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Stereocontrolled Synthesis of a Sphingomyelin Methylene Analogue as a Sphingomyelinase Inhibitor

Toshikazu Hakogi, Yoshiko Monden, Seiji Iwama, and Shigeo Katsumura*

School of Science, Kwansei Gakuin University, 1-1-155, Uegahara, Nishinomiya 662-8501, Japan

Katumura@kwansei.ac.jp

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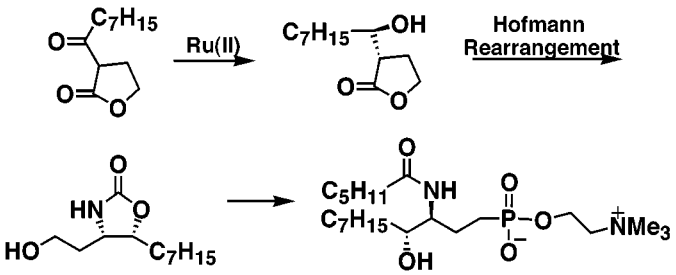
ABSTRACT



Efficient synthesis of a sphingomyelin methylene analogue, which was designed as a sphingomyelinase inhibitor, was stereoselectively achieved. The Hofmann rearrangement of the α-hydroxyethyl-β-hydroxy amide 4 followed by the intramolecular oxazolidinone ring formation was one of the key steps.

Sphingomyelinase (SMase) specifically catalyzes the hydrolysis of the phosphoester linkage of sphingomyelin to produce phosphoryl choline and ceramide, which has been known as a lipid second messenger in mammalian cell membranes and plays key roles in the cellular signal transmission pathway, in particular, as a signal transduction factor in cell differentiation and apoptosis derivation.[1] Hydrolysis of the *N*-acyl group of ceramide with ceramidase produces sphingosine. This unsaturated amino alcohol is well-known as a strong inhibitor of protein kinase C (Figure 1).[2] Although the significance of the sphingomyelin pathway, which is initiated by hydrolysis of sphingomyelin by sphingomyelinase, has been well-recognized, none of the three-dimensional structures of these important enzymes have been determined and their hydrolytic mechanism has not been well-defined. It is, therefore, a very attractive challenge to reveal the catalytic action mechanism of this important enzyme.

We had already established the method for the stereocontrolled synthesis of both D-*erythro*- and *threo*-sphingomyelin and provided both stereoisomers of the short-chain monodispersed sphingomyelin analogues. We then clarified that the initial velocities of the hydrolysis of D-*erythro* derivatives catalyzed by *B. cereus* SMase are more than 10 times faster than those of the D-*threo* isomers and that the double bond in the backbone skeleton would not be essential for the hydrolysis by this enzyme.[3]

Meanwhile, some inhibitors toward SMase have recently been reported, none of which, however, have the sphingolipid skeleton.[4] To elucidate the detailed catalytic mechanism of SMase, the development and the establishment of the method for supplying the sphingomyelin analogues, which competitively act at the catalytic site and strongly inhibit the hydrolytic ability of the enzyme, have strongly been desired.

(1) (a) Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1532. (b) Sawai, H.; Hannun, Y. A. *Chem. Phys. Lipids* **1999**, *102*, 141. (c) Kronke, M. *Chem. Phys. Lipids* **1999**, *102*, 157 and references cited therein.

(2) Hannun, Y. A.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 12604.

(3) Murakami, M.; Iwama, S.; Fujii, S.; Ikeda, K.; Katsumura, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1725.

