1-Deoxysphingolipids Encountered Exogenously and Made De Novo: Dangerous Mysteries Inside an Enigma*

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ABSTRACT

The traditional backbones of mammalian sphingolipids are 2-amino, 1, 3-diols made by serine palmitoyltransferase (SPT). Many organisms additionally produce non-traditional, cytotoxic 1-deoxysphingoid bases and, surprisingly, mammalian SPT biosynthesizes some of them, too (e.g., 1-deoxysphinganine from L-alanine). These are rapidly N-acylated to 1-deoxy-"ceramides" with very uncommon biophysical properties. The functions of 1-deoxysphingolipids are not known, but they are certainly dangerous as contributors to sensory and autonomic neuropathies when elevated by inherited SPT mutations, and they are noticeable in diabetes, non-alcoholic steatohepatitis, serine deficiencies and other disease. As components of food as well as made endogenously, these are new mysteries within an enigma.

EXAMPLES OF 1-DEOXY-SPHINGOID BASES

Sphinganine-analogue mycotoxins. The most extensively studied 1-deoxy-sphingoid bases are represented by fumonisin B1 (FB1, Fig. 1C). These sphinganine-analogue mycotoxins are

1 Abbreviations: 1-deoxyCer, 1-deoxyceramides (N-acyl-1-deoxysphingosine); 1-deoxyDH Cer, 1-deoxy-dihydroceramides (N-acyl-1-deoxysphinganine); 1-deoxy(DH)Cer, 1-deoxyceramides and 1-deoxy-dihydroceramides; 1-deoxySL, 1-deoxysphingo-lipids (all compounds lacking 1-hydroxyl on sphingoid base); Cer, ceramide; CerS, ceramide synthase; DH Cer, dihydroceramide; (DH) Cer, ceramides and dihydroceramides; FB1, fumonisin B1; HSAN1, hereditary sensory and autonomic neuropathy type I disease; PLP, Pyridoxal 5'-phosphate; S1P, sphingosine 1-phosphate; SM, sphingomyelin; SPT, serine palmitoyltransferase

The 2-amino, 1,3-diol moieties of sphingosine (Fig. 1A) were first described in a letter to the editors of The Journal of Biological Chemistry from H. E. Carter and colleagues in 1942, then as a full manuscript (1). The term “sphingolipid” was also proposed (2) for this category of compounds, building on the “sphingo-” morpheme chosen by J. L. W. Thudichum in naming “sphingosin” for “…the many enigmas which it presented to the inquirer…” (3).
produced by *Fusarium verticilloides* and related fungi (9) that infest maize and cause diseases of plants (10) and animals that consume contaminated food (11-14). Their major biochemical targets in both plants (10) and animals (11-13,15) are ceramide synthases (CerS), enzymes responsible for N-acylation of sphingoid bases (16,17). In addition to being inhibitors of CerS, fumonisins are N-acylated by CerS (18), as is the aminopentol backbone released from fumonisins when corn is treated with lye in preparation of masa (19). N-acyl-aminopentols also inhibit CerS.

Disruption of sphingolipid metabolism by fumonisins and related AAL-toxins (from *Alternaria alternata* (10)) induces plant programmed cell death pathways associated with defense and disease (20,21). This is thought to be a major reason that these mycotoxins are produced, but they might additionally provide protection against other inhabitants of the ecological niche of these fungi (22).

Fumonisin consumption causes a wide spectrum of animal disease: hepatotoxicity and hepatocarcinogenicity, renal toxicity, neurotoxicity, pulmonary edema (9,23), and for humans esophageal cancer (9,11-14) and probably birth defects (13,24,25). It is not surprising that they produce so many disorders because CerS inhibition causes buildup of highly bioactive compounds (sphinganine, sphinganine 1-phosphate, N-acetyl-sphinganine and others) and suppresses biosynthesis of Cer and complex sphingolipids, depending on the length of exposure and dosage (11,12). FB1 is often used as a tool to block Cer production and study Cer functions; however, the results must be interpreted with caution since this alters many other bioactive sphingolipids.

