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PREFACE

Amino sugars continue to play a major role in the overall carbohydrate field, and they present particular challenges for the synthetic chemist seeking to effect useful and controllable transformations toward targets of biological importance. Presented in this volume are two articles that provide complementary viewpoints on methodology for the manipulation of nitrogen functionality in sugar derivatives. The article by Karban and Kroutil (Prague) offers for the first time a comprehensive account of the chemistry of sugar aziridines (epimines), emphasizing preparative methods for introduction of the three-membered aziridine ring into a sugar framework, and on ring-opening reactions under controlled conditions to afford defined targets.

Glycosyl azides form the focus of the article by Gyorgydeák (Debrecen) and Thiem (Hamburg), furnishing a detailed survey of methods for introducing the azide group at the anomeric center, together with the wide range of transformations possible with this versatile and highly reactive functional group in both the monosaccharide framework and also in complex oligosaccharide structures related to glycopeptides and glycoproteins. Sadly, Zoltán Gyorgydeák, a prolific contributor to the carbohydrate literature and coauthor of the book "Monosaccharide Sugars" (1998, Academic Press) died after this article was completed.

When in 1928 Louis Malaprade described the oxidation of ethylene glycol and some other polyalcohols with periodic acid, he could never have envisaged the importance that this glycol-cleavage reaction was later to attain in the carbohydrate field. As a tool for determining structure in polysaccharides and glycoconjugates it now features in so many publications that it would be impossible to cover all of its applications in a single article. The fundamentals of the glycol-cleavage reaction, with both periodate and lead tetraacetate, in all types of carbohydrate structure, are surveyed authoritatively here by Perlin (Montreal), himself a pioneer who has made major contributions to our knowledge of the subject. His article provides a thorough basis for understanding the scope and potential limitations in applying the reaction, and gives a clear explanation of the widely used (but frequently misunderstood) Smith degradation procedure for structure determination.

The amino sugar theme reappears in a very different context in the article by Willis and Arya (Clemson, SC) dealing with the aminoglycoside antibiotics, some sixty years after the discovery of streptomycin and three decades after the

landmark articles in Volume 30 of this series by the Umezawa brothers who contributed so much to our knowledge of structure, synthesis, and mechanism of bacterial resistance to these antibiotics. The authors here explore the complex area of interaction of aminoglycosides with nucleic acids, from early concepts of ribosomal binding and disruption of the translation message to mRNA to our present recognition of the multiplicity of the interactions of these basic molecules with many forms of RNA and also DNA. This understanding has key significance in efforts to develop new, less toxic, therapeutic agents effective against resistant bacteria.

Many problems in current carbohydrate science require a multidisciplinary approach, as demonstrated here by the joint Spanish—German group coordinated by Jiménez-Barbero (Madrid) and a large group of coauthors, who address the question of noncovalent interactions between carbohydrates and proteins. In the article they explore both hydrophilic and hydrophobic interactions, as revealed by a wide range of experimental and computational methods, with particular emphasis on the defense proteins (lectins) of plants and their interactions with the chitin of fungi and other plant pathogens. They show that the lectins share a common structural motif (chitin-binding domain or hevein domain) involved in the defense mechanism, and provide a particularly useful model for studies at the atomic resolution level of the hydrogen-bonding and carbohydrate–aromatic interactions between proteins and carbohydrates.

The biographical article by Lundt and Bock (Copenhagen) gives an account of the life and work of Christian Pedersen, whose contributions on the use of anhydrous hydrogen fluoride as a solvent for studying the reactions of carbohydrates are particularly notable. In an era when most leaders in the field direct the work of large groups of coworkers, Pedersen harks back perhaps to the days of Emil Fischer in that he conducted much of his work with his own hands, and introduced many preparatively useful synthetic procedures without recourse to chromatography.

Much of the early scientific work of the late Aleksander Zamojski focused on the total synthesis of racemic monosaccharides, based on stereocontrolled reactions of substituted dihydropyrans obtained by Diels—Alder cycloaddition. His article in Volume 40 of this series details many of his early studies in this area. His colleagues Jarosz and Chmielewski (Warsaw) here offer a broad insight into Zamojski's contributions, which later extended into chiral structures, higher sugars, and oligosaccharides.

The passing in 2005 is noted of Nikolai Kochetkov, a major figure on the world carbohydrate scene. A detailed account of his life and scientific contributions is scheduled to appear in a later volume.

DEREK HORTON

Washington, DC
January 2006



Christiaan Pedersen



Christiaan Pedersen

CHRISTIAN PEDERSEN

1926–2003

In September 2003 the carbohydrate community lost one of its outstanding scientists in the field with the sudden passing away of Christian Pedersen.

Christian Pedersen was born in Vendsyssel, in the northern part of Denmark. After his basic education in a village school he later had to travel by bicycle and bus to the nearest high school in Aalborg to continue his education, which was not an everyday situation in the village environment where he and his younger sister grew up. In 1946, he obtained his high school certificate and went to Copenhagen to study science at the university. During those years an interest in organic chemistry emerged, and he specialized in heterocyclic chemistry under the guidance of Professor K. A. Jensen. Christian Pedersen obtained his master's degree in 1952, after which he went to London (1953–1954) to work together with Professor Adrian Albert at the National Australian University. Here the subject of his studies was again heterocyclic chemistry, especially the chemistry of pteridines and other azanaphthalenes.

Returning to Denmark, Christian was offered a temporary position at the University of Copenhagen, where he returned to the group of Professor K. A. Jensen and worked for two years (1954–1956). This collaboration resulted in a number of papers dealing with 5-membered heterocyclic compounds, and the study of thio acids and their derivatives resulted in five additional publications. In those years the foundation for an in-depth investigation into azoles was laid. In particular, the chemistry of 1,2,3-triazoles was to become the research profile of Christian Pedersen in subsequent years.

In those days tenured university positions were not regularly announced, and in 1956 Christian Pedersen was without a job. An old friend from the Technical University of Denmark (DTU) went to the professor of the organic department

and told him about a bright scientist he knew and who was interested in “doing some chemistry” in the laboratory. As a consequence Christian Pedersen obtained a permanent position, and in 1958 the first paper on 1,2,3-triazoles appeared: “Rearrangement of 4-Phenylazo-5-hydroxy-1,2,3-triazoles to Amides of 2-Phenyl-5-carboxytetrazole,” followed by a second paper in 1959, both having Pedersen as the only author. Young researchers had only their own hands and brain to rely on, and resources to build up a group were not easily available.

In 1958, a fellowship was open at the National Institute of Health, NIH, in Washington, DC, in the group of Dr. Hewitt G. Fletcher, Jr. Pedersen, with some reservation, applied with success for the fellowship, and this turned out to be a very fruitful scientific stay for two years (1958–1960) with Fletcher’s group. Dr. Fletcher was at that time a well-known carbohydrate chemist, being part of the younger generation of the renowned North American carbohydrate chemists, a field pioneered by C. S. Hudson and N. K. Richtmyer. The immediate reservation from Christian Pedersen’s point of view was his reluctance to enter into carbohydrate chemistry. Sugar molecules were judged difficult to handle, and since no efficient analytical tools and purification methods were available one was more or less dependent on crystallization of products for identification.

The main impact from these years was the introduction of anhydrous hydrogen fluoride both as a very efficient solvent and also an electrophilic reagent in carbohydrate chemistry. The first observation made in Fletcher’s group was the rearrangement of L-arabinopyranose tetrabenzoate to give 3,4-di-*O*-benzoyl- β -L-ribosepyranosyl fluoride. Fletcher had introduced the use of benzoyl groups as alternative protecting groups to the more commonly used acetyl groups, to afford sugar derivatives having improved crystallization properties. This made, for instance, the identification of the crystalline glycosyl fluorides more reliable and convenient.

The rearrangement of carbohydrate esters initiated by anhydrous hydrogen fluoride became a main research topic for many years. Although Christian Pedersen, upon his return to DTU in 1960, continued his studies of 1,2,3-triazoles in collaboration with Ph.D. students Mikael Begtrup and Carl Erik Olsen, his first paper on isomerization of penta-*O*-acetyl- β -D-glucopyranose with hydrogen fluoride appeared in 1962. This paper clearly showed the difficulties related to identification of the reaction products when no crystalline compounds were obtained directly. Predictable conversion into other known products was therefore necessary. Based on the isolated products derived from D-mannose and D-altrose, mechanistic considerations pointed to the involvement of dioxalonylium ions as intermediates in such rearrangement reactions. The tedious unraveling of

complicated reaction mixtures, however, became much more efficient upon the introduction in the early 1960s of thin-layer chromatography, both as an analytical method and as a preparative tool for isolation of pure compounds. In 1965, the Department of Organic Chemistry obtained a 60 MHz NMR instrument, and in combination with improved separation techniques, carbohydrate chemistry entered into a new era. It might not be realized completely by chemists today what a major step forward was reached by the introduction of such methods.

NMR spectroscopy as an analytical tool for determination of carbohydrate structures had at that time slowly burgeoned through the pioneering work by R. U. Lemieux. Of special interest for Christian Pedersen's work was the NMR spectroscopic properties of glycosyl fluorides, which resulted in several publications jointly with Laurance D. Hall.

The proposed involvement of dioxalylium ions in the rearrangements and ring contractions of sugar esters prompted Pedersen, very innovatively, to monitor these reactions directly by measuring ^1H NMR spectra of the mixtures directly in hydrogen fluoride. This was, of course, not without practical challenges, but with the help of an able technician, NMR tubes of Teflon were constructed. These tubes fitted into ordinary glass tubes and ^1H NMR spectra could thus be measured at various low temperatures. With this technique, he and his group clearly demonstrated formation of acetoxonium ions and their rearrangements during dissolution in anhydrous hydrogen fluoride, and the experimental results could thus be explained more convincingly.

A final proof of the existence of an acetoxonium ions in the ^1H NMR spectra was obtained by investigating the reaction of *cis*- and *trans*-1,2-diacetoxycyclohexanes. The *cis* compound reacted to give a dioxalylium ion, which was isolated as a tetrafluoroborate, identical with the salt earlier prepared by Winstein and coworkers. By contrast, the *trans* diacetate did not react at all. This experiment also provided direct demonstration of how the formation of acyloxonium ions may be initiated by the reaction of acylated sugars with hydrogen fluoride.

Dioxalynium ions had also been proposed as reactive intermediates by R. U. Lemieux, S. J. Angyal, H. G. Fletcher Jr. (among others), and later by H. Paulsen in analogous reactions of polyhydroxy compounds with electrophilic reagents, but now their existence was clearly proven.

In 1969, Christian Pedersen defended his *Doctor of Science* dissertation describing these rearrangements, and in the same year he received a full professorship at the DTU.

These studies on hydrogen fluoride reactions were continued, and another important result was the proof of differences in stability among acyloxonium

ions. The acetoxonium ions were shown to be less stable than the benzoxonium ions, and there were also differences in stability between substituted benzoxonium ions. This observation could be used for preparative purposes, since rearrangements in sugar esters could be directed by substituting the sugar at a certain position with a suitable acyl-protecting group, allowing formation of the more stable ion. Thus a rearrangement from an inexpensive sugar derivative to a more valuable target sugar could be designed.

The behavior of dioxallylium ions toward nucleophilic reagents was later investigated in collaboration together with Steffen Jacobsen. These ions were generated by hydride abstraction from sugar benzylidene acetals in acetonitrile. Pedersen's expertise and sustained interest in reactions of carbohydrates in anhydrous hydrogen fluoride led to a long collaboration with Dr. J. Defaye in Grenoble, France. He visited Defaye's laboratory every year in the summer period for one to three months starting in 1979 until his retirement in 1996. The studies performed with the French carbohydrate group were mainly focused on the behavior of different oligosaccharides in anhydrous hydrogen fluoride.

At the DTU, his research was continued from the late 1960s with studies of the behavior of unsaturated sugars and deoxy sugars in HF in conjunction with Ph.D. students Inge Lundt and Klaus Bock. Other electrophilic reagents were also investigated, including hydrogen bromide and hydrogen chloride as well as dibromomethyl methyl ether (with Poul Rasmussen). The most rewarding of these reagents was hydrogen bromide in acetic acid. It was found that treatment of a monoacylated *cis*-1,2-diol with hydrogen bromide in acetic acid afforded the *trans* bromoacyloxy compound. In sugar derivatives, bromine could only be introduced at the primary position of aldofuranoses, as it seemed that only exocyclic acyloxonium ions could be formed. These reactions were thus not of major preparative importance for reducing sugars.

In contrast, bromine could be introduced selectively in unprotected 1,5-anhydroalditols by way of acyloxonium ions. The prerequisite for the formation of such ions was the presence of a 1,2-*cis*-diol motif. This concept had previously been shown by B. T. Golding and coworkers through treatment of *cis* and *trans*-1,2-dihydroxycyclohexanes with hydrogen bromide in acetic acid. The *cis*-1,2-diol gave the *trans* bromo acetate, whereas no bromine was introduced into the *trans*-1, 2-diol under similar reaction conditions; only acetylation occurred. Pedersen showed furthermore that monobenzoylated *cis*- and *trans*-1,2-dihydroxycyclohexanes gave analogous results.

These observations initiated yet another main research topic from the Pedersen group, namely the investigations of reactions between aldonolactones and

hydrogen bromide in acetic acid. The first noteworthy results were presented in a plenary lecture at the International Carbohydrate Symposium in London in 1978, and the first paper on the preparation of bromodeoxyaldonic acids and lactones by Bock, Lundt, and Pedersen appeared in 1979. The chemistry of aldonolactones had not at that time been investigated in detail. With the facile and stereoselective preparation of α,ω -dibromo- α,ω -dideoxyaldonolactones, α -bromo- α -deoxyaldonolactones, or ω -bromo- ω -deoxyaldonolactones, the Pedersen group showed the high potential of these bromodeoxy lactones as useful chiral building blocks. The beauty and versatility of these new compounds resulted from the major difference in reactivity of the α -bromine and the primary ω -bromine atom, making selective reactions at these two positions possible. During subsequent years, the bromodeoxy aldonolactones, and the aldonolactones themselves, have shown great versatility for stereoselective synthesis, both in the carbohydrate field, giving access to otherwise difficultly obtainable sugars, and as chiral, enantiomerically pure building blocks in the broader sense within organic chemistry.

Throughout his scientific career, Christian Pedersen maintained his goal of using readily available starting materials and reagents, and avoiding protecting-group chemistry, in the quest for new methods, reactions, and principles for the preparation of valuable compounds, which most conveniently should be isolable without the need for chromatographic purification or separation. Convenient preparative methods for compounds of value for other scientists could thereby be delivered. The investigations on bromodeoxy lactones fulfilled to a large extent his criteria for valuable synthetic work. Thus, their treatment with aqueous base caused stereoselective rearrangements to yield aldonic lactones with inverted stereochemistry at one or more carbon centers. The mechanism of these rearrangements was elucidated by ^{13}C NMR spectroscopy. The formation of epoxides followed by Payne rearrangements clearly explained the observed inversions at specific stereocenters in the starting molecule.

When the bromodeoxy lactones were boiled in water tetrahydrofurans were formed, whereas catalytic hydrogenation caused deoxygenation at C-2 and/or at the primary position, while the acetylated aldonolactones could also be reduced to 3-deoxylactones after elimination and stereospecific reduction. Substitution by azide introduced nitrogen functions at C-2 or at both bromo-substituted carbons, and these compounds could be converted into the corresponding amino acids. The new molecules could furthermore be reduced to the corresponding aldoses or alditols.

The major difference between halogen substitution at C-2 in aldonolactones and in the corresponding aldose derivatives is the difference in reactivity. In the

lactone, the 2-position is the most reactive site in the molecule, whereas the same position in the sugar is less reactive. The chemistry of aldonolactones, initiated early by the Pedersen group is therefore of great synthetic value.

The use of bromodeoxy lactones as chiral building blocks for the stereoselective preparation of natural compounds was also explored. Thus, both (*S*)- and (*R*)-carnitine, (*S*)- and (*R*)-4-amino-3-hydroxybutanoic acid (GABOB), compounds of high relevance for the central nervous system, as well as muscaridine and 6-deoxyascorbic acid, were prepared by simple transformations of the bromodeoxy aldonolactones.

His last paper, which appeared in 1999 with Christian Pedersen as the only author, was on an improved preparation of *Leptosphaerin*, a marine fungal metabolite, using the very efficient and selective synthetic reactions of aldonolactones.

Christian Pedersen's scientific career coincided with the evolution of carbohydrate chemistry from a subject studied only by specialized, isolated carbohydrate groups to becoming a modern and accepted part of organic chemistry. This was mainly due to improved separation and analytical techniques, and especially the use of NMR spectroscopy as a powerful technique for structural elucidation of organic molecules in general, and in carbohydrate derivatives in particular.

Christian Pedersen, as a pioneer in Denmark, was able as early as in 1965 to attract a grant for a 60 MHz NMR instrument, followed in 1972 by Fourier-transform (FT) 90 MHz instrument for ^1H and ^{13}C NMR spectroscopy. The latter was the first instrument in Scandinavia able to record carbon NMR spectra by FT methodology only. This opened up a new world, the ^{13}C NMR spectra of sugars, which was immediately explored, and in 1973 a publication "Assignment of Anomeric Structure to Carbohydrates through Geminal ^{13}C – ^1H Coupling Constants" appeared from the group. This turned out to be one of Christian Pedersen's most cited papers, as it described an important new analytical tool for distinguishing between axially and equatorially oriented hydrogen atoms in tetrahydropyran rings.

The NMR studies were extended to the determination of ^{13}C –H long-range coupling constants as well as ^{13}C –F coupling constants in carbohydrate derivatives. Several such papers and two reviews in *Advances in Carbohydrate Chemistry and Biochemistry*, volumes 41 and 42, were published in conjunction with Klaus Bock.

Christian Pedersen was a person who loved "hands on" in research. He was continuously engaged in development of the NMR equipment at his institute

and was also the practical person who most often could bring an instrument to work again after a breakdown. The growing knowledge and experience of Klaus Bock also contributed to keeping the instruments running.

Pedersen's practical attitude was also reflected in the use of NMR methods for direct monitoring of many of the reactions studied. He solved the practical problems involved with reactions in liquid hydrogen fluoride, constituting a beautiful example of the direct observation of acyloxonium ions and their rearrangements. The base-induced rearrangements of bromodeoxy lactones were another example where insight and understanding was obtained by direct NMR monitoring of the reactions.

Christian Pedersen made his teaching obligation in organic chemistry at the university one of his most important tasks. He was a gifted and highly respected lecturer, who managed to present potentially boring aspects of organic chemistry in a lively way, most often accompanied by relevant experiments. His calm human nature was highly appreciated by the students. He always listened carefully to them and his office was always open for those students who wanted to dig deeper into the challenges of organic chemistry. He was thus instrumental in attracting many freshmen to specialize in organic chemistry later during their studies.

It was particularly the first-year courses, where Christian Pedersen had the important task of motivating young students, but he was also always interested in supervising master and doctoral students, where his outstanding capabilities as experimentalist were fully appreciated. This applied both to elementary reactions where he had hands-on experience in all aspects of his scientific contributions, but also when delicate handling of poisonous or otherwise dangerous chemicals were in question, where he was always at the frontline of the action. This was particularly true with his most important scientific contributions, the studies of acylated sugars in anhydrous hydrogen fluoride. It is a tribute to his experimental skill that he never had an accident during the many years he worked with anhydrous hydrogen fluoride. His hands-on experience was especially respected and admired by the students and colleagues, and it played a vital role in his association with the students over the years. In those years the quality of compounds prepared in the laboratories were labeled "professor quality" if they appeared as pure as the colorless crystals Christian Pedersen could deliver.

Christian Pedersen was likewise highly respected by colleagues both locally in the department, nationally during his many years of work for the Danish Natural Science Research Foundation, and particularly internationally for his solid scientific work, which was always very carefully documented in his publications.

He had a calm attitude and he was always prepared to listen to people and their problems, small or large. He would light his pipe and then sit back with his feet on the desk and listen. He never gave quick or easy solutions, but analyzed together with the person the alternatives in solving their problems and the potential consequences. In this way, he himself and on behalf of colleagues often avoided many unnecessary conflicts, although it gave him long working hours.

Even during the late part of his career, where more democratic university leadership methods had been introduced and where he had stepped back to let the next generation enjoy their experiences, he was often involved in decision processes by consultation with various personnel in the department, who all respected his integrity and sound judgment in complicated matters.

Christian Pedersen was a person who was at ease with himself, and particularly in his ability to combine human feelings with specific practical or more theoretical advice. These attributes made him a person who was always respected for his vision and insight. At the same time, he was a modest person who was always prepared to put other people before him if they could benefit from his support or insight.

He will be remembered as an inspiring teacher and colleague, and an honest and reliable person, while his dry humor made any interactions with him particularly enjoyable. Christian Pedersen will be remembered as the scientist who initiated the research field of carbohydrate chemistry in Denmark, and his original work has earned high international recognition and respect.

INGE LUNDT

KLAUS BOCK

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A. R. R. R.

ALEKSANDER WIEŚLAW ZAMOJSKI

1929–2004

Aleksander Wiesław Zamojski was born in Poznań on September 1, 1929. His father Szczepan was a medical doctor. Alex received his early education in elementary school in Smigiel near Poznań and then in Aleksandrów near Łódź. During the Second World War, when educational activity in Poland was significantly reduced by the invader, he attended secret private tuition in Łódź. In 1948, Alex graduated from high school with a special mathematics–physics program and began his chemical education in the Chemistry Department of the Technical University of Łódź. In 1952, he obtained the degree of engineer and then, in 1954, the M.Sc. degree in chemistry. The same year he moved to Warsaw, where he was appointed as a teaching assistant in the Chemistry Department of Warsaw University and started his Ph.D. program under the supervision of Professor Osman Achmatowicz on reactions of carbonyl cyanide, $\text{CO}(\text{CN})_2$. This very reactive compound had been synthesized by Professor Roman Małachowski before the Second World War. Alex demonstrated the extremely high reactivity of carbonyl cyanide as a heterodienophile in the Diels–Alder reaction, leading to dihydropyran derivatives. He also showed that diethyl mesoxalate was an even better dienophile. In 1959, he defended his Ph.D. thesis entitled ‘*Diene Reactions of Carbonyl Cyanide and Diethyl Mesoxalate*’. Between 1959 and 1961 he held a Rockefeller fellowship for work as a postdoctoral associate at the ETH in Zurich in the laboratory of the future Nobel Prize laureate Professor Vladimir Prelog. There he was engaged in a project directed toward determination of the structure of two antibiotics: narbomycin and lankacidin.

After returning to Poland, he began work as a senior research associate in the Department of Chemistry of Warsaw University under the direction of Professor Achmatowicz, and he continued the program that he had started with Prelog. In 1965, Dr. Zamojski defended his habilitation thesis entitled

'Structures of Narbomycin and Related Macrolide Antibiotics' and obtained the D.Sc. (habilitation) degree. That same year he moved to the newly formed Institute of Organic Chemistry of the Polish Academy of Sciences (IOC) in Warsaw, first as a Docent (Associate Professor) and from 1973 as a full Professor until the year of his retirement. In 1968, he succeeded Professor Osman Achmatowicz as head of the Department of Synthesis of Natural Products. After reorganization of the Institute and the departure of the alkaloid laboratory to Poznań, he became head of the laboratory responsible for the synthesis of mono- and oligo-saccharides. Between 1979 and 1982 he served as the Research Director of the Institute. After retirement at the age of seventy, he continued his association with the Institute as Professor Emeritus.

Professor Zamojski was very much engaged in the education of Ph.D. students in the Institute. He was a true scholar. Together with Dr. Osman Achmatowicz Jr., son of his former supervisor, he organized the first Ph.D. study program in Poland; located in the Institute, it resembled postgraduate studies in universities of the western hemisphere. At that time, a scientific career in Poland was based on the long-established way of doing a Ph.D. degree, usually taking many years. Study for the Ph.D. in the Institute of Organic Chemistry, even now, represents a model for training doctoral students, which deserves imitation elsewhere in Poland. As a part of the graduate students' training, he delivered a two-semester course on the stereochemistry of organic compounds. During the mid-1960s, he introduced stereochemistry into the advanced course of organic chemistry and was a popularizer of the celebrated books by Ernest Eliel, *'Stereochemistry of Carbon Compounds'* and *'Conformational Analysis'*. He was a founder of the Section of Stereochemistry of the Polish Chemical Society, which under his presidency (1973–1982) was a model of activity for other sections of the Society. From 1973 on, he organized the Schools on Stereochemistry, which took place in a beautiful palace in Jabłonna located close to Warsaw, where he and his coworkers taught a basic course of modern stereochemistry. At the end of each school, an eminent invited stereochemist delivered a lecture related to the program. In 1975, Alex organized the First National Symposium on Stereochemistry.

The first research project that Alex undertook in the Institute was directed to the chemistry of the macrolide antibiotic, erythromycin. Chemical modifications led to a new derivative, 8-hydroxyerythromycin, a patent on which was purchased by a major pharmaceutical company. At the same time, Alex initiated studies on the total synthesis of monosaccharides via the Diels–Alder adduct of 1-methoxy-1,3-butadiene and butyl glyoxylate. As Alex used to say *'I started to*

think about the concept of the synthesis as early as the beginning of the 1960s during my postdoctoral stay with Professor Prelog'. The total synthesis of sugars dominated the work of Zamojski's group for almost two decades, establishing his high position among the Polish, as well as international carbohydrate societies. The group continued research not only on the synthesis and transformation of Diels–Alder cycloadducts, but also in the study of the spectral properties and conformations of substituted dihydropyrans and the corresponding epoxides. The pioneering work of Professor Zamojski on the diastereoselective transformations of the cycloadducts allowed him to propose a new, original, and general method for the preparation of racemic monosaccharides. During early years of this program, he competed with Professor Robert Brown's group from Edmonton, Canada, which at the same time started on the total synthesis of racemic monosaccharides from acrolein dimer. In both methods the crucial step involved rearrangement of epoxides into allylic alcohols. Brown's group used a butyllithium-promoted rearrangement, whereas Alex performed a more versatile sequence of simple, high-yielding reactions that consisted of opening the epoxide with dimethylamine, followed by oxidation of the dimethylamino group to the *N*-oxide and finally a Cope degradation. Zamojski's total synthesis of monosaccharides was also the beginning of modern organic synthesis in Poland. Alex clearly demonstrated to the scientific community that the target compounds could be synthesized by a sequence of reactions in which every step has been very carefully planned, leading to the desired product with high stereoselectivity and in high yields. This project led his group to syntheses of all of the stereoisomeric methyl glycosides of pentoses, hexoses, and hexuronic acids, as well as many deoxy sugars, aminodeoxy sugars, components of aminoglycoside antibiotics, and higher sugars.

Together with Professor Osman Achmatowicz Jr., Alex elaborated another approach to monosaccharides that utilized furfuryl alcohols as the starting material. The crucial step of this method consisted in the oxidation of the furan ring, followed by rearrangement of the dihydrofuran skeleton into a dihydropyranone. The transformation, which became known as the Achmatowicz rearrangement, had major impact in the chemical literature since it provided syntheses, not only of a number of pentoses, hexoses, and 6-deoxyhexoses, but also an attractive new method in general organic synthesis. The Achmatowicz rearrangement and its many versions have been widely used in a variety of sophisticated syntheses of natural products.

Alex spent two sabbaticals (1971–1972 and 1984–1985) in Canada with Professor Walter Szarek and turned his attention to the newly discovered

Mitsunobu reaction; this was later applied widely in sugar chemistry by his collaborators Janusz Jurczak, Grzegorz Grynkiewicz, and Edward Grochowski. They discovered valuable new applications of the Mitsunobu reaction and explained mechanistic aspects.

His success in transforming the dihydropyran and furan skeletons into monosaccharides led Alex to study the photochemical reaction of furan with alkyl glyoxylates, opening up a new route to sugars. The Paterno-Büchi reaction, followed by cleavage of the oxetane ring, provided a convenient synthesis of 3-substituted furans, and this is still regarded as one of the best methods for preparing these molecules, which are otherwise accessible with difficulty. The preparation of racemic 3-deoxy-DL-streptose was the final success of this project.

Following developing research trends, Zamojski began studies on the synthesis of optically active monosaccharides. Early work with his Ph.D. student Janusz Jurczak was directed to chiral enantiomerically pure glyoxylates and to the diastereoselective formation of their Diels-Alder adducts. Although, the asymmetric inductions achieved at that time were relatively modest, this pioneering work provided a sound base for later successful investigations of this reaction performed by Professor Jurczak's group that involved separation of enantiomers, diastereoselective, and finally catalytic enantioselective cycloadditions, carried out under atmospheric and high-pressure conditions.

Based on the methodology for synthesis of racemic monosaccharides, Alex elaborated an entry to oligosaccharides, in particular rhamnobioses and rhamnotrioses. In the beginning of the 1990s, he also proposed a convenient strategy for the synthesis of the 11-carbon atom sugar tunicamine. In order to simplify the ^1H NMR spectra of per-*O*-benzyl derivatives, he performed an elegant synthesis of α,α -dideuteriobenzyl chloride and bromide, compounds used for the protection of the free hydroxyl groups in sugars. The NMR spectra of such deuterated compounds were much simpler than 'normal' benzylated molecules.

In the mid-1980s, Alex concentrated his efforts on the synthesis of sugars of bacterial origin. The first convenient method for preparation of *L-glycero-D-manno*-heptose, a sugar occurring in bacterial lipopolysaccharides, was realized in 1986. Synthesis of monophosphates of *L-glycero-D-manno*-heptose and methyl *L-glycero-D-manno*-heptopyranoside, as well as studies on hydrolysis and migration of the phosphate moiety, rationalized our understanding of the location of the phosphate group in bacterial heptoses. These investigations attracted the interest of many biochemists, and Alex started a close

collaboration with Professor Helmut Brade from the Borstel Research Center, Germany. Joint studies on the synthesis of biologically important oligoheptoses were sponsored by Polish Academy of Sciences and the Deutsche Forschungs Gemeinschaft.

A new project, related to complexes of cyclopentadienylcarbonyltriphenylphosphinacyliron(II) was initiated at the end of the 1980s. Alex was particularly interested in the reactivity toward electrophiles of anions generated from the acyl fragment. This led to elaboration of a new method for the synthesis of deoxy sugars from 'acyliron' and sugar aldehydes.

In the last period of his research activity, Alex was engaged in the elongation of monosaccharides by the reaction of *aldehydo*-sugars with C_1 -Grignard reagents, $ROCH_2MgCl$. Synthesis of higher deoxy sugars prompted him to investigate an entry to indolizidine-type imino sugars from an aminodeoxyoctitol derivative, and this led to a new synthesis of castanospermine epimers. Alex also developed a synthesis, free of unpleasant odors, of thio sugars and thioglycosides, which proceeded via sugar thiocyanates followed by their reaction with Grignard reagents.

The activity of Alex Zamojski was well recognized by the scientific community. He was twice invited as a "Visiting Scientist" to the Department of Chemistry, Queen's University, Kingston, Canada for cooperation with Professors J.K.N. Jones and W.A. Szarek. He delivered plenary and invited lectures at numerous international conferences, including the International Carbohydrate Symposia in Madison, Bratislava, Seville, and Sydney; the American Chemical Society meetings in Philadelphia and Montreal, and other symposia in Bratislava, Rotstock, Borstel, and Varna. He also presented a number of plenary lectures at the annual meetings of the Polish Chemical Society. He visited many universities in the USA, Canada, Germany, France, Hungary, Spain, Denmark, and Switzerland presenting important results from his own work.

Professor Zamojski supervised 19 Ph.D. students. Five of his co-workers accomplished habilitation (D.Sc.) and six of them became full professors. Two of his former students (Janusz Jurczak and Marek Chmielewski) were elected to the Polish Academy of Sciences. Professor Zamojski published about 200 scientific papers and was a coauthor of 13 patents. He also wrote 10 chapters and reviews, including a landmark article in Volume 40 of this Series. It should also be mentioned that Alex was a great master in the Polish language, and taught his students how to write and present consistently and clearly in their Ph.D. theses and scientific papers.

Aleksander Zamojski served on many committees of academic societies and the Polish Ministries of Science and of Education. He was member of the Presidium of the Polish Chemical Society (1976–1982), served as President of the Society (1988–1991), and was given honorary membership by the Society in 2000. He served as a member of the Council of Polish Scientific Societies and a member of the Executive Committee of the Federation of European Chemical Societies (FECS) (1992–1995 and 1998–2001). He was chairman of the Section of Chemistry of the State Committee for Scientific Research in 1992–1994, and in 1998 until November 2003. He was an expert and a member of the Committee for Popularization of Science of the State Committee for Scientific Research (1993–1994 and 1995–2000). He served on the Editorial Advisory Boards of: *Carbohydrate Research* (1976–1996), *Chemtracts—Organic Chemistry* (from 1989), and was vice-chairman and subsequently chairman of the Editorial Board of “*Wiadomości Chemiczne*” (1981–1990), written in Polish. In 1991, he founded and served as Editor in Chief of an informative journal ‘*Orbital*’ of the Polish Chemical Society. He represented Poland in the International Carbohydrate Organization (1976–2000), and was Polish representative to the carbohydrate group in COMECON (East European Carbohydrate Organization coordinated by Professor N.K. Kochetkov; 1976–1991).

He was Chairman of the Organizing Committee of the 7th European Carbohydrate Symposium (*EUROCARB 7*) held in Kraków in 1993, and then served as President of the European Carbohydrate Organization (1993–1995).

He was a member of many Research Councils of institutes of the Polish Academy of Sciences, in particular was Chairman of the Council of the Institute of Bioorganic Chemistry in Poznań (1993–1998) and a member of the councils of the Institutes: Organic Chemistry (Warsaw), Physical Chemistry (Warsaw), and Center of Molecular and Macromolecular Studies (Łódź).

Aleksander Zamojski’s achievements were recognized by many scientific awards, including the Polish Chemical Society Award (1956), the Award of the 3rd Division of the Polish Academy of Sciences (1972), four Awards of the Scientific Secretary of the Polish Academy of Sciences, and a prestigious Kostanecki Medal of the Polish Chemical Society (1984). In 1984, he was awarded the *Polonia Restituta* Cavalier Cross by the President of Poland.

Alex enjoyed personal contacts with his coworkers and students. All of them remember the daily 5 o’clock tea in the Institute, collective volleyball games, and traditional picnics organized every autumn 30 kilometers out of Warsaw, and eating and drinking while discussing day-to-day problems. With his broad interests in organic chemistry and spectroscopy of organic compounds, he

always had time for scientific discussions with his students and colleagues. In 1999, his 70th birthday was celebrated in Ustroń, a small resort located in southern Poland. A large group of his friends from Poland and abroad attended the event, presenting lectures on topics related to Alex's interests and enjoying a social program. After retirement at the age of 70, Alex remained active and full of energy. As a professor emeritus he still associated with the Institute, giving courses for Ph.D. students. He also collaborated with the State Committee for Scientific Research and was an active member of its two commissions. His rich and rewarding life ended in Warsaw on February 23, 2004 when he passed away at the age of 74 after losing a battle with cancer. Alex is survived by his wife, Barbara. Alex's son, Jan, from his first marriage, now resides in Germany with his family.

Professionally, he will be long remembered as a creative and enthusiastic scientist and inspiring teacher, for his contributions to carbohydrate chemistry, for his service to the Institute of Organic Chemistry of the Polish Academy of Sciences, to the Polish Chemical Society, to the State Committee for Scientific Research, and to the International and European Carbohydrate Organizations.

SŁAWOMIR JAROSZ
MAREK CHMIELEWSKI

CHEMISTRY OF CARBOHYDRATE AZIRIDINES

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ABBREVIATIONS

Ac	acetyl, CH ₃ CO-
Bz	benzoyl, C ₆ H ₅ CO-
NBz	<i>p</i> -nitrobenzoyl, NO ₂ -C ₆ H ₄ -CO-
MPh	<i>p</i> -methoxyphenyl, CH ₃ O-C ₆ H ₄ -

Ans	<i>p</i> -anisoyl, CH ₃ O-C ₆ H ₄ -CO-
Ts	tosyl, <i>p</i> -toluenesulfonyl
Ms	mesyl, methylsulfonyl
DNP	2,4-dinitrophenyl
NPh	<i>p</i> -nitrophenyl
BOC	<i>tert</i> -butoxycarbonyl
BSA	bis(trimethylsilyl)acetamide
Cbz	benzyloxycarbonyl
MMTrCl	<i>p</i> -methoxytrityl chloride

In tables, Y is used to highlight substitution at nitrogen atom of the aziridine ring instead of the general symbol R.

I. INTRODUCTION

This article deals with those derivatives in which an aziridine ring is fused to a pyranose or furanose ring or to an exocyclic part of a carbohydrate molecule. These compounds are termed carbohydrate aziridines or epimines. Carbohydrate aziridines do not occur naturally, although a few noncarbohydrate aziridines have been found among antibiotics.¹⁻⁴ Investigations on this class of compounds date back to 1960 when Christensen and Goodman reported the synthesis of methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino- α -D-allopyranoside.⁵ In the following decades, methods for the synthesis of carbohydrate aziridines were developed and their scope and limits investigated. Aziridine-ring cleavage, the most important reaction of carbohydrate aziridines, was first addressed in the pioneering work of Guthrie,^{6,7} Ali,⁸ Buss,⁹ and other researchers in an effort to develop appropriate reaction conditions and elucidate the stereochemistry of this reaction. Further research on the reactivity of aziridines was then often directed toward the synthesis of aziridines as synthetic intermediates and was rather nonsystematic until the past decade, when new insight was provided by studies focused on the ring cleavage of a selection of diversely *N*-substituted aziridines with several nucleophiles.¹⁰⁻¹³

The purpose of this chapter is to present the chemistry of carbohydrate aziridines, with the emphasis being placed on surveying preparative methods and ring-opening reactions. We have omitted spiroaziridines and alditol-based aziridines from this chapter. Literature has been surveyed up to the end of 2003. The chemistry of carbohydrate aziridines has not yet been treated in a specialized article.

II. METHODS FOR THE SYNTHESIS OF CARBOHYDRATE AZIRIDINES

From the mechanistic point of view, the reported syntheses of carbohydrate aziridines are based almost exclusively on S_N2 intramolecular nucleophilic substitution. The nucleophile is a nitrogen-containing group, often free or an *N*-substituted amino group, which can be generated *in situ* by reduction of an azido or cyano group, or by the Michael addition of amines to a double bond with appropriate substitution. The neighboring leaving group is typically an alkyl (aryl)sulfonyloxy group, or is generated *in situ*, which is the case with the Mitsunobu reaction. The aziridine-ring closure invariably proceeds with inversion of configuration at the atom bearing the leaving group. The stereochemistry of S_N2 nucleophilic substitution strongly favors the antiperiplanar disposition of the participating groups in the transition state. This requirement is properly met by the pyranose derivatives having *trans*-diaxial orientation of both substituents in the favored conformation. If the favored conformation of a pyranoid aziridine precursor has the *trans*-diequatorial arrangement, the formation of the aziridine may require more-severe reaction conditions, or it may be suppressed, often in favor of products of competing reactions resulting from the participation of other nucleophilic atoms in such ambident nucleophiles as *N*-acylamino, ureido, and thioureido groups.

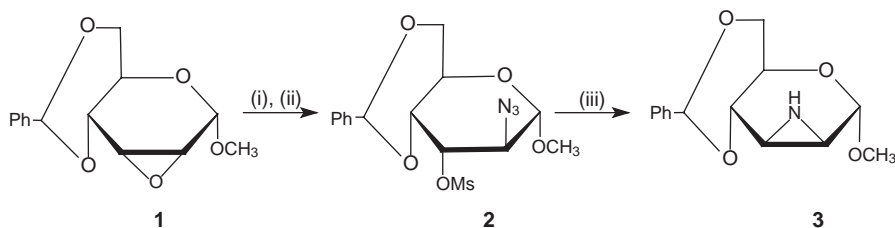
The aziridine-ring closure based on the Mitsunobu reaction, the Staudinger reaction, and isomerization of aminooxiranes also involves nucleophilic displacement as the key step of the reaction mechanism. Other reactions rarely reported in the synthesis of carbohydrate aziridines involve nonstandard,¹⁴ even unusual¹⁵ procedures lacking a general application.

1. Reduction of Azidosulfonates

This preparative method is one having the most general utility. It utilizes *in situ* reduction of suitable vicinal *trans*-azido sulfonates to intermediary amino sulfonates, which undergo base-induced cyclization to epimines. Elevated temperatures are often required to complete the cyclization. The reduction by hydrazine with Raney nickel originally used¹⁶⁻¹⁹ was later largely replaced by lithium aluminum hydride reduction in tetrahydrofuran or diethyl ether. Reduction by sodium borohydride,^{20,21} hydrogenation over Adams' catalyst,¹⁷ and reduction by tributyltin hydride²² have also been reported on occasion. Reductive cyclization by $LiAlH_4$, which is now the method of choice, proceeds

smoothly and usually without formation of undesirable by-products. Additional products of reductive cleavage of the aziridine ring^{23,24} have, however, occasionally been isolated when reduction by Raney nickel was employed.

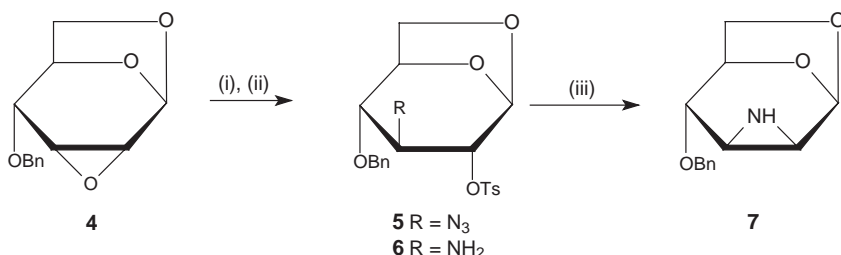
Precursor azido sulfonates in the pyranose series are usually prepared by the cleavage of suitable epoxides with azide, followed by mesylation or tosylation. This approach was used for the preparation^{16,25} of methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epiminohexopyranosides of the α -D-*manno*-, α -D-*allo*-, α -D-*gulo*-, β -D-*gulo*-, and α -D-*talo*-configurations using either Raney nickel or LiAlH₄ reduction. For example, methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino- α -D-mannopyranoside¹⁶ (**3**) was obtained in three steps from epoxide **1**. The reaction sequence involves azidolysis, tosylation, and Raney nickel reduction. Synthesis of the α -D-*allo*-,¹⁶ α -D-*gulo*-,²⁵ β -D-*gulo*-,²⁵ and α -D-*talo*-²⁵ epimines follows an analogous pattern with the oxirane ring being cleaved *trans*-diaxially by the azide anion. The low yield of the α -D-*talo* epimine (19%) was caused, at least in part, by hydrolysis of the tosylate during lithium aluminum hydride reduction.²⁵



(i) NaN₃, NH₄Cl; (ii) MsCl, pyridine; (iii) N₂H₄, Raney Ni, MeOH, refl., 83%

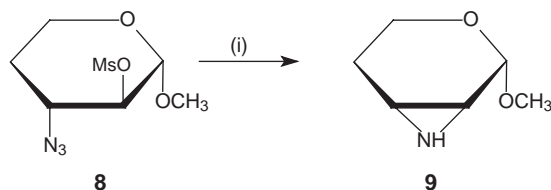
Reductive cyclization by lithium aluminum hydride as the key step has been employed for the preparation of *O*-benzylated 1,6-anhydro-2,3-dideoxy-2,3-epimino- and 1,6-anhydro-3,4-dideoxy-3,4-epimino- β -D-hexopyranoses having the *allo*-, *manno*-, *galacto*-, and *talo*-configurations from suitable 1,6:2,3- and 1,6:3,4-dianhydro- β -D-hexopyranoses.²⁶ Stereoselective *trans*-diaxial cleavage of the oxirane ring was effected by treatment with sodium azide and ammonium chloride in a 2-methoxyethanol–water mixture at 110–120 °C. Synthesis of 1,6-anhydro-4-*O*-benzyl-2,3-dideoxy-2,3-epimino- β -D-mannopyranose (**7**) from dianhydro derivative **4** illustrates this methodology. An alternative reduction by sodium borohydride in THF was also tested in the preparation of the 2,3-D-*allo*- and D-*manno*-epimines in this series.²¹ It provided a better yield (73%) than

LiAlH_4 for the former, whereas the latter was isolated in 56% yield together with 13% of the aminotosylate **6**. In both cases, boiling under reflux with MeOH was essential for complete cyclization.²¹



(i) NaN_3 , NH_4Cl , $\text{CH}_3\text{OCH}_2\text{CH}_2\text{OH}$, H_2O ; (ii) TsCl , pyridine; (iii) LiAlH_4 , THF, 60 %

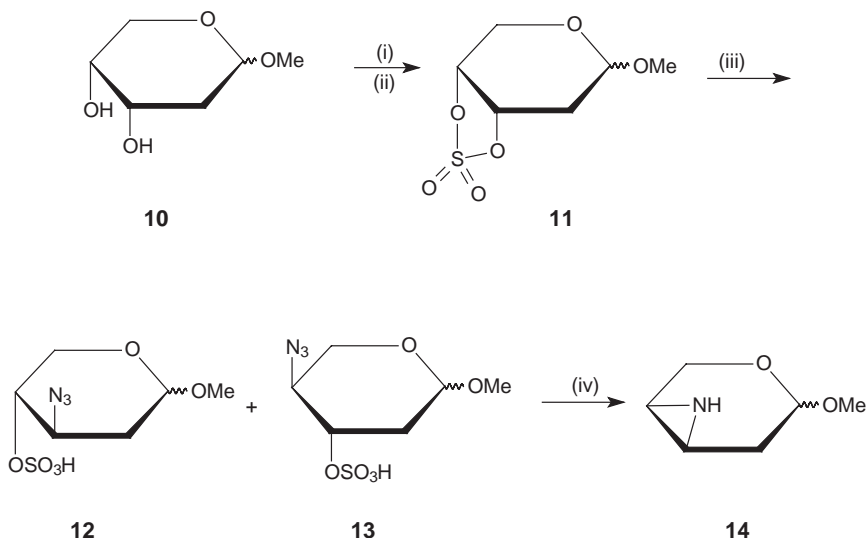
In a similar way Paulsen and Patt synthesized²⁷ benzyl 4-*O*-benzyl-2,3-dideoxy-2,3-epiminohexopyranosides of the β -D-*lyxo* and α -D-*ribo* configurations. Methyl 6-acetamido-2,3-*N*-acetylepimino-2,3,4,6-tetradeoxy- α -D-*ribo*-hexopyranoside²⁸ was prepared from the butyl ester of 2-methoxy-5,6-dihydro-(2*H*)-pyran-6-carboxylic acid in five steps. The reaction sequence involves ammonolysis of the ester group, epoxidization of the double bond, azidolysis of the epoxide, tosylation, and lithium aluminum hydride reduction. Methyl 2,3-epimino-2,3,4-trideoxy- α -DL-*erythro*-pentopyranoside (**9**) was synthesized²⁹ from methyl 3-azido-3,4-dideoxy-2-*O*-methylsulfonyl- α -DL-*threo*-pentopyranoside (**8**). According to the authors, formation of the aziridine did not occur on similar treatment of the corresponding β anomer. This is, however, a questionable result, since no description and outcome of the synthesis was given.



(i) LiAlH_4 , Et_2O , 69 %

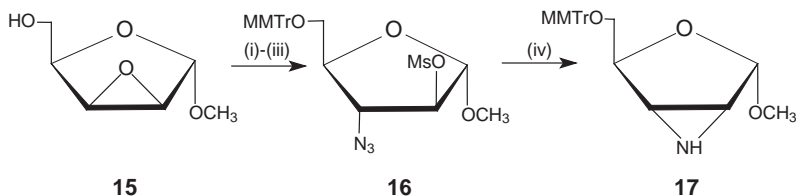
The use of sulfate esters was reported by Badalassi and coworkers.³⁰ The reaction sequence from ribopyranoside **10** to epimine **14** involves reaction with

SOCl_2 , oxidation to sulfate **11**, azidolysis to azides **12** and **13** and lithium aluminum hydride reduction.



(i) SOCl_2 , Et_3N ; (ii) NaIO_4 , RuCl_3 ; (iii) LiN_3 ; (iv) LiAlH_4

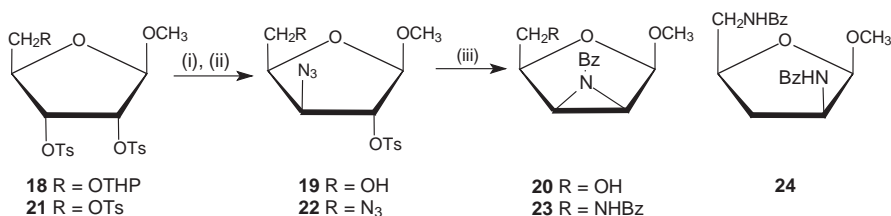
Synthesis of the starting *trans*-azido sulfonates in the furanose series employs either azidolysis of 2,3-anhydro derivatives^{31,32} or regioselective displacement of vicinal disulfonates. The former route was applied in the synthesis of methyl 2,3-dideoxy-2,3-epimino-5-*O*-(4-methoxytrityl)- α -D-ribofuranoside (**17**) from methyl 2,3-anhydro- α -D-lyxofuranoside³¹ (**15**) via azido compound **16**.



(i) NaN_3 , $(\text{NH}_4)_2\text{SO}_4$, EtOH , H_2O ; (ii) MMTrCl , pyridine; (iii) MsCl , pyridine; (iv) LiAlH_4 , Et_2O , 50%

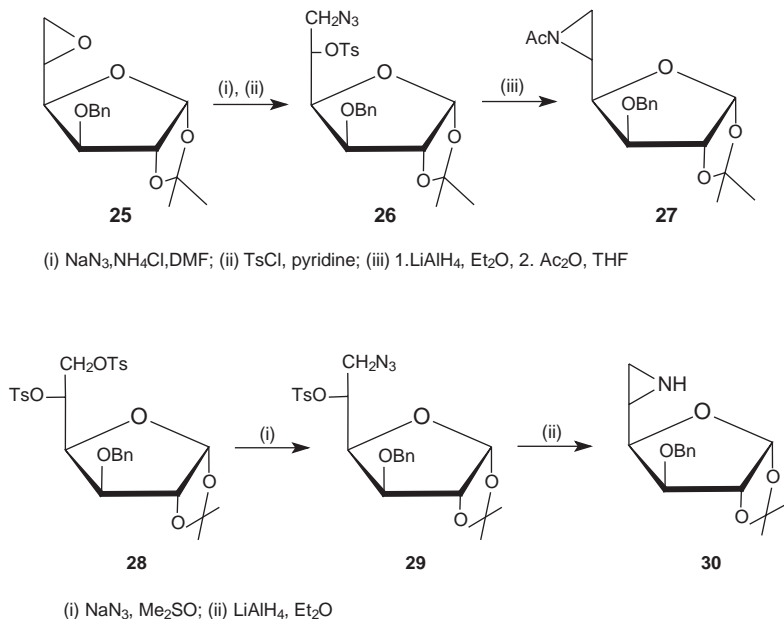
Similarly, methyl 2,3-dideoxy-2,3-epimino- α -D-erythrofuranoside was prepared from methyl 2,3-anhydro- β -L-erythrofuranoside.³²

Nucleophilic substitution of *cis*-2,3-disulfonates of ribofuranosides normally occurs³³ at position 3. For example, treatment of ditosylate **18** with NaN_3 in DMF, followed by removal of the tetrahydropyran group afforded 3-azidofuranoside **19**. Reductive cyclization by lithium aluminum hydride gave³⁴ epimine **20**. A similar reaction sequence has been accomplished in the 5-deoxy and 5-*O*-benzoyl series.³⁴ Diazido derivative **22**, prepared by azidolysis from tritosylate **21**, afforded epimine **23** on reaction with LiAlH_4 and subsequent benzoylation, whereas Raney nickel reduction gave compound **24**, most probably arising from reductive cleavage of the aziridine ring.^{23,33} A similar example of Raney nickel hydrogenolysis was observed in the pyranose series.⁹ Transformation of furanoid vicinal *cis*-ditosylates into epimines was also employed¹⁷ in the preparation of several 2,5-anhydro-3,4-epiminopentitols substituted at position 2.



(i) NaN_3 , DMF; (ii) AcOH, H_2O , 80 °C (for compd. **18**); (iii) 1. LiAlH_4 , THF, reflux, 2. benzoylation

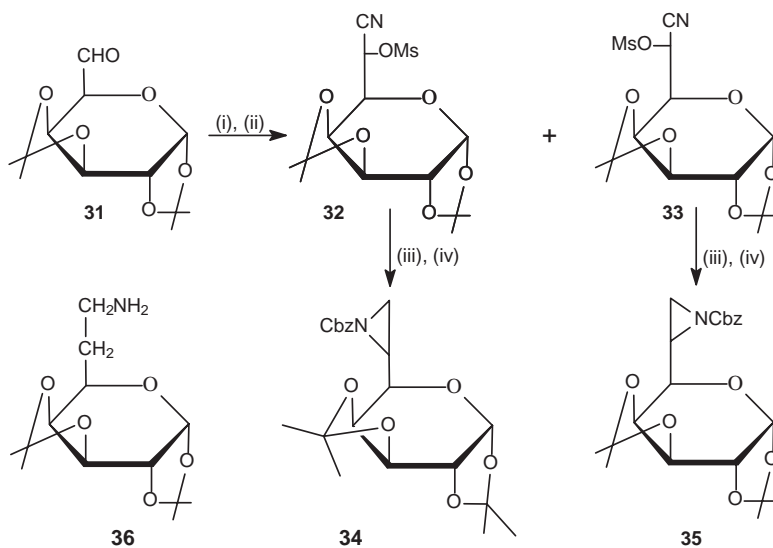
Saeki's group examined the possibility of synthesizing exocyclic monosaccharide epimines by reduction of azido sulfonates. Regioselective displacement of 5,6- and 6,7-disulfonates at the terminal carbon atom, or cleavage of 5,6-epoxides by azides also in the terminal position was used to introduce the azido group into the molecule. For instance, treatment of 5,6-anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene- β -L-idofuranose (**25**) with NaN_3 , followed by tosylation afforded azido tosylate **26**, which was converted into 5,6-acetylepimino-3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene- α -D-glucofuranose (**27**) by reaction with LiAlH_4 in diethyl ether and subsequent acetylation.³⁵ Its β -L-*ido* isomer **30** was obtained from ditosylate **28** by reaction with NaN_3 and lithium aluminum hydride reduction.³⁵ An alternative synthesis of this compound based on reduction of a cyanosulfonate has also been reported (see Section II.2). Benzyl 5,6-dideoxy-5,6-epimino-2,3-*O*-isopropylidene- α -D-mannopyranoside³⁶ and 6,7-dideoxy-6,7-epimino-1,2:3,4-di-*O*-isopropylidene-D-*glycero*- α -D-*galacto*-heptopyranose³⁷ were prepared by the same synthetic route from the corresponding terminal disulfonates.



2. Reduction of Cyanosulfonates

Substrates for this synthetic method are sulfonylated cyanhydrins obtained via elongation of the carbon chain by reaction of dialdoses with cyanides, followed by sulfonylation. The utility of this approach is therefore restricted to the synthesis of terminal epimines in the acyclic portion of a sugar molecule. Addition of cyanide anion gives rise to a mixture of two diastereomers that may be separated either as the cyanohydrins or following sulfonylation or aziridine-ring closure. Reductive cyclization is performed by reaction with lithium aluminum hydride. For example, treatment of dialdopyranose **31** with hydrogen cyanide in pyridine followed by mesylation gave³⁸ a mixture of diastereomers **32** and **33** in the ratio 1.0:1.7. The former compound was isolated by fractional recrystallization and the latter by column chromatography of the mother liquor. Reduction by lithium aluminum hydride afforded the corresponding epimines, which were isolated³⁸ as *N*-benzyloxycarbonyl derivatives **34** and **35** in moderate yields (54 and 50%). The same synthetic route was also applied with methyl 2,3,4-tri-*O*-benzyl- α -D-*gluco*-hexodialdo-1,5-pyranoside as the starting compound.³⁸ The reduction pattern of the 6-*O*-tosyl analogue of compound **33** was more

complex than that of **33**, and the 7-amino-6,7-dideoxy derivative **36** was formed under higher concentrations of the hydride and cyanotosylate.³⁷ Ichimura reported³⁹ synthesis of 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-5,6-epimino- β -L-idofuranose (**30**) from 3-*O*-benzyl-1,2-*O*-isopropylidene- α -D-*gluco*-pentodialdo-1,4-furanose by treatment with sodium cyanide and benzenesulfonyl chloride, followed by LiAlH₄ reduction. Catalytic hydrogenation of differently substituted 5-*O*-methanesulfonyl-D-glucofuranosiduronic nitriles was reported to afford 5,6-dideoxy-6-amino-hexofuranosides instead of the expected epimines.⁴⁰

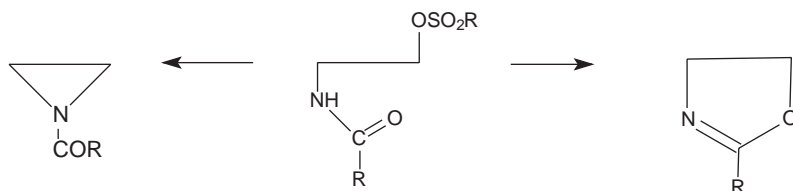


(i): HCN, pyridine; (ii): MsCl, pyridine; (iii): LAH, Et₂O, r.t.; (iv): CbzCl, 1,4-dioxane

3. Cyclization of *N*-Substituted Amino Derivatives

A vast majority of the amino derivatives used as substrates in the synthesis of carbohydrate aziridines are *N*-substituted, mostly as *N*-acylamines or *N*-aryl(alkyl)sulfonylamines. Reaction of free amines has rarely been reported^{18,41–43} in the carbohydrate field and difficult and incomplete cyclization was generally encountered.¹⁸ Paulsen and Stoye, however, reported spontaneous cyclization of 6-hydrazino-5-*O*-mesyl-D-hexofuranoses, obtained from 5,6-di-*O*-mesyl-hexofuranoses by treatment with hydrazine, into the *N*-amino-5,6-epimino derivatives.⁴⁴

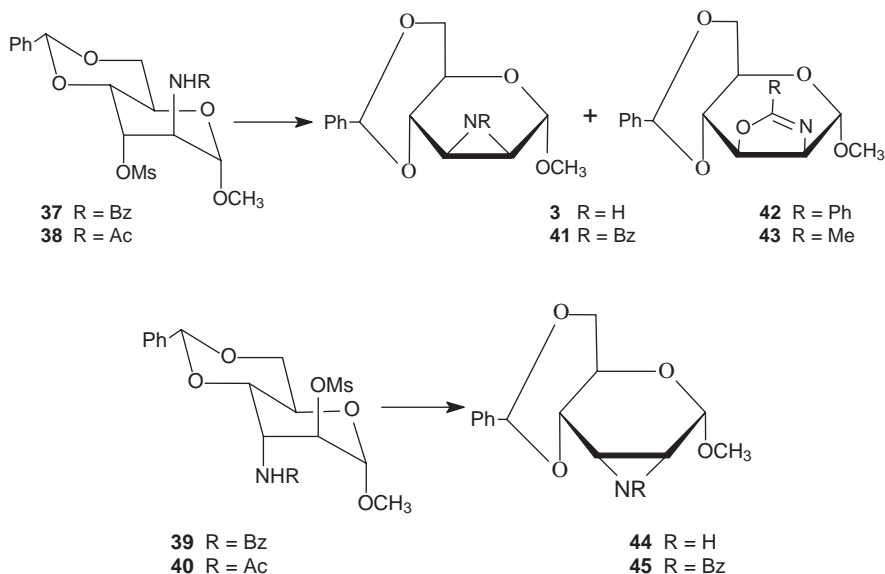
a. *N*-Acylamines.—Because the acylamino group has two nucleophilic atoms, nitrogen and oxygen, cyclization of acylaminosulfonates may give rise to either epimines or oxazolines:



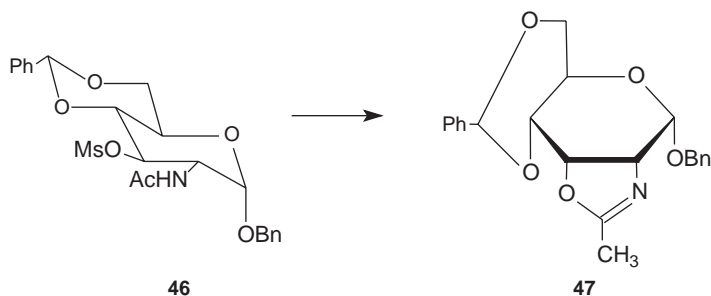
The epimine: oxazoline ratio depends on the nature of the substrate as well as on the reaction conditions, especially on the type of base catalyst, the temperature, and the solvent. In this respect, the formation of 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino-hexopyranosides has been studied in detail. Since the conformation of these derivatives is relatively fixed by 4,6-*O*-benzylidene ring, they provide a useful series for evaluation of the conformational effects involved in intramolecular cyclization to epimines. As far as reaction conditions are concerned, the results suggest that higher temperatures and a stronger base support epimine formation whereas oxazoline formation is favored under catalysis by weak bases or in neutral conditions.

Buss, Hough, and Richardson studied the base-induced cyclization of methyl 4,6-*O*-benzylidene- α -D-altropyranosides (**37**–**40**) into epimines and oxazolines.⁴⁵ Treatment of compound **37** with hot ethanolic sodium ethoxide⁴⁵ or potassium cyanide⁴⁶ in DMF at 100 °C resulted in rapid formation of a mixture of epimine **3** and oxazoline **42** in 2:1 ratio with overall yields of 81 or 74%, respectively. Treatment with sodium acetate gave⁴⁶ oxazoline **42** in 84% yield and only traces of epimine **3**. The *N*-acetyl derivative **38** reacted with sodium ethoxide in a similar way, affording⁴⁵ epimine **3** and oxazoline **43** (yields were not given). In contrast, compound **39** on treatment with hot sodium ethoxide gave only epimine **44** (72% yield). *N*-acetyl derivative **40** gave under these conditions low yield (35%) of the epimine **44**, but TLC did not indicate formation of the oxazoline or any other compound. When cyclizations of compounds **37** and **39** by the action of sodium ethoxide were performed at room temperature, the corresponding *N*-benzoyl epimines **41** and **45** were isolated in 38 and 29% yields, and apparently *N*-acylepimines are intermediates in the formation of free epimines. Hough and his coworkers explained the absence of oxazoline formation in the cyclizations of **39** and **40** as being due to the 1,3-diaxial interaction

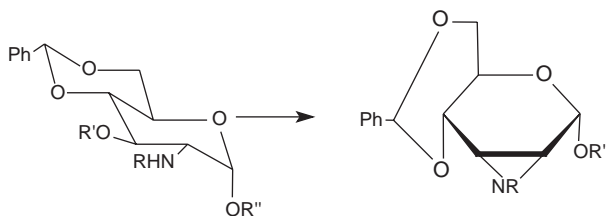
between the 3-acylamino- and 1-methoxy groups in 4C_1 (D) conformation, which prevent the *N*-acyl group from free rotation. Consequently, the carbonyl oxygen atom cannot attain a suitable position behind the departing 2-methanesulfonyl group, and the displacement leading to the oxazoline does not occur.⁴⁵ It is noteworthy that reduction by lithium aluminum hydride of compounds **37** and **39** afforded⁴⁵ free epimines **3** and **44** as the sole products in yields of 60 and 53%. Debenzoylation might have been the first step of these reactions, thus precluding formation of oxazolines.



The reaction pattern in the alkyl 4,6-*O*-benzylidene- α - and β -D-glucopyranosides frameworks is also complex. The participating groups possess the *trans* diequatorial disposition unfavorable to nucleophilic substitution, and a change of conformation is required to bring the acylamino and sulfonyloxy groups into antiperiplanar arrangement. Formation of oxazolines strongly depends on the reaction conditions, and occurs especially when such weak bases as sodium acetate or potassium cyanide are used. Thus acetamido mesylate **46**, on treatment with sodium azide or potassium cyanide in boiling DMF afforded⁴⁷ oxazoline **47** in 31 and 68% yields, respectively, instead of the expected products of direct nucleophilic substitution at C-3. Because the low yield in the reaction with sodium azide was not satisfactorily accounted for, epimine formation as a by-product cannot be excluded.



In contrast, compounds **48**, **46**, and **49** yielded^{8,47,48} epimines **44** and **53** respectively by boiling under reflux with sodium propoxide. These strong bases most probably support epimine formation by enhancing the nucleophilicity of the amide nitrogen atom.⁸ Formation of epimine **44** on reaction of **50** with sodium ethoxide was accompanied⁴⁹ by demesylation. A smooth cyclization of **50** to epimine **44** was achieved by refluxing with NaOH in 2-methoxyethanol.⁸ Derivatives **48** and **50** also gave^{8,49} epimine **44** in rather lower (60 and 44%) yields by reaction with lithium aluminum hydride whereas derivative **51** underwent⁴⁹ mainly reduction of the carbonyl group to compound **52**. The relatively high basicity of the fluoride anion effected rapid conversion of benzamido sulfonates **48** and **50** into *N*-benzoylepimine **54** by treatment with tetrabutylammonium fluoride in hexamethylphosphoric triamide (HMPT) or acetonitrile.^{50,51} The epimine **54** subsequently underwent aziridine-ring cleavage under these conditions giving mainly the 2-fluoro-3-benzamido- α -D-altroside (see Section IV.2).⁵¹ The benzyl glucoside **49** showed similar behavior on treatment with tetrabutylammonium fluoride in HMPT, giving *N*-benzoylepimine **55** as a reaction intermediate.⁴⁸



48 R = Bz, R' = Ts, R'' = CH₃

46 R = Ac, R' = Ms, R'' = Bn

49 R = Bz, R' = Ts, R'' = Bn

50 R = Bz, R' = Ms, R'' = CH₃

51 R = Ac, R' = Ms, R'' = CH₃

52 R = Et, R' = H, R'' = CH₃

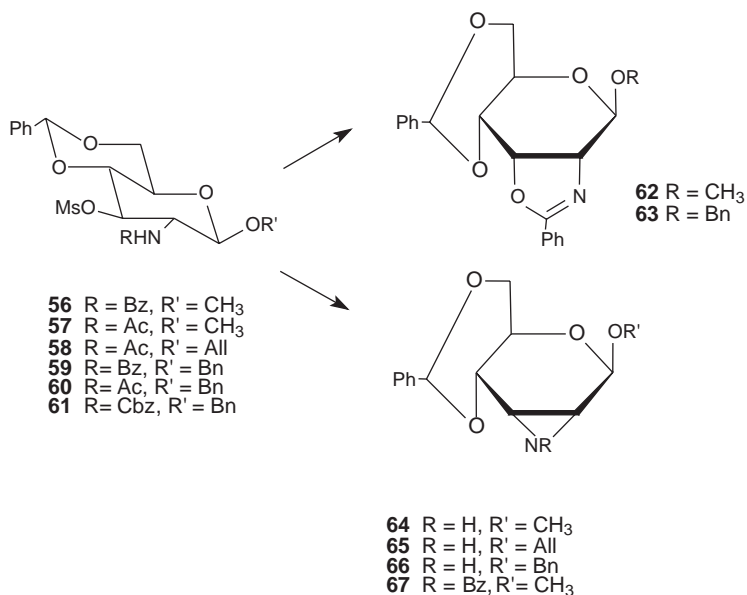
44 R = H, R' = CH₃

53 R = H, R' = Bn

54 R = Bz, R' = CH₃

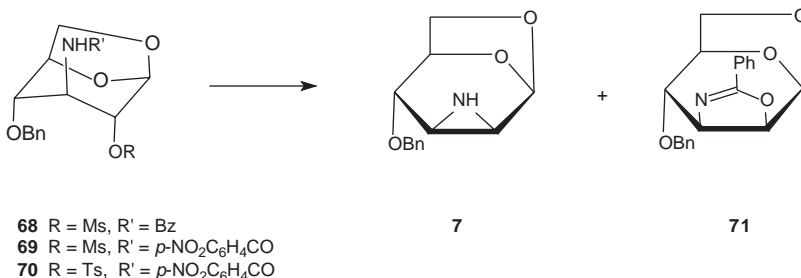
55 R = Bz, R' = Bn

In contrast to its α anomer **50**, the β -glucoside **56** yielded oxazoline **62** on treatment with sodium methoxide⁵² (later Meyer zu Reckendorf isolated 2–3% of the corresponding aziridine⁵³). The action of sodium 2-propoxide in boiling 1,4-dioxane on methyl glucosides **56** and **57** led,⁵⁴ however, to epimine **64**. Allyl glucoside⁵⁵ **58** and benzyl glucosides⁵⁶ **59–61** also afforded epimines (**65** or **66**, respectively) by refluxing with sodium 2-propoxide in 1,4-dioxane. On the other hand, the methyl glucoside⁴⁶ **56** afforded oxazoline **62** by treatment with sodium acetate in 2-methoxyethanol and benzyl glucosides⁵⁶ **59–61** under these conditions also cyclized to the corresponding five-membered rings: compound **59** to oxazoline **63**, and compound **61** to the corresponding oxazolidinone. Compound **60** afforded under these conditions a hydrolytic product of the methyl oxazoline presumably formed. Attempted replacement of the mesyloxy group of **56** by heating with KCN in *N,N*-dimethylformamide (DMF) resulted⁵³ in concomitant formation of oxazoline **62** and epimine **67** (17%).

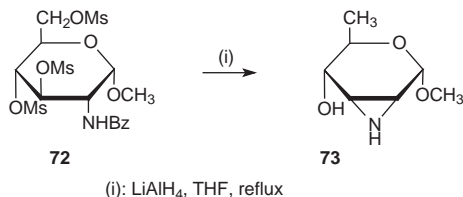


1,6-Anhydro-3-benzamido-4-*O*-benzyl-3-deoxy-2-*O*-methanesulfonyl- β -D-glucopyranose (**68**) reacted⁵⁷ with sodium 2-propoxide in 2-propanol to give a mixture of epimine **7** (65%) and oxazoline **71** (19%). Although participating groups in the starting compound **68** assume the conformationally fixed *trans*-diaxial arrangement, this fact does not prevent formation of the five-membered oxazoline ring. On the other hand, treatment of *p*-nitrobenzamido

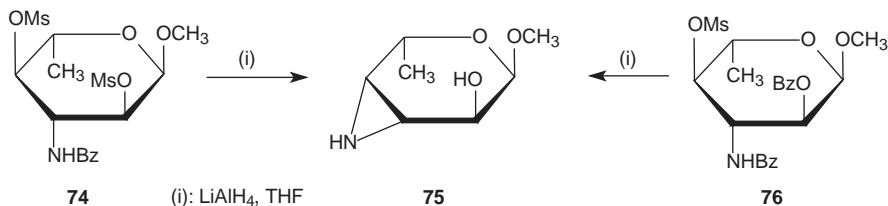
sulfonates **69** and **70** with 2-propoxide in 1,4-dioxane gave⁵⁷ exclusively the epimine **7** in 90 and 98% yields, respectively.



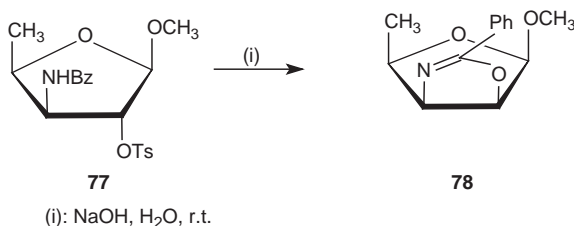
Gibbs and coworkers isolated methyl 2,3,6-trideoxy-2,3-epimino- α -D-allopyranoside (**73**) in 20% yield after treatment of derivative **72** with lithium aluminum hydride in tetrahydrofuran.⁵⁸ The structure of the product was confirmed by an independent synthesis from epimine **44**.



Barford and Richardson prepared⁵⁹ 3,4-epiminopyranoside **75** by lithium aluminum hydride reduction of methyl 3-benzamido-3,6-dideoxy-2,4-di-*O*-methanesulfonyl- α -L-glucopyranoside (**74**). The 3,4-position of the epimine ring was proved⁵⁹ by the synthesis of compound **75** from monomethanesulfonate **76**. Brimacombe and Rahman reported the synthesis of methyl 2,3,4,6-tetradeoxy-3,4-epimino-3-*C*-methyl- α -D-*ribo*-hexopyranoside from a trifluoroacetamido mesylate by treatment with sodium borohydride.⁶⁰



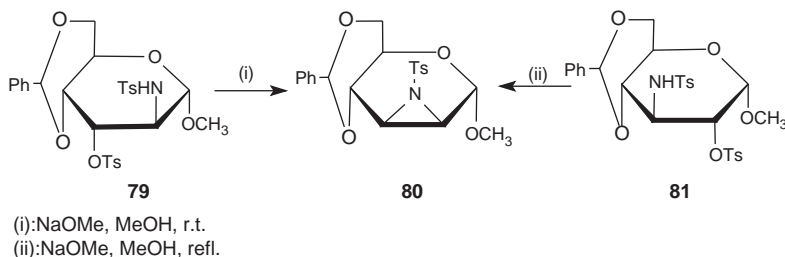
In an example from the furanose series, cyclization of methyl 3-benzamido-3,5-dideoxy-2-*O*-tosyl- β -D-xylofuranoside (**77**) in dilute sodium hydroxide was reported to afford oxazoline **78** rather than the corresponding epimine.³⁴



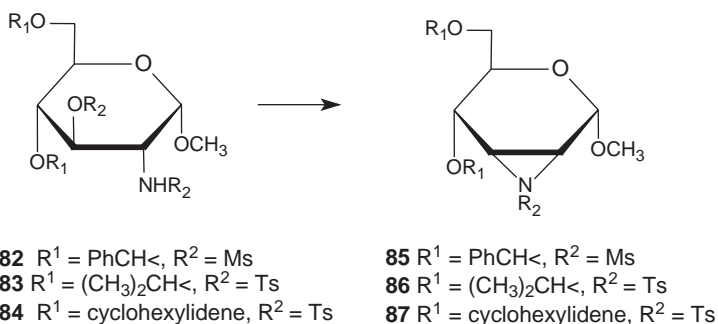
Attempts were made to prepare a terminal 5,6-epiminofuranose derivative by treatment of 6-benzamido-6-deoxy-1,2-*O*-isopropylidene-5-*O*-methanesulfonyl- α -D-glucufuranose with lithium aluminum hydride or sodium ethoxide. In both cases, only 6-benzamido-6-deoxy-1,2-*O*-isopropylidene- α -D-glucufuranose was obtained, indicating hydrolysis of the methanesulfonate.⁶¹

b. *N*-Aryl(alkyl)sulfonylamines.—In comparison with the preceding method, cyclization of vicinal sulfonamido-sulfonates has two advantages: formation of oxazolines is excluded, and the reaction proceeds more readily because of enhanced acidity of the N–H group and facile formation of the N[−] anion, which is considered the reactive species. Lower temperatures and weaker bases are therefore sufficient to induce cyclization.

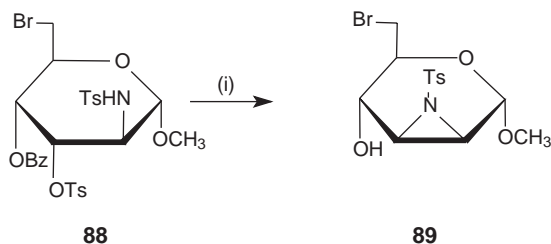
Cyclization of tosylates **79** and **81** to epimine **80** is illustrative of the steric effects involved, because α -D-altroside **79** cyclized at room temperature in sodium methoxide whereas the α -D-glucoside **81** required reflux.⁶² This discrepancy was rationalized by the fact that participating groups of **81** are in diequatorial relationship and additional energy is thus needed for ring distortion.



Methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-methanesulfonylepimino- α -D-allopyranoside (**85**) was prepared from dimesyl derivative **82** by boiling with sodium acetate in 2-methoxyethanol.⁸ The dibenzenesulfonyl analogue of **82** reacted in the same way with aqueous alkali to give methyl 4,6-*O*-benzylidene-2,3-benzenesulfonylepimino-2,3-dideoxy- α -D-allopyranoside.⁶³ Methyl 2,3-dideoxy-4,6-*O*-isopropylidene-2,3-tosylepimino- α -D-allopyranoside⁶⁴ (**86**) and its 4,6-*O*-cyclohexylidene analogue⁶⁵ **87** were obtained from ditosylates **83** and **84** by treatment with methanolic sodium hydroxide at 40 °C. An attempted substitution of the C-3 tosylate group by treatment of **84** with NaOAc, NaN₃, NaOBz, Bu₄NF, or LiNO₃ also resulted⁶⁵ in formation of epimine **87**. Reaction of **84** with alkali metal halides in *N,N*-dimethylformamide, however, effected the substitution.⁶⁵



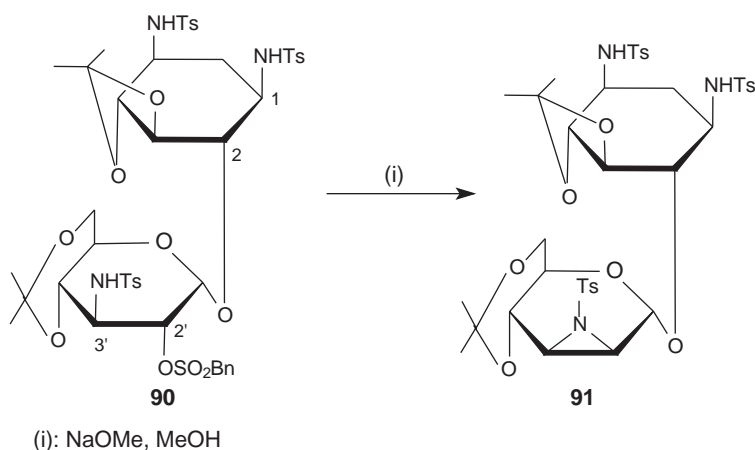
Hullar and Siskin obtained the *N*-tosylepimine **89** instead of the expected 2,6-imino derivative upon treatment of ditosylate **88** with sodium methoxide.⁶⁶



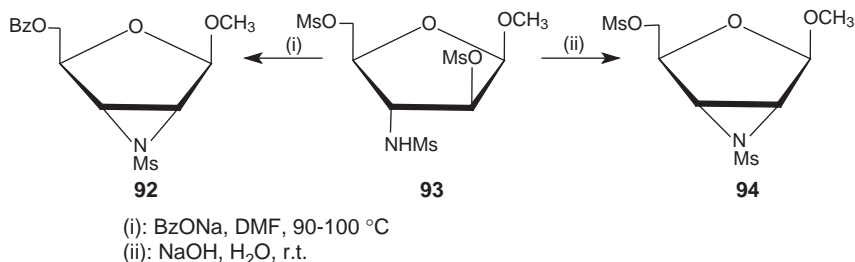
(i): NaOMe, MeOH, r.t.

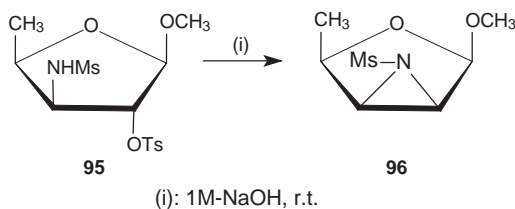
Application of this method for aziridine-ring closure was extended to the synthesis of epimino analogues of aminoglycosidic antibiotics.^{64,67-69} Kumar and coworkers have reported preparation of the 2'',3''-epimino analogue of

kanamycin B upon treatment of suitably protected 2''-*O*-mesyl-penta-*N*-tosylkanamycin B with sodium hydride in DMF, followed by detosylation in liquid ammonia⁶⁷ and deprotection. A synthesis of protected 2,3-tosylepiminokanamycin B was reported by Kobayashi and coworkers.⁶⁴ The same group also reported synthesis of 2-deoxy-6-*O*-(2,3-dideoxy-4,6-*O*-isopropylidene-2,3-*N*-tosylepimino- α -D-mannopyranosyl)-4,5-*O*-isopropylidene-1,3-di-*N*-tosylstreptamine (**91**) from compound **90** (a derivative of a component of kanamycin B) on treatment with sodium methoxide.⁶⁸

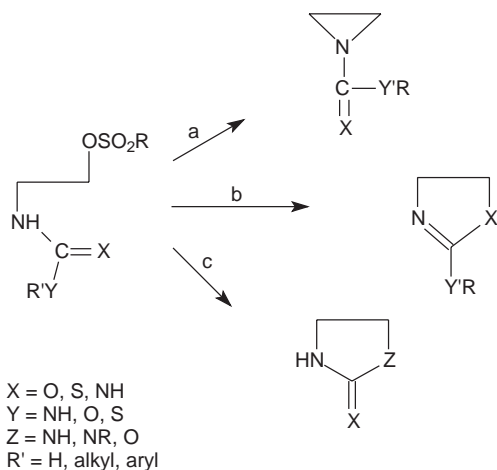


In the furanose series, the *D*-ribo epimines **92** and **94** were prepared from methyl 3-deoxy-3-methanesulfonamido-2,5-di-*O*-methanesulfonyl- β -D-arabinofuranoside (**93**) on treatment with sodium benzoate or sodium hydroxide,⁷⁰ and the *D*-lyxo epimine **96** by reaction³⁴ of methyl 3,5-dideoxy-3-methanesulfonamide-2-*O*-tosyl- β -D-xylofuranoside (**95**) with sodium hydroxide.





c. Derivatives of Urea, Thiourea, Carbamates and Thiocarbamates.—These derivatives contain complex groups, whose participation in intramolecular nucleophilic substitution may be generalized in the following scheme.



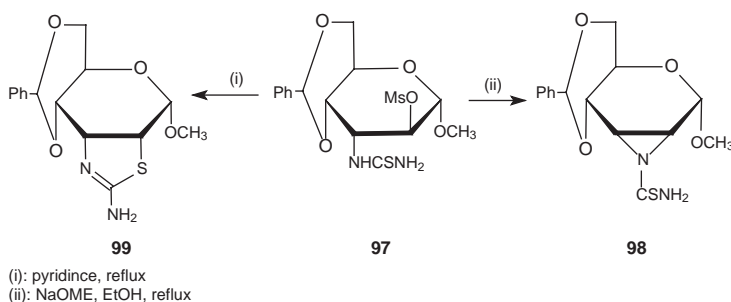
Up to three nucleophilic centers can take part in intramolecular displacement to give a three-membered aziridine ring (pathway **a**) or five-membered oxazoline, imidazoline, and thiazoline rings (pathway **b**), or oxazolidine and imidazolidine rings (pathway **c**). The complex nature of these reactions limits their general utility for the synthesis of carbohydrate aziridines, but the study of their course can, however, help to elucidate the mechanisms involved in complex neighboring group participation. Many of these reactions have been reviewed by Goodman⁷¹ in Volume 22 of this Series and this topic will be therefore discussed only briefly here.

Attention has been paid particularly to the reactions of methyl 4,6-*O*-benzylidene-hexopyranosides of the α - and β -D-*gluco* or α - and β -D-*altro* configurations. The results may be summarized in the two following rules:

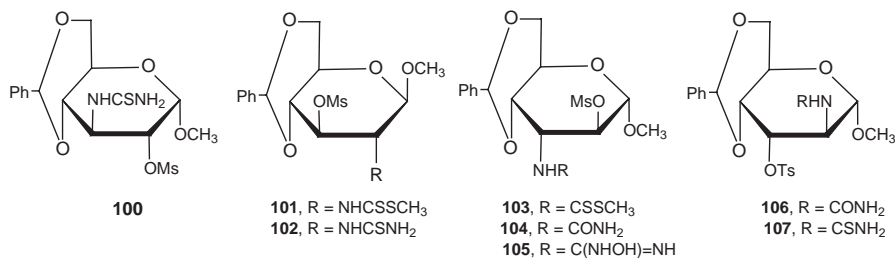
1. D-*altro* Derivatives provide epimines on treatment with methoxide and five-membered heterocycles on treatment with weak bases (typically pyridine).
2. D-*gluco* Derivatives provide five-membered heterocycles, regardless of the base used.

It is evident that unfavorable *trans*-diequatorial disposition of the participating groups of the D-*gluco* derivatives suppresses formation of the aziridine ring, whereas the D-*altro* derivatives can be deprotonated by strong bases to effect formation of the N⁻ anion, which is presumed to be the reactive species leading to the aziridine.

