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Chemical methods for selective cleavage of glycosidic bonds in the structural analysis of bacterial polysaccharides

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This review is devoted to methods for the selective cleavage of glycosidic bonds. The mechanisms of reactions underlying these methods are considered and examples of their practical application in the structural analysis of bacterial polysaccharides are given. Specific methods for the selective cleavage of polysaccharides, remaining relevant for researchers, include the Smith degradation based on destruction of monosaccharides containing vicinal diol groups, dephosphorylation of phosphate-containing polysaccharides with hydrofluoric acid and the hydrolytic cleavage of glycosyl phosphate bonds in the latter compounds. Non-specific methods, including partial acid hydrolysis, acetolysis and solvolysis with anhydrous organic (CF_3SO_3H, $MeSO_3H$, CF_3CO_2H) and inorganic (HF) acids do not make any specific demands on the composition and structure of the polysaccharide and are sensitive to its fine structural features. The review addesses the issue of stability of glycosidic bonds in various monosaccharides to reagents used for non-specific selective cleavage.
The bibliography includes 58 references.

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1. Introduction

Surface polysaccharides are common in bacteria. These are O-polysaccharides (OPSs) of Gram-negative bacteria, which represent polysaccharide chains of lipopolysaccharides (LPSs), cell-wall glycopolymers of Gram-positive bacteria (teichoic, teichuronic and teichulosonic acids) and capsular polysaccharides (CPSs) of both Gram-negative and Gram-positive bacteria. These polysaccharides carry specific functions associated with interactions with other biological systems, such as the immune system of the host organism and bacteriophages. Bacterial surface polysaccharides are antigens, the fine chemical structure of which is responsible for serological specificity of bacterial strains and is used for their classification required for diagnosis of infectious diseases and epidemiological monitoring. These compounds are employed as the basis for the development of vaccines and diagnostic agents.

Specific polysaccharides are the most structurally variable components of bacterial cells. They consist of various monosaccharides in the pyranose or furanose form, which are linked together in different sequences *via* α- or β-glycosidic bonds and are substituted at different positions. Such polysaccharides often include non-carbohydrate components (alcohols, amino alcohols, monobasic and dibasic acids, keto, hydroxy and amino acids, phosphate groups or, more rarely, sulfate groups). Most polysaccharides are heteropolymers, which are composed of regularly repeating linear or branched oligosaccharide units consisting of two to eight monosaccharide residues. This is a consequence of specific features of their biosynthesis involving the assembly followed by polymerization of oligosaccharide units.[1, 2] Post-polymerization modifications, such as glucosylation, O-acetylation and phosphorylation, are often non-stoichiometric and lead to the lack of regularity of polysaccharides. Homopolymers composed of the same monosaccharides are more rare. Their biosynthesis occurs as the transfer of monosaccharides, one by one, to a growing polysaccharide chain.[1, 3]

The structures of bacterial polysaccharides, mechanisms of their biosynthesis and specific physicochemical and

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Current research interests: structure and biological properties of bacterial antigen polysaccharides and lipopolysaccharides, development of methods for the structural analysis of complex carbohydrates.

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immunochemical properties are attracting continuous interest of chemists, biochemists, biophysicists, geneticists, immunologists and vaccinologists. A knowledge of the polysaccharide structure constitutes a chemical basis for the intraspecific classification of bacteria and is required for the identification of immunodeterminant regions of antigens, the description of characteristics of specific antibodies and the development of new vaccines and agents for diagnostics of bacterial infections.

Therefore, relevant methods for the structural analysis of polysaccharides are of great importance for research. Currently, 1D and 2D NMR techniques are the most efficient methods for this purpose.[4] These techniques allow the non-destructive identification of monosaccharide components, the determination of glycosidic bond configurations, substitution patterns and the sequence of monosaccharide residues in the repeating unit as well as identification and location of attachment sites of non-carbohydrate substituents. However, the limitation of this method is an insufficient resolution of NMR spectra primarily because of line broadening due to high viscosity of solutions of high-molecular-weight polysaccharides. An additional difficulty arises from a signal overlap, which is particularly typical of the analysis of irregular polymers giving series of signals with different intensities in NMR spectra. In these cases and often in order to independently confirm the structures, researchers apply chemical methods of analysis, such as the total acid hydrolysis followed by the identification of monomers, the methylation analysis to determine monosaccharide substitution patterns and the selective cleavage of glycosidic bonds. The latter approach is used to modify polysaccharides, in particular in order to transform irregular polymers into regular ones, and to prepare their oligosaccharide fragments, the structures of which allow one to reconstruct the structure of the starting polymer.

An advantage of oligosaccharides is that they can be isolated and purified by chromatographic methods. This is particularly important in studies of polysaccharides composed of different oligosaccharide units because this approach allows the preparation, separation and identification of the corresponding oligosaccharides. Besides, oligosaccharides generally give better resolved NMR spectra compared to the starting polymer and they can be studied by mass spectrometry. Oligosaccharide fragments of bacterial polysaccharides are also useful for immunochemical studies and are utilized as components of diagnostic agents and experimental vaccines.

Chemical methods for the selective cleavage of polysaccharides can be divided into specific and non-specific. Former methods rely on the presence of one or several monosaccharides of certain nature in the polysaccharide (*e.g.*, deamination using hexosamine, β-elimination using hexuronic acid or Smith degradation using monosaccharides containing the free vicinal diol group). The degradation leads to the cleavage of glycosidic bonds exclusively in these monosaccharides, whereas other bonds remain intact. The cleavage of phosphate-containing polysaccharides at the phosphate groups can also be assigned to specific methods.

Non-specific methods include partial hydrolysis with dilute acids, acetolysis and solvolysis with organic or inorganic anhydrous acids. These methods are based on the difference in the rate of cleavage (or, in other words, in stability) of glycosidic bonds of different monosaccharide components of polysaccharides.

Specific methods lead to a destruction of monosaccharide, in which the bonds are cleaved, whereas non-specific methods do not cause sugar degradation, and variation of the reaction conditions allows the preparation of larger or smaller oligosaccharide fragments depending on the task. This review addresses both types of chemical methods used for the selective cleavage.

A number of general reviews dealing with chemical methods for the selective cleavage of carbohydrates were published in the 1970–1980s (see, for example, Refs 5 and 6). In more recent years, reviews addressing the application of particular methods, such as solvolysis with anhydrous hydrofluoric or trifluoromethanesulfonic (triflic) acids, appeared.[7,8] For the sake of completeness, the present review includes a number of most typical examples of the use of methods considered previously and new data published in recent years, *e.g.*, the employment of trifluoroacetic acid as the reagent for solvolysis. Enzymatic methods for the selective cleavage of polysaccharides, which were covered in the earlier literature (see, for example, Refs 9 and 10), are beyond the scope of the present review.

The following designations and abbreviations are used:

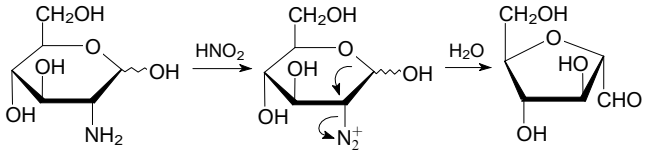
Am — acetimidoyl,
2,5anhMan — 2,5-anhydromannose,
CPS — capsular polysaccharide,
1dEry-ol — 1-deoxyerythritol,
8eLeg — 8-epilegionaminic acid (5,7-diamino-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*-non-2-ulosonic acid),
EtN — ethanolamine (2-aminoethanol),
f — furanose form of monosaccharide,
Fuc — fucose (6-deoxygalactose),
FucN — fucosamine (2-amino-2,6-dideoxygalactose),
FucN4N — 2,4-diamino-2,4-dideoxyfucose,
Fo — formyl,
Gal — galactose,
GalA — galacturonic acid,
GalN — galactosamine (2-amino-2-deoxygalactose),
GalNA — 2-amino-2-deoxygalacturonic acid,
Gal-ol — galactitol,
Glc — glucose,
GlcA — glucuronic acid,
GlcN3NA — 2,3-diamino-2,3-dideoxyglucuronic acid,
GlcN — glucosamine (2-amino-2-deoxyglucose),
Glc-ol — glucitol,
Gro — glycerol,
LPS — lipopolysaccharide,
Man — mannose,
ManN3NA — 2,3-diamino-2,3-dideoxymannuronic acid,
OPS — O-polysaccharide,
P — phosphate group,
p — pyranose form of monosaccharide,
Pse — pseudaminic acid (5,7-diamino-3,5,7,9-tetradeoxy-L-*glycero*-L-*manno*-non-2-ulosonic acid),
*R*3Hb — (*R*)-3-hydroxybutanoyl,
Rha — rhamnose (6-deoxymannose),
Rib — ribose,
Rib-ol — ribitol,
Thr-ol — threitol.
QuiN — quinovosamine (2-amino-2,6-dideoxyglucose),
Qui4N — 4-amino-4-deoxyquinovose,

2. Specific methods for selective cleavage

2.1. Deamination of hexosamines

Hexosamines are abundant components of bacterial polysaccharides. The most common hexosamines are 2-amino-2-deoxyhexopyranoses with *gluco* and *galacto* configurations, GlcN (glucosamine) and GalN (galactosamine). One of the methods for the selective cleavage of these polysaccharides is based on deamination of hexosamines with HNO_2, which is accompanied by the stereospecific Demjanov-type cationic rearrangement and pyranose ring contraction, the inversion of the configuration at the C(2) atom and the formation of 2,5-anhydromannose (from GlcN, as shown in Scheme 1) or 2,5-anhydrotalose (from GalN). 6-Deoxy derivatives of glucosamine and galactosamine, namely, QuiN (quinovosamine) and FucN (fucosamine), which are also present in many bacterial polysaccharides, show similar behaviour.

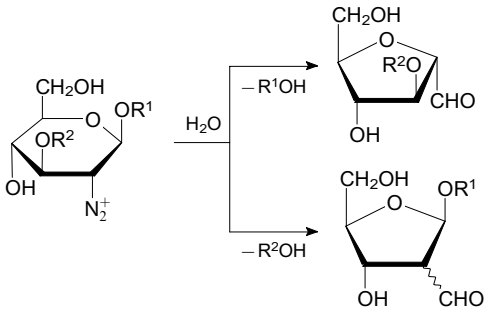
Scheme 1



The deamination of hexosamine glycosides affords hemiacetals of the corresponding 2,5-anhydrohexosides, which readily undergo glycosidic bond cleavage under deamination conditions to form free sugars (Scheme 2). This reaction also produces 2-C-formylpentofuranosides *via* the pyranose ring contraction following an alternative pathway. This reaction proceeds without glycosidic bond cleavage but is accompanied by the elimination of the substituent at the C(3) atom, if present.[11]

The deamination of mannosamine containing an amino group in the axial position is a more complex process. Its pathway depends on the reaction conditions,[5] and this reaction is not used for the selective cleavage of glycosidic bonds.

Scheme 2



In the majority of bacterial polysaccharides, hexosamine residues are present in the N-acylated (usually N-acetylated) form, and the preparation of polysaccharides containing free amino groups is a special problem. The acid hydrolysis is unsuitable for this purpose because N-deacylation requires rather severe conditions, resulting in the simultaneous glycosidic bond cleavage. The hydrazinolysis and alkaline hydrolysis are applicable to this task.

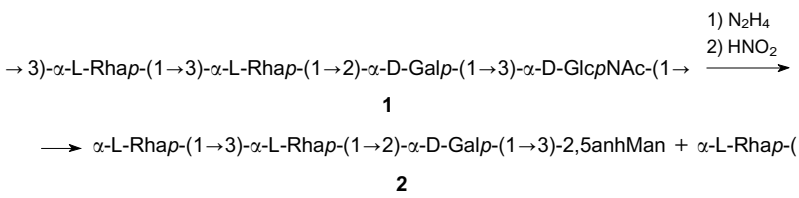
The N-deacetylation of both model glycosides and bacterial polysaccharides with anhydrous hydrazine in the presence of hydrazine sulfate at 105 °C for 10 h proceeded in nearly quantitative yield and was not accompanied by noticeable sugar degradation.[12] For example, OPS of *Shigella dysenteriae* type 1 (compound **1**) was successfully N-deacetylated by this method. The major product of the subsequent deamination was tetrasaccharide **2** bearing the 2,5-anhydromannose residue (2,5anhMan) at the reducing end (Scheme 3).[12] Besides, trisaccharide **3** was isolated in ~20% yield. The latter compound is produced by deamination *via* an alternative pathway through the formation of 2-*C*-formyl pentose (see above). The separation and structural analysis of these products were carried out after their reduction with sodium borohydride (borohydride reduction) to the corresponding oligosyl polyols. The latter reaction was necessary because of lability of 2,5-anhydromannose.

