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A facile regioselective synthesis of sphingosine 1-phosphate and ceramide 1-phosphate

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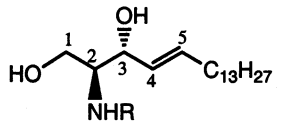
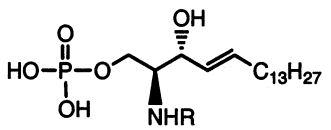
Abstract

A novel regioselective synthesis of D-*erythro*-sphingosine 1-phosphate and D-*erythro*-ceramide 1-phosphate is described. The synthesis is based upon (1) in situ oxidative phosphorylation of the primary hydroxyl group in *N*-Boc-D-*erythro*-sphingosine utilizing trimethyl phosphite and carbon tetrabromide in pyridine, (2) chemoselective deprotection of the 1-*O*-phosphate and/or 2-amino groups with trimethylsilyl bromide or chloride, and (3) introduction of the fatty acid moiety into sphingosine 1-phosphate dimethyl ester via base-catalyzed *N*-acylation.

Sphingosine (**1**), ceramide (**2**) and their phosphates **3** and **4** (Fig. 1) constitute a highly conserved, plasma-membrane derived set of molecular tools which eukaryotic cells use to trigger a variety of responses in order to regulate their growth, differentiation, apoptosis and migration.[1,2] Specifically, **3** opposes ceramide-mediated apoptosis through an intracellular action.[2a,e] It regulates the levels of calcium[3], cAMP[4] and induces angiogenesis[5] acting as a ligand of the G-protein-coupled cell surface receptors EDG-1, 3, 5, 6 and 8.[2a,6] The relative balance between the cellular concentrations of sphingolipids **1**–**4** is revealed to be crucial in regulating outcomes for cells.



1. D-*erythro*-sphingosine; R = H
2. Ceramide; R = $CO(CH_2)_nCH_3$



3. D-*erythro*-sphingosine 1-phosphate; R = H
4. Ceramide 1-phosphate; R = $CO(CH_2)_nCH_3$

Figure 1.

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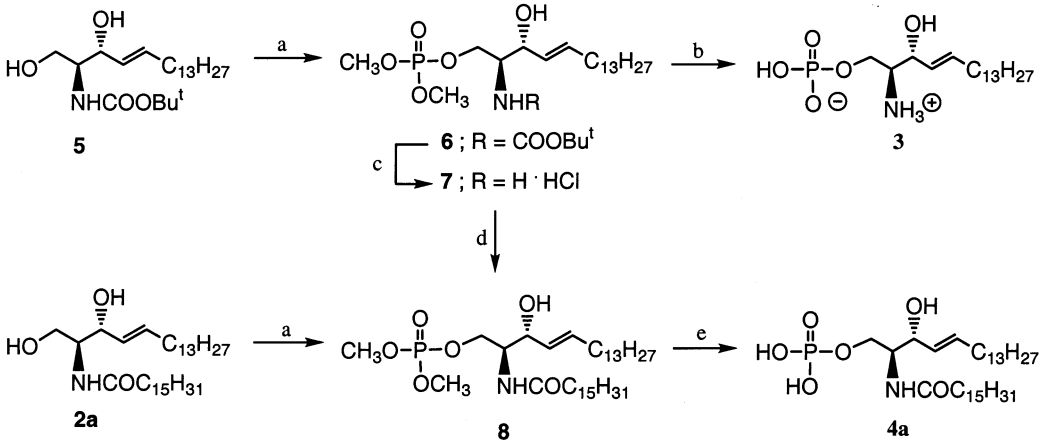
As a part of our program for the development of molecular probes to study sphingolipid-mediated signal transduction and cell regulation, we became interested in the synthesis of phosphates **3**, **4**, and their analogs. Since extremely diverse bioactivities of **1** and **2** are governed by their stereochemistry[7], an access to regioselective and racemization-free methods for their phosphorylation becomes critical.

To date, few enzymatic [8] and many more synthetic [9] methods for the preparation of **3** and **4** have been developed. Enzymatic methods usually provide small quantities of these phosphates and generally are used for the preparation of their radiolabeled analogs.[8a] Synthetic methods are based on P(III) and P(V) chemistry and, in most cases, they utilize specifically designed and not easily available, 2-*N*-, 3-*O*-diprotected sphingolipid precursors and require stringent reaction conditions.[9d,e,g–j] In addition, the reported syntheses are quite lengthy as the result of the necessary oxidation and deprotection steps following the 1-*O*-phosphitylation reaction. The most attractive synthetic approach to **3**, the specific 1-*O*-phosphorylation of *N*-Boc-D-*erythro*-sphingosine (**5**), utilizing phosphoramidate methodology,[9f] was recently reinvestigated by Li, Wilson and Schroepfer.[9k] However, the authors encountered numerous difficulties in carrying out this four-step synthesis. This method suffered from a low degree of consumption of **5** that was associated with the formation of side products during consecutive phosphitylation and oxidation steps. Under the best conditions the overall yield was only 32%. These disadvantages prompted us to develop a novel and practical synthesis of **3** and **4** from **5**.

