3.15 Glycophylogenetic Aspects of Lower Animals

S. Itonori and M. Sugita, Shiga University, Otsu, Japan
© 2007 Elsevier Ltd. All rights reserved.

3.15.1 Introduction

Glycolipid consists of a hydrophilic sugar head and a hydrophobic lipid tail, which localizes glycolipid in cell membrane. Differences in its lipid portion allow glycolipids to be classified mainly into two groups: one group in which the oligosaccharide is attached to diacylglycerol or related structures, called glycoglycerolipid; and the other in which the oligosaccharide is attached to ceramide consisting of sphingosine and fatty acid, called glycosphingolipid (GSL). Although many glycoglycerolipids are found in plants and bacteria, they exist also in vertebrates in small amounts. GSLs are widely distributed from the so-called lower animals to higher animals, and widely varied structures have been reported. In mammals, gangliosides (sialic acid-containing GSLs) are the major acidic GSLs in the central nervous system, and it is now well known that gangliosides play roles in neural functions, cell adhesion and proliferation, and signal transduction. Invertebrate GSLs, on the other hand, have only recently been observed to have some strong and unusual physiological activities. For example, it has been reported that αGalCer isolated from the sea sponge is a ligand for Vα14NKT cells and inhibits cancer progression and metastasis. This remarkable observation became possible with advances in the research on GSLs of lower animals. Furthermore, zwitterionic GSLs which are common in Nematoda induce cytokine secretion. This chapter describes the oligosaccharide structures of GSLs in lower animals (invertebrates), focusing on a molecular phylogeny based on the GSL structures. It also discusses invertebrate GSL structures, which are classified into neutral, acidic, polar, and zwitterionic types.

3.15.2 Preparation and Purification of Glycosphingolipids

The procedure for the preparation of invertebrate GSLs consists of lipid extraction from the tissue, removal or degradation of other lipids, fractionation of the neutral, acidic, polar, and zwitterionic GSLs, and finally purification of the individual components. Briefly, the acetone-dried material or the lyophilized tissues are extracted with mixtures of chloroform/methanol, chloroform/methanol/water, and/or propanol/hexane/water, and after alkaline hydrolysis of co-extracted saponifiable lipids, the GSL groups are separated by anion-exchange chromatography with DEAE- and

QAE-Sephadex columns. GSLs are fractionated into neutral, acidic, polar, and zwitterionic types on the basis of charged groups and polarity. However, the degree of the polarity in this case does not reflect physicochemical values but adsorption on ion-exchange resin during the separation. GSLs which are adsorbed to DEAE-Sephadex fractionated as the acidic GSLs, which contain sialic acid, uronic acid, sulfuric acid, or inositol phosphate. Acidic GSLs are also adsorbed to QAE-Sephadex. GSLs containing phosphoethanolamine or aminoethylphosphonate (C–P compound) cannot be adsorbed to DEAE-Sephadex, but can be adsorbed to QAE-Sephadex; therefore, it is isolated as the polar fraction. Zwitterionic GSLs do not bind to either ion-exchange resins, and are eluted as the neutral fraction. Using the acetylation method for separating GSLs from sphingomyelin which is a typical zwitterionic phospholipid, acetylated neutral and zwitterionic GSLs are separated by Florisil column chromatography. The individual GSL components may finally be obtained by Iatrobeads column chromatography and HPLC.

3.15.3 Nomenclature for GSL of Arthropoda and Mollusca

Although numerous GSLs have been assigned trivial names from their history, the nomenclature and abbreviations recommended by the IUPAC–IUB Joint Commission on Biochemical Nomenclature (JCBN) cover semisystematically the structures of most GSLs and are applied to vertebrate GSLs. From invertebrates, two GSL series with characteristic carbohydrate sequences have been demonstrated. The two series are the arthro-(derived from arthropod) series, and the mollu-(derived from mollusk) series, and these GSLs will be indicated by prefixes that relate to the chemical structures found in their carbohydrate moiety.

3.15.4 GSLs in Lower Animals

3.15.4.1 Protostomia

3.15.4.1.1 Arthropoda

Arthropoda are divided taxonomically into 11 classes and located at the top of the Protostomia. They are classified into Insecta such as mosquito, flies including *Drosophila melanogaster*, Crustacea such as shrimp and crab, and Diplopoda such as millipedes, etc. In particular, studies of *D. melanogaster* have led to great advances in developmental genetics and genome analysis.

3.15.4.1.1.1 Insecta

The first structural study of insect GSLs was reported in 1973 by Luukkonen *et al*. using cultured mosquito cells, *Aedes albopictus*.[1] The presence of glucosyl-, diglucosyl-, and mannnosylglucosylceramides was demonstrated in this dipteran insect. A systematic investigation of the insect GSL structures was reported in 1982, using the larvae of the greenbottle fly, *Lucilia caesar*, of Insecta.[2] In 1985, another systematic structural analysis of the dipteran insect, the pupae of the blowfly, *Calliphora vicina*, was reported; this kind of comparison of GSLs among different dipteran species and different developmental stages is important.[3] The GSLs and their profiles of the dipteran insects, *L. caesar* and *C. vicina*, are very similar, even when compared among different stages of development.[2–8] The insect GSLs have unbranched linear sugar chains, especially rich in hexosamines, up to nonahexoside, and have characteristic sequences, and positional and anomeric linkages. From these animals, a series of GSLs containing 1 mol of mannose have been characterized. A series of GSLs containing this characteristic oligosaccharide structure (GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-) has been named the 'arthro-series', derived from the name Arthropoda, because the structure was first encountered in this species. With the exception of Glcβ1-Cer and MacCer (Manβ1-4Glcβ1-Cer), arthro-series GSLs are entirely different from those known from any other animals at that time (see Section 3.15.4.1.2). And already the chemical synthesis of a valuable neutral arthro-GSL analog has been reported.[9]

Recently, non-arthro-series GSLs were identified from High Five^{TM} insect cells as the major neutral GSL (**Table 1**).[10] The High Five^{TM} cell line was developed and originated from the ovarian cells of the cabbage looper, *Trichoplusia ni* (Insecta: Lepidoptera). In the High Five^{TM} insect cell, the structures of the predominant tri- and tetraglycosylceramide were characterized as Galβ1-3Manβ1-4Glcβ1-Cer and GalNAcα1-4Galβ1-3Manβ1-4Glcβ1-Cer. The arthro-series At_3Cer (GlcNAcβ1-3Manβ1-4Glcβ1-Cer) was present, even though only as a minor component. The ceramide moieties in the dipteran and lepidoptera GSLs are also similar, being composed of d14:1 and d16:1 (tetradeca- and hexadeca-4-sphingenines) as the main sphingoids, and C18:0 (stearic acid), C20:0 (arachidic acid), and C22:0 (behenic acid) as the major fatty acids. Analysis of the GSL ceramide composition in the lower animals is

Table 1 Structures of glycosphingolipids found in Arthropoda

Structure	*[Abbreviation] and source*
Neutral GSL	
Glcβ1-Cer	[GlcCer] *Lucilia caesar* (L)[2] *Calliphora vicina* (P)[3] *Parafontaria laminata armigera*[33] High Five^{TM} cell[10]
Manβ1-4Glcβ1-Cer	[MacCer] *L. caesar* (L)[2] *C. vicina* (P)[3] *Euphausia superba*[24] *Macrobrachium nipponense*[24] *P. l. armigera*[33] High Five^{TM} cell[10]
GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[At$_3$Cer] *L. caesar* (L)[2] *C. vicina* (P)[3] *E. superba*[24] *M. nipponense*[24] *P. l. armigera*[33] High Five^{TM} cell[10]
Manβ1-4Glcβ1-Cer 3 \| Fucα1	*P. l. armigera*[33]
Galβ1-3Manβ1-4Glcβ1-Cer	High Five^{TM} cell[10]
GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[At$_4$Cer] *L. caesar* (L)[2] *C. vicina* (P)[4] High Five^{TM} cell[10]
GalNAcα1-4Galβ1-3Manβ1-4Glcβ1-Cer	High Five^{TM} cell[10]
GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[At$_5$Cer] *L. caesar* (L)[5] *C. vicina* (P)[4]
Galα1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[IV^{3}Galα-At$_4$Cer] *C. vicina* (P)[4]
Galβ1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[IV^{3}Galβ-At$_4$Cer] *C. vicina* (P)[6]
Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[At$_6$Cer] *C. vicina* (P)[4] *L. caesar* (L)[11]
GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[At$_7$Cer] *C. vicina* (P)[4] *L. caesar* (L) [11]
GalNAcβ1-3GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[At$_8$Cer] *L. caesar* (L)[7]

(continued)

Table 1 (continued)

Structure	*[Abbreviation] and source*
Galβ1-3GalNAcβ1-3GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[At$_9$Cer] *L. caesar* (L)[7]
Acidic GSL	
GlcAβ1-3Galβ1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[IV^{3}GlcAβ3Galβ-At$_4$Cer] *C. vicina* (P)[6]
GlcAβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[VI^{3}GlcAβ-At$_6$Cer] *L. caesar* (L) [11]
Polar GSL	
Etn*P* | 6 GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[III^6-Etn-*P*-At$_3$Cer] *L. caesar* (L)[19] *C. vicina* (P)[8]
Etn*P* | 6 GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[III^6-Etn-*P*-At$_4$Cer] *C. vicina* (P)[18,8] *L. caesar* (L)[19] *Drosophila melanogaster* (E)[12]
Etn*P* | 6 GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[III^6-Etn-*P*-At$_5$Cer] *C. vicina* (P)[18,8] *L. caesar* (L)[19] *D. melanogaster* (E)[12]
Etn*P* | 6 Galβ1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[IV^{3}Galβ-,III^6-Etn-*P*-At$_4$Cer] *C. vicina* (P)[8]
Etn*P* | 6 Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[III^6-Etn-*P*-At$_6$Cer] *L. caesar* (L)[19] *C. vicina* (P)[8] *D. melanogaster* (E)[12]
Etn*P* | 6 GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[III^6-Etn-*P*-At$_7$Cer] *L. caesar* (L)[19] *C. vicina* (P)[8] *D. melanogaster* (E)[12]
Etn*P* | 6 GalNAcβ1-3GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*L. caesar* (L)[19]
Etn*P* Etn*P* | | 6 6 GalNAcβ1-4GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*D. melanogaster* (E)[12]
Etn*P* | 6 Galβ1-3GalNAcβ1-3GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*L. caesar* (L)[19]

Table 1 (continued)

Structure	*[Abbreviation] and source*
EtnP | 6 GlcNAcβ1-3GalNAcβ1-3GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*L. caesar* (L)[19]
Acidic–polar GSL	
EtnP | 6 GlcAβ1-3Galβ1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[IV^{3}GlcAβ3Galβ-,III^6-Etn-*P*-At$_4$Cer] *C. vicina* (P)[6]
EtnP | 6 GlcAβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	[VI^{3}GlcAβ-,III^6-Etn-*P*-At$_6$Cer] *L. caesar* (L)[19] *D. melanogaster* (E)[12]
EtnP | 6 GlcAβ1-3Galβ1-3GalNAcβ1-4GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*D. melanogaster* (E)[12]
EtnP | 6 GlcAβ1-3Galβ1-3GalNAcβ1-4GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer EtnP 6	*D. melanogaster* (E)[12]
Phosphonoglycosphingolipid	
MAEPn-6Glcβ1-Cer	*E. superba*[30]
AEPn-4Glcβ1-Cer	*Erimacrus isenbeckii*[31]
MAEPn-4Glcβ1-Cer	*E. isenbeckii*[31]

Abbreviations: Etn*P*, 2-aminoethanolphospho-; MAEPn, (*N*-methyl-2-aminoethyl)hydroxyphosphoryl-; AEPn, (2-aminoethyl) hydroxyphosphoryl-.
E, embryo; L, larvae; P, pupae.

very important, because it provides one of the proofs that these GSLs are synthesized *de novo* and not derived directly from food or culture media.

As acidic GSLs, a glucuronic acid-containing GSL has been characterized as GlcAβ1-3At$_6$Cer, GlcAβ1-3Galβ1-3 GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer, and it is of interest as the functional counterpart of gangliosides, the sialic acid-containing acidic GSLs in vertebrates.[11] Up to date, six glucuronic acid-containing GSLs (with or without 2-aminoethylphosphate) were isolated from Insecta and their precise chemical structures have been characterized as containing up to nonahexosylceramide in *D. melanogaster*.[6,11,12] Two acidic GSLs were isolated from human cauda equina and characterized as sulfoglucuronylparagloboside and sulfoglucuronyllactosaminylparagloboside, which react with the serum IgM M-protein in some patients with neuropathy and plasma cell dyscrasia.[13–15] These acidic GSLs carry the HNK-1 epitope, SO$_3$-3GlcAβ1-3Galβ1-4GlcNAcβ-, on those structures. This M-protein binds weakly to the insect acidic GSL from *L. Caesar*. Using a monoclonal antibody CAF-I which recognizes acidic GSLs of the Calliphoridae, the presence of similar GSLs was demonstrated in the GSLs isolated from Diptera: *D. melanogaster* and Coleoptera:*Tenebrio molitor*, by thin-layer chromatography (TLC) immunostaining. Excellent reviews are available by Wiegandt *et al.* on the chemistry, structure, and immunological properties of insect GSLs.[16,17]

As polar GSLs, a series of phospho-GSLs have been characterized having phosphoethanolamine (Etn*P*) attached to the 6-hydroxyl group of GlcNAc moiety of arthro-series GSLs.[8,18,19] GSLs containing both uronic acid and Etn*P* have also been characterized. From *D. melanogaster*, GSL containing 2 mol of Etn*P* has been reported. Interestingly, Etn*P*

substitution on GlcNAc is more extensive in *Drosophila* embryo, suggesting a stage-specific GSL expression.[12] Investigation of GSLs in model organisms such as *D. melanogaster* enhances our understanding of GSL functions.[20] Recently, the *Drosophila* neurogenic genes, *egghead* and *brainiac*, have been shown to encode a β-1,4-mannosyltransferase and a β-1,3-*N*-acetylglucosaminyltransferase, respectively, essential for epithelial development during oogenesis and in the embryo.[21–23] These genes are predicted by *in vitro* analysis to control synthesis of the core GSL in Insecta such as At_3Cer. These studies demonstrate that GSLs are essential for development of complex organisms.[23]

3.15.4.1.1.2 Crustacea

The Antarctic krill, *Euphausia superba* of Crustacea, is a major food of whales, and the freshwater shrimp, *Macrobrachium nipponense*, is an animal inhabiting Lake Biwa. Arthro-series neutral GSLs were detected in these animals by chemical structural analysis and immunochemical methods. Anti-MacCer (Manβ1-4Glcβ1-Cer) antibody raised with MacCer isolated from spermatozoa of the freshwater bivalve, *Hyriopsis schlegelii*, and anti-At_3Cer antibody raised with At_3Cer isolated from the larvae of the green-bottle fly, *L. caesar*, were applied to the detection of crustacean GSLs by TLC-immunostaining and readily identified these characteristic structures.[24,25] In addition, cryostat sections of *M. nipponense* were stained with anti-At_3Cer antibody and results indicated that At_3Cer is localized in green gland, esophagus, and gill organs.[25] These studies show that specific antibodies against mannolipids should be useful in studying the distribution of these glycolipids in other animals of the invertebrate phyla.

In the shore (brown) shrimp, *Penaeus aztecus aztecus*, high concentrations of GlcCer were observed in the ventral nerve cord, brain, optic nerve, and antenna, but not in the non-neural tissue.[26,27] Ceramide composition of this lipid includes C14, C15, and C16 sphinganines and sphingenines, as well as significant amounts of C19 and C20 sphinganines, and the fatty acids are mainly composed of nonhydroxy ones with more than 22 carbons long.[28] These data closely resemble the ceramide composition of mammalian brain GalCer. In addition, crustacean GlcCer localizes in the sheath membranes surrounding axons of the ventral nerves of this animal. In the brine shrimp, *Artemia franciscana*, existence of a fucosylated GSL as a major neutral GSL has been predicted.[29]

The predominant GSL in the Antarctic krill, *E. superba*, is a phosphonoglucocerebroside in the polar GSL fraction. It was identified to be 6′-*O*-(*N*-methyl-2-aminoethylphosphonyl)Glcβ1-Cer (MAEPnGlcβCer).[30] The ceramide moiety was composed of tetradecasphingenine and octadecasphingatriene as the main sphingoids, and C22:1 and C24:1 fatty acids and their 2-hydroxy derivatives as the major fatty acids.

In the marine crab, *Erimacrus isenbeckii*, arthro-series GSLs were detected by TLC-immunostaining. The core structure of major GSLs is Galβ1-3Manβ1-4Glcβ1-. As polar GSLs in this animal, phosphonoglucocerebrosides (AEPn-4Glcβ1-Cer and MAEPn-4Glcβ1-Cer) have been characterized, in which 2-aminoethylphosphonate (AEPn) and its monomethyl derivative (MAEPn) are attached to the 4-hydroxyl group of glucose,[31] instead of 6-position of glucose as seen in *E. superba*.[30] Phosphonocerebrosides found in the animal kingdom are AEPn- and MAEPn-6Galβ1-Cer in the Mollusca, and MAEPn-6Glcβ1-Cer and AEPn- and MAEPn-4Glcβ1-Cer in the Arthropoda. The ceramide moieties of AEPn-4Glcβ1-Cer and MAEPn-4Glcβ1-Cer in *E. isenbeckii* were composed of tetradeca-4-sphingenine as the sole sphingoid, and C18:0, C20:0, C22:0, and C22:1 as the major fatty acids. By comparison, the ceramide moiety of MAEPn-6Glcβ1-Cer in *E. superba* was composed of tetradecasphingenine and octadecasphingatriene as characteristic sphingoids, and C22:1 and C24:1 acid and their 2-hydroxy derivatives as the major fatty acids.

