenzymatic procedure via deamination of CTP.[50] In all enzymatic approaches, the most convenient, ultimate phosphate donor is phosphoenolpyruvate (PEP), which can be generated in situ from D-3-phosphoglyceric acid (PGA),[58] chemically synthesized,[59] or purchased. Acetyl phosphate is also sometimes useful.[60]

Recently, a new synthesis of XTPs was developed, based on a one-pot reaction of unprotected but activated nucleosides with inorganic phosphates.[61]

Availability of the Sugars

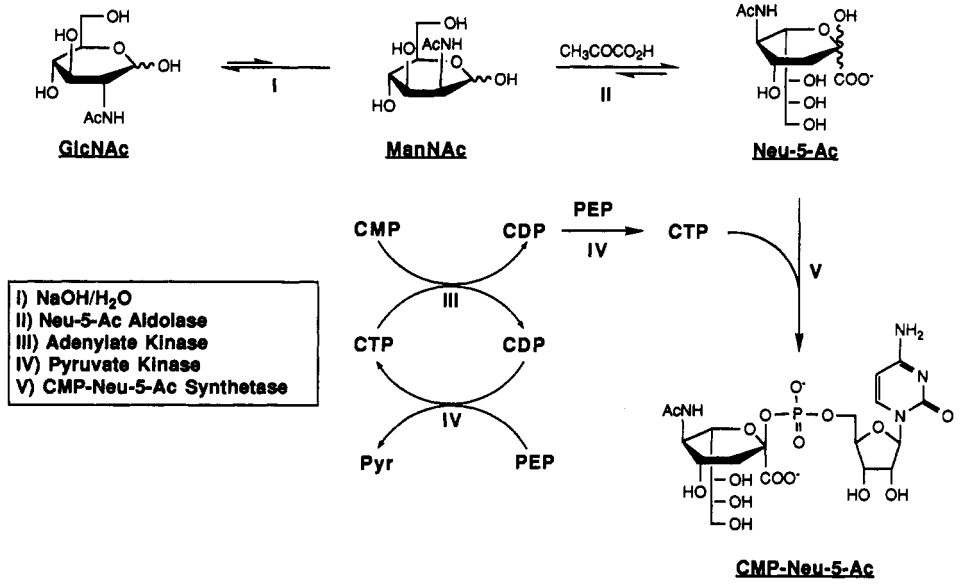
Seven of the sugars required for synthesis of the eight relevant mammalian nucleoside phosphate sugars are commercially available at reasonable prices: D-glucose (Glc), D-glucosamine (GlcN), D-galactose (Gal), D-galactosamine (GalN), D-*N*-acetylglucosamine (GlcNAc), D-mannose (Man), and L-fucose. 5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (*N*-acetylneuraminic acid, Neu-5-Ac) is also commercially available but is expensive (about $30 per 100 mg); for syntheses on a large scale involving Neu-5-Ac, most research laboratories will still require a preparation of this substance. A detailed review of synthetic approaches to Neu-5-Ac has been published recently.[62] Although the best available total synthesis of Neu-5-Ac from non-carbohydrate precursors is impressive for its chemical strategy,[63] it is of smaller practical importance. In our experience, the most useful preparation of Neu-5-Ac in gram quantities is the condensation of D-*N*-acetylmannosamine (in turn obtained by isomerization from GlcNAc) and pyruvate, catalyzed by *N*-acetylneuraminate pyruvate-lyase (NANA-aldolase, EC 4.1.3.3; Scheme III).[64] NANA-aldolase can be used in soluble form,[65] enclosed in membranes,[64] and in immobilized form.[66-68] A sophisticated enzyme membrane reactor has been described for large-scale syntheses.[69] An abundant natural source of Neu-5-Ac is the *Collocalia* mucoid, the nest-cementing glycoprotein substance of the Chinese swiftlet. Practical protocols have been developed to isolate Neu-5-Ac from these birds' nests (5%, w/w) in gram quantities.[70,71] Another large-scale preparation of Neu-5-Ac from chalaza and egg-yolk membrane has recently been reported.[72]

Preparations of the Nucleoside Phosphate Sugars

In this section we consider enzymatic, chemoenzymatic, and chemical preparations of sugar nucleotides. We emphasize enzymatic strategies, because they can, in principle, be coupled in situ to the reactions of the glycosyltransferases. It is useful to group the syntheses of biogenetically related nucleoside phosphate sugars together.

