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Synthesis of *erythro*-ω-Aminosphingosine and Preparation of an Affinity Column for Sphingosine Kinase Purification

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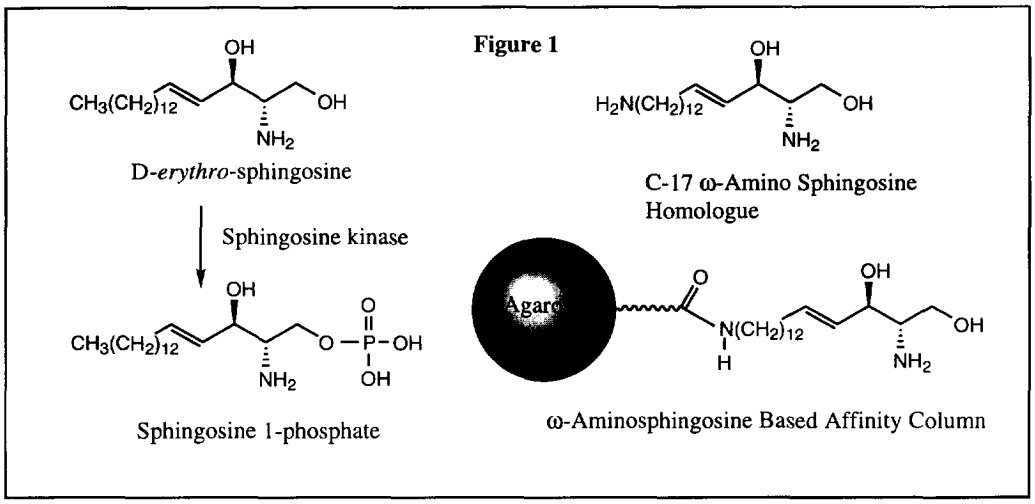
Abstract: The *de novo* synthesis of a C-17 *erythro*-ω-aminosphingosine homologue using L-serine is described together with its conjugation to Affi-Gel 10. Copyright © 1996 Elsevier Science Ltd

Phospholipids have long been recognized to serve as important constituents of the cell membrane, and more recently to provide a source of intracellular signaling molecules. Sphingosine and ceramide, metabolites of the complex sphingolipids, have been shown to play a role in intracellular signaling along with the other second messenger molecules such as diacylglycerol, phosphatidic acid, and inositol 1,4,5-trisphosphate. While sphingosine was first shown to be an important inhibitor of protein kinase C, sphingosine has also been found to regulate a host of cellular responses that are PKC-independent. Moreover, sphingosine-1-phosphate (SPP), a product formed by the action of sphingosine kinase on sphingosine, appears to be an important contributor to the intracellular signaling pathway that is connected to calcium release and regulation of cell growth induced by sphingosine.[1] The potent mitogen PDGF activates sphingosine kinase with concomitant increase in SPP levels in quiescent fibroblasts.[2a] SPP is also important for T cell proliferation since growth inhibition induced by ISP-1/myriocin, a new type of potent immunosuppressant with structural similarity to sphingosine that inhibits sphingolipid metabolism, was completely abolished by SPP or sphingosine.[2b] Moreover, an inhibitor of sphingosine kinase not only blocked production of SPP, but it also inhibited PDGF-induced cellular proliferation.[2a] Thus, sphingosine kinase might be a critical component in cell growth signaling pathways.

In an effort to learn more about sphingosine kinase, with the ultimate aim to discover various small molecule based activators or inhibitors of this enzyme, we felt that a substantial initial step in this direction would involve the isolation and purification of sphingosine kinase. While some efforts in this direction have been undertaken, no report of the successful isolation of the enzyme has been published.[3]

