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TANDEM CYCLIZATION OF PHYTOSPHINGOSINE

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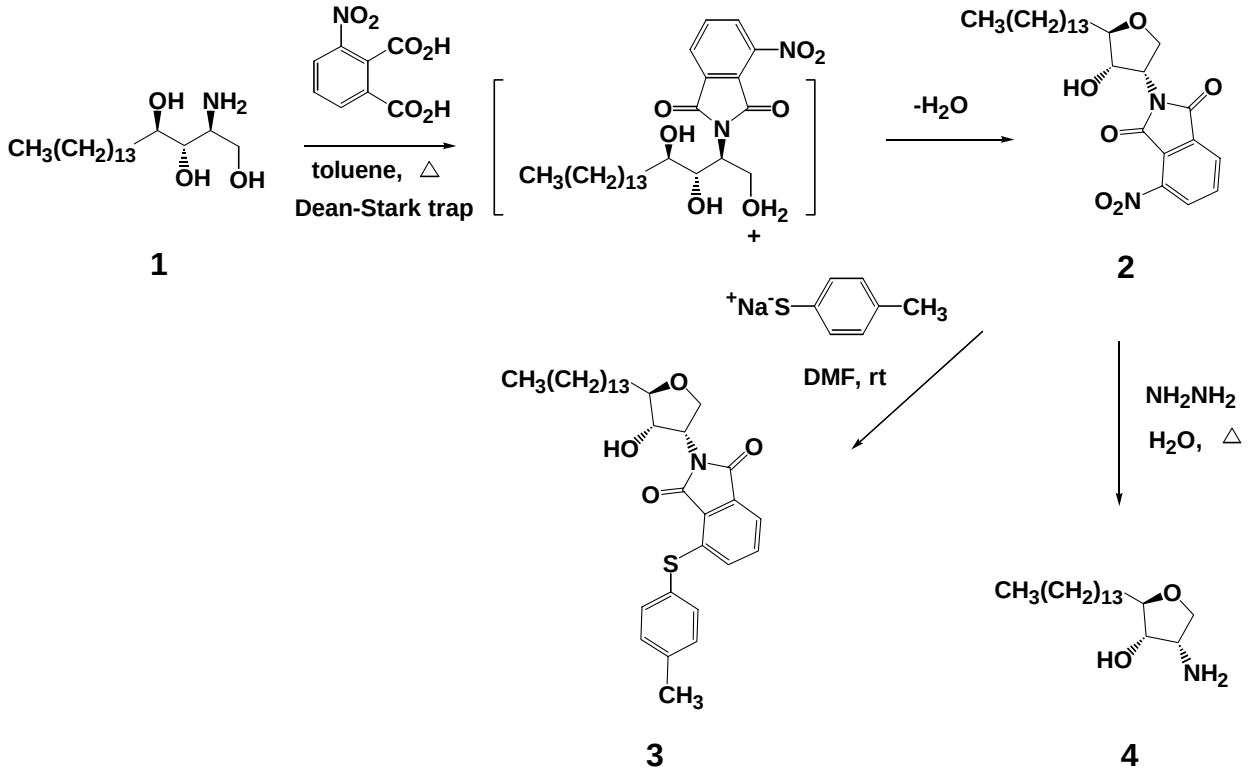
Abstract- Phytosphingosine is easily cyclized during the protection of amino group with phthalic acid derivatives to form trisubstituted tetrahydrofuran structure (**2**) by the attack of 4-hydroxyl group to the terminal carbon. The structure of compound (**2**) was confirmed by X-Ray crystallography and synthetic method.

Sphingolipids are ubiquitous membrane components of all eukaryotic cells and are abundantly located in all plasma membranes as well as in some intracellular organelles.[1] 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[2] and interestingly in some cancer cell-types.[3] 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.[4]

Sphingosine derivatives are very interesting not only for their biological functions, but also for synthetic aspects. Many attempts have been tried to synthesize sphingosine derivatives,[5] however, examples of modifications of sphingosine derivatives are very rare. In order to modify the structure of phytosphingosine, first we tried to protect its amino group by phthalic acid. A solution of phytosphingosine and phthalic acid in toluene was heated under reflux with removing moisture by Dean-Stark trap. From this reaction, we isolated white powder in 70% yield by pouring the concentrated mixture into cold methanol. The powder has unique spectrum in ^{1}H NMR but the peaks are too close to identify its structure by NOE experiment. Thus we decided to make a crystal to confirm its structure by X-Ray crystallography. Thus, phytosphingosine (**1**) was reacted with 3-nitrophthalic acid under the same reaction condition to obtain **2** as a white powder, which could not make crystals. After the nitro group of **2** was exchanged by *p*-tolylthiol, we could have yellow crystals of **3**, the structure of which was fortunately confirmed by X-Ray crystallography. Interestingly, the structure was cyclic ether as shown

