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Synthesis of a Nitrogen Analogue of Sphingomyelin as a Sphingomyelinase Inhibitor

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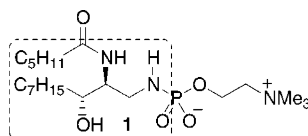
ABSTRACT



Continuous hydroxydiamine
via continuous Hofmann rearrangement & Crutius rearrangement

Sphingomyelin nitrogen analogue 1 was designed and synthesized as a sphingomyelinase inhibitor. The synthesis was established by continuous Hofmann rearrangement and Crutius rearrangement as key steps in constructing the 3-hydroxy-1,2-diamine structure in the backbone of 1. This analogue showed moderate inhibitory activity toward SMase isolated from *B. cereus.*

Sphingolipids are known as secondary lipid messengers in mammalian cells and cell membranes, and a great deal of attention has been devoted to studies of the biological processes regulated by sphingolipids.[1] It is now well accepted that the sphingolipids play key roles in the cellular signal transmission pathway. Ceramide, the primary sphingomyelin metabolite, is generated through the action of sphingomyelinase (SMase) and is believed to be an essential signal transduction factor in cell differentiation and in programmed cell death (apoptosis) derivation.[2] Sphingosine, the secondary metabolite of sphingomyelin, strongly inhibits protein kinase C (Figure 1).[3] Although the significance of the sphingomyelin pathway, which is initiated by hydrolysis of sphingomyelin by SMase, 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. Revealing the mechanism of the catalytic action of this important enzyme is, therefore, a very attractive challenge. Strong and selective sphingomyelinase inhibitors would contribute to a better understanding of both the roles of these enzymes and ceramide in signal transduction.[4] To elucidate the detailed catalytic mechanism of SMase, supplements of sphingomyelin analogues, which act competitively at the catalytic site and strongly inhibit the hydrolytic ability of the enzyme, are desired.

We have reported the synthesis of sphingomyelin carbon analogues, and these analogues actually showed moderate inhibitory activities toward the SMase from *B. cereus*.[5] The sphingomyelin nitrogen analogue **1** was then designed to

(1) For recent reviews on signal transduction mediated by sphingolipids, see; (a) Hannun, Y. A.; Luberto, C.; Argraves, K. M. *Biochemistry* **2001**, *40*, 4893. (b) Kolter, T.; Sandhoff, K. *Angew.* Chem., Int. Ed. **1999**, *38*, 1532 and references therein.

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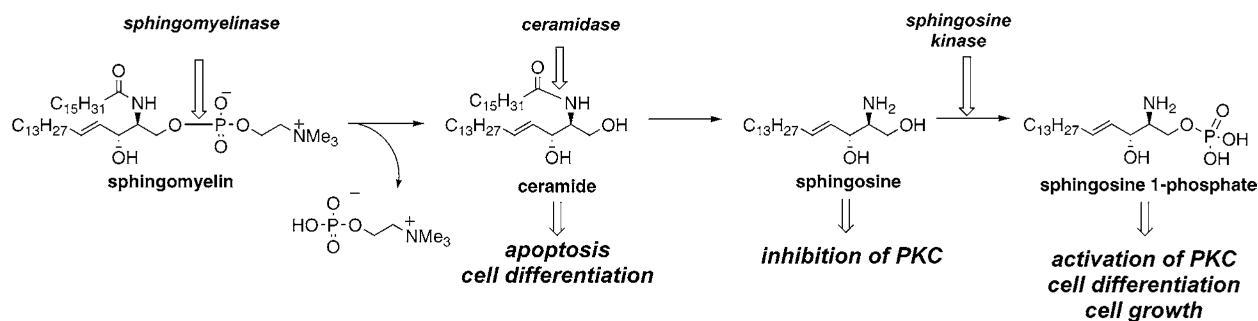


Figure 1. Sphingomyelin catabolism.

provide a desired stronger inhibitor of SMase. In this analogue **1**, one of the oxygen atoms, at which the sphingomyelin was hydrolyzed by SMase, was replaced with a nitrogen atom (Figure 2).

