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SPECIFIC TRITIUM LABELLING OF SPHINGOSINES AT THE 3-POSITION

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Summary: Azidosphingosines 4A,B are transformed with DDQ into the corresponding keto derivatives 6A,B. Reduction of the keto group with [^{3}H]$NaBH_4$ and then conversion of the azido group into the amino group by Staudinger reaction yields stereoselectively the D-erythro- and L-threo-sphingosines 9A,B, 10A,B, respectively.

Key words: Tritiated D-erythro- and L-threo-Azidosphingosines, Tritiated Sphingosines

INTRODUCTION

Glycosphingolipids possess as a constituent C_{18}-sphingosine [(2S,3R,4E)-2-amino-4-octadecen-1,3-diol] which is synthesized in vivo from serine and palmitoyl-CoA in a pyridoxalphosphatedependent reaction. Amide bond formation with a fatty acid leads to the ceramides. Subsequent stepwise glycosylation, first at the primary hydroxy group of ceramide and then at sugar residues, yields the glycosphingolipids (1).

Glycosphingolipids are constituents of the plasma membrane of vertebrates (2). At the cell surface cell-typical glycosphingolipid compositions are observed which vary upon viral transformation or oncogenesis (3-5). However, the biological function of glycos-

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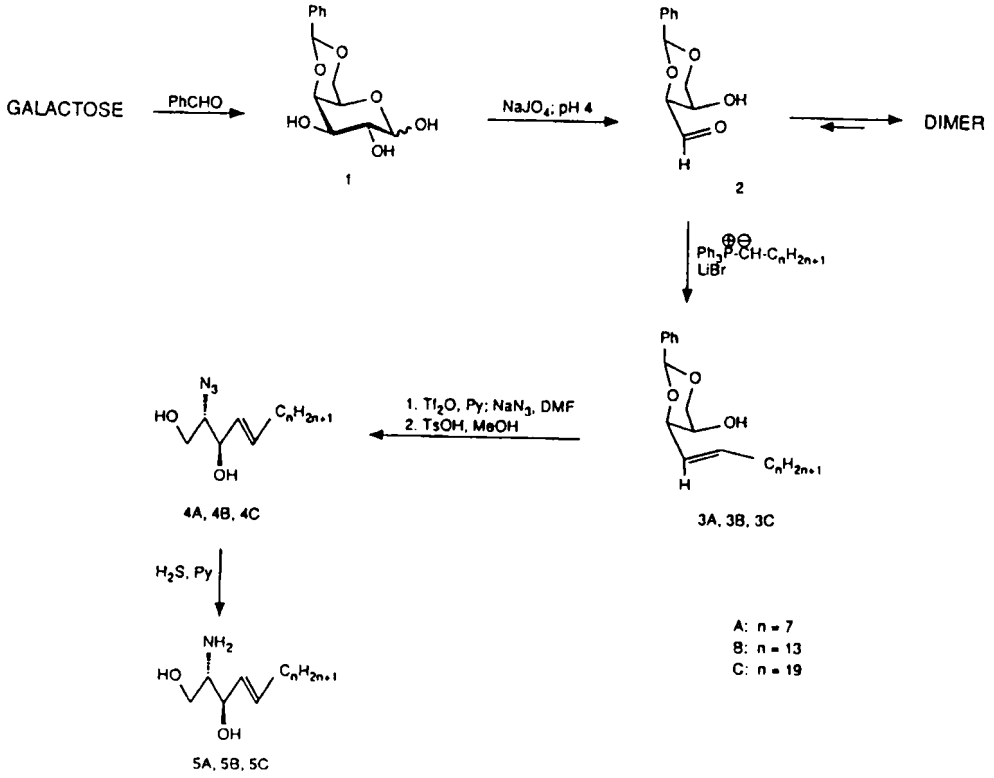
phingolipids is widely unknown (3,6). Sphingosine and sphingoids were recently found to be inhibitors of protein kinase C (7) and also regulatory properties in sphingosine biosynthesis were detected (8). For these studies and for the investigation of the physiological role of glycosphingolipids in general radioactively labelled sphingoids are required.

