CHAPTER - I

I N T R O D U C T I O N

CHEMISTRY OF PHYTOSPHINGOSINES AND SPHINGOSINES

CHEMISTRY OF PHYTOSPHINGOSINES AND SPHINGOSINES

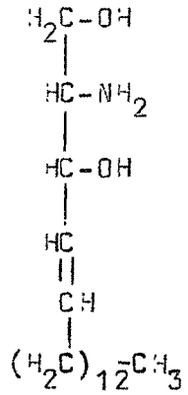
Abstract

This Chapter briefly reviews the chemistry of phytosphingosines and sphingosines which are closely related to guggultetrols. In guggultetrols, 2-amino-group of phytosphingosine (2-amino-1,3,4-octadecanetriol) is replaced by a hydroxyl group.

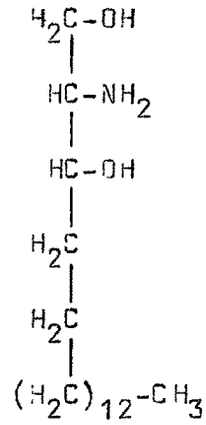
Sphingosines and Phytosphingosines

A) Occurrence

Spingosines* and phytosphingosines (4-D-hydroxysphinganine) occur in nature as glycosphingolipids of animal and plant origin. Although the first glycolipids were isolated from tissues of the central nervous systems of mammals, it was recognized many years ago that these groups of substances are general constituents of the animal kingdom (e.g. birds, fishes and insects), as well as micro-organisms, musnrooms and plant materials (such as, seeds and leaves).[1]



Spingosine (1)



Dihydrosphingosine (2)

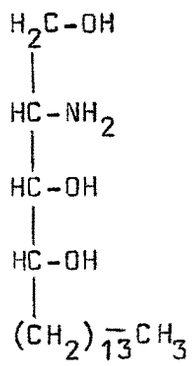
---

* The name "sphingosine" proposed by Thudichum[2], is derived from the Greek verb Sphingein, to bind or squeeze. This word is also etymologically related to the name of the mysterious, archaic statue called the Sphinx, the symbolic representation in Egyptian and Greek mythology of power and Law, and furthermore, of wisdom and omniscience.

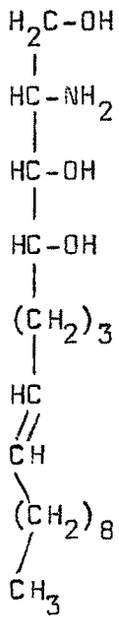
The first sphingosine preparation was obtained in 1880 by Thudicum[2] through hydrolytic cleavage of human brain material (mainly cerebroside mixtures). After the first structure representation of the long chain bases had been proved erroneous, Carter and co-workers[3] proposed the correct structure, namely 2-amino-4-octadecene-1,3-diol (1). The corresponding saturated derivative, dihydrosphingosine (sphinganine) (2), can be obtained by saturation of the allyl double bond of sphingosine with hydrogen. Dihydrosphingosine exists in nature in the form of N,1-O-disubstituted derivatives (e.g. dihydrocerebrosides and dihydrosulfatides).

Phytosphingosine (3) was first isolated from the mushroom Amanita muscaria by Zellner[4] in 1911 and has subsequently been isolated by other workers from various moulds.[5] In 1952, Oda[6] established that phytosphingosine was one of the eight stereoisomers of 2-amino-1,3,4-trihydroxy octadecane and in 1954, Carter and co-workers reported the isolation of phytosphingosine from plant seeds and arrived at the same structure independently. Karlsson[8] has shown that phytosphingosine is a major component of the long-chain bases of human kidney cerebroside and that it is also present in small quantities in human brain.

In 1963, Carter and Hendrickson[9] established by degradation that phytosphingosine has the D-ribo-configuration.



Phytosphingosine (3)



Dehydrophytosphingosine (4)

An unsaturated analog of phytosphingosine, dehydrosphingosine (4) was isolated by Carter and co-workers.[10] This base was shown by hydrogenation and IR. Studies to be identical to phytosphingosine except for the presence of a trans-double bond. The position of the double bond was established by degradative studies.[9]

The development of the chromatography, together with use of the mass spectrometer for analysis of the long chain amino alcohol mixtures obtained by hydrolysis of the zoo- and phyto-sphingolipids, led to the discovery of a number of natural sphingosines. In addition to the well-known C_{18}-

sphingosines and phytosphingosines, a number of similar natural sphingosines having C_{16}, C_{17}, C_{19}, C_{20} and C_{21} chains have been isolated.[11]

The sphingosine having a chain length of C_{21} carbon atoms was isolated from Chritidia fasciculata and characterized by Carter and co-workers.[1] It was found to be a branched chain, and is 19-methyl sphingosine.

Recently a bio-active sphingosine like teroenoid named aplidiasphingosine (2-amino-5,9,13,17-tetramethyl-8,-16-octadecadiene-1,3,14-triol) was isolated from a marine turnicate by Carter and K.C. Rinehart Jr.[12].

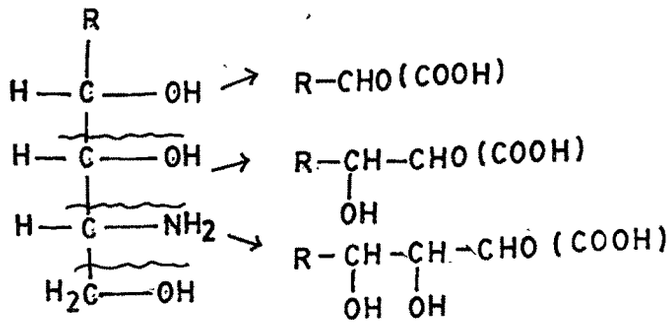
In Table I, the names of some sphingosines, dihydrosphingosines and phytosphingosines, that have been isolated by hydrolysis of sphingolipids of animal or plant origin, are given.

Table I. Natural Sphingosines and phytosphingosines

No.	No. of carbon atoms in chain	Structure	Ref.
1.	16	2-Amino-1,3-hexadecane diol	13
2.	16	2-Amino-4-trans-hexadecene-1,3-diol	13
3.	16	2-Amino-hexadecadiene-1,3-diol	14
4.	17	2-Amino-1,3-heptadecane diol	13
5.	17	2-Amino-4-trans-heptadecene-1,3-diol	13
6.	17	2-Amino-heptadecadiene-1,3-diol	14
7.	17	2-Amino-1,3,4-heptadecanetriol	14
8.	18	D-erythro-2-amino-1,3-octadecanediol (dihydrosphingosine)	15
9.	18	D-erythro-2-amino-4-trans-octadecene-1,3-diol (Sphingosine)	15
10.	18	2-Amino-4,14-trans,trans-octadecadiene-1,3-diol	14
11.	18	D-ribo-2-amino-1,3,4-octadecanetriol (Phytosphingosine)	9
12.	18	D-ribo-2-amino-8-trans-octadecene-1,3,4-triol (dehydrosphytosphingosine)	10
13.	19	2-Amino-1,3,4-nonadecane triol	14
14.	19	2-Amino-nonadecene-1,3,4-triol	14
15.	20	D-erythro-2-amino-4-trans-eicosane-1,3-diol	16
16.	20	2-Amino-1,3-eicosanediol	16
17.	20	2-Amino-1,3,4-cicosanetriol	17
18.	21	2-Amino-19-methyl-1,3,4-eicosanetriol	11

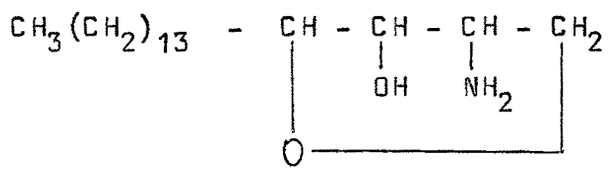
B) Stereochemical aspects of phytosphingosines and Sphingosines.

