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The Chemistry and Biology of 6-Hydroxyceramide, the Youngest Member of the Human Sphingolipid Family

Andrej Kováčik, Jaroslav Roh, and Kateřina Vávrová*[a]

Sphingolipids are crucial for the life of the cell. In land-dwelling mammals, they are equally important outside the cell—in the extracellular space of the skin barrier—because they prevent loss of water. Although a large body of research has elucidated many of the functions of sphingolipids, their extensive structural diversity remains intriguing. A new class of sphingolipids based on 6-hydroxylated sphingosine has recently been identified in human skin. Abnormal levels of these 6-hydroxylated ceramides have repeatedly been observed in atopic dermatitis; however, neither the biosynthesis nor the roles of these unique ceramide subclasses have been established in the human body. In this Minireview, we summarize the current knowledge of 6-hydroxyceramides, including their discovery, structure, stereochemistry, occurrence in healthy and diseased human epidermis, and synthetic approaches to 6-hydroxysphingosine and related ceramides.

Introduction

Sphingolipids (SLs) constitute a complex class of lipids found in mammalian cells. These structures were originally named after the sphinx from Greek mythology by J. L. W. Thudichum in 1884 because their nature and function were a riddle. SLs are present in all eukaryotic cells, where they act as both first and second messengers in cell signaling. In addition, they probably play an essential role in the formation and stability of membrane microdomains, such as lipid rafts, although this is a matter of debate.[1] They regulate several processes, including cell growth, metabolism, development, differentiation, aging, and death.[2] SLs also form a vital part of the epidermal permeability barrier located in the intercellular spaces of the uppermost layer of the skin, the stratum corneum (SC).[3]

SLs are most frequently formed from the long-chain amino diol sphingosine (S, Scheme 1). Acylation of the primary amino group of S by a fatty acid leads to the formation of a ceramide (Cer), which is a central molecule in SL metabolism. Further modification of the primary hydroxy group results in the formation of complex SLs, including glycosphingolipids, such as glucosylceramides (GlcCer), cerebrosides and gangliosides, which contain one or more saccharide units, and sphingomyelins (SM), which have phosphorylcholine or phosphorylethanolamine attached to the hydroxy group at position 1. These complex SLs can be hydrolyzed further to form Cer species. In addition, both S and Cer can be phosphorylated to yield S-1-phosphate and Cer-1-phosphate, respectively.[4]

Here, we focus on SLs based on the sphingoid base 6-hydroxysphingosine (H). This sphingoid base is likely the most enigmatic of the human SLs: it was discovered in the 1990s in the human epidermis, and its structure was only relatively recently completely elucidated, in 2003.[5] Although differences in Cer containing this base have been found in atopic dermatitis (see below), neither its biosynthesis nor its roles in the human body have been established thus far. Because 6-hydroxysphingosine is not commercially available (for more detailed research), we also summarize the synthetic approaches toward this base and related Cer.

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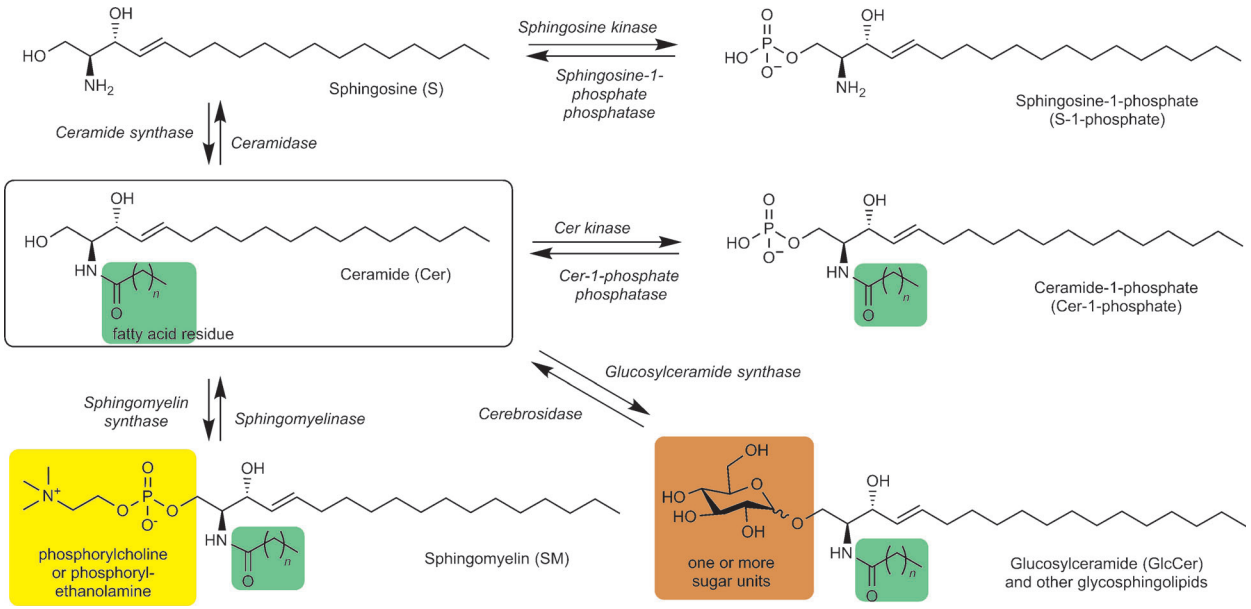
Sphingoid Bases in Mammalian Tissues: Nomenclature, Stereochemistry, and Biosynthesis

