Synthesis of 1-Substituted-phytosphingosine : Novel Protection of Phytosphingosine

Su Yeon Jo, Hyoung Cheul Kim, Seung Woo Woo, Min Jung Seo, Gehyeong Lee, and Hyoung Rae Kim*

Medicinal Science Division, Korea Research Institute of Chemical Technology, P.O. Box 107, Yusong, Daejeon 305-600, Korea
Received December 13, 2002

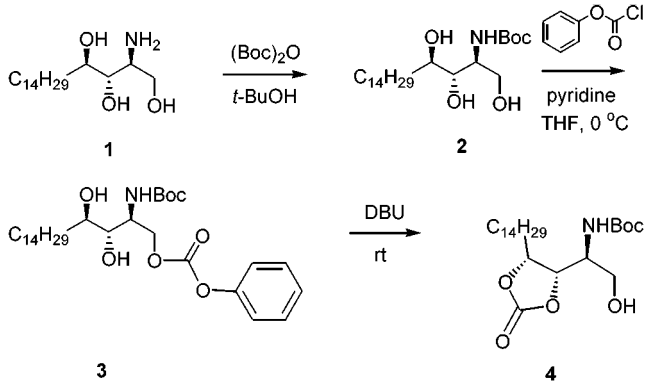
Key Words : Phytosphingosine, 1-Substituted-phytosphingosine, Phytosphingosine 1-phosphate, Sphingosine, Cyclic carbonate

Phytosphingosine, one of the major sphingosine derivatives was found in microorganisms, yeast, plants, and fungi as a major membrane component, and also found in many mammalian tissues[1] and interestingly in some cancer cell-types.[2] The roles of sphingosine derivatives in human cells have been enigmatic but they are recently proved to be essential in cell communications and regulation of cell growth.[3] Since the sphingosine derivatives are very interesting both biologically and synthetically, many attempts have been tried to synthesize sphingosine derivatives,[4] however, examples of modifications of sphingosine derivatives are very rare.[5,6]

In phytosphingosine (**1**), there exist two major parts, long linear hydrocarbon chain and very hydrophilic terminal with an amine and three hydroxyl groups. In order to modify the structure of phytosphingosine, proper protections for the amino and hydroxyl groups are essential. In this paper, we describe a novel protection of phytosphingosine and some example syntheses of new phytosphingosine derivatives.

Since phytosphingosine (**1**) itself has very low solubility in various organic solvents and water, first protection of amino group by di-*t*-butyl dicarbonate was only successfully performed in *t*-butyl alcohol. The resulting *N*-Boc-phytosphingosine (**2**) was easily separated as white precipitates in 94% yield by the evaporation of solvent and only washing with n-hexane several times. *N*-Boc-phytosphingosine (**2**) was reacted with phenyl chloroformate and pyridine at 0 °C in THF to afford phenyl carbonate **3** in good yield. To the reaction mixture was added 1.2 equiv of DBU at 25 °C, the carbonate at 1-position was migrated to 3-position, and then the 3,4-cyclic carbonate **4** was formed as a white solid in total 67% yield after purification by silica gel column chromatography (methylene chloride/methanol, 40/1) as shown in Scheme 1. Though excess phenyl chloroformate was used in pyridine, only **3** was obtained without any formation of byproducts. Phenyl carbonate **3** was stable in pyridine and it could not be converted to cyclic carbonate **4** without DBU at 25 °C.

We examined other bases to obtain 1,3-cyclic carbonate from **3**, but only **4** was obtained without any trace of 1,3-cyclic carbonate. Hydrolysis of **4** with potassium carbonate in 95% methanol quantitatively afforded *N*-Boc-phytosphingosine (**2**) and from these results the stereochemistry of **4** was confirmed. The spectroscopic data of all compounds were summarized in the references.[7] The compound is properly protected phytosphingosine derivative, which can

