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Synthesis of Cyclic Sphingosine 1,3-Phosphate (cSPP) Through a Photolytic Reaction

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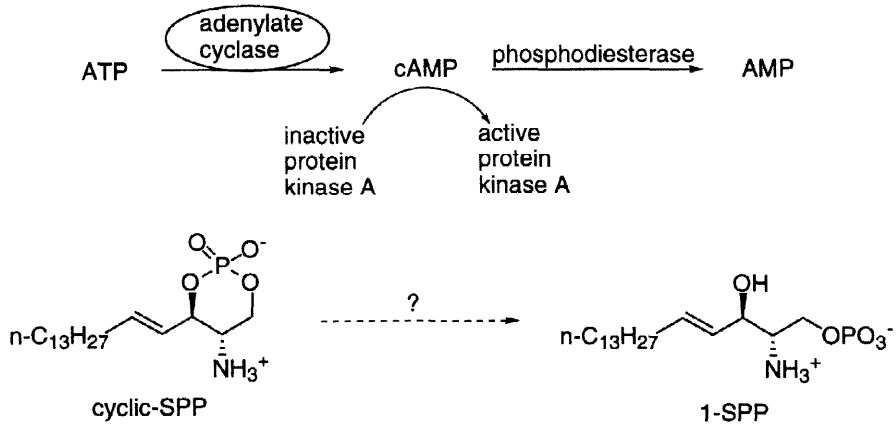
Abstract: The synthesis of cyclic sphingosine 1,3-phosphate through photolytic methodology is described starting from D-*erythro*-sphingosine. A bifunctional phosphorylating reagent, 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, is used to introduce the cyclic 1,3-phosphate moiety. This procedure generates cSPP in four steps and in 36% overall yield.

Sphingolipid metabolites, such as ceramide, sphingosine, and sphingosine-1-phosphate (SPP), are emerging as a novel class of lipid second messengers.[1] Ceramide, a sphingolipid generated by the hydrolysis of membrane-associated sphingomyelin, is an important regulatory participant of programmed cell death (apoptosis). Conversely, sphingosine and sphingosine-1-phosphate (SPP), metabolites of ceramide, induce mitogenesis and have been implicated as second messengers in cellular proliferation induced by platelet-derived growth factor and serum. It has been suggested that the dynamic balance between intracellular ceramide and SPP levels may determine the cell fate.[2] The signaling roles of ceramide and sphingosine produced through the degradation of membrane sphingolipids are currently receiving significant attention in the biochemical and biomedical research fields. The biological effects of SPP, such as calcium mobilization,[3] regulation of the levels of cAMP,[4] activation of mitogen-activated protein kinase,[5] and G-protein binding,[6] have been studied extensively. Moreover, the chemical synthesis[7-10] of sphingosine analogs has become fundamental to further advancing our understanding of sphingolipid-based signaling events.

Figure 1.