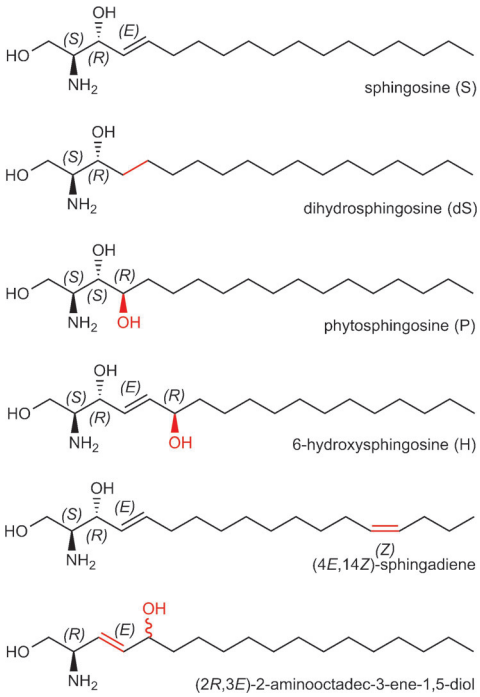
The building blocks of SLs are the sphingoid bases, which form a highly diverse class of long-chain amino alcohols found in many living organisms, including plants, fungi, and insects. Several sphingoid base species with a predominant chain length of 18 carbons are found in the human SL family. In SL chemistry, two short-hand nomenclature systems are used in addition to the official IUPAC names. In the first system, the number of hydroxy groups is expressed as "t" (tri-) or "d" (di-) followed by the numbers of carbons in the chain and of double bonds separated by a colon. The location and configuration of the double bonds is given as a prefix or suffix. Thus, sphingosine is referred to as 4*E*-d18:1 or d18:1$^{\Delta 4t}$. Accordingly, 6-hydroxysphingosine is designated 4*E*-t18:1 (or t18:1$^{\Delta 4t}$).[6] The skin Cer were previously termed Cer1 (the least polar), Cer2, etc., according to their mobility in TLC assays, until the development of the nomenclature of Motta et al.,[7] which was expanded by Robson et al.[8] and Masukawa et al.[9] In this second system, every structure is designated by the initial letter of the sphingoid base: S, dS (or DS), P, and H represent sphingosine, dihydrosphingosine, phytosphingosine, and 6-hydroxysphingosine, respectively. This system also includes a method for naming the fatty acid residues found in Cer: N, A, O, and EO represent nonhydroxylated acyl, α-hydroxyacyl, ω-hydroxyacyl, and ω-linoleyloxyacyl, respectively. Thus, for example, nonhy-



Scheme 1. Sphingolipid classes in mammalian tissues and their interconversion pathways.

droxy acylated sphingosine (formerly known as Cer2) is termed Cer NS, and α-hydroxyacyl phytosphingosine (Cer6) is called Cer AP. Motta nomenclature will be used throughout this Minireview.

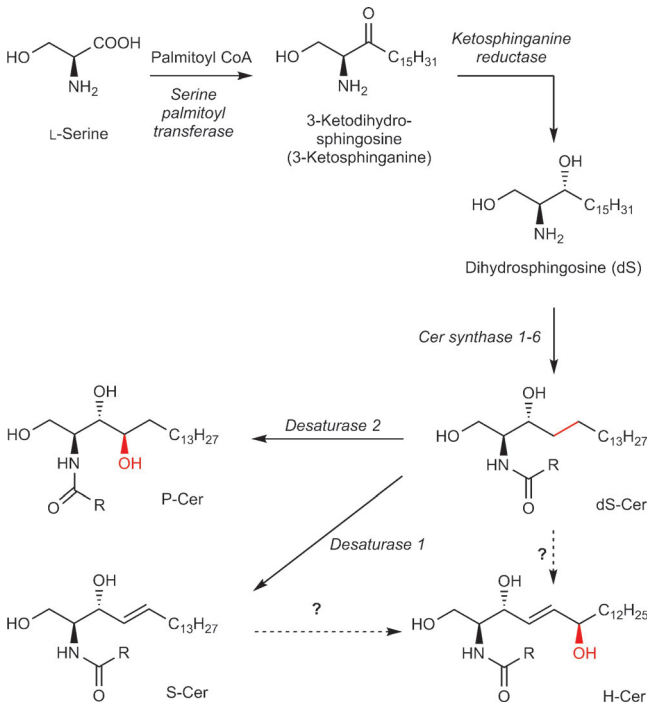
Typical human sphingoid amino alcohols (Scheme 2) include sphingosine (usually 4*E*-d18:1; S) and dihydrosphingosine (sphinganine, without the *trans* double bond between C-4 and C-5; d18:0; dS), which are ubiquitous in human tissues. Two other sphingoid bases contain additional hydroxy groups: phytosphingosine (4-hydroxysphinganine, t18:0; P) and its derivatives are widely distributed throughout the brain, kidney, skin, liver, and other tissues, whereas 6-hydroxysphingosine (4*E*-t18:1; H) has only been found in the skin.[8] Some atypical structures have also been described: (4*E*,14*Z*)-sphingadiene (sphingosine with a *cis* double bond at C-14; 4*E*,14*Z*-d18:2) is found in plasma, brain and aorta,[10] (2*R*,3*E*)-2-aminooctadec-3-ene-1,5-diol (5-hydroxy-(3*E*)-sphingosine, 5OH-3*E*-d18:1) in brain extracts,[11] and branched sphingoid bases in the atherosclerotic aorta.[12] Recently, a tetrahydroxylated sphingoid base (dihydroxysphinganine with unknown position of the fourth hydroxy; abbreviation "T") was detected in human epidermis.[13]