(a) Phytosphingosines - Carter and co-workers[9] established by degradative studies, that phytosphingosine has D-rib-configuration. Carbon-2 was shown to have D-configuration by the formation of N-benzoyl-L-serine after periodate oxidation of the N-benzoyl base. They have hoped to establish the configuration of carbon atoms 3 and 4 by partial periodate degradation and characterization of the mono- and di-hydroxy acid fractions. The possible oxidation products are shown below.



The main product of the per iodate-silver oxide oxidation was pentadecanoic acid. However, sufficient α-hydroxy palmitic acid was produced to determine its specific rotation and thus, establishing configuration as D-α-hydroxy palmitic acid. Thus, carbon-4 of phytosphingosine has the D-configuration as related to carbon-1. Insufficient amount of dihydroxy acid

was obtained to permit characterization. However, evidence concerning configuration of C-3 was obtained by their study of behaviour of N-benzyl anhydrophytosphingosine.



(5)

Anhydro phytosphingosine

In this derivative an N→O shift of benzoyl group under relatively mild conditions would be expected, only if the adjacent hydroxyl group is cis-to amide.

The behaviour of N-benzoyl anhydrophytosphingosine in 0.7N ethanolic HCl at room temperature was investigated. Migration of the benzoyl group occurred to the extent of 50% in 91 hrs and was complete in 15 days. These results establish that the hydroxyl and amino groups in anhydro-phytosphingosine bear a cis-relationship to each other, and that the same relationship exists in phytosphingosine, provided only that no epimerization occurs at carbon-3 during the formation of anhydrophytosphingosine. The fact that anhydrobase is obtained readily as a single isomer (cis), argues strongly

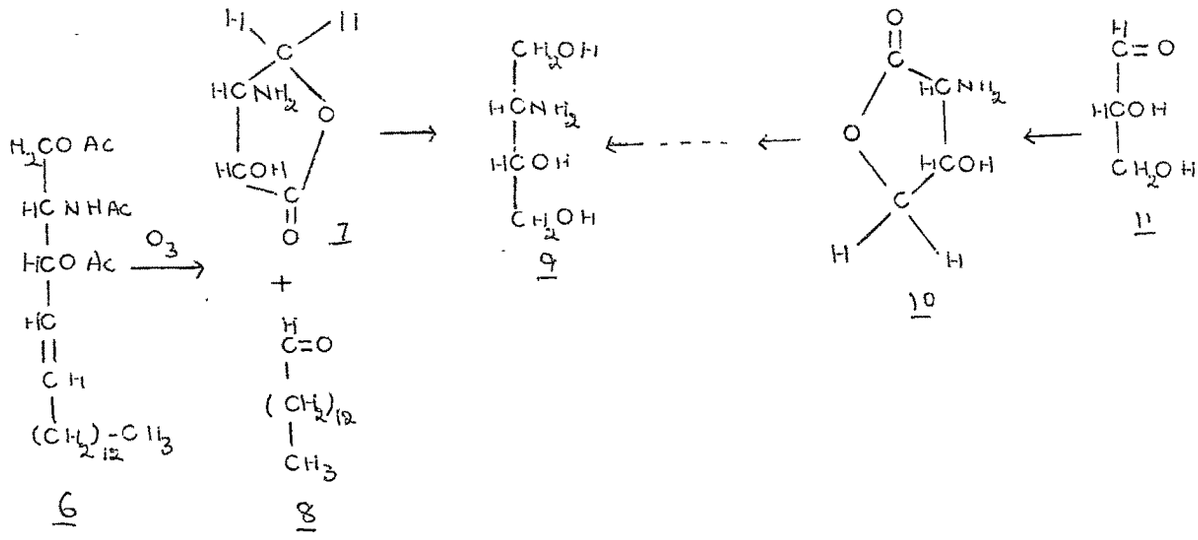
against this possibility as does also the stability towards acid hydrolysis of carbon-3 in dihydrosphingosines ceramides. Since the amino carbon of phytosphingosine has D-configuration, carbon-3 may also be assigned the D-structure. Thus the structure of phytosohingosine was established as

D-ribo-1,3,4-trihydroxy-2-amino-octadecane.

(b) Sphingosines. In sphingosine and dihydrosphingosine, the diestereoisomeric relationship of the amino and the 3-hydroxyl group is erythro; this was proved by degradation and synthesis.[18,19]

Configurational correlation of C_{18}-sphingosine with D-glucose (D-glyceraldehyde).

The degradation of N-acetyl-di-O-acetyl-C_{18}-sphingosine (6) with ozone gives[20,21] d-amino-2-hydroxybutyrolactone (7) and tetradecanal (8).

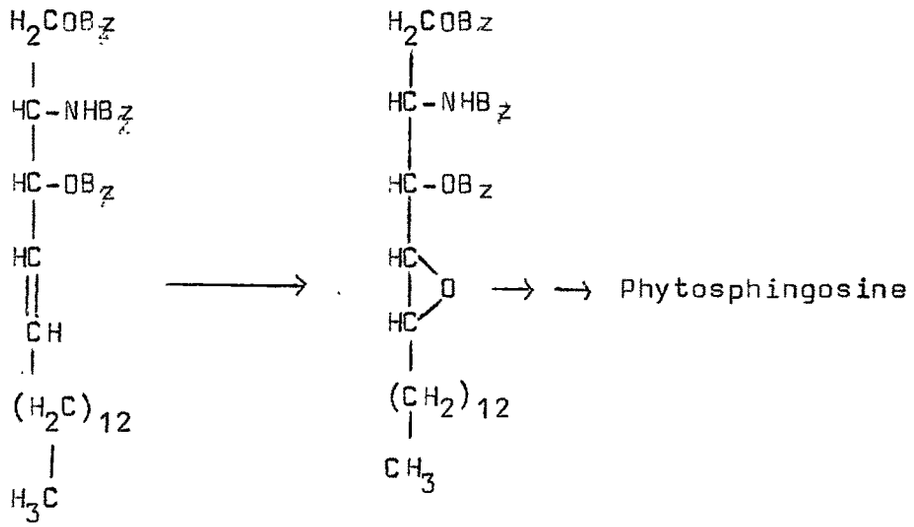


By a two step, catalytic hydrogenation, lactone (7) gives D-erythro-2-aminobutane-1,3,4-triol (9). The same product (9) was obtained by similar reduction of D-erythro-2-amino-3-hydroxybutyrolactone (10) which has been synthesised from D-glyceraldehyde (11) by the methods of Fischer and Fieldmann[22] and Hamel and Painter.[23] These results support the stereochemical proposals of Carter, Shapiro and Harrison.[19]

The trans-geometry of the sphingosine double bond was established with the help of IR spectrum[24,24a]. The spectrum of sphingosine has a characteristic; a well-defined trans-peak near 10.3μ(970 cm^{-1}).

