

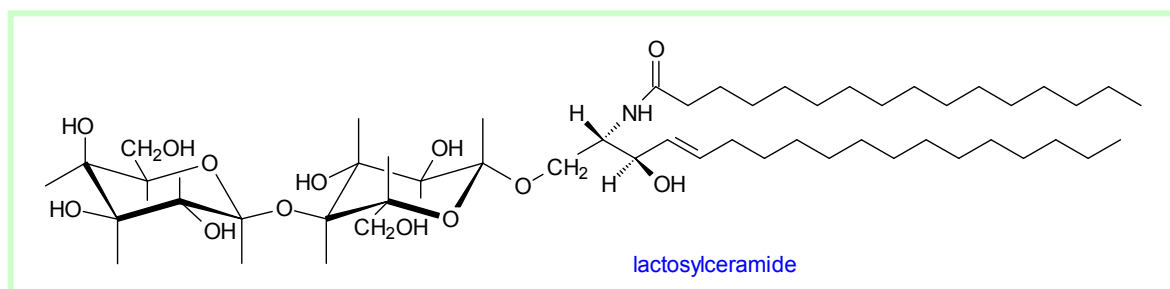
LACTOSYLCERAMIDE AND NON-ACIDIC OLIGOGLYCOSYLCERAMIDES

STRUCTURE, OCCURRENCE, BIOSYNTHESIS AND ANALYSIS

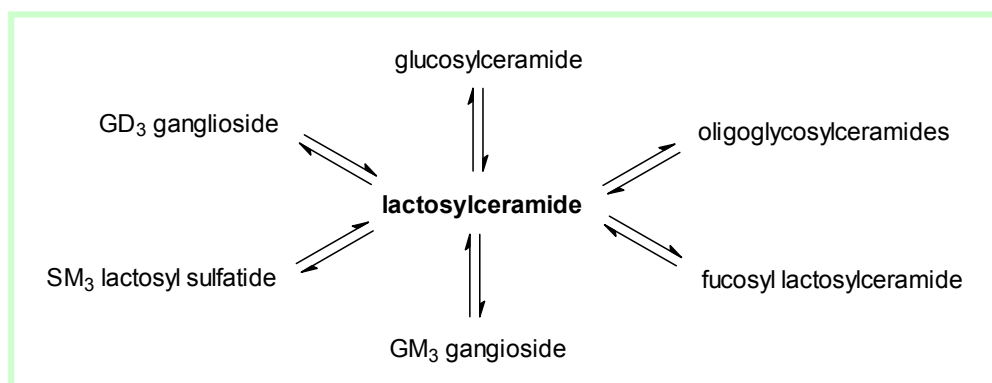
1. Lactosylceramide and Other Diosylceramides

Non-acidic di- and oligoglycosphingolipids, i.e. with two or more carbohydrate moieties attached to a ceramide unit, are vital components of cellular membranes of most eukaryotic organisms and some bacteria. Their abundance relative to other lipids is usually low other than in epithelial and neuronal cells, while the nature and proportions of the different glycolipid classes vary with the type of cell and the stage of growth. However, they are extremely important for the function of cells.

The most important and abundant of the diosylceramides is β -D-galactosyl-(1 \rightarrow 4)- β -D-glucosyl-(1 \leftrightarrow 1')-ceramide, more conveniently termed **lactosylceramide** (LacCer), using the trivial name of the disaccharide. In the early literature, it was termed 'cytolipin H'.



It is found in small amounts only in most animal tissues, but it has a number of significant biological functions and it is of great importance as the biosynthetic precursor of most of the neutral oligoglycosylceramides, sulfatides and gangliosides.



In animal tissues, biosynthesis of lactosylceramide involves addition of the second monosaccharides unit (galactose) as its nucleotide derivative to **monoglucosylceramide**, catalysed by a specific β -1,4-galactosyltransferase on the luminal side of the Golgi apparatus. The glucosylceramide precursor must first cross from the cytosolic side of the membrane, possibly via the action of a flippase. The lactosylceramide produced can be further glycosylated or transferred to the plasma membrane mainly by a vesicular mechanism that is poorly understood. It is also regenerated by the catabolism of many of the lipids for which it is the biosynthetic precursor.

Lactosylceramide may assist in stabilizing the plasma membrane and activating receptor molecules in the special micro-domains or **rafts**, as with the **cerebrosides**. In this environment, it has its own specialized function in the innate immunological system in that it is known to bind to specific bacteria, as with the oligoglycosylceramides (see below). Thus lactosylceramide forms such micro-domains on the plasma membrane of neutrophils and macrophages, which recognize, engulf and eliminate pathogens. It is known to have a role in the molecular mechanisms underlying these processes.

