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EFFICIENT SYNTHESIS OF SPHINGOSINE-1-PHOSPHONATE AND HOMO-SPHINGOSINE-1-PHOSPHONATE

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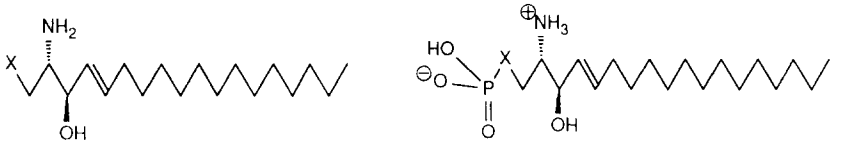
Abstract: Sphingosine can be selectively transformed into 2-N,3-O-protected 1-O-mesyl derivative **8**. Transformation into the bromide, Michaelis-Arbusov reaction with trimethyl phosphite, and then removal of all protective groups with LiOH afforded sphingosine-1-phosphonate (**4**) in high overall yield. Chain extension of **8** with KCN and ensuing reduction led to homosphingosine derivative **10** and also to homo-1-deoxysphingosine (**5**). 1-O-Mesylation of **10** led via the same sequence of reactions finally to homo-sphingosine-1-phosphonate (**6**). © 1997 Elsevier Science Ltd. All rights reserved.

Scheme 1



1: X = OH
3: X = H
5: X = CH_3

2: X = O
4: X = -
6: X = CH_2

Phosphosphingolipids, derivatives of sphingosine (Scheme 1, **1**), play an important role as membrane constituents. For instance, sphingomyelin was found in a variety of different cell types[1]. The phosphorylated metabolites of sphingomyelin, especially sphingosine-1-phosphate (**2**), sphingosine-1-phosphocholine (lysosphingomyelin), and ceramide-1-phosphate were found to participate in cell regulation and transmembrane signaling[2-5]. Recently, sphingosine-1-phosphate (**2**) has received special attention because it exhibits important second messenger properties[6]: the levels of **2** increase rapidly and transiently in response to fetal calf serum, platelet derived growth factor (PDGF)[7], and TPA[8]; it is a mitogen in several cell types[9], it is a strong inhibitor of cell motility and phagokinesis and it inhibits chemoinvasion of tumor cells[10]; **2** also induces platelet shape changes, aggregation, and intracellular calcium mobilisation, thus it may play a role in thrombosis, hemostasis and wound healing[11]. An efficient synthesis of **2** has been recently reported[12].

In order to separate the biological effect of **2** from that of sphingosine (**1**), availability of a hydrolytically stable structural analogue of **2** is highly desirable. Therefore, we initiated a program to synthesize the corresponding phosphonate **4**, which is a derivative of 1-deoxy-sphingosine (**3**). However, considering the