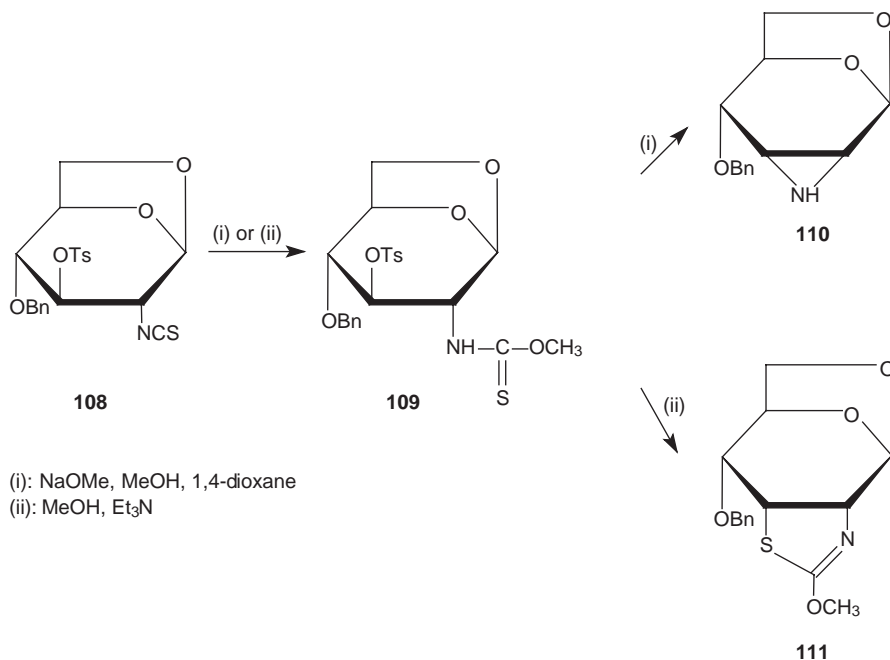
For instance, methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-methanesulfonyl-3-thioureido- α -D-altropyranoside (**97**) gave⁷² epimine **98** on treatment with sodium methoxide, but thiazoline derivative **99** was formed upon heating in pyridine. The corresponding derivative without the 4,6-*O*-benzylidene group also afforded an epimine on treatment with methoxide.⁷²



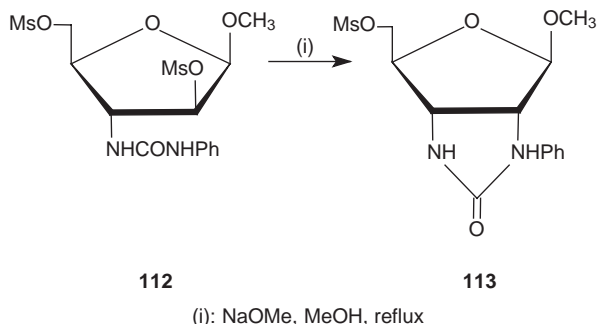
On the other hand, both D-glucosides **100** and **102** gave only thiazolines, in either refluxing pyridine or in methanolic methoxide.⁷³ Similarly, α -D-altropyranosides **103**,⁵ **104**,⁷⁴ **105**,⁷⁵ and **106**⁷⁶ reacted with sodium methoxide to afford the corresponding epimines, whereas thiazolines were produced from derivatives **103**⁵ and **107**⁷⁶ in refluxing pyridine. Dithiocarbamate **101** gave the corresponding thiazoline in pyridine and a mixture of 2-methoxy- and 2-methylthiothiazoline on treatment with methoxide.⁷⁷



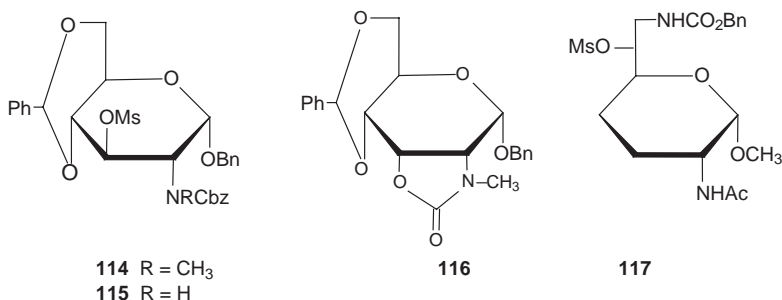
Analogously, 1,6-anhydro-4-*O*-benzyl-2-deoxy-2-isothiocyanato-3-*O*-tosyl- β -D-glucopyranose (**108**) cyclized by way of intermediary thiourethane **109** to give either the corresponding⁷⁸ epimine **110** in methanolic methoxide or the corresponding 2-methoxythiazoline **111** in methanolic triethylamine. For further examples of similar transformations see the review article in Ref. 71.



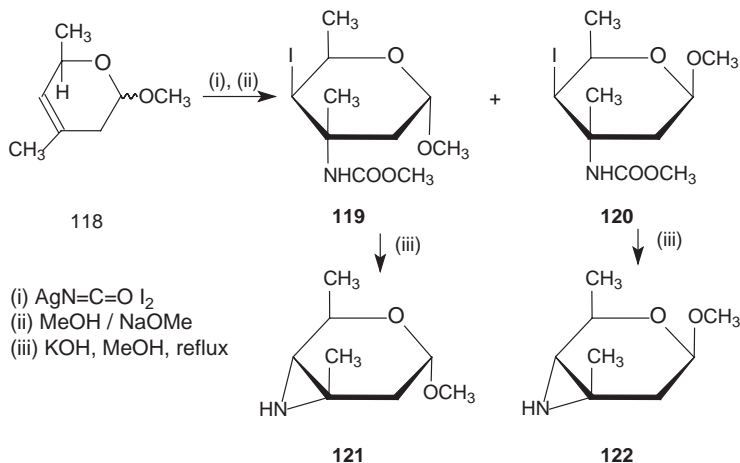
Reactions of the pyranosides just discussed did not afford imidazolidine or oxazolidine derivatives, but the furanoid phenylureido derivative **112**, however, cyclized to imidazolidinone **113** in refluxing methanolic methoxide (identification of the product was based only on infrared spectra and elemental analysis).⁷⁰



Cyclization of compounds containing the alkyl(aryl)oxycarbonylamino group vicinal to a sulfonate group or halogen may afford oxazolidinones. For example, compound **61** reacted with sodium 2-propoxide to give epimine **66**, whereas treatment with sodium acetate afforded the corresponding oxazolidinone.⁵⁶ Compound **114** gave oxazolidinone⁷⁹ **116** on heating with NaI in DMF, but compound **115** cyclized with sodium 2-propoxide to the free epimine.⁷⁹ Methyl 2-acetamido-2,3,4,6,7-pentadeoxy-6,7-epimino- β -L-*lyxo*-hexopyranoside was obtained from mesylate **117** on treatment with sodium isopropoxide.⁸⁰



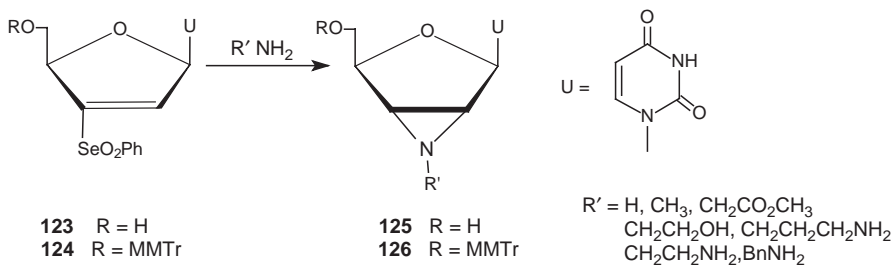
Kumar and coworkers have reported preparation of 2',3'-epimino analogues of the antibiotics neamine, ribostamine, and kanamycin B by reaction of suitably protected vicinal benzyloxycarbonylamino tosylates with NaH in *N,N*-dimethylformamide, followed by deprotection.⁶⁷ Iodo-carbamates **119** and **120** were obtained by addition of iodine isocyanate to 2-methoxy-4,6-dimethyl-3,6-dihydro-2*H*-pyrane **118** (a mixture of *cis* and *trans* isomers), followed by treatment with MeOH. Treatment of compound **119** and **120** with potassium hydroxide in refluxing methanol afforded⁸¹ epimines **121** and **122**.



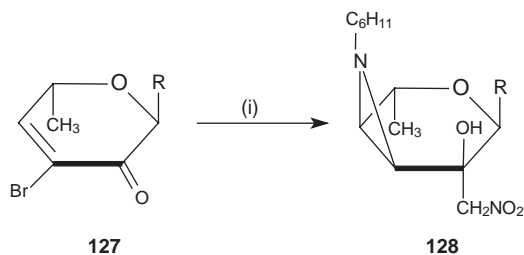
4. Cyclization Involving Michael Addition

This approach is based on nucleophilic addition of amines to a double bond activated by a suitable electron-withdrawing group. The resulting amino compounds undergo $\text{S}_{\text{N}}2$ displacement to give aziridines. The electron-withdrawing group is often employed as a leaving group as well.

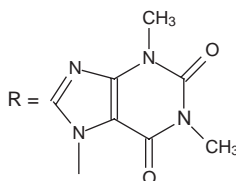
Thus 2',3'-ene-3'-phenylselenone nucleosides **123** and **124** add ammonia, methylamine, glycine methyl ester, 2-aminoethanol, 1,3-propanediamine, 1,2-ethanediamine, and benzylamine to give^{82,83} aziridines **125** and **126**.



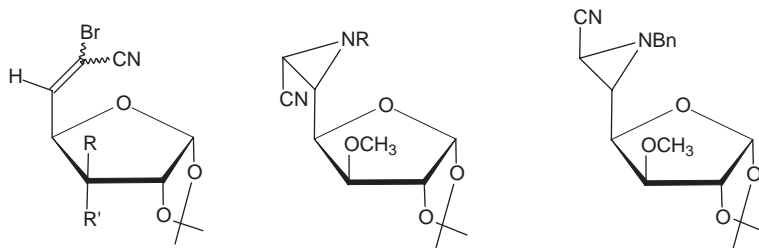
Bromoketonucleoside **127** adds⁸⁴ cyclohexylamine followed by nitromethane to form epimine **128**.



(i): $\text{C}_6\text{H}_{11}\text{NH}_2$, CH_3NO_2



The (*E*)-isomer of nitrile **129** adds⁸⁵ ammonia and primary amines (MeNH_2 , BnNH_2 , ethane-1,2-diamine and also 5-amino-5-deoxy-1,2-isopropylidene-3-*O*-methyl- α -D-xylofuranose) stereospecifically for each of the newly formed asymmetric carbon to give *cis*-(2*S*)-3-cyano-2-glycosylaziridines **132–136**. The (*Z*)-isomer of **129** adds benzylamine to give *trans*-(2*S*)-aziridine **137**. The stereospecificity of this addition is probably controlled by the steric hindrance of the *si* face at C-5, as shown by a substantial decrease in stereospecificity when compounds **130** and **131** add benzylamine.⁸⁵



129 $\text{R} = \text{OMe}$, $\text{R}' = \text{H}$

130 $\text{R} = \text{R}' = \text{H}$

131 $\text{R} = \text{H}$, $\text{R}' = \text{OMe}$

132 $\text{R} = \text{H}$

133 $\text{R} = \text{Me}$

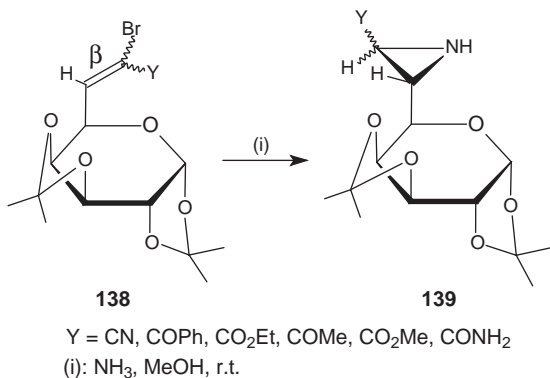
134 $\text{R} = \text{Bn}$

135 $\text{R} = \text{CH}_2\text{CH}_2\text{NH}_2$

136 $\text{R} = \text{H}_2\text{N-Glycose derivative}$

137

The stereospecific nucleophilic addition of ammonia at C- β from the *reside* of the bromoenoses **138** yielded aziridines **139**, sometimes as a mixture of *cis* and *trans* isomers.⁸⁶

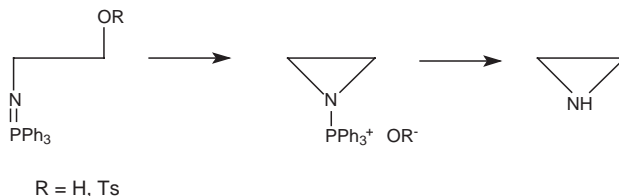


5. Staudinger-Type Cyclization

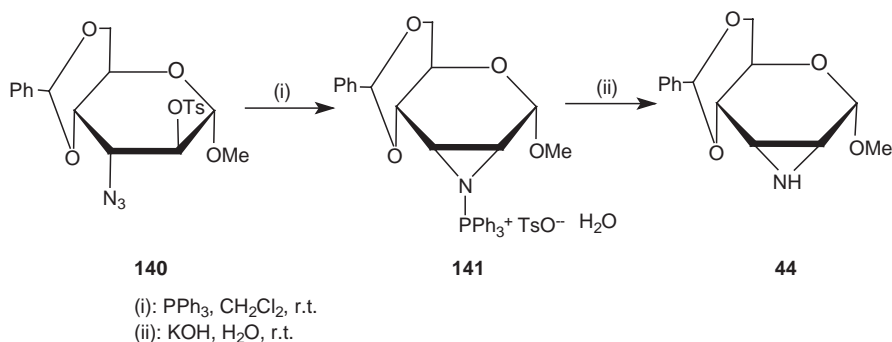
Organic azides react with tertiary alkyl(aryl)phosphines to give iminophosphoranes (phosphinimines):



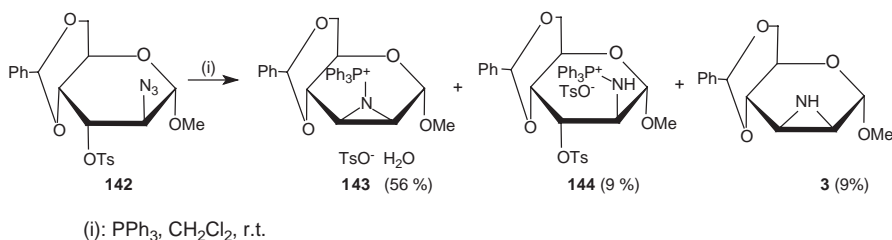
Iminophosphoranes having a vicinal hydroxy or sulfonyloxy group spontaneously undergo cyclization to form either aziridinylphosphonium hydroxides or sulfonates,⁸⁷⁻⁸⁹ or to produce aziridines directly. Cyclization proceeds with inversion of configuration at the carbon carrying the hydroxyl or sulfonyloxy group. The resultant phosphonium salts in some cases are labile and decompose under the reaction conditions to give free aziridines and triphenylphosphine oxide, or they may be converted into free aziridines by hot aqueous alkali.



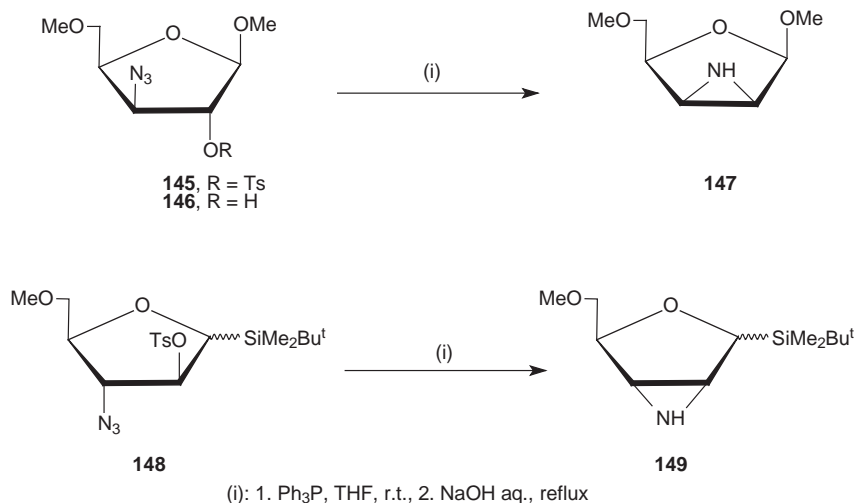
This reaction provides a useful alternative to the reduction of azido sulfonates by lithium aluminum hydride. Thus, treatment of azidotosylate **140** with PPh_3 in CH_2Cl_2 afforded⁹⁰ methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-(*N*-tri phenylphosphonioepimino)- α -D-alloside *p*-toluenesulfonate (**141**). The free epimine **44** was obtained from **141** on treatment with aqueous KOH.



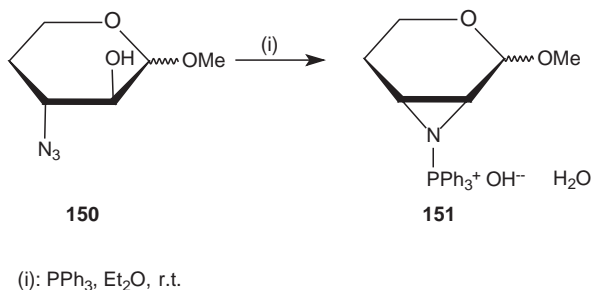
The reaction of azidotosylate **142** with PPh_3 was found to be more complex, depending on the solvent and particularly on the presence of water: in purified dry dichloromethane a mixture of phosphonioepimino salt **143**, free epimine **3** and another salt **144** was obtained, whereas in commercial-grade solvent only **144** (44%) and **3** (30%) could be isolated, and in dry 1,2-dichloroethane only phosphonioepimino salt **143** (96%) was formed. The authors assumed that, in the presence of traces of water an intermediate iminophosphorane reacts with **143** to give free epimine **3**, the phosphonioamino salt **144**, and triphenylphosphine oxide.⁹⁰ In the *gluco* analogue of **140**, where the reacting groups are *trans*-diequatorial, the reaction with PPh_3 afforded only an iminophosphorane. When the *O*-tosyl groups of **140** and **142** were replaced by *O*-acetyl groups imonophosphoranes were again the only products of the reaction.



Dubois and Dodd reported⁹¹ the use of this methodology for the synthesis of methyl 2,3-dideoxy-2,3-epimino-5-*O*-methyl- β -D-lyxofuranoside (**147**) and *tert*-butyldimethylsilyl 2,3-dideoxy-2,3-epimino-5-*O*-methyl- α,β -D-ribofuranoside (**149**) from azido tosylates **145** and **148**. Treatment of azido-alcohol **146** with PPh_3 afforded only the corresponding amino-alcohol.

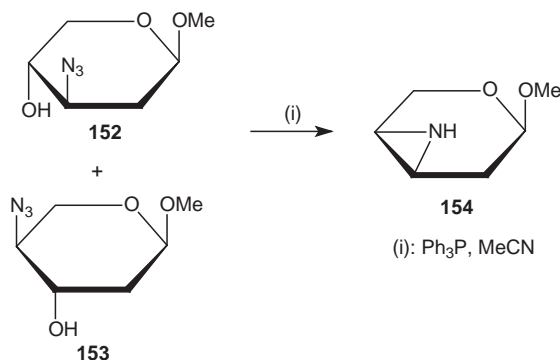


The reaction of azido alcohols was, however, effective with methyl 3-azido-3,4-dideoxy- α - and β -DL-*threo*-pentopyranosides (**150**), and yielded²⁹ epimine **151**.



Furthermore, treatment of the crude mixture of azido alcohols **152** and **153**, obtained by azidolysis of tosylate precursors, afforded aziridine **154** in high yield.⁹² Danishefsky and coworkers reported that reductive cyclization of an exocyclic azido mesylate in the presence of trimethyl phosphite, followed by

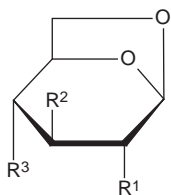
treatment with sodium hydride in tetrahydrofuran, afforded a phosphorylaziridine.⁹³



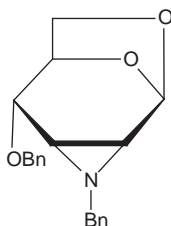
Appel reported that various aziridines may be obtained by the reaction of triphenylphosphine, carbon tetrachloride, and triethylamine with *N*-substituted vicinal amino alcohols.⁹⁴ Reaction of adjacent *p*-methoxybenzylamino and alcohol groups at the 2' and 3' positions of butirosin A and B under these conditions afforded epimino derivatives of both antibiotics.⁹⁵

6. Mitsunobu Reaction

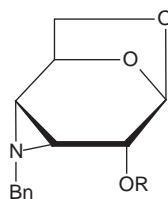
The synthetic potential of the Mitsunobu reaction⁹⁶ for the preparation of aliphatic aziridines from *N*-alkyl and *N*-acyl 2-amino alcohols has been demonstrated in a number of examples.^{97,98} The possibility of using the Mitsunobu reaction for preparation of sugar aziridines was explored in the synthesis of epimino derivatives of 1,6-anhydro- β -D-hexopyranoses.⁹⁹ Epimines **160–164** were obtained from *N*-benzylamino derivatives **155–159** by treatment with PPh_3 and diisopropyl azodicarboxylate in dry toluene at 0–5 °C. Compound **158** afforded solely the 3,4-epimine **163**, in accordance with the reactivity of the hydroxyl group decreasing in the order 4-OH > 3-OH > 2-OH. The starting benzylamino derivatives **155–159** were obtained by regioselective *trans*-diaxial cleavage of suitable 1,6:2,3- and 1,6:3,4-dianhydro- β -D-hexopyranoses with benzylamine. Dianhydro derivatives having the 2,3-oxirane ring *exo*-oriented (2,3-D-*allo* and D-*gulo* configurations), however, proved resistant toward cleavage of the oxirane ring by benzylamine.



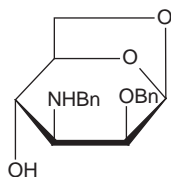
155 R¹ = NHBn, R² = OH, R³ = OBn
156 R¹ = OBn, R² = OH, R³ = NHBn
157 R¹ = OTs, R² = OH, R³ = NHBn
158 R¹ = OH, R² = NHBn, R³ = OH



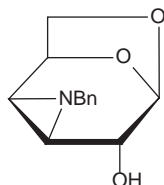
160



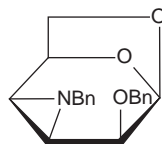
161 R = OBn
162 R = OTs



159

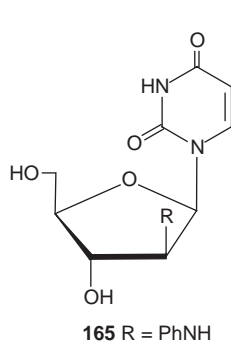


163

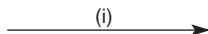


164

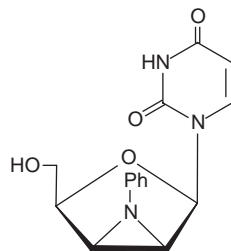
Minamoto and coworkers reported¹⁰⁰ use of the Mitsunobu reaction for the synthesis of 1-[2,3-dideoxy-2,3-(*N*-phenylepimino)-β-*D*-lyxofuranosyl]uracil (**166**) from compound **165**.



165 R = PhNH



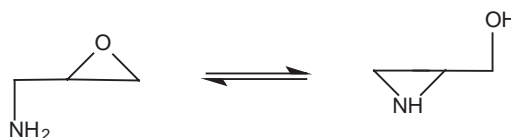
(i): PPh₃, DIAD, 1,4-dioxane, r.t.



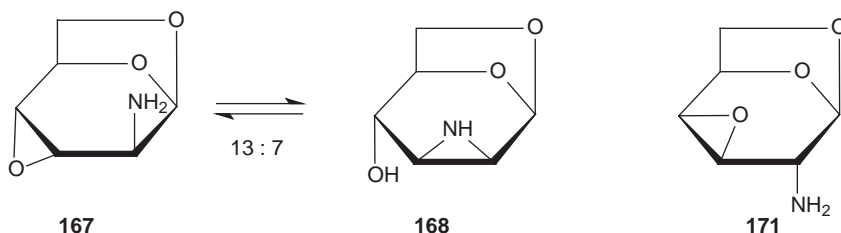
166

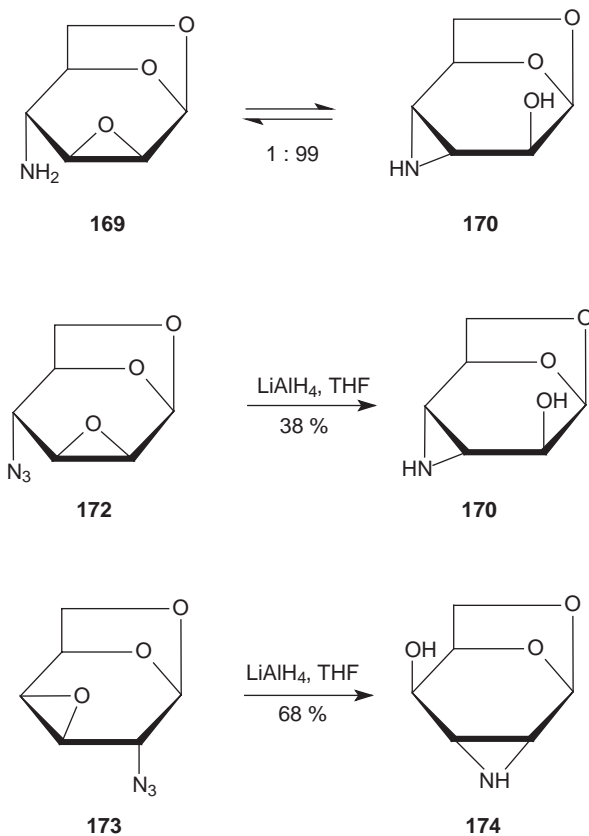
7. Isomerization of Vicinal Aminooxiranes

Vicinal *trans*-aminooxiranes isomerize at elevated temperature and/or acid–base catalysis to *trans*-hydroxyaziridines according to a general scheme:

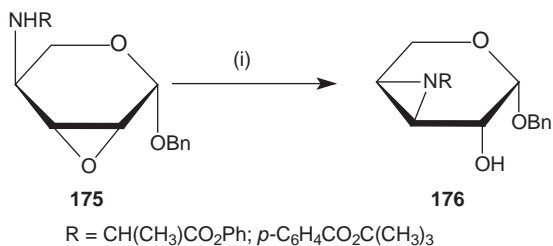


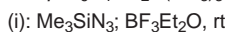
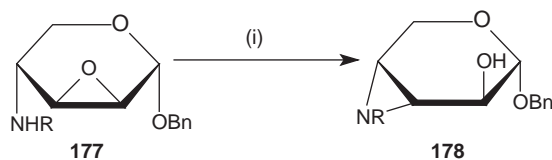
In general, the reaction is reversible and the composition of the equilibrium mixture can be significantly influenced by the stereochemistry of its components, as demonstrated¹⁰¹ in a study on the isomerization of 2-amino-1,6:3,4-dianhydro-2-deoxy- β -D-altropyranose (**167**) and 4-amino-1,6:2,3-dianhydro-4-deoxy- β -D-mannopyranose (**169**) into epimines **168** and **170** in water at 100 °C. The equilibrium ratio was found to be 13:7 for compounds **167** and **168** and 1:99 for compounds **169** and **170**, indicating a greater stability of the isomers having an *exo*-oriented three-membered ring¹⁰² and also the greater stability of the aziridine ring. Isomerization of an *N*-substituted derivative of mannopyranose derivative **169** was utilized in the synthesis of the pseudo-disaccharide acarviosin.¹⁰³ Attempted isomerization of the aminooxiranes **169** and **171** in aqueous 5% KOH resulted^{104,105} in concomitant hydrolytic cleavage of the oxirane ring, yielding 11 and 80% respectively of the corresponding hydrolytic products, and 82 and 20% of epimines **170** and **174** respectively. Lithium aluminum hydride reduction of azido epoxides **172** and **173** gave rise to aziridines **170** and **174**, apparently through isomerization of intermediate aminooxiranes.²⁶





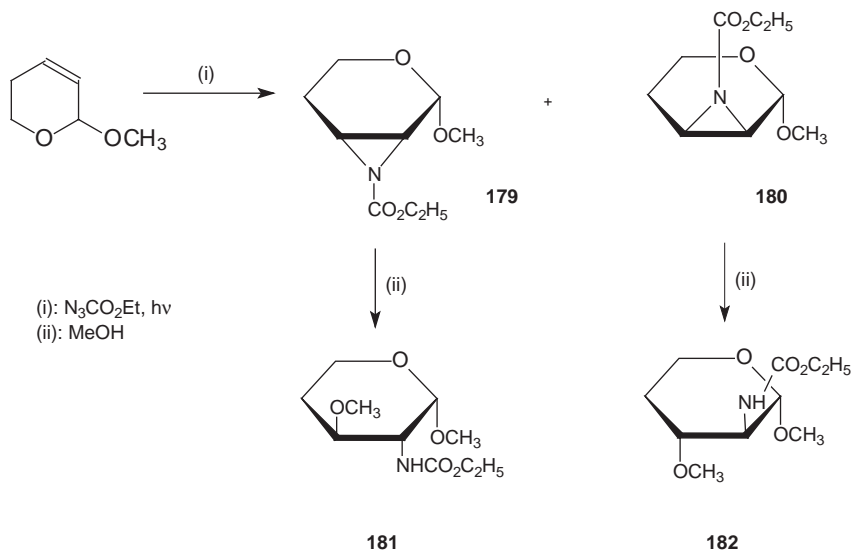
The *N*-substituted aminoderivatives **175** and **177** were reported^{106,107} to isomerize in trimethylsilyl azide under catalysis by boron trifluoride etherate to epimines **176** and **178**.





8. Addition of Nitrenes

Nitrenes are very reactive intermediates that readily react with alkenes to give aziridines. The stereochemistry of this aziridination and its use in synthetic chemistry has been the subject of several studies (see Refs. 108–112). In the carbohydrate field, the addition of a nitrene, generated by photolysis of ethyl azidoformate, to 2-methoxy-5,6-dihydro-(2*H*)-pyran was reported¹¹³ to afford aziridines **179** and **180** in 13:87 ratio (determined by GC); these products were, however, not isolated. Treatment of the crude reaction mixture with methanol gave ring-opening products **181** and **182**. The stereochemistry of the addition is controlled by the axial methoxy group, as indicated by the preponderant formation of aziridine **180**. Several other dihydropyran derivatives and glycals have been treated with ethyl azidoformate under photolytic conditions in the presence of aliphatic alcohols to yield products of alcoholysis of the supposed intermediary aziridines^{114,115} (for more details on the cleavage products see Section IV.5).



III. GENERAL PROPERTIES OF CARBOHYDRATE AZIRIDINES

Most epimino sugars are relatively stable in neutral or basic conditions. They are usually stable in the presence of such hydride reductive reagents as lithium aluminum hydride or sodium borohydride. They tend to undergo cleavage reactions or decompose in the presence of acids. *N*-Acyated aziridines are prone to lose the *N*-acyl substituent in the presence of nucleophiles and bases, and caution must be taken when nucleophilic cleavage of the aziridine ring of *N*-acylated epimines is to be performed.

The structure of epimino sugars may be demonstrated by spectroscopic means. NMR spectroscopy is the technique most frequently used for determining of the structure of epimines, particularly their conformation in solution. The existence of hydrogen bonds has been evidenced by IR spectroscopy.

NMR spectral parameters for alkyl 4,6-*O*-alkylidene-2,3-dideoxy-2,3-epimino-hexopyranosides having the *D*-*allo*^{47,48,55,72,116–119}, *D*-*gulo*,²⁵ and *D*-*mano*^{25,120} configurations have been reported in the literature. The chemical shifts of protons on carbons C-2 and C-3 of the aziridine ring range between 2.18–3.07 ppm for unsubstituted epimines, while *N*-substitution by electron-withdrawing groups leads to their increase (*N*-acetyl, 2.80–3.20 ppm, *N*-benzoyl, 3.01–3.32 ppm, and *N*-⁺PPh₃[−]OTs, 2.70–3.56 ppm, Ref. 118). The $J_{2,3}$ coupling constants are in the range 6.0–7.0 Hz.

A detailed study on the structure of 2,3- and 3,4-epimino derivatives of 1,6-anhydro- β -D-hexopyranoses has been made employing NMR and IR spectroscopy.^{26,121}

For the epimino derivatives of 1,6-anhydro- β -D-hexopyranoses, the ¹H and ¹³C NMR spectra have been measured in deuteriochloroform.²⁶ Chemical shifts of the aziridine ring hydrogens appear in the range 2.16–2.81 ppm for 2,3-epimines and 2.16–2.97 ppm for 3,4-epimines. The coupling constants of aziridine ring hydrogens, $J_{2,3}$ resp. $J_{3,4}$, appear in the range 5.0–6.6 Hz for 2,3-epimines and 5.2–6.4 ppm for 3,4-epimines. Chemical shifts of aziridine ring carbons appear in the range 26.5–38.8 ppm for 2,3-epimines and 27.2–35.5 ppm for 3,4-epimines.

Vibration bands belonging to the NH group of the aziridine ring in epimino derivatives of 1,6-anhydro- β -D-hexopyranoses have been observed by IR spectroscopy.²⁶ The free NH band was found in the 3321–3330 cm^{−1} range while for an NH group associated with an OBn group, with the oxygen atoms in the 1,6-anhydro bridge and with the tetrahydropyran ring, bands in the range 3301–3309, 3304–3309, and 3285–3295 cm^{−1}, respectively, were found.

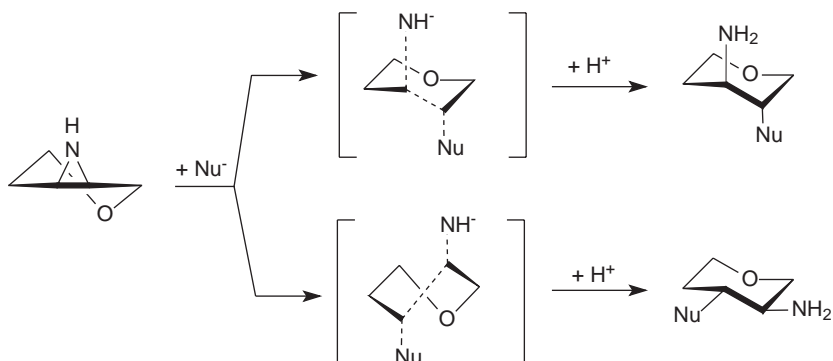
As with the dianhydro derivatives of hexopyranoses (cf. Refs. 102, 123), unsubstituted epimines can form equilibrium mixtures of vicinal aminooxiranes and hydroxyaziridines.¹⁰¹ The composition of the equilibrium depends on the stability of a three-membered ring, which is determined mainly by the orientation of the ring toward the 6,8-dioxabicyclo[3.2.1]octane skeleton.¹²² Rings that are 2,3-*endo* oriented have been found to be the most unstable (cf. Refs. 10, 124).

IV. REACTIONS OF CARBOHYDRATE AZIRIDINES

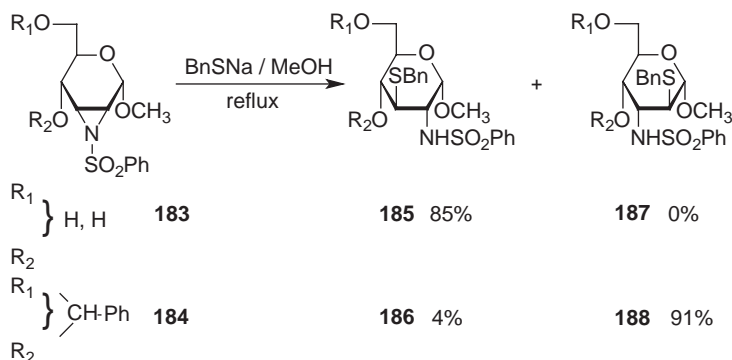
1. General Considerations on Reactivity

a. Stereochemistry of Aziridine-Ring Cleavage.—To perform cleavage of the aziridine ring, two problems have to be addressed: that of regioselectivity and the problem of aziridine-ring activation. Cleavage reactions of non-sugar aziridines with nucleophiles usually proceed with high degree of regioselectivity to give just one of the possible stereoisomers (for reviews see Refs. 125–127). Cleavage reactions of sugar aziridines are, on the other hand, also strongly influenced by the configuration of the starting aziridine and by its conformational flexibility. We have therefore decided to group sugar epimines into four classes according to the type of skeleton to which aziridine ring is fused; namely pyranoid epimines having rigid conformations, pyranoid epimines with flexible conformations, furanoid epimines, and exocyclic epimines.

Two different reaction pathways can be proposed for the reaction of pyranoid epimines:¹²⁸

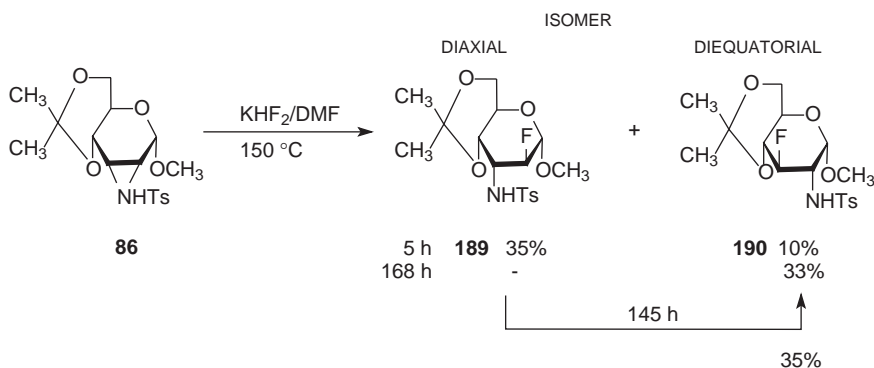


In general, two possible stereoisomers can be formed in the cleavage reaction. These two pathways involve either “*trans*-diaxial” or “*trans*-diequatorial” cleavage (compare Refs. 7, 8, 68). The pathway via a skew conformation of the tetrahydropyran ring is involved only if conformationally locked or biased pyranoid epimines are cleaved. It is supposed¹²⁹ that an “inner” S_N2 mechanism takes place and the regioselectivity is controlled primarily by the stereochemistry of nucleophilic substitution. The energy barrier between conformations of the tetrahydropyran ring in the reactant disfavors the formation of the diequatorial isomer. The favored formation of the diaxial isomer is often termed as the Fürst–Plattner rule.¹³⁰ However, this interpretation is valid only for aziridines with a sufficiently rigid conformation of the tetrahydropyran ring, such as 1,6-anhydro-β-D-hexopyranoses and alkyl 4,6-*O*-alkylidene-hexopyranosides. If the carbohydrate epimine exists in a flexible conformation, neither the transition state nor the stereochemistry of the cleavage product can be predicted from the conformation of the starting epimine. For example, the reaction of benzenesulfonylepimine **183** illustrates that absence of the 4,6-*O*-benzylidene group results in a complete change of the stereochemical outcome of the cleavage even though both derivatives **183–184** were shown to exist in the same conformation [^oH₅(D)].⁶³



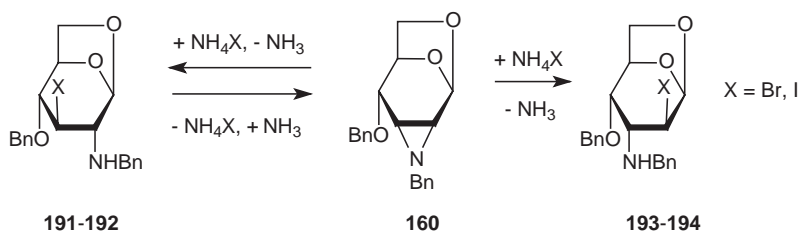
The Fürst–Plattner rule can be used only for predicting the regioselectivity of kinetically controlled cleavage reactions. If thermodynamic control takes place and the initially formed diaxial isomer is able reversibly to close back to the aziridine ring, the diequatorial isomer can begin to accumulate in the reaction mixture and is isolated either as the sole product^{10,131} or in a mixture^{64,68} with the diaxial isomer.

Such formation of both isomers of the cleavage products has been observed in the reactions with weak nucleophiles such as the hydrogendifluoride anion, which requires harsh reaction conditions to complete the cleavage.⁶⁴



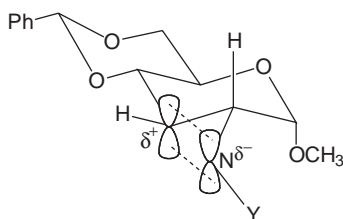
The diaxial isomer **189** was the main product at short times of reaction, whereas the diequatorial product **190** was formed exclusively after prolonged heating. Application of the same conditions to the diaxial isomer alone led to its conversion into the diequatorial isomer through the equilibrium between the diaxial isomer and the epimine. Rate constants for each reaction involved in the cleavage have been estimated.⁶⁴

Exclusive formation of the diequatorial isomers **193–194** has been observed in the reactions of 1,6-anhydro-4-*O*-benzyl-2,3-(*N*-benzylepimino)-2,3-dideoxy- β -D-allopyranose **160** with a mixture of ammonium and tetrabutylammonium bromide or iodide on heating in toluene at high temperature. In these instances, the corresponding diaxial isomers **191–192** were unstable and were formed only *in situ* (see Table X for details).¹⁰

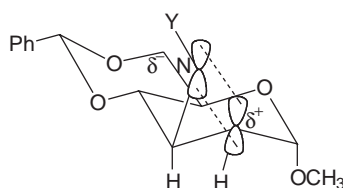


Another mechanism was proposed in the literature for the formation of the diequatorial product in reactions of methyl 4,6-*O*-benzylidene- α -D-hexopyranosides.⁸

This mechanism postulates an S_N1 -like transition state, which is formed by heterolysis of one of the C–N bonds in the aziridine ring. Fission of the bond can give rise to a carbocation at either C-2 or C-3 according to its relative stability. The adjacent acetal group destabilizes the ion only at C-2 and therefore the nucleophilic attack takes place at C-3. Because of the presence of the negatively charged nitrogen atom, which blocks one side of the carbonium ion, only *trans* isomer of the cleavage product is formed. This mechanism can take place for the reactions of *D-allo*-pyranosides, but not for the *D-manno* isomers due to the impossibility of stabilization of a carbonium ion. The authors were not able to explain why ring opening follows the S_N2 mechanism in some cases and S_N1 in the others.



D-allo

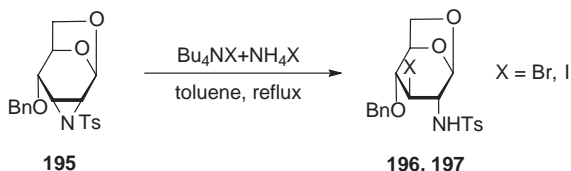


D-manno

b. Activation of the Aziridine Ring Toward Cleavage.—The reactivity of the aziridine ring with nucleophiles is lower than that of the oxirane ring,^{126,127} and successful cleavage requires either a powerful nucleophile or *N*-substitution of the aziridine nitrogen with an electron-withdrawing group, or both. Free, unsubstituted epimines have been utilized in only a few examples,²⁵ and mostly by-products with the ring unopened were formed instead of the cleavage products.⁸ The aziridine ring needs to be activated to achieve reasonable reaction yields of cleavage products. Unsubstituted epimines have been activated by protonation,^{9,132} quaternization¹³³ (mostly as *N,N*-dimethylaziridinium salts¹³²) or by the addition of a Lewis acid.¹³⁴ Another way used more often for the activation is *N*-substitution of the aziridine ring by sulfonyl-, acyl-, and alkoxycarbonyl-based substituents. The substitution promotes cleavage in two ways—it lowers the electron density in the ring and stabilizes negative charge on nitrogen after the cleavage, thus minimizing reversible ring closure. The substituents also influence the regioselectivity of ring cleavage. This is the main difference in nucleophilic cleavage of sugar aziridines as compared to their oxirane counterparts.

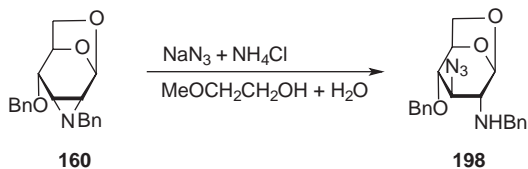
Changes in regioselectivity have been reported in the series of aziridine derivatives of 1,6-anhydro- β -D-hexopyranoses.¹⁰ *N*-Tosylepimine **195** of the *D-allo*

configuration gave the diaxial bromo- (**196**) or iodo- (**197**) derivative under the action of a mixture of ammonium and tetrabutylammonium bromide or iodide. In contrast, *N*-benzylepimine **160** under the same conditions produced diequatorial isomers of the *D-altro* configuration as the sole products.

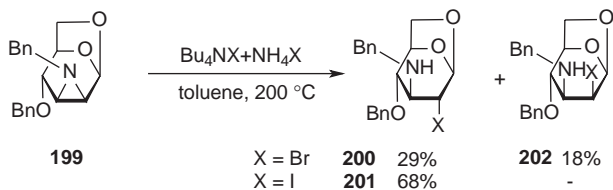


The reason for this different behavior is that the benzylamino group is a much more powerful nucleophile than the tosylamino group, and is able to cyclize and regenerate the aziridine ring. The equilibrium between the starting epimine and the diaxial isomer of the cleavage product could be established and consequently, after prolonged heating, the thermodynamically more stable diequatorial halo derivatives were formed as ultimate cleavage products.¹⁰

In the reaction of *N*-benzylepimine **160** with azide anion, *trans*-diaxial cleavage is favored since the azide anion is a poor leaving group, so that cyclization is not possible.



The configuration of the starting epimine can have a strong influence on feasibility of back-formation of the aziridine ring from the diaxial isomer of the cleavage product. This is particularly true for the reactions of *D-manno* epimine **199**. It has been reported in the literature (cf. Refs. 102, 135), that the cyclization of an oxirane^{124,136} or an aziridine¹⁰ ring in the 2,3-*endo* position on a 6,8-dioxabicyclo[3.2.1]octane skeleton proceeds rather slowly as compared to 2,3-*exo*-, 3,4-*exo*-, and 3,4-*endo* positions. Because of this reason, the cleavage reaction of *D-manno* epimine **199** does not produce diequatorial isomers, even though atoms with good leaving capability and the nucleophilic benzylamino group are present in the molecule. Instead, a mixture of isomers having the *D-gluco* and *D-manno* configurations is formed.



In conclusion, the *N*-substituent used for activation, the configuration of the epimine, and the nucleophile involved are three basic factors that determine the isomer composition of the cleavage products in reactions of pyranoid aziridines. The influence of reaction conditions (solvents and such promoters as Lewis acids) upon the regioselectivity is minimal, although they accelerate the cleavage itself or in cooperation with appropriate *N*-substitution. The regioselectivity of aziridine-ring cleavage is controlled through establishment of the equilibrium between the diaxial isomer and the starting epimine. If such an equilibrium is not established in the cleavage reaction, the regioselectivity for conformationally rigid epimines conforms to the Fürst–Plattner rule. Otherwise, the formation of diequatorial and yet other isomers must be taken into account. This hypothesis was experimentally confirmed^{10,11} for the cleavage reactions by nucleophiles of epimines derived from the 1,6-anhydro- β -D-hexopyranose skeleton, whereas for the cleavage reactions of epimines derived from alkyl 4,6-*O*-alkylidene-hexopyranosides, no rationalization of their regioselectivity has yet been reported because of the lack of experimental studies (cf. Refs. 64, 68).

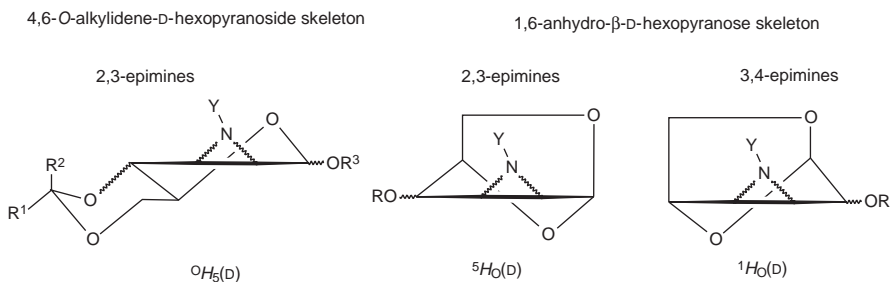
For reactions of furanoid epimines, the configuration of the epimine does not determine the regioselectivity. Other factors, such as steric interactions between nucleophile and epimine, or soft or hard character of the nucleophile, may control the regioselectivity.

Exocyclic aziridines react without the influence of a tetrahydropyran or tetrahydrofuran ring on the regioselectivity, and thus these epimines resemble non-sugar aziridines in their cleavage reactions.

2. Pyranose Aziridines

a. Derivatives of Fixed Conformation.—This class of sugar aziridines has been utilized most extensively in cleavage reactions on account of the possibility of predicting the product configuration by the Fürst–Plattner rule. There are two important families of pyranose monosaccharides possessing sufficiently rigid

skeleton conformations: the 1,6-anhydro- β -D-hexopyranoses and the alkyl 4,6-*O*-alkylidene-hexopyranosides. Their aziridine derivatives adopt half-chair [$^0H_5(D)$, $^5H_O(D)$, and $^1H_O(D)$] conformations.^{8,26}



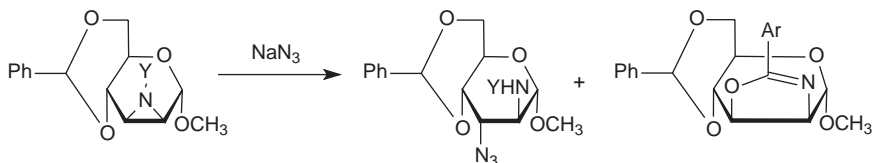
Cleavage reactions of various alkyl 4,6-*O*-alkylidene-2,3-dideoxy-2,3-epimino-hexopyranosides having the *D-allo*, *D-manno*, *D-ido*, and *D-galacto* configurations have been performed with azide anion, halo acids, and with ammonium halides.

Aziridine-ring cleavage of *N*-substituted methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino- α -D-mannopyranosides by either sodium azide alone or in admixture with ammonium chloride leads to the formation of the diaxial azido derivative and the corresponding oxazoline (see Table I for details).

It should be emphasized that both products originate from regioselective attack at carbon C-3 and the azide/oxazoline ratio decreases in the presence of ammonium chloride in the reaction mixture. The initially reported⁶ predominant formation of the oxazoline **42** in the reaction of *N*-benzoylepimine **41** with $\text{NaN}_3/\text{NH}_4\text{Cl}$ in DMF was later questioned by the same author because repetition⁷ of the experiment afforded solely the 3-azido altroside **206** in 80% yield. However, with no NH_4Cl present, the oxazoline (48%), along with the azido derivative (15%) was obtained.⁷ It was therefore assumed⁷ that NH_4Cl was not actually present in the reaction mixture. No conclusions on the regioselectivity of ring cleavage can be made for *N*-*p*-nitrobenzoylepimine **204** because of the low yields of identified products and decomposition of the starting epimine.⁷ There were some by-products found in the reaction mixtures, but they remained unseparated and uncharacterized.

The aziridine ring of methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino- α -D-talopyranoside **211** was opened by sodium azide with formation of the diaxial azido derivative (**212**) of the *ido* configuration in 26% yield, while the corresponding α -D-gulopyranoside did not react at all.²⁵

TABLE I
Cleavage Reactions of Free and *N*-Substituted Methyl 4,6-*O*-Benzylidene-2,3-dideoxy-2,3-epimino- α -D-mannopyranosides with Sodium Azide

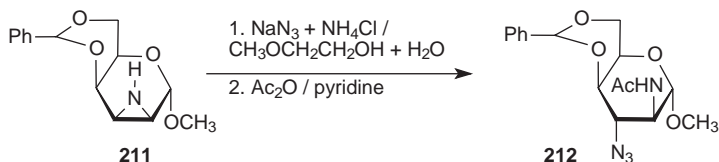


	Y	Reaction Conditions ^a		Yield (%)			Ref.
3	H	A	205	54		0 ^b	6
41	Bz	B	206	15	42	48	7
	Bz	C		20		36	6
	Bz	C		80		0	7
203	Ans	C	207	70		0	7
	Ans	B		< 10	210	47	7
204	NBz	C	208	45		0	7
	NBz	B		25		^c	7
80	Ts	C	209	63		0	6

^aA: $\text{NaN}_3 + \text{NH}_4\text{Cl} / \text{CH}_3\text{OCH}_2\text{CH}_2\text{OH} + \text{H}_2\text{O}$, reflux; B: $\text{NaN}_3 / \text{DMF}$, reflux; C: $\text{NaN}_3 + \text{NH}_4\text{Cl} / \text{DMF}$, reflux.

^bIn addition, 15% of the starting compound was isolated.

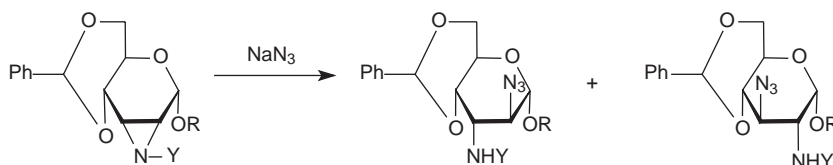
^cOther unseparated products present.



The results of the cleavage reactions of *N*-substituted alkyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino- α -D-allopyranosides are summarized in Table II.

The observed ratio of diaxial/diequatorial isomers is very little influenced by the presence of ammonium chloride in the reaction mixture in contrast to the marked effect of the *N*-substituent. The diaxial isomers predominate in most reactions, although the amounts of diequatorial isomers are sometimes significant. In the

TABLE II
Cleavage Reactions of Free and *N*-Substituted Methyl 4,6-*O*-Benzylidene-2,3-dideoxy-2,3-epimino- α -D-allopyranosides with Sodium Azide



	R	Y	Reaction Conditions ^a		Yield (%)		Ref.
44	Me	H	A	219	82	0	6
213	Me	Ac	C	220	64(Y = H) ^b	0	6
	Me	Ac	C		0	228 31 ^c	8
214	Me	Ts	C	221	41	0	6
85	Me	Ms	C	222	60	0	8
215	Me	DNP	C	223	30	0	6
45	Me	Bz	C		0	229 70 ^d	6
	Me	Bz	C	224	56	18	7
	Me	Bz	B		74	26 ^e	7
216	Me	Ans	C	225	71	230 29 ^e	7
	Me	Ans	B		65	35 ^e	7
217	Me	NBz	C	226	85	231 15 ^e	7
	Me	NBz	B		^f	^f	7
218	Bn	DNP	B	227	29	232 16	47

^aA: NaN₃ + NH₄Cl/CH₃OCH₂CH₂OH + H₂O, reflux; B: NaN₃/DMF, reflux; C: NaN₃ + NH₄Cl/-DMF, reflux.

^bDeacetylation before ring-opening.

^cIn addition, 12% of *N*-deacetylated epimine was isolated.

^dErroneous result, probably mixture of isomers.

^eIsomers ratio estimated from ¹H NMR spectrum of crude reaction mixture.

^fDecomposition of reaction mixture.

ring-cleavage reactions of *N*-acetylepimine **213**, Hough and coworkers reported⁸ formation of the diequatorial azido derivative **228** in 31% yield, accompanied by 12% of the *N*-deacetylated starting epimine, whereas Guthrie and coworkers found⁶ that on prolonged heating of the reaction mixture the aziridine ring was opened *trans*-diaxially after its *N*-deacetylation. The influence on regioselectivity of the presence or absence of an *N*-acetyl substituent is notable, although this preferential formation of the diequatorial isomer **228** was not rationalized at all.^{6,8}

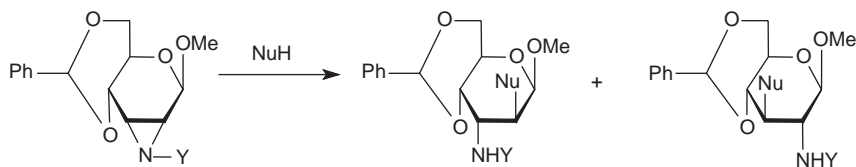
The claimed diequatorial cleavage of *N*-benzoylepimine **45** reported in Ref. 8 was later corrected.⁷ Guthrie and Williams in their contribution⁷ failed to reproduce the claim to the sole formation of the diequatorial isomer **229** in 70% yield. Instead, the cleavage of the *N*-benzoylepimine with $\text{NaN}_3/\text{NH}_4\text{Cl}$ in DMF afforded⁷ the *altro* (**224**) and the *gluco* (**229**) products in 56 and 18% yields, respectively. The authors therefore considered the previously claimed exclusive formation of the diequatorial isomer as an erroneous result.

Ring cleavage of the β anomer of *N*-benzoyl *allo*-epimine **67** gave preferential formation of diequatorial isomers as shown by Meyer zu Reckendorf⁵³ (see Table III).

It might be concluded that the configuration at the anomeric carbon atom reverses the regioselectivity of the ring cleavage, but we can only speculate about the reliability of these experiments⁵³ because only the yields of crude products were given. It seems that the products isolated were, in fact, mixtures of isomers (cf. Ref. 7). Other results^{47,54} for the cleavage of methyl 4,6-*O*-benzylidene-2,3-(*N*-2,4-dinitrophenylepimino)-2,3-dideoxy- β -D-allopyranoside (**233**) by sodium azide indicate formation of both isomers.

Only the diaxial isomer **240** was formed in the reaction of quaternized epimine **239** with sodium azide in *N,N*-dimethylformamide.¹³²

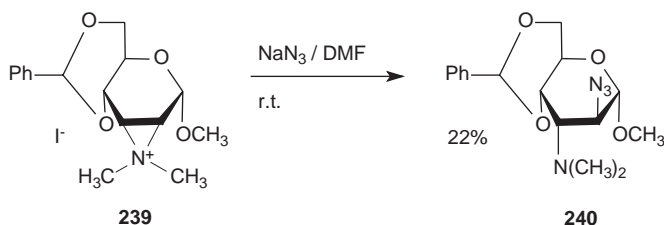
TABLE III
Cleavage Reactions of Methyl 4,6-*O*-Benzylidene-2,3-(*N*-benzoylepimino)-2,3-dideoxy- β -D-allopyranoside



	Nu	Y	Reaction Conditions		Yield (%)		Ref.
67	N_3	Bz	$\text{NaN}_3 + \text{NH}_4\text{Cl}/\text{DMF}$	0	235	89 ^a	53
	OH	Bz	$\text{Al}_2\text{O}_3/\text{benzene}$	0	236	17 ^b	53
	OAce	Bz	AcOK/DMF	0	237	86 ^a	53
233	N_3	DNP	$\text{NaN}_3 + \text{NH}_4\text{Cl}/\text{DMF}$	234	32	238	24 54

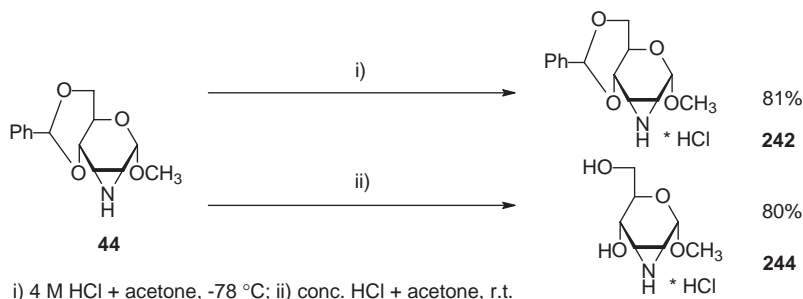
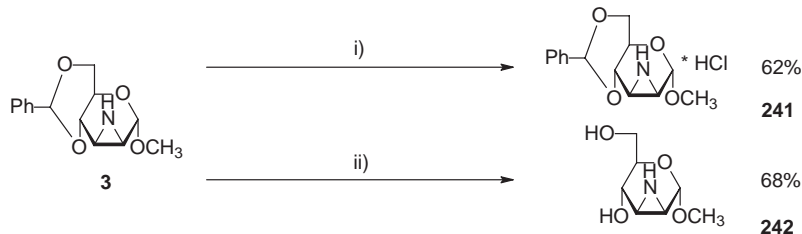
^aYield of crude product, yield after crystallization was not given.

^bIn addition, 23% of the starting compound and 42% of free epimine were isolated.



The reactions of methyl 4,6-benzylidene-2,3-dideoxy-2,3-epimino- α -D-manno- and allo-pyranosides with halo acids are complicated by acid-catalyzed removal of the benzylidene protecting group, either before or after the ring cleavage.

Free, unsubstituted *D-manno* epimine **3** gave only the hydrochloride salt **241**, without ring opening, when mixed with 4 M HCl in acetone at low temperature, whereas mixing with conc. HCl at room temperature led to deprotection of the benzylidene group, giving **242**. The same results were achieved in the reactions of *D-allo*-epimine **44** with HCl,⁸ leading to **243** and **244**.



i) 4 M HCl + acetone, -78°C ; ii) conc. HCl + acetone, r.t.

The results for cleavage reactions of *N*-substituted epimino derivatives of *D*-manno and *D*-allo configurations with halo acids are summarized in [Tables IV and V](#), respectively.

All products had configurations as predicted by the Fürst–Plattner rule, which implies that fission of the benzylidene group had to take place after aziridine-ring cleavage.

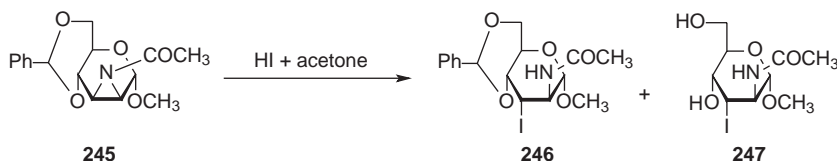
The reactions of *N*-substituted *D*-allo epimines with HCl and HI proceeded *trans*-diaxially in all cases where the biased conformation of the tetrahydropyran ring was maintained. In the reaction, where 4,6-*O*-benzylidene group was hydrolyzed prior to aziridine-ring cleavage, mixtures of stereoisomers were formed.¹³²

Cleavage reactions of epimino derivatives of methyl 4,6-*O*-benzylidene-2,3-epimino-2,3-dideoxy- α -*D*-allopypyranosides with ammonium halides led either to diaxial or diequatorial isomers, or mixtures of both ([Table VI](#)).

Free epimino derivative **44** afforded products of *trans*-diequatorial cleavage only, whereas *N*-substitution with an electron-withdrawing group led to the formation of mixtures of both stereoisomers, with the diaxial isomer predominant. In the cleavage of benzyl glycosides **53** and **255**, the authors proved¹³¹ the existence of an equilibrium between the diaxial isomer and the starting epimine, which led to preponderant formation of the diequatorial isomer.

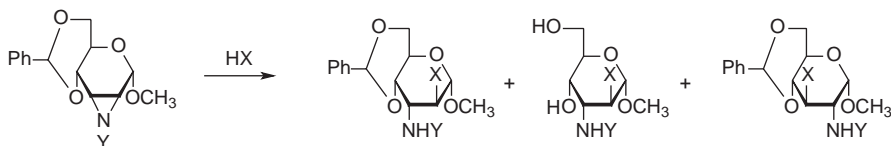
The *N*-benzoyl derivative **45** afforded a mixture of both isomers when treated with tetrabutylammonium fluoride. In the mixture, the diaxial isomer **265** preponderated, but its abundance depended on the solvent and slightly on the amount of the reagent ([Table VII](#)).

TABLE IV
Cleavage Reactions of Methyl 4,6-*O*-Benzylidene-2,3-(*N*-acetylepimino)-2,3-dideoxy- α -*D*-mannopyranoside with Hydroiodic Acid



Ref.	Reaction Temperature (°C)	Yield (%)	
9	-25	75	0
9	25	0	50

TABLE V
Cleavage Reactions of *N*-Substituted Methyl 4,6-*O*-Benzylidene-2,3-epimino-2,3-dideoxy- α -D-allopyranosides with Halo Acids



Ref.	Y	X	Reaction Conditions ^a		Yield (%)			
8	213	Ac	Cl	A	249	80	0	254 3
132		Ac	Cl	A		77	0	0
132		Ac	Cl	B		0	251 61	0
132		Ac	I	C	250	83	0	0
8	45	Bz	Cl	A		^b	^b	0
132		Bz	Cl	B		0	252 55	0
132	215	DNP	Cl	B		0	^c	0
133	248	Me	Cl	B		0	253 45	0

^aA: HCl/acetone, r.t.; B: HCl/acetone, reflux; C: HI/acetone, -25°C .

^bCompounds were identified, but no yields were given.

^cMixture of diaxial and diequatorial isomer without benzylidene group.

Benzene ring-substituted methyl 4,6-*O*-benzylidene-2,3-(*N*-aroylepimino)-2,3-dideoxy- α -D-mannopyranosides reacted with sodium iodide in *N,N*-dimethylformamide to form oxazolines, but they opened normally with sodium thiocyanate in 1,4-dioxane to form *trans*-diaxial products (Table VIII)¹³⁷.

Methyl 4,6-*O*-benzylidene-2,3-(*N*-tosylepimino)-2,3-dideoxy- α -D-mannopyranoside (**80**) was cleaved upon reaction with either sodium methoxide or hydroxide to afford the *trans*-diaxial product only. Under these conditions, unsubstituted epimine **3** did not react at all.¹³⁸

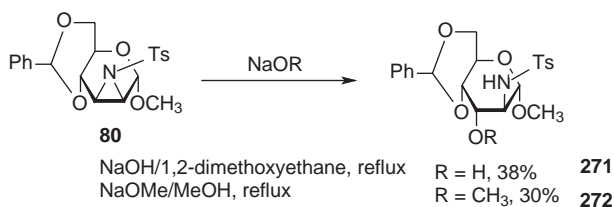
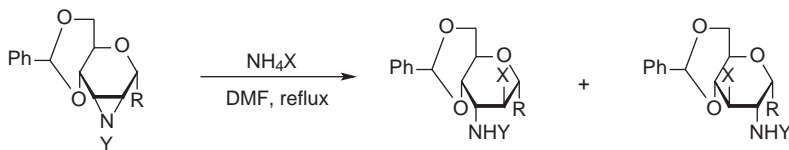


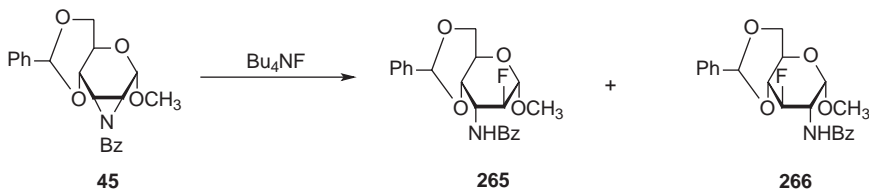
TABLE VI
Cleavage Reactions of Methyl and Benzyl 4,6-*O*-Benzylidene-2,3-epimino-2,3-dideoxy- α -D-allopyranosides with NH_4X



	Y		X	Yield (%)			Ref.
44	OMe	H	Cl	0	260	40	8
	OMe	H	Br	0		52	8
	OMe	H	I	0		54	8
213	OMe	Ac	Cl	256	42	261	8
45	OMe	Bz	Cl	257	35	262	8
215	OMe	DNP	Cl	258	29	263	8
53	OBn	H	Cl	0	260	52	131
255	OBn	^a	Cl	259	8	264	131

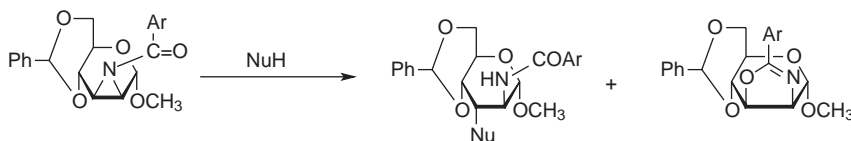
^aY = 3-Azido-3-deoxy-1,2-*O*-isopropylidene- α -D-glucofuranos-6-yl.

TABLE VII
Cleavage Reactions of Methyl 4,6-*O*-Benzylidene-2,3-(*N*-benzoylepimino)-2,3-dideoxy- α -D-allopyranoside with Tetrabutylammonium Fluoride



Ref.	Reaction Conditions	Yield (%)	
48	2.5 eq./HMPA, 80 °C	38	7
51	7.6 eq./CH ₃ CN, reflux	63	6
50	8.2 eq./HMPA, 85 °C	35	0

TABLE VIII
Cleavage Reactions of Methyl 4,6-*O*-Benzylidene-2,3-(*N*-aroylepimino)-2,3-dideoxy- α -D-mannopyranosides with NaI and NaSCN

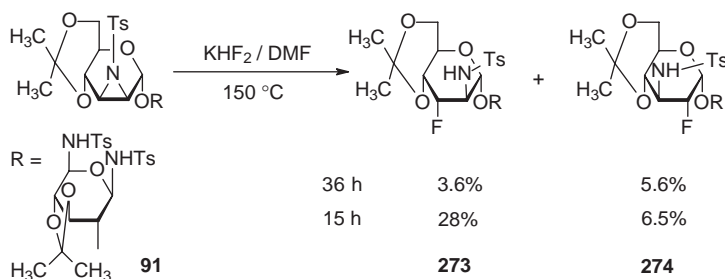


Nu		Ar	Reaction Conditions ^a		Yield (%)	
I	41	Ph	A	0	42	75 (40)
I	204	NPh	A	0	270	70 (38)
I	203	MPh	A	0	210	85 (20)
SCN	41	Ph	B	267	75	0
SCN	204	NPh	B	268	72	0
SCN	203	MPh	B	269	55	0

Note: Numbers given in parentheses are the yields of reactions without NaI added to the reaction mixture.

^aA: NaI/DMF, reflux; B: NaSCN/dioxane, reflux.

Kobayashi and coworkers reported⁶⁸ cleavage with potassium hydrogendifluoride of a streptamine derivative (**91**) containing a tosyl-substituted aziridine ring. Despite very low yields of the resultant fluoro tosylamides, the authors demonstrated the possibility of aziridine-ring cleavage in such a complex molecule. A mixture of diaxial and diequatorial isomers was formed in the cleavage, the diaxial isomer **273** preponderated in shorter-time reaction course, while prolonged heating led to its disappearance.

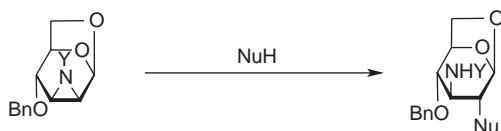


Cleavage reactions of *N*-benzyl-, *N*-*o*-nitrobenzenesulfonyl-, and *N*-tosylepimino- derivatives of 1,6-anhydro- β -D-hexopyranoses with azide and halide anions, halo acids, and benzyl-derived nucleophiles (amine, alcohol, thiol) have been studied extensively in the past decade.

The results of the reactions of 2,3-epimines having the *D-manno*, *D-allo*, and *D-talo* configurations are listed in [Tables IX, X, and XI](#), respectively.

All cleavage reactions of the epimines having the *D-manno* configuration afforded solely the diaxial isomers of the products, except for the reaction of *N*-benzyl derivative **199** with a mixture of Bu₄NBr and NH₄Br in toluene, wherein which 2-bromo derivative **202** of the opposite configuration on carbon C-2 was

TABLE IX
Cleavage Reactions of *N*-Substituted 1,6-Anhydro-4-*O*-benzyl-2,3-epimino-2,3-dideoxy- β -D-mannopyranoses



Y	Nu		Reaction Conditions ^a		Yield (%)	Ref.
Bn	Br	199	B	200	29 ^b	10
Bn	I		B	201	68	10
Bn	Br		C ^c	200	53 ^d	10
NBs	Cl	275	A	277	81	21
NBs	Br		B	278	77	21
NBs	I		B	279	78	21
NBs	F		D	280	59	21
Ts	Cl	276	A	281	83	10
Ts	Br		B	282	53	10
Ts	I		B	283	95	10
Ts	N ₃		E	284	87	10
Ts	BnO		F	285	70	11
Ts	BnS		G	286	78	11
Ts	BnNH		H	287	44	11

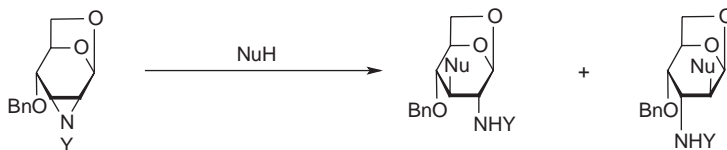
^aA: LiCl + NH₄Cl/Me₂SO; B: Bu₄NX + NH₄X/toluene; C: HX/MeOH + H₂O; D: Bu₄NHF₂; E: LiN₃ + CF₃COONH₄/Me₂SO; F: BnONa/Me₂SO; G: BnSNa/MeOH; H: BnNH₂.

^bIn addition, isomeric 2-bromo derivative **202** with *D-manno* configuration was isolated in 28% yield together with 30% of unreacted epimine **199**.

^cEtOH was used as the solvent.

^dIn addition, 24% of unreacted epimine was isolated.

TABLE X
Cleavage Reactions of *N*-Substituted 1,6-Anhydro-4-*O*-benzyl-2,3-epimino-2,3-dideoxy- β -D-allopyranoses



	Y	Nu	Reaction Conditions ^a	Yield (%)		Ref.
160	Bn	Br	B	0	193	69
	Bn	I	B	0	194	79
	Bn	Br	C ^b	191	50 ^c	0
	Bn	N ₃	D	198	94	0
288	NBs	Cl	C	289	66	0
	NBs	Br	C	290	66	0
	NBs	I	C	291	74	0
195	Ts	Cl	A	292	94	0
	Ts	Br	B	196	72 ^d	0
	Ts	I	B	197	83	0
	Ts	BnO	E	293	80.5	0
	Ts	BnS	F	294	62	0
	Ts	BnNH	G	295	69	0

^aA: LiCl + NH₄Cl/Me₂SO; B: Bu₄NX + NH₄X/toluene; C: HX/MeOH + H₂O; D: NaN₃ + NH₄Cl/CH₃OCH₂CH₂OH + H₂O; E: BnONa/Me₂SO; F: BnSNa/MeOH; G: BnNH₂.

^bEtOH was used as the solvent.

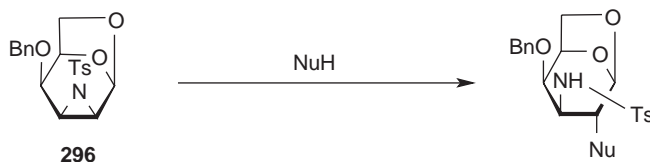
^cTogether with 24% of the epimine.

^dIn addition, 10% of unreacted epimine was isolated.

also formed. However, its formation was probably the result of a nucleophilic substitution of the diaxial cleavage product **200** by bromide and did not relate to the aziridine-ring cleavage.¹⁰

N-Benzylepimine **160** reacted with a mixture of ammonium and tetrabutylammonium bromide or iodide to give the respective diequatorial bromo (**193**) or iodo (**194**) derivatives as the sole products. This formation is the result of an equilibrium between the starting epimine and the diaxial halo derivative, which is formed initially by aziridine-ring cleavage. This equilibrium could be set up readily because of the instability of the diaxial halo derivatives and their tendency to undergo back-cyclization of the aziridine ring. To prove the existence of the equilibrium, the diaxial bromo derivative **191** was prepared by the action

TABLE XI
Cleavage Reactions of 1,6-Anhydro-4-*O*-benzyl-2,3-(*N*-tosylepimino)-2,3-dideoxy- β -D-talopyranose



Nu	Reaction Conditions ^a	Yield (%)	Ref.
BnO	A	297 91	11
BnS	B	298 70	11
BnNH	C	299 76	11

^aA: BnONa/Me₂SO; B: BnSNa/MeOH; C: BnNH₂.

of HBr on *N*-benzylepimine **160**, and was subjected to the cleavage with Bu₄NBr + NH₄Br mixture under the same conditions as the epimine. The instability of the diaxial bromo derivative was so great that it was not possible to isolate it as pure compound. Instead, only a mixture of its hydrobromide and the corresponding epimino derivative was isolated. Attempted removal of HBr from the hydrobromide by aqueous sodium hydrogencarbonate led to rapid and complete cyclization to the corresponding epimino derivative.¹⁰ However, the action of ammonium and tetrabutylammonium bromides in toluene on the mixture of the hydrobromide of the diaxial bromo derivative **191** and the corresponding *N*-benzylepimine **160** led to complete disappearance of **191** and the formation of diequatorial bromo derivative **193**. It was also demonstrated¹⁰ that **193** is stable and does not cyclize to the corresponding epimine **160** under these conditions. A pathway for the formation of diequatorial isomers of the cleavage products of epimino derivatives of 1,6-anhydro- β -D-hexopyranoses has also been demonstrated.¹⁰

Cleavage of the *N*-benzyl-, *N*-*o*-nitrobenzenesulfonyl, and *N*-tosyl-2,3-D-*allo*-epimines **160**, **195**, and **288**, with nucleophiles (azide anion and HBr) proceeds according to the Fürst–Plattner rule and only diaxial isomers are formed.

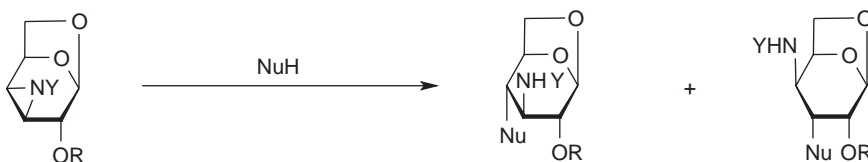
As for 2,3-epimines of the D-*talo* configuration, aziridine-ring opening reactions of *N*-tosylated epimine **296** with benzyl alcohol, benzyl amine, and α -toluenethiol have been reported in the literature.¹¹ All of these reactions proceeded *trans*-diaxially.

3,4-Epimines of the *D-galacto*, *D-allo*, and *D-talo* configurations have been treated with analogous nucleophiles as for the 2,3-epimines, and their cleavage reactions are summarized in Tables XII, XIII, and XIV.

All reactions mentioned in Table XII produced only diaxial isomers of the cleavage products, except for the reaction of *N*-benzylepimine **163** with a $\text{Bu}_4\text{NBr} + \text{NH}_4\text{Br}$ mixture, which gave the diequatorial bromo derivative **308**. Again, its formation is evidently the result of an equilibrium existing between diaxial isomer and the epimine, as already reported for the 2,3-*D-allo* epimine **160**.¹⁰

In the reactions of *N*-benzylepimine **161** with a mixture $\text{Bu}_4\text{NX} + \text{NH}_4\text{X}$ ($\text{X} = \text{Br}, \text{I}$) in toluene, the equilibrium between the diaxial isomer and the epimine was established¹⁰ and thus the diequatorial halo derivatives **319–320**

TABLE XII
Cleavage Reactions of *N*-Benzyl- and *N*-Tosyl-3,4-epimino Derivatives of 1,6-Anhydro- β -D-galactopyranose



	Y	R	Nu	Reaction Conditions ^a	Yield (%)		Ref.
163	Bn	H	Br	B	0	308	10
	Bn	H	I	B	^b		10
	Bn	H	Br	C	301 44 ^c		10
	Bn	H	N ₃	D	302 87.5		10
300	Ts	Bn	Cl	A	303 71.5	0	10
	Ts	Bn	Br	B	304 51 ^d	0	10
	Ts	Bn	I	B	305 77	0	10
	Ts	Bn	BnO	E	285 72	0	11
	Ts	Bn	BnS	F	306 97	0	11
	Ts	Bn	BnNH	G	307 97	0	11

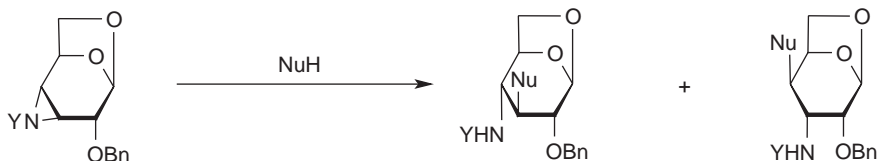
^aA: $\text{LiCl} + \text{NH}_4\text{Cl}/\text{Me}_2\text{SO}$; B: $\text{Bu}_4\text{NX} + \text{NH}_4\text{X}/\text{toluene}$; C: $\text{HBr}/\text{EtOH} + \text{H}_2\text{O}$; D: $\text{NaN}_3 + \text{NH}_4\text{Cl}/\text{CH}_3\text{OCH}_2\text{CH}_2\text{OH} + \text{H}_2\text{O}$; E: $\text{BnONa}/\text{Me}_2\text{SO}$; F: BnSNa/MeOH ; G: BnNH_2 .

^bDecomposition of the starting epimine.

^cTogether with 64% of the epimine.

^dIn addition, 25% of unreacted epimine was isolated.

TABLE XIII
Cleavage Reactions of *N*-Benzyl- and *N*-Tosyl-3,4-epimino Derivatives of 1,6-Anhydro- β -D-allopyranose



	Y	Nu	Reaction Conditions ^a	Yield (%)		Ref.
161	Bn	Br	B	0	319	92
	Bn	I	B	0	320	83
	Bn	Br	C	310	33 ^b	0
	Bn	N ₃	D	311	88	0
309	Ts	Cl	A	312	58 ^c	0
	Ts	Br	B	313	62 ^d	0
	Ts	I	B	314	73.5 ^e	0
	Ts	BnO	E	315	48 ^f	0
	Ts	BnO	F		74	0
	Ts	BnS	G	317	87	0
	Ts	BnNH	H	318	99	0
	Ts	BnNH	H			11

^aA: LiCl + NH₄Cl/Me₂SO; B: Bu₄NX + NH₄X/toluene; C: HBr/EtOH + H₂O; D: NaN₃ + NH₄Cl/CH₃OCH₂CH₂OH + H₂O; E: BnONa/Me₂SO; F: BnONa/BnOH; G: BnSNa/MeOH; H: BnNH₂.

^bTogether with 60% of the epimine.

^cIn addition, 25% of unreacted epimine was isolated.

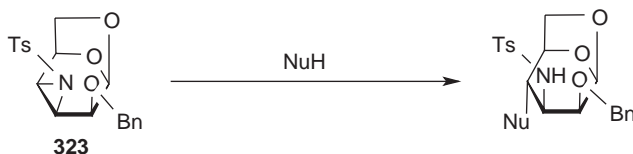
^dIn addition, 37% of unreacted epimine was isolated.

^eIn addition, 23% of unreacted epimine was isolated.

^fTogether with 32% of 1,6-anhydro-2-*O*-benzyl-3,4-dideoxy-4-(*N*-tosylamino)- β -D-*erythro*-hex-2-enopyranose (**321**).

were the final products. The course of the reaction of *N*-tosylepimine **309** with BnONa depends on the solvent. In benzyl alcohol, only the expected 2,3-di-*O*-benzyl derivative **315** was formed, but in dimethyl sulfoxide, the benzyloxy anion also acts as a base and along with the expected benzyl ether **315**, the unsaturated product **321** was also formed (Table XIII).¹¹ The possibility of such base-catalyzed rearrangement was verified by the reactions of *N*-tosylepimines having the D-*allo* and D-*talo* configurations with potassium *tert*-butoxide in tetrahydrofuran. These reactions produced hexenopyranoses **321**, **322**, and **324**.

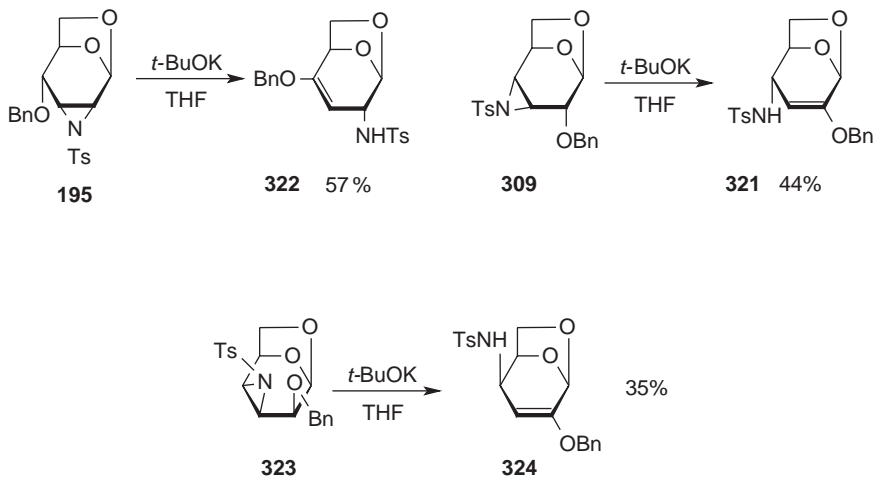
TABLE XIV
Cleavage Reactions of 1,6-Anhydro-2-*O*-benzyl-3,4-(*N*-tosylepipimino)-3,4-dideoxy- β -D-talopyranose



Nu	Reaction Conditions ^a	Yield (%)	Ref.
BnO	A	325 32 ^b	11
BnO	B	69	11
BnS	C	326 71.5	11
BnNH	D	327 96	11

^aA: BnONa/Me₂SO; B: BnONa/BnOH; C: BnSNa/MeOH; D: BnNH₂.

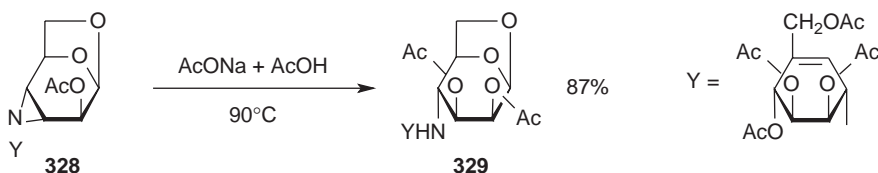
^bTogether with 48% of 1,6-anhydro-2-*O*-benzyl-3,4-dideoxy-4-(*N*-tosylamino)- β -D-*threo*-hex-2-enopyranose (**324**).



