Synthesis of Sphingomyelin and Ceramide 1-Phosphate from Ceramide without Protection of the Allylic Hydroxyl Group

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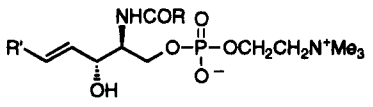
Sphingomyelin (**1**) is a major structural component of biological membranes and plasma lipoproteins.[1] Its interactions with cholesterol and other phospholipids have been studied extensively.[2,3] Sphingomyelin is also an important source of lipid second messengers that are generated in many cells when a variety of agonists activate a neutral sphingomyelinase in cell membranes, initiating the "sphingomyelin pathway."[4] Many sphingolipids, including ceramide and sphingosine 1-phosphate, have been shown recently to play key roles in cellular signal transduction pathways.[4] A phosphorylated form of ceramide, ceramide 1-phosphate (**2**), is equipotent to ceramide in inducing protein kinase activity.[5] Although this novel phospholipid has been found in rat brain synaptic vesicles[6a] and human leukemia cells,[6b,c] and appears to be hydrolyzed by a ceramide 1-phosphate phosphatase present in liver membranes,[6d] the physiological function of **2** in intercellular transduction processes has not yet been established.



R' = $C_{13}H_{27}$, R = C_7H_{15}

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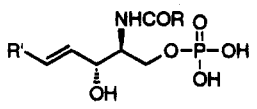

R' = $C_{13}H_{27}$, R = C_7H_{15}

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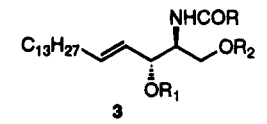
Sphingomyelin occurs naturally as a mixture of amide-linked fatty acyl chains (including very long saturated or monounsaturated fatty acyl chains) and long-chain (sphingoid) bases.[1] For the study of the interaction of sphingomyelins with membrane components such as cholesterol and proteins, chemically defined sphingomyelins are required. For further exploration of the biological functions of sphingomyelin and ceramide 1-phosphate, cell permeable short-chain analogs of these lipids are needed. The use of naturally occurring sphingomyelins as precursors of chemically defined sphingomyelins has two disadvantages. (1) Configurational instability at the allylic (C-3) position takes place during acid-catalyzed hydrolysis of the amide-linked chain in

Scheme 1[a]



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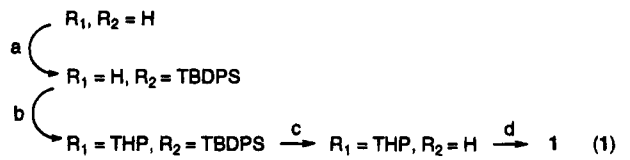


[a] Key: (a) imidazole, *t*-$BuPh_2SiCl$, CH_2Cl_2; (b) dihydropyran, *p*-TsOH, CH_2Cl_2; (c) *n*-Bu_4NF, THF; (d) (i) chloro(*N,N*-diisopropylamino)methoxyphosphine, Et_3N, $CHCl_3$; (ii) choline tosylate, tetrazole, CH_3CN–THF (1:1); (iii) *t*-BuOOH, THF; (iv) anhydrous Me_3N, toluene; (v) *p*-TsOH, MeOH.

1-butanol at 95 °C for 90 min[7a] and methanol at 70 °C for 20 h[7b] to give partially racemized sphingosylphosphocholine, which can be *N*-acylated with a desired fatty acid.[7c] (2) The backbone moiety of sphingomyelin, cerebrosides, and gangliosides is heterogeneous, consisting of several long-chain bases, such as C_{18}- and C_{20}-sphingosine, dihydrosphingosine (sphinganine), and phytosphingosine (4-D-hydroxysphinganine). To obtain sphingomyelin in homogeneous form, ceramides with different configurations and specific acyl chains have been generated from a variety of serine derivatives and utilized as precursors of sphingomyelin.[8]

