JOC Article

Synthesis of Sphingomyelin Carbon Analogues as Sphingomyelinase Inhibitors

Toshikazu Hakogi,[†] Yoshiko Monden,[†] Misako Taichi,[†] Seiji Iwama,[†] Shinobu Fujii,[‡] Kiyoshi Ikeda,[‡] and Shigeo Katsumura*,[†]

School of Science, Kwansei Gakuin University, Gakuen, Sanda, Hyogo 669-1337, Japan, and Department of Biochemistry, Osaka University of Pharmaceutical Sciences, Nasahara, Takatsuki, Osaka 569-1041, Japan

katsumura@ksc.kwansei.ac.jp

Received January 17, 2002

The highly efficient and stereocontrolled syntheses of sphingomyelin carbon analogues **1** and **2** were achieved by effectively utilizing Hofmann rearrangement of enantiomerically pure β-hydroxyamide **7**, which was prepared by an asymmetric hydrogenation of α-acyl-γ-butyrolactone **9** and ring opening with NH_3. Intermediary isocyanate **6** was selectively trapped with the vicinal hydroxy group in an intramolecular fashion to produce an oxazolidinone derivative, **5**. In the synthesis of a quite polar compound such as **1**, a convenient one-pot procedure of the introduction of a benzyloxycarbonyl group into the hydroxy group resulting from the oxazolidinone ring opening is another key point, because, in addition to the efficiency, this protecting group was easily removable by a simple procedure and workup at the final step. Both synthesized compounds **1** and **2** showed moderate inhibitory activity toward sphingomyelinase from *B. cereus*.

Introduction

Sphingolipids have been known as lipid second messengers in mammalian cells and cell membranes, and a great deal of attention has been devoted to the studies of the biological process regulated by sphingolipids.[1] Now, it has been well accepted that the sphingolipids play key roles in the cellular signal transmission pathway. Sphingomyelin, which is one of the key sphingolipids, is a ubiquitous constituent in animal tissue and has been known to occur in virtually every cell and in cell membranes. Ceramide and phosphoryl choline, which are the primary catabolites of sphingomyelin, are generated in the so-called sphingomyelin cycle through the action of either lysosomal acid sphingomyelinase (A-SMase) or membrane-bound neutral sphingomyelinase (N-SMase); ceramide is believed to display key roles as a signal transduction factor in cell differentiation and in programmed cell death (apoptosis) derivation.[2] Hydrolysis of the N-acyl group of ceramide with ceramidase produces sphingosine. This secondary catabolite 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 tertiary-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 mechanism of action of this important enzyme. Strong and selective sphingomyelinase inhibitors would contribute to a better understanding both of the roles of these enzymes and of ceramide in signal transduction. Some SMase inhibitors have recently been reported (Figure 2).[4] Among them, scyphostatin, natural product, has been paid much attention as a powerful inhibitor of N-SMase.[4b,c,f] Most recently, compound **Z** was designed and synthesized as the first selective irreversible inhibitor of N-SMase.[4a] Meanwhile, Bittman's group reported the preparation of sphingomyelin analogues modified at the C-1 and C-3 positions of the sphingosine backbone, and 3-*O*-methylsphingomyelin showed moderate inhibitory activity toward N-SMase.[5] To elucidate the detailed catalytic mechanism of SMase, the development of the methods 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.

* To whom correspondence should be addressed. Phone: 81-795-65-8314. Fax: 81-795-65-9077.

[†] Kwansei Gakuin University.

[‡] Osaka University of Pharmaceutical Sciences.

(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.

(2) (a) Sawai, H.; Hannun, Y. A. *Chem. Phys. Lipids* **1999**, *102*, 141. (b) Tilly, J. L.; Kolesnick, R. N. *Chem. Phys. Lipids* **1999**, *102*, 149. (c) Kronke, M. *Chem. Phys. Lipids* **1999**, *102*, 157. (d) Hannun, Y. A. *Science* **1996**, *274*, 1855 and references therein.

(3) Vankar, Y. D.; Schmidt, R. R. *Chem. Soc. Rev.* **2000**, *29*, 201.

(4) (a) Arenz, C. Giannis, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 1440. (b) Nara, F.; Tanaka, M.; Hosoya, T.; Suzuki-Konagai, K.; Ogita, T. *J. Antibiot.* **1999**, *52*, 525. (c) Nara, F.; Tanaka, M.; Masuda-Inoue, S.; Yamasato, Y.; Doi-Yoshioka, H.; Suzuki-Konagai, K.; Kumakura, S.; Ogita, T. *J. Antibiot.* **1999**, *52*, 531. (d) Uchida, R.; Tomoda, H.; Dong, Y.; Omura, S. *J. Antibiot.* **1999**, *52*, 572. (e) Tanaka, M.; Nara, F.; Yamasato, Y.; Masuda-Inoue, S.; Doi-Yoshioka, H.; Kumakura, S.; Enokita, R.; Ogita, T. *J. Antibiot.* **1999**, *52*, 670. (f) Tanaka, M.; Nara, F.; Suzuki-Konagai, K.; Hosoya, T.; Ogita, T. *J. Am. Chem. Soc.* **1997**, *119*, 7871.

(5) Lister, M. D.; Ruan, Z.-s.; Bittman, R. *Biochim. Biophys. Acta* **1995**, *1256*, 25.

10.1021/jo025529o CCC: $22.00
Published on Web 05/31/2002

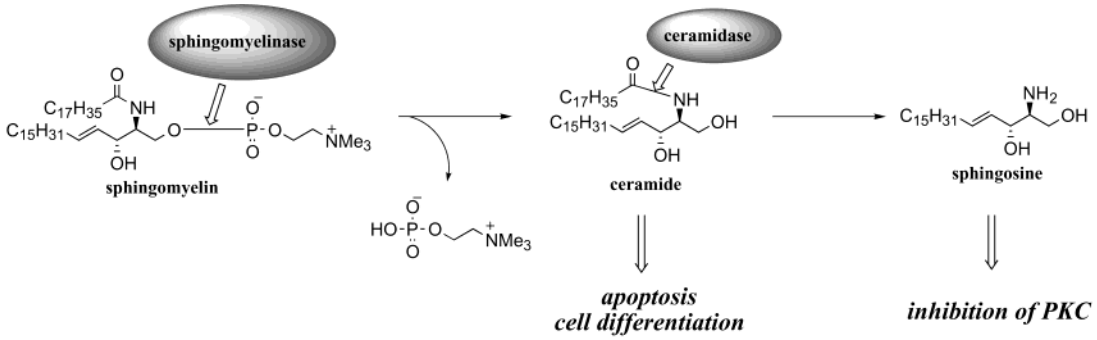


FIGURE 1. Sphingomyelin metabolism.

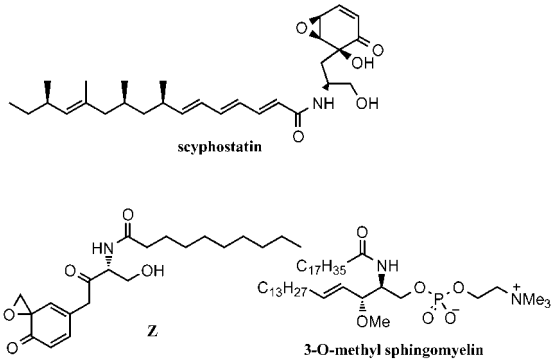


FIGURE 2. Reported inhibitors of sphingomyelinase.