In addition, it is believed that a number of pro-inflammatory factors activate lactosylceramide synthase to generate lactosylceramide, which in turn activates "oxygen-sensitive" signalling pathways that affect such cellular processes as proliferation, adhesion, migration and angiogenesis. Dysfunctions in these pathways can affect several diseases of the cardiovascular system, cancer and inflammatory states, so lactosylceramide metabolism is a potential target for new therapeutic treatments.

Lactosylsphingosine, i.e. the deacylated or lyso form, occurs naturally at low levels in brain where it may have some specific function.

A further diglycosylceramide, galabiosylceramide ($\text{Gal}\alpha 1\rightarrow 4\text{Gal}\beta 1-1'\text{Cer}$), has also been found in small amounts in kidney and pancreas, for example, and it is one of the lipids that accumulates in excessive amounts in Fabry's disease (see below). It is the precursor of the minor 'gala' series of oligoglycosylceramides. Other diosylceramides containing mannose units may be present in some primitive animal species and in plants. In addition, several insect species contain mactose ($\beta\text{Man}(1\rightarrow 4)\text{Glc}$) linked to ceramide, while a starfish has been found to contain gentiobiosyl- and cellobiosylceramide as well as lactosylceramide.

2. Non-Acidic Oligoglycosylceramides of Animal Tissues

Neutral oligoglycosylceramides with from three to more than twenty monosaccharide units in the chain have been detected in animal tissues ('megaloglycolipids' with up to 50 carbohydrate groups occur in erythrocytes). Of these, tri- to pentaglycosylceramides are often the most abundant or at least are most intensively studied. As of 2009, 172 such oligoglycosylceramides with variations in the carbohydrate chain had been characterized in vertebrates alone.

Table 1. Root names and structures of the oligoglycolipids

Root	Symbol	Root structure
ganglio	Gg	$\text{Gal}\beta 3\text{GalNAc}\beta 4\text{Gal}\beta 4\text{Glc-}$
lacto ^a	Lc	$\text{Gal}\beta 3\text{GlcNAc}\beta 3\text{Gal}\beta 4\text{Glc-}$
neolacto ^b	nLc	$\text{Gal}\beta 4\text{GlcNAc}\beta 3\text{Gal}\beta 4\text{Glc-}$
globo	Gb	$\text{GalNAc}\beta 3\text{Gal}\alpha 4\text{Gal}\beta 4\text{Glc-}$
isoglobo ^c	iGb	$\text{GalNAc}\beta 3\text{Gal}\alpha 3\text{Gal}\beta 4\text{Glc-}$
mollu	Mu	$\text{GlcNAc}\beta 2\text{Man}\alpha 3\text{Man}\beta 4\text{Glc-}$
arthro	At	$\text{GalNAc}\beta 4\text{GlcNAc}\beta 3\text{Man}\beta 4\text{Glc-}$

^a In this instance, 'lacto' does not refer to lactose

^b The prefix 'neo' is used here to denote a (1→4) vs (1→3) difference in the linkage position between the monosaccharide units IV and III.

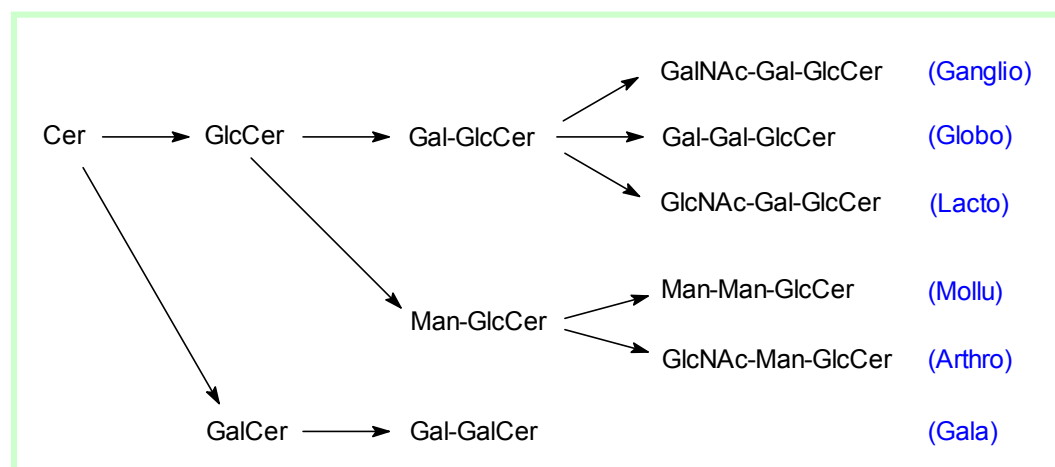
^c The prefix 'iso' is used here to denote a (1→3) vs (1→4) difference in the linkage position between the monosaccharide units III and II.