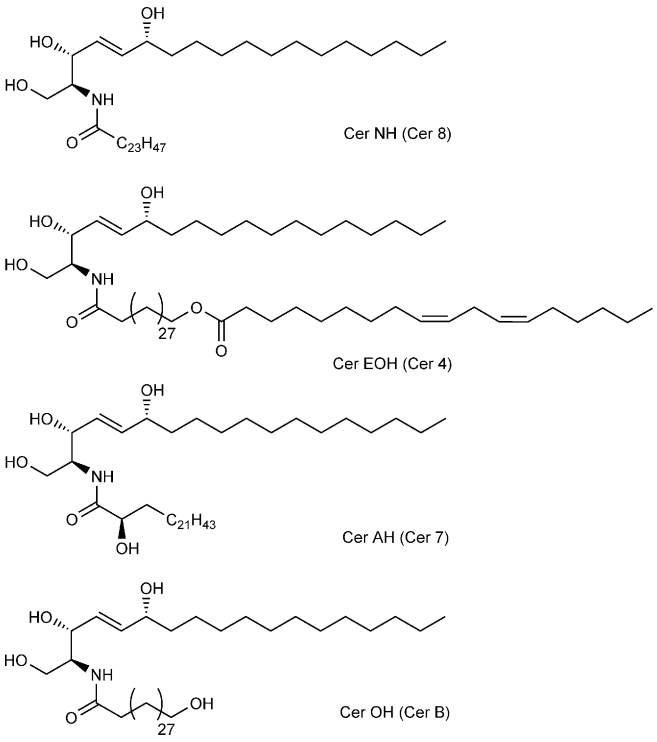
Scheme 2. Sphingoid bases in human tissues.

In terms of stereochemistry, all mammalian sphingoid bases share the D-*erythro* configuration, which is 2*S*,3*R* in S, dS, and H, but is 2*S*,3*S* in P because of a change in substituent priority. The additional hydroxy groups in P and H create further chiral centers, both of which have the *R*-configuration. Thus, the (4*R*)-hydroxy in a P molecule creates a D-*ribo* configuration. The (6*R*)-configuration in the skin H-Cer was established by a comparison of the ^{1}H NMR spectra of the synthesized acetyl derivatives of (6*S*)- and (6*R*)-H-Cer[5] with the acetylated derivatives of the naturally occurring H-Cer.[8]

In mammals, de novo synthesis of SLs primarily occurs in the endoplasmic reticulum; the pathway begins with condensation between L-serine and palmitoyl CoA, catalyzed by serine palmitoyl transferase (Scheme 3; for recent reviews see refs. [3a] and [14]). The product, 3-ketodihydrosphingosine (3-ketosphinganine), is reduced to dihydrosphingosine by ketosphinganine reductase and is subsequently N-acylated by Cer synthases 1–6 to form dihydroceramide (dS-Cer).[15] The (4*E*) double-bond is introduced by dS-Cer Δ4-desaturase (desaturase 1).[16] Biosynthesis of phytoceramide (P-Cer) is regulated by dS-Cer C-4 hydroxylase (desaturase 2).[17] The origin of the



Scheme 3. De novo Cer biosynthesis in the endoplasmic reticulum. Only dS is formed directly from L-serine; synthesis of the other sphingoid bases, S, P and, most likely, H, relies on their release from S-Cer, P-Cer, and H-Cer, respectively, through a salvage pathway.



Scheme 4. The structures of H-Cer subclasses identified in humans.[8,21]

unique 6-hydroxylated Cer (H-Cer) is not known. It seems that a hydroxylase similar to desaturase 2 might hydroxylate S-Cer, as the allylic position 6 is prone to oxidation; however, this reaction needs to be definitively established.

Discovery of H and H-Cer

The first instance of an unusual SL containing three hydroxy groups and a double bond was reported in 1989.[18] Hamanaka et al. found that one fraction of a preparation of epidermal GlcCer with ω-linoleyloxyacyl contained trihydroxyeicosasphingenine (t20:1) as a base. The third hydroxy group was suggested to be at position 4. Three years later, Wakita et al. found similar trihydroxysphingosines (t17:1, t18:1, and t20:1; termed phytosphingosines in that study) in the form of free long-chain sphingoid bases in samples isolated from the SC.[19] The unusual trihydroxysphingosines represented approximately 50% of free SC sphingoids, with 18 and 20 carbons as the most prevalent chain lengths. However, the positions of the functional groups of these structures were not specified.

The assignment of the additional hydroxy group to carbon 6 was achieved in 1994.[8] Downing's group studied human SC Cer, either extractable (at that time they were known as Cer 4 and Cer 7) or covalently bound to corneocytes (Cer B), containing this unusual sphingoid base. TLC on sodium arsenite plates excluded the possibility of the phytosphingosine hydroxylation pattern proposed in the above studies, and the multiplicity of the double bond proton resonances in the NMR spectra confirmed that the third hydroxy group is adjacent to the double bond (i.e., at position 6). The authors proposed the abbreviation "H" for this new base: thus, the Cer formerly known as 4, 7, and B were named Cer EOH, AH, and OH, respectively, according to the extended Motta nomenclature (Scheme 4).[7]

By comparison of the NMR spectra of these Cer with those of the previously identified GlcCer,[18] the authors also proposed that these SLs share the same 6-hydroxylated backbone and that the GlcCer isolated by Hamanaka et al. is a precursor for Cer EOH and Cer OH. Interestingly, the H-based SLs are not minor components of the skin lipid barrier; Cer AH and EOH represent 22 and 4%, respectively, of total SC Cers. Cer EOH is also a metabolic precursor of the covalent Cer OH, which represents 25% of bound lipids.

