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An Efficient Synthesis of Short-chain Sphingomyelin Analogs and Their Susceptibility to Hydrolysis Catalyzed by Sphingomyelinase

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Abstract: An efficient synthesis of (D)-*erythro*-sphingomyelin analogs was achieved *via* highly stereoselective reduction of 3-benzyl-4-(alkynyloxo)-oxazolidinone with diisobutylaluminum phenoxide reagent. The initial velocities of the hydrolysis of the (D)-*erythro*-derivatives catalyzed by *B. cereus* sphingomyelinase were more than 10 times faster than those of the (D)-*threo*-isomers. © 1997 Elsevier Science Ltd.

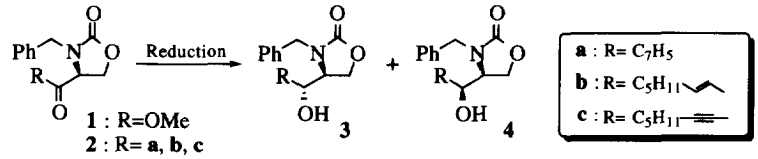
Sphingomyelin is a major component of biological membranes and plasma lipoproteins.[1] This phospholipid component is also an important source of ceramide, which has been generally accepted to be a lipid second messenger in cell membranes and plays key roles in cellular signal transmission pathway.[2] Although the significance of the sphingomyelin pathway,[3] which is initiated by hydrolysis of sphingomyelin to ceramide by sphingomyelinase, has been well recognized, hydrolysis of the phosphodiester bond to yield ceramide and phosphocholine is the only defined result in sphingomyelin degradation. For the study to elucidate the detailed catalytic mechanism of sphingomyelinase, development of water-soluble short-chain analogs of sphingomyelin and an efficient method for their synthesis have been strongly desired.[4]

In our previous synthesis of γ-hydroxy-β-amino alcohols from enantiomerically pure glycidol, we succeeded in the monoalkylation of ester **1** and highly *syn*-selective reduction of the resulting ketone with L-selectride®.[5] However, naturally occuring sphingosine and sphingomyelin are present in *erythro*-form having anti relative stereochemistry of its hydroxy and amino groups. Hence, Mitsunobu inversion of the hydroxy group generated by L-selectride® reduction was requisite to obtain the naturally occurring sphingosine.[6] In this paper, we would like to report a stereocontrolled straightforward method for synthesis of (D)-*erythro*-sphingomyelin, and synthesis of both *erythro* and *threo* short-chain analogs from the common compound. Furthermore, we also report that the initial velocities of the hydrolysis of (D)-*erythro* derivatives catalyzed by *B. cereus* sphingomyelinase were more than 10 times faster than those of (D)-*threo* isomers, and that the double bond in the backbone skeleton would not be essentially important for the hydrolysis by *B. cereus* sphingomyelinase.

An efficient synthesis to supply various kinds of sphingomyelin analogs is as follows. Independently prepared 1-heptyne, 1-heptene, and heptane lithium were used as alkylating agents to the ester **1** for the synthesis of short-chain analogs, and the corresponding ketones **2a, b,** and **c** were obtained under the conditions reported previously[5] in 76, 70, and 74% yield, respectively. After several attempts for the stereoselective reduction of the alkenyl ketone **2b** with numerous kinds of reducing agents,[7] we found that

reduction with diisobutylaluminum 2,6-di-*t*-butyl-4-methylphenoxide[8] gave the *anti*-stereoisomer **3b**,[9] mp 39.5-40.5 °C, with good stereoselectivity (11 : 1). Furthermore, the reduction of the alkynyl ketone **2c** with the same reagent was found to proceed with excellent selectivity (20 : 1) to give *anti*-isomer **3c**. The results of the reduction with diisobutylaluminum phenoxide and L-selectride® are listed in Table 1.

