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An Efficient Synthesis of Sphingosine-1-Phosphate

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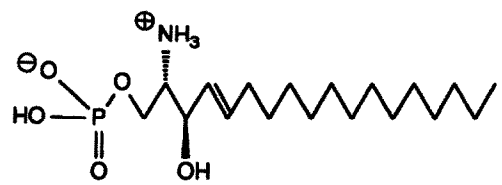
Abstract: The total synthesis of D-*erythro*-sphingosine-1-phosphate (2) via the phosphoramidite approach, starting from 3-O-TBDMS-protected D-*erythro*-azidosphingosine 3 is described.

Sphingosine (1) and related long chain sphingoid bases are building blocks of sphingolipids, which are important membrane constituents[1]. Interest in these intermediates of the sphingolipid metabolism increased, when sphingosine was found to inhibit strongly and specifically protein kinase C, a pivotal regulatory enzyme in cell growth[2]. In contrast, other results demonstrated, that sphingosine, at low concentrations, stimulates DNA synthesis and cell proliferation of quiescent cultures of Swiss 3T3 fibroblasts, in a protein kinase C independent way[3].

1

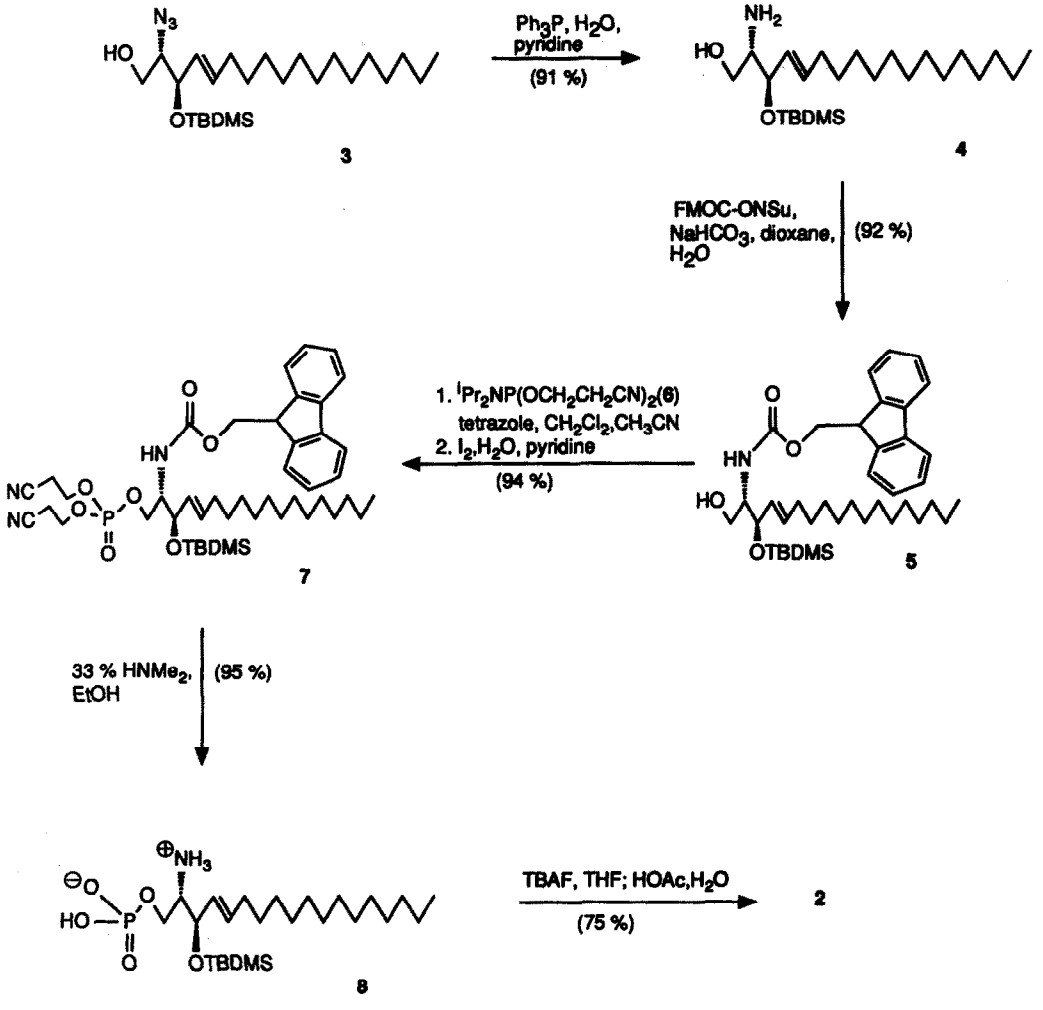


2

Recently metabolites of sphingosine have been shown to be produced in cells and to have potent effects on cell growth[4,5]. Thus, sphingosine-1-phosphate (2) which is produced from sphingosine by the action of a specific kinase[6], proved to be a very potent mitogen in Swiss 3T3 fibroblasts[5]. Furthermore, there is evidence that 2 induces a rapid and profound release of calcium from IP_3 - sensitive and insensitive intercellular pools in permeabilized smooth muscle cells[7] and that it is a very potent calcium-mobilizing agonist in viable 3T3 fibroblasts[5]. In order to examine the effects of this potential second messenger[8], a chemical synthesis is required which provides sufficient amounts of pure sphingosine-1-phosphate (2).

The synthetic attempts of Weiss[9] resulted in sphinganine-1-phosphate due to hydrogenolytic removal of O-benzyl protective groups at the phosphate moiety. In 1989 an enzymatic synthesis of 2 was reported[10]. In this preparation lysosphingomyelin was treated with phospholipase D, isolated from *Streptomyces chromofuscus*.

After purification by selective precipitation and differential extraction, milligram quantities of the D-*erythro*- and the L-*threo*-sphingosine-1-phosphate could be obtained. The occurrence of the unnatural L-*threo*-isomer was a result of the lysosphingomyelin used, which consisted of a mixture of the two isomers. We now report on a practical and short route to sphingosine-1-phosphate (**2**), starting from the 3-O-*tert*-butyldimethylsilyl (TBDMS) protected azidosphingosine **3**, using a