ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 331 (2001) 347–368

CARBOHYDRATE RESEARCH

Perspective

Glycosyl transfer: a history of the concept's development and view of its major contributions to biochemistry

Edward J. Hehre*

Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

Received 3 January 2001; accepted 16 January 2001

Abstract

Humans have fewer protein-coding genes than expected for all the inherent complexities of development. Supplementary factors include the post-translational modification of proteins by glycosylation. The latter term plus transglycosylation, glycosyltransferases, and 'to glycosylate' are used in biochemistry as though they always existed. Instead they have a history that can bring new insights in this science area to younger investigators. The present account describes five decades of findings and ideas on enzymic saccharide synthesis leading, finally to a rational theory that will surely continue to serve the biosciences well in the future. © 2001 Elsevier Science Ltd. All rights reserved.

Contents

* Tel.: +1-718-4302811; fax: +1-718-4308711.
E-mail address: vkalten@aecom.yu.edu (E.J. Hehre).

0008-6215/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0008-6215(01)00042-8

1. Introduction

There exists today an astonishingly large and cohesive body of information regarding the enzymic synthesis of the complex saccharides of living matter. A multitude of investigators helped create this vital information during the past half-century through their reports of newly discovered syntheses from nucleotide-activated glycosyl donors. Invariably, the responsible enzymes came to be recognized as transglycosylases and named as glycosyltransferases [EC 2.4], specifying the glycosyl donor and reaction product. Revealed thereby, was the commonality of the underlying chemical change (glycosyl transfer) effected in a vast range of saccharide syntheses. The indication that only a few closely related mechanisms may suffice to account for these diverse syntheses represents one of the major contributions of the glycosyl transfer concept to biological chemistry. Nucleotide diphospho-sugars, as direct precursors of most natural complex saccharides, form a further important common feature of these syntheses. However, since other types of glycosyl donors (dolichol-activated sugars, NAD^+, phosphoribosyl pyrophosphate, ribosyl phosphate, sucrose, or starch chains) are required for certain natural saccharide syntheses, the process based on glycosyl transfer activity is wider in scope.

Carbohydrate biochemistry was for many decades a field concerned solely with carbohydrate degradations. In recent decades it has expanded tremendously to become most significant and exciting; it now deals with the specific functional roles of different complex saccharides, often discovered following the latters' enzymic synthesis. The glycosyl transfer reaction model, proposed in 1951, thus contributed greatly to the enormous rise in importance of this sector of biological chemistry.

2. The course of development of the transglycosylation (glycosyl transfer) concept

This account reviews the findings and ideas reported by a succession of bioscientists during the first half of the twentieth century, attempting to define the process whereby particular enzymes bring about the synthesis of saccharides. It includes comments by a number of biochemists regarding what was achieved by one or another contributor. Often, the level of understanding existing at the time of comment was assigned to an author who clearly had proposed a different (often less advanced) view. Only the rare appraisal has been found to distort reported facts or proposals.

A multitude of investigators have used glycosyltransferase nomenclature for enzymes catalyzing natural complex saccharide synthesis — as students of carbohydrase mechanisms have used glycosyl transfer terminology — without mentioning the glycosyl transfer concept's origin. It did not spring fully formed from the brow of Zeus; yet no proper history of its development exists. Encouraged by colleagues, the present author has preprepared at career's end (and century's end) this account which aims to do justice to

the many scientists who had a part in the idea's evolution. The author's own involvement with saccharide synthesis began unexpectedly in 1939 as a new member of the Cornell Medical College faculty. A training project in immunochemistry† had quickly led to the unanticipated finding that bacterial dextrans cross-react, even at minute concentrations, with type 2 and 20 pneumococcus antisera.[4] This, and the fact that *L. mesenteroides* cultures in media containing sucrose often produce dextran in abundance but none when sucrose is replaced by other sugars,[4–7] begged for a one-time diversionary attempt at a cell-free synthesis by proteins extracted from a dextran-laden culture. The use of dextran's newly found serological reactivity, allowing its identification at a 1–2 μg/mL level, would be critical to judging the outcome of synthesis probes. Indeed, the very first trial of a sterile extract from an *L. mesenteroides* sucrose broth culture gave findings clearly indicative of cell-free dextran synthesis.

This sudden entry into the polysaccharide biosynthesis area came without awareness of what others may have accomplished. In any case, success with dextran synthesis led to a lifetime of work in carbohydrate enzymology and a clear view of how the glycosyl transfer concept evolved.

In this account, the findings and views reported by different investigators are presented in chronological order. The earliest studies were made with crude cellular extracts or emulsins known to hydrolyze certain saccharides. The hypothesis that hydrolysis-reversal could account for the formation of saccharides and glycosides was the first to be examined.

2.1. Saccharide biosynthesis in an era when carbohydrases were considered strictly limited to hydrolyzing their substrates

2.1.1. 1898–1911: Saccharide syntheses from hexoses by emulsins suggested that hydrolysis-reversal might be responsible.

Between 1898 and 1911, Croft-Hill and others reported disaccaride syntheses from hexoses, using maltase-rich extracts of dried yeast,[8–12] while Fisher and Armstrong used lactase from fermented milk (kefir).[13] van't Hoff and others[14–16] reacted almond emulsin with glucose plus glycerol or alkyl alcohols to form glucosides. Most of the observations were consistent with the idea of enzymically promoted synthesis by hydrolysis-reversal. However, the results did not provide clear evidence for the hypothesis, as the products (formed in scant amounts) were not those expected but rather their isomers. Also, the high concentrations of the sugars and other reactants used,[8–16] were far removed from those existing in living cells. Nevertheless, hydrolysis-reversal was reported to account for several disaccharide and glycoside syntheses and, in a strange way, for the formation of a polysaccharide by a crude bacterial extract studied at the end of this period.

2.1.2. 1910–1912: 'Nascent' levulose, formed on sucrose hydrolysis, was assumed to provide for levulan synthesis by hydrolysis-reversal.

Studies by Smith and Steel on levan formation by *Bacillus levanformans*[17] and by

† The desire for a career involving research in bioscience came from a remarkable book, 'Chemistry in Medicine',[1] in which pioneer researchers described the progress of their own experiments on insulin, thyroid and adrenal hormones, vitamins, etc. On graduation from Cornell Medical College, the author joined its science faculty. Teaching medical students was a prime and heavy responsibility, leaving just enough time to begin an exploratory project in immunochemistry — to learn whether fungi might form polysaccharides similar to those of the capsules of certain disease-producing bacteria. However, the rigidly controlled very first trial led to a serendipitous finding. The cleared fluid from a *Mucor* mold grown in a sucrose-plus-salts 'broth' gave a faint precipitate with alcohol. The recovered precipitate, tested vs. antisera to different bacterial polysaccharides, reacted with the antiserum to encapsulated type 2 pneumococcus. The reactive substance, however, proved not to be a product of the mold but an impurity of the sucrose in the medium. Nearly all reagent and food-grade sucrose was found to carry traces of the type 2 reactive impurity.[2,3] A bacterial origin was suspected when samples of sugar-cane juice from a sugar mill reacted strongly with type 2 antiserum and also yielded cultures of *Leuconostoc mesenteroides*, a species long known to produce much dextran from sucrose. Dextrans isolated from two *L. mesenteroides* cultures[4] were shown to be chemically like to those dextrans studied by others.[5–7] Both reacted strongly with type 2 and type 20 pneumococcus antisera. One (the serotype A dextran prototype), in addition reacted strongly with type 12 pneumococcus antibody; the other (serotype B) showed little or no type 12 reactivity.[4] In later studies of many dextrans, type 2 and type 20 reactivities were correlated with their α-(1→6) linkage content; type 12 reactivity with their α-(1→2) linkage content.

Beijerinck[‡] on laevulan formation by *B. mesentericus* and *B. megatherium*[19] established that, whereas cultures of these bacteria grown with sucrose or raffinose become opalescent and gummy, those grown with other common sugars (including fructose) gave no sign of gum formation. Based on their findings, and on the general belief that carbohydrases cleave saccharides by hydrolysis only, Smith and Steel[17] proposed that, in cultures, sucrose undergoes hydrolysis to yield an unknown 'nascent' form of fructose which is then immediately condensed by repeated reversals of hydrolysis to form the levan. This view was later put forward by Beijerinck[19,20] who went beyond studies with live cultures to make a number of qualitative findings which led him to conclude that an enzyme is responsible for the synthesis of laevulan from sucrose. This enzyme, named viscosaccharase, was found in a zone containing tiny slime droplets on and in the sucrose-agar surrounding *B. mesentericus* colonies. Placing small samples of this agar zone on a fresh agar plate containing 2–10% sucrose caused the development of slimy droplets on and into the plate in the absence of bacterial colonies. Steaming the samples abolished the slime-forming ability.

A crude synthesizing enzyme was prepared by filtering sucrose-broth cultures of *B. mesentericus* to remove most of the levulan and bacteria so as to obtain a culture filtrate poor in bacteria. Alcohol treatment of this fluid gave a precipitate described as containing much active enzyme, laevulan, and probably some bacteria. This preparation induced the formation of small droplets of gum on, and into, sucrose-agar plates without observable bacterial growth. Pieces of the slimy agar showed strong reducing power which, when the pieces were washed, emerged in the wash water indicating that a diffusable sugar accompanies the nondiffusable and readily hydrolyzed laevulan. The presence of invertase was doubted.

Beijerinck postulated that viscosaccharase promotes laevulan synthesis by way of 'laevulose im status nascens' which arises by sucrose hydrolysis and is immediately used for synthesis by hydrolysis-reversal.[19,20] In hindsight, it is clear that Beijerinck's[§] pioneering qualitative findings strongly indicated, but did not suffice to prove, that laevulan is synthesized by an enzyme independently of bacterial cells. However, his findings are significant for yet another reason. The synthesis was effective at concentrations of sucrose no higher than required by live bacterial cultures,[19,20] whereas all prior saccharide syntheses[8–16] were effective only with high levels of sugar and/or organic additive; that is, far from physiological conditions.

2.1.3. 1912–1920: Glycoside and disaccharide syntheses from hexoses via hydrolysis-reversal established, but only at lowered water concentration.

That carbohydrases can synthesize saccharides and glycosides from sugars by the reversal of hydrolysis was firmly established by Emil Bourquelot, Marc Bridel and their associates who demonstrated the formation of a wide range of well-defined glycosidic products from digests containing crude enzyme, a hexose, and 18–90% by weight of an alcohol

[‡] Martinius Beijerinck (1851–1931) was a prestigious pioneer microbiologist. According to one account,[18] he and J.H. van't Hoff were fellow students at Delft Polytechnical School and did some experiments together in their rooming house because of the school's poor facilities. Beijerinck later attended the University and received the doctorate in 1877. In 1885 he was appointed Professor (with newly built laboratories) at the Delft Polytechnical School. He retired in 1921, after much outstanding work on bacterial metabolism and tobacco mosaic virus. Evidently he was not a pleasant person, not sought out by students (Ref. 18 and other sources). Intriguing questions include whether Beijerinck knew of van't Hoff's works on enzymatic saccharide synthesis by hydrolysis-reversal and, if so, why were these not cited.

[§] Beijerinck's findings, not cited for 20 years, were confirmed in studies by Harrison et al.[21] who found that enzyme solutions, made as described by Beijerinck, do contain live bacterial cells whose numbers, however, decline during the synthesis. Dienes,[22] in a report that does not refer to either of the above works, erroneously attributed fructan synthesis from sucrose by material surrounding colonies of spore-forming bacilli to cell wall-less forms of the bacteria. Beijerinck reported that his active extracts could contain some live bacteria, but he was also well aware of the difference between reactions of enzymes and filterable agents capable of propagation. He had not only reported key studies on nitrogen fixation by numerous bacteria[18] but also, by demonstrating that the filterable agent of tobacco mosaic is basically different from bacteria, and came to be recognized by some as the father of virology.[23]

or glycol incubated together for weeks or months. Some 40 glycosides were recovered in crystalline form,[24–30] as were gentiobiose (β-D-glucopyranosyl-(1→6)-D-glucopyranose), cellobiose (β-D-glucopyranosyl-(1→4)-D-glucopyranose), and two galactobioses.[31–36] Each product was hydrolyzed by the enzyme used for its synthesis. Crude almond β-glucosidase and the β-galactosidase of kefir, gave rise to products of β configuration; the maltase of dried bottom yeast yielded α-glucosides. As noted above, all of these many syntheses required reaction mixtures in which the water concentration was significantly depressed by the presence of organic solvents. No evidence was presented to show that syntheses by hydrolysis-reversal occur to any significant degree under conditions found in living cells. Beijerinck's reports[19,20] of the effective synthesis of laevulan from sucrose by *B. mesentericus* extracts are not cited in any of the listed papers[24–36] from Bourquelot's laboratory.

