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Higher aldulosonic acids: components of bacterial glycans

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Recent data on the natural occurrence, chemistry, and biochemistry of C_8 and C_9 aldulosonic acids (3-deoxy-D-*manno*-oct-2-ulosonic acid, sialic acids, *N*-acyl derivatives of 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids, and some others) as well as on the structures and biological significance of bacterial glycans containing these higher acidic monosaccharides are summarized.

Cell-surface polysaccharides and glycoconjugates play an important role in bacterial life.[1–3] They are implicated in recognition of pathogens by the host immune system and determine the immunospecificity of bacteria. Various immunogenic forms of these biopolymers and their fragments are used as vaccine components. In Gram-negative bacteria, the outer leaflet of the outer membrane is composed mainly of the lipopolysaccharide (LPS), which consists of a polysaccharide chain (O-chain, O-antigen) attached to a lipid moiety (lipid A) *via* a large oligosaccharide called core. Gram-positive bacteria lack the outer membrane and their surface polysaccharides are linked either to the cell-wall peptidoglycan or to a lipid anchored to the inner plasma membrane. Extracellular glycans are found in both Gram-negative and Gram-positive bacteria. Some of them, such as capsular polysaccharides (CPS, K-antigens), are bound to the cell surface (*e.g.*, with the aid of a phosphatidic acid anchor) and form a protective capsule, whereas others are released to the environment. Finally, many bacterial proteins, such as pilin and flagellin, are glycosylated with mono- or oligosaccharides, and S-layer proteins bear polysaccharide chains.

Most bacterial polysaccharides are heteropolymers built up of oligosaccharide repeating units but some are homopolysaccharides. The glycopolymers are highly diverse in sugar composition and consist of both monosaccharides widely distributed in nature and uncommon sugars not found in other carbohydrates. One of the most intriguing constituents of bacterial glycans are higher aldulosonic acids, acidic C_8 and C_9 monosaccharides having carboxyl group at position 1 and keto group at position 2. Many

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of them are 3-deoxy and some are 3,9-dideoxy sugars, and one or several hydroxy groups may be replaced with N-acylated (most often N-acetylated) amino groups.

3-Deoxy-D-*manno*-oct-2-ulosonic acid (ketodeoxyoctonic acid, Kdo) is a common component of the LPS core, where one or several (up to four) residues of Kdo built the region proximal to lipid A. In the core of some bacteria, Kdo is partially replaced with D-*glycero*-D-*talo*-oct-2-ulosonic acid (ketooctonic acid, Ko). In addition, Kdo has been reported as a component of several bacterial O- and K-antigens. Many higher plants and some green algae, but not yeast and animals, also contain Kdo.[4] A well-known aldulosonic acid is 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (neuraminic acid, Neu), whose *N*-acetyl and *N*-glycolyl derivatives (sialic acids) are components of glycoconjugates of animals from the echinoderms upwards to humans and are critical for many physiological processes. In bacterial glycans, Neu is less common and, when present, is N-acetylated and often also O-acetylated. 3,5-Dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (ketodeoxynononic acid, Kdn) called also deaminoneuraminic acid occurs in both animal and bacterial glycans too. In contrast, sugars of another class presenting similarities with sialic acids, different isomers of 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid, were found only in bacteria.

Here, bacterial aldulosonic acids are considered in terms of natural occurrence, chemical properties, and biosynthesis. Bacterial polysaccharide structures containing these higher sugars are exemplified and their biological significance is discussed as well. As all major representatives of the aldulosonic acids have been surveyed during last decades, the emphasis in this review is made to the most recent data.

3-Deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) and related octulosonic acids

Three octulosonic acids occurring in bacterial carbohydrates are shown in Figure 1. From them Kdo is an obligatory component of the LPS, where it was discovered in 1959.[5]

One or several residues of Kdo or a related octulosonic acid constitute a negatively charged domain of the LPS proximal to lipid A, and the gycosidic linkage of the first Kdo residue connects the core and lipid A moieties (Figure 2, **1**–**5**). Most commonly this region is represented by the α-Kdo*p*-(2→4)-α-Kdo*p*-(2→ disaccharide **1** or α-Kdo*p* 4-phosphate.[5] It may be partially neutralized by a positively charged group, such as 2-aminoethyl phosphate or a 4-amino-4-deoxy-L-arabinose residue.

In *Yersinia*, *Acinetobacter*, *Burkholderia*, and some other bacteria, one of the Kdo residues in the inner region may be replaced (usually non-stoichiometrically) with Ko, the 3-hydroxy derivative of Kdo (Figure 2, **2** and **3**). In the core of *Shewanella* species, the only aldulosonic acid is the 8-amino derivative of

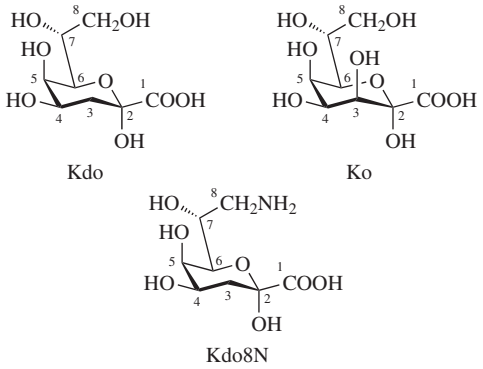


Figure 1 Structures of naturally occurring octulosonic acids: 3-deoxy-D *manno*-oct-2-ulosonic acid (Kdo), D-*glycero*-D-*talo*-oct-2-ulosonic acid (Ko), 8-amino-3,8-dideoxy-D-*manno*-oct-2-ulosonic acid (Kdo8N). Shown is the most favorable α-pyranose form with the equatorial carboxyl group.

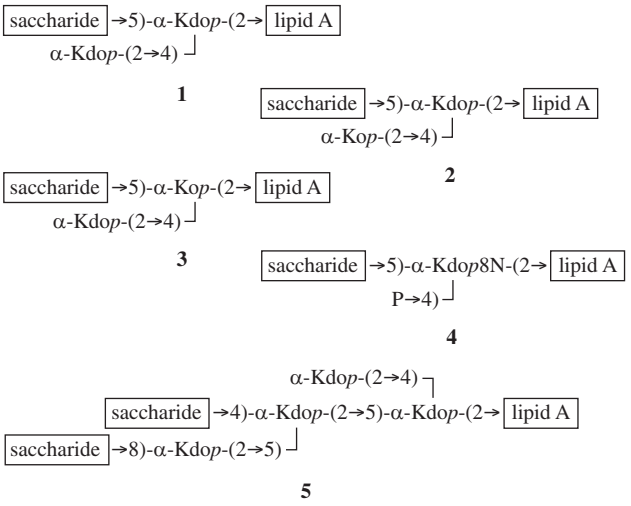


Figure 2 Examples of the inner core region structures of the LPS of *Escherichia coli*[6] (**1**), *Yersinia pestis*[7] (**2**), *Acinetobacter haemolyticus*[8] (**3**), *Shewanella algae*[9] (**4**), and *Acinetobacter baumannii*[10] (**5**).

Kdo (Kdo8N),[9,11] which is 4-phosphorylated (**4**).[9] As opposite to Kdo, neither Ko no Kdo8N have been reported in O-antigens or another surface polysaccharides.

