# Synthesis and Potent Antileukemic Activities of *N*-Lactylsphingosine and *N*-Lactyldihydrosphingosine

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*Received March 18, 2003*

**Abstract:** *N*-(*R*)- and *N*-(*S*)-Lactylsphingosine and their corresponding dihydrosphingosine derivatives were synthesized. The antileukemic activities of these compounds were measured by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay in human leukemia HL-60 cells. *N*-(*R*)- and *N*-(*S*)-Lactylsphingosine displayed higher activities than *N*-acetylsphingosine (C2-ceramide, a well-known apoptosis inducer), and their dihydrosphingosine derivatives had slight activities.

**Introduction.** Sphingolipids and glycosphingolipids are ubiquitous membrane components of essentially all eukaryotic cells and serve physiologically important roles in bioorganisms.[1] Ceramide (*N*-acyl-D-*erythro*-sphingosine, typically with acyl chain lengths of 16−24 carbon atoms), a metabolite or a precursor of sphingolipids, is an important molecule in the second messenger role of sphingolipid signaling, especially apoptosis.[2] Dihydroceramide, which lacks the 4,5-trans C=C bond, is biologically inactive.[3] Ceramide is generated by neutral or acidic sphingomyelinase via the so-called sphingomyelin cycle[4] in response to various extracellular agents and stress such as the antibody against Fas,[5] tumor necrosis factor α (TNF-α),[6] and ionizing radiation.[7] In addition, exogenously treated agents with some short-chain and cell-permeable ceramides such as *N*-acetyl-D-*erythro*-sphingosine (C2-Cer) or *N*-octanoyl-D-*erythro*-sphingosine (C8-Cer) can also induce apoptosis.[3a]

Recently, we reported the syntheses and antileukemic activities of new types of bioactive dihydroceramides, symbioramide[8] and its diastereomers[9] (Figure 1). Although symbioramides are dihydroceramide-type analogues, they showed moderate antileukemic activities against murine leukemia L-1210 cells but not against human leukemia HL-60 cells.[9] It is generally accepted that the chemical structure of ceramides is important for their biological efficiency, but most studies of the structure−activity correlation of ceramides have been concerned with the structures of sphingoid bases, not those of *N*-acyl chains. Because symbioramide has the unique *N*-acyl chain, we suggested that the acyl structure is also important for their bioactivities.

In this paper, we describe the synthesis and antileukemic activities of symbioramide-like and cell-permeable short-chain ceramide and dihydroceramide analogues with (*R*)- or (*S*)-lactic acid as an *N*-acyl group (Figure 1).

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**Synthesis of *N*-Lactylsphingosine and Dihydrosphingosine.** The synthetic approach to *N*-(*R*)- and *N*-(*S*)-lactylsphingosine [(*R*)-Lac-Cer and (*S*)-Lac-Cer, respectively] and their corresponding dihydrosphingosine derivatives [(*R*)-Lac-DHCer and (*S*)-Lac-DHCer, respectively] is outlined in Scheme 1. The preparation of dihydrosphingosine acetonide **2** from **1**[10] to facilitate the next amide formation was previously reported.[9] The corresponding sphingosine derivative **5** was also prepared by a similar method. The starting compound **3**[10] with a small amount of (*Z*)-isomer was synthesized according to our method, and after removal of the isopropylidene moiety of **5**, *N*-Boc-D-*erythro*-sphingosine **4** was purified by recrystallization. The obtained **4** was deprotected, and then the diol group of the resulting free sphingosine was protected as acetonide **5** (65% yield from **3**).

First, (*S*)-Lac-Cer was prepared because the *N*-acyl group of (*S*)-Lac-Cer is a naturally occurring inexpensive (*S*)-lactic acid. (*S*)-*O*-Acetyl-lactic acid was condensed with **5** in the presence of DCC and HOBt to give **8** with 56% yield. After hydrolysis of the acetyl group of **8** and then removal of acetonide protection, (*S*)-Lac-Cer was obtained with 61% yield from **5**. (*S*)-Lac-DHCer was also prepared from **2** using the same methodology.

(*R*)-Lactic acid is extremely expensive; therefore, relatively inexpensive (*R*)-lactic acid methyl ester was used for the syntheses of (*R*)-Lac-Cer and (*R*)-Lac-DHCer. After the protection of the secondary hydroxyl group of this ester with methoxymethyl chloride (MOMCl) in the presence of diisopropylethylamine and followed by hydrolysis of methyl ester, the O-protected acid **6** was obtained in 62% yield. The condensation of **6** with **5** was performed using the same method described above (86%). Deprotection of the obtained fully protected short-chain ceramide **10** was attempted with $BF_3 \cdot Et_2O$ in EtSH. However, the desirable (*R*)-Lac-Cer was not obtained, although this deprotection method of the MOM group was successful in a similar case of the syntheses of symbioramide derivatives[9] and in the case of **9** where (*R*)-Lac-DHCer was obtained in 76% yield. From the $^1H$ NMR analysis of this undesirable product, the presence of one EtS group was identified (further characterization of this compound was not conducted). Thus, other methods to deprotect the MOM group were employed. In consequence, the treatment of **10** with excess TMSBr in $CH_2Cl_2$ at −30 °C was the best way to obtain (*R*)-Lac-Cer in tolerable yield (78%).[11]