2.1.4. 1935: 'Nascent glucose', formed from salicin by hydrolysis, considered to provide efficient glucoside syntheses by hydrolysis-reversal.

In 1935, a seminal paper was published by Jacques Rabate,[37¶] an eleve (student) of Bridel, on the hydrolysis of salicin (2-(hydroxymethyl)-phenyl β-glucoside) by nearly insoluble enzyme preparations from leaves of the willow, *Salix purpurea*. Rabate followed changes in the optical rotation and reducing power of a 4% solution of salicin incubated with the *Salix* leaf powder. After 19 days, the isolated digest components comprised (a) residual substrate; (b) saligenol and glucose (the presumed hydrolytic products); and (c) a new compound clearly identified as salicyl β-glucoside. The latter amounted to 29% of the salicin used. In contrast, salicin incubated with almond emulsin was completely hydrolyzed; no salicyl glucoside was found. Moreover, a solution containing 0.05 M each of the hydrolytic products (glucose and saligenol) gave little or no salicyl glucoside when incubated with *Salix* enzyme or almond emulsin. At this point, Rabate concluded that the usual direct reversal of hydrolysis cannot explain the efficient synthesis of salicyl glucoside during salicin hydrolysis by the *Salix* enzyme. Further experiments were made on the actions of the *Salix* enzyme powder using piceoside (4-acetylphenyl β-D-glucopyranoside) as substrate as it allowed more exact determinations of the free glucose in digests. Studies of incubated digests of 3% piceoside plus 3% methanol showed that 57% of the utilized piceoside was converted to methyl β-D-glucoside by the *Salix* enzyme. It effected essentially no synthesis of methyl β-D-glucoside from glucose plus methanol.

In the end, Rabate assumed that the glucose liberated by the action of the *Salix* powder on salicin or piceoside differs from ordinary glucose. He reported that a nascent β-D-glucose provides for effective synthesis in an aqueous medium if a small amount of a primary or secondary alcohol is present at the moment of substrate hydrolysis; that ordinary glucose plus saligenol or piceatol is not used for synthesis. The idea of the release of 'glucose naissant' on hydrolysis of salicoside or piceoside was repeatedly stressed. No reference was made to Beijerinck's[19,20] parallel proposal of 'nascent laevulose' for the efficient synthesis of laevulan from sucrose (but not fructose) made 25 years earlier.

In later (1937–1938) works, Rabate[40,41] described the actions of an essentially water-insoluble enzyme preparation from the leaves of *Gaultheria procumbens*, an evergreen of the heath family. The findings generally paralleled those obtained with the *Salix* enzyme. Monotropitoside (*o*-methylsalicyl β-primeveroside) was completely hydrolyzed to form primeverose [β-D-xylopyranosyl-(1→6)-D-glucopyranose] plus methyl salicylate. After hydrolysis was complete, the addition of ethanol did not yield ethyl primeveroside. However, when the enzyme was incubated with monotropitoside in 10% ethanol, ethyl primeveroside amounting to 60% of the sub-

¶ This paper is a verbatim publication of Rabate's 1934 doctoral dissertation. Some personal facts about Rabate are provided by two close associates[38,39] who note that he was the 'eleve' (protege) of Bridel; also that Rabate was Courtois' 'cadet' (section chief). Rabate is said to have been killed in 1941 while serving with the French Liberation Forces.[38,39]

strate was synthesized (and recovered in crystalline form). 'Primeverose naissant' liberated on monotropitoside hydrolysis was repeatedly postulated to be part of the process; primeverose plus ethanol did not give the synthesis.

In a final (1940) article on heteroglycosidases,[42] Rabate reviews his work on β-glucoside synthesis by the *Salix* enzyme. With salicin as the substrate, hydrolysis is said to occur and the released glucose said to interact with the alcohol group of the released saligenol to form salicyl β-glucoside. In contrast, the salix enzyme hydrolyzes piceoside completely. However, when the reaction is carried out in water containing 4% vol methanol, methyl β-glucoside is formed in large amounts; on prolonged incubation the methyl glucoside is hydrolyzed. Rabate names the synthetic process 'conjugated heterosidification' and restates the idea that, in the course of phenolic glucoside hydrolysis, 'glucose naissant' is formed which is highly reactive — unlike ordinary glucose which fails to support glucoside synthesis under comparable conditions. The equations on pp. 574 and 597 of Rabate's 1935 paper[37] depict hydrolysis; those on pp. 573 and 598, the hydrolysis-reversal. The latter is again illustrated on p. 1825 of his 1940 review:

$$\underset{\text{glucose naissant}}{C_6H_{12}O_6} + CH_3OH \rightleftharpoons \underset{\text{methyl glucoside}}{C_6H_{11}O_5 - O - CH_3} + H_2O$$

Although his studies deal with alkyl-glycoside rather than fructan synthesis, with phenolic glucosides rather than sucrose as substrate, and with enzymes from plants rather than bacteria, Rabate proposed the same process as had Beijerinck to account for the observed syntheses. The virtually water-insoluble plant glycosidase preparations probably contained cellular fragments; while Beijerinck's *B. mesentericus* preparations doubtless contained bacterial cells. Each author's findings indicate the occurrence of efficient enzymic syntheses without significantly lowered water concentration.

How Rabate accounted for his findings allows his view of the process of saccharide synthesis to be placed on the path of ideas leading to the glycosyl transfer concept. Several authors[43–45] have stated that he named the process transglycosidation or transglycosylation, but that is not so. These terms were coined much later for saccharide syntheses not involving water as a reactant. Other authors[38,39,46–54] have reported that the transferase activity of hydrolases was first demonstrated by Rabate. They are correct in the sense that his findings are important first demonstrations of the ability of some plant β-glucosidases to catalyze what today would be called transfer reactions. However, the attribution of 'transferase activity'[38,39,46–54] implies that Rabate held that *Salix* and *Gaultheria* enzymes transfer sugar directly from the substrate in forming product; but his stated view[37,41,42] differs. Nowhere does he use such terms as transfer, exchange, donor, or acceptor.

2.2. Efficient saccharide syntheses without water as a reactant realized in independent studies between 1938 and 1943.

Relatively soon after Rabate's first papers, several authors reported the occurrence of different effective enzymic saccharide syntheses by more direct processes. Kiessling, the Coris, Freudenberg, Hehre, Hestrin and Miwa all independently judged that the particular reaction studied by each occurs without mediation by water as a reactant. Together, these interpretations represented a major departure from the decades-long paradigm that all saccharide and glycoside biosyntheses involve hydrolysis-reversal. Except for initial reports[55,56] that a starch-branching enzyme from potatoes converts amylose to amylopectin via hydrolytic cleavage to form a pseudoamylose intermediate ca. 20 units long, no later authors would assume that effective saccharide synthesis in water can occur by a process requiring hydrolysis-reversal.

2.2.1. 1938–1940: In vitro syntheses of amylopolysaccharides from glucose 1-phosphate represented as reversals of phosphorolysis.

The first and most widely promulgated of the new views arose from multiple independent demonstrations of efficient in vitro α-glucan syntheses from glucose 1-phosphate (Cori

ester) by phosphorylase. Synthesis by this process was in keeping with the phosphorolytic (rather than hydrolytic) mode of glycogen degradation effected in cellular extracts.

The stage was set for defining the latter process by Parnas's 1935 discovery that muscle extracts degrade glycogen to a stable glucose 6-phosphate product, with phosphate taking the place of water[57,58]. Cori and Cori, using a frog muscle extract activated with adenylic acid, found[59] that the glycogen phosphorolytic product is not glucose 6-phosphate but an acid-labile 'new ester', the 1-phosphate, that is converted to the 6-phosphate by a separate enzyme. Glucose 1-phosphate, identical with the chemically synthesized compound, was isolated from digests with frog and rabbit muscle phosphorylase; the latter enzyme was further shown to require activation by adenylic acid.[60,61]

With this as background, the years 1939–1940 saw the publication of a spate of studies that brought a sense of excitement to biological chemists on learning that in vitro enzymic syntheses of a vital class of α-glucans had been achieved. Reports from laboratories in Heidelberg, Prague, St. Louis and Cambridge described the formation of glycogen- or starch-type polysaccharides from Cori ester by the action of soluble phosphorylase preparations from yeast (Kiessling et al.), mammalian muscle and liver (Cori et al., Cori and Cori, Ostern and Holmes); peas and potatoes (Hanes).[62–71]

An unusual feature was soon noted about the synthesis catalyzed by adenylate-activated rabbit muscle and brain phosphorylase. That is, signs of a lag in initial glucan formation were observed, followed by the rapid production of polysaccharide and attainment of equilibrium.[65–67] The lag could be abolished by adding minute amounts of glycogen to the test mixture. Charles Hanes,[70] likewise found a pronounced induction period in the synthesis by potato phosphorylase. The delay was abolished by addition of a little starch. Maltose was also reported to be active,[70] but the possibility was not excluded that maltotriose, commonly present in maltose preparations, might be responsible.

Early evidence that the synthetic and phosphorolytic reactions are reversals of each other, leading to the same equilibrium state, was obtained for muscle and potato phosphorylase.[65–67,70] With the latter enzyme, the synthesis-favoring equilibrium at pH 6.4, showed 84% of the phosphate to be free.[70]. In addition, a puzzling characteristic of the glucan synthesized by muscle phosphorylase was its blue staining with iodine whereas muscle glycogen stains brown[65–67]. In contrast, the glucan formed by liver and heart extract gave the brown color typical of glycogen. Likewise, the product synthesized by potato phosphorylase stained blue, as does starch.[70] Kiessling[62] reported the synthesis of a glycogen-type product (staining brown with iodine) by yeast phosphorylase. This reaction reached the same equilibrium state from the synthetic and degradative directions, with 85% of the Cori ester converted to glucan. It is to be noted that none of these many 1939–1940 papers[62–71] made reference to the earlier reports[19,20,37,40–42] of effective in vitro saccharide syntheses; or to the then contemporaneous description of a nonhydrolytic reaction in which starch is the substrate.

2.2.2. 1939: Cyclodextrin formation from starch by B. macerans *amylase reported to occur by 'Umglucosidierung'.*

A second nonhydrolytic mode of saccharide formation was proposed by Karl Freudenberg et al.[72] in 1939 for the enzymic formation of cyclodextrins from starch. The study was primarily aimed at clarifying the spatial relations between the structures of starch and cyclodextrins. Unreported observations in Freudenberg's laboratory, and the recently published findings by Tilden and Hudson[73], showed that nonreducing Schardinger dextrins (cyclic oligosaccharides, the smallest now known to contain six (1→4)-linked α-D-glucopyranose units) are formed in very high yields from starch by the action of a cell-free enzyme from *B. macerans*. Using space-filling models, Freudenberg compared the structures of 5- and 6-membered cyclodextrins with the spatial arrangement of starch chains, and found what he considered to be the cyclodextrin precursors. This conclusion was based on Hanes'[74]

model of the screw or helical arrangement of starch chains, comprising an array of closely spaced turns or loops made up of some five to eight glucose units. This helical arrangement was envisioned as able to account for the sizes of the dextrins formed on starch hydrolysis by malt α-amylase; also for those of the nonreducing cyclodextrins formed by the *B. macerans* enzyme.

Freudenberg[72] gave few details about the synthesis process but proposed that, instead of the hydrolysis effected by α-amylase, the *B. macerans* enzyme promotes 'Umglucosidierung', a reaction in which a glucosidic linkage in a starch helical turn is cleaved and a new glucosidic linkage is formed to the nearby free end of the loop, unstitching a starch chain by forming closed rings. The terms donor, acceptor, exchange or transfer were not used but are implied in 'Umglucosidierung'. Freudenberg et al. were the first to use this term for an enzymic reaction, but R. Kuhn[75] had recently used it for a reaction in which a mixture of *p*-toluidin-glucoside and nitroxylidin refluxed in absolute ethanol led directly to nitroxylidin-glucoside and *p*-toluidin. Freudenberg[72] did not cite this report or those of amylopolysaccharide synthesis by phosphorylase.[62–71]

Freudenberg's proposal of 'Umglucosidierung' long remained uncited by students of cyclodextrin formation.[76–88] The Chemical Abstracts' summary[72] noted that the structural findings support Hanes' helical model of starch with the coils as cyclodextrin precursors, and also explain both hydrolysis by α-amylases and cyclodextrin formation by the *B. macerans* enzyme. However, the abstract failed to note that a new type of process ('Umglucosidierung') was put forth to account for the latter reaction.