In 1995, Stewart and Downing published another report in which they showed that a significant amount of H is present as a free base in human skin but that it is not found in pig epidermis.[20] The polar head structure of the sphingoid bases in ethanol-extracted lipids obtained from human volunteers was confirmed by ^{1}H NMR analysis, and chain-length distribution was determined by gas chromatography. These results showed that approximately 15% of the free sphingoid bases of human skin are 6-hydroxylated and have chain lengths of primarily 18 and 20 carbons. This is significantly less than the amount reported by Wakita et al.,[19] most likely because of the presence of extremely long dS compounds (chain lengths of 24–26 carbons account for 60% of the SC free bases), which were previously not detected.

The fourth class of H-Cer was found by the same authors in 1999.[21] They examined human skin specifically for Cer NH (nonhydroxy acyl 6-hydroxysphingosine; Scheme 4), because

its sphingoid base and fatty acid were present in other classes of epidermal Cer. Indeed, they managed to resolve what appeared to be a single spot on a TLC plate (which was previously assigned to Cer AS) into two components and proved that the second lipid was its isomer, Cer NH. This new lipid accounted for approximately 9% of total human SC Cer.

The role of H-Cer in the skin barrier

Thus far, the H-based SLs have only been found in human skin. These SLs include H-Cer, free H (a product of H-Cer hydrolysis), and H-GlcCer (which are deglucosylated at the stratum granulosum/SC interface). Interestingly, H-Cers are not common to all mammals: for example, they are found in dog but not in all dog breeds,[22] and none was detected in pig skin.[23] These structures most likely do not play a role in cell signaling; rather, they seem to be important components of the skin barrier. The skin of living organisms represents a barrier between the external and internal environments. It protects the body from pathogens, allergens, unwanted chemical substances, ultraviolet light, and mechanical damage. Skin also acts as a barrier to prevent the loss of water and electrolytes. Both of these barrier functions are necessary for survival on land and are localized to the SC (the uppermost layer of the skin).[3b,c]

The SC consists of corneocytes (keratinocytes from the basal layer of the epidermis) that are in the terminal phase of differentiation and are encapsulated by a cornified envelope of crosslinked proteins such as loricrin and involucrin.[24] Approximately 20% of the SC represents the lamellar lipid matrix, which fills the SC extracellular spaces and inhibits the movement of water and electrolytes.[25] This hydrophobic extracellular space consists of 50% (*w*/*w*) Cer, 25% cholesterol, and 15% fatty acids (molar ratio, ~1:1:1) and small amounts of other substances (cholesterol esters, cholesterol sulfate).[3b] Interestingly, no phospholipids have been found in the SC. For reviews of skin barrier lipids, see refs. [3], [14], and [26]. In addition to free lipids, the SC also contains several species of covalently bound lipids, including fatty acids, ω-hydroxy acids, and three types of protein-bound Cers. The covalent Cers are bound as esters at their ω-hydroxy groups to the carboxyl groups of the protein involucrin, which is found in the epidermal cornified envelope.[27]

Cer form an essential part of the skin barrier. For example, in rare cases of severe deficiency of GlcCer β-glucosidase (β-glucocerebrosidase), Cer are not released from GlcCer; infants with this condition exhibit abnormal skin function and die shortly after birth.[28] Although there is an alternative pathway that releases Cer NS and AS from the SM in the skin, the acid sphingomyelinase cannot compensate for the deficiency of GlcCer β-glucosidase or saposin C.[29] Minor alterations to Cer content and composition are not lethal but contribute to barrier defects associated with skin diseases such as atopic dermatitis and psoriasis.[26a,30] Notably, supplementation of Cer (or their analogues) seems to be a promising therapeutic approach in skin diseases with defective barrier function;[31] this avenue certainly deserves to be further explored.

The relationship between Cer structure and function in skin barrier homeostasis is still not fully understood. Twelve subclasses of free Cer have been identified in human skin: combinations of four sphingoid bases (S, dS, P, and H) and three acyl chain types (N, A, and EO).[9,32] Given the variation in chain length, hundreds of Cer species have been identified. H-class Cer comprises approximately 34% (*w*/*w*) of SC Cer (Table 1).[13,33] The total carbon number, which reflects the lengths of the chains, ranges from C_{32} to C_{52} for Cer NH and Cer AH, C_{48} to C_{54} in Cer OH, and C_{62} to C_{73} in Cer EOH. This is similar to S-Cer and P-Cer, although minor differences in the relative distributions of these Cer species exist.[13]

Table 1. Cer distribution in human SC [%, *w*/*w*] according to the most recent LC/MS studies.