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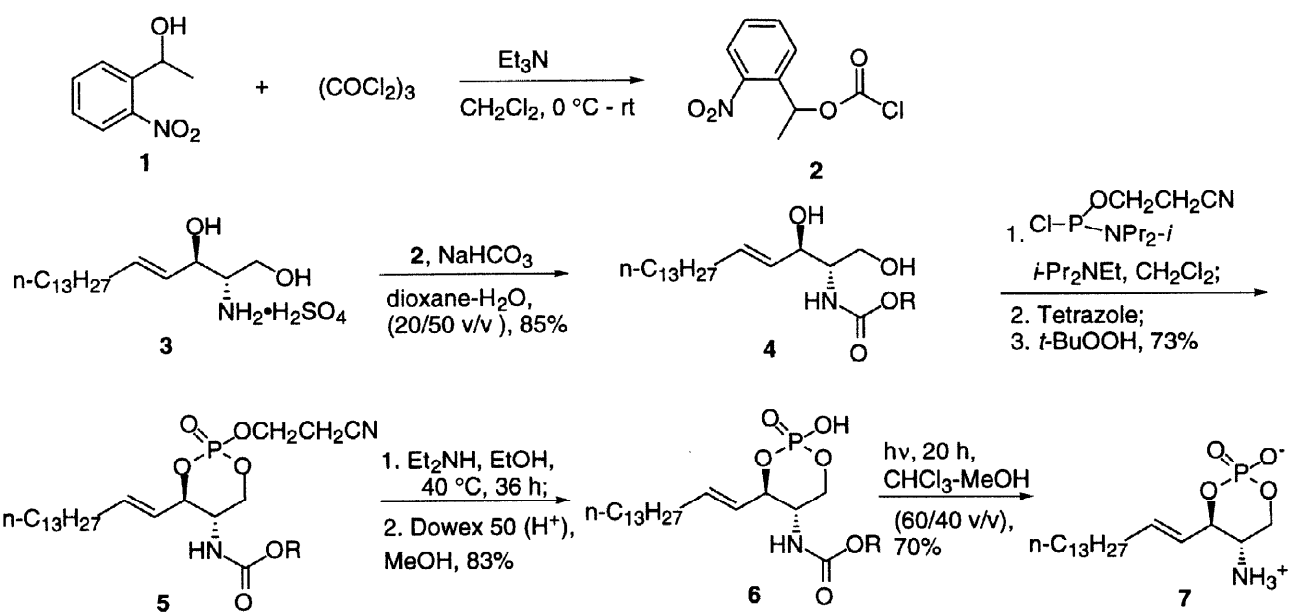
Due to its poor solubility, SPP is poorly able to penetrate through membranes and to be taken up by cells. Recently, we synthesized a more readily solubilized caged SPP.[11] The photolysis of caged SPP-loaded cells was shown to cause a significant increase in DNA synthesis. The use of caged SPP presents a novel approach to mimic signal-dependent activation of sphingosine kinase and to allow the rapid and controlled elevation of intracellular SPP levels. In the continuation of our efforts to learn more about the mechanism by which SPP modulates cellular survival, we chose to synthesize a cyclic sphingosine 1,3-phosphate. Although an analog of sphingomyelin, C6-ceramide-cyclic-1,3-phosphate, was reported in 1993,[12] cSPP has not been reported yet. cSPP is of interest due to the converse activity of SPP compared with ceramide. Through its cyclic phosphate moiety, cSPP resembles cAMP, which is catalytically synthesized by adenylate cyclase from ATP inside the cell. cAMP, in turn, triggers activation of protein kinase A and is eventually broken down to AMP by the action of a phosphodiesterase. Analogously, cSPP might be transformed in cells into SPP by means of ring opening (Figure 1). We present herein the first synthesis of cSPP through a photolytic methodology.

Unlike C6-ceramide-cyclic-1,3-phosphate, cSPP cannot be prepared via dehydration of 1-SPP with *N*, *N'*-dicyclohexylcarbodiimide.[12] The presence of the free amino group is a substantial challenge to its preparation. Therefore, the amino group of sphingosine was protected at the beginning and released in the final step to avoid interference with the cyclic phosphate moiety. Our initial attempts using Boc and Cbz as protecting groups were unsuccessful because byproducts were observed under the harsh conditions employed in the deprotection step, such as trifluoroacetic acid or concentrated HCl. The Fmoc group also proved impractical for this synthesis, as a complex mixture resulted on its attempted removal concurrently with the cyanoethyl group present on the phosphate when using diethylamine. Thus, the use of a photocleavable protecting group[13] came once again to our attention as a possible means for avoiding the present pitfalls. This type of light-sentitive protecting group has been used extensively in the synthesis of caged biologically active molecules (e.g., peptide,[14] glutamate,[15] sphingolipids,[11,16] ADP, and ATP[17]) designed in order to gain insights into fast biological processes.

Scheme 1.