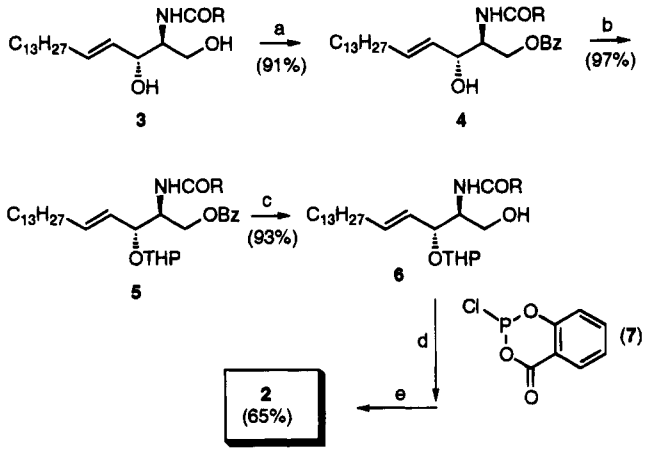
All of the published methods for selective insertion of the phosphocholine moiety into ceramide at C-1 to give sphingomyelin involve prior protection of the allylic hydroxyl group. Ceramides have been protected at C-3 as acetoxy,[9] benzoyl,[10] and pivaloyl[11] esters and as silyl[12] and tetrahydropyranyl[2,3] ethers. Equation 1 (Scheme 1) shows a common strategy for conversion of ceramide (**3**, $R_1 = R_2 = H$) into **1**, i.e., blocking of the C-1 hydroxyl group as a *tert*-butyldiphenylsilyl (TBDPS) ether, followed by protection of the C-3 hydroxyl group as a tetrahydropyranyl (THP) ether, desilylation, phosphitylation, choline insertion, phosphite oxidation, and deprotection of the THP group.[2,3] Obviously, this route suffers from the requirement of using multiple protection and deprotection steps and also from the occasional occurrence of migration of acyl protecting groups from C-3 to C-1 during deprotection reactions.[9,10b] Here we report a highly convenient preparation of two biologically relevant sphingolipids, sphingomyelin (**1**) and ceramide 1-phosphate (**2**), by a route involving phosphitylation without blocking of the allylic hydroxyl group of ceramide (**3**).

(1) For a review, see: Barenholz, Y.; Thompson, T. E. *Biochim. Biophys. Acta* **1980,** *604*, 129–158.
(2) Kan, C.-C.; Ruan, Z.-S.; Bittman, R. *Biochemistry* **1991,** *30*, 7759–7766.
(3) Grönberg, L.; Ruan, Z.-S.; Bittman, R.; Slotte, J. P. *Biochemistry* **1991,** *30*, 10746–10754.
(4) (a) Kolesnick, R. N.; Golde, D. W. *Cell* **1994,** *77*, 325–328. (b) Hannun, Y. A. *J. Biol. Chem.* **1994,** *269*, 3125–3128.
(5) Joseph, C. K.; Byun, H.-S.; Bittman, R.; Kolesnick, R. N. *J. Biol. Chem.* **1993,** *268*, 20002–20006.
(6) (a) Bajjalieh, S. M.; Martin, T. F. J.; Floor, E. *J. Biol. Chem.* **1989,** *264*, 14354–14360. (b) Dressler, K. A.; Kolesnick, R. N. *J. Biol. Chem.* **1990,** *265*, 14917–14921. (c) Kolesnick, R. N.; Hemer, M. R. *J. Biol. Chem.* **1990,** *265*, 18803–18808. (d) Boudker, O.; Futerman, A. H. *J. Biol. Chem.* **1993,** *268*, 22150–22155.
(7) (a) Kaller, H. *Biochem. Z.* **1961,** *334*, 451–456. (b) Gaver, R. C.; Sweeley, C. C. *J. Am. Oil Chem. Soc.* **1965,** *42*, 294–298. (c) Sripada, P. K.; Maulik, P. R.; Hamilton, J. A.; Shipley, G. G. *J. Lipid Res.* **1987,** *28*, 710–718.
(8) For a recent review of synthetic methods for sphingolipids, see: Byun, H.-S.; Bittman, R. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; pp 97–140.
(9) Crossman, M. W.; Hirschberg, C. B. *J. Lipid Res.* **1984,** *25*, 729–737.
(10) (a) Ohashi, K.; Kosai, S.; Arizuka, M.; Watanabe, T.; Yamagiwa, Y.; Kamikawa, T.; Kates, M. *Tetrahedron* **1989,** *45*, 2557–2570. (b) Bushnev, A. S.; Karpyshev, N. N.; Zvonkova, E. N.; Evstigneeva, R. P. *Soviet J. Bioorg. Chem.* **1980,** 1027–1030; *Chem. Abstr.* **1980,** *92*, 41295t.
(11) Peterson, M. A.; Polt, R. *J. Org. Chem.* **1993,** *58*, 4309–4314.
(12) (a) Tkaczuk, P.; Thornton, E. R. *J. Org. Chem.* **1981,** *46*, 4393–4398. (b) Nicolaou, K. C.; Caulfield, T.; Kataoka, H.; Kumazawa, T. *J. Am. Chem. Soc.* **1988,** *110*, 7910–7912. (c) Bruzik, K. S. *J. Chem. Soc., Perkin Trans. 1* **1988,** 423–431.