The first glycopolymers other than LPS that were found to contain Kdo were the CPS of intestinal bacteria *Escherichia coli* and the cause of meningococcal diseases *Neisseria meningitidis*.[5] Kdo is a component of several group II K-antigens of *E. coli*, whose structures have been summarized.[12] They are distinguished by a small (di- or trisaccharide) linear repeating units, which, in addition to Kdo or an *O*-acetyl derivative thereof, contain one or two residues of D-ribose (most common variants), two residues of L-rhamnose or one residue of GlcNAc or GalNAc. In most bacterial types, Kdo occurs as a β-pyranoside but an α-pyranoside in the K6 antigen or β-furanoside in the K74 and K95 antigens have been reported. The K24 antigen is a linear polymer consisting of partially O-acetylated α-Kdo*p* and glycerol phosphate.[13] The K-antigens of *N. meningitidis* in general are reminiscent of the *E. coli* group II CPS but only one from them, that of serogroup 29-e, contains Kdo in the →3)-α-D-Gal*p*NAc-(1→7)-β-Kdo*p*-(2→ repeating unit.[14] In group II[12] and possibly also group III[15] strains, Kdo may provide a linker between the reducing terminus of the CPS and the diacylglycerophosphate anchor.

Later, Kdo was found also in CPS of soil nitrogen-fixing bacteria of the rhizobia group, whose structures and involvement in establishing the rhizobium–legume symbiosis, particularly, their adhesive properties with respect to host plant cells, have been surveyed recently.[16] As *E. coli* group II K-antigens,[12] most of them are linear and have disaccharide repeating units containing Kdo and another monosaccharide (Man, Gal, 2-*O*-methylhexose, GlcNAc). Exceptionally, the CPS of *Sinorhizobium meliloti* 1021 produces a low-molecular mass (4–4.5 kDa) homopolymer of β-(2→7)-linked Kdo.[17]

More structures of extracellular polysaccharides (**6**–**10**) isolated from various Gram-negative bacteria are shown in Figure 3. From them, the CPS of a swine pathogen *Actinobacillus pleuropneumoniae*[18,19] (**6**) and *Moraxella nonliquefaciens* occurring in the human upper respiratory tract[20] (**7**) have disaccharide repeats and thus resemble the glycopolymers discussed above. The others (**8**–**10**) are built up of linear or branched tetrasaccharide or pentasaccharide repeating units.

In O-antigens, Kdo is less common and by now found only in three genera, including enteric bacteria *Providencia* and *Cronobacter* and marine bacteria *Pseudoalteromonas*. Structures of these polysaccharides are shown in Figure 3. From them, the O-antigens of *Providencia alcalifaciens*[26] (**11**) and *Pseudoalteromonas*

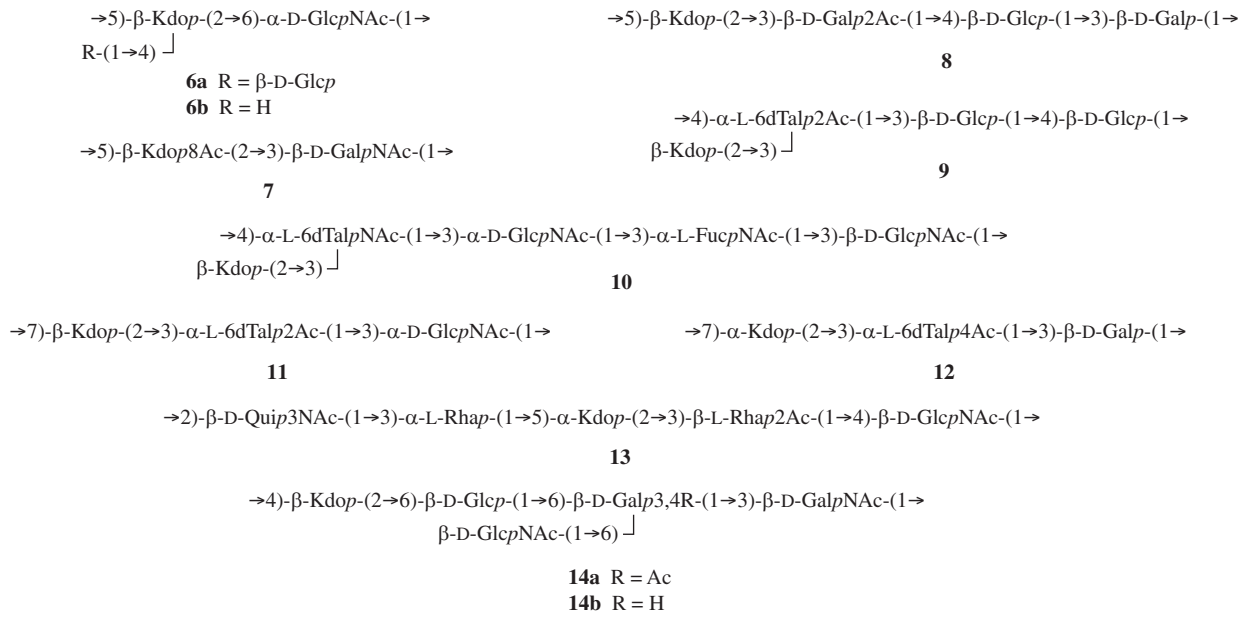


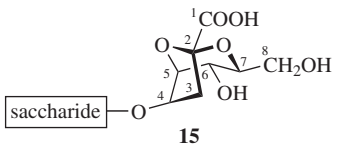
Figure 3 Structures of Kdo-containing extracellular (capsular) polysaccharides of *Actinobacillus pleuropneumoniae* serotypes 5b[18] (**6a**) and 5a[19] (**6b**), *Moraxella nonliquefaciens*[20] (**7**), *Burkholderia pseudomallei* 304b[21,22] and *Burkholderia cepacia* BTS13[23] (**8**), *Burkholderia caribensis* MWAP71[24] (**9**), *Pseudoalteromonas nigrifaciens* IAM 13010[25] (**10**) and O-antigens of *Providencia alcalifaciens* O36[26] (**11**), *Pseudoalteromonas flavipulchra* NCIMB 2033[27] (**12**), *Cronobacter sakazakii* G2706[28] (**13**) and G2704[28] (**14a**), and *Cronobacter malonaticus* 3267[29] (**14b**). 6dTal, 6-deoxytalose; Qui3NAc, 3-acetamido-3,6-dideoxyglucose.

flavipulchra[27] (**12**) have trisaccharide repeats of remarkably similar structures. The O-antigens of *Cronobacter* are built up of linear or branched pentasaccharide repeating units[28,29] (**13** and **14**). In some bacteria, a Kdo residue, being no component of the repeating unit, occurs at the reducing end of the O-antigen linking it to the LPS core.[30]

The Kdo linkage is highly labile towards acids and, as a result, the LPS molecule can be cleaved to the carbohydrate and lipid parts under mild acidic conditions. This facilitates significantly structural analysis of the LPS and enables biological evaluation of each LPS component. When a Kdo residue occurs between the O-antigen and the core, these parts of the LPS are separated by mild acid hydrolysis too. The glycosidic linkage of Ko is more stable and is not cleaved so easily.

When Kdo is present in the O-antigen repeating unit, delipidation of the LPS is accompanied by depolymerisation of the polysaccharide chain. Although upon the cleavage information on the position and configuration of the Kdo linkage is lost, partial acid hydrolysis is used in structural studies of Kdo-containing glycopolymers to obtain a single oligosaccharide when Kdo occurs in the main chain or a modified Kdo-lacking polysaccharide when Kdo is attached as a monosaccharide side chain, *e.g.*, as in extracellular polysaccharides **9** and **10** shown in Figure 3.