Based upon this notion, the synthesis of cyclic SPP started with D-*erythro*-sphingosine sulfate. First the amino group was transformed into the corresponding carbamate by reaction with the requisite chloroformate **2**, which was readily prepared by reaction of α-methyl-*o*-nitrobenzyl alcohol **1** with triphosgene.[18] 2-Cyanoethyl *N*, *N*-diisopropylchlorophosphoramidite was employed as a bifunctional phosphitylating reagent to furnish the cyclic phosphate moiety by reaction with either hydroxyl group of the diol **4** first in the presence of diisopropyl-ethylamine and by subsequent phosphitylation of the other hydroxyl group in the presence of tetrazole. Oxidation of the resulting cyclic phosphite to the corresponding phosphate **5** was performed in one pot with *t*-butyl hydroperoxide giving 73% overall yield after column chromatography. Phosphate deprotection with a solution of diethylamine (40% in ethanol) at 40 °C for 36 h provided cyclic phosphate **6** after filtration over Dowex® 50W (H^+). Finally, cSPP **7** was released by UV illumination of phosphate **6** using a Pen Ray® lamp through borosilicate glass in a mixed solvent of chloroform and methanol (60/40 v/v) for 20 h. ^{1}H NMR analysis of the crude product revealed that no isomerization of the double bond took place under these conditions. Compound **7** was then readily purified by thin-layer chromatography on silica gel 60 F_{254} with an R_f value of 0.31 in $CHCl_3$/MeOH (60/40 v/v) (detection by staining with a sulfuric acid solution of ammonium molybdate), and obtained as an amorphous white powder after lyophilization.

In contrast to 1-SPP, cSPP is soluble in most polar solvents such as MeOH and H_2O. Its structure was confirmed by NMR and mass spectral analysis.[19] All protons on C-1, C-2, and C-3 were assigned through ^{1}H-^{1}H COSY.[20] Additionally, three different $J_{P\text{-}H}$ values were observed upon comparing the ^{31}P-decoupled ^{1}H NMR with the coupled spectrum. Such differences result from the different dihedral angles among P, O, C-1 (C-3), and the corresponding protons, as well as their electronic environment; these couplings also indicate conformational rigidity within this part of the molecule. In the negative ion mode ESI mass spectrum, a peak at m/z 360.2 $(M\text{-}H)^-$ confirms the required molecular weight. The dimer $(2M\text{-}H)^-$ was also observed at 721.2.

In summary, we describe herein an efficient synthetic route to a new derivative of sphingosine phosphate. Biological studies to evaluate its activity are underway and will be published in due course.

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References and Notes.

1 For reviews, see: (a) Hannun, Y. A. *Science* **1996**, *274*, 1855. (b) Spiegel, S.; Merrill, A. H. Jr. *FASEB J.* **1996**, *10*, 1388. (c) Igarashi, Y. *J. Biochem.* (Japan) **1997**, *122*, 1080.

2 Cuvillier, O.; Pirianov, G.; Kleuser, B.; Vanek, P. G.; Coso, O. A.; Gutkind, S.; Spiegel, S. *Nature* **1996**, *381*, 800.

3 Mattie, M.; Brooker, G.; Spiegel, S. *J. Biol. Chem.* **1994**, *269*, 3181

4 Goodenite, K. A.; Mattie, M. E.; Berger, A.; Spiegel, S. *J. Biol. Chem.* **1995**, *270*, 10272.

5 Conway, A. M.; Pyne, N. J.; Pyne, S. *Biochem. Soc. Trans.* (England) **1997**, *25*, S585.

6 Lee, M.; Brocklyn, J. R. V.; Thangada, S.; Liu, C. H.; Hand, A. R.; Menzeleev, R.; Spiegel, S.; Hla, T. *Science* **1998**, *279*, 1552.

7 Ruan, F.; Sadahira, Y.; Hakomori, S.; Ifarashi, Y. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 973.

8 Kratzer, B.; Schmidt, R. R. *Tetrahedron Lett.* **1993**, *34*, 1761.

9 Boumendjel, A.; Miller, S. P. F. *J. Lipid Res.* **1994**, *35*, 2305.

10 (a) For a review, see: Byun, H.; Bittman, R. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; 97-140. For recent activities, see: (b) Nugent, T. C.; Hundlicky, T. *J. Org. Chem.* **1998**, *63*, 510. (c) Byun, H.; Sadlofsky, J. A.; Bittman, R. *J. Org. Chem.* **1998**, *63*, 2560, and references cited therein.