However, subsequent studies showed that this method is not versatile and the substitution position of hexosamine is one of the factors affecting the N-deacylation. Thus, the hydrazinolysis of CPS of the bacterium *Haemophilus influenzae* (100 °C, 8 h) resulted in the removal of N-acetyl groups only from *N*-acetylglucosamine and *N*-acetylmannosaminuronic acid substituted at position 4, whereas the residues of these monosaccharides substituted at position 3 remained almost intact upon N-deacetylation.[13]

Another N-deacetylation procedure involves the treatment of polysaccharides with a solution of NaOH in aqueous dimethyl sulfoxide in the presence of sodium thiophenoxide as the reducing agent, which is required to prevent oxidation with atmospheric oxygen (100 °C, 15 h).[14] This method was applied to quantitatively *N*-deacetylate *N*-acetylhexosamine residues in polysaccharides of the bacteria *Escherichia coli* and *Shigella flexneri*, the *N*-acyl groups of which are only partially removed by hydrazinolysis. It was mentioned that stability of acetamide bonds differs in different polysaccharides, thereby making it necessary to find special reaction conditions in each particular case.

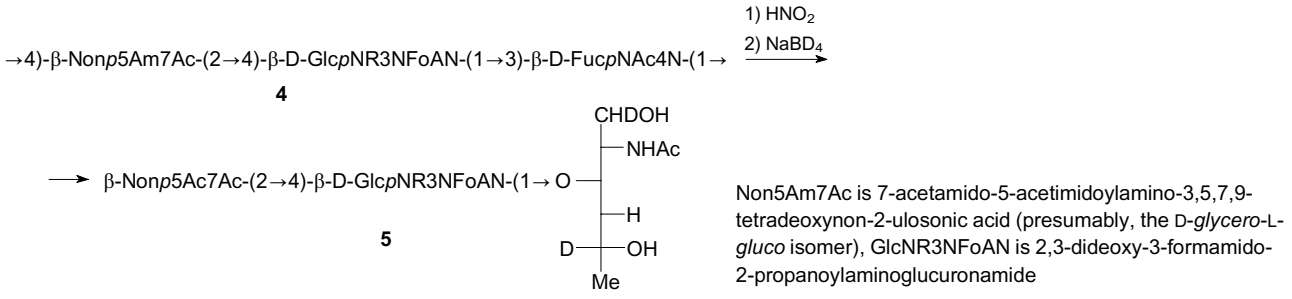
It should be noted that the N-deacylation–deamination method was widely used for the selective cleavage of polysaccharides until the end of the 1980s. In subsequent years, this method was almost not applied for polysaccharides

Scheme 3



Gal is galactose, Rha is rhamnose

Scheme 4



because it is difficult to achieve complete N-deacylation under relatively mild conditions, which should be used to prevent non-specific polymer degradation.

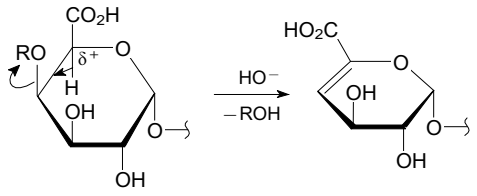
However, the deamination is still relevant for the determination of the structures of low-molecular-weight LPSs with a complex oligosaccharide chain (core) containing a hexosamine residue with a free amino group in the middle of the chain. This structural feature is typical, in particular, of LPSs of the bacteria of the genus *Proteus*.[15,16] For example, the presence of the GalN residue in the core made it possible to cleave LPSs under mild conditions and obtain the intact polysaccharide part from high-molecular-weight LPS of *P. mirabilis* O48 (see Ref. 15) and oligosaccharide, which corresponds to the outer region of the core, from low-molecular-weight LPS of *P. mirabilis* O6.[16]

The unique opportunity of performing selective cleavage is provided by the 2,4-diamino-2,4-dideoxyfucose residue (FucN4N), which is present in some bacterial polysaccharides in the mono-N-acetylated form with a free amino group at position 4. Thus, the deamination of OPS **4** from *Fusobacterium nucleatum* ATCC 23726 resulted in the transformation of FucNAc4N into 2-acetamido-2,4,6-trideoxyhex-5-ulose as the major product of the reaction accompanied by glycosidic bond cleavage, and the subsequent reduction with $NaBD_4$ afforded oligosaccharide **5** (Scheme 4).[17] In this case, the *N*-acetimidoyl group at the nonulosonic acid residue was hydrolyzed to the *N*-acetyl group.

2.2. Alkaline β-elimination

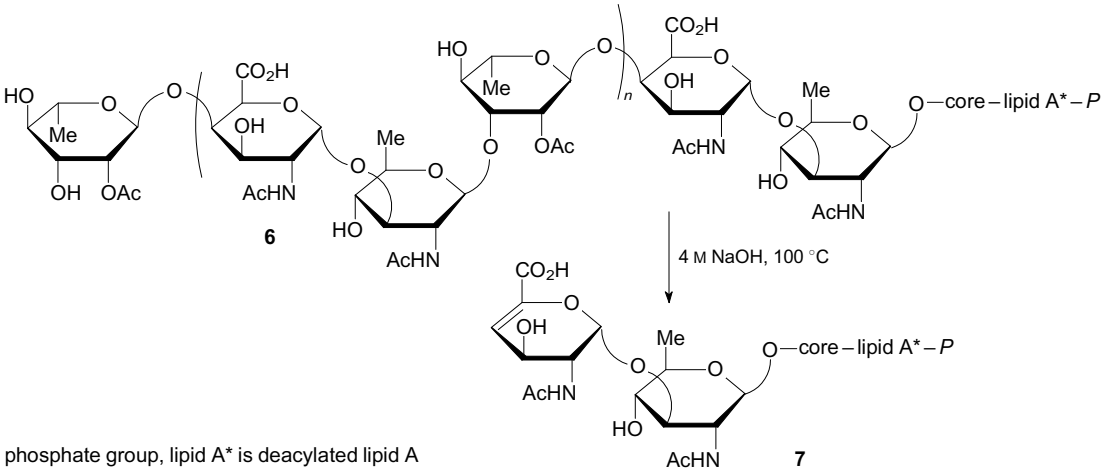
The β-elimination under alkaline conditions is a typical reaction of hexuronic acids and can be applied for the selective cleavage of polysaccharides. The necessary condition for the cleavage to occur *via* this mechanism is the presence of a substituent (usually monosaccharide) at position 4 of hexuronic acid. This reaction proceeding through the bimolecular β-elimination (E2) mechanism is facilitated by the negative inductive effect of the carboxyl group, which creates a partial positive charge on the C(5) atom, thereby increasing the H(5) proton mobility. The reaction produces unsaturated hexuronic acid with a double bond at position 4 (Scheme 5).

Scheme 5



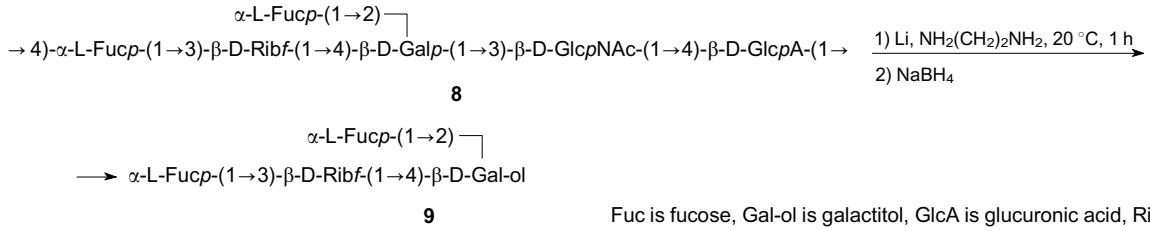
Under severe conditions required for the β-elimination in hexuronic acid containing a free carboxyl group (4 M NaOH or KOH, 100–120 °C), the resulting oligosaccharide having a free reducing end degrades in aqueous medium. However, the β-elimination can be applied to determine the structure of LPS protected at the reducing end by a phosphate group, which is stable under alkaline conditions. Thus, the treatment of *P. aeruginosa* O10a,10b LPS (**6**) with 4 M NaOH at 100 °C for 16 h afforded oligosaccharide **7** (Scheme 6).[18] The analysis of the latter compound allowed the determination of the structure of the core and the carbohydrate backbone of the lipid A and the identification of the attachment site of OPS to the core.

Scheme 6



P is phosphate group, lipid A* is deacylated lipid A

Scheme 7



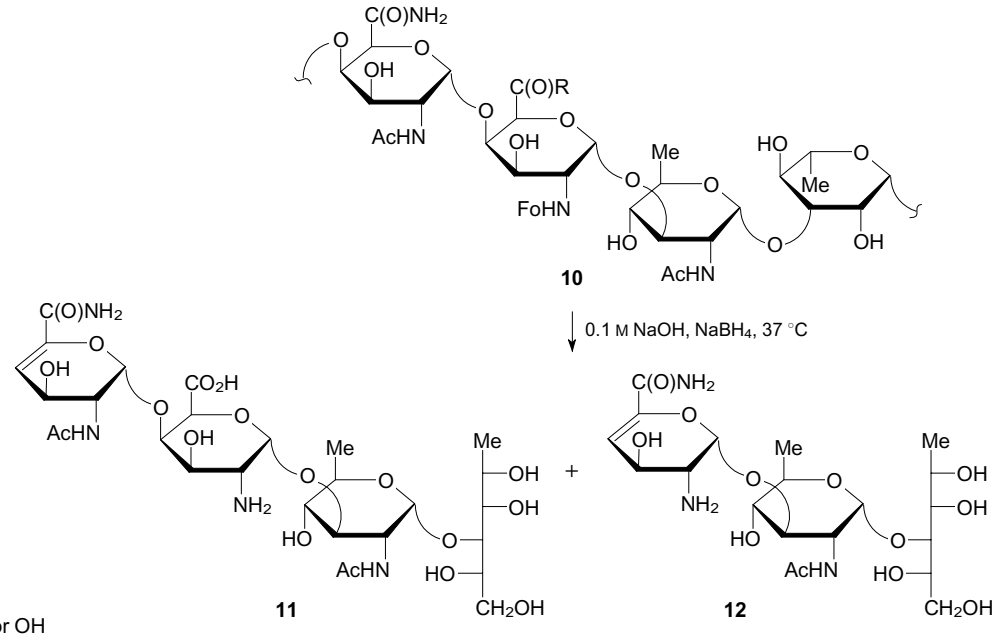
Fuc is fucose, Gal-ol is galactitol, GlcA is glucuronic acid, Rib is ribose

Oligosaccharide fragments can be prepared by the treatment of polysaccharides containing hexuronic acids with anhydrous bases. Thus, the reaction of OPS of the bacterium *Salmonella arizonae* O45 (compound **8**) with lithium in anhydrous ethylenediamine (20 °C, 1 h) followed by borohydride reduction gave branched tetrasaccharide **9** (Scheme 7).[19] This results from two sequential β-elimination reactions involving first the substituent at position 4 of the GlcA residue and then the substituent at position 3 of the GlcNAc residue at the reducing end of the resulting oligosaccharide. Under the reaction conditions, the unsaturated glucuronic acid residue was decomposed and removed upon the work-up of the reaction mixture.

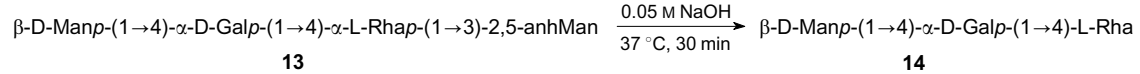
The absence of the negative charge on the carboxyl group of hexuronic acid significantly facilitates the nucleophilic attack on the HC(5) centre, due to which amides and esters of hexuronic acids much more readily undergo β-elimination than the free acids. This reaction accompanied by borohydride reduction of oligosaccharide products (0.1 M NaOH, 0.5 M $NaBH_4$, 37 °C, 24 h) was used to determine the structure of OPS of the bacterium *Pseudomonas aeruginosa* IID 1008 (**10**) containing *N*-acetylgalactosaminuronic acid amide (GalNAcA) and an approximately equal amounts of free *N*-formylgalactosaminuronic acid (GalNFoA) and its amide (Scheme 8).[20] Under these mild conditions, only hexuronic acid amides underwent β-elimination, and, as GalNFoA was amidated non-stoichiometrically, tetrasaccharide **11** and trisaccharide **12** were produced (the simultaneous alkaline N-deformylation of GalNFoA took place). Analysis of these oligosaccharides revealed the amidation pattern and allowed the identification of the sequence of different *N*-acyl derivatives of hexosaminuronic acid in OPS.