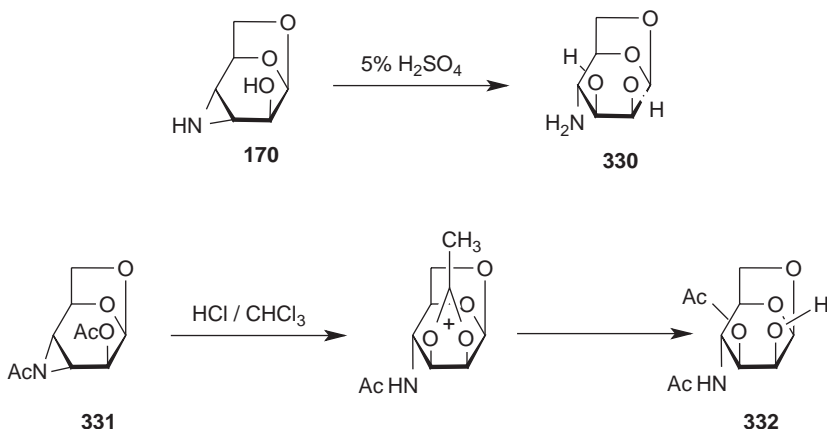
It has been found,¹¹ that only *N*-tosylepipimines with the *cis* arrangement of the OBn group and aziridine ring are able to form unsaturated hexenopyranoses via base-catalyzed abstraction of the hydrogen atom *trans*-oriented to the aziridine ring.

The *N*-tosylated 3,4-epimine of *D-talo* configuration (**323**) gave diaxial isomers in cleavage reactions with benzyl alcohol, benzylamine, and α -toluenethiol.¹¹

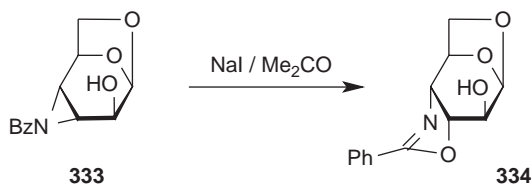
Ogawa and Sugizaki published¹⁰³ a cleavage reaction with acetate buffer of the epimino derivative of 1,6-anhydro- β -D-altropyranose having the aziridine ring *N*-substituted by a derivative of acarbose. The cleavage proceeded *trans*-diaxially.



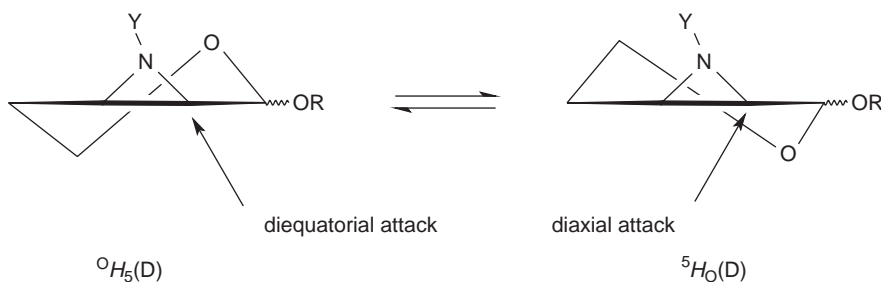
Epimines **170** and **331** were cleaved¹³⁹ by H_2SO_4 and HCl to afford 4-amino derivatives having the *D-manno* configuration.



The corresponding *N*-benzoyl epimine **333** gave the oxazolidine derivative **334** when treated with NaI in acetone.¹⁴⁰

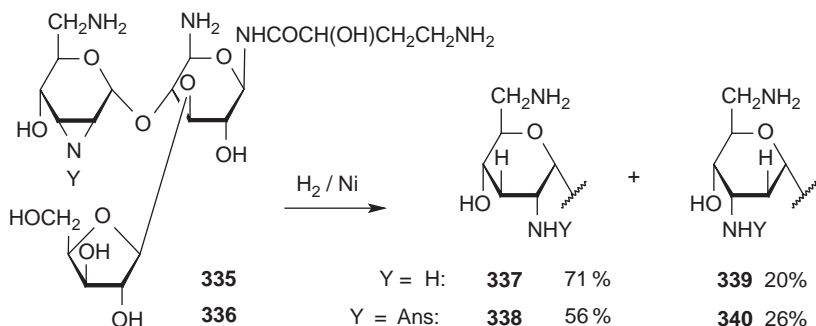


b. Derivatives of Flexible Conformation.—This class deals with epimines derived from hexopyranosides in which the conformation of tetrahydropyran ring is not fixed by any substituent present in the molecule. However, this does not mean that these epimines exist as mixtures of conformers; usually their favored conformation is 0H_5 , as documented by NMR measurements. Because there exist no factors that can stabilize the conformation in the cleavage reactions, the structures of the products cannot be predicted by the Fürst–Plattner rule. In the reaction of the epimine with a nucleophile, the 0H_5 half-chair conformation can be readily changed with minimal energy to the other half-chair, 5H_0 . If the configuration of the cleavage product is *trans*-diequatorial for the 0H_5 conformation, it is changed to *trans*-diequatorial for 5H_0 , and vice versa.



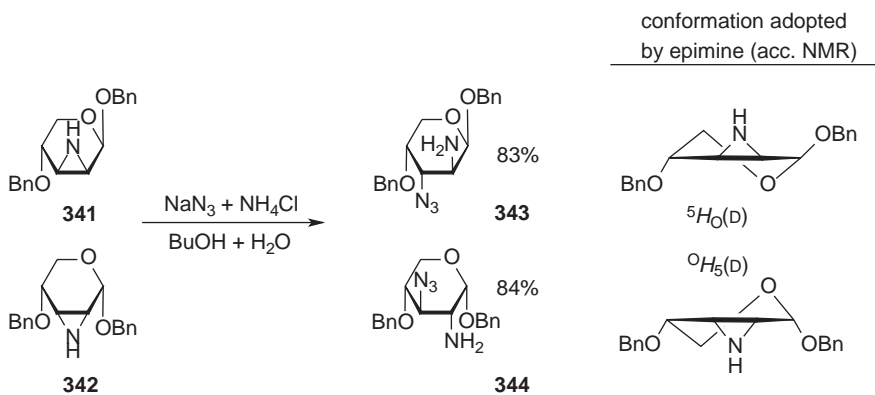
Diequatorial isomers can preponderate or be produced solely, without the reaction being controlled thermodynamically or without the involvement of a cationic (S_N1 -like) transition state. The formation of cleavage products with unpredictable configuration is, therefore, the result of cleavage reactions of such epimines. Other factors, such as steric interaction of the nucleophile with an acetal moiety, can play a role in causing the cleavage to be generally preferred at either carbon C-3 rather than C-2 irrespective of the configuration of the epimine.

Fukase and coworkers described⁹⁵ the deoxygenation of butirosins A and B via hydrogenation of their epimine-based derivatives over Raney nickel catalyst. Free (335) and *N*-anisoylated (336) epimines were cleaved by hydrogen to form mixtures of 3-deoxy- (337–338) and 2-deoxybutirosins (339–340) in 71:20 and 56:26% yields, respectively. The authors explained the regioselectivity in terms of less steric hindrance at C-2 than C-3 at the catalyst surface.



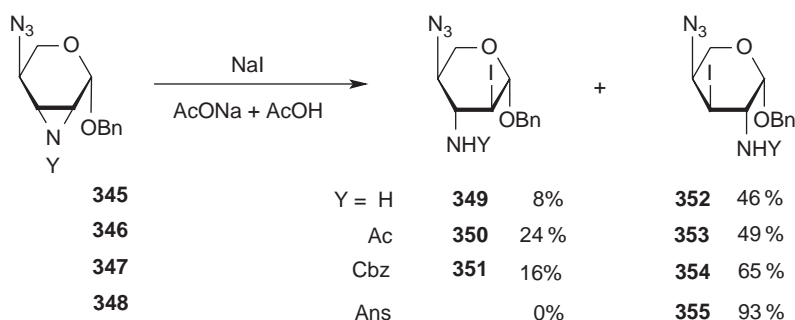
Similar product ratios of deoxybutyrosins (3-deoxy:2-deoxy = 5:1) have been observed by Okutani *et al.*⁴³ The corresponding epimino derivative (**335**) of butyrosin was prepared in 58% yield by nucleophilic attack of an amino group at C-2 displacing a 3-*O*-phosphoryl leaving-group in a 5:1 BSA–Me₃SiCl mixture.

Unsubstituted benzyl 4-*O*-benzyl-2,3-dideoxy-2,3-epimino- β -D-*lyxo*- (**341**) and - α -D-*ribo*-pyranoside (**342**) have been used by Paulsen and Patt in 1981 in cleavage reactions with azide.²⁷ By the action of a mixture of sodium azide and ammonium chloride in a butanol–water system, the aziridine-ring cleavage proceeded exclusively at C-3, thus exhibiting anti-Fürst–Plattner regioselectivity for both epimines. No explanation for this preference was given in the paper.

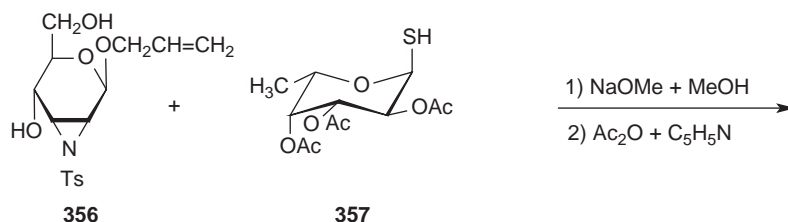


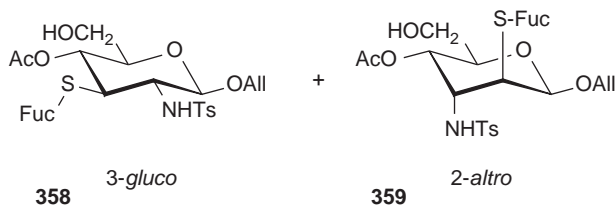
Hashimoto and coworkers published two papers dealing with nucleophilic cleavage of hexopyranoside-derived epimines. In the first one,⁴¹ the cleavage of

benzyl 4-azido-2,3,4-trideoxy-2,3-epimino- α -L-lyxopyranoside (**345**) and its derivatives *N*-substituted by acetyl (**346**), anisoyl (**348**), and benzyloxycarbonyl (**347**), was effected by sodium iodide in acetate buffer. The reactions led to mixtures of 2-iodo (**349–351**) and 3-iodo (**352–355**) derivatives with a high abundance of the latter. The regioselectivity was against the Fürst–Plattner rule, since it was found that the epimines adopted nearly exclusively the $^oH_5(L)$ conformation. The authors explained this regioselectivity in terms of dominance of the inductive effect of the anomeric carbon atom, which affects C-2 more than C-3, over the stereoelectronic effect (Fürst–Plattner rule). The introduction of an electron-withdrawing group onto the aziridine nitrogen atom facilitates cleavage at C-2 as a result of acceleration of the cleavage reaction. According to the authors, the acceleration caused a relative decrease of the electrostatic over the stereoelectronic effect in controlling the regioselectivity.



The second paper¹⁴¹ describes the cleavage of an *N*-tosylepimine (**356**) derived from allyl D-alloside by a 1-thio sugar (**357**), which afforded a mixture of 3-*gluco* (**358**, 63%) and 2-*altro* (**359**, 29%) isomers of the cleavage products. No explanation of the regioselectivity was reported.

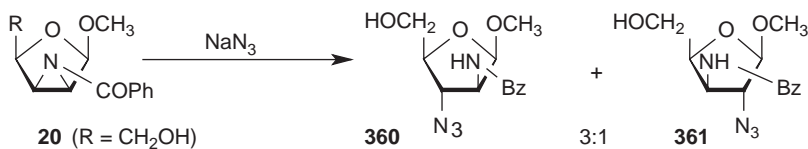




3. Furanose Aziridines

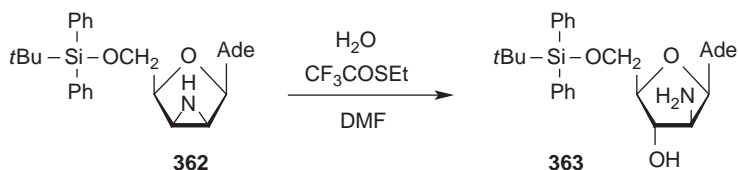
Aziridines derived from furanoses and furanosides have been less utilized in nucleophilic cleavage reactions than pyranose aziridines. Reactions affording nucleoside analogues have been the most important transformations. However, complex protecting-group manipulations have often been required and thus, for the majority of nucleosides, lactones were found to be more convenient and have been utilized more frequently than glycosides. The regioselectivity of cleavage reactions of aziridino lactones is different from that of epimino furanosides and has been rationalized¹² from the hard and soft nucleophile viewpoint. For some nucleophiles, an alternative pathway, namely cleavage of the lactone \rightarrow cleavage of the aziridine ring \rightarrow back-closure of the lactone ring, has been shown to be involved in cleavage reactions.^{12,13}

In 1969, the azidolysis of *N*-benzoyl-2,3-epimino derivative **20** by NaN_3 was published.¹⁷ The azidolysis afforded a mixture of regioisomers in which the 3-azido derivative **360** preponderated (3:1 ratio).

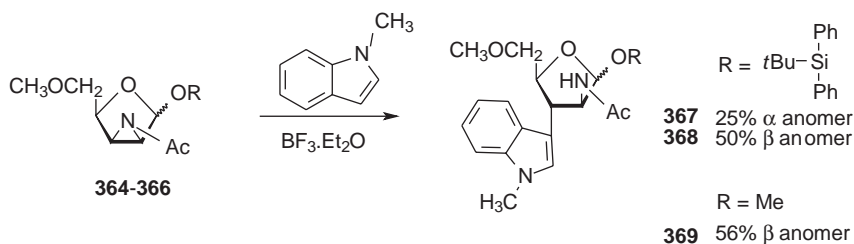


Other 2,3-epimino derivatives based on D-lyxose were also opened by sodium azide ($\text{R} = \text{CH}_2\text{NHBz}$,^{15,23,33} $\text{R} = \text{CH}_2\text{NHCbz}$,¹⁵ $\text{R} = \text{H}$ ³²), and the same regioselectivity was observed. Formation of the C-3 regioisomer corresponds to a cleavage pathway having minimum activation energy as documented by *ab initio* calculations.¹⁴²

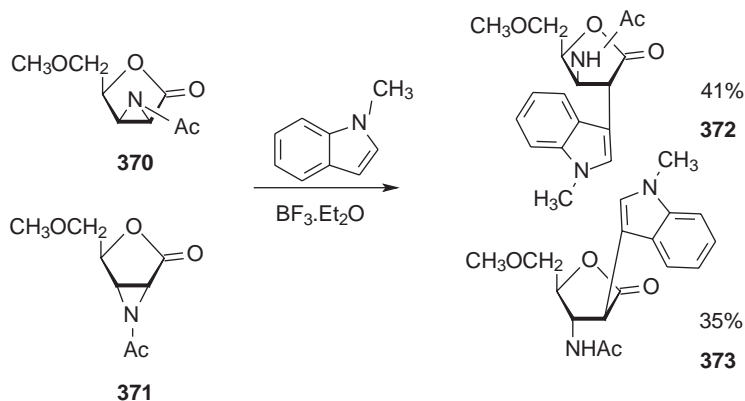
In 1978, Robins and Hawrelak reported¹⁴³ the preparation of a 2-amino analogue of adenosine via treatment of unsubstituted epimine **362** with *S*-ethyl trifluorothiolacetate in DMF. The cleavage was effected by H₂O after activation of the aziridine ring by *N*-trifluoroacetylation *in situ*. Cleavage proceeded regioselectively at C-3; no yield or detailed reaction conditions were given in the paper.



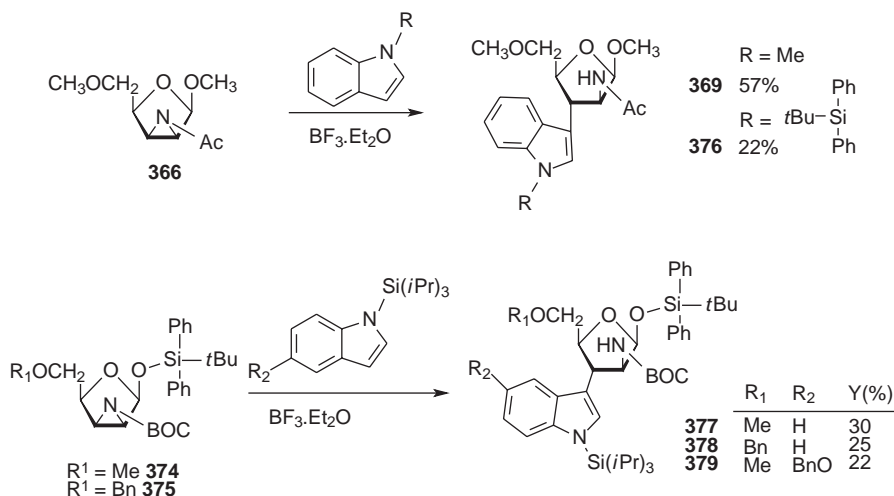
Dubois *et al.* performed¹³ the cleavage of *N*-acetylepimino derivatives of protected lyxofuranosides **364–366** with *N*-methylindole under BF₃·Et₂O catalysis.



The observed regioselective attack was at C-3, while the cleavage of the corresponding aziridino lactones proceeded exclusively at C-2, despite the configuration of the epimine (*D*-*lyxo* and *D*-*ribo*).

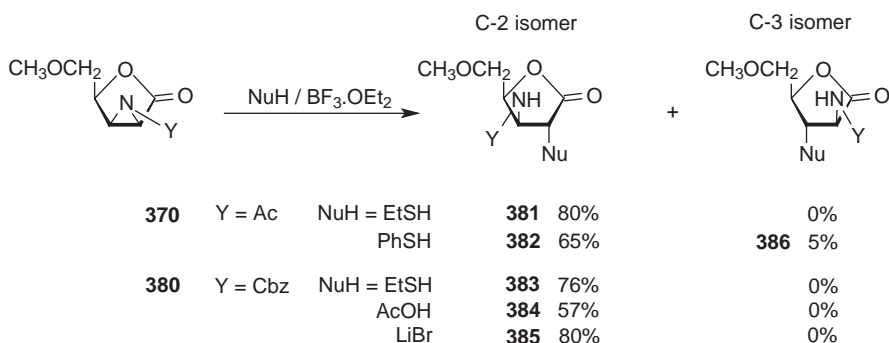


Cleavage reactions of similar *N*-acylepimines with indole and its derivatives have been reported by Hofmann *et al.*¹³⁴ The authors evaluated the reactivity of *N*-acetylated (**366**) and *N*-*tert*-butyloxycarbonylated (**374–375**) 2,3-epimines with indoles under $\text{BF}_3 \cdot \text{Et}_2\text{O}$ catalysis.

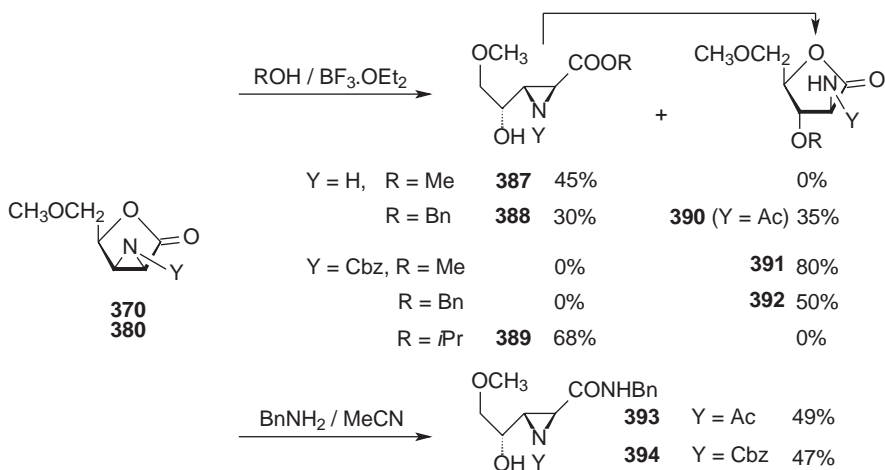


Although the yields were relatively low, complete regioselective attack at C-3 was achieved. Attempted cleavage by indole itself was unsuccessful.

Dauban and coworkers published a detailed study on the reactivity of 2,3-epimino-2,3-dideoxy-D-lyxono-1,4-lactone derivatives **370**, **380** with both soft (RSH , AcOH , LiBr) and hard (ROH , BnNH_2) nucleophiles.¹² Reactions of the epimines with soft nucleophiles proceeded by direct aziridine-ring cleavage to give predominantly C-2 regioisomers.



The authors compared the observed regioselectivity to the cleavage reactions of simple, non-sugar, aziridine carboxylates with the same nucleophiles, which were reported (cf. Refs. 125, 127, 144, 145) to afford mostly C-3 regioisomers. HSAB theory and semiempirical quantum mechanical MNDO calculations were used for such comparison of regioselectivity. The authors concluded that attack at C-2 of the aziridino lactones was favored over attack at C-3 if the cleavage reaction was under orbital control. It was true for the reactions with soft nucleophiles, whereas hard nucleophiles caused cleavage of the lactone ring first. Non-cyclic aziridine carboxylates thus formed further reacted either with another portion of the nucleophile to give C-3 regioisomers (in the case of ROH), or remained unreacted (as in the case of BnNH₂). Finally, back closure of the lactone ring occurred in some instances.

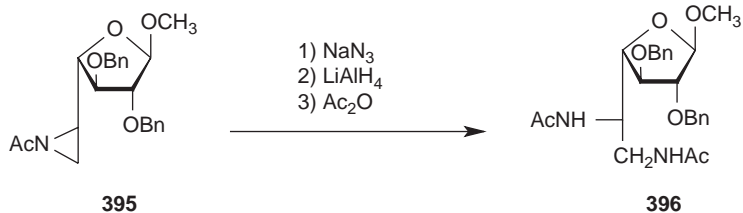


4. Exocyclic Aziridines

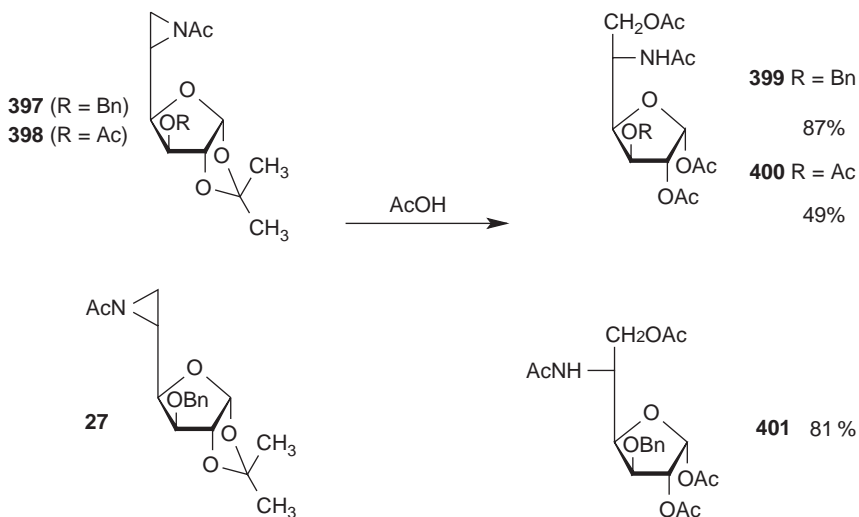
Cleavage of exocyclic aziridines proceeds almost exclusively at the terminal carbon atom of the aziridine ring.

In a series of papers,^{35,37,146–150} Saeki and coworkers described cleavage reactions by nucleophiles of 5,6-epimines derived from hexofuranosides or hexofuranoses, and 6,7-epimines derived from heptopyranoses.

Methyl 5,6-(*N*-acetylepimino)-2,3-di-*O*-benzyl-5,6-dideoxy- α -L-altrofuranside (**395**) gave the 5,6-diacetamido derivative **396** after reaction successively with sodium azide, LiAlH₄, and acetylation.³⁵ The yields of intermediary products and the regioselectivity of the cleavage were not reported.



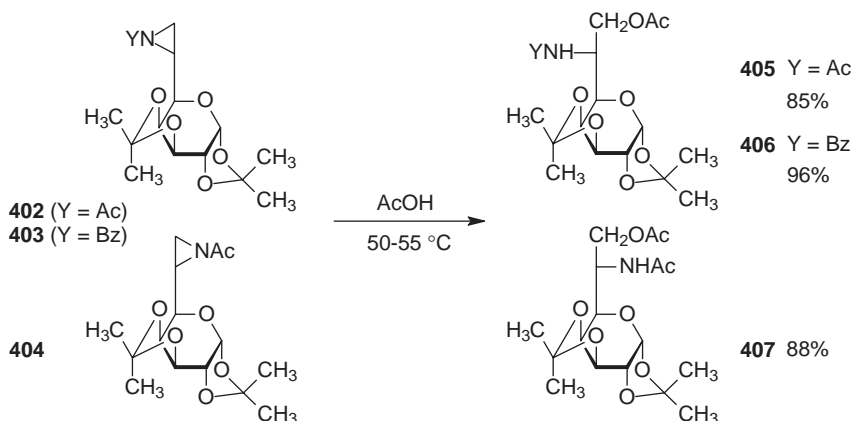
A synthesis of nojirimycin was accomplished by epimine cleavage.^{35,147,148} *N*-Acetylepimines **27** and **397** were treated with acetic acid at 60 °C to afford the 6-acetoxy derivatives **399–401** as intermediates in the synthesis.



Similar treatment of 3-*O*-acetyl epimine **398** with acetic acid was reported subsequently.¹⁴⁹

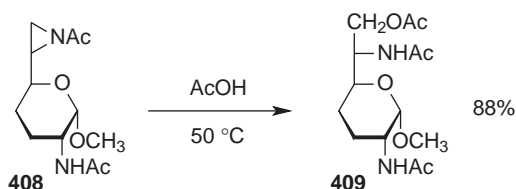
6,7-(*N*-Acetylepimino)-6,7-dideoxy-1,2:3,4-di-*O*-isopropylidene-D- and -L-glycero- α -D-galacto-heptopyranoses (**402** and **404**) have been prepared³⁸ and converted^{37,150} into 7-*O*-acetyl heptopyranose derivatives **405** and **407** by treatment with warm acetic acid. The cleavage seems to proceed only at the terminal position of the aziridine ring, although no yields of reaction products¹⁵⁰ or only yields of crude products³⁷ are provided in the original papers. In contrast to the

cleavage of 5,6-epimines of hexofuranoses (**27**, **397**, and **398**),^{147,149} both isopropylidene moieties remained intact during the reaction with acetic acid.

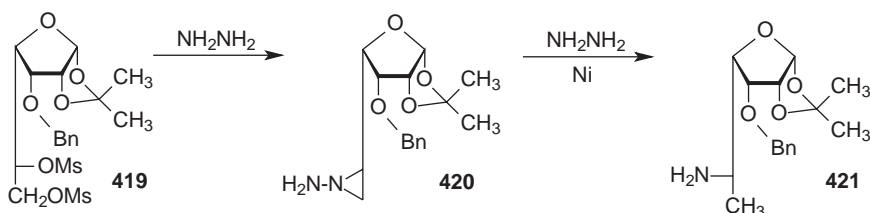
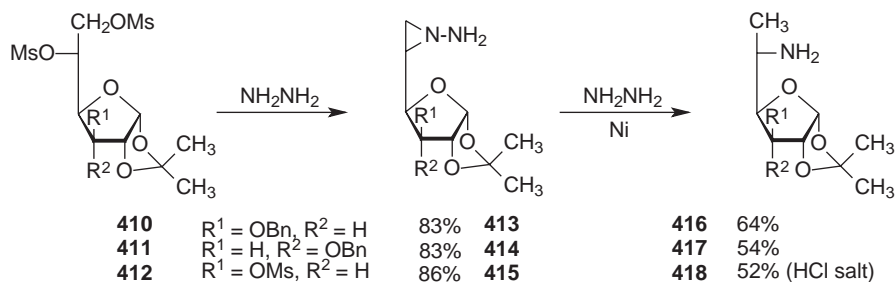


The cleavage of *N*-benzoylepimino derivative **403** with acetic acid, resulting in the formation of 7-acetoxy derivative **406**, was also reported in the later paper.³⁷

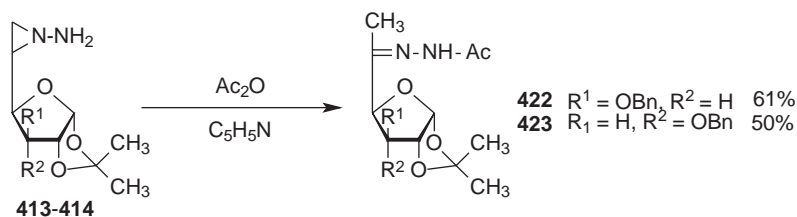
In the synthesis of 6-*epi*-purpurosamine, a component of the antibiotic fortimicin A, methyl 2-acetamido-6,7-(*N*-acetylepimino)-2,3,4,6,7-pentadeoxy- β -L-*lyxo*-heptopyranoside (**408**) reacted with acetic acid to give 6-acetoxy derivative **409** in 88% yield.⁸⁰



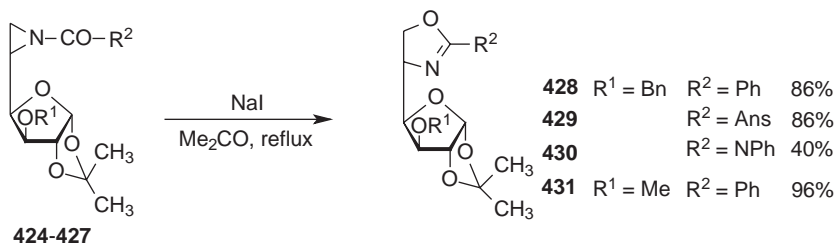
Preparations of *N*-aminoepimines and their catalytic reduction by hydrazine over Raney nickel have been described by Paulsen and Stoye.⁴⁴ Using 5,6-di-*O*-mesyl derivatives of 1,2-isopropylidene- α -D-gluco-, α -D-allo-, and α -D-gulofuranose (**410–412**), and compound **419**, reactions with anhydrous hydrazine afforded the corresponding *N*-aminoepimines **413–415**, and **420**, which were regioselectively transformed into 5-amino-5,6-dideoxy derivatives **416–418**, and **421**.



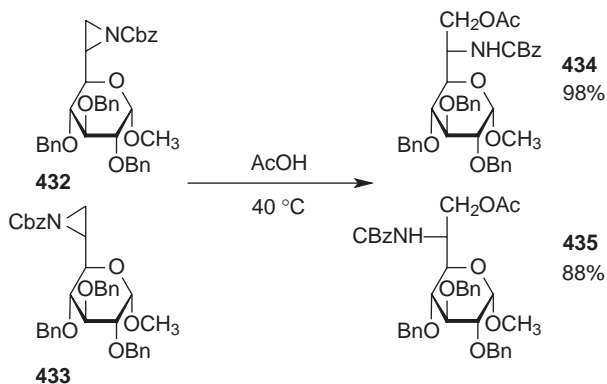
The *N*-aminoepimines **413–414** were also converted into *N*-acetylhydrazones of the corresponding glycoses **422–423** by a rearrangement of the aziridine ring during acetylation by Ac_2O in pyridine.⁴⁴



Iwakawa *et al.* described¹⁴⁰ in 1975 a rearrangement leading to oxazolidines **428–431** in the series of ring-substituted *N*-aroylepimines **424–427**. The rearrangement was induced by reaction of the epimines with sodium iodide in acetone.



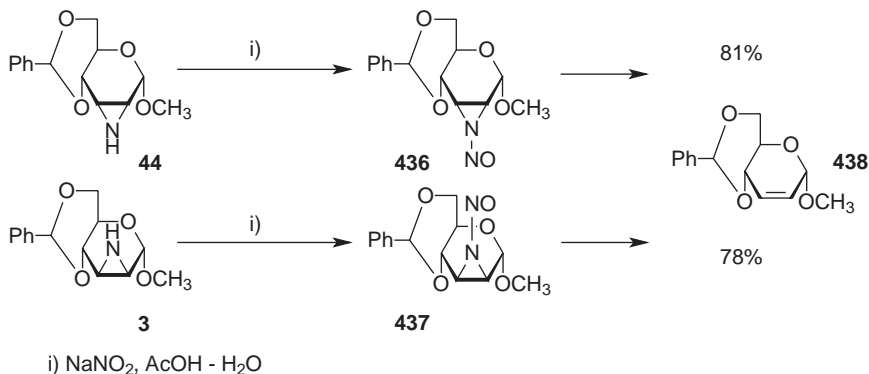
During the synthesis of destomic acid derivatives, Hashimoto *et al.* reported³⁸ successful cleavage of *N*-benzyloxycarbonylated 6,7-epimino heptopyranosides **432–433** with acetic acid under heating. The cleavage reactions regioselectively gave 7-acetoxy derivatives **434–435** in high yields (98 and 88%).



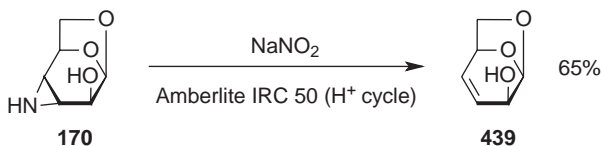
5. Miscellaneous Reactions of Sugar Aziridines

Epimines derived both from 1,6-anhydro- β -D-hexopyranoses and from methyl 4,6-*O*-benzylidenehexopyranosides have been utilized in deamination reactions by the action of nitrous acid. The reactions led predominantly to unsaturated pyranoses possessing a double bond in place of the aziridine ring.

The first attempts at the deamination of epimines were made by Guthrie and coworkers in 1966.¹⁵¹ Methyl 4,6-benzylidene-2,3-dideoxy-2,3-epimino- α -D-allo- (**44**) and manno-pyranosides (**3**) were converted into the corresponding hex-enopyranose **438** under the action of sodium nitrite in an acetic acid–water system. *N*-Nitrosoepimines **436–437** were isolated as the reaction intermediates (although in low yields).



The 1,6-anhydro- β -D-hex-3-enopyranose **439** was produced on deamination of 3,4-D-*altro*-epimine **170**.¹⁵²

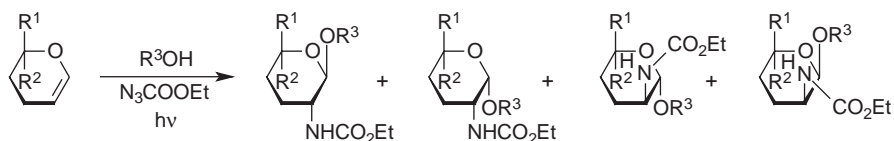


During 1981–1983, Kozłowska-Grams and Descotes published several papers^{113–115} dealing with the preparation of aminosaccharide derivatives from cyclic vinyl ethers and glycals. The key step was aziridination of the double bond by photochemically generated ethoxycarbonylnitrene, which produced 1,2-epimines as intermediates. The 1,2-epimines were cleaved *in situ* by the alcohol present in the reaction mixture to give vicinal alkoxy amines as mixture of stereoisomers.

Results for the aziridination of 5,6-dihydro-(2*H*)-pyran derivatives by irradiation of them in admixture with ethyl azidocarboxylate and methanol or 2-methyl-2-propanol are summarized in Table XV.

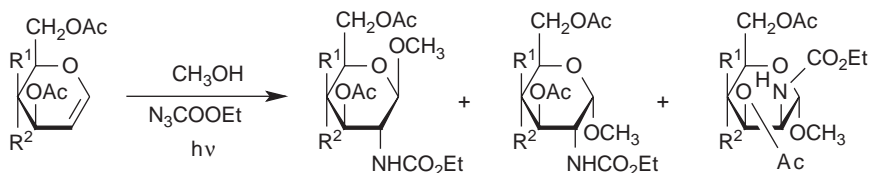
In these reactions, both *endo*- and *exo*-oriented 1,2-epimines were formed, although in different relative amounts, dependent upon the configuration at C-5. Cleavage of the epimines by an alcohol produced predominantly the *trans* isomers. The authors explained the formation of *cis* isomers by an $\text{S}_{\text{N}}1$ -like mechanism involving an oxocarbenium ion as intermediate.¹¹⁵

TABLE XV
Aziridination of Substituted 5,6-Dihydro-(2*H*)-pyran Derivatives in Alcoholic Medium



	R ¹	R ²	R ³	Yield (%)					
440	H	H	Me	443	53	447	10	—	—
	H	H	<i>t</i> Bu	444	69	448	7	—	—
441	CH ₂ OAc	H	Me	445	25	449	11	452	31
	CH ₂ OAc	H	<i>t</i> Bu	446	20	450	Traces	453	36
442	H	OCH ₃	<i>t</i> Bu	—	—	451	11	454	51
								455	18

Similar results were obtained for tri-*O*-acetyl-D-glucal and galactal.^{114,115}



456	R ¹ = OAc, R ² = H	458	26%	460	11%	462	5%
457	R ¹ = H, R ² = OAc	459	4%	461	34%	463	4%

It is noteworthy, that the aziridine ring was formed preferentially in the 1,2-*exo* position, regardless of the configuration at C-4.

In contrast to these reactions, attempts¹¹³ at aziridination of 2-methoxy-5,6-dihydro-(2*H*)-pyran led only to products of *trans* cleavage of the epimine. This regioselectivity can be explained by predominance of Fürst–Plattner-type cleavage over S_N1-type because of destabilization of the carbonium ion at C-3 by the acetal moiety.

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SYNTHESIS AND TRANSFORMATION OF GLYCOSYL AZIDES

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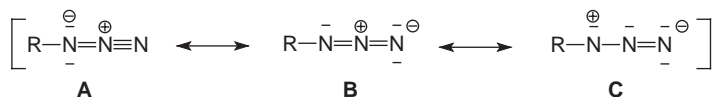
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I. INTRODUCTION

Owing to their functional group, glycosyl azides (general structure Glyc-N₃) constitute important and versatile derivatives for carbohydrate chemistry. Because

* Deceased October 20, 2005.

of the dipole character of organic azides (see resonance structures **A**, **B**, and **C**) they can function both as nucleophiles and electrophiles, and readily undergo dipolar cycloadditions. Further, as configurationally stable groups, azides are well suited as starting materials for formation of other nitrogen-containing functionalities, such as amines, amides, ureas, carbodiimides, and others.



The current article ties in with previous ones that were published in 1961¹ and 1993.² In the past decade there have been reports on a number of relevant preparative approaches and uses of anomeric glycosyl azides, which provide a plethora of synthetic options for carbohydrate chemistry.

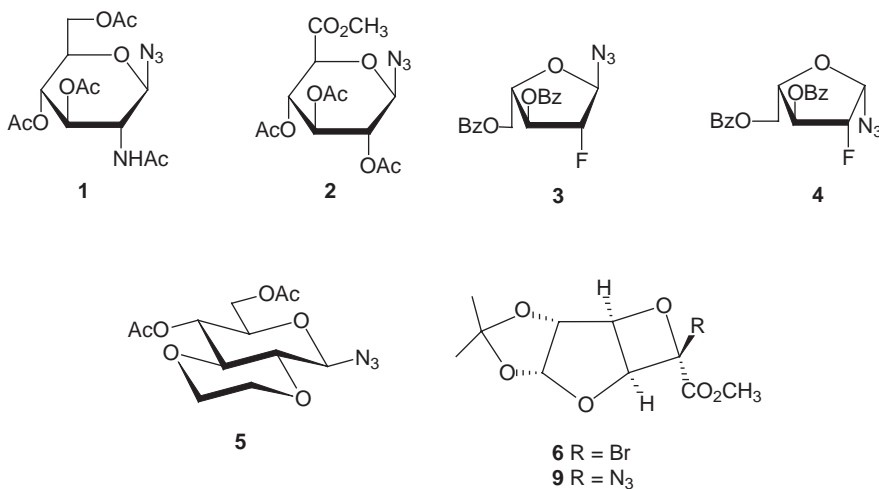
II. SYNTHESIS OF GLYCOSYL AZIDES

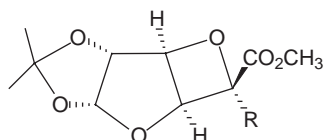
1. Synthesis of 1,2-*trans* Glycosyl Azides from Glycosyl Halides

This only method known up to 1974 for preparing glycosyl azides was from acylated glycosyl halides by treatment with sodium or silver azide. However, because of the reactivity of glycosyl halides with water, the very low solubility of sodium azide in organic solvents, and the thermal lability of silver azide there were considerable difficulties in preparing the corresponding glycosyl azides. Following the seminal studies of Bertho *et al.*,^{1,3-7} the acetylated glycosyl azides of the *D*-*gluco*, *D*-*galacto*, and 2-acetamido-2-deoxy-*D*-*gluco* series could be obtained, albeit by a somewhat laborious method. Thus 2,3-*O*-isopropylidene-5-*O*-trityl- α -*D*-ribofuranosyl azide was obtained in a moderate yield after a three-day reaction.⁸ In addition to a series of acetylated glycobiosyl azides,⁹ tri-*O*-acetyl- α -*D*-lyxofuranosyl¹⁰ and - β -*D*-ribofuranosyl¹¹ as well as 2,3,4-tri-*O*-acetyl- β -*D*-xylopyranosyl azides¹² could also be prepared by the same method. Introduction of such dipolar aprotic solvents as formamide and *N,N*-dimethylformamide (DMF) afforded a practical synthetic improvement for preparing 2,3,4,6-tetra-*O*-acetyl- β -*D*-galactopyranosyl azide and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -*D*-glucopyranosyl azide.^{15,13-16}

The silver azide method was nevertheless recommended by two research groups.¹⁷⁻¹⁹ A comparative study by Korytnyk *et al.* showed LiN_3 in DMF to be best suited for the formation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -*D*-glucopyranosyl azide (**1**) from the corresponding chloride¹⁹ (see also Refs.

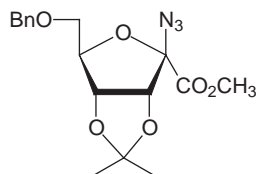
20–22). Methyl 1-azido-2,3,4-tri-*O*-acetyl-1-deoxy- β -D-glucopyranuronate (**2**) could also be obtained likewise.²³ The anomeric 3,5-di-*O*-benzyl-2-deoxy-2-fluoro- α - and β -L-arabinofuranosyl azides (**3** and **4**) were described as crystalline derivatives prepared from a corresponding glycosyl bromide with NaN_3 in DMF.²⁴ The anomeric 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl azides could be obtained in satisfactory yield following chromatographic separation.²⁵ Similarly, a bromide precursor could be transformed into 4,6-di-*O*-acetyl-2,3-*O*-ethylene- β -D-glucopyranosyl azide (**5**).²⁶ Treatment at room temperature of the 5-epimeric methyl 3,5-anhydro-5-bromo-1,2-*O*-isopropylidene- α -D-glucuronate (**6**) and - β -L-iduronate (**7**) smoothly gave the corresponding inverted azides **8** and **9**.²⁷ A further example of this bromide–azide exchange in pyranosyl derivatives was reported in the formation of methyl 2-azido-3,7-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-4,5-*O*-isopropylidene- β -D-*galacto*-2-heptulopyranosonate.²⁸ Based on the reaction of NaN_3 in DMF with a crude α -bromo-tetrahydrofurancarboxylic ester, Fleet *et al.* prepared additional “tetrahydrofuran α -azido esters”.²⁹ Methyl 2,5-anhydro-2-azido-6-*O*-benzyl-3,4-*O*-isopropylidene- β -D-*ribo*-2-hexulofuranosonate (**10**) was prepared from the α -bromide in 95% yield.³⁰ A study with both the *manno*-configured glycosyl azides **11a** and **11b** demonstrated that this method does not yield the inverted products, but always leads to the glycosyl azide having the 1,2-*trans* configuration.³¹ In all cases, reaction of the acylated (benzoylated or acetylated) mannopyranosyl bromides with NaN_3 in DMF exclusively led to α -mannopyranosyl azide derivative **11**.^{32–34}





7 R = Br

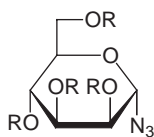
8 R = N₃



10

Ac = CH₃CO, Bz = C₆H₅CO, Bn = C₆H₅CH₂

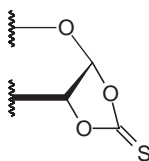
Treatment of protected, cyclic 1,2-sulfites (**12**) of monosaccharides with NaN₃ in DMF resulted in the formation of homogenous 1,2-*trans* azides **13**, with a free OH group in position 2.^{35,36} In this same reaction, a mixture of the intermediate *endo/exo* sulfites can be formed employing *N,N'*-thionyl-diimidazole, and further treatment can be done with the more soluble LiN₃.³⁷ A corresponding transformation of furanoside 1,2-thiocarbonates leads to the furanosyl azides.³⁸ Reaction of 2-levulinoylglycosyl halides with NaN₃ gave the corresponding azides, which in turn were converted into 3,4,6-tri-*O*-acetyl-β-D-gluco- and -galactopyranosyl azides.³⁹ After the removal of the levulinoyl group with hydrazine acetate, both the OH groups can be methylated by either diazomethane or methyl iodide-silver oxide. The 2-methyl ethers **14** and **15** can be also obtained from glucose and galactose 2-methyl ethers.⁹



11

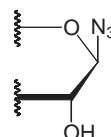
a R = Ac

b R = Bz

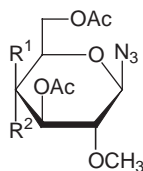


12

+ NaN₃ →



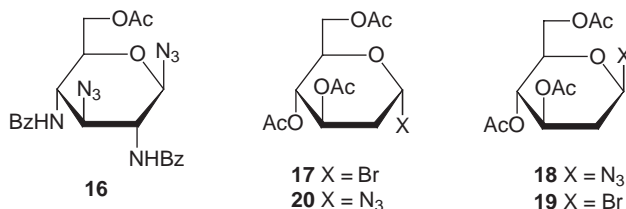
13



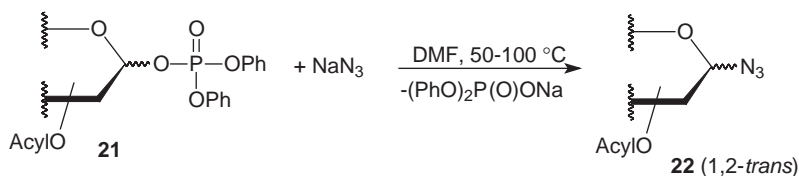
14 R¹ = H, R² = OAc

15 R¹ = OAc, R² = H

The 1,2-*trans* azide **16** can be obtained in crystalline form from 6-*O*-acetyl-3-azido-2,4-dibenzamido-2,3,4-trideoxy- α -D-glucopyranosyl chloride with LiN_3 in DMF.⁴⁰ Both the acetylated anomeric azides of 2-deoxy- α -D-*arabino*-hexopyranose can be obtained by this approach; however, reaction of the α -bromide **17** with azide must be performed at 5 °C, affording the inversion product **18** in excellent yield. Curiously, formation of the anomeric azide **20** from the bromide **19** was reported in only 45% yield.^{9,41}

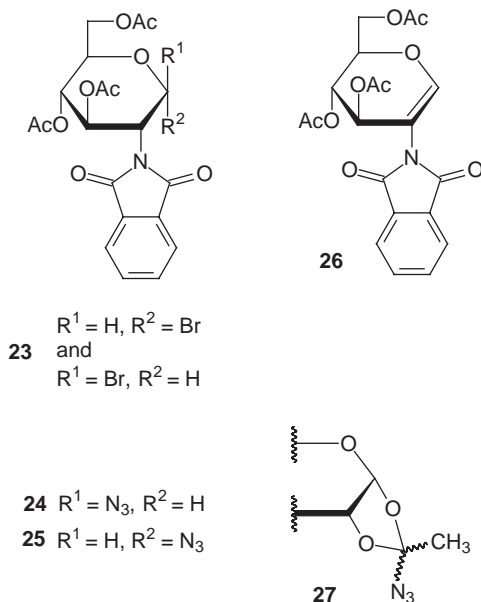


Glycosyl phosphate triesters of the D-*galacto*-, D-*gluco*-, D-*manno*-, 2-acetamido-2-deoxy-D-*gluco*-, 6-deoxy-*galacto*-, and 6-deoxy-L-*manno* configurations (**21**) have also been displaced by azide to give the otherwise more directly accessible 1,2-*trans* glycosyl azides **22**. When the anomeric phosphate triester group was synclinal to the adjacent C-2 substituent, displacement of the phosphate group by azide was facile.⁴²



Phase-transfer catalysis (PTC) has been used in preparing protected 1,2-*trans* glycopyranosyl azides.⁴³⁻⁵³ These reactions proceed at room temperature, employing catalysts such as benzyltriethylammonium chloride,⁴² tetrabutylammonium hydrogensulfate,⁴⁸ tetrabutylammonium bromide,⁴⁹ or Aliquat 236 (methyltrioctylammonium chloride).⁴⁴ Yields in these cases generally are above 90%, with different reaction times. Formation of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl- β -D-glucopyranosyl azide (**1**) was reported in good yield by several groups.^{45,52} The reaction of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- α , β -D-glucopyranosyl bromide (**23**) with NaN_3 gave both anomeric azides **24** and **25** along with the elimination product 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-2-

phthalimido-D-*arabino*-hex-1-enitol (**26**).⁴⁷ The formation of orthoesters under PTC-mediated azidation was reported by Roy *et al.*⁴⁸ Treatment of acetylated mannopyranosyl, rhamnopyranosyl, and fucopyranosyl bromides with NaN₃ leads to the formation of the expected glycosyl azides, together with a considerable amount of an *endo/exo* mixture of the 1,2-*O*-azidoethylene derivatives ("orthoesters", or specifically "orthoester azides") (**27**). This reaction under neighboring-group participation is summarized in Table I.

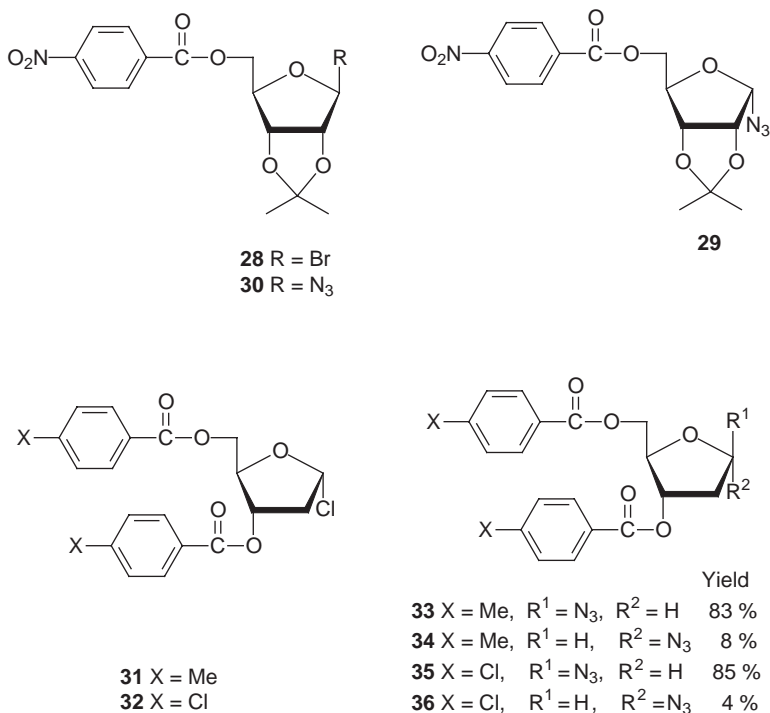


The role of the counter ion was studied by Canadian authors: employment of tetrabutylammonium chloride as catalyst in the reaction with NaN₃ resulted in anomerization of 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl chloride.⁵⁴ PTC-catalyzed reactions of furanosyl halides proceed much faster (~15 min) and with higher selectivity than with their pyranosyl counterparts.

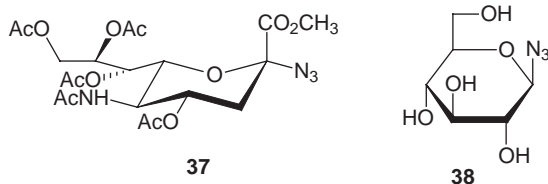
TABLE I
Products of Reaction of Acetylated Glycosyl Bromides with NaN₃

Configuration	1,2-Azide (%)	<i>endo/exo</i> Azidoethylene Derivative (%)
<i>manno</i>	50	37
6-Deoxy- <i>manno</i>	41	38
6-Deoxy- <i>galacto</i>	94	0

Thus 2,3-*O*-isopropylidene-5-*O*-(4-nitrobenzoyl)- β -D-ribofuranosyl bromide (**28**) undergoes rapid reaction and gives the α -azide **29** in good yield. From the mother liquor 0.6% of the corresponding β anomer **30** could be obtained. Formation of 2,3,5-tri-*O*-benzyl- α,β -arabinofuranosyl azide shows corresponding results. The reaction of 3,5-acylated 2-deoxy- α -D-*erythro*-pentofuranosyl chlorides (**31** and **32**) showed less convincing anomeric selectivities,⁵⁵ as shown in the products **33–36**. The use of CsN₃ in Me₂SO was recommended.



By treatment of the corresponding chloride with NaN₃ in 0.1 M NaOH solution, the sensitive methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-azido-3,5-deoxy- α -D-*glycero*-D-*galacto*-2-nonlopyranosid)onate (**37**) could be prepared by using the PTC method. A fast reaction was reported, giving **37** in 65–72% yield.⁵⁶ Treatment of unprotected α -D-glucopyranosyl fluoride¹ with calcium azide in aqueous methanol yielded β -D-glucopyranosyl azide (**38**).⁵⁷ A kinetic study showed that the transformation proceeds through a concerted bimolecular S_N2 (or A_ND_N) mechanism.⁵⁸



In a synthesis of precursors for anomeric pyranoid α -amino acids, the readily accessible 1-halo-glycopyranosyl cyanides **39** were treated with NaN_3 .⁵⁹ Detailed studies showed Me_2SO to be the most effective solvent, and the azido cyanides **40** (2-azido-2-deoxy-glycohept-2-ulopyranosonitriles) were obtained within 5 min; the reaction was slower in DMF. It was presumed that the reaction starts with a light-promoted single-electron transfer (SET) from the ion to the tertiary reaction center. A detailed course for the reaction was reported by the authors.⁵⁹ A fast transfer is required, since the nitrile function is able to attack azide anions in excess. In a similar manner the formamide derivative **41** undergoes this transformation successfully, giving **42** in 91% yield. The synthesis of 2,3,4-tri-*O*-acetyl-5-thio- β -D-xylopyranosyl azide (**44**) required a reaction time of 5 h from the corresponding bromide **43**.⁶⁰

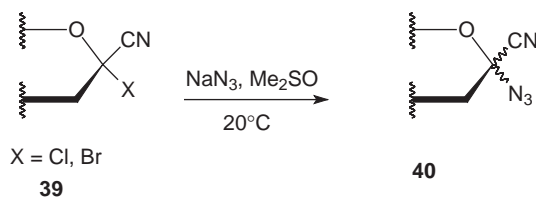
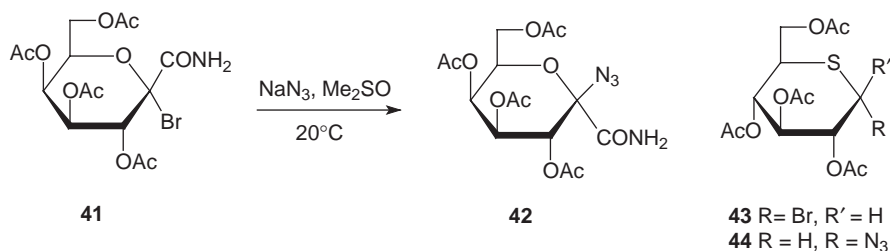


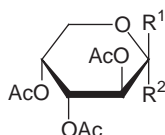
TABLE II
1-Bromo- β -D-glycopyranosyl Chloride Precursors

Compound	R ¹	R ²	R ³	R ⁴
45 (<i>gluco</i>)	OAc	H	OAc	H
46 (<i>manno</i>)	H	OAc	OAc	H
47 (<i>galacto</i>)	OAc	H	H	OAc



Studies by Descotes *et al.*^{61,62} showed the transformation of peracetylated 1-bromo-β-D-glycopyranosyl chlorides (**45–47**, Table II) into the corresponding 1,1-diazides **48–50**. Table III shows the yield to be lowest for the manno derivative, by both the PTC technique as well as in Me₂SO.⁶²

The conversion of methyl (3,4,5-tri-*O*-acetyl-β-D-*arabino*-hex-2-ulopyranosyl)onate bromide (**51**) by sodium azide in Me₂SO gave rise to the α-azide **52** in reasonable yield.⁶³ An alternative approach suggested omission of phase-transfer methods and avoidance of dipolar aprotic solvents, and allowing glycosyl halides to react with NaN₃ in aqueous acetone or acetonitrile.⁶⁴

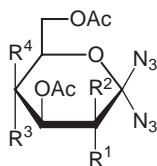


51 R¹ = Br, R² = CO₂CH₃

52 R¹ = CO₂CH₃, R² = N₃

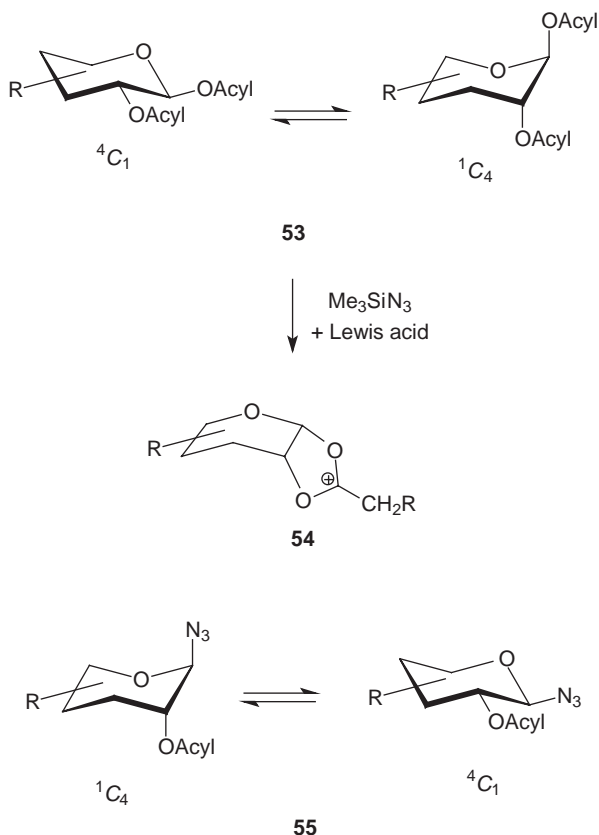
TABLE III
1,1-Diazido Products

Compound	In Me ₂ SO	PTC Method
48 (<i>gluco</i>)	61	82
49 (<i>manno</i>)	8	36
50 (<i>galacto</i>)	57	65

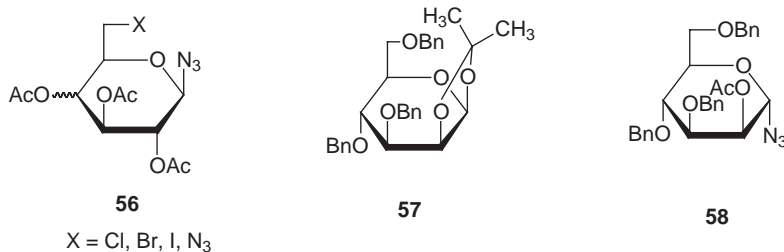


2. Use of Trimethylsilyl Azide for Synthesis of 1,2-*trans*-Glycosyl Azides

Following the discovery of trimethylsilyl azide and its application in organic synthesis,⁶⁵ this reagent was employed in carbohydrate chemistry. Trimethylsilyl azide proved to be an excellent azide donor and permitted direct conversion, under Lewis acid catalysis, of acylated monosaccharides and reducing disaccharides into glycosyl azides, thus eliminating the need for glycosyl halides. The high stereoselectivity observed in these reactions is due to the intermediate formation of acyloxonium ions⁶⁶ whose ring opening by the azide reactant yields 1,2-*trans* products. When the starting acylated saccharide has the 1,2-*cis* configuration, this process is presumably preceded by a Lewis acid-promoted anomerization,⁶⁷ as illustrated for the conversion **53** → **54** → **55**.

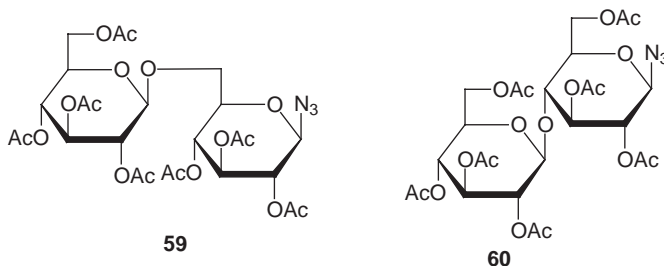


This technique is rather simple, no glycosyl halide is required, and the Lewis acid catalyst, for instance SnCl_4 , can be readily removed. Depending on the ring size, the transformation requires 20 min to 4 h. The four possible pentopyranosyl derivatives, seven of the eight hexopyranosyl derivatives,⁶⁷ three 6-deoxy compounds,⁶⁸ the acetylated α -L-rhamno-, α -L-talo-, and β -L-fuco-pyranosyl azides;^{68–70} and the 1,2-*trans* isomers of 4-deoxy-DL-*threo*- and *erythro*-pentopyranosyl azide have all been synthesized⁷¹ by this method. Later the β -D-*gluco* azide^{34,73} and the α -D-*manno* azide⁷⁴ were again reported. 6-Deoxy-6-halo- and 6-azido-6-deoxy-D-gluco- and -*galacto*-pyranosyl azides (**56**) could be advantageously prepared, using SnCl_4 catalysis.⁷⁵ These azides can, of course, be obtained from the appropriate 6-tosyl esters via nucleophilic displacement, preferably using the corresponding lithium salts.⁷⁵ Under these conditions, orthoesters also yield *trans*-pyranosyl azides. Thus, 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)- β -D-mannopyranose (**57**) was transformed into 2-*O*-acetyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl azide (**58**) in good yield.⁷⁶



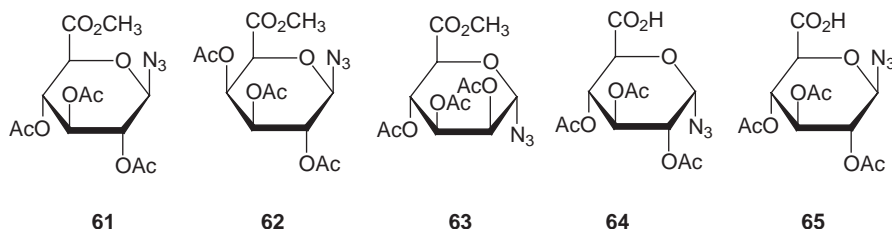
Benzoylated pyranoses also react smoothly with trimethylsilyl azide under SnCl_4 catalysis.^{31,49} Treatment of penta-*O*-benzoyl- α -D-mannopyranose yields the 1,2-*trans*-configured product, 2,3,4,6-tetra-*O*-benzoyl- α -D-mannopyranosyl azide (**11**), and not to the originally assumed β anomer.^{32–34}

The readily accessible peracetates of lactose, maltose, and melibiose are converted in good yields into the corresponding 1,2-*trans* glycobiosyl azide hepta-acetates⁷⁷ more conveniently than by the previously published procedure^{21,79} for the preparation of hepta-*O*-acetyl- β -cellobiosyl azide (**59**). Later this formation of hepta-*O*-acetyl- β -lactosyl azide (**60**)⁷⁷ was reported as a novel procedure,^{80,81} and the corresponding melibiosyl derivative⁷⁷ was again described.⁸² Compound **59** can also be obtained from an orthoester precursor with trimethylsilyl azide as already described,⁸³ but this route does not enjoy any particular preparative advantage.



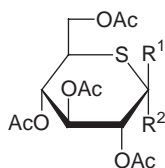
Uronic acid azides constitute important precursors for *N*-glycosyl amino acids and their various peptide derivatives, and may be formed by a comparable pathway. Thus, the treatment of acetyl-protected uronic acid esters with trimethylsilyl azide and SnCl_4 gives the desired acetylated 1,2-*trans* methyl (D-glycopyranosyl azide)uronates in crystalline form.⁸⁴ Whereas the yields for the gluco **61** and galacto derivatives **62** are satisfactory, the manno analog **63** was obtained in only 11% yield. Even with an excess of trimethylsilyl azide, the results could not be improved.⁸⁵

Reports on the transformation of 1,2,3,4-tetra-*O*-acetyl- β -D-glucopyranuronic acid with trimethylsilyl azide under SnCl_4 catalysis are ambiguous. According to Murphy *et al.*⁸⁶ the α -azide **64** was obtained, however Tóth *et al.*⁸⁷ reported the product to be the β derivative **65**. However, the first report⁸⁶ describes a partial ester hydrolysis of **61** with LiOH to give **65**, but the authors did not cite the earlier paper.⁸⁷



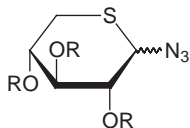
Under similar reaction conditions, treatment of the thio sugar 1,2,3,4,6-penta-*O*-acetyl-5-thio-D-glucopyranose was shown to give the 1,2-*trans* azide **66**, accompanied by minor amounts of the 1,2-*cis* product.⁸⁸ Another approach to **66** employs the α -bromide **67** and LiN_3 .⁸⁸ A protecting group at C-2, less prone to form an acetoxonium ion (compare **54**), results in the loss of stereoselectivity.⁸⁹

Thus, the reaction of 1,2,3,4-tetra-*O*-(2-methylpropanoyl)-5-thio-D-xylose with SnCl_4 in excess gave the mixture of azides **68** with $\beta:\alpha = 2.3:1$.^{89,90} It may be assumed that this outcome is influenced by the donor properties of the ring sulfur atom.⁹⁰

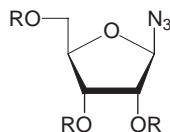


66 $R^1 = \text{N}_3$, $R^2 = \text{H}$

67 $R^1 = \text{H}$, $R^2 = \text{Br}$



68 $R = (\text{CH}_3)_2\text{CHCO}$

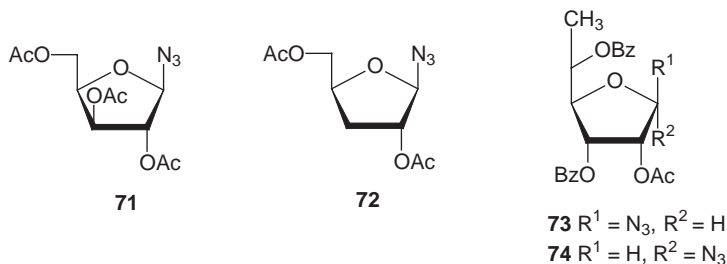


69 $R = \text{CH}_3\text{CO}$

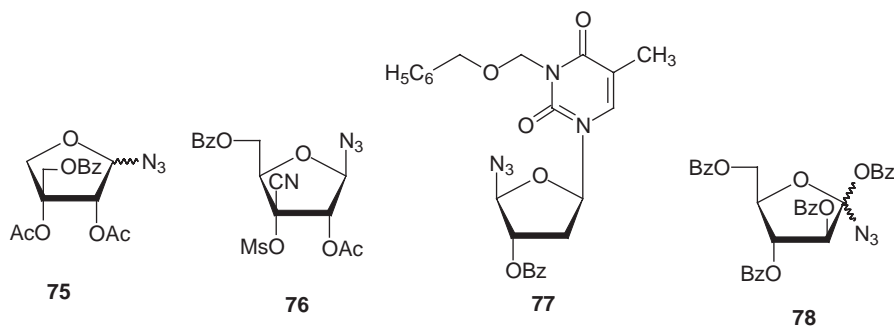
70 $R = \text{C}_6\text{H}_5\text{CO}$

The SnCl_4 -catalyzed reaction of trimethylsilyl azide with glycopyranosyl esters possessing a participating 2-*O*-acyl group proved to be the most efficient and reliable method for the preparation of 1,2-*trans* glycosyl azides.² The efficiency of this azidation approach in terms of chemical yield and selectivity makes it undoubtedly the method of choice; however, the best choice in selection of catalyst and the amount to be used is not so clear. The system $\text{SnCl}_4\text{--AgClO}_4$ in dichloromethane, or ytterbium triflate in nitromethane, in catalytic amounts was reported to give known glycosyl azides in excellent yields.⁹¹

Introduction of the azide function into furanosides merits a special remark. In all of these nucleophilic substitutions, the anomeric selectivity is unpredictable. Initial studies on the formation of furanosyl azides gave useful results.^{8,92–97} 2,3,5-Tri-*O*-acetyl- β -D-ribofuranosyl azide (**69**) was prepared in over 90% yield with a catalytic amount of SnCl_4 ,^{8,95} and a corresponding approach gave the tribenzoate **70**.^{93,95} Further studies showed a very facile anomerization with trimethylsilyl triflate as catalyst,^{92,94} and separation of the anomers was rather demanding. Štimac *et al.*⁹⁶ stated that protected 1,2-*trans* β -D-glycofuranosyl azides with the *ribo*-, *xylo*-, and 3-deoxy-*erythro*-pentose configurations were best prepared from the corresponding glycosyl esters using 0.05 equivalents of SnCl_4 , that is, under anomerization-free conditions. Azidation of methyl glycofuranosides proceeds with inferior and less-predictable selectivity, regardless of the starting anomeric configuration. As a consequence of these findings, 2,3,5-tri-*O*-acetyl- β -D-xylofuranosyl azide (**71**) could be obtained as the pure anomer. In case of the crude 2-*O*-acetyl-5-*O*-benzoyl-3-deoxy- β -D-*erythro*-pentofuranosyl azide (**72**) the ratio was $\beta:\alpha = 124:1$.⁹⁷

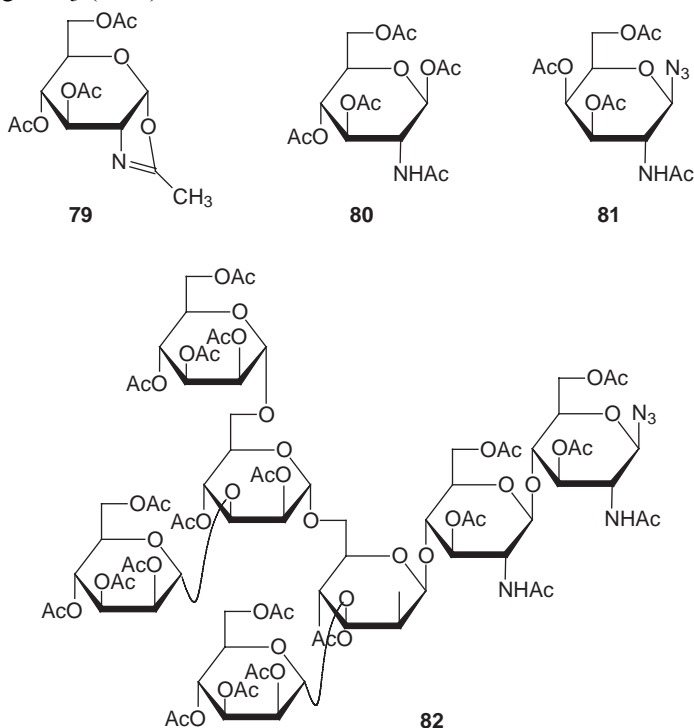


In contrast, azidation of 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-6-deoxy-*L*-talofuranose ($\alpha:\beta = 5:3$) after an extended reaction time leads to a 3:2 mixture of 2-*O*-acetyl-3,5-di-*O*-benzoyl-6-deoxy- α - and β -*L*-talofuranose (**73** and **74**).⁹⁴ Likewise, 2,3-di-*O*-acetyl-3'-*O*-benzoyl-*D*-apiofuranosyl azide (**75**) was obtained as a difficultly separable anomeric mixture, because equimolar amounts of trimethylsilyl triflate were used in the reaction.⁹⁸ The branched-chain structure, 2-*O*-acetyl-5-*O*-benzoyl-3-*C*-cyano-3-*O*-methylsulfonyl- β -*D*-ribofuranosyl azide (**76**), could be synthesized in excellent yield without anomerization.⁹⁹ The "inverse nucleoside" **77** was obtained as the anomerically pure azide from 1-[5'(*R,S*)-acetoxy-4'(*R*)-benzoyltetrahydrofuran-2'-yl]-3-benzylloxymethylthymine in 79% yield.¹⁰⁰ In another report, 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzoyl- α , β -*D*-fructofuranose was treated with trimethylsilyl azide and $TiCl_4$ to give 1,3,4,6-tetra-*O*-benzoyl-*D*-fructofuranosyl azide (**78**) as an anomeric mixture.^{101,102}



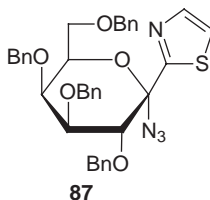
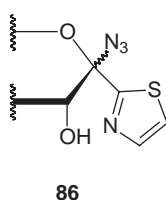
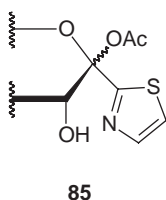
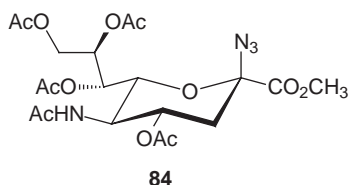
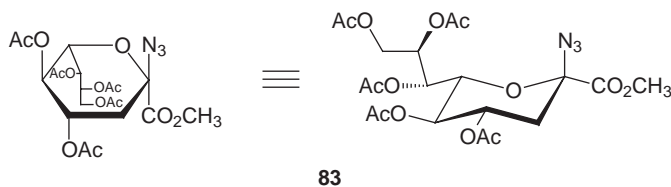
Trimethylsilyl azide is useful for opening the oxazoline ring. 2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy- α -*D*-glucopyrano)-[2,1-*d*]-2-oxazoline (**79**) gives the same *trans* azide (**1**) as that obtained¹⁰³ from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy- β -*D*-glucopyranose (**80**). In contrast, the corresponding α anomer of **80** does not yield azide **1**. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -*D*-galactopyranosyl

azide (**81**)¹⁰⁴ and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl azide (**24**) were also obtained readily by this method. Opening of pyrano[2,1-*d*]oxazolines with trimethylsilyl azide proved to be applicable to higher oligosaccharides as well.^{105,106} The heptasaccharide derivative (2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 \rightarrow 6)-[2,3,4,6-tetra-*O*-acetyl- α -D-annopyranosyl)-(1 \rightarrow 3)]-(2,4-di-*O*-acetyl- α -D-mannopyranosyl)-(1 \rightarrow 6)-[(2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 \rightarrow 3)-2,4-di-*O*-acetyl- β -D-mannopyranosyl)-(1 \rightarrow 4)]-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -D-glucopyranosyl)-(1 \rightarrow 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -D-glucopyranosyl azide (**82**) could be prepared from the corresponding oxazoline in better yield (42%) than from the glycosyl chloride employing LiN_3 (22%).¹⁰⁵

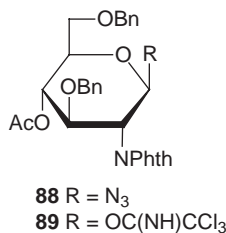


Methyl 4,5,7,8,9-penta-*O*-acetyl-2-azido-2,3-dideoxy-D-*glycero*- α - and β -D-*galacto*-2-nonulo-pyranosonates (**83**) were formed by treatment of the β acetate with trimethylsilyl azide in the presence of equimolar amounts of SnCl_4 . The poor yields reported are associated with problems in separation of the catalyst.¹⁰⁷ Formation of the neuraminic acid analog, namely methyl (5-acetamido-4,7,8,

9-tetra-*O*-acetyl-3,5-dideoxy- β -D-glycero- β -D-galacto-non-2-ulopyranosyl)onate-azide (**84**) succeeds in 86% yield with SnCl_4 as catalyst.¹⁰⁸ The same combination of reagents was used in the synthesis of the 6-thio analog of **84**.^{109,110} Other anomeric amino acid derivatives serving as precursors for the synthesis of hydantocidins were prepared employing this reaction,¹¹¹ thus furanoid and pyranoid ketolacetates of the type **85** were transformed into the azides **86** with varying stereoselectivity. The azides could in turn be converted into the desired anomeric amino acids. It should be noted that, with trimethylsilyl azide, both anomers of **86** led to the same product "1-azido-2,3,4,6-tetra-*O*-benzyl-1-(2-thiazolyl)- α -D-galactopyranoside" (**87**).¹¹¹

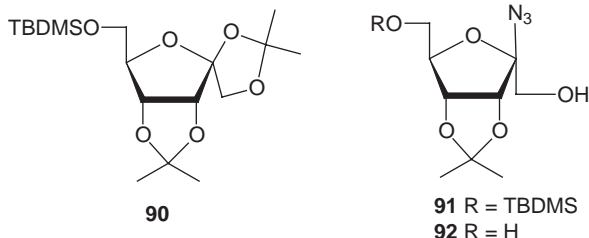


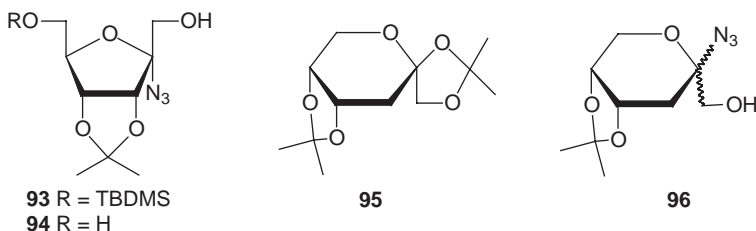
As a precursor to chitobiosyl derivatives, 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl azide (**88**)¹¹² was obtained from the trichloroacetimidate **89** and trimethylsilyl azide. The same azide (**88**) was prepared from the corresponding phenyl 1-thio-glycoside with trimethylsilyl azide and *N*-iodosuccinimide-trifluoroacetic acid.¹¹³ This modification was also employed with further benzylated derivatives.^{114,115}



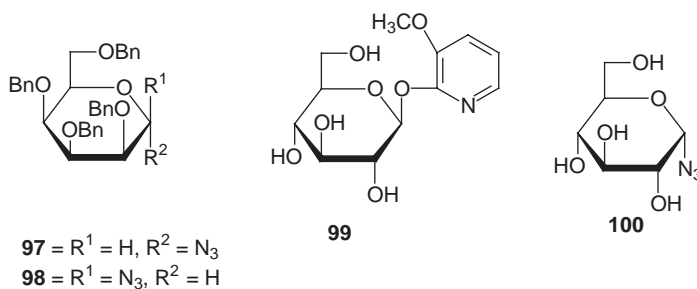
Hypervalent azidosilicate derivatives, prepared *in situ* by the reaction of trimethylsilyl azide with tetrabutylammonium fluoride, are effective sources of nucleophilic azide. Acetylated glycosyl chlorides, bromides, or trifluoroacetimidates^{116–119} react with this silicon reagent to give known glycosyl azides. Deca-*O*-acetyl- β -maltotriosyl azide could be formed by this method;⁸² however the configuration of the product remained ambiguous. The supposedly same derivative obtained with trimethylsilyl azide and BF₃·Et₂O was not compared with the aforementioned material, and the ¹H-NMR spectra were not identical.^{120,121} This method requires the preparation of glycosyl halides from the peracetylated compounds, and needs increased reaction times or elevated reaction temperatures.

In recent years, the Lewis acid-catalyzed azidation of protected sugar derivatives has been extended to more-complex starting materials. For instance, isopropylidene-protected ketose derivatives were treated with trimethylsilyl azide and a catalyst to give various ketosyl azides.^{122–124} Thus, the reaction of 6-*O*-*tert*-butyldimethylsilyl-1,2:3,4-di-*O*-isopropylidene- β -D-psicofuranose (**90**) gave a mixture of partially deprotected anomeric azides **91–94** in 54% overall yield. The corresponding transformation of 3-deoxy-1,2:4,5-di-*O*-isopropylidene- β -D-*erythro*-hex-2-ulopyranose (**95**) led to the azide mixture **96** in 75% yield.¹²³ 2,3,4,6-Tetra-*O*-pivaloyl- α -D-galactopyranosyl fluoride, with its sterically demanding pivaloyl groups and thus less-pronounced neighboring-group effects, reacts with trimethylsilyl azide and BF₃·Et₂O to give the anomers in ratio $\alpha:\beta = 3:1$.¹²⁵



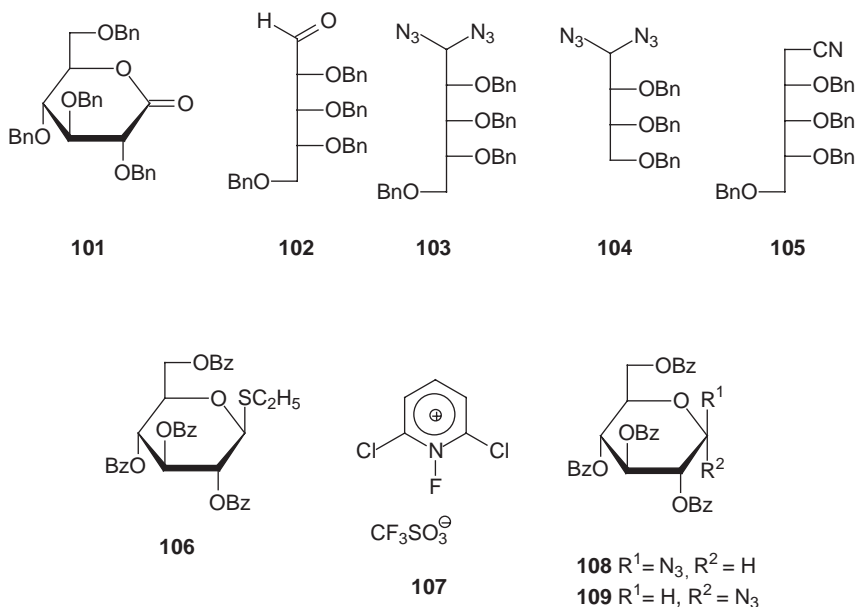


Such benzyl-protected pyranoses as 1-*O*-acetyl-2,3,5-tri-*O*-benzyl- β -D-ribofuranose¹²⁶ or 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl- α -D-mannopyranose¹²⁷ do not give single glycosyl azides but lead to anomeric mixtures. Thus 2,3,4,6-tetra-*O*-benzyl- α - (**97**) and - β -D-mannopyranosyl azide (**98**) were obtained in the ratio 1:1.2. In his book Hanessian¹²⁸ reports on a process based on the “remote activation concept”. For example, 3-methoxy-2-pyridyl β -D-glucopyranoside (**99**) reacts with trimethylsilyl azide and trimethylsilyl triflate in DMF, leading in virtually quantitative yield to the unprotected α -glycopyranosyl azide (**100**). (For the formation of **100** in an alternative way, see Ref. 75.)



2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosylidene diazide (**48**) could be obtained not only by substitution of the chlorobromo sugar **45** (see foregoing) but also, according to Praly *et al.*,^{61,62} alternatively by treatment of tetra-*O*-benzyl-D-glucuno-1,5-lactone (**101**) with trimethylsilyl azide and $BF_3 \cdot Et_2O$ for two days. Corresponding conditions were successfully used in the preparation of benzyl-protected 1,1-diazo aldoses^{129–131} from open-chain *O*-benzylated aldoses. In the reaction, the formation of three products could be observed.¹³¹ By treating 2,3,4,5-tetra-*O*-benzyl-aldehydo-D-ribose (**102**) with the foregoing combination of reagents at 0 °C in CH_2Cl_2 , the diazides **103** and **104**, and the ribonitrile **105** could be detected. According to their postulated reaction mechanism, a tri-

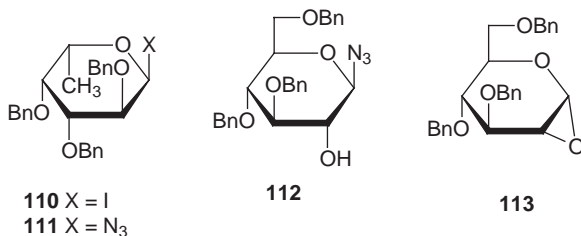
methylsilylated azide is obtained as the intermediate, which is subsequently degraded. The main product **103** was obtained in 35% yield. By treatment of the benzoylated 1-thioglucoside **106** with a sixfold excess of trimethylsilyl azide and 1-fluoropyridinium triflate (**107**) as catalyst, considerable anomerization was observed, leading to the 1,2-*trans* azide **108**⁴³ along with its anomer **109**.¹³²



For the direct formation of glycosyl azides from peracylated saccharides, the latter can be first transformed by trimethylsilyl iodide into the glycosyl iodides, which in turn show an increased reaction rate.^{133,134} With trimethylsilyl azide or tetramethylguanidinium azide^{135,136} the familiar 1,2-*trans* glycosyl azides are obtained. An exception was reported in the treatment of 2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl iodide (**110**) with (insoluble) NaN_3 in dichloromethane, which gave the α -azide **111** under retention of configuration.¹³⁸ Likewise, 1,2-*trans* azides are formed from 1,2-*trans* peracetates in CH_3CN with a large excess of $BF_3 \cdot Et_2O$ and NaN_3 at extended reaction times.¹³⁹ Another catalyst system developed by Gin *et al.* uses the hemiacetals in diphenyl sulfoxide and trifluoromethanesulfonic anhydride at $-45^\circ C$, and by treatment with trimethylsilyl azide as nucleophile a trisaccharidyl azide could be obtained.¹⁴⁰ In this

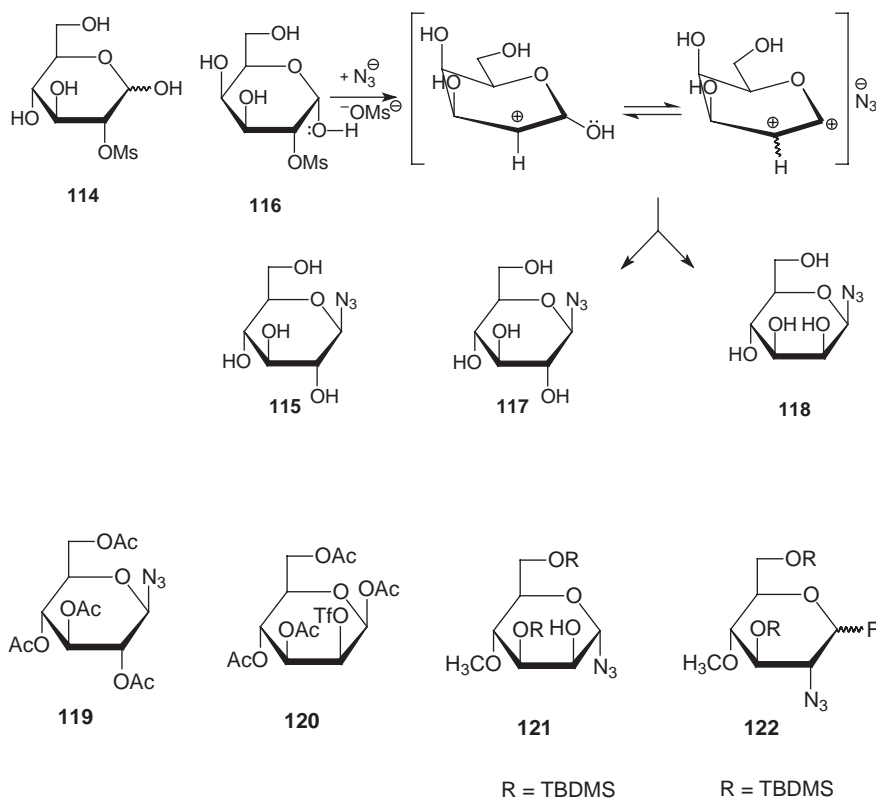
system, trimethylsilyl azide can also be used as nucleophile, and thus a trisaccharidyl azide could be obtained.¹⁴⁰

Opening of the epoxide **113** with tetrabutylammonium azide gave the 1,2-*trans* glycosyl azide **112**.¹⁴¹ Further epoxide openings employing “lithium azidohydridodiisobutylaluminum” in the *gluco*-, *galacto*-, and *allo*-series were reported in a short communication.¹⁴² Apparently, this nucleophile also attacks at the anomeric center, giving **112** in 73% yield.