(49) Hutchinson, D. W. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum: New York, 1991; Vol. 2, pp 81–160.
(50) Simon, E. S.; Grabowski, S.; Whitesides, G. M. *J. Org. Chem.* **1990**, *55*, 1834–1841.
(51) Chenault, H. K.; Simon, E. S.; Whitesides, G. M. In *Biotechnology and Genetic Engineering Reviews*; Russell, G. E., Ed.; Intercept: Wimborne, 1988; Vol. 6, pp 221–270.
(52) Kim, M.-J.; Whitesides, G. M. *Appl. Biochem. Biotechnol.* **1987**, *16*, 95–108.
(53) Crans, D. C.; Kazlauskas, R. J.; Hirschbein, B. L.; Wong, C.-W.; Abril, O.; Whitesides, G. M. *Methods Enzymol.* **1987**, *136*, 263–280.
(54) Hirschbein, B.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 3765–3766.
(55) David, S.; Auge, C. *Pure Appl. Chem.* **1987**, *59*, 1501–1508.
(56) Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron Lett.* **1988**, *29*, 1123–1126.
(57) Stiller, R.; Thiem, J. *Synlett* **1990**, 709–710.
(58) Simon, E. S.; Grabowski, S.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 8920–8921.
(59) Hirschbein, B. L.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 3765–3766.
(60) Crans, D. C.; Whitesides, G. M. *J. Org. Chem.* **1983**, *26*, 3130–3132.
(61) Mishra, N. C.; Broom, A. D. *J. Chem. Soc., Chem. Commun.* **1991**, 1276–1277.
(62) DeNinno, M. P. *Synthesis* **1991**, 583–593.
(63) Danishefsky, S. J.; DeNinno, M. P.; Chen, S. *J. Am. Chem. Soc.* **1988**, *110*, 3929–3940. Neu-5-Ac from glucose: Yamamoto, T.; Teshima, T.; Inami, K.; Shiba, T. *Tetrahedron Lett.* **1992**, *33*, 325–328.
(64) Bednarski, M. D.; Chenault, H. K.; Simon, E. S.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 1283–1285.
(65) Kim, M.-J.; Hennen, W. J.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 6481–6486.
(66) Auge, C.; David, S.; Gautheron, C. *Tetrahedron Lett.* **1984**, *25*, 4633–4634.
(67) Auge, C.; David, S.; Gautheron, C.; Veyrieres, A. *Tetrahedron Lett.* **1985**, *26*, 2439–2440.
(68) Auge, C.; David, S.; Gautheron, C.; Malleron, A.; Cavaye, B. *New J. Chem.* **1988**, *12*, 733–744.
(69) Kragl, U.; Gygax, D.; Ghsalba, O.; Wandrey, C. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 827–828.
(70) Czarniecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8273–8279.
(71) Roy, R.; Laferriere, C. A. *Can. J. Chem.* **1990**, *68*, 2045–2054.
(72) Juneja, L. R.; Koketsu, M.; Nishimoto, K.; Kim, M.; Yamamoto, T.; Hoh, T. *Carbohydr. Res.* **1991**, *214*, 179–185.

Scheme III
Generation of Neu-5-Ac from *N*-Acetylglucosamine and Coupling in Situ with CTP To Form CMP-Neu-5-Ac