In oligosaccharides isolated upon the cleavage of the Kdo linkages, the Kdo residue at the reducing may exist in different forms depending on the position of its substitution in the polysaccharide. In case of the 4-substitution, as in the *Cronobacter sakazakii* G2706 O-antigen **14a**, the Kdo residue is converted into the bicyclic 2,7-anhydro furanose derivative **15**.[28,29] Substitution at position 5 or 7 precludes formation of this derivative, and Kdo is released mainly as the most favorable α-pyranose with the equatorial carboxyl group[28] (Figure 1) independently of the initial configuration of the Kdo linkage.



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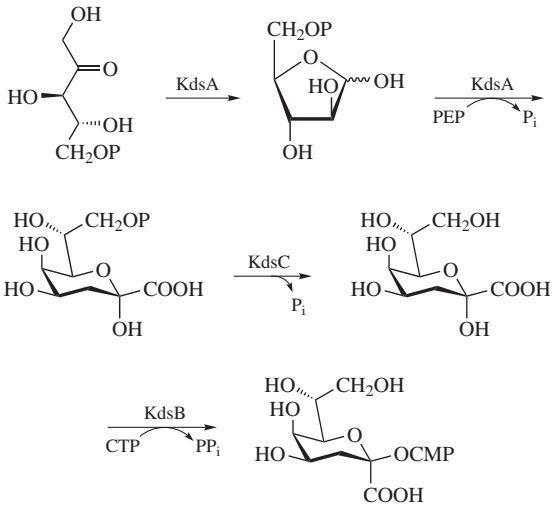
Biosynthetic pathway of Kdo that is similar in bacteria and plants is well studied[31] (Scheme 1). The rather short pathway starts from D-ribulose 5-phosphate, which is converted into D-arabinose 5-phosphate with the aid of D-arabinose-5-phosphate isomerase. Condensation of D-arabinose 5-phosphate with phosphoenolpyruvate is catalyzed by Kdo 8-phosphate synthase and affords Kdo 8-phosphate. Then, Kdo 8-phosphate phosphatase hydrolyzes Kdo 8-phosphate to Kdo and inorganic phosphate. Finally, the formation of the activated sugar nucleotide derivative Kdo cytidine monophosphate (CMP) from Kdo and cytidine triphosphate is catalyzed by CMP-Kdo synthetase. CMP-Kdo is the substrate of monofunctional or bifunctional glycosyltransferases, which sequentially add one or two Kdo residues to lipid A to initiate the core biosynthesis. In *Helicobacter pylori*, one of the two Kdo residues is then removed by specific Kdo hydrolase.[32] Ko is synthesized from Kdo by a unique Fe^{2+}/α-ketoglutarate/O_2-dependent



Scheme 1 Biosynthetic pathway of the nucleotide-activated derivative of Kdo.[31] CMP is cytidine monophosphate, CTP is cytidine triphosphate, PEP is phosphoenolpyruvate, P is phosphate, P_i is inorganic phosphate, PP_i is inorganic diphosphate.

Kdo 3-hydroxylase, probably after attachment of two Kdo residues to lipid A.[33]

3-Deoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (Kdn)

The natural occurrence of Kdn (Figure 4) was revealed for the first time in a polysialoglycoprotein of rainbow trout eggs in 1986.[34]

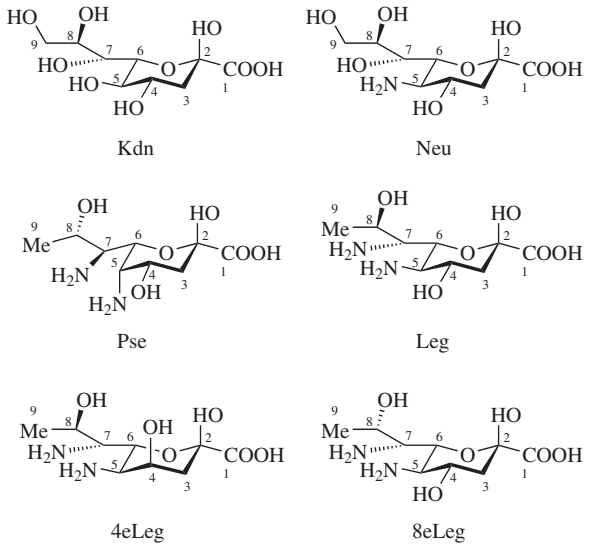


Figure 4 Structures of nonulosonic acids: 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (Kdn), 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (Neu), 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids with the L-*glycero*-L-*manno* (Pse), D-*glycero*-D-*galacto* (Leg), D-*glycero*-D-*talo* (4eLeg), and L-*glycero*-D-*galacto* (8eLeg) configurations. Shown is the most favorable pyranose form with the equatorial carboxyl group.

In bacteria, Kdn has been reported first as a component of the CPS of *Klebsiella ozaenae* K4,[35] a widespread pathogen that causes respiratory diseases in humans. The CPS has a branched pentasaccharide repeating unit **16** with a lateral Kdn residue (Figure 5). It could be released as a free sugar by mild acid hydrolysis of the CPS as easy as Kdo without depolymerisation of the main polysaccharide chain. For identification of Kdn methyl ester methyl glycoside released from the CPS by acidic methanolysis, the authentic sample was synthesized by deamination with HNO_2 of the corresponding derivative of neuraminic acid.[35]

5-*O*-Methyl derivative of Kdn has been identified in the CPS **17** of another Gram-negative bacterium *Sinorhizobium fredii* SVQ293, a thiamine autotrophic mutant of *S. fredii* HH103.[36] Remarkably, the CPS of the parental strain contains no Kdn and is a homopolymer of pseudaminic acid (see below).[38] The rhizobial CPS are believed to play an important role in the symbiotic bacterium-plant interaction and, particularly, in defining the nodulation host range.

Kdn-containing polysaccharides and an oligosaccharide called teichulosonic acids (**18**–**22**) have been found in cell walls of Gram-positive bacteria of the order Actinomycetales, first in phytopathogenic streptomycetes (Figure 5). They all have the main chain of (2→4)-linked Kdn residues (>20 in case of polysaccharides), which are substituted at position 8 or 9 or both with lateral residues of a hexose (Glc, Gal, 3-*O*-methylgalactose) or GlcNAc.[37] The presence of Kdn-containing compounds on the bacterial cell surface plays a key role in pathogenesis favoring the pathogen cell adhesion to the host plant in the initial stages of infection.

In animals, Kdn is synthesized by condensation of D-mannose 6-phosphate (from D-mannose) and phosphoenolpyruvate followed by dephosphorylation and then converted into the activated sugar nucleotide donor CMP-Kdn.[34] In bacteria, the Kdn synthesis pathway has not been elucidated and may differ from that in animals as the biosynthetic pathway of CMP-Neu5Ac does[39] (see next section).

5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (Neu5Ac)

Acyl derivatives of neuraminic acid called sialic acids have been known since early 1940s when Klenk isolated a Neu derivative by methanolysis of a submundibular-gland glycoprotein.[40] Sialic acid-containing bacterial carbohydrates were reported since late 1950s, and their roles in negative charge repulsion and inhibiting alternate complement pathway activation have been recognized.