In the horseshoe crab, *Limulus polyphemus*, cerebrosides and ganglioside were absent in the nervous system of this animal, despite the presence of glycerophospholipid and sphingomyelin as major components.[32]

3.15.4.1.1.3 Diplopoda

The periodical millipede, *Parafontaria laminata armigera* of Diplopoda, appears in outbreaks with an 8-year span and was studied in two development stages, namely the 7th instar and adult. The production by this animal of a poisonous gas, hydrogen cyanide, as a defense mechanism, has also been investigated. Its neutral GSLs have been identified as arthro-series with the detection of Glcβ1-Cer, Manβ1-4Glcβ1-Cer, and GlcNAcβ1-3Manβ1-4Glcβ1-Cer. A fucosylated mannolipid (Manβ1-4(Fucα1-3)Glcβ1-Cer) from this animal has a unique carbohydrate linkage characterized by a fucose residue attached to the reducing end glucose through an α1-3 linkage, allowing prediction of polyfucosylated GSL.[33] It is also noteworthy that in the fatty acid composition of Glcβ1-Cer, hydroxylated fatty acids at 2- and 3-positions comprise more than 70% of the total fatty acids; the existence of 3-hydroxy fatty acids in animal sphingolipid is so far unique.

3.15.4.1.2 Nematoda

Nematoda have included parasitic roundworms and *Caenorhabditis elegans* whose genome has been completely sequenced and cell lineage has been determined, etc. The porcine parasitic nematode, *Ascaris suum*, is a model

organism for immunological studies, such as infections involving human parasitic nematode, *Acanthocheilonema viteae*. The free-living nematode, *C. elegans*, is a conceptual model for biosynthetic studies using molecular genomics. Sometimes Nematoda is not considered to be a separate phylum, but should be one of the seven groups making the phylum Aschelminthes.

From 1975, the glycolipid components of the Nematoda have been verified by TLC with chemical detections in *Ascaris lumbricoides*,[34] *Dirofilaria immitis*,[35] *Trichuris globulosa*,[36] *Angiostrongylus cantonensis*,[37] and *Onchocerca gibsoni*.[38] A quantitative study of the incorporation of 1-[^{14}C]-acetate into lipids of the intestinal nematode parasite, *T. globulosa*, revealed the presence of GlcCer, as well as ganglioside and sulfatide. Ganglioside was present at very low concentrations, around 0.1% of total lipids.[36] In the Nematoda, *C. elegans*, GlcCer contained iso-branched d17:1 sphingosine as major sphingoid and 2-hydroxylated C20:0–C26:0 as major fatty acids.[39] Neutral GSLs from *A. suum* and *C. elegans* have been characterized as arthro-series GSLs (**Table 2**), which are also detected in Insecta of Arthropoda, as mentioned above.[40–42] The GSLs with longer oligosaccharides are characteristic of nematode, such as Galα1-3GalNAcβ1-4 GlcNAcβ1-3Manβ1-4Glcβ1-Cer. Phylogenetic analysis of 18*S* ribosomal DNA sequences indicates a close relationship between arthropods, nematodes, and all other moulting (ecdysis) phyla.[43]

As acidic GSLs, inositol phosphate-containing GSLs, so-called 'phytoglycolipids' have been characterized for the first time from Animalia.[44] This discovery accelerated research on phytoglycolipids in other species of lower animals. Phytoglycolipids or mycoglycolipids have been found to be widespread in plants, yeast, protozoans, fungi, nematoda, and annelida. This acidic GSL was detected in the intestine of *A. suum* by immunohistochemical staining with a polyclonal antiserum.[45] Another acidic GSL, sulfatide, was also found in *A. suum*, a compound which had not yet been reported in invertebrate. Sulfatide was detected in the hypodermis, contractile zone of somatic muscle cells, and the external musculature of the uterus by immunohistochemical staining.[45]

Zwitterionic GSLs of nematode have been characterized as a series of GSLs having phosphocholine (PC) attached to 6-hydroxyl of *N*-acetylglucosamine on their characteristic neutral GSLs, while, in contrast, insecta GSLs are substituted with Etn*P* on *N*-acetylglucosamine.[46–48] Interestingly, two major GSLs in *A. suum* were identified as Galα1-3GalNAcβ1-4(PC-6)GlcNAcβ1-3Manβ1-4Glcβ1-Cer (component A) and Galα1-3GalNAcβ1-4(PC-6)GlcNAcβ1-3(Etn*P*-6)Manβ1-4Glcβ1-Cer (component C).[49] It has been reported that these zwitterionic GSLs induce the release of pro-inflammatory monokines, such as tumor necrosis factor-α, interleukin 1, and interleukin 6, from human peripheral-blood mononuclear cells. Further investigation of immunomodulatory properties of the zwitterionic GSLs was performed using immune cells from BALB/c mice; it was found that *Ascaris* GSLs can modulate both innate and adaptive host immune responses by PC-dependent and independent mechanisms.[50] The zwitterionic PC substitution of nematode glycoconjugates has been shown to play an important role in host–parasite interaction by downregulation of lymphocyte proliferative responses.[51] Also, this epitope has been shown to induce the production of pro-inflammatory and T-helper-2-type cytokines and to promote dendritic cell maturation toward T-helper 2 cell development.[52]

These PC-containing zwitterionic GSLs were also determined to be highly conserved antigenic structures of parasitic nematodes, *A. suum* (host animal: porcine), *Setaria digitata* (equine), *Litomosoides sigmodontis* (mouse), *Onchocerca volvulus* (human), *A. viteae* (human), and even *C. elegans* (free living) using chemical structure determination or immunochemical detection.[48,53,54] In *C. elegans*, during embryonic and postembryonic development, the spatial and temporal expression of the PC epitope is located predominantly in seam cells and basement membranes. In early embryonic ontogenesis, the PC epitope was observed to be solely lipid bound, while in late embryonic and postembryonic development this epitope was observed to be both lipid and protein bound.[46]

Most systematic structural analysis of zwitterionic GSLs in the porcine parasitic nematode *A. suum* has been completed with excellent studies by Geyer *et al*.[55] The chemical synthesis of a valuable zwitterionic GSL analog has been reported.[56]

Molecular analysis of *C. elegans* genes during epithelial invagination and the formation of tubular structures during gastrulation, neurulation, and organogenesis identified the involvement of glycosyltransferase or glycoconjugate biosynthesis-related genes, and such studies are accumulating.[57] RNAi experiments targeting enzymes of GSL biosynthesis and choline metabolism in *C. elegans* have been performed.[58]

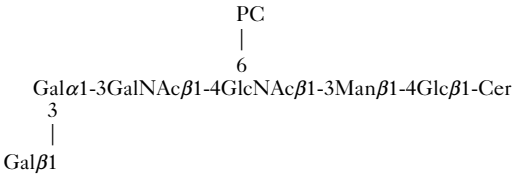
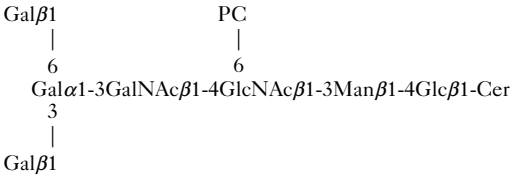
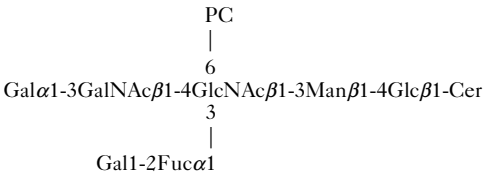
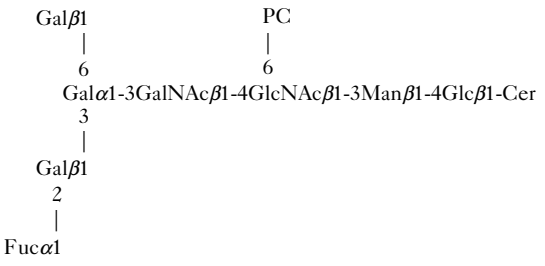
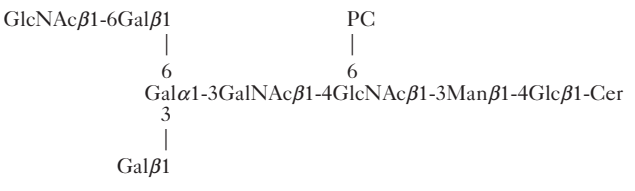
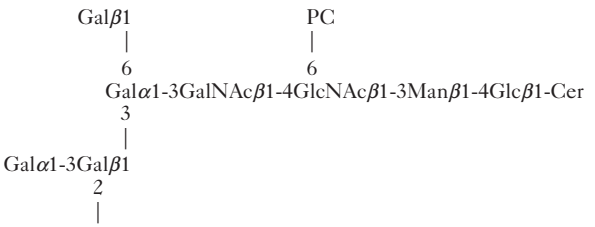
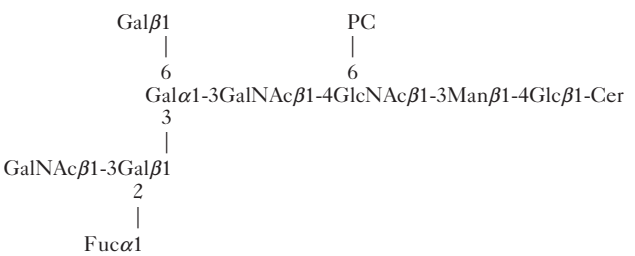
3.15.4.1.3 Platyhelminthes

Platyhelminthes are classified into Trematoda, Cestoda, Turbellaria, etc., and investigation of GSL structures of this phylum (**Table 3**) have focused on parasitic worms. An excellent review is also available by Dennis and Wiegandt on the chemistry, structure, and immunological properties of Platyhelminthes GSLs.[17]

Table 2 Structures of glycosphingolipids found in Nematoda

Structure	*Source*
Neutral GSL	
Glcβ1-Cer	*Ascaris suum*[41] *Caenorhabditis elegans*[39,42]
Manβ1-4Glcβ1-Cer	*A. suum*[41] *C. elegans*[39,42]
GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*A. suum*[41] *C. elegans*[39,42]
Galα1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*A. suum*[41]
Acidic GSL	
Galα1-2Ins-1-*P*-Cer	*A. suum*[44,45]
HSO_3-3Galβ1-Cer	*A. suum*[45]
Zwitterionic GSL	
PC | 6 GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*A. suum*[47,55] *Onchocerca volvulus*[48] *Acanthocheilonema viteae*[54]
PC Etn*P* | | 6 6 GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*A. suum*[55]
PC | 6 GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*C. elegans*[46] *A. suum*[47,55] *O. volvulus*[41]
PC Etn*P* | | 6 6 GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*A. suum*[55]
PC | 6 Galα1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*C. elegans*[46] *A. suum*[47,55] *O. volvulus*[41] *A. viteae*[54]
PC Etn*P* | | 6 6 Galα1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*A. suum*[49,55]
PC | 6 GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer 3 \| Fucα1	*A. suum*[55]
PC | 6 Galα1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer 3 \| Fucα1	*A. suum*[55]
Galβ1 PC \| \| 6 6 Galα1-3GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer	*A. suum*[47,55]

Table 2 (continued)

Structure	*Source*
	*C. elegans*[46] *A. suum*[47,55]
	*A. suum*[55]
	*A. suum*[55]
	*A. suum*[55]
	*A. suum*[55]
	*A. suum*[55]
	*A. suum*[55]

Abbreviations: PC, phosphocholine; Etn*P*, 2-aminoethanolphospho-.

Table 3 Structures of glycosphingolipids found in Platyhelminthes

Structure	*Source*
Trematoda	
Neutral GSL	
Galβ1-Cer	*Fasciola hepatica*[75] *Fasciola gigantica*[75] *Schistosoma mansoni*[67]
Glcβ1-Cer	*F. hepatica*[75] *F. gigantica*[75] *S. mansoni*[67]
Galβ1-4Glcβ1-Cer	*F. gigantica*[75]
GalNAcβ1-4Glcβ1-Cer	*S. mansoni*[62,65,67]
Galα1-4Galβ1-4Glcβ1-Cer	*F. hepatica* [75,76] *F. gigantica*[75]
Galα1-3Galβ1-4Glcβ1-Cer	*F. hepatica*[76]
Galβ1-6Galβ1-4Glcβ1-Cer	*F. hepatica*[76]
GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer	*S. mansoni*[65]
Galβ1-6Galα1-3Galβ1-4Glcβ1-Cer	*F. hepatica* [76]
Galβ1-6Galα1-4Galβ1-4Glcβ1-Cer	*F. hepatica*[76]
Galβ1-3GalNAc1-4Galβ1-4Glcβ1-Cer	*S. mansoni* (E)[66]
Galβ1-4GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer	*S. mansoni*[65]
Galβ1-6Galβ1-6Galα1-3Galβ1-4Glcβ1-Cer	*F. hepatica*[76]
Galβ1-6Galβ1-6Galα1-4Galβ1-4Glcβ1-Cer	*F. hepatica*[76]
GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer	*F. hepatica*[76]
GalNAcα1-3GalNAcβ1-4Galα1-3Galβ1-4Glcβ1-Cer	*F. hepatica*[76]
Fucα1-3GalNAcβ1-4GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer	*S. mansoni* (E) [66]
Galβ1-4GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer 3 \| Fucα1	*S. mansoni*[65]
Fucα1-3GalNAcβ1-4GlcNAcβ1-3GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer	*S. mansoni* (E) [66]
Galβ1-4GlcNAcβ1-3GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer 3 \| Fucα1	*S. mansoni*[65]
Fucα1-3Galβ1-4GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer 3 \| Fucα1	*S. mansoni*[65] *S. mansoni* (E) [66]
GalNAcβ1-4GlcNAcβ1-3GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer 3 \| Fucα1	*S. mansoni* (E) [66]
Fucα1-3GalNAcβ1-4GlcNAcβ1-4GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer 3 \| Fucα1	*S. mansoni* (E) [66]
Fucα1-3GalNAcβ1-4GlcNAcβ1-3GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer 3 \| Fucα1-2Fucα1	*S. mansoni* (E)[64,66]
Fucα1-3GalNAcβ1-4GlcNAcβ1-3GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer 3 \| Fucα1-2Fucα1-2Fucα1	*S. mansoni* (E) [64,66]
Fuc1-4GlcNAc1-2Fuc1-4GlcNAc1-2Fuc1-4GlcNAc1-2Fuc1-4GlcNAc1-3GalNAc1-3GalNAc1-4Glc1-Cer 3 3 3 3 | | | | Fuc1 Fuc1 Fuc1 Fuc1	*S. mansoni* (E)[63]

Table 3 (continued)

Structure	*Source*
Acidic GSL	
GlcNAcα1-HPO_3-6Galβ1-Cer	*F. hepatica*[78]
Cestoda	
Glcβ1-Cer	*Echinococcus multilocularis*[85] *Spirometra erinacei*[92]
Galβ1-Cer	*Spirometra mansonoides*[84] *E. multilocularis*[85,77] *Metroliasthes coturnix*[86] *Taenia crassiceps*[89] *Taenia solium cysticerci*[90] *Diphyllobothrium hottai*[94]
Glcβ1-3Galβ1-Cer	*D. hottai*[94]
Galα1-4Galβ1-Cer	*M. coturnix*[86]
Galβ1-6Galβ1-Cer	*M. coturnix*[86] *E. multilocularis*[77] *T. crassiceps*[89]
Galβ1-6Galβ1-6Galβ1-Cer	*M. coturnix*[86] *E. multilocularis*[77] *T. crassiceps*[89]
Fucα1-3Galβ1-6Galβ1-Cer	*E. multilocularis*[77] *D. hottai*[94]
Glcβ1-3Galβ1-Cer 6 | Galβ1	
Galβ1-6Galβ1-6Galβ1-6Galβ1-Cer	*M. coturnix*[86] *E. multilocularis*[77] *T. crassiceps*[89]
Galα1-4Galβ1-6Galβ1-6Galβ1-Cer	*T. crassiceps*[89]
Galβ1-6Galβ1-6Galβ1-Cer 3 | Fucα1	*E. multilocularis*[77]
Galβ1-4Glcβ1-3Galβ1-Cer 3 | Fucα1	*S. erinacei*[91] *D. hottai*[94]
Galβ1-4Glcβ1-3Galβ1-Cer 3 6 | | Fucα1 Galβ1	*S. erinacei*[93] *D. hottai*[94]

E, egg.

3.15.4.1.3.1 Trematoda

Schistosomiasis is a vascular parasitic disease caused by blood flukes of the genus *Schistosoma*. Although there is no good vaccine to prevent the disease, drug therapy is effective in most cases. This parasite has a complex life cycle which alternates between a definitive vertebrate host and an intermediate freshwater snail host. There are three major species infecting humans, *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium*. In 1970, the composition and biosynthesis of fatty acids and complex lipids were examined in the parasitic blood fluke, *S. mansoni*, and the free-living planarian *Dugesia dorotocephala*.[59] The report showed that *D. dorotocephala* contained two glycolipids, whereas no glycolipids were detectable in *S. mansoni*. The small amount of glycolipids found in *D. dorotocephala* was tentatively identified as cerebroside. GSL expression in the tegument has been shown by oxidation with galactose oxidase or periodate treatment followed by reduction with tritiated borohydride for *Schistosoma mansoni* schistosomula and adults.[60,61] In the parasitic blood fluke, *S. mansoni* of Trematoda, GalCer and GlcCer have been reported to be

major GSLs; no LacCer is biosynthesized by this animal.[62] Compositional and methylation analysis have demonstrated the presence of the 'schisto-'series, GalNAcβ1-4Glcβ1-Cer, as well as the likely presence of polyfucosylated GSL with a typical oligosaccharide structure.[63,64] GSLs from cercariae and eggs of this animal were analyzed in the form of their corresponding, pyridylaminated oligosaccharides, and over 10 oligosaccharide structures were characterized including Lewis X (Le^x), Galβ1-4(Fucα1-3)GlcNAcβ1- and pseudo-Lewis Y (Le^y), Fucα1-3Galβ1-4(Fucα1-3)GlcNAcβ1- epitopes.[65,66] The predominant ceramide species of these GSLs are composed of C16:0 fatty acid and C18 to -C20-phytosphingosines. An analysis of ceramide structures in the GSLs showed that monohexosylceramide and dihexosylceramide contained hydroxylated C16:0 as the major fatty acid in all the three life-cycle stages – adults, cercariae, and eggs.[67] Sphingoids were C18- and C20-phytosphingosines in egg ceramide monohexoside (CMH, GlcCer:GalCer=1:0.25), C18-sphinganine as well as C18-, C19-, and C20-phytosphingosines in cercarial CMH (GlcCer:GalCer=1:0.1), and C18- and C20-phytosphingosines as well as C18-sphingosine in adult CMH (GlcCer:GalCer=1:0.5), differing in stage-associated expression.