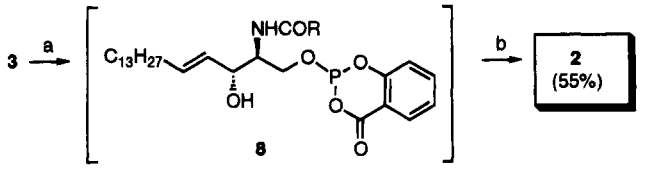
0022-3263/94/1959-6495$04.50/0

Scheme 2.[a] Synthesis of D-*erythro*-Ceramide 1-Phosphate (R = C_7H_{15})

Procedure A: With blocking of the C3-OH group



Procedure B: Without blocking of the C3-OH group



[a] Key: Procedure A: With blocking of the C3-OH group. Reagents: (a) benzoic anhydride, DMAP, CH_2Cl_2, rt; (b) (i) dihydropyran, *p*-TsOH, CH_2Cl_2, rt; (ii) $NaHCO_3$; (c) sodium methoxide, MeOH, rt; (d) **7**, Et_3N, CH_2Cl_2, 0 °C; (e) (i) Amberlite MB-3, THF/H_2O (9:1); (ii) *p*-TsOH, MeOH, rt; (iii) *t*-BuOOH, CH_2Cl_2. Procedure B: Without blocking of the C3-OH group. Reagents: (a) (i) *N,N*-diisopropylethylamine, 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (**7**), THF, −78 °C; (b) (i) H_2O, rt; (ii) Amberlite MB-3, THF/H_2O (9:1); (iii) *t*-BuOOH, CH_2Cl_2.

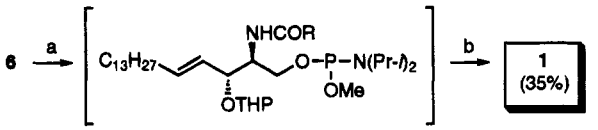
Results and Discussion

Synthesis of Ceramide 1-Phosphate (2) with Blocking of the C-3 Hydroxyl Group. Ceramide 1-phosphate (**2**) was prepared from 3-*O*-tetrahydropyranylceramide (**6**), which was obtained from **3** after a sequence of protection and deprotection steps (procedure A, Scheme 2). The primary hydroxyl group of ceramide (**3**) was protected as a benzoyl ester, and the allylic hydroxyl group was blocked as a THP ether to give **5**. The benzoyl group of **5** was converted to methyl benzoate by base-catalyzed methanolysis, giving **6**. Compounds **5** and **6** were obtained in pure form without chromatography or crystallization, since the excess of dihydropyran and methyl benzoate was readily removed under vacuum. Phosphitylation of **6** with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (**7**) followed by oxidation and deprotection of the THP group gave ceramide 1-phosphate (**2**) in 53% overall yield.

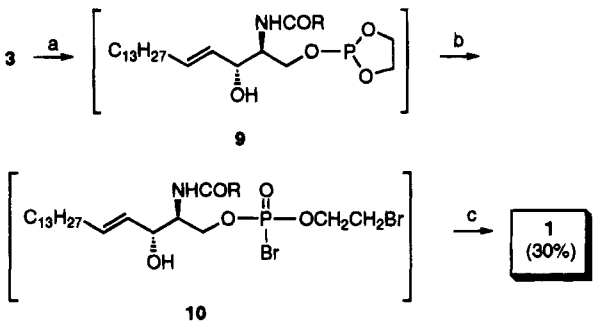
Synthesis of Ceramide 1-Phosphate (2) without Blocking of the C-3 Hydroxyl Group via Monophosphitylation. A more convenient preparation of ceramide 1-phosphate (**2**) is by selective monophosphitylation of the primary hydroxyl group of ceramide (**3**) (procedure B, Scheme 2). Ceramide (**3**) was treated with 3 equiv of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (**7**) in the presence of *N,N*-diisopropylethylamine in THF at −78 °C to provide phosphite **8**. Water was added to quench the excess **7** and to hydrolyze the salicylic acid group in phosphite **8**. After removal of salts from the reaction mixture by passing the mixture through an Amberlite MB-3 ion exchange resin, ceramide 1-phosphite was oxidized with *tert*-butyl hydroperoxide in methylene chloride to give ceramide 1-phosphate (**2**) in 55% overall yield from **3**. The physical properties of ceramide 1-phosphate (**2**) prepared by both methods are identical.