One of the sialic acid-containing bacterial glycopolymers discovered first was the K1 antigen of *E. coli*, an α-(2→8)-linked homopolymer of Neu5Ac called colominic acid, which in some strains is O-acetylated at position 7 or 9.[41,42] Colominic acid also is produced by *Neisseria meningitidis* serotype B, *Moraxella nonliquefaciens*, and *Mannheimia* (*Pasteurella*) *haemolytica* A2[41] but it is devoid of O-acetylation, a reason for the lack of cross-reactivity between group B meningococci antiserum and *E. coli* K1 antigen.[43] The CPS of *N. meningitidis* serotype C is a α-(2→9)-linked isomer of colominic acid with *O*-acetyl groups at position 7 or/and 8, whereas *E. coli* K92 antigen is a molecular hybrid between the two antigens with the alternating α-(2→8)- and α-(2→9)-linkages.[41,42] The hybrid and α-(2→9)-linked homopolymers are good immunogens, and the latter is used as a component of effective meningococcal vaccines. In contrast, the α-(2→8)-linked polysialic acid is poorly immunogenic in humans,[42] probably owing to a similarity to the polysialylated form of human neural cell adhesion glycoprotein.

The CPS of *N. meningitidis* serotypes W-135 and Y have the →4)-α-Neu*p*5Ac-(2→6)-β-D-Gal*p*-(1→ or →4)-α-Neup5Ac-

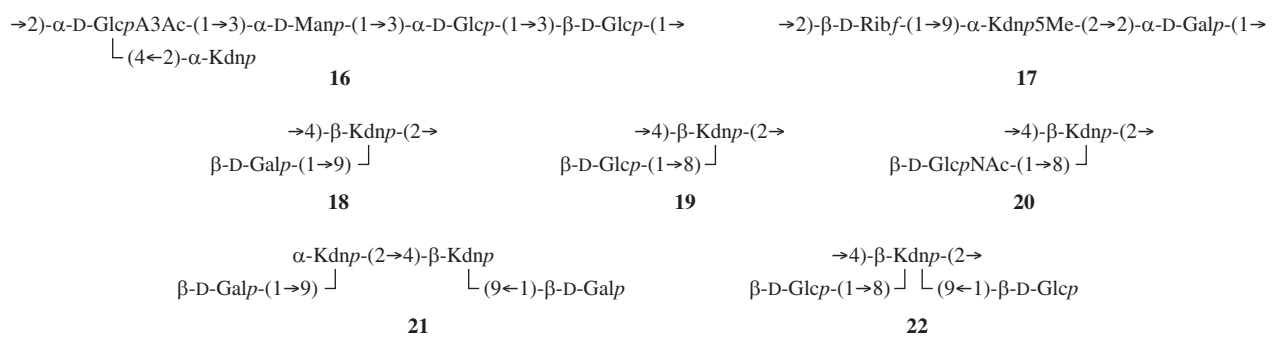


Figure 5 Structures of Kdn-containing CPS of *Klebsiella ozaenae* K4[35] (**16**), *Sinorhizobium fredii* SVQ293[36] (**17**) and cell-wall teichulosonic acids of actinomycetes[37] *Streptomyces scabei* (**18**), *Streptomyces setonii-griseus*, *Streptomyces* sp. VKM Ac-2090, *Streptomyces macrosporus* and *Arthrobacter protophormiae* VKM Ac-2104 (**19**), *Arthrobacter* spp. VKM Ac-2549 and VKM Ac-2550 (**20**), *Streptomyces* sp. VKM Ac-2274 (**21**), and *Brevibacterium casei* (**22**). In the teichulosonic acids **18** and **21**, parts of Gal residues are 3-O-methylated; in the oligosaccharide **21**, residues of Kdn and Gal may be O-acetylated with up to two *O*-acetyl groups per molecule.[37] GlcA, glucuronic acid.

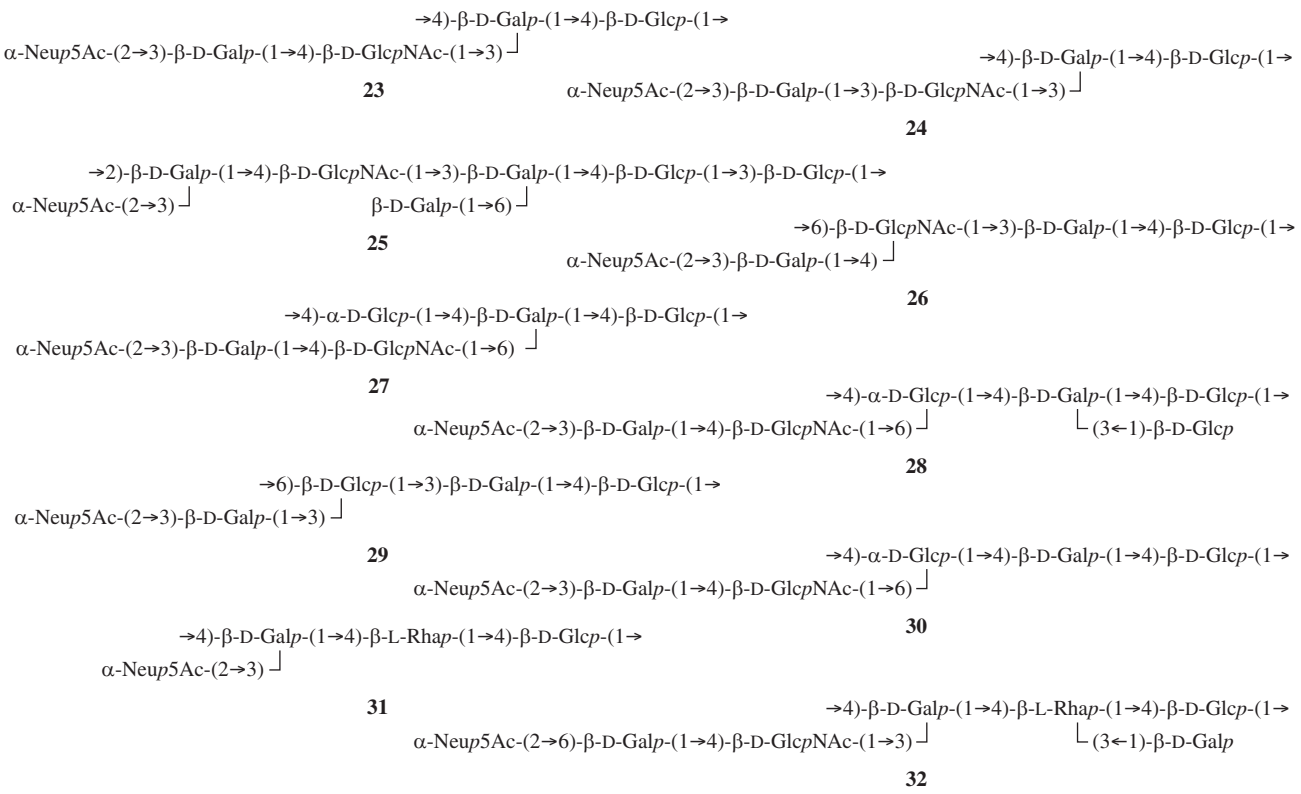


Figure 6 Structures of Neu5Ac-containing CPS of *Streptococcus agalactiae* (group B *Streptococcus*) serotypes Ia (**23**), Ib (**24**), and II–VIII[45] (**25**–**31**, respectively) and *Streptococcus suis*[47] (**32**). O-Acetylation of Neu5Ac in *S. agalactiae* is not shown.