Cer class	Cer profile [%]				
	[a]	[b]	[c]	[d]	Mean
NS	10.0[e]	7.3	7.8	7.4	7.5[g]
AS	5.6[f]	5.7	4.6	9.6	6.6[g]
EOS	4.4	4.5	2.9	6.5	4.6
NdS	10.0[e]	5.8	9.0	9.8	8.2[g]
AdS	5.6[f]	1.2	1.0	1.6	1.3[g]
EOdS	n.d.	n.d.	0.2	0.4	0.3[h]
NP	24.4	18.0	28.9	22.1	23.4
AP	14.9	18.9	15.7	8.8	14.6
EOP	1.1	0.7	1.0	1.1	1.0
NH	21.4	19.6	13.9	14.5	17.4
AH	14.3	16.7	12.5	10.8	13.6
EOH	3.4	1.9	2.6	4.3	3.1

[a] Masukawa et al. (2009),[33a] LC/MS analysis using 11 Cer standards. [b] Ishikawa et al. (2010),[33b] LC/MS analysis using 11 Cer standards. [c] Janssens et al. (2011),[33c] semiquantitative LC/MS using deuterated Cer NS and Cer EOS as standards. [d] t'Kindt et al.(2012),[13] semiquantitative LC/MS analysis using Cer NS, AS, NdS, and NP as standards. [e] Amount for both Cer NS and NdS. [f] Amount for both Cer AS and AdS. [g] Mean of [b]–[d]. [h] Mean of [c]–[d]. n.d.: not detected.

In general, Cer are extremely hydrophobic, thus explaining their central role in preventing desiccation. Unlike phospholipids, Cer are uncharged and do not extensively hydrate. Their rather small polar heads contribute to their packing ability in membrane systems; they create hydrogen bonding networks; and their lipophilic chains are saturated and very long (usually 24 carbons or more). The importance of Cer chain length for competent barrier function of the skin has recently been shown.[34] However, the importance of the individual functional groups of Cer and the reason that the skin needs so many Cer classes (including the more polar H-Cer) are not known. The additional hydroxy(s) could possibly serve to confer heterogeneity to the lipid mixture so that it can better resist external stressors, or it could have some specific function. Nevertheless, it is unclear why there is a need for additional hydroxy groups beyond those provided by P-Cer.

The arrangement of the SC lipids is equally important. Cer create arrays of interconnected multiple membranes that fill the SC intercellular spaces. Although the exact structure of the SC lipid lamellae is still under debate,[35] the importance of the

EO class of Cer, including Cer EOH, is widely accepted.[36] These extremely long Cer most likely act as molecular rivets connecting adjacent lipid lamellae and thus contribute significantly to the prevention of water loss. The role of Cer EOH is most likely similar to that of Cer EOS because of the unique structure: its ω-linoleyloxyacyl (usually 30–34 carbons) can span a bilayer and insert into an adjacent bilayer, thus increasing cohesion. Schreiner et al. confirmed this by analyzing skin obtained from young normal human volunteers (those with dry skin and those with aged skin), and found that the presence of Cer EOH is necessary for proper SC lipid organization.[37] In addition, Cer EOH is the metabolic precursor for covalent Cer OH.

One open question concerns Cer conformation in the SC (Figure 1): it is unclear whether a hairpin conformation with parallel saturated long chains pointing in the same direction

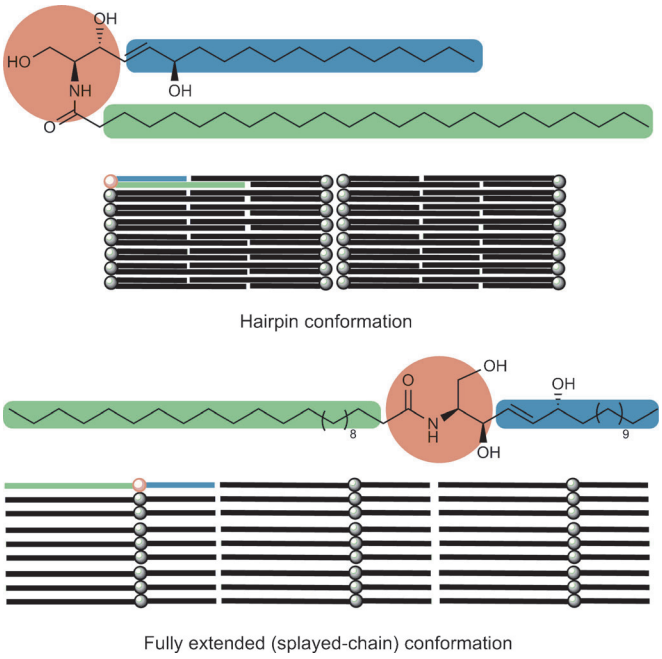


Figure 1. Two possible Cer conformations in the skin lipid barrier.

(similar to bilayer-forming lipids) is preferred, or whether a splayed-chain (or fully extended) conformation with the antiparallel chains is more advantageous for the cohesion of multiple lamellae.[38] GlcCer and SM (precursors of barrier Cer) have large highly hydrated, charged, polar heads and likely adopt a hairpin conformation. Upon Cer release from these pro-barrier lipids, conversion from the hairpin conformation to a fully extended form is nevertheless possible and is most likely driven by the difference in length between the amino alcohol (C_{18} predominant) and the fatty acid acyl (usually C_{24}).[38,39] Such chain "flipping" from hairpin to extended conformation was found to be relatively fast in Cer.[40] It is possible that both conformations occur in the SC and have distinct functions. The propensity to form either conformation might also be connected to the hydroxylation pattern of Cer. Based on infrared studies of model SC lipid membranes, it has been suggested that the driving force for the assembly of Cer NS with only two hydroxy groups is formation of very tight, orthorhombic chain packing and that an additional hydroxy group, either in the sphingoid base (Cer NP) or in the acyl (Cer AS) group, prevents tight chain packing but contributes to hydrogen bonding, which is the major cohesive force in these more polar Cer.[41] This strategy could also be employed by Cer NH, as the hydroxy group at position 6 could change the hydrogen-bonding pattern and enhance cohesion of the SC lipid lamellae. This behavior might be yet more pronounced in the case of Cer AH, which contains four hydroxy groups.