*Oceanin, Calyxin and other complex 1-deoxy-sphingoid bases.* Perhaps the most structurally amazing 1-deoxy-analogs are “two-headed”—i.e., appearing as if two sphingoid bases are connected tail-to-tail (see oceanapiside from *Oceanapia phillipensis* (26,27), Fig. 1C). These compounds often display antibacterial or antifungal activity, which might be their biologic function; many are cytotoxic for cancer cells (27-32). These compounds illustrate only a fraction of the sphingoid base biodiversity (5).

**Simple 1-deoxy-sphingoid bases—e.g., 1-deoxyphinganines and related compounds.** Many organisms have been known to produce simple 1-deoxy- and 1-deoxymethyl- sphingoid bases (5) (Fig. 1B and C) such as xestoaminol C from *Xestospongia sp.* and a methyl-branched 1-deoxyphinganinen(2-amino-14,16-dimethyl-octadecan-3-ol, 2-AOD-3-ol), produced by *Fusarium avenaceum*, a fungus found on grains and fruit (33,34). 1-Deoxyphinganine (Fig. 1B) was initially named spisulosine when isolated from the edible Stimpson’s surf clam, or Atlantic surf clam (*Spisula polynyma*), during a screen for anti-cancer compounds (35). Being cytotoxic for cancer cells in culture (36,37), it has been evaluated in phase I clinical trials, which will be described later in this minireview.

**MAMMALIAN PRODUCTION OF 1-DEOXYSPHINGOID BASES**

Considering the unusual structural features and cytotoxicity of 1-deoxyphingoid bases, it came as a surprise when mammals, including humans, were found to produce them by two independent lines of investigation published at approximately the same time (6-8). One study discovered that mutations in the initial enzyme of traditional sphingoid base biosynthesis (serine palmitoyltransferase, SPT) that cause hereditary sensory and autonomic neuropathy type I disease (HSAN1) allow SPT to utilize L-Ala and glycine to make 1-deoxysphinganine and 1-(deoxymethyl)sphinganine, respectively (7), which are neurotoxic when added to dorsal root ganglia neuron cultures (8).

The other study (6) characterized 1-deoxysphinganine as a previously noticed (38), but unidentified, compound that accumulates when cells in culture or animals are exposed to FB1. It was shown to be produced in substantial amounts from L-Ala by wild-type SPT, and was probably been overlooked previously because it is mainly present as N-acyl-metabolites (e.g., 1-deoxydihydroceramides, 1-deoxyDHCer) unless CerS is inhibited.

**Background information about SPT.** SPT is a family of pyridoxal 5'-phosphate (PLP)-dependent isozymes that catalyze the reaction displayed in Fig. 2 (39,40). It’s proposed mechanism is typical
for the alpha-oxoamine synthase (AOS) family (41), and some of the main features are: formation of a Schiff base between PLP and an active site Lys (called an “internal aldimine”); displacement of Lys when an amino acid substrate is bound (forming the “external aldimine”); orientation of the amino acid-PLP imine in a configuration described as the “Dunathan intermediate” to facilitate abstraction of the amino acid α-proton forming a quinoid intermediate; carbon-carbon bond formation between the amino acid α-carbon and a fatty acyl-CoA, displacing CoASH; decarboxylation of this β-unsaturated intermediate to form a product external ketimine; protonation of the ketimine to form the external aldimine of the 3-keto-sphingoid base, which is released to regenerate the enzyme PLP-internal aldimine. The traditional reaction catalyzed by SPT utilizes L-Ser as the substrate to make 3-ketosphinganine (in red), and the analogous reaction with L-Ala (green) produces 1-deoxysphinganine and with glycine, 1-(deoxymethyl)sphinganine (not shown).

Mammalian SPT appears to be comprised of heterotrimeric isoforms that share an SPTLC1 (sometimes referred to as SPT1 or hLCB1) subunit combined with either SPTLC2 (also called SPT2 or hLCB2a) or SPTLC3 (also called SPT3, LBC3 or hLCB2b) subunit and one of two highly-related isoforms of a third “small subunit” (in humans, ssSPTa and ssSPTb) (40). The active site Lys (Fig. 2) resides in the SPTLC2/SPTLC3 subunit.