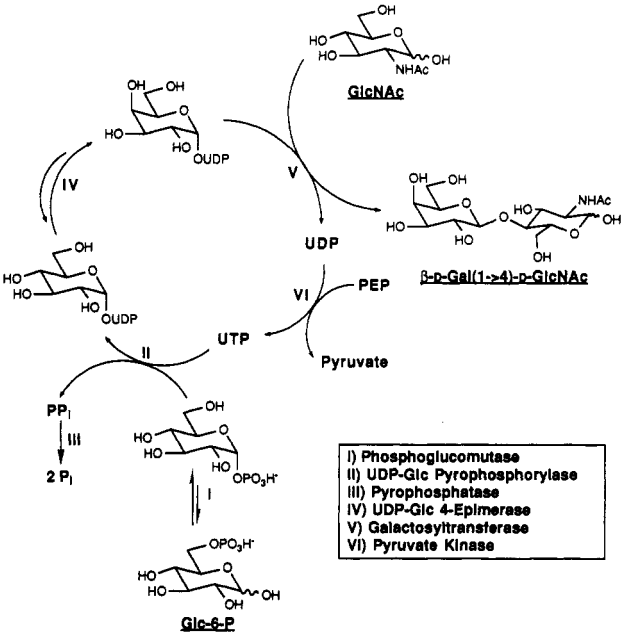


UDP-Glc, UDP-Gal, and UDP-GlcUA. The enzymatic syntheses of UDP-Glc starting from Glc or Glc-6-P can be easily performed in vitro, since the three enzymes required—hexokinase (EC 2.7.1.1), phosphoglucomutase (EC 2.7.5.1), and UDP-Glc pyrophosphorylase (EC 2.7.7.9)—are commercially available at a moderate price and are stable. The in situ preparation of UDP-Glc has been scaled up to 40 mmol[73] and has been conducted with immobilized enzymes, starting with yeast RNA as the source of UTP.[54]

UDP-Gal can be obtained from UDP-Glc by an epimerization at C-4 catalyzed by UDP-Glc 4-epimerase (EC 5.1.3.2). The thermodynamically unfavorable equilibrium between UDP-Gal and UDP-Glc (1/3.8),[74] however, makes it necessary to drive the reaction in favor of the formation of UDP-Gal. This approach was illustrated by the synthesis of *N*-acetyllactosamine,[73] in which UDP-Gal was withdrawn from the equilibrium by glycosyl transfer, using GlcNAc as the acceptor (Scheme IV). UDP-Gal has also been obtained by using a group of enzymes active in the catabolism of Gal in vivo:[75] UMP was transferred from UDP-Glc to Gal-α-1-P by Gal-1-P uridyltransferase (EC 2.7.7.12). The practicality of this strategy was demonstrated by a straightforward preparation of Gal-α-1-P, using crude enzyme extracts from commercial dried yeast cells and using acetylphosphate as the ultimate phosphate donor.[75] UDP-Glc was regenerated in situ from Glc-α-1-P and UTP.

UDP-GlcUA is synthesized in vivo by oxidation of UDP-Glc by a nicotinamide-dependent UDP-Glc dehydrogenase (EC 1.1.1.22). The commercially available enzyme preparation from bovine liver is expensive ($20 per 1 U), relatively unstable, and only useful for microgram-scale preparations of UDP-GlcUA.[76] UDP-Glc dehydrogenase can, however, be isolated relatively simply from whole bovine liver (450 U was obtained from 4.5 kg of frozen liver). This source of the enzyme makes the synthesis of UDP-GlcUA on a gram scale practical.[77] A crude homogenate from guinea pig liver has been used as a multicatalyst system to generate UDP-GlcUA in situ for the enzymatic synthesis of β-D-glucuronides.[78]

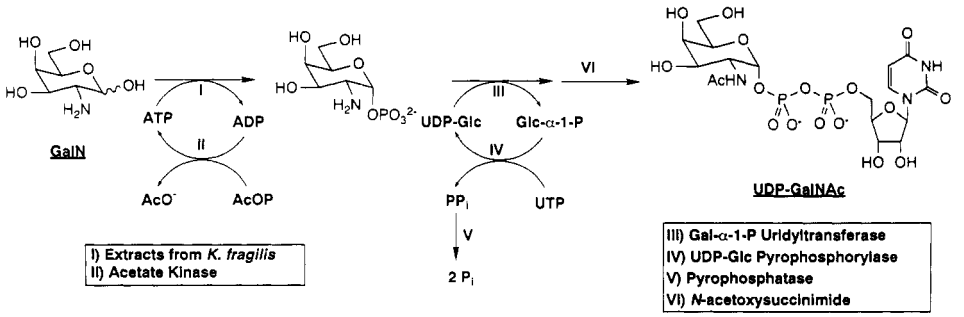
Scheme IV
Synthesis of *N*-Acetyllactosamine