In a number of synthetic efforts for these biologically significant and enantiomerically pure sphingolipids,[6] we had also established a method for the stereocontrolled synthesis of sphingomyelin and provided all stereoisomers of the short-chain monodispersed sphingomyelin analogues from the chiral oxazolidinone synthon,[7] which had been easily prepared from enantiomerically pure glycidol.[8] 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. Meanwhile, phosphonate analogues, in which the labile P−O−C bond is displaced by a stable P−CH_2−C bond, have been explored as phosphate surrogates[9] and are often utilized as useful bioorganic tools;[10] Martin and co-workers reported the phospholipase C (PLC) substrate analogues, in which the hydrolyzed phosphonate oxygen was replaced by methylene, difluoromethylene, NH, and S groups, and reported that some of them strongly inhibited the hydrolytic ability of PLC.[11]

We then designed the substrate analogues **1** and **2** as inhibitor candidates on the basis of both our previous results obtained on sphingomyelin analogues and the results reported by Martin's group.[11] In analogues **1** and **2**, one of the oxygen atoms of the phosphoester, at which sphingomyelin is hydrolyzed by the enzyme, is replaced by either a methylene or ethylene group, and the relative configuration of the asymmetric centers is the (D)-*erythro* (3*S*,4*R*) form. In addition, the double bond in the backbone skeleton is saturated (Figure 3). In this paper, we describe in detail the highly efficient stereocontrolled syntheses of the short chain substrate methylene analogue **1**[12] and ethylene analogue **2** and their inhibitory activities toward *B. cereus* SMase. [13]

Results and Discussion

Our plan to synthesize these kinds of substrate analogues substituted by carbon groups is shown in Scheme 1. The sphingomyelin carbon analogues **1** and **2** might be, respectively, derived from phosphonate ceramide analogues **3** and **4** by hydrolysis of the phosphonyl ester followed by introduction of a choline group. Both ceramide phosphonate analogues might be synthesized from the 2-substituted oxazolidinone **5** by introduction of a phosphoryl group and then oxazolidinone ring opening. To achieve the efficient synthesis of **1**, therefore, a convenient method for the preparation of *erythro* amino alcohol derivative **5** was required. The Hofmann rear-

(6) Synthesis of sphingomyelin; see review: (a) Weis, A. L. *Chem. Phys. Lipids* **1999**, *102*, 3. (b) Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, 1075. (c) Byun, H.-S.; Erukulla, R. K.; Bittman, R. *J. Org. Chem.* **1994**, *59*, 6495. (d) Kratzer, B.; Mayer, T. G.; Schmidt, R. R. *Tetrahedron Lett.* **1993**, *34*, 6881. (e) Dong, Z.; Butcher, J. A., Jr. *Tetrahedron Lett.* **1991**, *32*, 5291. (f) Bruzik, K. S. *J. Chem. Soc., Perkin Trans. 1* **1988**, 423. Recent syntheses of enantiomerically pure sphingosine and ceramide, see: (g) He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7618. (h) He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7627. (i) Corey E. J.; Choi, S. *Tetrahedron Lett.* **2000**, *41*, 2765. Synthesis of sphingolipid derivatives, see: (j) Triola, G.; Febrias, G.; Llebaria, A. *Angew. Chem., Int. Ed.* **2001**, *40*, 1960. (k) Chun, J.; He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7634. (l) He, L.; Byun, H.-S.; Smit, J.; Wilschut, J.; Bittman, R. *J. Am. Chem. Soc.* **1999**, *121*, 3897. (m) Dondoni, A.; Perrone, D.; Turturici, E. *J. Org. Chem.* **1999**, *64*, 5557. (p) Byun, H.-S.; Sadlofsky, J. A.; Bittman, R. *J. Org. Chem.* **1998**, *63*, 2560.

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

(8) Katsumura, S.; Yamamoto, N.; Morita, M.; Han; Q. *Tetrahedron: Asymmetry* **1994**, *5*, 161.

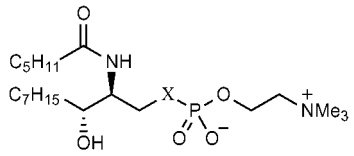
(9) For reviews on phosphonates, see: (a) Wiemer, D. F. *Tetrahedron* **1997**, *53*, 16609. (b) Engel, R. *Chem. Rev.* **1977**, *77*, 349.

(10) For recent examples, see: (a) Berkowitz, D. B.; Bose, M.; Pfannenstiel, T. J.; Doukov, T. *J. Org. Chem.* **2000**, *65*, 4498. (b) Viera de Almeida, M.; Cleophax, J.; GateauOlesker, A.; Prestat, G.; Dubreuil, D.; Gero, S. D. *Tetrahedron* **1999**, *55*, 12997. (c) Billault, I.; Vasella, A. *Helv. Chim. Acta* **1999**, *82*, 1137. (d) Kitanuma, H.; Shimazaki, K.; Takahashi, N.; Takahashi, K.; Niihata, S.; Aoki, Y.; Hamada, K.; Matsushita, H.; Nishi, Y. *Tetrahedron* **1999**, *55*, 25559. (e) Sachpatzidis, A.; Dealwins, C.; Lubetsky, J.; Liang, P. H.; Anderson, K. S.; Lolis, E. *Biochemistry* **1999**, *38*, 12665. (f) Arni, R. K.; Watanabe, L.; Ward, R. J.; Kreiman, R. J.; Kumar, K.; Walz, F. G., Jr. *Biochemistry* **1999**, *38*, 2452.

(11) (a) Martin, S. F.; Josey, J. A.; Wong, Y. L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805. (b) Martin, S. F.; Wong, Y. L.; Wagman, A. S. *J. Org. Chem.* **1994**, *59*, 4821.

(12) Hakogi, T.; Monden, Y.; Iwama, S.; Katsumura, S. *Org. Lett.* **2000**, *2*, 2627.

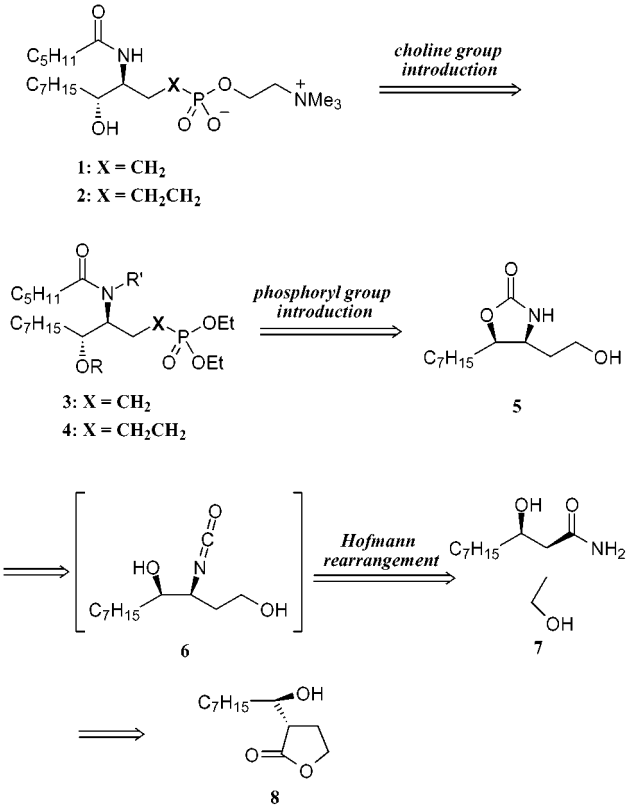
(13) During our study, a CF_2 analogue having a phenyl derivative in the backbone skeleton was reported: Yokomatsu, T.; Takeuchi, H.; Akiyama, T.; Shibuya, S.; Kominato, T.; Soeda, S.; Shimeno, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1277.