The SPTLC2/SPTLC3 subunit influences the specificity for the acyl-CoA substrate (42) in a manner that also depends on the ssSPT isoform (43,44). That is, from studies in which cells were transfected with these isoforms in different combinations (43,44): SPTLC1/SPTLC2/ssSPTa had a clear preference for palmitoyl-CoA (the precursor for the 18-carbon-chain length sphingoid bases); SPTLC1/SPTLC2/ssSPTb utilized both palmitoyl-CoA and stearoyl-CoA (the latter producing 20-carbon-chain length sphingoid bases); SPTLC1/SPTLC3/ssSPTa had a clear preference for palmitoyl-CoA (the precursor for the 18-carbon-chain length sphingoid bases); SPTLC1/SPTLC3/ssSPTb utilized myristoyl-CoA and palmitoyl-CoA (the former producing 16-carbon-chain length sphingoid bases); and SPTLC1/SPTLC3/ssSPTb, which seems to use a wide range of chain length fatty acyl-CoA’s. Another level of regulation involves ORMLD proteins, which have been proposed to help control flux through the pathway (45-48).
SPTLC2 at this site as a regulator of 1-deoxySL synthesis by wild-type SPT.

To determine if lowering 1-deoxySL might be clinically beneficial, Garofano et al. (56) fed a 10% L-Ser-enriched diet to mice bearing a transgene expressing C133W SPTLC1 and 1-deoxySL decreased significantly, reaching the levels of mice with wild-type SPT within 2 to 4 days. Mice on the L-Ser-enriched diet were also protected from neurodegeneration (measured by mechanical sensitivity and motor performance), and retained neurological function up to 15 months of age; untreated mice developed neuropathy by that age. In contrast to these favorable responses, mice fed a 10% L-Ala diet had elevated 1-deoxySL and developed severe peripheral neuropathy. A pilot study with HSAN1 patients also found that L-Ser supplementation reduced 1-deoxySL levels, and a clinical trial is ongoing (https://clinicaltrials.gov).

Production of 1-deoxysphingoid bases by wild-type SPT. In the other early study, 1-deoxySL were identified as products of wild-type SPT by mass spectrometry (6), which characterized both the free 1-deoxysphinganine in cells incubated with FB1 and the N-acyl-derivatives when CerS was not inhibited. This acylation might explain why these compounds have been previously overlooked since they are somewhat difficult to detect in a background of Cer and other neutral lipids.

Wild-type SPT was proven to be the source because biosynthesis of 1-deoxySL from L-Ala was absent in CHO-LyB cells and reappeared when the normal SPT1 subunit was restored (6). The amounts of 1-deoxysphinganine made by wild-type SPT can be quite substantial—for examples: about half of the level of sphinganine in LLC-PK1 cells after ~4 days in culture with FB1; Vero cells have high basal 1-deoxySL, which might be due to these cells depleting L-Ser in the medium (57); and RAW264.7 cells (58) have essentially equal amounts of 1-deoxyDHCer and Cer after 4 days in culture, and are also known (59) to deplete the culture medium of L-Ser while accumulating L-Ala and glycine.

There has not yet been an explanation for why wild-type SPT is somewhat “promiscuous” (to use a term applied to mutant SPT) (44) in utilizing three amino acids as substrates, nor if this might occur with other alpha-oxoamine synthase family members (40). The sidechains of L-Ala and Gly are smaller than the hydroxymethyl-group of L-Ser and could fit in the same binding pocket. The favoring of L-Ser appears to be due to an interaction between the sidechain hydroxyl of L-Ser and the 5’-phosphate of PLP, both for substrate binding and optimal catalytic efficiency (60).

Since amino acid availability is an important factor in the amounts of 1-deoxySL that are made, it would be interesting to know more about other factors that are thought to influence L-Ser utilization for lipid synthesis, such as SERINC (61) and, at least for yeast, CHA1, which codes for an L-Ser deamidase/dehydratase that regulates sphingolipid levels by limiting available L-Ser (and perhaps vice versa) (62).

METABOLISM AND TRAFFICKING

1-Deoxysphingoid base metabolism. Most publications on 1-deoxySL have described them as total “1-deoxysphinganines” or 1-deoxySL rather than as individual molecular species because they have been quantified after acid hydrolysis to release the free sphingoid bases. As noted above, when specific molecular species were analyzed by LC-MS/MS, the majority of traditional and 1-deoxy-sphingoid bases are N-acyl-derivatives (Fig. 3) (4).