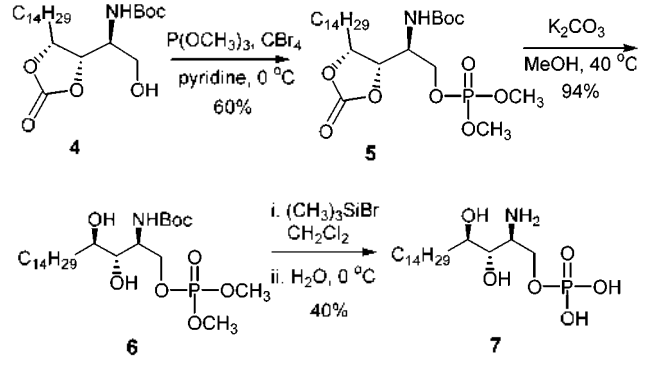


Scheme 1

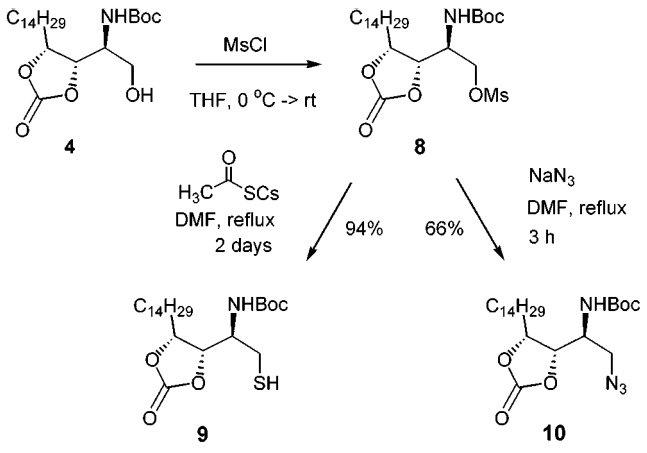
be used as a starting material for the derivatizations of phytosphingosine at 1-position.

To explore the substitutions at 1-position of phytosphingosine, first we tried to prepare phytosphingosine-1-phosphate (**7**) as shown in Scheme 2. The cyclic carbonate **4** was reacted with trimethyl phosphite and carbon tetrabromide at 0 °C in pyridine to afford the phosphate **5** in 60% yield, and the removal of carbonate group of **5** were easily performed by potassium carbonate in 95% methanol at 40 °C within 1 h to give **6** as a pale yellow oil in 94% yield. *N*-Boc group and methyl ester of **6** were removed simultaneously by 5 equiv of bromotrimethylsilane[5b] followed by addition of small water to afford **7** as a white precipitate in 40% yield. The spectral data of **7** showed good coincidence with those in the references.[6]

Next we mesylated the cyclic carbonate **4**, and the resulting **8** was reacted with nucleophiles such as sodium



Scheme 2



Scheme 3

azide and cesium thioacetate (Scheme 3). **8** was reacted with 1.2 equiv of cesium thioacetate in DMF at 25 °C for 2 days, and addition of small amount of water to the reaction mixture gave thiol **9** as white precipitates in 94% yield, which could be identified by upfield shift of C-1 protons (3.01 and 3.29 ppm) compared with **4** (3.73 and 3.91 ppm) in ^{1}H NMR. Similarly, **8** was reacted with 1.2 equiv of sodium azide in DMF with heating for 3 h, and addition of small amount of water to the reaction mixture gave azide **10** as pale yellow precipitates in 66% yield, which also could be identified by upfield shift of C-1 protons (3.42 and 3.50 ppm) compared with **4** (3.73 and 3.91 ppm) in ^{1}H NMR. Interestingly, when electron-withdrawing groups such as esters, carbonates, and sulfonates were substituted at C-1, C-2, C-3, or C-4 in phytosphingosine, protons at those positions appeared over 4 ppm, while unsubstituted hydroxyl groups and amine in phytosphingosine appeared below 4 ppm in ^{1}H NMR spectrum.

In summary, phytosphingosine was protected by the formation of cyclic carbonate in two steps, which could be useful for the derivatizations of 1-position of phytosphingosine. Phytosphingosine-1-phosphate and other derivatives of phytosphingosine were synthesized from the phytosphingosine derivatives protected with cyclic carbonates.