1: X = CH_2
2: X = CH_2CH_2

FIGURE 3. Designed inhibitors of sphingomyelinase.

SCHEME 1. Retrosynthesis

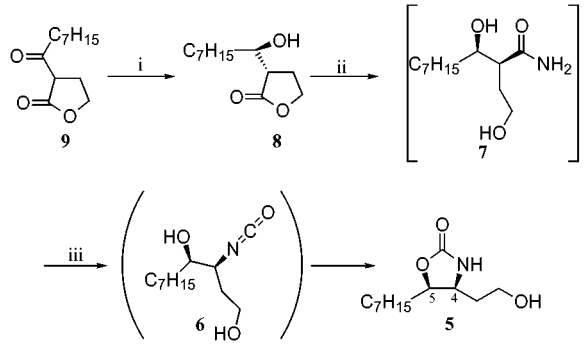


1: X = CH_2
2: X = CH_2CH_2
3: X = CH_2
4: X = CH_2CH_2
5
6
7
8

rangement followed by the intramolecular oxazolidinone ring formation strategy was very attractive for this.[14] Thus, stereospecific oxazolidinone formation resulting from an intramolecular trap with the vicinal hydroxy group of reactive isocyanate **6**, which would be produced by the Hofmann rearrangement of an amide such as **7**, would be a very preferable strategy for the synthesis of **5**. Amide **7** would be easily prepared from enantiomerically pure γ-butyrolactone derivative **8**, which possesses a hydroxyalkyl group at the α-position.

We then started the synthesis with reduction of α-acyl-γ-butyrolactone **9** by the method of Noyori's asymmetric hydrogenation.[15] Thus, hydrogenation of β-ketoester **9** quantitatively yielded the corresponding alcohol, **8**, even if the reaction is on more than a 20-gram scale, in the presence of a catalytic amount of (*R*)-BINAP-$RuCl_2$ in

SCHEME 2. Synthesis of 2-Substituted Oxazolidinone[a]



9
8
7
6
5

[a] Reagents and conditions: (i) H_2, cat. (*R*)-BINAP-$RuCl_2$, CH_2Cl_2, 60 °C, 100 atm, 10 days, 99% de; (ii) concentrated NH_3, DME; (iii) AgOAc, NBS, DMF, 77% for 2 steps.

CH_2Cl_2 under 100 atm pressure of hydrogen at 60 °C for 10 days according to the literature.[16] The diastereoselectivity of **8** was determined to be 98% de by ^{1}H NMR, and the enantioselectivity was determined to be 95% ee by HPLC analysis using a chiral column after benzylation of the secondary hydroxyl group under neutral conditions.[17] With enantiomerically pure alcohol **8** in hand, our attention turned to construction of the amino alcohol equivalent. Alcohol **8** was then treated with a large excess of aqueous NH_3 to yield amide **7**. The Hofmann rearrangement of **7** by treatment with silver acetate and NBS in DMF followed by intramolecular cyclization successfully proceeded and produced substituted oxazolidinone **5**, which was easily purified by recrystallization, in 77% yield from alcohol **8** (Scheme 2). The vicinal coupling constant of the ^{1}H NMR of the oxazolidinone ring thus generated was $J_{4,5} = 7.8$ Hz and was compatible with a *cis*-orientation of the two substituents.[18] The results obviously meant that the intermediary isocyanate resulting from the Hofmann rearrangement was selectively trapped with the secondary hydroxyl group spontaneously to produce the five-membered oxazolidinone ring with retention of the stereochemistry.[19] Thus, the desired protected amino alcohol **5** was efficiently synthesized.

The next subject was introduction of a phosphoryl group and then a choline group. After bromination of the primary alcohol in **5** with carbon tetrabromide and triphenylphosphine, the phospholyl group was introduced by the Arbuzov reaction[20] with triethyl phosphite under reflux. The reaction mixture was directly evaporated and was reacted with *tert*-butoxycarbonyl anhydride to produce quantitatively *N*-*tert*-butoxycarbonyl derivative **12**. Considering the quite strong polarity of the molecule after introduction of a choline group at the phosphoryl group, the protecting group of the secondary hydroxyl

(14) (a) Murakami, M.; Hinoue, K.; Nakagawa, K.; Monden, Y.; Furukawa, Y.; Katsumura, S. *Heterocycles* **2001**, *54*, 77. (b) Simons, S. S., Jr. *J. Org. Chem.* **1973**, *38*, 414.

(15) (a) Noyori, R.; Ikeda, T.; Ohkuma, T.; Widhalm, M.; Kitamura, M.; Takaya, H.; Akutagawa, S.; Sayo, N.; Saito, T.; Taketomi, T.; Kumobayashi, H. *J. Am. Chem. Soc.* **1989**, *111*, 9134. (b) Kitamura, M.; Tokunaga, M.; Ohkuma, T.; Noyori, R. *Org. Synth.* **1993**, *71*, 1.

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

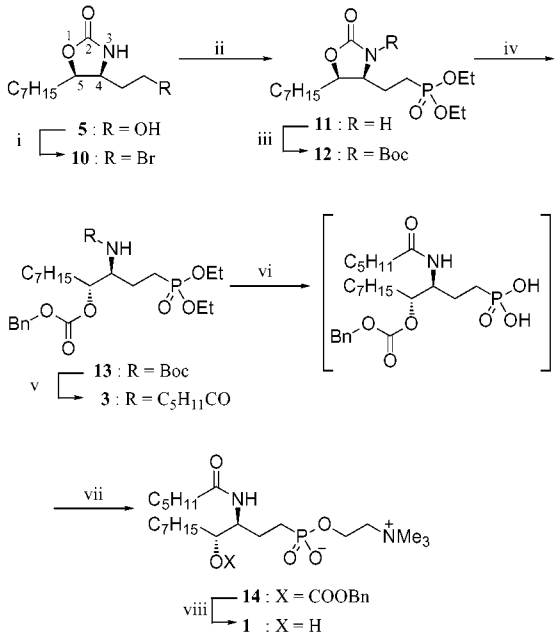
(17) Hatakeyama, S.; Mori, H.; Kitano, K.; Yamada, H.; Nishizawa, M. *Tetrahedron Lett.* **1994**, *35*, 4367.

(18) Sakaitani, M.; Ohfune, Y. *J. Am. Chem. Soc.* **1990**, *112*, 1150.

(19) 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.

(20) Arbuzov, B. A. *Pure Appl. Chem.* **1964**, *9*, 307.