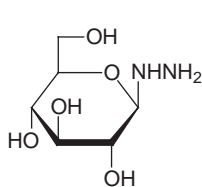
3. Synthesis of 1,2-*trans*-Glycopyranosyl Azides by Intramolecular Rearrangement

Attempts to displace the methanesulfonyloxy group by azide¹⁴³ in 2-*O*-methylsulfonyl- β -mannopyranose (**114**) resulted in formation of the β -D-glucopyranosyl azide (**115**) in 87% yield. In the case of the 2-*O*-mesyl derivative **116**, attack of the azide anion from both sides was observed. Thus, the β -D-galactopyranosyl azide **117** plus somewhat more of the β -D-talopyranosyl azide (**118**) were formed, and they were identified in the form of their peracetates (such as **119**).¹⁴³ These findings were supported by the transformation of 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethylsulfonyl- β -D-mannopyranose (**120**) with NaN₃ to give **119** in about 20% yield.¹⁴⁴ It was suggested¹⁴⁴ that, in an S_N1 reaction, a carbocation is first generated and is then converted into the epimeric mixture of products under nucleophilic attack by the azide ion. A similar transformation was observed when a mannosyl azide (**121**) unsubstituted at position 2 underwent rearrangement into 2-azido-2-deoxy-D-glucopyranosyl fluoride (**122**) under the action of diethylaminosulfur trifluoride (DAST).¹⁴⁵ Dutch authors were able to observe not only the rearrangement product but also the “normal” substitution product in case of a fucose (6-deoxygalacto)-configured substrate.⁷⁰

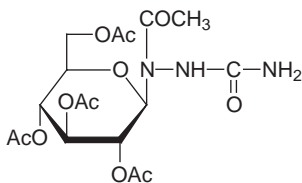


Another possible route to glycosyl azides consists in the reaction of HNO_2 with glycosylhydrazines, which exist in equilibrium between the open-chain hydrazone form and the cyclic hydrazone form.¹⁴⁶ This equilibrium is shifted toward the hydrazone form **123** for the D-glucose derivatives. Williams *et al.* showed that the reaction with HNO_2 gave in addition to D-glucose, the well-known azide **115**. Treatment of the semicarbazide derivative **124** of D-glucose with HNO_2 also gave 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl azide (**115**).¹⁴⁷

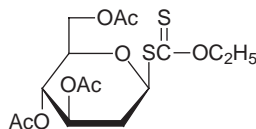
The azide radical can be generated thermally by treatment of ethylsulfonyl azide at 100 °C.¹⁴⁸ This can be coupled with the 3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl radical obtained in turn from the xanthate **125** to give the α -D-arabino-hexopyranosyl azide **15** in good yield.^{148,149}



123



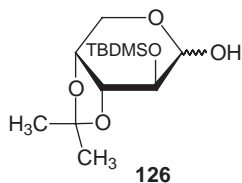
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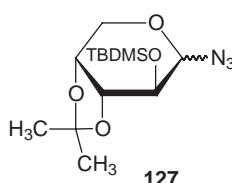
125

4. Synthesis of Glycosyl Azides from Glycosyl Derivatives with an Unprotected Anomeric Center, Employing Phospho-Organic Compounds

The transformation of alcohols into derivatives carrying a nucleophilic group, by employing the reagent combination triphenylphosphine, azodicarbonic acid ester, and a nucleophile (NuH) (Mitsunobu reaction)^{150–152} can be used to form various glycosyl azides. Work-up of is often difficult because of the required chromatographic removal of triphenylphosphine oxide and the corresponding hydrazinodicarbonic acid ester. Protected furanoses with a free anomeric center reacted under Mitsunobu conditions to give glycofuranosyl azides, using thermolysis of azoimide as the source for azide. The following anomeric mixtures were employed as starting materials:⁹² 5-*O-tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-D-ribofuranose, 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose, 2-*O-tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-β-D-ribofuranose, 4-*O-tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranose, and 2-*O-tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-D-arabinopyranose. For the pair of anomers **126** (α:β ~1.4:1), the resultant mixture of anomeric azides **127** had a different ratio (α:β = 1.96:1). The authors subsequently preferred to use the method mentioned earlier (see Section II.3) for the preparation of **127**.⁹⁴



126

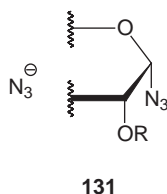
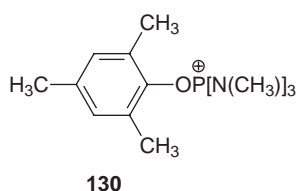
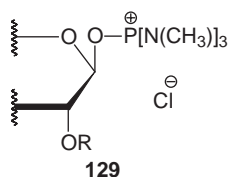
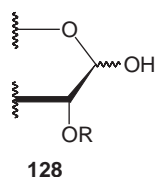


127

For free monosaccharides, a mixture of three components was employed to obtain unprotected azides. Based on such a concept and literature data, French

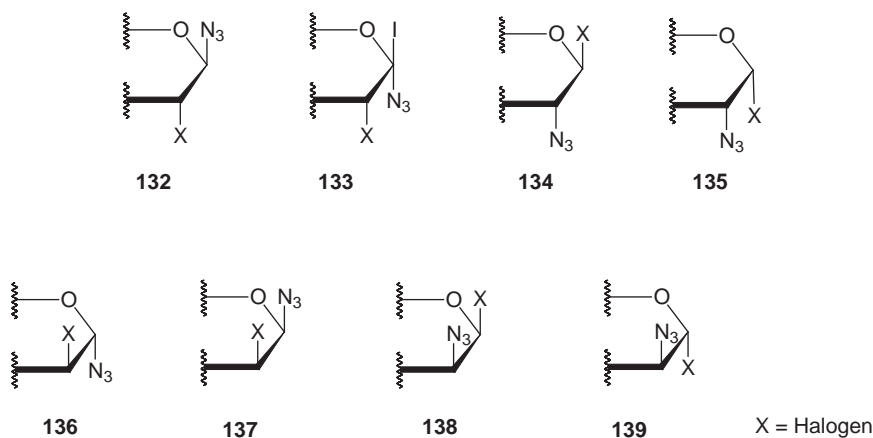
authors¹⁵³ suggested the use of *N*-chlorosuccinimide–triphenylphosphine–lithium azide in DMF. *N*-chlorosuccinimide preferentially forms the anomeric chlorides (however, not *N*-protected amino sugars), and this in turn yields the 1,2-*trans* azide, isolated as its peracetate after chromatography. In all instances the 1,2-*cis* azide is formed.¹⁵³

Sugars having a free anomeric OH group (**128**) react with tris(dimethylamino)phosphine in CCl_4 to give alkoxytris(dimethylamino)phosphonium chlorides (**129**) with 1,2-*trans* stereochemistry. These reactive oxyphosphonium salts can be converted¹⁵⁴ at -10°C under kinetic control into 1,2-*cis*-glycofuranosyl azides (**131**) by using the reagent mesityloxytrisdimethylaminophosphonium azide (**130**) (commercially not available). By this reaction, 2,3:5,6-di-*O*-isopropylidene- β -D-mannofuranosyl azide was obtained in 65% yield. The same azide was obtained crystalline by the Mitsunobu method and in 75% yield.⁹²

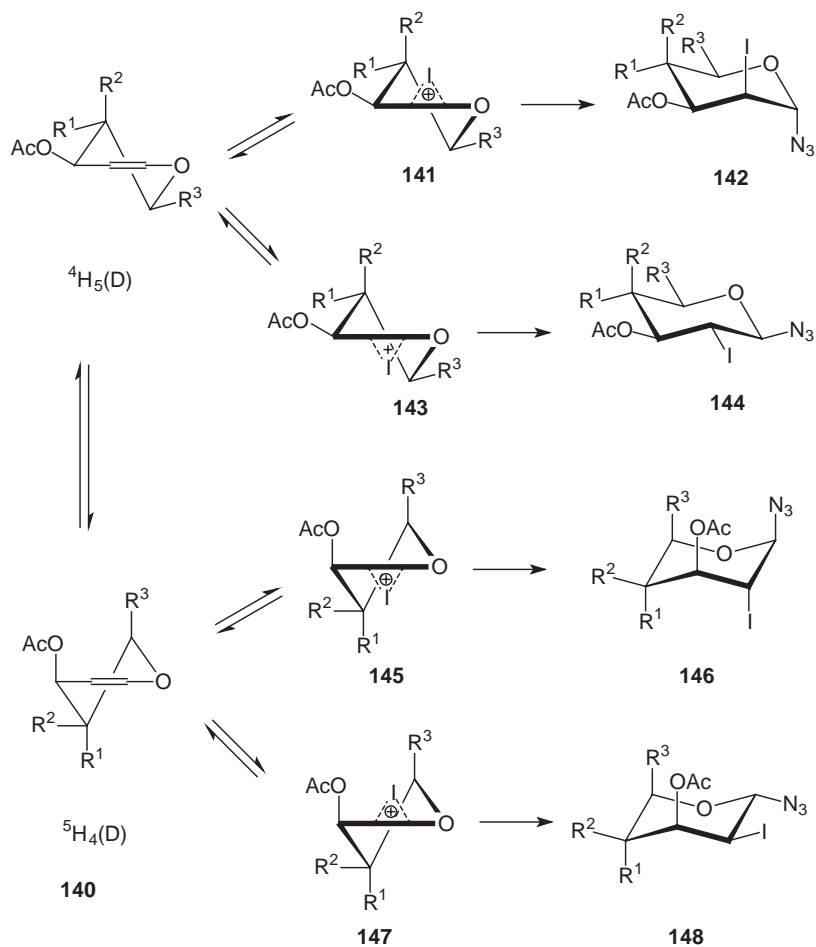


5. Glycols as Starting Materials for Synthesis of 2-Halo-Substituted Glycosyl Azides

The quest for efficient methods to prepare 2-aminohexoses prompted the first investigations based on glycols. It is generally known that trisubstituted alkenes (to which glycols are related) react with such unsymmetrical reagents as halo azides, leading in principle to a mixture of eight isomers **132–139**.



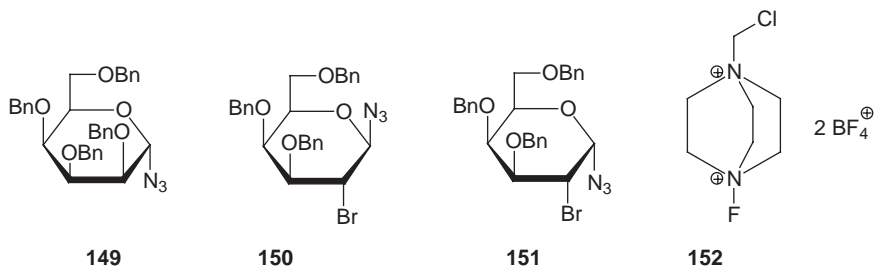
Nevertheless, a high degree of regio- and stereo-selectivity in these reactions may be expected if all factors (steric and energetic) governing the mode of addition are considered. Polarization of halo azides requires ionic conditions and yields glycosyl azides, whereas radical conditions yields 2-azido-2-deoxyaldoses. The radical conditions employed by Khorlin^{155,156} (2.0–2.5 equivalents of chloro azide, -20°C , 3 h in nitromethane) gave 1,2-*trans* chloro azides in only moderate yields; thus 3,4,6-tri-*O*-acetyl-2-chloro-2-deoxy- β -D-glucopyranosyl azide and 3,4,6-tri-*O*-acetyl-2-chloro-2-deoxy- α -D-mannopyranosyl azide could be isolated from the same reaction mixture in yields of 17% and 26%, respectively. Higher regioselectivity could be achieved by using the iodo azide, while ionic addition to **140** at 0°C in acetonitrile or ethyl acetate for 2 h yielded the 1,2-*trans*-2-deoxy-2-iodo-glycosyl azides **142**, **144**, **146** and **148**.¹⁵⁷ Although separation of the α and β anomers of the 1,2-*trans* products from acetylated glycals required an additional step,¹⁵⁸ the benzylated and methoxymethylated glycals, on the other hand, gave 1,2-*trans* 2-iodo azides in good overall yield. It was suggested¹⁵⁸ that the 2-iodoglycosyl azides are formed from glycals via the cyclic iodonium intermediates **141**, **143**, **145**, and **147** (see also Ref. 159). Another reagent described for haloazidation of glycals is tetrabutylammonium [di(acyloxy)bromate]¹⁶⁰ plus trimethylsilyl azide, which is directly added to a solution of the protected glycal. Tri-*O*-benzyl-D-galactal thus gives 3,4,6-tri-*O*-benzyl-2-bromo-2-deoxy- α -D-talopyranosyl azide (**149**), 3,4,6-tri-*O*-benzyl-2-bromo-2-deoxy- β -D-galactopyranosyl azide (**150**), and 3,4,6-tri-*O*-benzyl-2-bromo-2-deoxy- α -D-galactopyranosyl azide (**151**) in the ratio of 1.3:1.7:1. For azidoiodination, a polymer-supported reagent was developed and tested with protected galactal and fucal derivatives.¹⁶¹



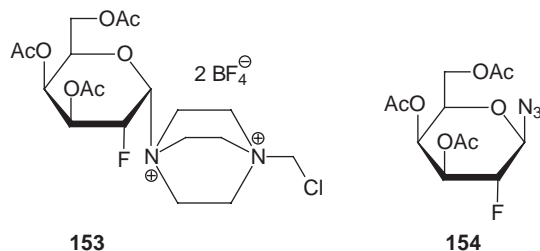
a $R^1 = \text{OAc}$, $R^2 = \text{H}$, $R^3 = \text{CH}_2\text{OAc}$

b $R^1 = \text{H}$, $R^2 = \text{OAc}$, $R^3 = \text{CH}_2\text{OAc}$

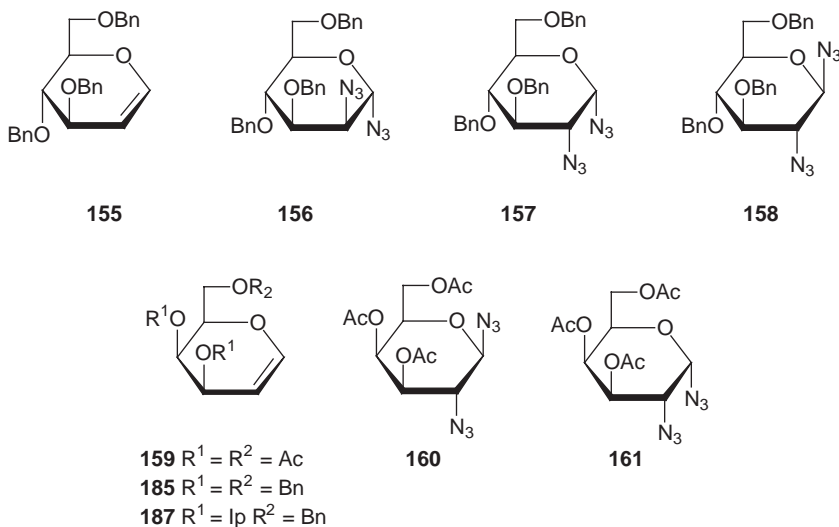
c $R^1 = \text{OAc}$, $R^2 = \text{H}$, $R^3 = \text{H}$



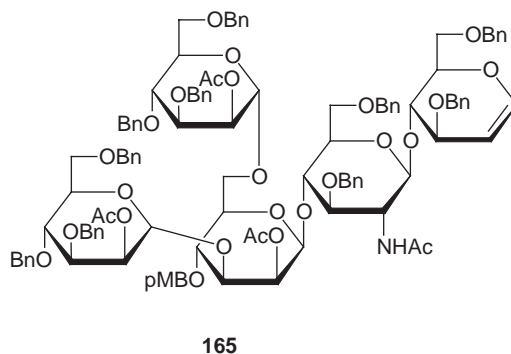
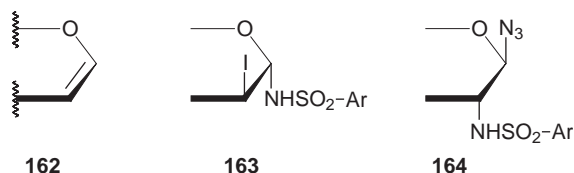
The electrophilic fluoridation reagent SelectfluorTM [**152**, 1-chloromethyl-4-fluoro-1,4-diazabicyclo[2.2.2]octane bis(tetrafluoroborate)] was allowed to react with tri-*O*-acetyl-D-galactal, and the resulting salt **153** {1-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro- α -D-galactopyranosyl)-4-chloromethyl-1,4-diazabicyclo[2.2.2]octane bis(tetrafluoroborate)} reacted with NaN₃ affording the 1,2-*trans* fluoro azide **154**.¹⁶²

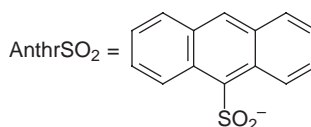
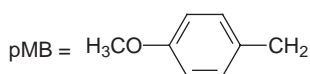
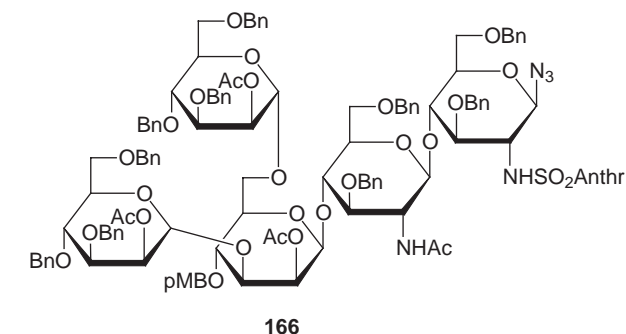


Feasibility studies for the formation of azidoglycosyl azides were performed with dihydropyran as model compound.¹⁶³ Glycals react with Mn(OAc)₃ · 2H₂O and NaN₃ in 9:1 acetonitrile–trifluoroacetic acid to give 1,2-diazides in > 80% yield. Azide addition to 3,4,6-tri-*O*-benzyl-D-glucal (**155**) at 0 °C affords 81% of an inseparable 41:32:27 mixture of **156**, **157**, and **158**. A similar reaction with tri-*O*-acetyl-D-galactal (**159**) yields 83% of a 2:3 mixture of **160** and **161**. The preparation of the latter 1,2-*cis* azide (**161**) was previously reported by another method.¹⁰³

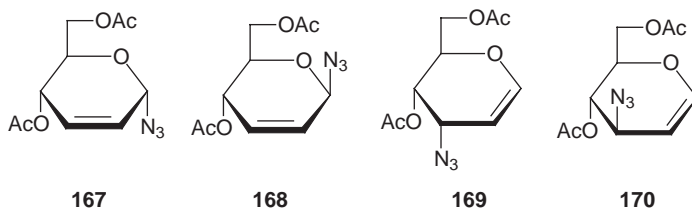


A useful transformation of pyranoid glycals by Danishefsky *et al.*^{164–168} leads to 1,2-*trans* 2-deoxy-2-sulfonylaminoglycopyranosyl azides. In this two-stage procedure, the glycal **162** first undergoes iodosulfonamidation to give the 1,2-dideoxy-2-iodo-1-*N*-sulfonylamido-glycopyranose (**163**). By the addition of NaN₃ in DMF, this compound in turn undergoes a rearrangement to form the 1,2-*trans* 2-deoxy-2-sulfonamido-glycopyranosyl azide **164**.^{164–168} There is no requirement for silver salt promoters to effect displacement of the iodine. In the first step, anhydrous conditions and cooling are necessary, and addition of the required aromatic sulfonamide to **162** is performed with I(coll)₂ClO₄ in dichloromethane. Formation of **164** required stoichiometric amounts of NaN₃. This principle was also demonstrated for a pentasaccharide glycal **165**, which could be transformed into the 1,2-*trans* azide **166** (AnthrSO₂ = anthracene-5-sulfonyl) using tetrabutylammonium azide.¹⁶⁸ Of particular interest is the subsequent transformation of the sulfonamido group into an acetamido function. Furthermore, this method could be performed as a solid-supported process.¹⁶⁶





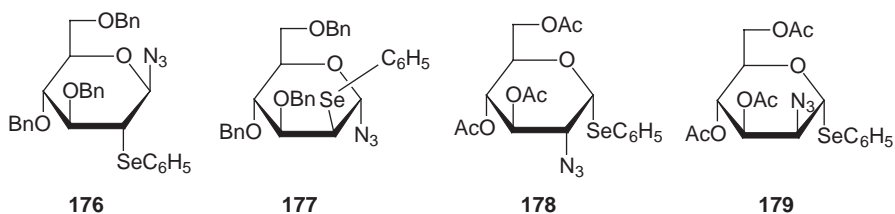
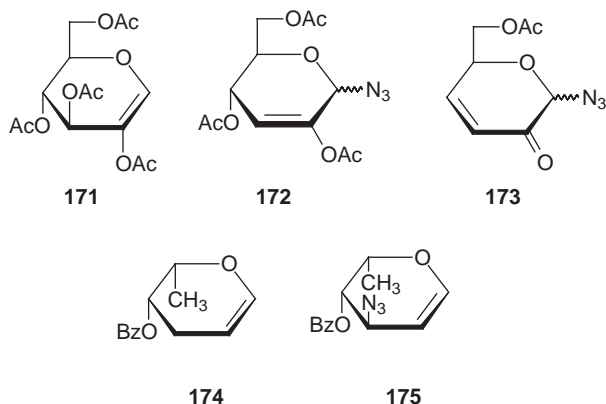
In 1978, Heyns and Hohlweg reported the reaction of 3,4,6-tri-*O*-acetyl-D-glucal (**140a**) with NaN₃ in the presence of three equiv. of boron trifluoride diethyl etherate,¹⁶⁹ and described the sigmatropic interconversion of the products between the 2,3-unsaturated glycosyl azides and the 3-azido-3-deoxyglycals. Later this reaction was examined in detail, and also the use of Me₃SiN₃ and trimethylsilyl triflate or ytterbium triflate studied.¹⁷⁰ As reaction products, the azides of the “pseudoglycal” (4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl azides **167** and **168**) and the 3-azido-3-deoxyglycals **169** and **170** were obtained. In contrast, Indian authors observed only the formation of **167** and **168** in their InBr₃-Sc(OTf)₃ and ZrCl₄-catalyzed reactions.^{171–173}



Reaction of 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol (tetra-*O*-acetyl-hydroxyglucal, **171**) with trimethylsilyl azide and the aforementioned Lewis acid catalysts leads exclusively to a mixture of 2,4,6-tri-*O*-acetyl-3-deoxy-

α/β -D-*erythro*-hex-2-enopyranosyl azide (**172**) and 6-O-acetyl-3,4-dideoxy- α/β -D-*glycero*-hex-3-en-2-ulopyranosyl azide (**173**).¹⁷⁰

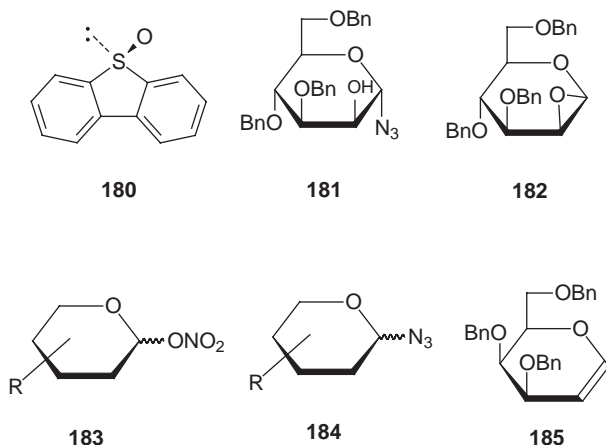
It should be indicated that glycals undergo allylic azidation if trimethylsilyl azide and an iodine(III) reagent are employed. Thus the 3,6-dideoxy-L-*threo*-glycal **174** is transformed into the 3-azido-3,6-dideoxyglycal derivative **175**.^{174,175}



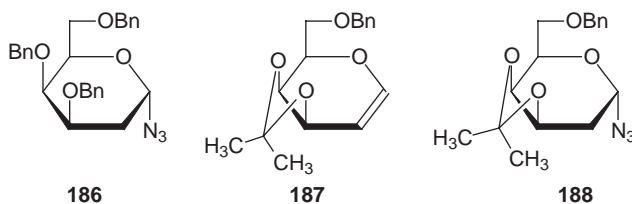
Glycals are suitable precursors for 1,2-*trans* phenylselenyl azides, and in 1994 two different preparative approaches were reported.^{176,177} 3,4,6-Tri-O-benzyl-2-deoxy-2-(phenylseleno)- β -D-glucopyranosyl azide (**176**) and the α -D-mannopyranosyl azide (**177**) were prepared by two different methods: trimethylsilyl azide + *N*-phenylselenophthalimide + tetrabutylammonium fluoride in dichloromethane at room temperature,¹⁷⁶ or sodium azide + phenylselenenyl chloride in *N,N*-dimethylformamide.¹⁷⁷ The physical data of these compounds differ considerably between the two reports and so the results remain somewhat dubious. When D-glucal triacetate **140a** was treated with (diacetoxyiodo)benzene and sodium azide in the presence of diphenyl diselenide at room temperature, an

inseparable mixture of phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-seleno- α -D-glucopyranose (**178**) and the manno isomer **179** was obtained in 91% yield.¹⁷⁷

A 2-hydroxyglycosylation reaction employing the reagent combination of a diaryl sulfoxide and triflic anhydride offers a novel method for assembly from a glycal, whereby a hydroxyl functionality is stereoselectively installed at the 2-position of a glycal donor with concomitant glycosylation of a nucleophilic acceptor.¹⁷⁸ The 2-hydroxyglycosyl azide **112** was formed from 3,4,6-tri-*O*-benzyl-D-glucal in this reaction when Ph₂SO and NaN₃ in methanol was used. A complementary method for 2-hydroxyglycosylation, generating α -mannopyranosides from glucal donors, is available when dibenzothiophene-5-oxide (**180**) is employed as the sulfoxide reagent. The formation of 3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl azide (**181**) and of dibenzothiophene was closely monitored by ¹⁸O-isotopic marking, and interpreted via intermediate formation of the manno epoxide **182**.



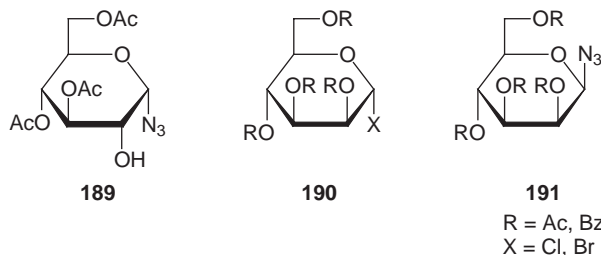
A reagent system comprising trimethylsilyl azide and trimethylsilyl nitrate permits conversion of glycals into 2-deoxyglycopyranosyl azides in one step and in good yields.¹⁷⁹ Intermediate formation of an anomeric mixture of glycosyl nitrates (**183**) occurs, and in the strict absence of water the reaction with trimethylsilyl azide gives a mixture of 2-deoxy azides (**184**). For galacto derivatives there is anomeric-selectivity: 3,4,6-tri-*O*-benzyl-D-galactal (**185**) gives the 2-deoxy- α -D-*lyxo* azide **186**, and 6-*O*-benzyl-3,4-*O*-isopropylidene-D-galactal (**187**) leads to the 2-deoxy- α -D-*lyxo* azide **188** in the ¹C₄ conformation.¹⁷⁹ All of these derivatives show large positive rotations, supporting the α configuration.^{31,67}

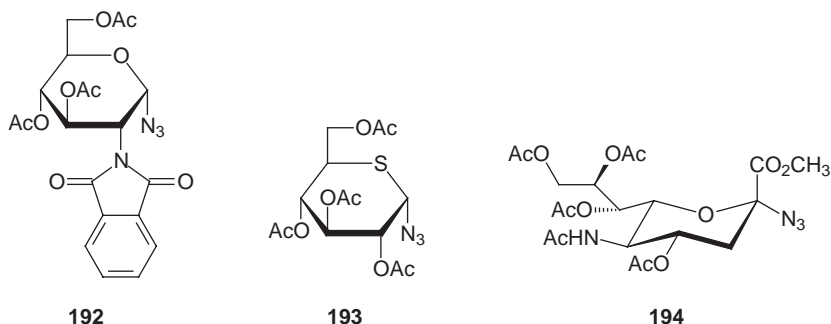


6. 1,2-*cis* Glycosyl Azides

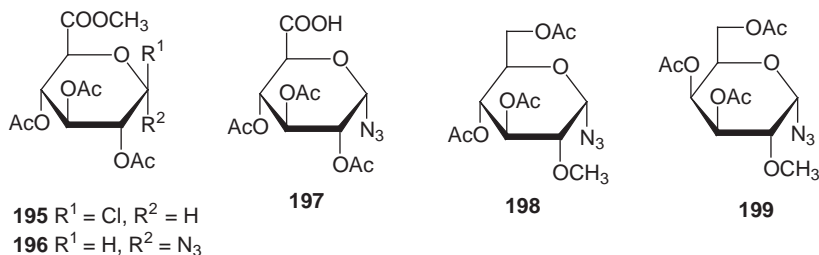
3,4,6-Tri-*O*-acetyl- α -D-glucopyranosyl azide (**189**), obtained from Brigl's chloride (3,4,6-tri-*O*-acetyl-2-trichloroacetyl- β -D-glucopyranosyl chloride) by selective removal of the trichloroacetyl group¹⁷ has long been the only representative of this class of compounds.

It has been found that, under certain conditions, acylated glycosyl halides react with alkali metal azides with inversion by an S_N2 process. Thus, 1,2-*cis* azides may be prepared by reaction of the thermodynamically less stable 1,2-*trans* per-*O*-acylglycopyranosyl halides, such as **190**, with NaN_3 in hexamethylphosphoric acid triamide (hexamethylphosphoramide, HMPA) at room temperature. After a short time, anomerically pure products can be isolated by dilution of the mixture with water. An example is provided³¹ by the synthesis of the β -mannopyranosyl derivatives **191**. This method proved useful for synthesis of 1,2-*cis* hexopyranosyl azides,^{75,103,180–182} 1,2-*cis*-6-deoxy-hexopyranosyl azides,⁶⁸ 1,2-*cis*-2-acylamino-2-deoxy-hexopyranosyl azides¹⁰³ [such as 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- α -D-glucopyranosyl azide, **192**], and 1,2-*cis* glycobiosyl azides.⁷⁷ The four possible 1,2-*cis*-D-pentopyranosyl azides¹⁸³ have also been obtained in this way. This nucleophilic displacement thus allows the preparation of such 1,2-*cis* azides as 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranosyl azide (**193**) and the peracetylated α -neuraminyl azide **194**,¹⁰⁸ the free form of which is used to crosslink and precipitate *Limax flavus* lectin.¹⁸⁴ Formation of the azide, starting with glycosyl halide **28**, was compared by two different methods. In HMPA, the product (**29**) of inversion prevails over the product (**30**) of retention by a ratio of 17:3, and under phase-transfer reaction conditions the ratio of 49:1 was observed.⁵⁵





By this method in HMPA, a 1,2-*cis* azidouronic acid derivative was prepared.⁸⁴ The yield of methyl (2,3,4-tri-*O*-acetyl- α -D-glucopyranosyl azide)uronate (**196**) was 56% starting with the β -chloride **195**, and its further transformation into the corresponding acid **197** was also described.⁸⁶



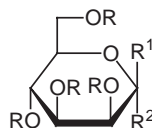
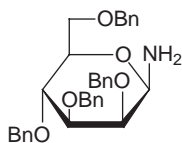
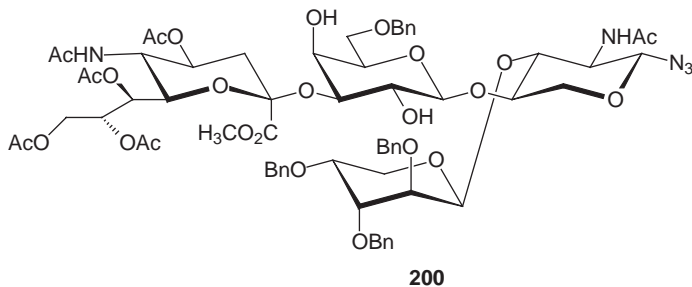
3,4,6-Tri-*O*-acetyl-2-*O*-methyl- α -D-gluco- (**198**) and -galactopyranosyl azide (**199**) are formed from the corresponding peracetates with trimethylsilyl azide under catalysis by SnCl_4 .³⁹ 2,3,4,6-Tetra-*O*-benzyl- α -D-glucopyranosyl azide has been obtained in two different ways.^{49,185} With other ether substituents, as for example in the formation of 2,3,4,6-tetra-*O*-(5-phthalimido-3-oxapentyl)-D-glucopyranosyl azide, no α -selectivity could be observed.¹⁸⁶

III. TRANSFORMATION OF GLYCOSYL AZIDES

1. Reduction of Glycosyl Azides

Reduction of protected glycosyl azides under mild conditions (hydrogenation at atmospheric pressure and room temperature using PtO_2 ^{12,5,72} or Raney

nickel^{13,187} catalysts) leads to the formation of glycosylamines.¹⁸⁸ No clear rule is yet evident for predicting the anomeric outcome. A preference for β -glycosylamine formation is illustrated in the reduction of 2,3,4-tri-*O*-acetyl- β -D-xylopyranosyl azide,^{5,189} 2,3,4,6-tetra-*O*-pivaloyl- β -D-galactopyranosyl azide,^{16,125} 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)- β -D-glucopyranosyl azide⁸¹ and the “arabino sialyl Lewis^x azide” **200**¹⁹⁰ (Raney-Ni), 2-acetamido-6-*O*-acetyl-2-deoxy- β -D-glucopyranosyl azide,¹⁹¹ hepta-*O*-acetylchitobiosyl azide,⁴⁴ 2,3,4,6-tetra-*O*-benzyl- β -D-glucopyranosyl azide,¹⁹² and 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl azide (**11a**). The reduction of the α -manno azides **11** is accompanied by anomerization^{23,193} (see also Ref. 76). Such anomerizations can be misleading in attempts to deduce the configuration of the azide from that of the reduction product.^{32–34} The benzyl derivative **201** was prepared by hydrogenation of the corresponding 2,3,4,6-tetra-*O*-benzyl- β -D-mannopyranosyl azide in the presence of Lindlar catalyst, but when the anomeric α -azide was subjected^{127,194} to similar reaction conditions, rapid anomerization took place, affording **201** as the sole product.¹⁹⁵ However, hydrogenation of 2,3,4,6-tetra-*O*-*tert*-butyldimethylsilyl- α -D-mannopyranosyl azide (**202**) with H₂/Pd-C gives mainly the α anomer, with **203**:**204** = 2:1.¹⁹⁶



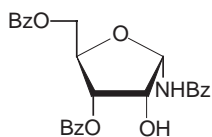
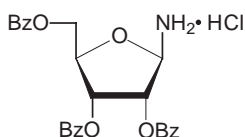
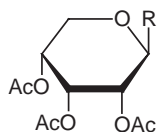
R = TBDMS

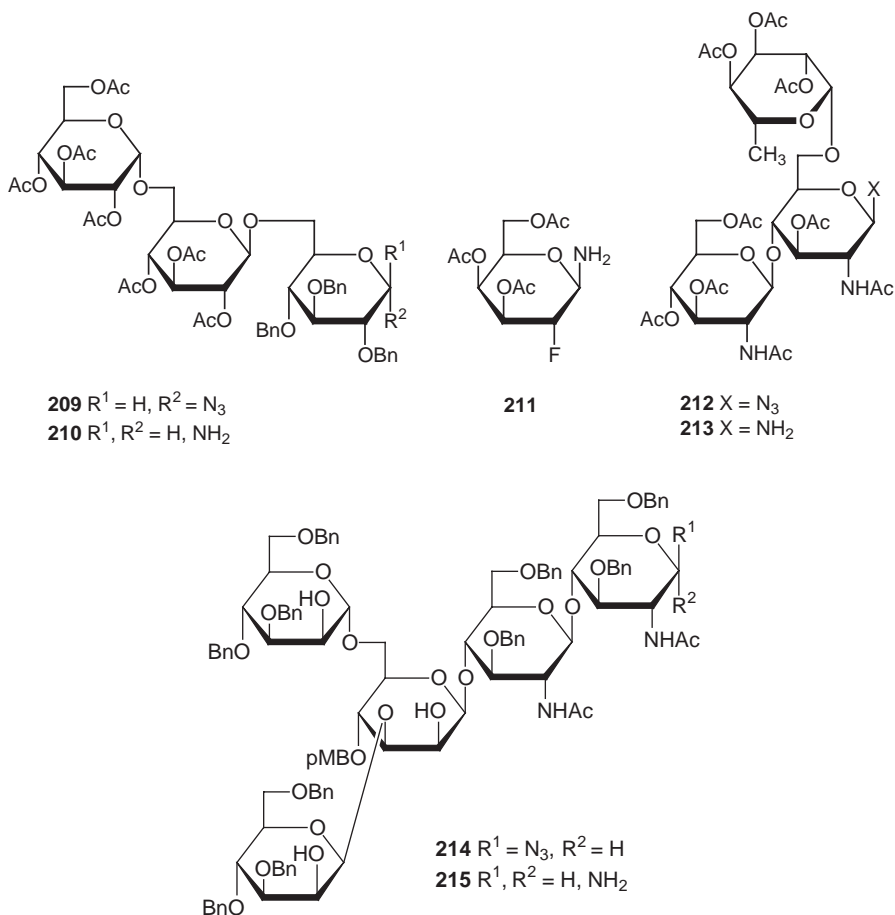
202 R¹ = N₃, R² = H

203 R¹ = NH₂, R² = H

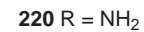
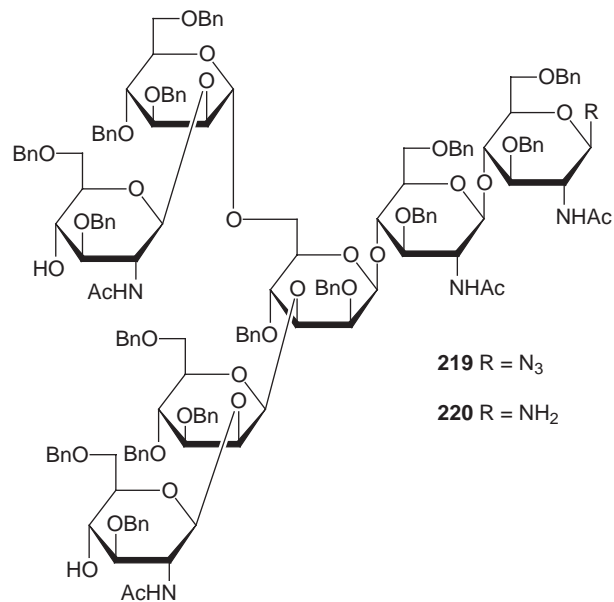
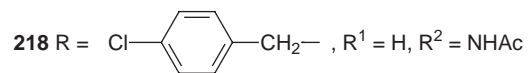
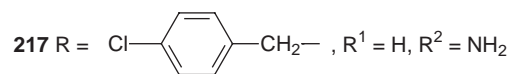
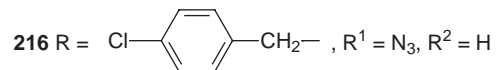
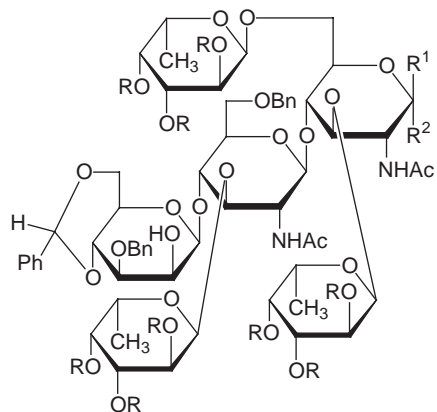
204 R¹ = H, R² = NH₂

Reduction¹⁹⁷ of tri-*O*-benzoyl- β -D-ribofuranosyl azide (**70**) was accompanied by *O* \rightarrow *N* acyl migration and anomerization, yielding the α amide **205** (the desired amine HCl salt **206** could be obtained by precipitation), and the reduction of 2,3,4-tri-*O*-acetyl- β -D-ribofuranosyl azide (**207**) yielded a mixture of products from which the *O,N*-peracetate **208** was isolated (see also Ref. 95). To obtain an important azido disaccharide, chitobiosyl azide, various *O*-protecting groups, and hydrogenation conditions have been studied.^{198–200} In none of these reactions could anomerization be entirely prevented, and the best means found for reduction was by Raney nickel in ethanol, to give β : α = 91:9. Reduction with Lindlar catalyst of the α -azido trisaccharide **209** gave (2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 \rightarrow 6)-(2,3,4-tri-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 6)-2,3-di-*O*-benzyl- α,β -D-glucopyranosylamine (**210**) (α : β = 5:1), and even the product of peracetylation could not be separated into the desired anomers.²⁰¹ In the hydrogenation of 2,3,4,6-tetra-*O*-benzyl- α -D-glucopyranosyl azide with Lindlar catalyst, the reaction could be stopped at the stage of pure α amine without any further cleavage of benzyl groups or anomerization.¹⁸¹ Subsequent reports on the reduction of azido disaccharides with H_2/PtO_2 gave less-convincing results.^{202,203} Some 1,2-*trans* glycosaminyl azides could be satisfactorily reduced to give the corresponding amines in good yields,^{21,204} as in the reduction of the 1,2-*trans* 2-deoxy-2-fluoro azide **154** with Adams catalyst to give the β amine **211**.²⁰⁵ In relation to the synthesis of glycoproteins and neoglycoproteins, reduction of the fucosylated azide **212** to give **213** was a key step, which could be effected with Raney nickel in 95% yield.²⁰⁶ The pentasaccharide **214** was hydrogenated with the same catalyst, but this resulted in the formation of an anomeric mixture **215**.²⁰⁷ At this stage the overall picture appears ambiguous in judging the outcome of catalytic hydrogenation of oligosaccharides having a combination of different protecting groups.

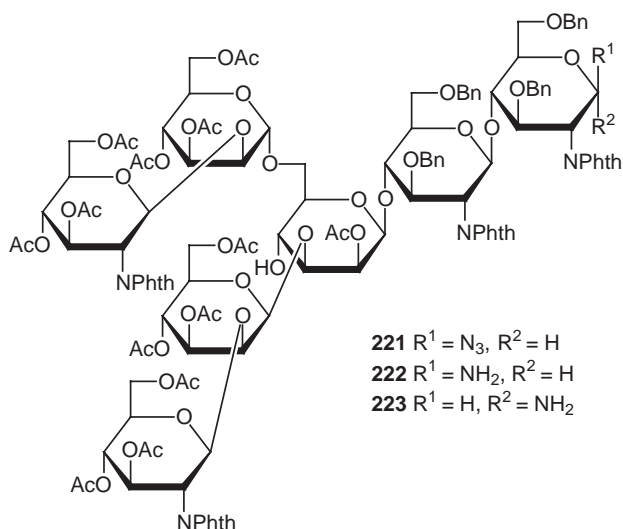
**205****206****207** R = N_3 **208** R = NHAc



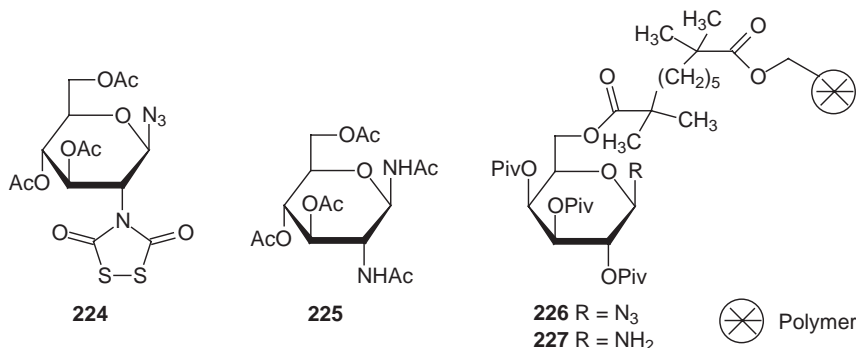
Swedish authors²⁰⁸ prepared a trifucosylated N-linked hexasaccharide component of a glycoprotein from *Haemonchus contortus*, and selected *p*-chlorobenzyl as the protecting group. Catalytic hydrogenation with $Pd(OH)_2$ of the azide **216** surprisingly gave the 1,2-*cis* amine **217**, obtained and characterized as the α -acetamide **218**. In most examples studied, the various azides were precursors to be reduced to the corresponding amines, which were required in the synthesis of N-glycoproteins and -peptides. (For further examples see Refs. 209–220.) The *O*-benzyl-*N*-acetyl-protected 1,2-*trans* azido heptasaccharide **219** could be reduced with Lindlar catalyst to give the key compound **220** without anomerization.²¹¹ A detailed review covering this particular research and further aspects has appeared.²²¹



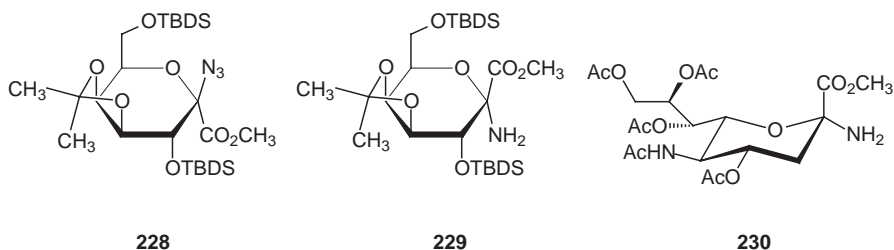
A later-developed method to obtain amines from azides consists in their reduction with propane-1,3-dithiol,^{9,222–225} employing ethyldiisopropylamine as a base. The transformations reported proceeded rapidly and resulted in 1,2-*trans* selectivity. Unverzagt²²³ described the reduction of the azido heptasaccharide **221** by this dithiol method, to give exclusively the β amine **222**. In contrast, reduction of **221** by Raney nickel resulted in both anomers **222** and **223**, with $\beta:\alpha = 7:3$. A corresponding reduction of the azido octasaccharide gave a 52% yield;²²⁶ however, a low yield of 35% was observed in the reduction of the thio sugar azide **68** with propane-1,3-dithiol.⁹⁰

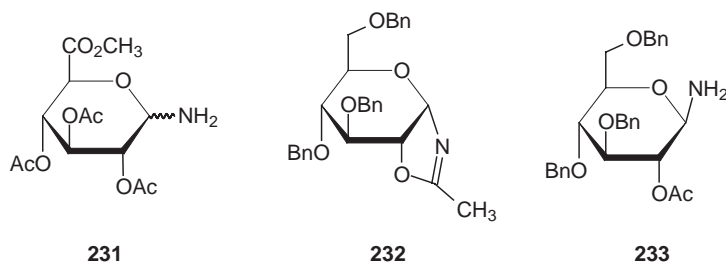


An extensive study revealed that the *N*-dithiasuccinyl-protected azide **224** offers a major advantage in the synthesis of *N*-glycans.²²⁵ Efficient reduction of the *N*-dithiasuccinyl- and azido-functionality in **224** could be achieved, either in solution by utilizing simultaneous *in situ* reduction with Zn in THF–AcOH–Ac₂O, or on solid phase upon treatment with ethyldiisopropylamine and an excess of dithiothreitol, propane-1,3-dithiol, or 2-mercapto-*N*-methylacetamide leading²²² to the known **1** or **225**.



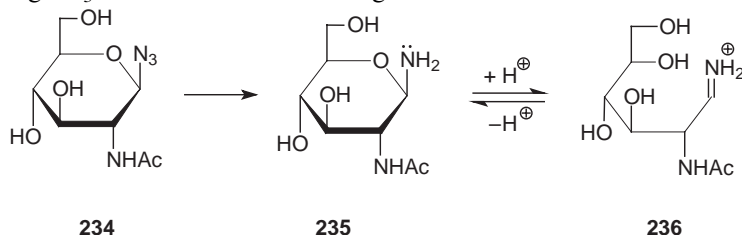
Reduction of the polymer-linked 1,2-*trans* azide **226** with complex hydrides was not successful. Under the conditions of the Staudinger reaction (see Section III.3), extensive anomerization occurred. However, the reaction of **225** with five equivalents of propane-1,3-dithiol and triethylamine in DMF proved efficient for the formation of the solid-phase-linked amine **227**. This reduction proceeded quantitatively without anomerization.²²⁴ Glycosyl azides with a quaternary anomeric center were also treated under these reductive conditions; however, the results are not unambiguous.^{28,111} Reduction of methyl 2-azido-3,7-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-4,5-*O*-isopropylidene- β -D-galacto-2-heptulopyranosonate²⁸ (**228**) under Pd/C catalysis with hydrogen in methanol gave the α -galacto amine **229** in 46% isolated yield; however, the product obtained was not pure. Reduction of the α -azido neuraminic acid ester **194**^{56,108} following the same method²²⁷ gave the amine **230** with exclusive retention of anomeric configuration. Reduction of the azido uronic acid ester **61** performed with H₂/PtO₂²²⁸ yielded the anomeric mixture of amines **231**, with the highest β : α ratio of 4:1 at -15°C .

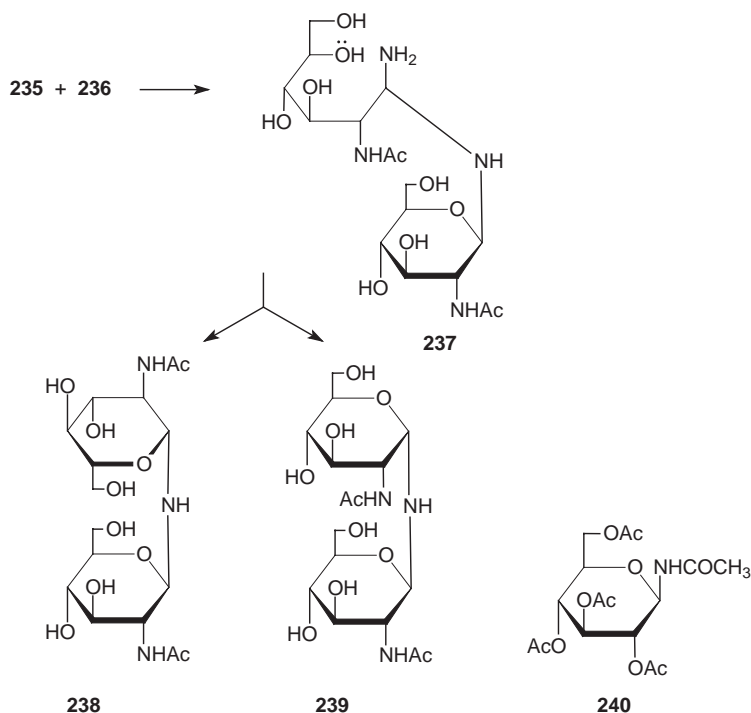




Anomerization occurs during the preparation of α -D-glucopyranosylamine and its 6-*O*-glycosides via hydrogenation of the appropriate azides;^{180,209,229} the 1,2-*trans* anomers are formed no matter which protective groups and catalysts are used.^{209,229} The anomeric amine group favors the equatorial orientation, indicating operation of the reverse anomeric effect^{189,192} (see also Ref. 230). It is noteworthy that the formation of a β amine (233) in the reaction of oxazoline²³¹ 232 may be attributed to the stronger reverse anomeric effect of a protonated amino group.^{180,232}

In addition to anomerization, dimer formation with the elimination of ammonia is frequently observed in these reactions. For instance, formation of the diglycosylamines 238 and 239 was observed^{17,18,20,21,233,234} during synthesis of the amine 235, an important intermediate in the synthesis of glycopeptides. A probable mechanism for dimer formation¹⁹ is shown in schemes 234–239. Initially, the resulting amine 235 in methanol is converted into the acyclic immonium intermediate 236, which then reacts with a second molecule of 234 to give the intermediate 237. The latter undergoes ring closure, with elimination of the amino group at the anomeric carbon atom as ammonia, giving the β,β and α,β dimers 238 and 239. For formation of other dimers see Refs. 235–236. A combined reagent of acetic anhydride and chlorotrimethylsilane may be used for transformation of 119 into the acetamide 240.²³⁷ Miethchen *et al.* were able to realize the radical reduction of glycosyl azides in average to good yields by employing Bu₃SnH and AIBN and long-reaction times.²³⁸



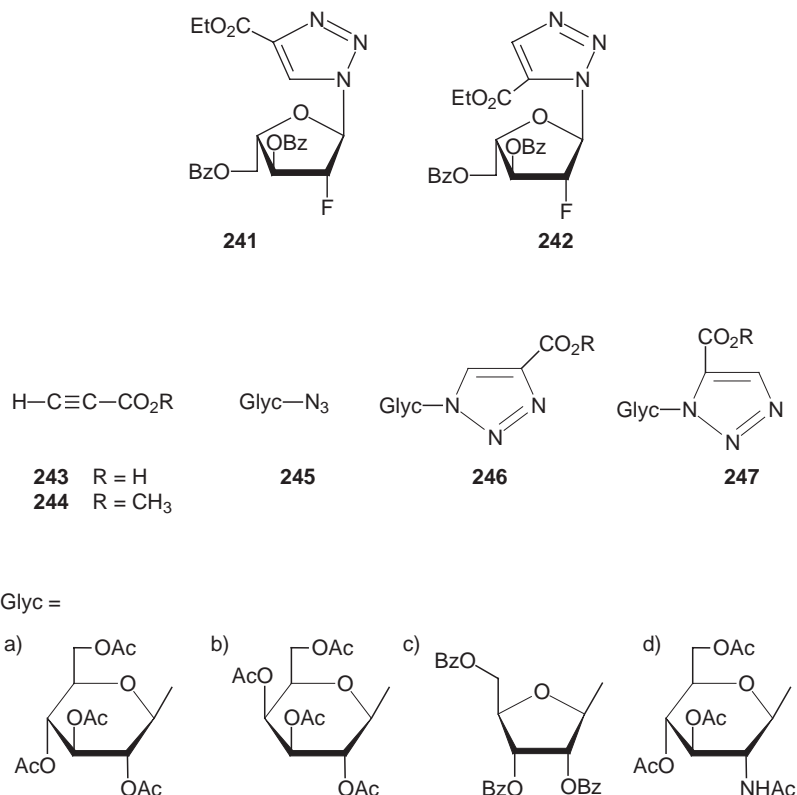


The Ru(III)-promoted formation of the amide bond permits the synthesis of amides^{239,240} from azides and thio acids at room temperature, and the reaction is applicable to less-reactive azides. Thus the azide, **119** as a model compound, in methanol solution, was transformed in 80% yield into the corresponding acetamide **240** by the action of thioacetic acid (2.5 eq.) and 2,6-lutidine.²⁴⁰

2. [1,3]-Cycloaddition Reactions of Glycosyl Azides

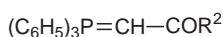
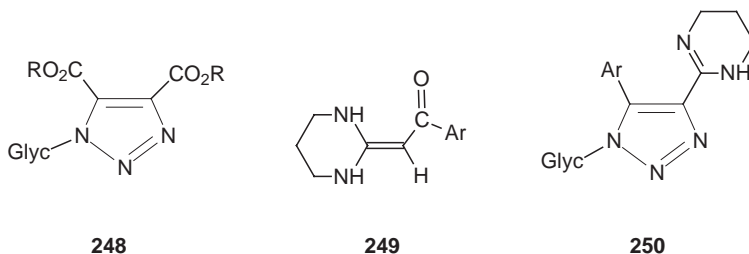
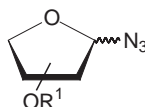
The 1,3-dipolar character of the azido group has been previously exploited^{1,2} for [2 + 3]-cycloaddition reactions of glycosyl azides with compounds containing triple bonds. It is known that formation of 1,4-disubstituted 1,2,3-triazoles is favored over the 1,5-disubstituted ones. Motivated mainly by pharmacological considerations (in order to obtain compounds with cytostatic properties), syntheses of a great number of 1-*N*-glycosyl-1,2,3-triazole derivatives have been reported.^{78,92,94,98,189,241–265}

Addition of ethyl propiolate to furanosyl azide **3** in toluene gives **241**²⁴ as the prevailing cycloadduct, which can be readily separated from the regio-isomer **242** (ratio 23:10). 1,2-*trans* Glycopyranosyl or glycofuranosyl azides (**245**) were treated with methyl propiolate (**244**) or propiolic acid (**243**) to yield a pair of isomeric 1,2,3-triazoles²⁴⁴ (**246** and **247**).



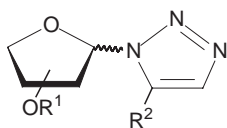
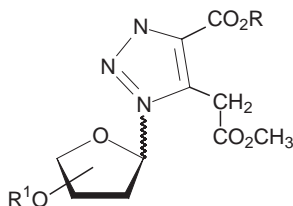
At elevated temperature, the azides **245** react with acetylenedicarbonic esters leading to 4,5-dicarboxylic acid esters (**248**).^{189,254,260} Dipolar additions to azide **119** were performed with ketene aminals, for instance, with the ketene aminal **249**, acetylated 4,5-disubstituted 1,2,3-triazole glycosides (**250**) resulted. The synthesis of 5-substituted α - or β -pentofuranosyl 1,2,3-triazole derivatives (**252**) via 1,3-dipolar cycloadditions of the β -oxoalkylidenephosphorane **251** to furanosyl azides (**245**) with the elimination of triphenylphosphine oxide has also been described.^{92,253} The cyclization and subsequent rearrangement of azides

with cyanoacetamide according to Dimroth,^{250,254,256} resulting in the formation of N³-glycosyl derivatives of 1,2,3-triazolo[4,5-*d*]pyrimidin-7-ones, has already been discussed. These studies were subsequently extended by treating a series of pyranosyl and furanosyl azides (**252**) with dimethyl 3-oxopentanedioate under K₂CO₃ catalysis in Me₂SO.²⁵⁸ According to these results, no anomerization occurred (compare the previously reported rearrangement and Dimroth conditions^{250,254,256}) and the products had the structure **254**.

**251****252**

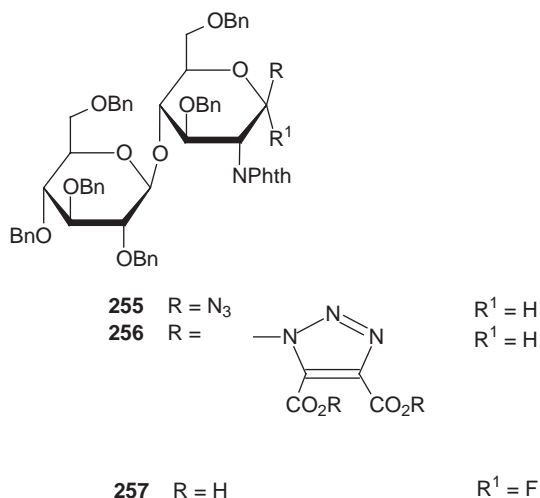
R¹ = Bz

R² = H, CH₃, CH₂Hal, CO₂Alkyl

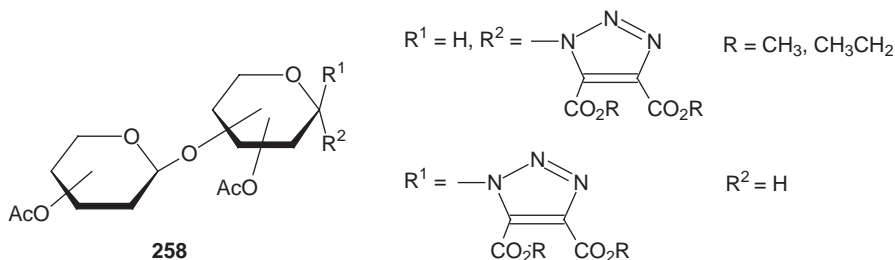
**253****254**

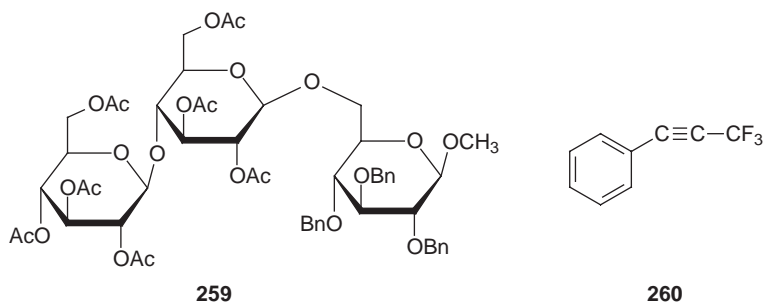
1-*N*-Glycosyl-1,2,3-triazoles react with HF in pyridine to give glycosyl fluorides, which, as shown by Kunz *et al.*, can be employed for oligosaccharide synthesis.^{257,266} This is exemplified for the protected lactosaminyl azide **255**,

where the cycloaddition product **256** and HF react to give the fluoride precursor (**257**) of **255**.

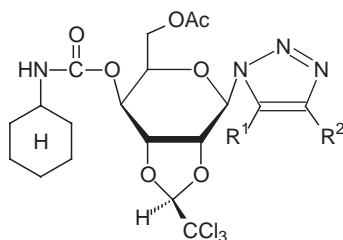


1,3-Dipolar cycloaddition of the acetylated 1,2-*trans* and 1,2-*cis* cellobiosyl, lactosyl, maltosyl, and melibiosyl azides with various acetylenedicarboxylic acid esters gives the corresponding 1-*N*-glycobiosyl-1,2,3-triazoles, which have been used as glycosyl donors for the synthesis of glycosides of 1,2-*trans* glycobioses.²⁶⁷ According to these studies, the configuration of the resulting glycosyl triazoles (**258** α and **258** β) obtained by [3+2] Huisgen cyclization is inconsequential en route to the glycosylation product, because exclusive formation of the 1,2-*trans* product [such as methyl (2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-(2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl)-(1 \rightarrow 6)-tri-*O*-benzyl- β -D-glucopyranoside, **259**] was observed. Reaction of the aglycon with 1-*N*-glycosyl-1,2,3-triazoles (**258**) proceeds fast at room temperature with Me_3SiOTf catalyst.



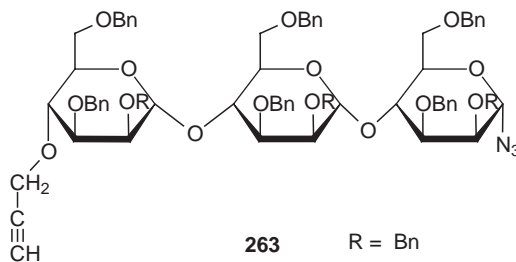


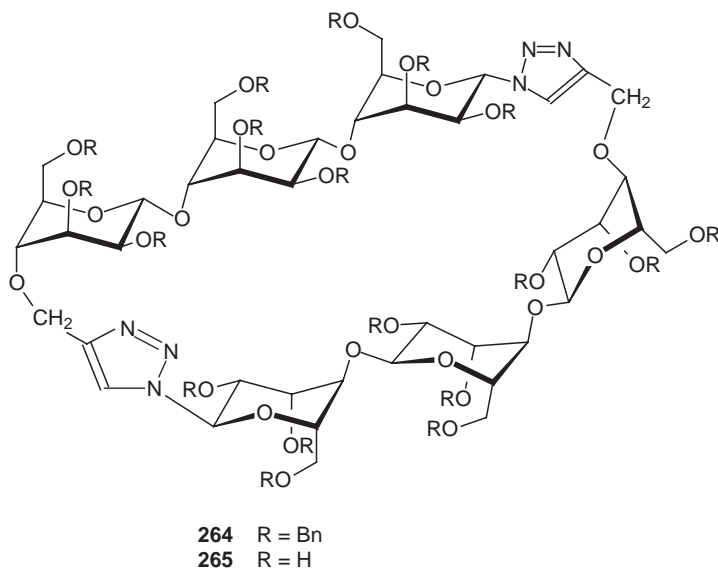
Cycloaddition of specially substituted glycosyl azides is of interest. Miethchen *et al.*²⁶¹ used 3,3,3-trifluoropropynylbenzene (**260**) with a protected glycosyl azide and isolated both regioisomeric 1-(β -D-gulopyranosyl)-1,2,3-triazole derivatives **261** and **262**.



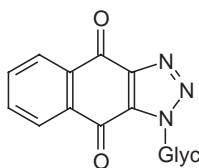
		Yield/%
261	$R^1 = C_6H_5, R^2 = CF_3$	47
262	$R^1 = CF_3, R^2 = C_6H_5$	31

A remarkable intermolecular cycloaddition has been reported.¹⁴⁰ The α -trisaccharide azide **263**, obtained by the trimethylsilyl method and carrying a suitable propynyl function at C-4''' was treated under CuI–DBU-catalyzed cyclization conditions. The cyclodextrin-resembling cyclodimer **264** was obtained as the main product in 80% yield, hydrogenolysis of which gave the water-soluble cyclodextrin analog **265**. The formation of a corresponding cyclotrimer in 15% yield was also observed.





Dipolar cycloaddition of glycosyl azides **245** to 1,4-naphthoquinones has been observed. At room temperature, the 1-glycosylnaphtho[2,3-*d*]triazole-4,9-dione (**266**) was formed selectively in low yield; at elevated temperatures decomposition of the cycloadduct took place²⁶⁸ and several transformation products of **266** were identified.

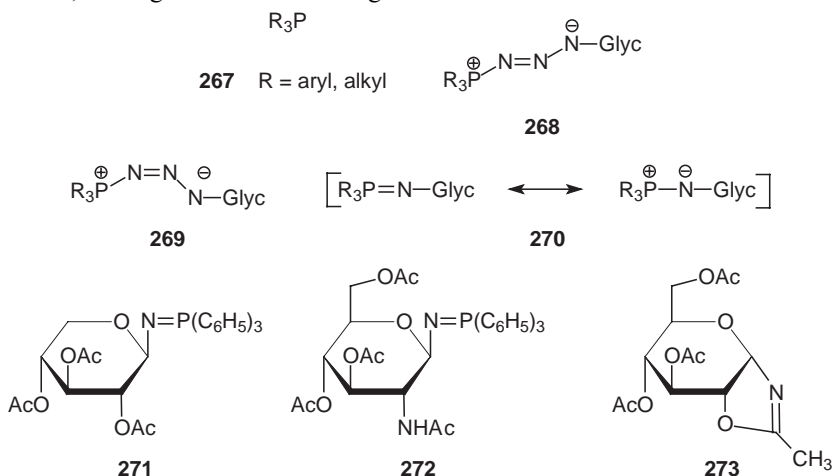


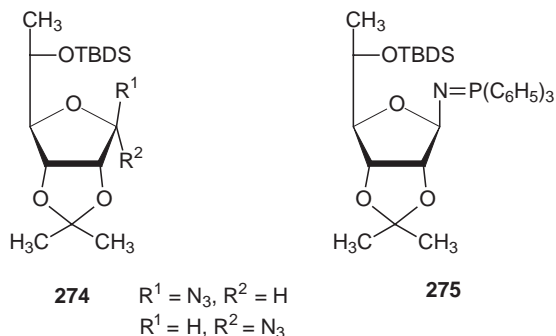
266

Spanish authors^{268a} added a glycosyl azide to benzyne and obtained the *N*-1-glycosyl derivative of benzotriazole. In an interesting extension, the incorporation of oligosaccharides into [60]fullerene via cycloaddition was described by researchers of Nagoya University. Cycloaddition of acetylated glycopyranosyl azides (galacto-, gluco-, lacto-, malto-, and maltotriosyl) in boiling chlorobenzene gave a mixture of two inseparable *N*-glycopyranosyl [5,6]-azafulleroid isomers in moderate yield.²⁶⁹

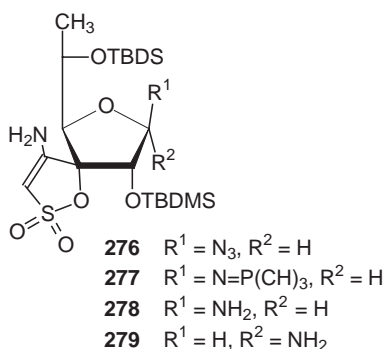
3. Reaction of Glycosyl Azides with Phosphines, Leading to Amines, Amides, and Schiff Bases

The Staudinger reaction^{270–272} of protected glycosyl azides with triarylphosphines, leading to glycosylphosphinimines (iminophosphoranes, iminophosphines, λ^5 -phosphazenes) has found widespread use.^{189,273,274} This reaction of azides with such trivalent phosphorus compounds as phosphines and phosphites has been of major impact on the transformations and characterization of this class of compounds, and has had important applications in carbohydrate chemistry. The reaction of azides with triaryl or trialkyl phosphines and phosphites proceeds stepwise, as indicated in the accompanying schemes. First, the azide **245** and phosphine **267** give a phosphazide (triazaphosphadiene) **268**, preferentially in the *cis* form **269**. This intermediate undergoes expulsion of nitrogen to give the iminophosphorane **270**, a phosphorus–nitrogen ylide. Reports indicate that in case of triarylphosphines (**267**), the inductive effect of the pyranose ring contributes to the stabilization of the negative charge at the anomeric nitrogen atom.²⁷⁵ The few reactions performed at room temperature gave such phosphinimines as 2,3,4-tri-*O*-acetyl- β -D-xylopyranosylphosphineimine (**271**),¹⁸⁹ the hydrochloride salt of which is also stable. The same applies to amino sugar derivative **272**,²⁷⁶ obtained from azide **1**; again the perchlorate salt of **272** is crystalline. Heating the phosphinimine **272**⁸² gives the well-known glucopyranoxazoline **273**. Another transformation with a non-acylated starting material results in anomerization: both the α - and β -azides **274** yield the same phosphinimine **275**, having the α -L-*talo* configuration.⁹⁴



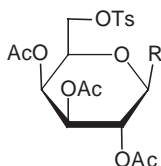


In some instances, unprotected glycosyl azides are also suitable²⁷⁷ precursors for phosphinimines which, in contrast to vicinal azidoalcohols,^{278–281} do not cyclize to oxazaphospholidines but are converted into glycosylamines by treatment according to the Zemplén²⁷⁴ condition or by using ammonia.^{99,282} Because of their ylide structure, where the nitrogen atom bears a negative charge, the phosphinimines show a marked anomeric effect, as demonstrated¹⁸⁹ with various acetylated pentopyranosyl derivatives. As a consequence of their ylide character, the reactions of phosphinimines often result in anomeric mixtures and/or isomerized products.^{283–285} The bicyclic 1,2-*trans* ribo azide **276** with trimethylphosphine gives a phosphinimine **277**, which reacts in turn with ammonia to give mainly the glycosylamine **278**; the α anomer **279** was not characterized.²⁸⁵

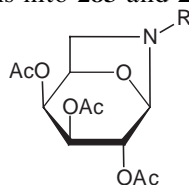


Phosphinimines obtained from 1,2-*trans* 6-*O*-*p*-tolylsulfonylhexopyranosyl azides rearrange to 6-amino-1,6-anhydro-6-deoxyhexoses, which are stable as acylated derivatives.^{68,286} As intermediates, the tosylate salts could be

isolated.²⁸⁶ The 6-tosylated phosphinimine **281** derived from the β -galacto azide **280** cyclizes in solution and the resulting amorphous 6-amino-1,6-anhydro compound **282** was identified with conversions into **283** and **284**.

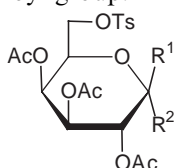


280 $R = N_3$
281 $R = N=P(C_6H_5)_3$

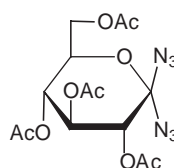


282 $R = \overset{\oplus}{P}(C_6H_5)_3 TsO^\ominus$
283 $R = H$
284 $R = AC$

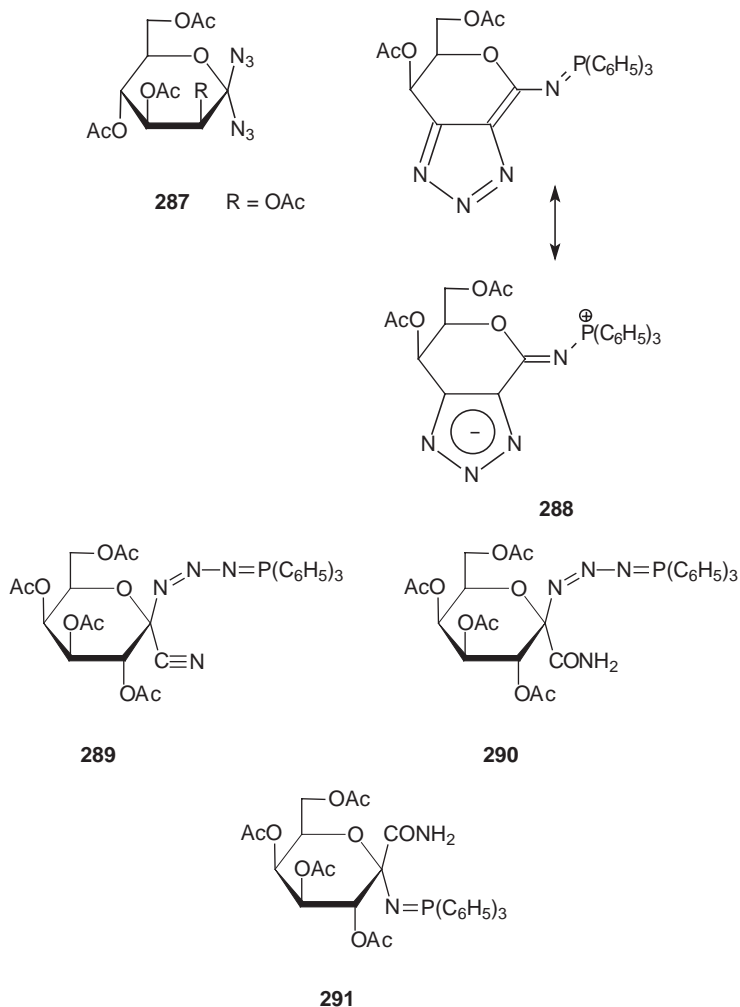
The anomeric phosphazides (**285** α and β) prepared employing tris(dimethylamino)phosphine were structurally identified in solution.²⁸⁷ The Staudinger reaction of glycopyranosylidene 1,1-diazides (**48–50**) led to resonance-stabilized iminophosphoranes of 6,7-dihydro[3,4-*d*]triazole.²⁸⁸ This transformation involves β -elimination of acetic acid and cycloaddition of azide ion to the resulting 2,3-double bond. The proposed mechanism was supported by the finding that both the gluco (**286**) and the manno starting material (**287**) afford the same chiral heterobicycle **288** on treatment with triphenylphosphine. A corresponding fragmentation reaction had been previously observed in the reaction of (1*R*)-2,3,4,6-tetra-*O*-acetyl-1-azido-D-galactopyranosyl cyanide **40** with triphenylphosphine.²⁸⁹ In this example, the first step consists the formation of the crystalline 1-(3-triphenylphosphazido)- β -D-*galacto*-hept-2-ulopyranonitrile (**289**), which also forms a salt with perchloric acid. In case of the carboxamido azide **42**, the resultant phosphinimine was obtained as anomeric mixture. Both anomers (**290** and **291**) are stable in the neat form, but in chloroform solution they anomerize to give a 15:85 mixture. The preponderance of the α anomer (**291**) in the equilibrium mixture may be explained by the strong anomeric effect of the phosphinimino group¹⁸⁹ and the reverse anomeric effect of the carbamoyl group.²⁹⁰



285 $R^1 = H, R^2 = N=N-N=P(NMe_2)_3$
 $R^1 = N=N-N=P(NMe_2)_3, R^2 = H$

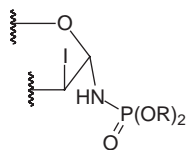
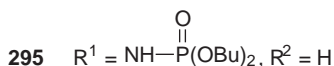
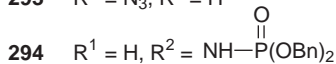
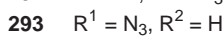
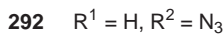
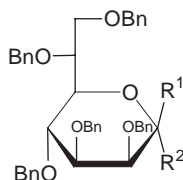


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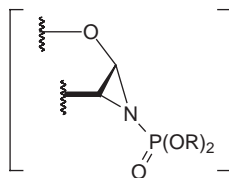
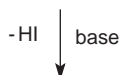


The reaction of glycosyl azides with phosphates has proved less popular, but it was first studied with acetylated pentopyranosyl azides¹⁸⁹ and the products are glycosyl phosphoramidates (glycosyl amidophosphates). Paulsen *et al.*²⁹¹ demonstrated this transformation with *D-manno*-heptose derivatives. The α -azide **292** and triphenylphosphite give the phosphoramidate **294**, and the β anomer **293** with tributylphosphite leads to β -dibutyl (2,3,4,6,7-penta-*O*-benzyl-*D*-glycero- β -*D-manno*-heptopyranosylamido)phosphate (**295**). Additional isosteres of glycosyl phosphates were later reported to be the first access to this group of

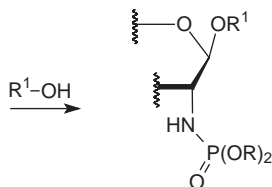
compounds from glycosyl azides and trimethyl phosphite.²⁹² The mild conditions used in these reactions have been applied to the 2-deoxy-2-halo azides **141–148** and it was found¹⁵⁷ that the 2-iodo phosphoramidates **296** obtained can be readily isolated as well-characterized compounds. These proved to be useful starting materials, since their reaction with alcohols in the presence of base leads, via *N*-aziridinophosphoric esters (**297**), to the formation of 1,2-*trans* 2-deoxy-2-phosphoramido-glycopyranosides (**298**).^{157,158,293,294}



296

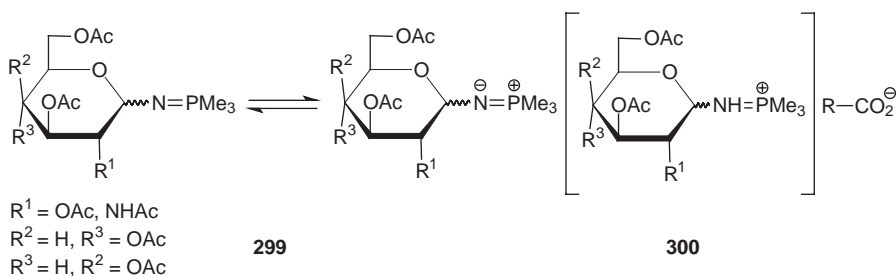


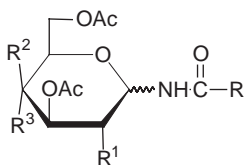
297



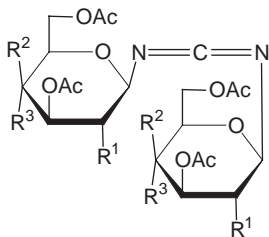
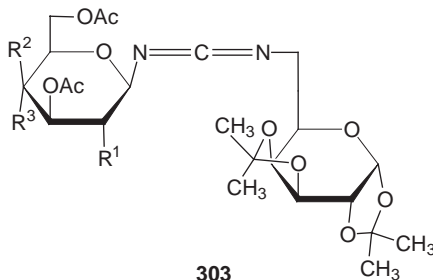
298

The most important transformations of glycosyl phosphinimines are their behavior on acylation. Depending on the structure of the $P=N$ component (phosphazide or phosphinimine) derived from the azide and the trivalent phosphorus compound, various modes of acylation may occur, as described by Kosower *et al.*²⁹⁵ The results are partially contradictory: the acylation of phosphinimines is dependent on the acid derivative used, the properties of the phosphine, and the reaction temperature. Formation of the amide from an azide, a phosphine, and an activated acid derivative is presumed to proceed in the so-called Staudinger reaction,^{270–272} when first a triazaphosphadiene intermediate,²⁹⁵ and then a *P*-triaryl/(alkyl)-iminophosphorane is produced. The iminophosphorane reacts with an acyl chloride or anhydride to yield an iminophosphonium salt, which then forms the oxazaphosphetane. The latter undergoes an electrocyclic fragmentation to form the phosphine oxide and chloroimines and (E)-(Z)-chloroimines which are hydrolyzed to the acylamido compound. This set of reactions constitutes, according to Kosower,²⁹⁵ the iminophosphorane pathway (for details see Refs. 287 and 295). In practice, glycosyl azides are transformed into the intermediate product employing triphenylphosphine,^{94,305} 2-diphenylphosphino phenyl acetate,¹³⁸ tributylphosphine,^{297–300,301,303} triethylphosphine,^{296,301,304} or trimethylphosphine.^{287,306} The intermediate in turn was acylated with anhydrides,^{94,298,299} acid chlorides,^{297,302,305} acids,^{287,304} or protected amino acids^{287,296,300,301,303,306} (see also Ref. 138). In this context, trimethylphosphine has twofold advantages, because it furnishes the smallest phosphinimino group and the resulting trimethylphosphine oxide can be removed by aqueous extraction. Starting with anomerically pure trimethylphosphinimines (**299**) with the gluco, galacto, or 2-acetamido-2-deoxy-gluco configurations, the reaction with acid RCO_2H gives the corresponding salt **300** which is cleaved to Me_3PO and acylamide **301**. With regard to anomerization and its suppression, see Refs. 138 and 287.



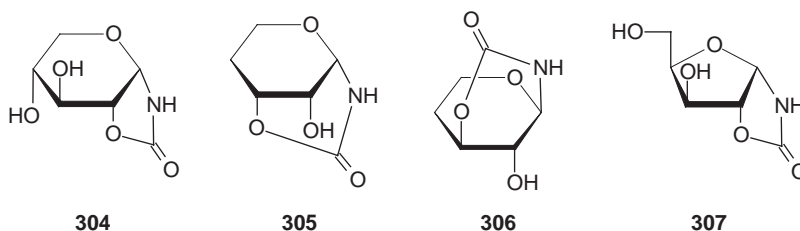
**301**

A further use of the phosphinimines **299** is in the synthesis of Schiff bases or their cyclic tautomers. Glycosylphosphinimines are suitable starting material for the formation of various glycosylated carbodiimide and cyanamide derivatives. It is well known that phosphinimines of the general structure **271** do not react with CO_2 or CS_2 to give iso(thio)cyanates, because additional **271** would react further to give glycosyl carbodiimides.^{273,276,283} Thus, the sparingly soluble bis(2-acetamido-3,4,6-tri-*O*-acetyl- β -D-glucopyranosyl)carbodiimide (**302**) can be readily obtained,²⁸⁴ and corresponding syntheses of unsymmetrically structured compounds were recorded.^{275,284} It is also possible for **271** to react with glycopyranosyl isothiocyanates to give other unsymmetrical derivatives such as **303**, in which the carbodiimide carries two different carbohydrate residues.³⁰⁷ In this synthesis of carbodiimides, use of the trimethyl derivative **299** was advantageous. Its reaction with CS_2 in dichloromethane at room temperature leads to the rapid formation of the symmetrical carbodiimide of the type **302**, and the liquid phosphine oxide is readily removed.³⁰⁸

**302****303**

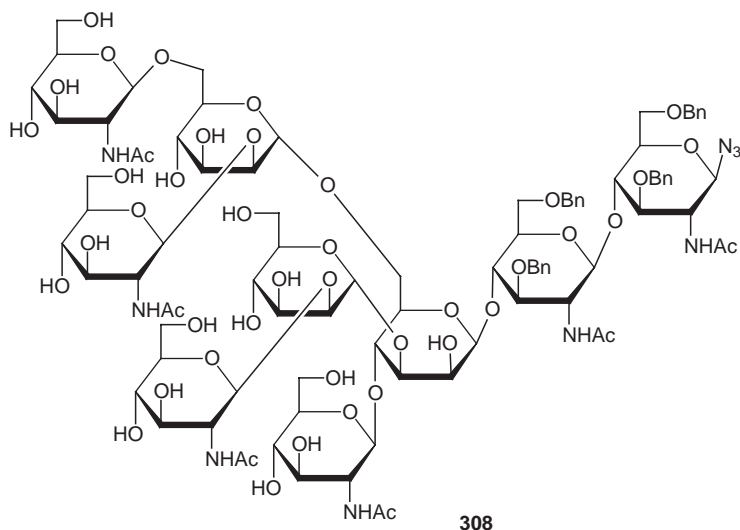
Unprotected glycosylphosphinimines^{276,277,309} cannot be transformed with CO_2 into the corresponding carbodiimides. Depending on the stereochemistry of the glycosyl azide as starting material, the intermediate carbodiimide is able to react with sterically favored hydroxyl groups to give cyclic glycofuranosyl or

pyranosyl carbonates. Thus, in the reaction of α -D-xylopyranosyl azide¹⁸³ with triphenylphosphine and CO₂, the products **304–307** could be isolated.³⁰⁹ An isocyanate intermediate is assumed to be responsible for the inversion products, for instance in the formation of the 1-*N*,3-*O*-carbonyl- α -D-ribofuranosylamine **305** (see a related PM3 semiempirical quantum chemical computation study³¹⁰).