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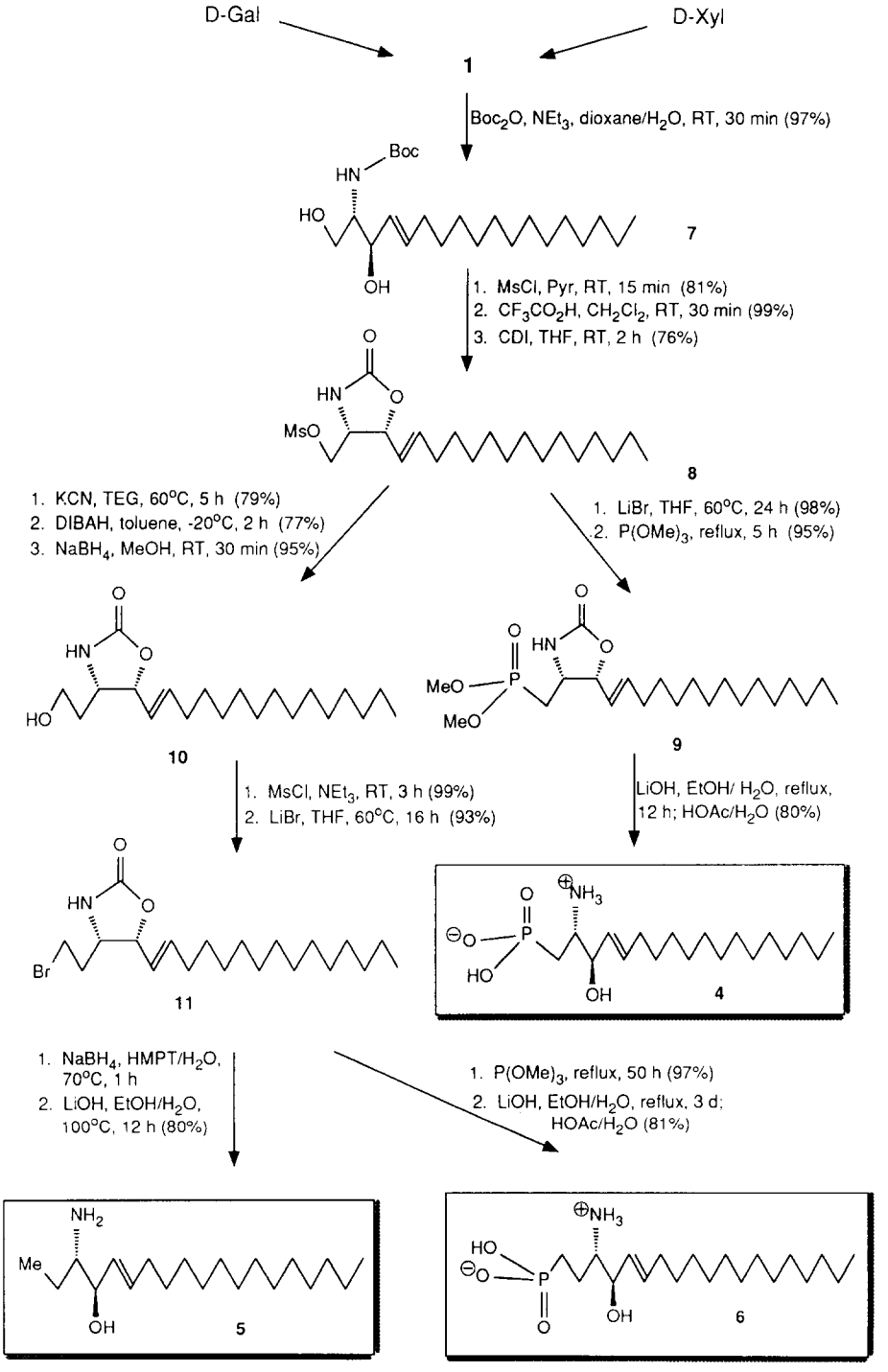
Scheme 2


D-Gal
D-Xyl
1
Boc2O, NEt3, dioxane/H2O, RT, 30 min (97%)
Boc
HN
HO
OH
7
1. MsCl, Pyr, RT, 15 min (81%)
2. CF3CO2H, CH2Cl2, RT, 30 min (99%)
3. CDI, THF, RT, 2 h (76%)
O
HN
O
MsO
8
1. KCN, TEG, 60°C, 5 h (79%)
2. DIBAH, toluene, -20°C, 2 h (77%)
3. NaBH4, MeOH, RT, 30 min (95%)
1. LiBr, THF, 60°C, 24 h (98%)
2. P(OMe)3, reflux, 5 h (95%)
HO
10
MeO
P
MeO
9
1. MsCl, NEt3, RT, 3 h (99%)
2. LiBr, THF, 60°C, 16 h (93%)
LiOH, EtOH/ H2O, reflux,
12 h; HOAc/H2O (80%)
Br
11
⊕NH3
⊖O
HO
4
1. NaBH4, HMPT/H2O,
70°C, 1 h
2. LiOH, EtOH/H2O,
100°C, 12 h (80%)
1. P(OMe)3, reflux, 50 h (97%)
2. LiOH, EtOH/H2O, reflux, 3 d;
HOAc/H2O (81%)
NH2
Me
OH
5
⊕NH3
HO
⊖O
6

distance between the functional groups in **2**, homo-sphingosine-1-phosphonate **6**, derived from deoxy-homo-sphingosine **5**, may be biologically even more active. Efficient syntheses for compounds **4-6** are described in this paper. To our knowledge only the sphinganine analogue of **4** has been synthesized as racemic mixture[13].