In 1945, Freudenberg's proposal of this direct process of cyclodextrin formation was adopted by Cori[83] who termed the reaction an exchange of one glycosidic bond for another without mentioning the equivalent earlier proposal of 'Umglucosidierung'.[72] French et al.[87] credited Cori with interpreting the reaction, and also ignored Freudenberg's[72] 'Umglucosidierung' idea until many years later.[89] Hassid[90] held that, while Freudenberg's 'is perhaps the simplest hypothesis for the mechanism of Schardinger dextrin formation, there is no evidence for the mechanism.' The original failure to fully describe the basis of the proposed process[72] may have delayed the proposal's acceptance. Yet, the proximity of one end of a cleaved starch loop to its other (free) and offers a credible reason for envisioning a loop-opening and ring-closure occurring together at a catalytic site. Further, since starch–*B. macerans* enzyme mixtures produce no significant change in reducing power,[73] hydrolysis need not be considered a factor in the large conversion to nonreducing cyclodextrins. A later review[91] states that Freudenberg in 1939 interpreted cyclodextrin formation by *B. macerans* amylase as a transglycosylation. However, the latter term was first proposed for the enzyme's reactions only in 1951;[92] and received experimental verification much later.[93]

2.2.3. 1942: Glucoside synthesis by plant extracts that hydrolyze β-glucosides reported to occur by 'Umglucosidierung'.

In 1942, Tomoh Miwa et al.** in a paper published in Japanese[94] expanded Rabate's[37,40] observations by showing that *soluble* β-glucosidase extracts from *Salix* leaves promote substantial methyl β-glucoside synthesis as well as the hydrolysis of phenyl β-glucoside in digests containing 6% methanol. In contrast to Rabate,[37] Miwa et al. considered that the synthesis occurs by 'Umglucosidierung', a term adopted to parallel German usage of 'Umaminierung' and 'Umphosphorylierung' for similar types of transformations. This paper, with its new findings and independent proposal of a nonhydrolytic process for β-glu-

** Tomoh Miwa (1899–1979) was the first to describe efficient methyl glucoside synthesis by soluble β-glucosidases as nonhydrolytic reactions, and to use the terms donator and acceptor for the reactants. His suggestion that β-glucoside-hydrolyzing enzymes from different plants catalyze 'Umglucosidierung'[94] was made before the equivalent 'exchange of one glycosidic bond for another' had been advanced.[83] Close friendship began in 1964–1965 during the author's sabbatical at the Tokyo University of Education (Professor Miwa, then its President) to continue work on dextransucrase with one of his young associates, Dr Hiroshi Suzuki who had recently served for 2 years as a research associate in the author's laboratory. A memorial of Professor Miwa has been recorded by one of his students, Professor K. Nisizawa.[94]

coside synthesis by soluble (purifiable) plant extracts, was long lost to the West because of the War and use of the Japanese language and characters and thus had no impact on Western views about the process of saccharide biosynthesis.

Determination of both the free phenol and glucose formed in enzyme–phenyl glucoside–methanol test mixtures allowed Miwa et al.[94] to calculate substrate utilized (from liberated phenol), and methyl glucoside synthesized (the difference between glucose expected if the utilized substrate were fully hydrolyzed, and the free glucose found). Miwa et al. were apparently unaware of the prior use of 'Umglucosidierung' for cyclodextrin synthesis from starch;[72] their concern was with glycoside syntheses by plant β-glucosidases. They employed the terms donator, acceptor and 'ten-i' (equivalent in 1942 to 'change' or 'exchange'); these terms had not yet been introduced in Western papers on saccharide biosynthesis.

2.2.4. 1941–1943: Dextran and levan synthesis from sucrose by bacterial enzymes shown to be direct and not mediated by water or phosphate.

In 1941–1942, the present author reported findings demonstrating the enzymic synthesis of dextran from sucrose.[95,96] The first positive experimental results were obtained without awareness of the various recent demonstrations of in vitro syntheses of glycogen- and starch-type α-glucans from glucose 1-phosphate.[62–71] The latter studies were being intensively pursued in several laboratories since this synthesis reaction appeared to represent the enzymic process responsible for forming the α-glucans present in eukaryotic cells. Demonstration of the cell-free synthesis of a related α-glucan (dextran) from sucrose indicated the existence of a synthesis process other than that promoted by phosphorylase.

The enzyme preparations used in the author's first studies[95,96] were derived from sucrose-broth cultures of a strain of *L. mesenteroides* of serotype B† that produced much water-soluble dextran. Almost all dextran-free extracts (assayed serologically) were prepared by shaking the dextran-laden cultures with chloroform, then recovering the emulsion of chloroform droplets trapped between solvent phases (a procedure used to remove traces of protein in polysaccharide purification). The collected chloroform droplets were repeatedly washed by suspension in water, sedimented for treatment with ethanol to remove chloroform. Solutions of the scant residue in water were rendered sterile and free of cells by Berkfeld filtration. Reaction mixtures were prepared using sterile buffered solutions of enzyme plus a sample of crystalline sucrose nearly free of the usual dextran impurity,[2,3†] then incubated under aseptic conditions and shown to remain sterile by sensitive cultural tests.

The findings reported in 1941–1942[95,96] demonstrated that incubated mixtures of enzyme with sucrose (but not glucose or other common sugars) became increasingly opalescent; increasingly turbid when treated with 1.5 volumes of alcohol; and progressively more reactive with anti-dextran antibodies as well as those against type 2 pneumococcus capsular polysaccharide (but not with various control sera). Successive samples of the mixture also contained increasing concentrations of a reducing sugar reacting as fructose. Its measured levels corresponded closely to those found for acid hydrolyzates of the alcohol precipitated dextran from the same samples. The correspondence indicated that the observed reaction corresponds to the conversion of X molecules of sucrose into X molecules of fructose plus high polymer dextran having an average of X anhydroglucose units per molecule. The polysaccharide recovered from two large enzyme–sucrose test mixtures amounted to 0.5 and 0.7 g; each had the physicochemical and serological properties characteristic of the dextran recovered from a culture of the *Leuconostoc* strain from which the enzyme was obtained.[96] Dextran formation from sucrose by the cell-free extracts was more rapid in the range of pH 4.0–6.0 than at pH 7.0 or above. Activity was lost on brief heating at 55–60° at pH 5.6 or 7.0.

The demonstration of in vitro synthesis of dextran from sucrose was confirmed by Stacey[97] who compared its significance to that of Hanes' discovery of starch synthesis by phosphorylase. It was also well cited during

the next few years[54,98–101] and recalled long thereafter.[102–104] An immediate need was to learn if dextran synthesis by *L. mesenteroides* extracts might be mediated by glucose 1-phosphate which reportedly is required for the synthesis of related starch-type α-glucans by phosphorylase preparations.[62–71] A report by Russian scientists[105] had recently indicated that *L. mesenteroides* elaborates a phosphorylase that catalyzes the formation of glucose 1-phosphate from sucrose.

A direct comparison was therefore made between the actions of a *Leuconostoc* enzyme (prepared as detailed above) and crude potato phosphorylase on 0.1 M sucrose and 0.1 M glucose 1-phosphate. Concurrently, incubated sterile test mixtures and controls were analyzed together. At 96 h, the leuconostoc enzyme–sucrose mixture produced abundant dextran (assayed serologically) and 6.2 mg/mL of fructose; no phosphate was detected using tests of high sensitivity. The potato phosphorylase acted on glucose 1-phosphate to form abundant starch-like polysaccharide (staining blue–black with iodine) and 6.4 mg/mL of inorganic phosphate; it did not use sucrose to form any detectable polysaccharide. The *Leuconostoc* extract's inaction on glucose 1-phosphate shows that the latter compound does not mediate dextran synthesis which, instead, occurs directly from sucrose.[95,96,106] Furthermore, although each substrate was used by one enzyme but not the other, it was pointed out that both substrates have a structural feature in common; i.e., 'an unusually acid-labile glucosido' moiety that supplies the repeating unit of the polysaccharide in each case.[106]

The 1943–1944 studies of Shlomo Hestrin[††] and his associates[98,108–110] clearly established the cell-free conversion of sucrose to levan and glucose by sterile levansucrase preparations from autolyzates of *Aerobacter levanicum*. The findings indicated that the levan-forming reaction parallels that reported for dextran formation.[95,96] The product, recovered in gram quantities from enzyme–sucrose mixtures, had overall properties similar to those reported for bacterial levans. It was nondialyzable and nonreducing, gave turbid and viscous solutions in water and was readily hydrolyzed to fructose by dilute acid. Galactose was not liberated during levan formation from raffinose, showing that polymer synthesis from this fructose-ended trisaccharide is not mediated by sucrose.[110]

The reactions with sucrose and raffinose were exceptional in releasing more reducing sugar than expected from a direct formation of levan plus glucose (or melibiose). The excess reducing sugar included fructose and this was first assumed to be the result of some substrate hydrolysis, allowing the possibility that levan synthesis might somehow depend on sucrose or raffinose hydrolysis. However, although it is accompanied by excess reducing sugar, levan synthesis was shown not to be associated with any reduction of total carbohydrate, or inhibited by poisons of glycolysis or respiration. That is, the synthesis appeared to be independent of any energy-yielding sugar dismutation. These indications that levan synthesis is not energetically coupled to substrate hydrolysis[98] were supported by the analogy with dextran formation in which no association with hydrolysis had been observed.[95,96] Evidence that the direct precursor of levan is not fructofuranose or fructopyranose indicated[98,110] that reversal of hydrolysis cannot describe levan formation.

The failure of levan synthesis to occur with several known phosphate esters of sugars further suggested that phosphate participation is highly improbable.[98] Its nonpartcipation in the comparable synthesis of dextran had recently been demonstrated.[106] Moreover, the finding that dialyzed levan-forming enzyme was free from both detectable inorganic phosphate and levan-hydrolyzing activity also opposed a role for phosphorus or water involvement in the synthesis. Among the general conclusions, reached by Hestrin and Avineri-Shapiro in 1944,[98] was that there is a striking analogy between levan formation

[††] Shlomo Hestrin (1914–1962) was a good friend and colleague. His early death was a great loss to science. A brief biography has been reported by M. Schramm,[107] along with a list of Professor Hestrin's works. He proposed the names dextransucrase and levansucrase[108] to conform with the term viscosaccharase coined by Beijerinck for the enzyme reported to form laevulan from sucrose; noted early that dextran and levan syntheses fit ill with any recognized class of reactions involving carbohydrates.

from sucrose and glycogen formation from glucose 1-phosphate. Among the similarities noted was that each synthesis proceeds without the desmolysis of sugar at relatively low concentrations of substrate;[98] that levan formation does not occur from a simple sugar but from a glycosidic derivative having a higher 'polymerative energy' (as also recognized in dextran synthesis from sucrose[95,106]).

Prominent biochemists[111–113,107‡‡] judged these demonstrations of the cell-free enzymic syntheses of dextran and levan to be among the important recent advances in carbohydrate chemistry and enzymology. They were specifically cited in one case[107] for establishing the occurrence of polysaccharide synthesis from an immediate precursor that is not a phosphate ester. These syntheses showed unequivocally that, contrary to widespread belief, phosphorolysis-reversal is not the sole process where-by polysaccharides may be formed in nature.

2.2.5. 1943–1944: Enzyme-catalyzed polysaccharide syntheses require substrates with an 'unusually labile (reactive) glycosido residue'.

The demonstrations that the in vitro syntheses of dextran and levan arise directly from sucrose, while those of amylopolysaccharides require glucose 1-phosphate, provided an alternative to the prevailing belief that the latter type of substrate is the key to efficient synthesis. The new focus is on those feature(s) of substrates that enables them to be effecient precursors of natural homopolysaccharides. Since sucrose (but not glucose and/or fructose) supports dextran and levan synthesis with no need of an external source of energy,[95,96,98] Hestrin and Avineri-Shapiro emphasized that sucrose has a higher 'energy' for polymerization than a free sugar.[98] The finding that sucrose but not glucose 1-phosphate is used by dextransucrase (and the reverse for phosphorylase) indicates that, in polysaccharide syntheses by these enzymes, and by levansucrase, the effective substrate in each case contains an 'unusually acid-labile glycosido group' which furnishes the repeating unit of the polysaccharide.[106]

2.3. Proposed processes of saccharide biosythesis: transglycosylation (glycosyl transfer) adopted as the chemically appropriate model

2.3.1. 1941–1944: New findings on saccharide syntheses from glucose 1-phosphate emphasize the need of phosphorylases for synthesis.