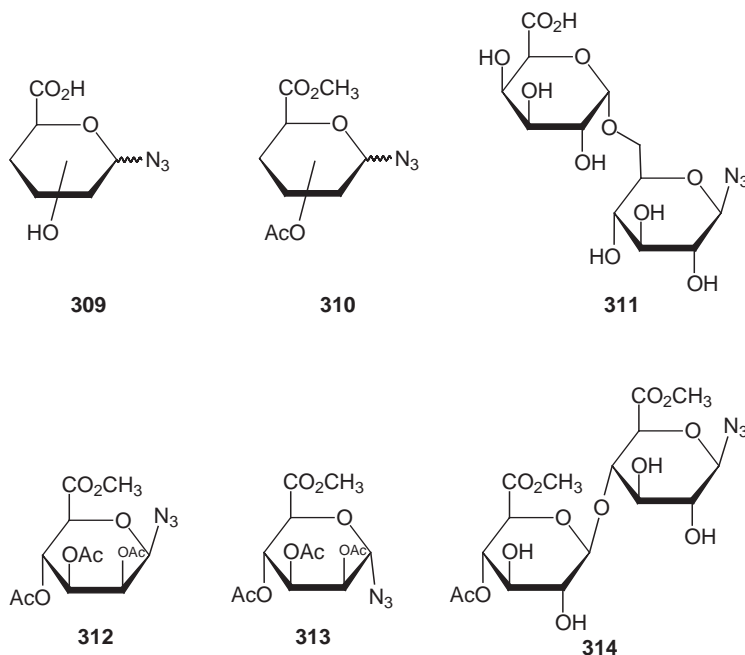
4. Unprotected and Partially Protected Glycosyl Azides as Starting Materials for *N*-Glycans

Glycosyl azides can, in general, be subjected to standard protection–deprotection operations commonly used in carbohydrate chemistry. Unprotected, free hydroxylated glycosyl azides are generally obtained from their acylated derivatives by Zemplén deacylation;^{1,9,31,39,68,69,75,180,203,311} in some cases ammonia,²² guanidine,²⁰⁸ or triethylamine^{19,312} are used. Stronger bases may effect loss of the azido function, as shown by the formation of 1,6-anhydro- β -D-glucopyranose from β -D-glucopyranosyl azide (**38**) under the action of barium hydroxide.¹ Various protecting derivatives, such as benzylidene,^{9,203,274,313} *p*-methoxybenzylidene,^{47,208,253} isopropylidene,^{39,68,314,315} 6-*O*-trityl,^{202,212,316} partially acylated,^{39,47,191,216,225,273,311} and partially^{201,208,253,313} or fully benzylated^{49,181,192,317} glycosyl azides have been synthesized for use in the synthesis of *N*-glycans^{52,76,80,105,112–114,128,165,182,190,191,201–203,207–213,216–218,226,294,311–313,316–323} (see also Ref. 128). These syntheses profit from the fact that the azide functions as a quasi-protecting group during all protection and deprotection steps in glycosylation synthesis. Currently, the highly branched non-asaccharide derivative **308** constitutes the glycosyl azide with most sugar residues.³²²

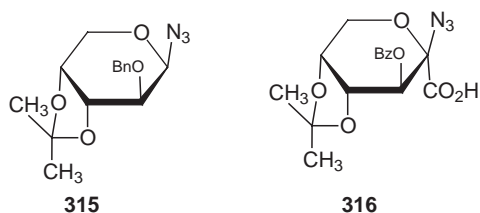


5. Oxidation of Glycosyl Azides

In unprotected glycosyl azides, the terminal hydroxymethyl group can be oxidized to give modified uronic acids if efficient oxidation procedures, such as the TEMPO-catalyzed oxidation with NaOCl, are employed.⁸⁴ In aqueous sodium hydrogencarbonate, the transformation proceeds at 5 °C sufficiently rapidly to give the azido uronic acid **309**, which in turn can be converted into the methyl ester, and then by acetylation to the peracetylated azido uronic acid ester **310**. As an alternative reagent $\text{Ca}(\text{OCl})_2$ can be employed,³²⁴ and a repeat of the approach¹³⁷ confirmed the reported results. Reversed-phase HPLC permits isolation of such primary reaction products as β -azido melbiouronic acid **311**.¹³⁷ Corresponding reactions have been used for synthesis of the anomeric azido mannouronic esters **312** and **313** in moderate yield.⁸⁴ β -D-Glucopyranosyl azide, and cellobiosyl, maltosyl, and lactosyl azides have been converted into the corresponding uronic acids in good yield by TEMPO-mediated anodic oxidation. The anode proves to be an alternative to other cooxidants in TEMPO-oxidations, and is compatible with azido groups.³²⁵ Schäfer *et al.*³²⁵ prepared the azido diuronic acid dimethyl ester **314** from cellobiosyl azide in 63% yield.

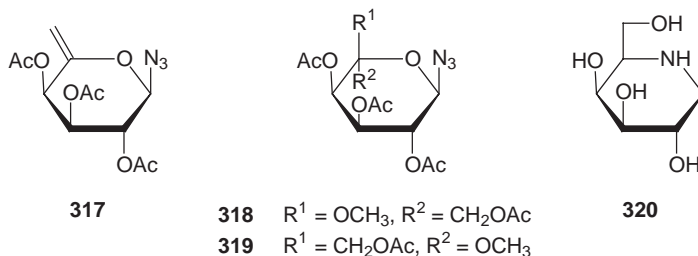


Some glycosyl azides having such stable groups as benzyl^{326,327} have been oxidized with RuCl_3 and NaIO_4 .³²⁸ Evidently, in such cases, oxidation of the hydroxymethyl group to the carboxyl function is accompanied by transformation of the benzyl ethers into benzoates. Thus, 3-*O*-benzyl-3,4-*O*-isopropylidene- β -D-fructopyranosyl azide (**315**) reacted to give the 2-azido-3-*O*-benzoyl-D-fructopyranuronic acid derivative **316**.



Epoxidation of the hex-5-enopyranosyl azide **317**⁷⁵ with *m*-chloroperoxybenzoic acid in methanol led to the formation of 5-*C*-methoxy- α -L-*altro*- (**318**) and - β -D-*galacto* azides (**319**), and their further transformation resulted in

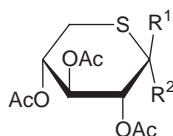
1D-deoxy-galactostatin **320**.³²⁹



6. Photochemical and Thermochemical Formations of Glycosyl Azides

The photochemistry of free and acetylated glycopyranosyl azides has been studied. β -Glucopyranosyl azide (**115**) and α -D-mannopyranosyl azide were found to afford in good yield, on irradiation with UV light, the next lower aldose, D-arabinose. In the case of β -maltosyl azide and β -D-ribofuranosyl azide the formation of an intermediate was observed which, on standing in the dark, reverted back to starting material.⁹

Both α and β anomers of 5-thio-D-xylopyranosyl azide (**321**) lead upon thermolysis (yield 60%) to the tetrahydrothiazepine **322**, which arises from a ring expansion.⁶⁰



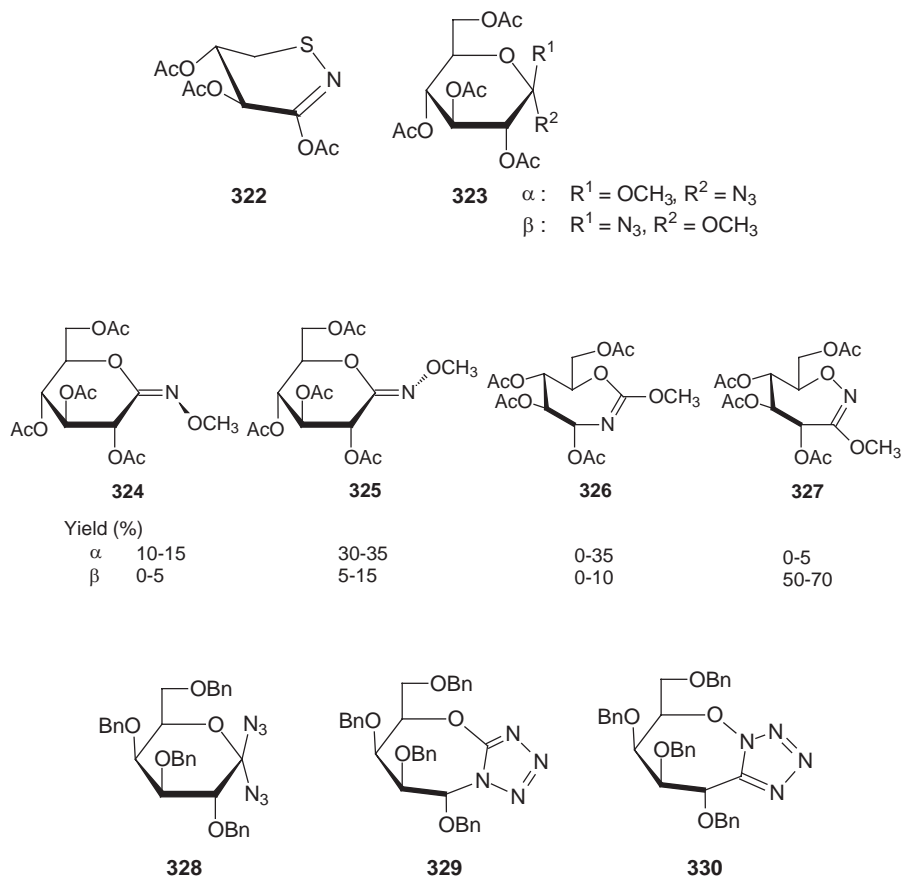
321

α : $R^1 = \text{H}, R^2 = \text{N}_3$

β : $R^1 = \text{N}_3, R^2 = \text{H}$

The photochemical transformations of anomeric diazides, 1-methoxy azides, and cyano azides were studied by French^{123,330–334} and Japanese^{129,130} research groups. A nitrene proved to be the key intermediate. Its stability is dependent on stereochemical and structural features of the molecule, and in particular the anomeric configuration exerts an important influence. Thus for the 1-methoxy azides **323 α** and **323 β** it was shown that both migration of the methoxy group as well as the Beckmann rearrangement proceed differently for the anomers,

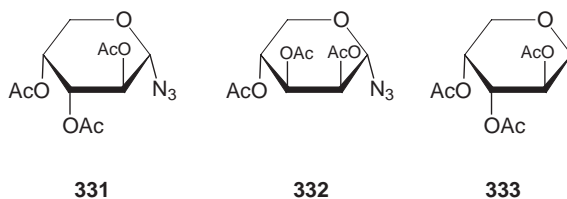
resulting in different product ratios of compounds **324–327**. Thermolysis of the perbenzylated D-galactopyranosylidene diazide **328** afforded¹³⁰ the corresponding 6-oxa-1,5-pentamethylenetetrazole **329** via the sugar azido nitrene, while photolysis of diazide **328** gave compound **329** together with the corresponding 10-oxa-1,5-pentamethylenetetrazole **330**.



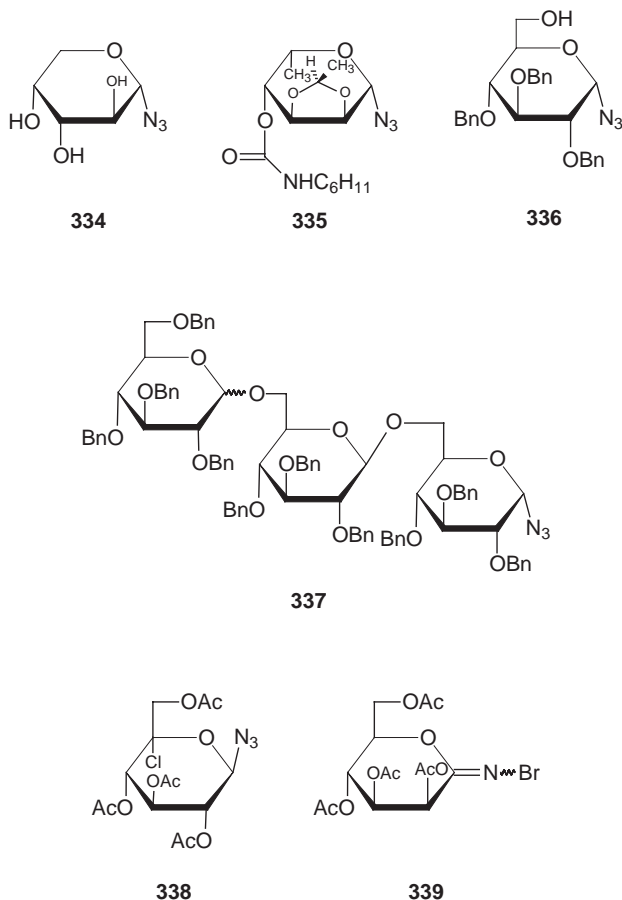
7. Further Reactions of Glycosyl Azides

The reaction of aldopentopyranosyl azides with hydrazine leads to 1,5-anhydrosugars. The initial formation of an intermediate carbanion is presumed, and this picks up a proton to give a deoxy sugar. The chirality at one carbon

atom is lost in this reaction, so that 2,3,4-tri-*O*-acetyl- α -D-arabinopyranosyl azide (**331**) and the α -D-lyxopyranosyl azide **332** yield the same 2,3,4-tri-*O*-acetyl-1,5-anhydro-D-arabinitol (**333**) upon treatment with hydrazine followed by acetylation.³³⁵ Attempts to treat peralkylated glycosides under conditions of the trimethylsilyl azide procedure led to the isolation of peralkylated glyconolactones, and peralkylated glycosyl azides were the presumed intermediates.³³⁶



Glycopyranosyl azides (from D-arabinose, L-fucopyranose, D-mannopyranose, and D-galactose) were epimerized in good yields by heating with chloral and *N,N'*-dicyclohexylcarbodiimide (DCC) in 1,2-dichloroethane. The respective products, epimerized at the C-3 atom, have the D-*lyxo*, L-*gulo*, D-*altro*, and D-*gulo* configurations. The azide function was not attacked by DCC. Employing this transformation, β -D-fucopyranosyl azide (**334**)^{68,69} was converted into 4-*O*-cyclohexylcarbamoyl-6-deoxy-2,3-*O*-(2,2,2-trichloroethylidene)- β -L-gulopyranosyl azide (**335**) as the endo isomer in 70% yield. Glycosylation of 2,3,4-tri-*O*-benzyl- α -D-glucopyranosyl azide (**336**)³¹² with 2,3,4,6-tetra-*O*-benzyl- α -D-glucopyranosyl bromide was successfully accomplished under high pressure (8 kbar), and the trisaccharide analogue **337** was prepared similarly to give an anomeric mixture (α : β 9:1) of **337** in 69% yield.³³⁷ Halogenations of glycosyl azides have been reported several times.^{338–341} In an extended study it was demonstrated that treatment of **245a** with SO₂Cl₂ under radical conditions gave the 5-chloro derivative **338**.³³⁸ A series of protected glycosyl azides were treated with an excess of *N*-bromosuccinimide under irradiation, leading to the corresponding (moderately stable) glycosyl bromoimines in almost quantitative yields, except for the less-reactive peracetylated α -D-glucopyranosyl azide. With the acetylated anomeric mannopyranosyl azides there was little discrimination in the reaction and both anomers gave bromoimine **339**,³⁴⁰ which upon reaction with Zn/Ag-graphite gave aldononitriles.³⁴¹

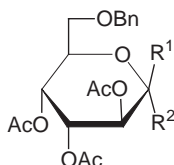


IV. STRUCTURAL STUDIES OF GLYCOSYL AZIDES

The foregoing sections have discussed the chemical properties and transformations of glycosyl azides. This section is devoted to the results concerning structural elucidation and conformational studies of glycosyl azides.

During the past 40 years, X-ray structures have been determined for some glycosyl azides. Thus, the structure of 2,3,4-tri-*O*-acetyl- α -D-arabinopyranosyl azide (**340**) provides evidence³⁴² that the azido group is oriented toward the ring

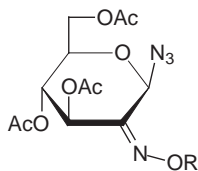
oxygen atom, with a torsion angle O-5-C-1...N-1-N-2 of 75.6° , and thus the exo-anomeric effect of an azido group is demonstrated to be of the same magnitude as the effect of an alkoxy group. This assignment was compared with calculation by the semi-empirical quantum-chemical PCILO method. The lowest minima found were in the range showing the exo-anomeric effect.³⁴³



340 $R^1 = \text{H}, R^2 = \text{N}_3$

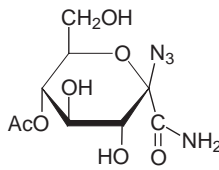
341 $R^1 = \text{N}_3, R^2 = \text{H}$

Similar observations and conclusions were obtained for the xylo azide³⁴⁴ The β -arabino anomer **341**³⁴⁵ shows a torsion angle O-5-C-1-N-1-N-2 of -39.8° , which is smaller than in the compound **340**. Both of these structural studies reinforce the occurrence of an exo-anomeric effect for both anomers. Although in solution the conformation of derivative **341** was determined to be 4C_1 , in the crystalline state the 1C_4 form is adopted.³⁴⁵ Further X-ray crystal structures of 2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl azide³⁴⁶ (**245b**), 3,4,6-tri-*O*-acetyl-2-deoxy-2-hydroximino- β -D-*arabino*-hexopyranosyl azide (**342**),³⁴⁷ and its 2-acetyl derivative **343**³⁴⁸ have been reported. A nitrogen-containing inhibitor of muscle glycogen phosphorylase b (GPb) was complexed with the enzyme and subjected to X-ray structural studies. The β -azide **38** proved not to function as an inhibitor, however its structural analog having a quaternary anomeric center C-(1-azido- α -D-glucopyranosyl)formamide (**344**) showed considerable inhibition.³⁴⁹



342 $R = \text{H}$

343 $R = \text{CH}_3\text{CO}$



344

Authors from Slovenia^{350,351} and Hungary³⁵² studied mass-spectroscopic fragmentation of glycosyl azides by the EI, FAB, and MIKE methods. It was observed that the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in the ion source is favorable for producing the protonated nitrene form. The protonated nitrene shows a new type of ring-expansion rearrangement. The abundance of the $[\text{M} + \text{H} - \text{N}_2]^+$ ion makes identification of the anomeric configuration of the azido group possible.³⁵²

Conformational studies of glycosyl azides, in particular of pyranosyl derivatives, demonstrate that the azido group behaves like the *O*-acetyl group as far as the anomeric effect is concerned.¹⁸⁹ These experimental results show that the dipolar character of the azido group correlates well with the presumed steric (or dipole-dipole or *N-N* type) and electronic (or conjugative, back-donation or $\text{N} \rightarrow \sigma$ type) interactions governing the anomeric effect.³⁵³⁻³⁵⁶

Further conformational studies of these compounds have made use of the azide chromophore, and the circular dichroism (CD) of glycosyl azides has been studied.⁶⁷ Application of the azide octant rule³⁵⁶ predicts a negative Cotton effect for α -glycosyl azides, no matter whether the conformation of the pyranose ring is $^1\text{C}_4$ or $^4\text{C}_1$. For β anomers, a positive Cotton effect was predicted; both were confirmed by experiment. CD spectra have been also recorded for glycopyranosyl azides substituted at the anomeric position by amido, azido, or methoxy groups. Application of the octant rule for the interpretation of the sign for the long-wavelength azide band allowed the conformation of the azido group in each mono azido derivative investigated to be established.³⁵⁷

Another extended report on ^1H - and ^{13}C -NMR spectroscopic characterization of a series of variously protected as well as unprotected glycosyl azides has been published.⁶⁸

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GLYCOL-CLEAVAGE OXIDATION[☆]

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[☆]This article provides a comprehensive account of the principles of the glycol-cleavage reaction, and especially the fundamentals of its applications with carbohydrates. It would be beyond the scope of a single chapter to address all of the myriad examples where the reaction has been used for the elucidation of structure in oligosaccharides, polysaccharides, and glycoconjugates. Forthcoming articles in this series will address in detail the use of periodate oxidation as a component of protocols for structure elucidation that employ a range of spectroscopic methods and micro-scale procedures.

I. INTRODUCTION

Carbohydrates provide a profusion of compounds that contain hydroxyl groups on two or more adjacent carbon atoms, and the fact that this type of carbon-carbon bond generally undergoes oxidative scission selectively and quantitatively has been a major factor contributing to the current status of carbohydrate chemistry. The cleavage reaction was discovered by Malaprade,^{1,2} who observed that polyols are rapidly oxidized by periodate ion. Criegee³ subsequently found that lead tetraacetate cleaves 1,2-diols, and Fleury and Lange⁴ reported that the success of Malaprade's reaction depends on the presence of contiguous hydroxyl groups in the compound.

For many purposes, these oxidants are interchangeable; however, the fact that periodate functions best in water, and lead tetraacetate in organic solvents, makes glycol-cleavage oxidation possible with all types of carbohydrates and derivatives.^{5,6} For favorable examples, the behavior of the two reagents toward a given compound is sufficiently different to provide complementary information.

Oxidative glycol cleavage is one of the most widely used methods for determining constitution, usually in combination with such physical methods as NMR spectroscopy and mass spectroscopy. In addition, it furnishes a general, degradative method for the preparation of compounds in the aldotriose, aldotetrose, and aldopentose series, and in the synthesis of a wide range of sugars and derivatives, including isotopically labeled compounds.

Several reviews of glycol-cleavage oxidations have appeared.⁵⁻⁹

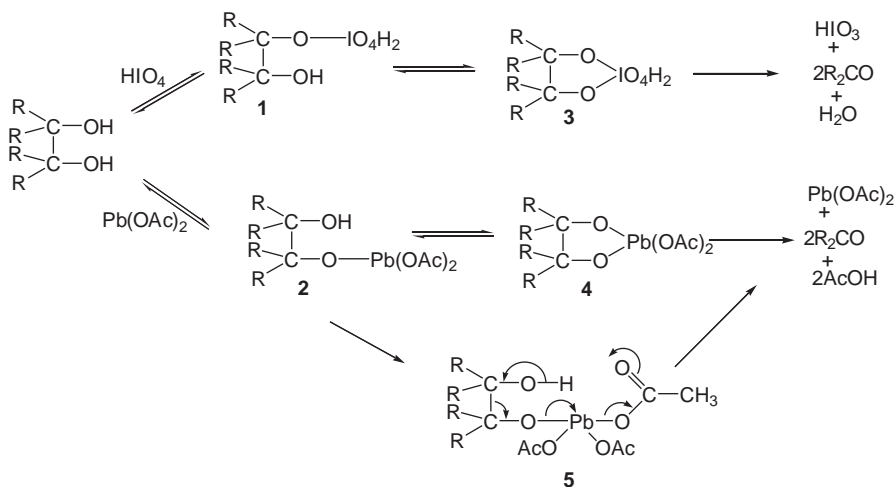
II. CHARACTERISTICS OF PERIODATE AND LEAD TETRAACETATE OXIDATIONS

1. Mechanisms of Glycol Cleavage

The high selectivity of periodate and lead tetraacetate as glycol-cleaving oxidants is attributed mainly to the ability of the central atom of the reagent to complex with a 1,2-diol and effect a two-electron transfer.¹⁰ That an intermediate complex is formed has been shown for periodate by pH¹⁻¹¹ and ultraviolet spectral¹² changes, and, for both oxidants, less directly by consideration of the reaction kinetics.⁹⁻¹³

Based on the original proposals of Criegee *et al.*,¹³ it is generally considered that the cleavage reaction involves reversible formation of a cyclic complex or

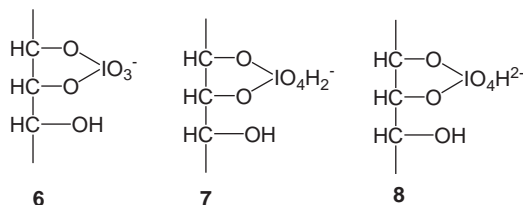
intermediate (**3** or **4**), via an acyclic ester (**1** or **2**), and that the intermediate decomposes via a cyclic transition-state (**5**) to the products.



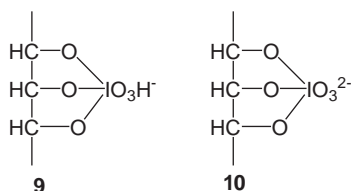
Criegee *et al.* also suggested that the planar conformation of the cyclic complex is optimal for cleavage, because the most reactive compounds are those in which the adjacent hydroxyl groups are virtually eclipsed, as in 1,2-acenaphthanediol,¹³ methyl 2,6-anhydro- α -D-altropyranoside,¹⁴ and *cis*-1,2-camphanediol.¹⁵ Slightly less reactive are diols that may be able to attain the eclipsed condition with little strain—for instance, *cis*-1,2-cyclopentanediol¹⁶ and *cis*-2,3-tetrahydrofurandiols.¹⁷ Equally consistent with the mechanism of Criegee and coworkers are (a) the inert behavior of antiparallel *trans*-1,2-diols,^{15,16,18,19} which clearly cannot form a complex of type **3** or **4**, and (b) the intermediate oxidation rates found for six-membered rings and acyclic 1,2-diols, which would be expected to form a puckered rather than a planar complex. Generally, the *cis* isomers of these cyclic diols are more reactive than the *trans*,^{13,19–21} presumably because there is lessened internal strain and a closer approach to coplanarity when the *a,e* hydroxyl groups of the former isomer coordinate with the oxidant.^{13,10,22} Similarly, *threo*-1,2 diols are usually oxidized faster than the *erythro* isomers,^{19,25–30} and the former also appear better able to accommodate a cyclic complex.^{21–24}

Direct, kinetic evidence for the formation of cyclic intermediates is available in only a limited number of instances, largely through studies by Bunton and colleagues,^{9,11,12,21} who have shown that the singly charged anions **6** and **7** are transient intermediates in periodate cleavage. The concentrations of **6** and **7** are

at the maxima at pH 4 to 5, a pH range that is also maximal for the rate of oxidation. Most probably the entity that actually decomposes to products is the dehydrated form **6**. The doubly charged intermediate **8** preponderates at higher pH values, but it should be inert, because it cannot likewise suffer dehydration; hence, the oxidation rate is low in alkaline media.



The detection of stable periodate complexes having a tridentate structure also supports the concept of the cyclic intermediate. These complexes are formed at neutral and high pH by α -D-ribopyranose and other *cis,cis*-1,2,3-triols,²³ and by such compounds as 1,2-*O*-isopropylidene- α -D-glucofuranose, O-3, O-5, and O-6 of which coordinate with the iodine atom of the reagent.³⁰ There is evidence for two types of tridentate complex (**9** and **10**), corresponding to **7** and **8**, respectively, and their stability appears to be consistent with Bunton's findings, because neither can be converted into a dehydrated form.³⁰



The ease of complex formation is not the sole factor governing oxidation rates. Reactions proceeding through a planar, cyclic complex should favor concerted electron transfer and, therefore, concerted bond-breaking, which would accelerate the decomposition of the complex to products.⁹ A clear demonstration of the relative importance of the *decomposition* step is found in the reaction between the periodate dianion ($\text{H}_3\text{IO}_6^{2-}$) and the isomeric 1,2-cyclohexanediols or 2,3-butanediols; in these examples, strong, nonbonded interactions cause the rate of decomposition of the intermediate to products to be a more decisive factor than the equilibrium constant for its formation.²¹

The glycol-cleaving action of periodate is generally more consistent with the concept of a five-membered ring intermediate than is the action of lead tetraacetate, because the latter reagent can oxidize a number of diols incapable of forming a type **4** complex^{16,19} and can effect oxidation under one set of conditions but not another.^{31,32} Such seeming inconsistencies as these were rationalized by Criegee's suggestion¹⁹ that other pathways are possible within the general framework. For example, a concerted displacement of electrons (as in **5**) can be envisaged for the lead complex **2**. In addition, **2** may decompose by proton transfer to a Lewis base, as suggested by the fact that lead tetraacetate oxidations are base-catalyzed.³³ A wider selection of pathways open to the lead tetraacetate reagent is thus consistent with the fact that it promotes a number of oxidations in addition to glycol cleavage.³⁴

Rates of periodate oxidation have also been shown to be solvent-dependent; solvents having unhindered, basic oxygen atoms,³⁵ such as *N,N*-dimethylformamide, slow down cleavage reactions³⁶ by altering the conformation of the diol, or by competitive complexation with the oxidant, whereas 1,4-dioxane enhances the rate of oxidation of simple diols.

2. Some General Properties of Cleavage Reactions: Experimental Methods³⁷

In addition to *vic*-diols, other 1,2-dioxygenated groups—2-hydroxyaldehydes,^{1,3} 1,2-dicarbonyl compounds,³⁸ α -hydroxy and α -keto acids^{39–40}—and α -amino alcohols⁴¹ are oxidatively cleaved both by periodate and by lead tetraacetate; however, lead tetraacetate oxidizes α -hydroxy acids much more readily than does periodate,^{40,42,43} and both reagents attack 2-hydroxyaldehydes and 1,2-dicarbonyl compounds relatively slowly.^{40,42,44–47} Mechanistically, these reactions appear to resemble glycol scission.⁹

A terminal 1,2-diol yields formaldehyde,^{1–3,48} whereas the carboxylic derivatives yield carbon dioxide.^{39,49} Several methods are available for detecting and determining these important products in reaction mixtures: formaldehyde may be determined as a sparingly soluble methone,⁵⁰ by its highly specific color reaction with chromotropic acid,⁵¹ or polarographically⁵²; conventional, manometric techniques are utilized for measurement of the carbon dioxide^{49,53} from carboxylic acids.

Formic acid is produced from a 1,2,3-triol grouping. As this acid is stable toward periodate, it is readily determined by volumetric^{52,54} or potentiometric⁵⁵ titration of the reaction mixture, by highly sensitive spectrophotometric methods,⁵⁶ or manometrically.⁵⁷ Lead tetraacetate slowly oxidizes formic acid to carbon dioxide, which may complicate the stoichiometry of the glycol-cleavage

reaction.^{39,44} Corrected values for uptake of oxidant may be obtained⁵⁷ by introducing potassium acetate to catalyze this secondary oxidation (and the rate of glycol cleavage as well)^{33,57} and measuring the liberated carbon dioxide manometrically. Cleavage of an aldehydic α -hydroxy hemiacetal group leads to a formic ester, whereas a ketonic α -hydroxy hemiacetal group may yield an ester of either glycolic or glyoxylic acid (see Section V.2).

Nuclear magnetic resonance spectroscopy may provide a convenient measure of the formaldehyde⁵⁸ or formic acid⁵⁹ produced in some periodate oxidations, and may also permit differentiation between free formic acid and that bound as formic esters;⁵⁹ deuterium oxide is a convenient solvent for these determinations. Mechanical, automated techniques for determining the amounts of various oxidation products have been developed.⁶⁰

Most of the common types of substituent groups—esters, acetals, ethers—are stable to conditions normally used in glycol-cleavage oxidations. Notable exceptions are thio derivatives (see Section II.3). Phenolic constituents of *C*-glycosyl derivatives⁶¹ and other naturally occurring saccharides⁶² may also undergo oxidation; however, the phenolic groups may be adequately stabilized by alkylation.⁶¹ With partially acylated sugars, the danger exists that ester migration might occur in the oxidizing medium and lead to inconclusive results. Such a possibility was specifically considered in regard to certain cleavage reactions, but no evidence for migration was obtained,^{46,63,64} nor has an authentic example of acyl migration accompanying oxidation been reported;⁶⁵ however, the general need for caution with potentially labile substituents is illustrated by the observation that the acetal group of 1,4-anhydro-2,3-*O*-benzylidene-D-mannitol migrates to O-5,O-6 as the compound dissolves in acetic acid, so that treatment with lead tetraacetate effects glycol cleavage in the latter anhydro acetal.⁶⁶ Trityl groups may also be hydrolyzed slowly under the same conditions.^{67,68} The oxidation of conduritol oxide to 2,3-epoxysuccinaldehyde⁶⁹ indicates that an oxirane ring is stable. Nevertheless, there is evidence that such a ring can be opened, and recycled through an adjacent position, by lead tetraacetate, as suggested by Criegee and Fiedler⁷⁰ to explain the unexpectedly high rate of oxidation of *trans*-3,4-epoxy-1,2-cyclobutanediol.

The consumption of oxidant is most generally determined by volumetric methods—periodate by titration with arsenite,⁷¹ and both oxidants by iodimetry.^{72,73} Spectrophotometric methods are also frequently used for determining⁷⁴ periodate^{75–77} and lead tetraacetate,^{78,79} and are especially useful for micro-oxidations or highly dilute reaction mixtures.⁷⁹ Spectrophotometric determination of the extent of conversion of the violet dye tris[2,4,6-tris(2-pyridyl)-1,3,5-triazino]iron(II) into its colorless ferric state reportedly⁸⁰ provides a means of quantitating nanomole amounts of unreacted periodate, and it has been coupled

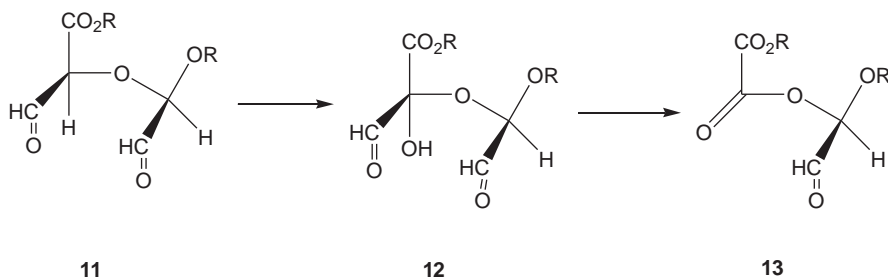
with online electrochemical detection. Polarographic analysis has also been shown to be a practical⁸¹ method for measurement of concentrations of periodate (and iodate), and other possibilities include the use of ion-selective electrodes,⁸² and microcalorimetry.⁸³ The latter technique has been used to study the kinetics of periodate oxidation of various monosaccharides and to determine the activation parameters for the reaction,⁸⁴ and the approach has been extended to a range of polysaccharides.⁸⁵ The relatively high periodate oxidation rates, as measured by isothermal microcalorimetry, of reducing residues in dextran oligomers are analogous to selectivities observed with lead tetraacetate.⁸⁵

Periodate and lead tetraacetate both decompose quite rapidly at elevated temperatures and, therefore, are employed at room (or lower) temperature; in addition, periodate oxidations are best conducted in the absence of light.⁸⁶

3. Oxidations Other Than Glycol Cleavage: "Overoxidation"

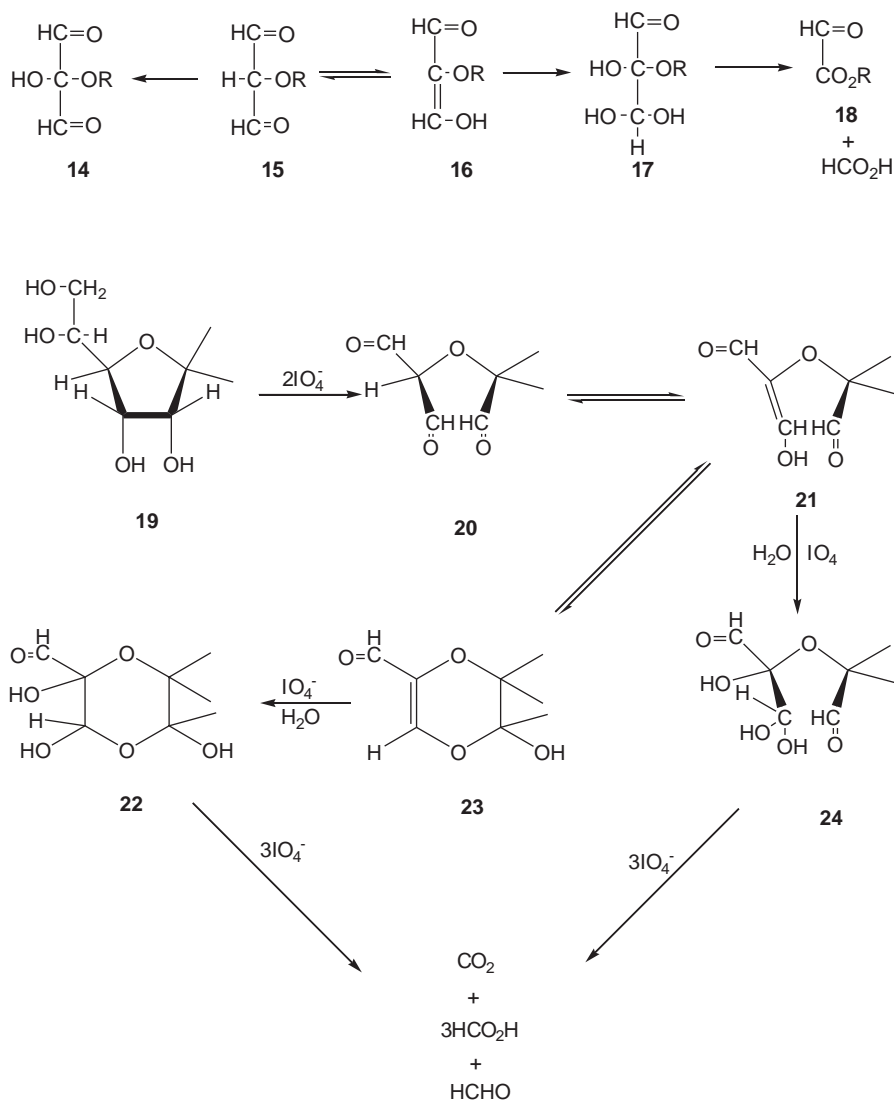
Although periodate and lead tetraacetate are among the most highly specific of oxidants, they nevertheless promote a number of oxidations in the carbohydrate series besides glycol cleavage.

A problem frequently encountered is "overoxidation" or "non-Malapradian" oxidation. Most examples of this kind of behavior involve the formation, as a product of the cleavage reaction, of tartronaldehyde and related compounds containing an "active" hydrogen atom. Glycuronic acids and some other carboxylic derivatives are subject to extensive overoxidation⁸⁷⁻⁹⁰ attributable to instability⁹¹ of the product of normal scission—for instance, **11**, or to dehydrogenation at C-5. Similarly, substituted tartronaldehydes (**12**), which are formed during oxidation of hexofuranosides,^{44,48} partially substituted sugars,⁹²⁻⁹⁴ oligosaccharides (see Section V.3), and polysaccharides (see Section VI.1), are readily degraded oxidatively.



Overoxidation by periodate may involve direct hydroxylation as the first step^{88,91,95} (as in the oxidation of hydrocarbons),⁹⁶ with the resulting hydroxy-aldehyde (**12** or **14**) being cleaved in the conventional way; however, hydroxylation

of the enolic form **16** is the more probable pathway.⁹⁷⁻¹⁰⁰ Direct evidence for the latter possibility is provided by the finding¹⁰⁰ that the trialdehyde (**20**) produced from 1,4-anhydro-D-allitol (**19**) enolizes to yield **23**. The latter, which has been isolated as a crystalline compound, is oxidized to the same products as are obtained without interrupting the reaction,¹⁰⁰ probably via **22** or **21** and **24**.



A similar route has been suggested to account for the formation of glyoxylic acid (**18**, R = H) and methyl glyoxylate (**18**, R = Me) during the periodate oxidation of inositols⁹⁷ and *O*-methylinositols,⁹⁹ respectively. Formation of these carboxy derivatives was depicted^{97,99} by sequences involving enolization to such reductones as **16**, which are hydroxylated to **17** and then cleaved oxidatively.

Direct hydroxylation is a probable step in the overoxidation phase observed during the periodate oxidation of glycals; attack presumably occurs at the allylic position adjacent to the carbonyl group of the initial product formed by cleavage of the 3,4-diol grouping.¹⁰¹

Products formed in the overoxidation of such compounds with lead tetraacetate have not been so well characterized, although acetoxylation might be expected to occur.^{43,73,102} Some compounds examined as models of these activated methine groups, or as possible products of glycol cleavage—for instance, 2,4-pentanedione and formic, glycolic, and oxalic acids—are readily oxidized by lead tetraacetate^{13,39,43,44,57} but not by periodate.^{5,103}

Effective *O*-demethylation has been observed to occur in the periodate oxidation of 3-deoxy-4-*O*-methylaldosulosonic acids, which produce 2-oxobutanedioic acid and give a positive test with thiobarbituric acid.¹⁰⁴

N-Substituted derivatives in the amino sugar series show a variety of oxidative characteristics. Whereas *N*-acetylation or *N*-benzoylation prevents cleavage of a 1,2-amino alcohol,^{74,105–110} an *N*-ethoxycarbonyl¹¹¹ or *N*-*p*-tolylsulfonyl group¹⁰⁶ may permit oxidation to the imine (R-CH = N-CO₂Et, or R-CH = N-SO₂C₇H₇-*p*, respectively). Nonspecific oxidation has been observed with some *N*-methyl derivatives, the extent of the “anomalous” reaction being pH-dependent.¹¹² The dimethyl ether of methyl amosaminide is probably *N*-demethylated, because 1 mol of it reduces 2.3 mol of periodate to yield 1 mol of formaldehyde.¹¹² Cleavage of the α -hydroxydimethylamino group of desosamine has also been reported,¹¹³ whereas methyl mycaminoside is resistant¹¹⁴ to periodate at pH 4.5. “Anomalous” results were also obtained with 3-amino-3-deoxyribofuranosides, which consume 2 mol (not the expected 1 mol) of periodate per mole.¹¹⁵ An interesting steric effect is encountered in the periodate oxidation of methyl 2-amino-4,6-*O*-benzylidene-2-deoxy- α -D-altropyranoside at pH 6.9 (but not at pH 4), as the corresponding 2,3-diol is not attacked.¹¹⁶

Re-examination of the conditions of reaction of the conformationally fixed 2-amino-2-deoxy-2-altropyranoside derivative and the 3-amino-3-deoxy isomer revealed that they slowly undergo C-2–C-3 cleavage, whereas the 2,3-diol is unreactive¹¹⁷; the latter amino sugars are subject to overoxidation, whereas stereoisomeric 2-amino-4,6-*O*-benzylidene-2-deoxyaldopyranosides, which are

oxidized much faster, consume the stoichiometric amount of the oxidant.¹¹⁸ Several 1,2-*O*-isopropylidenefuranos-3-ulose 3-*p*-nitrophenylhydrazones have been converted into the corresponding geminal azo alcohols in which O-2 is *trans* to O-3 by treatment with lead tetraacetate.¹¹⁹

The ability of periodate and lead tetraacetate to oxidize sulfides may produce unexpected results when the effects of these reagents are used to examine thio sugars and derivatives. Some alkyl and aryl 1-thioglycosides show complex stoichiometry,^{120–123} sometimes accompanied by the release of iodine, whereas the corresponding sulfones undergo normal glycol cleavage under suitable conditions.¹²⁰ The sulfur atoms of sugar dithioacetals are relatively stable in a solvent of low dielectric constant (commonly, benzene), but *S*-oxidation is rapid in a more-polar solvent (such as acetic acid) and may take precedence over glycol cleavage.^{124,125} By contrast, 1 mol of 1,2-*D*-isopropylidene- α -*D*-glucofuranose 5,6-thionocarbonate consumes only 0.5 mol of lead tetraacetate in acetic acid, but over 2 mol in pyridine.¹²⁶ Cyclic thioethers appear to be less reactive; 1 mol of methyl 5-thio- α -*D*-ribofuranoside reportedly¹²⁷ reduces 3 mol of periodate.

Overoxidation accompanied by release of inorganic phosphate or sulfate has been observed in the periodate oxidation of some hexose monophosphates¹²⁸ or monosulfates,^{129,130} although the ester hydrolysis is largely obviated by use of dilute reaction mixtures. The products of the periodate oxidation of *D*-glucose 2-sulfate and *D*-galactose 2-sulfate suggest that the acyclic form of each is oxidized; 3-sulfates are, however, oxidized normally.¹³¹

Instances of *apparent* oxidation sometimes occur that are actually attributable to analytical difficulties. Thus, 1 mol of methyl 2-deoxy- α -*D*-xylo-hexopyranoside appears to consume 2 mol of periodate, not one, as measured by the arsenite method, because the methylene group of the oxidation product is iodinated during the back-titration.¹³² Also, some tridentate periodate complexes are decomposed only slowly by arsenite, resulting in spurious uptake values.^{25–30} A 3,6-anhydro-4,5-*O*-isopropylideneoctitol of unspecified configuration at C-2 and C-7 was reported to undergo stoichiometric oxidation by periodate to afford 2,5-anhydro-3,4-isopropylidene-DL-allose directly;¹³³ however, re-examination of this reaction revealed that the actual precursor in the reported oxidation is a heptitol, and that oxidation of the octitol gives the (expected) 2,5-anhydroheptoseptanose.¹³⁴

4. Vicinal Diols Resistant to Glycol Cleavage

Periodate and lead tetraacetate are routinely used to test for the presence or absence of a 1,2-diol grouping. Nevertheless, a relatively large number of

examples of marked resistance to cleavage is known. This consideration attaches some uncertainty to the absolute validity of a negative oxidation test, particularly for a complex compound.

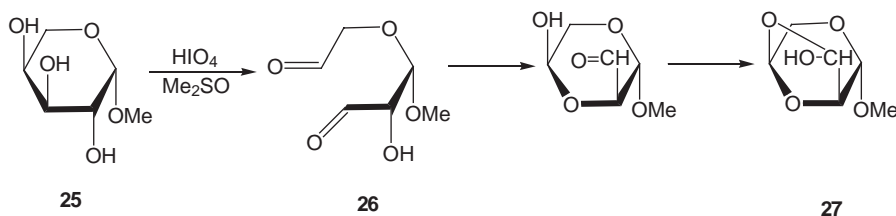
1,6-Anhydro- β -D-glucofuranose was mentioned (in Section II.1) as an α -glycol that is resistant to cleavage, in accord with Criegee's cyclic intermediate mechanism. The D-*galacto* isomer behaves similarly,^{135,136} and related inert compounds are 2,7-anhydroheptulofuranoses that contain a 3,4-*trans*-diol grouping.¹³² Other examples are methyl 4,6-*O*-benzylidene- α -D-altropyranoside,¹¹⁶ 2,6-anhydro- β -D-fructofuranose,³¹ and 1,4-anhydro-*epi*-inositol;¹³⁸ the last two are sterically analogous to *trans*-2,3-camphanediol.¹⁵ Each of these is a bicyclic, fused- or bridged-ring compound in which the inactive *trans*-diol grouping appears to be held rigidly, so that the minimum dihedral angle between the two C-O bonds probably exceeds 100°; however, these compounds are oxidized by lead tetraacetate in pyridine solution³¹ (a particularly vigorous form of the oxidant⁶⁸) perhaps by way of an acyclic mechanism (see Section II.1). In several instances, the oxidation products have been characterized as those expected from normal cleavage,^{31,137,138} a matter of some importance, because this solvent-modified oxidant may effect oxidation of single hydroxyl groups.^{139,140}

Formation of a highly stable, tridentate complex may prevent cleavage when periodate is used above pH 7, although few classes of sugar compounds can satisfy the steric requirements for this kind of complexing. Thus far, the effect has been observed with α -D-ribopyranose, α -D-allopyranose, β -D-lyxopyranose, and several inositols—compounds affording a 1*a*,2*e*,3*a*-triol system—and with certain aldohexofuranose derivatives;³⁰ in glucofuranoses the complex involves O-3, O-5, and O-6, and in galactofuranoses, O-2, O-5, and O-6.

There are several instances in which inductive effects undoubtedly suppress reactivity markedly. Thus, arabinono- and xylono-3,4-lactones are virtually unaffected by lead tetraacetate,⁴⁸ possibly because inductive electron withdrawal depresses the nucleophilicity of O-2 or destabilizes the Pb-O bond in the intermediate complex.¹⁴¹ Inertness of a 2,3-*trans*-diol grouping within a lactone ring is evidenced in the observation that D-galactono-1,4-lactone is oxidized by periodate solely at C-5 and C-6.¹⁴² Similarly, an electron-withdrawing substituent (such as a sulfonyloxy group) can markedly retard scission of an adjacent α -glycol grouping; for instance, the rate of oxidation of methyl 2-*O*-*p*-tolylsulfonyl- α -D-glucopyranoside is one thousandth that of the corresponding 2-*O*-methyl-D-glucoside,¹¹⁶ although steric factors may also contribute substantially to the difference in rates. Hence, sulfonyl derivatives of unknown structure, or compounds containing similar deactivating (and perhaps, bulky)

substituents, may give a spurious, negative test, unless the reaction time is sufficiently long and the concentration of oxidant adequately high.

A combination of inductive and steric effects may also account for the fact that phenyl β -D-glucopyranoside (but not the α anomer) rapidly consumes only one molar equivalent of periodate, instead of the theoretical two; this oxidation is specific for the 3,4-diol grouping.¹⁴³ Methyl 6-O-trityl- α -D-mannopyranoside consumes only one molar equivalent of lead tetraacetate in benzene, but there is partial cleavage at both C-2-C-3 and C-3-C-4.¹⁴⁴ A pentose analog, methyl β -L-arabinopyranoside (**25**), likewise reacts with dilute¹⁴⁵ solutions of periodate in dimethyl sulfoxide to reduce one molar equivalent of oxidant; in the absence of the large substituent on C-5, however, cleavage occurs primarily at the C-3-C-4 bond.¹⁴⁶ It was proposed¹⁴⁶ that this underoxidation arises because of intramolecular blocking of the 2-hydroxyl group of the initial dialdehyde (**26**) by its incorporation into a 1,4-dioxane derivative that undergoes a second ring closure to afford the 3,6,8-trioxabicyclo[3.2.1]octane derivative **27**; the latter, unequivocal, spectroscopic characterization of the crystalline acetate of **27** provided support for this mechanism.¹⁴⁷ Analogous internal cyclization reactions accompany the oxidation of methyl α - and β -D-galactopyranoside under similar conditions.¹⁴⁸



Even in highly polar solvents, the relatively low reactivity of hydroxy aldehydes^{44,46} may lead to marked underoxidation of polyhydric alcohols. Thus, 4, 6-O-ethylidene-D-mannose (and -D-galactose) and D-glucofuranose 5,6-carbonate each consume 2 mol of lead tetraacetate per mole in acetic acid when the concentration is 10 mM, but, in more-dilute solution (100 μM), take up only 1 mol at a significant rate.⁴⁶ This behavior was attributed to the minimal reactivity of the aldehydo-pentose formic esters that are produced by initial cleavage of the α -hydroxy hemiacetal group.

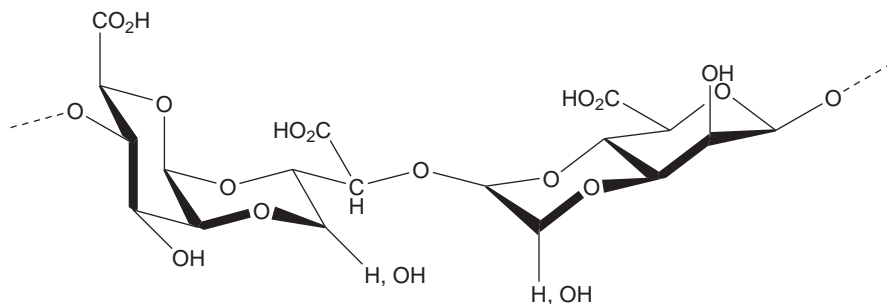
In other instances of apparent underoxidation, formate groups elaborated during the reaction serve as protecting substituents. Some examples are

discussed in Section V.2 in connection with reducing-sugar oxidations. An extreme example may be mentioned here, namely *D-erythro-L-galacto*-octose:¹⁴⁹ although this sugar has five *vic*-diol groupings, 1 mol reduces only 2 M equivalents of lead tetraacetate readily, because stepwise degradation converts it into a 2,3-(or 2,4-) di-*O*-formyl-*D*-glucopyranose, which lacks a free 1,2-diol grouping.

Internal glycosyl residues of many oligosaccharides, particularly those linked to adjacent residues by (1 → 4) bonds and containing a 2,3-*trans*-diol grouping¹⁵⁰ (see Section V.3), are highly resistant to cleavage by lead tetraacetate in acetic acid, even under catalyzed conditions. A resistant 1,2-glycol was encountered¹⁵¹ in the sophorosyl residue of a partially acetylated glycolipid; although 1 mol of the compound consumed only 1 mol of periodate or lead tetraacetate under the usual conditions, a second *vic*-diol grouping was detected by oxidation with lead tetraacetate in pyridine. A di-*D*-fructofuranose 1,1':2,2'-dianhydride¹⁵² provides an unusual example of unreactivity in an oligosaccharide derivative; the 3,4-diol grouping of the β-*D*-glycosyl residue in the "disaccharide" is attacked at a markedly low rate by periodate, whereas the α-*D*-glycosyl residue is oxidized rapidly. This striking difference in reactivity is a consequence of local, structural rigidity, which constrains the diol grouping of the resistant residue to subtend an unfavorably large angle of about 150°. Oxidation of chondroitin and dermatan sulfates, and of heparin and heparan sulfate, by aqueous periodic acid at pH 3 and pH 7 effects selective cleavage of the *L*-iduronic acid residues and/or the *D*-glucuronic acid residues, depending also on the identity of the neighboring amino sugar residues.^{153,154}

In the study of polysaccharides, it is sometimes difficult to determine whether certain residues are resistant to oxidation for steric reasons, or because of the presence of (1 → 3) bonds or multiple linkages. This difficulty is compounded by the possibility of overoxidation, which militates against the use of greatly extended reaction periods. Poor solubility of certain polysaccharides (as well as of some compounds of low molecular weight) may strongly retard oxidation. Most probably this contributes to the relative inertness toward lead tetraacetate of suspensions of polysaccharides in acetic acid or pyridine;⁶ however, as cellulose is readily oxidized in water by periodate under heterogeneous conditions,^{155,156} such surface effects as wetting or adsorption must play an important role. Interesting examples of underoxidation are observed for alginates,^{157,158} xylans,¹⁵⁹ and amylose,^{158,160,161} attributable to inter-residue hemiacetal formation as oxidation progresses. This would protect approximately every third glycosyl residue in a linear (1 → 4)-linked polysaccharide (as in **28** derived from alginic acid¹⁵⁹); the results of sequential applications of the Smith degradation

(see Section VI.2) to amylose are in accordance with this formulation.¹⁵⁸ An analogous influence of inter-residue hemiacetals formed during the course of oxidation is observed in the periodate cleavage reaction of (C-6)-oxycellulose¹⁶² and of sodium and methyl pectates.¹⁶³ Solvent-induced, conformational effects have also been implicated as affecting the rates of such oxidations.³⁶



28

III. ACYCLIC ALDITOLS AND CYCLITOLS

Oxidative scission of 1,2-diol groupings in acyclic compounds is generally rapid and quantitative, providing an excellent means for structural examination of such partially substituted derivatives of alditols as esters^{63,141,164,165} and acetals.¹⁶⁶⁻¹⁶⁸ An early, highly fruitful application in this series was the finding that the common di-*O*-isopropylidene-*D*-mannitol is the 1,2:5,6-diacetal, shown by the fact that it consumes 1 mol of oxidant per mole and yields 2,3-*O*-isopropylidene-*D*-glyceraldehyde.¹⁶⁶ Reduction of this triose acetal affords 1,2-*O*-isopropylidene-*L*-glycerol, a starting compound for the synthesis of numerous, naturally occurring glycerides and phospholipids;¹⁶⁹ periodate oxidation of 1,6-dideoxy-1,6-difluoro-2,5-*O*-methylene-*D*-mannitol is a key step in the stereo-specific synthesis of 1-deoxy-1-fluoro-*L*-glycerol.¹⁷⁰ Similarly, glycol-cleavage oxidation was used to determine the structure of 1,3-*O*-benzylidene-*D* (or *L*)-arabinitol; although the product, 2,4-*O*-benzylidene-*D* (or *L*)-threose, it provides perhaps the best route to *D*- or *L*-threose and derivatives.¹⁷¹

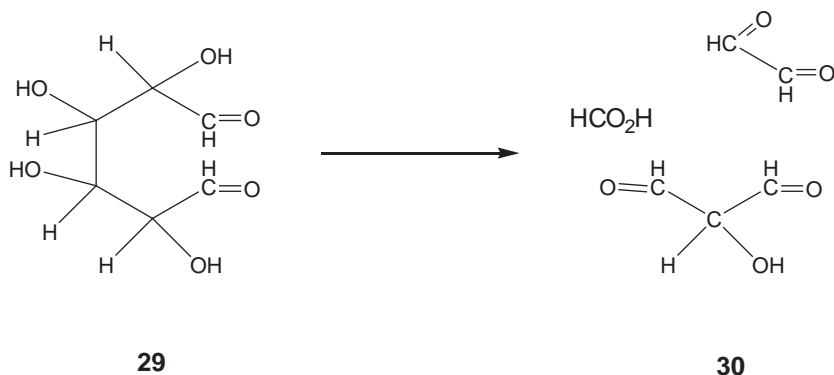
In acyclic alditols, a *vic*-diol grouping consisting of secondary hydroxyl groups is usually oxidized more readily than one containing a primary and a

secondary hydroxyl group,^{24,172} and the *threo* (*trans*) configuration is particularly vulnerable.^{17,23,24,172,173} This difference can be used to afford information about the branching patterns of O-linked glycoprotein oligosaccharides.¹⁷³ Consequently, initial attack of periodate on a polyhydric alcohol such as mannitol mainly cleaves the 3,4-*threo* diol grouping and yields a glyceraldehyde, whereas galactitol is mainly split at C-2-C-3 and affords a threose.^{24,172} Subsequent oxidation of one mole degrades these (as well as the primary products from other hexitols) to the expected, normal end products—2 moles of formaldehyde and 4 moles of formic acid. When lead tetraacetate in acetic acid is used, underoxidation is sometimes observed.¹⁷⁴ This occurs most noticeably with galactitol, presumably because the intermediate tetrose is oxidized as a cyclic sugar in this medium and yields a stable formic ester. In these reactions, account must also be taken of the lead tetraacetate consumed in the (slower) oxidation of formic acid.^{57,175}

Usually, although not invariably, *vic*-diol groupings including a tertiary hydroxyl group are relatively unreactive.^{19,176}

The oxidation of an inositol differs notably from that of an acyclic hexitol; in principle, the main difference to be expected is the production of formic acid instead of formaldehyde. Thus, inositols show an *over-consumption* of periodate;^{177,178} that is, 1 mole consumes ~6.7 moles of oxidant, not 6.0, and gives close to 1 mole of carbon dioxide, and only ~5 moles of formic acid, not 6. These results were accounted for in the following way:^{45,99,179} the initial product is a hexodialdose (**29**), the glycol (but not hydroxyaldehyde) groupings of which are randomly split to yield glyoxal and tartronaldehyde (**30**). The latter then reacts as the tautomeric reductone (**16**, R = H) (as in the overoxidation route depicted in Section II.3), yielding glyoxylic acid (**18**, R = H) and, ultimately, carbon dioxide.

Although this pathway appears to be general for the inositols, the rates of oxidation vary widely; *cis*-inositol (all-*cis*) reacts about two hundred times as fast as the *scyllo* isomer (all-*trans*). For several of the most reactive isomers, the rates are higher than would be anticipated for a *cis*-diol of a cyclitol molecule, and this enhancement is attributed to excess steric strain caused by mutual repulsion of axially attached hydroxyl groups in these compounds.¹⁸⁰ The rule that, in the cyclohexane series, *cis*-diols react more rapidly than *trans* isomers with periodate and lead tetraacetate, appears to hold generally for inositols and their derivatives,^{180,181} including inosamines;¹⁸² it has been utilized for assigning configuration.^{181,182} Overoxidation of inositols is reportedly suppressed at 0 °C in 0.1 M sulfuric acid solution.²⁸

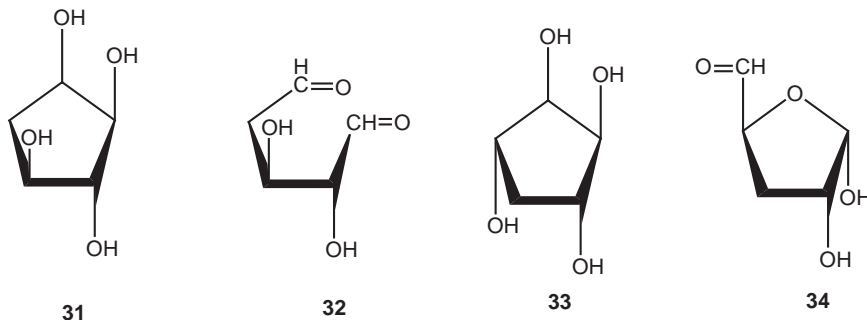


As formic esters are not products of these periodate oxidations,⁹⁹ a hexodialdose, such as **29**, must react in an acyclic form; however, 1,3-di-*O*-methyl-*myo*-inositol shows exceptional behavior, in that it affords a high yield of a formic ester, which must be derived by cleavage of a furanose form of the intermediate hexodialdose.¹⁸³ Cyclization of the latter is regarded as being favored by the all-*trans* arrangement of groups on its furanose ring.¹⁷⁹

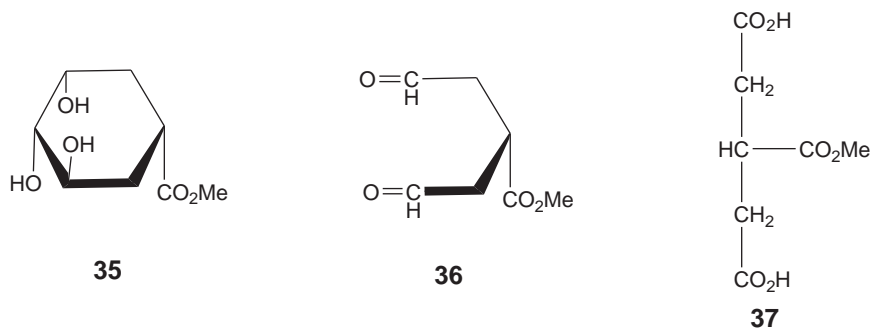
The influence of cyclization of intermediate dialdehydes on the course of reaction is also evident during the oxidation of cyclopentanetetrols with lead tetraacetate at high dilution in acetic acid.¹⁸⁴ For example, the *cis*-diol of the (1,2,4/3) isomer (**31**) rapidly consumes 1 mol of oxidant per mole; subsequent attack on the intermediate pentodialdose **32** is slow, because it can involve only the relatively inactive 2-hydroxyaldehyde group of this acyclic form or a six-membered ring hemialdal form. By contrast, the (1,2/3,5) isomer (**33**) rapidly consumes 2 mol of lead tetraacetate per mole, undoubtedly because the derived dialdehyde can be oxidized in the reactive furanose form **34**. The 1,2- and 2,3-*cis*-diol groupings of the (1,2,3/4) isomer provide two possibilities for initial cleavage, one leading to **32** and the other to **34**; hence, this isomer exhibits a rate of oxidant uptake that is intermediate between those for **31** and **33**. A unique situation is afforded by the (1,2/3,4) isomer, which contains two highly reactive *vic*-diol groupings situated on the same ring, independent of each other; however, only one of these groupings can be cleaved rapidly, and reduction of a second molar equivalent of lead tetraacetate is slow, because, in both instances, the dialdose produced corresponds to an epimer of **32**, which can form an unreactive, furanose ring to protect the hydroxyl group adjacent to the uncyclized aldehyde group.

Of the cyclohexanetetrols, 1 mol of the *ortho* (1,2,3,4) isomers reduces the expected 3 mol of oxidant, whereas *para* (1,2,4,5) isomers show overoxidation,

because of the concomitant formation of malonaldehyde¹⁸⁵ from C-2, C-3, and C-4. Although three of the *meta* (1,2,3,5) isomers undergo a normal uptake of oxidant, the (1,2,3/5) isomer is, as yet inexplicably, overoxidized.¹⁸⁶



Oxidative interconversion of cyclitols and derivatives into acyclic, dicarboxylic acids has been widely used for structural elucidation in this series.¹⁷⁹ Early applications of the procedure helped determination of the constitution of shikimic and quinic acids;¹⁸⁷ for example, cleavage of the 1,2,3-triol grouping of methyl dihydroshikimate (**35**) produced a dialdehyde (**36**), which was oxidized¹⁸⁷ with bromine water to the (known) degradation end product, tricarballic acid (**37**). This approach to structural problems has proved particularly successful for determining the configuration of glycosides and related compounds.

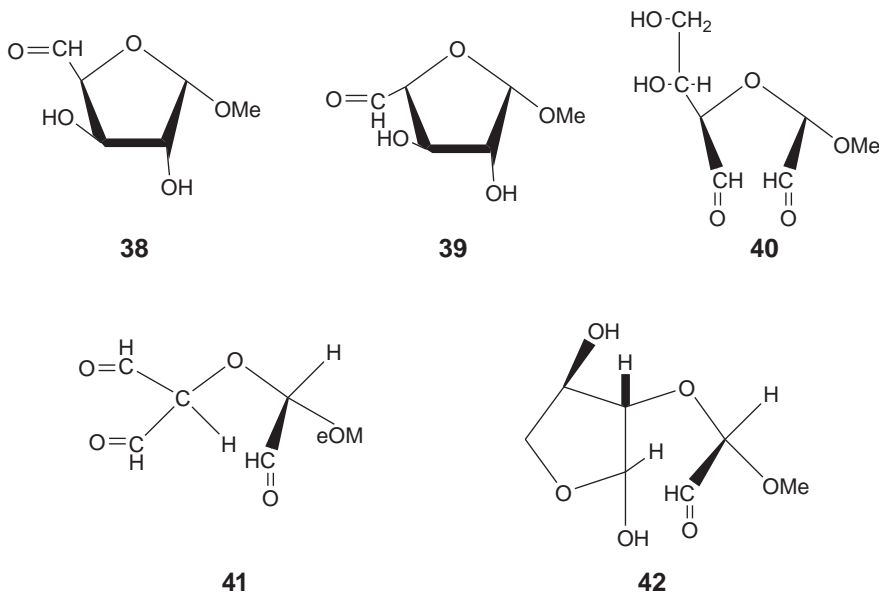


IV. GLYCOSIDES AND RELATED ALICYCLIC COMPOUNDS

1. Reaction Characteristics; Configurational Relationships

Criegee found that 1 mol of ethyl α -D-glucofuranoside rapidly reduces 1 mol of lead tetraacetate, and yields about 1 mol of formaldehyde, whereas 1 mol of methyl

α -D-glucopyranoside gives no formaldehyde, even after 2 mol of oxidant have been reduced.⁴⁸ This difference constitutes a general means for distinguishing between five- and six-membered ring forms of various compounds. The exocyclic 5,6-diol grouping of a D-galactofuranoside is also cleaved more rapidly than the *trans* 2,3-diol grouping within the ring;¹⁸⁸ periodate shows the same effect, which has been utilized in descending from aldohexofuranosyl to aldopentofuranosyl derivatives.¹⁸⁹⁻¹⁹² If further oxidation of the 5-aldehyde derivative (**38**, D-*gluco* isomer, or **39**, D-*galacto* isomer) is allowed to proceed, the trialdehyde **41** is obtained which, having a tartronaldehyde residue, is overoxidized (see Section II.3). Because of its highly reactive 2,3-*cis*-diol grouping, methyl α -D-mannofuranoside is mainly cleaved at the C-2-C-3 bond by lead tetraacetate.^{39,144} Further oxidation is retarded, presumably because the resulting dialdehyde (**40**) adopts a stable, cyclic form (such as **42**). These stages are not evident in the oxidation of the D-mannoside by periodate, which appears to yield the trialdehyde **41** readily,⁵⁴ although the extreme reactivity of the 2,3-diol grouping probably accounts for the fact that this glycoside fails³⁰ to form a tridentate periodate complex at high pH.

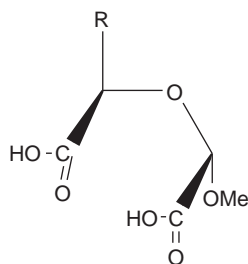


Configurationally related 1,4-anhydrohexitols show closely analogous oxidation patterns, and this fact aided greatly in their characterization.^{193,194}

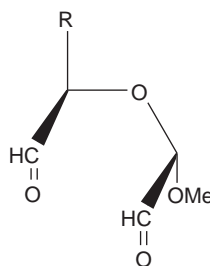
Rates of cleavage of the 2,3-diol grouping of furanosides are, of course, very much higher for *cis* than for *trans* isomers. In addition, anomers are oxidized by

lead tetraacetate at substantially different rates, the more reactive anomer having the 1-alkoxyl and 2-hydroxyl groups in *cis* relationship. For example, the rate for methyl β -D-threofuranoside is six times that of the α anomer,¹⁹⁵ whereas that for methyl α -D-erythrofuranside is 3.5 times that of the β anomer.⁷⁹ The relative reactivities of the erythrosides toward lead tetraacetate contrast with their behavior toward tetraborate, as it is the β anomer which complexes more extensively.¹⁹⁶ If these borate complexes serve as reasonable models for the cyclic lead complexes,¹³ the enhanced rate of oxidation of the α anomer can be attributed to a relatively accelerated decomposition of its intermediate, because of greater internal repulsions, rather than to greater ease of complexing with the oxidant (see Section II.1).

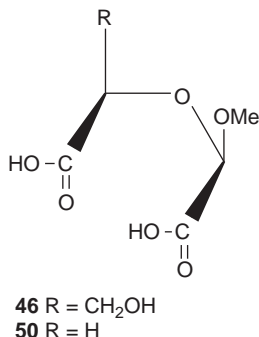
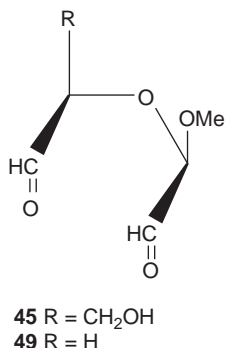
Glycol-cleavage oxidation of glycopyranosides involves a well-defined stoichiometry under suitable conditions. Methyl α -D-glucopyranoside, for example, consumes 2 molar equivalents of periodate, yielding the syrupy dialdehyde **44** plus a mole of formic acid. The same reaction is accomplished by lead tetraacetate,⁴⁴ although due allowance must be made for the side-consumption of oxidant by the formic acid.⁵⁷ An elaborate study of oxidation products from glycosides permitted Jackson and Hudson¹⁹⁷⁻²⁰⁰ to correlate the ring size and the configuration of a whole series of such compounds with known structures and provided a general approach applicable to related types of compounds. Thus, 1 mol of certain methyl glycosides prepared from D-galactose, D-gulose, and D-mannose were all converted by oxidation with 2 mol of periodate into **44**. This was demonstrated by optical rotatory measurements and, more satisfactorily, by converting each product by hypobromite oxidation into a common, crystalline salt of the dicarboxylic acid **43**. The parent glycosides were thereby shown to be α -D-pyranosides. Similarly, a series of β -D-glycosides was correlated sterically by preparation²⁰¹ of the isomeric dialdehyde **45**, and a salt of the dicarboxylic acid **46**.



43 R = CH₂OH
47 R = H



44 R = CH₂OH
48 R = H
51 R = CH₂CHO



Oxidation of 1 mol of the anomeric methyl aldopentopyranosides with 2 mol of periodate¹⁹⁷ or lead tetraacetate^{190–202} yields one of a pair of dialdehydes, a dextrorotatory product **48** being derived from α -D- or β -L-glycosides and levorotatory **49** from β -D or α -L isomers.¹⁹⁷ In turn, hypobromite oxidation affords either dicarboxylic acid **47** or **50**. Additional possibilities for configurational correlation accrue from the fact that a methyl aldotetrofuranoside similarly yields **48** or **49**, whereas a methyl aldopentofuranoside gives **44** or **45** as the final product.

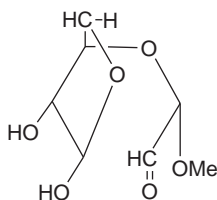
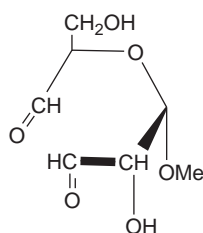
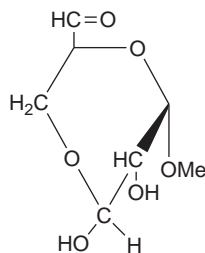
Extension of Jackson and Hudson's approach is also feasible with 2-keto pyranosides, nucleosides,^{192–203} some types of anhydrides,^{204,205} nonreducing and reducing disaccharides,¹⁶¹ and glycosylamines.^{108,207,208}

Sometimes a better basis for such correlations is provided by reducing the dialdehyde,^{209,210} which affords products containing fewer asymmetric centers; furthermore, these polyhydric alcohols frequently yield crystalline derivatives.

Configurational relationships may also be deduced by degrading the glycol-cleavage product to a known fragment. For example, noviose and mycarose were shown to be members of the L series, because glycosides of these antibiotic sugars, on successive periodate cleavage, bromine oxidation, and hydrolysis afforded (–)-3-hydroxy-2-methoxy-3-methylbutyric acid²¹¹ and L-lactic acid,²¹² respectively. A related application is found in the preparation²¹³ of (+)-[1-²H] ethanol, which was generated by a sequence of reactions initiated by periodate cleavage of the C-3–C-4 bond of butyl β -D-[5-²H]xylopyranoside of established stereochemistry, to give the deuterated dialdehyde. In turn, the xyloside was used²¹⁴ in determining the absolute configuration of the 6-carbinol group of methyl β -D-[6-²H]galactopyranoside, which involved lead tetracetate oxidation of the [6-²H]aldohexose to the four-carbon homologue, D-[4-²H]threose.

Although stereoisomeric aldopyranosides are frequently oxidized to common end products, the rates at which these transformations take place differ in a

substantial, but generally explicable, way.^{44,215} When two hydroxyl groups of the 2,3,4-triol grouping have a *cis(a,e)* orientation, the first mole of oxidant per mole is reduced faster than when only *trans*-glycol groupings are present. Among the hexopyranosides, a *cis*-2,3-diol, as in methyl α -D-mannopyranoside, can give rise preferentially to a product (**52**) that contains an even more reactive, five-membered ring diol, causing an overall, rapid uptake of a second mole per mole. This second step is clearly evident in lead tetraacetate oxidations because the formic ester **51** is formed,⁵⁷ and not **44**. Favored cleavage at C-3-C-4, such as would be expected with methyl α -D-galactopyranoside, results in a much slower reduction of the second mole per mole. In this instance, the initial cleavage product **53** cannot develop so reactive a type of *vic*-diol as is present in **52**. However, as shown by the fact that the D-galactoside also gives **51** in high yield, reaction probably proceeds⁵⁷ largely via the hemiacetal **54**.

**52****53****54**

An understanding of the oxidation characteristics of hexopyranosides has been useful in establishing the stereochemistry of 1,5-anhydrohexitols.^{215,216}

A diversity of conformational and configurational effects on the rate of periodate oxidation is evident with bicyclic derivatives of glycosides.^{116,117} In a series of 4,6-*O*-benzylidene aldohexopyranosides, for example, the higher reactivity associated with an *a,e* over an *e,e* orientation for the diol grouping is much enhanced when there is greater ring flexibility, as when the ring junction is *cis* (not *trans*). Also, since the inertness of a D-*altro* isomer (see Section II.4) can be attributed to a diaxial 2,3-diol grouping (and, hence, a $^4C_1(D)$ conformation), the observed oxidation of a *cis*-fused D-*ido* isomer implied a $^1C_4(D)$ conformation and a diequatorial 2,3-diol grouping in the latter. Anomeric configurational influences are more evident in compounds of this class¹¹⁶ than among the parent glycosides.^{44,215} Rate constants for the α anomers of the pairs examined are greater than those for the β anomers; this was attributed to greater steric interference with formation of the 2,3-periodate complex by an equatorial C-1 substituent.¹¹⁶ Conformational information was also deduced from the observation that

2,6-anhydro-1-deoxy-1,1-bis(ethylsulfonyl)-D-allitol undergoes rapid oxidation by periodate; the ${}^4C_1(D)$ conformation, which would have the *a,e,a*-triol arrangement and, thus, would complex rather than oxidize, was logically excluded as a major contributor to the conformational equilibrium.²¹⁷

The fact that a glycopyranoside consumes more oxidant than a glycofuranoside, and also that it yields formic acid, finds routine application for the determination of ring size, not only of glycosides but of related classes of compounds. For example, a ready confirmation of the furanoid structure of the D-ribosyl group in uridine and cytidine was afforded by the observation^{203,218} that only 1 mol of periodate per mole is consumed; thymidine and 2'-deoxycytidine, however, consume no oxidant, which is consistent with the presence of a furanoid ring in these 2'-deoxynucleosides.²¹⁹ Periodate oxidation of adenosine monophosphate has also received attention,²²⁰ oxidized adenosine triphosphate has been utilized²²¹ as a potential affinity-labeling reagent, and modification of the 3' terminus of t-RNA has been described.²²²

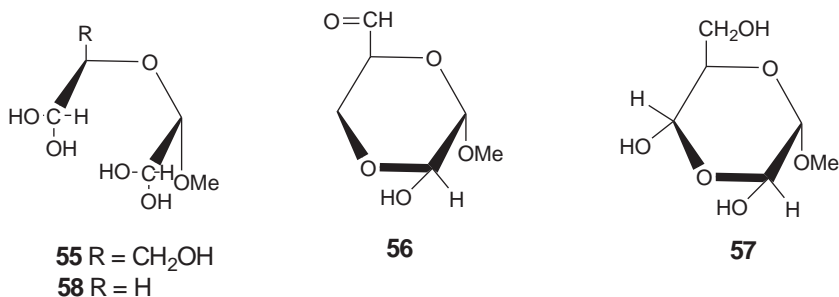
Successful applications of these criteria of ring size (as well as of the production of formaldehyde) to naturally occurring glycosides, oligosaccharides, and polysaccharides (see Sections V.3 and VI) are numerous. Although a number of interesting examples are found in the chemistry of C-glycosyl compounds, results in this series are sometimes inconclusive.^{61,223}

A glycosyl residue of a disaccharide or higher oligosaccharide usually shows oxidation behavior similar to that of a structurally related simple glycoside. This fact has been utilized in determining the sequence of linkages in solanose.²²⁴ Also, it is sometimes possible to open one particular glycosyl ring selectively in the presence of others in the same molecule. For example, the D-galactopyranosyl residues of raffinose and stachyose are oxidized by periodate much faster than the D-glucopyranosyl and L-fructofuranosyl groups of these oligosaccharides. This behavior permits selective removal of the oxidized fragments from the intact sucrose moiety.²²⁵ The components of sucrose itself also show widely different reactivities, just as do other glycosides of D-glucopyranose and D-fructofuranose. Lead tetraacetate selectively oxidizes the D-fructofuranosyl group, whereas periodate attacks the D-glucopyranosyl group much more readily, affording two different partially oxidized sucroses.²²⁶ The course of periodate oxidation of sucrose has been the subject of a high-performance liquid chromatographic study.²²⁷

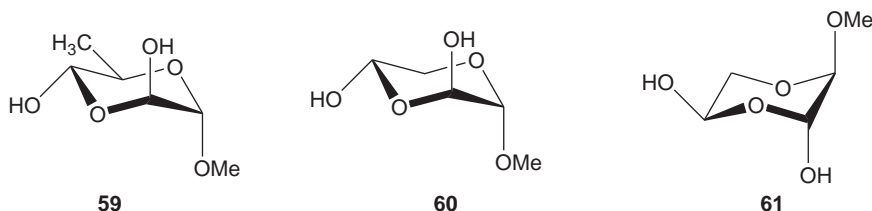
2. Dialdehydes

Dialdehydes formed by glycol cleavage of glycosides and other cyclic derivatives are capable of existing in a variety of modifications, depending on such

conditions as the solvent and the type of reaction to which they are subjected.²²⁸ In water they may exist^{228,229} as a hydrated, acyclic dialdehyde (such as **55**), as an internal hemiacetal (**56**), or as a hemialdal²³⁰ (**57**); further cyclization of **56** is also possible in principle (see **28**, Section II.4). Formation of **57** requires the addition of the elements of a molecule of water,²³¹ and this has been envisaged²²⁸ as proceeding via formation of an intermediate monoaldehydic *gem*-diol.



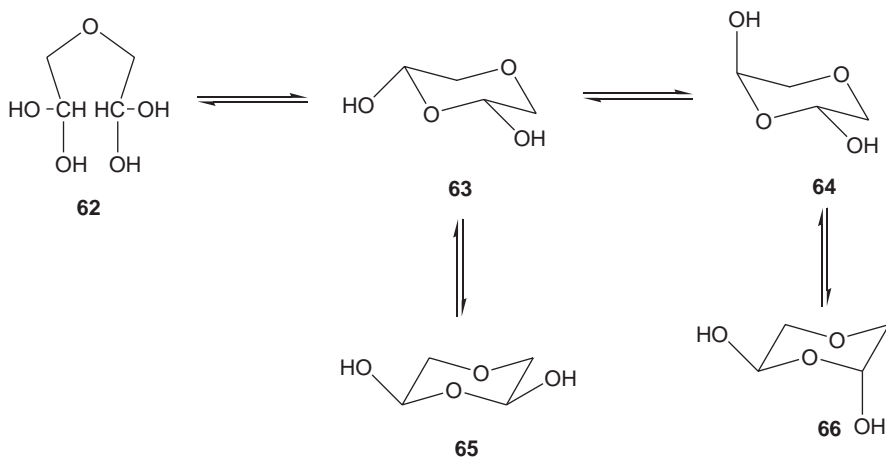
Relatively few dialdehydes are thus far known in crystalline form. The first reported example is that obtained from methyl α -L-rhamnopyranoside and related 6-deoxyglycosides;¹⁹⁷ its elemental composition is that of a dialdehyde monohydrate, but it has been found to possess a hemialdal structure, as it affords a bis(*p*-nitrobenzoate) and a dimethyl ether.²³² Similarly, NMR spectroscopy showed that the compound contains two hydroxyl groups, and indicated that it exists almost exclusively as **59** in solution.²³³



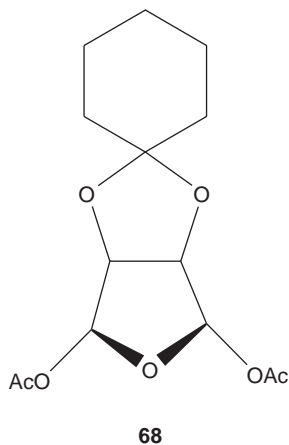
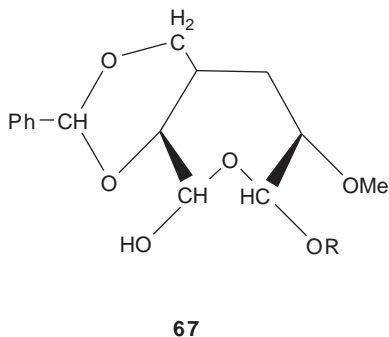
For similar reasons, the dialdehydes **48** and **49**, obtained from methyl aldopentopyranosides or aldotetrofuranosides, can be formulated as hemialdals;^{228,234,235} according to NMR-spectral data, however, they exist in deuterium oxide as an almost 1:1 mixture of the acyclic hydrated dialdehyde (**58** or as the β anomer) and the hemialdal (**60** or **61**) form. They occur almost exclusively as the latter form in such solvents as dimethyl sulfoxide or pyridine.²³⁵

The acyclic, hydrated form of the dialdehyde **62**, prepared from a 1,5-anhydropentitol (or 1,4-anhydrotetritol), is also moderately stable in aqueous solution, in which it exists in equilibrium with two hemialdal forms.²³³ One hemialdal contains equatorially attached hydroxyl groups and may be represented

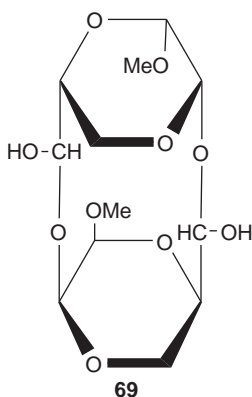
as an equilibrium between **63** and **65**, whereas the other contains one equatorial and one axial hydroxyl group, and is depicted by the interconverting chair forms **64** and **66**. As was found with **55**, the acyclic form is displaced by the hemialdals in dimethyl sulfoxide or pyridine.