UDP-GlcNAc and UDP-GalNAc. The aminodeoxy sugars GlcNAc and GalNAc play a central role in glycoproteins as the groups that link the oligosaccharide and the polypeptide chain.[2,3] In spite of their biochemical importance, no purely enzymatic synthesis suitable for in situ generation of UDP-GlcNAc and UDP-GalNAc has been reported to date. Enzymatic preparations have been hampered by the limited

(73) Wong, C.-H.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 5416–5418.
(74) Barman, T. E. *Enzyme Handbook*; Springer: New York, 1985; Vol. 2, p 821.
(75) Heidlas, J. E.; Lees, W. J.; Whitesides, G. M. *J. Org. Chem.* **1992**, *57*, 152–157.
(76) Simon, E. S.; Toone, E. J.; Ostroff, G.; Bednarski, M. D.; Whitesides, G. M. *Methods Enzymol.* **1989**, *179*, 275–287.
(77) Toone, E. J.; Simon, E. S.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 5603–5606.
(78) Gygax, D.; Spies, P.; Winkler, T.; Pfarr, U. *Tetrahedron* **1991**, *28*, 5119–5122.

Scheme V
Synthesis of UDP-*N*-Acetylgalactosamine



availability and laborious preparations of the required enzymes.

Three chemoenzymatic procedures for the synthesis of UDP-GlcNAc have been described recently: (i) Crude enzyme extracts from dried yeast cells of *Candida utilis* (Sigma) were used to isomerize chemically prepared glucosamine 6-phosphate (GlcN-6-P) to GlcN-α-1-P and then to couple this GlcN-α-1-P in situ with UTP to afford UDP-GlcNAc on a gram scale.[79] (ii) UDP-GlcNAc pyrophosphorylase (EC 2.7.7.23) was isolated from calf liver and used to catalyze the condensation between chemically synthesized GlcNAc-α-1-P and UTP.[80] (iii) The broad substrate specificity of the enzymes active in the biosynthesis of UDP-Glc (hexokinase, phosphoglucomutase, UDP-Glc pyrophosphorylase) was exploited to prepare UDP-GlcN from GlcN and UTP in an enzyme hollow fiber reactor; UDP-GlcN was subsequently chemically acetylated.[81] In the case of UDP-GlcNAc, nonbiological syntheses are competitive with these enzymatic methods. A chemical approach, which was used on a gram scale, proceeded in five steps in an overall yield of 15% from pentaacetylglucosamine.[79] (This investigation also provided practical improvements of the "morpholidate procedure",[82] which is still the standard method for the chemical preparation of nucleoside diphosphate sugars, using protected sugar α-1-phosphates and XMP-morpholidates as its key intermediates.)

Although the required enzymes are not commercially available, a straightforward large-scale synthesis of UDP-GalNAc has been described (Scheme V).[75] This strategy employs enzymes that are active in vivo in the metabolism of D-galactose. In the first step, GalN-α-1-P was conveniently obtained from GalN using crude enzyme extracts from galactose-adapted yeast. In the second step, UDP-GalN was prepared by a UMP transfer from UDP-Glc to GalN-α-1-P, catalyzed by Gal-1-P uridyltransferase (EC 2.7.7.12). UDP-Glc was regenerated in situ from Glc-α-1-P and UTP using UDP-Glc pyrophosphorylase (EC 2.7.7.9) as catalyst. The driving force for the system was the hydrolysis of the pyrophosphate released by pyrophosphatase (EC 3.6.1.1). Finally, UDP-GalN was acetylated using *N*-acetoxysuccinimide to afford UDP-GalNAc.

GDP-Man and GDP-Fuc. GDP-Man and GDP-Fuc are related biogenetically and are interconvertible in vivo by an oxidoreductase system.[83,84] This enzymatic interconversion is, however, not synthetically practical.