The cleavage of CPS of the bacterium *Klebsiella* type 81 fully methylated at glucuronic acid methyl ester residues using dimethylsulfinyl anion followed by borohydride reduction, acid hydrolysis and gas-liquid chromatography–electron ionization mass spectrometry analysis of partially methylated monosaccharides provided significant data on the polymer structure.[21] On the other hand, the determination of the structures of oligosaccharide derivatives, which are formed by the cleavage of methylated polysaccharides, by NMR spectroscopy is a more difficult task compared to free oligosaccharides, and this technique is little used in structural analysis.

Scheme 8



Scheme 9



Man is mannose

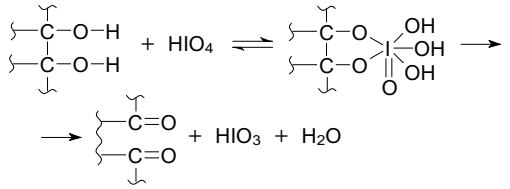
Under alkaline conditions, the β-elimination readily proceeds in 2,5-anhydrohexoses substituted at position 3, which is also employed in the structural analysis. Thus, the mild alkaline treatment of the major deamination product of *E. coli* O75 polysaccharide (compound **13**) afforded oligosaccharide **14** in high yield (Scheme 9).[22] This product is identical to the side product of deamination through the formation of 2-*C*-formylpentose.

2.3. Smith degradation

The polysaccharide structure can be determined using Smith degradation provided that the polysaccharide contains at least one monosaccharide unit bearing the free vicinal diol group. The method involves periodate oxidation of polysaccharide accompanied by the carbon–carbon bond cleavage in this group, borohydride reduction of the resulting polyaldehyde to the polyhydroxy derivative and selective acid hydrolysis under mild conditions at the acetal bond of the oxidized monosaccharide.[23] The oxidation is quantitative and proceeds through the reversible formation of an intermediate compound with a five-membered iodine-containing ring (Scheme 10).

The Smith degradation may involve only the modification of branched polysaccharide without its depolymerization. An example is the cleavage of *E. coli* O43 OPS (**15**) composed of pentasaccharide repeating units, which led to oxidation and subsequent elimination of the fucose residue in the side chain to form linear polysaccharide **16** composed of tetrasaccharide repeating units (Scheme 11).[24]

Scheme 10

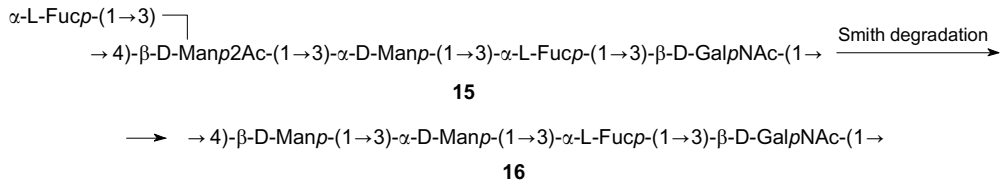


If the α-glycol group is present in the monosaccharide ring of one of the main chain residues of a polysaccharide, the Smith degradation affords oligosaccharide (or oligosaccharides) containing a glycerol (derived from 6-substituted aldoses), glyceraldehyde (from 2-substituted aldoses), erythritol or threitol (from 4-substituted hexopyranoses, 5- or 6-substituted hexofuranoses) residue at the reducing end. For example, the galactose residue in the side chain along with the 4-substituted rhamnose residue in the main chain are subjected to degradation in *E. coli* O39 OPS (**17**) to form oligosaccharide **18** bearing 1-deoxyerythritol (1dEry-ol) as the aglycone (Scheme 12).[25]

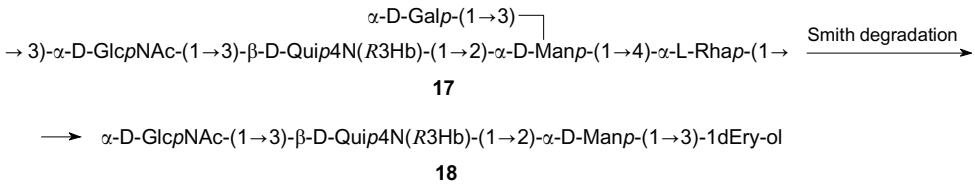
Smith degradation products may contain a new α-glycol group, which was absent in the starting polysaccharide and, in this case, they can undergo repeated Smith degradation. Thus, Smith degradation of branched *E. coli* O7 OPS (**19**) resulted in its oxidation at galactose residues in the main chain and rhamnose residue in the side chain to give linear trisaccharide (**20**) bearing threitol (Thr-ol) at the reducing end (Scheme 13).[26] The repeated Smith degradation of this trisaccharide led to oxidation of the terminal GlcNAc residue and the mannose residue that lost one of substituents to form compound **21**, which is 4-acetamido-4,6-dideoxyglucose glycoside (Qui4NAc) bearing the glycerol residue (Gro).

The Smith degradation is an efficient method for analysis of structurally heterogeneous polysaccharides. An example is the cleavage of OPS of the bacterium *Pseudomonas syringae* pv. *ribicola*[27] consisting of two types of pentasaccharide repeating units, the major (**22**) and minor

Scheme 11

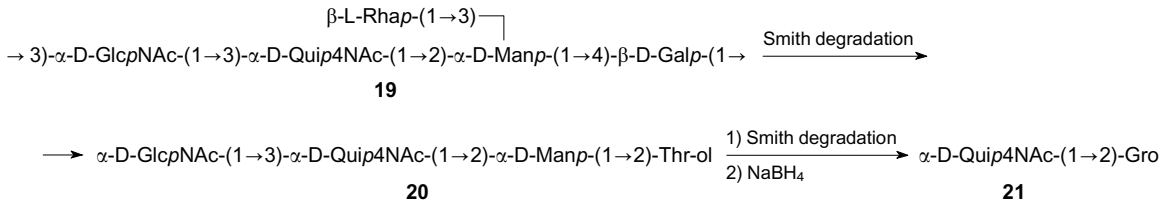


Scheme 12

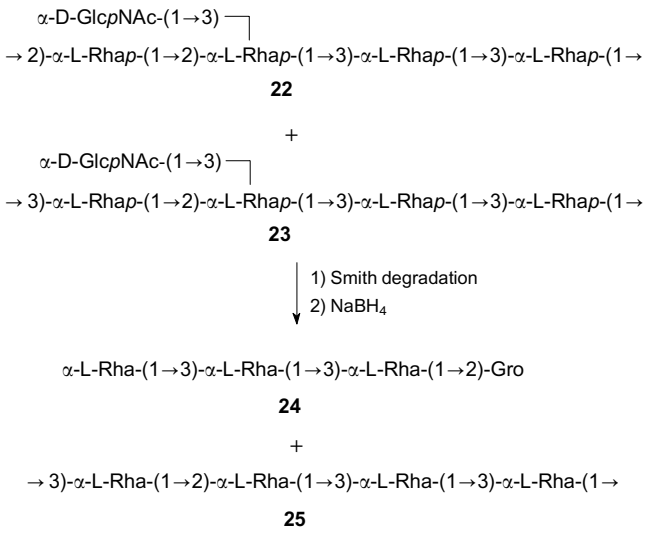


Qui*p*4N(*R*3Hb) is 4-[(*R*)-3-hydroxybutanoylamino]-4-deoxyquinovose

Scheme 13



Scheme 14



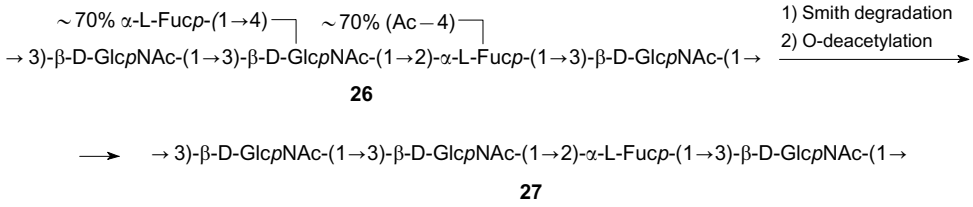
(**23**) ones. The Smith degradation followed by borohydride reduction resulted in the transformation of the units of compound **22** into oligosaccharide **24** and the modification of the units of derivative **23** giving linear structure **25** (Scheme 14).

A comparison of the structures of the starting irregular polysaccharide and the modified regular polysaccharide obtained by Smith degradation provides insight into the factors responsible for irregularity. For example, the application of this method to irregular *E. albertii* O2 OPS (**26**) composed of fucosylated branched pentasaccharide repeating units and non-fucosylated linear tetrasaccharide repeating units resulted in the elimination of the side fucose residue and the formation, after O-deacetylation, of modified regular linear polysaccharide **27** composed of tetrasaccharide repeating units (Scheme 15).[28]

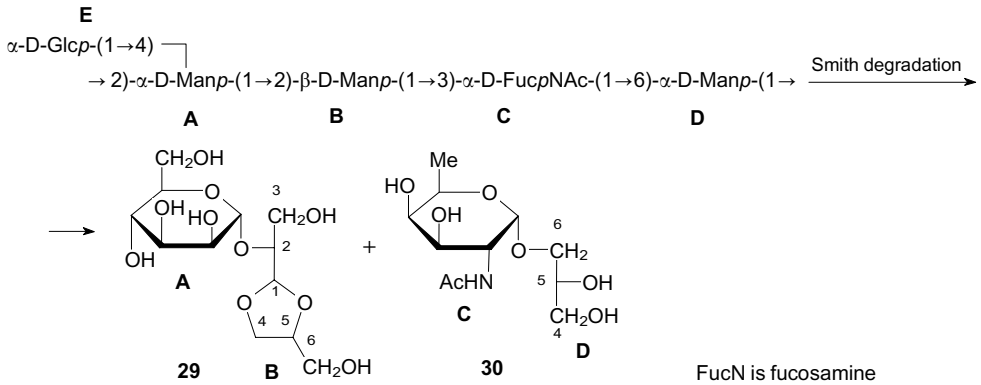
Although in most cases the Smith degradation proceeds smoothly, it should be taken into account that this procedure can afford side products, such as cyclic acetals consisting of oxidized monosaccharide units. For example, the Smith degradation of OPS of the bacterium *Enterobacter cloacae* O11 (**28**) resulted in the formation of oligosaccharides **29** and **30** from the main chain (residues **A**–**D**) (Scheme 16).[29] It should be noted that oligosaccharide **30** contained the expected aglycone (glycerol) derived from the 6-substituted mannose residue (**D**), whereas the aglycone in oligosaccharide **29** was formed *via* transacetalization in the cleaved 2-substituted mannose residue (**B**).