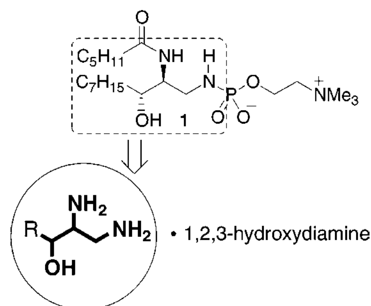


Figure 2. Designed SMase inhibitor.

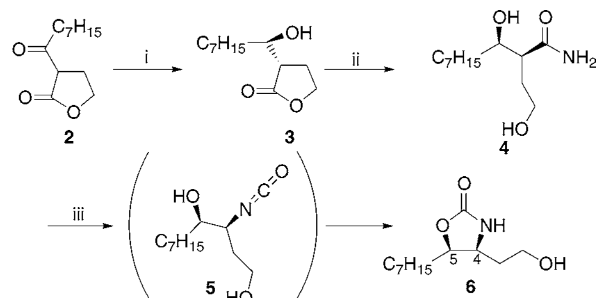
The backbone skeleton of **1** possesses a characteristic structure, which consists of continuous vicinal amino alcohol and vicinal diamine as illustrated in Figure 2. A 1,2-diamino moiety can be found in various natural products possessing valuable biological properties.[6] We planned to utilize an amino alcohol **6**, which had been used as an intermediate for the synthesis of carbon analogues, as a precursor of the desired 1,2-diamine moiety. Diamine construction utilizing continuous rearrangement reactions, such as the Hofmann and the Crutius rearrangements, was very attractive. To examine the latter rearrangement, oxazolidinone **6** was first synthesized via Hofmann rearrangement by following the previously reported procedure (Scheme 1).[5]

The carboxylic acid derivative for the Crutius rearrangement was synthesized from compound **6**. Thus, after protection of the primary hydroxyl group of **6**, hydrolysis of the oxazolidinone ring of **7** by treatment with potassium hydroxide in ethanol under reflux provided amino alcohol **8** in good yield. A long-chain acyl group, which decreased the polarity compared with that of the compound with the acetyl group, was introduced to the nitrogen to afford amide **9** under the Shotten−Baumann condition followed by removal of the tetrahydropyranyl group to give alcohol **11**. Jones oxidation gave the desired carboxylic acid **12** in good yield.

The Crutius rearrangement of compound **12** with diphenyl phosphoryl azide (DPPA)[7] in the presence of triethylamine in toluene proceeded smoothly at 60 °C, and gave the corresponding imidazolidinone **13** almost quantitatively. In this reaction, the intermediary isocyanate was successfully trapped with the vicinal amide nitrogen to afford the corresponding cyclic imidazolidinone. In the literature, few examples can be found that use this intramolecular imidazolidinone formation method in natural product synthesis.[8] Obviously, these reactions are very useful for the construction of the protected 1,2-diamine moiety (Scheme 2).

Since the method for stereoselective construction of the desired 1,2-diamino moiety was established by utilizing continuous rearrangements, the next objective was the completion of the synthesis of the nitrogen analogue **1**. When monocyclic imidazolidinone **13** was used for the synthesis of **1**, it was necessary to hydrolyze the imidazolidinone ring accompanied by differentiation of the resulting two nitrogen atoms by introduction of different kinds of protecting groups. Hydrolysis of imidazolidinone has usually needed vigorous

