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Synthesis of an Inhibitor of Sphingosine-1-Phosphate Lyase

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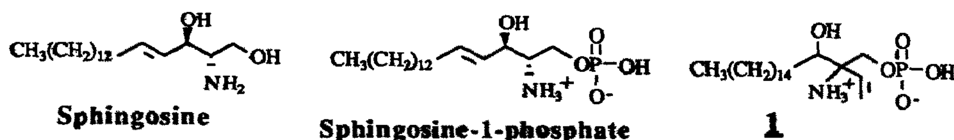
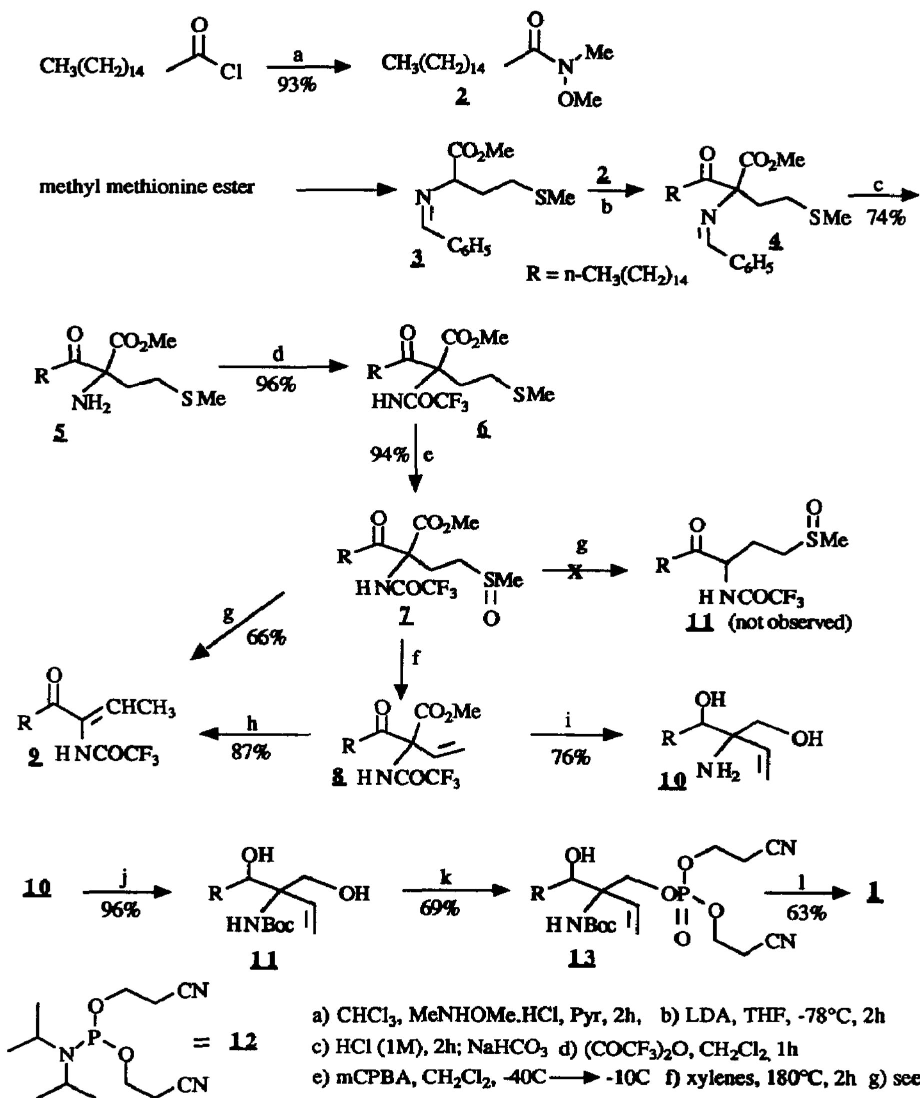
Abstract: The 2-vinyl dihydrosphingosine-1-phosphate **1** was synthesized and tested for inhibition of sphingosine-1-phosphate lyase. The carbon skeleton was prepared by acylation of a methionine imine with N-methoxy-N-methyl palmitamide. The vinyl group was generated by thermal sulfoxide elimination. Reduction of both carbonyl groups and deprotection gave 2-vinyl dihydrosphingosine. The phosphate group was introduced by means of bis(2-cyanoethyl)-N,N-diisopropyl-phosphoramidite as monofunctional phosphitylation reagent.