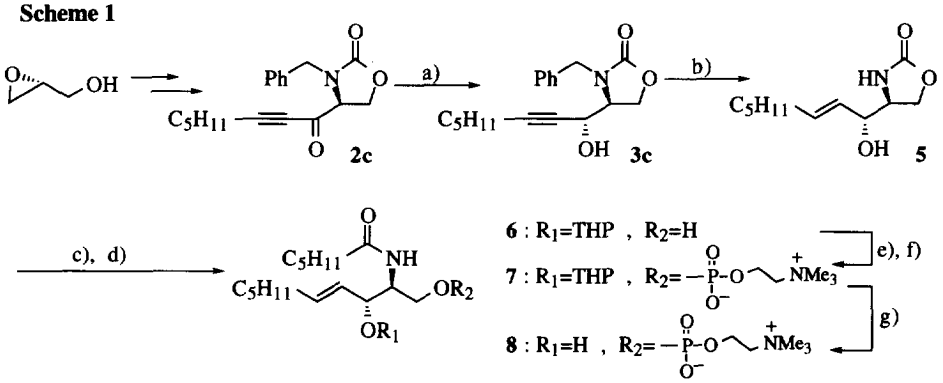
Table 1 Stereoserective reduction of ketone 2



Ketone	Products	Aluminum reagent [a,b]		L-Selectride® [c]	
		Ratio of [b] 3 : 4	Yield (%) [e]	Ratio of [b] 3 : 4	Yield (%) [e]
2a	**3a , 4a**	5 : 1	96	1 : 13	98
2b	**3b , 4b**	11 : 1	91	1 : > 20	93
2c	**3c , 4c**	20 : 1	88	3 : 4	95

[a] Diisobutylaluminum 2,6-di-*t*-butyl-4-methylphenoxide. [b] The reactions were performed using 2 equiv. of the reagent in toluene at 0 °C for 15 minutes. [c] The reactions were performed in tetrahydrofuran at -78 °C for 15 minutes. [d] The ratio was determined by ^{1}H NMR. [e] Isolated yield.

Scheme 1



Reagents and Conditions: a) diisobutylaluminum 2,6-di-*t*-butyl-4-methylphenoxide, toluene, 0 °C; b) Li, liq. NH_3, THF, reflux; c) dihydropyran, PPTS, CH_2Cl_2; d) 6N NaOH, dioxane-H_2O, reflux, then $(C_5H_{11}CO)_2O$, room temp.; e) 2-chloro-2-oxo-1,3,2-dioxaphospholane, DMAP, Et_3N, benzene, room temp.; f) Me_3N, DMF, 70 °C; g) TsOH, MeOH, room temp.

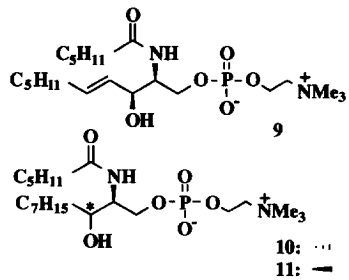
The *anti*-selective reduction of the alkynyl ketone made it possible to efficiently synthesize (D)-*erythro*-sphingomyelin derivatives as described in Scheme 1. Thus, removing the benzyl group and reduction of the alkynyl group to E-olefin of the alcohol **3c** was effective with lithium in liquid ammonia (79% yield). Protection

of the obtained alcohol **5**, mp 49.0-50.0 °C, with a tetrahydropyranyl group (96% yield) followed by hydrolysis of the oxazolidinone ring with aqueous sodium hydroxide, and then successive acylation of the generated amino group gave alcohol **6** (87% yield for two steps), which was reacted with cyclic chlorophosphate[10] and then trimethylamine in DMF successfully to afford phosphorylcholine **7** (54% yield for two steps). Acid treatment of **7** gave short-chain sphigomyelin **8** (91% yield).[11,12] In addition, the (D)-*threo* isomer **9**[13] was synthesized *via* L-selectride® reduction of **2b**, and both *erythro*- and *threo*-derivatives having a saturated alkyl group in the backbone skeleton, **10** and **11**, were also synthesized by similar reaction sequences. Thus, an effective method to provide sphingomyelin derivatives was established.

In order to test the ability of the synthesized sphingomyelin analogs to be hydrolyzed, initial velocities of their hydrolysis catalyzed by sphingomyelinase from *Bacillus cereus* were measured in the presence of a magnesium ion by using pH-stat method at 25 °C, pH 7.0, and ionic strength 0.2.[14] The final concentrations of the enzyme, magnesium ion, and substrate were 1.3×10^{-8} M, 1.3×10^{-2} M, and 3.0×10^{-3} M, respectively. The results of the hydrolysis are shown in Table 2. It was found that (D)-*erythro* derivative **8** was hydrolyzed more than 10 times faster than the (D)-*threo* isomer **9**, and the saturated-chain analogs, **10** and **11**, were also hydrolyzed in a similar manner to the case of the unsaturated compounds, **8** and **9**. Thus, *erythro* stereochemistry is important as a substrate for sphingomyelinase, and the double bond would not be essentially important for the hydrolysis catalyzed by *B. cereus* sphigomyelinase.