This problem can be overcome by methylation of free hydroxy groups before the mild hydrolysis of the oxidized and reduced polysaccharide, which excludes the possibility of intramolecular transacetalization.[30]

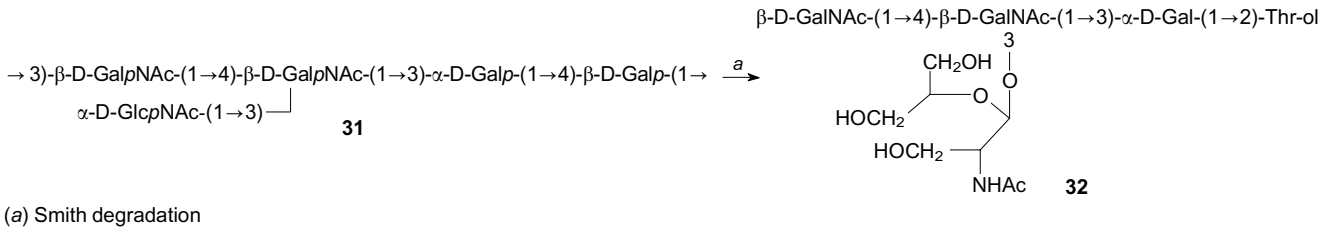
Scheme 15



Scheme 16



Scheme 17



(*a*) Smith degradation

The hydrolysis of the glycosidic bond of the oxidized monosaccharide residue is another difficulty encountered in Smith degradation. This residue cannot be removed without the cleavage of the glycosidic bonds of non-oxidized monosaccharides. Thus, Smith degradation of OPS of the bacterium *Citrobacter* sp. O32 (**31**) gave oligosaccharide **32** bearing the threitol residue at the end. The side chain of **32** contained the degraded terminal GlcNAc residue, which was not cleaved upon mild acid hydrolysis apparently due to steric hindrance (Scheme 17).[31]

2.4. Cleavage of phosphate-containing polysaccharides

Teichoic acids of Gram-positive bacteria are well-known phosphate-containing polysaccharides. Biopolymers built up in a similar way are present also in Gram-negative bacteria (OPSs and CPSs) and other organisms. Two types of phosphate-containing polysaccharides are known. The phosphate group can attach the 2-aminoethanol (EtN) or polyol moiety as the side substituent or incorporate a polyol in the main chain of the polysaccharide by linking it to one of monosaccharides. Glycerol or ribitol (Rib-ol) are most common polyols but erythritol and arabitol were also found (see the Bacterial Carbohydrate Structure Database accessed *via* the Internet at http://csdb.glycoscience.ru/bacterial). In another type of polysaccharides, the phosphate group links two monosaccharides, one of which forms a glycosyl phosphate bond.

2.4.1. Dephosphorylation with hydrofluoric acid

This approach is based on the phosphate cleavage with hydrofluoric acid and is applied in studies of phosphate-containing polysaccharides of the first type. The reaction proceeds through the P–F bond formation accompanied by P–O bond cleavage. The cleavage is usually performed using commercial 48% HF at room temperature or on cooling to 4 °C, but more concentrated solutions and the anhydrous acid can also be employed. Generally, glycosidic bonds remain intact when treated with this reagent.

If the phosphoethanolamine or a polyol residue involved in the polysaccharide is attached through the phosphate group as the side substituent, the reaction affords dephosphorylated polysaccharide. Thus, the treatment of *Proteus mirabils* O41 OPS (**33**) with 48% hydrofluoric acid resulted in elimination of all three substituents attached through phosphate groups to form modified polysaccharide **34** (Scheme 18).[32]

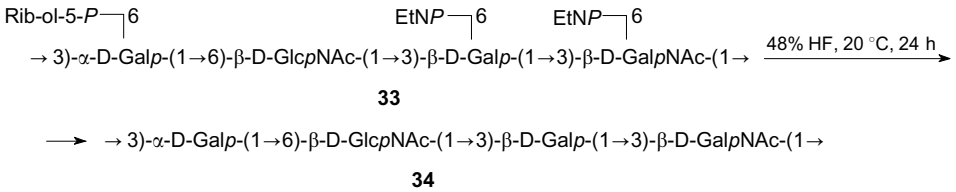
Another example is the elimination of glycero-2-phosphate upon the treatment of *E. coli* O33 OPS (**35**) with 48% HF giving dephosphorylated polysaccharide **36** (Scheme 19).[33]

If polyol phosphate is present in the main chain, the dephosphorylation is accompanied by depolymerization of the polysaccharide to form an oligosyl polyol. An example is the cleavage of *E. coli* O28ab OPS (**37**) giving trisaccharide glycoside **38** bearing the glycerol residue (Scheme 20).[34]

2.4.2. Hydrolytic cleavage of glycosyl phosphates

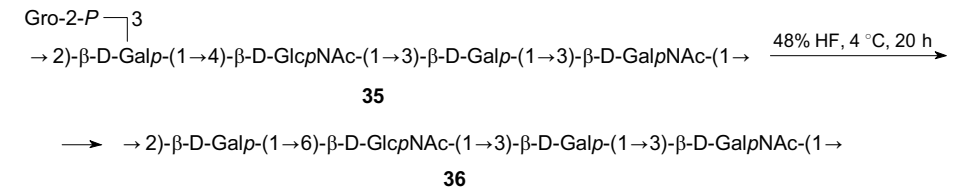
The glycosyl phosphate bond is characterized by high acid lability and is readily cleaved under mild acidic conditions, in which glycosidic bonds of other monosaccharides remain intact [exceptions are bonds in keto sugars and β-(1 → 6)-bonds in *N*-acetylhexosamines]. In this case, the phosphate group is preserved in the resulting oligosaccharide at the monosaccharide residue to which it is attached by an ester linkage. For example, OPS of *Shigella boydii* type 13 (compound **39**) is depolymerized through the glycosyl phosphate bond cleavage even under conditions that are used for the elimination of the carbohydrate part of LPS from the lipid moiety (Scheme 21). The resulting oligosaccharide is subjected to borohydride reduction to the corresponding oligosyl polyol **40** through the transformation of the glucose residue at the reducing end into glucitol (Glc-ol).[35] This modified product is more convenient for NMR

Scheme 18

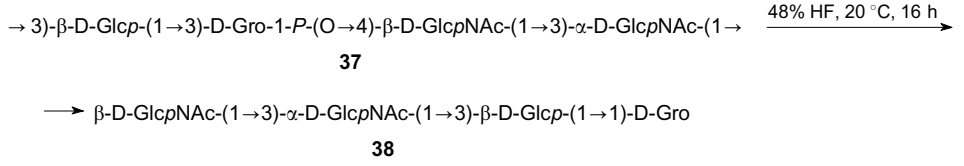


Rib-ol is ribitol, EtN is ethanolamine

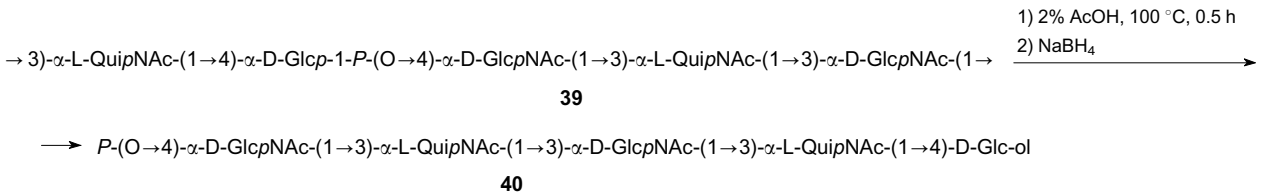
Scheme 19



Scheme 20



Scheme 21



Glc-ol is glucitol, Quip*N* is quinovosamine

study compared to the starting reducing oligosaccharide, which gives two series of signals in NMR spectra due to the presence of the glucose residue as α- and β-anomers.

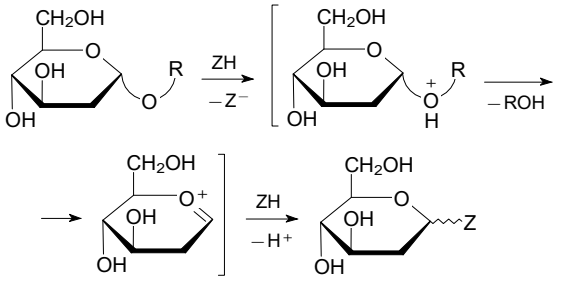
The phosphodiester bond cleavage giving monophosphates can be performed using hydrolysis under alkaline conditions.[6] The drawback of this approach is that the reaction is accompanied by phosphate group migration through the formation of cyclic phosphate, which does not allow one to reliably determine the position of this group in the starting polysaccharide.

3. Non-specific methods for selective cleavage

Non-specific methods for selective cleavage, which are used both to modify polysaccharides and prepare oligosaccharide fragments or complex monosaccharides, include the partial acid hydrolysis, acetolysis and solvolysis with protic acids. The preparation of oligosaccharides by these methods relies on the difference in the rate of cleavage (corresponding to different stability) of glycosidic bonds of monosaccharide components of polysaccharides (hexoses, 6-deoxyhexoses, hexosamines, hexuronic and 3-deoxyaldulosonic acids, *etc.*), thereby making it possible to perform complete or nearly complete cleavage of one or several bonds in the polysaccharide, with other bonds being almost intact.

The solvolytic (in the particular case, hydrolytic) cleavage of glycosidic bonds occurs through the protonation of the anomeric oxygen atom followed by the cleavage of the bond between this atom and the C(1) atom and the release of the aglycone as an alcohol to form a glycosyl cation. The latter reacts with the solvolytic reagent to give the free monosaccharide in the case of hydrolysis and glycosyl fluoride or esters in the case of solvolysis with hydrofluoric acid or organic acids, respectively (Scheme 22).

Scheme 22



3.1. Partial acid hydrolysis

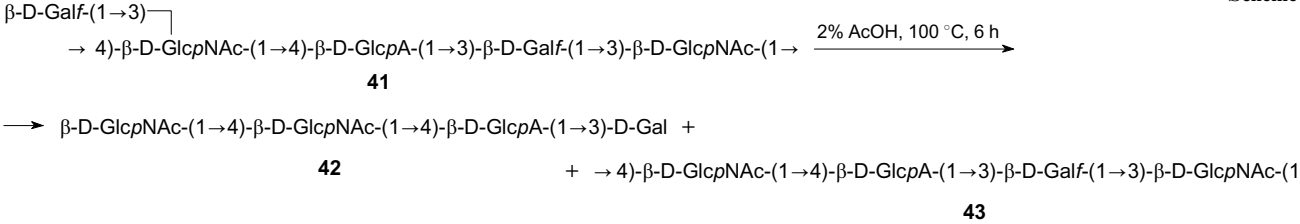
The partial acid hydrolysis is most commonly used [6] for the selective cleavage. This is performed with different organic and inorganic acids, such as dilute AcOH, CF_3CO_2H, H_2SO_4, HCl, HF and so on. The drawbacks of this method are low selectivity with polysaccharides lacking monosaccharides with highly acid-stable or acid-labile glycosidic linkages and the possible cleavage of amide bonds in N-acylated amino sugars and hexuronic acid amides under insufficiently mild hydrolysis conditions.

Glycosidic bonds of hexofuranoses, 3,6-dideoxyhexopyranoses, 3-deoxyaldulosonic acids and, unexpectedly, β-(1→6)-bonds [but not α-(1→6)-bonds] of *N*-acetylhexosamines are the most acid-labile bonds, which allows one to easily prepare oligosaccharide fragments containing such monosaccharides. For instance, the depolymerization of branched OPS of *E. coli* O170 (**41**) with dilute acetic acid proceeded with exceptional selectivity at glycosidic bonds of most β-D-galactofuranose residues (β-D-Gal*f*) to form tetrasaccharide **42** and modified linear polysaccharide **43** (Scheme 23).[36]

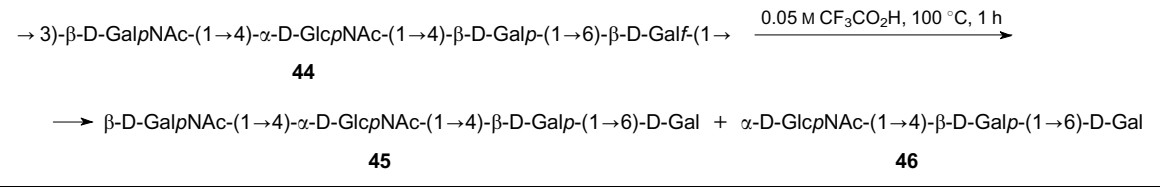
Another example is the treatment of O-deacetylated OPS of *E. albertii* O5 (**44**) composed of tetrasaccharide repeating units with 0.05 M trifluoroacetic acid, resulting in the cleavage of all bonds of β-D-Gal*f* and some bonds of β-D-Gal*p*NAc to form oligosaccharides **45** and **46** (Scheme 24).[37]

Ketosidic bonds of 3-deoxynonulosonic acid derivatives (neuraminic, pseudaminic, legionaminic, *etc.*) are easily cleaved under conditions in which the linkages of other monosaccharides remain intact. For example, when treated with dilute acetic acid at 100 °C, OPS of *Enterobacter cloacae* C5529 (**47**) undergoes depolymerization at the bond of di-*N*-acetylpseudaminic acid (Pse5Ac7Ac) to give tetrasaccharide **48**, which corresponds to the repeating unit of OPS, in high yield (Scheme 25).[29] Under these mild