11 Qiao, L.; Kozikowski, A. P.; Olivera, A.; Spiegel, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 711.

12 Boudker, O.; Futerman, A. H. *J. Biol. Chem.* **1993**, *268*, 22150.

13 Pillai, V. N. R. *Synthesis* **1980**, 1.

14 Walker, J. W.; Gilbert, S. H.; Drummond, R. M.; Yamada, M.; Sreekumar, R.; Carraway, R. E.; Ikebe, M.; Fay, F. S. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1568.

15 Rossi, F. M.; Margulis, M.; Tang, C. M.; Kao, J. P. *J. Biol. Chem.* **1997**, *272*, 32933.

16 Zehavi, U. *Chem. Phys. Lipids* **1997**, *90*, 55.

17 Broustovetsky, N.; Bamberg, E.; Gropp, T.; Klingenberg, M. *Biochemistry* **1997**, *36*, 13865.

18 To a solution of 1.98 g of triphosgene (20 mmol) (**LACHRYMATOR**) in 10 mL of CH_2Cl_2 at 0 °C under N_2, was added slowly a solution of 1.67 g of α-methyl-*o*-nitrobenzyl alcohol (10 mmol) and 1.28 mL of Et_3N (10 mmol) in 10 mL of CH_2Cl_2. The resulting mixture was stirred at 0 °C for another hour before filtration through Celite. The filtrate was concentrated in a well-ventilated hood affording desired chloroformate **2**, which was used directly in the next step without further purification.

19 Data for cyclic SPP: $[\alpha]_D = +19.7°$ (c = 0.53 in $CHCl_3$/MeOH 1/1 (v/v)) ^1H NMR ($CDCl_3/CD_3OD$ 1/1 (v/v), TMS) δ 5.87 (dt, 1H, J = 15.3 Hz (d), 6.6 Hz (t)), 5.30 (dd, 1H, J = 15.3, 7.8 Hz), 4.39 (dt, 1H, $J_{P\text{-}H}$ = 2.4 Hz (d), $J_{H\text{-}H}$ = 8.1 Hz (t), H-3), 4.15 (ddd, 1H, $J_{H\text{-}H}$ = 4.5, 10.8 Hz, $J_{P\text{-}H}$ = 20.7 Hz, H-1), 3.98 (dt, 1H, $J_{P\text{-}H}$ = 3.9 Hz (d), $J_{H\text{-}H}$ = 10.8 Hz (t), H-1), 2.87 (br s, 1H, H-2), 2.10 (q, 1H, J = 6.9 Hz), 1.43-1.20 (br s, 23H), 0.89 (t, 3H, J = 6.3 Hz); ^{13}C NMR ($CDCl_3/CD_3OD$ 1/1 (v/v), TMS) δ 137.63, 127.60 (d, J = 9.3 Hz), 82.81, 69.13, 50.73, 32.72, 32.37, 30.12, 30.09, 29.94, 29.79, 29.70, 29.46, 23.09, 14.23; ^{31}P NMR ($CDCl_3/CD_3OD$ 1/1 (v/v), 85% H_3PO_4) δ −1.63; ESI-MS (negative ion mode): *m/z* 360.2 $(M\text{-}H)^-$, 721.2 $(2M\text{-}H)^-$. FAB-HRMS: calcd for $C_{18}H_{37}NO_4P$ $(M+H)^+$, 362.2460; found, 362.2446.

20 ^1H-^1H COSY ($CDCl_3/CD_3OD$ 1/1 (v/v), TMS) δ 2.87 correlates with δ 4.39, δ 4.15, and δ 3.98; δ 4.39 correlates with δ 5.30 and δ 2.87 only; δ 4.15 and δ 3.98 correlate with each other, and with δ 2.87.