Table 2 **Initial velocities of the hydrolysis of short-chain sphingomyelin analogs catalyzed by *B.cereus* sphingomyelinase**

Substrate	v (μmol / min / mg)
8 : erythro (2S, 3R)	136.31 ± 15.92
9 : threo (2S, 3S)	11.17 ± 1.08
10 : erythro-saturated	109.15 ± 2.04
11 : threo-saturated	5.08 ± 4.51



Further studies on the effects of the absolute stereochemistry and the length of the alkyl chain in sphingomyelin for hydrolysis are in progress.

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9. **3b:** mp 39.5-40.5 °C; $[\alpha]_D^{23}$ -14.7° (c 1.28, $CHCl_3$); ^{1}H-NMR(400MHz, $CDCl_3$) δ 0.87(3H, t, 7.1Hz), 1.22-1.38(6H, m), 2.01(2H, dt, J=7.3, 7.1Hz), 2.64(1H, d, J=2.9Hz), 3.65(1H, ddd, J=9.0, 6.1, 2.9Hz), 4.16(1H, dd, J=9.0, 9.0Hz), 4.27(1H, dd, J=8.8, 6.1Hz), 4.30(1H, d, J=15.4Hz), 4.32(1H, br s), 4.77(1H, d, J=15.1Hz), 5.26(1H, ddt, J=15.4, 5.9, 1.5Hz), 5.79(1H, dtd, J=15.4, 6.8, 1.2Hz), 7.30-7.38(5H, m); ^{13}C-NMR (100MHz, $CDCl_3$) δ 13.9, 22.4, 28.5, 31.2, 32.2, 46.4, 58.4, 62.7, 69.3, 126.2, 127.9, 128.0, 128.9, 135.2, 136.1, 159.2; IR(cm^{-1}, neat) 3416, 2928, 2860, 1738.
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12. **8:** $[\alpha]_D^{28}$ 9.60° (c 1.13, $CHCl_3$: CH_3OH / 1:1); ^{1}H-NMR(400MHz, CD_3OD) δ 0.91(6H, m), 1.31-1.40(10H, m), 1.59(2H, tt, J=7.1, 7.1Hz), 2.02(2H, dt, J=7.1, 7.1Hz), 3.22(9H, s), 3.64(2H, br t, J=4.5Hz), 3.95-3.98(2H, m), 4.03-4.27(2H, m), 4.85(2H, br s), 5.45(1H, dd, J=15.2, 7.6Hz), 5.70(1H, dt, J=15.1, 7.3Hz); ^{13}C-NMR (100MHz, CD_3OD) δ 14.3, 14.4, 23.5, 23.6, 27.0, 30.0, 32.56, 32.63, 33.4, 37.3, 54.7, 55.3, 55.35, 55.43, 60.39, 60.44, 65.76, 65.81, 67.5, 72.6, 131.2, 135.0, 175.9; FAB-HR-MS 451.2945(M+H)$^+$ calcd for $C_{21}H_{43}O_6N_2P$ 451.2926.
13. **9:** $[\alpha]_D^{28}$ -9.53° (c 1.28, $CHCl_3$: CH_3OH / 1:1); ^{1}H-NMR(400MHz, CD_3OD) δ 0.91(6H, q, J=6.8Hz), 1.27-1.42(10H, br m), 1.56-1.64(2H, br m), 2.01(2H, dt, J=7.1, 6.8Hz), 2.15-2.27(2H, m), 3.22(9H, s), 3.64(2H, m), 3.83(1H, dt, J=6.3, 6.3Hz), 3.96-4.01(1H, m), 4.02-4.06(1H, m), 4.24-4.29(2H, br m), 4.30-4.32(1H, br m), 5.44(1H, ddt, J=15.4, 6.1, 1.2Hz), 5.73(1H, dtd, J=15.4, 6.8, 1.2Hz); ^{13}C-NMR (100MHz, CD_3OD) δ 14.3, 14.4, 23.5, 23.6, 26.9, 30.0, 32.6, 33.4, 37.2, 54.65, 54.68, 54.72, 55.3, 55.4, 60.4, 60.5, 65.1, 65.2, 67.5, 71.0, 130.7, 133.7, 176.4; FAB-HR-MS 451.2937(M+H)$^+$ calcd for $C_{21}H_{43}O_6N_2P$ 451.2926.
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