SCHEME 3. Synthesis of the Methylene Analogue[a]

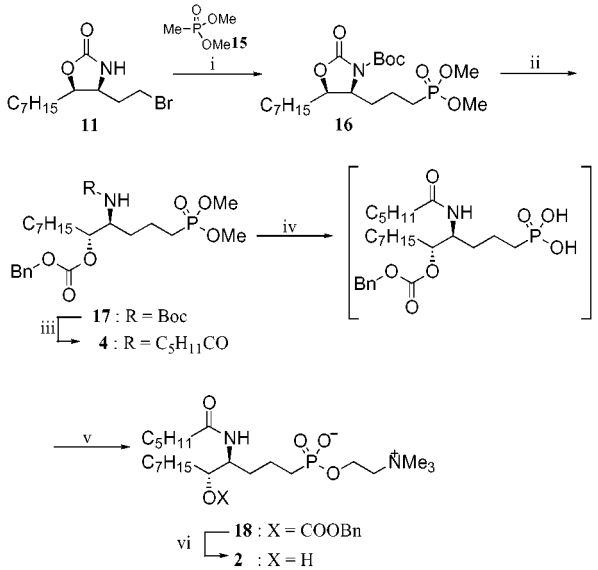


[a] Reagents and conditions: (i) CBr_4, PPh_3, CH_2Cl_2, 0 °C; (ii) $(EtO)_3P$, reflux; (iii) Boc_2O, DMAP, Et_3N, DMF, 0 °C, quant. for 3 steps; (iv) BnOH, Cs_2CO_3, THF, 81%; (v) (a) TFA, CH_2Cl_2, 0 °C; (b) aqueous 1 N NaOH, $CHCl_3$; (c) $C_5H_{11}COCl$, 85%; (vi) TMSBr, CH_2Cl_2, then MeOH; (vii) choline chloride, CCl_3CN, pyr., 50 °C, 17% for 2 steps; (viii) H_2, Pd−C, MeOH, 77%.

group resulting from the oxazolidinone ring opening should be selected. It should be removed by hydrogenolysis, which workup requires only simple filtration. Fortunately, reaction of **12** with cesium carbonate in the presence of benzyl alcohol in THF successfully proceeded, and benzyl carbonate was introduced to the secondary alcohol resulting from the opening of the oxazolidinone ring, which was activated with the *tert*-butoxycarbonyl group.[21] After removing the *tert*-butoxycarbonyl group of **13** by an acid treatment, the introduction of the acyl group at the resulting amino group produced the desired *O*-benzyloxycarbonyl-*N*-acylphosphonate **3** in 71% yield in two steps. The final part of the synthesis of **1** was the introduction of a choline group. Treatment of **3** with bromotrimethylsilane in CH_2Cl_2 produced the corresponding silylester, which was continuously stirred in methanol to complete the hydrolysis[22] and was followed by the reaction with choline chloride and trichloroacetonitrile in pyridine[23] without any purification. The desired choline derivative, **14**, was obtained in 16% yield after purification by reverse HPLC. The major byproduct of this reaction was the vinyl ester resulting from the elimination of trimethylamine. Finally, the benzyloxycarbonyl group of the secondary hydroxyl group was removed by hydrogenation over Pd−C, and the synthesis of (3*S*,4*R*) sphingomyelin methylene analogue **1** was thus achieved (Scheme 3).

Next, our interest is the synthesis of ethylene analogue **2**. To construct the backbone skeleton of **2**, we planned

SCHEME 4. Synthesis of the Ethylene Analogue[a]



[a] Reagents and conditions: (i) **16**, *n*-BuLi, THF, −78 °C, then Boc_2O, 63%; (ii) BnOH, Cs_2CO_3, THF, 57%; (iii) (a) TFA, CH_2Cl_2, 0 °C; (b) aqueous 1 N NaOH, $CHCl_3$; (c) $C_5H_{11}COCl$, 79%; (iv) TMSBr, CH_2Cl_2, then MeOH; (v) choline chloride, CCl_3CN, pyr., 50 °C, 17% for 2 steps; (vi) H_2, Pd−C, MeOH, 89%.

to react bromide **10** with a lithium anion of methyldimethylphosphonate, **15**. Unfortunately, the desired product was not obtained by the usual extraction procedures because of the strong polarity of the products. We then tried to introduce a *tert*-butoxycarbonyl group into the oxazolidinone nitrogen. Thus, the reaction mixture of the lithium anion of **15** with bromide **10** was successively treated with di-*tert*-butyl dicarbonate to produce the desired **16** in 63% yield. The convenient one-pot procedure was again tried for the oxazolidinone ring opening followed by the protection of the alcohol generated with a benzyloxycarbonyl group. Thus, compound **16** was treated with cesium carbonate in the presence of benzyl alcohol in THF to successfully produce **17** in 57% yield. The *tert*-butoxycarbonyl group was removed with a trifluoroacetic acid treatment, and then, a hexanoyl group was introduced into the resulting amine by treatment with sodium hydroxide and hexanoyl chloride in chloroform to give *N*-acyl derivative **4** in 79% yield. Hydrolysis of the phosphonate ester of **4** by treatment with trimethylsilyl bromide and then methanol gave the corresponding acid, which was reacted without any purification with choline chloride and trichloroacetonitrile in pyridine at 50 °C for 2 days. The desired choline derivative, **18**, was obtained in 16% yield after purification by reversed phase HPLC. Finally, the benzyloxycarbonyl group of the secondary hydroxyl group was removed by hydrogenation over Pd−C, and the synthesis of (3*S*,4*R*) sphingomyelin ethylene analogue **2** was achieved (Scheme 4). Thus, effective and stereocontrolled methods of providing the substrate carbon analogues **1** and **2** were established.

We tested the ability of compounds **1** and **2** to inhibit SMase from *B. cereus*. Enzyme activity was measured three times at 37 °C and ionic strength 0.2 with a buffer of 50 mM Tris-HCl buffer (pH 7.5) in the presence of 10 mM $MgCl_2$. The concentrations of SMase and 2-hexade-

(21) Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185.
(22) (a) Pergament, I.; Srebnik, M. *Tetrahedron Lett.* **1999**, *40*, 3895. (b) McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc., Chem. Commun.* **1979**, 739.
(23) 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.

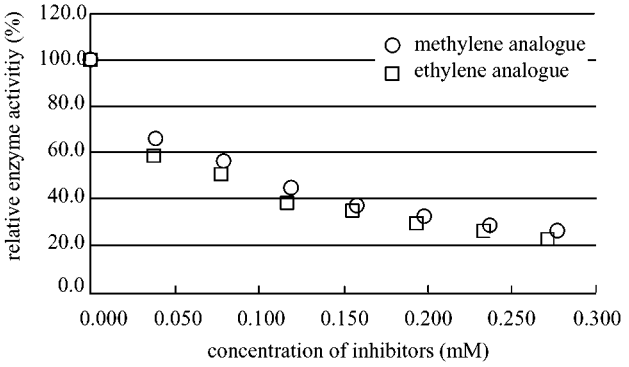


FIGURE 4. Inhibition activities.

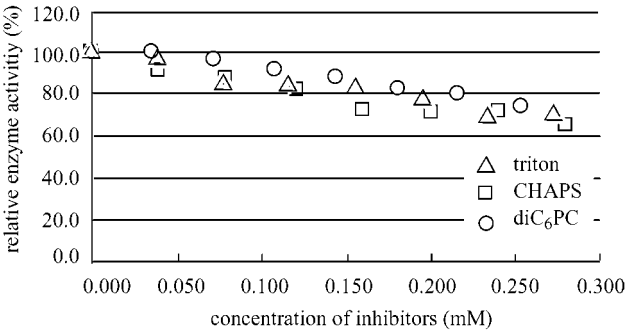


FIGURE 5. Results by detergents.

canoylamino-4-nitrophenylphosphocholine used as the substrate are 6.0×10^{-9} M and 1.0 mM, respectively.[24] As shown in Figure 4, compounds **1** and **2** inhibited the activity of SMase, and their respective IC_{50} values were approximately 120 and 78 μM. The experiments were conducted exactly as shown in Figure 4. To exclude the factors for whether the inhibitions by compounds **1** and **2** are due to the surface dilution,[25] we examined the effects of three types of amphiphiles (two detergents and one phospholipid) on the activity of SMase. Under the present experimental conditions, 2-hexadecanoylamino-4-nitrophenylphosphocholine used as a substrate is in a micellar state. Because compounds **1** and **2** are kinds of amphiphiles having the same length of aliphatic hydrocarbon chain as those of dihexanoylphosphatidylcholine (diC_6PC), they are thought to have similar inhibitory power because of the surface dilution. These three types of amphiphiles inhibited the enzyme activity to a lesser extent as shown in Figure 5. The observed inhibitions by compounds **1** and **2** were much greater than those by three types of amphiphiles. It was thus concluded that the inhibitions of SMase by compounds **1** and **2** were caused by the specific binding of these compounds to the substrate binding site of the enzyme, although the effect of surface dilution was partly involved.