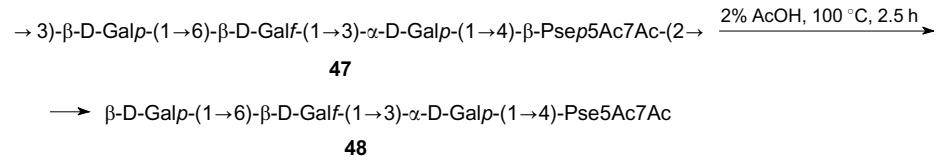
Scheme 23



Scheme 24



Scheme 25



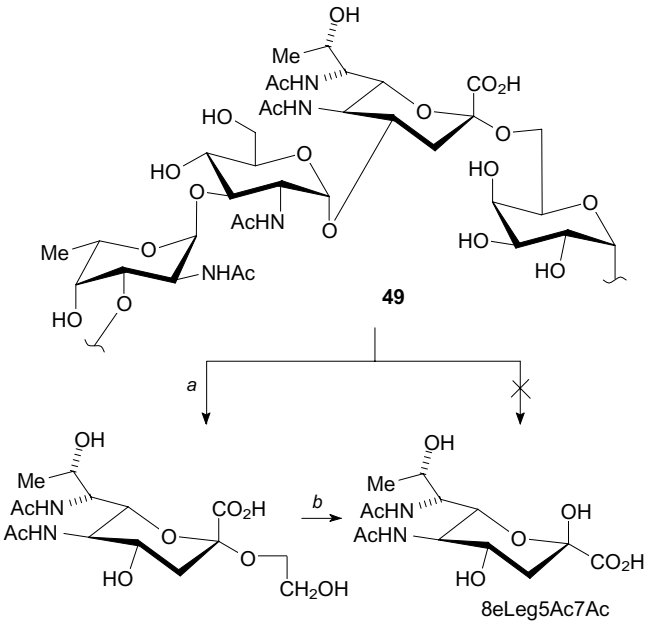
Pse is 5,7-diamino-3,5,7,9-tetradeoxy-L-*glycero*-L-*manno*-non-2-ulosonic (pseudaminic) acid

conditions, another acid-labile bond in OPS (galactofuranosidic bond) remained almost intact.

In some cases, the cleavage of oligosaccharides allows the isolation of complex monosaccharides or their derivatives, which are impossible to obtain directly from the starting polysaccharides, and their subsequent identification. Thus, a combination of three sequential Smith degradations and mild acid hydrolysis of the resulting glycoside made it possible to isolate and then identify di-*N*-acetyl-8-epilegionaminic acid (8eLeg5Ac7Ac) involved as a component in *E. coli* O108 OPS (**49**) (Scheme 26).[38] The direct preparation of this 3-deoxynonulosonic acid by the partial acid hydrolysis of OPS was hindered by its degradation under the conditions required for the cleavage of the glycosidic bond of the GlcNAc residue attached to 8eLeg5Ac7Ac.

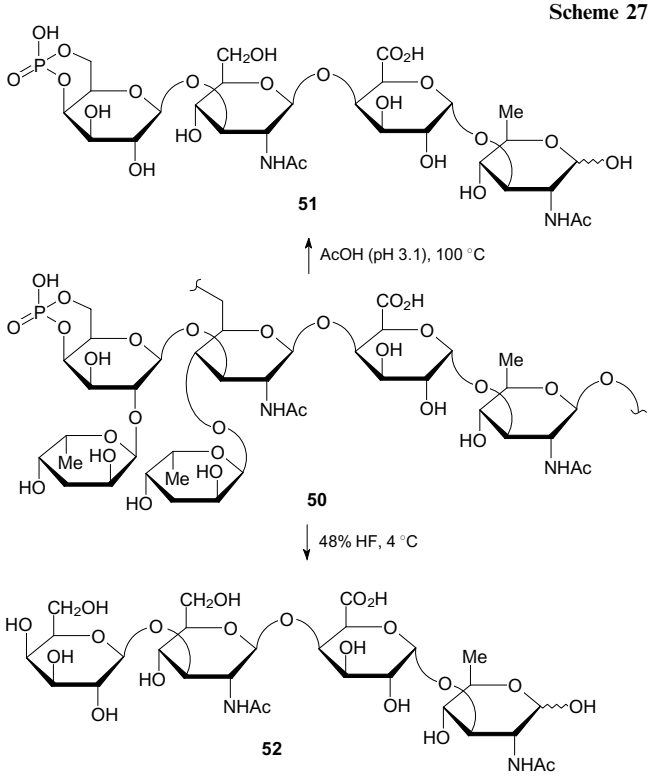
The treatment of CPS of the bacterium *Vibrio cholerae* O139 (**50**) with dilute acetic acid (pH 3.1) at 100 °C resulted in not only the expected elimination of 3,6-dideoxy-L-*xylo*-hexose (colitose) residues in the side chains, but also depolymerization of the polysaccharide giving tetrasaccharide **51** through the cleavage of the β-(1→6)-bond of the residue Qui*p*NAc in the backbone of the polysaccharide (Scheme 27).[39] This cleavage is not incidental. Other examples of acid lability of the β-(1→6)-bond of *N*-acetylhexosamines are also known.

Scheme 26



(*a*) Smith degradation (3 times), (*b*) 2% AcOH, 100 °C, 2 h

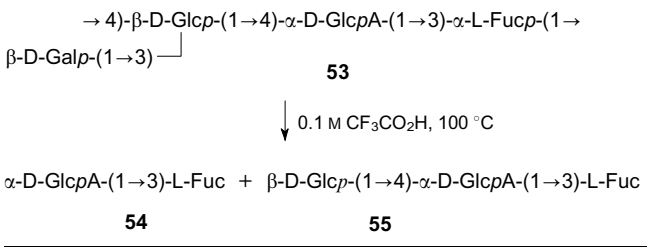
Scheme 27



Acid-labile glycosidic bonds can be cleaved with hydrofluoric acid (generally 48%), which is used for carbohydrate dephosphorylation at 0–4 °C. Thus, the treatment of *V. cholerae* O139 CPS (**50**) with this reagent is not limited to dephosphorylation but gives tetrasaccharide **52**, a dephosphorylated analogue of tetrasaccharide **51** (see Scheme 27).[39]

On the other hand, polysaccharides containing monosaccharides with highly acid-stable glycosidic bonds, such as hexuronic acids, can be subjected to efficient selective cleavage. For instance, the partial acid hydrolysis of *E. coli* K27 CPS (**53**) affords disaccharide **54** bearing the GlcA residue at the non-reducing end along with a smaller amount of trisaccharide **55** (Scheme 28).[40]

Scheme 28



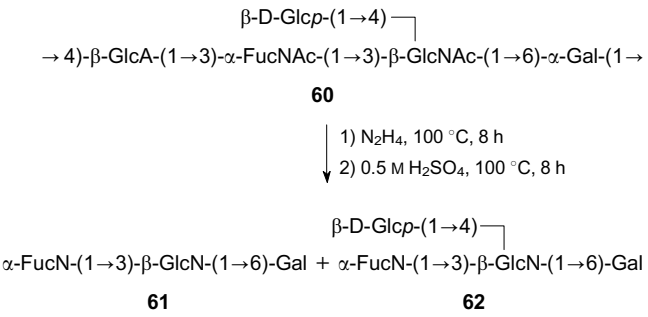
Hexuronic acid stabilizes not only its own but also adjacent glycosidic bonds, as was demonstrated by the mild acid hydrolysis of OPS of *Shigella boydii* type 5 (**56**) containing one GlcA residue in the hexasaccharide repeating unit.[41] This process affords trisaccharide **57** and two complementary tetrasaccharides **58** and **59** (Scheme 29).

Hexosamine glycosides bearing a free amino group at position 2 are highly stable to acid hydrolysis. This is attributed to the protonation of this amino group, resulting in electrostatic repulsion, which prevents protonation of glycosidic oxygen as the initial step of glycosidic bond hydrolysis. The acid hydrolysis of polysaccharide containing such hexosamine leads to the cleavage of all glycosidic bonds except for the bond of the hexosamine and the formation of disaccharide bearing amino sugar at the non-reducing end. However, amino groups of hexosamines in bacterial polysaccharides are generally N-acetylated and their release is a necessary but difficult problem (the issues associated with N-deacetylation of amino sugars were discussed above in Section 2.1 devoted to deamination of hexosamines). Nevertheless, examples of the successful utilization of this property of hexosamines for the selective cleavage of glycosidic bonds are known.

The sequential arrangement of several hexosamines gives rise not to disaccharides but to larger oligosaccharides. For example, the acid hydrolysis of N-deacetylated CPS of *Escherichia coli* K87 (**60**) afforded trisaccharide **61** (Scheme 30)[42] along with tetrasaccharides **62**. The formation of the latter is apparently attributable to stabilization of the glycosidic bond of the side-chain glucopyranose residue by two spatially proximal protonated amino groups of GlcN and FucN residues.

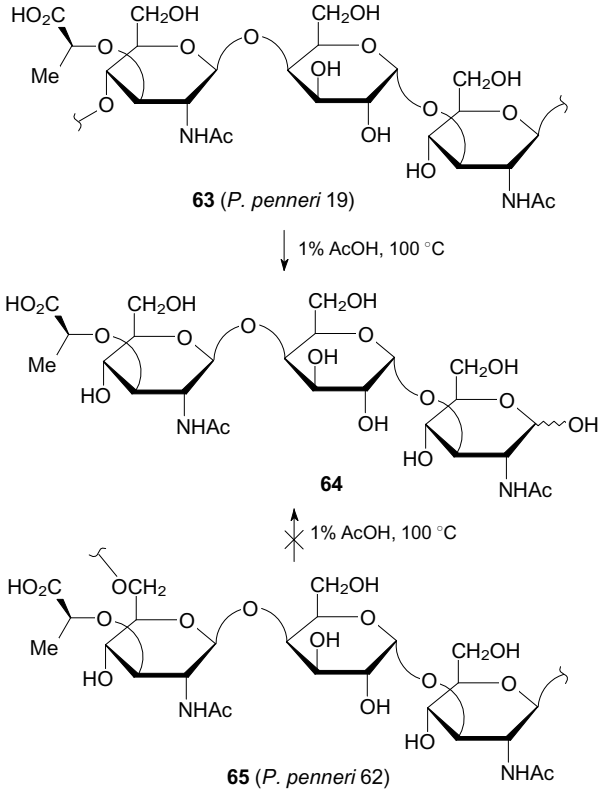
The behaviour of polysaccharides towards hydrolytic cleavage may be determined by their fine structural features.

Scheme 30

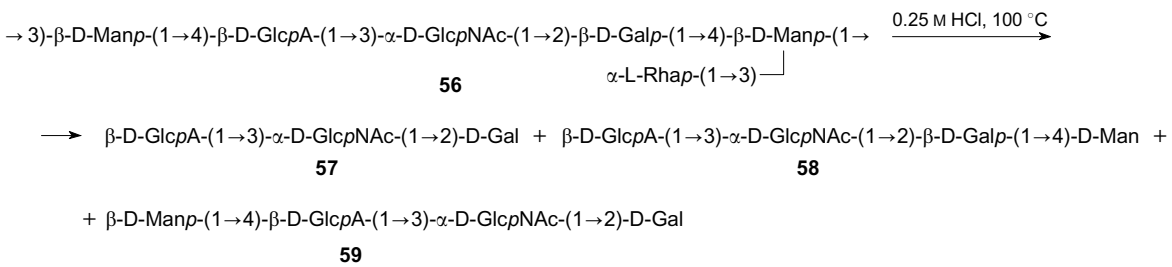


For instance, under conditions of mild acid degradation of LPS (1% AcOH, 100 °C, 4 h), *Proteus penneri* 19 OPS (**63**) is selectively cleaved at the glycosidic bond of the residue β-D-Glc*p*NAc to give trisaccharide **64** corresponding to the repeating unit of OPS and also its oligomer homologues (Scheme 31).[43] Meanwhile, *P. penneri* 62 OPS (**65**), which differs only by the attachment site of the residue β-D-Glc*p*NAc to the *N*-acetylisomuramic acid residue (at

Scheme 31



Scheme 29



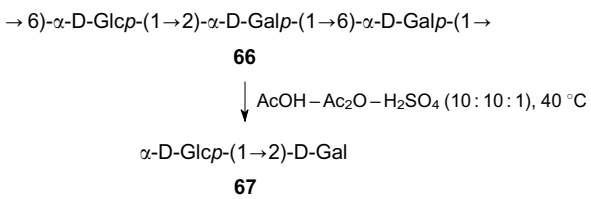
position 6 rather than 4 found in OPS of *P. penneri* 19), is stable under these conditions.[43] This difference can be attributed to the spatial proximity of the residue β-D-Glc*p*NAc in OPS of *P. penneri* 19 to the lactic acid moiety of *N*-acetylisomuramic acid, thereby allowing its carboxyl group to be involved in the glycosidic bond hydrolysis.