Seven main series are recognized from structural and biosynthetic relationships. As the systematic names tend to become rather cumbersome, semi-systematic names are usually recommended in which trivial names for a “root” structures are used as a prefix. The recommended root names and structures are listed in **Table 1**. In each instance, the primary unit linked to ceramide is glucose (Glc), with galactose (Gal), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc) or mannose (Man) as the other monosaccharides units.

The name of a given glycosphingolipid is then composed of (root name)(root size)osylceramide. Thus, lactotetraosylceramide or LcOse₄Cer designates the second structure listed in Table 1 linked to a ceramide. When it is necessary to refer to specific glycosyl residues, Roman numerals are used, counting from that nearest the ceramide (i.e. I to IV in this example). As further monosaccharides units are added to or substituted for these basic structures, or when branches occur, the nomenclature becomes increasingly complex, taking us into the realm of the specialist. The IUPAC-IUB recommendations cited below should then be consulted.

Fucolipids are oligoglycolipids in any of the above series in which a fucose (Fuc) residue substitutes for one of the usual carbohydrate residues. In addition, certain of the oligoglycolipids exist as **lipid sulfates**, and others are linked to sialic acid residues, i.e. **gangliosides**, (discussed elsewhere on this site).

The pathways for the biosynthesis of neutral oligoglycosphingolipids are illustrated below. Glucosyl- and galactosylceramide synthesised on the cytosolic side of the endoplasmic reticulum and early Golgi membranes are transferred to the luminal side of the Golgi, where further glycosylation occurs through the activity of a variety of distinct glycosyltransferases, with differing substrate specificities. Some of these appear to recognize only the carbohydrate portion of the molecule, but others appear to respond to the nature of the ceramide backbone. In the most important of these, the first step is the synthesis of lactosylceramide catalysed by galactosyltransferase I (discussed above), before further monosaccharides are added sequentially by reactions catalysed by specific glycosyltransferases.



Literally hundreds of different neutral oligoglycosphingolipids have been found in various organs and species of animals, with further complexity being added by the nature of the ceramide unit. In any given cell type, the number of different glycosylceramides may be relatively small, but their nature and compositions may be characteristic and in some way related to the function of the cell. This composition tends to change substantially as an animal develops. Comprehensive descriptions of all the innumerable complex oligoglycosylceramides are impossible here. A few selected examples of particular biological importance are discussed below.

Often with these sphingoglycolipids, it has been the nature of the carbohydrate moiety that has received most study, as this is usually presumed to carry the primary biochemical function. Data on the nature of the fatty acid and long-chain base constituents are relatively sparse, though it

appears that these tend to reflect their biosynthetic origins and resemble those of the precursor **glucosylceramides** (see the relevant web page). In general, as might be expected, the fatty acids are long-chain saturated and monoenoic in nature, but an exception is in the testicular lipids where there are distinctive fucolipids containing polyunsaturated fatty acids; these lipids appear to be essential for spermatogenesis and fertility in male mice.

There is an increasing body of evidence to suggest that the composition of the ceramide unit is biologically important. At the very least, the composition of the ceramide unit ensures that the lipid takes its correct place in the plasma membrane, and with the hydroxy fatty acid constituents providing an additional and apparently essential hydrogen bonding capacity. Similarly, binding of various bacteria, viruses and antibodies to glycolipids seems to require specific ceramide compositions, often requiring the presence of hydroxy fatty acids, for example.

While the lipid moiety is essential, the structure of the carbohydrate component is indeed critical for many of the functions of oligoglycosphingolipids, 80-90% of which occur on the plasma membrane exclusively facing into the extra-cellular space. In particular, glycolipids are important components of the body's immune defense system, either in haptenic reactivity or in antibody-producing potency, i.e. as cellular immunogens or antigens. Certain glycolipids are involved in the antigenicity of blood group determinants, while others bind to specific toxins or bacteria. For example, *Propionibacterium*, which causes a disease of the skin, binds to the lactosyl moiety of glycosphingolipids, as does the organism responsible for gonorrhoea. Some glycosphingolipids function as receptors for cellular recognition, and they can be specific for particular tissues or tumours. Antibodies to specific glycolipids have been implicated in certain diseases of the autoimmune system. In addition, glycosphingolipids can modify the activity of membrane receptors, such as those for insulin, and epidermal and nerve growth factors.