Sphingolipids constitute a large class of biologicaly important compounds. Both glycosides (cerebrosides, gangliosides) and non-glycosylated derivatives (sphingomyelin, ceramides) are important constituents of cellular membranes [1]. The principal component of sphingolipids is almost invariably the long chain base, sphingosine. Recently several reports have shown sphingosine to be a potent inhibitor of the regulatory enzyme protein kinase C (PKC) both *in vivo* and *in vitro* [2]. In addition sphingosine-1-phosphate causes rapid translocation of calcium from intracellular stores and a regulatory role has been suggested for this compound [3].



Previously it was believed that the major role of sphingosine-1-phosphate was its involvement in the catabolic pathway for sphingosine. In this pathway sphingosine-1-phosphate is produced from sphingosine by the action of a specific kinase in the cytoplasm [4]. Sphingosine-1-phosphate is then cleaved to 2-hexadecenal and phosphoryl ethanolamine by sphingosine-1-phosphate lyase (aldolase) located in the endoplasmic reticulum [5]. The preparation of irreversible inhibitors would aid in the isolation and purification of the lyase. A second use for enzyme inhibitors would be to provide information on the biological effects of blocking sphingosine catabolism *in vivo*. Our approach to the design of inhibitors is based upon the facts that sphingosine-1-phosphate lyase is a pyridoxal phosphate (PLP) dependent enzyme [6], and that many PLP-dependent amino acid decarboxylases are inhibited by α-amino derivatives that possess an unsaturation in the β,δ position [7]. In this paper we describe the synthesis of **1** as a structural analog of sphingosine. To the best of our knowledge only one inhibitor of sphingosine-1-phosphate lyase has been described in the literature. This is a phosphonate analog which acts as a competitive inhibitor, and is highly toxic [8].



a) $CHCl_3$, MeNHOMe.HCl, Pyr, 2h, b) LDA, THF, -78°C, 2h
c) HCl (1M), 2h; $NaHCO_3$ d) $(COCF_3)_2O$, CH_2Cl_2, 1h
e) mCPBA, CH_2Cl_2, -40C ⟶ -10C f) xylenes, 180°C, 2h g) see table I
h) xylenes, 190°C, 2h i) $Ca(BH_4)_2$, EtOH:THF 1:1, 20h j) $(Boc)_2O$, DMAP, 3h
k) 1- 12, CH_2Cl_2, tetrazole, 1 h 2- I_2 (0.4M in pyridine:H_2O:CH_2Cl_2 3:1:1), 15 min
l) 1- TFAA, CH_2Cl_2, 30 min 2- Sat. ammonia in MeOH, 40°C, 5h

Treatment of palmitoyl chloride with N,O-dimethylhydroxylamine hydrochloride in the presence of pyridine according to Weinreb [9a] lead to amide 2 . Acylation with Schiff base 3, derived from methionine, following the procedure first reported by Weinreb and recently employed in the synthesis of mono and polyketo esters [9b] yielded the keto ester 4. The condensation was accomplished by adding a solution of the amide to the lithium enolate of Schiff base 3 in THF at -70°C. The product was isolated after hydrolysis of the intermediate chelate. The reaction was clean (TLC, NMR) with few side products, although a small amount of

unreacted amide was detected. Owing to its instability to distillation and chromatography (partial hydrolysis of the Schiff base occurred), the imine **4** was usually not purified. After removal of the benzylidene group with HCl (1M, ether), subsequent protection of the free amino group with trifluoroacetic anhydride provided the amide **6**.

Oxidation of **6** with m-chloroperbenzoic acid (mCPBA) afforded the sulfoxide **7** as a mixture of 2 diastereoisomers, which were not separated. The thermolysis of **7** was carried out at 180°C in xylenes. After removal of the unreacted sulfoxide by short column chromatography, compound **8** was isolated in 66% yield. Surprisingly we have observed significant formation of **9**, which is the by-product of decarbomethoxylation and isomerization. Previous reports on the synthesis of vinylglycine and its derivatives have discussed their sensitivity towards olefinic isomerization [10], although in these cases no decarboxylation was observed. A very similar decarboxylation with isomerization was reported following saponification of a β,δ- unsaturated keto ester [11]. It is likely that **9** is formed from **7** by initial sulfoxide elimination followed by decarbomethoxylation and isomerization. This hypothesis is supported by the facts that isolated vinyl keto ester **8** was completely converted to **9** by heating at 190°C for 2h, and that the sulfoxide **11** has never been detected among the reaction products. The reaction parameters of the thermolysis (temperature, time, base as sulfenic acid trapping agent) were optimized in order to obtain maximum rate of conversion with minimum formation of **9**. Results obtained under various conditions are summarized in table 1.