(c) Stereochemical correlation of phytosphingosines with C_{18}-sphingosine.

The structure and stereochemical correlation of C_{18}-sphingosine with C_{18}-phytosphingosine was made by Prostenik and co-workers[25] and by Weiss.[26] Tribenzyl sphingosine (12) was oxidised with peroxy acids, giving the corresponding epoxide (13). The reductive opening of the oxirane ring led to the ribo-2-amino-1,3,4-octadecane triol derivative, which after hydrolysis and hydrogenation of the protective groups, proved to be identical with natural C_{18}-phytosphingosine.



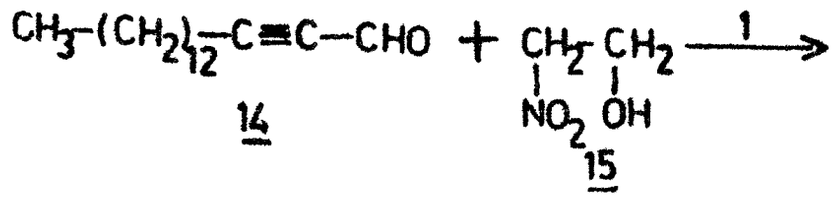
(12) (13)

C) Synthesis of Sphingosines and Phytosphingosines

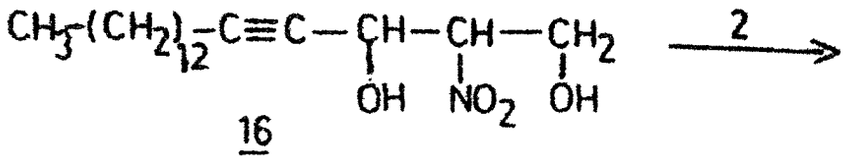
a) Sphingosine

There are several reports of the synthesis of racemic erythro-sphingosines and D-erythro-sphingosines. Of these, some very interesting synthesis are described below:

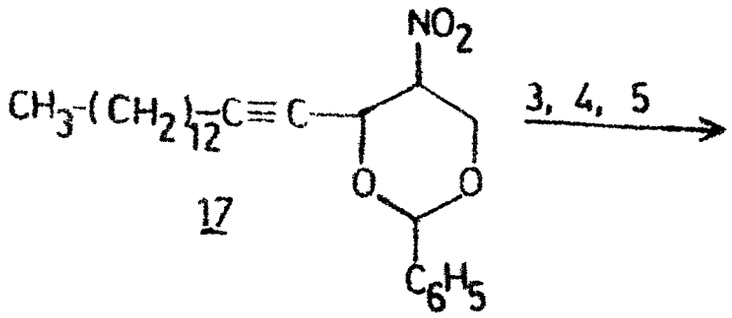
1) Synthesis of racemic erythro-sphingosine. Racemic erythro- and threo- sphingosines were synthesised from 2-hexadecyn-1-al (14) by C.A. Grob and F. Gadient[27] (Fig. 1). 1,3-Dihydroxy-2-nitro-4-octadecyne (16) was prepared by the base catalysed



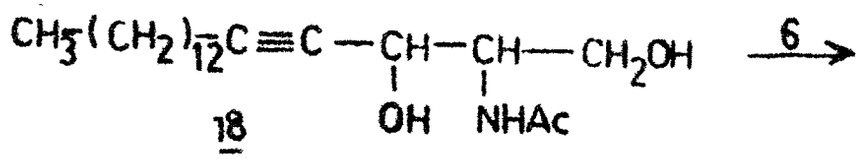
14 15



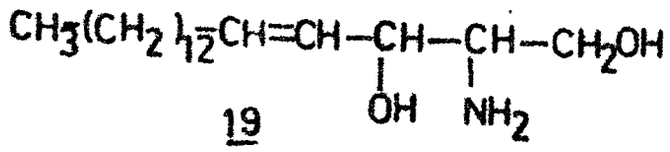
16



17



18



19

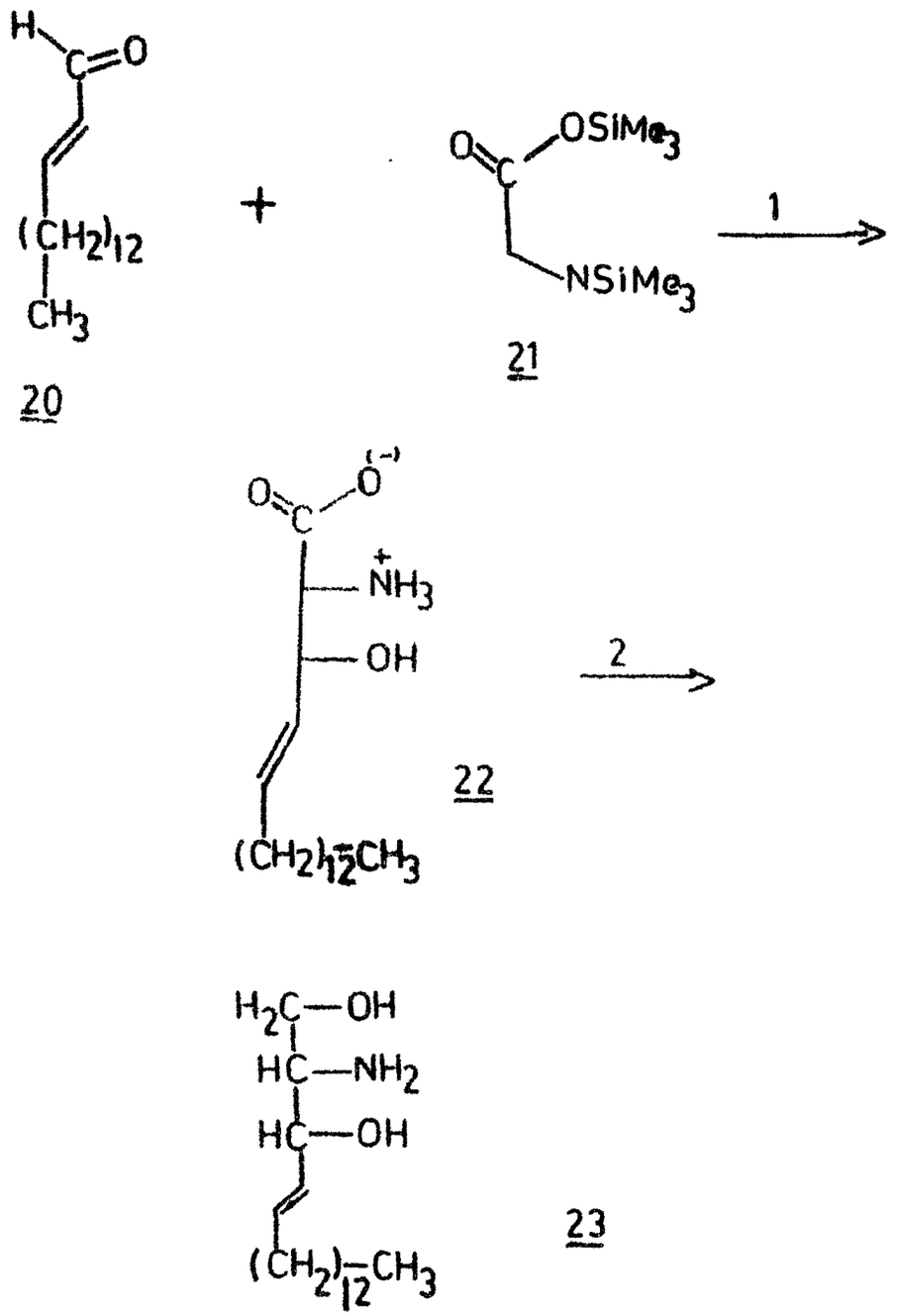
Reagents: 1. base, 2. Benzaldehyde/$ZnCl_2$,
3. Al(Hg), 4. Ac_2O/pyridine,
5. H^+, 6. Na/n-buOH.