**Antileukemic Activities.** To study the influence of (*R*)- and (*S*)-lactyl groups of ceramide analogues, the activities of cell death (cell death % = 100 − living cell %) by treatment of Lac-Cer and Lac-DHCer were compared with the activities of C2-Cer and C2-DHCer, positive and negative controls, respectively, by MTT assay according to our method.[9] Human leukemia HL-60 cells were treated with 20 μM of each short-chain ceramide analogues for 6 h. All ceramide analogues with a C=C bond showed highly antileukemic activities (Figure 2). In particular, both (*R*)-Lac-Cer and (*S*)-Lac-Cer were more effective than C2-Cer, which was considered as a potent apoptosis inducer against HL-60

10.1021/jm030125p CCC: $25.00 
Published on Web 07/03/2003

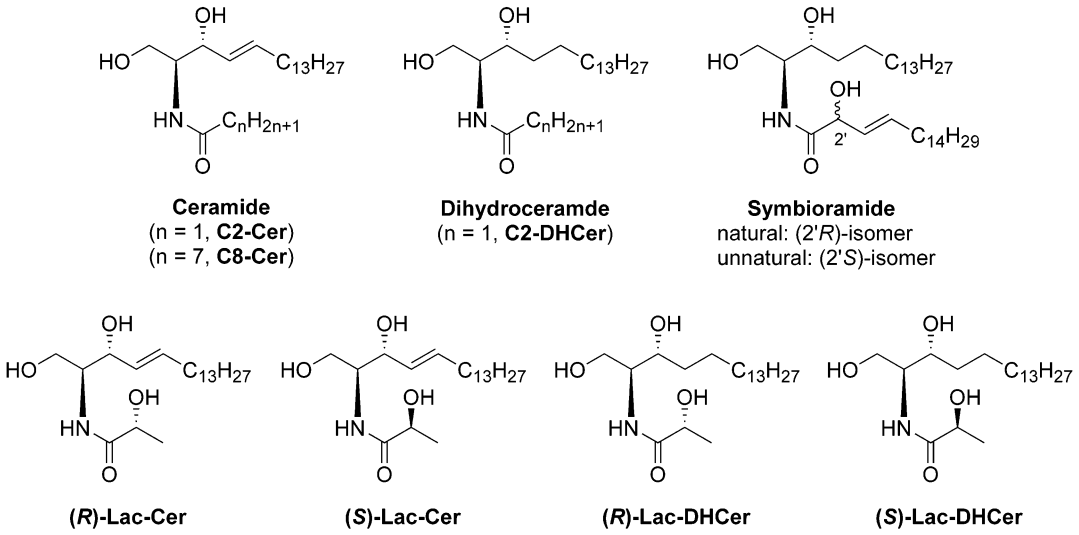
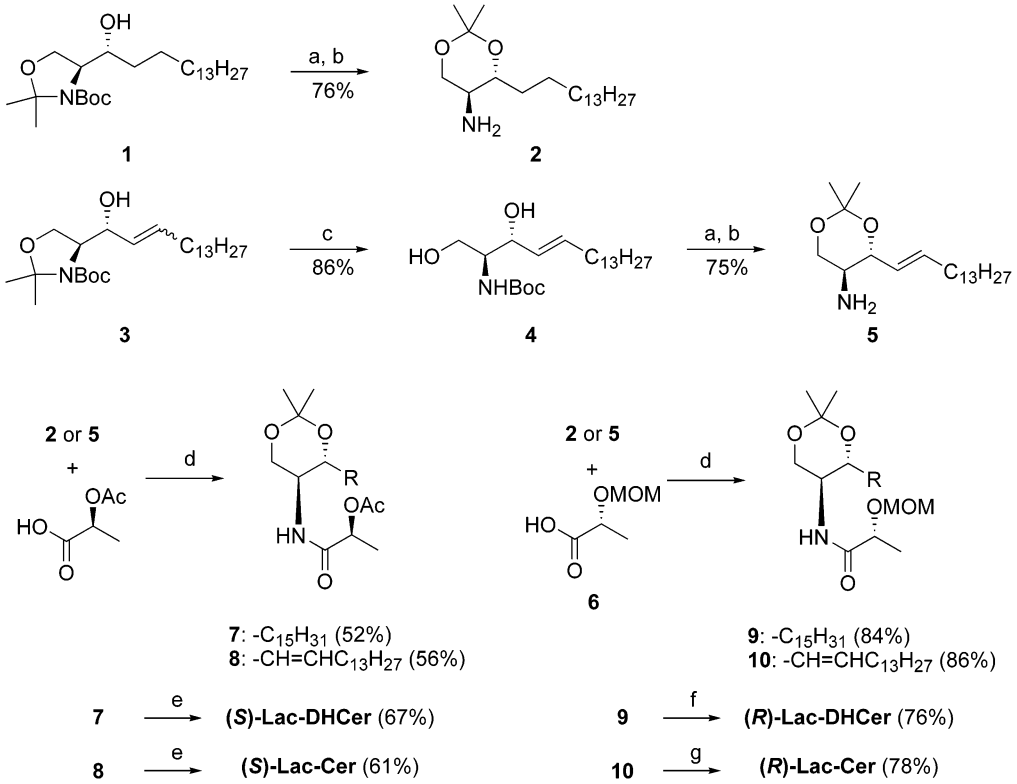