phosphitamide approach which is based upon the monofunctional phosphitylation reagent bis(2-cyanoethoxy)(diisopropylamino)phosphine[11] (**6**). We chose this method, because it was successfully applied to the phosphorylation of biomolecules like amino acids, peptides, DNA-fragments[11], carbohydrates[12] and with bifunctional phosphitylation reagents in the synthesis of sphingomyelin[13] and ceramide phosphoinositol derivatives[14].



The readily available azide **3**[15] was converted to the amine **4** with two equivalents of triphenylphosphine and an excess of water in pyridine, according to a procedure described by Z. Dong and J.A. Butcher Jr.[16]. Selective, base labile protection of the amino group was achieved by treatment of the amine **4** with 9-fluorenylmethylsuccinimidyl carbonate[17] in the system dioxane/water/$NaHCO_3$, to yield compound **5**[18] in 91%. The FMOC-derivative **5** (1 eq) was now condensed with the monofunctional phosphitylation reagent **6** (1.6 eq) in the solvent system dichloromethane/acetonitrile (1:1) under a nitrogen atmosphere in the presence of freshly sublimated tetrazole (2.0 eq). TLC-analysis of the reaction mixture after 30 min revealed the absence of **5** and the formation of a product with higher R_f-value. The phosphite intermediate thus obtained was oxidized in situ by successive addition of iodine (0.4 m solution in pyridine/water/dichloromethane, 3:1:1), to afford the phosphotriester **7**[19] in 94% yield. Treatment of **7** with a solution of dimethylamine (33% in dry ethanol) at 45 °C for three days caused quantitative removal of the FMOC and both 2-cyanoethyl groups, respectively, to afford compound **8**. The ^{1}H-NMR spectra of **8** in $CDCl_3$ showed broad signals due to the strong amphiphilic character of this molecule (betaine **8** was insoluble in more polar solvents like MeOH or DMSO). Deprotection of the allylic hydroxy group was accomplished by using tetrabutylammonium fluoride (TBAF) (1.2 eq) in THF at 45 °C for three days to give the desired sphingosine-1-phosphate (**2**). Further purification appeared to be very difficult, because of the low solubility of **2** in most organic and inorganic solvents[10]. However, addition of water to a THF solution of **2**, ensuing solution of the precipitate in hot glacial acetic acid, reprecipitation by addition of water, and successive washing of the residue with water, acetone and ether furnished pure sphingosine-1-phosphate **2** in 75% yield which had R_F and MS data in accordance with literature values[10,20].