3.2. Acetolysis

The acetolysis occurs upon the treatment of a polysaccharide with a mixture of acetic anhydride and sulfuric acid, sometimes in the presence of acetic acid. This results in the formation of O-acetylated sugars, which can be easily saponified to a mixture of free monosaccharides and oligosaccharides. The selectivity of the cleavage is observed for different types of glycosidic bonds, including those formed by monosaccharides of the same or similar kind or nature. Thus, (1→6)-bonds are cleaved by acetolysis more easily compared to other bonds regardless of their configuration.

The acetolysis is rarely used in the structural analysis of bacterial polysaccharides. However, in some cases it allows the preparation of fragments inaccessible by other methods. For example, the acetolysis of polysaccharide of the bacterium *Acetobacter methanolicus* MB 58 (**66**) gave disaccharide **67** *via* selective cleavage of (1→6)-bonds of two galactose residues with the retention of the (1→2)-bond of the glucose residue (Scheme 32).[44]

Scheme 32



3.3. Solvolysis with anhydrous acids

The solvolysis of polysaccharides with anhydrous protic acids under selected conditions can be performed with fairly high selectivity and without significant sugar degradation.[8] Anhydrous hydrofluoric acid was the first solvolytic reagent used for the selective cleavage of polysaccharides in the 1980s. However, high toxicity and problems of handling of HF (b.p. 19 °C), which requires specialized equipment, forced researchers to search for alternative, safer and more convenient, solvolytic reagents. Trifluoromethanesulfonic (triflic) acid was tested and put into practice in the beginning of the 2000s; then trifluoroacetic acid was applied in the mid-2010s.

3.3.1. Solvolysis with hydrofluoric acid

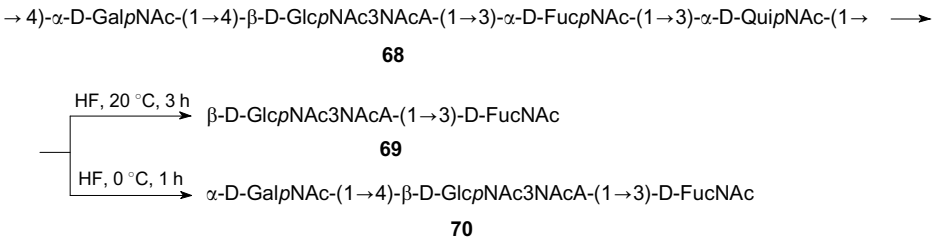
The solvolysis of glycosidic bonds with anhydrous hydrofluoric acid proceeds through the formation of glycosyl fluorides, which are easily hydrolyzed by treatment of the reaction mixture with water. An exception are glycosyl fluorides glycosylated at position 2, which can be isolated and analyzed.[7] The treatment with this reagent leads to the cleavage of polysaccharides at the bonds of pentoses, hexoses, 6-deoxyhexoses and N-acylated 6-deoxyhexosamines, while the bonds of N-acylated diaminohexoses, hexuronic and 3-deoxyaldulosonic acids remain intact.[7] Glycosidic bonds of *N*-acylhexosamines are intermediate in stability to HF, which makes it possible to perform the targeted solvolysis by varying the reaction conditions. For example, the solvolysis of OPS of *Pseudomonas aeruginosa* O6 (**68**) at 20 °C for 3 h resulted in the cleavage of all glycosidic bonds, except for the bond of 2,3-diacetamido-2,3-dideoxyglucuronic acid (GlcNAc3NAcA), giving disaccharide **69**, which bears this monosaccharide at the non-reducing end (Scheme 33). Under milder conditions (0 °C, 1 h), only glycosidic bonds of 6-deoxyhexosamines (QuiNAc and FucNAc) were cleaved, while the more stable bond of GalNAc was retained, resulting in the formation of trisaccharide **70**.[45]

The selective cleavage of glycosidic bonds of 6-deoxyhexoses in the presence of hexoses can be achieved by performing solvolysis at lower temperature. Thus, when treated with HF at −40 °C, extracellular polysaccharide of *P. aeruginosa* (**71**) is cleaved at the glycosidic bonds of rhamnose residues to give pentasaccharide **72**, which corresponds to the repeating unit of the polysaccharide, as the only product (Scheme 34).[46]

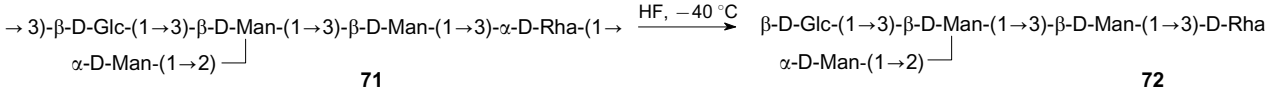
The solvolysis of *E. coli* O86 OPS (**73**) is an example of the difference in stability of α- and β-glycosidic bonds. Only the β-glycosidic bond of the bonds of two GalNAc backbone residues of this polysaccharide was cleaved upon the treatment with HF at 50 °C, resulting in the formation of a series of oligosaccharides **74**–**76**, from di- to tetrasaccharides (Scheme 35).[47]

Glycosides of oligosaccharide or complex monosaccharides can be prepared in the presence of lower alcohols

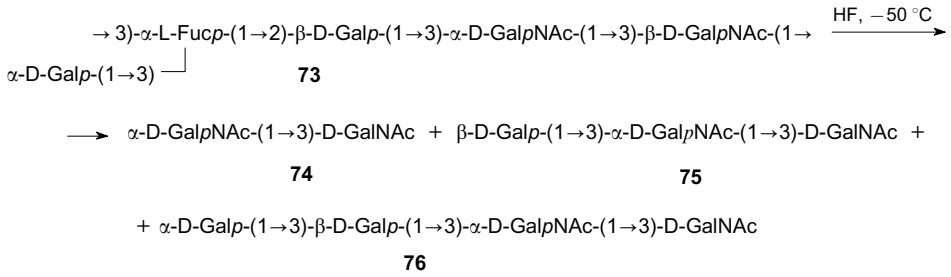
Scheme 33



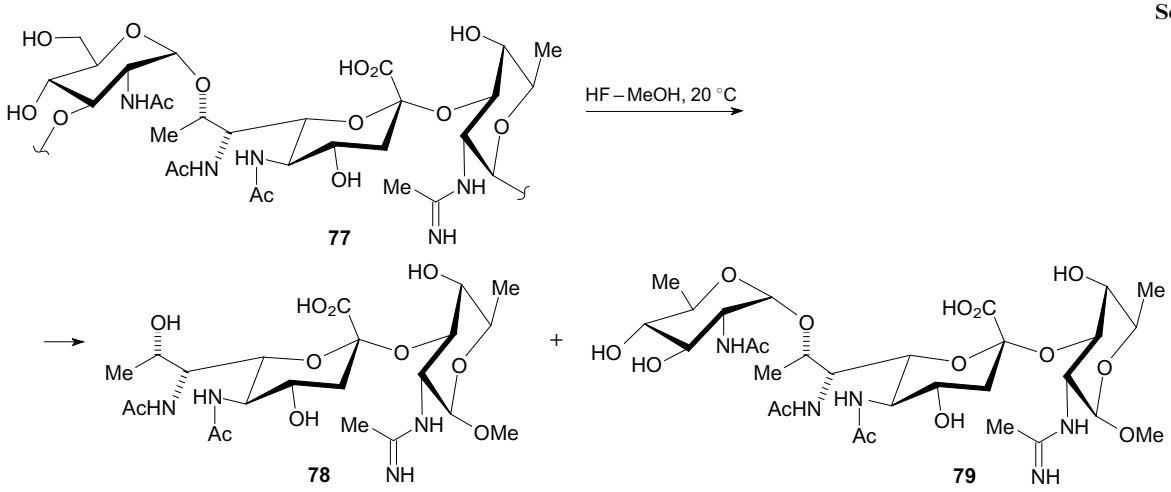
Scheme 34



Scheme 35



Scheme 36



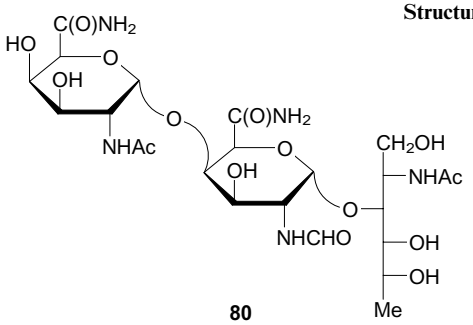
(usually methanol). These products are more convenient for isolation by high-performance liquid chromatography and subsequent NMR spectroscopic analysis. For example, the solvolysis of *P. aeruginosa* O13 OPS (**77**) with anhydrous HF in methanol led to the cleavage of glycosidic bonds of one or both 6-deoxyamino sugar derivatives (D-QuiN and L-FucN), while the bond of di-*N*-acetylpseudaminic acid was stable (Scheme 36).[48] This gave rise to methyl glycosides of disaccharide (**78**) and trisaccharide (**79**).

This approach was applied to analyze amidated derivatives of D-galactosaminuronic acid (D-GalNA) in a series of five structurally related OPSs of *P. aeruginosa* O4 (apart from the degree of amidation, they differ in the substitution position of the Rha residue, the configuration of the glycosidic bond of the QuiNAc residue and the position of the O-acetyl group) (Table 1).[49] The products of HF solvolysis were reduced with $NaBH_4$ and separated by anion-exchange chromatography. The degree of amidation of

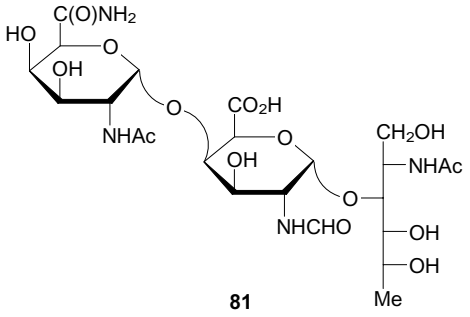
Table 1. Structures and the degrees of amidation of structurally related OPSs of *P. aeruginosa* O6 and immunotype 1.

Serotype	OPS structure
O6a,b	(H$_2$N–6)┐ ~10% (H$_2$N–6)┐ → 4)-α-D-Gal*p*NAcA-(1→4)-α-D-Gal*p*NFoA-(1→3)-α-D-Qui*p*NAc-(1→2)-α-L-Rha*p*-(1→ (AcO–3)┘
O6a,c	(H$_2$N–6)┐ → 4)-α-D-Gal*p*NAcA-(1→4)-α-D-Gal*p*NFoA-(1→3)-α-D-Qui*p*NAc-(1→3)-α-L-Rha*p*-(1→
O6a,d	(H$_2$N–6)┐ ~10% (H$_2$N–6)┐ → 4)-α-D-Gal*p*NAcA-(1→4)-α-D-Gal*p*NFoA-(1→3)-β-D-Qui*p*NAc-(1→3)-α-L-Rha*p*-(1→ (AcO–3)┘
O6a	(H$_2$N–6)┐ → 4)-α-D-Gal*p*NAcA-(1→4)-α-D-Gal*p*NFoA-(1→3)-α-D-Qui*p*NAc-(1→3)-α-L-Rha*p*-(1→ (AcO–3)┘
Immunotype 1	(H$_2$N–6)┐ (H$_2$N–6)┐ → 4)-α-D-Gal*p*NAcA-(1→4)-α-D-Gal*p*NFoA-(1→3)-α-D-Qui*p*NAc-(1→2)-α-L-Rha*p*-(1→ (AcO–3)┘

Structures 80, 81



80



81

N-acylated GalNA residues was evaluated from the ratio of neutral (**80**) to acidic (**81**) trisaccharides. It was shown that the N-acetylated residue (GalNAcA) is present exclusively in the amide form in all OPSs, while the degree of amidation of the N-formylated residue (GalNFoA) varies in a broad range (see Table 1).