In 1941, the starch-type glucan synthesized in vitro by potato phosphorylase, shown earlier to stain blue with iodine and to be hydrolyzed by β-amylase,[70] was investigated structurally. Hydrolysis of the permethylated compound yielded 2,3,6-tri-*O*-methyl glucose plus 1.5% 1,2,3,6-tetra-*O*-methyl glucose, showing the synthesized glucan to be a linear $(1\rightarrow4)$-linked chain of at least 90 glucose units long — similar to one component of potato starch.[114] In 1942–1943 Green et al.[115] crystallized rabbit muscle phosphorylase as an adenylic acid complex and found the purified enzyme to be active in the synthetic direction only after addition of a trace of glycogen. This so-called 'primer' was considered to serve as a cosubstrate in the synthetic direction of the reaction catalyzed by the enzyme.[116] Further, the glucan formed by crystalline muscle phosphorylase was shown to be structurally similar to that formed by potato phosphorylase; that is, to amylose rather than glycogen. On hydrolysis, the permethylated glucan yielded predominantly 2,3,6-tri-*O*-methylglucose plus 0.6% of tetra-*O*-methylglucose, indicative of a linear chain of some 200 $(1\rightarrow4)$-linked glucose units.[117] Indications were found[118] that the muscle phosphorylase, when supplemented with a liver or heart extract lacking phosphorylase activity but containing a possibly diastase-like enzyme, synthesizes a product resembling glycogen.

In 1943, Michael Doudoroff et al.[119,120] reported that dry preparations of the bacterium *Pseudomonas saccharophila* bring about the phosphorolysis of sucrose to form glucose 1-phosphate plus fructose. This phosphorolyzing ability of *P. saccharophila* was discovered

‡‡ In 1947 Sumner and Somers[111] noted that four notable advances in enzymology had been made since 1931; the second of these was, 'The reversible enzymatic synthesis of polysaccharides, such as starch and glycogen, and the synthesis of bacterial levan and dextran.'

independently of a reported finding that *L. mesenteroides* has that ability.[105] The *P. saccharophila* cell preparations were, in addition, found to act on glucose 1-phosphate plus fructose to form a product judged to be sucrose[119,120] Preparations of the responsible enzyme, sucrose phosphorylase, were obtained relatively free of invertase and phosphatase. They appeared to be specific for sucrose and not to require a coenzyme for the reversible phosphorolytic process. The equilibrium constant for phosphorolysis was found to be about 0.05 at pH 6.6; 0.09 at pH 5.8.[120]

As the data indicating that the synthesized sugar is sucrose were not sufficient to fully establish that point, the product formed by purified sucrose phosphorylase acting on glucose 1-phosphate plus fructose was subsequently isolated and identified as sucrose by X-ray diffraction and rate of hydrolysis by acid — each in comparison with authentic sucrose.[121] The synthesized product's sucrose structure was confirmed by the identity of its peracetylated derivative with sucrose octaacetate. Finally, sucrose phosphorlyase was assumed to act on α-D-glucose 1-phosphate with retention of configuration, based on analogy with the known behavior of muscle and potato phosphorylase. This assumed behavior was said to establish that the glucose moiety of sucrose is of α anomeric configuration.[121] However, it appears simply to confirm what prior enzymic studies had indicated.[122–124]

The successful enzymatic synthesis of sucrose following the failed attempts of others to achieve a reproducible synthesis of this sugar by enzymic or chemical means, gave this work[121] well deserved special appeal, and thereby bolstered the idea of the requirement of phosphorylases for saccharide synthesis. A further 1944 report[125] on sucrose phosphorylase showed that it also acts on glucose 1-phosphate in the presence of L-sorbose or D-xylulose to form two new analogs of sucrose.

2.3.2. 1945–1946: Syntheses viewed as exchange of a phosphate ester bond for a glycosidic bond, or the exchange of one glycosidic bond for another.

The year 1945 marked the first time that investigators of the syntheses catalyzed by phosphorylases acknowledged that enzymically catalyzed saccharide syntheses not involving phosphorus had been demonstrated. At a Symposium on 'The Formation of Disaccharides, Polysaccharides and Nucleosides', Carl Cori[83] in a brief overall introduction presented bimolecular equations for the reactions of polysaccharide phosphorylase, sucrose phosphorylase and a recently reported nucleoside phosphorylase [126]; also for the theoretically reversible reactions of dextransucrase and levan-sucrase. The original reports of findings and interpretations were not cited in any case. Phosphorylases were viewed as catalyzing the exchange of an ester bond on carbon atom 1 for a glycosidic bond at this carbon atom; dextransucrase and levansucrase as promoting the exchange of one glycosidic bond for another. Cyclodextrin formation from starch was accepted as a special example of the exchange of one glycosidic bond for another without noting that Freudenberg had proposed the equivalent process of 'Umglucosidierung'[72] or providing any additional evidence. Hassid[90] contended in his Symposium presentation that Freudenberg had no evidence for this mechanism, as noted earlier.

Carl Cori considered that dextran and levan synthesis occur by a mechanism analogous to that of starch–glycogen synthesis,[83] but did not mention the telling point that high acid-lability (or 'polymerization energy') characterizes the substrates of all three syntheses. Glucose 1-phosphate was, instead, confidently set apart as a glycan precursor in a wide range of living forms: 'A sufficient number of cases has been tested to expect that wherever polysaccharides belonging to the starch and glycogen class are found, glucose 1-phosphate will be the substrate from which they are formed'.[83]

In another of the 1945 symposium reports, Michael Doudoroff[128] reviewed recent findings[129] on the synthesis of sucrose and two analogous disaccharides by sucrose phosphorylase. The latter compounds, called 'α-D-glucosido-α-L-sorboside' and 'α-D-glucosido-β-D-ketoxyloside', were nonreducing, easily hydrolyzed by acid and gave the Raybin color reaction for sucrose.[130,131] Doudoroff[128] com-

mented favorably on the earlier demonstrated enzymic syntheses of dextran[96,106] and levan[98] from sucrose without involvement of phosphate or phosphate esters. He concurred with Cori's[83] view that these two syntheses appear to represent the exchange of an already existing glycosidic linkage for a new glycosidic linkage; noted that a polysaccharide and a reducing sugar by-product in equivalent amounts characterizes dextran formation, and suggested that the residues of some saccharides may serve a function similar to that of the phosphate ester group of glucose 1-phosphate in forming polysaccharides. Beyond the two polysaccharide syntheses known to occur without phosphate,[106,98,132] a new reaction having special relevance to Cori's view of the need for glucose 1-phosphate in starch–glycogen synthesis[83] was found by Hehre and Hamilton.[133] Washed cells of a species of *Neisseria* bacteria were shown to form large amounts of amylopectin- or glycogen-type polysaccharide from sucrose; trace amounts from glucose 1-phosphate (suppressed in the presence of high phosphate concentrations without lessening the synthesis from sucrose); and none from glucose, fructose, maltose or other sugars.[133] The polysaccharide stains red–purple with iodine, is readily hydrolyzed by α-amylase and partly degraded by β-amylase and potato phosphorylase. It is hydrolyzed by HCl to form glucose at the same rate as amylopectin and four to five times faster than dextran. The washed bacteria convert sucrose to glucan plus fructose in equimolar proportions, as found[96,132] in the synthesis of dextran from sucrose. A different enzyme, extracted from potatoes, was reported in Britain to produce amylopectin from amylose,[55,56] apparently without phosphate mediation; it is known as the Q or branching enzyme.

2.3.3. 1947–1950: Sucrose phosphorylase reported to seemingly belong to the same (transglycosidase) class as dextransucrase and levansucrase.

In 1947, Doudoroff et al.[134] reported convincing evidence that sucrose phosphorylase exhibits a type of catalytic activity not previously known for any phosphorylase. Nearly phosphate-free preparations of the enzyme were shown to interconvert sucrose and an analog, glucosido-sorboside; also, to catalyze the synthesis of sucrose directly from glucosidoketoxyloside plus fructose. Because of the ability to synthesize saccharides from sucrose-type substrates as well as from glucose 1-phosphate, a new view of the enzyme's catalytic scope and mechanism was put forth. Sucrose phosphorylase was described as a versatile transglucosidase, apparently of the same class of enzymes as dextransucrase and levansucrase. The authors proposed that enzymes of the general type that catalyze the exchange of glycosidic bonds be called 'transglycosidases'.[134] The reversible phosphorolysis of sucrose was assumed to consist of the following reactions:

$$\text{glucose-1-fructoside} + \text{enzyme} \overset{\text{sucrose}}{\rightleftharpoons} \text{glucose-enzyme} + \text{fructose}$$

$$\text{glucose-enzyme} + \text{phosphate} \rightleftharpoons \text{glucose-1-phosphate} + \text{enzyme}$$

Direct disaccharide interconversion was similarly accounted for, e.g.,

$$\text{'glucose-1-ketoxyloside'} + \text{enzyme} \rightleftharpoons \text{glucose-enzyme} + \text{'ketoxylose'}$$

$$\text{glucose-enzyme} + \text{fructose} \rightleftharpoons \text{glucose-1-fructoside} + \text{enzyme}$$

The assumption that sucrose phosphorylase acts on sucrose, sucrose analogs, or glucose 1-phosphate to form a glucose-enzyme intermediate that reacts with fructose, 'ketoxylose' or phosphate[134] is an attractive but vaguely defined idea compared to Koshland's later[135,136] association of nucleophilic displacement(s) with transfer reactions. Of greater present interest is the place of the study by Doudoroff et al.[134] on the path leading to the concept of glycosyl transfer.

The present author felt slight disappointment on reading this excellent experimental demonstration of the broad catalytic scope of sucrose phosphorylase. This enzyme, originally believed to require glucose 1-phosphate or inorganic phosphate in order to act,[119] is now reported[134] to apparently belong to the same class as dextransucrase and levansucrase. Yet, the prior relevant articles were not cited

except by way of other references.[128,137] Bell[138] called attention to the precedent syntheses of dextran and levan without need for phosphate, citing the first of these.[96]

In 1948 and 1949 three insightful reviews concerning saccharide synthesis appeared,[139–141] as well as several reports of significant new experimental findings. Each review makes use of brief notations, such as 'glucose transfer' and 'transfer reaction', for syntheses other than by hydrolysis-reversal. That of Hassid and Doudoroff[139] notes that sucrose phosphorylase 'acts to transfer glucose to suitable acceptors' in accord with their recently revised name for the enzyme.[134] A brief discussion of dextran and levan synthesis from sucrose observes that, since the energy of the glycosidic bond in sucrose is similar to that of the C1–O–P bond in glucose 1-phosphate, sucrose can be used for polysaccharide production. Not stated is that it had already been pointed out that the precursor of each of the known enzymically produced glycans contains the polymer's repeating unit as a very labile, high 'energy' glycosido residue.

Hassid and Doudoroff[139] indicated their support for Cori's view[83] that glycogen and starch in nature are formed by phosphorylase acting on glucose 1-phosphate. However, two experimental observations raised doubt about this projection. New studies by Hehre and Hamilton[142,143] brought further evidence that *Neisseria perflava* elaborates an enzyme (amylosucrase) that acts specifically on sucrose without phosphate mediation to form an amylopectin-like α-glucan plus fructose. Soon thereafter, Monod and Torriani[144,145] and, independently, Doudoroff et al.,[146] reported that certain strains of *Escherichia coli* produce amylomaltase, an enzyme that converts maltose to a mixture of linear dextrins plus glucose in a reaction without phosphate intervention.

In a 1948 review, Hestrin[140] illustrates starch phosphorolysis with a figure adapted from Parnas[58] — (also used without citation by Cori et al.[60] and Hassid et al.[147]) — that assumes cleavage of the reactive glucose unit of the substrate at the C1–O bond. Hestrin[140] states that the reactions of dextransucrase, levansucrase, sucrose phosphoryase and polysaccharide phosphorylase represent transfers of glycosyl groups; but no reason or evidence is given for assuming that cleavage by these enzymes occurs at one rather than the other of the two bonds, C′1–O–C, that make up a glycosidic linkage.