A chemoenzymatic procedure for the synthesis of GDP-Man used GDP-Man pyrophosphorylase (EC 2.7.7.13) for the coupling of chemically prepared Man-α-1-P and GTP.[50] This procedure should be useful, even for synthesis on a large scale, since GDP-Man pyrophosphorylase can be readily prepared in crude extracts from brewer's yeast,[85] and Man-α-1-P is accessible by a standard phosphorylation method.[86]

No practical enzymatic synthesis has been developed for GDP-Fuc. A minor catabolic sequence exists for exogenous L-Fuc in vivo that completes the anabolic route from GDP-Man to GDP-Fuc. Fucokinase (EC 2.7.1.52) catalyzes the formation of L-fucose β-1-phosphate (Fuc-β-1-P), which is subsequently transformed into GDP-Fuc by GDP-fucose pyrophosphorylase (EC 2.7.7.30).[87,88] (We note that, in contrast to the nucleoside diphosphate sugars derived from the D series of sugars, the anomeric configuration of the L-fucose moiety in the naturally occurring and biologically active GDP-Fuc is the β.) An enzymatic preparation of GDP-Fuc based on the enzymes of this catabolic sequence has been reported, but without experimental details.[89]

In chemical approaches to GDP-Fuc, the major problem in the synthesis of Fuc-β-1-P was to control the stereochemistry at C-1.[90] A recent strategy took advantage of a neighboring benzoyl group at C-2, participating in a Koenigs–Knorr-like glycosidation, to direct the formation in favor of the β-anomer. The usefulness of this five-step synthesis has been demonstrated on a 15-mmol scale in a procedure requiring only one chromatographic purification.[91]

CMP-Neu-5-Ac. This compound is generated in vivo by direct coupling between Neu-5-Ac and CTP cata-

(79) Heidlas, J. E.; Lees, W. J.; Pale, P.; Whitesides, G. M. *J. Org. Chem.* **1992**, *57*, 146–151.
(80) Korf, U.; Thimm, J.; Thiem, J. *Synlett* **1991**, 313–314.
(81) Ropp, P. A.; Cheng, P.-W. *Anal. Biochem.* **1990**, *187*, 104–108.
(82) Roseman, S.; Distler, J. J.; Moffat, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 659–663.
(83) Ginsburg, V. *J. Biol. Chem.* **1960**, *235*, 2196–2201.
(84) Ginsburg, V. *J. Biol. Chem.* **1961**, *236*, 2389–2393.
(85) Munch-Petersen, A. *Methods Enzymol.* **1962**, *5*, 171–174.
(86) Warren, D. C.; Jeanloz, R. W. *Biochemistry* **1973**, *12*, 5031–5037.
(87) Ishihara, H.; Massaro, D. J.; Heath, E. C. *J. Biol. Chem.* **1968**, *243*, 1103–1109.
(88) Ishihara, H.; Heath, E. C. *J. Biol. Chem.* **1968**, *243*, 1110–1115.
(89) Zehavi, U.; Thiem, J. In *Enzymes in Carbohydrate Synthesis*; Bednarski, M. D., Simon, E. S., Eds.; ACS Symposium Series No. 466; American Chemical Society: Washington, DC, 1991; pp 90–98.
(90) Nunez, H. A.; O'Connor, V.; Rosevar, P. R.; Barker, R. *Can. J. Chem.* **1981**, *59*, 2086–2095.
(91) Adelhorst, K.; Whitesides, G. M. *Carbohydr. Chem.*, in press. Hindgaul, O.; Gokhale, U. B.; Palcic, M. M. *Can. J. Chem.* **1990**, *68*, 1063–1070. Schmidt, R. R.; Wegmann, B.; Jung, K.-H. *Liebigs Ann. Chem.* **1991**, 121–124. Veeneman, G. H.; Broxterman, H. J. G.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1991**, *32*, 6175–6178. Recent additional information on GDP-fucose can be found in the following: Schmidt, R. R.; Braun, H.; Jung, K.-H. *Tetrahedron Lett.* **1992**, *33*, 1585–1588.
(92) Kean, E.; Roseman, S. *Methods Enzymol.* **1966**, *8*, 208–215.

Table I
Examples of Uses of Nucleoside Phosphates and Glycosyl Transferases

enzyme(s)	donor or acceptor sugar	examples of use	refs
β-1,4-galactosyltransferase	UDP-Gal, UDP-Glc, UDP-GlcN	-used to prepare natural and unnatural oligosaccharides	111, 112
		-branched penta- and hexasaccharides	113
N-acetylglucosaminyltransferase	8-(methoxycarbonyl)octylglycosides	-isolation of products from reversed-phase chromatographic supports	116
		-preparative-scale reactions employing polymeric carriers	89
Gal-β-1,4-GlcNAc-α,2,6-sialyltransferase	CMP-NeuAc	-resialylation of cell-surface oligosaccharides	117
Gal-β-1,3-GalNAc-α,2,3-sialyltransferase		-preparative-scale syntheses of α-2,3(6)-sialyllactose or α-2,3(6)-sialyllactosamine	76, 97, 118
Gal-β-1,3-GlcNAc-α,2,6-sialyltransferase		-synthesis of a bacterial oligosaccharides	121
		-related preparations using inhibitory UDP removal and CMP-NeuAc regeneration	119, 120