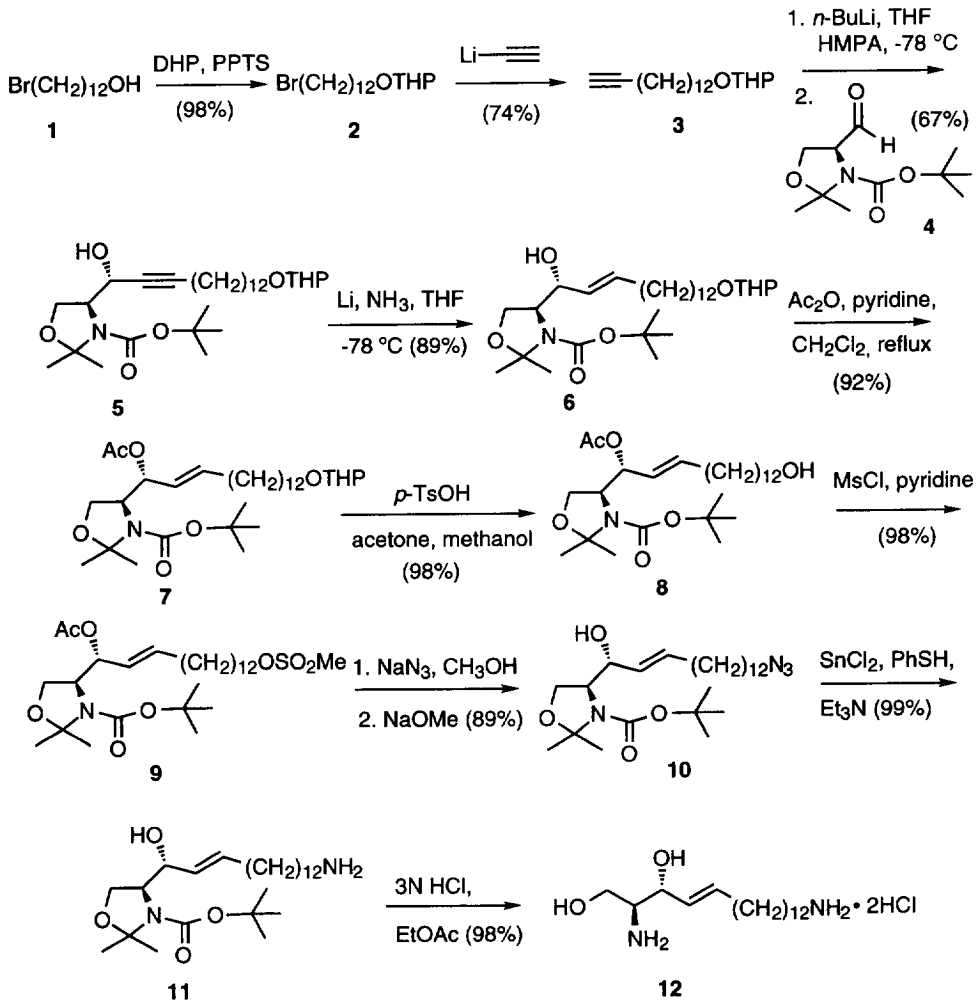
In this communication we wish to report our preliminary results toward this problem. Specifically, we detail the *de novo* synthesis of a C-17 ω-aminosphingosine homologue and its coupling to the derivatized crosslinked agarose gel bead support Affi-Gel 10 to create an affinity column.[4] This commercially available gel contains a neutral 10-atom spacer arm bearing a carboxylic acid function activated as its N-hydroxysuccinimide ester and thus is eminently suitable for reaction with the aminosphingosine homologue.[5] Our determination to introduce a second amino group into our sphingosine analogue as a functional handle was in part made on the basis of the availability of several affinity supports bearing N-hydroxysuccinimide activated esters.



The synthesis of ω-aminosphingosine was initiated from 12-bromododecanol **1** (Scheme 1). Reaction of this alcohol with dihydropyran under the catalysis of pyridinium *p*-toluenesulfonate provided the desired THP ether **2** in 98% yield.[6] Reaction of this intermediate with lithium acetylide ethylenediamine complex in DMSO afforded the acetylene **3** in 74% yield after column chromatography.[7] Lithiation of the acetylene in turn with *n*-butyllithium at -78 °C in a mixture of THF and HMPA and subsequent addition of aldehyde **4**, prepared from L-serine according to the procedure of Garner,[8] gave the alkynol **5** in 67% yield after column chromatography.[9] Reduction of alkynol **5** with excess lithium in liquid ammonia at -78 °C afforded the desired trans-alcohol **6**.[9] Next, we chose to bring about the selective removal of the THP protecting group in **6**, a transformation which was complicated somewhat by the presence of the acetonide. Although the BOC group proved to be stable to the various acidic conditions that were examined, the acetonide ring was invariably opened preferentially under conditions which included, *inter alia*, treatment with *p*-TsOH in methanol,[10] 0.2N HCl in a mixture of THF and H_2O,[11] or acetic acid in a mixture of THF and water[12] at various temperatures.[13] Finally, this problem was resolved by adoption of a two step reaction sequence. Acetylation of the hydroxyl group with acetic anhydride/pyridine gave the acetylated THP ether **7**. Treatment of acetate **7** with *p*-TsOH in a mixture of acetone and methanol afforded the desired primary alcohol **8** in 87% yield. Next, transformation of the free hydroxyl group to an amino group was required. Mesylation of alcohol **8** with methanesulfonyl chloride and pyridine in methylene chloride at room temperature provided the mesylate **9** in 98% yield. Nucleophilic displacement with sodium azide in methanol at reflux followed by removal of the acetate group with sodium methoxide in methanol generated **10** in 89% yield. Attempts to bring about reduction of the azide group of **10** with $SnCl_2$[14] or by hydrogenation[15] using Lindlar's catalyst were ineffective. However, a reagent mixture comprised of one equivalent of tin(II) chloride, three equivalents of triethylamine, and three equivalents of thiophenol worked nicely to provide the primary amine **11** in 99% yield after chromatography using 10% methanol in chloroform.[16] Complete deprotection to the title compound **12**[17] was then accomplished in 98% yield by simple treatment with 3N HCl in ethyl acetate for 2 hours. To our knowledge, this is the first report of the synthesis of a sphingosine derivative containing a terminal amino group.