Scheme 3.[a] Synthesis of *N*-Octanoyl-D-*erythro*-sphingomyelin (R = C_7H_{15})

Procedure A: From 3-O-protected ceramide



Procedure B: From unprotected ceramide



[a] Key: Procedure A: From 3-*O*-protected ceramide. Reagents: (a) (i) chloro(*N,N*-diisopropylamino)methoxyphosphine, Et_3N, $CHCl_3$, 0 °C; (b) (i) choline tosylate, tetrazole, rt; (ii) *t*-BuOOH, THF; (iii) anhydrous Me_3N, toluene, 60 °C; (iv) *p*-TsOH, MeOH, rt. Procedure B: From unprotected ceramide. Reagents: (a) (i) *N,N*-diisopropylethylamine, ethylene chlorophosphite, −20 °C, THF; (b) (i) MeOH; (ii) Br_2, −20 °C; (c) (i) H_2O; (ii) aqueous Me_3N, CH_3CN/*i*-PrOH/$CHCl_3$ (1.5:1.5:0.9).

Synthesis of Sphingomyelin (1) with and without Blocking of the C-3 Hydroxyl Group. Phosphocholine insertion into 3-protected ceramides such as **6** has been achieved by using various phosphitylation and phosphorylation procedures,[2,3] but the yields are poor and the route is long. Procedure A (Scheme 3) shows a typical result of this approach; sphingomyelin (**1**) was prepared from protected ceramide **6** in 35% overall yield. In contrast, we found that unprotected ceramide (**3**) reacted with ethylene chlorophosphite (procedure B, Scheme 3) to give **1** in 30% overall yield, as outlined below. Thus procedure B represents a short and convenient route to **1** from unprotected **3**. The low yield for the conversion of *N*-acylsphingosine (**3**) into **1** may arise from removal of the NH or OH proton followed by cyclization during conversion of **10** into **1**. Alternatively, an unfavorable intramolecular hydrogen bonding pattern in *N*-acylsphingosine (**3**)[11,13] may account for the low reactivity toward phosphitylation. Polt et al. postulated that such hydrogen bonding results in a diminished nucleophilicity of the 1-hydroxyl group of *N*-acylsphingosine (**3**), which was offered as an explanation of the relative lack of reactivity of **3** as a glycosyl acceptor in cerebroside synthesis.[13]

Sphingomyelin (**1**) was synthesized via procedure B (Scheme 3) as follows. C8-Ceramide (**3**) was first reacted with 3 equiv of ethylene chlorophosphite in the presence

(13) Polt, R.; Szabó, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **1992**, *114*, 10249−10258.

of 3 equiv of *N,N*-diisopropylethylamine in methylene chloride at −20 °C to give cyclic phosphite **9**. Methanol was added to quench the excess unreacted ethylene chlorophosphite. The cyclic phosphite **9** was oxidized and opened with bromine[14] at −20 °C. In this reaction the cyclic phosphite ester **9** was converted to (2-bromoethyl)-phosphate ester **10**. At −20 °C excess water was added to hydrolyze the P−Br bond, in order to avoid cyclization with the allylic hydroxyl group or the amide NH group of ceramide derivative **10**. The solvents were removed, and the 1-(2′-bromoethyl) phosphate ester derivative of ceramide was quaternized with aqueous trimethylamine in acetonitrile−2-propanol−chloroform to give sphingomyelin (**1**). This method has the advantage of brevity over the existing methods, which involve a series of protection and deprotection reactions, and the overall yields are also almost the same.