Our method successfully utilizes the synthetic potential of three chemoselective reactions for regioselective functionalization of sphingosine, and it expands the repertoire of available mild deprotection conditions for this class of compounds. The first reaction is the in situ oxidative phosphorylation of alcohols with $P(OCH_3)_3$ and CBr_4 in pyridine, introduced by Oza and Corcoran.[10] The second is the silane-induced cleavage of esters and/or carbamates,[11] and the third involves the *N*-acylation of vicinal aminoalcohols with carboxylic acid derivatives catalyzed by bases.[7a,12]



Scheme 1. Reagents and conditions: (a) $P(OCH_3)_3$, CBr_4, pyridine, ~4°C–rt, N_2, 2 h; (b) (1) $(CH_3)SiBr$, CH_2Cl_2, 0°C–rt, N_2, 1 h; (2) H_2O; (c) $(CH_3)_3SiCl$, MeOH, 0°C–rt, N_2, 24 h; (d) $C_{15}H_{31}COCl$, Et_3N, EtOH/CH_2Cl_2 (1:2, v/v), ~4°C–rt, N_2, 1 h; (e) (1) $(CH_3)_3SiBr$, CH_3CN, 0°C–rt, N_2, 4 h; (2) H_2O

Our synthesis commences with the versatile synthon **6**,[†] which was prepared with excellent regioselectivity (>95%, vide infra) by treatment of **5** with 1.4 equiv. of trimethyl phosphite and 1.25 equiv. of carbon tetrabromide in dry pyridine (Scheme 1). Under these conditions, compound **6** is prepared in 72% yield, with 12% of unreacted **5** remaining. According to our results,[13] and consistent with Oza's and Corcoran's observations for 1,2-diols,[10] **5** is selectively phosphorylated at the primary hydroxyl group. Successive $(CH_3)_3SiBr$-mediated deprotection of the phosphate ester and the 2-amino groups in **6** gave the target compound[14] **3** in 73% yield, after recrystallization from THF/water (2:1, v/v). On the other hand, when $(CH_3)_3SiCl$ was used instead of $(CH_3)_3SiBr$ and the deprotection reaction of **6** was performed in anhydrous MeOH,[11e] the cleavage of the C–N bond in the carbamate moiety proceeded exclusively, and compound **7**[‡] was obtained in 85% yield after recrystallization from EtOAc/hexane (1:2, v/v).

Amide **2**, unlike the carbamate **5**, proved to be much less susceptible toward phosphorylation with $P(OCH_3)_3$.[15] Under the same phosphorylation conditions, C-16 ceramide (**2a**) gave the corresponding 1-phosphate dimethyl ester[13] **8**[§] with a yield of 20%. Attempts to increase consumption of **2a** by the use of greater amounts of $P(OCH_3)_3$ and expanding the reaction time were moderately successful, and they led to a substantial increase in the formation of the less polar byproducts, including a product of 1,3-diphosphorylation of **2a** ($R_f = 0.81$), in regard to compound **8** ($R_f = 0.63$). To circumvent this problem we have utilized **7** as a key intermediate and developed a four-step synthesis of **4a** from **5**. Thus, *N*-acylation of **7** with palmitic acid chloride, performed in the presence of triethylamine, provided **8** in 90% yield. Treatment of **8** with $(CH_3)_3SiBr$ smoothly afforded **4a** in 72% yield.[14]

In summary, we have developed a highly regioselective, convenient and efficient synthesis of the model phosphates **3** and **4a**. This synthesis should be amenable to the preparation of regio- and stereoisomers of **3** and **4a** and their analogs with ease. Further work is in progress and will be reported in due course.

[†] Selected physical data. Compound **6**: $R_f = 0.58$ (12:1, $CHCl_3$:CH_3OH); mp 36–37°C; $[\alpha]_D^{24} = +4.3°$ ($c = 1.0$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.75 (dtd, 15.4, 6.8, 1.2 Hz, H-5), 5.49 (ddt, 15.4, 6.8, 1.4 Hz, H-4), 5.01 (d, 7.6 Hz, NH), 4.32 (ddd, 10.7, 7.8, 4.8 Hz, H_a-1), 4.11 (m, 2H, H_b-1, H-3), 3.79 (d, 2.3 Hz, 3H, OCH_3), 3.77 (m, 4H, H-2 and OCH_3), 2.75 (bs, 2H, OH), 2.02 (q, 2H, 7.0 Hz, H-6), 1.42 (s, 9H, $C(CH_3)_3$), 1.35 (m, 2H, H-7), 1.25 (m, 20H), 0.87 (t, 3H, 7.1 Hz, CH_3); ^{31}P NMR (500 MHz, $CDCl_3$, $CH_2[P(O)(OH)_2]_2$) δ −16.4; FAB-MS (positive ion mode) m/z 508.3 ($[M+H]^+$, 100), 490.3 ($[M+H]^+-H_2O$, 10), 452.3 (29), 434.3 (59), 408.3 ($[M+H]^+-COOC_4H_9$, 99), 326.3 (71); calcd for $C_{25}H_{50}NO_7P$ m/z 507.3.