In this pathway, the initially formed 3-keto-intermediates are rapidly reduced and N-acylated, followed by addition a headgroup (dihydroSM, etc.), desaturation to produce the backbone double bond (making Cer), then addition of headgroups or hydrolyzed to sphingosine, which can be reacylated or converted to sphingosine 1-phosphate (S1P), which is cleaved to ethanolamine phosphate and hexadecenal (hexadecanal from sphinganine 1-phosphate). There are also reports of N-methylation of some sphingoid bases (63,64).

The early steps of this pathway appear to be similar for the 1-deoxy-sphingoid bases (Fig. 3). The kinetics parameters for N-acylation of various sphingoid base variants have been compared using rat liver microsomes (19). The apparent Km for 1-deoxysphinganine (2 mM) is somewhat higher than for sphinganine (0.5 mM), but the Vmax are similar. Individual CerS have not been analyzed,
but the N-acyl-chain length distribution of 1-deoxy(DH)Cer of different types of cells suggest that most or all of the CerS accommodate these compounds (65). Little is known about desaturation of 1-deoxy(DH)Cer; likewise, the possibility of alternative metabolites, such as N-methylated species, has not been explored. Turnover by lyase cleavage (Fig. 3) would appear to be unavailable to 1-deoxySL unless S1P lyase, or another enzyme, can catalyze an analogous reaction with 1-deoxysphingoid bases.

1-Deoxydihydroceramide trafficking. The intracellular trafficking of Cer has been studied using fluorescently labeled analogs, such as N-(4-nitrobenzo-2-oxa-1,3-diazole-aminocaproyl)-sphingosine (C₆-NBD-Cer) (66), which is rapidly taken up by cells in culture and fluorescence is seen first in multiple intracellular compartments (the plasma membrane, ER, nuclear envelope, and mitochondria), then the Golgi apparatus becomes intensely fluorescent concomitant with its metabolism to NBD-SM and NBD-GlcCer, which appear at the plasma membrane after longer times. In contrast, C₆-NBD-1-deoxyDHCer (67) was neither metabolized nor labeled the Golgi apparatus and plasma membrane, even after prolonged incubation. Thus, the 1-deoxySL do not appear to undergo the typical trafficking of traditional Cer, which is in agreement with similar studies with a 1-methoxy-analog (68).

The 1-deoxySL in plasma (69,70) appear to be associated mainly with lipoproteins (71), which might be of hepatic origin (70).

CELLULAR EFFECTS OF 1-DEOXY-SPHINGOLIPIDS

Some of the earliest findings with 1-deoxysphinganine were that it has diverse effects on cell growth and survival: sometimes stimulating cell proliferation (for Swiss 3T3 cells at 1 µM) (72); inhibiting growth (for Vero cells at ~2 µM), possibly due to disruption of actin stress fibers through inactivation of Rho (35), and for the human glioblastoma cell line SHG-44 (73); and often displaying cytotoxicity at low micromolar concentrations for DU145 and LLCPK1 cells (6), MDA MB 468 cells (65), and PC-3 and LNCaP cells (37), as examples. The cytotoxicity has been proposed to have several causes: stimulation of de novo synthesis of Cer and PKCζ activation (37); and an atypical cell death program with activation of caspase 3 and 12 and altered phosphorylation of p53 (36). ER stress might also have a role in 1-deoxySL-mediated apoptosis (44,74). Effects on insulin-producing cells (75) include compromised glucose-stimulated insulin secretion, intracellular accumulation of filamentous actin, activation of Rac1, increased CerS5 expression and morphologic changes characteristic of senescent, necrotic, and apoptotic cells.

Other reported effects of 1-deoxySL are sphingosine kinase 1 inhibition (and/or its proteasomal degradation) (76) and perturbation of membrane structure because 1-deoxy(DH)Cer are poorly miscible with other lipids (some 1-deoxySL are not even capable of forming monolayers at the air-water interface) (58). The latter might contribute to the formation of lipid bodies in cells accumulating 1-deoxySL (77). Another intriguing finding is that 1-deoxy-(DH)Cer have been reported to be one of the endogenous ligands for human CD1b antigen-presenting molecules (78).