Experimental Section

General Procedure. The melting points are uncorrected. Optical rotations were measured in a cell of 1-dm pathlength. Silica gel GF TLC plates of 0.25-mm thickness were used to monitor reactions, with visualization by charring using 10% sulfuric acid in ethanol. Column chromatography was carried out with silica gel 60 (230−400 ASTM mesh).

Chemicals. 2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, chloro(*N,N*-diisopropylamino)methoxyphosphine, 3,4-dihydro-2*H*-pyran, anhydrous and 45% aqueous trimethylamine, triethylamine, *p*-toluenesulfonic acid (*p*-TsOH), 1*H*-tetrazole, *tert*-butyl hydroperoxide, Amberlite MB-3, and TMD-8 were purchased from Aldrich. 4-(Dimethylamino)pyridine (DMAP) was obtained from Fluka. Ethylene chlorophosphite was purchased from Lancaster. Benzoic anhydride and *N,N*-diisopropylethylamine were obtained from Sigma. D-*erythro*-Sphingosine was prepared by the coupling reaction of Garner aldehyde[15] with lithium pentadecyne in the presence of hexamethylphosphoramide,[16] followed by Birch reduction (lithium, ethylamine).[17] *N*-Octanoyl-D-*erythro*-sphingosine (**3**) was prepared by reaction of D-*erythro*-sphingosine with *p*-nitrophenyl octanoate in THF at rt. The solvents were dried as follows: Tetrahydrofuran was refluxed over sodium benzophenone ketyl radical for several hours and then distilled and used immediately. Methylene chloride was refluxed and distilled from CaH_2 and then stored over CaH_2. Chloroform was refluxed and distilled over P_2O_5.

***N*-Octanoyl-1-*O*-benzoyl-D-*erythro*-sphingosine (4).** To a solution of 426 mg (1.0 mmol) of ceramide (**3**) in 10 mL of dry CH_2Cl_2 were added benzoic anhydride (250 mg, 1.11 mmol) and DMAP (2 mg, 16 μmol). After the reaction was stirred at rt under N_2 for 24 h, 100 mL of EtOAc was added. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and dried over Na_2SO_4. The residue was purified by flash chromatography (elution with hexane−EtOAc 4:1) to give 483 mg (91%) of benzoyl-protected ceramide **4** as a white solid: mp 56−57 °C; R_f 0.46 (hexane−EtOAc 2:1); $[\alpha]^{26}_D$ −3.30° (*c* 40.0, $CHCl_3$); IR (film) 3424, 3285, 1725, 1643 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.9−8.1 (m, 2H), 7.2−7.6 (m, 3H), 6.10 (d, 1H, *J* = 7.6 Hz), 5.76 (dt, 1H, *J* = 15.0, 8.0 Hz), 5.53 (dd, 1H, *J* = 15.0, 6.9 Hz), 4.2−4.6 (m, 4H), 3.17 (br s, 1H), 2.18 (t, 2H, *J* = 7.8 Hz), 1.25 (br s, 32H), 0.87 (t, 6H, *J* = 6.7 Hz). Anal. Calcd for $C_{33}H_{55}O_4N$: C, 74.81; H, 10.46; N, 2.64. Found: C, 74.94; H, 10.55; N, 2.67.

***N*-Octanoyl-1-*O*-benzoyl-3-*O*-tetrahydropyranyl-D-*erythro*-sphingosine (5).** To a solution of 400 mg (0.76 mmol) of 1-*O*-benzoyl-C8-ceramide (**4**) in 10 mL of dry CH_2Cl_2 were added 184 mg (0.2 mL, 2.19 mmol) of freshly distilled dihydropyran and a few crystals of *p*-TsOH monohydrate. The reaction mixture was stirred for 2 h at rt, quenched by the addition of 20 mg of $NaHCO_3$, and filtered through a pad of silica gel. The solvent was removed under reduced pressure to give 450 mg (97%) of a diastereomeric mixture[18] of 3-*O*-protected ceramide **5** as a white solid, which was used without further purification: mp 43−44 °C; R_f 0.73, 0.69 (hexane−EtOAc 2:1); $[\alpha]^{26}_D$ −2.88° (*c* 30.0, $CHCl_3$); IR (film) 3284, 1725, 1643 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.9−8.1 (m, 2H), 7.3−7.6 (m, 3H), 6.22, 5.84 (a pair of d, 1H, *J* = 8.0 Hz), 5.75 (dt, 1H, *J* =14.5, 7.0 Hz), 5.53, 5.38 (a pair of dd, 1H, *J* = 15.4, 7.0 Hz), 4.73−4.71 (m, 1H), 4.57−4.21 (m, 4H), 3.92−3.79 (m, 1H), 3.50−3.42 (m, 1H), 2.15 (t, 2H, *J* = 7.6 Hz), 2.05−1.01 (m, 38H), 0.87 (t, 6H, *J* = 6.8 Hz). Anal. Calcd for $C_{38}H_{63}O_5N$: C, 74.34; H, 10.34; N, 2.28. Found: C, 74.31; H, 10.41; N, 2.16.