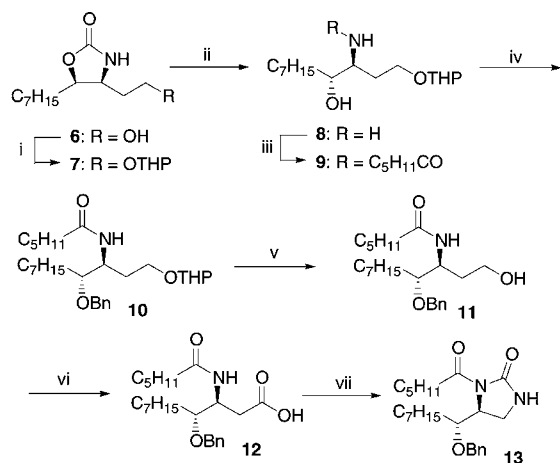
Scheme 1. Synthesis of Oxazolidinone[a]



[a] Reagents and conditions: (i) H_2, cat. (R)-BINAP-$RuCl_2$, 60 °C, 100 atm, 10 days, 99%, 98% de; (ii) conc NH_3, DME; (Iiii) AgOAc, NBS, DMF, 77% for 2 steps.

(6) Lucet, D.; Gall, T. L.; Mioskowski, C. *Angew. Chem., Int. Ed.* **1998**, *37*, 2580 and references therein.

Scheme 2. Synthesis of Imidazolidinone[a]

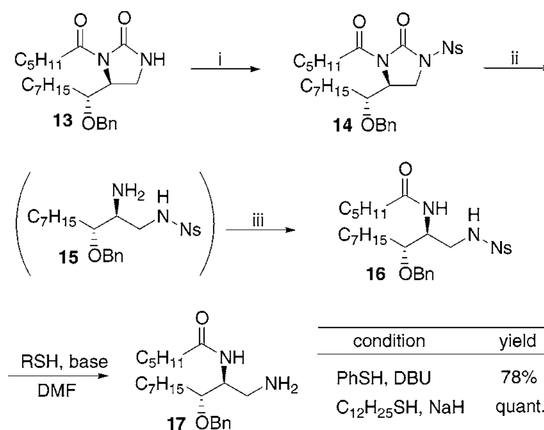


[a] Reagents and conditions: (i) DHP, PPTS, CH_2Cl_2, quant; (ii) KOH, EtOH, reflux, 87%; (iii) $C_5H_{11}COCl$, K_2CO_3, THF, H_2O, 0 °C, quant; (iv) BnBr, NaH, DMF, rt, 87%; (v) TsOH, MeOH, 99%; (vi) Jones oxid., acetone, 0 °C, 98%; (vii) DPPA, Et_3N, toluene, 60 °C, 96%.

reaction conditions such as treatment with concentrated hydrochloric acid or saturated barium hydroxide under reflux for long periods.[9] If imidazolidinone could be easily hydrolyzed under mild conditions, this heterocycle could be used as a more useful synthetic intermediate. In the case of the hydrolysis of an oxazolidinone ring, introduction of a *tert*-butoxycarbonyl group at the nitrogen has been very effective, and cesium carbonate treatment of oxazolidinone derivatives in methanol at room temperature has produced the desired hydrolyzed product in good yield.[10] In the case of the imidazolidinone ring, however, introduction of a *tert*-butoxycarbonyl group was not effective, and that group was easily removed under the same reaction conditions. When the nosyl group (4-nitrobenzenesulfonate), which is hydrolytically stable and easily removed by a treatment with thiophenol in the presence of potassium carbonate,[11] was introduced to the imidazolidinone nitrogen, the imidazolidinone ring was easily cleaved with potassium hydroxide in ethanol to produce primary amine **15** at room temperature. In this reaction, the *N*-acyl group was first removed and then subsequent hydrolysis of the imidazolidinone ring proceeded to give the amine **15**. The acyl group was introduced again into the resulting primary amine to produce the desired amide **16**.

Although the removal of the nosyl group of **16** was realized by treatment with thiophenol and DBU in DMF in 78% yield as reported,[11] we were unwilling to proceed with scaling up of this reaction because of the extremely unpleasant odor of the thiophenol. Odorless decanethiol[12] was therefore used for the removal of the nosyl group. Thus, treatment of nosylamide **16** with decanethiol and sodium hydride in DMF successfully afforded amine **17** in quantitative yield (Scheme 3).