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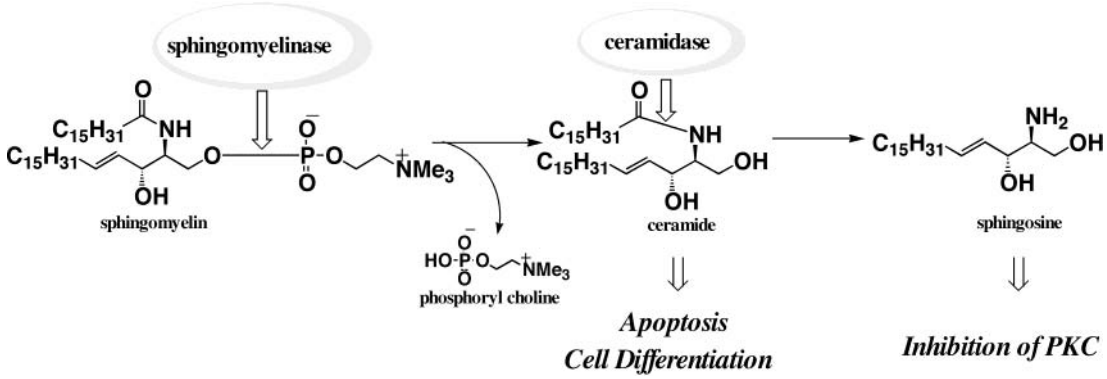

Figure 1.

To this end, we designed the substrate analogue **1** as an inhibitor candidate on the basis of the results reported for the phospholipase C inhibitors by Martin's group[5] and our previous results obtained on sphingomyelin analogues.[3] In the analogue **1**, one of the oxygen atoms of the phosphoester, at which sphingomyelin is hydrolyzed by the enzyme, is replaced by a methylene group, and the stereochemistry of the asymmetric centers must be in D-*erythro* form, (3*S*,4*R*), and in addition, the double bond in the backbone skeleton is saturated. In this paper, we describe the highly efficient stereocontrolled synthesis of the short chain substrate methylene analogue **1** (Figure 2).

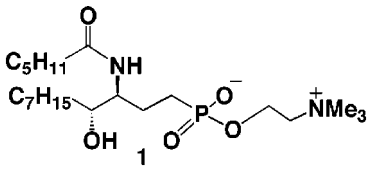

Figure 2. The methylene analogue that is designed as a SMase inhibitor.

To achieve the efficient synthesis of **1**, a convenient method for the preparation of an *erythro* amino alcohol derivative such as **5** was required. Stereospecific oxazolidinone formation resulting from an intramolecular trap with the vicinal hydroxy group of the reactive isocyanate, which was produced by the Hofmann rearrangement of an amide such as **4**, was a very attractive strategy for the synthesis of **5**.

We then started the synthesis with reduction of α-acyl-γ-butyrolactone **2** by the method of Noyori's asymmetric hydrogenation.[6] Thus, hydrogenation of the β-ketoester **2** quantitatively yielded the corresponding alcohol in the presence of a catalytic amount of (*R*)-BINAP−$RuCl_2$ in CH_2Cl_2 under 100 atomospheres pressure of hydrogen at 60 °C for 10 days according to the literature.[7] The diastereoselectivity and enantioselectivity of **3** were determined to be 98% de by ^{1}H NMR and 95% ee by HPLC after benzylation under acidic conditions.[8] With enantiomerically pure alcohol **3** in hand, our attention turned to construction of the amino alcohol equivalent. After the lactone ring of **3** was opened by treatment with a large excess of aqueous NH_3, we tried to react the obtained amide **4** with silver acetate and *N*-bromosuccinimide in DMF. The Hofmann rearrangement of **4** followed by the intramolecular cyclization successfully proceeded and produced the substituted oxazolidinone **5** in 54% yield in two steps. The intermediary isocyanate resulting from the Hofmann rearrangement was selectively trapped with the secondary hydroxy group to spontaneously produce the five-membered oxazolidinone ring with retention of the stereochemisry.[9] Thus, the protected amino alcohol was efficiently synthesized.

The next subjects were the introduction of a phosphoryl group, protection of the secondary hydroxy group resulting from opening the oxazolidinone ring, and then introduction of an acyl group at the amino group. After bromination of the primary alcohol in **5** with carbon tetrabromide and triphenylphosphine, the phosphoryl group was successfully introduced by the Arbuzov reaction with triethyl phosphite under reflux to yield the corresponding phosphoric ester **6**, the carbamate nitrogen of which was activated by the introduction of a *tert*-butyloxycarbonyl group without any purification of the reaction mixture to give **7** quantitatively.