***N*-Octanoyl-3-*O*-tetrahydropyranyl-D-*erythro*-sphingosine (6).** To a solution of 400 mg (0.65 mmol) of 1-*O*-benzoyl-3-*O*-tetrahydropyranylceramide (**5**) in 20 mL of dry MeOH was added catalytic sodium methoxide in MeOH (prepared from 2 mL of MeOH and 10 mg (0.44 mmol) of sodium). After being stirred for 24 h at rt, the reaction mixture was diluted with 40 mL of ether and filtered through a pad of silica gel. The filtrate was concentrated under reduced pressure and then dried over high vacuum to give 308 mg (93%) of diastereomeric 3-*O*-tetrahydropyranylceramide **6** as a white solid, which was used without further purification: mp 42−43 °C; R_f 0.28, 0.17 (hexane−EtOAc 1:1); $[\alpha]^{25}_D$ −5.35° (*c* 25.0, $CHCl_3$); IR (film) 3424, 3307, 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.38, 6.19 (a pair of d, 1H, *J* = 7.5 Hz), 5.73 (dt, 1H, *J* = 15.5, 6.8 Hz), 5.48, 5.37 (a pair of dd, 1H, *J* = 15.5, 6.8 Hz), 3.3−4.7 (m, 12H), 1.9−2.3 (m, 6H), 1.26 (br s, 32H), 0.88 (t, 6H, *J* = 6.8 Hz). Anal. Calcd for $C_{31}H_{59}O_4N$: C, 73.04; H, 11.66; N, 2.75. Found: C, 73.27; H, 11.80; N, 2.85.

D-*erythro*-Ceramide 1-Phosphate (2). This compound was prepared by using two different procedures (Scheme 2). **Procedure A.** To a solution of 306 mg (0.60 mmol) of diastereomeric 3-*O*-tetrahydropyranylceramide **6** were added 145 mg (0.2 mL, 1.43 mmol) of triethylamine and 243 mg (1.2 mmol) of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (**7**) in 5 mL of dry CH_2Cl_2. The reaction mixture was stirred for 2 h at 0 °C and then was quenched by the addition of 10 mL of THF−H_2O (9:1). After the solvents were removed under reduced pressure, the residue was dissolved in 5 mL of THF−H_2O (9:1) and passed through an Amberlite MB-3 column, which was previously equilibrated with the same solvent system. The crude material was dissolved in 10 mL of MeOH, and the THP group was removed by adding a few crystals of *p*-TsOH monohydrate. The mixture was stirred for 2 h at rt. After the solvents were removed under reduced pressure, the residue was dissolved in 10 mL of CH_2Cl_2 and treated with 0.25 mL (0.75 mmol, a 3.0 M solution in 2,2,4-trimethylpentane) of anhydrous *tert*-butyl hydroperoxide to give crude ceramide 1-phosphate. The residue was purified by column chromatography, eluting with $CHCl_3$−MeOH−glacial AcOH (65:15:3), to give 196 mg (65%) of **2**, which on lyophilization from C_6H_6 gave ceramide 1-phosphate (**2**) as a white solid: mp 105−108 °C; $[\alpha]^{25}_D$ −1.75° (*c* 10.0, $CHCl_3$); R_f 0.17 ($CHCl_3$−MeOH−glacial AcOH 65:15:5). **Procedure B.** To a solution of 107 mg (0.25 mmol) of ceramide **3** and 148 mg (0.2 mL, 1.2 mmol) of *N,N*-diisopropylethylamine in 45 mL of dry THF was added 152 mg (0.75 mmol) of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (**7**) in 5 mL of THF at −78 °C. After 3 h the reaction mixture was quenched by the addition of 5 mL of H_2O and warmed to rt. The reaction mixture was passed through an Amberlite MB-3 column (elution with THF−H_2O 9:1) and concentrated under reduced pressure. The residue was dissolved in 10 mL of CH_2Cl_2 and treated with 0.11 mL (0.33 mmol, a 3.0 M solution in 2,2,4-trimethylpentane) of anhydrous *tert*-butyl hydroperoxide to give crude ceramide 1-phosphate. Purification as described above gave 69 mg (55%) of **2**, which on lyophilization from C_6H_6 gave a white solid: mp 105−108 °C; R_f 0.17 ($CHCl_3$−MeOH−glacial AcOH 65:15:5) [lit.[19] for mixed-chain ceramide 1-phosphate R_f 0.06 ($CHCl_3$−MeOH−AcOH 65:15:5)]; R_f 0.73 ($CHCl_3$−MeOH−glacial AcOH−H_2O 60:20:6:1) [lit.[10b] for C18-ceramide 1-phosphate (disodium salt) R_f 0.40 ($CHCl_3$−MeOH−AcOH−H_2O 60:20:6:1)]; R_f 0.72 ($CHCl_3$−MeOH−glacial AcOH−