The present author's 1948 review of polysaccharide synthesis[141] illustrates how closely the serological activity of dextrans formed enzymically matches that of the serotype A and B dextrans† from cultures of the various strains that yielded the enzymes. The review also questioned the validity of the terms, '1-ester', 'ester-bond', or 'phosphate-ester', commonly used in referring to glucose 1-phosphate, noting that the latter resembles a glycoside or mixed acetal more closely than a true ester as it is more readily hydrolyzed by acid than alkali. Its susceptibility to acid relative to the ester, glucose 6-phosphate, had long been known.[60]

The idea that enzymic saccharide syntheses occur by transfer reactions raises the question of exactly what is transferred. To state that a transglucosidase[134] catalyzes glucose transfer does not suffice to define the transferred moiety. Findings of fundamental importance bearing on this problem were reported by Cohn[148] who determined the point of cleavage of glucose 1-phosphate in the reversible phosphorolysis of glycogen by muscle phosphorylase and KH_2PO_4 containing ^{18}O; also in the phosphate exchange reaction catalyzed by sucrose phosphorylase acting on glucose 1-phosphate and KH_2PO_4 containing ^{18}O. When equilibrium was reached in each case, the atom% excess ^{18}O was determined for the inorganic phosphate and also for the inorganic phosphate obtained by hydrolyzing the glucose 1-phosphate component with acid (in which the inorganic phosphate retains the oxygen).

The results establish that C1–O bond cleavage occurs in both phosphorylase reactions. A diagram of the sucrose phosphorylase exchange reaction depicts an α-D-glucopyranosyl unit linked to an enzyme via an atom of undetermined nature. However, Cohn[148] does not name the unit or use the term *glucosyl* in the functional sense. She notes[148] that glucose 1-phosphate, as already suggested,[141] is by no means a typical ester.

Soon after Doudoroff et al.[134] redefined sucrose phosphorylase as a transglucosidase, the term transglucosidation was applied to the action on starch of the cyclodextrin-forming enzyme of *B. macerans*[149] and to the glycogen–starch branching enzymes.[150–152] In addition, newly observed 'transfer reactions' catalyzed by various *glycosidases* were generally reported as transglycosidations[153–171] — at least until 1955.[§§] Ironically, the initial spread of this terminology arose from work with glycosidases though these were excluded from the class of transglycosidases.[134] Thus, the acceptance of transglycosidation owed much to Rabate's[37,40–42] findings that insoluble glucoside-hydrolyzing preparations from willow leaves promote efficient glucoside syntheses, and to Miwa et al.,[94] who reported such syntheses by soluble β-glucoside-hydrolyzing enzymes from many plants[94] and later called the responsible enzyme glucotransferase.[171] Reports of transfer reactions by various glycosidases became commonplace as the use of paper and column chromatography widened.

The early acceptance of the transglycosidation model[134] was also helped by two 1950 reviews by Hassid and Doudoroff,[183,184] mainly on their work on disaccharide synthesis, and by another in 1951 by Hassid et al.[185] on phosphorylases. The latter review included a discussion of Kalckar's studies on inosine and guanosine phosphorolysis[126,127] but did not state (nor had Kalckar[126,127]) that the exchange between the C1–N bond of a nucleoside and the C1–O bond of ribose 1-phosphate shows the transferred moiety to be a *ribosyl* group. Again, Hassid et al.[185] did not mention (nor had Cohn[148]) that the cleavage of the C1–O bond of glucose 1-phosphate by muscle and sucrose phosphorylases shows the *glucosyl* group to be transferred. Neither used the term 'glucosyl'.[148,185,186]

2.3.4. 1951: Transglycosylation, with glycosyl as the functional group, proposed as the chemically appropriate model for saccharide biosynthesis.

The present author, aware of the glycosyl moiety as a well-defined structural unit used in naming carbohydrates,[187,188] saw it also as a functional group in enzyme-catalyzed saccharide synthesis. To express the synthesis process as one of transglycosylation appeared chemically sound. The reasoning included recognition that a glycosyl group is transferred in the reactions described by Kalckar[126] and by Cohn[148]; and that glycosyloxy transfer, implied by the term transglycosidation,[134,185] had not been demonstrated. The idea to replace the latter by transglycosylation was informally presented[¶¶] to a few colleagues, then proposed in a 1951 review of polysaccharide biosynthesis.[92]

Early comments about the reported proposal differed widely. Several authors[191,192,47] would continue to use glycoside exchange or transglycosidation. Bourne[193] noted that 'it remains to be seen which term is the more accurate in portraying the functions of other enzymes of this class'. Bacon's response[194] was more positive, stating that, 'despite present uncertainty, it seems reasonable to follow Hehre and to refer to the transfer of fructose residues as 'transfructosylation''. Another positive early reaction was that of Hoffmann-Ostenhof who, in assembling a new classification of enzymes,[195] accepted the term transglycosidases[92] as probably better than transglycosidases[134] or transcarbohydrases as he had earlier proposed. Class III enzymes in

[§§] After 1955, most of the many new transfer reactions catalyzed by glycosidase preparations would be recorded as transglycosylations.[172–182]

[¶¶] One of the author's first opportunities to meet with other carbohydrate chemists and enzymologists came when he attended the 1949 Starch Round Table, an annual 4-day informal meeting of some 40–60 invited scientists from the US and Canada. At this and subsequent Round Tables he met and was able to discuss in a casual way matters of mutual interest with Drs Carl and Gerty Cori, W.Z. (Zev) Hassid, C.S. Hudson, Karl Link, Fred Smith, Mel Wolfrom and many others. At the 1950 meeting, for example, he met together with Carl Cori and 'Huddy' (Hudson) and presented the idea of replacing 'transglycosidation' by 'transglycosylation'. Both said simply that the idea was novel. Also, in 1949–1950 the author was invited to lecture at The Enzyme Club (Columbia University) and the Department of Chemistry (Fordham University) whose chairman, F.F. Nord, then offered an invitation to prepare a review of enzymic polysaccharide syntheses for *Advances in Enzymology* of which he was editor. At a later meeting of The Enzyme Club, with Michael Doudoroff as speaker, the opportunity arose to meet him and Mildred Cohn and to present the idea of transglycosylation. Both encouraged its formal proposal, but Michael Doudoroff indicated that Dr Hassid would continue using transglycosidation. Brief memoirs of Carl and Gerty Cori[189] and of Professor Hassid[190] exist.

the overall projected system[195] were designated 'Transglycosylases' (Ref. 92).

Koshland's articles on the stereochemistry and mechanism of enzymatic reactions[135,136] did not use 'transfer', 'transglucosidation' or 'transglycosylation' to keep from disturbing current nomenclature.*** Reactions were presented as substitutions or displacements at the carbon ether oxygen bond. He did not refer to the suggestion[92] that transglycosylase and transglycosylation would be the chemically appropriate terms.

Herman Kalckar's 1954 review of the mechanism of transglycosidation[47] retained this term for the reaction process but used 'glycosyl' terms for reaction components and attributed this usage to authors[126,127,144,148] who did not employ glycosyl terminology in the reports cited. Hassid's 1954 review[192] also referred to the process as transglycosidation but now the term 'glucosyl' (as in 'glucosyl–enzyme') in the static sense. Neither review mentioned the present author's proposal[92] to replace transglycosidation with transglycosylation.

An early use of glycosyl transfer terminology came in the course of describing an unusual synthesis of dextran, promoted by the enzyme dextrandextrinase from *Acetobacter capsulatum*.[196] This enzyme was considered to act by successively transferring glucosyl units from α-(1→4)-linked dextrins to form highly polymerized serotype B dextran. The enzyme product was predominantly α-(1→6)-linked as shown by optical rotation and periodate oxidation findings. Hestrin[197] later verified that levansucrase and dextransucrase act by transglycosylation. He showed, by using ^{18}O, that the former enzyme cleaves the glucopyranosyl C1–O bond; levansucrase, the fructofuranosyl C-2–O bond of sucrose.

From 1956 on, the transglycosylation concept would gain general acceptance among biochemists.[172–182,198–203,207†††] Most important, this concept was in place at the onset of the explosive growth of newly discovered enzymic syntheses of naturally occurring complex saccharides. A 1960 review by Leloir[201] illustrates how the glycosyl transfer model efficiently articulates saccharide syntheses from nucleotide diphospho sugars with earlier syntheses requiring other substrates. Biochemists felt excitement when the syntheses of trehalose phosphate,[208,209] sucrose and sucrose phosphate,[210–212] and β-(1→3)-glucan (callose)[213] were reported. An intriguing finding was that glucosyltransferases, induced in T-even phages by growth in *E. coli*, use host UDPG to glucosylate the hydroxymethylcytosine groups in newly assembled phage DNA, protecting the latter from degradation by the bacterial host's nucleases.[214,215] Also the 1995 synthesis of orotidine phosphate from phosphoribosyl diphosphate plus orotate[216] was soon seen to be a glycosyl transfer. By 1957 Kornberg[217] adopted the transglycosylation concept, without citing its origin. That concept[92] con-

*** Having heard that Dr Koshland would publish a review on enzymic reaction mechanisms, the author wrote to ask for a reprint and also commented that a review on polysaccharide synthesis was *in press* and would recommend a change in terminology from transglycosidation to transglycosylation. Koshland replied that he had just begun to prepare his review and that the suggested change in nomenclature would be an improvement.

††† Leloir et al.[201] state: 'In all the cases which have been studied, the bond broken during the transfer reactions is that between the carbon atom, and oxygen. That is, a glycosyl group is transferred and hence the name *transglycosylase* and not *transglycosidase* which would imply the breaking of the bond beyond the oxygen.[92,136] Miwa states[199] 'Dr Hehre proposed that these reactions should be designated as transglucosylation and transfrucosylation, respectively, … because a glucosyl or fructosyl residue is transferred. Now the term, transglycosylation is widely accepted.' Hassid and Neufeld[202] note that: 'Hehre[92] pointed out that, since it is the *glycosyl* group (which does not include the hemiacetal oxygen) rather than the *glycosido* group which is transferred in such reactions, the terms 'transglycosylase' for the enzyme and 'transglycosylation' for the reaction mechanism would be more appropriate. These terms have been accepted by investigators in the field of carbohydrate chemistry.' In two reviews, Hassid[204,205] made statements leaving the impression that the transglycosylation concept was proposed in Ref. 134, the sole citation in each statement. Florkin,[206] misguided by this impression gathered from Ref. 204, stated that 'Doudoroff et al.[134] introduced the terms transglyosylase and transglycosylation instead of transglycosidase and transglycosidation.' He must not have read Ref. 134. Pazur[203] writes: 'The development of the concept of glycosyl transfer (Ref. 92) has been an important factor contributing to our modern views on pathways of biosynthesis of polysaccharides.' Dedonder[207] states: 'the concept of transglycosylation is linked to the discovery by Hehre[92]… The terms 'transglycosylases' and 'transglycosylation' have been adopted instead of 'transglycosidases' and 'transglycosidation' suggested by Doudoroff et al.,[134] since it is the glycosyl group (which does not include the hemiacetal oxygen) rather than the glycoside group which is transferred in such reactions as has been pointed out by Hehre (Ref. 92).'

tributed to his recognition[218,219] of the synthesis from 2-deoxynucleotide triphosphates (plus a DNA chain) of a complementary DNA chain by an enzyme that transfers nucleotidyl rather than glycosyl groups.

3. The expanded glycosyl transfer concept: glycosylation proposed and used as the paradigm of all carbohydrase actions

Evident from the foregoing is the critical role filled during the past five decades by the glycosyl transfer concept[92] through its articulation of the syntheses of a vast range of complex saccharides (e.g., glycoproteins and glycolipids) that serve a wide range of newly recognized metabolic functions. Further great contributions to carbohydrate biochemistry have come in the last two decades through the use of a widened form of the glycosyl transfer concept. Koshland[135,136,220] had discussed together the mechanisms of transfer enzymes (esterases, glycosidases, phosphatases, transglycosidases, peptidases) since their reactions whether hydrolytic or synthetic are, in chemical terms, displacement reactions. A more specific model for use in carbohydrate enzymology, suggested by Hehre in 1960,[221] and elaborated on in 1971–1973,[222,223] proposed that transglycosylases + glycosidases could be called catalysts of glycosylation. The chemical change assumed to be effected by these enzymes was illustrated by a compact equation[222] and defined as *glycosylation*:[223]

$$\text{glycosyl}{-}\text{X} + \text{H}{-}\text{X}' \rightleftharpoons \text{glycosyl}{-}\text{X}' + \text{H}{-}\text{X}$$

This expression, which emphasizes the functionality of the glycosyl group, indicated for the first time that a compound need have only the ability to bind appropriately at an active site, and to furnish a glycosyl moiety in exchange for a proton, to serve as a substrate. It embraces Bourquelot's and later evidence for the synthetic, as well as, hydrolytic reactions of glycosidases; also all complex saccharide syntheses catalyzed by transglycosylases of [EC 2.4].

