Tetrahedron Letters,Vol.29,No.25,pp 3037-3040,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

A STEREOSELECTIVE SYNTHESIS OF SPHINGOSINE, A PROTEIN KINASE C INHIBITOR.

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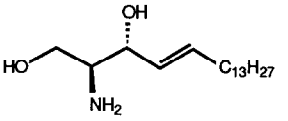
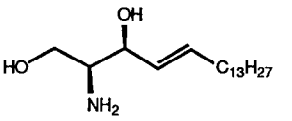
Summary: A stereoselective synthesis of each of the four enantiomers of sphingosine from either L- or D-serine is reported here. The key steps in the sequence involve: (a) the diastereoselective addition of 1-lithiopentadecyne to aldehyde 4 and (b) Mitsunobu inversion of propargyl alcohol 5.

Sphingolipids (e.g., ceramides, sphingomyelin, gangliosides) constitute a broad class of biologically important compounds. The backbone component of sphingolipids is almost invariably the long chain base, sphingosine, which is usually present as its D(+)-*erythro* isomer (2S,3R).[1] Recent reports have shown sphingosine to be a potent inhibitor of protein kinase C (PKC) *in vivo* and *in vitro*.[2] Because of the pivotal role which PKC plays in modulating cell growth, cell differentiation and signal transduction, we thought it would be useful to develop a synthetic route to each of the sphingosine enantiomers which not only permits the stereoselective production and biological assay of these materials, but also provides the flexibility to do extensive structure / function modifications.[3]



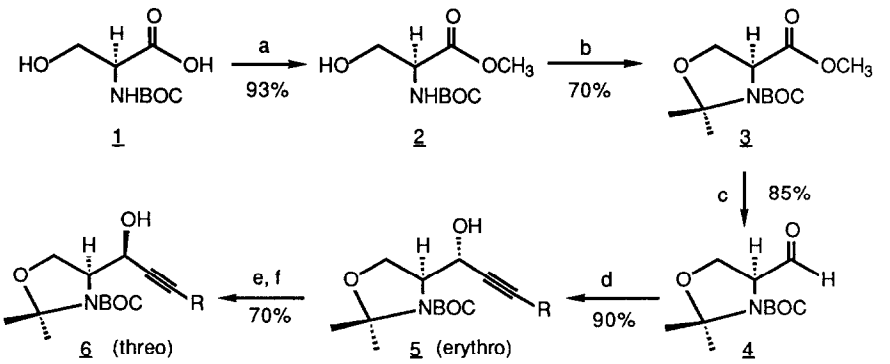
D(+)- *erythro* sphingosine



L(-)- *threo* sphingosine

The synthetic approach which we have utilized is shown in **Scheme 1**.[4] Starting from either N-BOC-D- or L-serine (BOC = t-butyloxycarbonyl), one can carry out a series of standard synthetic manipulations to produce the protected serine methyl ester 3 in 62% overall yield. DIBAL reduction of 3 in toluene at -78°C gave aldehyde 4 in 85% yield. As noted previously by Garner,[5] all of the intermediates in the conversion of 1 to 4 can be produced without significant amounts of racemization. We have found that compound 4 is an extremely useful intermediate for the preparation of each of the enantiomers of sphingosine on a multigram scale.

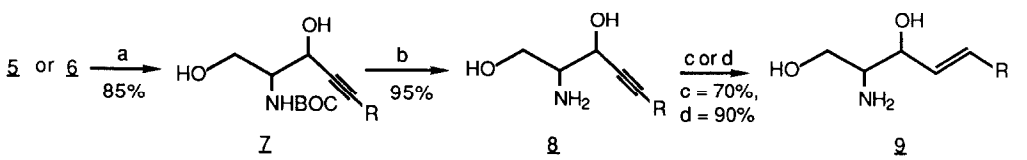
Scheme 1



a. CH_2N_2; b. H^+, $(CH_3)_2C(OCH_3)_2$; c. DIBAL; d. Li—≡—$C_{13}H_{27}$; e. PPh_3 / DEAD / $PhCO_2H$; f. KOH.