A seven-membered (1,4-dioxepan) ring hemialdal (**67**) is obtained by glycol cleavage of the 2,3-diol group of methyl 4,6-*O*-benzylidene- α -D-aldohexopyranosides.^{236–239} In common with other hemialdals, this compound is readily converted into a monoalcoholate,²³⁸ the favored location of the alkoxy group being shown (**67**, R = alkyl).²³⁹ Oxidation of 1,2-*O*-cyclohexylidene-*myo*-inositol yields an *erythro*-tetrodialdose derivative that appears to exist as a mixture of isomeric, five-membered ring hemialdals. A crystalline hemialdal diacetate, prepared in high yield, was shown²⁴⁰ to have structure **68**.



Dialdehydes obtained from methyl aldohexopyranosides and aldopentofuranosides (for instance, **44**, which is formed from α -D anomers) may assume a multitude of structures, because, in addition to acyclic and hemialdal forms, they can exist as internal hemiacetals.^{145,234,241–245} A crystalline, dimeric form of the latter type (**69**) has been characterized.²⁴⁵ However, **44** affords different derivatives that represent various types of structures, and that can, therefore, best be described as a complex, equilibrium mixture of several forms.²²⁸ Condensation of the dialdehyde derived from uridine with benzoylhydrazine effected replacement of the bridging oxygen atom of the hemialdal unit to afford an *N*-benzamidomorpholine derivative.²⁴⁶



Several other classes of dialdehydes prepared from monosaccharide derivatives, oligosaccharides, and polysaccharides are known.²²⁸ A good deal of early interest centered on those obtained by periodate oxidation of cellulose and starch,^{155,156,158,247–253} as they constitute a novel, chemically modified form of these important polymers; the dialdehydic polymers themselves were later developed as materials of commercial significance.^{228,254} Isolated examples of marginal activity in tests for inhibition of carcinoma have been reported for some periodate-oxidized derivatives.²⁵⁵ In addition, dialdehydes are starting materials for several useful syntheses; a particularly fruitful example is the synthesis of 3-amino-3-deoxyaldo-pentoses and -hexoses, in which the recyclization step is achieved by condensing both the carbonyl groups with *one* molecule of nitromethane.²⁵⁶ A related type of application is the synthesis of 1,4-oxathianes, which involves incorporation of a sulfur atom into the ring generated from the glycol-cleavage product.²⁵⁷

V. REDUCING SUGARS

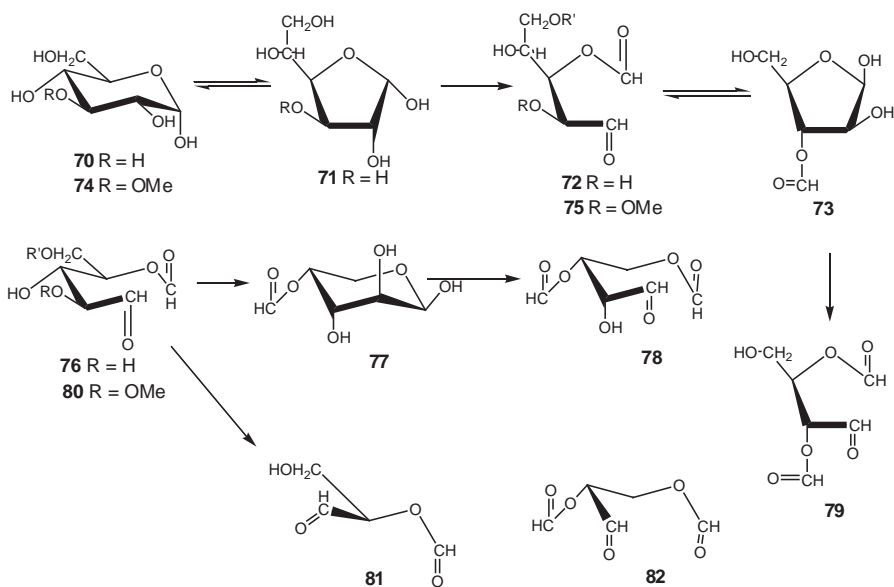
1. Introduction

In the oxidation of reducing sugars, periodate and lead tetraacetate frequently act quite differently. These differences can usually be traced to differences in the rates of oxidation of (*a*) the various forms that the sugars assume in solution and (*b*) the intermediates produced by the initial cleavage. In turn, these differences may arise merely because each of the oxidants is not normally used in the same solvent system as the other. Because anomeric pyranose–furanose ring–aldehyde interconversions are faster in acetic acid than scissions of most kinds of *vic*-diols, a straightforward, overall course of reaction is sustained for most sugars; in water, however, the rates of oxidation often sufficiently exceed the rates of the tautomeric changes to produce a markedly different, sometimes highly complex outcome.

2. Monosaccharides and Partially Substituted Derivatives

The sugars behave as their cyclic forms toward lead tetraacetate in acetic acid.^{46,48,149} Oxidation primarily involves α -hydroxy hemiacetal groups, and it results in stepwise shortening of the carbon chain.^{46,149,258,259†} For example, the reaction of D-glucose (**70**) may be depicted as follows: (*a*) initial cleavage at the C-1–C-2 bond of α -D-glucofuranose (**71**) yields 3-*O*-formyl-D-arabinose (**72**), which, as the β -furanose (**73**), (*b*) is degraded to 2,3-di-*O*-formyl-D-erythrose (**79**); consecutively, steps (*a*) and (*b*) are much faster than the rate of oxidation of either α - or β -D-glucopyranose. Also, oxidation of 3-*O*-formyl-*aldehyde*-D-arabinose (**72**) is a slower process than its cyclization to a particularly reactive ring form, such as **73**. Hence, several equilibrium displacements are maintained in this sequence in such a way that the reaction follows essentially a single pathway. Of the two steps (*a*) and (*b*), the former is implicated as rate controlling by the fact that changes in the concentration of reactants have relatively little effect on the oxidation rate.⁴⁶

† Attack on the hydroxy hemiacetal should be favored by stabilizing the developing carbonyl group in the transition state through electron release from the ring-oxygen atom,⁶ and also by steric factors.^{5,46}



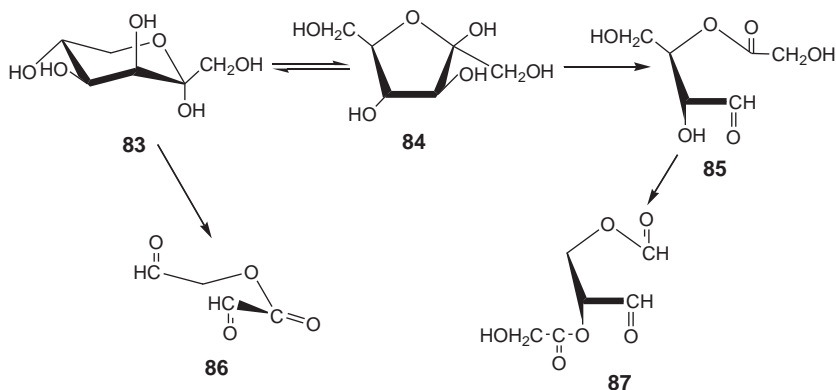
By contrast, periodate oxidation of D-glucose in water is initiated chiefly at C-1-C-2 of the pyranose form.^{99,260-264} As anomerization is relatively slow, both anomers are oxidized, but the β anomer is the less reactive and is also cleaved at other glycol groupings.⁹⁹ Intermediate products, such as 4-*O*-formyl-D-arabinose (**76**), are probably oxidized faster than they can cyclize. The overall result is a moderately good yield of 2-*O*-formyl-D-glyceraldehyde (**81**) when 3 molar proportions of periodate are used,²⁶⁰⁻²⁶³ although this ester is accompanied by smaller quantities of the other products possible, which range from formaldehyde to pentoses, accompanied by unoxidized D-glucose.²⁶¹

These differences between the two oxidants and the influence of solvent are emphasized in the unimolar oxidation of 3-*O*-methyl-D-glucose (**74**), which, with each, yields a mono-*O*-formyl-2-*O*-methyl-D-arabinose,^{46,59,265} as shown most clearly⁵⁹ with 3-*O*-methyl-D-[5-²H]glucose, periodate cleavage gives the 4-formate (**80**, R' = H), whereas the 3-formate (**75**, R' = H) is produced by lead tetraacetate in acetic acid.⁴⁶⁻⁵⁹ That the solvent is probably the main differentiating factor was found on comparing the oxidation of the 6-trityl ether of **74** in acetic acid with that in benzene;⁴⁶ in the latter medium, cleavage of the 1,2-diol of the pyranose is fast enough to obviate a furanose pathway almost completely, and the main product is 4-*O*-formyl-2-*O*-methyl-5-*O*-trityl-D-arabinose (**80**, R' = Tr), whereas in acetic acid the 3-formate (**75**, R' = Tr) is obtained.

The oxidation of one mole of D-mannose by lead tetraacetate in acetic acid, which involves rapid reduction of 2.8 molar proportions of oxidant,^{46,149} is consistent with a principal reaction pathway in which the pyranose is degraded stepwise; 4-*O*-formyl-D-arabinose (**76** \leftrightarrow **77**) is produced first, further oxidized to 3,4-di-*O*-formyl-D-erythrose (**78**), and finally degraded to 2,3-di-*O*-formyl-D-glyceraldehyde (**82**). In aqueous periodate solution, however, cleavage at positions other than the α -hydroxy-hemiacetal grouping is a prominent reaction, and the behavior of D-mannose is very similar to that of D-glucose.⁹⁹

The other aldohexoses, and the aldopentoses, are characterized by rapid values of uptake of lead tetraacetate ranging between those for D-glucose and D-mannose.^{46,149} These intermediate levels of oxidation reflect differing proportions of furanose and pyranose pathways comprising the overall reactions. Periodate-oxidation data for these sugars are generally consistent with almost exclusive attack of pyranose forms, this behavior being most clearly evident with the aldopentoses.²⁶³ Glycol-cleavage characteristics have, to some extent, been correlated with conformational²⁵⁻²⁹ and configurational⁴⁶ properties of the sugars.

Gross differences between the action of periodate and lead tetraacetate are found also in the way in which they oxidize ketoses. The main pathway for periodate oxidation involves cleavage of the 1,2-diol to a glyoxylic ester^{49,263,266}—for instance, **86** from D-fructose (**83**); the latter probably reacts in the pyranose form, whereas results for L-sorbose are more compatible with oxidation of a furanose form.²⁶³ Lead tetraacetate cleaves the 2,3- α -hydroxy hemiacetal group almost exclusively, yielding a glycolic ester.^{43,149,267,268} The reaction pathway for D-fructose may be depicted¹⁴⁹ by the sequence: 2-fructofuranose (**84**) \rightarrow 3-*O*-glycolyl-D-erythrose (**85**) \rightarrow 3-*O*-formyl-2-*O*-glycolyl- β -D-glyceraldehyde (**87**). L-Sorbose gives the corresponding L-glyceraldehyde derivative,²⁶⁷ and D-*altro*-heptulose affords the diester of D-erythrose.⁴³



Even higher sugars that contain an exocyclic *vic*-diol or 1,2,3-triol group are specifically attacked by lead tetraacetate at the anomeric center and are degraded stepwise, as shown by the oxidation of heptoses,¹⁴⁹ an octose,¹⁴⁹ 2-octuloses,^{268,269} and a 2-nonulose.²⁷⁰ The products are always stable formates or formate-glycolates. Periodate, however, rapidly cleaves exocyclic diol groupings,²⁷¹ initiating a different overall reaction course that involves more-extensive oxidation.

As illustrated with 3-*O*-methyl-D-glucose (**74**), introduction of a substituent group at O-3 of an aldose results in a controlled, limited oxidation, regardless of the oxidant used.^{59,265,272–276} Similar behavior is shown by 3-amino-3-deoxyaldoses in which the amino group is acetylated or permethylated.¹¹⁴ A substituent on O-4 confines the oxidation largely to the 1,2,3-triol grouping. When an aldose contains a substituent on O-2, it is highly resistant toward lead tetraacetate^{272–276} and relatively unreactive toward periodate; however, with each of these classes of derivatives, the normal reaction may be obscured by the use of prolonged reaction periods or severe conditions (high concentration, elevated temperature). Nonselective oxidation, which is thus promoted, may be further accentuated by the formation of substituted tartronaldehydes as intermediate products (see Section II.3).

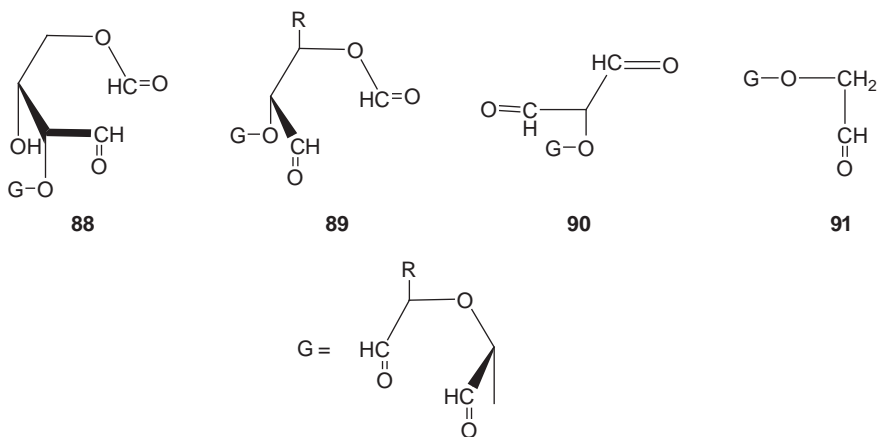
The course of oxidation is essentially unaltered^{275–278} for an aldohexose containing a substituent on O-6; however, a strongly electron-withdrawing group may promote overoxidation by destabilizing formic ester intermediates or by activating neighboring positions.^{263,277,278} Thus, although D-*erythro*- and D-*threo*-tetruronic acids can be prepared by lead tetraacetate oxidation of D-glucuronic acid and D-galacturonic acid, respectively, overoxidation is more pronounced than in the reaction of the corresponding aldohexoses.²⁷⁷ Similarly, D-erythrose 4-phosphate may be obtained by oxidizing D-glucose 6-phosphate with 2 molar proportions of lead tetraacetate,^{278,280} but the stoichiometry of these reactions is less precise than that for neutral derivatives, and the experimental conditions can exert a profound effect on the composition of the products.^{149,278,288}

3. Oligosaccharides

The oxidation behavior of reducing oligosaccharides is essentially a combination of the patterns exhibited by monosubstituted derivatives of monosaccharides and by glycosides. In general, these merged patterns are sufficiently characteristic under suitable conditions to permit unambiguous characterization

of oligosaccharides. Ideally, reducing disaccharides containing aldopentopyranose ($R = H$ in **88**, **89**, and **G**) or aldohexopyranose ($R = CH_2OH$ in **88**, **89** and **G**) residues show the following characteristics (moles per mole):^{6,73,275,276,280-287§}

- (a) A (1→3)-linked disaccharide is converted into **88** with concomitant reduction of 3 mol of oxidant and liberation of 1 mol of formic acid.
- (b) A (1→4)-linked disaccharide is degraded to **89**, which is accompanied by reduction of 4 mol of oxidant and liberation of 2 mol of formic acid; in the lead tetraacetate oxidation of a hexose (reducing) residue C-1 and C-2 both become formic ester groups, and only one equivalent of free acid is obtained.
- (c) A (1→2)-linked disaccharide is degraded to **90**. Again, the (nonreducing) glycosyl group consumes 2 mol of oxidant, and yields 1 mol of formic acid; the reducing residue consumes 2 mol (pentose) or 3 mol (hexose) of oxidant, and yields 1 mol (pentose) or 2 mol (hexose) of formic acid, *plus* 1 mol of formaldehyde from the primary alcohol group. When the reducing residue is that of a 6-deoxyaldohexose, acetaldehyde is liberated.²⁸⁵
- (d) A (1→5)-linked (pentose) or (1→6)-linked (hexose) disaccharide is degraded to **91**, the uptake of oxidant being 5, or 6 moles, and the yield of formic acid four, or 5 mol, respectively.²⁸⁸



§ These characteristics are observed when lead tetraacetate is used in aqueous acetic acid containing potassium acetate.^{275,276} In glacial acetic acid, the degree of oxidation is much lower, because the reaction of the reducing glucose residues resembles that of monosaccharides (Section V.2).²⁷⁶

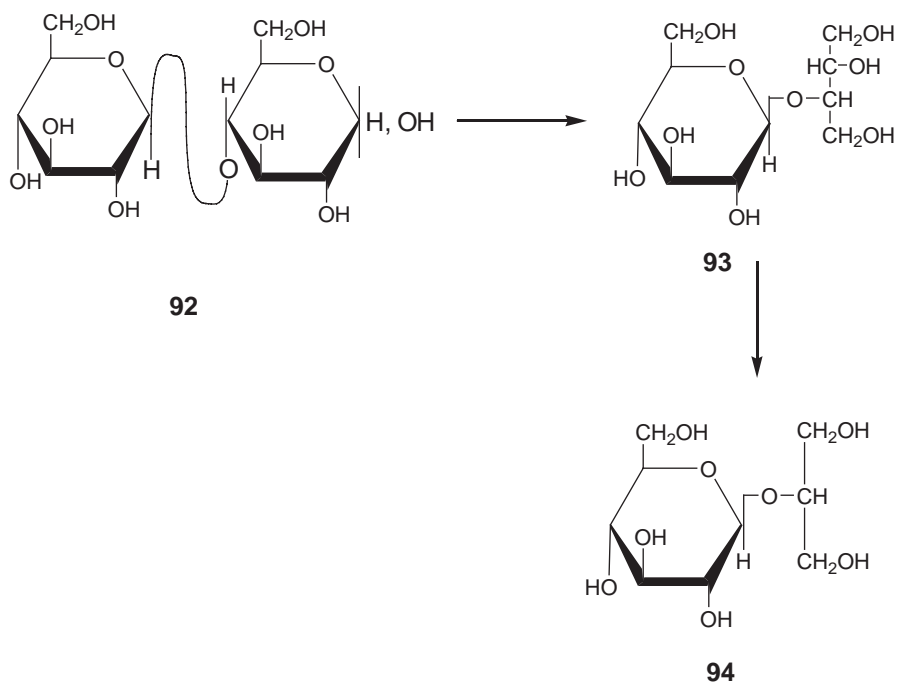
In practice, the results obtained are frequently complicated by overoxidation²⁸⁸ (see Section II.3). An early, comparative study of the periodate oxidation of maltose and isomaltose showed that the latter, a (1 → 6)-linked disaccharide, behaves as outlined in category (*d*), whereas the (1 → 4)-linked biose is extensively overoxidized; this information, however, differentiated between the two possible structures.²⁸³ The overoxidation phase can be attributed to hydrolysis of the formate group of **89**, followed by cleavage of a 2-substituted tartronaldehyde, which is subject to further attack. Similarly, the ester group of product **88** can suffer hydrolysis, resulting in a spuriously high uptake of oxidant by (1 → 3)-linked disaccharides; however, overoxidation is minimized when conditions are such (low temperature, controlled pH, low concentration of oxidant) as to stabilize formate groups. Because these groups are relatively stable in acetic acid, and because their formation is promoted under the reaction conditions, overoxidation of disaccharides is usually not a serious problem when lead tetraacetate is used.^{206,275} When the 4-substituted reducing residue is a pentose, the product **89** is a glyceraldehyde moiety, which is not degraded further. The (1 → 2)-linked disaccharide is disposed toward overoxidation under all conditions because it yields a tartronaldehyde derivative (**90**) directly; this can be prevented, however, by initial reduction to the alditol, which is oxidized instead to a stable, 2-substituted glyceraldehyde.^{206,285} Another approach for preventing the overoxidation of reducing oligosaccharides is by their conversion into 1,5-anhydroalditol derivatives.²⁸⁹

With disaccharides containing a 2-amino-2-deoxyaldose reducing residue, it is advantageous to use the *N*-acetyl derivative,^{290,291} or perhaps better still, the *N*-acetyl derivative of the disaccharide alditol. The oxidation products formed from these derivatives do not become significantly overoxidized, and hence, the stoichiometry of the reaction is clearly indicative of the linkage position.^{288–291} For example, 1 mol of a 3-substituted 2-amino-2-deoxyaldohexose residue consumes 2 mol of oxidant, and yields 1 mol each of formic acid and formaldehyde, whereas the 4-substituted isomer consumes only 1 mol of oxidant and releases only 1 mol of formaldehyde.²⁹⁰

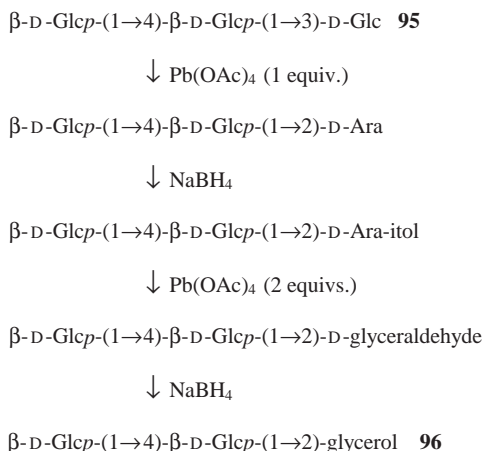
Oxidations involving 2-ketose or glycuronic acid residues also give satisfactory data,²⁰⁶ the acids being best examined in the ester form²⁹² in order to minimize overoxidation. Periodate oxidation is effective as well for structural elucidation of sialic acid-containing oligosaccharides.²⁹³

A different approach to structural elucidation—namely, selective degradation—is made possible by the markedly disparate rates at which units may be oxidized;^{117,161,220,221} usually, the reducing residue is the most susceptible. For example, treatment of cellobiose (**92**) with 2 mol of lead tetraacetate per mole

yields 3,4-di-*O*-formyl-2-*O*- β -D-glucopyranosyl-D-erythrose, which has been characterized²⁰⁶ by conversion into 2-*O*- β -D-glucopyranosyl-D-erythritol (**93**). A further, selective attack is feasible owing to the fact that the alditol residue of **93** is oxidized more rapidly than the glycosyl group. Hence, treatment of **93** with 1 M proportion of oxidant produces 2-*O*- β -D-glucopyranosyl-L-glyceraldehyde, which is readily characterized²⁰⁶ by reduction to 2-*O*- β -D-glucopyranosylglycerol (**94**). The same sequence of reactions converts maltose into the anomer of **94**, 2-*O*- α -D-glucopyranosylglycerol,²⁷⁶ and related sequences afforded anomeric pairs of 2-*O*-glycosylglycerols containing D-galactosyl, D-mannosyl, *Q*-xylosyl, and L-arabinosyl groups.²⁷⁶⁻²⁹⁴ Such compounds served as reference materials for establishing the configuration of a number of disaccharides (prior to the advent of NMR spectroscopy).

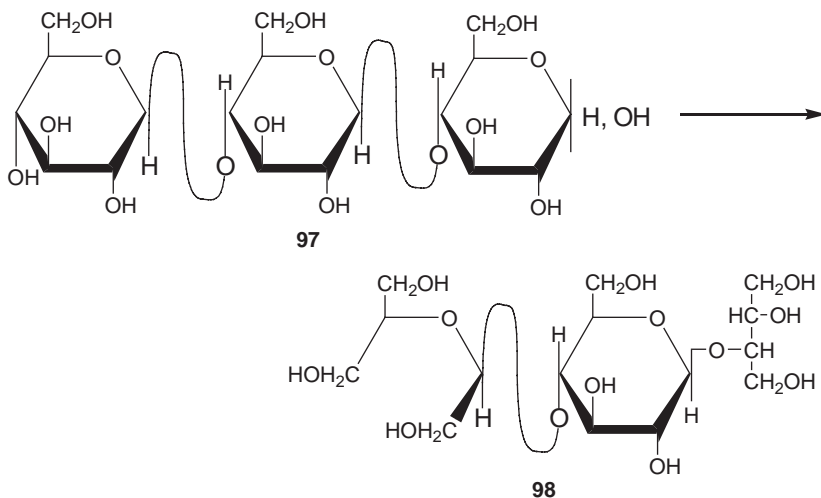


This degradative technique is also applicable to higher oligosaccharides. For example, a trisaccharide was characterized as *O*- β -D-glucopyranosyl-(1 \rightarrow 4)-*O*- β -D-glucopyranosyl-(1 \rightarrow 3)-D-glucose (**95**) by the following series of reactions.²⁹⁵

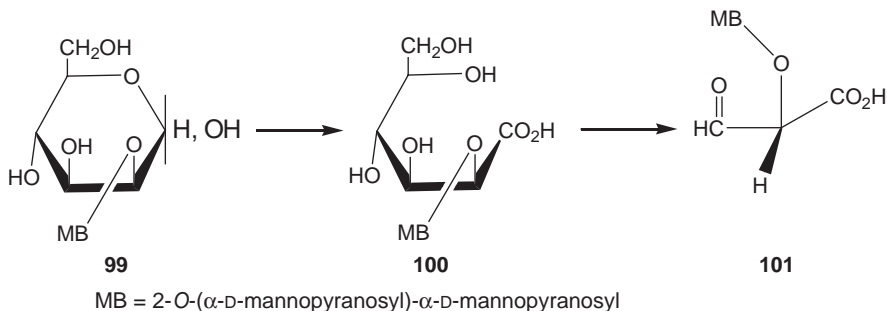


Identification of the final product as 2-*O*- β -cellobiosylglycerol (**96**) was accomplished by comparing it with **96** prepared from cellotriose by the sequence used for degradation of **92** to **94**. Similarly, the tetrasaccharide *O*- β -D-glucopyranosyl-(1 \rightarrow 4)-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)-*O*- β -D-glucopyranosyl-(1 \rightarrow 3)-D-glucose was identified by its conversion into 2-*O*- β -cellotriosylglycerol by the same sequence of reactions as used to degrade **95** to **96**. As the cellotriosylglycerol is also obtainable from authentic cellotetraose by selective degradation (as in **92–94**), the linkage positions and anomeric configurations for the tetrasaccharide were established simultaneously.²⁹⁵

Glycol-cleavage oxidation may sometimes provide a means for preferentially removing the nonreducing group of a trisaccharide or higher oligosaccharide;^{225,296,297} this can be achieved when the end group is more readily oxidizable than the internal residues, a situation that is frequently encountered.¹⁵⁰ For example, oxidation of cellotriose (**97**) with 4 molar equivalents of lead tetraacetate, reduction, and partial hydrolysis^{298,299} of the resultant polyhydric alcohol acetal **98** with acid affords²⁹⁵ **93**. In the same way, β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 4)-D-glucose was degraded to 2-*O*- β -cellobiosyl-D-erythritol (also prepared from cellotriose).²⁹⁵ Selective removal of the nonreducing group from trisaccharides was also achieved in other series^{297,300,301} (sometimes by the removal of the initially produced dialdehyde unit through the action of phenylhydrazine or alkali^{225,296,297}), illustrating the general utility of this approach for structural studies.



The reducing residue of the trisaccharide α -D-mannopyranosyl-(1 \rightarrow 2)- α -D-mannopyranosyl-(1 \rightarrow 2)-D-mannose (**99**) was selectively detached after lead tetraacetate oxidation;²⁹⁷ the nonreducing group was converted (via **100**, in several steps) into the 2-substituted triouronic acid **101**, which was hydrolyzed with dilute acid to 2-*O*- α -D-mannopyranosyl-D-mannose. Another example involves degradation of manninotriose to 6-*O*- α -D-galactopyranosyl-D-galactose by oxidation with 2 M proportions of lead tetraacetate and subsequent alkaline hydrolysis of the 4-substituted L-erythrose.³⁰² Another procedure³⁰³ for removing the reducing residue of oligosaccharides, for example a trisaccharide, consists of reduction to the alditol, selective oxidation by lead tetraacetate of a *threo* diol (see Section III) in the acyclic component (analogous to **100** \rightarrow **101**), and then removal of the 2-substituted aldehydic fragment with hydrazine to give the disaccharide hydrazone, from which the reducing disaccharide is generated.



Mixed detecting reagents containing periodate are used in chromatographic analysis of oligosaccharides and polysaccharides. Periodate combined with alkaline silver nitrate is employed to detect nonreducing sugars³⁰⁴ as well as glycoproteins,³⁰⁵ and a periodate–Schiff reagent mixture is reported to produce specific color reactions from which linkage positions may be determined.³⁰⁶ Much structural and biochemical work related to 3-deoxy-D-manno-2-octulosonic acid (Kdo) is based on assays with periodate–thiobarbituric acid, in which malonaldehyde is an intermediate.^{307,308}

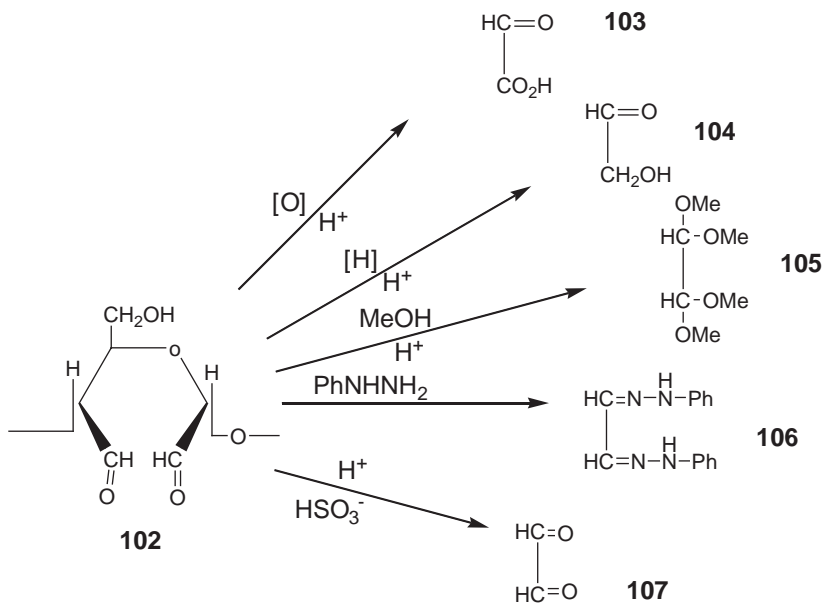
VI. POLYSACCHARIDES

1. Oxidation Patterns

Periodate oxidation is a standard method⁷ for determining various structural features of polysaccharides; its earliest applications helped define fundamental structures for cellulose,^{155,156,247–249,252,253} starch,^{156,251,309–311} glycogen,^{54,55,312–314} and xylan.^{249,315} It has been used to examine virtually every polysaccharide that has been studied, usually in conjunction with classical methylation procedures and NMR spectroscopy. A standard method for structural analysis of glycoproteins involves treatment with alkaline borohydride, which cleaves the protein–carbohydrate linkage and releases the latter as a mixture of oligosaccharide alditols. The latter can then be separated chromatographically and be subjected to the periodate oxidation, methylation, and NMR analysis procedures just mentioned, together with additional characterization of fragments by fast atom bombardment mass spectrometry.^{316,317}

Characteristic patterns of oxidation are readily recognized for glycosyl residues that are joined by different types of linkage. An aldohexopyranosyl residue bonded to adjacent residues through O-1 and O-4 reduces 1 molar proportion of periodate.¹⁵⁵ Bromine oxidation of the resulting dialdehyde (**102**), followed by acid hydrolysis, yields D-erythronic and glyoxylic acids (**103**), showing that the C-2–C-3 bond is cleaved initially.²⁴⁹ Similarly, reduction of **102**, and acid hydrolysis, gives erythritol and glycolaldehyde (**104**).^{249,250,318} More-direct demonstrations of the cleavage point in **102** are afforded by methanolysis, which converts¹⁵⁶ the glyoxal residue into the volatile tetramethyl acetal **105**, or by treatment with phenylhydrazine, which affords glyoxal bis(phenylhydrazone) (**106**) and tetralose phenylosazone (see Section VI.2).³¹⁹ Although the dialdehyde is relatively resistant to aqueous mineral acid, glyoxal (**107**) and D-erythrose are readily liberated by

hydrolysis with sulfurous acid,³²⁰ and their corresponding dithioacetals also may be obtained.³²¹ Additionally, **102** undergoes facile β -elimination in the presence of alkali, a property that largely accounts for the marked alkali lability of periodate-oxidized cellulose and starch.³²²⁻³²⁵ Several structures (of the kind considered in Sections II.4 and IV.2 for dialdehydes of low molecular weight) may be proposed for **102**, but the polymeric structure can also accommodate hemiacetal cross-linked to an adjacent glycosyl residue or polymer molecule^{157-162,228,229} (see Section II.4).



A (1 \rightarrow 2)-linked aldohexopyranosyl residue also consumes 1 mol of periodate per mole of residues. However, this type of linkage is readily distinguished from the (1 \rightarrow 4) by the fact that fragmentation of the dialdehyde by the various reactions applied to **102** affords two 3-carbon fragments, not a 2- and a 4-carbon fragment.

In the aldopentopyranosyl series, (1 \rightarrow 4)- and (1 \rightarrow 2)-linked residues also reduce only one mole of oxidant per mole, but the liberated products of cleavage are characteristically different; therefore, examination of the resultant dialdehydes, or of products derived from them, is necessary for differentiation between these two possibilities.

A (1 → 3)-linked hexopyranosyl residue contains no *vic*-diol grouping and accordingly is not oxidized.

When the linkage to an aldohexopyranosyl residue is through O-6, three contiguous hydroxyl groups are available for oxidation. Consequently, such a residue is distinguishable from the others considered, as it consumes two molecules of oxidant and releases one molecule of formic acid per residue that reacts. A detailed kinetic analysis of such systems has been presented.³²⁶

Although the stoichiometry of these oxidations is independent of the configuration, the rates of reaction differ widely.³²⁷⁻³²⁹ In the (1 → 4)-linked D-*gluco* series, for example, α anomers reduce periodate much faster than β anomers. In this respect, their behavior is related, not to that of simple glycosides (which show only small differences in the rate between the anomers), but to that of conformationally rigid, fused-ring derivatives of the glycosides.¹¹⁶ Within a given group, there may be large differences in the response to oxidation, indicative of variations in fine structure (as seen with amylopectins); glycogens, however, show remarkably uniform behavior. The (1 → 3)-linked residues in nigeran or oat glucan, which are themselves unoxidized, appear³²⁷ to suppress the reactivity of the (1 → 4) residues present. For nigeran,³³⁰ this effect is attributable³²⁷ to inter-residual hemiacetal formation (see Section III.4); an associated departure from second-order oxidation kinetics is a reflection of the fact that the 4-linked residues occur mainly as isolated singlets. As would be expected, residues having the *manno* configuration (2,3-*cis*-diol) oxidize at much higher rates than their *gluco* epimers.

Periodate in aqueous perchloric acid oxidizes the glycuronic acid residues of glycosaminoglycuronans with sufficient selectivity that the reagent has been proposed³³¹ for histochemical classification. Several other histochemical applications of periodate for the detection of carbohydrates^{332,333} and sialic acids specifically,³³⁴ have been described.

Under proper conditions, periodate oxidation of cellulose affords products rich in carboxylic acid groups;³³⁵ evaluation of the carboxyl content of oxycellulose can be complicated by processes of lactonization and by the presence of acidic, enediol groupings.³³⁶

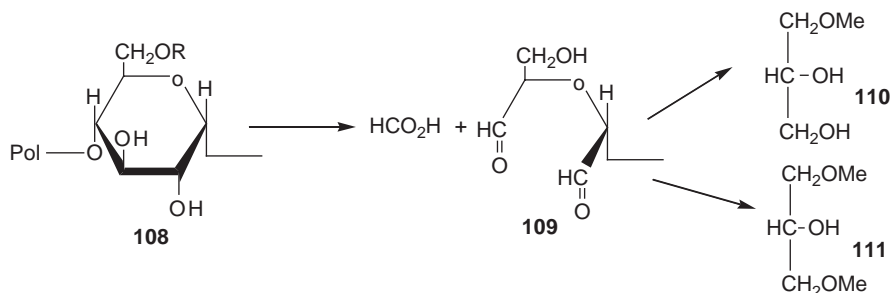
Lead tetraacetate has been used little for oxidation of polysaccharides.⁶ Its ineffectiveness for this purpose probably stems mainly from the fact that the organic media commonly employed for the reagent are unable to dissolve polysaccharides. However, the use of dimethyl sulfoxide (containing about 10% of acetic acid), a good solvent for many polysaccharides, has permitted satisfactory

oxidations to be conducted with lead tetraacetate.³³⁷ Data thus obtained generally correspond closely to those obtained by periodate oxidation; however, the reaction rates are generally higher, and a more satisfactory recovery of the oxidized product appears to be feasible when dimethyl sulfoxide–acetic acid is the solvent.³³⁷

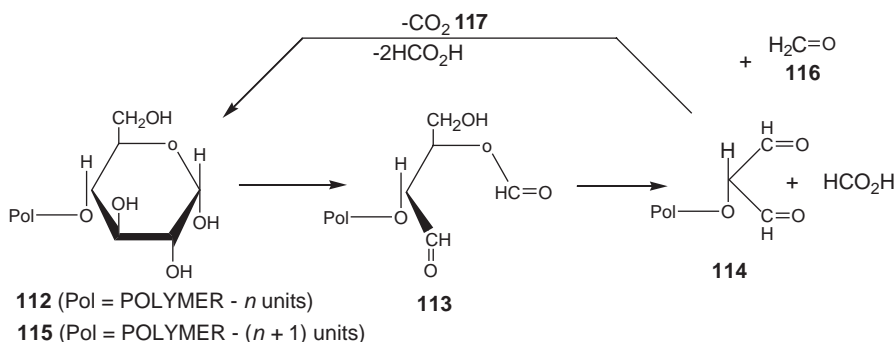
Such polysaccharides as cellulose and amylose serve as ideal precursors for the preparation of novel, stereoregular, polymers through glycol-cleavage oxidation, and then reduction. Thus, cellulose affords^{338,339} a macromolecule based on 2,3-*O*-(2-hydroxyethylidene)erythritol residues. An improved procedure for the preparation of polymers of this class consists³⁴⁰ of stepwise oxidation and reduction, which minimizes interference from intra-residual hemiacetal formation on the periodate reaction. Applied to cyclodextrins, the combination of oxidation–reduction gives rise³⁴¹ to macro crown-ether-like compounds. The conversion of starch³⁴² and maltodextrin³⁴³ dialdehydes into the corresponding poly(dicarboxylic) acids, affords polymers that strongly complex calcium ions. Alternatively, by reductive animation of dextran dialdehydes, materials suitable as polymer supports for antitumor drugs are obtained.³⁴⁴

2. End-Group Analysis

Nonreducing groups of polysaccharides, either in the aldo-pento- or -hexopyranose series (for instance, **108**, R = H) are oxidized by 2 mol of periodate per mole, yielding formic acid. This phenomenon provides the basis for a widely exploited method of estimating molecular weight;⁵⁴ similarly, branched polysaccharides yield formic acid in proportion to the ratio of terminal to nonterminal residues in the average repeating unit. The method has been of particular value in comparing various samples of glycogen,^{54,55,312,345–347} amylopectin,^{348,349} and dextran.^{350–352} Application of this end-group analysis requires, however, a knowledge of the amount of formic acid released from other structural components of the molecule. A (1 → 6)-linked hexopyranosyloxy residue (such as **108**, R = a glycol group) is one such additional source of formic acid requiring independent characterization, possibly by reduction of the dialdehyde **109** (R = a glycosyl group) and methylation of the derived polyhydric alcohol;³⁵³ subsequent hydrolysis of the latter affords 1-*O*-methyl-D-glycerol (**110**), whereas the oxidized end group (**109**, R = H) simultaneously affords 1,3-di-*O*-methylglycerol (**111**).



Formic acid is also produced from the reducing residue (as in the oxidation of **112** to **113**) in much the same way as for oligosaccharides (see Section V.3). The attendant problem of overoxidation is, therefore, encountered in oxidation of polysaccharides when the malonaldehyde-derived structures (**114**) are a product of the reaction of the reducing residue. Because the other residues are oxidized relatively slowly, overoxidation can proceed in the interim, exposing a succession of new, reducing residues (such as **115**) as the degradation proceeds along the chain. Because the release of formaldehyde (**116**) and carbon dioxide (**117**) during these sequential steps approximates a reaction of zero order, overoxidation occurs at a linear rate, which can be corrected for by back-extrapolating the rate plot of the acid produced.



The amount of formaldehyde (**116**) liberated^{288,354,355} provides a good index of the extent of overoxidation, because **116** is derived from the primary alcohol group of degraded, reducing residues (**112**–**114**). The rate at which the formaldehyde is

produced is proportional to the number of reducing residues,³⁵⁶ so that a measure of the rate of overoxidation may itself provide an independent estimate of the degree of polymerization. Similarly, if the oxidative erosion process is arrested by formation of a nonoxidizable fragment (for instance, ROCH_2CHO , which would be produced by a 6-linked aldohexosyl group), the yield of formaldehyde at that stage determines the location of the stable structure in the polysaccharide chain.^{354,355}

3. Fragmentation Analysis

As outlined in Section VI.1, the linkage position of an individual sugar residue may be determined when glycol-cleavage oxidation gives a dialdehyde that is convertible into recognizable fragments of the original polysaccharide. Dialdehyde **102**, for example, representing a 4-linked aldohexopyranosyl residue, gives a 2- and a 4-carbon fragment, whereas the dialdehyde obtained from the corresponding 2-linked residue yields two 3-carbon segments. By contrast, a residue that is (1→3) linked, or has no *vic*-diol group because of branching or the presence of an ester or ether substituent, or by deoxygenation, appears ultimately as an intact monosaccharide (or derivative). Consequently, polysaccharides can yield a wide variety of fragmentation products, ranging from a relatively few species for a highly stereoregular polymer, to a large array from one with a complex architecture. Overall, fragmentation analysis amounts to an alternative to classical methylation analysis.

Much attention has been given to finding efficient procedures for the dismantling of oxidized polysaccharides, and also to methods for the separation and characterization of product mixtures. Of the fragmentation procedures employed (see Section VI.1), that^{249,251,318} in which the dialdehyde is reduced, and the polyol formed is subjected to total acid hydrolysis, is the most widely applied. (Although the procedure is sometimes referred to as the “Smith degradation”, this term is more commonly reserved for *selective* hydrolysis of the polyol under milder acidic conditions (see Section VI.4.) In one variation,³⁵⁷ the dialdehyde is methylated prior to reduction with borohydride, which differentiates free hydroxyl groups in the oxidized polymer from those formed upon reduction of the aldehydes.

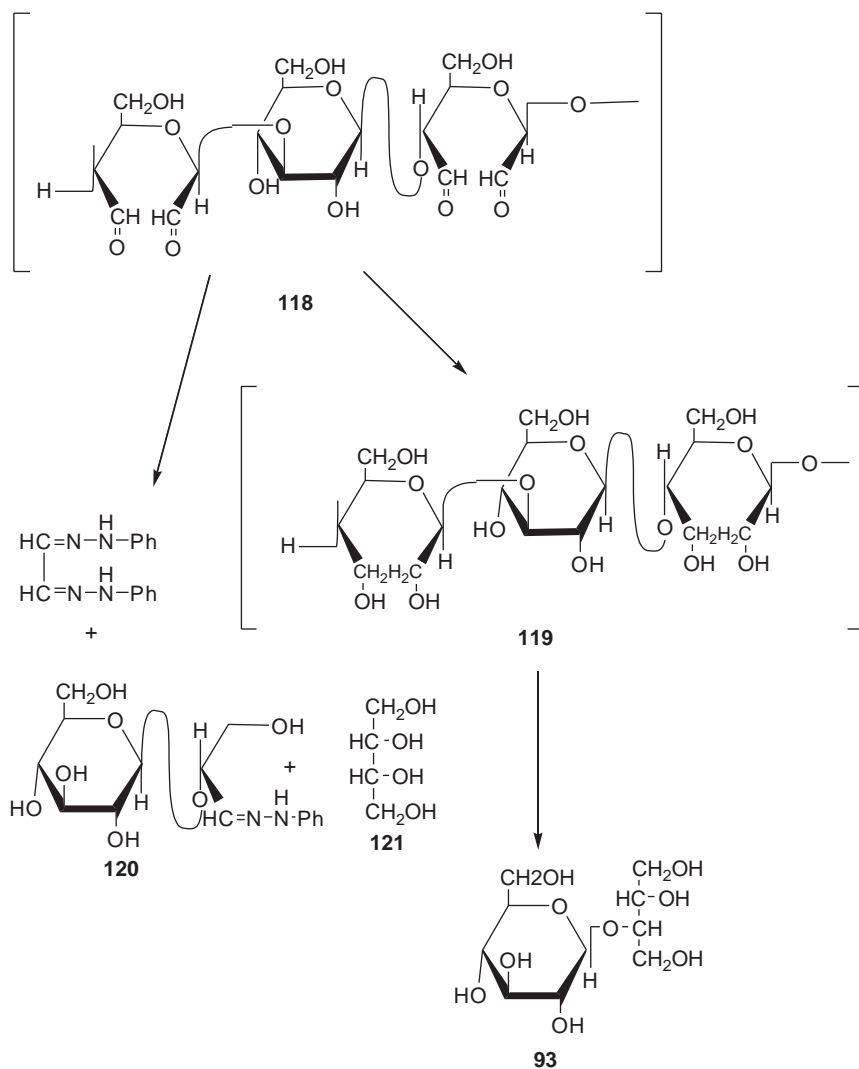
Various chromatographic methods are utilized for analysis of the fragmentation products. Of them, gas-liquid chromatography as per(trimethylsilyl) ethers,

under properly calibrated conditions, provides³⁵⁸ a quantitative measure. Enzymatic assay is also feasible for the determination of such products as glycerol^{359,360} and erythritol.³⁵⁹ A diminution in the possible number of fragments (various ring forms, anomers) was reported³⁶¹ to occur on oximation of the fragmentation products prior to etherification, and methanolysis prior to per(trimethylsilyl)ation has also been recommended.³⁶² Additional simplification derives^{363,364} from complete hydrolysis with acid, oximation, and forcing treatment with pyridine-acetic anhydride, which converts the fragmentation products into aldononitrile peracetates that are amenable to GLC analysis. Similarly, mixtures of peracetylated alditols, prepared by a sequence of oxidation, reduction, hydrolysis, a second reduction, and acetylation, are suitably analyzed by GLC (see for example Ref. 284). In some instances,³⁶⁵ high-performance liquid chromatography is effectively utilized. Irrespective of the procedural variation used, the identification of fractions separated chromatographically is greatly facilitated by mass spectrometric analysis,³⁶⁶ usually employed in tandem with a gas-liquid, or high-performance liquid chromatograph.

4. The Barry and Smith Regioselective Degradation Procedures

As noted in Section VI.3, polysaccharides may contain residues that do not have *vic*-diol groupings and hence are inert to glycol-cleaving oxidants. Degradation of the partially oxidized polymers by selective removal of oxidized residues then facilitates study of the resistant parts of the molecules. Likewise, deliberate treatment of a polysaccharide with less than the theoretical amount of oxidant, and recovery of the unoxidized portions, affords a means of fragmenting the polymer.

Selective removal of oxidized residues (as in **118**) may be effected in two principal ways: treatment with phenylhydrazine in hot, dilute acetic acid, according to Barry,³¹⁹ promotes rupture of the glycosidic bond with formation of glyoxal bis(phenylhydrazone) (**106**), but leaves the unoxidized residue (**120**) intact; alternatively, reduction of **118** converts the dialdehyde residues into polyhydric alcohols (**119**) which, being acetals, are hydrolyzed by the acid far faster than the glycopyranosyl group. The latter approach, developed by Smith and co-workers,^{298,299} is the more satisfactory of the two (experimentally) and is far more frequently applied, but both procedures have provided detailed insight into the fine structure of many highly complex polysaccharides.



An early application³⁶⁷ of the Barry procedure showed that snail galactan contains (1 \rightarrow 3) and (1 \rightarrow 6) linkages dispersed in a highly complex, dichotomous way. A succession of Barry degradations was applied, each degradation yielding a polymeric residue comprising only about half of the starting material, but still resembling the parent polysaccharide.

The value of the degradative approach is emphasized by the contrasting behavior of another type of (1 → 3), (1 → 6)-linked D-glucopolysaccharide.^{368,369} On the basis of the results of methylation analysis and periodate oxidation alone, the D-glucan could be regarded as structurally related to the snail galactan. However, degradation of the oxidized D-glucan afforded a polymeric residue that consumed very little periodate, and, hence, was affected little by a second degradation. Therefore, the (1 → 3) linkages are confined to a linear backbone of residues, whereas those of the (1 → 6) kind bind terminal glycosyl groups to the main chain.

Several other polysaccharides³⁷⁰⁻³⁷⁵ show a stepwise response similar to that of snail galactan, in that polymeric products decreasing in size were isolated from successive Barry degradations. In these polysaccharides also, the periodate-resistant residues are assembled without interruption over large regions of a highly ramified molecule.

The degradative methods readily differentiate between these types of polysaccharides and those in which the nonoxidized residues are distributed more uniformly. For example, a xylan (from *Rhodomenia*), known to contain about equal proportions of (1 → 3) and (1 → 4) linkages, yields no polymeric products but gives D-threo-pentulose phenylosazone, showing that the linkages alternate uniformly along the polymer chain.³⁷⁶ Similarly, nigeran was found to possess the same type of structure, but based on D-glucose; under the conditions of the Barry degradation it yielded D-arabino-hexulose phenylosazone,³⁷⁷ whereas it was degraded²⁹⁹ by the Smith method to 2-O-α-D-glucopyranosyl-D-erythritol (93). The arrangement of branching in beet arabinan and in certain arabinoxylans was determined in an analogous way. In these pentosans, the L-arabinofuranosyl residues are attached through O-3 (and sometimes O-2) of the residues in the main, pentosan chain, so that only residues constituting branch points are unoxidized by periodate. As the Barry degradation affords 3-O-α-L-arabinofuranosyl-1,3-dihydroxy-2-propanone phenylosazone from the arabinan, the branches of this polymer are on isolated arabinosyl residues;³⁷⁸ the oxidized arabinoxylans, however, afford osazones of xylose, xylobiose, and xylotriose,³⁷⁹ or, by the Smith procedure, 2-O-β-D-xylopyranosylglycerol and the corresponding di- and tri-D-xylosyl derivatives,³⁸⁰ showing that the arabinosyl branches occur variously on isolated, adjacent, and three consecutive xylosyl residues. Also in accord with these branching arrangements determined with both degradation procedures is the finding³⁷⁹ that controlled hydrolysis of periodate-oxidized wheat arabinoxylan released xylose, xylobiose, and xylotriose. That is, although dialdehydes are remarkably stable in mineral acids,^{381,382} they are readily hydrolyzed by sulfurous acid.³⁸³

Another example of the type of information obtainable is the degradation of a β -D-glucan from oat flour. Detection of 2-*O*- β -D-glucopyranosyl-D-erythritol (**93**) as a major product shows that (1 \rightarrow 3)- and (1 \rightarrow 4)-linked residues alternate, as in **118**. Erythritol (**121**) was another major product, which indicates that two (or more) adjacent, (1 \rightarrow 4)-linked D-glucosyl residues are also present. In addition, small proportions of 2-*O*- β -laminarabiosyl-D-erythritol and higher oligosaccharides of the series were formed, proving that the glucan contains occasional sequences of two, three, and more, consecutive, 3-*O*-substituted β -D-glucopyranosyl residues flanked by (1 \rightarrow 4) links.^{298,299}

A reduction in the yield of such principal products as **93** may occur²⁹⁹ during the hydrolysis step through the acid-catalyzed formation of acetals between the alditol moiety and the glycolaldehyde released, a side-reaction that appears to be more severe with α -glycans.³⁸⁴ However, this complication is avoidable³⁸⁵ by methylating the polyol before partial hydrolysis, because it blocks the hydroxyl groups that, otherwise, would be engaged in acetal formation.

Conditions for selective hydrolysis do not apply uniformly to all polyols because of variations in stability among their acetal structures. This has frequently meant³⁸⁴⁻³⁹¹ that the acid strength chosen was based on preliminary experiments. GLC methods³⁵⁸ and gel permeation chromatography³⁹² have been proposed as means of selecting optimum conditions for the release of glycosyl alditols and residual oligosaccharide or polysaccharide, and the rate at which formaldehyde is produced by periodate oxidation of the hydrolysis mixture offers³⁹² another diagnostic method. Methanolysis, rather than hydrolysis, has been advanced³⁹³ as an experimental variation that can reduce the amount of artifact formation associated with the use of aqueous acid.

Numerous examples further illustrate the great value of the Smith degradation in determinations of the fine structure of polysaccharides. They include studies on arabinoxylans,³⁸⁰ mesquite gum,³⁸⁶ an exocellular yeast mannan,³⁸⁷ and a type-specific bacterial polysaccharide.³⁹⁴ Branching patterns in complex types of glycoproteins from several different origins have been elucidated,³⁸⁹ and detailed structures of gum exudates,^{390,395,396} seed polysaccharides,³⁹⁷ and pectic substances,^{398,399} including the location of *O*-acetyl groups present,³⁹⁹ have been described. Information about sequences of residues in heparin^{400,401} and heparan sulfate,⁴⁰¹ and in dermatan sulfate, and chondroitin 6- and 4-sulfates,⁴⁰² has been obtained by combined application of selective oxidation with the Smith degradation, and alkaline β -elimination reactions of the polymer dialdehydes, to obtain periodate-resistant oligosaccharide segments.

Extensive kinetic data for periodate oxidation of a large group of polysaccharides, which indicate substantial rate differences for various types of residues in different locales, have been presented³²⁸ as a basis for effecting regioselective applications of the Smith degradation. Another type of variation on the classical procedure of Smith involves multiple degradations in series which, for example, has been utilized with the carcinoembryonic antigen glycoprotein,⁴⁰² as well as the stem bromelain glycopeptide.⁴⁰³ Often, both the mild acid hydrolysis conditions of the Smith degradation and total acid hydrolysis (Section VI.3) are used to examine polyols derived from oxidized polysaccharides, as represented³⁹¹ by sequencing studies of sugar residues in a heteroglycan of *Staphylococcus faecalis*.

5. Spectroscopic Methods in Perspective

Historically, developments in chromatography led to a marked growth in research on polysaccharides and carbohydrate-containing biopolymers, which commonly entailed glycol-cleavage oxidation. With time, NMR spectroscopy and mass spectroscopy have acquired complementary roles of ever-increasing importance in studies on molecular structure.

For determining the configuration of glycosidic linkages, NMR spectroscopy is preferable to the earlier approach that pairs glycol cleavage with optical rotation (Section IV), especially for molecules larger than a disaccharide. The spectra by contrast, which are acquired with relative ease, routinely afford assignments of anomeric configuration in oligo- to polysaccharides, namely, according to the orientation of their individual anomeric protons as defined by spin-spin coupling values of $^3J_{H-1, H-2}$ ⁴⁰⁴ or $^1J_{C-1}$.⁴⁰⁵ Furthermore, the NMR spectra furnish information about linkage position and residue sequence.

Mass spectroscopy facilitates the identification of products of glycol-cleavage oxidations in general, and is useful especially in conjunction with such complex procedures as the Smith degradation (Section VI.4). Mass spectroscopy is combined with periodate oxidation of polysaccharides in a notably different fashion⁴⁰⁶ through the use of fast-atom bombardment mass spectrometry. By observing fragmentation directed exclusively to oxidized or unoxidized residues, both linkage and sequence information are obtained with nanomole quantities of polymer.

Increasingly, investigations on structure bring together various combinations of glycol-cleavage oxidation with methylation, enzymatic, and spectroscopic

methods for the characterization of polysaccharides, as well as other carbohydrate-containing macromolecules, in ever-greater detail. Examples are found in structural studies on sulfated L-galactofucan⁴⁰⁷ from a tunicate, glycoproteins⁴⁰⁸ from *Clostridium* and *Bacteroides*, a glycosaminoglycan⁴⁰⁹ from squid ink, glycosphingolipids⁴¹⁰ from bovine brain, cell-wall polysaccharides⁴¹¹ of a *Chlorophyta* green alga, core oligosaccharides⁴¹² of bovine submaxillary mucin, an exopolysaccharide⁴¹³ from *Streptococcus thermophilus*, and an arabinoxyran⁴¹⁴ from *Sonolika* wheat, and *Streptococcus* heteroglycans.⁴²⁵

VII. APPLICATION TO BIOPOLYMERS

Aside from its role in the structure determination of polysaccharides, glycol-cleavage oxidation is applied widely in studies on various carbohydrate-containing macromolecules of biological interest. In most of the following examples the oxidation is used to alter sugar residues selectively, either to focus attention on the biochemical nature of the carbohydrate part, the protein, or some other constituent.

Biopolymers are commonly subjected to oxidation by periodate for site-specific modification or immobilization for use in analytical procedures. By controlled oxidation of a rabbit antibody (IgG),^{415,416} the number of sites attacked may be varied and optimized for individual assays. Immobilized monoclonal antibodies (against CPA and HRP) are obtained⁴¹⁷ when oxidized sugar residues are coupled with amino or hydrazine derivatives of a suitable matrix. Chemically related⁴¹⁸ is a site-specific conjugation of selected glycoproteins following oxidation, by introducing stable hexanedioic-dihydrazone bonds between dialdehyde structures in, for example, alkaline phosphatase, with retention of activity. A carbohydrate component of a glycoprotein in the outer membrane of enterotoxigenic *Escherichia coli*, provides⁴¹⁹ a site detection of the biopolymer through oxidation and labeling with a hydrazide-conjugated agent.

Results obtained⁴²⁰ from the reaction of periodate with oligosaccharide structures in erythropoietin are consistent with the proposal that antibodies directed against the recombinant hormone are "anti-carbohydrate". That oligosaccharide sequences are involved in the binding of human spermatozoa to a glycoprotein of the zona pellucida, is indicated^{334,421} by a marked decrease in binding that accompanies selective periodate oxidation of terminal sialic acid residues of the glycoprotein.

Low molecular weight heparin modified by oxidation followed by borohydride reduction offers distinctive advantages for the prevention of thrombosis in a variety of clinical contexts.⁴²²

Alkaline β -elimination at glycol-cleavage dialdehydic sites in glycoproteins is an effective means for selective removal of the O-glycosidically linked carbohydrate side chains. Applied⁴²³ to human mucin glycoprotein, the peptide core is exposed in a less-degraded state than by conventional partial acid hydrolysis. This approach is also useful⁴²⁴ in unmasking mucin gene products in tissue sections embedded in paraffin. Bovine submaxillary mucin, without prior oxidation, directly yields an array of oligosaccharide fragments upon treatment with alkaline borohydride.⁴¹² Oxidation of these fragments with lead tetraacetate is characterized³⁰³ by cleavage patterns diagnostic for the positions of substitution of the core structure in the mucin. An alternative possibility for the separation of carbohydrate from glycoprotein is the Smith degradation (Section VI.4). Three successive applications of this procedure fully removed the oligosaccharide chains of stem bromelain, a proteolytic enzyme in pineapple stems. Notably, it appears that none of the sugar residues is essential for catalysis by this enzyme.⁴²⁶

Periodate-oxidized yeast glucomannan is found⁴²⁷ to enhance the thermal stability of the levansucrase of *B. natto*, perhaps due to an intermolecular association between the polymer and the enzyme.

A measure of the numerical sequences of residues in a wheat arabinoxylan that accommodate enzymolysis by a *Streptomyces* xylanase, is given by the pattern of periodate oxidation of the hydrolysis fragments. For the observed release of xylobiose, for example, the sequence required is at least four consecutive D-xylopyranose residues having no L-arabinofuranosyl branches.⁴²⁸

Polysaccharides from soil contain considerable proportions of (1 \rightarrow 4)-linked glucose and xylose residues that resist periodate oxidation, for both chemical and physical reasons that remain unsolved.⁴²⁹

An early use of periodate in an approach to a complex macromolecular problem consists of selective removal of cellulose from intimate association with lignin in wood.⁴³⁰ Repeated treatment of the fibrous matrix by oxidation followed by hydrolysis of the dialdehyde cellulose in water under reflux, affords lignin preparations referred to as "periodate lignin" on "Purves lignin".

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AN EXPANDING VIEW OF AMINOGLYCOSIDE–NUCLEIC ACID RECOGNITION

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I. INTRODUCTION

The origin of aminoglycoside antibiotics began with streptomycin 60 years ago.¹ Isolated from *Actinomyces griseus*, streptomycin immediately found applications for the treatment against the tuberculosis-causing microorganism *Mycobacterium tuberculosis*.² This was the second antibiotic (after penicillin) to be used clinically. Throughout the coming years, a number of aminoglycosides were discovered (Table I),³ with varying potencies for treating infections. However, therapeutic applications were diminished by the emergence of resistance to aminoglycosides by bacteria. Coupled with adverse side effects, such as renal toxicity and ototoxicity, aminoglycoside antibiotic applications toward infectious diseases were placed on the back burner in favor of less harmful antibiotics such as β -lactams. Only within the past decade has the area become increasingly intriguing again, due largely to chemical derivatization, a deeper understanding of resistance mechanisms, and structural information on aminoglycoside activity. Also, though widely known for binding to prokaryotic ribosomal RNA, aminoglycosides have more recently shown affinity for other nucleic acid targets. This opens the door to a new area of therapeutic applications. Outlined in the forthcoming paragraphs is the history of aminoglycosides, from their discovery, structural elucidation, and mechanism of action, to current knowledge of mechanisms of action and resistance, toxicity, and novel nucleic acid targets. Owing to a number of recent reviews pertaining to some of these areas, particular emphasis will be placed on the recently discovered aminoglycoside targets and their potential therapeutic applications.

TABLE I
Aminoglycoside Antibiotic Classes and their Source Organism³

Aminoglycoside	Organism
Kanamycin	<i>Streptomyces kanamyceticus</i>
Streptomycin	<i>S. griseus</i>
Gentamicin	<i>Micromonospora purpurea</i>
Spectinomycin	<i>S. spectabilis</i>
Butirosin	<i>Bacillus circulans</i>
Tobramycin	<i>S. tenebrarius</i>
Neomycin	<i>S. fradiae</i>
Amikacin	Semisynthetic derivative of kanamycin
Netilmicin	Semisynthetic derivative of sisomicin
Isepamicin	Semisynthetic derivative of gentamicin B

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1. Early Years

Early structural studies of aminoglycosides relied on chemical degradation experiments.⁴ The chemical structure of streptidine (the central aglycon in streptomycin) was determined in 1946.⁵ Some years later, the streptose moiety was characterized.⁶ The absolute configuration was confirmed during this period,⁷ and the absolute structure completed the structural investigations by 1968.⁸ The development of such powerful techniques as NMR, X-ray crystallography, and mass spectrometry paved the way for structural elucidation of the large population of aminoglycosides known to date. All natural or semisynthetic aminoglycosides share a similar structural motif. Consisting of two to four rings of cyclitols (saturated six-membered carbon rings), pentoses, or hexoses linked by glycosidic bonds, aminoglycosides are unique in the carbohydrate class by their substitutions of amino groups at various ring positions. The amino groups are responsible for the basic nature of the aminoglycoside. Most amines have pK_a values greater than 7, protonating them at physiological pH. The pH of each amine varies, as illustrated by NMR studies of the neomycin class.^{9,10} The ionic nature, coupled with the presence of hydroxyl groups at other ring positions, is also responsible for the high hydrophilicity of aminoglycosides. A number of reviews on the structural characteristics and chemistry of aminoglycosides are available.^{11,12} Representative structures of aminoglycosides are illustrated in Fig. 1.

After its discovery, streptomycin became the first antibiotic for tuberculosis treatment.¹³ Early on, it was known to be a basic molecule with nucleic acid-binding properties.¹⁴ It was eventually shown to prevent protein synthesis,¹⁵ but its bactericidal properties were yet to be explained. In 1959, it was proposed that ribosomes were the primary target for streptomycin.¹⁶ However, this was controversial due to beliefs that membrane damage was the sole process.^{17,18} Davies and others were later able to show that streptomycin binds within the 30S subunit of the 70S ribosome,^{19,20} illustrating that indeed the mechanism of action was probably ribosome related. Davies quickly thereafter showed resounding evidence that streptomycin disrupts the fidelity of translation between the ribosome and mRNA, producing proteins of inefficient function.²¹ It was proposed that this “flooding” of non-productive proteins affects other cellular functions, which leads to cell death. To this day, a complete understanding of the bactericidal effect is lacking, though much progress has been made (discussed in a later section). The *in vitro* studies of the 1960s were punctuated with evidence of *in vivo* codon misreading by the late 1970s.²²

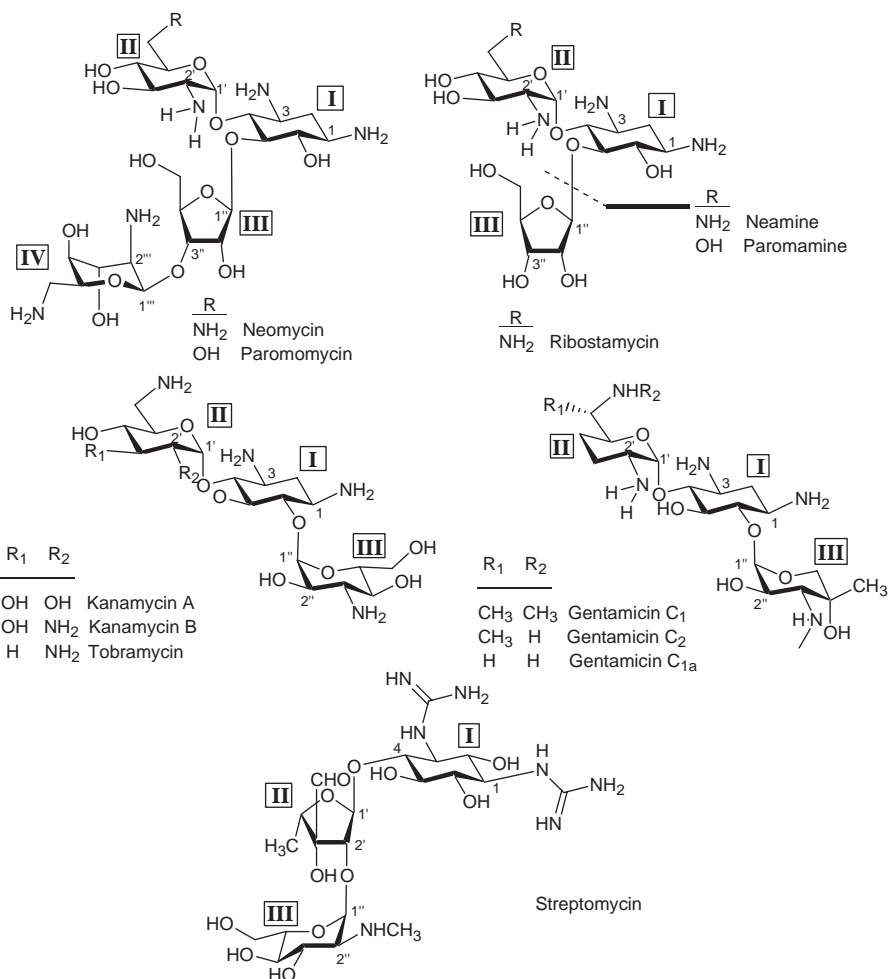


FIG. 1. Representative chemical structures of aminoglycoside antibiotics.

II. MECHANISM OF ACTION

1. Pre-Ribosomal Binding: Cellular Membrane Permeation

Before eliciting action on its ribosomal target, aminoglycosides must first penetrate the cell membrane, which, upon evaluation of the ionic properties of

aminoglycosides, would seem unlikely due to the lipophilic nature of the cell wall. Like many steps in the bactericidal activity of aminoglycosides, a detailed picture of the uptake of these cationic ligands is incompletely resolved. Aminoglycoside uptake is believed to involve a self-promoted process^{23–25} characterized by three steps.²⁶ The first phase is characterized by outer-membrane binding by the aminoglycoside, most probably to negatively charged pockets of lipopolysaccharides, phospholipids, or other membrane proteins possessing negatively charged residues (in Gram-negative bacteria). Membrane binding in Gram-positive bacteria probably involves phospholipid or teichoic acid interactions in the first phase. It is this surface binding that is believed to displace divalent cations that bridge adjacent lipopolysaccharide molecules, causing a disruption in the membrane integrity, and an enhancement in permeability for the aminoglycoside.²⁷

The second phase is proposed to involve protrusion of the inner membrane, which is concentration-dependent due to the requirement of a threshold potential for the transmembrane step.^{28,29} This step is also energy-dependent, and requires a proton motive force (Δp) according to the chemiosmotic hypothesis.³⁰ Since aerobically grown bacteria generate a Δp by a membrane-bound respiratory chain with oxygen as the terminal acceptor, anaerobic bacteria lack an acceptor, therefore compromising the requirement of energy for uptake to occur. Numerous results have shown that anaerobic bacteria display an absence of aminoglycoside uptake.^{29,31,32} Alternative terminal electron acceptors (such as nitrates) in the presence of anaerobic bacteria have been shown to promote aminoglycoside uptake.^{33,34} The third step, also energy-dependent, is believed to involve minimal uptake of aminoglycoside into the cell, which results in ribosomal binding and non-functional protein synthesis.³⁵ The incorporation of these newly synthesized proteins into the inner membrane potentially disrupts the membrane integrity, sparking further aminoglycoside “leaking” into the cell, which triggers more ribosome binding and eventual membrane disruption for further ligand uptake.³⁶ The ultimate consequence of this uptake phase is an irreversible saturation of all ribosomes, which leads to cell death. A model of the uptake mechanism and lethal action is shown in Fig. 2.³⁵

2. Ribosomal RNA Binding

As mentioned in the introductory paragraph, the mechanism of aminoglycoside (streptomycin) action toward bacteria was found to involve ribosome binding. In their landmark paper, Davies and coworkers showed that

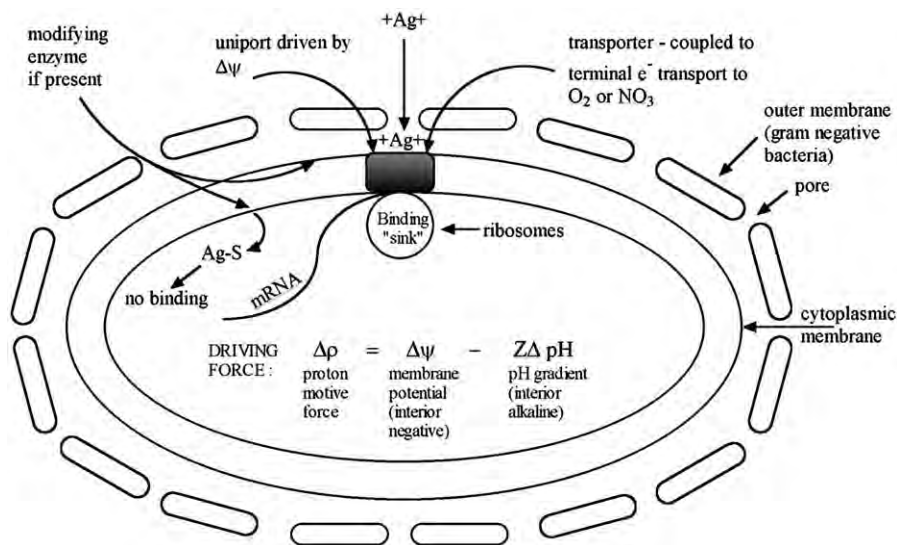


FIG. 2. Model of aminoglycoside (Ag) uptake provided by Bryan and Kwan.³⁵ Ag^+ represents polycationic aminoglycoside; $Ag-S$ represents enzymatically modified aminoglycoside. Reprinted with permission, Copyright 1983 American Society for Microbiology.

aminoglycosides such as streptomycin, kanamycin, and neomycins induce mis-readings during polypeptide synthesis.²¹ This finding led to the general belief that aminoglycosides binding to the ribosome disrupts the rRNA conformation in such a way that accurate recognition between the codon of mRNA and the anticodon of tRNA is lost.

The binding site of aminoglycosides was ultimately discovered to be the 16S rRNA aminoacyl site (A-site) of the small ribosomal subunit (30S) in prokaryotic bacteria.³⁷ Studies in the 1980s largely relied on enzymatic footprinting, but advances in NMR and crystallographic techniques have vastly widened the views. As a result of structural elucidations of the complexes, both in solution and in crystal form, there is a much clearer picture of the aminoglycoside-RNA interaction.

Early structural studies using NMR provided a clear picture of a 27-nucleotide RNA mimic of the A-site bound to paromomycin,³⁸ as well as to neamine, ribostamine, and neomycin.³⁹ All showed similar contacts to the RNA, particularly with rings I and II, with the exception of neamine, which indicated two different orientations of binding by two amines in its deoxystreptamine ring

(ring II). In the case of paromomycin, binding was shown to occur in the RNA major groove, with ring I bound within a bulged helical structure composed of the A1408–A1493 base pair as well as the unpaired base A1492 (Fig. 3). Sequence-specific contacts were also shown to involve the U1406–U1495 base pair and both G1491 and G1494. Non-specific electrostatic interactions were the primary binding modes of rings III and IV. These findings suggested that recognition is driven by rings I and II, and supported earlier findings that neamine

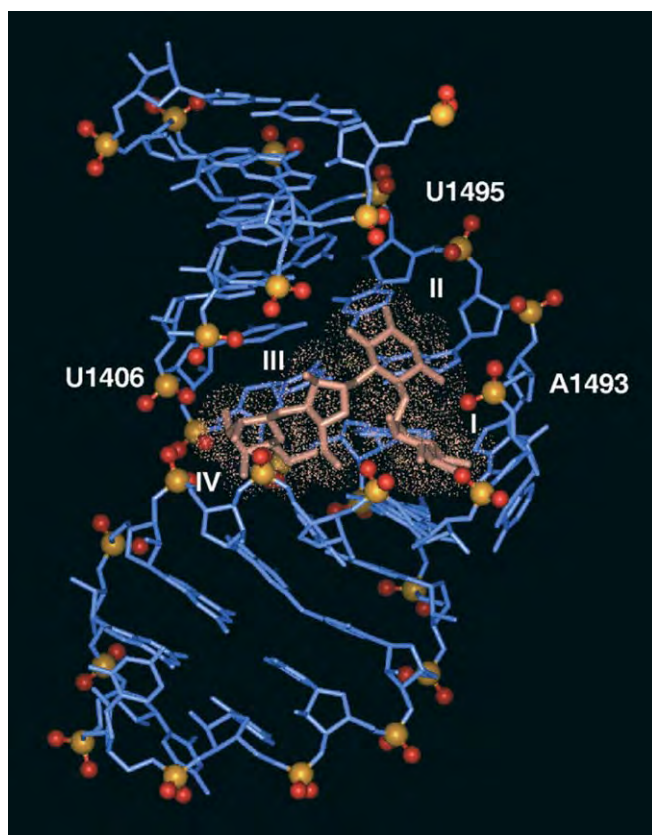


FIG. 3. Solution structure of the A-site RNA–paromomycin complex determined by Puglisi and coworkers.³⁸ Paromomycin (represented in light brown) is bound within the major groove of RNA (shown in blue). Key contacts are labeled (U1495, U1406, A1493) and the van der Waals surface of paromomycin is depicted with a haze pattern. The rings of paromomycin are labeled as illustrated in Fig. 1. Reprinted with permission, Copyright 1996 American Association for the Advancement of Science.

(the simplest aminoglycoside, possessing rings I and II only) binds 16S rRNA and induces miscoding. More recent results with deoxystreptamine demonstrate that it is more simply this structure that modulates the recognition of the RNA by targeting specific G–U and G–G steps in the major groove.⁴⁰

Recent crystallographic studies of the 30S subunit complexed with different aminoglycosides have provided valuable insight into the translation process at the molecular level.⁴¹ Accurate translation relies heavily on the efficient distinction of codons in mRNA with anticodon loops of cognate aminoacyl-tRNA by forming a mini-helical structure. When this structure is formed, two adenines (A1492 and A1493) from the A-site flip out to interact with the two end base pairs of the mRNA–tRNA formed minihelix. This molecular “switch” has been shown to irreversibly determine the fate of subsequent translation steps.⁴² As described for the NMR structure and found in these crystal studies, aminoglycoside (for example paromomycin) binding displays specific interactions with both of these bases. Binding by aminoglycosides stabilizes this conformation of the A-site such that the stabilities of cognate tRNA–mRNA complexes and near-cognate tRNA–mRNA complexes are very similar. The end result of this energetic indistinction is misreading of translation due to ribosome binding to near-cognate forms. Oligonucleotide RNA binding by various aminoglycosides, such as paromomycin⁴³ and tobramycin,⁴⁴ has shown a close resemblance to its 30S subunit counterpart. It is also worth noting that the interactions between paromomycin and the A-site were in strong agreement with the earlier NMR structure. A more refined NMR structure of the paromomycin–RNA interaction with a detailed comparison of similarities to the X-ray structure has also been reported.⁴⁵

The recently described interactions are not universal among the aminoglycoside family. Aminoglycosides not possessing a 2-deoxystreptamine ring have been shown to display different modes of binding from those already mentioned. Particular aminoglycosides include streptomycin, which consists of a streptidine ring instead of a 2-deoxystreptamine as its aminocyclitol, and spectinomycin, a non-aminoglycoside aminocyclitol.

Streptomycin is unique among the aminoglycosides because its binding involves RNA and protein interactions. Key interactions in its binding include bases U14, C526, G527, A913, A914, C1490, G1491 in the 16S RNA as well as Lys45 of protein S12.⁴¹ Conformational switches in 30S during translation have been shown to occur, which delegate proteins S5 and S12 to prompt a change in base-pairing schemes in helix 27. Of the two conformations, one is responsible for hyperaccurate translation, whereas the other is considered error prone (*ram*,

for ribosomal *ambiguity*). Streptomycin binding is believed to involve preference for the *ram* state, which results in a more indistinguishable interaction between cognate and near-cognate tRNAs. This stabilization by streptomycin is also believed to restrict the transition of *ram* state back to the hyperaccurate state, disrupting any possible proofreading step within the mRNA–tRNA complex.⁴¹

Spectinomycin is unique in that its binding results in a bacteriostatic effect, contrary to other bactericidal aminoglycosides.⁴⁶ It binds in the minor groove at the end of helix 34 in 16S rRNA, with close proximity to helix H28 and a protein S5 loop (Fig. 4).⁴¹ Its primary action is inhibition of translocation of peptidyl-tRNA from the A-site to the P-site. Crystal structures have shown that spectinomycin binding to the end of helix 34 sterically hinders ribosomal movement, restricting any necessary conformational changes in the helix.⁴¹ Therefore, misreading does not occur in this case of aminoglycoside binding, which may explain its bacteriostaticity.

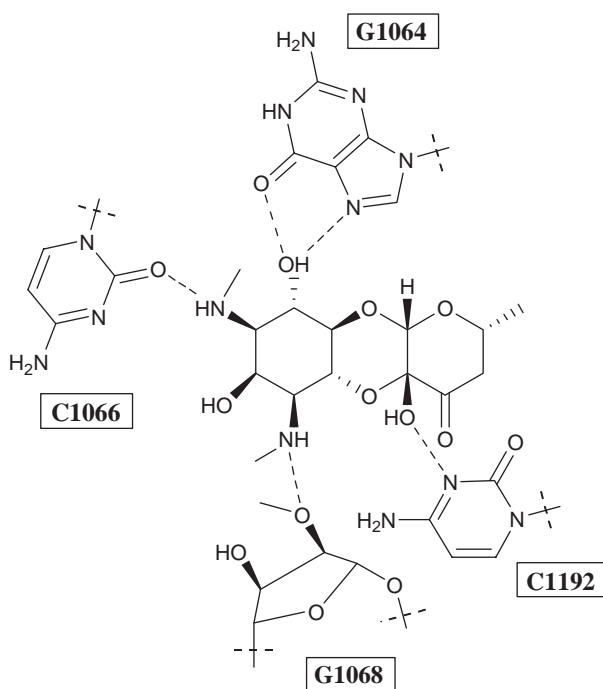


FIG. 4. Spectinomycin interactions in the minor groove of 16S rRNA.⁴¹ Contacts of spectinomycin include C1066, G1068, C1192, and G1064. Reprinted with permission, Copyright 2000 Nature Publishing Group (<http://www.nature.com/>).

III. MAJOR ISSUES IN AMINOGLYCOSIDE THERAPEUTIC APPLICATIONS

1. Toxicity

Aminoglycosides have long been known to elicit toxic side effects with their treatment. Within a year of the first clinical use of streptomycin, both nephrotoxicity (kidneys) and ototoxicity (inner ear) were observed.⁴⁷ Extensive research to understand the reasons for toxicity has led to a number of hypotheses on the mechanism of aminoglycoside-induced toxicity in the ear and kidney. The strongest evidence of aminoglycoside action at the molecular level comes from Schacht, who has proposed a free-radical pathway for aminoglycoside action.⁴⁸ Experiments showed that free-radical scavengers, such as glutathione, inhibit the death of aminoglycoside-exposed cells.^{49,50} Substantial support came later in NMR experiments showing that gentamicin can chelate iron and form an oxygen-reactive species,⁵¹ catalyzed by the Fe^{2+} -gentamicin complex, which can ultimately lead to hydroxyl radicals.⁵² The overall reaction is believed to involve 3 steps: (1) formation of the Fe^{2+} -gentamicin complex, which can then (2) activate O_2 , with reduction to superoxide by an electron donor, and (3) further chain reaction, forming other free-radical species, leading to cell damage. The proposed mechanism for free-radical-induced ototoxicity is illustrated in Fig. 5.⁵³

Polyphosphoinositides have also been suggested to play a role in the biochemical event associated with aminoglycoside toxicity. Aminoglycosides have shown high affinity for polyphosphoinositides, such that they have been referred to as pseudoreceptors.⁵⁴ Polyphosphoinositides lie within cell membranes, and are negatively charged phospholipids that function as substrates in providing diacylglycerol and inositol triphosphate as intracellular messengers. These lipids are predominately composed of arachidonic acids. Recent studies have shown that arachidonic acid acts as an electron donor in the aminoglycoside-catalyzed reaction (Fig. 5), supporting early evidence that aminoglycoside binding to polyphosphoinositides could be the reason for their ototoxic effects.^{55,56} Likewise, the hypothesis for iron involvement is supported by evidence showing that ototoxicity in guinea pigs is enhanced by iron supplements, and prevented by treatment with antioxidants and iron chelating agents.⁵⁷ Further support for the free-radical mechanism lies with the observed attenuation of aminoglycoside-induced toxicity in inner ear tissues by treatment with the antioxidant glutathione as well as with the observed enhancement of toxicity when glutathione levels are depleted.^{49,50,58} Mice overexpressing the antioxidant enzyme superoxide dismutase have been shown to resist kanamycin-induced hearing loss.⁵⁹

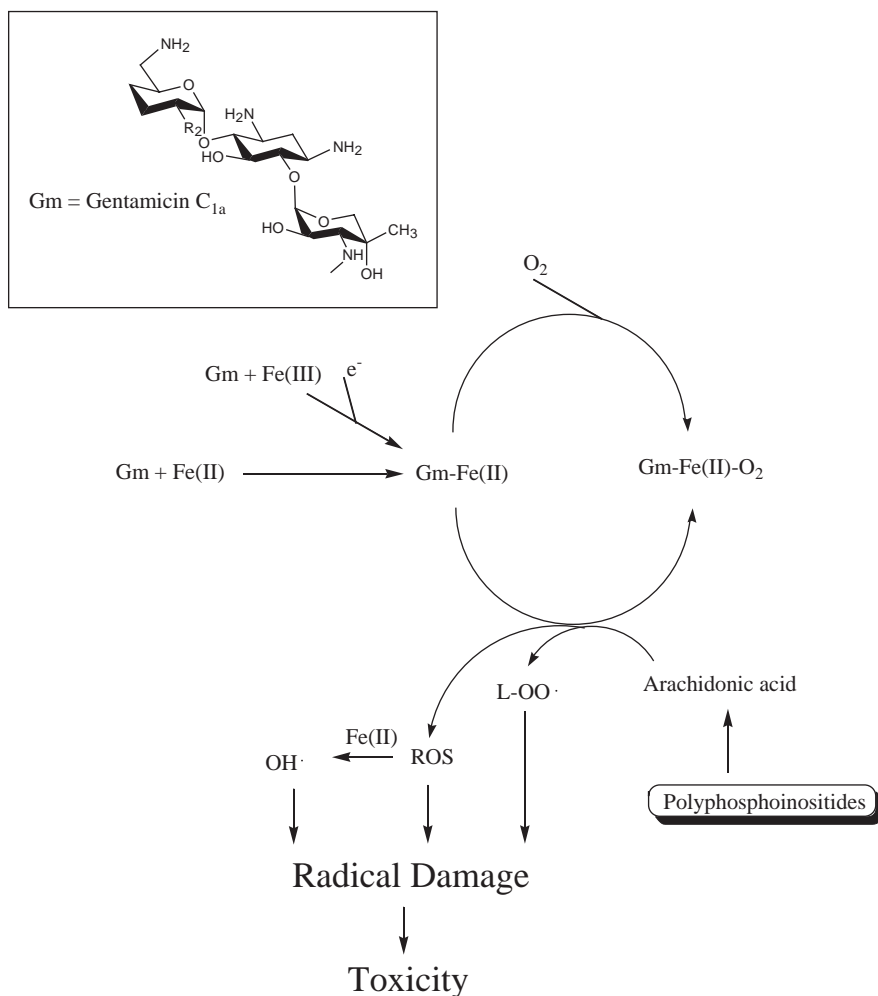


FIG. 5. Proposed free-radical pathway attributed to gentamicin-induced ototoxicity.⁵³ Steps include (1) gentamicin–Fe(III) complex, (2) O₂ activation and reduction to lipid peroxide (L-OO radical) and superoxide (ROS) by an electron donor (arachidonic acid), and (3) free-radical formation and damage. Reprinted with permission, Copyright 1997 UMI.