The oligoglycosylceramides of erythrocytes have attracted particular interest, as they are important for cellular interactions including blood-type determinants. The main glycosphingolipid found in human erythrocytes is a tetrahexoside, often abbreviated to Gb4 or GbOse₄Cer, which was termed '**globoside**' (as it was first obtained as a globular, birefringent precipitate) and gave the root name to the globo-series (**Table 2**).

Table 2. Structures of some of the important oligoglycosylceramides of the globo-series

Gal α 4Gal β 4Glc β 1Cer	Gb3
Gal α 3Gal β 4Glc β 1Cer	Iso-Gb3
GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	Gb4
GalNAc β 3Gal α 3Gal β 4Glc β 1Cer	Iso-Gb4
GalNAc α 3GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	Forsman
Gal β 3GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	Gb5 (SSEA-3a)
Fuc α 2Gal β 3GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	globo-H (SSEA-3b)

The triose from which this is derived, Gb3 or GbOse₃Cer, is also a significant component of human erythrocytes, and this structure is the inner core of all globo-series oligoglycosylceramides. It binds specifically to the verotoxins of *Escherichia coli* (the nature of the ceramide component is also important in this instance) and Shiga toxin, and this lipid has also been implicated in host cell interactions with the human immunodeficiency virus (HIV). Isoglobotriaosylceramide (iso-Gb3) is a stimulatory antigen to both mouse and human Natural Killer T cells and is believed to be involved in controlling the responses of these cells to infections, malignancy, and autoimmunity.

A pentaosyl-ceramide of the globo-series (GbOse₅Cer with an additional terminal α -*N*-acetylgalactosamine) is the Forssman antigen, and it is of special interest in that it is species specific. Some animals (e.g. horse, cat, dog) are Forssman-positive and others (e.g. rat, pig) are Forssman-negative and lack the glycolipid. Although humans have been considered to belong to the latter group, certain populations have been found with this glycolipid in the intestinal mucosa. In addition, there are further globo-series glycolipids with various alternative carbohydrate moieties attached to the terminal GalNAc of Gb₄, which have been isolated from human embryonal carcinoma and are believed to be functionally involved in the development of stem cells.

Oligoglycosylceramides of the lacto-series tend to exist in forms that are heavily substituted with fucose moieties and are associated with blood group A, B, H and other activities. At least 15 types are known with both linear and branched structures. Most of these lipids function as antigens, and some are associated with specific carcinomas. Some have distinctive ceramide structures including phytosphingosine and hydroxy acids.

Cats, dogs and horses contain a glycolipid similar to globosides but with sialic acid replacing the galactosamine; this has been termed 'hematoside'. **Gangliosides** based on the globo- and lacto-root structure in general are discussed elsewhere on this site.

Mucolipids, i.e. oligoglycosylceramides with di- to hexagalactosyl glycosides (some with branch structures) linked to glucosylceramide, are found predominantly in the gastrointestinal mucosa.

Mannosylglucosylceramide (Man β 1-4Glc β 1-cer) is the precursor diosylceramide for the complex glycosphingolipids in *Drosophila*, widely studied as an insect model. *N*-Acetylgalactosamine residues are then added sequentially, the first via a β 1-3 linkage and subsequently via β 1-4 linkages. The long-chain bases in this instance tend to be C₁₄ and C₁₆ in chain-length.

Parasitic nematodes contain distinctive zwitterionic oligoglycosphingolipids of the arthro series with phosphocholine moieties attached as highly conserved, antigenic glycolipid markers. The glycosphingolipids of the pig parasitic nematode, *Ascaris suum*, have been most studied, and they are characterized by the phosphodiester-bound phosphocholine substituent linked to C6 of a central *N*-acetylglucosamine residue. In addition, some of these glycosphingolipids carry phosphorylethanolamine linked to C6 of an adjacent mannose residue. The ceramide moiety contains (*R*)-2-hydroxytetracosanoic acid as the main fatty acid component and C₁₇ *iso*-branched sphingosine and sphinganine bases.