Fig. 1

condensation of (14) and nitroethanol (15). Reaction of the diol (16) with benzaldehyde in presence of zinc chloride furnished 2 benzal derivatives (17), differing only with respect to the configuration of the phenyl group at position (2) of the 1,3-dioxane ring. Both compounds yield 1,3-dihydroxy-2-acetylamino-4-octadecyne (18), on reduction of the nitro group with amalgamated aluminium followed by acetylation and mild acidic hydrolysis of the cyclic acid. Partial reduction of the triple bond in compound (18) by sodium in boiling n-butanol afforded racemic threo-sphingosine. erythro-Sphingosine was prepared by epimerizing the nitro-group in compound (17) by treating it with alcoholic sodium ethoxide, and following the same sequence of reactions as described for racemic threo-isomer. Both racemic threo- and erythro-sphingosines were characterized as triacetyl derivatives.

Richards R. Schmidt and Rudolf Klager have reported diastereo-selective synthesis of D,L-sphingosine from C_{16} aldehyde and glycine[28] (Fig. 2).

The α,β-unsaturated C_{16}-aldehyde (20) was allowed to react with α-carbanian of the protected glycine (21) to give erythro-configurated, unsaturated β-hydroxy-α-amino acid exclusively. (22) was reduced with LAH to D,L-sphingosine in 90% yield.



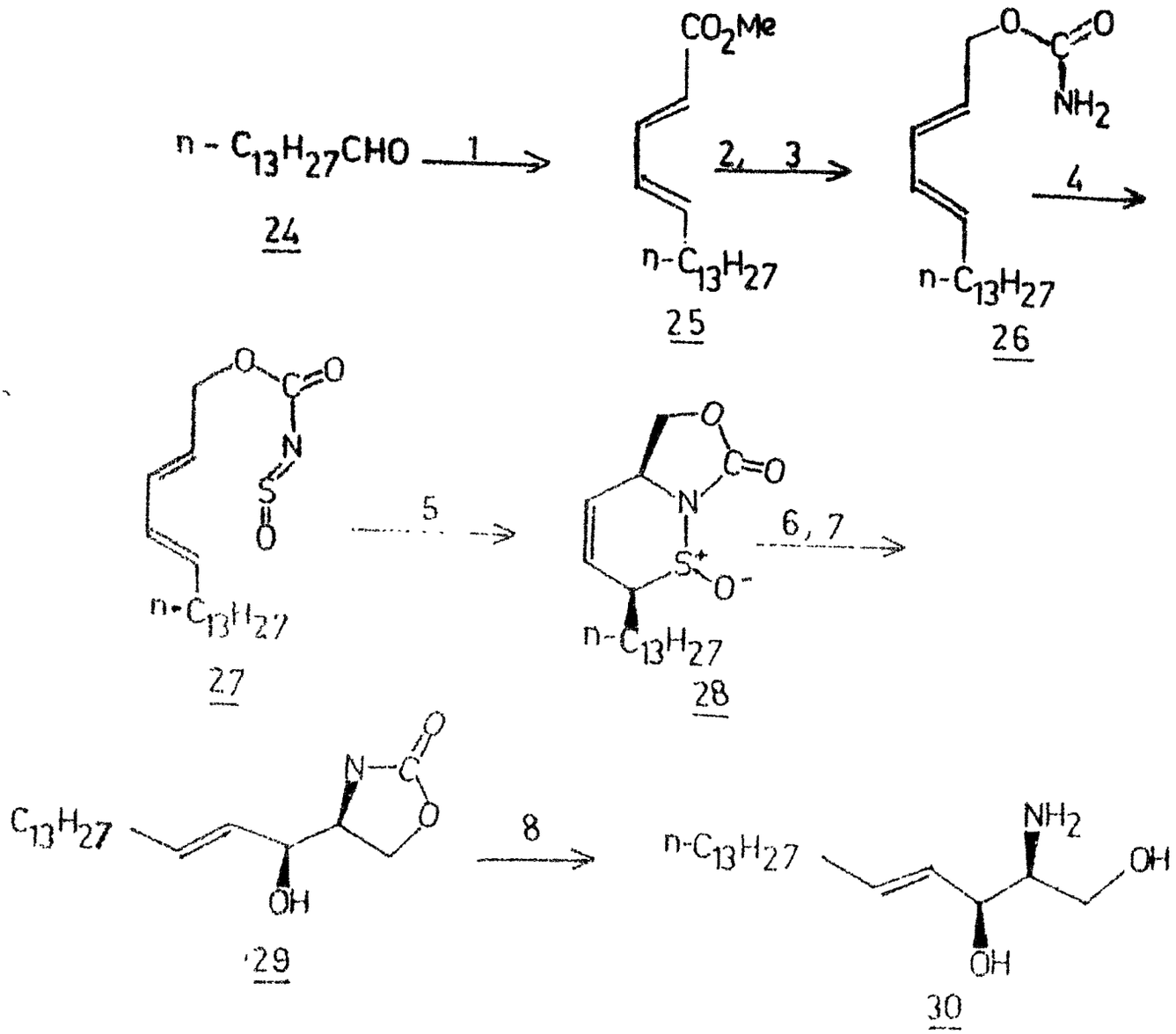
Reagents: 1. LDA/THF, 2. LAH/THF.