3.3.2. Solvolysis with sulfonic acids

The stability of glycosidic bonds of different sugars towards CF_3SO_3H decreases in the same order as towards anhydrous HF: 5,7-diamino-3,5,7,9-tetradeoxynonulosinic acids > 2,3-diamino-2,3-dideoxyuronic acids ≈ 2,4-diamino-2,4,6-trideoxyhexoses > hexuronic acids ≈ 2-amino-2-deoxyhexoses > 2-amino-2,6-dideoxyhexoses ≈ hexoses > 6-deoxyhexoses (assuming all amino sugars are in the N-acylated form).[8]

The selectivity of the cleavage can be observed in the case of different configurations at the anomeric centre. For example, the glycosidic bond of β-GlcA is more stable than that of β-GlcNAc but is less stable than the bond of α-GlcNAc, as demonstrated by OPSs of two *Proteus vulgaris* strains. Thus, the solvolysis of *P. vulgaris* O4 OPS (**82**) with triflic acid cleaves all glycosidic bonds except for the bond of β-GlcA and affords disaccharide **83** bearing this monosaccharide at the non-reducing end (Scheme 37).[50]

On the contrary, the solvolysis of *P. vulgaris* O46 OPS (**84**) gave disaccharide (**85**) containing the α-GlcNAc residue at the non-reducing end (Scheme 38).[51] *O*-Acetyl groups of this OPS, like those in *P. vulgaris* O4 OPS (**82**), were completely removed by solvolysis.

Trifluoromethanesulfonic acid has high solvolytic activity and cleaves many glycosidic bonds that are stable towards other reagents (HF, CF_3CO_2H). For example, polysaccharide of the marine bacterium *Pseudoalteromonas* sp. KMM 634 (**86**) is stable towards anhydrous HF, but depolymerizes during the solvolysis with CF_3SO_3H at 4 °C.[52] In this polysaccharide, glycosidic bonds of the GlcA residue and the 2,3-diamino-2,3-dideoxyglucuronic acid derivative (GlcN3NA) are completely cleaved, while the bond of the 2,3-diamino-2,3-dideoxymannuronic acid derivative (ManN3NA) is stable and the bond of the 2,4-diamino-2,4,6-trideoxyglucose derivative is cleaved in approximately half of the residues (Scheme 39). The resulting mixture contains approximately equal amounts of disaccharide **87** and trisaccharide **88**. A significant difference in the behaviour of monosaccharides of one class (ManN3NA and GlcN3NA derivatives) indicates that the sugar configuration is one of the key factors responsible for stability of glycosidic bonds.

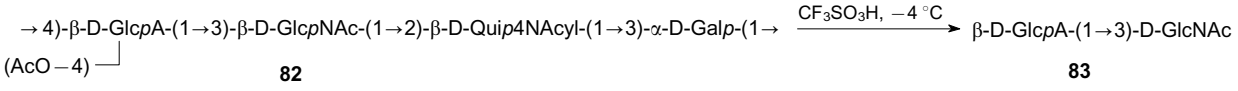
The selective cleavage of *P. vulgaris* O39 OPS (**89**) with CF_3SO_3H demonstrates that the targeted solvolytic cleavage can be achieved by varying the reaction conditions. The solvolysis of **89** at 20 °C for 16 h gave disaccharide derivative **90**, while trisaccharide **91** was produced at −4 °C within 2 h (Scheme 40).[53]

The use of $MeSO_3H$ instead of CF_3SO_3H under the same conditions (20 °C, 16 h) resulted in the formation of a mixture of oligosaccharides **90** and **91** in a ratio of 2 : 1.[53] Hence, $MeSO_3H$ is a milder solvolytic reagent compared to CF_3SO_3H. The glycosidic bond of the pseudaminic acid derivative involved in *P. vulgaris* O39 OPS (**89**) is stable to sulfonic acid like to other solvolytic reagents.

3.3.3. Solvolysis with trifluoroacetic acid

The solvolysis of OPS with CF_3CO_2H affords glycosyl trifluroacetates, as demonstrated by the experiment with methyl α-L-rhamnopyranoside. The latter was transformed into 1-*O*-trifluoroacetyl-α-L-rhamnopyranose in 50% yield upon the treatment with CF_3CO_2H at 60 °C for 30 min.[54] The aqueous treatment of the solvolysis products after the removal of the acid in an air flow gives free oligosaccharides or modified polysaccharides. Glycosidic bonds of furanosides, 6-deoxyhexopyranosides (α-Rha*p*, α-Fuc*p*) and 2-acetamido-2-deoxy-β-hexopyranosides (β-Glc*p*NAc, β-Gal*p*NAc) are the least stable to CF_3CO_2H, whereas the

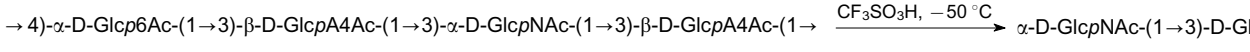
Scheme 37



82 **83**

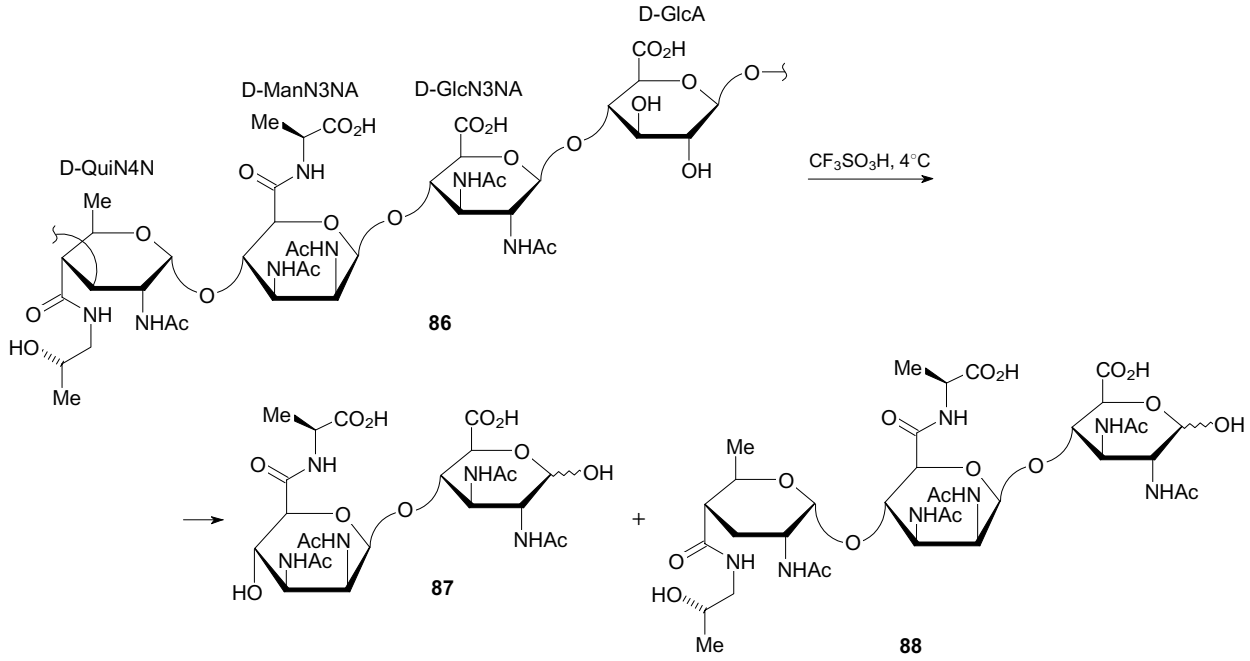
Acyl = MeCH(OH)CH_2C(O)NHCH(Me)C(O)

Scheme 38

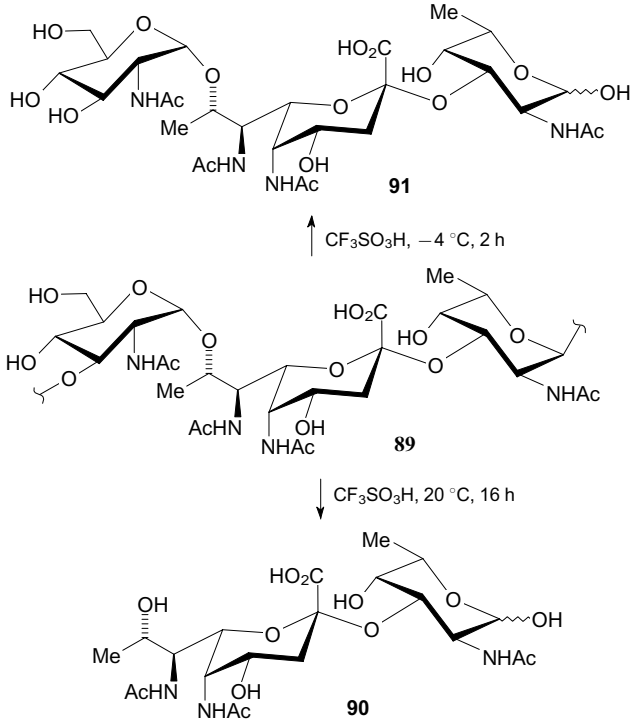


84 **85**

Scheme 39



Scheme 40



bonds of hexopyranoses, 2-acetamido-2-deoxy-α-hexopyranoses and hexuronic acids are stable at temperatures of 50 °C and below.[54, 55]

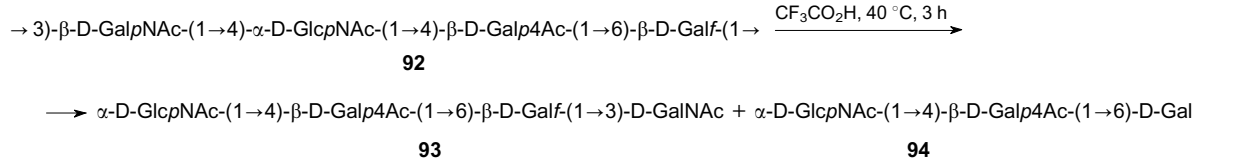
For example, the solvolysis of *E. albertii* O5 OPS (**92**) led to the cleavage of all bonds of β-D-Gal*p*NAc and part of the bonds of β-D-Gal*f* to form oligosaccharides **93** and **94** (Scheme 41).[37] Interestingly, the partial acid hydrolysis of this OPS resulted in the cleavage of all bonds of β-D-Gal*f* and part of the bonds of β-D-Gal*p*NA giving the same trisaccharide **94** (compound **46** from O-deacetylated OPS) but different tetrasaccharide **45** (see Scheme 24 in Section 3.1).

In the solvolysis of *E. cloacae* O3 OPS (G3421) (**95**), α-rhamnopyranoside bonds were more readily cleaved, whereas the β-rhamnopyranoside bond was stable, resulting in the formation of oligosaccharide **96** bearing the rhamnose residue at the reducing end (Scheme 42).[56]

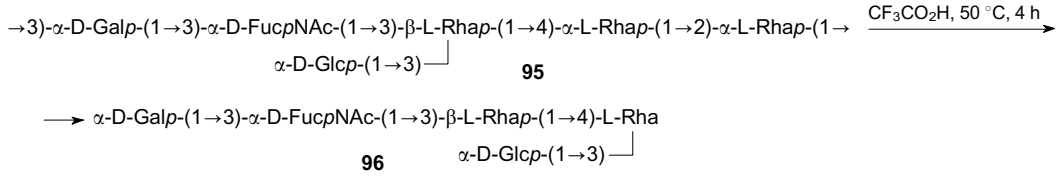
In the solvolysis of *E. coli* O80 OPS (G3422) (**97**) under milder conditions (40 °C, 1 h), the bonds of the side-chain residues α-Fuc*p* and the backbone residues β-Glc*p*NAc and β-Gal*p*NAc were cleaved to form two disaccharides, compounds **98** and **99** (Scheme 43).[57]

The solvolysis of OPS of the untyped strain *E. coli* F17 (**100**) provided insight into the origin of irregularity of this polysaccharide. When treated with CF_3CO_2H, the polysaccharide was selectively cleaved at the glycosidic bonds of α-Rha*p*, while the bonds of other monosaccharides (α- and β-Glc*p*, α-Man*p*, α-Gal*p*, β-Glc*p*NAc) were stable. The solvolysis afforded hexasaccharide **101** and pentasaccharide