There are numerous reports relating to the immunochemical characterization of schistosomiasis concerning the carbohydrate determinants in both glycoproteins and glycolipids of adult worms and their eggs.[68] It is notable that Le^x, also termed CD15 (leucocyte cluster of differentiation antigen 15),[69,70] SSEA I[71] (stage-specific embryonic antigen I), or fucose-containing epitope, is shared by the parasite and the mammalian host as the case of keyhole limpet hemocyanin.[72] Furthermore, Le^x and pseudo-Le^y GSL are stage-specifically expressed by the cercarial life-cycle stage, and not by the adult or egg, and these characteristic epitopes are recognized by the DC-specific C-type lectin, DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin, CD209).[73,74]

Fascioliasis is a chronic disease of veterinary and medical importance in domestic animals and humans. Neutral GSLs from the liver fluke, *Fasciola hepatica* and *F. gigantica*, were isolated and characterized as GalCer, GlcCer, LacCer, and globotriaosylceramide (Galα1-4Galβ1-4Glcβ1-Cer), which is designated as P^k-blood group antigen or CD77.[75] Additionally, isoglobotriaosylceramide (Galα1-3Galβ1-4Glcβ1-Cer) and Forssman antigen (GalNAcα1-3GalNAcβ1-3/4Galα1-4/3Galβ1-4Glcβ1-Cer) were isolated as mammalian-type GSL species.[76] Furthermore, highly antigenic GSLs were characterized as Galβ1–6Gal-terminating with globo- and isoglobo-series core structures. These GSLs account for cestode serological cross-reactivity found in human *Echinococcus granulosus* (cestode) infection sera.[77]

The acidic GSL from this animal was isolated and characterized as GlcNAcα1-HPO_3-6Gal1-Cer.[21] This GSL was shown to be highly antigenic and strongly recognized by both animal and human *F. hepatica* infection sera. And the antigenic determinant GlcNAcα1-HPO_3-might have a potential in the serodiagnosis of *F. hepatica* infections from the result of enzyme-linked immunosorbent inhibition assay (ELISA). The ceramide moieties of most GSLs from this animal were composed of C18- and C20-phytosphingosines as the main sphingoids, and 2-hydroxylated C18:0 as the major fatty acid.[78]

3.15.4.1.3.2 Cestoda

Since 1941, the glycolipid components of the Cestoda have been verified by TLC with chemical detection in *Cysticercus fasciolaris*,[79] *Taenia taeniaeformis*,[80] *E. granulosus*,[81] *Echinococcus multilocularis*,[82] *Taenia crassiceps*, *Taenia solium*, and *Taenia saginata*.[83] In 1987, hydroxylated GalCer was isolated from the tegument of the adult and the plerocercoid larve of a pseudo-phyllidean cestode, *Spirometra mansonoides*.[84] The predominant ceramide species of GSL are composed of C18:0 and 2-hydroxylated C18:0 fatty acid and dihydrosphingosine and phytosphingosine. Monohexosyleramides of *E. multilocularis* have been isolated and characterized as GalCer and GlcCer with C16:0 and C26:0 fatty acids and their hydroxylated derivatives, and sphingosine and phytosphingosine as major sphingolipids.[85]

The combination of liquid chromatography and fast atom bombardment mass spectrometry (FAB-MS) has recently been applied to GSL structural analysis. Most neutral GSLs from cestoda seem to be classified in the neogala-series GSL. The major GSLs of the cestode, *Metroliasthes coturnix*, were isolated and characterized as a series of mono-, di-, tri-, and tetra galactosylceramides.[86] These were further characterized as Galα1-4GalCer, Galβ1-6Galβ1-6GalCer, and Galβ1-6Galβ1-6Galβ1-6GalCer. These ceramides were predominantly composed of C26:0 fatty acid and C18–C20 sphinganine and phytosphingosine. Neutral GSLs in the metacestode stage of the parasite, *E. multilocularis*, were investigated and characterized as four neogala-series GSLs, including the two fucose-containing GSLs, Fucα1-3Galβ1-6GalCer and Galβ1-6(Fucα1-3)Galβ1-6GalCer.[77] Ceramides contained sphinganine and either non-hydroxy fatty acids with C16, C18, C26, and C28, or hydroxyl fatty acids with C16 and C18. An immunological study has reported that these neutral GSLs inhibit human peripheral blood mononuclear cell proliferation.[87] In this animal, several ganglioside species were also found in low quantities and identified after preparative high-performance TLC.[88] From metacestode of the fox tapeworm, *T. crassiceps*, the three simplest GSLs have been isolated and determined to consist of two neogala-series GSLs and an elongated tetrahexosylceramide, Galα1-4Galβ1-6Galβ1-6Galβ1-6GalCer.[89] The major ceramide fatty acids have particularly long chains, predominantly

hexacosanoic and octacosanoic acids. From the human tapeworm, *T. solium*, GalCer was identified and was composed mainly of phytosphinganine and C16–C24 fatty acids with predominated 2-hydroxylated derivatives. Immunoreactivities to this GSL were observed in human sera and cerebrospinal fluids.[90]

From the plerocercoids of the tapeworms, *Spirometra erinacei*, neutral GSLs were isolated and characterized as Galβ1-4(Fucα1-3)Glcβ1-3Galβ1-Cer and Galβ1-4(Fucα1-3)Glcβ1-3(Galβ1-6)Galβ1-Cer.[91–93] These characteristic 'spirometo-series' GSLs were also found in *Diphyllobothrium hottai* in both adult worm and plerocercoid.[94] A monoclonal antibody established against Galβ1-4(Fucα1-3)Glcβ1-3Galβ1-Cer reacted with Galβ1-4(Fucα1-3)Glcβ1-3(Galβ1-6)Galβ1-Cer and also cross-reacted with the SSEA I antigen.[95] Immunohistochemical staining with this antibody showed the epitope located in the tegument of *S. erinacei* and the inner surface of bothria of *D. hottai*.[94] The ceramide was composed of sphinganine and phytosphingosine as major sphingoids, and C16:0–C28:0, their unsaturated derivatives, and C28:1 as major fatty acids. Analysis of the ceramide moieties of monohexosylceramide showed that the glucocerebrosides of plerocercoids contained only C18:0 fatty acid, whereas those of adult tapeworm contained varying ceramide moieties.

3.15.4.1.4 Annelida

Although Annelida is classified into Oligochaeta, Polychaeta, Hirudinea, etc., their habitable environments are as different as land, freshwater, brackish water, and marine. From the viewpoint of medical usage, earthworm and leech have been used in Chinese medicine. It might be expected that compounds derived from them have pharmacological functions. One set of annelida GSLs was examined for fruiting-inducing activity and antifungal properties; zwitterionic GSLs with two or three double bonds in the sphingosine have the inducing activity (**Table 4**).[96] A synthetic zwitterionic GSL was found to inhibit histamine release.[97,98]

3.15.4.1.4.1 Oligochaeta

In 1985, the lipid composition of the ventral nerves of earthworm, *Lumbricus terrestris* of Oligochaeta, was investigated as part of a systematic study of the evolution of the nervous system.[28] Neither GalCer nor sulfatide, both of which are considered to be markers for myelin, were present, while only traces of GlcCer were found. Neutral GSLs in the earthworm, *Pheretima* sp. (*P. hilgendorfi* and *P. aspergillum*), have been characterized as LacCer (Galβ1-4Glcβ1-Cer), which is the major GSL in vertebrates; gala-series (Galβ1-6Galβ1-), which is the major GSL in Mollusca sea snails, and oligosaccharide structures with Glc or Man linked α1-4 to gala-series.[99–101]

From this animal, a series of GSLs containing PC have been characterized as zwitterionic GSLs.[102] It can be said that this discovery has had great impact on other researchers in this field, leading to discoveries of PC-containing GSLs in other Annelida or Nematoda. Also nine zwitterionic GSLs were isolated from the earthworm, *P. asiatica*.[103] The ceramide moieties of these GSLs consisted of C22:0, C23:0, and C24:0 as major fatty acids, and branched octadeca- and nonadeca-4-sphingenines and octadeca-4-sphingenine as the main sphingoids. Considering the similarity of the ceramide components in both neutral and zwitterionic GSLs, it appears likely that the zwitterionic GSLs in the earthworm are biochemically derived from the neutral GSLs. Neutral and zwitterionic GSL analogs were chemically synthesized and assayed in an *in vitro* histamine release-inhibition test. Two zwitterionic GSLs appeared to act as inhibitors of histamine release from rat basophilic leukemia cells (RBL-2H3).[98]

The lipid composition of GSLs of the earthworm *L. terrestris* was reported by other researchers, including cerebrosides and sulfatides containing both glucose and galactose, gangliosides containing glucosamine and sialic acid, and sphingomyelin as well as glycerophospholipids.[104]

Sphingomyelin, a widespread PC-containing sphingolipid and a constituent of membranes, is absent in *L. terrestris* ventral nerve,[28] and it is interesting to speculate that PC-containing GSLs may have sphingomyelin-like functions in earthworms. It is also interesting that the purified protein, lysenin, from the coelomic fluid of the earthworm *Eisenia foetida* induced erythrocyte lysis and bound specifically to sphingomyelin.[105,106]

3.15.4.1.4.2 Polychaeta

From the marine annelid, *Marphysa sanguinea*, and *Neanthes diversicolor*, also of Polychaeta, up to 14 GSLs were isolated and completely characterized as PC-containing zwitterionic GSLs.[107–109] This was the first discovery of PC-containing GSLs in nature.[107] The ceramide consisted of C16:0, C17:0, and C18:0 fatty acids and sphingosine, (4*E*,8*E*)-sphigadiene, and (4*E*,8*E*,10*E*)-sphingatriene. From the marine annelid, *Pseudopotamilla occelata*, also of Polychaeta, GSLs have been characterized as follows: Galβ1-Cer, Galα1-4Galβ1-Cer, LacCer, and amino-CTH (GlcNAcβ1-3Galβ1-4Glcβ1-Cer) (the latter two structures being also found in vertebrate GSLs), as well as neutral

Table 4 Structures of glycosphingolipids found in Annelida

Structure	*Source*
Oligochaeta	
Neutral GSL	
Galβ1-Cer	*Lumbricus terrestris*[28]
	Pheretima hilgendorfi[102]
	Pheretima aspergillum[101]
Glcβ1-Cer	*P. hilgendorfi*[101]
	P. aspergillum[101]
Galβ1-6Galβ1-Cer	*P. hilgendorfi*[101,102]
	P. aspergillum[101]
Galβ1-4Glcβ1-Cer	*P. hilgendorfi*[101]
	P. aspergillum[101]
Galβ1-6Galβ1-6Galβ1-Cer	*P. hilgendorfi*[101,102]
	P. aspergillum[101]
Glcα1-4Galβ1-6Galβ1-Cer	*P. hilgendorfi*[101]
	P. aspergillum[101]
Manα1-4Galβ1-6Galβ1-Cer	*P. hilgendorfi*[101]
	P. aspergillum[101]
Galβ1-6Galβ1-6Galβ1-6Galβ1-Cer	*P. hilgendorfi*[101]
	P. aspergillum[101]
Glcα1-4Galβ1-6Galβ1-6Galβ1-Cer	*P. hilgendorfi*[101]
	P. aspergillum[101]
Galα1-6Galβ1-6Galβ1-Cer 4 \| Manα1	*P. hilgendorfi*[101]
Glcα1-4Galβ1-6Galβ1-6Galβ1-Cer 4 \| Glcα1	*P. hilgendorfi*[101]
Zwitterionic GSL	
PC-6Galβ1-Cer	*P. hilgendorfi*[98,101]
	P. aspergillum[101]
	Pheretima asiatica[103]
PC-6Galβ1-6Galβ1-Cer	*P. hilgendorfi*[98,101]
	P. aspergillum[101]
	P. asiatica[103]
PC-6Galβ1-6Galβ1-6Galβ1-Cer	*P. hilgendorfi*[98,100,101]
	P. aspergillum[101]
PC-6Galβ1-6Galβ1-Cer 4 \| Manα1	*P. hilgendorfi*[100,101]
	P. aspergillum[101]
Polychaeta	
Neutral GSL	
Glcβ1-Cer	*Pseudopotamilla occelata*[110]
Galβ1-Cer	*P. occelata*[110]
	Tylorrhynchus heterochetus[112]
Galα1-4Galβ1-Cer	*P. occelata*[110]
Galβ1-4Glcβ1-Cer	*P. occelata*[110]
GlcNAcβ1-3Galβ1-4Glcβ1-Cer	*P. occelata*[110]
Xylβ1-4Fucα1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer	*P. occelata*[111]
Gal2Meα1-3Fucα1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer	*P. occelata*[111]
Xylβ1-4Fucα1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer 3 \| Gal2Meα1	*P. occelata*[111]

Table 4 (continued)

Structure	*Source*
Acidic GSL	
Ins-1-*P*-Cer	*T. heterochetus*[112]
InsMe-1-*P*-Cer	*T. heterochetus*[112]
Manα1-2Ins-1-*P*-Cer	*T. heterochetus*[112]
Man-InsMe-1-*P*-Cer	*T. heterochetus*[112]
Fucα1-5Ins-1-*P*-Cer	*T. heterochetus*[112]
Fuc-InsMe-1-*P*-Cer	*T. heterochetus*[112]
Fucα1-5Ins-1-*P*-Cer 2 \| Manα1	*T. heterochetus*[112]
Zwitterionic GSL	
PC-6Galβ1-Cer	*Neanthes diversicolor*[107,108,109] *T. heterochetus*[112]
Hirudinea	
Neutral GSL	
Galβ1-Cer	*Hirudo nipponica*[114]
Galα1-6Galβ1-Cer	*H. nipponica*[114]
Galα1-6Galα1-6Galβ1-Cer	*H. nipponica*[113,114]
Galα1-6Galβ1-6Galβ1-Cer	*H. nipponica*[113]
Galα1-6Galα1-6Galα1-6Galβ1-Cer	*H. nipponica*[114]
Zwitterionic GSL	
PC-6Galβ1-Cer	*H. nipponica*[114]
PC-6Galβ1-6Galβ1-Cer	*H. nipponica*[114]

Abbreviation: PC, phosphocholine.

GSLs containing xylose and methyl sugar with a branching fucose.[110,111] Although no zwitterionic GSLs have been detected, acidic GSLs containing inositol phosphate and mannose have been characterized.

From the brackish water lugworm, *Tylorrhynchus heterochetus* of Polychaeta, the simple neutral GSL, Galβ1-Cer, has been characterized, as well as a zwitterionic GSL in which PC is attached to cerebroside.[112] As an acidic GSL, a group of inositol phosphate-containing GSL has been found in which fucose and mannose are linked to inositol.[113] Furthermore, existence of methyl-inositol has been shown for the first time as a component in a complex lipid, although the linkage position of the methyl group to inositol has not been determined yet.

3.15.4.1.4.3 Hirudinea

From the freshwater leech, *Hirudo nipponica* of Hirudinea, neutral GSL containing two Galα1-6 core series with different anomeric configurations have been characterized as Galα1-6Gal and Galβ1-6Gal.[114,115] From the leech, *Hirudo medicinalis*, ceramide of GalβCer contained an unusual polyunsaturated sphingosine analog and C25:2, C27:1, C27:3, C28:3, C29:3, C30:3, and C33:3 and hydroxylated C27:2 fatty acids.[116] It would be interesting to know whether the GSLs of this animal could be cleaved by ceramide glycanase or endoglycoceramidase. This enzyme is a GSL-specific enzyme that hydrolyzes the glycosidic linkage between oligosaccharides and ceramides and has been found in both prokaryotes and eukaryotes, that is, actinomycetes, bacteria, leech,[117,118] short-necked clam, earthworm,[119] jellyfish,[120] and hydra.[29] Endoglycoceramidase from the hydra, *Hydra magnipapillata*, has quite low activity to invertebrate GSLs, especially Galβ1-6Galβ1-6Galβ1-Cer from the sea snail.

The presence of zwitterionic GSLs has been found as a common feature of earthworm *Pheretima* sp., and GSLs containing PC linked to GlcNAc have also been purified from leech.[112] The ceramide moieties of these GSLs consisted of C16:0, C18:0, C22:0, and C24:0 as major fatty acids, and dihydroxy-(d18:1, d19:1, and d22:3) and trihydroxy-(t18:0 and t19:0) sphinganines.