The syntheses of sphingosine derivatives **4-6** are based on C_{18}-sphingosine (**1**) which is available from very different starting materials[14]. The efficiency of the D-galactose or D-xylose based synthesis was reason to use this approach[15]. N-Protection of **1** with di-*tert*-butyl-dicarbonate (Boc_2O) in the presence of triethylamine afforded **7** (Scheme 2). Regioselective mesylation with methanesulfonyl chloride (Ms-Cl) in pyridine at the primary hydroxy group, then acid catalyzed removal of the Boc group and treatment with carbonyl-diimidazole (CDI) led to cyclic urethane **8** in high yield. Exchange of the mesyloxy group by bromide with LiBr in THF and then performing a Michaelis-Arbusov reaction with trimethyl phosphite afforded dimethyl phosphonate **9** in 95% yield. Treatment of **9** with LiOH in ethanol/water led to ester and urethane cleavage; after protonation with acetic acid target molecule **4** was obtained as solid material in 80% yield. The structural assignment is based on NMR (^1H, ^{13}C, ^{31}P) and MS data[16] and on elemental analysis.

For the synthesis of target molecules **5** and **6**, C_1-chain extension of **1** was required. To this aim intermediate **8** was treated with KCN in triethyleneglycol (TEG) as solvent. Reduction of the cyano group with DIBAH in toluene at -20 °C afforded after hydrolysis the corresponding aldehyde which gave on reduction with $NaBH_4$ the desired homo-sphingosine derivative **10** in high overall yield. Treatment of **10** with MsCl in pyridine and then with LiBr furnished bromide **11** in practically quantitative yield. Hydrogenolysis of the carbon-bromine bond with $NaBH_4$ in HMPT and then LiOH treatment led to loss of the urethane moiety, thus furnishing **5**[16] in very high yield. Michaelis-Arbusov reaction of **11** with trimethylphosphite gave the corresponding dimethyl phosphonate which on treatment with LiOH and protonation with acetic acid afforded target molecule **6** as solid material; **6** was again characterized by NMR and MS data[16] and by elemental analysis. The biological studies with target molecules **5** and **6** are under investigation[17].

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References and Notes

1. Barenholz, Y., Thomson, T.E., *Biochim. Biophys. Acta* **1980**, *604*, 129-158; Barenholz, Y., Gatt, S. in *Phospholipids* (Hawthorne, J.N., Ansell, G.B., Eds.), Elsevier Biochemical Press, Amsterdam **1982**, pp. 129-177.
2. Hannun, Y.A., Bell, R.M., *Science* **1989**, *243*, 500-507; Hannun, Y.A., *J. Biol. Chem.* **1994**, *269*, 3125-3128.
3. Olivera, A., Spiegel, S., *Glyconjugate J.* **1994**, *9*, 110-117; Spiegel, S., Milstien, S., *J. Membr. Biol.* **1995**, *146*, 225-237.
4. Merill, A.H., Jones, D.D., *Biochim. Biophys. Acta* **1990**, *1004*, 1-12.
5. Kolesnick, R., Fuks, Z., *J. Exp. Med.* **1995**, *181*, 1949-1952.

6. Cuvillier, O., Pirianov, G., Klenser, B., Vanek, P.G., Coso, O.A., Gutkind, J.S., Spiegel, S., *Nature* **1996**, *381*, 800-805; Berger, A., Bittman, R., Schmidt, R.R., Spiegel, S., *Molec. Pharmacol.*, submitted.

7. Olivera, A., Spiegel, S., *Nature* **1993**, *365*, 557-560.

8. Mazurek, N., Megidish, T., Hakomori, S.-i., Igarashi, Y., *Biochem. Biophys. Res. Commun.* **1994**, *198*, 1-9.

9. Goodemate, K.A., Mattie, M.A., Berger, A., Spiegel, S., *J. Biol. Chem.* **1995**, *270*, 10272-10275; Miyake, Y., Kozutsumi, Y., Nakamura, S., Fujita, T., Kawasaki, T., *Biochem. Biophys. Res. Commun.* **1995**, *211*, 396-403.

10. Sadahira, Y., Ruan, F., Hakomori, S.-i., Igarashi, Y., *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9686-9690.