(2→6)-β-D-Glc*p*-(1→ disaccharide repeating units, respectively, the latter being mono-O-acetylated.[41,44] The K9 antigen of *E. coli* is a Neu5Ac-containing linear heteropolymer with a tetrasaccharide repeating unit.[12]

Neu5Ac is the terminal sugar in the side chains of the CPS of all nine serotypes of *Streptococcus agalactiae* (group B *Streptococcus*), a common cause of bacterial sepsis and meningitis among newborn infants. Eight serotypes possess a conserved basic motif of a variable Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)/(1→3)-β-D-GlcNAcp/β-D-Glc*p* trisaccharide (Figure 6, **23**–**30**); in a more distantly related serotype VIII, a trisaccharide is interrupted by a β-L-Rha*p* residue (**31**).[45] The striking conservation of Neu*p*5Ac-(2→3)-β-D-Gal*p* suggests that this structural element is central to the antiphagocytic function of the CPS. In all *S. agalactiae* serotypes, Neu5Ac is O-acetylated at various levels,[46] an *O*-acetyl group first appearing at position 7 and then migrating spontaneously to position 9 at pH above 7.0 or below 3.0. O-Acetylation of Neu5Ac may protect these and other microorganisms in the environments where other bacteria express sialidases, and could be an important factor in their potential virulence.

The CPS of *Streptococcus suis* has the same backbone sequence as *S. agalactiae* serotype VIII and a Neu5Ac→Gal→GlcNAc→Gal sequence in common with five other serotypes but differs in the linkage between Neu5Ac and Gal (2→6 rather than 2→3) (Figure 6, **32**).[47]

In LPS, Neu5Ac may occur as a component of both O-antigen and core. The first sialic acid-containing O-antigen structure established was that of *Hafnia alvei* 2.[48] It has an unusually large branched octasaccharide repeating unit including one Neu5Ac residue in the main chain (Figure 7, **33**). The O-antigen **34** of another *H. alvei* strain reclassified later to *Escherichia albertii* has a side-chain Neu5Ac residue.[49] A group of structurally similar

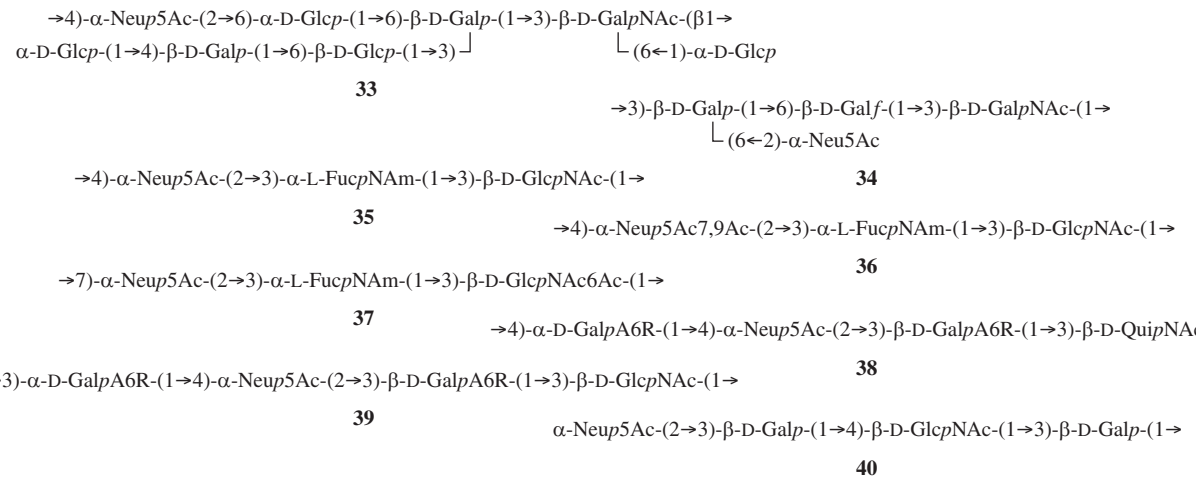


Figure 7 Structures of Neu5Ac-containing O-antigens of *Hafnia alvei* 2[48] (**33**), *Escherichia albertii* (former *Hafnia alvei*)[49] (**34**), *E. coli* O145[50] (**35**), *Salmonella* Toucra O48[50,51] (**36**), *Citrobacter braakii* O37[52] (**37**), *Vibrio cholerae* H11[53] (**38**), *Shewanella algae* 48055[54] (**39**), and *Haemophilus influenzae*[55] (**40**). R indicates N-linked 2-aminopropane-1,2-diol, FucNAm is 2-acetimidoylamino-2,6-dideoxygalactose, QuiNAc is 2-acetamido-2,6-dideoxyglucose.

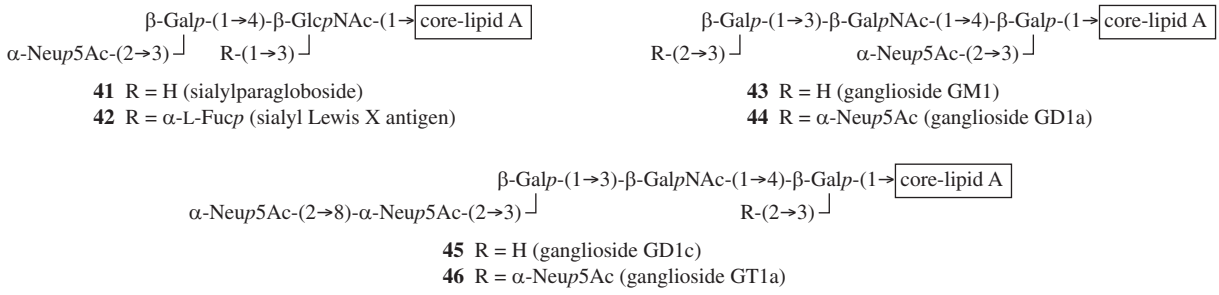


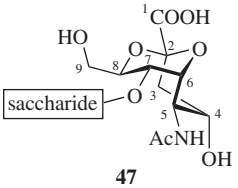
Figure 8 Examples of the sialylated outer core region structures of the LPS of *Neisseria meningitidis*[57] (**41**), *Helicobacter pylori*[58] (**42**), and *Campylobacter jejuni*[56] (**43**–**46**), which mimic human glycoconjugate structures indicated in parentheses.

Neu5Ac-containing O-antigens **35**–**37** have been found in closely related enterobacteria *Salmonella enterica*, *E. coli*, and *Citrobacter*.[50–52] Their trisaccharide repeating units have the same sugar composition and differ only in the position of substitution of Neu5Ac (4 or 7) and the O-acetylation pattern (Figure 7). More Neu5Ac-containing *E. coli* O-antigen structures are available from the ECODAB database at http://www.casper.organ.su.se/ECODAB/. Similar structures **38** and **39** have been reported for taxonomically remote bacteria *Vibrio cholerae*[53] and *Shewanella algae*,[54] which both are distinguished by the presence of another uncommon polysaccharide component, amide of D-galacturonic acid with 2-aminopropane-1,2-diol.

Haemophilus influenzae is perceived to lack any O-antigen, but when grown on a solid medium enriched in sialic acid, some *H. influenzae* strains synthesize an LPS, in which tetrasaccharide **40** is attached *en bloc* to the core and may be considered as a single O-antigen unit.[55] This glycofrom is coexpressed with another structure, in which the terminal Neu5Ac residue is replaced with a 2-aminoethyl phosphate-bearing GalNAc residue.