Several interesting studies of gangliosides in this animal have been reported.[121] The monoclonal antibody, A2B5, recognizing vertebrate gangliosides, also recognizes embryonic cells in the medicinal leech, *Hirudo medicinalis*, and

demonstrates that the expression of epitope is regulated in a time- and space-dependent way, and A2B5-positive glycolipids could be isolated from embryonic leeches. The effects of antiganglioside antibodies on the electrical activity of Retzius neurons in the leech have been studied using antiganglioside antiserum prepared by the immunization of rabbits with total bovine brain gangliosides.[122]

3.15.4.1.5 Mollusca

Mollusca are classified into Bivalvia such as the bivalves, *Corbicula sandai* and *H. schlegelii* from Lake Biwa, and Gastropoda such as sea snail, *Turbo cornutus*, and sea hare, *Aplysia kurodai*, and Cephalopoda such as squid and octopus, etc. A review is also available by Hori and Sugita on the chemistry, structure, and immunological properties of Mollusca GSLs.[123]

3.15.4.1.5.1 Bivalvia

As early as 1959, a study reported an unusual glycolipid from oyster of Bivalvia.[124] The glycolipids of several species belonging to Bivalvia were later verified by TLC with chemical detections.[125] This study showed the presence of cerebroside-like glycolipid and very polar glycolipids, and the absence of ganglioside like most invertebrate animals. GSL structural analysis has shown the occurrence of 3-*O*-methylfucose in shellfish, *C. sandai*,[126] and branched sugar structures containing 3-*O*-methylgalactosamine in oyster glycolipid.[127,128] From the bivalve, *H. schlegelii*[129–134] and *C. sandai*,[135–138] a group of GSL containing mannose (mannnolipid) has been characterized by chemical analysis. The composition and structure of invertebrate GSLs as compared to those of vertebrate GSLs are indeed remarkably different (**Table 5**). Their characteristic core oligosaccharide structure (GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-) has been termed 'mollu-series', derived from the name Mollusca. It is different from the mannolipid of Arthropoda, which was investigated later. In Mollusca neutral GSLs, fucose is located in the middle of oligosaccharide structure and the structures are rich in methyl sugars such as 3-*O*-methylxylose, 3-*O*-methylgalactosamine, and 3-*O*-methyfucose.[139] Furthermore, it has been shown clearly that *O*-methyl sugar exists in the nonreducing end of the oligosaccharide sequence. In the seawater bivalve, *Meretrix lusoria*, neutral GSL composition is very similar to that of the freshwater bivalves.[140] Using antisera against Mu_4Cer, the GSL antigens are limited to certain taxonomic orders of the shellfish species tested, namely to *H. schlegeli*, *Cristaria plicata*, and *Inversidens reiniana* (order Palaeoheterodonta), and to *C. sandai* and *M. lusoria* (order Heterodonta).[141]

The existence of an acidic GSL containing uronic acid (4-*O*-methylglucuronic acid) has been clarified for the first time in Protostomia animals, and research has also shown the presence of this acidic GSL in the taxonomically related Palaeoheterodonta, such as *H. schlegeli*, *Anodonta woodiana*, and *C. plicata*.[142] Moreover, immunohistochemical observations have shown clearly that this acidic GSL exists only on the surface of sperm.[143] In contrast, Etn*P*-containing polar GSL exists only in egg of *H. schlegelii* and another bivalve, *C. sandai*.[144] This is supported by the result that antiserum against this acidic antigen agglutinates spermatozoa of three kinds of freshwater bivalves, *H. schlegeli*, *A. woodiana*, and *C. plicata*, but does not agglutinate those of *C. sandai*.[143]

3.15.4.1.5.2 Gastropoda

Gastropoda includes as minor classes the sea snail, sea hare, etc. GSLs from the sea snail, *T. cornutus* of Gastropoda, contain a series of GSLs in which galactose is the only sugar. These neutral GSLs are the so-called gala-6-(neogala) series, Galβ1-6Galβ1-6Galβ1-6Galβ1- sequence (**Table 5**).[145] GSL belonging to the gala-6-series have been found to occur commonly in sea snails, *Monodonta labio*, *Chlorostoma argyrostoma turbinatum*, and *Nerita albicilla*.[146] From the sea abalone, *Haliotis japonica*, another series of neutral GSL structures has been characterized as fucose-containing lactosyl derivatives.[147] Particular attention has been paid to GSLs of sea snail, because polar GSLs contain C–P compounds such as aminoethylphosphonate (AEPn), and/or its monomethylated derivative (MAEPn) on galactosylceramide (phosphonoglycosphingolipid; PnGSL).[148–151] The distribution of these PnGSL's with various sugar chains has been investigated by FAB-MS as applied to snail GSL.[152]

More than 70 natural sphingoids, including sphingosine, phytosphingosine, monoenoic, and dienoic sphingoid, have been found in the ceramide moiety of sea snail GSLs. A trienoic sphingoid, octadecaphinga-4,8,10-trienine, was found in sphingolipids obtained from some gastropods (*M. labio*, *Cellana eucosmia*, *C. a. tubinatum*, and *Pugilina ternatana*), pelecypods (*Ostrea gigas*), coelenterates (*Anthopleura midori*), and brachiopoda (*Lingula unguis*). Furthermore, evidence has also been found for a nonadecasphigatrienine occurring in *P. ternatana*.[153]

Table 5 Structures of glycosphingolipids found in Mollusca and Brachiopoda

Structure	*Source*
Mollusca	
Bivalvia	
Neutral GSL	
Glcβ1-Cer	*Hyriopsis schlegelii* (E)[134]
	H. schlegelii (S)[129]
	Meretrix lusoria[140]
Galβ1-Cer	*H. schlegelii* (E) [134]
	H. schlegelii (S) [129]
	M. lusoria[140]
Manβ1-Cer	*H. schlegelii* (H)[132]
Galβ1-4Glcβ1-Cer	*H. schlegelii* (H)[133]
	H. schlegelii (E) [134]
	M. lusoria[140]
Galβ1-4Galβ1-Cer	*H. schlegelii* (S)[131]
	H. schlegelii (H) [133]
	H. schlegelii (E) [134]
Manβ1-4Glcβ1-Cer	*Corbicula sandai*[135]
	H. schlegelii (S) [131]
	H. schlegelii (H) [133]
	H. schlegelii (E) [134]
	M. lusoria[140]
Manβ1-2Manβ1-Cer	*H. schlegelii* (H) [133]
Galα1-3Manβ1-4Glcβ1-Cer	*M. lusoria*[140]
Manα1-4Manβ1-4Glcβ1-Cer	*C. sandai*[136]
Manα1-3Manβ1-4Glcβ1-Cer	*H. schlegelii* (S) [131]
	H. schlegelii (H) [133]
	H. schlegelii (E) [134]
	M. lusoria[140]
Manα1-3Manβ1-2Manβ1-Cer	*H. schlegelii* (H) [133]
GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-Cer	*H. schlegelii* (S)[137]
	H. schlegelii (E) [134]
	M. lusoria[140]
Manα1-2?Manα1-3Manβ1-4Glcβ1-Cer	*H. schlegelii* (E) [134]
Manα1-3Manβ1-4Glcβ1-Cer 2 \| Galβ1	*H. schlegelii* (E) [134]
Manα1-3Manβ1-4Glcβ1-Cer 2 \| Xylβ1	*H. schlegelii* (S) [131] *H. schlegelii* (E) [134]
GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-Cer 2 \| Xylβ1	*H. schlegelii* (S)[130] *M. lusoria*[140]
Gal4Meβ1-3GalNAcβ1-3Fucα1-4GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-Cer 2 \| Xylβ1	*C. sandai*[138]
Fuc3Meα1-2Xyl3Meβ1 \| 4 Fucα1-4GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-Cer 3 2 \| \| GalNAc3Meα1 Xylβ1	*H. schlegelii* (S)[139] *M. lusoria*[140]

(continued)

Table 5 (continued)

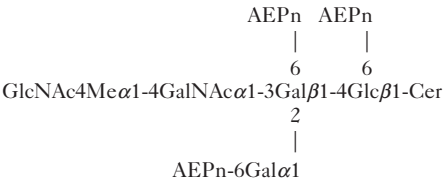
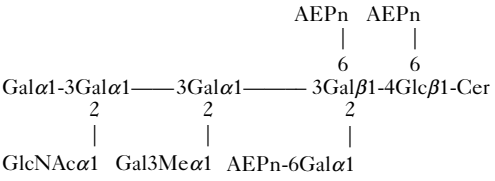
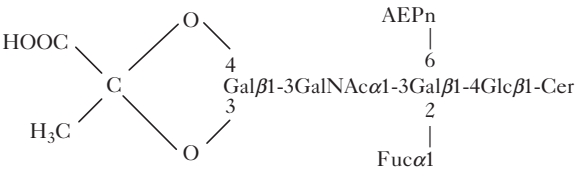
Structure	*Source*
Fuc3Meα1 \| 2 GlcNAc1-3Gal1-4GalNAc1-3Galβ1-3Galβ1-4Glcβ1-Cer 2 \| GalNAc3Me1-3Gal1 2 \| Fucα1	*Osteria gigas*[127,128]
Acidic GSL	
GlcA4Meβ1 \| 4 Fucα1-4GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-Cer 3 2 \| \| GalNAc3Meα1 Xylβ1	*H. schlegelii* (S)[142] *M. lusoria*[140]
Polar GSL	
EtnP | 6 Gal4Meβ1-3GalNAcβ1-3Fucα1-4GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-Cer 2 \| Xylβ1	*C. sandai*[144]
Gastropoda	
Neutral GSL	
Galβ1-Cer	*Chlorostoma argyrostoma turbinatum*[146]
Galβ1-6Galβ1-Cer	*Turbo cornutus*[145] *C. a. turbinatum*[146]
Galβ1-6Galβ1-6Galβ1-Cer	*T. cornutus*[145] *C. a. turbinatum*[146]
Galβ1-6Galβ1-6Galβ1-6Galβ1-Cer	*C. a. turbinatum*[146]
Fucα1-3GalNAcα1 \| 3 Galβ1-4Galβ1-Cer 2 \| Fucα1	*Haliotis japonica*[147]
Polar GSL	
AEPn-6Galβ1-Cer	*T. cornutus*[149] *Monodonta labio*[150]
MAEPn-6Galβ1-Cer	*T. cornutus*[149] *M. labio* [150] *C. a. turbinatum*[148,146]
MAEPn-6Galβ1-6Galβ1-6Galβ1-Cer	*T. cornutus*[151]
MAEPn | 6 Fuc1-2Gal3Me1-3GalNAc1-3Gal1-4Glc-Cer 2 | Gal1	*C. a. turbinatum*[152]

Table 5 (continued)

Structure	*Source*
MAEPn | 6 Gal3Me1-3GalNAc1-3Gal1-3GalNAc1-3Gal1-4Glc-Cer 2 2 2 | | | Fuc1 Fuc1 Gal1	*C. a. turbinatum*[152]
Opisthobranchia	
Neutral GSL	
Glcβ1-Cer	*Aplysia juliana*[168]
Galβ1-Cer	*A. juliana*[168]
Galβ1-4Glcβ1-Cer	*A. juliana*[168]
Galα1-2Galβ1-4Glcβ1-Cer	*A. juliana*[168]
Fucα1-2Galβ1-4Glcβ1-Cer	*A. juliana*[168]
GalNAcα1-3Galβ1-4Glcβ1-Cer 2 \| Galα1	*A. juliana*[168]
Polar GSL	
AEPn | 6 Galα1-2Galβ1-4Glcβ1-Cer	*A. juliana*[169]
Gal3Meα1-3GalNAcα1-3Galβ1-4Glcβ1-Cer 2 \| AEPn-6Galα1	*Dolabella auricularia*[170]
Gal3Meβ1-3GalNAcα1-3Galβ1-4Glcβ1-Cer 2 \| AEPn-6Galα1	*Aplysia kurodai*[158]
AEPn | 6 Gal3Meβ1-3GalNAcα1-3Galβ1-4Glcβ1-Cer 2 \| AEPn-6Galα1	*A. kurodai*[157]
AEPn AEPn | | 6 6 Gal3Meα1-3Galβ1-4Glcβ1-Cer 2 \| AEPn-6Galα1	*A. kurodai*[161]
AEPn AEPn | | 6 6 Gal3Meβ1-3GalNAcα1-3Galα1-4Glcβ1-Cer 2 \| AEPn-6Galα1	*A. kurodai*[164]

(continued)

Table 5 (continued)

Structure	*Source*
	*A. kurodai*[159]
	*A. kurodai*[160]
	*A. kurodai*[163]
Brachiopoda	
Glcβ1-Cer	*Lingula unguis*[173]
Manβ1-4Glcβ1-Cer	*L. unguis*[173]
Manα1-3Manβ1-4Glcβ1-Cer	*L. unguis*[173]
GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-Cer	*L. unguis*[173]
GlcNAcβ1-4GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-Cer	*L. unguis*[173]

Abbreviations: Etn*P*, 2-aminoethanolphospho-; MAEPn, (*N*-methyl-2-aminoethyl)hydroxyphosphoryl-; AEPn, (2-aminoethyl) hydroxyphosphoryl-.
E, egg; G, gonad; H, hepatopancreas; S, sperm.

The sea hare, *A. kurodai* of Gastropoda, has been investigated with interest in the comparison of lipid content in the nervous system between sea hare and vertebrates. It has also been used in investigation of tissue-specific distribution of GSLs, for example, in nervous tissue, skin, and egg.[154,155] A group of GSLs containing 1–3 mol of AEPn with a GalNAcα1-3Galβ1-4Glcβ1- sequence were found in the skin,[156–159] GSLs with lactosyl core in the egg,[160,161] and acidic GSLs containing pyruvic acid in the nerve fibers.[162,163] Furthermore, some of these GSLs in sea hare contain methylated sugars such as 3-*O*-metylgalactose and 4-*O*-methyl-*N*-acetylglucosamine, which are unique among Mollusca GSLs. Unlike the PnGSL's of sea snail, those of sea hare contain predominantly AEPn rather than MAEPn.[164] In the sea hare, the PnGSL containing pyruvic acid has been localized in nerve bundles.[165,166] This restricted expression suggests that the PnGSL may have some neurobiological function.[167] The GSLs containing C–P compounds are called phosphonoglycolipids and research on those particular GSLs now forms one field in glycobiology. Neutral GSLs and PnGSL's were isolated from other sea hares, *Aplysia juliana* and *Dolabella auricularia*, and shown to have a lactosyl core.[168–170]

3.15.4.1.5.3 Cephalopoda

Acidic GSLs were isolated from hepatopancreatic tissues of the marine squid, *Todarodes pacificus*, and the pacific octopus, *Octopus vulgaris* of Cephalopoda.[171,172] These resorcinol-positive acidic lipids were found to be reactive with A2B5 monoclonal antibody, which reacts to c-series gangliosides. Using chemical analysis, two major acidic lipids were identified as GT3 and GQ1c. Immunochemically, these acidic lipids were distributed in hepatopancreas, cerebral ganglion, and eye lens at different concentrations.

3.15.4.1.6 Brachiopoda

Because Brachiopoda morphologically resemble clams, they have long been classified as Mollusca. The lamp shell, *Lingula unguis* of Brachiopoda, with its morphological resemblance to bivalves, has sometimes been included

among the Molluscoidea; this resemblance, plus the similarity of its GSL oligosaccharide core structure to that of Mollusca, are interesting factors in considering their evolution. During the Paleozoic era, they were extremely abundant. Since many of their fossils have been discovered, the existing Brachiopoda is called 'living fossil'. From this animal, a group of neutral GSLs containing the mollu-series have been characterized.[173] Like the GSLs in Mollusca, GSLs of this animal with longer oligosaccharides contain a branching fucose and O-methylated sugars (**Table 5**).

3.15.4.2 Deuterostomia

3.15.4.2.1 Echinodermata

Echinodermata are divided taxonomically into five classes, namely Echinoidea (sea urchin), Asteroidea (starfish), Holothuroidea (sea cucumber), Crinoidea (feather star), and Ophiuroidea (brittle star). Echinodermata contain several unique GSLs.[125]

All Echinodermata contain only glucocerebroside (Glcβ1-Cer) as the monoglycosylceramide.[174,175,178–184,186–200] A diverse array of neutral GSLs is found in the sea urchin and starfish species. Eggs of the sea urchin, *Anthocidaris crassispina*, contain melibiosylceramide[174] as the sole diglycosylceramide, and the novel trihexosylceramide[175] and difucosylated GSL[176,177] have been reported in eggs of another sea urchin, *Hemicentrotus pulcherrimus*. This raises the question whether, in, for example, *A. crassispina* and *H. pulcherrimus*, structural differences in their GSLs might be related to morphological differences in their embryonic development.[175] Spermatozoa of the starfish, *Asterias amurensis*, contain three dihexosylceramides: lactosylceramide and two diglucosylceramides, namely gentiobiosylceramide and cellobiosylceramide.[185]

The sphingoids are generally composed of mixtures of phytosphingosines with both branched and linear chains, and the fatty acids of mixtures of normal, monounsaturated, and 2-hydroxy fatty acids. GSLs isolated from the starfish, *A. amurensis*,[184] *Ophidiaster ophidianus*,[186] and *Cosmasterias lurida*;[188] the sea cucumber, *Pentacta australis*;[194] and the sea urchin, *Temnopleurus toreumaticus*,[178] contain (4*E*,8*E*,10*E*)-2-amino-4,8,10-octadecatriene-1,3-diol (d18:3) and (4*E*,8*E*,10*E*)-2-amino-9-methyl-4,8,10-octadecatriene-1,3-diol (d19:3) as sphingoid components (**Table 6**).

3.15.4.2.1.1 Echinoidea (sea urchin)

Acidic GSLs, gangliosides, in the sea urchins contain (besides sialic acid) only glucose as the neutral sugar component. Systematic studies of sea urchin gangliosides suggest a common carbohydrate backbone composed of glucose and sialic acid, attached to Glcβ1-Cer at position C6.

It seems worthwhile to note the clear contrast between the egg and sperm gangliosides of *A. crassispina* in their constituents as so far elucidated.[201,202] The egg gangliosides are more hydroxylated than the sperm ones in this species: sialic acid is exclusively NeuGc in the egg gangliosides, but only NeuAc in the sperm ones; sphingoids are exclusively phytosphingosines in the egg ones but sphingenines in the sperm ones; fatty acids are mostly 2-hydroxy fatty acids in the egg ones while there is no hydroxyl fatty acid in the sperm ones. As the 'more-hydroxylated' gangliosides are also found in gonads[203] and embryos[204] which contain somatic cells, the 'less-hydroxylated' gangliosides might be characteristic of spermatozoa.