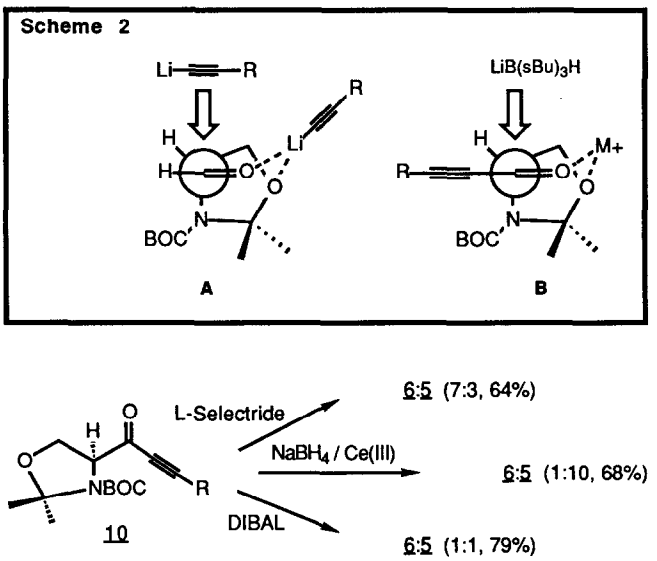
Addition of the lithium salt of pentadecyne in THF (-78°C to 0°C) produced alkynol 5 as a 9:1 mixture of *erythro*- and *threo*-isomers, respectively, in 90% isolated yield. These materials were readily separated by chromatography on silica gel (hexane / ethyl acetate, 2:1). Conversion of 5 to dehydrosphingosine, 8, could be achieved using the deprotection sequence shown below.[6]

Our first attempts at converting 8 to 9 using lithium aluminum hydride in refluxing THF proceeded only in modest yield.[7] We therefore examined the alternative dissolving metal reduction conditions. Exposure of 8 to a refluxing solution of excess lithium in liquid ammonia / THF (4:1) for 7 hours resulted in the quantitative recovery of a 9:1 mixture of 9 and 8, respectively. Pure *erythro*-sphingosine, 9, could be obtained by recrystallization of the mixture from hexane. While the dissolving metal reduction worked well on a small scale (10 - 100 mg), attempts to repeat this reaction on a 1 -5 gram scale required significantly longer reaction times (> 24 hours). Because of the problems associated with maintaining relatively constant amounts of ammonia during the large scale dissolving metal reductions, we reexamined the lithium aluminum hydride reduction. By simply switching solvents from refluxing THF (BP = 67°C) to refluxing DME (dimethoxyethane, BP = 85°C) for 12 hours, we could consistently obtain yields of 70% on scales up to 5 grams.



a. p-TsOH, MeOH; b. HCl / EtOAc; c. excess $LiAlH_4$ / DME / Δ ; d. Na / liq. NH_3 / THF (reflux).

We rationalize the *erythro*-selectivity observed in the conversion of 4 to 5 via the β-chelation-controlled model depicted in **Scheme 2A**. In principle, one ought to be able to use this β-chelation as a means of inverting the stereochemistry of the C-3 alcohol to obtain the corresponding *threo*-isomer by converting 5 to its corresponding ketone 10 (Swern oxidation, 80 % yield), followed by subsequent reduction. In the event, exposure of 10 to L-Selectride resulted in a disappointing 7:3 *threo/erythro* ratio. Reduction of 10 with either DIBAL or sodium borohydride / Ce(III) gave *threo/erythro* ratios of 1:1 and 1:10, respectively. While the origins of the observed selectivies remain unsubstantiated, we speculate that the cases which proceed with low selectivity are simply the result of competitive α-chelation control with the urethane nitrogen or γ-chelation control with the urethane oxygen. In order to obtain the *threo*-product, we turned to Mistunobu inversion of the C-3 alcohol.[8] Under these conditions, *erythro* alcohol 5 could be cleanly isomerized to its corresponding *threo*-isomer 6 in 70% yield. Compound 6 could then be converted to *threo*-sphingosine in yields which were consistently within 2% of those obtained in the *erythro* series.



In conclusion, we have developed a synthesis which is suitable for the preparation of each of the four enantiomers of sphingosine on a multigram scale. In addition, this sequence permits easy modification of head group functionality (e.g., substitution at nitrogen or oxygen) and hydrophobicity (by addition of alkyne of different chain lengths to 4) as well as radioisotopic substitution in the conversion of 8 to 9. These results, as well as the bioassays on the inhibitory activity of each enantiomer toward protein kinase C, will be reported in the near future.[9]

Acknowledgement: Financial support by the National Science Foundation (DCB-871083) and the Winship Cancer Center of Emory University is gratefully acknowledged.

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Note Added In Proof: After submission of this manuscript, we became aware of a recently published article which uses a similar strategy to prepare the BOC-derivative of D(+)-*erythro*-dehydrosphingosine. Many of the observations made in it nicely complement those discussed above. For further details, see: P. Herold, *Helv. Chim. Acta*, **71**, 354 (1988).

(Received in USA 6 April 1988)