The expanded glycosyl transfer model and its simple precise nomenclature has been a major factor underlying the phenomenal growth of carbohydrate biochemistry. It called early attention to and helped resolve a sinestral relationship that existed between the views of carbohydrate biochemists and chemists regarding the process of saccharide and glycoside synthesis.[‡‡‡] Moreover, for more than two decades this model[222,224] and its equivalent used by Sinnott[227] proved to be a highly productive paradigm-allowing the rapid growth of mechanistic information among glycosidases and transglycosylases through the general use of such chemically precise terms as 'to glycosylate' or 'deglycosylate' a compound. Important findings which were facilitated include Withers' identifications of the catalytic nucleophile (and strong indications of a glycosyl–enzyme intermediate) in reactions promoted by various 'retaining' enzymes. Glycosyl fluorides, including those of the 'wrong' configuration (and also certain prochiral (glycal-type) compounds), have provided alternative views of the mechanism of certain 'retaining' enzymes (e.g., glycogen phosphorylase), or inferred from the near-identity of the transition state structure of some 'retaining' and 'inverting' glycosidases. Reviews dealing at least in part with these nonglycosidic substrates and their use as probes of the catalytic scope, stereochemistry and mechanisms have appeared at intervals since 1973.[224,228–234] Recently it was shown[235] for the first time that glycosyl fluorides are substrates for glycosyltransferases which, until now, were believed to require nucleotide-

[‡‡‡] By 1973, it was clear that glycosylation is the likely basis of all carbohydrase-catalyzed reactions.[224] Yet a contrary process of glycoside formation had repeatedly been put forth in reports on Rules of Carbohydrate Nomenclature, first in 1948 by an American Chemical Society Committee[188]; again, 20 years later, by an IUPAC Commission.[225] These reports defined glycosides as mixed acetals resulting from replacement of the hydrogen atom of the anomeric hydroxyl group of a sugar by a group. X, from an alcohol or phenol (X–OH). Yet, in 1969, methyl α- and β-D-glucopyranoside had been shown to undergo hydrolysis by acid or the appropriate glycosidase with C1–O bond cleavage[221] so that the reverse (glycoside synthesis) would necessarily also involve cleavage of the C1–O bond of glucose in reaction with methanol. Also, by 1969, abundant evidence had shown that the process underlying various enzymic synthesis and hydrolysis reactions is one of nucleophilic displacement (glycosylation). Hehre et al.[224] expressed doubt that any glycoside in Nature had arisen via reactions of the type proposed by the above Committees. The current (revised) IUPAC Commission report[226] no longer includes the earlier erroneous proposal.

diphospho-sugar substrates. The importance of this new finding is that it provides a well-tested and much needed probe into the mechanisms of the multitude of biologically important syntheses catalyzed by these enzymes.

The concise terminology of the extended glycosyl transfer model[224] continues to allow information gained with glycosidases to help advance understanding of the biologically significant nucleotide-diphospho-glycosyltransferases. Five such enzymes (one a member of a very large family that is responsible for glycosylating diverse proteins and lipids) were recently described in terms of their crystal structures. These structures were solved[236–241] by employing crystallographic techniques already used for many glucosidases. As the structures of many more NDP-type enzymes become available, comparison with the reported structures of various glycosidases and long-known glycosyltransferases will bring new insights into their catalytic mechanisms and allow the rational design of potentially therapeutic or agriculturally useful enzyme inhibitors. Several fundamental questions also may be resolved by such comparisons. Are the paired catalytic groups, typically positioned in glycosidases so that the distance between them is smaller in 'retaining' than in 'inverting' enzymes, also found in the NDP-type glycosyltransferases? Do the latter enzymes possess structural features that govern the orientation of acceptors to the catalytic center and hence the enzyme's stereospecific behavior, as they evidently do for various ligand-complexed glycosidases and long familiar glycosyltransferases?[242]

In summation, the concept of enzymically catalyzed glycosyl transfer has served biochemistry extremely well during the last half-century. It has integrated, in a chemically meaningful way, an enormous range of syntheses of complex saccharides that have sophisticated functional roles in living matter. Additional great contributions have been made via the widened glucosyl transfer concept which has greatly facilitated the transfer of information obtained with glycosidases to help advance knowledge of the mechanisms and of the factors underlying the stereochemical behavior of glycosylases. In view of its major contributions to the rise of modern carbohydrate biochemistry, the glycosyl transfer concept surely deserves to have the history of its development recorded as the century ends.

Acknowledgements

Grateful thanks are due to several long-time colleagues and friends: Professor Fred Brewer (Albert Einstein College of Medicine) and Professor David S. Feingold (University of Pittsburgh School of Medicine) for encouraging this project and providing helpful comments on the manuscript; to Professor Jochen Lehmann (Freiburg University) for help with a section of Freudenberg's paper on cyclodextrin formation from starch, and Professor Gentaro Okada (Shizuoka University) for translations of sections of the 1942 article by Miwa et al. on the synthesis of alkyl β-glucosides by soluble plant enzymes.

References

1. *Chemistry in Medicine*; Steglitz, J., Ed.; The Chemical Foundation: New York, 1929; p. 757.
2. Neill, J. M.; Hehre, E. J.; Sugg, J. Y.; Jaffe, E. *J. Exp. Med.* **1939**, *70*, 427–442.
3. Neill, J. M.; Sugg, J. Y.; Hehre, E. J.; Jaffe, E. *Am. J. Hyg.* **1941**, *34*, 65–78.
4. Sugg, J. Y.; Hehre, E. J. *J. Immunol.* **1942**, *43*, 119–128.
5. Fowler, F. L.; Buckland, I. K.; Brauns, F.; Hibbert, H. *Can. J. Res.* **1937**, *15B*, 486–497.
6. Peat, S.; Schlucterer, E.; Stacey, M. *J. Chem. Soc.* **1939**, 581–585.
7. Hassid, W. Z.; Barker, H. A. *J. Biol. Chem.* **1940**, *134*, 163–170.
8. Hill, A. C. *J. Chem. Soc.* **1898**, *43*, 634–658.
9. Emmerling, O. *Chem. Ber.* **1901**, *34*, 600 2206–2207, 3810–3811.
10. Hill, A. C. *Chem. Ber.* **1901**, *34*, 1380–1384.
11. Hill, A. *J. Chem. Soc.* **1903**, *83*, 578–598.
12. Armstrong, E.-F. *Proc. R. Soc. B* **1905**, *76*, 592–599.
13. Fisher, E.; Armstrong, E.-F. *Chem. Ber.* **1902**, *35*, 3144–3153.
14. van't Hoff, J. A. *Sitz. d. k. Preus. Akad. Wiss.* **1910**, *48*, 963–970.
15. Visser, A. W. *Z. Phys. Chem.* **1905**, *52*, 257.
16. Bayliss, W. M. *J. Physiol.* **1911**, *43*, 455–466.
17. Smith, R. G.; Steel, T. *J. Soc. Chem. Ind.* **1902**, *21*, 1381–1385.
18. Lechevelier, H. A.; Solotorovsky, M. *Three Centuries of Microbiology*; McGraw Hill: New York, 1965; pp. 275–289.

19. Beijerinck, M. W. In Koninkl. Acad. v. Wetensch., Proc. Sect. Sci. XII part 2 (English edition); Amsterdam, 1910, pp. 635–640, 795–798.
20. Beijerinck, M. W. *Folia Microbiol.* **1912**, *1*, 377–401; *Chem. Abstr.* **1912**, *8*, 944.
21. Harrison, F. C.; Tarr, H. L. A.; Hibbert, H. *Can. J. Res.* **1930**, *3*, 449–463.
22. Dienes, L. *J. Infect. Dis.* **1935**, *57*, 22–45.
23. Lwoff, A. In *The Viruses*; Burnet, F. M.; Stanley, W. M., Eds.; Academic: New York, 1959; Vol. 3, p. 1888.
24. Bourquelot, E.; Bridel, M. *J. Pharm. Chim.* **1912**, *5*, 388–392.
25. Bourquelot, E..; Bridel, M. *J. Pharm. Chim.* **1912**, *6*, 56–62 97–103, 164–169, 193–199, 298–301, 442–445.
26. Bourquelot, E.; Bridel, M. *J. Pharm. Chim.* **1913**, *8*, 489 547–553.
27. Bourquelot, E.; Bridel, M.; Aubry, A. *J. Pharm. Chim.* **1913**, *8*, 411.
28. Bourquelot, E. *J. Pharm. Chim.* **1914**, *10*, 361–412.
29. Bourquelot, E. *Ann. Chim.* **1915**, *3*, 287.
30. Bourquelot, E. *Rev. Gen. Sci.* **1920**, *31*, 745–752.
31. Bourquelot, E.; Herissey, H.; Coirre, J. *C. R. Acad. Sci.* **1914**, *157*, 732–734.
32. Bourquelot, E.; Aubry, A. *J. Pharm. Chim.* **1916**, *14*, 65–78.
33. Bourquelot, E.; Bridel, M.; Aubry, A. *J. Pharm. Chim.* **1917**, *16*, 353.
34. Bourquelot, E.; Aubry, A. *C. R. Acad. Sci.* **1917**, *164*, 443–445, 521–523; *Chem. Abstr.* **1917**, *11*, 1645, 1954.
35. Bourquelot, E.; Bridel, M. *C. R. Acad. Sci.* **1919**, *168*, 253–256, 1016–1019; *Chem. Abstr.*, **1919**, *13*, 1209–1210.
36. Bridel, M. *Bull. Soc. Chim. Biol.* **1922**, *4*, 329–354.
37. Rabate, J. *Bull. Soc. Chim. Biol.* **1935**, *17*, 572–601.
38. Fleury, P.; Courtois, J.-E. *Bull. Soc. Chim. Biol.* **1959**, *41*, 933–957 esp. 949.
39. Courtois, J.-E. *Bull. Soc. Chim. Biol.* **1863**, *42*, 1865 1960.
40. Rabate, J. *C. R. Acad. Sci.* **1937**, *204*, 153–155.
41. Rabate, J. *Bull. Soc. Chim. Biol.* **1938**, *20*, 449–453.
42. Rabate, J. In *Die Methoden d. Fermentforschung*; Baumann, E.; Myrbäck, K., Eds., 5th ed.; Thieme: Leipzig, 1940; pp. 1818–1827 esp. 1823–1827.
43. Bacon, J. S. D. *Ann. Rep. Prog. Chem.* **1954**, *50*, 281–287 esp. 281.
44. Hassid, W. Z.; Ballou, C. E. In *The Carbohydrates*; Pigman, W., Ed.; Academic: New York, 1957; pp. 478–535 esp. 528.
45. Stanek, J.; Černý, M.; Pacak, J. *The Oligosaccharides*; Czechoslavakia Academy of Science: Prague, 1965; p. 122.
46. Miwa, T.; Takano, K.; Mafune, K.; Furutani, S. *Proc. Jpn. Acad.* **1948**, *25*, 111–115.
47. Kalckar, H. M. In *The Mechanism of Enzyme Action*; McElroy, W. D.; Glass, B., Eds.; Johns Hopkins: Baltimore, 1954; pp. 675–739 esp. 677.
48. Koshland, Jr., D. E.; Stein, S. S. *J. Biol. Chem.* **1954**, *208*, 139–148.
49. Morton, R. K. *Discuss. Faraday Soc.* **1955**, *20*, 149–156 esp. 149.
50. Courtois, J.-E.; Leclerc, M. *Bull. Soc. Chim. Biol.* **1956**, *38*, 365–375.
51. Stone, B. A. In *Biochemists Handbook*; Long, C., Ed.; van Nostrand: New York, 1961; p. 224.
52. Courtois, J.-E.; Perles, R. In *Traite de Biochimie Générale*; Boulanger, P.; Polonovski, J., Eds.; Masson: Paris, 1964; Vol. 2, pp. 224–395 esp. 303.
53. Courtois, J.-E.; Percheron, F. In *The Carbohydrates*; Pigman, W.; Horton, D., Eds., 2nd ed.; Academic: New York, 1970; pp. 213–240.
54. Dedonder, R. *Ann. Rev. Biochem.* **1961**, *30*, 347–382 esp. 347.
55. Bourne, E. J.; Macey, A.; Peat, S. *J. Chem. Soc.* **1945**, 882–888.
56. Peat, S.; Bourne, E. J.; Barker, S. *Nature* **1948**, *161*, 127–128.
57. Parnas, J. K.; Baranowski, T. *C. R. Soc. Biol.* **1935**, *120*, 307–310.
58. Parnas, J.-K. *Ergeb. Enzymforsh.* **1937**, *6*, 57–110 esp. 69, 81–82.
59. Cori, C. F.; Cori, G. T. *Proc. Soc. Exp. Biol. Med.* **1936**, *34*, 702–705.
60. Cori, C. F.; Colowick, S. P.; Cori, G. T. *J. Biol. Chem.* **1938**, *123*, 375–380.
61. Cori, G. T.; Colowick, S. P.; Cori, C. F. *J. Biol. Chem.* **1938**, *123*, 381–389.
62. Kiessling, W. *Naturwissenschaften* **1939**, *27*, 129–130.
63. Kiessling, W. *Biochem. Z.* **1939**, *302*, 50–72.
64. Schäffner, A.; Specht, H. *Naturwissenschaften* **1938**, *26*, 494–495.
65. Cori, C. F.; Schmidt, H.; Cori, G. T. *Science* **1939**, 464–465.
66. Cori, G. T.; Cori, C. F.; Schmidt, G. *J. Biol. Chem.* **1939**, *129*, 629–639.
67. Cori, G. T.; Cori, C. F. *J. Biol. Chem.* **1939**, *131*, 397–398.
68. Cori, G. T.; Cori, C. F. *J. Biol. Chem.* **1940**, *135*, 733–756.
69. Ostern, P.; Holmes, E. *Nature* **1939**, *144*, 34.
70. Hanes, C. S. *Nature* **1940**, *145*, 348–349.
71. Hanes, C. S. *Proc. R. Soc. (Lond.)* **1940**, *B129*, 174–208.
72. Freudenberg, K.; Schaaf, E.; Dumpert, G.; Ploetz, T. *Naturwissenschaften* **1939**, *27*, 850–853; *Chem. Abstr.* **1940**, *34*, 2632.
73. Tilden, E. B.; Hudson, C. S. *J. Am. Chem. Soc.* **1939**, *61*, 2900–2902.
74. Hanes, C. S. *New Phytol.* **1937**, *36*, 189–239.
75. Kuhn, R.; Dansi, A. *Ber. Deutsch. Chem. Ges.* **1936**, *69*, 1745–1754.
76. Tilden, E. B.; Hudson, C. S. *J. Bact.* **1942**, *43*, 527–544.
77. Tilden, E. B.; Hudson, C. S. *J. Am. Chem. Soc.* **1942**, *64*, 1432–1433.
78. McClenehan, W. S.; Tilden, E. B.; Hudson, C. S. *J. Am. Chem. Soc.* **1942**, *64*, 2139–2144.
79. Kerr, R. W. *J. Am. Chem. Soc.* **1942**, *64*, 3044–3045.
80. French, D.; Rundle, R. E. *J. Am. Chem. Soc.* **1942**, *64*, 1651–1653.
81. Wilson, Jr., E. J.; Schoch, T. J.; Hudson, C. S. *J. Am. Chem. Soc.* **1943**, *65*, 1380–1383.
82. Meyer, K. H. *Adv. Enzymol.* **1943**, *3*, 109–135.
83. Cori, C. F. *Fed. Proc.* **1945**, *4*, 226.
84. Myrbäck, K.; Györling, L.-G. *Arkiv Kemi., Minerol., Geol.* **1945**, *A20*, 1–13 esp. 2.
85. Myrbäck, K.; Järneström, T. *Arkiv. Kemi.* **1948**, *1*, 129–143 esp. 129–130.
86. Myrbäck, K. *Adv. Carbohydr. Chem.* **1948**, *3*, 252–310.
87. French, D.; Pazur, J.; Levine, M. L.; Norberg, E. *J. Am. Chem. Soc.* **1948**, *70*, 3145.
88. Barker, S. A.; Bourne, E. J. *Q. Rev. Chem. Soc.* **1963**, *VII*, 56–83, esp. 64.
89. French, D. *Adv. Carbohydr. Chem.* **1957**, *12*, 189–260 esp. 225.
90. Hassid, W. Z. *Fed. Proc.* **1945**, *4*, 227–241.