The finding of sulfated gangliosides is especially noteworthy in that the sulfate group exclusively resides on the C8 of the nonreducing terminal residues of oligo- and/or polysialyl chains[202–205] and that sulfation appears to be a termination signal for elongation of oligosialyl chains.[205]

Some gangliosides purified from the sea urchins are potent haptens and induce an immune response, and the antiserum obtained provides a useful reagent for the immunological analysis of the organization, distribution, localization, and function of GSL molecules in cell membranes.[206–211]

3.15.4.2.1.2 Asteroidea (starfish)

Sialic acid appears at not terminal but internal position in many starfish gangliosides[212–214,216,218–222,225] and binds to the glycolyl group of the penultimate sialic acid,[215,217,228,230] and some gangliosides of the starfish carry arabinose residues in furanose form as the terminal sugar substituting at positions C3 or C6 of the penultimate galactose.[212–214,216,221,222]

Table 6 Structures of glycosphingolipids found in Echinodermata and Urochordata

Structure	*Source*
Echinodermata	
Neutral GSL	
Sea urchin	
Glcβ1-Cer	*Anthocidaris crassispina* (E)[174]
	Hemicentrotus pulcherrimus (E)[175]
	Temnopleurus toreumaticus[178]
Galα1-6Glcβ1-Cer	*A. crassispina* (E) [174]
Galβ1-6Galβ1-6Glcβ1-Cer	*H. pulcherrimus* (E) [175]
Fucα1-3GalNAcβ1 | 4 GlcNAcβ1-4Glcβ1-Cer 3 | Fucα1	*H. pulcherrimus* (E)[176,177]
Starfish	
Glcβ1-Cer	*Asterias rubens*[179,180]
	Asterias pectinifera[181]
	Acanthaster planci[182]
	Astropecten latespinosus[183]
	Asterias amurensis (S)[184]
	Ophidiaster ophidianus[186]
	Pentaceraster regulus[187]
	Cosmasterias lurida[188]
	Allostichaster inaequalis (G, body wall)[189]
	Luidia maculate[190]
	Anasterias minuta[191]
	Linckia laevigata[192]
Galβ1-4Glcβ1-Cer	*A. pectinifera*[181]
	A. planci[182]
	A. amurensis (S)[185]
Glcβ1-6Glcβ1-Cer	*A. amurensis* (S) [185]
Glcβ1-4Glcβ1-Cer	*A. amurensis* (S) [185]
Sea cucumber	
Glcβ1-Cer	*Cucumaria echinata*[193,195]
	Pentacta australis[194]
	Holothuria pervicax[196]
	Stichopus japonicus[197]
	H. leucospilota[198]
Feather star	
Glcβ1-Cer	*Comanthus japonica* (27)[199]
Acidic GSL	
Sea urchin	
NeuAcα2-6Glcβ1-Cer	*Anthocidaris crassispina* (S)[201]
NeuGcα2-6Glcβ1-Cer	*A. crassispina* (E)[202]
NeuAcα2-8NeuAcα2-6Glcβ1-Cer	*A. crassispina* (S) [201]
NeuAc2-6Glc1-8NeuAc2-6Glc-Cer	*Strongylocentrotus intermedius* (E)[200]
NeuAc2-8NeuAc2-6Glc1-6Glc-Cer	*S. intermedius* (E) [200]
NeuGc2-8NeuGc2-6Glc1-6Glc-Cer	*S. intermedius* (E) [200]
NeuGcα2-6Glcβ1-8NeuGc2-6Glcβ1-Cer	*S. intermedius* (E, embryo) [200,204]
HSO_3-8NeuGcα2-6Glcβ1-Cer	*A. crassispina* (E) [202]
	Echinocardium cordatum (G)[203]
HSO_3-8NeuAcα2-6Glcβ1-Cer	*Hemicentrous pulcherrimus* (S)[205]
HSO_3-8NeuAcα2-8NeuAcα2-6Glcβ1-Cer	*H. pulcherrimus* (S) [205]
HSO_3-8NeuGcα2-6Glcβ1-8NeuGcα2-6Glcβ1-Cer	*S. intermedius* (embryo)[204]

Table 6 (continued)

Structure	*Source*
HSO_3-8NeuAcα2-8NeuAcα2-8NeuAcα2-6Glcβ1-Cer	*H. pulcherrimus* (S) [205]
HSO_3-8NeuAcα2-8NeuAcα2-8NeuAcα2-8NeuAcα2-6Glcβ1-Cer	*H. pulcherrimus* (S) [205]
Starfish	
NeuGc8Meα2-3Galβ1-4Glcβ1-Cer	*Aphelasrias japonica* (H)[217]
	Luidia maculate[229]
NeuAc8Meα2-3Galβ1-4Glcβ1-Cer	*L. maculate*[229]
NeuAcα2-3Galβ1-4Glcβ1-Cer	*L. maculate*[227]
Gal*f*β1-4NeuAcα2-3Galβ1-4Glcβ1-Cer	*Acanthaster planci*[219]
NeuGc8Me2-3GalNAc1-3Gal1-4Glc-Cer	*Asterias rubens*[224]
NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ1-Cer	*L. maculate*[230]
NeuGc8Meα2-11NeuGc8Meα2-3Galβ1-4Glcβ1-Cer	*A. japonica* (H)[217]
NeuAc8Meα2-11NeuGcα2-3Galβ1-4Glcβ1-Cer	*Linckia laevigata*[228]
Ara*p*β1-6Gal*p*β1-4NeuGc2-3Galβ1-4Glcβ1-Cer	*Asterina pectinifera*[213]
Ara*p*β1-6Gal*p*β1-4NeuGc8Me2-3Galβ1-4Glcβ1-Cer	*A. pectinifera*[213]
L-Ara*f*α1-3Galα1-4NeuAcα2-3Galβ1-4Glcβ1-Cer	*A. pectinifera*[221]
	Astropecten latespinosus[222]
NeuAcα2-9NeuAcα2-3GalNAcβ1-3Galβ1-4Glcβ1-Cer	*Evasterias retifera* (H)[215]
Gal*f*β1-3Gal*p*α1-4NeuAcα2-3Galβ1-4Glcβ1-Cer	*A. planci*[218,225]
Fuc*f*β1-4Gal*p*α1-4NeuAcα2-3Galβ1-4Glcβ1-Cer	*A. planci*[220]
NeuGc8Me2 | 6 NeuGc8Me2-3GalNAcβ1-3Galβ1-4Glcβ1-Cer	*Asterias amurensis* (H) [215]
Gal*p*β1 | 8 Ara*f*,*p*1-6Gal*p*β1-4NeuGc2-3Galβ1-4Glcβ1-Cer	*A. rubens*[223] *A. planci*[218,225]
L-Ara*f*α1 | 4 L-Ara*f*α1-3Galα1-4NeuAcα2-3Galβ1-4Glcβ1-Cer	*A. pectinifera*[212]
L-Ara*f*α1-3Galα1-4NeuAcα2-3Galβ1-4Glcβ1-Cer	*A. pectinifera*[221]
L-Ara*f*α1-3Galα1 | 6 L-Ara*f*α1-3Galα1-4NeuAcα2-3Galβ1-4Glcβ1-Cer	*A. pectinifera*[221]
Ara*f*1-3Galα1 | 6 Ara*f*1-3Galβ1-4NeuGcα2-3Galβ1-4Glcβ1-Cer	*Patiria pectinifera* (H)[214]
Ara*f*1-3Galα1-4NeuGc8Me2-3Gal1-3Gal1-4NeuAc2-3Galβ1-4Glcβ1-Cer	*P. pectinifera* (H)[216]
HSO_3-3Galβ1-4Galβ1-4Glcβ1-Cer	*L. maculate*[226]
Sea cucumber	
NeuGcα2-6Glcβ1-Cer	*Stichopus japonicus*[232]
	Holothuria leucospilota[234]
	Stichopus chloronotus[235]
NeuGcα2-4NeuAcα2-6Glcβ1-Cer	*Holothuria pervicax*[231]
	H. leucospilota[234]
Fucα1-11NeuGcα2-6Glcβ1-Cer	*S. chloronotus*[235]
Fucα1-8NeuGcα2-4NeuAcα2-6Glcβ1-Cer	*H. pervicax*[231]
Fucα1-11NeuGcα2-4NeuAcα2-6Glcβ1-Cer	*H. leucospilota*[234]
Fucα1-4NeuAcα2-11NeuGcα2-4NeuAcα2-6Glcβ1-Cer	*H. pervicax*[233]
HSO_3-8NeuGcα2-6Glcβ1-Cer	*Cucumaria echinata*[195]

(continued)

Table 6 (continued)

Structure	*Source*
HSO_3-4NeuAcα2-6Glcβ1-Cer	*H. pervicax*[231]
HSO_3-8NeuGcα2-6Glcβ1-Cer	*S. chloronotus*[235]
Feather star	
Ins-1-*P*-Cer	*Comanthus japonica*[236]
NeuGc9Meα2-3Ins-1-*P*-Cer	*C. japonica*[237]
NeuGc9Meα2-11NeuGc9Meα2-3Ins-1-*P*-Cer	*C. japonica*[237]
NeuGc9Meα2-11NeuGc9Meα2-11NeuGc9Meα2-3Ins-1-*P*-Cer	*C. japonica*[238]
Brittle star	
NeuGcα2-6Glcβ1-Cer	*Ophiocoma scolopendrina*[239]
NeuGcα2-8NeuAcα2-6Glcβ1-Cer	*O. scolopendrina*[239]
NeuGcα2-8NeuGcα2-6Glcβ1-Cer	*O. scolopendrina*[239]
HSO_3-8NeuAcα2-6Glcβ1-Cer	*O. scolopendrina* [239]
Urochordata	
Glcβ1-Cer	*Botrillus leachii*[240]
	Phallusia fumigate[241]
Galβ1-4Glcβ1-Cer 3 | Fucα1	*Microcosmus sulcatus*[242]

E, egg; G, gonad; H, hepatopancreas; S, sperm.

Since the starfish gangliosides predominantly contain *N*-glycolyl-8-*O*-methylneuraminic acid (Neu Gc8Me)[213,215–217,224,229] as a sialic acid, it would be interesting to know the biosynthesis of this methylated sialic acid, that is to study sialate 8-*O*-methyltransferase and *N*-acetylneuraminic acid monooxygenase in these starfish species.

The disialogangliosides in two starfish species, *Aphelasrias japonica*[217] and *Linckia laevigata*,[228] have the additional NeuGc or NeuGc8Me residues at the subterminal position to which the terminal sialic acid residue is linked through the hydroxyl group (the position C11) of the glycolic acid unit.

In general, sialic acids are bound to galactose residue in sugar chains of the gangliosides. Amino sugar-containing gangliosides have been isolated from the starfish species, *A. amurensis*,[215] *Evasterias retifera*,[215] and *Asterias rubens*,[223,224] in which a sialic acid residue is attached to *N*-acetylgalactosamine. In the *A. amurensis*[215] gangliosides, two NeuGc8Me residues are linked to one *N*-acetylgalactosamine residue at C3 and C6 having a highly unusual type of positioning of the sialic acid residues. In the *E. retifera*[215] gangliosides, the NeuAc residues are joined by 2-9 linkage, unusual for gangliosides. In the *Acanthaster planic*[218,220,225] gangliosides, their oligosaccharide moieties are different in the terminal monosaccharide moieties, characteristically having a terminal furanose-type sugar unit. The terminal β-Gal*f* is linked to C3 of α-Gal*p*, however, the terminal β-Fuc*f* is linked to C4 of α-Gal*p*. This difference in terminal sugar linkages seems to be derived from the coexistence of different glycosyltransferases, namely β-1,3-galactofuranosyltransferase and β-1,4-fucofuranosyltransferase. A sulfatide having a trisaccharide chain has been isolated from the *Luidia maculate*.[226] Systematic studies of the sea urchin gangliosides suggest a common carbohydrate backbone composed of glucose and sialic acid, attached to GlcCer at position C6; on the other hand, while common structures are indeed evident in gangliosides of the starfish species, it appears that no common structural type of ganglioside is characteristic for starfish as a whole (**Table 6**).

3.15.4.2.1.3 Holothuroidea, Crinoidea, and Ophiuroidea (sea cucumber, feather star, and brittle star)

Higuchi and his colleagues have been engaged in systematic studies of acidic GSLs including gangliosides of the sea cucumber,[195,231,232–235] feather star[236,237,238] and brittle star.[239] In the sea cucumber and brittle star gangliosides, the sialic acid (NeuAc and/or NeuGc) and sialyloligosaccharide residues are linked to the position C6 of the glucocerebrosides; and some gangliosides of the sea cucumber carry fucose residues as the terminal sugar substituting at positions C-4,-8, or-11 (the hydroxyl group of the glycolic acid unit) of the penultimate sialic acid residues.[231,233–235]

Three sea cucumber species, *Cucumaria echinata*,[195] *Holothuria pervicax*,[231] and *Stichopus chloronotus*,[235] and the brittle star, *Ophiocoma scolopendrina*,[239] contain a sulfated ganglioside with a common structure.

The feather star, *Comanthus japonica*, contains an inositolphosphoceramide[236] which has been found in plants and protostomia, and mono-, di-, and trisialoglycosylinositolphosphoceramides.[237,238] Furthermore, the presence of the 9-*O*-methyl-*N*-glycolylneuraminic acid residues is also unique in the naturally occurring gangliosides.[237,238]

3.15.4.2.2 Urochordata

TLC analysis showed the presence of cerebroside-like glycolipids and high-polarity glycolipids, but no gangliosides in the sea ascidian, *Halocynthia roretzi*, *Halocynthia aurantium*, and *Styela clava*, although this animal is classified in Deuterostomia.[125] The chemical structures of GSLs (see **Table 6**) have been characterized as Glcβ1-Cer from the ascidian, *Botrillus leachii* [240] and *Phallusia fumigate*,[241] as well as Galβ1-4(Fucα1-3)Glcβ1-Cer from *Microcosmus sulcatus*.[242] The ceramide components predominantly consisted of 2-hydroxylated fatty acids and phytosphingosine-type sphingoids. Ascidian is a good model organism for understanding vertebrate development, because it belongs to a chordate and its cell lineage can be traced. Furthermore, a large amount of genome-related data have been accumulated, including genome sequences and gene expression patterns.

3.15.4.3 Cnidaria

In 1970, the glycolipid components of 50 species of marine invertebrates were identified by TLC with chemical detections and the monosaccharide contents of the lipid extracts were analyzed.[243] This study showed the presence of cerebroside-like glycolipid and high-polarity glycolipids in most invertebrate animals; ganglioside was absent, although ganglioside-like lipids are present in Echinodermata. The presence of glycolipids has been shown in the sea anemones, *Metridium* s. *fimbriatum* and *Anthopleura* sp., and the jellyfish, *Aurelia aurita* of Cnidaria. In the sea anemone, *Metridium senile*, cerebroside has been characterized as GlcβCer with 2-hydroxylated C16:0 and C20:0 acids as major fatty acids and 9-methyl-sphingadiene as major sphingoid (**Table 7**).[244]

3.15.4.4 Porifera

Sponges of Porifera, the simplest and earliest multicellular organisms, have been well studied as models for cell-recognition and adhesion mechanisms. Interestingly, carbohydrate self-recognition is involved in marine sponge cellular adhesion.[245] Sponges have long been recognized as a rich source of novel lipids including GSLs, hence numerous papers have been reported from diverse chemical and pharmacological research areas. Cerebroside-like glycolipid was found in the sea sponge, *Halicloma aqueducta*, *Halichondria panacea*, and *Myxilla incrustans* of Porifera.[243] A mixture of cerebrosides was isolated from the lipids of sea sponge, *Chondrilla nucula*, and GlcCer was characterized to contain long-chain bases and 2-hydroxy fatty acids.[246] Cerebrosides were found in the sponge, *Chondropsis* sp., and identified as Galβ1-Cer[247] and from *Haliclona* sp. and *H. panicea* as Glcβ1-Cer (**Table 7**).[248,249] From *Amphimedon viridis*, GlcNα1-Cer and GlcNβ1-Cer were found and named as amphicerebrosides.[248] Digalactosylceramide was found in *Halichondria japonica* and characterized as Galα1-4Galβ1-Cer, using FAB-MS, IR, ^{1}H-NMR analysis and chemical methods.[250]

During screening of natural products for antitumor and immunostimulatory activities, especially marine sponge, it was found that GSL from an Okinawan sponge, *Agelas mauritianus*, showed high *in vivo* antitumor activity against murine B16 melanoma and enhanced the mixed lymphocyte reaction (MLR) *in vitro*.[251–255] This compound was named agelasphin GSL and is α-galactosylceramide, α-GalCer (Galα1-Cer); its synthetic analog is known as KRN7000. After testing of various synthetic analogs for biological activities, KRN7000 was found to be a potent agent to stimulate Vα14NKT cells and was also identified as a ligand for invariant T-cell antigen receptor of Vα14NKT cell.[256–258] The ceramide components influence activities through modification of presentation by CD1d molecules; the synthetic analog has C26:0 fatty acid and phytosphingosine.[256,259] Numerous studies have been reported, which are summarized by excellent reviews,[260,261] especially focusing on the role of Galα1-Cer-reactive invariant NKT cell in controlling autoimmune response, prevention of parasite infection,[262] and abortion.[263]

The structural analysis of sponge GSLs has accompanied the investigation of their biological activities. There are antifungal activities of GlcNAcβCer from *Halichondria cylindrata*,[264] and immunostimulatory activities of di- and triglycosylceramides from four *Agelas* species,[265] *Stylissa frabeliformis*, and *Axinella damicornis*,[266] as well as nitric oxide release inhibitor activity of triglycosylceramides from *Aplysinella rhax*.[267] Prenylated ($(CH_3)_2C{=}CH{-}$) GSLs featuring a cyclopropane-containing alkyl chain have been isolated from *Ectyoplasia ferox* and *Plakortis simplex*.[268,269] GlcβCer GSL is found in *Iotrochota baculifera*,[270] Galα1-6Glcβ1-Cer in *Amphimedon* sp.,[271] Galα1-Cer-based GSLs in *Agelas clathrodes* [272] and *A. damicornis* [273] with various ceramide species. GSL structures of Porifera are summarized in **Table 7**.