Besides catalytic hydrogenation of the CC-double bond with tritium (9,10), radioactively labelled sphingoids are obtained by selective oxidation of the allylic hydroxy group and subsequent reduction of the keto group with sodium boro[^{3}H]hydride (11-14). In this paper the readily accessible 2-azidosphingosines (15,16) are employed as starting materials for specific labelling which are themselves interesting compounds for biological testing (8).

Scheme 1



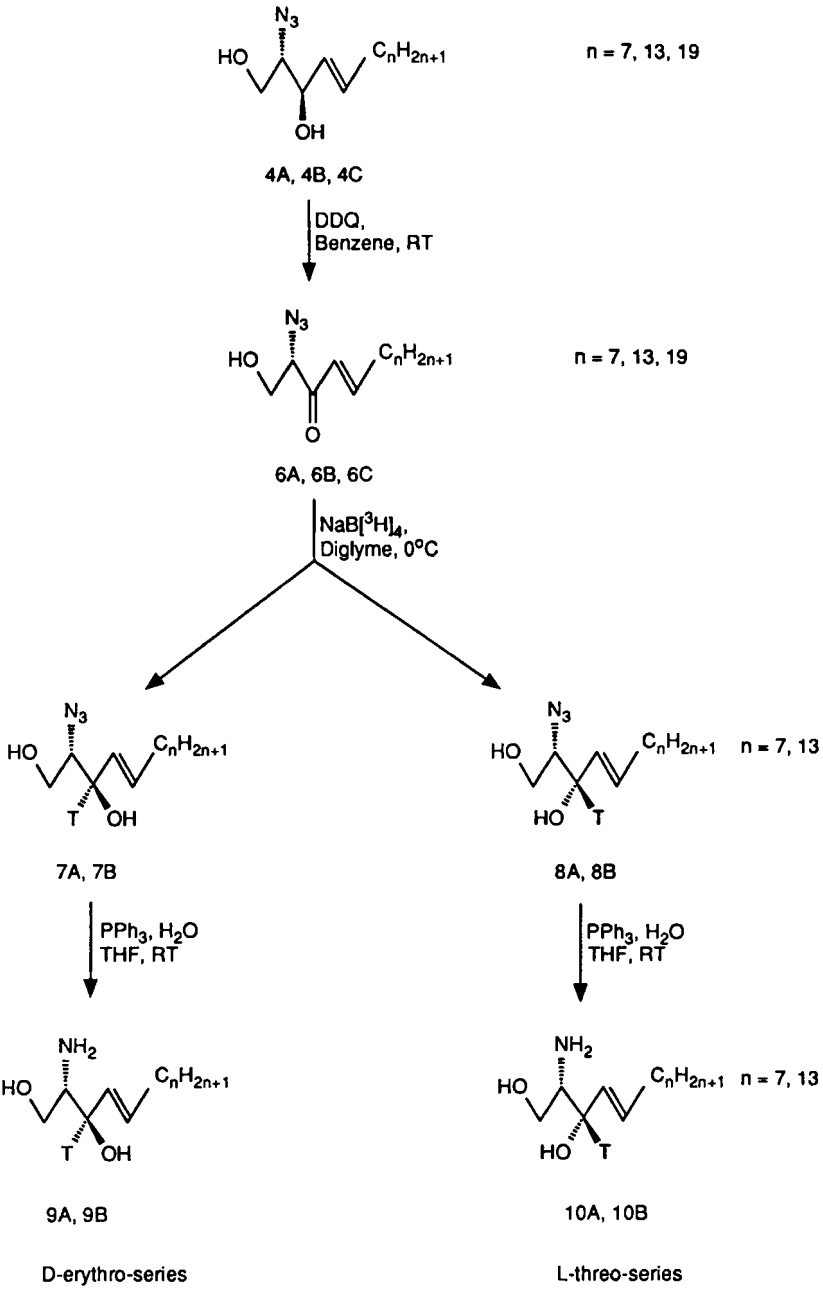
RESULTS AND DISCUSSION

2,4-O-Protected D-threose 2 (Scheme 1)is readily obtained in a two step procedure from D-galactose, following a previously published procedure (15,16); then Wittig-reaction in presence of lithium bromide yields trans-enetriols 3A-C having varying chain length (C_{12}, C_{18}, C_{24}). Activation of the 2-hydroxy group by treatment with trifluoromethane sulfonic anhydride, then azide group introduction, and finally acid catalyzed removal of the 1,3-O-benzylidene group furnishes azidosphingosines 4A-C, which are readily converted into the corresponding sphingosines 5A-C (17).

Various oxidizing reagents were investigated for the selective transformation of compounds 4A-C into the corresponding 3-ketosphingosine derivatives 6A-C (Scheme 2). The best yields were obtained with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in benzene at room temperature (17). This reagent proved to be particularly suited for mild and selective transformations of allylic alcohols into α,β-unsaturated ketones (18,19). Racemisation was not observed under the reaction conditions.