In coupling **12** to the gel, it was of course desired that only the terminal amino group react with the N-hydroxysuccinimide ester appendage. This seemed likely given the fact that the secondary amino group is not only sterically less accessible, but additionally, this group is internally hydrogen bonded. To test for such selectivity, we simply exposed the dihydrochloride salt **12** to one equivalent of N-hydroxysucciminimide-

Scheme 1. Synthesis of ω–Aminosphingosine

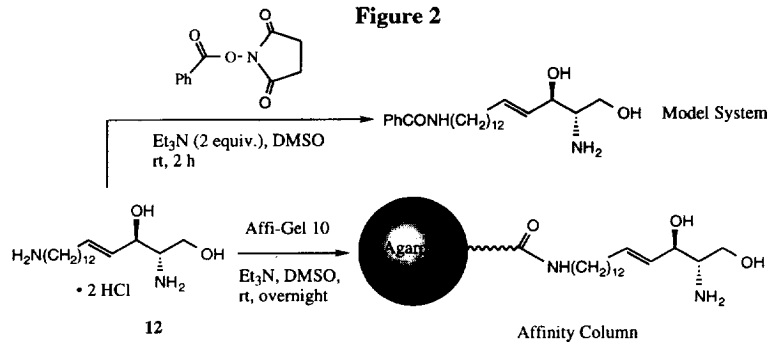


1 **2** **3** **4** **5** **6** **7** **8** **9** **10** **11** **12**

activated benzoic acid as a model system. This reaction was carried out in the presence of two equivalents of triethylamine at room temperature in DMSO as solvent (Figure 2). After a reaction time of 2 hours, we were able to isolate in 95% yield the desired benzamide derivative in which only the terminal amino group had been acylated. The obtention of this product serves to validate the notion that **12** would couple to the affinity gel predominantly in the required fashion.

To prepare the affinity matrix required for isolation of sphingosine kinase, an anhydrous coupling protocol was employed. Affi-Gel 10 was stirred with an excess of ω-aminosphingosine dihydrochloride and triethylamine in DMSO at room temperature overnight (gel was ninhydrin positive). In some cases, blocking of any unreacted groups on the gel was brought about by further treatment with ethanolamine. Preliminary results appear to show that this affinity column may be suitable for enzyme purification, for partially purified kinase was found to bind avidly to the column. Further details of the affinity purification will be published separately.

Figure 2



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4. Affi-Gel 10 was purchased from Bio-Rad Laboratories.
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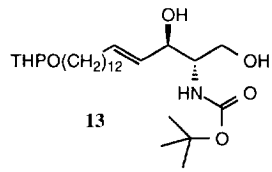
13. Without prior protection of the allylic hydroxyl group, compounds **13** and **14** were the only two products isolated when the THP ether **6** was subjected to the acidic conditions mentioned in the text.



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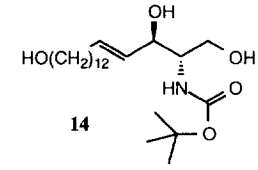

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17. The physical and spectral data for **12** are as follow: $[\alpha]^{20}_D$ = -11° (*c* 0.35, water); mp (sublimes); ^{1}H NMR (D_2O) δ 1.15-1.45 (m, 18H), 1.66 (m, 2H), 2.08 (m, 2H), 3.00 (t, 2H, *J* = 7.5 Hz), 3.36 (ddd, 1H, *J* = 4, 5.5, 10 Hz), 3.73, 3.90 (ABq, 2H, J_{AB} = 12.5 Hz, both d with *J* = 8 and 4 Hz, respectively), 4.36 (t, 1H, *J* = 6.5 Hz), 5.50 (dd, 1H, *J* = 7.5, 15.5 Hz), 5.94 (dt, 1H, *J* = 15.5 Hz (d), 7 Hz (t)).

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