Fig. 2

Very recently Ravi S. Garigipati and Steven M. Weinreb have reported[29] stereospecific synthesis of racemic threo- and erythro-sphingosines. threo-Sphingosine was synthesised from myristic aldehyde (Fig. 3). Myristic aldehyde (24) was converted into (E,E)-carbamate (26) in three steps, which upon treatment with thionyl chloride/ pyridine generated sulfinyl carbamate (27). This compound cleanly cyclized at room temperature to afford adduct (28). Treatment of (28) with phenylmagnesium bromide followed by trimethylphosphite gave (29) exclusively which on hydrolysis gave racemic threo-sphingosine completely free of the erythro-isomer.

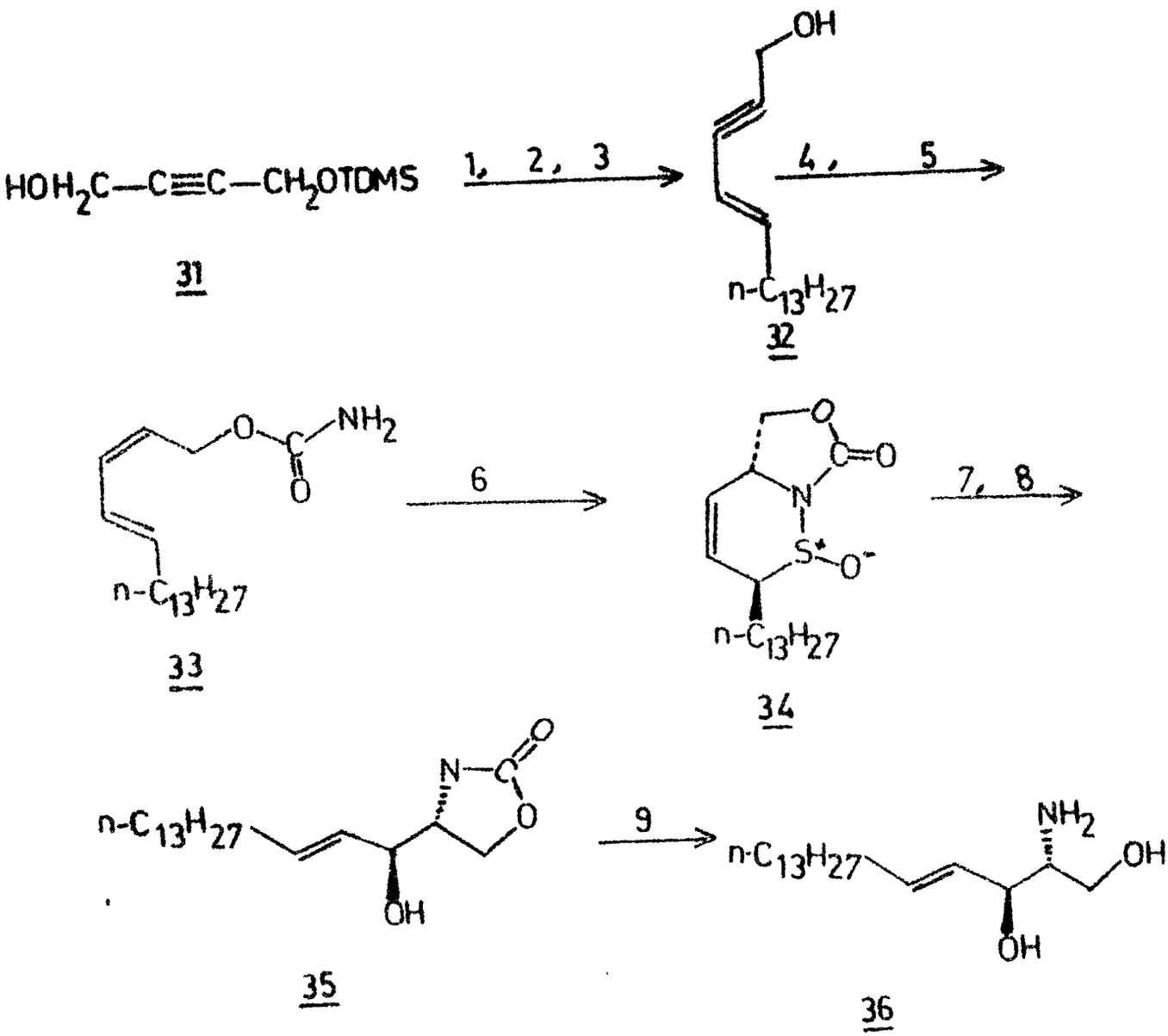
The (E,Z)-carbamate (33) (Fig. 4) needed for erythro-sphingosine was prepared as shown in Fig. 4. Intramolecular Diels-Alder cycloaddition of N-sulfinylcarbamate derived from (33) was slow but cleanly gave the desired adduct (34). From (34), racemic erythro-sphingosine was obtained in a similar way as that of threo-isomer

1) Synthesis of D-erythro-sphingosine: L-Serine was the starting material for the synthesis of enantiomerically pure D-erythro-sphingosine reported by Newman.[30] The synthesis involves the simple conversion of the commercially available L-serine, whose chiral centre corresponds to that of



Reagents: 1. $(MeO)_2P(O)CH_2CH{=}CHCO_2Me$, LDA/THF, 2. LTA/$Et_2O$,
3. NaOCN, TFA/Et_2O, 4. $SOCl_2$/Py, PhMe,
5. Room temperature, 6. PhMgBr/THF, 7. $(MeO)_3P$/MeOH,
8. $Ba(OH)_2$, dioxane/H_2O.