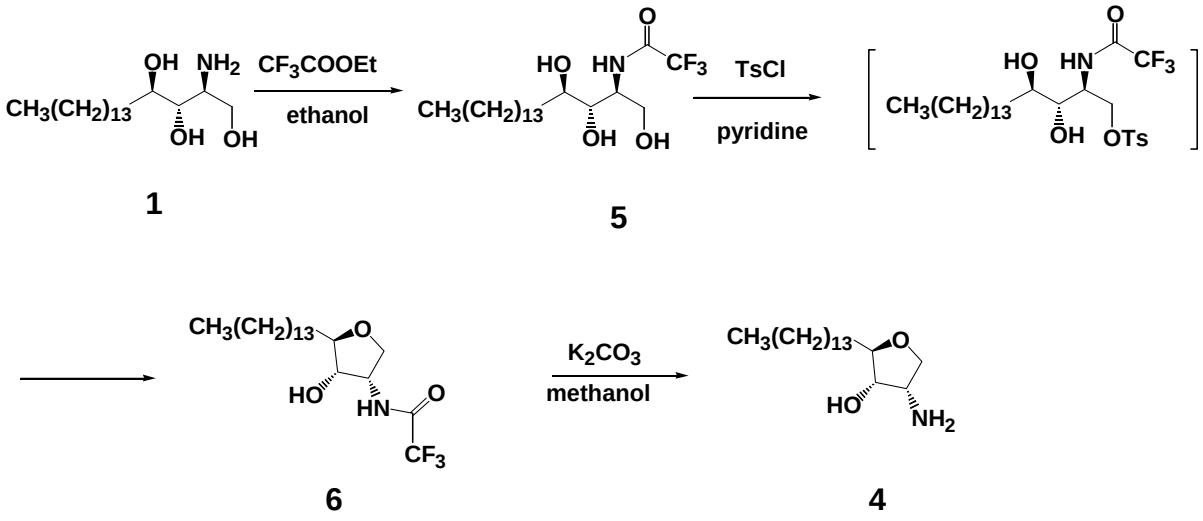
in Scheme 1.

As the structure of phytosphingosine has not been confirmed by X-Ray crystallography, we examined which hydroxyl group was left in the cyclization process. As shown in Scheme 2, phytosphingosine was protected with ethyl trifluoroacetate in ethanol to give *N*-trifluoroacetylphytosphingosine (**5**). During the tosylation of **5**, cyclization took place and we isolated only **6** in excellent yield. After deprotection of **6** by potassium carbonate in methanol, we found the ^{1}H NMR spectrum of **4** was identical with that of **4** in Scheme 1. From these results, we could realize that 4-hydroxyl group attacked to the terminal carbon to form the tetrahydrofuran ring with retention of the stereocenter on C-4 in Scheme 1 and in addition, the 4-hydroxyl group of phytosphingosine has considerable nucleophilicity.



Scheme 1

As a conclusion, phytosphingosine is easily cyclized by heating with phthalic acid derivatives in toluene to form trisubstituted tetrahydrofuran structure (**2**) by the attack of 4-hydroxyl group to the terminal carbon, the structure of which could be proved by X-Ray crystallography and synthetic method.



Scheme 2

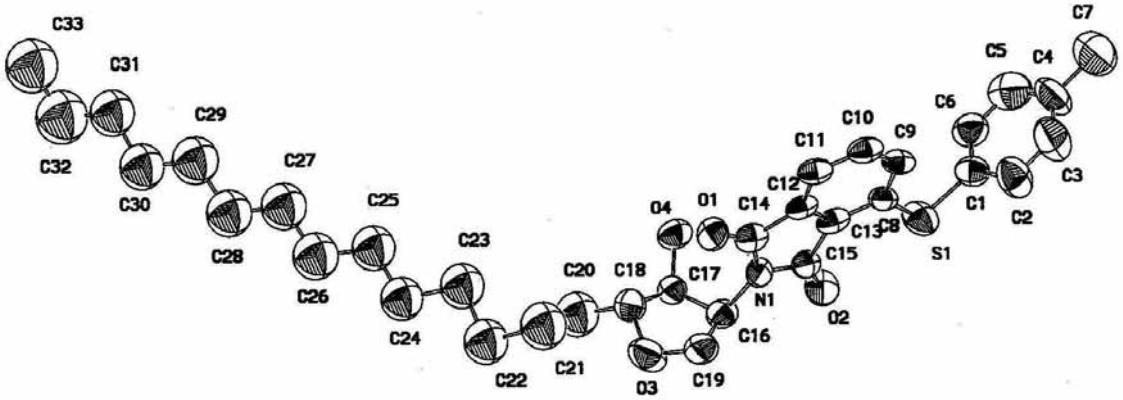


Figure 1. X-Ray Structure of **3**.