91. Clarke, R. J.; Coates, J. H.; Lincoln, S. F. *Adv. Carbohydr. Chem. Biochem.* **1988**, *46*, 205–249 esp. 215–217.
92. Hehre, E. J. *Adv. Enzymol.* **1951**, *11*, 297–337.
93. Hehre, E. J.; Mizokami, K.; Kitahata, S. *Dempun Kagaku (Jpn. J. Starch Sci.)* **1983**, *30*, 76–82.
94. Miwa, T.; Mafune, K.; Furuya, T. *Igaku to Seibutsugaku (Medicine Biol.)* **1942**, *1*, 80–84 (in Japanese). Nisizawa K. *Sorui (Jpn. J. Phycol.)* **1980**, *28*, 201–204.
95. Hehre, E. J. *Science* **1941**, *93*, 237–238.
96. Hehre, E. J.; Sugg, J. Y. *J. Exp. Med.* **1942**, *75*, 339–353.
97. Stacey, M. *Nature* **1942**, *149*, 639.
98. Hestrin, S.; Avineri-Shapiro, S. *Biochem. J.* **1944**, *38*, 2–10.
99. Lineweaver, H.; Jansen, A. F. *Ann. Rev. Biochem.* **1945**, *14*, 69–90.
100. Doudoroff, M. *Fed. Proc.* **1945**, *4*, 241–247.
101. Kalckar, H. M. *Nature* **1947**, *160*, 143–147.
102. Hassid, W. Z.; Doudoroff, M. *Adv. Carbohydr. Chem. Biochem.* **1950**, *5*, 29–48.
103. Stodola, F. H. *Chemical Transformations by Microorganisms*; Wiley: New York, 1971; p. 71.
104. Mooser, G. In *Mechanisms of Catalysis*; Sigman, D. S., Ed. The enzymes, 3rd ed.; Academic: New York, 1992; Vol. 20, p. 210.
105. Kagan, B. O.; Latker, S. N.; Zfasman, E. M. *Biokhimiya* **1942**, *7*, 93–108 (In Russian).
106. Hehre, E. J. *Proc. Soc. Biol. Med.* **1943**, *54*, 240–241.
107. Schramm, M. *Bull. Res. Counc. Isr.* **1963**, *IIA*, 243–244 (Issue dedicated to the memory of Professor Shlomo Hestrin).
108. Hestrin, S.; Avineri-Shapiro, S.; Aschner, M. *Biochem. J.* **1943**, *37*, 450–456.
109. Hestrin, S.; Avineri-Shapiro, S. *Nature* **1943**, *152*, 49–50.
110. Hestrin, S. *Nature* **1944**, *154*, 581.
111. Sumner, J. B.; Summers, G. F. *Chemistry and Methods of Enzymes*, 2nd ed.; Academic: New York, 1947, xiv.
112. Evans, T. H.; Hibbert, H. *Adv. Carbohydr. Chem.* **1946**, *2*, 204–233.
113. Whistler, R. L.; Smart, C. L. *Polysaccharide Chemistry*; Academic: New York, 1953; p. 378, 385.
114. Haworth, W. N.; Heath, R. L.; Peat, S. *J. Chem. Soc.* **1942**, 55–58.
115. Green, A. A.; Cori, G. T.; Cori, C. F. *J. Biol. Chem.* **1942**, *142*, 447–448.
116. Green, A. A.; Cori, G. T.; Cori, C. F. *J. Biol. Chem.* **1943**, *151*, 21–29.
117. Hassid, W. Z.; Cori, G. T.; McCready, R. M. *J. Biol. Chem.* **1943**, *148*, 89–96.
118. Cori, G. T.; Cori, C. F. *J. Biol. Chem.* **1943**, *151*, 57–63.
119. Doudoroff, M.; Kaplan, N.; Hassid, W. Z. *J. Biol. Chem.* **1943**, *148*, 67–75.
120. Doudoroff, M. *J. Biol. Chem.* **1943**, *151*, 351–361.
121. Hassid, W. Z.; Doudoroff, M.; Barker, H. A. *J. Am. Chem. Soc.* **1944**, *66*, 1416–1419.
122. Hudson, C. S. *J. Am. Chem. Soc.* **1908**, *30*, 1564–1583.
123. Weidenhagen, R. *Ergeb. Enzymforsch.* **1933**, *2*, 90–103.
124. Lemieux, R. U.; Huber, G. *J. Am. Chem. Soc.* **1956**, *78*, 4117–4119.
125. Doudoroff, M.; Hassid, W. Z.; Barker, H. A. *Science* **1944**, *100*, 315–316.
126. Kalckar, H. M. *Fed. Proc.* **1945**, *4*, 248–252.
127. Kalckar, H. M. *J. Biol. Chem.* **1947**, *167*, 477–486.
128. Doudoroff, M. *Fed. Proc.* **1945**, *4*, 241–247.
129. Hassid, W. Z.; Doudoroff, M.; Barker, H. A.; Dore, W. H. *J. Am. Chem. Soc.* **1945**, *67*, 1465–1467.
130. Raybin, H. W. *J. Am. Chem. Soc.* **1933**, *55*, 2603–2604.
131. Raybin, H. W. *J. Am. Chem. Soc.* **1937**, *59*, 1402–1403.
132. Hehre, E. J. *J. Biol. Chem.* **1946**, *163*, 221–233.
133. Hehre, E. J.; Hamilton, D. M. *J. Biol. Chem.* **1946**, *166*, 777–778.
134. Doudoroff, M.; Barker, H. A.; Hassid, W. Z. *J. Biol. Chem.* **1947**, *168*, 725–732.
135. Koshland, Jr., D. E. *Biol. Rev. Cambridge Phil. Soc.* **1953**, *28*, 416–436.
136. Koshland, Jr., D. E. In *The Mechanism of Enzyme Action*; McElroy, W. D.; Glass, B., Eds.; John Hopkins: Baltimore, 1954; pp. 608–641.
137. Doudoroff, M.; O'Neal, R. *J. Biol. Chem.* **1945**, *159*, 585–592.
138. Bell, D. J. *Ann. Rep. Chem. Soc.* **1947**, *44*, 217–226.
139. Hassid, W. Z.; Doudoroff, M. *Fortschr. Chem. Org. Naturstoffe* **1948**, *5*, 101–127.
140. Hestrin, S. *Brewer's Digest* **1948**, *23*, 1–4 8.
141. Hehre, E. J. *Trans. New York Acad. Sci., Ser. 11* **1948**, *10*, 188–198.
142. Hehre, E. J.; Hamilton, D. M. *J. Bact.* **1948**, *55*, 197–208.
143. Hehre, E. J.; Hamilton, D. M.; Carlson, A. S. *J. Biol. Chem.* **1949**, *177*, 267–279.
144. Monod, J.; Torriani, A.-M. *C. R. Acad. Sci.* **1948**, *227*, 240–241.
145. Torriani, A.-M.; Monod, J. *C. R. Acad. Sci.* **1949**, *228*, 718–720.
146. Doudoroff, M.; Hassid, W. Z.; Putman, E. W.; Potter, A. L.; Lederberg, J. *J. Biol. Chem.* **1949**, *179*, 921–934.
147. Hassid, W. Z.; Doudoroff, M.; Barker, H. A. *Arch. Biochem. Biophys.* **1947**, *14*, 29–37.
148. Cohn, M. *J. Biol. Chem.* **1949**, *180*, 771–781.
149. Myrbäck, K.; Neumüller, G. In *The Enzymes*, *Part 1*; Sumner, J. B.; Myrbäck, K., Eds.; Academic: New York, 1950; Vol. 1, pp. 653–724.
150. Barker, S. A.; Bourne, E. J.; Peat, S. *J. Chem. Soc.* **1949**, 1712–1716.
151. Peat, S. *Adv. Enzymol.* **1951**, *11*, 339–375.
152. Larner, J. *J. Biol. Chem.* **1953**, *202*, 491–503.
153. Bacon, J. S. D.; Edelman, J. *Arch. Biochem. Biophys.* **1950**, *28*, 467–468.
154. Blanchard, P. H.; Albon, N. *Arch. Biochem. Biophys.* **1950**, *29*, 220–222.
155. Takano, K.; Miwa, T. *J. Biochem.* **1950**, *37*, 435–444.
156. Fisher, E. H.; Kohtes, J.; Fellig, J. *Helv. Chim. Acta* **1951**, *34*, 1132–1138.
157. Wallenfels, K. *Naturwissenschaften* **1951**, *38*, 306–307.
158. Pazur, J. H.; French, D. *J. Biol. Chem.* **1952**, *196*, 265–272.
159. Whitby, L. G. *Biochem. J.* **1952**, *50*, 433–438.
160. Pazur, J. H. *J. Biol. Chem.* **1952**, *199*, 217–225.
161. Aronson, M. *Arch. Biochem. Biophys.* **1952**, *39*, 370–378.
162. Pan, S. C.; Nicholson, L. W.; Kolachov, P. *Arch. Biochem. Biophys.* **1953**, *42*, 406–420.
163. White, Jr., J. W.; Maher, J. *Arch. Biochem. Biophys.* **1953**, *42*, 360–367.
164. Wallenfels, K.; Bernt, E. *Liebigs Ann.* **1953**, *584*, 63–85.
165. Roberts, H. R.; McFarren, E. F. *Arch. Biochem. Biophys.* **1953**, *43*, 233–234.
166. Pazur, J. H. *J. Am. Chem. Soc.* **1953**, *75*, 6323–6324.
167. Takano, K.; Miwa, T. *J. Biochem.* **1953**, *40*, 471–476.
168. Giri, K. V.; Narasimha Rao, P. L.; Saroja, K.; Venkataraman, R. *Naturwissenschaften* **1953**, *40*, 484–485.