(14) For insertion of the phosphocholine moiety into primary alcohols via phosphitylation and ring opening/oxidation with bromine, see: Erukulla, R. K.; Byun, H.-S.; Bittman, R. *Tetrahedron Lett.* **1994,** 5783−5784.

(15) Garner, P.; Park, J. M. *Org. Synth.* **1991,** *70,* 18−26.

(16) Herold, P. *Helv. Chim. Acta* **1988,** *71,* 354−362.

(17) Garner, P.; Park, J. M.; Malecki, E. *J. Org. Chem.* **1988,** *53,* 4395−4398.

(18) The mixture of diastereomers results from the presence of the additional chirality due to the tetrahydropyranyl group.

(19) Okazaki, T.; Bielawska, A.; Bell, R. M.; Hannun, Y. A. *J. Biol. Chem.* **1990,** *265,* 15823−15831.

15 mM $CaCl_2$ 60:35:2:4) [lit.[6d] for C6-ceramide 1-phosphate (potassium salt) R_f 0.3 ($CHCl_3$–MeOH–AcOH–15 mM $CaCl_2$ 60:35:2:4)]; R_f 0.49 ($CHCl_3$–MeOH–H_2O 65:25:4); R_f 0.62 ($CHCl_3$–MeOH–8.6 N NH_4OH 65:35:8); $[\alpha]^{25}_D$ −1.75° (*c* 10.0, $CHCl_3$); IR (film) 3307 and 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.3–6.4 (br m), 3.0–4.0 (br m), 1.7–2.3 (br m), 1.25 (br s), 0.88 (t, *J* = 6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$–CD_3OD) δ 13.5, 14.5, 18.7, 19.2, 22.2, 22.3, 25.0, 28.8, 29.3, 30.1, 31.4, 31.5, 35.5, 47.7 and 49.0 (C-2, partially obscured by CD_3OD), 65.6 (C-1), 72.6 (C-3), 128.0–128.7 (C-5), 130.6–132.2 (C-4), 167.3–167.8 (C=O). The FAB mass spectrum gave $(M - H)^+$ 506. Anal. Calcd for $C_{26}H_{52}O_6NP$: C, 61.76; H, 10.36; N, 2.77; P, 6.13. Found: C, 61.84; H, 10.26; N, 2.68; P, 6.28.