lyzed by CMP-Neu-5-Ac synthetase (EC 2.7.7.43). Until recently, there were no practical chemical approaches to CMP-Neu-5-Ac. Although the first use of CMP-Neu-5-Ac synthetase for the in vitro synthesis of CMP-Neu-5-Ac dates back to 1966[93] and various publications on this topic have appeared since,[93-98] only recent progress has made possible the synthesis of this important compound on large scales. The most practical approach to CMP-Neu-5-Ac is probably a one-pot synthesis from *N*-acetylmannosamine, pyruvate, and CMP using several coupled enzymatic reactions (Scheme III).[64,99] This system, in which the enzymes can be reused by the "MEEC" technique (membrane-enclosed enzymatic catalysis),[64] has been successfully applied in the preparation of 1.2 g of CMP-Neu-5-Ac. A similar synthesis of CMP-Neu-5-Ac used the enzymes immobilized in agarose.[100] The major drawback of all these syntheses was the need for the laborious purification of CMP-Neu-5-Ac synthetase from calf brain. This enzyme has now been cloned and overexpressed in *Escherichia coli*[101] and will be commercially available in the near future.

Examples of Use. The number of oligosaccharides that have been synthesized using enzymatic methods is increasing; these syntheses have been reviewed.[29,46,89,102-104] Table I lists examples. This table is illustrative and makes no pretense of completeness.

Table II
Summary of Best Procedures for Preparations of Nucleoside Phosphate Sugars on Scales >1 g

nucleoside phosphate sugar	"best" procedure(s)[a]	ref(s)
UDP-Glc	Glc or Glc-6-P (E)	54, 73
UDP-Gal	UDP-Glc (E)	73
	Gal/Gal-1-P (E)	75
UDP-Man	Man/Man-1-P (CE)	50
GDP-Fuc	Fuc/Fuc-1-P (C)	91
UDP-GlcNAc	GlcN/GlcN-6-P (CE)	79
	GlcNAc/GlcNAc-1-P (CE)	80
	GlcNAc/GlcNAc-1-P (C)	79
UDP-GalNAc	GalN/GalN-1-P (CE)	75
UDP-GlcUA	UDP-Glc (E)	77, 78
CMP-Neu-5-Ac	Neu-5-Ac (E)	99

[a] The "best" procedure is that recommended on the basis of our experience for use in laboratories involved in carbohydrate chemistry. The entry in this column is the precursor or precursors. E indicates a purely enzymatic process, C a nonbiological ("chemical") process, and CE a combination of enzymatic and chemical steps ("chemoenzymatic").

Conclusions

The object of this Account has been to demonstrate that all of the eight nucleoside phosphate sugars, used