Table 7 Structures of glycosphingolipids found in Cnidaria and Porifera

Structure	*Source*
Cnidaria	
Glcβ1-Cer	*Metridium senile*[244]
Porifera	
Galα1-Cer	*Agelas mauritianus*[251]
	Agelas clathrodes[272]
Galβ1-Cer	*Chondropsis sp*[247]
	Halichondria japonica[250]
Glcβ1-Cer	*Halicona* sp.[248]
	Halichondria panicea[249]
	Iotrochota baculifera[270]
GlcNAcβ1-Cer	*Halichondria cylindrata*[264]
GlcNα1-Cer	*Amphimedon viridis*[248]
GlcNβ1-Cer	*A. viridis*[248]
Galα1-2Galα1-Cer	*Agelas conifera*[265]
	Unidentified sponge[266]
Galα1-4Galβ1-Cer	*H. japonica*[250]
Glcα1-2Galα1-Cer	*Agelas longissima*[265]
Galfβ1-3Galα1-Cer	*A. clathrodes*[265]
	Agelas axisera[266]
Galα1-6Glcβ1-Cer	*Amphimedon* sp.[271]
GalNAcα1-3Galα1-Cer	*A. clathrodes*[265]
GlcNAcα1-4Galα1-Cer Galβ1-Cer	*Axinella damicornis*[273]
	Ectyoplasia ferox[268]
$(CH_3)_2C$=CH-2 Glcβ1-6Galβ1-Cer	*Plakortis simplex*[269]
	E. ferox[268]
$(CH_3)_2C$=CH-2 GalNAcβ1-4GlcNAcβ1-Cer 3 | Fucα1	*Aplysinella rhax*[267]
GalNAcα1-3Galα1-Cer 2 | Glcα1	*Stylissa frabeliformis*[266]
GalNAcα1-6Galα1-Cer 2 | Galα1	*Agelas dispar*[265]
Rhaα1-3GalNAcβ1-6Galα1-2Galα1-Cer	*A. clathrodes*[271]

3.15.5 Conclusions

When animals diverged evolutionarily in ancient times but retained morphological and embryological similarities, they could be arranged at a similar place on the taxonomic tree based on their morphology. Better understanding of molecular phylogeny as determined by comparison of genes using molecular biology, as well as other new approaches, has been aimed and new lines of evidence are accumulating in this research field. Molecular phylogeny mainly collects information by comparison of ubiquitous protein molecules or their nucleic acid sequences. Glycosyltransferases responsible for unique invertebrate GSL biosynthesis would be excellent molecules for this purpose. As suggested in this chapter, it is important that information on species' characteristic GSL structures is incorporated into the phylogenitc tree along with molecular phylogeny using ubiquitous molecules, and the hoped-for result will be a new type of molecular phylogeny. The varieties of characteristic GSL structures help to confer onto various species the diversity they show. Extraction and characterization of GSLs are part of the search and discovery process for identifying previously unknown natural products, from invertebrate sources, having antitumor or immunoreactive properties. Characterization of ceramide composition is also important as shown by Galα1-Cer, because ceramides

influence the way of presentation by CD1d molecules. The complex of GSL molecules is increasingly recognized to play an important part in the interactions between animal and animal, parasite and host, and invertebrate and vertebrate, probably involving their presence and functions at cell surfaces. Research in these areas is now expanding in the scientific community and is benefiting greatly from various medical and pharmacological contributions. Investigation of GSLs in model organisms such as *D. melanogaster* and *C. elegans*, both of which are genome-sequenced species, should lead to interesting new findings. In fact, studies of the remarkable structures of invertebrate GSLs could provide a new approach to the field of genomics. Such recent research on GSLs of model organisms such as *D. melanogaster*, and its results, actually point in the direction of a new research field, 'glycogenomics'.

Glossary

αGalCer α-Galactosylceramide was discovered in the marine sponge during a screen for antitumor agents. This structure has a different anomeric configuration from the cerebroside which ubiquitously exists in most animals including the mammal.
arthro-series Named for the characteristic oligosaccharide structure (GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-), derived from the name Arthropoda.
cerebroside Monohexosylceramide has been found as a major glycosphingolipid in brain tissue. In general, cerebroside is defined as galactosylceremide, but occasionally indicates glucosylceramide as well galactocerebroside (a precursor of sulfatide) which is prominent in brain, and as well as glucocerebroside (a precursor of ganglioside and other glycosphingolipid) which is prominent in other tissues, are used for differentiating brain and other tissues.
fucolipid Glycosphingolipids contain fucose as sugar components, and fucose typically is found on branches attached to the core sugar structure.
mannolipid Glycosphingolipids contain 1 or 2 mol mannose as sugar components, and are found in Protostomia animals.
mollu-series Named for the characteristic oligosaccharide structure (GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-), derived from the name Mollusca.
phosphonoglycosphingolipid Glycosphingolipids contain a C–P compound which has carbon and phosphorus chemical C–P bond, such as [(2-aminoethyl)hydroxyphosphoryl].
sphingoid A component which, combined with a fatty acid, makes up ceramide; typically, this is one of the long chain bases. Sphingosine is the most commonly occurring sphingoid structure [(2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol].
zwitterionic glycosphingolipid The meaning is similar to amphoteric glycosphingolipid. First, it was defined as a glycosphingolipid containing 2-aminoethanolphosphate (phosphoethanolamine); later, phosphocholine (PC) was also discovered in this lipid class. Like neutral glycolipids, PC-containing lipids show no adsorption to anion-exchange resin, but do show a more polar migration than neutral glycolipid on thin layer chromatography.

References

1. Luukkonen, A.; Brummer-Korvenkontio, M.; Renkonen, O. *Biochim. Biophys. Acta* **1973**, *326*, 256–261.
2. Sugita, M.; Nishida, M.; Hori, T. *J. Biochem.* **1982**, *92*, 327–334.
3. Dennis, R. D.; Geyer, R.; Egge, H.; Menges, H.; Stirm, S.; Wiegandt, H. *Eur. J. Biochem.* **1985**, *146*, 51–58.
4. Dennis, R. D.; Geyer, R.; Egge, H.; Peter-Katalinic, J.; Li, S. C.; Stirm, S.; Wiegandt, H. *J. Biol. Chem.* **1985**, *260*, 5370–5375.
5. Sugita, M.; Iwasaki, Y.; Hori, T. *J. Biochem.* **1982**, *92*, 881–887.
6. Weske, B.; Dennis, R. D.; Helling, F.; Keller, M.; Nores, G. A.; Peter-Katalinic, J.; Egge, H.; Dabrowski, U.; Wiegandt, H. *Eur. J. Biochem.* **1990**, *191*, 379–388.
7. Sugita, M.; Inagaki, F.; Naito, H.; Hori, T. *J. Biochem.* **1990**, *107*, 899–903.
8. Helling, F.; Dennis, R. D.; Weske, B.; Nores, G.; Peter-Katalinic, J.; Dabrowski, U.; Egge, H.; Wiegandt, H. *Eur. J. Biochem.* **1991**, *200*, 409–421.
9. Ohtsuka, I.; Hada, N.; Sugita, M.; Takeda, T. *Carbohydr. Res.* **2002**, *337*, 2037–2047.
10. Fuller, M. D.; Schwientek, T.; Wandall, H. H.; Pedersen, J. W.; Clausen, H.; Levery, S. B. *Glycobiology* **2005**, *15*, 1286–1301.
11. Sugita, M.; Itonori, S.; Inagaki, F.; Hori, T. *J. Biol. Chem.* **1989**, *264*, 15028–15033.
12. Seppo, A.; Moreland, M.; Schweingruber, H.; Tiemeyer, M. *Eur. J. Biochem.* **2000**, *267*, 3549–3558.
13. Chou, D. K. H.; Ilyas, A. A.; Evans, J. E.; Costello, C.; Quarles, R. H.; Jungalwala, F. B. *J. Biol. Chem.* **1986**, *261*, 11717–11725.
14. Chou, D. K. H.; Schwarting, G. A.; Evans, J. E.; Jungalwala, F. B. *J. Neurochem.* **1987**, *49*, 865–873.
15. Ariga, T.; Kohriyama, T.; Freddo, L.; Latov, N.; Saito, M.; Kon, K.; Ando, S.; Suzuki, M.; Hemling, M. E.; Rinehart, K. L.; Kusunoki, S.; Yu, R. K. *J. Biol. Chem.* **1987**, *262*, 848–853.
16. Wiegandt, H. *Biochim. Biophys. Acta* **1992**, *1123*, 117–126.
17. Dennis, R. D.; Wiegandt, H. *Adv. Lipid Res.* **1993**, *26*, 321–351.
18. Dabrowski, U.; Dabrowski, J.; Helling, F.; Wiegandt, H. *J. Biol. Chem.* **1990**, *265*, 9737–9743.
19. Itonori, S.; Nishizawa, M.; Suzuki, M.; Inagaki, F.; Hori, T.; Sugita, M. *J. Biochem.* **1991**, *110*, 479–485.
20. Kohyama-Koganeya, A.; Sasamura, T.; Oshima, E.; Suzuki, E.; Nishihara, S.; Ueda, R.; Hirabayashi, Y. *J. Biol. Chem.* **2004**, *279*, 35995–36002.
21. Schwientek, T.; Keck, B.; Levery, S. B.; Jensen, M. A.; Pedersen, J. W.; Wandall, H. H.; Stroud, M.; Cohen, S. M.; Amado, M.; Clausen, H. *J. Biol. Chem.* **2002**, *277*, 32421–32429.
22. Wandall, H. H.; Pedersen, J. W.; Park, C.; Levery, S. B.; Pizette, S.; Cohen, S. M.; Schwientek, T.; Clausen, H. *J. Biol. Chem.* **2003**, *278*, 1411–1414.

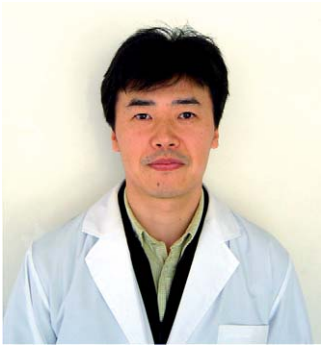
23. Wandall, H. H.; Pizette, S.; Pedersen, J. W.; Eichert, H.; Levery, S. B.; Mandel, U.; Cohen, S. M.; Clausen, H. *J. Biol. Chem.* **2005**, *280*, 4858–4863.
24. Itonori, S.; Hiratsuka, M.; Sonku, N.; Tsuji, H.; Itasaka, O.; Hori, T.; Sugita, M. *Biochim. Biophys. Acta* **1992**, *1123*, 263–268.
25. Sugita, M.; Hayata, C.; Hori, T.; Morikawa, A.; Miwa, S. *J. Jpn. Oil Chem. Soc.* **1993**, *42*, 649–655.
26. Shimomura, K.; Hanjura, S.; Ki, P. F.; Kishimoto, Y. *Science* **1983**, *220*, 1392–1393.
27. Okamura, N.; Stoskopf, M.; Hendricks, F.; Kishimoto, Y. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 6779–6782.
28. Okamura, N.; Stoskopf, M.; Yamaguchi, H.; Kishimoto, Y. *J. Neurochem.* **1985**, *45*, 1875–1879.
29. Horibata, Y.; Sakaguchi, K.; Okino, N.; Iida, H.; Inagaki, M.; Fujisawa, T.; Hama, Y.; Ito, M. *J. Biol. Chem.* **2004**, *279*, 33379–33389.
30. Itonori, S.; Kamemura, K.; Narushima, K.; Sonku, N.; Itasaka, O.; Hori, T.; Sugita, M. *Biochim. Biophys. Acta* **1991**, *1081*, 321–327.
31. Kimura, K.; Itonori, S.; Hada, N.; Itasaka, O.; Dulaney, J. T.; Takeda, T.; Sugita, M. *J. Oleo Sci.* **2002**, *51*, 83–91.
32. Lee, R. F.; Gonsoulin, F. *Comp. Biochem. Physiol. B* **1979**, *64*, 375–379.
33. Sugita, M.; Hayata, C.; Yoshida, T.; Suzuki, M.; Suzuki, A.; Takeda, T.; Hori, T.; Nakatani, F. *Biochim. Biophys. Acta* **1994**, *1215*, 163–169.
34. Ehrlich, I.; Hrzenjak, T. *Vet. Arh.* **1975**, *45*, 129–133.
35. Hutchison, W. F.; Turner, A. C.; Grayson, D. P.; White, H. B., Jr. *Comp. Biochem. Physiol. B* **1976**, *53*, 495–497.
36. Sarwal, R.; Sanyal, S. N.; Khera, S. *J. Helminthol.* **1989**, *63*, 287–297.
37. Kwong, A. Y.; Wong, P. C.; Ko, R. C. *Comp. Biochem. Physiol. B* **1990**, *95*, 193–197.
38. Maloney, M. D.; Semprevivo, L. H. *Parasitol. Res.* **1991**, *77*, 294–300.
39. Chitwood, D. J.; Lusby, W. R.; Thompson, M. J.; Kochansky, J. P.; Howarth, O. W. *Lipids* **1995**, *30*, 567–573.
40. Dennis, R. D.; Baumeister, S.; Smuda, C.; Lochnit, C.; Waider, T.; Geyer, E. *Parasitology* **1995**, *110*, 611–623.
41. Lochnit, G.; Dennis, R. D.; Zahringer, U.; Geyer, R. *Glycoconj. J.* **1997**, *14*, 389–399.
42. Gerdt, S.; Lochnit, G.; Dennis, R. D.; Geyer, R. *Glycobiology* **1997**, *7*, 265–275.
43. Aguinaldo, A. M.; Turbeville, J. M.; Linford, L. S.; Rivera, M. C.; Garey, J. R.; Raff, R. A.; Lake, J. A. *Nature* **1997**, *387*, 489–493.
44. Sugita, M.; Mizunoma, T.; Aoki, K.; Dulaney, J. T.; Inagaki, F.; Suzuki, M.; Suzuki, A.; Ichikawa, S.; Kushida, K.; Ohta, S.; Kurimoto, A. *Biochim. Biophys. Acta* **1996**, *1302*, 185–192.
45. Lochnit, G.; Nispel, S.; Dennis, R. D.; Geyer, R. *Glycobiology* **1998**, *8*, 891–899.
46. Gerdt, S.; Dennis, R. D.; Borgonie, G.; Schnabel, R.; Geyer, R. *Eur. J. Biochem.* **1999**, *266*, 952–963.
47. Kurimoto, A.; Kawakami, Y.; Dulaney, J. T.; Miyake, A.; Sugita, M. *J. Jpn. Oil Chem. Soc.* **2000**, *49*, 127–135.
48. Wuhrer, M.; Rickhoff, S.; Dennis, R. D.; Lochnit, G.; Soboslay, P. T.; Baumeister, S.; Geyer, R. *Biochem. J.* **2000**, *348*, 417–423.
49. Lochnit, G.; Dennis, R. D.; Ulmer, A. J.; Geyer, R. *J. Biol. Chem.* **1998**, *273*, 466–474.
50. Deehan, M. R.; Goodridge, H. S.; Blair, D.; Lochnit, G.; Dennis, R. D.; Geyer, R.; Harnett, M. M.; Harnett, W. *Parasite Immunol.* **2002**, *24*, 463–469.
51. Lochnit, G.; Dennis, R. D.; Geyer, R. *Biol. Chem.* **2000**, *381*, 839–847.
52. Whelan, M.; Harnett, M. M.; Houston, K. M.; Patel, V.; Harnett, W.; Rigley, K. P. *J. Immunol.* **2000**, *164*, 6453–6460.
53. Lochnit, G.; Dennis, R. D.; Muntefehr, H.; Nispel, S.; Geyer, R. *Parasitology* **2001**, *122*, 359–370.
54. Houston, K. M.; Lochnit, G.; Geyer, R.; Harnett, W. *Mol. Biochem. Parasitol.* **2002**, *123*, 55–66.
55. Friedl, C. H.; Lochnit, G.; Zahringer, U.; Bahr, U.; Geyer, R. *Biochem. J.* **2003**, *369*, 89–102.
56. Ohtsuka, I.; Hada, N.; Ohtaka, H.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **2002**, *50*, 600–604.
57. Herman, T.; Horvitz, H. R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 974–979.
58. Lochnit, G.; Bongaarts, R.; Geyer, R. *Int. J. Parasitol.* **2005**, *35*, 911–923.
59. Meyer, F.; Meyer, H.; Bueding, E. *Biochim. Biophys. Acta* **1970**, *210*, 257–266.
60. Samuelson, J. C.; Caulfield, J. P. *J. Cell Biol.* **1982**, *94*, 363–369.
61. Naiki, M.; Ramasamy, R.; Ochanda, J. O.; Maina, G. *Jpn. J. Vet. Sci.* **1985**, *47*, 777–786.
62. Makaaru, C. K.; Damian, R. T.; Smith, D. F.; Cummings, R. D. *J. Biol. Chem.* **1992**, *267*, 2251–2257.
63. Levery, S. B.; Weiss, J. B.; Salyan, M. E.; Roberts, C. E.; Hakomori, S.; Magnani, J. L.; Strand, M. *J. Biol. Chem.* **1992**, *267*, 5542–5551.
64. Khoo, K. H.; Chatterjee, D.; Caulfield, J. P.; Morris, H. R.; Dell, A. *Glycobiology* **1997**, *7*, 653–661.
65. Wuhrer, M.; Dennis, R. D.; Doenhoff, M. J.; Lochnit, G.; Geyer, R. *Glycobiology* **2000**, *10*, 89–101.
66. Wuhrer, M.; Kantelhardt, S. R.; Dennis, R. D.; Doenhoff, M. J.; Lochnit, G.; Geyer, R. *Eur. J. Biochem.* **2002**, *269*, 481–493.
67. Wuhrer, M.; Dennis, R. D.; Doenhoff, M. J.; Geyer, R. *Biochim. Biophys. Acta* **2000**, *1524*, 155–161.
68. Cummings, R. D.; Nyame, A. K. *FASEB J.* **1996**, *10*, 838–848.
69. Wuhrer, M.; Dennis, R. D.; Doenhoff, M. J.; Bickle, Q.; Lochnit, G.; Geyer, R. *Mol. Biochem. Parasitol.* **1999**, *103*, 155–169.
70. Robijn, M. L.; Wuhrer, M.; Kornelis, D.; Deelder, A. M.; Geyer, R.; Hokke, C. H. *Parasitology* **2005**, *130*, 67–77.
71. Ko, A. I.; Drager, U. C.; Harn, D. A. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 4159–4163.
72. Wuhrer, M.; Dennis, R. D.; Doenhoff, M. J.; Geyer, R. *Mol. Biochem. Parasitol.* **2000**, *110*, 237–246.
73. Van Liempt, E.; Imberty, A.; Bank, C. M.; van Vliet, S. J.; Van Kooyk, Y.; Geijtenbeek, T. B.; Van Die, I. *J. Biol. Chem.* **2004**, *279*, 33161–33167.
74. Meyer, S.; Van Liempt, E.; Imberty, A.; Van Kooyk, Y.; Geyer, H.; Geyer, R.; Van Die, I. *J. Biol. Chem.* **2005**, *280*, 37349–37359.
75. Wuhrer, M.; Berkefeld, C.; Dennis, R. D.; Idris, M. A.; Geyer, R. *Biol. Chem.* **2001**, *382*, 195–207.
76. Wuhrer, M.; Grimm, C.; Dennis, R. D.; Idris, M. A.; Geyer, R. *Glycobiology* **2004**, *14*, 115–126.
77. Persat, F.; Bouhours, J. F.; Mojon, M.; Petavy, A. F. *J. Biol. Chem.* **1992**, *267*, 8764–8769.
78. Wuhrer, M.; Grimm, C.; Zahringer, U.; Dennis, R. D.; Berkefeld, C. M.; Idris, M. A.; Geyer, R. *Glycobiology* **2003**, *13*, 129–137.
79. Lesuk, A.; Anderson, R. J. *J. Biol. Chem.* **1941**, *139*, 457–469.
80. Mills, G. L.; Coley, S. C.; Williams, J. F. *J. Parasitol.* **1983**, *69*, 850–856.
81. Richards, K. S.; Ilderton, E.; Yardley, H. J. *Comp. Biochem. Physiol.* **1987**, *86*, 209–212.
82. Persat, F.; Bouhours, J. F.; Mojon, M.; Petavy, A. F. *Mol. Biochem. Parasitol.* **1990**, *38*, 97–103.
83. Baumeister, S.; Dennis, R. D.; Kunz, J.; Wiegandt, H.; Geyer, E. *Mol. Biochem. Parasitol.* **1992**, *53*, 53–61.
84. Singh, B. N.; Costello, C. E.; Levery, S. B.; Walenga, R. W.; Beach, D. H.; Mueller, J. F.; Holz, G. G. *Mol. Biochem. Parasitol.* **1987**, *26*, 99–111.
85. Persat, F.; Bouhours, J. F.; Mojon, M.; Petavy, A. F. *Mol. Biochem. Parasitol.* **1990**, *41*, 1–6.
86. Nishimura, K.; Suzuki, A.; Kino, H. *Biochim. Biophys. Acta* **1991**, *1086*, 141–150.
87. Persat, F.; Vincent, C.; Schmitt, D.; Mojon, M. *Infect. Immun.* **1996**, *64*, 3682–3687.
88. Persat, F.; Bouhours, J. F.; Petavy, A. F.; Mojon, M. *Biochim. Biophys. Acta* **1994**, *1225*, 297–303.