Table 1. Dehydrosulfenylation of 7

Solvent (base)	Temp°C/Time (h)	Yield of 8/9
diglyme (DIPEA)* n=2	145 / 2	0 / 46
diglyme (DIPEA)* n=2	160 / 3.5	8.5 / 50
diglyme (CaCO3) n=1	160 / 2	0 / 45
xylenes n=1	135 / 5.5	9 / 0
xylenes n=4	180 / 2	66 / 10
xylenes n=1	200 / 2	0 / 34

*DIPEA=diisopropylethylamine
n = number of repetitions
Ratio of olefinic products was determined by separation of **8** and **9**

The intermediate **8** was smothly reduced and deprotected in one step to the aminodiol **1** by means of calcium borohydride (prepared *in situ* from $CaCl_2$ and $NaBH_4$) [12]. Vinyl-dihydrosphingosine **10** was isolated as an inseparable mixture of two diastereoisomers. The phosphate derivative of **10** was prepared by using the monofunctional phosphitylation reagent **12**. This reagent was successfuly applied to the phosphorylation of biomolecules especially oligonucleotides [13]. The N-Boc derivative **11** was condensed with 1 eq of **12** in CH_2Cl_2 and in the presence of 1H-tetrazole. The phosphite intermediate was oxidized *in situ* by addition of iodine (0.4 M in pyridine:water:dichloromethane 3:1:1) to give the phosphotriester **13** in 69% yield. Treatment of **13** with trifluoroacetic acid in CH_2Cl_2 resulted in complete removal of the Boc group, the free amine was not isolated. Treatment with a saturated solution of ammonia in methanol for 5 h at 40°C caused complete removal of the two β-cyanoethyl groups. Crude **1** was purified by selective precipitation as follows. The solution (10 mL, obtained from 300 mg of **13**) was evaporated and crude **1** was

dissolved in 5 mL of hot acetic acid, the solution was cooled to 0°C and 2 mL of water were added. The solution was kept at 0°C for 10 min and centrifuged, the supernatant was removed, this step was repeated again. The final pellet was washed with 3 mL of cold water, 5 mL of diethyl ether, dried under vacuum for 5 h, to yield pure **1** as a white solid and as a mixture of two diastereoisomers. Further separation of diastereoisomers appeared to be very difficult due to the low ΔR_f value. Although natural sphingosine has the 2S, 3R configuration, it is difficult to predict which isomer of compound **1** would be most active against sphingosine-1-phosphate lyase. Once the most active stereoisomer has been identified, selectivity could be introduced into the synthesis during the acylation of the methionine enolate [14] and at the ketone reduction step.

Biological experiments (*in vitro*) using a rat liver microsomal preparation as enzyme source show that compound **1** is a potent inhibitor of sphingosine-1-phosphate lyase (IC_{50} = 2.4 μM).

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Compound 1: ^1H NMR (CD_3OD, 300 MHz): 6.14 (dd, J_1 = 10Hz, J_2 = 16.5Hz, 0.5H); 5.80 (dd, J_1 = 10Hz, J_2 = 16.5Hz, 0.5H); 5.50 (m, 2H); 4.5-4.26 (m, 2H); 3.90 (dd, J_1 = 10Hz, J_2 = 21Hz, 1H); 1.63-1.22 (m, 28H); 0.9 (t, J = 7Hz, 3H). Neg.Ion.FAB-MS m/z 406 (M-1, 5); 388 (74); 97 (33); 79 (100). Anal. Calc for $C_{20}H_{42}NO_5P$: C 58.94, H 10.38, N 3.43, P 7.60; Found: C 58.63, H 10.19, N 3.38, P 7.25. Full experimental details and spectral data are available from the authors upon request.

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