As a cautionary note, it is difficult to determine what the “normal” functions of 1-deoxSL are because studies with cells in culture begin with cells that probably already contain abnormally high 1-deoxySL since they are present in serum and/or produced by the cells themselves, due to the tendency of many cell lines to deplete L-Ser and accumulate L-Ala in the medium.

1-DEOXY-SPHINGOID BASES AND OTHER DISEASE

Diabetes. In common with HSAN1, one of the clinical complications of diabetes mellitus is sensory neuropathy; therefore, connections between 1-deoxySL and diabetes have been explored. A case-control study of plasma from healthy and diabetic individuals found that 1-deoxySL were higher in the diabetic group, which also displayed lower plasma Ser (71). 1-DeoxySL have been found to be elevated in plasma from subjects with metabolic syndrome (79) and type 2 diabetes (80) (levels in type 1 diabetes did not differ from controls). 1-DeoxySL were also examined as possible predictive biomarkers for type 2 diabetes (81) in a prospective cohort with 339 individuals who were followed for a period of...
1-Deoxysphingolipids: mysteries in an enigma

8 years, and were elevated in patients with metabolic syndrome, impaired fasting glucose, and type 2 diabetes, and for patients who developed diabetes during the follow-up period. 1-DeoxySL were found to be significantly elevated in plasma from patients with distal sensorimotor polyneuropathy, a frequent, disabling complication of diabetes mellitus, and were detectable in early disease stages but did not correlate with the clinical course (82).

In analogy to the studies conducted with an animal model for HSAN1, L-Ser supplementation has been tested in diabetic STZ rats (81). This intervention not only lowered plasma 1-deoxySL but also improved mechanical sensitivity, in agreement with the hypothesis that 1-deoxySL are involved in the pathology of diabetic neuropathy and L-Ser supplementation might be clinically beneficial.

It is worth mention that plasma L-Ala is elevated following glucose ingestion (83), and fructose ingestion has an even greater effect on plasma L-Ala concentration (84).

Non-alcoholic fatty liver disease, especially non-alcoholic steatohepatitis (NASH). Nonalcoholic fatty liver disease is associated with metabolic syndrome and is becoming one of the most common forms of liver disease worldwide. It is thought to progress from relatively benign stages to steatohepatitis (NASH), which can develop into end-stage liver disease, cirrhosis, and sometimes hepatocellular carcinoma. A recent double-blinded study of plasma, liver biopsies and urinary lipids from 88 subjects with liver histology categorized as normal, steatotic, NASH or cirrhotic (70) found that a diverse panel of 20 plasma lipids and aqueous metabolites separated these states by linear discriminant analysis, with the compounds that gave the greatest distinction between NASH and steatosis including the 1-deoxy-DHCer. A possible explanation for this association might be L-Ser deficiency that has been reported for NASH (85).

Defective Ser biosynthesis. L-Ser is made de novo by a pathway initiated by D-3-phosphoglycerate dehydrogenase (PHGDH), and mice carrying a brain-specific deletion of Pchgdl have been used to study the effects of defects in this pathway on 1-deoxySL (77). The mice displayed reductions in both L- and D-Ser and elevation of 1-deoxySL that were associated with mild microcephaly and atrophy of the forebrain, including the cerebral cortex and hippocampus. No significant changes in traditional Cer and SL were noted. Since humans with genetic defects in this enzyme exhibit Ser deficiency and severe neurological symptoms, these results raise the possibility that 1-deoxySL might be involved in the central neurological symptoms (77).

Tumor Necrosis Factor (TNF)-dependent toxicity via caspase signaling in dopaminergic neurons. Dopaminergic neurons in the ventral midbrain selectively degenerate in Parkinson’s disease and TNF can increase neuronal cell death. TNF treatment of dopaminergic neurons has been found to increase 1-deoxySL, which reduce cell viability and inhibit neurite outgrowth and branching in primary when added exogenously (86). Therefore, induction of de novo biosynthesis of 1-deoxySL might be involved in the neurotoxicity of TNF for dopaminergic neurons.