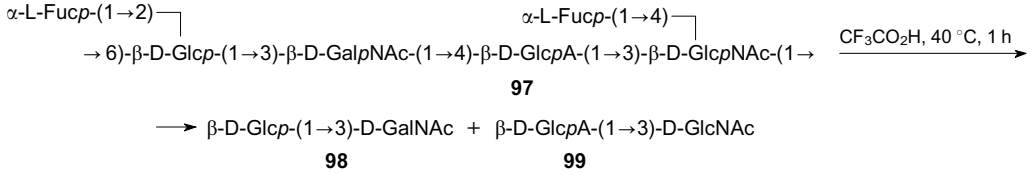
Scheme 41



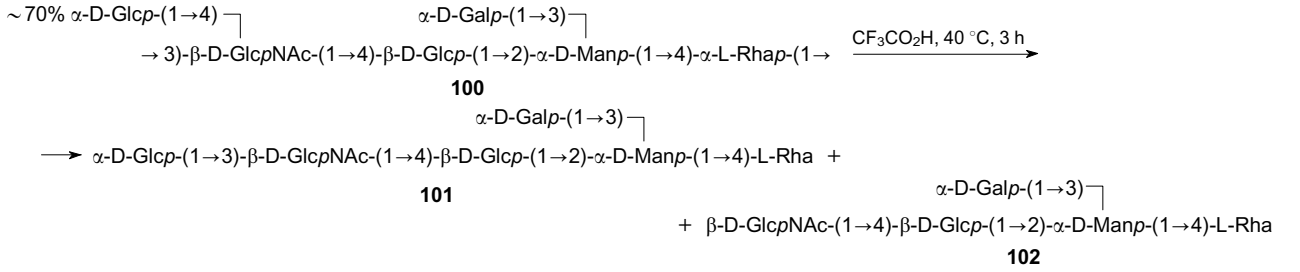
Scheme 42



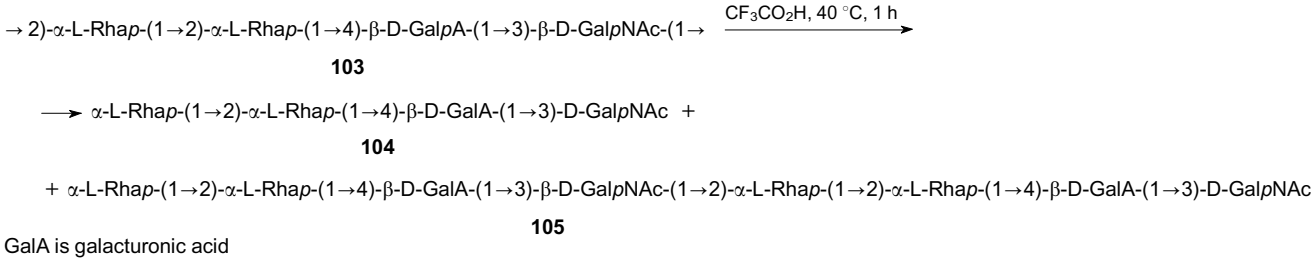
Scheme 43



Scheme 44



Scheme 45

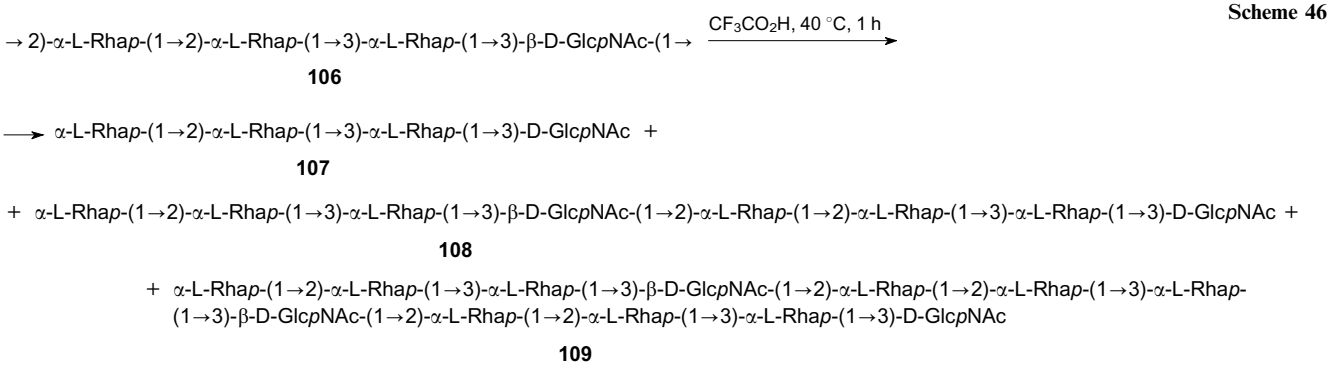


GalA is galacturonic acid

102 in a ratio of ~2 : 1 corresponding to glucosylated and non-glycosylated repeating units of OPS (Scheme 44).[58] These oligosaccharides were separated by gel chromatography and identified by 2D NMR spectroscopic techniques. It was shown that ~70% of the repeating units in the starting OPS contain the side-chain residue α-Glc*p* and, consequently, the irregularity of OPS is due to nonstoichiometric glucosylation.

It was shown that the solvolysis with CF_3CO_2H is applicable to the preparation of oligosaccharides from OPS of causative agents of shigellosis (bacillary dysentery) *Shigella flexneri* types Y, 2a, 3a and 6 as potential components of conjugate anti-shigellosis vaccines.[55] These OPSs contain the α-L-Rha*p* and β-D-Glc*p*NAc or β-D-Gal*p*NAc residues, the bonds of which are potentially labile towards CF_3CO_2H. In OPS of type 6 (**103**), the bond of β-D-Gal*p*-

Scheme 46



NAc proved to be the least stable. This bond was selectively cleaved under mild conditions giving approximately equal amounts of tetrasaccharide **104** and octasaccharide **105**, which correspond to the monomer and dimer of the repeating unit, respectively (Scheme 45).

Under these conditions, OPSs of *S. flexneri* types Y and 2a were predominantly cleaved at the bond of the 3-substituted α-Rha*p* residue to give monomers, dimers and trimers of the repeating units. The bonds of the (1→2)-linked α-Rha*p* residues in these OPSs, like those in OPSs of type 6 (see above), were retained. For example, tetrasaccharide **107**, octasaccharide **108** and dodecasaccharide **109** were obtained from OPS of type Y (compound **106**) (Scheme 46).[55]

The solvolysis of OPS of type 3a was more difficult to perform, evidently because of O-acetylation of the 3-substituted α-L-Rha*p* residue at position 2.[55] This OPS gave a mixture of oligosaccharides higher than the trimer. The use of more drastic conditions of solvolysis led to significant non-specific cleavage of other glycosidic bonds.

4. Conclusion

Currently, the Smith degradation based on the destruction of monosaccharides with vicinal diol groups is still the relevant specific method for the selective cleavage of polysaccharides; the dephosphorylation with hydrofluoric acid (generally 48%) and hydrolytic cleavage of glycosyl phosphate bonds under mild acidic conditions are applied for phosphate-containing polysaccharides. When only side substituents attached by a glycosidic bond or through the phosphate group undergo degradation, the process affords modified polysaccharides, whereas the involvement of backbone components of the polysaccharide leads to depolymerization giving oligosaccharide (or oligosaccharides). The hydrolytic cleavage of polysaccharides containing glycosyl phosphate bonds in the backbone gives oligosaccharide monophosphates.

The Smith degradation is a convenient tool for the analysis of structurally heterogeneous polysaccharides composed of different repeating units, which show different behaviours towards periodate oxidation. The latter can be applied to eliminate irregularity in branched polysaccharides associated with the nonstoichiometric content of a side-chain monosaccharide.

Other specific methods used for the cleavage, such as HNO_2 deamination of hexosamines with free amino groups and alkaline β-elimination in hexuronic acids bearing a substituent at position 4, have lost their importance due mainly to difficulties of performing necessary preceding modifications, such as the N-deacetylation in the former case and the transformation of free acids into amides in the latter one. Meanwhile, the deamination is still employed to cleave carbohydrates containing amino sugars with a free amino group. Anhydrous bases, *e.g.*, lithium in ethylenediamine, can be considered as alternative reagents for β-elimination in hexuronic acids.

Although non-specific methods used for the glycosidic bond cleavage are generally characterized by lower selectivity compared to specific methods, they do not place particular demands on the polysaccharide composition and structure. Their advantage is sensitivity to fine structural features of polysaccharides, including differences in the configuration and substitution patterns of monosaccharide residues. The targeted solvolytic (in particular, hydrolytic) cleavage can be performed by varying the reaction conditions.

The solvolysis with anhydrous reagents does not cause degradation of acid-labile monosaccharides, the glycosidic centre of which is released upon the glycosidic bond cleavage. In this case, the amide bonds are retained, thereby providing access to N-acylated amino sugars and hexuronic acid amides as monomers or components of oligosaccharide fragments. Due to these advantages, the selective solvolysis is widely used in the structural analysis and can also be employed to prepare components of diagnostic agents and vaccines from bacterial polysaccharides.

The details and features of the glycosidic bond cleavage by means of partial acid hydrolysis, acetolysis and solvolysis with hydrofluoric, trilfluoroacetic and sulfonic acids were revealed. The most efficient selective cleavage is achieved for polysaccharides, the glycosidic bonds of which exhibiti significantly higher stability (*e.g.*, bonds of hexuronic acids and hexosamines with a free amino group) or, on the contrary, higher lability (bonds of 3,6-dideoxyhexoses, monosaccharides in the furanose form, N-acylated β-hexosamines) compared to other glycosidic bonds. The bonds of 3-deoxyaldulosonic acids behave differently when treated with different reagents. Thus, they are easily cleaved by mild acid hydrolysis but are stable to solvolysis with anhydrous acids.

The available data allow the selection of an appropriate reagent and the prediction of the cleavage pattern of the polysaccharide in each particular case. The use of one of the two reagents having different solvolytic activity (trifluoromethanesulfonic or trifluoroacetic acid) makes it possible to obtain oligosaccharide fragments of virtually any heteropolysaccharide containing monosaccharides with both highly stable and highly labile glycosidic bonds, respectively.

In conclusion, let us address the product yields of the reactions employed for the selective cleavage of glycosidic bonds. Although these reactions lead to complete depolymerization of the polysaccharides under consideration, the yields of the target products are often low. Apart from losses during the isolation of oligosaccharides (generally by gel permeation chromatography, sometimes followed by high-performance liquid chromatography), which may be up to 50%, low yields are due to intricate reaction patterns.

Thus, the Smith degradation is complicated by transacetalization and incomplete hydrolysis of the glycosidic bond of the oxidized monosaccharide (see Section 2.3). The dephosphorylation with hydrofluoric acid and mild acid hydrolysis of glycosyl phosphate bonds are complicated by the hydrolytic cleavage of acid-labile glycosidic bonds (see Section 2.4). The deamination of 2-amino sugars proceeds *via* two pathways (see Section 2.1); however, the resulting oligosaccharides contain complementary data on the structure of the starting polysaccharide and, consequently, can be considered as the target products. The alkaline β-elimination in hexuronic acid amides gives rise to one or several products depending on whether the amidation is stoichiometric or not (see Section 3.2), but these components are rare in bacterial polysaccharides.

Non-specific method used for the selective cleavage gave target products in high yields when the glycosidic bonds of one type of monosaccharides are completely cleaved under the conditions in which the bonds of other monosaccharide

units are completely stable. For example, the hydrolytic cleavage of glycosidic bonds of nonulosonic acid derivatives is strictly selective (see Section 3.1). The predominant cleavage of glycosidic bonds of particular monosaccharides affords more or less complex mixtures of oligosaccharides and sometimes of monosaccharides. For instance, the partial solvolysis with trifluoroacetic acid generally gives individual oligosaccharides in yields less than 30% and sometimes even less than 10%.[54] The optimal conditions for the cleavage can be selected using mass spectrometry analysis of the resulting mixture. The structures of the isolated oligosaccharides can be determined by modern highly sensitive 2D NMR spectroscopic techniques even when the quantity of oligosaccharides is less than 1 mg.

Therefore, high yields of the target reaction products are not as critical for analytical chemistry as for synthetic chemistry, although they are desirable because they simplify the isolation of the products and allow a decrease in the amount of the compound required for analysis. What is important is that the resulting oligosaccharide (or oligosaccharides) corresponds to the repeating unit of the polymer under study and is not derived from the non-repeating oligosaccharide region, which can be attached to a particular end of bacterial polysaccharide composed of regularly repeating oligosaccharide units. This is clearly evident from a comparison of the NMR spectra of the starting polysaccharide and the isolated oligosaccharide product.

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