EXPERIMENTAL

General Methods: Unless otherwise specified, all reagents were purchased from commercial sources and were used without further purification. Melting points were obtained with a Thomas Hoover capillary melting point apparatus and are not corrected. IR spectra were recorded on a Mattson Genesis II FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AM-300. All chemical shifts

are reported in ppm downfield from internal tetramethylsilane and coupling constants are given in Hz. MS spectra were recorded by EI method and HRMS spectra were measured on a Jeol JMX-DX 303 mass spectrometer. Elemental analysess were performed with a Carlo Erba EA1108 analyzer at the Organic Chemistry Research Center in Sogang University. Optical rotations were measured on a Rudolph Research Autopol III polarimeter. Chromatographic separations were carried out on a silica gel column (Merck silica gel 60).

Synthesis of 2. Reaction of Phytosphingosine with 3-Nitrophthalic Acid: Phytosphingosine (3.17 g, 10.0 mmol) and 3-nitrophthalic acid (2.12 g, 10.0 mmol) were placed in a 250 mL round-bottomed flask equipped with Dean-Stark trap and condenser. Toluene (100 mL) was added and the reaction mixture was heated under reflux for 3 h. The reaction mixture was concentrated by a rotary evaporator and cold methanol cooled in a freezer poured into the residue to form white precipitates of **2**. The precipitates were filtered and washed with cold methanol several times to give 3.01 g (67%) of **2**.

mp 101.0-109.8 ^{o}C. $[\alpha]^{23}_{D}$ +54.0 ^{o} (c 0.001, EtOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J*=7.0 Hz, 3H), 1.25 (br, 25H), 1.56 (m, 2H), 3.88-3.91 (m, 1H), 3.95-4.02 (m, 1H) 4.18 (t, *J*=9.0 Hz, 1H), 4.36 (t, *J*=9.0 Hz, 1H), 4.82 (q, *J*=8.2 Hz, 1H), 7.93-7.97 (m, 1H). 8.10-8.14 (m, 2H). IR (cm^{-1}, KBr) 3449 (OH), 2918, 2850, 1767, 1728, 1553, 1402, 1358, 1121. Anal. Calcd for $C_{26}H_{38}N_2O_2$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.84; H, 8.11; N, 5.89.

Synthesis of 3. Reaction of 2 with *p*-Tolylthiol: Sodium hydride (60% in mineral oil, 0.084 g, 2.2 mmol) was added to the solution of *p*-tolylthiol (0.248 g, 2.0 mmol) dissolved in anhydrous DMF (20 mL) with good stirring. After 10 min stirring at 25 ^{o}C, a solution of **2** (0.94 g, 2.0 mmol) in DMF (1 mL) was added to the mixture. After the reaction mixture was stirred for 2 h at 25 ^{o}C, it was poured into cold water, extracted with methylene chloride (50 mL). The organic layer was washed with 1 N HCl (50 mL) and brine (50 mL), dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated by a rotary evaporator and the residue was purified by silica gel column chromatography (methylene chloride/hexane, 5/1) to afford **3** (0.74 g, 75%) as yellow crystals.

mp 127.0-127.5 ^{o}C. $[\alpha]^{23}_{D}$ -2.1 ^{o} (c 0.002, EtOH). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.89 (t, *J*=6.3 Hz, 3H), 1.31 (br, 26H), 2.47 (s, 3H), 3.97-4.00 (m, 2H), 4.25 (t, *J*=8.7 Hz, 1H) 4.61 (t, *J*=8.9 Hz, 1H), 4.78 (q, *J*=8.1 Hz, 1H), 6.98 (m, 1H), 7.32-7.46 (m, 3H), 7.53-7.56 (m, 4H). IR (cm^{-1}, KBr) 3526 (-OH), 2921, 2851, 2360, 2342, 1766, 1694, 1458, 1397, 1340. MS m/z (rel. int.) 197 (6.67), 253 (9.66), 269 (100), 296 (12.37), 309 (6.45), 351 (1.89), 442 (0.61), 478 (0.43), 518 (0.15), 533 (8.13), 551 (M^{+}, 2.15). HRMS calcd for $C_{33}H_{45}NO_4S$ 551.3069, found 551.3073. Anal. Calcd for $C_{33}H_{45}NO_4S$: C, 71.83; H, 8.22; N, 2.54. Found: C, 71.83; H, 8.21; N, 2.60.