89. Dennis, R. D.; Baumeister, S.; Geyer, R.; Peter-Katalinic, J.; Hartmann, R.; Egge, H.; Geyer, E.; Wiegandt, H. *Eur. J. Biochem.* **1992**, *207*, 1053–1062.
90. Lopez-Marin, L. M.; Montrozier, H.; Lemassu, A.; Garcia, E.; Segura, E.; Daffe, M. *Mol. Biochem. Parasitol.* **2002**, *119*, 33–42.
91. Kawakami, Y.; Nakamura, K.; Kojima, H.; Suzuki, M.; Inagaki, F.; Suzuki, A.; Sonoki, S.; Uchida, A.; Murata, Y.; Tamai, Y. *J. Biochem.* **1993**, *114*, 677–683.
92. Kawakami, Y.; Kojima, H.; Nakamura, K.; Suzuki, M.; Uchida, A.; Murata, Y.; Tamai, Y. *Lipids* **1995**, *30*, 333–337.
93. Kawakami, Y.; Nakamura, K.; Kojima, H.; Suzuki, M.; Inagaki, F.; Suzuki, A.; Ikuta, J.; Uchida, A.; Murata, Y.; Tamai, Y. *Eur. J. Biochem.* **1996**, *239*, 905–911.
94. Iriko, H.; Nakamura, K.; Kojima, H.; Iida-Tanaka, N.; Kasama, T.; Kawakami, Y.; Ishizuka, I.; Uchida, A.; Murata, Y.; Tamai, Y. *Eur. J. Biochem.* **2002**, *269*, 3549–3559.
95. Yanagisawa, M.; Kojima, H.; Kawakami, Y.; Iriko, H.; Nakamura, T.; Nakamura, K.; Uchida, A.; Murata, Y.; Tamai, Y. *Mol. Biochem. Parasitol.* **1999**, *102*, 225–235.
96. Tanaka, R.; Ishizaki, H.; Kawano, S.; Okuda, H.; Miyahara, K.; Noda, N. *Chem. Pharm. Bull.* **1997**, *45*, 1702–1704.
97. Hada, N.; Matsusaki, A.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **1999**, *47*, 1265–1268.
98. Hada, N.; Sat, K.; Sakushima, J. I.; Goda, Y.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **2001**, *49*, 1464–1467.
99. Sugita, M.; Mizunoma, T.; Inagaki, F.; Suzuki, M.; Suzuki, A.; Hori, T.; Nakatani, F.; Ohta, S. *J. Jpn. Oil Chem. Soc.* **1994**, *43*, 1052–1061.
100. Sugita, M.; Fujii, H.; Dulaney, J. T.; Inagaki, F.; Suzuki, M.; Suzuki, A.; Ohta, S. *Biochim. Biophys. Acta* **1995**, *1259*, 220–226.
101. Sugita, M.; Ohta, S.; Morikawa, A.; Dulaney, J. T.; Ichikawa, S.; Kushida, K.; Inagaki, F.; Suzuki, M.; Suzuki, A. *J. Jpn. Oil Chem. Soc.* **1997**, *46*, 755–766.
102. Sugita, M.; Fujii, H.; Inagaki, F.; Suzuki, M.; Hayata, C.; Hori, T. *J. Biol. Chem.* **1992**, *267*, 22595–22598.
103. Tanaka, R.; Miyahara, K.; Noda, N. *Chem. Pharm. Bull.* **1996**, *44*, 1152–1156.
104. Albro, P. W.; Schroeder, J. L.; Corbett, J. T. *Lipids* **1992**, *27*, 136–143.
105. Yamaji, A.; Sekizawa, Y.; Emoto, K.; Sakuraba, H.; Inoue, K.; Kobayashi, H.; Umeda, M. *J. Biol. Chem.* **1998**, *273*, 5300–5306.
106. Yamaji-Hasegawa, A.; Makino, A.; Baba, T.; Senoh, Y.; Kimura-Suda, H.; Sato, S. B.; Terada, N.; Ohno, S.; Kiyokawa, E.; Umeda, M.; Kobayashi, T. *J. Biol. Chem.* **2003**, *278*, 22762–22770.
107. Noda, N.; Tanaka, R.; Miyahara, K.; Kawasaki, T. *Tetrahedron Lett.* **1992**, *33*, 7527–7530.
108. Noda, N.; Tanaka, R.; Miyahara, K.; Kawasaki, T. *Biochim. Biophys. Acta* **1993**, *1169*, 30–38.
109. Noda, N.; Tanaka, R.; Tsujino, K.; Takasaki, Y.; Nakano, M.; Nishi, M.; Miyahara, K. *J. Biochem.* **1994**, *116*, 435–442.
110. Sugita, M.; Yamane, N.; Hamana, H.; Sasaki, K.; Dulaney, J. T. *J. Jpn. Oil Chem. Soc.* **1999**, *48*, 671–679.
111. Itonori, S.; Hamana, H.; Hada, N.; Takeda, T.; Dulaney, J. T.; Sugita, M. *J. Oleo Sci.* **2001**, *50*, 537–544.
112. Itonori, S.; Sugita, M. *Trends Glycosci. Glycotech.* **2005**, *17*, 15–25.
113. Sugita, M.; Miwa, S.; Aoki, J.; Dulaney, J. T.; Ichikawa, S.; Inagaki, F.; Suzuki, M. *J. Jpn. Oil Chem. Soc.* **2000**, *49*, 33–43.
114. Noda, N.; Tanaka, R.; Miyahara, K.; Sukamoto, T. *Chem. Pharm. Bull.* **1996**, *44*, 895–899.
115. Sugita, M.; Morikawa, A.; Dulaney, J. T.; Okada, A. *J. Jpn. Oil Chem. Soc.* **1996**, *45*, 731–740.
116. Zipser, B.; Bradford, J.; Hollingsworth, R. I. *Carbohydr. Res.* **1998**, *308*, 47–55.
117. Li, S. C.; DeGasperi, R.; Muldrey, J. E.; Li, Y. T. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 346–352.
118. Zhou, B.; Li, S. C.; Laine, R. A.; Huang, R. T.; Li, Y. T. *J. Biol. Chem.* **1989**, *264*, 12272–12277.
119. Carter, B. Z.; Li, S. C.; Li, Y. T. *Biochem. J.* **1992**, *285*, 619–623.
120. Horibata, Y.; Okino, N.; Ichinose, S.; Omori, A.; Ito, M. *J. Biol. Chem.* **2000**, *275*, 31297–31304.
121. Jellies, J.; Kopp, D. M.; Geisert, E. E., Jr. *Dev. Biol.* **1993**, *159*, 691–705.
122. Sergeeva, S. S.; Zapryanova, E.; Sotnikov, S.; Deleva, D.; Fil'chev, A. *Neurosci. Behav. Physiol.* **2004**, *34*, 579–585.
123. Hori, T.; Sugita, M. *Prog. Lipid Res.* **1993**, *32*, 25–45.
124. Nakazawa, Y. *J. Biochem.* **1959**, *46*, 1579–1585.
125. Vaskovsky, V. E.; Kostetsky, E. Y.; Svetashev, V. I.; Zhukova, I. G.; Smirnova, G. P. *Comp. Biochem. Physiol.* **1970**, *34*, 163–177.
126. Itasaka, O. *J. Biochem.* **1966**, *60*, 435–438.
127. Matsubara, T.; Hayashi, A. *J. Biochem.* **1973**, *74*, 853–856.
128. Matsubara, T.; Hayashi, A. *J. Biochem.* **1978**, *83*, 1195–1197.
129. Higashi, S.; Hori, T. *Biochim. Biophys. Acta* **1968**, *152*, 568–575.
130. Hori, T.; Takeda, H.; Sugita, M.; Itasaka, O. *J. Biochem.* **1977**, *82*, 1281–1285.
131. Sugita, M.; Yamamoto, T.; Masuda, S.; Itasaka, O.; Hori, T. *J. Biochem.* **1981**, *90*, 1529–1535.
132. Hori, T.; Sugita, M.; Shimizu, H. *Biochim. Biophys. Acta* **1981**, *665*, 170–173.
133. Itasaka, O.; Sugita, M.; Kataoka, H.; Hori, T. *Biochim. Biophys. Acta* **1983**, *751*, 8–13.
134. Sugita, M.; Nakano, Y.; Nose, T.; Itasaka, O.; Hori, T. *J. Biochem.* **1984**, *95*, 47–55.
135. Sugita, M.; Shirai, S.; Itasaka, O.; Hori, T. *J. Biochem.* **1975**, *77*, 125–130.
136. Itasaka, O.; Sugita, M.; Yoshizaki, H.; Hori, T. *J. Biochem.* **1976**, *80*, 935–936.
137. Hori, T.; Sugita, M.; Kanbayashi, J.; Itasaka, O. *J. Biochem.* **1977**, *81*, 107–114.
138. Hori, T.; Sugita, M.; Ando, S.; Tsukada, K.; Shiota, K.; Tsuzuki, M.; Itasaka, O. *J. Biol. Chem.* **1983**, *258*, 2239–2245.
139. Hori, T.; Sugita, M.; Ando, S.; Kuwahara, M.; Kumauchi, K.; Sugie, E.; Itasaka, O. *J. Biol. Chem.* **1981**, *256*, 10979–10985.
140. Sugita, M.; Nakae, H.; Yamamura, T.; Takamiya, Y.; Itasaka, O.; Hori, T. *J. Biochem.* **1985**, *98*, 27–34.
141. Sugita, M.; Sanai, Y.; Itonori, S.; Hori, T. *Biochim. Biophys. Acta* **1988**, *962*, 159–165.
142. Itasaka, O.; Kosuga, M.; Okayama, M.; Hori, T. *Biochim. Biophys. Acta* **1983**, *750*, 440–446.
143. Sugita, M.; Inoue, T.; Itasaka, O.; Hori, T. *J. Biochem.* **1984**, *95*, 737–742.
144. Itasaka, O.; Hori, T. *J. Biochem.* **1979**, *85*, 1469–1481.
145. Matsubara, T.; Hayashi, A. *J. Biochem.* **1981**, *89*, 645–650.
146. Matsubara, T.; Hayashi, A. *J. Biochem.* **1986**, *99*, 1401–1408.
147. Matsubara, T.; Hayashi, A. *Biochem. Biophys. Acta* **1982**, *711*, 551–553.
148. Hayashi, A.; Matsuura, F. *Chem. Phys. Lipids* **1978**, *22*, 9–23.
149. Matsuura, F. *Chem. Phys. Lipids* **1979**, *19*, 223–242.
150. Matsuura, F. *J. Biochem.* **1979**, *85*, 433–441.

