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Design, Synthesis, and Evaluation of β-Galactosylceramide Mimics Promoting β-Glucocerebrosidase Activity in Keratinocytes

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Abstract—We have established an efficient synthesis of mimics of β-galactosylceramide (β-GalCer) increasing a β-glucocerebrosidase (β-GlcCer'ase) activity that associates with the skin barrier function. Among the synthetic β-GalCer analogues (**6a–6e**) described herein, compound **6e** exhibited a potent effect on the activation of β-GlcCer'ase function in vitro and reduced the transepidermal water loss (TEWL) level in a UVB-induced barrier disrupted mice model. These findings indicated that compound **6e** could be useful for cosmetics and medicines to improve skin barrier function.

β-Galactosylceramide (β-GalCer) is one of the simplest glycosphingolipids having a lipophilic sphingosine and a hydrophilic galactose moiety attached via an ether linkage to sphingosine. β-GalCer is found abundantly in the myelin sheath of the central and peripheral nervous system,[1,2] and acts as cell surface receptors for bacterial toxins and viruses.[3,4]

Recently, we have reported that β-GalCer promoted the activity of β-glucocerebrosidase (β-GlcCer'ase) in epidermis and resulted in the increase of the ceramide (Cer) levels.[5] This enzyme plays a critical role for the formation and maintenance of the epidermal permeability barrier, by which β-glucosylceramide (β-GlcCer) localized in epidermis is hydrolyzed to Cers that is essential for the formation of lamellae structure in the stratum corneum.[6,7] Moreover, we found that acylceramide[8] (Cer1), an important lipid in preventing various skin diseases, such as dry skin, atopic dermatitis and psoriasis, is only produced by β-GlcCer'ase not but by sphingomyelinase.[9] Actually, it has been demonstrated that β-GalCer increased not only the in vitro β-GlcCer'ase activity but also the in vivo Cer level of stratum corneum to improve the resistance against the above skin diseases.[5,10]

Cer is a natural product and Cer mimics have been applied for cosmetics. However, it is very difficult to utilize these compounds widely. Because the general synthesis of biologically active ceramides involves two important aspects; one is the establishement of the chirality and the other the construction of the *trans* double bond, so a practical synthesis for active ceramides has been still studied.[11–15] Therefore, our attention has been focused on the galactose–attached Cer mimics for increasing the β-GlcCer'ase activity. In this study, we designed the structural assembly using the galactose and the Cer mimic lead from L-serine, in view of the structural diversity, for example we have applied easily the various fatty amines or fatty acids in the synthetic strategy, as shown in Figure 1.

Here, we describe the synthesis of β-GalCer analogues **6a**–**6e** as the general method. As shown in Scheme 1, β-D-galactose penta acetate **1** was derived into the

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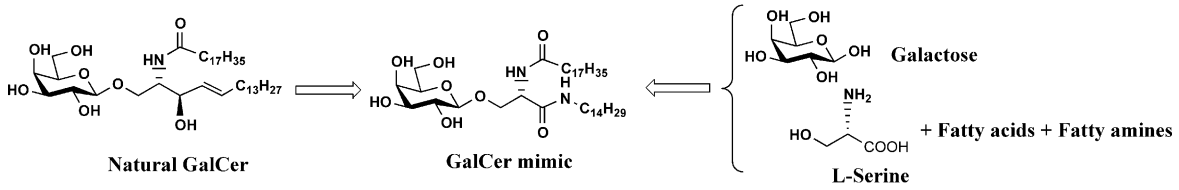
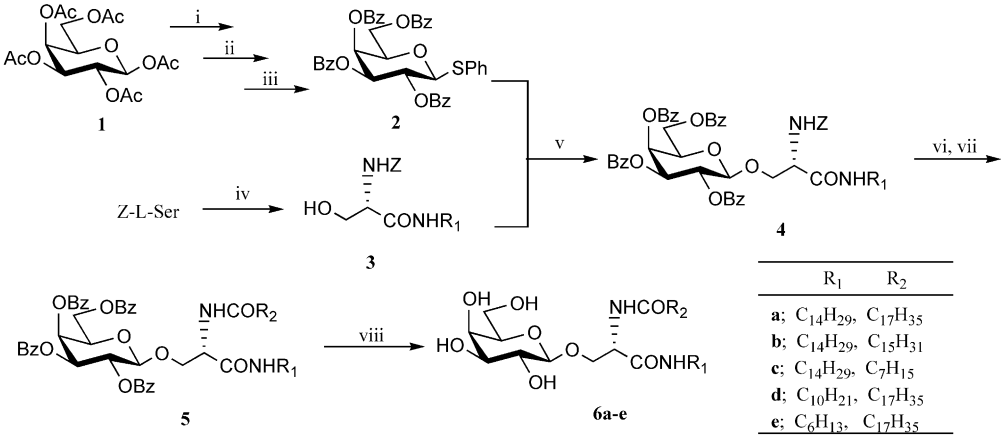


Figure 1.