Synthesis of 4. Removal of Phthalimide Group in 2: Compound (**2**) (0.94 g, 2 mmol) in aqueous

hydrazine solution (35%, 5 mL + 5mL of ethanol) was heated under reflux for 2 h. The reaction mixture was poured into ice water and stood for 10 min to form precipitates. The precipitates were filtered, washed with cold water, and dried under vacuum. The pale brown solid was purified by silica gel column chromatography (methylene chloride/methanol, 20/1) to give **4** (0.37 g, 62%) as white solid.

mp 103.5-104.2 ^{o}C. $[\alpha]^{23}_{D}$ +14.8 ^{o} (c 0.002, EtOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*=6.3 Hz, 3H), 1.18-1.33 (br, 24H), 1.52-1.60 (m, 2H), 2.05 (br, 5H), 3.36 (q_{AB}, *J*=8.7 Hz, 1H), 3.45 (q, *J*=6.3 Hz, 1H), 3.58-3.62 (m, 2H), 4.20 (q_{AB}, *J*=8.7 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.114, 22.679, 25.851, 29.350, 29.550, 29.585, 29.648, 29.662, 31.910, 33.712, 52.585, 73.141, 74.737, 85.217. HRMS calcd for $C_{18}H_{37}NO_2$ 299.2824, found 299.2821. Anal. Calcd for $C_{18}H_{37}NO_2$: C, 72.19; H, 12.45; N, 4.68. Found: C, 72.14; H, 12.51; N, 4.67.

Synthesis of 5. *N*-Trifluoroactylation of Phytosphingosine: To the solution of phytosphingosine (10.50 g, 0.03 mol) in anhydrous ethanol (100 mL) was slowly added ethyl trifluoroacetate (5.35 mL, 0.045 mol) and the reaction mixture was stirred overnight at 25 ^{o}C. Crushed ice was added to the reaction mixture to form white precipitates, which were filtered, washed with cold water, and dried under vacuum to give **5** (11.15 g, 90%).

mp 99.4-101.7 ^{o}C. $[\alpha]^{23}_{D}$ –2.0 ^{o} (c 0.002, EtOH). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, *J*=6.3 Hz, 3H), 1.10-2.21 (br, 24H), 2.19-2.32 (br, 1H), 3.42-3.61 (m, 2H), 3.63-3.84 (m, 4H) 4.10-4.21 (m, 2H), 4.23-4.38 (m, 1H), 7.59-7.65 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 10.45, 23.55, 25.58, 28.92, 29.93, 30.51, 31.05, 32.73, 39.85 39.87, 39.90, 39.93, 39.95, 39.97, 40.01, 52.43, 61.33, 73.03, 75.07, 208.03. IR (cm^{-1}, KBr) 3289 (-OH), 2918, 2849, 1698, 1209, 1184.

Synthesis of 6. Cyclization of *N*-Trifluoroactylphytosphingosine (5): To a solution of *N*-trifluoroacetylphytosphingosine (**5**, 413 mg, 1 mmol) in pyridine (2 mL) was added *p*-toluenesulfonyl chloride (285 mg, 1.5 mmol) portionwisely and the reaction mixture was stirred overnight at 25 ^{o}C. The reaction mixture was poured into cold water (20 mL) and extracted with methylene chloride (20 mL x 2). The combined organic layer was washed with 1 N HCl (20 mL x 2) and saturated $NaHCO_3$. The organic layer was dried over anhydrous magnesium sulfate and concentrated by a rotary evaporator to give pale yellow oil. The oil was purified by silica gel column chromatography (methylene chloride/methanol, 40/1) to give **6** (296 mg, 75%) as white solid.

mp 82.5-83.1 ^{o}C. $[\alpha]^{23}_{D}$ –17.5 ^{o} (c 0.0008, EtOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*=6.3 Hz, 3H), 1.18-1.33 (br, 24H), 1.50-1.58 (m, 2H), 2.18 (d, *J*=4.9 Hz, 1H), 3.70 (q_{AB}, *J*=9.0 Hz, 1H), 3.69-3.80 (m, 1H), 4.02-4.09 (m, H), 4.25 (q_{AB}, *J*=9.0 Hz, 1H), 4.30-4.40 (m, 1H), 6.95 (br, 1H).^{13}C NMR (125 MHz, $CDCl_3$) δ 14.115, 22.682, 25.695, 29.353, 29.493, 29.543, 29.625, 29.647, 29.673, 31.911, 33.105, 51.604, 69.981, 74.240, 85.531.

Syntheis of 4. Deprotection of 6: To a solution of **6** (0.07 g, 0.17 mmol) in methanol (20 mL) was added well-ground potassium carbonate (0.70 g, excess) and the reaction mixture was stirred overnight at 25 ^{o}C. The reaction mixture was poured into cold water (50 mL) to form white precipitates, which was filtered, washed with cold water, and dried under vacuum to give **4** (0.05 g, 94%) as white solid. Spectra of ^{1}H NMR and ^{13}C NMR were identical with those prepared from **2**.

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