Reduction of the 3-keto group withr tritium introduction should not lead to concomitant removal of the CC-double bond and/or the azido group. To this aim treatment with [^{3}H]$NaBH_4$ in diglyme as solvent was carried out providing cleanly a mixture of 3-tritiated D-erythro- and L-threo-azidosphingosine 7A,B and 8A,B, respectively, which could be separated by thin-layer chromatography on silica gel. Racemisation was not observed under the reaction conditions. Staudinger reaction (20) with each of these compounds employing a modified one-pot procedure (21) in tetrahydrofuran/water and 1.5 equivalents of triphenylphosphine at room temperature furnished the tritiated sphingosines 9A,B, 10A,B in high yield and high specific activity. Purification was again performed by thin-layer chromatography, thus concluding a convenient tritium labelling procedure of sphingosines at the 3-position.

Scheme 2


N3
HO
CnH2n+1
OH
n = 7, 13, 19
4A, 4B, 4C
DDQ,
Benzene, RT
N3
HO
CnH2n+1
O
n = 7, 13, 19
6A, 6B, 6C
NaB[3H]4,
Diglyme, 0°C
N3
HO
CnH2n+1
T
OH
7A, 7B
N3
HO
CnH2n+1
HO
T
n = 7, 13
8A, 8B
PPh3, H2O
THF, RT
PPh3, H2O
THF, RT
NH2
HO
CnH2n+1
T
OH
9A, 9B
NH2
HO
CnH2n+1
HO
T
n = 7, 13
10A, 10B
D-erythro-series
L-threo-series

EXPERIMENTAL PART

Material and general procedures

^{1}H-NMR spectra were performed on a Jeol JNM-GX 400 instrument using tetramethylsilane as internal standard. Optical rotation was determined with a polarimeter Perkin-Elmer 241 MC (monochromatic sodium-light; D-line, 589.3 nm). Melting points are uncorrected. R_F values refer to TLC performed on silica gel (Merk, 60 F_{254}) with the solvent systems noted. Preparative TLC was performed with silica gel on glass (Merck, 60). Column chromatography was performed under normal pressure with silica gel (Merck, 63-200 µm) or with reversed-phase silica gel (Merck, "LiChroprep" RP-18, 40-63 µm), under medium pressure with silica gel (Merck, "LiChroprep" Si60, 40-63 µm) and by flash chromatography with silica gel (Merck, 40-63 µm). All reagents were of analytical reagent grade or better.