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18. ^{1}H-NMR data (250 MHz, $CDCl_3$) δ = 0.01, 0.03 (2 s, 6 H, $Si[CH_3]_2$), 0.85 (m, 12 H, CH_3, $SiC[CH_3]_3$), 1.24 (m, 22 H, 11 CH_2), 2.01 (m, 2 H, H-$6_{a,b}$), 2.93 (d, J = 9.4 Hz, 1 H, OH), 3.55-3.63 (m, 2 H, H-1_a, H-2), 4.05 (m, $J_{1a,1b}$ = 11.1 Hz, 1 H, H-1_b), 4.20 (t, J = 6.7 Hz, 1 H, $NCOOCH_2CH$), 4.37 (d, J = 6.7 Hz, 2 H, $NCOOCH_2CH$), 4.47 (m, 1 H, H-3), 5.40-5.49 (dd, $J_{3,4}$ = 5.9 Hz, $J_{4,5}$ = 15.5 Hz, 1 H, H-4), 5.56 (d, J = 7.9 Hz, 1 H, NH), 5.66-5.76 (dt, $J_{4,5}$ = 15.5 Hz, $J_{5,6}$ = 6.6 Hz, 1 H, H-5), 7.26-7.76 (m, 8 H, aryl). Anal. Calc. for $C_{39}H_{61}N_1O_4Si_1$: C 73.65, H 9.66, N 2.2; Found: C 73.58, H 9.80, N 2.0.

19. ^{1}H-NMR data (250 MHz, $CDCl_3$) δ = 0.01, 0.03 (2 s, 6 H, $Si[CH_3]_2$), 0.85 (m, 12 H, CH_3, $SiC[CH_3]_3$), 1.24 (m, 22 H, 11 CH_2), 2.01 (m, 2 H, H-$6_{a,b}$), 2.67-2.73 (m, 4 H, 2 CH_2CN), 3.87 (m, 1 H, H-2), 4.13-4.32 (m, 9 H, H-1_a, H-1_b, $NCOOCH_2$, $NCOOCH_2CH$, 2 CH_2CH_2CN), 4.40 (dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 7.2 Hz, 1 H, H-3), 5.16 (d, J = 9.3 Hz, 1 H, NH), 5.37 (dd, $J_{3,4}$ = 7.2 Hz, $J_{4,5}$ = 15.3 Hz, 1 H, H-4), 5.65 (dt, $J_{4,5}$ = 15.3 Hz, $J_{5,6}$ = 6.6 Hz, 1 H, H-5), 7.26-7.75 (m, 8 H, aryl).

^{31}P-NMR data (161 MHz, $CDCl_3$) δ_P = - 1.25.

Anal. Calc. for $C_{45}H_{68}N_3O_7P_1Si_1$: C 65.74, H 8.33, N 5.11; Found: C 65.66, H 8.44, N 5.00.

20. ^{1}H-NMR data (250 MHz, CD_3COOD) δ = 0.87 (t, J = 6.9 Hz, 3 H, CH_3), 1.28 (m, 22 H, 11 CH_2), 2.04 (m, 2 H, H-$6_{a,b}$), 3.67 (m, 1 H, H-2), 4.24 (m, 2 H, H-$1_{a,b}$), 4.46 (dd, $J_{2,3}$ = $J_{3,4}$ = 6.6 Hz, 1 H, H-3), 5.49-5.58 (dd, $J_{3,4}$ = 6.6 Hz, $J_{4,5}$ = 15.3 Hz, 1 H, H-4), 5.85-5.96 (dt, $J_{4,5}$ = 15.3 Hz, $J_{5,6}$ = 6.4 Hz, 1 H, H-5).

^{31}P-NMR data (161 MHz, CD_3COOD) δ_P = 2.42; mass spectrum (FAB), m/z 380 (MH^+); R_F = 0.48 [n-butanol]/acetic acid/water (3:1:1)] (Lit.[10]: mass spectrum (FAB), m/z 380 (MH^+); R_F = 0.48 [n-butanol/acetic acid/water (3:1:1)]).

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