References and Notes

1. (a) Karlsson, K. A. *Acta Chem. Scand.* **1964**, *18*, 2295. (b) Karlsson, K. A. *Acta Chem. Scand.* **1964**, *18*, 2297. (c) Karlsson, K. A.; Samuelsson, B. E.; Steen, G. O. *Acta Chem. Scand.* **1968**, *22*, 1361. (d) Wertz, P. W.; Miethke, M. C.; Long, S. A.; Strauss, J. S.; Downing, D. T. *J. Invest. Dermatol.* **1985**, *84*, 410. (e) Schmidt, R. P. In *Liposome Dermatics*; Braun-Falco, O. H.; Corting, C.; Maibach, H. I., Eds.; Springer-Verlag: Berlin, Heidelberg, 1992; pp 44-56. (f) Barenholz, Y.; Gatt, S. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 319. (g) Takamatsu, K.; Mikami, M.; Kikuchi, K.; Nozawa, S.; Iwamori, M. *Biochim. Biophys. Acta* **1992**, *1165*, 177. (h) Okabe, K.; Keenan, R. W.; Schmidt, G. *Biochem. Biophys. Res. Commun.* **1968**, *31*, 137. (i) Vance, D. E.; Sweeley, C. C. *J. Lipid Res.* **1967**, *8*, 621.
2. Kanfer, J. N.; Hakomori, S. In *Handbook of Lipid Research, Vol. Sphingolipid Biochemistry*; Hakomori, S., Ed.; Plenum Press: New York, 1983.
3. (a) Lee, Y. J.; Hannun, Y. A.; Obeid, L. M. *J. Biol. Chem.* **1996**, *271*, 13169. (b) Hannun, Y. A. *J. Biol. Chem.* **1994**, *269*, 3125. (c) Gomez-Munoz, A.; Waggoner, D. W.; O'Brien, L.; Brindley, D. N. *J. Biol. Chem.* **1995**, *270*, 26318. (d) Merill Jr., A. H. *Nutr. Rev.* **1992**, *50*, 78. (e) Merill Jr., A. H.; Schmelz, E.-M.; Dillehsy, D. L.; Spiegel, S.; Shayman, J. A.; Schroeder, J. J.; Riley, R. T.; Voss, K. A.; Wang, E. *Toxicol. Appl. Pharmacol.* **1997**, *142*, 208. (f) Merill Jr., A. H. *J. Bioenerg. Biomembr.* **1991**, *23*, 83.
4. (a) Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, 1075. (b) He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7618. (c) Chun, J.; He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7634.
5. (a) Jo, S. Y.; Kim, H. C.; Jeon, D. J.; Kim, H. R. *Heterocycles* **2001**, *55*, 1127. (b) Szule, Z. M.; Hannun, Y. A.; Bielawska, A. *Tetrahedron Lett.* **2000**, *41*, 7821.
6. (a) Boumendjel, A.; Miller, S. P. F. *J. Lipid Res.* **1994**, *35*, 2305. (b) Li, S.; Wilson, W. K.; Schroepfer, Jr. G. J. *J. Lipid Res.* **1999**, *40*, 117.
7. **2**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 6.5 Hz), 1.22-1.31 (m, 24H), 1.42 (s, 9H), 1.50-1.58 (m, 1H), 1.60-1.71 (m, 1H), 3.61-3.67 (m, 3H), 3.72-3.76 (m, 2H), 3.86 (dd, 2H, J = 11.2, 2.8 Hz), 4.17 (br, 1H), 5.24 (d, 1H, J = 8.3 Hz, NH); IR (NaCl) 3326.4, 2916.9, 2847.9, 1167.3, 1545.2, 1481.9, 1356.6, 1252.2, 1171.1, 1057.9 cm^{-1}; MS m/z (CI, relative intensity) 418 (M^+, 39), 390 (6), 362 (87), 346 (29), 318 (100). **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 6.9 Hz), 1.22-1.31 (m, 24H), 1.42 (s, 9H), 1.46-1.52 (m, 1H), 1.58-1.70 (m, 1H), 2.57 (br, 1H), 3.12 (br, 1H), 3.61-3.64 (m, 2H), 4.06-4.07 (m, 1H), 4.46 (dd, 2H, J = 10.0, 3.2 Hz), 5.23 (d, 1H, J = 9.1 Hz, NH), 7.14-7.15 (m, 2H), 7.20-7.24 (m, 1H), 7.33-7.36 (m, 2H); IR (NaCl) 2924.1, 2853.0, 1765.5, 1689.2, 1366.2, 1249.2, 1166.3, 1047.2 cm^{-1}; MS m/z (CI, relative intensity) 416 (M -PhOC=O, 9), 388 (89), 370 (5), 344 (9), 326 (12), 95 (100). **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 6.5 Hz), 1.22-1.31 (m, 24H), 1.42 (s, 9H), 1.56-1.72 (m, 2H), 2.72 (br, 1H), 3.73 (d, 1H, J = 4.9 Hz), 3.91 (d, 1H, J = 4.9 Hz), 3.96-3.99 (m, 1H), 4.68-4.71 (m, 2H), 4.97 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.08, 22.65, 23.15, 25.50, 28.53, 29.10, 29.32, 29.39, 29.49, 29.60, 29.62, 29.65, 29.66, 31.88, 48.55, 61.53, 77.52, 80.32, 154.70, 170.53; IR (NaCl) 3298.9, 2918.5, 1806.1, 1657.5, 1562.0, 1462.2, 1372.0, 1058.1 cm^{-1}; MS m/z (CI, relative intensity) 416 (M^+-C=O, 23), 388 (100), 370 (77), 344 (17), 326 (25), 95 (62); Anal. Calcd for $C_{21}H_{41}NO_6$: C, 64.98; H, 10.22; N, 3.16. Found: C, 65.01; H, 10.32; N, 3.08. **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 6.9 Hz), 1.18-1.29 (m, 24H), 1.43 (s, 9H), 1.50-1.57 (m, 2H), 3.79 (d, 3H, J = 2.6 Hz), 3.82 (d, 3H, J = 2.6 Hz), 4.27-4.29 (m, 1H), 4.26-4.30 (m, 1H), 4.68-4.75 (m, 2H), 5.24 (d, 1H, J = 9.5 Hz, NH); MS m/z (relative intensity) 554 (M^+, 11), 539 (24), 524 (98), 480 (37), 436 (8), 370 (14), 326 (16), 155 (100). **6**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 6.9 Hz), 1.18-1.26 (m, 24H), 1.41 (s, 9H), 1.48-1.55 (m, 2H), 3.40 (br, 2H), 3.55-3.68 (m, 2H), 3.72 (s, 3H), 3.78 (s, 3H), 3.81-3.98 (m, 1H), 4.16-4.21 (m, 1H), 4.23-4.40 (m, 1H), 5.42 (d, 1H, J = 4.5 Hz, NH). **8**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 6.9 Hz), 1.22-1.28 (m, 24H), 1.40 (s, 9H), 1.57-1.71 (m, 2H), 3.05 (s, 3H), 4.16-4.23 (m, 1H), 4.36 (dd, 2H, J = 5.3, 10.4 Hz), 4.64 (t, 1H, J = 10.0 Hz), 4.71 (dd, 1H, J = 3.4, 9.2 Hz), 4.96 (d, 1H, J = 5.3 Hz, NH); IR (NaCl) 3359.4, 2925.5, 2854.1, 2360.5, 2341.2, 1810.8, 1717.9, 1523.5, 1365.4, 1174.4, 1050.2 cm^{-1}. **9**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 6.9 Hz), 1.23-1.29 (m, 24H), 1.43 (s, 9H), 1.69-1.82 (m, 2H), 3.01 (dd, 1H, J = 9.5, 14.2 Hz), 3.29 (dd, 1H, J = 2.9, 14.2 Hz), 4.02-4.10 (br, 1H, NH), 4.57-4.65 (m, 2H), 4.69 (dd, 1H, J = 7.3, 13.8 Hz); IR (NaCl) 2920.3, 2870.0, 2359.0, 2339.9, 1808.1, 1696.4, 763.0 cm^{-1}. **10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 7.1 Hz), 1.23-1.32 (m, 24H), 1.50-1.65 (m, 2H), 1.57 (s, 9H), 3.42 (dd, 1H, J = 6.4, 12.4 Hz), 3.50 (dd, 1H, J = 5.0, 12.4 Hz), 3.52-3.57 (m, 1H), 3.89-3.94 (m, 1H), 4.18 (dd, 1H, J = 3.3, 5.3 Hz), 5.47 (br, 1H, NH); IR (NaCl) 2922.1, 2851.3, 2359.3, 2101.0, 1810.0, 1715.6, 749.1 cm^{-1}; MS m/z (CI, relative intensity) 441 (M^+-C=O, 11), 413 (M^+-C=O-N_2, 100), 370 (5), 370 (9), 338 (8), 312 (35).