Several reports have mentioned the possibilities of toxicity stemming from other pathways. For example, inhibition of protein synthesis may lead to undesirable cellular events that lead to toxicity. Aminoglycosides have also been shown to inhibit Klenow DNA polymerase as well as poly(A)-specific

ribonuclease, both metalloenzymes, most probably by the displacement of crucial divalent metal ions.⁶⁰ There is also evidence that nucleic acid-metabolizing enzymes, such as Taq DNA polymerase and T7 RNA polymerase can be inhibited by the aminoglycoside neomycin.⁶⁰ Earlier studies have indicated neomycin binding to such enzymes as DNA polymerase I⁶¹ and DNase I of *E. coli*.⁶² Phospholipase C, also a metalloenzyme, can also be inhibited by aminoglycosides.⁶³ Inhibition of dopamine regulating P-type Ca^{2+} channels by neomycin can also be considered a toxic pathway.⁶⁴ Lastly, aminoglycosides have been shown to bind triple-helical DNA,⁶⁵ which are known to exist in biological systems as potential regulators of gene expression.⁶⁶

A serious form of ototoxicity by aminoglycosides has been linked to heredity.^{67,68} A mutation in mitochondrial DNA has been linked to hereditary deafness. The end result of the mutation is an A to G transition at the 1555 position in mitochondrial 12S rRNA.⁶⁹ The transition results in a new (Watson–Crick) base pair, which alters the RNA conformation such that it adopts a structure similar to that of *E. coli* 16S rRNA. Substantial support from a biophysical perspective comes from work in Rando's group. They have illustrated, using a fluorescence-based binding assay, that the mutation in RNA, from A to G, results in an observed binding (K_d in the low micromolar range) by aminoglycosides (Fig. 6),⁷⁰ while wild-type human mitochondrial 12S RNA displayed no binding by aminoglycosides. These results gave quantitative support to the hypothesis that the mutation is likely responsible for aminoglycoside-induced deafness.

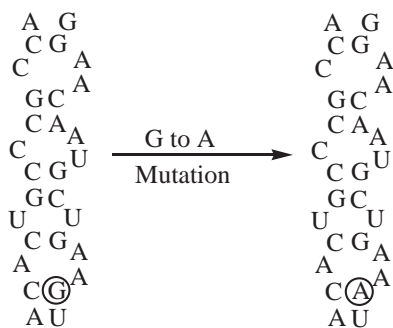


FIG. 6. Secondary structure of the decoding region of *E. coli* 16S rRNA and the 1491 mutation to eukaryotic RNA (right).⁷⁰ The mutation at 1491 is circled. Reprinted with permission, Copyright 1997 American Chemical Society.

2. Combating Toxicity

Much like the ambiguity in mechanisms of toxicity, the prevention of toxic effects by aminoglycosides has not been limited to one approach. Growth factors and neurotrophins have been used in preventing ototoxicity in a number of cell-culture studies.⁷¹ Small molecules have also been shown to inactivate apoptotic neuroresponses⁷² and protect hair cells from aminoglycoside-treated cultures. (Hair cells of the inner ear are the most sensitive to aminoglycoside exposure and their damage most often results in hearing loss.) Other enzyme inhibitors, such as those that inhibit c-Jun-N-terminal kinase (JNK), have been shown to protect hair cells from aminoglycoside exposure. Upstream activators of JNK, such as the GTPases Rac and Cdc42, have more recently been shown to disrupt JNK formation by their glycosylation by *Clostridium difficile* toxin B, the end result being a rescue of hair cells.⁷³ Caspase, an enzyme that catalyzes apoptotic death, has also been inhibited to prevent hair-cell death from gentamicin exposure.^{74–76} A relatively new and exciting approach, gene therapy, has been utilized to enhance expression of genes responsible for the synthesis of protective agents.⁷⁷ When considering the free-radical mechanism of induced toxicity, the application of antioxidants seems the most viable approach. Numerous reports, as already mentioned, have indicated that successful preservation of hair cells is achieved with the administration of antioxidants.⁷⁸

Recent research has also identified megalin deficiency as a protection mechanism from renal toxicity.⁷⁹ Megalin, a receptor that is expressed on the surface of the proximal tubular epithelium, acts somewhat as a filter for low-molecular-weight protein passage,^{80,81} and is largely thought to be responsible for aminoglycoside uptake in the kidney.^{82,83} Numerous accounts have illustrated that megalin deficiencies, by aid of antagonists such as a receptor-associated protein⁸² and maleate,⁸⁴ result in diminished toxicity effects, though marginally effective in terms of therapeutic applications. A more recent report indicated that genetically induced megalin deficiencies negatively correlate with renal accumulation of aminoglycosides, providing sound evidence that megalin is the sole pathway for aminoglycoside uptake (Fig. 7).⁸⁵ Therefore, megalin has been suggested as a drug target for nephrotoxicity prevention.

3. Aminoglycoside Resistance

A second challenge in the area of applied therapeutics for aminoglycosides is their bacterial resistance. A number of different biochemical pathways are

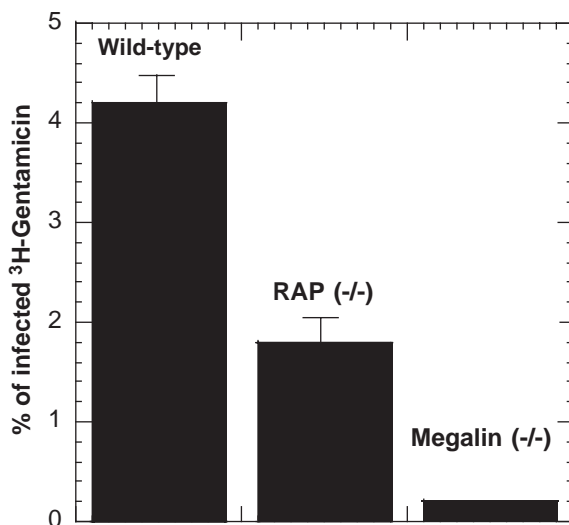


FIG. 7. Megalin deficiency results in diminished gentamicin uptake in mice. RAP represents a receptor-associated protein, a megalin antagonist.⁸⁵ Complete megalin deficiency (far right) results in complete absence of aminoglycoside uptake. Reprinted with permission, Copyright 2002 The American Society for Biochemistry and Molecular Biology.

known for effecting resistance, such as reduced uptake of the aminoglycoside, rRNA mutations, ribosomal protein mutations, enzymatic derivatization of rRNA, and enzymatic derivatization of aminoglycosides. A number of reviews dealing with aminoglycoside resistance have been published over the past few years, warranting a brief description in this section.^{86–88} The reduced uptake pathway relies on a change in the chemistry of the aminoglycoside–membrane interactions, thereby halting permeation of the aminoglycoside into the cell or discharging any aminoglycoside that made its way into the cell. The diminished permeability is usually a result from a change in transmembrane potential, likely effected by chromosomal mutations. Aminoglycoside discharge is due to the generation of efflux pumps,^{89–95} which are coordinated by proteins for the extrusion of ligand from the cell, halting the saturation of the rRNA binding. The proteins in these pumps are, again, products of mutations. The other pathways of resistance, mutation-induced rRNA and proteins, and enzymatic modifications of rRNA and aminoglycosides, all rely on, as their name implies, changes in sequence or chemical structure which give rise to diminished recognition of the aminoglycoside to its natural target RNA A-site. A considerable amount of

focus has been placed on enzymatic aminoglycoside modification, as evident in the number of reviews published over the past few years.^{87,96}

4. Fighting Resistance with Aminoglycoside Derivatives

Significant progress has been made in the fight against aminoglycoside resistance by the development of novel molecules that mimic aminoglycosides and show either increased cellular delivery or binding to the target A-site without acting as substrates for resistance enzymes. Mobashery and colleagues have synthesized novel antibiotics with considerable antibacterial activity and resistance to enzymatic modifications.⁹⁷ Compounds **2** and **3** (Fig. 8) showed a great

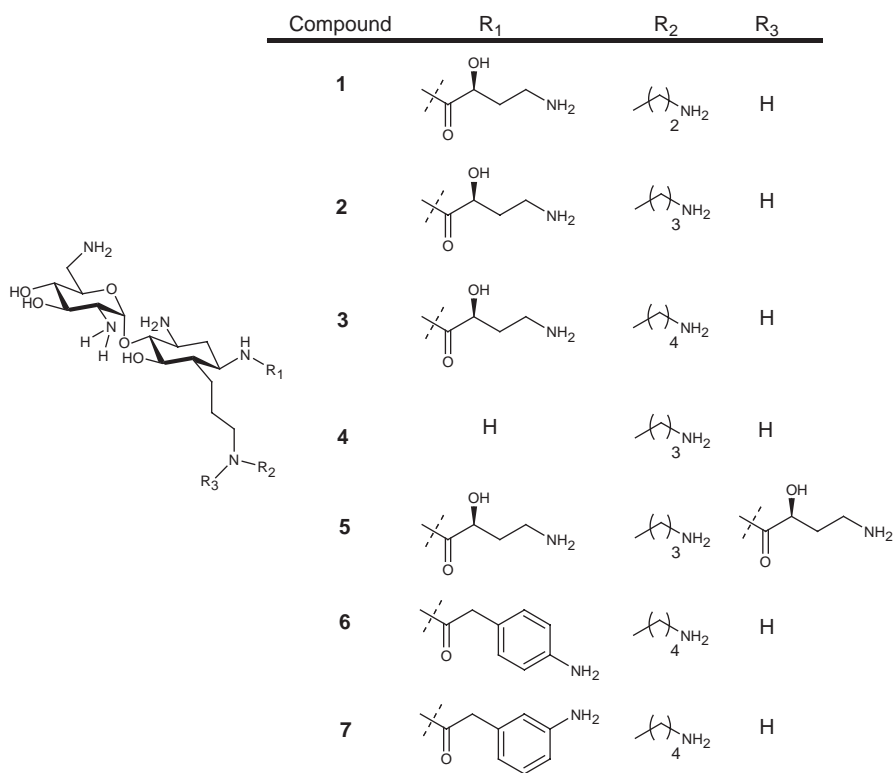


FIG. 8. Novel aminoglycoside mimics developed by Mobashery and coworkers.⁹⁷

improvement in activity against an *E. coli* strain hyperexpressing aminoglycoside-modifying enzymes (Table II). The enzymes of poor binding include those of the aminoglycoside phosphotransferase (APH) or aminoglycoside acetyltransferase (AAC) classification.⁹⁸ The novel derivatives **2** and **3** also displayed increased activity over β -lactam antibiotics such as ampicillin and imipenem (Fig. 9). Kinetic studies further supported the enzyme-resistant properties of both these compounds, along with other novel derivatives (compounds **6** and **7**).

Wong has also utilized aminoglycoside mimics with significant RNA binding and antimicrobial properties.^{99,100} Among these are compounds **8** and **9** (Fig. 10),¹⁰⁰ which have shown K_d values of 0.26 and 10 μ M, respectively, in the binding to an 16S A-site model.¹⁰¹ However, the applications for resistance to enzymatic modifications came with a more recent development, that of dimeric aminoglycosides, which consist of two neamine moieties separated by an appropriate linker (of both glycol and alkyl nature, Fig. 11).¹⁰² These novel aminoglycosides were found to be active against several aminoglycoside-resistant bacterial strains.

The neamine dimers also displayed enhanced binding to a model A-site, and showed that neamine binds in a 2:1 fashion to the RNA A-site. The application of such novel compounds is certainly substantiated by characteristic activity

TABLE II
Activity of Novel Aminoglycosides 1–4 and Comparison with Other Selected Aminoglycosides and β -Lactam Antibiotics⁹⁷

Bacterial strains	MIC (g/mL)					1	2	3	4
	Neamine	Kanamycin	Ampicillin	Ceftazidime	Imipenem				
<i>Escherichia coli</i> JM83	64	4	4	0.03	0.06	8	2	1	32
<i>E. coli</i> JM83 (APH(3')-I)	8000	2000	16,000	0.5	0.25	32	8	8	> 512
<i>E. coli</i> JM83 (AAC6'/APH2'')	2000	500	8000	0.5	2	32	8	8	> 512
<i>Serratia marcescens</i> ATCC13880	16	8	32	0.25	0.5	32	8	4	64
<i>Enterobacter cloacae</i> ATCC3047	64	8	1000	2	0.5	8	2	2	16
<i>Pseudomonas aeruginosa</i> 66	500	32	> 500	64	0.25	8	2	2	> 512
<i>P. aeruginosa</i> C43	4000	1000	> 500	128	32	64	4	2	> 512
<i>Staphylococcus aureus</i> 3	64	4	4	2	< 0.03	2	0.5	0.5	4
<i>Enterococcus faecium</i> 119	1000	64	1	> 128	0.5	256	32	32	> 512

Note: The bacterial strains range from Gram-negative bacteria (*E. coli*, *S. marcescens* ATCC13880, *E. cloacae* ATCC3047, and *P. aeruginosa* 66 and C43) to Gram-positive cocci (*S. aureus* 3 and *Enterococcus faecium* 119) that show resistance for aminoglycosides. Reprinted with permission, Copyright 2002 American Chemical Society.

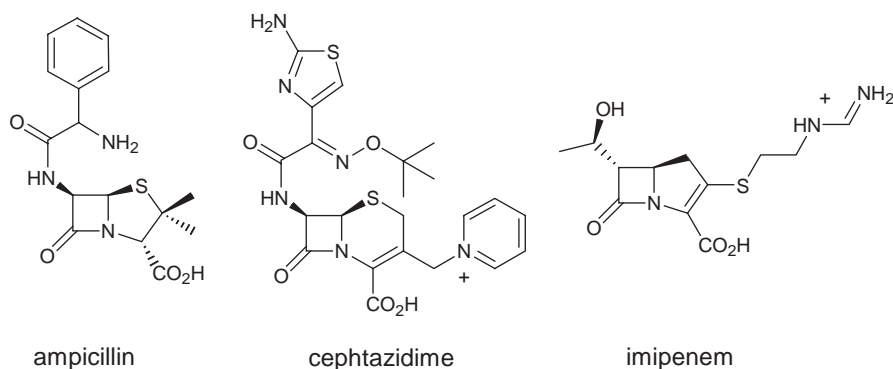


FIG. 9. Structures of some β -lactam antibiotics used for comparison studies with compounds 1–7.

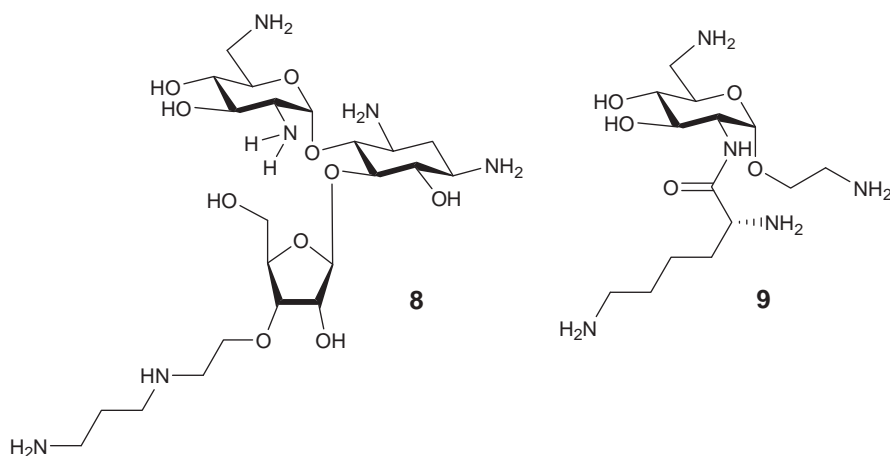
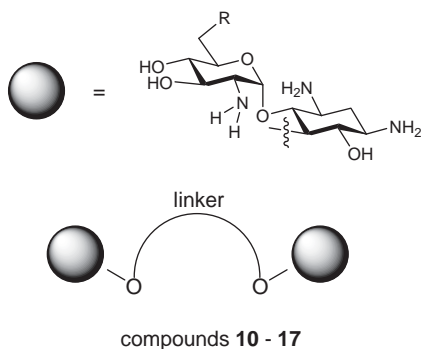


FIG. 10. Novel aminoglycoside mimics developed by Wong and coworkers.¹⁰⁰

toward such resistant bacteria, and future developments and applications are sure to surface in the literature.

Other work has approached the design of active agents in an opposite sense: by developing molecules similar to aminoglycosides to inhibit aminoglycoside-modifying enzymes. By constructing a microarray of aminoglycosides and derivatives, Seeberger's group has probed their binding to enzymes known for aminoglycoside derivatization, specifically 2'-acetyltransferase [AAC(2')] from *M. tuberculosis*, and 6'-acetyltransferase [AAC(6')] from *Salmonella enterica*.¹⁰³



Cmpd	Linker	K _d (μM)	<i>S.</i> <i>aureus</i> ATCC 27853	<i>E.</i> <i>coli</i> ATCC 25922	<i>P.</i> <i>aeruginosa</i> ATCC 27853
10	(CH ₂) ₂ O(CH ₂) ₃ O(CH ₂) ₂	1.1	31	125	>250
11	(CH ₂) ₃ O(CH ₂) ₂ O(CH ₂) ₃	3.6	125	>250	>250
12	(CH ₂) ₂ [O(CH ₂) ₂] ₂ O(CH ₂) ₂	1.8	62.5	>250	>250
13	(CH ₂) ₃ O(CH ₂) ₄ O(CH ₂) ₃	3.3	>250	>250	>250
14	(CH ₂) ₃ [O(CH ₂) ₂] ₂ O(CH ₂) ₃	6.0	>250	>250	>250
15	(CH ₂) ₂ [O(CH ₂) ₂] ₃ O(CH ₂) ₂	1.4	62.5	>250	>250
16	(CH ₂) ₁₀	5.3	>250	>250	>250
17	(CH ₂) ₁₂	7.7	>250	>250	>250

FIG. 11. Various neamine dimers and their respective activities with aminoglycoside deactivating enzymes.¹⁰² The numbers for each strain represent minimum inhibitory concentrations. Reprinted with permission, Copyright 2004 Wiley-VCH Verlag GmbH & Co. KGaA.

By guanidylating the amino groups of several aminoglycosides, an enhancement in binding affinity for the enzymes was observed, giving opportunity to such derivatives as potential inhibitors of such protein nuisances. As an example, a guanidinoribostamycin derivative (Fig. 12) was shown to exhibit a nearly eight-fold enhancement in binding over ribostamycin itself (Table III). The guanidylation of amines in such aminoglycosides as neomycin and tobramycin was previously shown to increase the cellular uptake of such aminoglycosides over their parent counterpart in eukaryotes.¹⁰⁴ The implications for this increased uptake could extend to potentially understanding and combating resistance mechanisms that affect the self-promoted uptake of ligand.

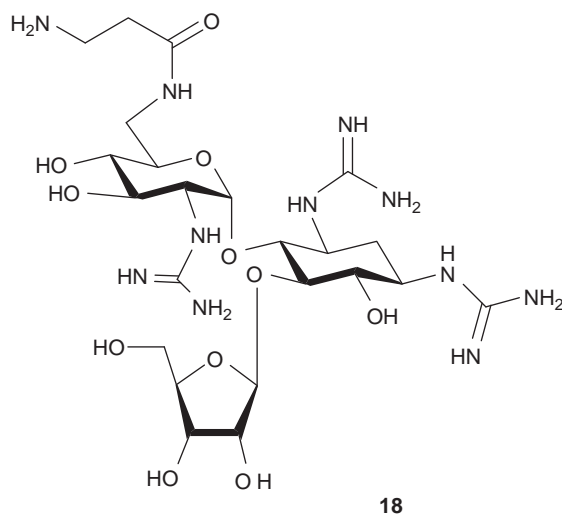
FIG. 12. Structure of a guanidino-derivatized ribostamycin.¹⁰³

TABLE III
Activity of Guanidino-Functionalized Aminoglycosides with Resistance Enzymes

Aminoglycoside	AAC (6')	AAC (2')
Kanamycin A	2.1	1.1
Kanamycin B	1.6	5.5
Neomycin	3.2	2.5
Ribostamycin	7.5	6.7
Paromomycin	1.4	1.1
Lividomycin	1.1	1.8

Note: The numbers represent the enhancement in activity of the guanidine derivatives over the parent aminoglycoside.¹⁰³ Reprinted with permission, Copyright 2004 Wiley-VCH Verlag GmbH & Co. KGaA.

IV. EXPERIMENTAL TECHNIQUES FOR PROBING AMINOGLYCOSIDE–RNA INTERACTIONS

As briefly stated in an earlier paragraph, earlier insight on aminoglycoside interactions with the 16S rRNA A-site was gathered using chemical footprinting techniques. Such advancements paved the way for more detailed studies involving NMR and X-ray crystallographic structure studies. More-modern techniques,

such as isothermal titration calorimetry and surface plasmon resonance, have provided useful thermodynamic and kinetic data, thus widening the surface of knowledge pertaining to the driving force for aminoglycoside recognition. Outlined next is an overview of such techniques, with emphasis on the usefulness each brings to the scientific community in regards to understanding the motivations behind aminoglycoside–RNA interactions. Specific examples are introduced, by no means emphasizing each as the only example in the literature. Spectroscopic and footprinting techniques are not reviewed for brevity. However, powerful structural and newer biophysical techniques will be discussed.

1. NMR

NMR studies were the first to give a detailed picture of the interaction between an aminoglycoside (paromomycin) with the 16S A-site. Since then, a great deal of information has been obtained using NMR, from structural comparisons of eukaryotic and prokaryotic A-sites,^{105,106} to aminoglycoside binding to aminoglycoside-modifying enzymes.^{107–112} Patel has conducted extensive research involving RNA aptamer complexes with numerous biological ligands for identifying common structural characteristics of RNA in their recognition to such ligands as aminoglycosides.^{113,114} Abbott Laboratories, using an NMR-based screening assay,¹¹⁵ have recently shown that aminoglycosides of very different chemical structure can bind the A-site with binding affinities in the low micromolar range.

A particularly noteworthy structural study comes from Puglisi's group involving the origins of aminoglycoside specificity for prokaryotic ribosomes. By changing a single nucleotide in a model A-site, A1408 to G1408 (a characteristic difference between prokaryotic and eukaryotic RNA), reduced affinity of aminoglycosides is noticeable.¹⁰⁵ Their earlier studies indicated a base pairing between A1408 and A1493 to be a critical part of the binding affinity of paromomycin,³⁸ providing impetus for a eukaryotic structural study. This base pair displaces somewhat nearby adenines (A1492 and A1494) toward the minor groove, and creates a pocket for ring I of paromomycin. In the eukaryotic RNA, however, this absence of adenine displacement, due to the guanine replacement, provides for a diminished interaction with paromomycin's ring I (Fig. 13). Paromomycin was found to bind the major groove of both RNAs; however, ring I was more solvent-exposed in eukaryotic RNA. It is the lack of interaction in ring I that prevents the necessary conformational change that attracts specific interactions between other rings within paromomycin. More specifically, the

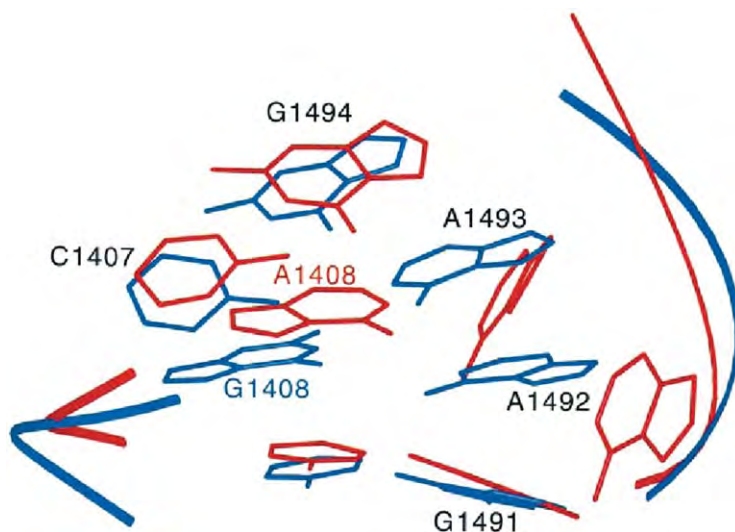


FIG. 13. NMR-derived structures of paromomycin interactions with prokaryotic (red) and eukaryotic (blue) RNA indicating conformational differences between the two.¹⁰⁵ A notable difference is in the 1408–1492 base pair, which is responsible for paromomycin ring I binding. Ribbons represent the phosphodiester backbone. Paromomycin is left out of the picture for clarity. Reprinted with permission, Copyright 2001 Elsevier.

required contacts to position ring II for G1494 and U1406–U1495 binding (which is required for rings III and IV to contact the phosphodiester backbone) is disrupted by this single-nucleotide change.

2. X-Ray Crystallography

Several insightful crystal structures have been solved within the past few years. Some notable examples are the ribosomal 30S subunit of *Thermus thermophilus* in its free¹¹⁶ and paromomycin-complexed form,⁴¹ cognate¹¹⁷ and near-cognate forms¹¹⁸ of the tRNA–mRNA complexes of this 30S subunit with paromomycin bound to the A-site, and deoxystreptamine-containing aminoglycosides such as tobramycin⁴⁴ and geneticin (Fig. 14)¹¹⁹ bound to A-site oligomers. As with NMR studies, there are several examples investigating aminoglycoside interactions with resistance enzymes. The reader is referred to a recent review by Vicens and Westhof for a detailed discussion of recent X-ray structures and their potential impacts on the future of aminoglycoside recognition.¹²⁰

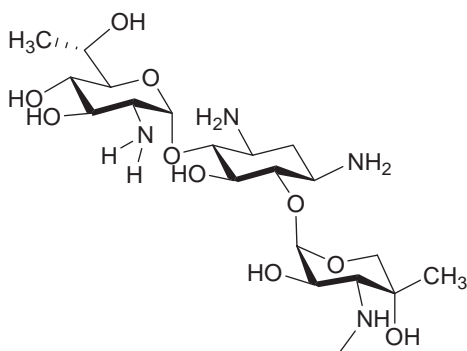


FIG. 14. Chemical structure of geneticin.

3. Isothermal Titration Calorimetry

Isothermal titration calorimetry (ITC) has become a valuable technique for investigating ligand–substrate interactions. As opposed to other structural techniques such as NMR and crystallography, ITC provides detail of the ligand–substrate interaction from a thermodynamic perspective. A typical experiment involves the titration of a concentrated ligand solution (aminoglycoside) into a sample chamber cell containing substrate (RNA). The binding event, accompanied by heat changes, results in heat-burst curves that can be integrated for each titration to yield the injection heats. The subtraction of heat changes accompanied with ligand into buffer alone as well as buffer into substrate is necessary (but usually negligible) to provide an exact value for aminoglycoside–RNA binding only. The ΔH for each ligand:substrate ratio can then be plotted and fit theoretically to give values such as ΔH , ΔS , n (stoichiometry), and K_a for the interaction (see Table IV for an example). To date, there is just a handful of studies that use ITC to investigate aminoglycoside interactions. A particularly attractive study involves the binding of neomycin-class antibiotics to a 16S rRNA A-site model (Table IV).¹²¹ In this study, it was found that aminoglycoside binding to the RNA is linked to an uptake of protons by the drug's amino groups upon binding. This event was supported by the fact that binding enthalpy became more exothermic (indicative of a favorable interaction) when pH was increased (Table V). Also, utilizing ΔH data from ITC and T_m values from UV thermal denaturation studies, it was found that the binding affinity decreased (K_a values became lower) as the pH increased, as may be expected due to the loss of cationic nature as the pH is raised. Other useful

TABLE IV
Thermodynamic Parameters for Paromomycin Binding to RNA A-Site Model at pH 7.0, Determined by Using ITC

Binding Site	K_1 (M^{-1})	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)
1	$2.7 \pm 0.5 \times 10^8$	-17.0 ± 0.1	-5.5 ± 0.2	-11.5 ± 0.1
2	$3.2 \pm 0.5 \times 10^6$	-11.9 ± 0.2	-3.0 ± 0.3	-8.9 ± 0.1
3	$1.4 \pm 0.1 \times 10^5$	-14.3 ± 0.3	-7.3 ± 0.4	-7.0 ± 0.1

Note: The binding isotherm was theoretically fitted to a three-sequential binding-site model.¹²¹ Reprinted with permission, Copyright 2002 American Chemical Society.

TABLE V
Comparison of pH-Dependent Binding Enthalpies for Three Aminoglycosides with an RNA A-Site Model121

Aminoglycoside	ΔH (kcal/mol)	
	pH 6.0	pH 7.0
Neomycin	-9.6 ± 0.1	-20.0 ± 0.1
Paromomycin	-6.1 ± 0.1	-17.0 ± 0.1
Ribostamycin	-6.9 ± 0.1	-12.0 ± 0.2

Note: Values were determined using ITC. Reprinted with permission, Copyright 2002 American Chemical Society.

information from these pH and salt-dependent ITC studies indicated that neomycin is the strongest binding aminoglycoside, probably due to the highest number of amino groups. Furthermore, these results also suggest that such enhancement in binding is linked to enthalpic terms. The salt-dependent studies also suggested that at least three protonated amines bind the host RNA in an electrostatic fashion. Further studies implementing ^{15}N NMR indicated the specific protonated amines responsible for binding.⁹

More recently, Pilch's group has shown that intrinsic heat capacity changes (ΔC_p , determined by ITC analysis at different temperatures) can indicate whether distinct (and necessary) conformational changes are induced by aminoglycosides, specifically the displacement of A1492 and A1493 residues.¹²² They found that eukaryotic rRNA, lacking the neighboring adenine residues, yield ΔC_p values close to zero for paromomycin binding ($\Delta C_p = -5 \pm 26$ cal/mol K), whereas prokaryotic rRNA, possessing the adenines at the 1492 and 1493 positions, display a large ΔC_p (-162 ± 5 cal/mol K) for paromomycin

binding. These findings, and a more recent report,¹²³ support NMR structural studies comparing prokaryotic and eukaryotic rRNA binding to paromomycin.

Other ITC-based aminoglycoside studies involve binding to duplex RNA,¹²⁴ hybrid RNA/DNA duplexes,¹²⁵ DNA, and resistance enzymes such as phosphotransferases¹²⁶ and acetyltransferases.¹²⁷ Such information provides a basis for future analysis of rationally designed molecules for targeting RNA, and is critical for understanding the molecular forces behind aminoglycoside recognition of the A-site and how they compare with other competing substrates (such as resistance enzymes).

4. Surface Plasmon Resonance

Valuable thermodynamic and kinetic data from ligand–substrate interactions can also be gathered using surface plasmon resonance (SPR). A general description of a typical SPR experiment consists of immobilization of a 5'-biotinylated RNA aptamer onto a streptavidin-coated sensorchip. This is followed by introduction of ligand solution, which upon binding, results in a change in refractive index of the RNA-bound sensorchip. Changes in refractive index can be monitored to convey the ligand–RNA interactions in real time.¹⁰¹ Wong's group has provided the majority of research in the area of aminoglycoside–RNA interactions monitored by SPR, particularly with the 16S A-site and other novel RNA targets.^{99–102,128,129} The reader is therefore referred to a recent publication focusing on the utility of SPR for such interactions.¹³⁰

V. NOVEL TARGETS FOR AMINOGLYCOSIDE RECOGNITION

Over the past decade, several nucleic acid structures other than the 16S rRNA A-site have been discovered as aminoglycoside targets. Virtually all of these novel targets are RNA structures, and this infidelity of aminoglycosides for various RNA structures has been the subject of numerous reviews.^{131–133} Over the past few years, not only has a deeper understanding of RNA recognition been grasped, but the list of nucleic acid structures that bind aminoglycosides has been expanded to include DNA and proteins. Outlined next are the variety of targets, other than the 16S A-site, that have been discovered for their binding to aminoglycosides. These include RNA targets such HIV-1 RNA, ribozymes, mRNA, and tRNA. Novel DNA targets include both DNA and hybrid RNA/DNA duplexes and triplexes. The new discovery of aminoglycoside binding to proteins such as the Anthrax lethal factor will also be addressed.¹³⁴ The array of

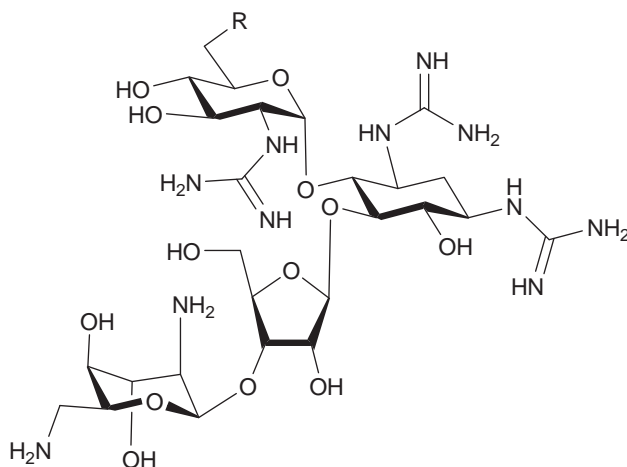
studies discussed in the forthcoming sections rely primarily on the techniques already described, so limited detail will be on the experimental technique.

1. RNA

a. HIV-1 RNA.—RNA targets that play key roles in transcription of the HIV genome include the trans-activating region (TAR) and the Rev response element (RRE). Both RNA regions are responsible for recognition of proteins that assist in transcription. The RRE is responsible for binding the Rev protein. This protein is responsible for facilitating the transport of HIV RNA out of the host cell nucleus without exposure to splicing agents. The prevention of splicing retains the complete HIV strand that is required for further replication of viral particles. The HIV-1 Tat protein binds TAR RNA, a required interaction for the efficient transcription of the full-length viral genome. A more recently discovered HIV RNA target is the packaging region (Ψ element), which is a site for RNA dimerization and nucleocapsid recognition, required events for the viral life cycle. Therefore, one can envision the potential that aminoglycoside-based recognition has in combating AIDS.

Among the aminoglycosides, neomycin B is the most effective at inhibiting Rev protein recognition of RRE. Quantitative studies of neomycin binding to constructs of RNA similar to the RRE decoding region gave strong indication of the necessity of non-duplex RNA forms.¹³⁵ By utilizing a fluorescent-labeled paromomycin structure for binding various constructs, K_d values for several aminoglycosides have been determined using a competition assay monitored by fluorescence. Neomycin showed the strongest binding, with K_d values in the sub micromolar range. The binding was shown to decrease as the number of non-canonical base pairs and/or bulges decreased.¹³⁵ This finding agreed with earlier methods and painted a uniform picture of the structural requirements for high-affinity binding by neomycin.

However, around this time Wong and coworkers, using SPR, demonstrated that up to three molecules may bind such constructs at one time, therefore suggesting more than one binding site.¹⁰¹ This phenomenon gained further support in later stopped-flow fluorescence studies.¹³⁶ More recently, novel aminoglycoside-based ligands have been developed and exhibit enhanced binding to RRE. Aminoglycoside dimers such as neo-neo¹³⁷ (Fig. 15) have been shown to bind the RRE region nearly 20-fold more strongly than monomeric neomycin, further suggesting a secondary binding site for neomycin (Fig. 16).¹³⁸ An excellent review was recently reported incorporating such dimers and other novel



- 20** guanidino-paromomycin R = OH
21 guanidino-neomycin BR = NH(C=NH)NH₂

FIG. 17. Structures of guanidinoglycosides with potent HIV inhibition activity.¹³⁹

shown to inhibit HIV replication nearly 100 times greater than parent aminoglycosides (Fig. 17).¹⁴⁰

TAR binding by aminoglycosides, like RRE, has received its share of attention as a potential anti-HIV area. The TAR element consists of the first 59 bases in the primary HIV-1 transcript, adopting a hairpin structure with a UCU bulge four base pairs below the loop of the hairpin.^{141,142} A construct of the TAR element is shown in Fig. 18.¹⁴³ Neomycin has been found to be a non-competitive inhibitor of Tat by binding the lower stem of TAR and disrupting the conformation such that the neighboring site becomes inadequate for Tat recognition.^{144,145} An interesting electron paramagnetic resonance study has suggested the possibility of a guanidinoneomycin binding at the site of Tat, in contrast to that of neomycin B.¹⁴³ Aminoglycoside–arginine conjugates have been shown to also bind TAR, and with greater affinity than RRE (5 nM vs. 23 nM).^{146–150} A recently developed peptide nucleic acid (PNA)–neamine conjugate has been shown to inhibit viral synthesis as well as hydrolyze the RRE target.¹⁵¹

An RNA target in HIV later discovered is the ψ element, responsible for RNA dimerization and packaging, two necessary functions for viral perpetuation.¹⁵² Little is known as yet regarding the exact binding site within the large RNA.

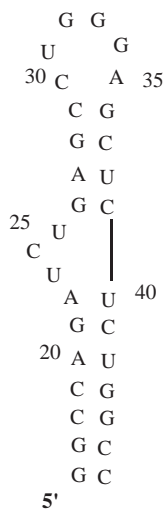


FIG. 18. Secondary structure of a construct of the TAR element of HIV-1 found to bind aminoglycosides.¹⁴³

Footprinting and spectroscopic results have indicated multiple binding sites for such aminoglycosides as neomycin,¹⁵³ paromomycin, and guanidinoneomycin.^{154,155} Significantly different results were obtained for a neomycin–neomycin dimer as well as a neomycin–acridine conjugate, demonstrating recognition differences that may potentially be exploited in future studies.¹⁵⁵

b. Ribozymes.—Aminoglycoside antibiotics have been shown to bind preferentially to ribozymes and inhibit their activity. Among these are the hammerhead, hairpin, RNase P, group I intron, and the hepatitis delta virus ribozymes. In all cases, the cationic nature of aminoglycosides plays an important role. Therefore, aminoglycosides such as neomycin, which possesses six amino groups, five of which are protonated at physiological pH, display the strongest binding to such ribozymes. Most studies mentioned here therefore consider neomycin or neomycin derivatives.

The hammerhead ribozyme is a small RNA that catalyzes specific RNA cleavage in the sugar–phosphate backbone. The function of this RNA strongly relies on Mg^{2+} placement to maintain structural integrity. Aminoglycoside action in inhibition of hammerhead ribozyme function has been shown to involve displacement of these necessary Mg^{2+} ions.¹⁵⁶ Structural studies of the hammerhead ribozyme bound by neomycin have indicated that the charged ammonium groups of neomycin are at similar sites of divalent Mg^{2+} ions (a model is

depicted in Fig. 19).^{157,158} Moreover, neomycin has been shown to displace five Mg^{2+} ions upon binding to the RNA, so all ammonium ions in neomycin are essential for binding. An increase in pH (above 8) has been shown to significantly reduce the inhibition properties of neomycin, further validating the concept that charge is a definite requirement for strong binding.¹⁵⁶ Modified aminoglycosides containing an extra amino group have shown that increased cationic charge results in increased binding and inhibition.¹⁵⁹ However, the number of charges can go too far; dimeric aminoglycosides,^{159,160} possessing upwards of +10 charge, showed no profound increase in activity, suggesting

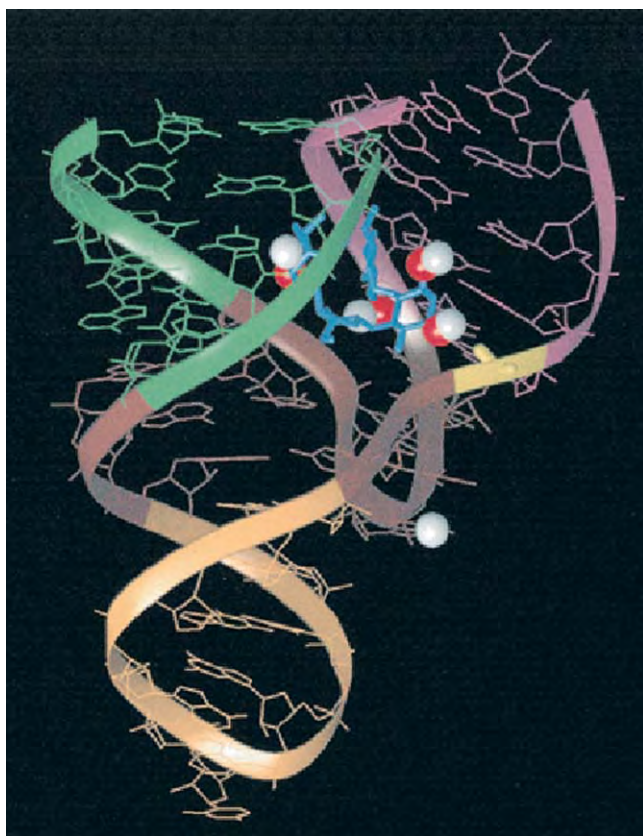


FIG. 19. Neomycin binding to the hammerhead ribozyme.¹⁵⁷ Protonated amino groups of neomycin (in blue) are shown as red spheres and are compared with the positions of Mg^{2+} ions (white spheres). The phosphate cleavage site is depicted in yellow. Reprinted with permission, Copyright 1998 Elsevier.

that discrete binding pockets are present and can be satisfied with approximately six well-placed electrostatic interactions.¹⁶¹

Like most ribozymes, the hepatitis δ virus (HDV) ribozyme requires divalent cations and self-cleaves to generate a 2',3'-cyclic phosphate at the 5' end and 3' fragment containing a 5'-hydroxyl group.¹⁶² Also characteristic among ribozymes in regard to neomycin binding and inhibitory activity, displacement of crucial Mg^{2+} within the RNA is the most likely explanation.^{163,164} Footprinting experiments have indicated two binding sites for neomycin binding, one near the catalytic core and one at the end of stem IV.¹⁶⁴ The catalytic core binding is the probable cause for inhibitory activity, given the fact that other aminoglycosides bind the HDV ribozyme but show no inhibition,¹⁶⁴ and that stem IV can be removed and catalytic activity is still maintained.

Neomycin has also been shown to inhibit hairpin ribozyme activity, but to a weaker extent than other catalytic RNA such as those already mentioned.¹⁶⁵ However, the aminoglycoside 5-epi-sisomicin (Fig. 20) has shown notable activity, with inhibition constants in the sub-micromolar range. Interestingly, ribozyme cleavage is promoted with aminoglycosides in the absence of Mg^{2+} . The same observation was made with such linear polyamines as spermine,¹⁶⁵ suggesting that the cleavage step is not necessarily dependent on charge and shape complementarity as it would seem to be with aminoglycosides.

Aminoglycosides are also known to inhibit group I intron splicing.¹⁶⁶ Footprinting studies have indicated that neomycin, as it does with other ribozymes, most probably displaces metal ions to elicit its action in inhibiting splicing.¹⁶⁷ Detailed mutational studies, coupled with molecular modeling, have shown that displacement of two Mg^{2+} ions is required for inhibition.¹⁶⁸

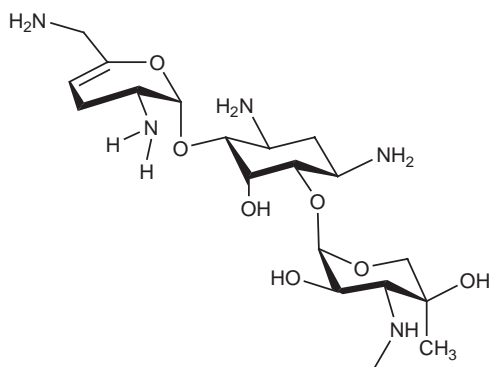


FIG. 20. Chemical structure of 5-*epi*-sisomicin.

A more recently discovered ribozyme that binds neomycin, RNase P is responsible for the maturation at the 5' end of all tRNA in both prokaryotes and eukaryotes.¹⁶⁹ Bacterial RNase P consists of a small RNA and protein subunit, of which the RNA acts as the catalyst in the cleavage reaction. Like the group 1 intron ribozyme, inhibition of activity by such aminoglycosides as neomycin is suggested to occur as a result of the displacement of two important Mg^{2+} ions.^{170,171,172}

c. tRNA.—One may infer at this point that aminoglycosides bind to a variety of RNA, all of which play different roles biologically. The list extends with evidence that aminoglycosides bind tRNA.^{173–175} Chemical and enzymatic footprinting analysis of tRNA^{Phe} with such aminoglycosides as neomycin and dimeric neomycin has indicated that binding sites probably exist in duplex regions adjacent to loop or bulges as well as loops themselves. Specific interactions include the anticodon stem and the junction of the T ψ C and D loops.¹⁷⁴ A more recent X-ray study has shown that neomycin's primary binding site is in the major groove adjacent to the D loop (Fig. 21), containing six potential hydrogen bond interactions.¹⁷⁵ Comparisons of the neomycin–tRNA^{Phe} crystal structure with other tRNA^{Phe} crystal structures with either Pb^{2+} or Mg^{2+} indicate a noteworthy resemblance to the placement of cations (the protonated amines of neomycin). The binding of neomycin to tRNA is therefore believed to involve the displacement of divalent metal ions, a similar phenomenon to that observed with ribozymes.

d. mRNA.—A number of aminoglycosides have been shown to specifically bind an RNA construct corresponding to the mRNA site for thymidylate synthase (TS). Thymidylate synthase catalyzes the reductive methylation of 2'-deoxyuridine 5'-monophosphate (dUMP) to form thymidine monophosphate, which is a critical reaction within the DNA synthesis cycle.¹⁷⁶ Thus, it has become a target for such chemotherapeutic agents as 5-fluorouracil. Among the RNA constructs known for TS binding is its own mRNA. Aminoglycoside binding to TS mRNA involves an internal CC bubble structure that coincidentally is thought to be important for efficient translation.¹⁷⁷ Other than the TS mRNA construct, other structures containing an internal CC bubble were shown to attract aminoglycoside binding, validating a structural preference of aminoglycosides.¹⁷⁷

e. RNA Triplex.—Though it is the first triple–helical nucleic acid structure reported,¹⁷⁸ the RNA triplex has received little attention when compared with other RNA structures or DNA triplexes (discussed later) for that matter. Since a large number of important RNA targets consist of duplex motifs, the

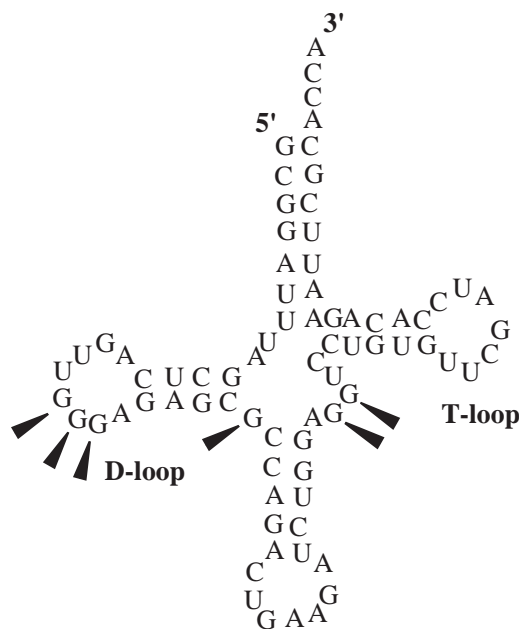


FIG. 21. Secondary structure of the aminoglycoside-binding region of tRNA^{Phe}. Dashes indicate neomycin and dimeric neomycin interactions.¹⁷⁵ Reprinted with permission, Copyright 2001 Nature Publishing Group (<http://www.nature.com/>).

introduction of a third strand to the duplex, to form a triplex, has obvious implications for inhibiting protein function at their recognition sites. Likewise, single-stranded RNA can be targeted by circular or foldback triplex-forming oligonucleotides (TFOs), which intramolecularly form duplex structures.^{179–182} Triplex formation is limited to homopyrimidine or homopurine stretches, which in turn limit its therapeutic applicability. Nevertheless, potential exists with knowledge of the RNA primary sequence. One example of an important RNA sequence for TFO targeting has been the 5' non-coding region of hepatitis C viral RNA, which has been shown to form a triple-helical structure in the presence of Mg²⁺ and the polyamine spermidine.¹⁸³

More recently, aminoglycosides have been shown to significantly stabilize RNA triplex structures. Among the aminoglycosides, and as with many other RNA-binding studies, neomycin was found to be the most significant RNA triplex-stabilizing aminoglycoside.⁶⁵ More notably, neomycin was shown to be the most significant RNA triplex-stabilizing agent among all known ligands,

with the exception of ellipticine. Thus, another RNA structure was found to bind aminoglycosides, further emphasizing the binding infidelity of aminoglycosides.

2. DNA Triplex

The association of homopyrimidine•homopurine stretches of duplex DNA are known to be targets for triplex formation by major-groove association of a TFO.¹⁸⁴ TFO recognition of duplex DNA can be exploited in a variety of ways, such as by inducing transcription inhibition, site-directed mutagenesis, or recombination. Another attractive feature of triplex DNA is the feature of H-DNA, an intramolecular-forming triplex, found in biological systems. H-DNA formation is found within mirror repeats of homopyrimidine•homopurine stretches in plasmid DNA, in which triplex formation requires a negative supercoiling (dissociation of symmetrical duplex stretch with folding back of a single strand to form triplex).⁶⁶ The constrained, bent DNA conformation that occurs upon H-DNA formation is often observed with regulatory proteins, and therefore the formation of such structures may represent a form of molecular switch in controlling gene expression. The targeting of triplex DNA is thus of obvious interest. However, triplex formation is thermodynamically and kinetically less favorable than duplex–TFO dissociation. Therefore, the driving force for utilizing TFO-based recognition for therapeutic purposes is the development or discovery of ligands that stabilize and kinetically favor the formation of triplex structures in a specific fashion.

Neomycin, among a series of aminoglycoside antibiotics studied, has been shown to significantly stabilize DNA triplexes.^{65,185–188} Neomycin was also shown to enhance the rate of TFO–duplex association.⁶⁵ The binding and stabilization of DNA triplexes by neomycin is unique among other triplex-stabilizing ligands in that no DNA duplex binding occurs. Molecular modeling has suggested neomycin binding within the Watson–Hoogsteen groove, and that it is neomycin's charge and shape complementarity that drives triplex recognition over duplex (Fig. 22).¹⁸⁶ All previously discovered triplex-stabilizing ligands also displayed some degree of duplex stabilization as well. Moreover, neomycin is the first-groove binding ligand to exhibit DNA triplex stabilization (the absence of a fused, planar ring system eliminates the structural possibilities for intercalation). This exciting finding was the first example of DNA-based nucleic acid recognition by aminoglycosides. The list of nucleic acid structures that aminoglycosides bind, however, did not end with the DNA triplex.

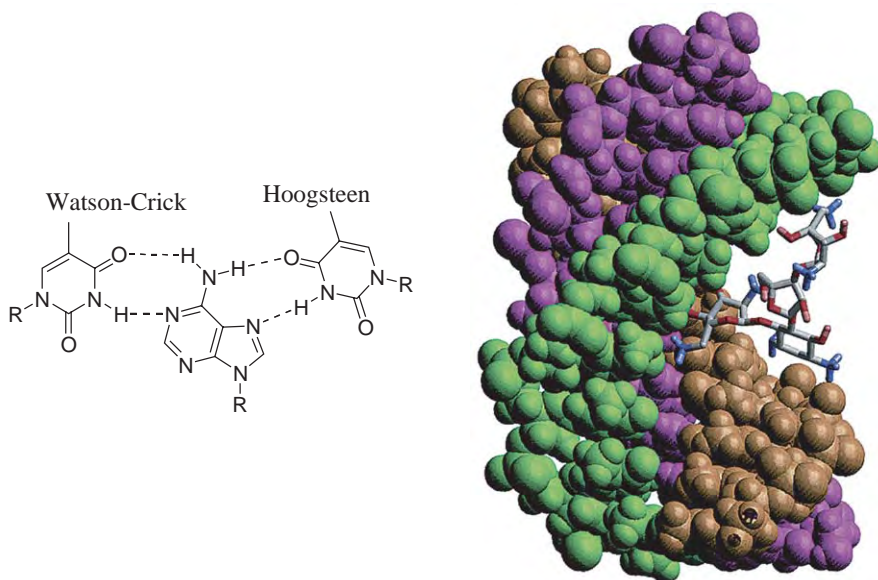


FIG. 22. (left) Base pairing in a T•A•T triplex; grooves (from TFO binding in the major groove) are indicated; (right) computer model of neomycin bound to the Watson–Hoogsteen groove of a DNA triplex.¹⁸⁶ Ring I of neomycin (see Fig. 1 for structure and ring designations) rests in the groove center, while protonated amines of rings II and IV assist in bridging the two pyrimidine strands of the triplex. The model is reprinted with permission, Copyright 2003 American Chemical Society.

3. DNA/RNA Hybrids

a. Hybrid Duplex.—DNA/RNA hybrid duplexes are biologically relevant due to their recognition by such enzymes as RNase H and reverse transcriptase.^{189,190} Recognition of such structures by small molecules therefore has potential in antiviral applications. Earlier mutational studies indicated that genetic deactivation of the RNase H activity of HIV-1 reverse transcriptase (RT) results in non-infectious virus particles,¹⁹¹ and thus the importance of RNase H is obvious. Targeting crucial RNase H-based interactions is a pathway for developing anti-HIV agents. It is even more attractive when considering that RNA/DNA hybrids formed during the reverse-transcription process are not associated with a high mutation frequency, as RT¹⁹² and protease inhibitors^{193–196} (both AIDS therapeutic agents) are.

Aminoglycosides were recently shown to bind DNA/RNA hybrids related to HIV-1.^{125,197} Using a combination of cleavage, calorimetric, and spectroscopic studies, paromomycin was shown to significantly stabilize octomeric hybrid duplexes with binding affinities up to 200-fold higher than the control DNA duplexes.¹²⁵ Significant inhibition of cleavage by RNase H was also observed. A later study, involving hybrid duplexes that mimic RNase H substrates at both early and late stages of the reverse transcription process, has involved other aminoglycosides, namely neomycin and ribostamycin.¹⁹⁷ The key structural differences between these are the following (see Fig. 1 for structures): neomycin possesses an amine at the 6' position (ring I), whereas paromomycin contains a hydroxyl group; ribostamycin is similar to neomycin, but lacks ring IV. The activity of these three aminoglycosides were in the order: neomycin > paromomycin > ribostamycin. The activity thus correlates with the amount of charge on each aminoglycoside (neomycin has six protonated amines, paromomycin has five, and ribostamycin has three. Under these conditions (pH 6.0), the 3-position amine (ring II) is protonated. The correlation of charge with binding is a common theme in aminoglycoside binding, and emphasizes the potential problems in achieving binding specificity. Nevertheless, the utilization of aminoglycosides to HIV-1-based hybrid duplexes offers an exciting new area to explore in efforts to combat viral infections.

b. Hybrid Triplex.—DNA/RNA hybrid duplexes can be targeted by TFOs to form a hybrid triplex structure. The TFO in hybrid triplex can consist of a DNA or RNA strand complementary to either strand of the duplex¹⁹⁸ (consider the examples poly(rA)•2poly(dT) and 2poly(rA)•poly(dT) that have been shown to exist).¹⁹⁹ As with small ligands that bind hybrid duplex, TFOs may produce similar results concerning the prevention of key biological events involving hybrid structures. In fact, stable hybrid triplex formation has been shown to inhibit RNA polymerase,¹⁹⁸ RNase,²⁰⁰ and DNase I.²⁰⁰ However, the formation of such triplex structures requires molar salt concentrations. Recent studies have circumvented this requirement by introducing neomycin. Neomycin was shown to induce the hybrid triplex structures poly(rA)•2poly(dT) and 2poly(rA)•poly(dT) using a series of spectroscopic techniques.²⁰¹ The induction and binding of this groove-binding ligand occurred at low micromolar neomycin concentrations and low millimolar sodium concentrations. In concert with binding to hybrid duplex structures, a common theme started to emerge regarding the structural preference of such aminoglycosides as neomycin. Not only does neomycin binding occur with complex RNA structures, but to triple-helical DNA and hybrid duplexes and triplexes. What, then, do such structures have in common?

4. A-Form Nucleic Acids

Competition dialysis has recently been utilized to explore neomycin's binding preference among a number of different nucleic acid structures.^{202(a,b)} It was found that, as expected, neomycin binds RNA structures, including a 16S A-site construct and RNA duplexes and triplexes. However, other non-RNA structures were found to bind neomycin. These included not just DNA/RNA hybrids and DNA triplexes, but also tetraplex structures and the poly(dG)•poly(dC) duplex. The initial feeling from these experiments was that, as expected, neomycin's promiscuity for binding different nucleic acid structures were not an exception to this assay. However, a deeper investigation of the literature elicited an exciting discovery. Though known for RNA, all "unexpected" structures that displayed binding in the competition assay have been shown to possess A-like conformations. For example, cations such as aminoglycosides have been shown to induce the B-A transition in dG•dC rich sequences such as poly(dG)•poly(dC),^{203,204} and CD studies have shown tetraplexes to possess A-like conformations.²⁰⁵ These results unraveled the ties that held RNA structures together as the specific site for aminoglycoside recognition. While not questioning the mode of action of aminoglycosides to rRNA, the chemical principles behind aminoglycoside–nucleic acid binding warrants concern. It is not just RNA, but A-like conformations of nucleic acids that such aminoglycosides as neomycin prefer to bind. A clear representation of neomycin binding to an A-form structure, compared with B-DNA, is depicted in Fig. 23.

5. B-DNA

The recognition of DNA by aminoglycosides was recently found to include B-DNA.^{206,207} Previous studies involving triplex DNA–aminoglycoside interactions showed no stabilization of duplex DNA. By utilizing neomycin intermediates^{208,209} for synthetic conjugation with B-DNA-binding ligands such as Hoechst 33258, significant stabilization of DNA duplexes was observed.²⁰⁶ Spectroscopic studies, along with molecular modeling, have suggested a dual interaction between this neomycin–Hoechst 33258 conjugate (Fig. 24) and the different grooves of DNA (Fig. 25). The DNA binding of the Hoechst moiety is likely within the minor groove of an A_nT_n tract, which then docks the neomycin moiety, separated by an alkyl linker, within the major groove. Even more recently a neomycin–neomycin dimer has shown promise in preferential binding of A/T stretches of B-DNA within the major groove.²⁰⁷ These studies underscore

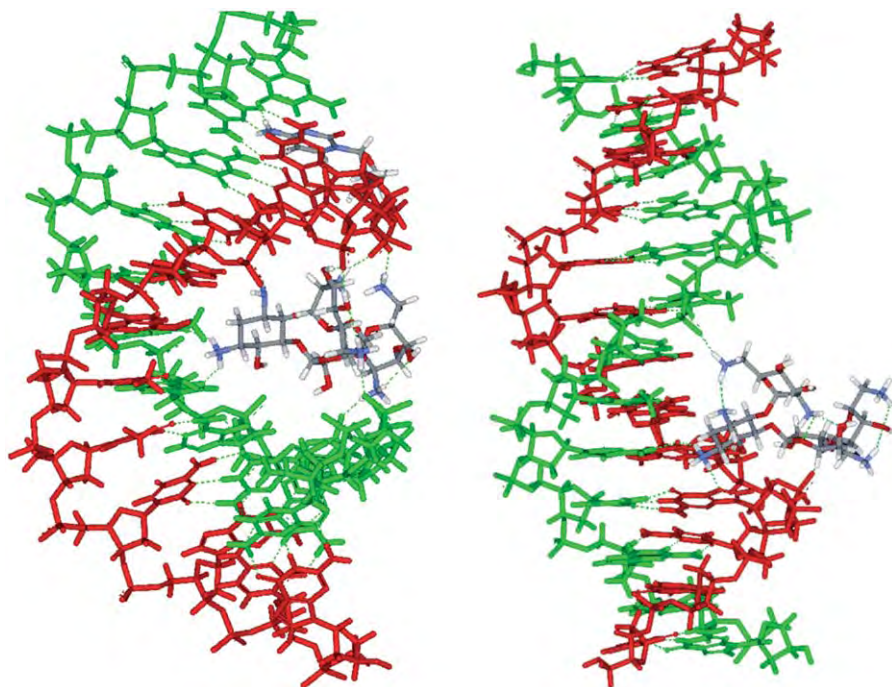


FIG. 23. Computer-generated models of neomycin binding to A-form DNA (left) compared with B-DNA (right). Note the groove complementarity of neomycin with the A-DNA major groove as compared with the B-DNA groove.

the utility of recent synthetic developments in neomycin derivatization for the applicability in DNA recognition endeavors. Furthermore, these novel molecules are the lone examples of aminoglycosides that exhibit B-DNA stabilization. Such work warrants structural investigation which surely will be insightful for the development of recognition principles that govern aminoglycoside–nucleic acid interactions.

6. Aminoglycosides as Cleaving Agents

The hydroxyl groups in aminoglycosides have been exploited as metal donors in their complexation with copper. Such “metalloaminoglycosides” as Cu^{2+} –kanamycin (Fig. 26) has shown promising cleavage activity with both RNA and DNA. Efficient and specific cleavage of an RNA aptamer has been achieved at

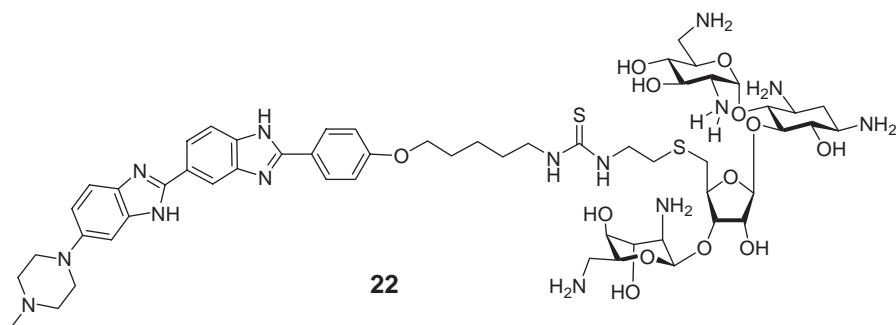


FIG. 24. Structure of a neomycin–Hoechst 33258 conjugate that binds B-DNA.²⁰⁶

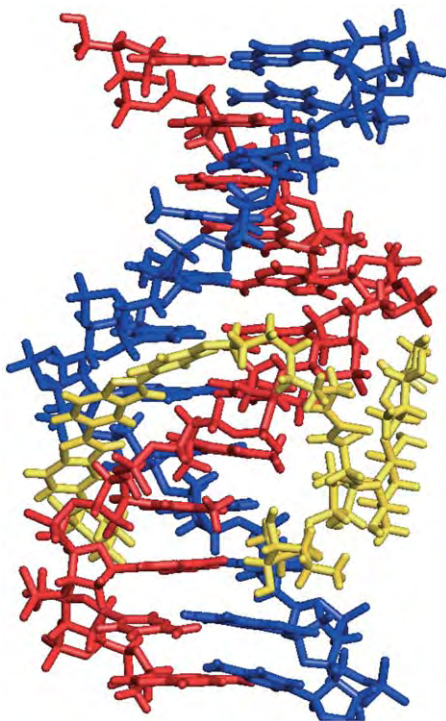


FIG. 25. Computer model of the neomycin–Hoechst 33258 conjugate bound to both grooves of d(CGAAATTTGCG)₂.²⁰⁶ The conjugate is depicted in yellow, and DNA strands are in blue and red. Reprinted with permission, Copyright 2003 American Chemical Society.

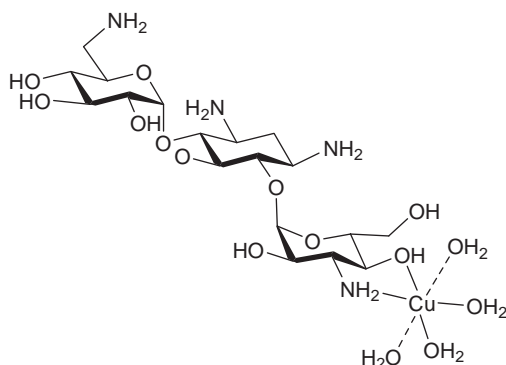


FIG. 26. Structure of a Cu^{2+} –Kanamycin complex with nucleic acid cleavage properties.²¹⁰

physiological pH and temperature by a Cu^{2+} –kanamycin complex.²¹⁰ The absence of cleavage of other RNA structures such as poly(C) and poly(A)•poly(U) confirmed the specificity of the kanamycin complex. Because RNA binding is structure specific and not sequence specific, such structural recognition by these aminoglycoside complexes has advantages over artificial ribonucleases based on oligonucleotide–Lewis acid conjugates. Further *in vivo* studies have indicated that targeted cleavage can occur at concentrations where aminoglycosides alone show translation inhibition.²¹⁰ Cu^{2+} –kanamycin complexes have also been shown to catalytically cleave DNA via oxidative (Fig. 27) and hydrolytic pathways.^{211,212} Both Cu^{2+} –kanamycin and Cu^{2+} –neamine complexes were also shown to display a greater than million-fold rate enhancement of DNA cleavage, approaching that of enzymes.²¹¹

7. Other Aminoglycoside Targets: The Anthrax Lethal Factor

A recent discovery out of Wong's laboratory involves the inhibition of the Anthrax Lethal Factor protein, one of three plasmid-encoded proteins responsible for anthrax development.¹³⁴ Among a library of 3000 compounds studied, neomycin was found as the most potent in inhibitory activity ($K_i = 7$ nM). Interestingly, a further comparison of dimeric neomycin derivatives (see Fig. 11) indicated that neomycin was still the most potent, though dimeric aminoglycosides bind the 16S A-site more strongly. These interesting findings further illustrate the potential for utilizing aminoglycoside-based structures for targeting not just RNA, but DNA and proteins as well. As with the rest of the structural

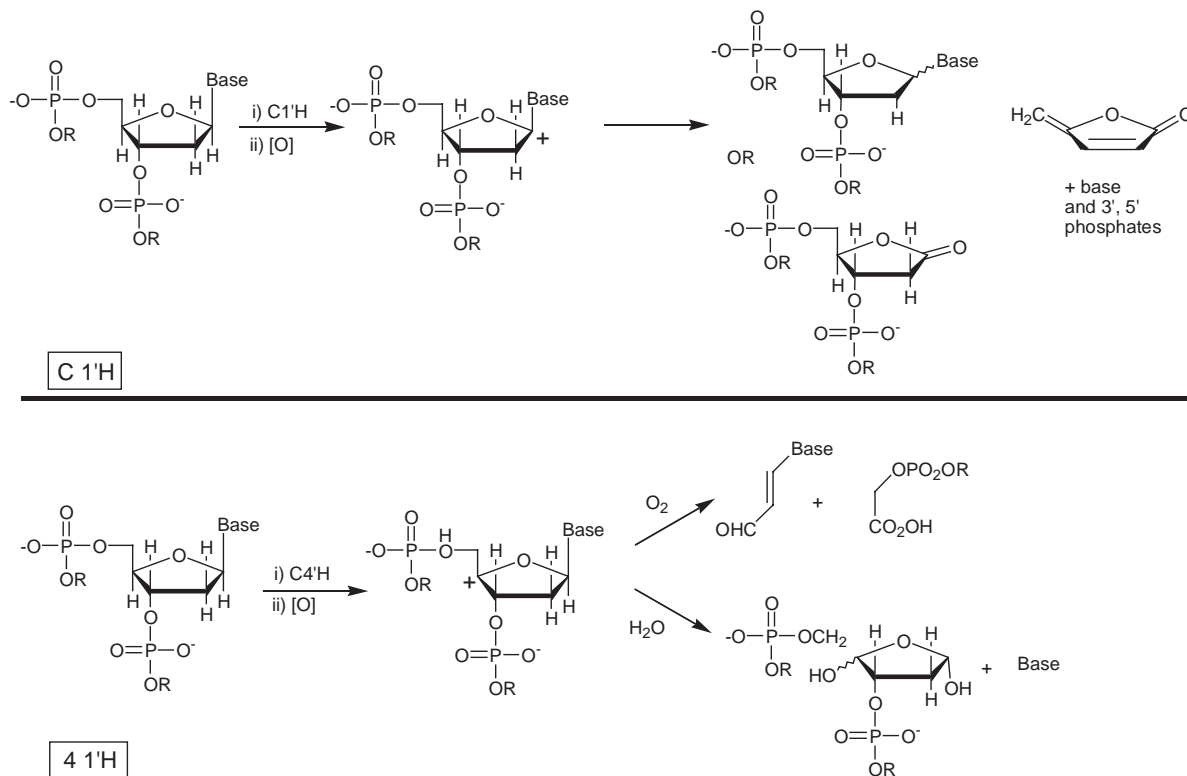


FIG. 27. Oxidative cleavage of DNA by C 1'H (top) or C 4'H (bottom) pathways by Cu^{2+} -aminoglycosides.²¹² Reprinted with permission, Copyright 2001 Royal Society of Chemistry.

targets for neomycin, both charge and shape complementarity to the protease active site contributes to its binding.

VI. CONCLUSION

Since the discoveries that aminoglycosides induce codon misreading by binding to the aminoacyl site of the bacterial ribosome's 30S subunit, a generous amount of insight has been gathered in regards to their mechanisms of action, toxicity, and resistance. There still remains an incomplete understanding of the mechanisms of toxicity and resistance. The ever-expanding list of structures to which aminoglycosides bind prompts anticipation of multiple pathways for toxicity to occur. For example, could neomycin binding to critical triple-helical H-DNA be one of the primary factors for toxicity? The generalization that aminoglycosides favor A-like conformations of nucleic acids is sure to prompt further structure-activity studies of aminoglycoside-based molecules. Additionally, chemical modification can exploit aminoglycoside structure and charge in binding to otherwise unnoticed structures such as B-DNA. Such advanced knowledge of aminoglycoside binding could have promising impacts on the fate of future developments of aminoglycoside-based drugs with novel biological targets, as well as with reduced side effects and resistance.

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HEVEIN DOMAINS: AN ATTRACTIVE MODEL TO STUDY CARBOHYDRATE-PROTEIN INTERACTIONS AT ATOMIC RESOLUTION

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I. INTRODUCTION

The elucidation of the mechanisms that govern how oligosaccharides are accommodated in the binding sites of lectins, antibodies, and enzymes is currently a topic of major interest.¹ It is obvious from the emerging wide functionality of this type of molecular recognition that a detailed knowledge of the structural, dynamic, and energetic features of the complex when carbohydrates are bound to lectins (non-enzymatic carbohydrate-binding proteins) and enzymes is indeed relevant,² and that the adoption of an interdisciplinary approach to study this phenomenon in depth is an essential precondition.³ The concerted use of a variety of an array of biophysical, spectroscopic, and biochemical techniques, together with access to synthetically prepared oligosaccharides, and analogs thereof, or glycomimetic peptides,⁴ as well as to natural and “designed” protein domains is of paramount importance.⁵ The accrued knowledge, thereby will help in devising guidelines for the design of new classes of pharmaceuticals, based on exploiting the sugar code.⁵ X-Ray, NMR, microcalorimetry, and other biophysical experimental techniques are among the methods that are becoming more widely adopted to gain access to detailed structural and thermodynamic information.⁶

II. PROTEIN–CARBOHYDRATE INTERACTIONS: A GENERAL VIEW

From a chemical viewpoint, prominent aspects of carbohydrate structure relevant for intermolecular interactions are the distinct patterns of (hydrophilic) hydroxyl groups and apolar (hydrophobic) aliphatic C–H regions (patches). Because of this amphiphatic character of the oligosaccharide, various kinds of forces may be involved in the process of its recognition by a given protein.⁷ Not only are polar forces involved in carbohydrate recognition: depending on the stereochemistry of the monomer constituents of the oligosaccharide chain, the presence of a number of rather apolar C–H groups in fact constitutes zones for which hydrogen bonds are not responsible for binding.⁸ When an apolar face of a monosaccharide shows three or more C–H groups close in space (as in D-galactose), it has been found that the corresponding surface can come into contact with a delocalized π -electron cloud of an aromatic ring of an amino acid side chain, and additional affinity-enhancing factors can be envisaged.⁹ Although the exact nature and origin of this interaction is still under investigation,¹⁰ it may be proposed that the mutual shielding of the non-polar surfaces

from bulk water by ligand contact is entropically favorable,¹¹ and that the electrostatic interaction between the positive net charge of the C–H groups and the quadrupole created by the π -system of the aromatic ring makes a favorable enthalpic contribution.¹² Probably, the polarizability of the aromatic electrons and the polarizing nature of the C–H vector leads to an attractive force. Indeed, equivalent features are also found in other protein–ligand complexes, and have been proposed to account for an important portion of the driving force in ligand-accommodating mechanisms.¹³ This role provides an intriguing argument for the necessity for tryptophan among the set of proteinogenic amino acids. Apart from these thermodynamic factors, it should be noted that the proper orientation of the C–H vectors and the aromatic rings maximizes the generation of net enthalpic gain, and thereby improves the conformational/-structural selection.¹⁴

In order to elucidate the details of ligand recognition, the use of X-ray diffraction and NMR spectroscopic analysis, alone or in combination with computations, provides a further means for the study of these interactions. Table I summarizes the techniques used and the information obtained.

III. THE HEVEIN DOMAIN: BASIC FEATURES AND BIOLOGICAL RELEVANCE

Among the various biological processes in which carbohydrates are involved as biochemical signals,¹⁵ it is noteworthy that many plants harbor defense proteins (lectins) against pathogenic attack. These proteins are able to bind to chitin, a β -(1 \rightarrow 4)-linked *N*-acetylglucosamine (GlcNAc) polysaccharide.¹⁶ This natural biopolymer is a key structural component of the cell wall of fungi and of the exoskeleton of such invertebrates as insects, nematodes, and arthropods. Direct binding to the saccharide can occur for the respective lectin, while a particular domain can also be instrumental for chitin-degrading enzymes. The antifungal activity of plant chitinases is largely restricted to those chitinases that contain such a non-catalytic, plant-specific, chitin-binding domain (ChBD, also termed a hevein domain).¹⁷ This domain displays a common structural motif of 30–43 residues rich in glycine and cysteine residues in highly conserved positions and organized around a four-disulfide core.¹⁷ The hevein domain is present in several lectins,¹⁸ as in hevein itself¹⁹ and its natural variant, pseudohevein,²⁰ in the *Urtica dioica* agglutinin (UDA),²¹ wheat-germ agglutinin (WGA),²² and Ac-AMP antimicrobial peptides²³ (Scheme 1; Table II). New members of this group are still being detected, as the example of the smallest pokeweed (*Phytolacca*

TABLE I
Techniques used to study protein-carbohydrate interactions

Biophysical technique	Information provided	References
NMR	3D structure, binding affinity (titration), thermodynamics, binding epitope (titration), conformation, oligomerization state (DOSY)	41–47, 49–53, 59, 80, 86, 92–98, 105, 111, 113, 114, 117, 118
X-ray diffraction (crystal)	3D structure, binding epitopes, conformation, oligomerization state	9, 17, 22, 24, 32–40, 78, 79, 89, 91, 99, 102, 110
Calorimetry	Binding affinity, thermodynamics, stoichiometry	31, 50, 65–70, 81
Laser-photo-CIDNP	Binding affinity, conformational change, involvement of photo-reactive residues	61, 63, 64
Fluorescence	Binding affinity, binding site, thermodynamics	49, 70, 83, 100, 101
IR	Binding affinity	74
Small angle X-ray and neutron scattering	Shape alteration, oligomerization state	75
Modeling	3D structure, estimations for binding affinity, binding epitope, conformation	44, 49, 76, 77

americana) PL-D attests.²⁴ An extensive list of sequence homologs of hevein domains can be found at the CAZY database, within the carbohydrate-binding module family 18 (see <http://afmb.cnrs-mrs.fr/CAZY/>).

This chitin-binding motif can also be found in the enzymes mentioned with antifungal activity, such as class I chitinases.²⁵ Its biological significance is probably related to the catalytic properties of the protein, whose centers are optionally positioned to effect hydrolysis. Growth of the fungus is thus probably limited by the degradation of fungal cell walls caused by the hydrolytic action of the enzyme. In addition, small chitin-binding proteins that contain the hevein domain, such as WGA, hevein itself or Ac-AMP peptides, have also been shown to exhibit a remarkable antifungal and insecticidal activity, even though they do not have any known enzymatic activity.²⁵ Moreover, there is a medical interest in these domains, since they have been related to allergy problems, especially to latex allergy²⁶ and to the so-called fruit-latex syndrome.²⁷ In consequence, several attempts have been made to define the distinct conformational epitope triggering these allergy features.²⁸ Prominent structural features of these

HEVEIN**ACAMP-2****WGA-B****UDA-VI A**

SCHEME 1. Sequence homologs of hevein domains.

proteins are the strict conservation of six cysteines, three glycines, and the key residues for sugar binding at relative positions 19, 21, 23, and 30.

The small size of hevein (43 residues), and the ease of its availability by biochemical purification or methods of peptide synthesis make this domain an excellent model system for the study of carbohydrate recognition by proteins. Herein, and taking the hevein domain as a model, we focus on the study of those molecular-recognition features relevant for the interactions between carbohydrates and proteins. We detail all of the techniques that are instrumental for tackling this problem, and how these can strategically be combined in an efficient manner. Particular emphasis is placed on the acquisition and analysis of data at atomic resolution (by NMR²⁹ and/or X-ray^{7,30}), and how these structural data relate with thermodynamic³¹ and kinetic information in reaching an understanding of the forces and interactions that play decisive roles in the interactions between carbohydrates and proteins.

TABLE II
Hevein domains included in this article and their origin

Domain	Source	References
Hevein	latex (<i>Hevea brasiliensis</i>)	39
Hevein	elderberry	46
Truncated hevein	solid phase synthesis	49
Pseudohevein	latex	50
WGA	Wheat germ (<i>Triticum vulgare</i>)	17
WGA-B	recombinant	51
WGA mutants	recombinant	102
UDA	<i>Urtica dioica</i>	36
Ac-AMP2	<i>Amaranthus caudatus</i>	52
Ac-AMP2 mutants	solid phase synthesis	9
cbML1, cbML2, cbML3	mistletoe, <i>Viscum album</i> L.	107
AVR4 elicitor	<i>Cladosporium fulvum</i>	108
EAFP1 and EAFP2	<i>Eucommia ulmoides</i> Oliv.	109
Ee-CBP	<i>Euonymus europaeus</i> L.	48
PL-D	<i>Phytolacca americana</i>	24
Tachycitin	<i>Tachypleus tridentatus</i>	113
Scarabaecin	Beetle (<i>Oryctes rhinoceros</i>)	117
Tachystatin	<i>Tachypleus tridentatus</i>	118

IV. SUGAR–HEVEIN INTERACTIONS: BASIC TECHNIQUES

1. X-Ray

A variety of methods can be employed to characterize protein–carbohydrate interactions. Obviously, X-ray diffraction is particularly useful to describe the architecture of the complete complex, but this application necessitates the obtaining of crystals of the proteins, a task that is not easy, particularly for relatively small domains. Several three-dimensional (3D) structures of hevein domains have been solved by X-ray methods, and these are detailed in Table III. Starting with the pioneering work of C. S. Wright on WGA,^{32–35} other proteins having hevein domains, such as UDA,^{36–38} AcAMP2,²³ pokeweed lectin,²⁴ and hevein itself^{39,40} have been characterized similarly. Except for the first X-ray structure of hevein,³⁹ the polypeptide backbone of these structures invariably displays a similar architecture, with root-mean-square deviations (rmsd) below 2 Å among the various structures. Importantly, all structures show a cluster of three aromatic residues and one serine, which forms the basic carbohydrate-binding domain (see later).

TABLE III
X-Ray structures reported to date

Domain	Source	References
Hevein	latex (<i>Hevea brasiliensis</i>)	39, 40
WGA and mutants	wheat germ (<i>Triticum vulgare</i>)	17, 22, 32–35, 78, 79, 89, 91, 99, 102
UDA	<i>Urtica dioica</i>	36–38
EAFP2	<i>Eucommia ulmoides</i> Oliv.	110
PL-D	<i>Phytolacca americana</i>	24

2. NMR

NMR spectroscopy has also been employed to deduce the 3D architecture of these domains. A series of studies on latex hevein,^{41–45} elderberry hevein,⁴⁶ five-disulfide-containing heveins,⁴⁷ a truncated hevein of 32 amino acids,⁴⁹ pseudohevein,⁵⁰ the B domain of WGA,⁵¹ as well as natural AcAMP2⁵² and related Ac-AMP2⁵³ peptides have been completed (Table IV). Basically, the 3D structures of these small proteins are again very similar by direct comparison, and are also essentially identical to the solid-state structures described by X-ray.

a. Chemical-Shift Perturbation. Titration NMR.—NMR can be readily employed for detecting binding and then for gaining insight into the structures at atomic resolution. The measurements of chemical-shift perturbations induced by the association process provide a means to monitor the binding process in titrations (Fig. 1).⁵⁴ Provided that the NMR spectrum of the protein in the free state has been totally or partially assigned, this method provides a semiquantitative estimation of the location of the lectin's binding site.⁵⁵ Thus, the binding of carbohydrates to hevein domains can be monitored by recording ¹H-NMR spectra of a series of samples at various sugar concentrations while maintaining the concentration of protein constant during the experiments. Thus, the chemical shifts of the protein protons are monitored by NMR in the presence of increasing amounts of GlcNAc-containing oligosaccharides. Using this protocol, it is straightforward to detect complexes between hevein domains and the corresponding carbohydrates, and, in addition, the alterations in chemical shifts may be used to determine the equilibrium association constants, K_a 's, following simple equations.⁵⁶

In most of the examples reported to date, from mono- to tetrasaccharides, the observed effects on chemical shifts and line broadening indicate that the interaction is basically rapid on the chemical-shift NMR timescale.⁵⁶ For extended chitooligosaccharides, the process takes place more slowly.⁴⁵ Association

TABLE IV
NMR structures reported to date

Domain	Source	References
Hevein	latex (<i>Hevea brasiliensis</i>)	41–43, 45
Hevein	elderberry	46
Truncated hevein	solid phase synthesis	49
Pseudohevein	latex	50
WGA-B	recombinant	51
Ac-AMP2	<i>Amaranthus caudatus</i>	52
Ac-AMP2 mutants	Solid phase synthesis	53
AVR4 elicitor	<i>Cladosporium fulvum</i>	108
EAFP2	<i>Eucommia ulmoides</i> Oliv.	47
Tachycitin	<i>Tachypleus tridentatus</i>	113
Scarabaeicin	Beetle (<i>Oryctes rhinoceros</i>)	117
Tachystatin	<i>Tachypleus tridentatus</i>	118

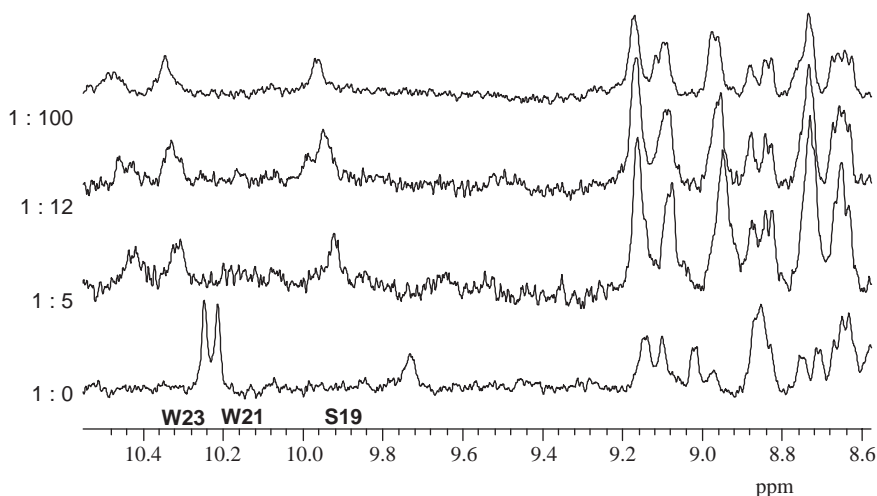


FIG. 1. NMR-titration experiments permit the detection of binding as well as delineating the protein region responsible for interaction with the sugar. The different spectra illustrate the variations in chemical shifts of a hevein domain upon addition of increasing molar amounts of (GlcNAc)₃. Distinct key resonances are labeled.

constants for the binding of a variety of GlcNAc-containing sugars to several hevein domains are given in Table V, and selected examples of the type of spectra acquired are shown in Fig. 1.