The radiochemical sodium boro[^{3}H]hydride was supplied by Amersham (specific activity: 9.76 Ci/mmol = 256.9 mCi/mg). The tritium determinations were made with a Packard liquid scintillation counter (1900 CA). The radioactivity on the plates was scanned with a Berthold radiochromatogram scanner (TLC-Linear Analyzer LB 2821).

Dehydrogenation of the 3-hydroxy group of compounds 4A,B,C. General Procedure.

To a solution of 1.9 mmol 4 in 20 ml dry benzene was added at room temperature and under nitrogen-atmosphere 2.3 mmol 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). After stirring for 8-10 days at +20°C the reaction mixture was evaporated under reduced pressure (10 torr) and the residue dissolved in petroleum ether (200 ml). The organic layer was washed with sodium hydroxide (5%

aqueous solution, 30 ml), with a saturated solution of ammonium chloride (30 ml) and with brine (30 ml). The petroleum ether layer was dried over anhydrous magnesium sulfate and evaporated. The oily residue was purified by flash chromatography (silica gel; dichloromethane/methanol, 95:5).

(2S,4E)-2-Azido-4-dodecen-1-ol-3-on (6A)

Yield 286 mg (63%); colourless oil; TLC (silica gel; dichloromethane/methanol, 95:5): R_F = 0.54; $[\alpha]^{22}$ = -2.9° (c = 1, $CHCl_3$).- ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.09 (dt, 1H, CH=C<u>H</u>-CH_2, J = 6.8 Hz, J = 7.1 Hz, J = 15.6 Hz), 6.35 (d, 1H, C<u>H</u>=CH-CH_2, J = 15.9 Hz), 4.16-4.13 (m, 1H, C<u>H</u>-N_3), 4.01-3.92 (m, 2H, O-C<u>H_2</u>), 2.27 (q, 2H, CH=CH-C<u>H_2</u>, J = 7.1 Hz), 2.35-2.04 (m, 1H, O<u>H</u>), 1.49-1.46 (m, 2H, CH=CH-CH_2-C<u>H_2</u>), 1.40-1.08 (m, 8H, 4 C<u>H_2</u>), 0.89 (t, 3H, C<u>H_3</u>, J = 6.4 Hz).

$C_{12}H_{21}N_3O_2$ (239.32) Calc.: C 60.22 H 8.85 N 17.55
Found: C 59.71 H 8.91 N 17.00

(2S,4E)-2-Azido-4-octadecen-1-ol-3-on (6B)

Yield 393 mg (65 %); TLC (silica gel; dichloromethane/methanol, 95:5): R_F = 0.62; mp. 39.5-40.5°C; $[\alpha]^{20}$ = -3.2° (c = 1, $CHCl_3$).- ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.09 (dt, 1H, CH=C<u>H</u>-CH_2, J = 6.8 Hz, J = 6.8 Hz, J = 15.9 Hz), 6.36 (d, 1H, C<u>H</u>=CH-CH_2, J = 15.6 Hz), 4.15 (t, 1H, C<u>H</u>-N_3, J = 4.9 Hz), 4.03-3.89 (m, 2H, O-C<u>H_2</u>), 2.30-2.24 (m, 3H, CH=CH-C<u>H_2</u>, O<u>H</u>), 1.49-1.46 (m, 2H, CH=CH-CH_2-C<u>H_2</u>), 1.37-1.15 (m, 20H, 10 C<u>H_2</u>), 0.88 (t, 3H, C<u>H_3</u>, J = 6.8 Hz).