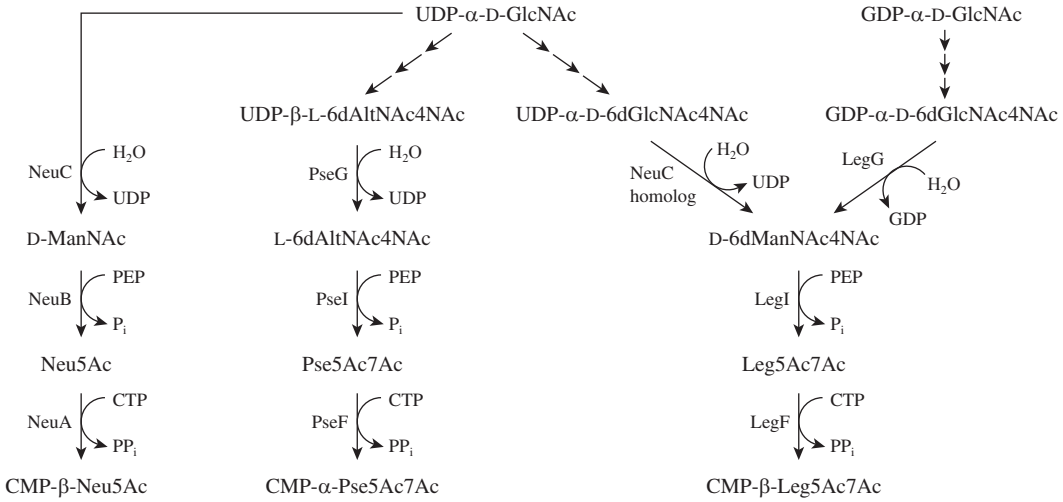
In LPS of many human and animal pathogens, such as *N. meningitidis*, *Neisseria gonorrhoeae*, *H. influenzae*, *Haemophilus ducreyi*, *Histophilus somni*, *Campylobacter jejuni*, and *Helicobacter pylori*, the core bears one or more terminal Neu5Ac residues, a paradigm of molecular mimicry of host glycoconjugate structures by LPS (examples **41**–**46** are shown in Figure 8). This trait plays a role in the pathogenesis helping the bacteria to evade host immune defenses and facilitating adherence to and entry into mucosal epithelial cells utilizing human cell receptors. In some cases, it is responsible for autoimmune diseases. Particularly, mimicry of human gangliosides GM1 and GD1a by the LPS of *C. jejuni* induces production of anti-GM1 and anti-GD1a IgG autoantibodies, which disrupt lipid rafts, paranodal or nodal structures, and ion channel clusters in peripheral motor nerves and cause acute motor axonal neuropathy, a subtype of Guillain-Barré syndrome.[56] Infection with *C. jejuni* strains that express GT1a-like or GD1c-like structures on the LPS may induce anti-GQ1b IgG autoantibodies associated with Fisher syndrome characterized by a weakness of the eye muscles. A determinant of the clinical syndromes manifested after infection by the two groups of the bacteria is gene polymorphism responsible for synthesis of various sialylated LPS forms.[56]

The glycosidic linkage of Neu5Ac is labile against both mild acid hydrolysis[50] and solvolysis with anhydrous hydrogen fluoride.[59] Cleavage with the latter reagent converts 7-substituted Neu5Ac into the 2,8-anhydro pyranose derivative **47**.[59]



47

In bacteria, biosyntheis of Neu5Ac starts from GlcNAc uridine diphosphate (UDP-GlcNAc), which is converted to ManNAc by the action of hydrolyzing UDP-GlcNAc 2-epimerase[39] (Scheme 2). Condensation of ManNAc with phosphoenolpyruvate is followed by activation as CMP-Neu5Ac, both steps being catalyzed by the corresponding synthases. This pathway is different from the Neu5Ac biosynthetic pathway in animals, which includes two additional steps: 6-phosphorylation of ManNAc and dephos-



Scheme 2 Biosynthetic pathways of the CMP-activated derivatives of NeuAc,[39] Pse5Ac7Ac,[61,62] and Leg5Ac7Ac.[63,64] The enzymes involved are indicated to the left of arrows. 6dAltNAc4NAc, 6dGlcNAc4NAc, 6dManNAc4NAc indicate 2,4-diacetamido-2,4,6-trideoxyaltrose, -glucose, -mannose; CMP is cytidine monophosphate; CTP is cytidine triphosphate; GDP is guanidine diphosphate; UDP is uridine diphosphate; PEP is phosphoenolpyruvate; P_i is inorganic phosphate, PP_i is inorganic diphosphate.

→4)-α-8eLeg5Ac7Ac-(2→4)-α-D-Gal*p*-(1→3)-α-L-Fuc*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→

48

→4)-α-8eLeg*p*5Ac7Ac8Ac-(2→4)-β-D-Glc*p*-(1→3)-α-D-Glc*p*NAc-(1→

49

→8)-α-8eLeg*p*5Ac7Ac-(2→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*A-(1→3)-α-D-Glc*p*NAc-(1→

50

→8)-α-Leg*p*5Ac7DAla-(2→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→

51

→4)-α-4eLeg*p*5Ac7Ac-(2→4)-β-D-Glc*p*A3Ac-(1→3)-β-D-Gal*p*NAc-(1→

52

→3)-α-D-Gal*p*-(1→6)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*A-(1→3)-β-D-Qui*p*NAc-(1→
└(6←2)-β-Pse*p*5Ac7Ac

→4)-β-Pse*p*5Ac7Ac-(2→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→

53

54

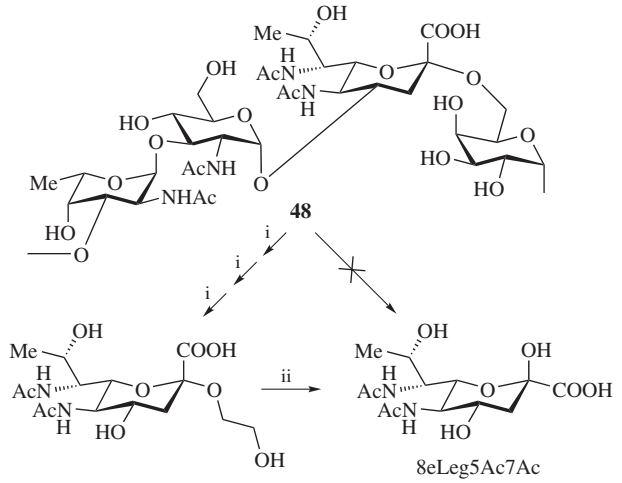
Figure 9 Structures of 8eLeg-containing O-antigens of *E. coli* O108[68,71] (**48**), *E. coli* O61[69] (**49**), *Providencia stuartii* O20[70] (**50**); Leg-containing O-antigen of *E. coli* O161[72] (**51**); 4eLeg-containing O-antigen of *Shewanella japonica* KMM 3601[73] (**52**); and Pse-containing O-antigens of *Pseudoalteromonas atlantica* IAM 14165[76] (**53**), and *Cellulophaga fucicola*[77] (**54**). GlcA is glucuronic acid, GalA is glalacturonic acid, Leg5Ac7DAla is 5-*N*-acetyl-7-*N*-(D-alanyl)legionaminic acid.

phorylation of the condensation product Neu5Ac 9-phosphate. The O-acetylation of Neu5Ac is carried out by specific *O*-acetyltransferases and, in *S. agalactiae*, is modulated by an *O*-acetylesterase prior to polymerization to the CPS.[60]

Derivatives of 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids

First representatives of this class were discovered in the LPS of *Pseudomonas aeruginosa* and *Shigella boydii* in the authors' laboratory in 1984.[65] By now, four isomers of 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids with the L-*glycero*-L-*manno* (Pse), D-*glycero*-D-*galacto* (Leg), D-*glycero*-D-*talo* (4eLeg), and L-*glycero*-D-*galacto* (8eLeg) configurations have been identified (Figure 4). In 2003, a comprehensive review was published summarizing data on these monosaccharides known by that time.[66] Since then, more bacterial polysaccharides and glycoproteins have been reported to contain them and biosynthetic pathways of Pse and Leg have been elucidated as shown below.