Scheme 3. Synthesis of Primary Amine[a]



[a] Reagents and conditions: (i) NsCl, LDA, THF, 0 °C, 87%; (ii) KOH, EtOH, H_2O; (iii) $C_5H_{11}COCl$, K_2CO_3, THF, H_2O, 70% for 2 steps.

For the synthesis of nitrogen analogue **1**, the remaining objectives were introduction of the phosphoryl choline moiety to primary amine **17** and the removal of the benzyl group. Introduction of a phosphoryl group was examined. Unfortunately, treatment of amine **17** with 2-bromoethylchloromethyl phosphite in THF followed by oxidation of the resulting phosphite to the corresponding phosphate with hydrogen peroxide according to literature[13] gave a complex mixture. After various trials, phosphorylation of **17** with the reagent **A**,[14] which was easily prepared in situ by the reaction of methyl phosphorodichloridate with lithium 2-bromoethoxide, readily proceeded in the presence of diisopropylethylamine in THF to produce **18**. Compound **18** thus obtained was treated with anhydrous trimethylamine in toluene in a sealed tube[13] to give *O*-protected nitrogen analogue **19** in 25% yield after purification by reverse-phase HPLC, and the starting primary amine **17**, which would be generated by hydrolysis, was also recovered in 20% yield after flash chromatography. Finally, removal of the benzyl group of **19** was achieved by hydrogenolysis with a catalytic amount of Pd-black and one portion of formic acid, and the nitrogen analogue **1** was successfully produced. Purification by reverse-phase HPLC afforded pure nitrogen analogue **1**. Thus, the convenient synthesis of the nitrogen analogue **1** was achieved (Scheme 4).[15]

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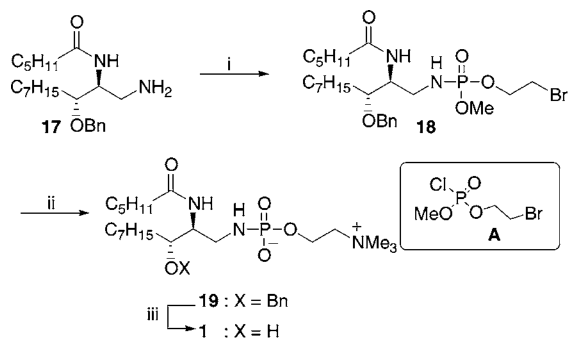
Scheme 4. Synthesis of Sphingomyelin Nitrogen Analogue **1**[a]



[a] Reagents and conditions: (i) 2-bromoethanol, *n*BuLi, MeO-P(O)Cl_2, *i*Pr_2NEt, THF, 64%; (ii) Me_3N, DMF, 60 °C, 25%; (iii) H_2, HCO_2H, Pd-black, MeOH, 27%.

The synthesized substrate analogue **1** showed moderate inhibitory activity toward SMase isolated from *B. cereus.*

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **13**, **17**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) **Data for 1**: $[\alpha]^{25.0}_{D}$ 23.3 (*c* 0.29, CH_3OH); IR (NaCl neat) 3331, 2924, 1638, 1202, 1055 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 4.14−4.21 (m, 2H), 3.67−3.73 (m, 1H), 3.58−3.64 (m, 3H), 3.22 (s, 9H), 3.02−3.16 (m, 2H), 2.22 (dt, J = 3.7, 8.6 Hz, 2H), 1.23−1.70 (m, 18H), 0.91 (t, J = 7.1 Hz, 3H), 0.89 (t, J = 7.1 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.2, 72.0, 67.7 (m), 59.5 (d, J_{C-P} = 5.0 Hz), 56.7 (d, J_{C-P} = 3.3 Hz), 54.7 (m), 42.8, 37.4, 34.7, 33.0, 32.6, 30.8, 30.4, 27.0, 26.9, 23.7, 23.5, 14.4, 14.3.