Scheme 1. Reagents and condition: (i) PhSH, BF_3OEt_2, $CHCl_3$ (85%); (ii) NaOMe, MeOH (quant); (iii) BzCl, Pyr. (quant); (iv) R_1NH_2, WSC, HOBt, DMF (quant); (v) NIS, TfOH, $CHCl_3$ (75%); (vi) $Pd(OH)_2$, H_2, dioxane–MeOH (quant); (vii) R_2COOH, WSC, HOBt, DMF (quant); (viii) NaOMe, MeOH (quant).

thioglycoside donor having better reactivity as the leaving group for the accessible glycosylation reaction, followed by the change of the protecting groups from acetate to benzoyl groups for the prevention of the *ortho*-ester formation to afford compound **2**. Glycosylation[16,17] of **2** and **3** in the presence of *N*-iodosuccinimide (NIS) and trifluoromethansulfonic acid (TfOH) gave the key intermediate, β-glycoside **4** in 75% yield. The glycosylation step to compound **4** proceeded stereoselectively based on the neighboring effect of the C-2 benzoyl group of **2** to afford only β-anomer **4**. Removal of the benzyloxycarbonyl group of compound **4** with $Pd(OH)_2$, followed by the introduction of stearic acid gave the precursor **5** in good yields. Finally, debenzoylation of **5** gave quantitatively the desired β-GalCer analogues **6a**–**e**.

The β-GlcCer'ase activity in cultured human keratinocytes was determined according to our previous paper.[5] First of all, to examine the structure–activity relationship (SAR) regarding the length of alkyl chain on fatty acids (R_2), the effects of compounds **6a**–**e** on the β-GlcCer'ase activities in cultured keratinocytes were investigated as shown in Table 1.

Among compounds **6a**–**c**, compound **6c** exhibited weaker activity than that of natural β-GalCer. It might be due to their shorter alkyl chain of R_2 moiety. Next, we investigated the SAR regarding the alkyl chain lengths of alkyl amines (R_1). As shown in Table 1, compound **6e** ($R_1 = C_6H_{13}$) had stronger activity than those of compounds **6a** and **6d** ($R_1 = C_{10}H_{21}$, $C_{14}H_{29}$). Optimization of the length of R_1 is under investigation.

The in vitro activity of the most active compound **6e** was examined using UVB- induced barrier disrupted mice. As shown in Table 2, the topical application of compound **6e** significantly reduced the transepidermal water loss (TEWL)[19] compared to that GalCer. This might be due to the difference of bioavailability including skin permeability or biological half life.

These findings suggest that the L-serine unit modified by alkyl amine and acylalkyl groups with a moderate chain length can mimic the ceramide moiety of natural GalCer to maintain similar biological activities. Compound **6e** could be useful for improving skin barrier in cosmetic and medicinal fields. Optimization of compound **6e** is in progress.

Table 1. Effects of β-GalCer analogues **6a**–**e** on β-GlcCer'ase activity in cultured normal human keratinocytes

Compd	β-GlcCer'ase activity ($n = 5$)
Control	1.00 ± 0.06
GalCer	1.65 ± 0.17**
6a	1.24 ± 0.07
6b	1.37 ± 0.12
6c	0.87 ± 0.03
6d	1.20 ± 0.10
6e	1.42 ± 0.11*

Normal human keratinocytes (NHKs) were cultured with GalCer or compounds **6a**–**e** (5 μM) or vehicle for 4 days. After extracting enzyme from the cells, the β-GlcCer'ase activity was measured as nmol 4-methylumbeliferone synthesized/min/mg of protein. Activity was expressed as ratio to control. Each value represents the means of five determinations. (*$P < 0.05$, **$P < 0.001$; respectively, vs control) The Dunnett's test was used for comparisons between control and experimental groups.[18]

Table 2. Effects of the GalCer analogue **6e** on UVB-induced murine epidermal barrier disruption

Compd	TEWL
Control (3 days)	5.55±0.68
GalCer	5.24±1.48
6e	2.01±0.44 **
Control (4 days)	6.05±0.43
GalCer	3.68±0.28* ($n=4$)
6e	2.19±0.39***

GalCer, compound **6e** or vehicle (10 μL/cm^2) was applied daily to dosal skin of mice before irradiation and for 2 days after irradiation (0.15 J/cm^2). TEWL was expressed as ratio to control water loss before application of each compound. Each value represents the means±SEM of four or five animals. (*$P<0.05$, **$P<0.01$, ***$P<0.001$, respectively, vs each control).

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