(24) Levade, T.; Salvayre, R.; Douste-Blazy, L. *J. Neurochem.* **1983**, *40*, 1762.

(25) Most phospholipases including SMase show interesting but complex kinetics of hydrolysis, because their substrates are not monodispersed and exist in a two-dimensional array. The catalytic mechanisms of phospholipases are complicated by an additional binding event that may be separated from formation of the Michaelis complex with substrate. This additional factor is mainly due to the binding of enzymes to the aggregated surface, which can be affected by the structure of substrates, kinds of detergents, or other amphiphiles. When the aggregated substrates (e.g., micelles) at a constant concentration are diluted with detergents or other amphiphiles, surface dilution refers to the observation that the specific activities of phospholipases decrease. This phenomenon is attributed to the existing state of substrates and not enzymes.

Experimental Section

All commercially available reagents were used without further purification. All solvents were used after distillation. Tetrahydrofuran and diethyl ether were refluxed over and distilled from sodium. Dichloromethane was refluxed over and distilled from P_2O_5. Dimethylformamide (DMF) was distilled from CaH_2 under reduced pressure. Preparative separation was usually performed by column chromatography on silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded, and chemical shifts were represented as δ-values relative to the internal standard TMS. IR spectra were recorded on a Fourier Transform infrared spectrometer.

2-(1-Oxooctyl)-4-butanolide (9). To a solution of lithium diisopropylamide prepared from di-*iso*-propylamine (9.7 mL, 69.6 mmol) and 1.6 N *n*-butyllithium in hexane (39.8 mL, 63.9 mmol) in THF (58 mL) was added a solution of γ-butyrolactone (5.00 g, 58.1 mmol) in THF (116 mL) at −78 °C, and the reaction mixture was stirred for 30 min at the same temperature. To this mixture was added dropwise a solution of octanal (10.9 mL, 69.7 mmol) in THF (116 mL) at −78 °C. After the reaction mixture was stirred for an additional 1 h, an aqueous 2 N HCl solution was added, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 16% to 50% ethyl acetate in hexane) gave 2-(1-hydroxyoctyl)-4-butanolide (11.6 g, 93%) as a colorless oil. To a solution of the alcohol (9.01 g, 42.0 mmol) obtained in acetone (210 mL) was added Jones reagent at 0 °C until the color of the reaction mixture turned yellow. After the reaction mixture was stirred for 5 min at the same temperature, brine was added, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 11% to 17% ethyl acetate in hexane) gave **9** (8.21 g, 92.0%) as a colorless oil: IR (NaCl neat) 2930, 1771, 1725, 1410, 1377 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.39 (ddd, J = 6.4, 8.1, 9.0 Hz, 1H), 4.32 (ddd, J = 6.3, 8.1, 8.8 Hz, 1H), 3.69 (dd, J = 6.3, 9.3 Hz, 1H), 2.96 (ddd, J = 7.3, 7.6, 18.1 Hz, 1H), 2.78 (tdd, J = 6.3, 8.1, 12.9 Hz, 1H), 2.61 (ddd, J = 7.1, 7.6, 17.8 Hz, 1H), 2.32 (m, 1H), 1.55−1.67 (m, 2H), 1.20−1.36 (m, 8H), 0.88 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.6, 172.8, 67.5, 52.2, 42.2, 31.6, 29.0, 28.9, 23.9, 23.2, 22.6, 14.0; FAB HRMS *m/z* calcd for $C_{12}H_{21}O_3$ ($M^+ + H$) 213.1491, found 213.1519.

(2*S*)-[(1*R*)-Hydroxyoctyl]-4-butanolide (8). (*R*)-BINAP-$RuCl_2$ catalyst was prepared in situ from (*R*)-BINAP (212 mg, 0.341 mmol) and benzene ruthenium chloride dimer (86 mg, 0.171 mmol) in degassed DMF (1.0 mL) at 100 °C for 10 min under argon atmosphere.[15b] A solution of **9** (24.2 g, 113.8 mmol) and (*R*)-BINAP-$RuCl_2$ catalyst prepared in degassed CH_2Cl_2 (23 mL) was stirred in an autoclave under 100 atm of hydrogen at 60 °C for 10 days. Column chromatography on silica gel (from 11% to 17% ethyl acetate in hexane) gave **8** (24.1 g, 99.0%, 94.8% ee, 94.3% de) as a colorless oil. Its enantiomeric excess was determined by HPLC analysis using a chiral column (Daicel chiralcel OD) after benzylation of the secondary hydroxy group according to the procedure reported in the literature:[17] $[\alpha]_D^{27.0}$ −6.87 (c = 1.06, $CHCl_3$); IR (NaCl neat) 3482, 2901, 1782 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.40 (ddd, J = 2.9, 8.8, 9.0 Hz, 1H), 4.23 (ddd, J = 7.3, 9.0, 9.5 Hz, 1H), 4.18 (m, 1H), 2.68 (ddd, J = 2.9, 9.3, 10.2 Hz, 1H), 2.39 (ddd, J = 9.5, 12.4, 19.3 Hz, 1H), 2.16−2.24 (m, 2H), 1.72 (br s, 1H), 1.29−1.57 (m, 11H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.8, 69.2, 67.1, 45.5, 34.7, 31.7, 29.3, 29.2, 25.8, 22.6, 21.8, 14.0; FAB HRMS *m/z* calcd for $C_{12}H_{23}O_3$ ($M^+ + H$) 232.1913, found 232.1905.

(4*S*)-(2-Hydroxyethyl)-(5*R*)-heptyloxazolidinone (5). To a solution of **8** (5.0 g, 23.3 mmol) in DME (23 mL) was added 30% aqueous ammonia (35.3 mL, 600 mmol) at room temperature. After the reaction mixture was stirred for 14.5 h, solvents were removed in vacuo to give crude **7** as a solid. To a solution of crude **7** thus obtained in DMF (46.7 mL) were added *N*-bromosuccinimide (5.4 g, 30.3 mmol) and silver acetate (5.06 g, 30.3 mmol) at room temperature. After being stirred for 3 h, the reaction mixture was diluted with methanol, and the precipitates which appeared were removed by filtration. The filtrate was concentrated in vacuo, an aqueous 2 N HCl solution was added to the residue, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with a 10% sodium hydrogen sulfite solution and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give crystals, which were purified by recrystallization from ethyl acetate to produce **5** (4.15 g, 77.8% for 2 steps): mp 96.0−96.5 °C; $[\alpha]_D^{26.0}$ −0.84 (c = 0.84, $CHCl_3$); IR (KBr disk) 3362, 3260, 2927, 1724 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.13 (br s, 1H), 4.60 (ddd, J = 3.4, 7.8, 10.0 Hz, 1H), 3.96 (ddd, J = 3.4, 7.8, 10.0 Hz, 1H), 3.89 (ddd, J = 4.6, 5.6, 10.7 Hz, 1H), 3.76 (ddd, J = 4.6, 7.8, 10.7 Hz, 1H), 1.85 (br s, 1H), 1.66−1.79 (m, 3H), 1.47−1.62 (m, 2H), 1.24−1.41 (m, 9H), 0.88 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.5, 80.1, 60.7, 57.5, 54.9, 54.5, 31.7, 31.5, 30.5, 29.5, 29.3, 29.0, 25.8, 22.6, 14.0; FAB HRMS m/z calcd for $C_{12}H_{24}NO_3$ (M^+ + H) 230.1756, found 230.1808.