The last member of the H-Cer family, Cer OH, was formerly known as Cer B and is one of the covalent lipids. It is attached to the outer corneocyte surface at its ω-hydroxy group, and, together with the other lipids, it forms a lipid monolayer known as the covalent lipid envelope.[27b] The chains of their bases (S and H) range in length from C_{12} to C_{22}, and their acyl chain lengths vary between C_{28} and C_{36}; this gives 67 covalently bound ω-hydroxy Cer SC species.[42] These lipids most likely serve as templates to orient the free lipids and link them to corneocytes, thus preventing the formation of a permeable boundary.[43]

H and H-Cer in Skin Diseases

In 1992, differences in the skin concentrations of the newly discovered trihydroxysphingenines (later termed "H") in hyperkeratotic skin conditions were described. In particular, the concentration of free t20:1 was five times lower in psoriatic patients than in healthy individuals.[19] In addition, several studies demonstrated changes in epidermal lipid metabolism[44] and generally less SC Cer in atopic dermatitis.[45] The levels of the individual Cer in atopic skin differ between studies (some authors did not detect any changes between healthy and diseased skin),[46] and such comparisons are complicated by the fact that some authors report amounts as absolute values (e.g., lipid amount per mg SC protein), while other authors compare the relative abundances (% of found Cer). However, lower amounts of H-Cer classes in atopic skin (relative to that in healthy skin) are consistently observed.

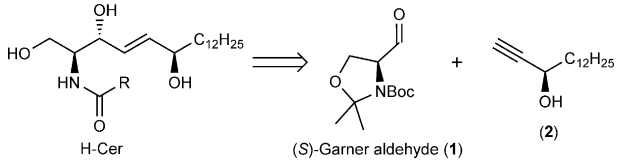
In 1999, Bleck et al. found lower levels of several Cer classes, including Cer AH, in nonlesional atopic eczema compared to healthy skin. One year later, differences were found in the X-ray diffraction patterns of SC lipids between dry and normal skin; these were linked to a deficiency of Cer EOH and EOS in patients with dry skin.[37] These results were confirmed by Macheleidt et al., who found attenuated de novo synthesis of Cer EOH (and also of Cer NP) in lesional atopic biopsies compared to healthy skin.[47] In a study focused on filaggrin mutations,[48] lower levels of Cer EOH were found in atopic eczema compared to normal skin.

The recent development of HPLC assays using highly sensitive MS detection for Cer quantification has further supported these results. Ishikawa et al. reported that lesional atopic skin had lower levels of Cer NH and Cer EOH (as well as of Cer NP, Cer EOS, and Cer EOP) per SC protein compared to healthy skin, whereas the levels of Cer AS were higher.[33b] Janssens

et al. studied patients with atopic skin without the most prevalent filaggrin mutations and did not detect any differences in H-Cer levels, except for lower Cer EOH levels in two patients (different X-ray diffraction patterns).[33c] In a later study, patients with filaggrin mutations were included: significant lower Cer NH and Cer EOH levels (together with Cer NP and Cer EOP) were accompanied by higher levels of Cer AH, Cer AS and Cer NS in atopic patients.[49] Nevertheless, other studies have suggested that the filaggrin and Cer differences in atopic dermatitis are unrelated features of this disease.[50]

Synthesis of H and H-Cer

The major limitation to a better understanding of the function of H-Cer in healthy human epidermis and its role in skin barrier pathologies is that these Cer are currently not commercially available and their isolation from the SC in sufficient amounts is extremely difficult. Currently, only three papers have addressed the synthesis of H and H-Cer:[51] all three synthetic approaches rely on the reaction of (*S*)-Garner aldehyde (protected L-serinal, **1**) with a long-chain propargylic alcohol **2** (Scheme 5).

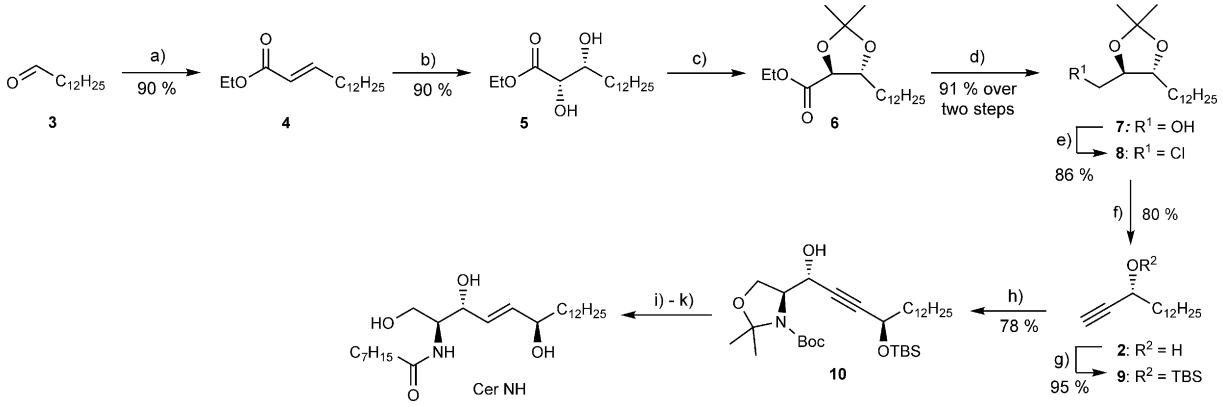


Scheme 5. Synthetic strategy for H and H-Cer.