169. Giri, K. V.; Nigam, V. N.; Srinivasan, K. S. *Nature* **1954**, *173*, 953–954.
170. Giri, K. V.; Nagabhushanam, A.; Nigam, V. N.; Belavadi, B. *Science* **1955**, *121*, 898–899.
171. Miwa, T.; Takano, K.; Mafune, K.; Furutani, S. *Proc. Jpn. Acad.* **1949**, *25*, 111–115.
172. Miller, K. D.; Copeland, W. H. *Biochim. Biophys. Acta* **1956**, *22*, 193–194.
173. Miwa, T.; Takano, K.; Yasumura, A.; Suzuki, H.; Isizawa, K. *Symp. Enzyme Chem. (Jpn.)* **1956**, *11*, 225–232.
174. Suzuki, H. *Sci. Rep., Toyko Kyoiku Daigaku, Sect. B* **1957**, *8*, 80–94.
175. Sawai, T. *J. Biochem.* **1958**, *45*, 50–56.
176. Miller, K. D. *J. Biol. Chem.* **1958**, *231*, 987–995.
177. Miller, K. D.; Copeland, W. H. *J. Biol. Chem.* **1958**, *231*, 999–1008.
178. Stetten, M. R. *J. Am. Chem. Soc.* **1959**, *81*, 1437–1447.
179. Petrova, A. N. *Enzymologia* **1959**, *21*, 23–31.
180. Miwa, T.; Takeshita, M.; Nakamura, S. *Biochim. Biophys. Acta* **1960**, *37*, 541–542.
181. Matsubara, S. *J. Biochem.* **1961**, *49*, 226–231.
182. Sawai, T.; Hehre, E. *J. Bacteriol. Proc.* **1960**, 161; *J. Biol. Chem.* **1962**, *237*, 2047–2052.
183. Hassid, W. Z.; Doudoroff, M. *Adv. Carbohydr. Chem.* **1950**, *5*, 29–48.
184. Hassid, W. Z.; Doudoroff, M. *Adv. Enzymol.* **1950**, *10*, 123–143 mistakenly attribute the enzymic formation of dextran from sucrose to Fowler et al.[5] and Kagan et al.[105].
185. Hassid, W. Z.; Doudoroff, M.; Barker, H. A. In *Enzymes, Part 2*; Sumner, J. B.; Myrbäck, K., Eds.; Academic: New York, 1951; Vol. 1, pp. 1014–1039.
186. Hassid, W. Z. In *Phosphorus Metabolism*; McElroy, W. D.; Glass, B., Eds.; Johns Hopkins: Baltimore, 1951; Vol. 1, pp. 11–42.
187. Pictet, A.; Castan, P. *Helv. Chim. Acta* **1921**, *4*, 319–324.
188. American Chemical Society, Committee Report on Rules of Carbohydrate Nomenclature. *Chem. Eng. News* **1948**, *26*, 1623–1626.
189. Cohn, M. In *Creative Couples in the Sciences*; Pychior, H. M.; Slack, N. G.; Abir-Am, P. G., Eds.; Rutgers University: New Brunswick, NJ, 1996; pp. 72–84 304–305.
190. Ballou, C.; Barker, H. A. *Natl. Acad. Sci.* **1979**, *50*, 187–230.
191. French, D.; Levine, M. L.; Norberg, E.; Nordin, P.; Pazur, J. H.; Wild, G. M. *J. Am. Chem. Soc.* **1954**, *76*, 2387–2390.
192. Hassid, W. Z. In *Chemical Pathways of Metabolism*; Greenberg, D., Ed., 1st ed.; Academic: New York, 1954; Vol. 1, pp. 235–275 esp. 245, 251.
193. Bourne, E. J.; Biochemistry Society Symposium no. II; Cambridge University, 1953; pp. 3–16.
194. Bacon, J. S. D. *Ann. Rep. Progr. Chem.* **1954**, *50*, 281–287.
195. Hoffmann-Ostenhof, O. *Adv. Enzymol.* **1953**, *14*, 219–260.
196. Hehre, E. J. *J. Biol. Chem.* **1951**, *192*, 161–174.
197. Hestrin, S. In *Biological Structure and Function*; Goodwin, T. W.; Lindberg, O., Eds.; Academic: London, 1961; Vol. 1, pp. 315–325.
198. Hestrin, S.; Feingold, D. S.; Avigad, G. *Biochem. J.* **1956**, *64*, 340–351.
199. Miwa, T. *(Tampaku, Kakusan, Kohso (Protein, Nucleic Acid and Enzyme)* **1957**, *2*, 35–41 (in Japanese).
200. Whistler, R. L.; Corbett, W. M. In *The Carbohydrates*; Pigman, W., Ed.; Academic: New York, 1957; pp. 641–708 esp. 703.
201. Leloir, L. F.; Cardini, C. E.; Cabib, E. In *Comparative Biochemistry*; Florkin, M.; Mason, H. S., Eds.; Academic: New York, 1960; Vol. 2, pp. 97–138.
202. Hassid, W. Z.; Neufeld, E. F. In *The Enzymes*; Boyer, P. D.; Lardy, H.; Myrbäck, K., Eds.; Academic: New York, 1962; Vol. 6, pp. 277–315.
203. Pazur, J. H. In *Starch: Chemistry and Technology*; Whistler, R.; Paschall, E. F., Eds.; Academic: New York, 1965; pp. 133–173.
204. Hassid, W. Z. In *The Structure and Bisynthesis of Macromolecules*; Bell, D. J.; Grant J. K., Eds.; Biochemical Society Symposia, No. 21, 1962; pp. 63–79, esp. 64.
205. Hassid, W. Z. In *Metabolic Pathways*; Greenberg, D. M., Ed., 3rd ed.; Academic: New York, 1967; Vol. 1, pp. 307–393 esp. 315.
206. Florkin, M. In *Comparative Biochemistry*; Florkin, M.; Stotz, E. H., Eds.; Elsevier: New York, 1979; Vol. 33A, pp. 141–169 esp. 143.
207. Dedonder, R. In *Biochemistry of the Glycosidic Linkage*; Piras, R.; Pontes, H. G., Eds.; Academic: New York, 1972; p. 23.
208. Leloir, L. F.; Cabib, E. *J. Am. Chem. Soc.* **1953**, *75*, 5445.
209. Cabib, E.; Leloir, L. F. *J. Biol. Chem.* **1958**, *231*, 259–275.
210. Leloir, L. F.; Cardini, C. E. *J. Am. Chem. Soc.* **1953**, *75*, 6084.
211. Leloir, L. F.; Cardini, C. E.; Chiriboga, J. *J. Biol. Chem.* **1955**, *214*, 149–155.
212. Cardini, C. E.; Leloir, L. F. *J. Biol. Chem.* **1955**, *214*, 157–165.
213. Feingold, D. S.; Neufeld, E. F.; Hassid, W. Z. *J. Biol. Chem.* **1958**, *233*, 783–788.
214. Kornberg, S. R.; Zimmerman, S. B.; Kornberg, A. *J. Biol. Chem.* **1961**, *236*, 1487–1493.
215. Josse, J.; Kornberg, A. *J. Biol. Chem.* **1962**, *237*, 1968–1972.
216. Lieberman, I.; Kornberg, A.; Simms, E. S. *J. Biol. Chem.* **1955**, *215*, 403–415.
217. Kornberg, A. *Adv. Enzymol.* **1957**, *18*, 191–237.
218. Lehman, I. R.; Bessman, M. J.; Simms, E. S.; Kornberg, A *J. Biol. Chem.* **1958**, *233*, 163–170.
219. Schachman, H. K.; Adler, J.; Radding, C. M.; Lehman, I. R.; Kornberg, A. *J. Biol. Chem.* **1960**, *235*, 3242–3253.
220. Koshland, Jr., D. E. In *The Enzymes*; Boyer, P. D.; Lardy, H.; Myrbäck, K., Eds., 2nd ed.; Academic: New York, 1959; Vol. 1, pp. 305–346.
221. Bunton, C. A.; Lewis, T. A.; Llewellyn, D. R.; Tistram, H.; Vernon, C. A. *Nature* **1954**, *174*, 560.
222. Hehre, E. J. *Bull. Soc. Chim. Biol.* **1960**, *42*, 1713–1714.
223. Hehre, E. J.; Genghof, D. S.; Okada, G. *Arch. Biochem. Biophys.* **1971**, *142*, 382–393.
224. Hehre, E. J.; Okada, G.; Genghof, D. S. In *Advanced Chemical Series*; Gould, R. F., Ed. Carbohydrates in solution. 1973; Vol. 117, pp. 309–333.
225. IUPAC Commission on the Nomenclature of Organic Chemistry (CNOC) and IUPAC-IUB Commission on Biochemical Nomenclature (CBN). *Eur. J. Biochem.* **1971**, *21*, 455–477.
226. IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (JCBN). *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 1–90.

227. Sinnott, M. L. In *The Chemistry of Enzyme Action*; Page, M. I., Ed.; Elsevier: Amsterdam, 1984; pp. 389–431.
228. Hehre, E. J. *Denpun Kagaku (Jpn. J. Starch Sci.)* **1989**, *36*, 197–205.
229. Hehre, E. J. In *Enzymatic Degradation of Insoluble Carbohydrates*; Saddler, J. N.; Penner, M. H., Eds.; ACS Symposium Series 618; American Chemical Society: Washington, DC, 1995; pp. 66–78.
230. Sinnott, M. L. In *Enzyme Mechanisms*; Page, M. I.; Williams, A., Eds.; Royal Society of Chemistry: London, 1987; pp. 259–287.
231. Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171–1202.
232. Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319–384.
233. Chiba, S. In *Enzyme Chemistry and Molecular Biology of Amylases and Related Enzymes*; Amylase Research Society of Japan, Ed.; CRC: Tokyo, 1994; pp. 68–82.
234. Davies, G.; Sinnott, M. L.; Withers, S. G. In *Comprehensive Biological Catalysis*; Sinnott, M. L., Ed.; Academic: New York, 1998; Vol. 1, pp. 119–209.
235. Lougheed, B.; Ly, H. D.; Wakarchuk, W. W.; Withers, S. G. *J. Biol. Chem.* **1999**, *274*, 37717–37722.
236. Vrielink, A.; Rüger, W.; Driessen, H. P. C.; Freemont, P. S. *EMBO J.* **1994**, *13*, 3413–3422.
237. Morera, S.; Imberty, A.; Aschke-Sonnenborn, U.; Rüger, W.; Freemont, P. S. *J. Mol. Biol.* **1999**, *292*, 717–730.
238. Charnock, S. J.; Davies, G. J. *Biochemistry* **1999**, *38*, 6380–6385.
239. Gastinel, L. N.; Cambillau, C.; Bourne, Y. *EMBO J.* **1999**, *18*, 3546–3557.
240. Ha, S.; Walker, D.; Shi, Y.; Walker, S. *Protein Sci.* **2000**, *9*, 1045–1052.
241. Ünligel, U. M.; Rini, J. M. *Curr. Opin. Struct. Biol.* **2000**, *10*, 510–517.
242. Hehre, E. J. *Adv. Carbohydr. Chem. Biochem.* **1999**, *55*, 265–310; Abstr 214th National Meeting of the American Chemical Society: Las Vegas, NV, September 8, 1997; CARB 5.