(93) Hultsch, E.; Reutter, W.; Decker, K. *Biochem. Biophys. Acta* **1972**, *273*, 132–140.
(94) Haverkamp, J.; Beau, J. M.; Schauer, R. *Hoppe-Seyler's Z. Physiol. Chem.* **1972**, *353*, 883–886.
(95) van den Eijnden, D. H.; van Dijk, W. *Hoppe-Seyler's Z. Physiol. Chem.* **1972**, *353*, 1817–1820.
(96) Higa, H. H.; Paulson, J. C. *J. Biol. Chem.* **1985**, *260*, 8838–8849.
(97) Thiem, J.; Treder, W. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1096–1097.
(98) Thiem, J.; Stangier, P. *Liebigs Ann. Chem.* **1990**, 1101–1105.
(99) Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159–7163.
(100) Auge, C.; Gautheron, C. *Tetrahedron Lett.* **1988**, *29*, 789–790.
(101) Shames, S. L.; Simon, E. S.; Christopher, C. W.; Schmid, W.; Whitesides, G. M. *Glycobiology* **1991**, *1*, 187–191.
(102) Nilsson, K. G. I. In *Enzymes in Carbohydrate Synthesis*; Bednarski, M. D., Simon, E. S., Eds.; ACS Symposium Series No. 466; American Chemical Society: Washington, DC, 1991; pp 51–62.
(103) Gygax, D.; Hammel, M.; Schneider, R.; Berger, E. G.; Stierlin, H. In *Enzymes in Carbohydrate Synthesis*; Bednarski, M. D., Simon, E. S., Eds.; ACS Symposium Series No. 466; Americal Chemical Society: Washington, DC, 1991; pp 79–89.
(104) Stangier, P.; Thiem, J. In *Enzymes in Carbohydrate Synthesis*; Bednarski, M. D., Simon, E. S., Eds.; ACS Symposium Series No. 466; American Chemical Society: Washington, DC, 1991; pp 63–78.
(105) Beyer, T. A.; Sadler, J. E.; Rearick, J. I.; Paulson, J. C.; Hill, R. L. *Adv. Enzymol.* **1981**, *52*, 23–173.
(106) Sadler, J. E.; Beyer, T. A.; Oppenheimer, C. L.; Paulson, J. C.; Prieels, J.-P.; Rearick, J. I.; Hill, R. L. *Methods Enzymol.* **1982**, *83*, 458–514.
(107) Hagopian, A.; Eylar, E. H. *Arch. Biochem. Biophys.* **1968**, *128*, 422–433.
(108) Palcic, M. M.; Hindsgaul, O. *Glycobiology* **1991**, *1*, 205–209.
(109) Palcic, M. M.; Srivastava, O. P.; Hindsgaul, O. *Carbohydr. Res.* **1987**, *159*, 315–324.
(110) Thiem, J.; Wiemann, T. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 80–82.
(111) Hindsgaul, O.; Kaur, K. J.; Gokhale, U. B.; Srivastava, G.; Alton, G.; Palcic, M. M. In *Enzymes in Carbohydrate Synthesis*; Bednarski, M. D., Simon, E. S., Eds.; ACS Symposium Series No. 466; American Chemical Society: Washington, DC, 1991; pp 38–62.
(112) Wong, C.-H.; Krach, T.; Gautheron-Le Narvor, C.; Ichikawa, Y.; Look, G. C.; Gaeta, F.; Thompson, D.; Nicolaou, K. C. *Tetrahedron Lett.* **1991**, *32*, 4867–4870.
(113) Auge, C.; Mathieu, C.; Merienne, C. *Carbohydr. Res.* **1986**, *151*, 147–156.
(114) Tahir, S. H.; Hindsgaul, O. *Can. J. Chem.* **1986**, *64*, 1771–1780.
(115) Srivastava, O. P.; Hindsgaul, O. *Carbohydr. Res.* **1988**, *179*, 137–161.
(116) Kaur, K. J.; Alton, G.; Hindsgaul, O. *Carbohydr. Res.* **1991**, *210*, 145–153.
(117) Sebasan, S.; Paulson, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 2068–2080.
(118) Auge, C.; Fernandez-Fernandez, R.; Gautheron, C. *Carbohydr. Res.* **1990**, *200*, 257–268.
(119) Unverzagt, C.; Kunz, H.; Paulson, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 9308–9309.
(120) Ichikawa, Y.; Shen, G.-J.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 4698–4700.
(121) Pozsgay, V.; Brisson, J.-R.; Jennings, H. J.; Allen, S.; Paulson, J. C. *J. Org. Chem.* **1991**, *56*, 3377–3385.

in vivo by mammalian glycosyltransferases in the Leloir pathway, are now accessible by practical synthetic approaches. The sugar moieties are either commercially available at acceptable prices or, in case of Neu-5-Ac, accessible by convenient methods. Procedures for synthesis of the nucleoside phosphate sugars, based on enzymatic or chemoenzymatic methodologies, are now practical in gram quantities and larger (Table II). Although not all of these syntheses are trivial, and some presuppose substantial synthetic expertise, the availability of the nucleoside phosphate sugars is no longer the factor that limits the application of glycosyltransferases in preparative carbohydrate chemistry. At present, the major limitations are access to a broad spectra of glycosyltransferases and limited knowledge on the breadth of the substrate specificity for the enzymes that are available.