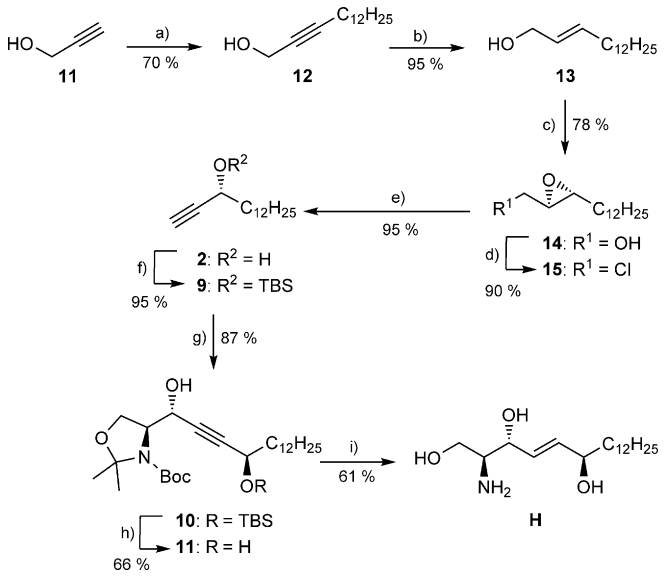
The first asymmetric synthesis of (6*R*)- and (6*S*)-Cer NH (with an N-octanoyl chain, for ease of handling) was published by Bittman's group in 2003.[51a] First, they attempted to prepare the chiral propargylic alcohol by reacting tridecanal with a protected acetylide, followed by oxidation of the product to form an ynone and asymmetric reduction; however, *ee* was low. Thus, they chose a pathway that used asymmetric dihydroxylation and double elimination (Scheme 6). The Horner–Wadsworth–Emmons reaction of tridecanal **3** with ethyl 2-(diisopropoxyphosphoryl)acetate afforded α,β-unsaturated ester **4**, which was dihydroxylated with AD-mix-β to yield diol ester **5**. The vicinal hydroxy groups were protected using 2,2-dimethoxypropane and the resulting acetonide ester **6** was reduced to alcohol **7**, which was converted through a Mitsunobu reaction into chloride **8**. The acetylenic alcohol **2** was obtained from chloride **8** by double elimination with *n*-butyllithium. For the next step, the free hydroxy group of alcohol **2** was protected with a *tert*-butyldimethylsilyl (TBS) group to yield **9**. The authors also described the synthesis of the (*S*)-enantiomer of the propargylic alcohol by using asymmetric dihydroxylation of allylic chloride followed by double elimination. Next, treatment of the chiral silyl ether **9** with butyllithium and alkynylation of (*S*)-Garner aldehyde **1** in the presence of hexamethylphosphoramide (HMPA) gave alcohol **10**, which was converted to the target Cer NH in three steps: hydrolysis with HCl in dioxane, *N*-acylation with *p*-nitrophenyl octanoate, and Birch reduction of the triple bond (Scheme 6).

Yadav et al. reported the total synthesis of the (6*R*)- and (6*S*)-diastereomers of H, also by double elimination reactions (Scheme 7).[51b] First, propargyl alcohol **11** was treated with dodecyl bromide to generate propargylic alcohol **12**, which was reduced with $LiAlH_4$ to (*E*)-allylic alcohol **13**. This was subjected to Sharpless asymmetric epoxidation to yield epoxy alcohol **14**, which was converted to epoxy chloride **15**. Propargylic alcohol **2** was prepared by treatment of **15** with $LiNH_2$ in liquid NH_3 and was protected using TBSCl to generate ether **9**. This was converted into the target H by reaction with Garner aldehyde **1**, deprotection, and reduction by $LiAlH_4$.

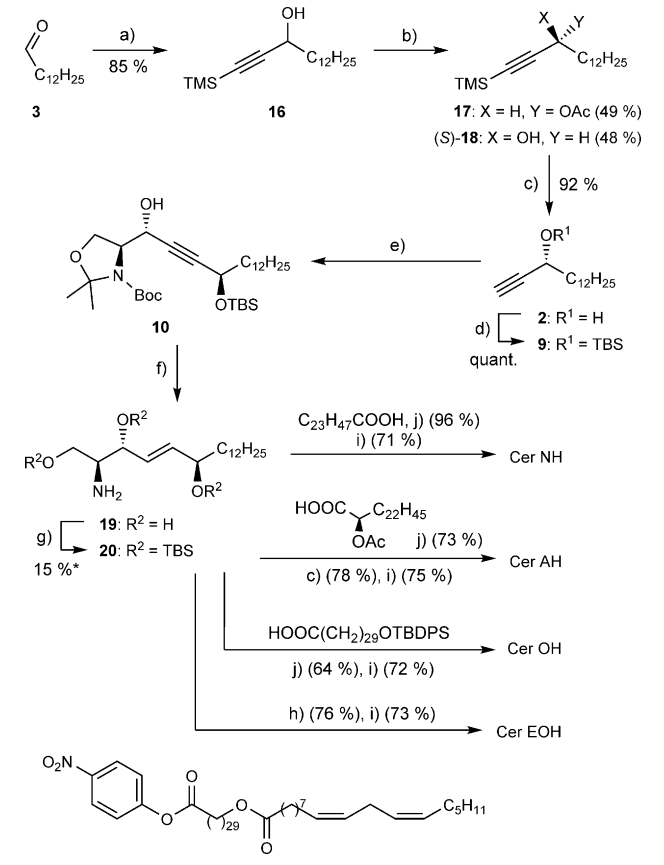
Another approach to the key propargylic alcohol is enzymatic resolution, as used by Masuda and Mori in the synthesis of human skin Cer NH, Cer AH, Cer EOH, and Cer OH.[51c] Treatment of tridecanal **3** with trimethylsilyl acetylide produced racemic alcohol **16** (Scheme 8). Then, by using lipase to acetylate only the *R* isomer into ester **17**, they were able to separate it chromatographically from unreacted *S* alcohol (*S*)-**18** in a single step. The protecting groups were removed, and the hydroxy of **2** was protected with a TBS group. Treatment of silyl alkynide **9** with (*S*)-Garner aldehyde **1** generated **10**, which