**Figure 1.** Structures of ceramide derivatives and novel *N*-lactylsphingosine analogues.

**Scheme 1.** Synthetic Plans of *N*-(*R*)- and (*S*)-Lactylsphingosine Analogues[a]




[a] (a) TFA-$H_2O$, room temp; (b) PPTS, $(Me)_2C(OMe)_2$, benzene, reflux; (c) *p*-TsOH, MeOH, room temp; (d) DCC, HOBt, $CH_2Cl_2$, room temp; (e) $K_2CO_3$, MeOH, room temp then *p*-TsOH, MeOH, room temp; (f) $BF_3-Et_2O$, EtSH, room temp; (g) TMSBr (6 equiv), $CH_2Cl_2$, −30 °C.

cells.[12] Interestingly, (*S*)-Lac-Cer, with a naturally occurring (*S*)-lactic acid, showed a higher activity than the (*R*)-isomer. Two dihydroceramide-type analogues [(*R*)-Lac-DHCer and (*S*)-Lac-DHCer] also showed slight antileukemic activities, although C2-DHCer showed no cell death. The order of potency is as follows: (*S*)-Lac-Cer > (*R*)-Lac-Cer > C2-Cer > (*R*)-Lac-DHCer = (*S*)-Lac-DHCer ≫ C2-DHCer. Recently, we reported that the unnatural-type (2′*S*)-isomer of symbioramide was more active than natural-type (2′*R*)-isomer.[9] These results indicate that the introduction of the (2′*S*)-hydroxyl group into the *N*-acyl chain enhanced the antileukemic activity of the ceramide analogue, although the reason for these high activities of *N*-lactyl derivatives against HL-60 cells is not clear at present.

Furthermore, DNA fragmentation by Lac-Cer was observed with 20 $\mu$M after 8 h. Figure 3 shows that cells, when stimulated with C2-Cer, (*S*)-Lac-Cer, and (*R*)-Lac-Cer, displayed a large quantity of DNA fragmentation. On the other hand, (*S*)-Lac-DHCer and (*R*)-Lac-DHCer resulted in a small quantity of DNA fragmentation. A good correlation between cell death and DNA fragmentation by Lac-Cer was found. These observations raise the possibility that these *N*-lactyl-derivative-induced cell deaths are apoptosis. In future, we intend to measure antineoplastic activities against other cancer cell lines

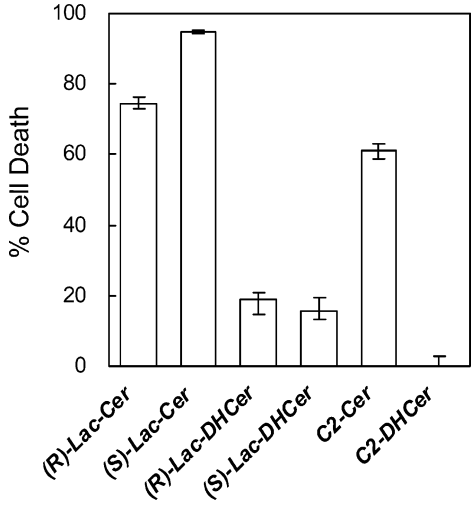



**Figure 2.** Percent cell death of HL-60 cells after treatment with 20 $\mu$M of each short-chain ceramide analogue for 6 h.

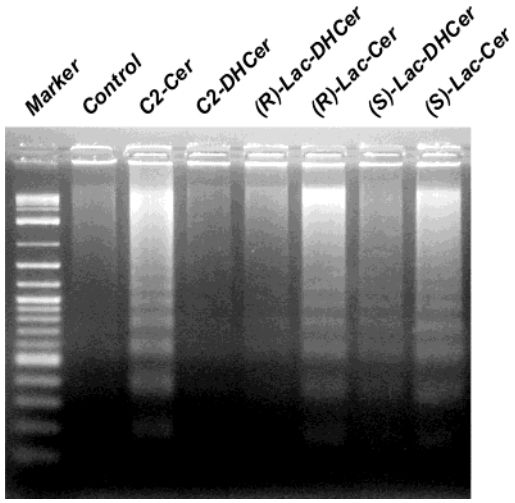



**Figure 3.** Agarose gel electrophoresis of DNA following treatment of HL-60 cells with short-chain ceramide and dihydroceramide analogues.

and to evaluate caspase activity to determine whether these Lac-Cer-induced cell deaths in HL-60 cells are an apoptotic process as well as C2-Cer-induced apoptosis.

**Supporting Information Available:** Characterization data of four *N*-lactyl compounds (*R*)-Lac-Cer, (*S*)-Lac-Cer, (*R*)-Lac-DHCer, and (*S*)-Lac-DHCer. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM030125P