Fig. 3

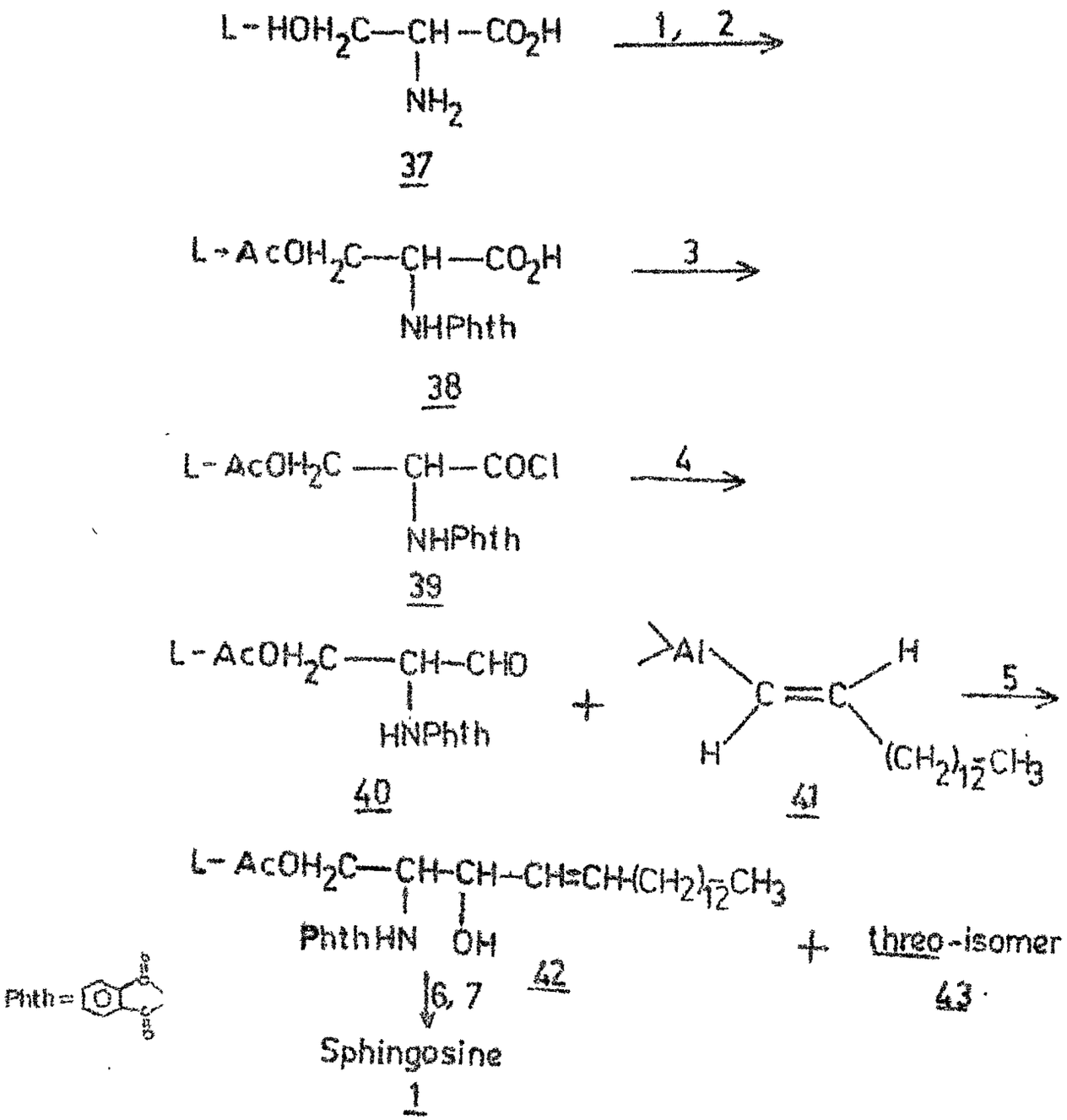


Reagents: 1. $BaMnO_4/CH_2Cl_2$, 2. $n\text{-}C_{13}H_{27}CH_2\overset{+}{P}Ph_3\bar{B}r$, n-BuLi/THF,
3. 3.5 N.HCl, 4. H_2/Lindlar catalyst, PhMe,
5. NaOCN, TFA/Et_2O, 6. $SOCl_2$/Py, PhMe, 7. PhMgBr/THF,
8. $(MeO)_3P$/MeOH, 9. $Ba(OH)_2$, glyme/H_2O

Fig. 4

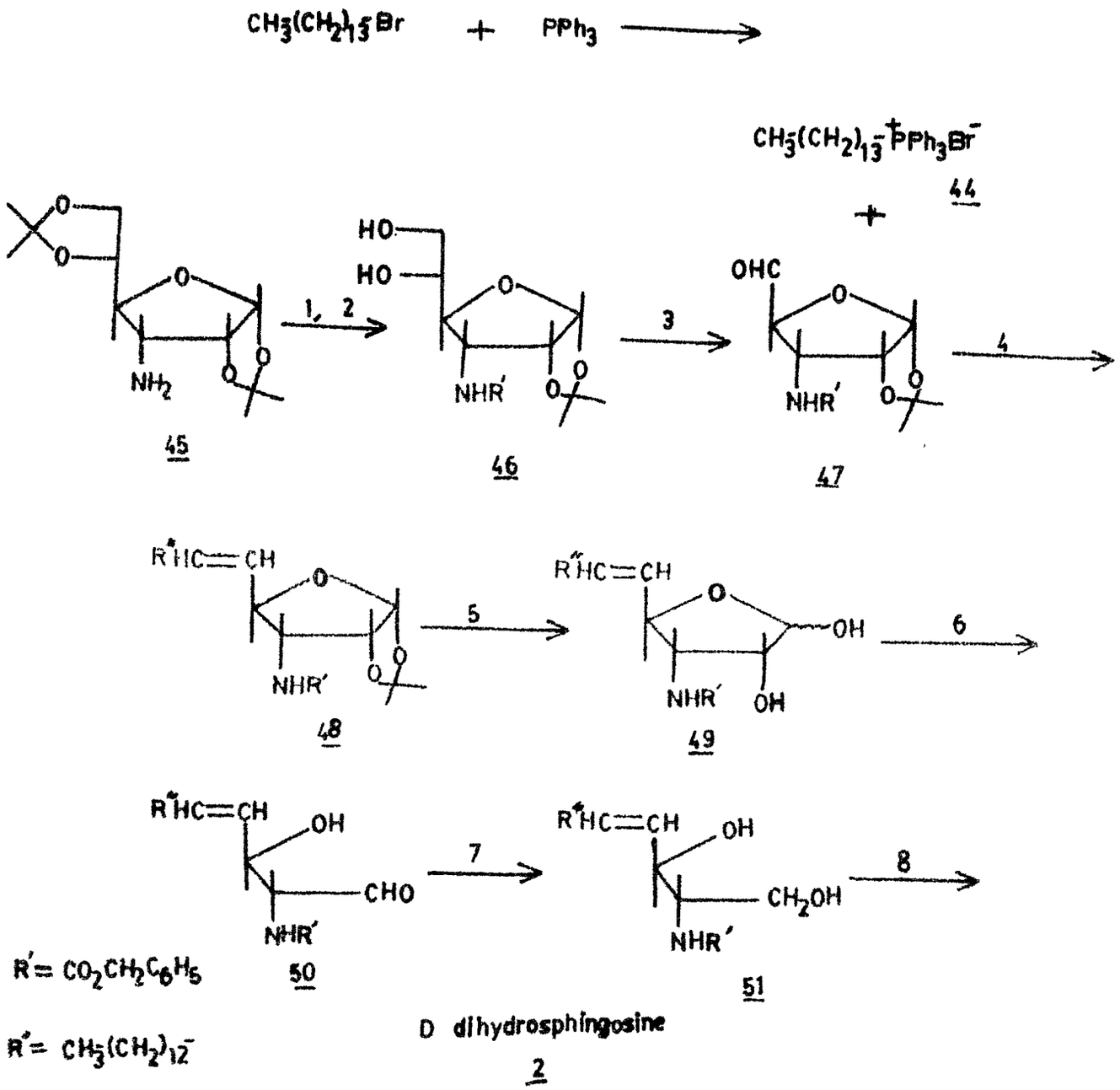
sphingosine, to the L-aldehyde (40) by N-ohthaloylation, O-acetylation, acid chloride formation and catalytical hydrogenation as outlined in Fig. 5. Addition of chiral aldehyde (40) to trans-petadecenyl-di-isobutylalane (41) furnished directly D-erythro-O-acetyl N-naohthaloyl sphingosine (42) along with the unnatural isomer (43) (4:1). These isomers were readily separated by partition chromatography. 42 was converted to D-erythro-sphingosine by deprotecting the protecting groups.