Appreciation of the biological importance of glycoconjugates is growing rapidly. We believe that the resulting demand for synthetic materials in fundamental and applied research and the unique ability of enzymatic methods to carry out synthetic modification of delicate biological structures (proteins, organelles, living cells) will encourage the application of glycosyltransferases to the synthesis of more complex carbohydrate structures. Enzymatic glycosyl transfers can offer an efficient complement to nonbiological synthetic chemistry for the syntheses of structures such as N-linked glycoproteins (via dolichol-bound intermediates), O-linked glycoproteins, and glycolipids.

This research was supported by the National Institutes of Health (GM 30367). J.E.H. was supported by a postdoctoral fellowship granted by the Deutsche Forschungsgemeinschaft in 1991. K.W.W. was an NSF postdoctoral fellow (CHE-90-02653).

Mechanism of Alkene Epoxidation by Iron, Chromium, and Manganese Higher Valent Oxo-Metalloporphyrins

DRAZEN OSTOVIC† and THOMAS C. BRUICE*,‡

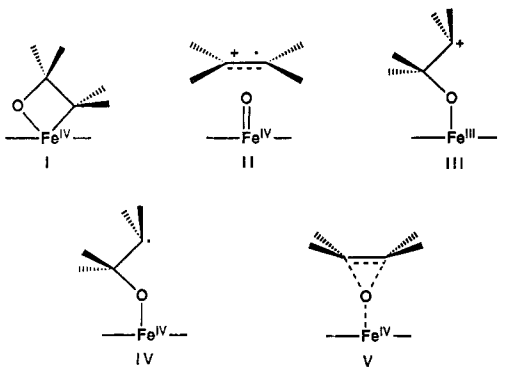
Merck Research Laboratories, West Point, Pennsylvania 19486, and Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106

Received December 3, 1991

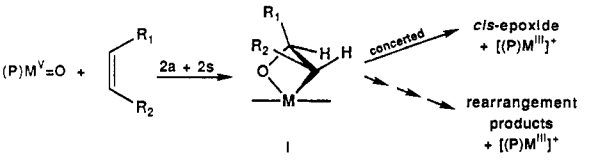
Cytochrome P-450 enzymes catalyze a number of important metabolic reactions. This large class of enzymes also includes members whose role is the oxidative degradation of xenobiotic compounds and unwanted products of normal metabolism. Cytochrome P-450 enzymes catalyze, among other reactions, the epoxidation of alkenes.[1] A reasonable assumption is that the oxidant is an enzyme-bound hypervalent iron-oxo porphyrin. From this assumption ensued investigations directed toward the elucidation of the mechanisms of oxidation of alkenes by such species. We present here an account of our studies using iron, chromium, and manganese tetraphenylporphyrins.[2]

Deliberations concerning the mechanism of epoxidation have revolved around questions of the intermediacy of metallaoxetane I,[3-5] π-radical cation II,[6-10] carbocation III,[7-13] and carbon radical IV[14-20] as opposed to concerted "oxene" insertion (V)[21-24] (Chart I).

Chart I



Scheme I



The stereochemistry of epoxide products relates directly to the mechanism of epoxidation. The nature of

Drazen Ostovic was born in Croatia in 1956. He received his B.S. degree in pharmacy from the University of Zagreb (1979), working with Prof. Stanko Borcic, and his Ph.D. in chemistry from the University of Minnesota (1985), working with Prof. Maurice M. Kreevoy. He carried out postdoctoral work in Prof. Thomas C. Bruice's laboratory at the University of California at Santa Barbara. Since 1989 he has been working in the Pharmaceutical Chemistry Group at Merck Research Laboratories.

After dropping out of high school to serve in the military, Thomas C. Bruice attended the University of Southern California (B.S., 1950; Ph.D., 1954) and received his postdoctoral training at UCLA. Prior to moving to the University of California at Santa Barbara in 1964, Professor Bruice held faculty positions at Yale, Johns Hopkins, and Cornell Universities. His research has involved the study of the mechanisms of reactions of biochemical interest; it has been recognized by a number of awards.

† Merck Research Laboratories.
‡ University of California at Santa Barbara.

(1) *Cytochrome P-450; Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986.

0001-4842/92/0125-0314$03.00/0