Whereas derivatives of Pse, Leg, and 4eLeg had been isolated before their final stereochemical assignment in 2001,[66,67] a derivative of 8eLeg was obtained in the free state and unambiguously identified only recently.[68] For this purpose, the O-antigen of *E. coli* O108 **48** was subjected to three sequential Smith degradations followed by mild acid hydrolysis (Scheme 3), and the isolated 8eLeg5Ac7Ac was compared with the synthetic counterpart.[66]



Scheme 3 Isolation of 8eLeg5Ac7Ac in the free state from the O-antigen **48** of *E. coli* O108.[68] *Reagents and conditions*: i, Smith degradation including periodate oxidation, borohydride reduction and mild acid hydrolysis with 0.1 M NaOAc–HOAc buffer at pH 4.3 and 100 °C; ii, mild acid hydrolysis with 2% HOAc at 100 °C.

The O-antigens of *E. coli* O61[69] (**49**) and *Providencia stuartii* O20[70] (**50**) contain the same di-*N*-acetyl derivative of 8eLeg as the O-antigen **48** of *E. coli* O108,[68,71] whereas the 5-*N*-acetyl-7-*N*-(D-alanyl) derivative of Leg is a component of the O-antigen **51** of *E. coli* O161[72] (Figure 9). The O-antigen **52** of a marine bacterium *Shewanella japonica* was the first heteropolysaccharide reported to contain a 4eLeg derivative.[73] Earlier, 4eLeg had been known only as the monomer of α-(2→4)-linked homopolysaccharides of *Legionella pneumophila* non-1 serogroups,[74] a feature dividing them from serogroup 1 characterized by a similar homopolysaccharide of Leg.[67,75]

Pse5Ac7Ac has been reported in the O-antigens of two other marine bacteria, *Pseudoalteromonas atlantica* (**53**)[76] and *Cellulophaga fucicola* (**54**)[77] (Figure 9). In the former polysaccharide, the Pse derivative is linked as a side chain and could be easily released in the free state by mild acid hydrolysis.[76] When in the main chain of heteropolysaccharides, most likely, Pse and the other isomers occupy the non-reducing end of the polymer.

Pse-containing teichulosonic acids occur in cell walls of some actinomycetes[37] (Figure 10). They are distinguished by 7-N-acylation of Pse with a 4-hydroxybutanoyl group in *Kribella* spp. (**55**–**57**) or a 3,4-dihydroxybutanoyl group in a sporangial actinomycete *Actinoplanes utahensis* (**58**), in all bacteria the *N*-acyl groups being 4-O-glycosylated with a neighbouring monosaccharide residue. A lateral monosaccharide substituent (D-Gal, 3-*O*-methyl or 2,3-di-*O*-methyl derivative of D-Gal, L-Rha) may

→OCH$_2$CH$_2$CH$_2$CO–7)-β-Pse5NAc-(2→
R-(1→4)┘

55 R = H, α-D-Gal*p*3Me or α-D-Gal*p*2,3Me

A →OCH$_2$CH$_2$CH$_2$CO–7)-β-Pse5NAc-(2→

B →3)-β-D-Gal*p*-(1→OCH$_2$CH$_2$CH$_2$CO–7)-β-Pse5NAc-(2→
R-(1→4)┘

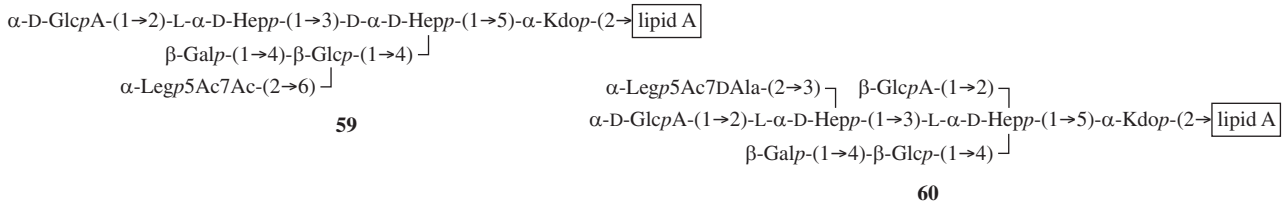
56 R = H, α-D-Gal*p*3Me or α-D-Gal*p*2,3Me
57 R = α-D-Gal*p*3Me, α-D-Gal*p*2,3Me or β-L-Rha*p*

A →OCH$_2$CH(OH)CH$_2$CO–7)-β-Pse5NAc-(2→

B →3)-β-D-Glc*p*-(1→OCH$_2$CH(OH)CH$_2$CO–7)-β-Pse5NAc-(2→
R-(1→4)┘

58 R = α-D-Gal*p* (major) or H (minor)

Figure 10 Structures of Pse-containing cell-wall teichulosonic acids of actinomycetes[37] *Kribbella* sp. VKM Ac-2541 (**55**), *Kribbella* spp. VKM Ac-2500, Ac-2568, Ac-2572, Ac-2575 (**56**), *Kribbella* sp. VKM Ac-2527 (**57**), and *Actinoplanes utahensis* VKM Ac-674 (**58**). Gal3Me and Gal2,3Me indicate 3-*O*-methylgalactose and 2,3-di-*O*-methylgalactose, respectively.



59 **60**

Figure 11 Structures of Leg-containing dephosphorylated core oligosaccharides of the LPS of *Vibrio parahaemolyticus* O2[79,80] (**59**) and KX-V212[78,79] (**60**). Leg5Ac7DAla is 5-*N*-acetyl-7-*N*-(D-alanyl)legionaminic acid.

be linked to O4 of Pse. In most strains studied, the teichulosonic acids are irregular and consist of repeating units of two types with the main chain composed of either a Pse derivative only (type **A**) or Pse and another monosaccharide (type **B**).

The LPS core oligosaccharides of two strains of *Vibrio parahaemolyticus*, a causative agent of sea-food-bone poisoning, include derivatives of Leg at different positions (Figure 11, **59** and **60**). These bacteria are devoid of any O-antigen, and their serological relatedness is due to similarities in the LPS core, including the presence of terminal Leg derivatives.[78,79] Structures of bacterial oligo- and polysaccharides containing 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids studied earlier have been summarized in the previous review.[66]

Campylobacter jejuni, *Campylobacter coli*, and *Helicobacter pylori* are flagellated, motile, Gram-negative bacteria that colonize the gastrointestinal tract of people worldwide, and *H. pylori* is often responsible for gastric and duodenal ulcers. Their flagellins are heavily O-glycosylated with nonulosonic acids, which is important for the development of a functional flagella filament necessary for invasiveness of these microorganisms. Targeted metabolomics analysis of the *Campylobacter* species revealed various CMP-activated *N*-acetyl, *N*-acetimidoyl (Am), and *N*-(*E*/*Z*-*N*-methylacetimidoyl) (AmMe) derivatives of Pse and Leg (Pse5Ac7Ac, Pse5Ac7Am, Leg5Ac7Ac, Leg5Am7Ac, Leg5AmMe7Ac),[81,82] as well as such unique derivative as 7-*N*-acetyl(or acetimidoyl)-5-*N*-(2,3-di-*O*-methylglyceroyl)pseudaminic acid.[82] In *Clostridium botulinum*, a Gram-positive spore forming anaerobe that cause a symmetrical paralysis called botulism, flagellin is glycosylated with an unusual Leg derivative, 7-*N*-acetyl-5-*N*-(*N*-methyl-5-glutamyl)legionaminic acid.[83]

The CMP-Pse5Ac7Ac biosynthetic pathway has been identified in *C. jejuni*[61] and *H. pylori*[62] (Scheme 2). It includes six enzymes, from which three first convert UDP-D-GlcNAc into UDP-2,4-diacetamido-2,4,6-trideoxy-L-altrose. Then, UDP-sugar hydrolase (nucleotidase) removes the UDP group, and Pse5Ac7Ac synthase catalyzes the condensation of the product with phosphoenolpyruvate giving rise to Pse5Ac7Ac, which is converted into CMP-Pse5Ac7Ac by a cytidylyltransferase (CMP-Pse5Ac7Ac synthetase).