(4*S*)-(2-Bromoethyl)-(5*R*)-heptyloxazolidinone (10). To a solution of **5** (1.0 g, 4.36 mmol) in CH_2Cl_2 (22 mL) were added triphenylphosphine (4.58 g, 17.4 mmol) and carbon tetrabromide (2.89 g, 8.72 mmol) at 0 °C. After the reaction mixture was stirred for 30 min, the solvent was removed in vacuo. Column chromatography on silica gel (from 25% to 50% ethyl acetate in hexane) gave corresponding bromide **10** (1.27 g, 100%) as crystals: mp 76.5−77.5 °C; $[\alpha]_D^{16.5}$ −41.03 (c = 1.12, $CHCl_3$); IR (KBr disk) 3268, 2922 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.96 (br s, 1H), 4.64 (ddd, J = 3.7, 7.6, 9.8 Hz, 1H), 4.00 (m, 1H), 3.55 (ddd, J = 4.9, 6.1, 10.5 Hz, 1H), 3.41 (ddd, J = 5.9, 9.3, 10.5 Hz, 1H), 1.95−2.09 (m, 2H), 1.72 (m, 1H), 1.48−1.61 (m, 2H), 1.20−1.42 (m, 9H), 0.88 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.8, 80.1, 53.9, 32.7, 31.7, 29.3, 29.242, 29.0, 25.9, 22.6, 14.0.

Diethyl 2-[3-*N*-*tert*-butyloxycarbonyl-(5*R*)-heptyl-(4*S*)-oxazolidinonyl]ethylphosphonate (12). A solution of **10** (1.27 g, 4.36 mmol) in triethyl phosphite (3.74 mL, 21.8 mmol) was stirred for 3 h under reflux, and then, the excess of triethyl phosphite was removed in vacuo to give crude **11**, which was used without further purification. To a solution of **11** thus obtained in DMF (22 mL) were added *N*,*N*-(dimethylamino)-pyridine (160 mg, 1.31 mmol), triethylamine (0.92 mL, 6.55 mmol), and di-*tert*-butyl dicarbonate (1.43 g, 6.54 mmol) at 0 °C. After the reaction mixture was stirred at room temperature for 45 min, a saturated ammonium chloride solution was added, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 50% to 75% ethyl acetate in hexane) gave **12** (1.96 g, 100% for two steps) as a colorless oil: $[\alpha]_D^{25.0}$ +25.80 (c = 1.11, $CHCl_3$); IR (NaCl neat) 2932, 1723, 1254, 1026 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.48 (m, 1H), 4.27 (m, 1H), 4.05−4.15 (m, 4H), 2.09 (m, 1H), 1.70−1.92 (m, 4H), 1.49−1.63 (m, 2H), 1.55 (s, 9H), 1.33 (t, J = 7.1 Hz, 6H), 1.20−1.38 (m, 9H), 0.89 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.8, 149.4, 84.0, 78.3, 61.8 (J_{C-P} = 5.8 Hz), 61.7 (J_{C-P} = 5.8 Hz), 57.9 (J_{C-P} = 19.9 Hz), 31.6, 29.2, 29.0, 28.5, 27.9, 25.8, 22.6, 22.5, 21.9, 21.9, 21.5, 16.5, 16.4, 14.0; FAB HRMS m/z calcd for $C_{16}H_{33}NO_5P$ (M^+ + H − $(CH_3)_3COCO$−) 350.2096, found 350.2082.

Diethyl (4*R*)-Benzyloxycarbonyloxy-(3*S*)-*N*-*tert*-butyloxycarbonylaminoundecylphosphonate (13). To a solution of **12** (100 mg, 0.22 mmol) in THF (2.2 mL) were added benzyl alcohol (0.05 mL, 0.44 mmol) and cesium carbonate (14 mg, 0.044 mmol) at room temperature. After the reaction mixture was stirred for 3 h, an aqueous 2 N HCl solution was added at 0 °C, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 33% to 67% ethyl acetate in hexane) gave **13** (100 mg, 80.6%) as a colorless oil: $[\alpha]_D^{25.0}$ −0.67 (c = 0.94, $CHCl_3$); IR (NaCl neat) 3277, 2930, 1746, 1713, 1256, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.31−7.40 (m, 5H), 5.17 (s, 2H), 4.75 (ddd, J = 4.1, 4.4, 8.3 Hz, 1H), 4.65 (d, J = 9.8 Hz, 1H), 4.02−4.15 (m, 4H), 3.80 (m, 1H), 1.47−1.93 (m, 10H), 1.43 (s, 9H), 1.31 (t, J = 7.1 Hz, 6H), 1.20−1.32 (m, 6H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.6, 155.0, 135.2, 128.6, 128.5, 128.2, 80.4, 79.6, 69.7, 61.6 (J_{C-P} = 6.6 Hz), 61.6 (J_{C-P} = 6.6 Hz), 52.9 (J_{C-P} = 17.3 Hz), 31.7, 30.6, 29.3, 29.0, 28.3, 25.3, 22.6, 22.4 (J_{C-P} = 142.4 Hz), 16.4, 16.4, 14.0; FAB HRMS m/z calcd for $C_{28}H_{49}NO_8P$ (M^+ + H) 558.3197, found 558.3196.

Diethyl (4*R*)-Benzyloxycarbonyloxy-(3*S*)-*N*-(1-oxohexyl)aminoundecylphosphonate (3). To a solution of **13** (807 mg, 1.45 mmol) in CH_2Cl_2 (7.2 mL) was added trifluoroacetic acid (2.9 mL) dropwise at 0 °C. After being stirred for 3 h, the reaction mixture was poured into a mixture of an aqueous 1 N NaOH solution and $CHCl_3$ at 0 °C, and the resulting mixture was stirred for 5 min at the same temperature. To this mixture was added hexanoic chloride (0.40 mL, 2.89 mmol) at 0 °C. After the reaction mixture was stirred for an additional 15 min, a saturated NH_4Cl solution was added, and the resulting mixture was extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 50% to 65% ethyl acetate in hexane) gave **3** (701 mg, 84.5%) as a colorless oil: $[\alpha]_D^{25.0}$ −1.18 (c = 0.81, $CHCl_3$); IR (NaCl neat) 3281, 2930, 1746, 1651, 1256, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32−7.39 (m, 5H), 5.97 (d, J = 8.8 Hz, 1H), 5.15 (s, 2H), 4.74 (m, 1H), 4.05−4.20 (m, 5H), 2.14 (t, J = 7.6 Hz, 2H), 1.15−1.91 (m, 28H), 0.89 (t, J = 6.8 Hz, 3H), 0.87 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 155.1, 135.2, 128.6, 128.5, 128.2, 80.5, 69.7, 61.7, 51.2 (J_{C-P} = 14.8 Hz), 36.7, 31.7, 31.4, 31.1, 29.3, 29.0, 25.4, 25.3, 22.6, 22.4, 22.3 (J_{C-P} = 141.4 Hz), 16.4, 14.0, 13.9; FAB HRMS m/z calcd for $C_{28}H_{51}NO_7P$ (M^+ + H) 556.3403, found 556.3409.