[‡] Compound **7**: $R_f = 0.34$ ($CHCl_3$:CH_3OH:$[(CH_3)_2CH]_2NC_2H_5$, 10:1:0.01); mp 82–83°C (>88°C decomp.); $[\alpha]_D^{24} = -4.0°$ ($c = 1.0$, $CHCl_3$); ^1H NMR (CD_3OD) δ 5.89 (dtd, 15.4, 6.8, 1.1 Hz, H-5), 5.48 (ddt, 15.4, 6.7, 1.4 Hz, H-5), 4.30 (m, 2H, H_a-1 and H-3), 4.20 (ddd, 11.3, 7.9, 6.0 Hz, H_b-1), 3.84 (d, 3.0 Hz, OCH_3), 3.80 (d, 3.0 Hz, OCH_3), 3.47 (m, 1H, H-2), 2.11 (m, 2H, H-6), 1.43 (m, 2H, H-7), 1.29 (m, 20H), 0.89 (t, 7.0 Hz, CH_3); ^{13}C NMR (CD_3OD) δ 137.2 (C-5), 128.2 (C-4), 70.7 (C-3), 65.7 (C-1), 56.7 (C-2), 55.8 ($2OCH_3$), 33.4 (C-6), 33.1, 31.0, 30.9 (3C), 30.7, 30.6, 30.5, 30.2(C-7), 23.8, 14.5 (CH_3); ^{31}P NMR (CD_3OD, $CH_2[P(O)(OH)_2]_2$) δ −18.1.

[§] Compound **8**: $R_f = 0.63$ (12:1, $CHCl_3$:CH_3OH); mp 61–62°C; ^1H NMR (500 MHz, CD_3OD) δ 5.72 (dtd, 15.4, 6.8, 1.2 Hz, H-5), 5.42 (ddt, 15.4, 6.8, 1.4 Hz, 4-H), 7.6 Hz, 4.10 (m, 2H, H-1), 4.02 (m, 2H, H-2, H-3), 3.78 (d, 3H, 3.0 Hz, OCH_3), 3.75 (d, 3H, 3.0 Hz, OCH_3), 2.18 (t, 2H, 7.3 Hz, $NCOCH_2$), 2.03 (m, 2H, H-6), 1.58 (m, 2H, $HNCOCH_2CH_2$), 1.38 (m, 2H, H-7), 1.27 (m, 44H), 0.90 (t, 6H, 7.4 Hz, CH_3); ^{31}P NMR (500 MHz, CD_3OD, $CH_2[P(O)(OH)_2]_2$) δ −17.8; EI-MS (99:1, CH_3OH:CH_3COOH) m/z 1291($2M^+$, 43), 1290 ($[2M]-H]^+$, 30), 1052 ($[2M]^+-COC_{15}H_{31}$, 15), 744 (55), 646.4 ($[M+H]^+$, 25), 645.3 (M^+, 68), 627.6 ($[M^+-H_2O$, 28), 519.8 (30); calcd for $C_{36}H_{72}NO_6P$ m/z 645.5.

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13. To confirm selectivity of this reaction the formed phosphate ester was acylated with an excess of *S*-(+)-MTPA chloride in pyridine[10,16] resulting in a large downfield shift of the methine H-3 proton ($\Delta\delta_{CDCl_3} = 1.33$ ppm) and a small upfield shift of the methylene H-1 protons ($\Delta\delta_{CDCl_3} = 0.30$ (H_a-1) and 0.10 (H_b-1) ppm, respectively). In addition, the recorded ^1H and ^{13}C NMR spectra of the 3-*O*-MTPA derivative of **6** showed only one set of ^1H and ^{13}C resonance signals.
14. Compounds **3** and **4a** were identical with or match the reported data with respect to TLC, NMR and MS.[9f,g,k]
15. The lower reactivity of the primary hydroxyl group of ceramide toward electrophiles (glucosyl donors) was also observed, see: Matt, M. A.; Polt, R. *J. Org. Chem.* **1993**, *58*, 4309–4314, and references cited therein.
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