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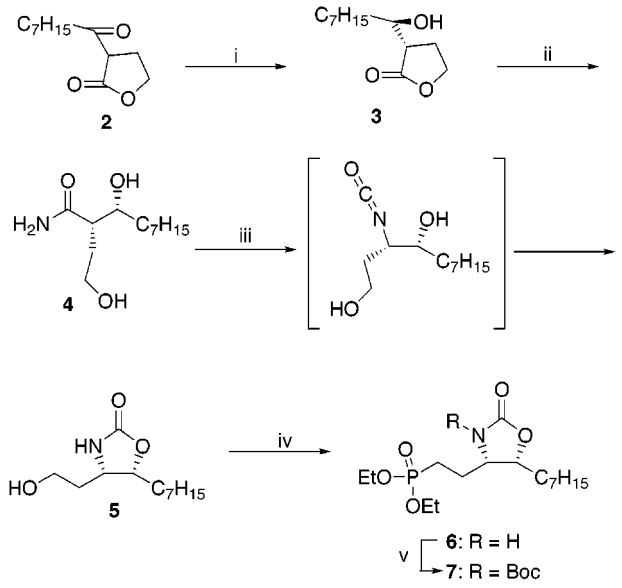
(7) Nishizawa, M.; Garcia, D. M.; Minagawa, R.; Noguchi, Y.; Imagawa, H.; Yamada, H.; Watanabe, R.; Yoo, Y. C.; Azuma, I. *Synlett* **1996**, 452.

(8) Hatakeyama, S.; Mori, H.; Kitano, K.; Yamada, H.; Nishizawa, M. *Tetrahedron Lett.* **1994**, *35*, 4367.

(9) Recently, the Curtius rearrangement for the similar oxazolidinone formation was reported. (a) Pais, G. C. G.; Maier, M. E. *J. Org. Chem.* **1999**, *64*, 4551. (b) Ghosh, A. K.; Hussain, K. A.; Fidanze, S. *J. Org. Chem.* **1997**, *62*, 6080. (c) Ghosh, A. K.; Liu, W. *J. Org. Chem.* **1996**, *61*, 6175.

Scheme 1[a]



[a] Reagents and conditions: (i) cat. (*R*)-BINAP−$RuCl_2$, H_2, 60 °C, 100 atm, 10 days, CH_2Cl_2 (99%, 95% ee, 98% de); (ii) concentrated NH_3 aq., DME; (iii) AgOAc, NBS (64% for two steps); (iv) (a) CBr_4, PPh_3, CH_2Cl_2, (b) $(EtO)_3P$; v, Boc_2O, DMAP, Et_3N, DMF (quant. for three steps).