(4*R*)-Benzyloxycarbonyloxy-(3*S*)-*N*-(1-oxohexyl)aminoundecylphosphonocholine (14). To a solution of **3** (209 mg, 0.365 mmol) in CH_2Cl_2 (0.4 mL) was added a solution of trimethylsilyl bromide (0.48 mL, 3.65 mmol) in CH_2Cl_2 (0.7 mL) dropwise at room temperature. After the reaction mixture was stirred for 3 h at the same temperature, the solvent was removed in vacuo. The resulting crude silylester was dissolved in MeOH (5 mL), and the solution was stirred for an additional 1 h to complete the hydrolysis of the silylester. Then, the solvent was removed in vacuo to give the crude phosphonic acid. To a suspension of the crude phosphonic acid thus obtained and choline chloride (234 mg, 1.68 mmol) in pyridine (9.86 mL) was added trichloroacetonitrile (1.57 mL, 15.70 mmol) at 60 °C. After the reaction mixture was stirred for 8 h, the solvent was removed in vacuo. The precipitates that appeared were removed by filtration through Celite, and the filtrate was concentrated in vacuo. Column chromatography on silica gel (from 12.5% methanol in chloroform to 4.3% water and 26.6% methanol in chloroform) gave **14** as a slightly brownish foam. It was purified again by HPLC (50% acetonitrile in water; flow rate, 2 mL/min; retention time, 29 min; column, Develosil C8-5, 10 × 250, 216 nm detection) to afford pure **14** (37 mg, 16.1%) as a colorless foam: IR (NaCl neat) 3387, 2928, 1738, 1649 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.31−7.39 (m, 5H), 5.17 (d, J = 12.2 Hz, 1H), 5.12 (d, J = 12.2 Hz, 1H), 4.76 (m, 1H), 4.23 (m, 2H), 4.07 (m, 1H), 3.59 (m, 2H), 3.20 (s, 9H), 2.18 (t, J = 7.1 Hz, 2H), 1.87 (m, 1H), 1.46−1.69 (m, 7H), 1.22−1.36 (m, 14H), 0.91 (t, J = 6.8 Hz, 3H),

0.89 (t, $J = 6.8$ Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.0, 155.0, 135.0, 128.0, 127.9, 127.6, 79.4, 68.9, 66.3, 57.1, 57.0, 53.1, 51.8, 51.6, 25.5, 31.3, 30.9, 29.7, 28.8, 28.7, 25.3, 24.9, 23.8, 22.6, 22.4, 22.1, 21.9, 12.9, 12.8; FAB HRMS *m/z* calcd for $C_{30}H_{54}N_2O_7P$ ($M^+ + H$) 585.3671, found 585.3673.

(4*R*)-Hydroxy-(3*S*)-*N*-(1-oxohexyl)aminoundecylphosphonocholine (1). To a solution of **14** (37 mg, 0.058 mmol) in methanol (0.58 mL) was added palladium on carbon (7 mg). After the reaction mixture was stirred under H_2 atmosphere for 1 h, the catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give residues, from which title compound **1** (20 mg, 76.9%) was obtained as a soluble component in MeOH (1 mL) as a foam: $[\alpha]_D^{26.0}$ +4.65 ($c = 0.72$, MeOH); IR (KBr disk) 3420, 2928, 1642, 1053 cm^{-1}; ^{1}H 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; FAB HRMS *m/z* calcd for $C_{22}H_{48}N_2O_5P$ ($M^+ + H$) 451.3303, found 451.3271.

Dimethyl 3-[3-*N*-*tert*-butyloxycarbonyl-(5*R*)-heptyl-(4*S*)-oxazolidinonyl]propylphosphonate (16). To a solution of dimethyl methylphosphonate **15** (0.85 mL, 7.85 mmol) in THF (7.8 mL) was added a solution of 1.6 N *n*-butyllithium in hexane (4.9 mL, 7.84 mmol) at −78 °C. After the reaction mixture was stirred for 30 min at −78 °C, a solution of **10** (765 mg, 2.62 mmol) in THF (5.2 mL) was added dropwise at the same temperature. After the reaction mixture was stirred for 1 h, di-*tert*-butyl dicarbonate (1.71 g, 7.84 mmol) was added at the same temperature, and the resulting mixture was stirred for an additional 20 min. The reaction was quenched with a saturated NH_4Cl solution, and then, the solvents were removed in vacuo. Chloroform was added to the obtained residue, and the chloroform layer was decanted, dried over $MgSO_4$, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 50% to 75% ethyl acetate in hexane) gave **16** (714 mg, 62.5%) as a colorless oil: $[\alpha]_D^{23.0}$ +15.24 ($c = 0.592$, $CHCl_3$); IR (NaCl neat) 2930, 1815, 1740, 1717, 1248, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.47 (m, 1H), 4.21 (q, $J = 7.6$ Hz, 1H), 3.75 (s, 3H), 3.72 (s, 3H), 1.63−1.92 (m, 9H), 1.55 (s, 9H), 1.24−1.45 (m, 9H), 0.89 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.0, 149.5, 83.8, 57.6, 52.3, 31.6, 29.2, 29.0, 28.5, 27.9, 25.8, 23.8, 22.5, 18.93, 18.88, 14.0; FAB HRMS *m/z* calcd for $C_{15}H_{31}NO_5P$ ($M^+ + H - (CH_3)_3COCO-$) 336.1940, found 336.1978.

Dimethyl (5*R*)-Benzyloxycarbonyloxy-(4*S*)-*N*-*tert*-butyloxycarbonylamindodecylphosphonate (17). To a solution of **16** (100 mg, 0.23 mmol) in THF (1.15 mL) were added benzyl alcohol (0.12 mL, 1.15 mmol) and cesium carbonate (164 mg, 0.504 mmol) at room temperature. After the reaction mixture was stirred for 4 h, an aqueous 2 N HCl solution was added at 0 °C, and then, the solvents were removed in vacuo. Chloroform was added to the obtained residue, and the chloroform layer was decanted, dried over $MgSO_4$, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 33% to 60% ethyl acetate in hexane) gave **17** (70 mg, 56.5%) as a colorless oil: $[\alpha]_D^{23.0}$ −10.35 ($c = 0.578$, $CHCl_3$); IR (NaCl neat) 3277, 2930, 1743, 1711, 1458, 1259, 1172, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32−7.39 (m, 5H), 5.16 (s, 2H), 4.73 (ddd, $J = 4.2$, 4.4, 8.5 Hz, 1H), 4.58 (d, $J = 9.8$ Hz, 1H), 3.79 (m, 1H), 3.73 (s, 3H), 3.70 (s, 3H), 1.47−1.85 (m, 9H), 1.43 (s, 9H), 1.24−1.39 (m, 9H), 0.87 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.6, 155.0, 135.2, 128.54, 128.47, 128.2, 80.7, 79.5, 69.6, 52.3, 51.8, 31.7, 30.6, 29.9 ($J_{C-P} = 16.4$ Hz), 29.3, 29.0, 28.3, 27.7 ($J_{C-P} = 29.6$ Hz), 25.3, 24.1 ($J_{C-P} = 142.4$ Hz), 22.5, 18.9, 14.0; FAB HRMS *m/z* calcd for $C_{27}H_{47}NO_8P$ ($M^+ + H$) 544.3039, found 544.3019.