***N*-Octanoyl-D-*erythro*-sphingomyelin (1).** This compound was prepared by using two different procedures (Scheme 3). **Procedure A.** *N*-Octanoyl-D-*erythro*-sphingomyelin (**1**) was prepared from 3-*O*-tetrahydropyranylceramide (**6**) by using a previously described phosphitylation procedure.[12c] To a solution of 75 mg (0.15 mmol) of diastereomeric 3-*O*-tetrahydropyranylceramide (**6**) in 10 mL of dry $CHCl_3$ at 0 °C were added 30 mg (42 μL, 0.30 mmol) of triethylamine and 35.6 mg (0.18 mmol) of chloro(*N*,*N*-diisopropylamino)methoxyphosphine. The reaction mixture was stirred for 15 min at 0 °C, and the progress of the reaction was monitored by TLC (hexane–EtOAc 1:1). The mixture was concentrated to dryness under vacuum. The residue was dissolved in CH_3CN–THF (1:1), and 31.5 mg (0.45 mmol) of 1*H*-tetrazole and 124 mg (0.45 mmol) of dry choline tosylate were added. After being stirred at rt for 3 h the reaction mixture was again concentrated to dryness, the residue was dissolved in THF, and 22 mg (0.3 mmol) of *tert*-butyl hydroperoxide was added. After 2 h, 10 mL of EtOAc was added, and the resulting solution was washed with 1 M triethylammonium hydrogen carbonate buffer (pH 7.5) to remove excess tetrazole and choline tosylate. The organic phase was concentrated to dryness, and the crude material was lyophilized from C_6H_6 (5 mL). The crude material was dissolved in 5 mL of dry toluene and treated with 0.5 mL of anhydrous trimethylamine in a pressure tube for 12 h at 60 °C. After the *O*-demethylation reaction was completed, the solvents were removed and the crude product was dissolved in dry MeOH and treated with a catalytic amount of *p*-TsOH at rt overnight in order to deprotect the C3-hydroxyl group. The product was purified by column chromatography (first elution with 25% MeOH in $CHCl_3$, then with $CHCl_3$–MeOH–H_2O 65:25:4) to give 31 mg of sphingomyelin (**1**); the overall yield from 3-*O*-tetrahydropyranylceramide (**6**) was 35%: R_f 0.24 ($CHCl_3$–MeOH–H_2O 65:25:4); $[\alpha]^{25}_D$ −5.5° (*c* 1.40, $CHCl_3$). **Procedure B.** To a solution of 60 mg (0.14 mmol) of ceramide (**3**) in 5 mL of THF were added 72 mg (98 μL, 0.74 mmol) of *N*,*N*-diisopropylethylamine and 53 mg (38 μL, 0.42 mmol) of ethylene chlorophosphite at −20 °C. After the mixture had been stirred for 20 min at −20 °C, 11 μL (0.28 mmol) of MeOH was added to quench the reaction. The mixture was stirred for 15 min, and then 67 mg (0.42 mmol) of Br_2 was added at −20 °C. After 5 min, 0.5 mL (27.8 mmol) of H_2O was added, and the reaction mixture was allowed to warm to rt. After the solvent was removed under reduced pressure, the residue was dissolved in 3 mL of CH_3CN–*i*-PrOH–$CHCl_3$ (1.5/1.5/0.9), and 3 mL of 45% aqueous trimethylamine was added. After the mixture had been stirred at rt overnight, the solvents were removed under reduced pressure and the residue was dissolved in a minimum volume of THF–H_2O (9:1) and passed through a column of TMD-8 ion exchange resin (elution with THF–H_2O, 9:1). The fractions containing the crude product were pooled and concentrated under reduced pressure. The residue was lyophilized to afford **1** as a white powder which was purified by column chromatography (first elution with 25% MeOH in $CHCl_3$ and then with $CHCl_3$–MeOH–H_2O 65:25:4), giving 25 mg (30%) of **1** as a white powder: R_f 0.22 ($CHCl_3$–MeOH–H_2O 65:25:4); bovine-brain sphingomyelin gave R_f 0.24 ($CHCl_3$–MeOH–H_2O 65:25:4); $[\alpha]^{25}_D$ −5.04° (*c* 0.35, $CHCl_3$:CH_3OH 1:1); ^1H NMR ($CDCl_3$) δ 5.68 (dt, 1H, *J* = 15.4 Hz, 6.7 Hz), 5.45 (dd, 1H, *J* = 15.4 Hz, 6.7 Hz), 4.29 (m, 4H), 3.93–3.66 (m, 4H), 3.28 (s, 9H), 2.30 (t, 2H, *J* = 7.0 Hz), 1.97 (m, 2H), 1.53 (m, 2H), 1.25 (m, 32H), 0.85–0.91 (t, 6H, *J* = 6.0 Hz); HRMS [FAB, $(M - H)^+$] calcd for $C_{31}H_{64}PN_2O_6$ 591.4502, found 591.4514.

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