As with the other sphingolipids, the neutral oligoglycosylceramides are concentrated in the specific region of membranes known as '**rafts**'. This is especially true of the brush border of intestinal cells, where much of the digestion and absorption of nutrients occurs. Many of the main enzymes are localized in rafts with little cholesterol but high concentrations of glucosylceramide and oligoglycosylceramides, such as lactosylceramide, globotriaosylceramide and ganglioside G_{M3}, which are resistant to pancreatic enzymes, bile salts and the products of digestion. Surprisingly, phytosphingosine accounts for 70% of the sphingoid bases, and a high proportion of the fatty acids are hydroxylated in this instance.

3. Oligoglycosylceramides of Plants and Microorganisms

In plants, elongation of glucosylceramide occurs to form two series of oligoglycosylceramides with either mannosyl or galactosyl units by mechanisms that are presumably related to those in animals. In the mannosyl series, up to four mannosyl units may be added via β 1 \rightarrow 4 linkages; addition of a 1-4 linked β -D-glucopyranosyl unit terminates the elongation step after 1, 2 or 3 mannose units have been coupled together. This results in a series of di-, tri-, tetra- and pentaglycosylceramides, terminating in either a glucose or mannose unit. The second series appears to involve linkage of up to three D-galactopyranosyl residues to the primary

glucosylceramide unit, although not all members of the series have been characterized. These complex lipids are found mainly as constituents of the endoplasmic reticulum, Golgi, tonoplasts and plasma membrane, but little is known of their functions in plants.

The fatty acid and sphingoid base compositions tend to reflect their biosynthetic origins and resemble those of the precursor **monoglycosylceramides** (see the web pages). Thus, the fatty acids are mainly saturated 2-D-hydroxy acids with 14 to 26 carbon atoms, although some species differences are found. Phytosphingosines (including 8-*cis/trans* isomers) and various 4*t*,8*c/t*-sphingadienes are the main long-chain bases.

A tetraglycosylceramide has been isolated from *Neurospora crassa*, and monoglycosylceramides are frequently reported from fungi and yeasts, but in general complex **glycosylinositol phosphorylceramides** appear to take the place of neutral oligoglycosylceramides. The bacterial genus *Sphingomonas* contains a tetraglycosylceramide α -D-Man ρ (1 \rightarrow 2)- α -D-Galp-(1 \rightarrow 6)- α -D-GlcpN-(1 \rightarrow 4)- α -D-GlcpA(1 \rightarrow 1)Cer, i.e. with an α - rather than a β -linkage to the ceramide unit, in addition to a cerebroside analogue.

4. Catabolism of Non-Acidic Oligoglycosylceramides

As discussed in the web page dealing with monoglycosylceramides, oligoglycosphingolipids are degraded in lysosomal compartments within the cell by water-soluble hydrolases. In brief, sections of plasma membrane containing glycosphingolipids intended for degradation are endocytosed and transported to the lysosomes where exohydrolases cleave sugar residues sequentially from the non-reducing end of glycoconjugates. The cleaved fragments, i.e. the sugar residues and ceramide, with the latter further hydrolysed to fatty acids and sphingoid bases, can then leave the lysosomes and be degraded further or re-enter the biosynthetic pathways.

If any of the ten different hydrolase enzymes is deficient, the corresponding lipid substrate accumulates and is stored in the lysosomal compartment, sometimes to a considerable extent as in the inherited sphingolipid storage diseases, such as **Fabry's disease**. This is a genetic disorder (X-linked) arising from the absence of the lysosomal enzyme ceramide trihexosidase, which catalyses the hydrolytic cleavage of the terminal galactose molecule. This leads to deposition of excessive amounts of glycosphingolipids with terminal galactose units, such as globotriaosylceramide and galabiosylceramide in tissues, especially in the kidney, heart and brain. Enzyme replacement therapy is now making a significant contribution to ameliorating the affects of the disease.

An alternative pathway in certain bacteria and leeches involves the action of a ceramide glycanase to cleave the β -glucosidic linkage between glucose and ceramide, which results in the formation of ceramide and an oligosaccharide. This enzyme has proved of value in the structural analysis of glycosphingolipids.

4. Analysis

High-performance thin-layer chromatography is widely used for separation of various types of oligoglycolipids, based on the number and to some extent the type of monosaccharide units, though high-performance liquid chromatography (sometimes after benzylation) is also employed for the purpose. Mass spectrometry is the main method for identifying and sequencing the carbohydrate chains, with invaluable assistance from nuclear magnetic resonance spectroscopy. Indeed, modern mass spectrometric methods, especially with electrospray ionization and matrix-assisted laser desorption/ionization (MALDI), appear to have greatly eased the technical problems of structural analysis.

Recommended Reading

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