1-DEOXY-SPHINGOIDS AS THERAPEUTIC AGENTS?

Clinical trials with atypical sphingoid bases. The first structural variant that was evaluated in a phase I clinical trial (87) was Safingol (L-threo-sphinganine), which has 2S,3S stereochemistry as found in fumonisins (Fig. 1C). This is not a 1-deoxySL but inhibits sphingosine kinase and affects some of the same targets as 1-deoxysphinganine (88). The maximum tolerated dose was 840 mg/m² (~1 to 2 g based on adult body surface areas of ~1.5 to 2 m²) administered i.v. over 120 min, with the dose-limiting toxicity attributed to hepatic enzyme elevation. Plasma S1P was reduced for Safingol doses of 750 to 930 mg/m². Of the 37 patients that were evaluated for response, 6 were reported to have some degree of disease stabilization, and one with adrenal cortical cancer had regression of liver and lung metastases.

Several phase I clinical trials have been conducted with 1-deoxysphinganine (named “ES-285”) and relevant findings from two will be mentioned here. From dose-escalating studies (89,90), the maximum tolerated dose was ~200 mg/m² (i.e., ~0.3 to 0.4 g for body surface areas of 1.5 to 2 m², respectively). The dose-limiting
toxicities were relatively consistent for all the studies: hepatic and neurological toxicity as well as injection site reactions. One patient who received 8 infusions of ES-285 at 128 mg/m² developed numbness of the face, hands, and feet that worsened rapidly to neuropathy, pain, and general weakness that was assessed to contribute to his death (90). Clinical development of ES-285 as a single agent was discontinued due to its questionable safety profile and limited antitumor activity. Noteworthy from these trials was the similarity in the adverse effects and the neuropathies that have been associated with elevations in 1-deoxySL produced de novo.

Animal studies with a synthetic 1-deoxy-sphingoid base. A synthetic 1-deoxysphingoid base, named Enigmol (Fig. 4), displayed tumor suppression with little toxicity when administered to mouse models for colon and prostate cancer (91,92). Enigmol is not phosphorylated and is poorly N-acylated (93), and one of the most interesting findings from these in vivo studies was a high oral bioavailability versus traditional sphingoid bases. The likely explanation for this difference, which might apply to other 1-deoxy-sphingoid bases, is shown in Fig. 4. Traditional sphingoid bases are readily taken up by intestinal cells but mainly phosphorylated and degraded (94), which limits their effectiveness against colon cancer targets, but cleavage reduces the likelihood that the intermediate S1P will promote carcinogenesis (94,95). Lacking the 1-hydroxyl group, 1-deoxysphingoid bases (at least, as exemplified by Enigmol) are absorbed, escape phosphorylation and degradation, and appear in blood and tissues (91). Another factor affecting the absorption of these compounds is efflux via P-glycoprotein (96). All in all, the possible uptake of 1-deoxySL from food highlights the need for a better understanding of their effect(s) on health.

Other clinical applications? Sphingolipids and sphingoid base-like compounds, including derivatives of such compounds, have been suggested to offer promise as anti-bacterial (97) and antifungal (98) drugs and for other disease (99,100).

CONCLUSIONS AND FUTURE PERSPECTIVES

The capacity to make 1-deoxy-sphingoid bases was once thought to be the purview of organisms that make bizarre secondary metabolites, but is now clearly established to be a process shared by mammals, including humans. This leads one to wonder if these compounds are made as accidents of a sloppy de novo biosynthesis pathway, or to perform biological functions? And, considering their widespread occurrence, how much is present in food and to what extent does the diet can affect tissue 1-deoxySL? Since these compounds can be toxic (but possibly beneficial), how many ways do they impact health? These are some of the intriguing mysteries to be solved for this branch of a family of compounds long known for their enigmas.