11. Yatomi, Y., Ruan, F., Hakomori, S.-i., Igarashi, Y., *Blood* **1995**, *86*, 193-202.

12. Kratzer, B., Schmidt, R.R., *Liebigs Ann. Chem.* **1995**, 957-963; and references therein.

13. Stoffel, W., Grol, M., *Chem. Phys. Lipids* **1974**, *13*, 372-388.

14. Devant, R.M., *Kontakte* **1992**(3) 11-30; Schmidt, R.R., Bär, T., Wild, R., *Synthesis* **1995**, 868-876.

15. Schmidt, R.R., Zimmermann, P., *Tetrahedron Lett.* **1986**, *27*, 481-484; *Liebigs Ann. Chem.* **1988**, 663-667.

16. Selected physical data: **4**: TLC(n-butanol/acetic acid/water 5:1:1). R_f = 0.42, $[\alpha]_D$ -2.6° (c 0.5 acetic acid), mp. = 190°C (decomp.). ^{1}H NMR (250 MHz, CD_3COOD): δ 0.79 (t, 3 H, H-18), δ 1.18-1.35 (m, 22 H, 11 CH_2), δ 1.90-2.05 (m, 4 H, H-1_a, -1_b, -6_a, -6_b), δ 3.73-3.81 (m, 1 H, H-2), δ 4.32-4.38 (m, 1 H, H-3), δ 5.40 (dd, $J_{3,4}$ = 6.3 Hz, $J_{4,5}$ = 15.5 Hz, 1 H, H-4), δ 5.79 (ddd, $J_{5,6a}$ = 6.8 Hz, $J_{5,6b}$ = 6.8 Hz, 1 H, H-5). ^{13}C NMR (151 MHz, CD_3COOD): δ 14.40 (C-18), δ 27.00 (d, $J_{1,P}$ = 135.5 Hz, C-1), δ 33.20 (C-6), δ 53.47 (C-2), δ 73.13 (d, $J_{3,P}$ = 16.2 Hz, C-3), δ 126.59 (C-4), δ 137.36 (C-5). ^{31}P NMR (162 MHz, CD_3COOD): δ 26.25. FAB-MS (negative mode): Matrix: dimethylsulfoxide/nitrobenzylalcohol/glycerol 1:1:1, m/z (%): 362 (40) [M-H$^+$]. $C_{18}H_{38}NO_4P$ (363.5): Anal. Calc. for C 59.48, H 10.54, N 3.85; Found C 59.02, H 10.24, N 4.08. **5**: δ 0.73-0.95 (m, 6 H, H-1, H-19), δ 1.07-1.55 (m, 24 H, H-2_a,- 2_b, 11 CH_2), δ 1.93-2.01 (m, 2 H, H-7_a, -7_b), δ 2.98 (m, 1 H, H-3), δ 4.22 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 6.3 Hz, 1 H, H-4), δ 5.29 (dddd, $J_{5,6}$ = 15.3 Hz, $J_{5,7a}$ < 1.0 Hz, $J_{5,7b}$ < 1.0 Hz, 1 H, H-5), δ 5.79 (ddd, $J_{6,7a}$ = 6.7 Hz, $J_{6,7b}$ = 6.7 Hz, 1 H, H-6). **6**: TLC (n-butanol/acetic acid/water 5:1:1): R_f = 0.41, $[\alpha]_D$ -1.0° (c 0.2 in acetic acid), mp. = 150°C (decomp.). ^{1}H NMR (250 MHz, CD_3COOD): δ 0.79 (t, 3 H, H-19), δ 1.15-1.39 (m, 22 H, 11 CH_2), δ 1.70-2.06 (m, 6 H, H-1_a, -1_b, -2_a, -2_b, -7_a, -7_b), δ 3.42-3.52 (m, 1 H, H-3), δ 4.29-4.33 (m, 1 H, H-4), δ 5.43 (dd, $J_{4,5}$ = 6.6 Hz, $J_{5,6}$ = 15.7 Hz, 1 H, H-5), δ 5.79 (ddd, $J_{6,7a}$ = 6.7 Hz, $J_{6,7b}$ = 6.7 Hz, 1 H, H-6). ^{13}C NMR (151 MHz, CD_3COOD): δ 14.41 (C-19), δ 27.5 (d, $J_{1,P}$ = 136.0 Hz, C-1), δ 32.94 (C-7), δ 58.06 (C-3), δ = 73.21 (C-4), δ 126.67 (C-5), δ 137.25 (C-6). ^{31}P NMR (162 NMz, CD_3COOD): δ 30.78. FAB-MS (negative mode): Matrix: dimethylsulfoxide/nitrobenzylalcohol/glycerol 1:1:1, m/z (%): 376 (50) [M-H$^+$], 753 (5) [(2M)-H$^+$]. $C_{19}H_{40}NO_4P$ (377.5): Anal. Calc. for C 60.45, H 10.68, N 3.71; Found C 60.16, H 10.36, N 3.80.

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