Scheme 6. Reagents and conditions: a) $(iPrO)_2P(O)CH_2CO_2Et/Et_3N/LiBr/THF$; b) AD-mix-β/$MeSO_2NH_2$/*t*BuOH/$H_2O$ (1:1); c) $Me_2C(OMe)_2$/*p*-TsOH/CH_2Cl_2; d) $(iBu_2AlH)_2$/THF/0 °C; e) *N*-chlorosuccinimide/Ph_3P/CH_2Cl_2/0 °C to RT; f) BuLi/HMPA/THF/−42 °C to RT; g) TBSCl/imidazole/DMF; h) **1**, BuLi/HMPA/THF/−78 °C to RT; i) 1 M HCl/dioxane/100 °C; j) $p\text{-}O_2NPhOOCC_7H_{15}$/THF (80%, two steps); k) $Li/EtNH_2$/−78 °C (88%).



Scheme 7. Reagents and conditions: a) $C_{12}H_{25}Br/LiNH_2$/liquid NH_3/−33 °C; b) $LiAlH_4$/THF/0 °C to reflux; c) (−)-diethyltartrate/Ti(*i*OPr)$_4$/*tert*-butylhydroperoxide/CH_2Cl_2/−33 °C; d) $Ph_3P/CCl_4/NaHCO_3$/reflux; e) $LiNH_2$/liquid NH_3; f) TBSCl/imidazole/4-(dimethylamino)pyridine/CH_2Cl_2/0 °C; g) **1**, BuLi/HMPA/THF/−78 °C; h) 1 M HCl/THF (1:1)/reflux/16 h; i) $LiAlH_4$/THF/RT/4 h.



Scheme 8. Reagents and conditions: a) TMSC≡CH/BuLi/THF/−78 °C; b) lipase/CH_2=CHOAc/(*i*Pr)$_2$O/RT; c) K_2CO_3/MeOH/RT; d) TBSCl/imidazole/DMF/RT; e) **1**, BuLi/HMPA/THF/−78 °C; f) i: Bu_4NF/THF/RT, ii: Li/$EtNH_2$/THF/−78 °C; g) TBSOTf/2,6-lutidine/CH_2Cl_2/RT; h) C_5H_5N; i) Bu_4NF/THF/RT; j) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/1-hydroxybenzotriazole/CH_2Cl_2/RT. * yield over three steps.

was deprotected, reduced, and again protected with TBS to form **20**. N-acylation yielded the target Cer.

Outlook

SLs are critical for the life of cells. They are equally important outside the cell in the extracellular spaces of the skin barrier because they prevent water loss in land-dwelling mammals. Although a large body of work has elucidated many of the functions of SLs, their extensive structural diversity is still intriguing. Both 6-hydroxylated sphingosine bases and H-Cers have been identified relatively recently and have only been found in the skin; this is most likely the reason that the origin and function of these SL subclasses are not fully understood. The reasons for the hydroxylation of lipids that evolved as extremely hydrophobic components of the skin barrier are not yet known. Determining the manner in which the hydroxy group at position 6 influences the basic biophysical properties of such ceramides in skin lipid membranes (compared to their sphingosine and phytosphingosine counterparts), the manner in which these ceramides assemble with cholesterol, fatty acids, and other ceramides, and how such lipid assemblies resist water loss might help to understand their roles in the skin barrier. Determining which enzymes are responsible for the generation of 6-hydroxylated SLs, what compounds serve as their substrates, and how these processes are regulated are also areas requiring future research. Because 6-hydroxylated ceramides share the allylic hydroxy group of sphingosine-based ceramides (necessary for Cer cell signaling), it is tempting to question whether these lipids actually only occur in the skin. Future improvements in analytical methods might determine that they are ubiquitous, despite the fact that they are most likely present in only small amounts. However, given the significant proportions of 6-hydroxyceramides in skin-barrier lipids and the different content in atopic dermatitis, these lipids certainly deserve attention. Future research should address the following: identifying the factors that lead to these ceramide abnormalities, determining whether these are primary or secondary changes, and elucidating barrier-repair strategies for these processes. Future efforts should certainly be directed toward the development of reliable synthetic procedures for these lipids to make them widely available for biochemical and biophysical studies and for use as standards in quantitative analysis.

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Keywords: ceramides · lipids · membranes · skin · sphingolipids

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