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Figure legends

**Figure 1. Representative sphingoid bases and 1-deoxysphingoid bases.** Panel A shows three of the traditional sphingoid bases of mammalian sphingolipids: sphingosine, sphinganine and 4-hydroxysphinganine (phytosphingosine). There is also some degree of variation in the alkyl chain length, branching and number of additional double bonds and hydroxyls (not shown). Panel B shows two simple 1-deoxy-sphingoid bases that are produced by mammals and other organisms, and these are also known to vary in chain length and double bonds. Panel C shows some of the broader structural variation in 1-deoxy-sphingoid bases produced by other organisms. For all of the panels, these structures have been highlighted in red to display the portions that are derived biosynthetically from serine, in green for alanine and brown for glycine. Some additional information for the compounds has been given in parentheses (stereochemistry and alternative names and abbreviations). For more information, see the text and reference (5).

**Figure 2. Scheme for the utilization of L-Serine or L-Alanine for 3-ketosphingoid base biosynthesis by serine palmitoyltransferase.** This diagram has been modified from reference (39) to illustrate the proposed catalytic mechanism for this enzyme and how the intermediates involved in the condensation of L-Ser to make 3-ketosphinganine could plausibly be substituted by L-Ala to make 1-deoxy-3-ketosphinganine with minor variations in the active site chemistry.

**Figure 3. Abbreviated pathway for the biosynthesis and turnover of 1-deoxy-sphingoid bases and traditional sphingoid bases.** This scheme summarizes the steps of de novo biosynthesis of traditional sphingoid bases (sphinganine and sphingosine) in red, as well as their turnover via phosphorylation and cleavage. In green is shown the analogous metabolic steps—as far as they are thought to occur—for 1-deoxysphinganine (produced from alanine) and 1-(deoxymethyl)sphinganine (produced from glycine, not shown). The dashed line indicates the known N-methylation of sphingoid bases, which might also occur for 1-deoxy-sphingoid bases, but this has not yet been established. For more information, see the text and reference (4).

**Figure 4. A schematic representation of the intestinal uptake, metabolism, effects on intestinal cells, and transport to blood and tissues of traditional sphingoid bases (sphingosine and sphinganine) and a synthetic 1-deoxy-sphingoid base (Enigmol).** As shown on the right, traditional sphingoid bases are absorbed well from the lumen of the intestinal tract but most is phosphorylated by sphingosine kinase (SphK) and degraded by S1P lyase. Nutritional studies have shown suppression of colon cancer by dietary sphingolipids, probably through the sphingoid base before phosphorylation and cleavage (94); however, if S1P accumulates (for example, due to defective S1P lyase) this can promote cancer (95). Shown on the left are findings with the synthetic 1-deoxy-sphingoid base Enigmol, which is absorbed more efficiently and transferred to blood and tissues, presumably because it cannot undergo phosphorylation and cleavage. Enigmol has also been shown to suppress intestinal tumorigenesis prostate cancer in animal models (91,92).
1-Deoxysphingolipids: mysteries in an enigma

**Prototypic sphingoid base backbones of mammalian sphingolipids**

1-hydroxy, 2-amino-moiety from serine highlighted in red

D-erythro-sphingosine (2S,3R, d18:1)

**Examples of naturally occurring 1-deoxy-sphingoid bases**

1-deoxy, 2-amino-moiety from alanine highlighted in green

1-deoxysphinganine (m18:0) (spisulosine, ES-285)

1-deoxymethylsphinganine (m17:0)

4-hydroxy-sphinganine (4-t18:0)

D-erythro-sphinganine (d18:0)

Fumonisin B₁ (2S, 3S)

Xestoaminol C

Oceanapiside

2-Amino-14,16-dimethyloctadecan-3-ol

Figure 1
Figure 2
Biosynthesis and turnover of 1-deoxy- vs 1-hydroxy- sphingoid bases and metabolites

1-Deoxysphingolipids: mysteries in an enigma

Figure 3

An analogous pathway with Glycine produces 1-(deoxymethy)-sphingolipids
** Other fatty acyl-CoA’s that are sometimes utilized: Myristoyl- and Stearoyl-CoA
Transport of 1-deoxy- vs 1-hydroxy- sphingoid bases: Lessons from Enigmol vs Sphingosine

Intracellular (e.g., intestinal cells)

Extracellular (e.g., intestinal lumen)

To or from circulation and other tissues

Figure 4
1-Deoxy sphingolipids Encountered Exogenously and Made De Novo: Dangerous Mysteries inside an Enigma
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