Dimethyl (5*R*)-Benzyloxycarbonyloxy-(4*S*)-*N*-(1-oxohexyl)aminododecylphosphonate (4). To a solution of **17** (317 mg, 0.58 mmol) in CH_2Cl_2 (2.9 mL) was added trifluoroacetic acid (1.2 mL) dropwise at 0 °C. After being stirred for 3 h, the reaction mixture was poured into a mixture of aqueous 1 N NaOH (5 mL) and $CHCl_3$ (5 mL) at 0 °C, and the resulting mixture was stirred for another 5 min at the same temperature. To this mixture was added hexanoic chloride (0.16 mL, 1.16 mmol) at 0 °C, and the mixture was stirred for 15 min. A saturated NH_4Cl solution was added, and the resulting mixture was extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude products. Column chromatography on silica gel (from 50% to 75% ethyl acetate in hexane) gave **4** (249 mg, 78.8%) as a colorless oil: $[\alpha]_D^{25.0}$ −14.40 ($c = 0.414$, $CHCl_3$); IR (NaCl neat) 3379, 2930, 1744, 1651, 1545, 1458, 1381, 1259, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32−7.39 (m, 5H), 5.71 (br d, $J = 8.8$ Hz, 1H), 5.16 (s, 2H), 4.72 (ddd, $J = 3.7$, 4.2, 7.8 Hz, 1H), 4.15 (m, 1H), 3.72 (s, 3H), 3.70 (s, 3H), 2.13 (t, $J = 7.3$ Hz, 2H), 1.40−1.90 (m, 10H), 1.20−1.40 (m, 14H), 0.89 (t, $J = 7.1$ Hz, 3H), 0.87 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1, 155.1, 135.2, 128.6, 128.5, 128.2, 80.9, 69.7, 52.3, 50.2, 36.7, 31.6, 31.3, 31.0, 29.3, 29.1, 29.0, 25.4, 25.3, 24.0 ($J_{C-P} = 139.9$ Hz), 22.4, 22.3, 19.0, 14.0, 13.9; FAB HRMS *m/z* calcd for $C_{28}H_{49}NO_7P$ ($M^+ + H$) 542.3246, found 542.3288.

(5*R*)-Benzyloxycarbonyloxy-(4*S*)-*N*-(1-oxohexyl)aminododecylphosphonocholine (18). To a solution of **4** (507 mg, 0.93 mmol) in CH_2Cl_2 (0.9 mL) was added a solution of TMSBr (1.2 mL, 9.34 mmol) in CH_2Cl_2 (1.9 mL) dropwise at room temperature. After the reaction mixture was stirred for 25 min, the solvent was removed in vacuo. The resulting crude silylester was dissolved in MeOH (5 mL), and the solution was stirred for an additional 1 h at room temperature to complete the hydrolysis of the silylester. Then, the solvent was removed in vacuo to give the crude phosphonic acid. To a suspension of the crude phosphonic acid thus obtained and choline chloride (1.09 g, 7.81 mmol) in pyridine (78 mL) was added trichloroacetonitrile (3.4 mL, 33.6 mmol) at 50 °C. After the reaction mixture was stirred for 40 h, the solvent was removed in vacuo. The precipitates that appeared were removed by filtration through Celite, and the filtrate was concentrated in vacuo. Column chromatography on silica gel (from 14% methanol in chloroform to 4.3% water and 26.6% methanol in chloroform) gave **18** as a slightly brownish foam. It was purified again by HPLC (50% acetonitrile in water; flow rate, 2 mL/min; retention time, 31 min; column, Develosil C8-5, 10 × 250, 216 nm detection) to afford pure **18** (89 mg, 17.0%) as a colorless foam: $[\alpha]_D^{20.5}$ −5.06 ($c = 0.937$, MeOH); IR (NaCl neat) 3368, 2928, 1742, 1647, 1545, 1460, 1261, 1188, 1053, 966 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.31−7.39 (m, 5H), 5.16 (d, $J = 12.4$ Hz, 1H), 5.12 (d, $J = 12.4$ Hz, 1H), 4.74 (m, 1H), 4.23 (m, 2H), 4.04 (m, 1H), 3.58 (m, 2H), 3.20 (s, 9H), 2.17 (t, $J = 7.1$ Hz, 2H), 1.46−1.70 (m, 10H), 1.22−1.36 (m, 14H), 0.91 (t, $J = 6.8$ Hz, 3H), 0.89 (t, $J = 6.8$ Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.37, 156.61, 137.24, 129.61, 129.46, 129.16, 81.19, 70.47, 67.83 (m), 58.52 (d, $J_{c-p} = 4.96$ Hz), 54.73, 54.70, 54.66, 52.26, 37.11, 32.88, 32.51, 31.30, 30.86 (d, $J_{c-p} = 14.88$ Hz), 30.37, 30.23, 27.67 (d, $J_{c-p} = 135.64$ Hz), 23.67, 23.46, 21.56 (d, $J_{c-p} = 4.13$ Hz), 14.43, 14.34; FAB HRMS *m/z* calcd for $C_{31}H_{55}N_2O_7P$ ($M^+ + H$) 599.3825, found 599.3870.

(5*R*)-Hydroxy-(4*S*)-*N*-(1-oxohexyl)aminododecylphosphonocholine (2). To a solution of **18** (80 mg, 0.13 mmol) in methanol (2.7 mL) was added palladium on carbon (16 mg). After the reaction mixture was stirred under H_2 atmosphere for 1 h, the catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give residues, from which title compound **2** (54 mg, 88.5%) was obtained as a soluble component in MeOH (1 mL) as a foam: $[\alpha]_D^{21.0}$ −9.12 ($c = 0.353$, MeOH); IR (NaCl neat) 3420, 2928, 1637, 1051 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 4.24 (m, 2H), 3.74 (m, 1H), 3.60 (m, 2H), 3.44 (ddd, $J = 2.4$, 5.9, 7.3 Hz, 1H), 3.22 (s, 9H), 2.20 (t, $J = 7.6$ Hz, 2H), 1.22−1.74 (m, 24H), 0.92 (t, $J = 7.1$ Hz, 3H), 0.90 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ

176.2, 74.9, 67.88 (J_{C-P} = 6.6 Hz), 67.86 (J_{C-P} = 6.6 Hz), 58.6, 55.0, 54.72, 54.69, 54.65, 37.3, 34.8, 33.0, 32.7, 31.9 (J_{C-P} = 15.7 Hz), 30.8, 30.4, 28.0 (J_{C-P} = 135.6 Hz), 27.0, 26.9, 23.7, 23.5, 21.5, 21.4, 14.4, 14.3; FAB HRMS *m*/*z* calcd for $C_{27}H_{47}NO_8P$ ($M^+ + H$) 465.3457, found 465.3456.

Acknowledgment. We thank Professors M. Nishizawa at Tokushima Bunri University and H. Yamada at Kwansei Gakuin University for their kind advice on Noyori's asymmetric hydrogenation. This research is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. T.H. is grateful to the JSPS for a Research Fellowship for Young Scientists.

Supporting Information Available: Selected 400 MHz ^{1}H NMR and ^{13}C NMR spectra of **1**, **2**, and the synthetic intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

JO025529O