3) Synthesis of D-dihydrosphingosine. Stereospecific synthesis of D-dihydrosphingosine was reported by Elmer J. Reist and Pamela H. Christie[31] (Fig. 6). They started with the easily available 3-amino-3-deoxy-1,2,5,6-di-O-isopropylidene α-D-allofuranose (45). The reaction of 6-benzyloxy carboxylamino-2,2-dimethyl-5-formyl-3a,5,6,6a-tetrahydro - D-ribofuro 2,3-d dioxolane (47) prepared in three steps (Fig. 6) from 3-amino-3-deoxy-1,2,5,6-di-O-isopropylidene α -D-allowfuranose (45), with the Wittig reagent, prepared from tetradecyl triphenylphosphonium bromide (44) gave the cis- and trans-olefin mixture (48). The olefin mixture was deacetonated, and resulting gylcol (49) was cleaved with sodium metaperiodate, and then reduced with sodium borohydride to give Crystalline-2-benzoyloxycarboxylamino-D-erythro-Δ^4-octadecene-1,3-diol (51). Hydrogenation of (51) over



Reagents: 1. $PhthNCHCO_2Et/Na_2CO_3$, 2. Ac_2O, 3. $SOCl_2/C_6H_6$
4. H_2/C_6H_6, 5. room temperature, 6. $MeOH/H^+$,
7. $NH_2-NH_2/EtOH$.

Fig. 5



Reagents: 1. $ClCO_2CH_2C_6H_5/Py$, 2. 75% aq. AcOH, 3. $NaIO_4$/ 50% aq. MeOH, 4. $PhLi/C_6H_6$, 5. 8% aq. AcOH, 6. $NaIO_4$/MeOH, 7. $NaBH_4$/MeOH, 8. H_2,Pd/C/AcOH.

Fig. 6

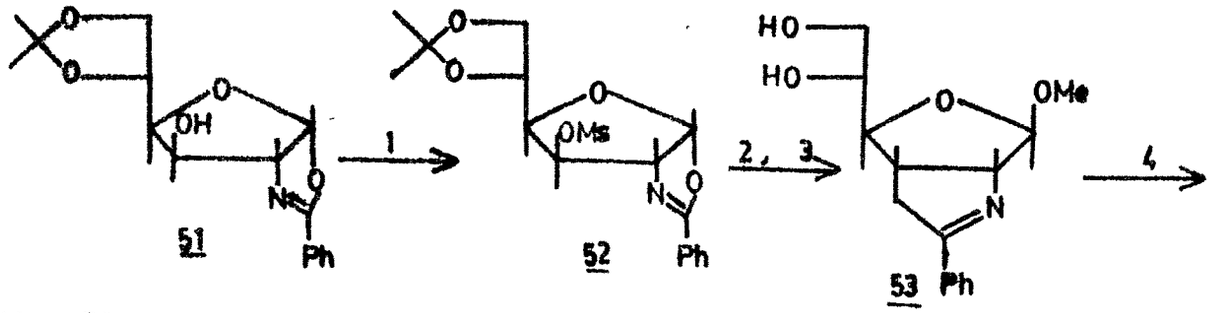
palladium/charcoal, reductively deblocked the amino and saturated the olefin to give D-dihydrosphingosine (2).

b) Synthesis of Phytosphingosine

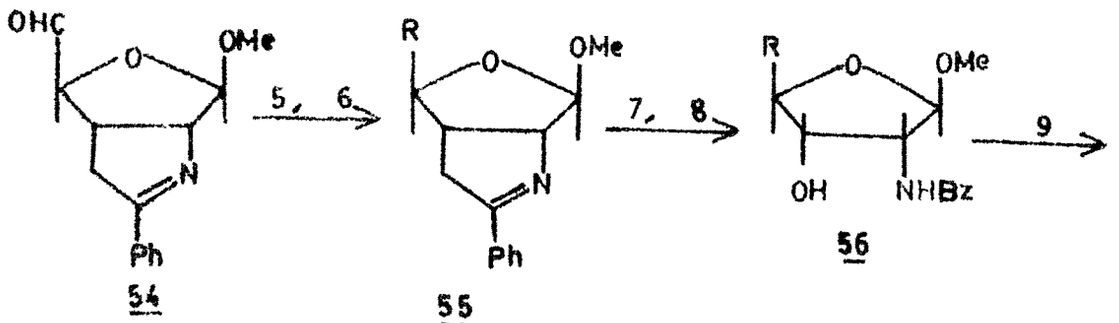
Gigg and Gigg[32] have published impressive work on the synthesis of phytosphingosine starting from the oxazoline (51) which was readily prepared,[33] from 2-benzamido-2-deoxy-D-glucose. Oxazoline methyl sulfonate (52) was converted to the glycol oxazoline (53) by treating it with methanolic hydrogen chloride and then with excess of sodium methoxide. Glycol was cleaved to aldehyde (54) which on condensation with the Wittig reagent prepared from triphenyl-n-tridecylphosphonium bromide gave the olefins. Olefins were hydrogenated over palladium on charcoal to furnish (55). The oxazoline ring of the compound (55) was opened by acid and subsequent treatment of the resulting product with base gave the amide (56) which was treated with 0.25 NHCl in dioxane and $NaBH_4$ reduction of the resulting sugar (57) furnished N-benzoyl-phytosphingosine.