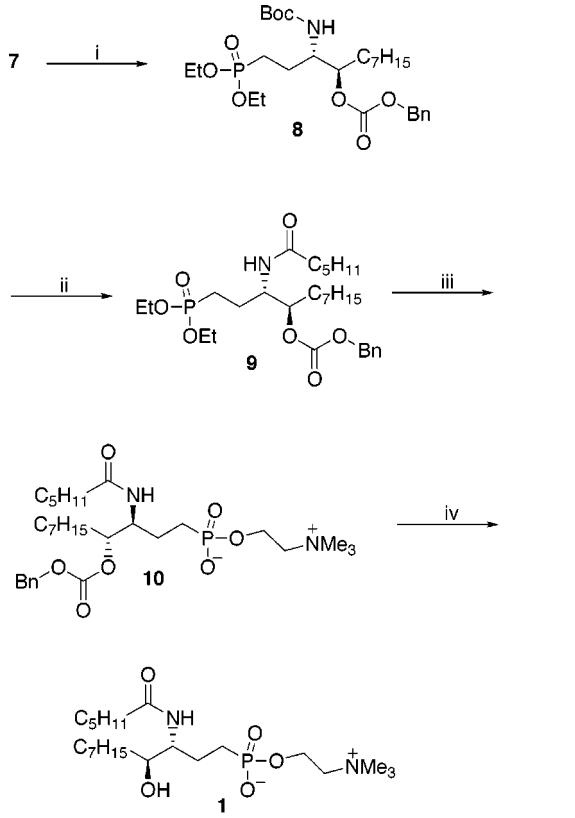
Considering the quite strong polarity of the molecule after introduction of a choline group at the phosphoryl group, the protecting group of the secondary hydroxy group resulting from the oxazolodinone ring opening should be chosen. It should be removed by hydrogenolysis, which workup requires only simple filtration. Fortunately, treatment of **7** with cesium carbonate[10] in the presence of benzyl alcohol in THF successfully produced benzyl carbonate **8** in 81% yield, which was followed by acid treatment to remove the Boc group and the introduction of the acyl group at the resulting amino group to produce the desired *O*-benzyloxycarbonyl-*N*-acylphosphonate **9** in 71% yield in two steps. The final part of the synthesis of **1** was the introduction of a choline group. Treatment of **9** with bromotrimethylsilane in CH_2Cl_2 produced the corresponding silylester, which was continuously refluxed in 10% aqueous THF to complete the hydrolysis and was followed by the reaction with choline chloride and trichloroacetonitrile in pyridine without any purification.[11] The desired choline derivative **10** was obtained in 16% yield and was purified by reverse HPLC.[12] Finally, the benzyloxycarbonyl group of the secondary hydroxy group was removed by hydrogenation over Pd−C, and the synthesis of the (3*S*,4*R*) sphingomyelin methylene analogue **1** was achieved.[13]

(10) Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185.

(11) Thomas, B. N.; Corcoran, R. C.; Cotant, C. L.; Lindemann, C. M.; Kirsch, J. E.; Persichini, P. J. *J. Am. Chem. Soc.* **1998**, *120*, 12178.

(12) The major byproduct of this reaction was the vinyl ester resulting from the elimination of trimethylamine.

Scheme 2[a]



[a] Reagents and conditions: (i) Cs_2CO_3, benzyl alcohol, THF (81%); (ii) (a) 30% TFA, CH_2Cl_2, (b) 1 N NaOH, (c) $C_5H_{11}COCl$ (71% for two steps); (iii) (a) TMSBr, CH_2Cl_2, (b) 10% aq. THF, reflux, (c) choline chloride, CCl_3CN, pyr., 60 °C (16%); (iv) cat. Pd/C, H_2, MeOH (77%).

Thus, an effective and stereocontrolled method to provide the substrate analogue **1** was established. The synthesized substrate analogue (3*S*,4*R*)-**1** showed moderate inhibitory activity toward SMase isolated from *B. cereus*.[14]

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(13) **Data for 1:** ^1H NMR (CD_3OD, 400 MHz) δ 4.25 (m, 2H), 3.73 (ddd, J = 3.2, 6.3, 9.5 Hz, 1H), 3.61 (m, 2H), 3.44 (m, 1H), 3.22 (s, 9H), 2.21 (t, J = 7.6 Hz, 2H), 1.97 (m, 1H), 1.30−1.66 (m, 21H), 0.92 (t, J = 6.6 Hz, 3H), 0.90 (t, J = 7.1 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.7, 73.0, 66.3, 57.1 (J_{C-P} = 4.9 Hz), 54.6, 54.5, 53.1, 35.7, 33.2, 31.5, 31.1, 29.2, 28.8, 25.4, 25.3, 23.8, 23.4, 22.4, 22.1, 21.9, 12.9, 12.8; IR (KBr disk) 3420, 2928, 1642, 1053 cm^{-1}

(14) The test was carried out under the conditions of 10 mM $MgCl_2$, 50 mM Tris-HCl buffer pH 7.5, 6.0×10^{-9} M SMase, 1 mM 2-hexadecanoylamino-4-nitrophenylphosphocholine as a substrate, and ionic strength 0.2. The detailed results will be reported elsewhere.