These chemical-shift perturbations harbor structural information. All of the significant changes in chemical shifts of the protein affect those residues located

TABLE V
Representative Data on Association Constants of Sugar Binding to Hevein Domains as Measured by Different Methods

Domain	Ligand	Method	Binding Constant (M^{-1})	ΔH° (kcal mol $^{-1}$)	References
Hevein	(GlcNAc)	NMR	30	—	42, 43
	(GlcNAc) ₂	NMR/ITC	600	−6.3	42, 43
	(GlcNAc) ₃	NMR/ITC	11,500/8800	−8.3	42, 43, 45
	(GlcNAc) ₄	ITC	10,900	−9.5	45
	(GlcNAc) ₅	ITC	474,000	−9.6	45
C-truncated hevein(HEV32)	(GlcNAc) ₃	NMR/ Fluorescence	7700	−15.0	49
Pseudohevein	(GlcNAc) ₃	NMR/ITC	4800	−8.8	50
WGA	(GlcNAc) ₂	ITC	5300	−15.6	69
	(GlcNAc) ₃	ITC	11,100	−19.4	69
	(GlcNAc) ₄	ITC	12,300	−19.3	69
	(GlcNAc) ₅	ITC	19,100	−18.2	69
	Sialyl-lactose	NMR		−13.3	92, 93, 105
WGA-B	(GlcNAc) ₃	NMR	1100	−9.3	51
UDA	(GlcNAc) ₂	ITC	2440	−4.1	68, 70
	(GlcNAc) ₃	ITC	7550	−18.3	68, 70
	(GlcNAc) ₄	ITC	17,800	−19.6	68, 70
	(GlcNAc) ₅	ITC	4460	−17.8	68, 70
	(GlcNAc) ₃	NMR	1200	−15.2	53
Ac-AMP2	(GlcNAc) ₃	NMR	1700	−12.9	53
Ac-AMP2 Phe18Trp	(GlcNAc) ₃	NMR	3500	−15.3	53
Ac-AMP2 Phe 18NaphthylAla	(GlcNAc) ₃	NMR	400	−10.8	53
Ac-AMP2 Phe18(4-F-Phe), Phe20(4-F-Phe)	(GlcNAc) ₃	NMR			

around the proposed sugar-binding site. From scrutiny of Fig. 2, it is evident that the binding process exerts a negligible effect on the chemical shifts of protons in residues far from the recognition site. Moreover, this experimental observation argues against the existence of a secondary binding site, but provides evidence for the presence of an extended binding site that can accommodate oligosaccharides having five or more GlcNAc units. Indeed, additional protein-carbohydrate interactions can be observed when chemical-shift differences are compared for oligosaccharides with different sizes. One example is the comparison between the hevein-(GlcNAc)₃ and hevein-(GlcNAc)₅ complexes.⁴⁵ When this methodology is extended to two-dimensional (2D)-spectroscopy, the existence of duplicate signals provides additional evidence for the involvement of dynamic phenomena. The hevein-(GlcNAc)₅ also affords evidence for the existence of at least two different types of complexes in solution.⁴⁵

b. DOSY.—The term diffusion ordered spectroscopy (DOSY)⁵⁷ describes a method of molecular-size determination through the measurement of diffusion coefficients ($\log D$). In the study of ligand-receptor interactions,⁵⁸ the $\log D$ values of ligands can be measured in the presence and absence of receptor. The measured $\log D$ values are a weighted average of receptor-bound and free ligand. Therefore, ligands whose NMR signals showed increase in apparent molecular weight signified a receptor-bound population, while ligands that do not bind to the receptor exhibited no change in $\log D$. This approach has also been used to characterize the binding of hevein to different oligosaccharides (Fig. 3; Table VI).⁵⁹

c. NOE-Based Analysis.—Nuclear Overhauser enhancement (NOE)-type experiments provide information on proximity between pairs of protons.⁶⁰ These proton pairs may belong to the protein or to the carbohydrate, and thus provide information on intra- or inter-molecular short distances. Analysis of the nuclear Overhauser enhancement spectroscopy (NOESY) type of spectra is therefore a great asset for deducing the 3D structure of hevein domains in solution, and for elucidating the bound conformation of the sugar, and is also of salient importance for characterizing the 3D structure of the binding site of the complex, in this case using the intermolecular sugar-protein NOEs as molecular rulers (Fig. 4).

Moreover, closer inspection of the NOESY-type EXSY (EXchange Spectroscopy) experiments is likely to provide evidence for the existence of more than one type of 1:1 protein-sugar complex in solution.⁴⁵ For instance, two sets of signals, undergoing slow exchange, can be observed for the NH protons of several residues of hevein upon binding to the pentamer of GlcNAc. NOESY

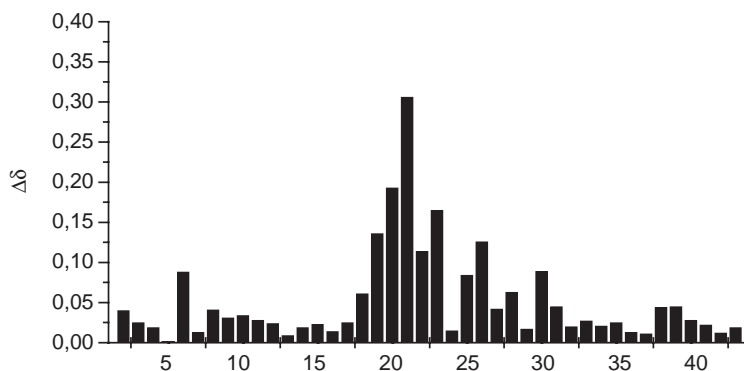


FIG. 2. Chemical-shift perturbation analysis of a hevein domain. The bars indicate the maximum chemical shift differences for the backbone protons between free hevein and the hevein-(GlcNAc)₃ complex.

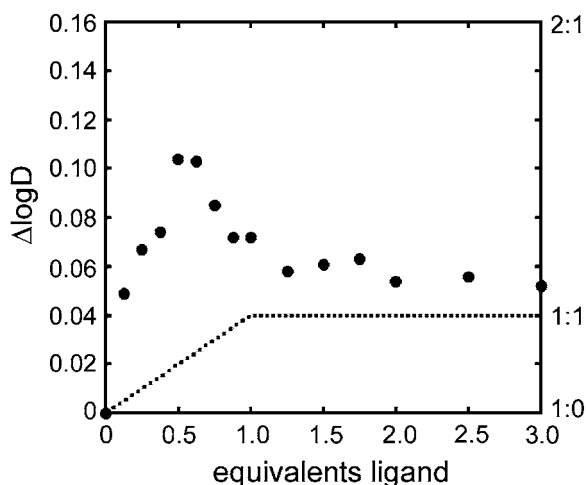


FIG. 3. Titration data obtained by DOSY experiments for (GlcNAc)₆ when added in portions to a hevein domain-containing solution. The plot is of the change in log D ($\Delta \log D$) as a function of stoichiometrically added (GlcNAc)₆ to a hevein domain containing solution. The predicted $\Delta \log D$ values for free hevein (1:0), as well as 1:1 and 2:1 protein-oligosaccharide complexes, are indicated in the right part of the panel.

experiments carried out at different protein-(GlcNAc)₅ ratios reveal that the relative intensities of both sets of signals is dependent on the protein-(GlcNAc)₅ ratio, up to 1:2. No further change was observed for higher molar fractions of (GlcNAc)₅. This observation reveals that, even when the protein is saturated

TABLE VI
Representative studies on the oligomerization state of hevein domains in the ligand-free and ligand-containing states, as determined by different techniques

Domain	Number of Hevein Domains	Ligand-Free State	Bound to	Ligand-Containing State	Method	References
Hevein (latex)	one	monomer	(GlcNAc) ₁₋₄	monomer	NMR ITC	45, 59
	one	monomer	(GlcNAc) ₅₋₈	dimer	NMR, Analytical Ultracentrifugation	45, 59
Hevein (elderberry)	one	monomer	(GlcNAc) ₃	dimer	NMR	46
C-truncated hevein (HEV32)	one	monomer	(GlcNAc) ₃	monomer	NMR	49
Pseudohevein	one	monomer	(GlcNAc) ₃	monomer	NMR ITC	50
WGA	four	dimer	(GlcNAc) ₂	dimer	X-ray	78, 99
		dimer	Sialyloligosaccharides	dimer	X-ray	34, 79
WGA-B	one	monomer	(GlcNAc) ₃	monomer	NMR ITC	51
UDA	two	monomer	(GlcNAc) ₃	monomer	X-ray	37
		monomer	(GlcNAc) ₃	dimer	X-ray	36
		monomer	(GlcNAc) ₃	monomer	NMR	52, 53
Ac-AMP2	one	monomer	(GlcNAc) ₃	monomer	NMR	53
Ac-AMP2 Phe18Trp		monomer	(GlcNAc) ₃	monomer	NMR	53
Ac-AMP2 Phe18NaphthylAla		monomer	(GlcNAc) ₃	monomer	NMR	53
Ac-AMP2 Phe18(4-F-Phe), Phe20(4-F-Phe)		monomer	(GlcNAc) ₃	monomer	NMR	53

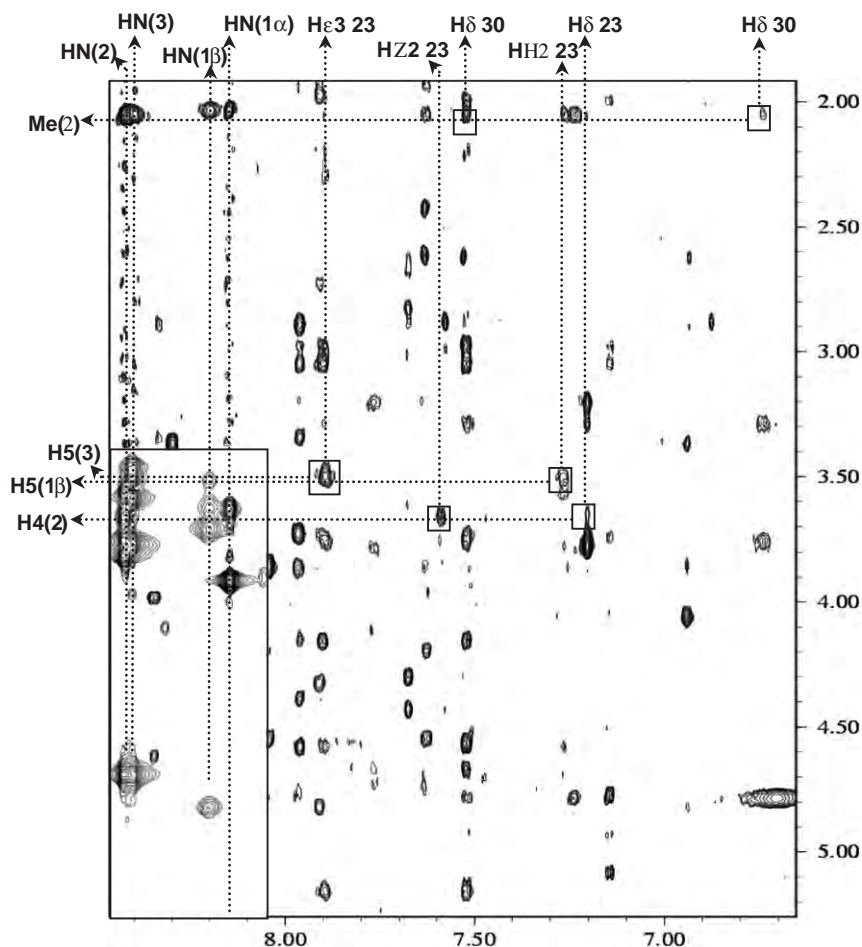


FIG. 4. Section of the NOESY spectrum for a complex between (GlcNAc)₃ and a hevein domain. Key intermolecular protein-sugar NOEs are highlighted. These NOE peaks permit location of the position of the binding site and to gauge the orientation of the sugar in the 3D structure of the complex.

with sugar, there still remains a certain amount of complex in solution for which a particular region remains unaffected by the binding. This fact probably indicates that (GlcNAc)₅ cannot fully cover all possible GlcNAc subsites in the extended binding-pocket of hevein in a simultaneous manner. Alternatively, since the three internal GlcNAc residues of the pentasaccharide are basically

identical from the structural viewpoint, more than one mutually exclusive binding site is possible, with the same interaction energy.

3. Laser Photo-CIDNP

Chitooligosaccharide recognition by hevein domains invariably involves the side chains of tyrosine and/or tryptophan residues. These moieties are able to produce chemically induced, dynamic nuclear polarization (CIDNP) signals after laser irradiation in the presence of a suitable radical pair-generating dye.⁶¹ Evidently, elicitation of such a response in proteins implies accessibility of the respective groups to the light-absorbing dye.⁶² This technique is therefore suitable for monitoring surface properties of a hevein-like receptor and the effect of ligand binding, provided that the laser photo-CIDNP-reactive Tyr and Trp amino acids are affected. Using this methodology, the region of space between the aromatic Tyr and Trp residues of a panel of hevein domains and chitooligomers has been explored by this shielding effect.^{63,64} The relevant data are gathered in Table VII.

4. Titration Microcalorimetry

Differential scanning calorimetry (DSC)⁶⁵ or isothermal titration microcalorimetry (ITC)⁶⁶ are the techniques of choice for quantitative analysis of the thermodynamic features of lectin domains and their sugar-binding abilities. In particular, ITC has been employed for analyzing the binding of chitooligosaccharides to hevein domains and has demonstrated unambiguously that, in all cases, the process is enthalpically driven, while the entropy change opposes binding.^{42–45,67–70} The relevant examples that have been studied are given in Table V.

As a typical example, ITC data on UDA, which contains two hevein-like domains per monomer with two non-identical-interacting sites (one for each

TABLE VII
Representative Studies on Application of Laser Photo-CIDNP, Fluorescence, and IR to Monitor Ligand Interaction with Hevein Domains

Domain	Ligand	Method	References
Hevein and hevein32	(GlcNAc) _{3, 6}	Fluorescence	59
Hevein, pseudohevein, WGA-B, UDA	(GlcNAc) ₃	Laser photo-CIDNP	63, 64
UDA	(GlcNAc) _{2–5}	Fluorescence	70
WGA	(GlcNAc) ₃	IR	74

domain),^{36,37} have shown that each site is composed of three subsites, each binding to a GlcNAc residue.⁷⁰ The thermodynamic parameters obtained show that, while chitobiose has two independent non-interacting sites, chitotriose, chitotetraose, and chitopentaose have two interacting sites on each monomer of UDA, forming a sandwich-like structure (see later). In particular, values of the binding constant increase by almost a factor of 7 in going from chitobiose to chitotriose, indicating the existence of at least three subsites in the combining site of UDA. On the other hand, according to the ITC data, the binding constant for chitotetraose and chitopentaose increases without any further enhancement in the values of ΔH , an indication that, for oligomers larger than chitotriose, the interaction is favored entropically.

The experimental ITC data are recorded using a titration calorimeter. Microliter amounts of the ligand in solution are added by means of a rotating stirrer-syringe to the solution of protein contained in a cell of \approx milliliter volume. Alternatively, microliter amounts of the protein solution may be added to the solution of ligand placed in the cell. The thermodynamic binding parameters are then calculated by analyzing the data through non-linear fitting with relatively simple software. The cumulative heat effect (Q) during the titration process for a simple set of binding sites is given by:

$$Q = M_t V_0 n v \Delta H$$

where M_t is the macromolecule concentration in the calorimetric cell characterized by the working volume V_0 , n the number of binding sites per protein in the given set with a binding enthalpy of ΔH , and v the fractional saturation of each type of site which can be related to the apparent association constant (K') and to the total ligand concentration (L_T):

$$K' = \frac{v}{[(1-v)L]}$$

$$L_T = L + M_t n v$$

5. Fluorescence and IR

Other biophysical methods, such as fluorescence can be used to monitor sugar binding to lectins.⁷¹ The presence of Trp units bound in the hevein domain provides a key point for spectroscopic probing of hevein-sugar interactions, and fluorescence can be used to detect and measure the binding affinity of lectin

domains to chitin oligomers.^{70,72} All relevant data for measuring interactions with fluorescence are compiled in Table VII. For these experiments, several aliquots of chitoooligosaccharide solution are added to the protein solutions, and data are recorded at the best λ_{em} value, which provides the maximum difference between the spectra of bound- and free protein. Corrections are made for protein dilution. Titration data are then fitted by least squares using a curve-fitting routine that corrects for bound-protein concentration.

In hevein domains containing both Tyr and Trp residues, fluorescence resonance energy transfer (FRET)⁷³ is expected to provide a large contribution to the fluorescence intensity, as Tyr30 (a FRET donor) is positioned 10–15 Å from Trp21 and Trp23 (FRET acceptors). As a key example, by comparing data for ligand-bound latex hevein and its C-terminally truncated polypeptide analog (HEV32)⁴⁹ their similar fluorescence spectra revealed that, in their complexes, their aromatic residues have similar degrees of solvent exposure, relative inter-residue distances, and orientations. However, this is not the case for the free entities, since their fluorescence spectra are distinct, showing a longer wavelength λ_{max} (by 1.4 nm) and larger degree of solvent quenching in HEV32 as compared to hevein, indicating different distances and/or orientations of the FRET donor and acceptors.⁴⁹

On a parallel basis, infrared (IR) spectroscopy may indicate variation of the secondary structure of the polypeptide between the free and bound states. For instance, the conformational changes in WGA induced by GlcNAc-bearing liposomes or GlcNAc oligomers have been studied by IR differential spectroscopy. According to the IR data, GlcNAc binding to WGA resulted in a decrease of turns and α -helices, with the concomitant appearance of β -sheets. While describing changes in the secondary structure, these rather global data should be considered only qualitative.⁷⁴ Nevertheless, the technique is helpful for monitoring ligand-induced changes. An emerging application concerns small-angle X-ray and neutron scattering, which is able to detect shape alterations in lectins.⁷⁵

6. Analytical Ultracentrifugation

Analytical ultracentrifugation experiments determine shape parameters and the average molecular weight of large molecules in solution, and can thus be used to deduce the stoichiometry of the complexes between hevein domains and chitoooligosaccharides.⁴⁵ Detailed experimental conditions for performing such experiments are outside the scope of this chapter, but may be found in Ref. 45. Such experiments, together with NMR DOSY data,⁵⁹ have been of significant

utility for verifying the existence of complexes having distinct stoichiometries for hevein bound to small oligosaccharides and for the same domain bound to longer oligomers (above 5 units, see Table VI).

7. Molecular Modeling

Modern molecular modeling protocols are very useful for predicting the conformational and dynamic behavior of biomolecules.⁷⁶ In the case of hevein domains, various molecular modeling methods have been used, either to compute the 3D structure of a new domain (based on homology methods), to compare the modeled structure of pseudohevein with that deduced by NMR,⁵⁰ or to study hevein mutants in attempts to deduce the 3D features of the allergenic epitope of hevein.²⁸ Additionally, molecular dynamics (MD) has demonstrated the presence of different types of complexes in solution.^{76,77} In this solution case, the MD data compared very satisfactorily with conclusions derived from NMR measurements, showing that a chitoooligomer is able to move on the surface of the (relatively flat) extended binding-pocket of hevein, thereby occupying different binding subsites. In addition, it is shown that a chitohexamer at least is necessary to span all possible interactions with the various hevein subsites.⁷⁷ Methods of statistical analysis were also applied in order to define the principal overall motions in the complexes, and to show how the different ligands in the simulations affect the protein motions.^{76,77} Also, comparison between computationally derived hevein models with experimental parameters obtained by laser photo-CIDNP experiments (see foregoing) has shown a reasonable degree of agreement between the two data sets. Indeed, strong internal dynamics of the Tyr and Trp residues in the binding site have already been inferred by a combined laser photo-CIDNP modeling study.^{63,64} Last but not least, the use of docking protocols followed by MD methods has also been instrumental for understanding the binding affinities of modified oligosaccharides having ManNAc and GalNAc units,⁸⁰ as well as deducing the 3D structure of a sugar-complexed, truncated hevein domain, in the absence of sufficient experimental data on protein–chitotriose NOE-based distance constraints.⁴⁹

V. STRUCTURE OF THE HEVEIN–SACCHARIDE COMPLEXES

1. Single Domains: Hevein

Several complexes of single hevein domains bound to oligosaccharide ligands have been studied at atomic resolution and their binding-energy features analyzed.

a. Features of the Recognition Process at the Atomic Level.—The location of the chitoooligosaccharide-binding site of hevein has been deduced from chemical-shift perturbation analysis (Fig. 2) and further confirmed by the presence of a number of unambiguous intermolecular protein–carbohydrate NOEs (see foregoing, Fig. 4).⁴⁵ Laser photo-CIDNP methods also demonstrated the presence of Tyr and Trp residues in the sugar-binding site.^{63,64}

The $\Delta\delta$ (complex-free) values establish that the binding site for short chitoooligosaccharides (up to 4 GlcNAc units) is located between the residues 18 and 30, since their corresponding ¹H NMR signals are subject to major changes in chemical shifts upon interaction with the sugars. In contrast, chemical-shift perturbations in other hevein regions are observed upon addition of the chitin pentasaccharide. Thus, the induced $\Delta\delta(\delta_{\text{free}} - \delta_{\text{bound}})$ for the hevein–(GlcNAc)₅ complex were compared with the corresponding values for the hevein–(GlcNAc)₃ complex, in order to characterize any additional amino acid residue involved in sugar recognition (Fig. 2). Although the residues most affected are located between S19 and Y30, in both cases, clear differences in the induced chemical-shift changes are evident between both the complexes. Fittingly, the NH group of C24 presents a large difference ($\delta > 0.2$ ppm) between the free and bound states for the hevein–(GlcNAc)₅ complex. In contrast, the corresponding $\Delta\delta$ value for the protein–trisaccharide complex is almost negligible. In addition, the region located between K10 and L16 generates very significant δ increments, but only when the interaction takes place with the pentasaccharide. This experimental observation suggests, as already mentioned, that other protein regions are involved in sugar binding when long GlcNAc oligomers are used as ligands.⁴⁵

The NMR structures of several hevein–(GlcNAc)_{*n*} complexes in solution have been constructed by NOE analysis.^{41–45} Several hundred protein–protein NOEs, as well as several protein–sugar NOEs, were measured and used as limiting constraints in a simulated annealing protocol to determine the 3D structure of the hevein–sugar complexes. Moreover, a hydrogen bond between the hydroxyl group of Ser19 and the carbonyl group of one of the GlcNAc residues has consistently been detected and is included as additional constraint. Examples of the number of NOEs and statistics of the complexes are given in Table VIII. The resulting 3D structures of the protein–carbohydrate complexes are fairly well defined (Fig. 5) and indicate that the protein experiences only slight changes in its conformation when interacting with the disaccharide. Indeed, with regard to the NMR structure of the free protein, no significant changes in the protein NOEs were observed, indicating that carbohydrate-induced conformational

TABLE VIII
Statistics of the NMR-Based Complexes between Hevein Domains and (GlcNAc)₃

Domain	Average RMSD Backbone	Average RMSD Backbone Core	Average RMSD Heavy atoms	References
Hevein	0.92	0.60	1.27	43
WGA B	1.05	0.86	2.00	51
Pseudohevein	1.14	0.89	1.74	50
Hevein32	0.79	—	1.60	49
Elderberry hevein	1.27	1.01	1.93	46
Ac-AMP2	0.69	0.39	1.91	52
Ac-AMP2W	0.70	0.60	1.54	53
Ac-AMP2Naph	0.83	0.42	1.93	53
Ac-AMP2F	0.84	—	1.96	53

Note: Deviations are smaller than ± 0.43 Å.

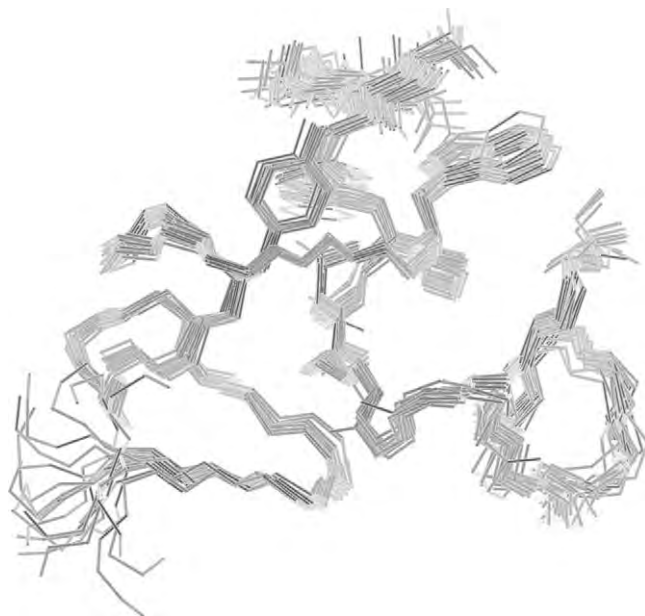


FIG. 5. Superimposition of 20 NMR structures for the hevein-(GlcNAc)₂ complex. The contact site between the protein and the sugar is positioned at the top of the figure.

changes are small. As an example, the rmsd of the average backbone in 20 refined structures for the hevein-(GlcNAc)₅ complex was 0.055 nm, whereas the heavy atom rmsd was 0.116 nm.

The backbone maintains the same topology in the free and bound states, and minor movements are observed in the lateral chains of the amino acids that form the binding site. Study of the 3D structure of hevein with (GlcNAc)₂ indicated that both GlcNAc residues make interactions with several lateral chains of the protein: the non-reducing acetamido methyl group engages in non-polar contacts with the aromatic Tyr30 and Trp21 residues, and, in addition, there are key hydrogen bonds which confer stability on the complex: one between Ser19 and the acetamido group of the non-reducing sugar and a second one involving 3-OH and Tyr30. An additional interaction is observed between the less-polar α -face of the reducing GlcNAc moiety and the plane of the indole ring of Trp21. Additional evidence for stacking interaction between the lateral chain of Trp21 with the reducing end came from the observation of strong shielding of several protons of the indole ring of Trp21 in the complex of hevein with *p*-nitrophenyl 2-acetamido-2-deoxy- β -D-glucopyranoside (**1b**), as well as from the upfield shifting of the *O*-methyl group of methyl β -chitobioside (**2b**) in the presence of hevein in comparison with that measured for the free sugar.

Comparisons have been made of the 3D structure of hevein in solution with the structures reported for other hevein domains, including WGA⁵¹ and hevein itself in the solid state.^{39,40} Despite differences in the number and nature of several amino acid residues, the polypeptide conformation has also been compared with the NMR-derived structure of a smaller antifungal peptide (30 amino acids) termed Ac-AMP2.⁵² The interactions just described have also been observed in the crystal structures of WGA-chitobiose⁷⁸ and WGA-sialyllactose⁷⁹ (see later). In all cases, the obtained conformations and intermolecular protein-sugar interactions are indeed similar (see later), regardless of the experimental method used to determine the 3D structure.

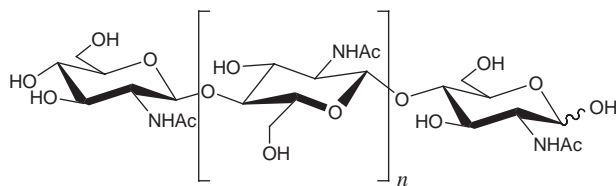
This combination of van der Waals and hydrogen-bond interactions is adequate to explain the basic features of the interaction between hevein and chito-oligosaccharides. Indeed, this type of structure is fairly stable, as demonstrated by its persistence in water-dimethyl sulfoxide (Me₂SO) mixtures.⁴⁴ Thus NMR-spectroscopic measurements demonstrated complexation between hevein and (GlcNAc)₃, albeit with progressively diminished affinity by more than 1.5 orders of magnitude, in mixtures of water and up to 50% Me₂SO.⁴⁴

Hevein provides a suitable model for verifying the minimum chito-oligosaccharide-binding domain. Based on the structure of the natural antifungal polypeptide, AcAMP-2, a 32-residue, truncated hevein lacking 11 C-terminal amino acids was synthesized by solid-phase methodology and correctly folded with 3 cysteine bridge pairs; it was termed HEV32.⁴⁹ The NMR structure of ligand-bound HEV32 in aqueous solution proved to be highly similar to the NMR structure of ligand-bound hevein. MD simulations, with explicit inclusion of the solvent molecules, were performed in order to monitor the changes in side-chain conformation of the binding site of both HEV32 and hevein upon interaction with ligands. HEV32 provides a simple molecular model for studying protein-carbohydrate interactions and also for understanding the physiological relevance of small native hevein domains lacking C-terminal residues.

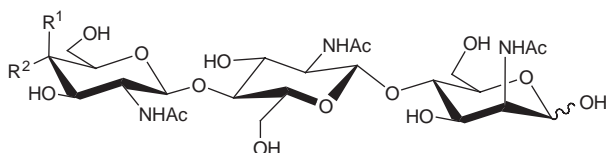
As regards ligand selection, hevein is also able to accommodate acetamido sugars other than residues containing GlcNAc.⁸⁰ NMR observations give strong indication that other analogs of chitotriose (**3**) modified at either the reducing end (**3b**, with ManNAc instead of GlcNAc), or at the non-reducing end (**3c**, as **3b** with GalNAc instead of GlcNAc) do not modify the mode of binding of the saccharide to hevein. Nevertheless, the association constants demonstrate that binding of chitotriose is better than that of **3b**, and that the binding of **3b** is favored with respect to that of **3c**.

b. Thermodynamics.—The binding affinities and thermodynamic parameters for chito-oligosaccharide binding to hevein have been determined by several methods.^{41–45,68–70} As typically observed for lectin-saccharide interactions, the processes are enthalpy-driven, while entropy opposes binding.⁶⁶ A summary is given in Table V.

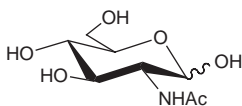
Apolar and polar interactions contribute to the complexation process, stabilizing the orientation of the sugar rings through formation of hydrogen bonds and by stacking interactions with aromatic side chains. The structural view obtained in solution therefore agrees perfectly with the insights generated from the equilibrium thermodynamic parameters. The variations in binding constants may be explained in structural terms: the minimum sugar size that can be bound by hevein is *N*-acetyl-D-glucosamine (**1**), the parent monosaccharide, whose binding is stabilized by non-polar forces involving Trp23 and Tyr30 and by hydrogen bonds involving Ser19 and the hydroxyl group of Tyr30. Binding of the β anomer is probably favored over the α analog, since the 1-hydroxyl group of this anomer would make unfavorable contacts with Trp23.



$n = 0$	2
$n = 1$	3
$n = 2$	4
$n = 3$	5
$n = 4$	6
$n = 6$	8

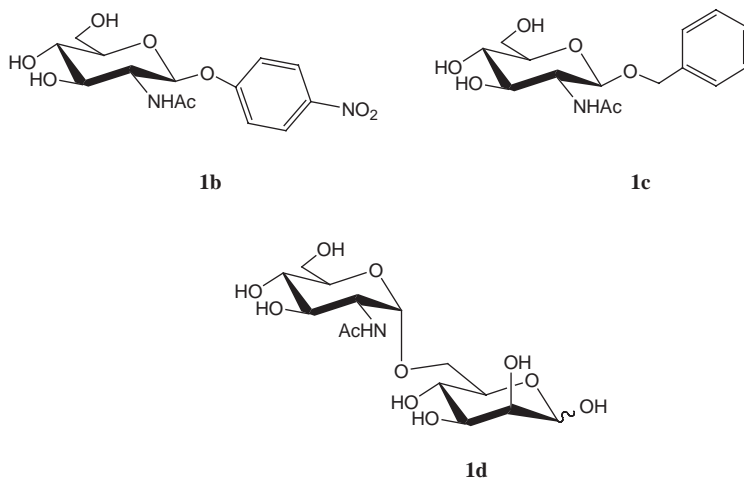


$R^1 = H, R^2 = OH$	3b
$R^1 = H, R^2 = H$	3c

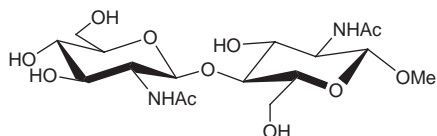
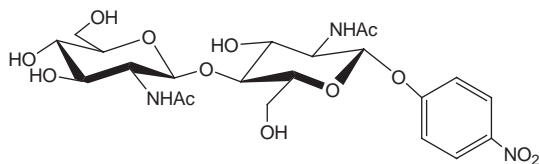
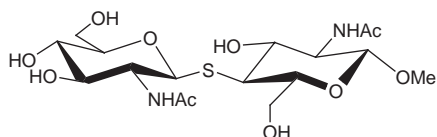
**1**

The binding constant for *p*-nitrophenyl 2-acetamido-2-deoxy- β -D-glucopyranoside (**1b**) is one order of magnitude higher than that of the free monosaccharide, because of its being locked in the β -anomeric configuration and through the additional stabilization provided by the interaction of the Trp21 indole ring and the *p*-nitrophenyl moiety of the glycoside.⁴³ On the other hand, the benzyl glycoside (**1c**) has a binding constant similar to that of the reducing sugar, indicating that the orientation of the phenyl ring is not appropriate for stabilizing aromatic–aromatic interactions. In addition, on account of the high flexibility of the benzyl derivative, loss of entropy upon binding can also be

assumed to be responsible for the small association constant experimentally observed.



Despite the mixture of anomers present in chitobiose (**2**) and the fact that only the β anomer is effectively bound by hevein, the binding constant of chitobiose is nevertheless higher than that of **1b**. It is probable that both entropic effects, due to the intrinsically higher flexibility of the *p*-nitrophenyl derivative with respect to the disaccharide, as well as stronger van der Waals interactions provided by the 3D shape of the pyranose chair may account for the improved binding. The use of methyl β -chitobioside (**2b**) enhances the binding. Both compounds restrict the anomeric configuration to the favored β orientation, and the presence of additional non-polar interactions between the *O*-methyl group and the extended surface of Trp21 are probably the key factors in this instance. A further increase in binding is observed when *p*-nitrophenyl β -chitobioside (**2c**) is employed. Although the corresponding changes in energy (between methyl β - and *p*-nitrophenyl β -chitobioside) are small, the aromatic–aromatic interaction that exists in this case is expected to be favored over the *O*-methyl–aromatic interaction that takes place for methyl β -chitobioside. In addition, the higher affinities deduced for the β -linked disaccharide with respect to **1** and α -GlcNAc-(1 \rightarrow 6)-Man (**1d**) can be explained by favorable stacking of the second β -linked GlcNAc moiety and Trp21.^{43,45}

**2b****2c****2d**

The binding constant found for chitotriose (**3**) is even higher, probably as a result of the better van der Waals contacts established between the rather large surface area of the Trp21 indole ring and the pyranose chair. In addition, the flexibility of the *p*-nitrophenyl derivative is also expected to be higher than that of the trisaccharide and, therefore, the comparatively high association constant measured in this instance also appears to have a component of entropic origin.

In contrast to the behavior reported for the binding of hevein to these short oligomers [up to (GlcNAc)₃], for which the exchange rate is fast on the chemical-shift timescale, the exchange rate in the case of (GlcNAc)₅ between the free and bound states of hevein at the same temperature is in the intermediate-slow regime.⁴⁵ In fact, at the binding site, there is a clear effect of the temperature on the shape and number of signals in the aromatic region of the hevein–(GlcNAc)₅ complex. Two different signals are observed for the aromatic protons Hε1 and Hε2 of residue Y30 at 5 °C. At higher temperatures, both singlets coalesce into

one averaged signal at 25 °C. In contrast, only one averaged signal is observed for both protons in the experiments corresponding to hevein in the free state at 5 °C. The fact that the rotation of Y30 is slow on the chemical-shift timescale, but only when complexed to the pentasaccharide, reflects freezing of the spatial orientation of Y30 side chain as a consequence of its interaction with the carbohydrate. The restriction of flexibility of the hevein side chains due to sugar binding probably has a significant effect on the entropic balance of the recognition process. This effect has in fact, been considered by several authors as the main origin of the entropy:enthalpy compensation phenomenon usually observed in protein–sugar interactions.^{7,66,76} Additionally, exchange cross-peaks were also observed for protons at the indole ring of W21, a residue that is also directly involved in sugar recognition. This fact also points to the existence of two orientations for this aromatic system in the complexes.

In a parallel study, Garcia-Hernández *et al.* have used ITC to characterize by calorimetry the association of hevein with the β -(1 \rightarrow 4) dimer and trimer of *N*-acetylglucosamine (GlcNAc).⁶⁷ Considering the changes in polar- and apolar-accessible surface areas resulting from complex formation, they propose that the experimental binding heat capacities may be explained adequately by means of parameters used in protein-unfolding studies. These findings resemble the convergence observed in protein-folding events; however, the average of decreased enthalpies for lectin–carbohydrate associations is generally higher than that for the folding of proteins. Analysis of hydrogen bonds present at lectin–carbohydrate interfaces revealed geometries closer to ideal values than those observed in protein structures.⁸¹ Thus, the formation of a network of more energetic hydrogen bonds might well explain the high association enthalpies of lectin–carbohydrate systems.

Finally, the affinities of the truncated HEV32 for small chitin fragments in the forms of *N,N',N''*-triacylchitotriose (**3**) (millimolar) and *N,N',N'',N''',N''''*-hexaacetylchitohexaose (**6**) (micromolar)—as measured by NMR and fluorescence methods, are comparable with those of native hevein. As usual, the HEV32–ligand-binding process is enthalpy-driven, while entropy opposes binding.⁴⁹ There is an enthalpy–entropy compensation phenomenon that can be ascribed to a better binding mode of Trp21 of HEV32 to the sugar when the C-terminus of hevein is lacking, but this binding ability is counterbalanced by a major reorientation of Trp21 when passing from the free to the bound states, with concomitant entropy loss. Indeed, as already mentioned, MD calculations also support the concept that the Trp21 side-chain orientation of HEV32 in the free form differs from that in the bound state, in agreement with the fluorescence data.

c. Multivalency Effects.—Binding constants for lectin–saccharide interactions are usually in the low micromolar range.² Indeed, these types of weak affinities play a role in nature and a number of monomeric sugar–lectin binding processes can multiply cooperatively to produce strong polyvalent interactions. This principle is exploited, for example, in the design of glycoclusters for applications as blocking reagents.⁸² In the hevein case, the three-subsite model of interaction presented here cannot explain the thermodynamic features of the recognition process for (GlcNAc)₄ and especially for (GlcNAc)₅. For tetrasaccharide binding ($n = 4$), a further increase in ΔH of $\sim 1 \text{ kcal mol}^{-1}$ is observed in comparison to the trisaccharide ($n = 3$).^{43,45} In contrast to the observed behavior for shorter oligomers, this favorable $\Delta\Delta H$ is almost completely counterbalanced by the entropic contribution, thus leading to a negligible increase in the association constant, K_a . According to the structural model just described, Fig. 6 shows that the reducing end of the tetrasaccharide is completely exposed to the solvent and does not make any contact with the protein.

The observed $\Delta\Delta H$ value cannot, therefore, be satisfactorily explained. Moreover, the ITC data indicate a sharp increase in the K_a value when pentasaccharide binding is monitored.⁴⁵ The K_a increased from $11,000 \text{ M}^{-1}$ (tetra) to more than $450,000 \text{ M}^{-1}$ (penta). In this case, and opposite to the observed behavior for $n = 1$ –4, it was not possible to obtain a perfect fit of the experimental ITC

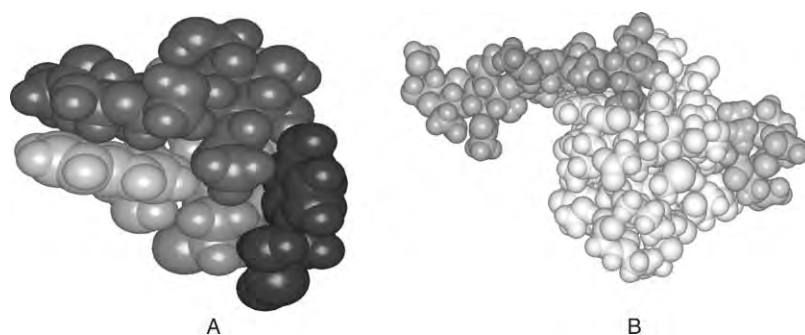


FIG. 6. Model of the interaction of hevein with chitoooligosaccharides. On the left side (A), a schematic overview of the binding site of hevein complexed to (GlcNAc)₂ is illustrated. The non-reducing unit of the disaccharide interacts with Trp23 and Tyr30. On the right side (B), interaction of an extended oligosaccharide is depicted, which follows the general rule deduced from the complex with the disaccharide. For saccharide ligands longer than four GlcNAc residues, the reducing end is exposed to the solvent and does not contact the protein. The NMR structure of hevein can be found in the protein data bank with code 1HEV. The X-ray structure is 1Q9B, while a truncated analog HEV32 is given the pdb code 1TW0.

curves by assuming a pure 1:1 stoichiometry. This result strongly suggests for pentasaccharide association the existence of higher-order complexes in solution, a hypothesis that was tested by using analytical ultracentrifugation and DOSY NMR methods for (GlcNAc)₅, (GlcNAc)₆, and (GlcNAc)₈ binding.^{45,59} For (GlcNAc)₈ (**8**), the average molecular weight corresponds to a 2:1 complex.

The observed dependence of the average molecular weight of the complexes on the protein–ligand ratio employed, and on the length of the oligosaccharide chain strongly suggests the presence of protein–ligand complexes in solution with 2:1 stoichiometry. Probably, for chitin fragments greater than tetrasaccharide, the carbohydrate chain may offer more than one binding site, thus allowing interaction with two hevein molecules. The nature of multivalent interactions between hevein and long (GlcNAc)_{*n*} oligomers was thereby elucidated, at least in part.⁴⁵

2. Pseudohevein

a. Features of the Recognition Process at the Atomic Level.—Pseudohevein differs from hevein in a number of amino acid residues, especially in the mutation of Trp21 to Tyr21 at the binding site.⁵⁰ Chemical-shift perturbation analysis⁵⁰ and laser photo-CIDNP methods⁶¹ have verified that despite this modification, the 18–30 lectin region is involved in molecular recognition of the chitooligosaccharides. NOESY experiments in water solution indicated a refined 3D structure of the pseudohevein–(GlcNAc)₃ complex that is very similar to that of hevein.⁵⁰ The NOE data also imply that two different binding modes of the trisaccharide within the pseudohevein-binding site are probable, differing only in the relative position of the trisaccharide with respect to Trp23, and furnishing structural explanation for the lectin's capacity to target chitin (see later) or even such other disaccharides, such as *N*-acetyllactosamine, having Gal instead of GlcNAc at the non-reducing end. In all cases, and as for hevein, hydrogen bonds and van der Waals contacts confer stability to the complexes.

For this single domain, surface-accessibility values for the key residues have been derived from the NMR data and also from MD-generated models.⁵⁰ These were found to be dependent on the force field and the type of modeling procedure used. For instance, from the NMR structures, the average surface value for Tyr21 is 89.9 Å², while that calculated by the GROMOS force field is 89.1 Å², providing a nearly complete match to the experimentally determined parameters. For Trp23, the GROMOS-derived average accessibility value is 129.6 Å², while for Tyr30 the value is only 33.1 Å². In contrast, the NMR

average values are 98.8 \AA^2 for Trp23 and 47.4 \AA^2 for Tyr30, showing this residue to be the less accessible one. Therefore, although the modeling can qualitatively reflect the NMR-based trend, a close correspondence between experimental and calculated data sets was not found for any protocol used. Modeling with different force fields⁷⁶ is thus helpful to estimate the actual changes of molecular parameters, but its predictive accuracy requires further refinement to reach an optimal level.

b. Thermodynamics.—Both NMR and isothermal titration calorimetry have allowed determination of the thermodynamic parameters of the binding of pseudohevein to $(\text{GlcNAc})_3$.⁵⁰ As with hevein itself, the association process is enthalpically driven, while entropy opposes binding. In relation to hevein, the Trp/Tyr substitution in the binding pocket has only a small effect on the free energy and enthalpy of binding, thus indicating that Nature may provide either Tyr or Trp rings to interact effectively with GlcNAc moieties, without significantly affecting the structural or energetic features of the binding process.

3. Wheat-Germ Agglutinin B Domain (WGA-B)

a. Features of the Recognition Process at the Atomic Level.—The B domain of WGA (WGA-B) has been prepared by recombinant techniques.^{51,63} This B domain also differs from hevein in a number of amino acids, but the significant modifications involve the two key tryptophan residues in the binding site (Trp21 and Trp23 in hevein), which are mutated to Tyr moieties. Again, the specific interaction of WGA-B with N,N,N' -triacetylchitotriose was analyzed by ^1H -NMR chemical-shift perturbation analysis⁵¹ and laser photo-CIDNP⁶³ methods. The results again confirmed the involvement of the three Tyr aromatic residues in the protein–sugar interaction. The NMR-based, experimentally derived NOESY constraints were processed in a refinement protocol that included restrained MD in order to determine the refined solution conformation of this protein–carbohydrate complex. With regard to the NMR structure of the free protein, no significant changes in the protein NOEs were observed, indicating that carbohydrate-induced conformational changes are small. In this case, the average rmsd of the backbone in the 35 refined structures was 1.05 \AA , while the heavy atom rmsd was 2.10 \AA . In fact, the lack of substantial changes in the pattern of the protein–protein NOEs appears to be a consequence of the superficial location of the lectin-binding site and of the strategic positioning of the aromatic residues involved in sugar recognition.

As a matter of fact, the orientation of the aromatic residues in the binding site of free WGA (the parent lectin that includes four hevein domains, see later), as deduced by X-ray analysis,^{78,79} is basically equivalent to that observed in the NMR-based WGA-B–chitotriose complex.⁵¹ Comparison of the 3D structure of WGA-B in solution with those deduced for other hevein domains again revealed a great similarity of their conformations at both the backbone and the side-chain level, including the orientation of the amino acid residues at the binding site. These observations strongly suggest that very minor changes indeed are required to accommodate the sugar moiety in the binding site of hevein domains. As already mentioned for pseudohevein, two different binding modes of the trisaccharide within the WGA-B binding-site are possible, with hydrogen bonds and van der Waals contacts conferring stability on both complexes.^{49–51,53}

As mentioned for pseudohevein, the only difference between both possible structures of the complex lies in the relative position of the trisaccharide with respect to the binding site: the non-reducing end occupies different protein sub-sites, with both modeling and experimental data supporting their existence. In the upper-left structure in Fig. 7, the acetamido methyl group at the non-reducing end is engaged in non-polar contacts with two aromatic residues: Tyr34 (Tyr30 in hevein) and Tyr27 (Trp23 in hevein), and, in addition, there are important hydrogen bonds that confer stability to the complex: one between the terminal non-reducing sugar acetamido group and Ser23 (Ser19 in hevein) and a second one involving 3-OH of the same sugar residue and Tyr34. In fact, the signal of the hydroxyl group of Ser23 moves downfield when the carbohydrate is added to the solution in the NMR tube containing the protein, and then broadens and disappears below the noise level.

Two additional CH– π -type interactions are observed, one between the non-reducing sugar and Tyr27 (Trp23 in hevein) and a second one between the central moiety and Tyr25, which are in agreement with the presence of this complex (Fig. 7). In a second complex upper-right, arising through the shift of one sugar unit, the reducing and the central GlcNAc residues make contacts with the aromatic amino acids implicated in binding. In this binding mode, the central sugar unit interacts with Ser23, Tyr27, and Tyr34 (in contrast with upper-left complex, for which the interaction was provided by the non-reducing end), while the reducing residue makes contacts with Tyr25.

b. Thermodynamics.—Isothermal titration calorimetry and NMR techniques have been used to measure binding constants for the association of β -linked GlcNAc oligomers to WGA-B, and the entropy and enthalpy of binding have

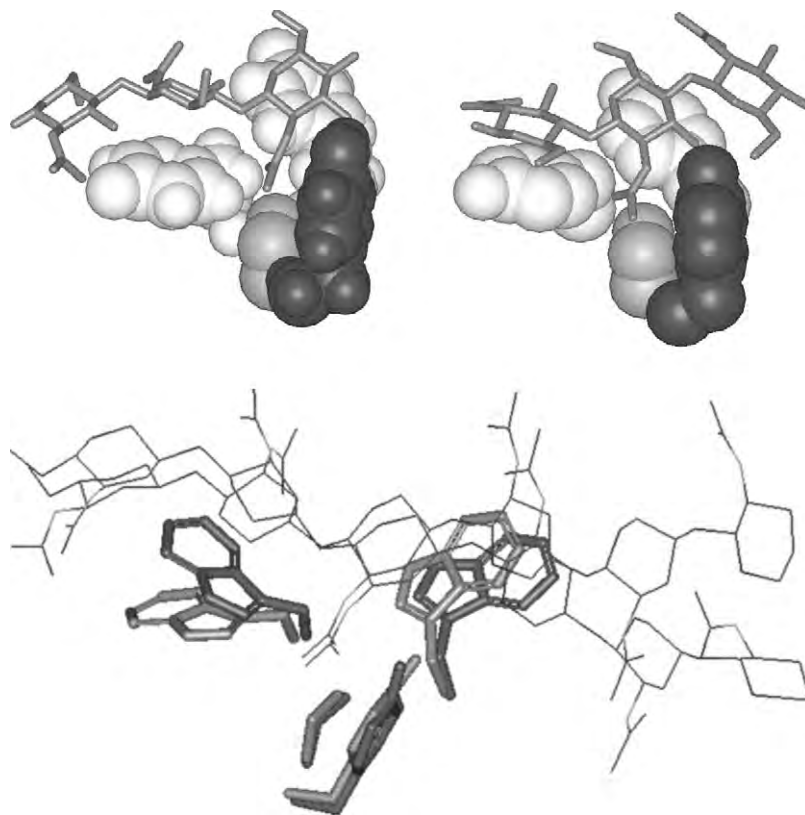


FIG. 7. Two different complexes are possible when the trisaccharide (GlcNAc)₃ interacts with hevein domains. Either the non-reducing end (left) or the central GlcNAc unit (right) can interact with Ser19, Trp23, and Tyr30. The existence of this mode versatility makes it possible for hevein domains to interact with extended oligosaccharide chains (lower illustration).

been determined⁵¹. The driving force for the binding process is provided by a negative ΔH , which is partially compensated by negative ΔS . These negative signs indicate that hydrogen bonding and van der Waals forces are the major interactions stabilizing the complex. The values obtained were roughly 10 times lower than published binding constants for the four-domain WGA molecule,^{83–85,69} which are in the micromolar range. Clearly, high-affinity binding requires auxiliary contacts from across the dimer interface, particularly in the case of *N*-acetylated sialosides.³³

4. AcAMP-2-type Domains

a. Features of the Recognition Process at the Atomic Level.—The interaction between Ac-AMP2 a lectin-like small protein of only 30 amino acids and three disulfide bridges, and having antimicrobial and antifungal activity isolated from *Amaranthus caudatus*, and *N,N',N''*-triacylchitotriose has been studied by NMR (protein database, pdb code 1MMC).⁸⁶ The most pronounced shifts were observed mainly in the C-terminal half of the sequence, and involve the aromatic residues Phe18, Tyr20, and Tyr27, together with their surrounding residues, as well as the N-terminal Val-Gly-Glu segment. In a second paper, the same group reported the NMR conformation of free Ac-AMP2 in water.⁵² The solution structure of Ac-AMP2 shows a backbone rmsd for the well-defined Glu3-Cys28 segment of 0.69 Å. Indeed, this structure is very similar to the equivalent regions of hevein domains. The free-solution structure complexes of Ac-AMP2 mutants, in which Phe18 has been changed to either Trp, naphthylalanine, and 4-fluorophenylalanine have been studied by NMR and by MD simulations.⁵³ The polypeptide structure is very similar between all mutants and is also basically identical to that of wild-type Ac-AMP2, with backbone rmsd values smaller than 1 Å. As found for the truncated HEV32, a major rearrangement of the aromatic residue at relative position 18 (Ac-AMP2, corresponding to residue 21 in hevein) takes place when passing from the free to the bound state. The ¹H and ¹⁹F NMR data are compatible with two different binding modes, as previously discussed for pseudohevein and WGA-B.^{50–53}

b. Thermodynamics.—The thermodynamic parameters of the interaction of Ac-AMP2 with *N,N',N''*-triacylchitotriose have been determined by a van't Hoff analysis of the binding constants measured at different temperatures.^{53,86} Although the van't Hoff analysis of the data may only yield a fair estimate, as deduced for the other hevein domains, the process is—as commonly found—enthalpy driven, while entropy opposes binding. The association constant at 300 K amounts to $\sim 1200 \text{ M}^{-1}$, while the binding enthalpy is of the order of $-50.1 \text{ kJ mol}^{-1}$. The Ac-AMP2 molecule has served as a valuable scaffold for verifying the importance of CH- π interactions in the molecular recognition of carbohydrates by protein receptors.^{9,87} Mutations of Phe18 of Ac-AMP2 to residues having larger aromatic rings, namely Trp, β -(1-naphthyl)alanine, or β -(2-naphthyl)alanine, enhanced the affinity, whereas the mutation of Tyr20 to Trp diminished it, in contrast to the observations for the hevein-pseudohevein pair.⁸⁷ Deactivation of the aromatic cloud by a fluorine atom, through

transforming Phe to 4-fluorophenylalanine, also provided a twofold decrease in the binding affinity to chitotriose.^{49,87} Thus, the affinity of a hevein domain for chitoooligosaccharide binding might be enhanced by adjusting the size and chemical nature of the aromatic residues involved in the interaction. The single replacement of any aromatic residue of Ac-AMP2 by Ala resulted in a significant diminution in affinity, suggesting the importance of the complete set of three aromatic residues in the ligand-binding site.⁸⁷

5. Multiple Domains: Wheat-Germ Agglutinin (WGA)

WGA exhibits specificity toward GlcNAc and NeuAc, and interacts with sialylated cell-surface receptors, as enzymatic removal of NeuAc from non-reducing terminal positions of receptor oligosaccharides impairs the binding of WGA.^{17,32,35,88}

The specific ligand (target) for WGA in erythrocytes is glycophorin A, the well-studied sialoglycoprotein.⁸⁹ WGA has been extensively characterized in terms of its molecular structure (see next paragraph). The physiologically active protein is a homodimer, of which three isoforms are present (WGA1, WGA2, and WGA3) in hexaploid wheat (*Triticum aestivum*).⁹⁰

a. X-Ray Analysis. Features of the Recognition Process at the Atomic Level.—High-resolution crystal structures have been determined for WGA1 and WGA2, both in the free state and in complexes with various saccharides.^{17,32,33,35,78,79,88,89,91} The molecular structure is highly stable because of the presence of 64 disulfide-linked cysteine residues distributed over eight hevein domains (with four identically folded domains per monomer). The presence of this fourfold sequence repeat comprised of hevein domains is in accord with the notion that the molecule evolved by gene duplication and fusion.²² The two polypeptide chains are associated in a “head-to-tail” manner, so that neighboring domains in the dimer interface obey quasi twofold relationships across the dimer interface. Consequently, and due to this arrangement, WGA deviates from the properties of other lectins in that it presents more than one carbohydrate-binding site per monomer. Indeed, each of the four domains that constitute the monomer is a carbohydrate-recognition domain (CRD). As found in hevein domains, their binding sites are composed of a shallow surface pocket characterized by three quasi-conserved aromatic amino acids and one conserved serine in one of the domains. However, in contrast to the monomeric hevein domains, there is additionally a non-conserved region that consists of one or two polar residues on the contacting domain of the second monomer. The binding

interactions for both NeuAc- and GlcNAc-containing oligosaccharides have been carefully analyzed.

(i) *Sialic Acid Binding.* The crystal complexes of WGA for the terminal non-reducing NeuAc residues of sialyllactose and the T5 sialoglycopeptide of glycophorin A^{33,79} were analyzed at three different sites, and those for bound (GlcNAc)₂ at four different sites.¹⁷ For NeuAc, there are two high-affinity sites. Both sites are generally occupied in the asymmetric WGA1–T5 crystal complex, where they participate in cross-linking the bivalent tetrasaccharide. Moreover, both sugars (GlcNAc and NeuAc) were observed to bind in one of the sites in all crystal complexes examined. This site has a highly favorable binding environment, allowing ligand stabilization through three or four hydrogen bond interactions, mainly involving a glutamic acid residue (Fig. 8). Additional contacts involving Tyr 66 may also be observed.⁷⁹ These structures may be readily reconciled with the earlier binding data for which thermodynamic and kinetic parameters were obtained from NMR measurements.⁹²

(ii) *(GlcNAc)_n Binding.* The four binding environments for WGA complexed with chitobiose also suggest the existence of high- and low-affinity sites. There are notable differences with respect to the sialic acid-containing complexes (see Fig. 8). NeuAc lacks an OH group at C-3 and thus can come into close contact with a polar region on domain B1 (Ala71–Glu72). Decreased binding affinity in one of the sites has been attributed to the absence of a third aromatic side chain at relative domain position 23, replaced by a Ser residue (Ser152). The other domains show Tyr 23, Tyr66, and Phe109 at this point. Nevertheless, the corresponding carbohydrate–aromatic interaction can be partially replaced by a strong hydrogen bond between Ser152 and the 3-OH group of GlcNAc, and an additional polar contact takes place between the 4-OH group and a second carboxylate group (Asp129).

In addition,⁹⁹ the interactions of WGA with the β -GlcNAc-(1→6)-Gal sequence have been investigated by ITC and X-ray crystallography. β -GlcNAc-(1→6)-Gal exhibited an affinity higher than β -GlcNAc-(1→4)-GlcNAc, while β -Gal-(1→6)-GlcNAc showed much lower affinity than β -GlcNAc-(1→4)-GlcNAc (Fig. 9) to all WGA isolectins. X-ray structural analyses of crystals of the glutaraldehyde-cross-linked WGA isolectin 3 in complexes with β -GlcNAc-(1→4)-GlcNAc, β -GlcNAc-(1→6)-Gal, and β -GlcNAc-(1→6)- β -Gal-(1→4)-GlcNAc (Fig. 10) were also performed, showing that the two disaccharides exhibited basically similar binding modes to each other, in contact with side chains of two aromatic residues, Tyr64 and His66. Interestingly, the conformations of the ligands in the two primary binding sites were not always

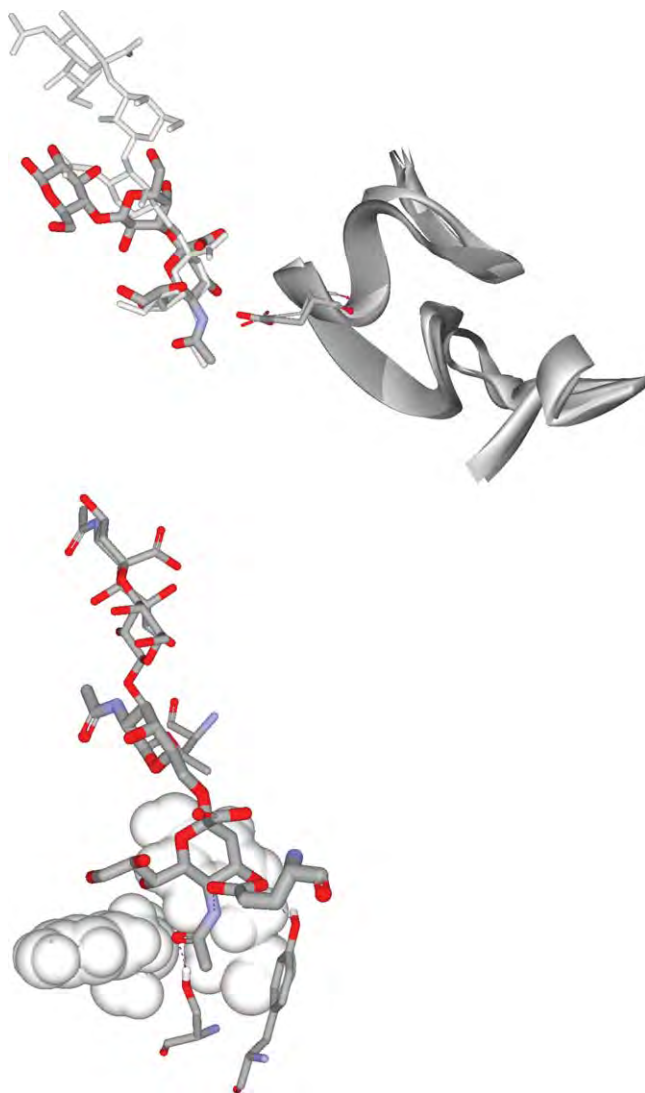


FIG. 8. Modes of binding of sialylated oligosaccharides to WGA according to published X-ray structures (pdb codes, 2WGC, 2CWG, ND8, 1WGC). The top figure shows a superposition of the binding modes of (2→3)- and (2→6)-linked sialyl oligosaccharides. The orientation of Glu72 preferably establishes hydrogen bonds with the sugar moiety. Aromatic-carbohydrate interactions are present for the α -(2→6)-linked sialylgalactose-containing oligosaccharide (lower portion).

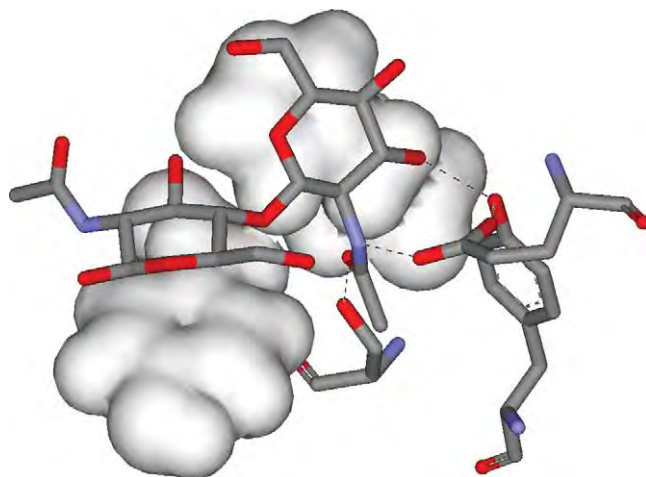


FIG. 9. The mode of binding of (GlcNAc)₂ to one of the hevein domains of WGA, as deduced from the published crystal structure (pdb code ND9). Hydrogen bonds and carbohydrate–aromatic interactions are salient for the complex formation and its stability.

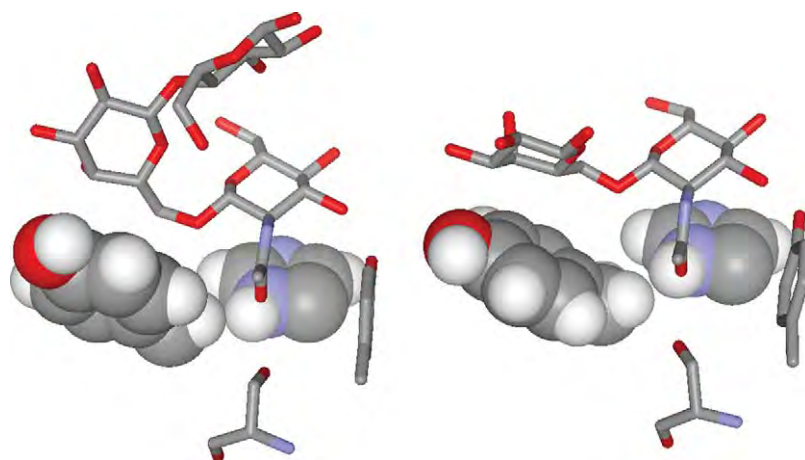


FIG. 10. The mode of binding of β -GlcNAc-(1 \rightarrow 6)-Gal (right) and β -GlcNAc-(1 \rightarrow 4)-GlcNAc (left) to one of the hevein domains of WGA, as deduced from the published crystal structures. Hydrogen bonds and carbohydrate–aromatic interactions are most important for complex formation and stability. As depicted, the two compounds differ in extent of contact formation, the β -GlcNAc-(1 \rightarrow 6)-Gal disaccharide (right) surpassing the corresponding β -GlcNAc-(1 \rightarrow 6)- β -Gal-(1 \rightarrow 4)-GlcNAc trisaccharide in this respect (left) (pdb codes, 1K7T, 1K7V).

identical. The β -GlcNAc-(1 \rightarrow 4)-Gal moiety maintained a large conformational flexibility even during interaction with WGA. As typical of hevein domains, the hydrogen bond between Ser62 and the non-reducing end GlcNAc was always observed, regardless of the ligand type, underscoring the key role of this interaction.⁹⁹ In addition, CH- π interactions involving Tyr64, His66, and Tyr73 were suggested to play an essential role in determining the ligand-binding conformation in the complexes studied.^{9,99}

(iii) *The Role of Water.* It is well known that ordered water plays a significant role in stabilizing bound saccharides.⁷ However, the X-ray results for WGA demonstrate that reasonable and informative molecular models of the oligosaccharide-WGA binding sites can be obtained without explicitly modeling water. Several water molecules could be refined successfully in the two high-resolution sialyloligosaccharide complexes.^{34,79} Although the overall patterns of H-bonded water molecules differ in the three sites, there is one common, well-ordered water molecule that solvates the 4-OH group in all sites. The location of this water molecule is well determined, with a low B-factor, and is tetrahedrally stabilized through other contacts with the protein. A number of other water molecules are also present in the two high-affinity sites, suggesting that they may play important roles in complex stabilization.

(iv) *NMR Studies.* NMR methods have been used to determine the conformational features of the binding of saccharides to WGA.⁹² Intermolecular nuclear Overhauser effects were observed for methyl β -chitobioside at the WGA-sugar-binding site, observations that—combined with modeling—support the conclusion that H-2 and the *N*-acetyl methyl protons are in close vicinity to protons of Tyr64, Tyr73, and of Tyr159.⁹³ Since the earliest investigations, WGA has been the subject of a variety of one-dimensional (1D) NMR studies aimed at investigating the specificity,⁹⁴ dynamics,⁹⁵ mode of binding,⁹⁶ and orientation of oligosaccharides in the binding pocket. Additional studies have been performed to deduce the bound conformation of the 4-*S*-thio analog (**2d**) of (GlcNAc)₂ to WGA.⁹⁷ More recently, a flexible disaccharide glycoside, β -D-GlcpNAc-(1 \rightarrow 6)- α -D-Manp-OMe, has been used as a ligand. Although molecular modeling of this disaccharide in the binding sites of the lectin indicated that several conformations could be adopted in the bound state,⁹⁸ NMR data confirmed the existence of a conformational selection process, so that one conformation having the *gt* conformation of the hydroxymethyl group and a negative sign for the ψ torsion angle is indeed favored for binding by the lectin.⁹⁸

b. Thermodynamics.—The aromatic pocket typical of hevein domains forms the main portion of the binding site, and may suffice for minimal binding. Several studies have focused on the interaction of WGA with chitooligosaccharides, using various techniques, including nanosecond-pulse fluorimetry.¹⁰⁰ An initial NMR study focused on the interaction of *N*-trifluoroacetylglucosamine with WGA was performed by ¹H and ¹⁹F NMR together with fluorescence spectroscopy.¹⁰¹ This technique was also used in a former study to measure the binding features of the interaction.⁷³ The energetics of association of WGA with GlcNAc and its β -(1 \rightarrow 4) oligomers were then measured by using ITC.⁶⁹ Association constants of 0.4, 5.3, 11.1, 12.3, and 19.1 mM⁻¹, and enthalpies of binding of -6.1, -15.6, -19.4, -19.3, and -18.2 kcal mol⁻¹ were obtained at 299 K for the titration of WGA with GlcNAc, (GlcNAc)₂, (GlcNAc)₃, (GlcNAc)₄, and (GlcNAc)₅, respectively. The term $T\Delta S$ was always negative, indicating that the binding process is enthalpically driven. Titrations of WGA performed at pH 4.5 did not differ significantly from those performed at pH 7.0, suggesting that no groups having a p*K*_a value in this range are directly involved in the binding event. Also, performing the titration in a buffer system having a higher enthalpy of protonation did not change the enthalpy of binding, confirming that there is no net protonation or deprotonation when WGA binds GlcNAc residues at pH 7. A model of four independent binding sites was found to adequately describe the binding curves, except for (GlcNAc)₄, which exhibited positive cooperativity.

Mutations at the key aromatic residues of WGA have also been performed.¹⁰² As an elegant way to clarify the role of the amino acid residue at position 30 (hevein numbering) of WGA2 in sugar binding, two WGA2 variants, each containing a mutation, either Tyr73 \rightarrow Phe (domain B) or Phe116 \rightarrow Tyr (domain C), were produced. The binding activity for (GlcNAc)₃ and the 3D structure of these mutants were characterized by comparison with the properties of wild-type WGA2. Equilibrium dialysis experiments using (GlcNAc)₃ indicated that the Tyr73 \rightarrow Phe mutation decreased the overall sugar-binding activity at two different pH values (5.9 and 4.7), because of the abolition of the hydrogen bond between the tyrosine hydroxyl group and a hydroxyl group on the oligosaccharide. In contrast, the Phe116 \rightarrow Tyr mutation increased the overall chitooligosaccharide-binding activity at pH 5.9, but diminished this activity at pH 4.7 without changing the number of sugar-binding sites.¹⁰²

Regarding the sialic acid binding already mentioned,⁹² the thermodynamic parameters that characterize the binding of WGA I to α -(2 \rightarrow 3) sialyllactose

have been determined by several techniques, including NMR. Moreover, the free energies of binding for the WGA–chitooligosaccharide interaction have been estimated by flexible docking techniques,¹⁰³ and compared to those free energies of binding experimentally obtained in cell-binding studies. It was shown that the predicted binding site, ligand orientation, and details of the binding mode were in perfect agreement with the known crystal structure of WGA with the sialoglycopeptide just mentioned.³³ Furthermore, an excellent linear correlation of the predicted binding free-energies was found between those deduced by the authors and other data already published.^{103,104} In both instances, predicted energies were within 1.0 kJ mol^{-1} of the experimental value. By using NMR, the equilibrium constant K , and the dissociation rate constant, k_{off} , have also been determined. A large entropy barrier to binding was detectable, with $\Delta H^\circ = -13.3 \pm 1.0 \text{ kcal mol}^{-1}$ and $\Delta S^\circ = -31.9 \pm 2.4 \text{ cal mol}^{-1} \text{ K}^{-1}$. From the kinetic viewpoint, an Arrhenius plot of the effect of temperature on the dissociation rate (k_{off}) and the plot of $\ln(k_{\text{off}}/T)$ vs. $1/T$ indicated that the transition complex constituted an unfavorable energy state as compared to the dissociated molecules, with an activation energy (EA) of $+18.0 \text{ kcal mol}^{-1}$ and enthalpy and entropy of dissociation values of $+17.4 \pm 0.3 \text{ kcal mol}^{-1}$ and $+13.4 \pm 1.2 \text{ cal mol}^{-1} \text{ K}^{-1}$, respectively. The driving force for this binding reaction is the large negative ΔH° with a small enthalpic barrier to association ($\Delta H^a = +4.1 \text{ kcal mol}^{-1}$).^{92,105}

6. *Urtica dioica* Agglutinin (UDA)

UDA, the plant lectin from rhizomes of the stinging nettle, is comprised of two covalently linked hevein domains. The interaction of UDA with chitooligosaccharides has been studied by multiple methods, including X-ray, NMR titration data, laser photo-CIDNP methods, titration microcalorimetry, and also fluorescence measurements.

a. Features of the Recognition Process at the Atomic Level.—Experimentally, the shape and intensity of CIDNP signals of UDA have been determined both in the absence and in the presence of specific glycoligands.^{61,63,64} When the carbohydrate ligand is bound, laser photo-CIDNP signals of side-chain protons of tyrosine, tryptophan, and histidine residues were altered, indicating their role in sugar binding. In the case of UDA, the appearance of a new tryptophan signal upon ligand binding was interpreted as an indication for a conformational change of the corresponding indole ring. The binding of N,N',N'' -triacylchitotriose to UDA has also been investigated by standard ^1H NMR spectroscopy

methods.¹⁰⁶ It was shown that carbohydrate-induced perturbations occur in one domain of UDA at trisaccharide concentrations below equimolar. Residues in the second domain were shifted at increased carbohydrate concentrations. These data confirm the presence of two binding sites of non-identical affinities per UDA monomer. The qualitative analysis of the 2D NOESY spectra indicated that UDA contains two short stretches of antiparallel β -sheets, similar to those of hevein domains.

Several studies of UDA have applied X-ray crystallography methods to determine its 3D structure in the free and bound states. For the isolectin I (among the seven individual isolectins), a 1.66 Å resolution structure is available.³⁸ In the free state, the crystals belong to the space group P2(1), and the asymmetric unit contains two molecules related by local twofold symmetry. The molecule consists of two hevein-like chitin-binding domains lacking defined secondary structure, and the typical four disulfide bonds in each domain maintain the tertiary structure. The backbone structure and the sugar-binding sites of the two independent molecules are essentially identical. In the crystal of the free structure, the C-terminal domains bind Zn^{2+} ions at the sugar-binding site. Owing to their location near a pseudo-twofold axis, the two zinc ions link the two independent molecules in a tail-to-tail arrangement; thus, His47 of molecule 1 and His67 of molecule 2 coordinate the first zinc ion, while the second zinc ion links Asp75 of molecule 1 and His47 of molecule 2.

Furthermore, the crystal structure of UDA has also been determined in complexation with N,N',N'' -triacylchitotriose (**3**) and N,N',N'',N''' -tetraacylchitotetraose (**4**) at 1.90 and 1.40 Å resolution, respectively.³⁷ Each of the two hevein-like domains harbors a saccharide-binding site (Fig. 11). As is typical for hevein domains, one serine and three aromatic residues at each site form the principal contacts with the ligand. Multiple modes of binding of the oligosaccharide are found. The binding site in the N-terminal domain can accommodate any residue of a chitoooligosaccharide, whereas that of the C-terminal domain is specific for residues at the non-reducing terminus of the ligand. Binding of natural ligands leads to mitogenicity of T cells with intrafamily selectivity, establishing this lectin's capacity to serve as a superantigen (for details, see Ref. 106). It has been shown previously that oligomers of GlcNAc inhibit the activity of UDA as a so-called superantigen. By binding to glycans on the MHC molecule, and also to glycans on the T-cell receptor (TCR), the presence of two saccharide-binding sites observed in the structure of UDA suggests that this property might arise from the simultaneous fixation of glycans on the TCR and MHC molecules of the T cell and antigen-presenting cell, respectively.³⁷ The well-defined spacing

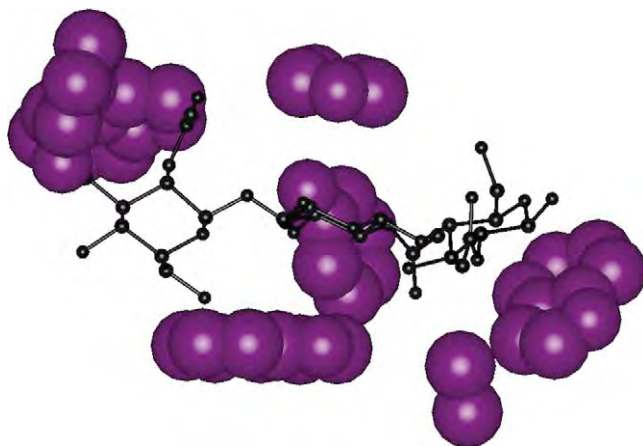


FIG. 11. UDA-VI forms a 2:2 protein–sugar complex with (GlcNAc)₃. The mode of sugar binding for UDA VI differs partially from that of UDA I (Fig. 12). One (GlcNAc)₃ trisaccharide is sandwiched between two different UDA-VI molecules, as shown in the figure. This positioning accounts for extensive aromatic–carbohydrate contacts (pdb codes, 1EHD, 1EHH, 1EIS, 1ENM).

between the two binding sites of UDA is probably a key factor in determining the specificity for lymphocytes.³⁷

An additional study has been performed on isolectin VI.³⁶ Herein, it was observed that, although the sequence similarity of the two domains is not high (42%), their backbone structures are well superposed, except for certain loop regions. The chitin-binding sites are located on the molecular surface at both ends of the dumbbell-shaped molecule. This crystal of UDA-VI complexed with (GlcNAc)₃ contains two independent molecules, forming a 2:2 protein–sugar complex.³⁶ The mode of sugar binding for UDA-VI is different in part from that in UDA I (Fig. 12). One (GlcNAc)₃ molecule is sandwiched between two independent UDA-VI molecules (Fig. 11), and the other sugar molecule is also sandwiched by one UDA-VI molecule and symmetry related to another one. Here, the sugar-binding site of the N-terminal domain consists of three subsites accommodating (GlcNAc)₃, while two (GlcNAc) residues are bound to the C-terminal domain. Nevertheless, in each sugar-binding site, three aromatic amino acid residues and one serine residue participate in the (GlcNAc)₃ binding. The sugar rings bound to two subsites are stacked to the side-chain groups of tryptophan or histidine, and a tyrosine residue is in face-to-face contact with an acetamido group, to which the hydroxyl group of a serine residue is hydrogen-bonded. The third subsite of the N-terminal domain binds a (GlcNAc) moiety with hydrogen bonds only.

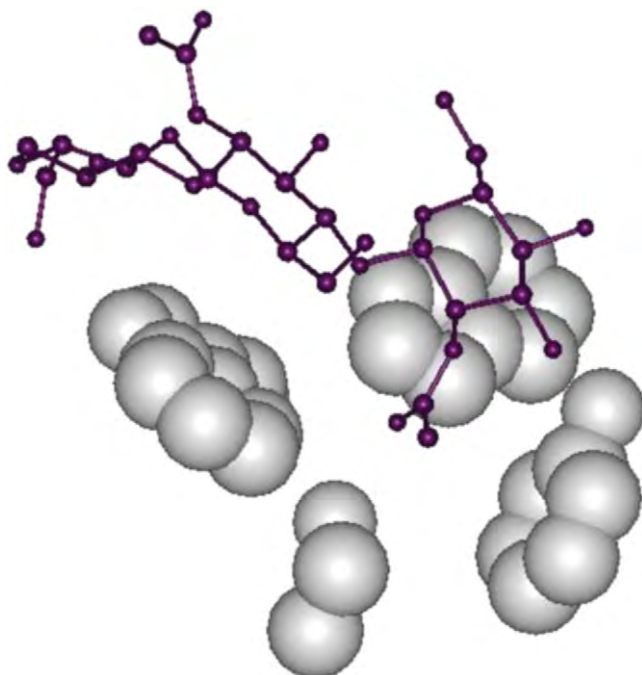


FIG. 12. The crystal structure of UDA I in complex with the ligand (GlcNAc)₃ is typical of hevein domains (pdb codes, 1EHD, 1EHH, 1EIS, 1ENM). Hydrogen bonds and carbohydrate–aromatic interactions provide stability for the complex.

b. Thermodynamics.—The binding of chitooligosaccharides to UDA has been extensively studied by the ITC. Based on experimental data, each site is composed of three subsites, each binding to a sugar residue.⁶⁸ The thermodynamic parameters obtained showed that, while chitobiose has two independent non-interacting sites, chitotriose, chitotetraose, and chitopentaose have two interacting sites on each monomer of UDA. Values of the binding affinity increase by almost a factor of 7 in going from chitobiose to chitotriose, indicating the existence of three subsites in the combining site of UDA. The binding constants for chitotetraose and chitopentaose increase without any further enhancement in the values of ΔH , indicating that, for oligomers larger than chitotriose, the interaction is entropically favored. In a parallel study,⁷⁰ the same interaction process was studied by fluorescence titration and isothermal titration microcalorimetry. It was proposed that UDA possesses one preferential binding site whose presence can be demonstrated calorimetrically. This site is composed of

three subsites, each subsite accommodating one GlcNAc residue. The interaction is enthalpically driven, and the binding area of UDA is characterized by a ΔH of interaction for a given oligosaccharide considerably smaller than that of WGA. Relatively high ΔC_p values of the UDA–carbohydrate interactions and the very favorable entropy term compared to WGA (in agreement with the report just mentioned), suggest that binding of the carbohydrate ligands by UDA is attributable to the notion that UDA has a higher hydrophobic component than that of WGA.

7. Other Domains

Other chitin-binding lectin isoforms, termed cbML1, cbML2, and cbML3, have been recently isolated from extracts of mistletoe (*Viscum album L.*),¹⁰⁷ and these contain two hevein-like domains linked by an intermolecular disulfide bond. The cbML sequence shows 55% identity to hevein. On the basis of the NMR data on hevein,^{41,43} the 3D structure of cbML3 was modeled, showing that the 26 sequence changes between cbML3 and hevein can be accommodated with only minor perturbation in the folding of the main chain. However, comparison of the primary structures of cbML3 and hevein have pinpointed differences in the loop region of the molecule and the potential interface region of cbML3, supporting dimer formation. Nevertheless, the high-affinity chitin-binding site appears to be highly conserved.

Muraki and coworkers examined the affinity of chemically prepared hevein domains for chitin.⁸⁷ An intact binding domain, CBP20-N, showed a higher affinity than a C-terminal truncated domain, Ac-AMP2. Curiously, the formation of a pyroglutamate residue from the N-terminal Gln of CBP20-N increased the affinity.⁸⁷ On the other hand, the chitin-binding site of the AVR4 elicitor of *Cladosporium fulvum* toward chitotriose units presents a novel binding site on the folding scaffold shared between the invertebrate and the plant chitin-binding domain.¹⁰⁸ The K_d , ΔH , and ΔS values obtained for the interaction between AVR4 and chitooligomers are comparable with those obtained for hevein. However, the binding site of AVR4 is larger than that of hevein, that is, AVR4 interacts strictly with chitotriose, whereas hevein can also interact with GlcNAc. Moreover, the binding of additional AVR4 molecules to chitin occurs through positive cooperative protein–protein interactions. By this mechanism, AVR4 is likely to shield chitin effectively on the fungal cell wall, preventing the cell wall from being degraded by plant chitinases.

Other domains having a five-disulfide motif have also been purified. Two antifungal peptides, named EAFP1 and EAFP2, have been isolated from the bark of *Eucommia ulmoides* Oliv.¹⁰⁹ Each of the sequences consists of 41 residues with an N-terminal residue of pyroglutamic acid. They show characteristics of the hevein domain and exhibit chitin-binding properties similar to those of the previously identified hevein-like peptides. The inhibitory activity of EAFP1 and EAFP2 is effective on both chitin-containing and chitin-free fungi. The crystal structure of EAFP2 at atomic resolution¹¹⁰ has been determined, and shows that its general fold of EAFP2 is composed of a 3(10) helix (Cys3-Arg6), an α -helix (Ala27-Cys31), and a three-stranded antiparallel β -sheet (Cys16-Ser18, Cys23-Ser25, and Cys35-Cys37), and it is cross-linked by five-disulfide bridges. Residues 11–30 adopt a conformation similar to the chitin-binding domain in the hevein-like proteins, and features a hydrophobic surface that embraces a chitin-binding site (Tyr20, 22, 29, and Ser18). The distinct disulfide bridge Cys7-Cys37 connects the N-terminal 10 residues with the C-terminal segment 35–41 to form a cationic surface that distributes all four positively charged residues, Arg6, 9, 36, and 40. The 3D structure of EAFP2 in aqueous solution has also been determined by NMR,⁴⁷ and shows a close resemblance to the solid-state conformation.

Moreover, a small (45 amino acid) antifungal polypeptide (Ee-CBP) has been isolated from the bark of the spindle tree (*Euonymus europaeus* L.); it has a primary structure very similar to the hevein domain, but with five disulfide bonds.⁴⁸ Ee-CBP is a potent antimicrobial protein, exhibiting IC(50)-values as low as $1 \mu\text{g mL}^{-1}$ for the fungus *Botrytis cinerea*. Indeed, Ee-CBP is a stronger inhibitor of fungal growth than Ac-AMP2 from *A. caudatus* seeds, a compound considered to be one of the most potent antifungal hevein-type plant proteins. With 84 or 82 amino acids, the pokeweed (*P. americana*) lectins PL-D1/D2 are recent entries to the list of hevein domain-coding agglutinins that have been structurally defined (protein database, pdb codes 1ULM, 1UHA).²⁴ Because differences in lymphocyte binding were observed, these two isolectins (having two chitin-binding domains, indicating one putative lectin site per domain) are models for relating the rather minor difference, namely detection of Leu83 and Thr84, to their disparate activity.²⁴ Of particular note, and interpreted as an example of convergent evolution, hevein-like domains are also present in peptides of invertebrates.^{111,112} The structure of tachycitin (protein database, pdb code 1DQC), a 73-residue polypeptide having antimicrobial activity and present in the hemocyte of the horseshoe crab (*Tachypleus tridentatus*), is fairly similar to that of hevein, although it differs in the nature of several key residues.¹¹³

Other putative hevein domains have been characterized from spider venom (protein database, pdb code 1QK7),¹¹⁴ the protozoan parasite *Trypanosoma cruzi*¹¹⁵ and from oat (*Avena sativa*) seeds,¹¹⁶ but their chitin-binding properties have not yet been characterized. Regarding scarabaecin¹¹⁷ or tachystatin A,¹¹⁸ it is noteworthy that these domains differ in the length of the primary sequence and the number of disulfide bonds from those in hevein or tachycitin, but they might still be able to provide a chitin-binding site.

VI. CONCLUSIONS AND PERSPECTIVES

The hevein domain is an attractive model for studying carbohydrate–protein interactions at atomic resolution. Hydrogen bonds and carbohydrate–aromatic interactions provide stability and selectivity for complex formation. Every carbohydrate–aromatic interaction stabilizes the complex by $\sim 1\text{--}2\text{ kcal mol}^{-1}$. No significant variations exist from the enthalpy viewpoint between Trp and Tyr for binding, but increasing the electron density on the aromatic ring enhances the affinity for GlcNAc moieties. Moreover, hevein domains also provide the basis of a simple structural model for assessing the importance of multivalency in recognizing sugars. Further modifications in the nature of the aromatic rings and in the key hydrogen bond donors and acceptors, should eventually permit reliable prediction of carbohydrate–protein interactions governed by these contacts.

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