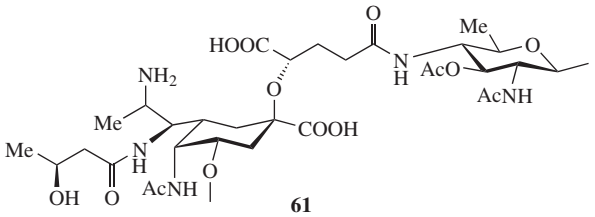
The biosynthetic pathway of Leg5Ac7Ac has been studied in the Legionnaire's disease bacterium *Legionella pneumophila* and found to be similar to the Pse5Ac7Ac pathway with a few exceptions[63] (Scheme 2). Particularly, the hydrolyzing enzyme that removes UDP from UDP-2,4-diacetamido-2,4,6-trideoxy-D-glucose possesses both nucleotidase and 2-epimerase activity and produces 2,4-diacetamido-2,4,6-trideoxy-D-mannose. *C. jejuni* has developed two pathways for CMP-Leg5Ac7Ac, one employing UDP-D-GlcNAc as the precursor and being the same as in *L. pneumophila*,[84] and the other using GDP-D-GlcNAc and differing only in the nucleotide group[64] (Scheme 2). CMP-Leg5Ac7Ac can be modified to CMP-Leg5Am7Ac by acetamidino synthase and then to CMP-Leg5AmMe7Ac by acetamidino *N*-methyltransferase,[82] whereas other uncommon *N*-acyl groups, such as *N*-(D-alanyl), evidently are incorporated to a C_6-precursor at an earlier step of biosynthesis.

A similar biosynthetic pathway of CMP-8eLeg5Ac7Ac in *E. coli* O108 has been proposed based on the homology of the corresponding genes[71] but has not been confirmed biochemically yet.

Other nonulosonic acids

An unusual 3-hydroxylated derivative, 5,7-diamino-5,7,9-trideoxynon-2-ulosonic acid bearing one *N*-acetyl group and one *N*-(3-hydroxybutanoyl) group, has been found in the O-antigen of a phytopathogen *Pseudomonas corrugate*, the causal agent of tomato pith necrosis.[85] Although a glycoside of this sugar was isolated by two consecutive Smith degradations of the polysaccharide, neither its configuration nor distribution of the *N*-acyl groups was determined. The O-antigen structure of this bacterium also remains to be established.

The polysaccharide chain of the LPS of a fish pathogen *Tenacibaculum maritimum* (former *Flexibacter maritimus*) includes a derivative of another unique higher sugar 5,7,8-triamino-3,5,7,8,9-pentadeoxynon-2-ulosonic acid.[86] It carries an *N*-acetyl group at position 5 and an *N*-[(*S*)-3-hydroxybutanoyl] group at position 7 whereas the amino group at position 8 is not acylated. The C-4–C-7 fragment of the nonulosonic acid has the β-L-*manno* configuration but the configuration at C-8 remains unknown; hence, the sugar is either the L-*glycero*-L-*manno* isomer (and is thus the 8-amino derivative of Pse) or the D-*glycero*-L-*manno* isomer. The O-antigen has a disaccharide repeating unit **61**, in which the nonulosonic acid is linked to the *N*-[(*S*)-2-hydroxy-5-glutaryl] group on the neighbouring 2,4-diamio-2,4,6-trideoxy-D-glucose residue.[86]



61

In conclusion, higher aldulosonic acids are important virulence determinants of bacterial pathogens. Kdo is an indispensable component of the LPS, a glycoconjugate present in the overwhelming majority of Gram-negative bacteria. Kdo-lacking bacterial mutants are significant impaired in growth ability and virulence. Sialic acids are not thought to be common in prokaryotes but, when present, may be important for bacterial life. In pathogens or commensals, Neu5Ac masks underlying antigenic structures on the cell surface such as O-antigens and can help the bacteria to avoid host defenses by sending to innate immune cells a false 'self signal'.[87] The molecular mimicry of host structures may be associated with autoimmune diseases. Homopolysaccharides of neuraminic and legionaminic acids are important for virulence of such human-specific pathogens as *Neisseria meningitidis* and *Legionella pneumophila*. 5,7-Diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid-containing polysaccharides on the cell surface significantly contribute to the immunospecificity and extend the

antigenic diversity of bacteria. Glycosylation with these unique higher sugars is essential for full functioning of flagella necessary for motility of *Helicobacter pylori* and *Campylobacter* species, a factor enabling these microorganisms to colonize the gastric and intestinal mucosa.

Biosynthetic pathways of aldulosonic acids are conserved in bacteria. Homologous enzymes catalyze common steps in each pathway, including the condensation of the C_5 or C_6 sugar precursors with phosphoenolpyruvate and the activation of the resultant octulosonic or nonulosonic acids as the CMP derivatives (Scheme 2). Earlier steps in the biosynthesis of nonulosonic acids are also similar; particularly, the C_6 sugar intermediates are released from their nucleotide-activated derivatives with or without 2-epimerization. Furthermore, the C_6 sugar precursors of 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids are synthesized from UDP-GlcNAc or GDP-GlcNAc by similar pathways, including 4,6-dehydratation (with or without 5-epimerization) followed by reductive 4-amination and N-acylation.

A long-standing paradigm considers the sialic acid biosynthetic pathway as a late innovation of deuterostomes (vertebrates, ascidians, and echinoderms) adapted later by their pathogens and symbionts. However, recent examination of ~1000 sequenced microbial genomes indicated that biosynthetic pathways of nonulosonic acids including sialic acids are far more widely distributed than previously realized.[88] Particularly, it has been found that expression of neuraminic acid is not limited to bacteria that associate with animals. This finding and a similarity of proteins involved in biosynthesis of different nonulosonic acids indicate that the old paradigm of the 'unique' sialic acid pathway of animals should be reconsidered in favour of a view that it was a much more ancient innovation by prokaryotes.

The α-(2→9)-linked polysialic acid generated during bacterial growth is used as a component of effective vaccines for protection against group C meningococci. In contrast, the α-(2→8)-linked Neu5Ac homopolymer is not immunogenic in humans, and no vaccine inducing protection against colominic acid-producing bacteria is currently available. In order to overcome this immunological tolerance different strategy are being developed, including modification of the N-acetylation pattern and conjugation with tetanus toxoid or therapeutic proteins (ref. 43 and refs. cited therein). Polysialic acids are also applied in drug delivery and drug selection.[41]

As the biosynthetic pathway of Kdo is sufficiently conserved among diverse medically important bacteria and no analogue counterpart is present in humans, the key enzymes involved in the biogenesis of Kdo are optimal targets for the development of novel therapeutics for combating bacterial pathogens and reducing the selection pressure on the total microflora. During the last decades, a number of inhibitors of these enzymes have been developed; some of these compounds have antibacterial properties, while others show excellent *in vitro* activity and are undergoing further investigation.[89] These achievements exemplify medical implications of fundamental studies of bacterial aldulosonic acid.

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