151. Hayashi, A.; Matsubara, T. *Biochim. Biophys. Acta* **1989**, *1006*, 89–96.
152. Hayashi, A.; Matsubara, T. In *New Vistas in Glycolipids Research*; Makita, A., Handa, S., Taketomi, T., Nagai, Y., Eds.; Plenum: New York, 1982; pp 103–114.
153. Matsubara, T.; Hayashi, A. *Prog. Lipid Res.* **1991**, *30*, 301–322.
154. Hayashi, A.; Matsubara, T.; Mishima, T. *Chem. Phys. Lipids* **1990**, *52*, 171–178.
155. Komai, Y.; Matsukawa, S.; Satake, M. *Biochim. Biophys. Acta* **1973**, *316*, 271–281.
156. Araki, S.; Komai, Y.; Satake, M. *J. Biochem.* **1980**, *87*, 503–510.
157. Araki, S.; Satake, M.; Ando, S.; Hayashi, A.; Fujii, N. *J. Biol. Chem.* **1986**, *261*, 5138–5144.
158. Araki, S.; Abe, S.; Ando, S.; Fujii, N.; Satake, M. *J. Biochem.* **1987**, *101*, 145–152.
159. Araki, S.; Abe, S.; Odani, S.; Ando, S.; Fujii, N.; Satake, M. *J. Biol. Chem.* **1987**, *262*, 14141–14145.
160. Araki, S.; Yamada, S.; Abe, S.; Waki, H.; Kon, K.; Itonori, S.; Sugita, M.; Ando, S. *J. Biochem.* **2001**, *129*, 93–100.
161. Yamada, S.; Araki, S.; Abe, S.; Kon, K.; Ando, S.; Satake, M. *J. Biochem.* **1995**, *117*, 794–799.
162. Araki, S.; Satake, M. *Neurosci. Lett.* **1981**, *22*, 179–182.
163. Araki, S.; Abe, S.; Ando, S.; Kon, K.; Fujiwara, N.; Satake, M. *J. Biol. Chem.* **1989**, *264*, 19922–19927.
164. Abe, S.; Araki, S.; Satake, M.; Fujiwara, N.; Kon, K.; Ando, S. *J. Biol. Chem.* **1991**, *266*, 9939–9943.
165. Watanabe, Y.; Abe, S.; Araki, S.; Kumanishi, T.; Satake, M. *J. Biochem.* **1989**, *106*, 972–976.
166. Abe, S.; Watanabe, Y.; Araki, S.; Kumanishi, T.; Satake, M. *J. Biochem.* **1988**, *104*, 220–226.
167. Beitinger, H.; Schifferer, F.; Sugita, M.; Araki, S.; Satake, M.; Mobius, D.; Rahmann, H. *J. Biochem.* **1989**, *105*, 664–669.
168. Yamaguchi, Y.; Konda, K.; Hayashi, A. *Biochim. Biophys. Acta* **1992**, *1165*, 110–118.
169. Yamaguchi, Y.; Ohta, M.; Hayashi, A. *Biochim. Biophys. Acta* **1992**, *1165*, 160–166.
170. Matsubara, T.; Hayashi, A. *Biochim. Biophys. Acta* **1993**, *1166*, 55–63.
171. Saito, M.; Kitamura, H.; Sugiyama, K. *Biochim. Biophys. Acta* **2001**, *1511*, 271–280.
172. Saito, M.; Kitamura, H.; Sugiyama, K. *Comp. Biochem. Physiol. B* **2002**, *131*, 433–441.
173. Aoki, K.; Sugiyama, S.; Dulaney, J. T.; Itonori, S.; Sugita, M. *J. Oleo Sci.* **2002**, *51*, 463–472.
174. Kubo, H.; Irie, A.; Inagaki, F.; Hoshi, M. *J. Biochem.* **1988**, *104*, 755–760.
175. Kubo, H.; Jiang, G. J.; Irie, A.; Morita, M.; Matsubara, T.; Hoshi, M. *J. Biochem.* **1992**, *111*, 726–731.
176. Kubo, H.; Jiang, G. J.; Irie, A.; Suzuki, M.; Inagaki, F.; Hoshi, M. *J. Biochem.* **1992**, *112*, 281–285.
177. Inagaki, F.; Tate, S.; Kubo, H.; Hoshi, M. *J. Biochem.* **1992**, *112*, 286–289.
178. Babu, U. V.; Bhandari, S. P. S.; Grag, H. S. *J. Nat. Prod.* **1997**, *60*, 732–734.
179. Karlsson, K. A. *Chem. Phys. Lipids* **1970**, *5*, 6–43.
180. Björkman, L. R.; Karlsson, K. A.; Pascher, I.; Samuelsson, B. E. *Biochim. Biophys. Acta* **1972**, *270*, 260–265.
181. Sugita, M. *J. Biochem.* **1977**, *82*, 1307–1312.
182. Kawano, Y.; Higuchi, R.; Isobe, R.; Komori, T. *Liebigs Ann. Chem.* **1988**, 1181–1183.
183. Higuchi, R.; Kagoshima, M.; Komori, T. *Liebigs Ann. Chem.* **1990**, 659–663.
184. Irie, A.; Kubo, H.; Hoshi, M. *J. Biochem.* **1990**, *107*, 578–586.
185. Irie, A.; Kubo, H.; Inagaki, F.; Hoshi, M. *J. Biochem.* **1990**, *108*, 531–536.
186. Jin, W.; Rinehart, K. L.; Jares-Erijiman, E. A. *J. Org. Chem.* **1994**, *59*, 144–147.
187. Venkannababu, U.; Bhandari, S. P. S.; Grag, H. S. *Liebigs Ann. Chem.* **1997**, 1245–1247.
188. Maier, M. S.; Kuriss, A.; Seldes, A. M. *Lipids* **1998**, *33*, 825–827.
189. Diaz de Vivar, M. E.; Seldes, A. M.; Maier, M. S. *Lipids* **2002**, *37*, 597–603.
190. Kawatate, S.; Nakamura, K.; Inagaki, M.; Higuchi, R. *Chem. Pharm. Bull.* **2002**, *50*, 1091–1096.
191. Chludil, H. D.; Seldes, A. M.; Maier, M. S. *Z. Naturforsch. C* **2003**, *58*, 433–440.
192. Maruta, T.; Saito, T.; Inagaki, M.; Shibata, O.; Higuchi, R. *Chem. Pharm. Bull.* **2005**, *53*, 1255–1258.
193. Higuchi, R.; Inagaki, K.; Togawa, T.; Miyamoto, T.; Komori, T. *Liebigs Ann. Chem.* **1994**, 79–81.
194. Higuchi, R.; Inagaki, M.; Togawa, K.; Miyamoto, T.; Komori, T. *Liebigs Ann. Chem.* **1994**, 653–658.
195. Yamada, K.; Hara, E.; Miyamoto, T.; Higuchi, R.; Isobe, R.; Honda, S. *Eur. J. Org. Chem.* **1998**, 371–378.
196. Yamada, K.; Sasaki, K.; Harada, Y.; Isobe, R.; Higuchi, R. *Chem. Pharm. Bull.* **2002**, *50*, 1467–1470.
197. Kisa, F.; Yamada, K.; Kaneko, M.; Inagaki, M.; Higuchi, R. *Chem. Pharm. Bull.* **2005**, *53*, 382–386.
198. Yamada, K.; Wada, N.; Onaka, H.; Matsubara, R.; Isobe, R.; Inagaki, M.; Higuchi, R. *Chem. Pharm. Bull.* **2005**, *53*, 788–791.
199. Inagaki, M.; Oyama, A.; Arao, K.; Higuchi, R. *Chem. Pharm. Bull.* **2004**, *52*, 1307–1311.
200. Kochetkov, N. K.; Zhukova, I. G.; Smirnova, G. P.; Glukhoded, I. S. *Biochim. Biophys. Acta* **1973**, *326*, 74–83.
201. Hoshi, M.; Nagai, Y. *Biochim. Biophys. Acta* **1975**, *388*, 152–162.
202. Kubo, H.; Irie, A.; Inagaki, F.; Hoshi, M. *J. Biochem.* **1990**, *108*, 185–192.
203. Kochetkov, N. K.; Smirnova, G. P.; Chekareva, N. V. *Biochim. Biophys. Acta* **1976**, *424*, 274–283.
204. Prokazova, N. V.; Mikhailov, A. T.; Kocharov, S. L.; Malchenko, L. A.; Zvezdina, N. D.; Buznikov, G.; Bergelson, L. D. *Eur. J. Biochem.* **1981**, *115*, 671–677.
205. Ijuin, T.; Kitajima, K.; Song, Y.; Kitazume, S.; Inoue, S.; Haslam, S. M.; Morris, H. R.; Dell, A.; Inoue, Y. *Glycoconj. J.* **1996**, *13*, 401–413.
206. Mikhailov, A. T.; Prokazova, N. V.; Zvezdina, N. D.; Kocharov, S. L.; Malchenko, L. A.; Buznikov, G. A.; Bergelson, L. D. *Differentiation* **1981**, *18*, 43–50.
207. Zvezdina, N. D.; Sadvkova, K. A.; Martvnova, L. E.; Prokazova, N. V.; Mikhailov, A. T.; Buznikov, G. A.; Bergelson, L. D. *Eur. J. Biochem.* **1989**, *186*, 189–194.
208. Kubo, H.; Hoshi, M. *J. Biochem.* **1990**, *108*, 193–199.
209. Shogomori, H.; Chiba, K.; Kubo, H.; Hoshi, M. *Zygote* **1993**, *1*, 215–223.
210. Shogomori, H.; Chiba, K.; Hoshi, M. *Glycobiology* **1997**, *7*, 391–398.
211. Nezuo, M.; Shogomori, H.; Hoshi, M.; Yamamoto, T.; Teshima, T.; Shiba, T.; Chiba, K. *Glycobiology* **2000**, *10*, 1243–1247.
212. Sugita, M. *J. Biochem.* **1979**, *86*, 289–300.
213. Sugita, M. *J. Biochem.* **1979**, *86*, 765–772.
214. Smirnova, G. P.; Kochetkov, N. K. *Biochim. Biophys. Acta* **1980**, *618*, 486–495.

215. Kochetkov, N. K.; Smirnova, G. P.; Glukhoded, I. S. *Biochim. Biophys. Acta* **1982**, *712*, 650–658.
216. Kochetkov, N. K.; Smirnova, G. P. *Biochim. Biophys. Acta* **1983**, *759*, 192–198.
217. Smirnova, G. P.; Kochetkov, N. K.; Sadovskaya, V. L. *Biochim. Biophys. Acta* **1987**, *920*, 47–55.
218. Kawano, Y.; Higuchi, R.; Komori, T. *Liebigs Ann. Chem.* **1990**, 43–50.
219. Smirnova, G. P. *Bioorg. Khim.* **1990**, *16*, 830–838.
220. Miyamoto, T.; Yamamoto, A.; Wakabayashi, M.; Nagaregawa, Y.; Inagaki, M.; Higuchi, R.; Iha, M.; Teruya, K. *Eur. J. Org. Chem.* **2000**, 2295–2301.
221. Higuchi, R.; Inagaki, K.; Natori, T.; Komori, T.; Kawajiri, S. *Liebigs Ann. Chem.* **1991**, 1–10.
222. Higuchi, R.; Matsumoto, S.; Isobe, R.; Miyamoto, T. *Tetrahedron* **1995**, *51*, 8961–8968.
223. Smirnova, G. P.; Glukhoded, I. S.; Kochetkov, N. K. *Bioorg. Khim.* **1988**, *14*, 636–641.
224. Muralikrishna, G.; Reuter, G.; Peter-Katalinic, J.; Egge, H.; Hanisch, F. G.; Siebert, H. C.; Schauer, R. *Carbohydr. Res.* **1992**, *236*, 321–326.
225. Miyamoto, T.; Inagaki, M.; Isobe, R.; Tanaka, Y.; Higuchi, R.; Iha, M.; Teruya, K. *Liebigs Ann. Chem.* **1997**, 931–936.
226. Kawatake, S.; Inagaki, M.; Isobe, R.; Miyamoto, T.; Higuchi, R. *Liebigs Ann. Chem.* **1997**, 1797–1800.
227. Kawatake, S.; Inagaki, M.; Miyamoto, T.; Isobe, R.; Higuchi, R. *Eur. J. Org. Chem.* **1999**, 765–769.
228. Inagaki, M.; Isobe, R.; Higuchi, R. *Eur. J. Org. Chem.* **1999**, 771–774.
229. Kawatake, S.; Inagaki, M.; Isobe, R.; Miyamoto, T.; Higuchi, R. *Chem. Pharm. Bull.* **2002**, *50*, 1386–1389.
230. Kawatake, S.; Inagaki, M.; Isobe, R.; Miyamoto, T.; Higuchi, R. *Chem. Pharm. Bull.* **2004**, *52*, 1002–1004.
231. Yamada, K.; Harada, Y.; Nagaregawa, Y.; Miyamoto, T.; Isobe, R.; Higuchi, R. *Eur. J. Org. Chem.* **1998**, 2519–2525.
232. Kaneko, M.; Kisa, F.; Yamada, K.; Miyamoto, T.; Higuchi, R. *Eur. J. Org. Chem.* **1999**, 3171–3174.
233. Yamada, K.; Harada, Y.; Miyamoto, T.; Isobe, R.; Higuchi, R. *Chem. Pharm. Bull.* **2000**, *48*, 157–159.
234. Yamada, K.; Matsubara, R.; Kaneko, M.; Miyamoto, T.; Higuchi, R. *Chem. Pharm. Bull.* **2001**, *49*, 447–452.
235. Yamada, K.; Hamada, A.; Kisa, F.; Miyamoto, T.; Higuchi, R. *Chem. Pharm. Bull.* **2003**, *51*, 46–52.
236. Arao, K.; Inagaki, M.; Higuchi, R. *Chem. Pharm. Bull.* **1999**, *47*, 687–689.
237. Arao, K.; Inagaki, M.; Higuchi, R. *Chem. Pharm. Bull.* **2001**, *49*, 695–698.
238. Arao, K.; Inagaki, M.; Miyamoto, T.; Higuchi, R. *Chem. Pharm. Bull.* **2004**, *52*, 1140–1142.
239. Inagaki, M.; Shibai, M.; Isobe, R.; Higuchi, R. *Chem. Pharm. Bull.* **2001**, *49*, 1521–1525.
240. Aiello, A.; Fattorusso, E.; Menna, M. *Biochem. Syst. Ecol.* **1996**, *24*, 521–529.
241. Duran, R.; Zubia, E.; Ortega, M. J.; Naranjo, S.; Javier, S. *Tetrahedron* **1998**, *54*, 14597–14602.
242. Aiello, A.; Fattorusso, E.; Mangoni, A.; Menna, M. *Eur. J. Org. Chem.* **2002**, 1047–1050.
243. Joseph, J. D. *Prog. Lipid Res.* **1979**, *18*, 1–30.
244. Karlsson, K. A.; Leffler, H.; Samuelsson, B. E. *Biochim. Biophys. Acta* **1979**, *574*, 79–93.
245. Haseley, S. R.; Vermeer, H. J.; Kamerling, J. P.; Vliegenthart, J. F. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9419–9424.
246. Schmitz, F. J.; McDonald, F. J. *J. Lipid Res.* **1974**, *15*, 158–164.
247. Endo, M.; Nakagawa, M.; Hamamoto, Y. *Pure Appl. Chem.* **1986**, *58*, 387–394.
248. Hirsch, S.; Kashman, Y. *Tetrahedron* **1989**, *45*, 3897–3906.
249. Nagle, D. G.; McClatchey, W. C.; Gerwick, W. H. *J. Nat. Prod.* **1992**, *55*, 1013–1017.
250. Hayashi, A.; Nishimura, Y.; Matsubara, T. *Biochim. Biophys. Acta* **1991**, *1083*, 179–186.
251. Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591–5592.
252. Akimoto, K.; Natori, T.; Morita, M. *Tetrahedron Lett.* **1993**, *34*, 5593–5596.
253. Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771–2784.
254. Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176–2187.
255. Kobayashi, E.; Motoki, K.; Natori, T.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Biol. Pharm. Bull.* **1996**, *19*, 350–353.
256. Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626–1629.
257. Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Sato, H.; Kondo, E.; Harada, M.; Koseki, H.; Nakayama, T.; Tanaka, Y.; Taniguchi, M. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 5690–5693.
258. Burdin, N.; Brossay, L.; Koezuka, Y.; Smiley, S. T.; Grusby, M. J.; Gui, M.; Taniguchi, M.; Hayakawa, K.; Kronenberg, M. *J. Immunol.* **1998**, *161*, 3271–3281.
259. Prigozy, T. I.; Naidenko, O.; Qasba, P.; Elewaut, D.; Brossay, L.; Khurana, A.; Natori, T.; Koezuka, Y.; Kulkarni, A.; Kronenberg, M. *Science* **2001**, *291*, 664–667.
260. Taniguchi, M.; Harada, M.; Kojo, S.; Nakayama, T.; Wakao, H. *Annu. Rev. Immunol.* **2003**, *21*, 483–513.
261. Van Kaer, L. *Nat. Rev. Immunol.* **2005**, *5*, 31–42.
262. Gonzalez-Aseguinolaza, G.; de Oliveira, C.; Tomaska, M.; Hong, S.; Bruna-Romero, O.; Nakayama, T.; Taniguchi, M.; Bendelac, A.; Van Kaer, L.; Koezuka, Y.; Tsuji, M. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8461–8466.
263. Ito, K.; Karasawa, M.; Kawano, T.; Akasaka, T.; Koseki, H.; Akutsu, Y.; Kondo, E.; Sekiya, S.; Sekikawa, K.; Harada, M.; Yamashita, M.; Nakayama, T.; Taniguchi, M. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 740–744.
264. Li, H. Y.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1995**, *51*, 2273–2280.
265. Costantino, V.; Fattorusso, E.; Mangoni, A.; Di Rosa, M.; Inaro, A.; Maffia, P. *Tetrahedron* **1996**, *52*, 1573–1578.
266. Uchimura, A.; Shimizu, T.; Nakajima, M.; Ueno, H.; Motoki, K.; Fukushima, H.; Natori, T.; Koezuka, Y. *Bioorg. Med. Chem.* **1997**, *5*, 1447–1452.
267. Borbone, N.; De Marino, S.; Iorizzi, M.; Zollo, F.; Debitus, C.; Ianaro, A.; Pisano, B. *Eur. J. Org. Chem.* **2001**, *24*, 4651–4656.
268. Costantino, V.; Fattorusso, E.; Mangoni, A. *Tetrahedron* **2000**, *56*, 5953–5957.
269. Mori, K.; Tashiro, T.; Akasaka, K.; Ohrui, H.; Fattorusso, E. *Tetrahedron Lett.* **2002**, *43*, 3719–3722.
270. Muralidhar, P.; Krishna, N.; Kumar, M. M.; Rao, C. B.; Rao, D. V. *Chem. Pharm. Bull.* **2003**, *51*, 1193–1195.
271. Emura, C.; Higuchi, R.; Miyamoto, T.; Van Soest, R. W. *J. Org. Chem.* **2005**, *70*, 3031–3038.
272. Costantino, V.; Fattorusso, E.; Imperatore, C.; Mangoni, A. *J. Org. Chem.* **2004**, *69*, 1174–1179.
273. Costantino, V.; D'Esposito, M.; Fattorusso, E.; Mangoni, A.; Basilico, N.; Parapini, S.; Taramelli, D. *J. Med. Chem.*. **2005**, *48*, 7411–7417.

Biographical Sketch



Saki Itonori studied chemistry at the Faculty of Liberal Arts & Education, Shiga University, under the direction of Prof. Mutsumi Sugita and graduated in 1988. He joined as a research student in the group of Prof. Yoshitaka Nagai at the Faculty of Medicine, The University of Tokyo, for two years. He received his Ph.D. from the Faculty of Agriculture, The University of Tokyo, in 1996 under the direction of Prof. Tomoya Ogawa. He was a postdoctoral fellow at Tulane University Medical Center (Prof. Yu-Teh Li Lab.) from 1996 to 1997 and at Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine (Prof. Ronald L. Schnaar Lab.) from 1997 to 2000. During two years at JHU, he was granted a postdoctoral fellowship for research abroad from the Japan Society for the Promotion of Science. He was a lecturer (2000–02), an associate professor (2002–07), and since 2007 he is a professor at the Department of Chemistry, Faculty of Liberal Arts & Education, Shiga University.

Mutsumi Sugita studied chemistry at the Faculty of Liberal Arts & Education, Shiga University, under the direction of Emeritus Prof. Taro Hori (deceased), and graduated in 1967. Since 1967, he was an assistant at Shiga University and a researcher at Department of Chemistry, Faculty of Science, Kyoto University (Emeritus Prof. Hirohiko Katsuki Lab. (deceased)), in 1969. He was a researcher at the Department of Neurology, Massachusetts General Hospital, in 1971. He received his Ph.D. from the Faculty of Science, Kyoto University, in 1980. He joined as a researcher in the group of Prof. Yoshitaka Nagai at the Institute of Medical Science, The University of Tokyo, in 1981. He was a lecturer (1973–76), an associate professor (1976–85), and since 1985, is a professor at the Department of Chemistry, Faculty of Liberal Arts & Education, Shiga University.