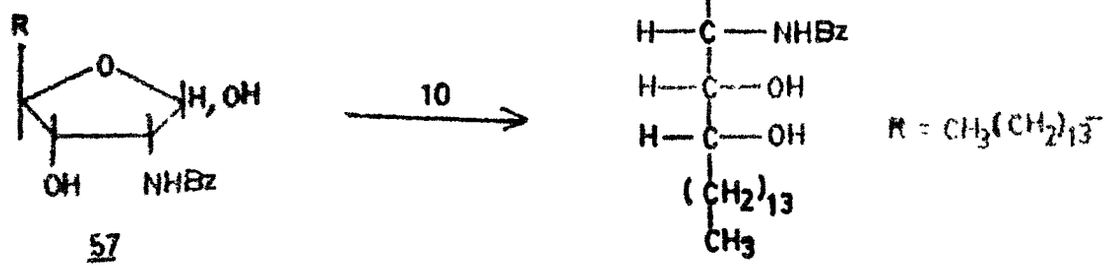
D. Biosynthetic pathway of Sphingosine

The precursors in the biosynthesis of sphingosines are,



Ms = SO_2Me

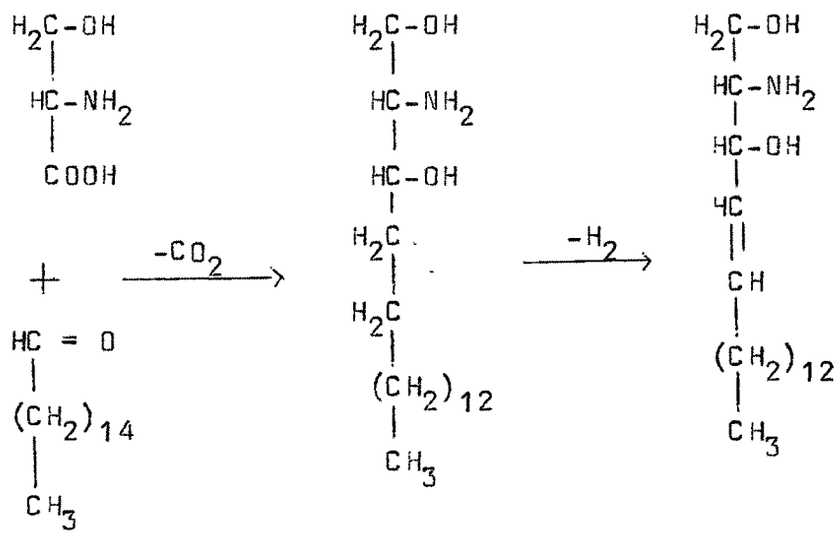




Reagents: 1. $MeSO_2Cl$/Py, 2. Methanolic HCl, 3. NaOMe,
4. 50% aq. HIO_4/MeOH, 5. $CH_3(CH_2)_{12}{}^{+}Pph_3Br$/PhLi,THF,
6. 10% Pd/C/AcOH, 7. N.HCl/MeOH, 8. K_2CO_3,
9. 0.25 N.HCl/dioxane, 10. $NaBH_4$/MeOH.

Fig. 7

according to Brady and associates[34], L-serine and pamitaldehyde. These compounds give dihydrosphingosine (2) which is catalytically dehydrogenated by flavo-proteins to give sphingosine (1).



(2)

Biosynthetic pathway of Sphingosine

References

1. J. Kiss, in Advances in Carbohydrate chemistry (edited by Melville L. Wolfrom and R. Stuart Tipson), Vol. 24 pp. 382. Academic Press, New York(1969).

2. J.L.W. Thudichum, A Treatise on the Chemical Constituents of the brain; based through out upon original researchers. Bailliere, Tindall and Cox, London (1884).

3. H.E. Carter, F. Glick, W. Norris and G.E. Phillips, J. Biol. Chem. 142, 449 (1942); 170, 285 (1947).

4. J. Zellner, Monatsh, 32, 133 (1911).

5. F. Reindel, A. Weickmann, S. Picard, K. Luber and P. Turula, Annalen, 544, 116 (1940).

6. T. Oda, J. Pharm. Soc. Jap. 72, 142 (1952).

7. H.E. Carter, W.D. Celmer, W.E.M. Lands, K.L. Mueller and H.H. Tomizawa, J. Biol. Chem. 206, 613 (1954).

8. K.A. Karlsson, Acta Chem. Scand. 18, 2397 (1964).

9. H.E. Carter and H.S. Hendrickson, Biochemistry 2, 389 (1963).

10. H.E. Carter, D.S. Galanos, H.S. Hendrickson, Barbara Janu, Teissi Nakayama, Yasuo Nakayawa and Nichols, J. Am. Oil. Chem. Soc. 39, 107 (1962).

11. E. Klenk and R.T.C. Huang, Z. Physiol. Chem. 349, 451 (1968).

12. G.T. Carter and K.L. Rinehart Jr., J. Am. Chem. Soc. 100, 7441 (1978).

13. K.A. Karlsson, Acta Chem. Scand. 18, 239 (1964).

14. K.A. Karlsson and G.A.L. Holm. Acta Chem. Scand. 19 2423 (1965).

15. H. Thierfelder and E. Klenk, "Die Chemie der Cerebroside and Phosphatide". Verlag Springers, Berlin (1930).

16. K.A. Karlsson, Acta Chem. Scand. 18, 565 (1964).

17. M. Protenik and N.Z. Stanacer, Chem. Ber. 91, 961 (1958).

18. For a review on Structural Investigation of C_{18}-sphingosine and C_{18}-dihydrosphingosine, see. C.A. Crob, Record Chem. Progr. 18, 15 (1957).

19. H.E. Carter, D. Shapiro and F.B. Harrison, J. Am. Chem. Soc. 75, 1007 (1953).

20. F. Kiss, G. Fodor, and D. Banfi, Chem. Ind. (London), 517 (1954); Helv. Chim. Acta 37, 1471 (1954)

21. E. Klenk and H. Faillard, Z. Physiol. Chem. 204, 48 (1955).

22. H.O.L. Fischer and F. Feldmann, Ber. 65, 1211 (1932).

23. E.E. Hamel and E.P. Painter, J. Am. Chem. Soc. 75, 1362 (1953).

24. G. Marinetti and E. Stotz, J. Am. Chem. Soc. 76, 1347 (1954). [a] K. Mislow, J. Am. Chem. Soc. 74 5155 (1952).

25) M. Prostenic, B. Majhofer-Orescanin, B. Reis-Lesic and N.Z. Stanacev, Tetrahedron 21, 651 (1965).

26. B. Weiss, Biochemistry 4, 686 (1965).

27. C.A. Grob and F. Gadient, Chem. Ind. (London), 660 (1956).

28. R.R. Schmidt and R. Klager, Angew. Chem. Int. Edu. (Eng.). 210 (1982).

29. R.S. Garigipati and S. Weinreb, J. Am. Chem. Soc. 105, 4499 (1983).

30. H. Newman, J. Am. Chem. Soc. 95, 4098 (1973).

31. Elmer J. Reist and Pamela H. Christie, J. Org. Chem. 35, 3521 (1970).

32. J. Gigg, Roy Gigg, and C.D. Warren, J. Chem. Soc. 1872 (1966).

33. S. Konstas, I. Photaki and L. Zervas, Chem. Ber. 92, 1288 (1959).

34. R.M. Burton, M.A. Sodd and R.O. Brady, J. Biol. Chem. 233, 1053 (1958).