$C_{18}H_{33}N_3O_2$ (323.48) Calc.: C 66.83 H 10.28 N 12.99
Found: C 66.75 H 10.20 N 13.00

(2S,4E)-2-Azido-4-tetracosen-1-ol-3-on (6C)

Yield 503 mg (65%); TLC (silica gel; dichloromethane/methanol, 95:5): R_F = 0.64; mp. 55-56°C; $[\alpha]^{20}$ = -3.8° (c = 1, $CHCl_3$).- ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.09 (dt, 1H, CH=C<u>H</u>-CH_2, J = 6.8 Hz, J = 7.1

Hz, J = 15.6 Hz), 6.36 (d, 1H, C<u>H</u>=CH-CH_2, J = 15.6 Hz), 4.14 (t, 1H, C<u>H</u>-N_3, J = 4.6 Hz), 3.99-3.94 (m, 2H, O-C<u>H_2</u>), 2.30-2.24 (m, 2H, CH=CH-C<u>H_2</u>), 2.11 (t, 1H, O<u>H</u>, J = 6.5 Hz), 1.53-1.46 (m, 2H, CH=CH-CH_2C<u>H_2</u>), 1.36-1.00 (m, 32H, 16 C<u>H_2</u>), 0.88 (t, 3H, C<u>H_3</u>, J = 6.8 Hz).

$C_{24}H_{45}N_3O_2$ (407.64) Calc.: C 70.72 H 11.12 N 10.31

Found: C 70.47 H 11.05 N 10.20

<u>Specific tritium labeling of compounds 6A,B at the 3-position.</u>

(2S,3R,4E)-[3-^{3}H]-2-Azido-4-dodecen-1,3-diol (<u>7A</u>)

(2S,3S,4E)-[3-^{3}H]-2-Azido-4-dodecen-1,3-diol (<u>8A</u>)

4.1 mg (17 µmol)of compound <u>6A</u>, dissolved in 500 µl of dry diglyme, were frozen under argon-atmosphere by liquid nitrogen. This freezing procedure was repeated after addition of 500 µl dry diglyme. 50 mCi (4 µmol) sodium boro[^{3}H]hydride in 100 µl dry diglyme were added to the frozen solution. The reaction mixture was allowed warm to 0°C and after further 6-8 h, it was kept for 2 days at +5°C. For work-up, the reaction mixture was poured into 20 ml water and 20 ml methanol. Exchangeable tritium was removed by washing through reversed-phase silica gel with 400 ml of water. The tritiated product was eluated successively with 250 ml methanol and 300 ml methanol/chloroform (1:1). The solvent was evaporated to dryness by a nitrogen-stream and the remaining solid was dissolved in chloroform. The obtained labelled diastereomers (D-erythro-<u>7A</u> and L-threo-<u>8A</u>) were separated and purified by preparative TLC (silica gel; petroleum ether/ethyl acetate, 1:1). The purity of the compounds was checked by TLC and by radiochromatogram scanning.

The labelled products were TLC identical to authentic unlabelled material.

D-erythro-7A: Yield: 1.6 mg (39%); TLC (silica gel; petroleum ether/ethyl acetate, 1:1): R_F = 0.52; specific radioactivity: 2500 mCi/mmol.

L-threo-8A: Yield: 2.2 mg (54%); TLC (silica gel; petroleum ether/ethyl acetate, 1:1): R_F = 0.61; specific radioactivity: 5000 mCi/mmol.

(2S,3R,4E)-[3-^{3}H]-2-Azido-4-octadecen-1,3-diol (7B)

(2S,3S,4E)-[3-^{3}H]-2-Azido-4-octadecen-1,3-diol (8B)

These compounds were obtained from 5.5 mg (17 µmol) 6B and 50 mCi (4 µmol) sodium boro[^{3}H]hydride as described for 7A and 8A. The labelled compounds were separated and purified by preparative TLC (silica gel; petroleum ether/ethyl acetate, 1:1). Analysis by TLC and radiochromatogram scanning indicated the purity. The labelled products were TLC identical to authentic unlabelled material.

D-erythro-7B: Yield: 1.6 mg (29%); TLC (silica gel; petroleum ether/ethyl acetate, 1:1): R_F = 0.57; specific radioactivity: 2900 mCi/mmol.

L-threo-8B: Yield: 2.7 mg (49%); TLC (silica gel; petroleum ether/ethyl acetate, 1:1): R_F = 0.65; specific radioactivity: 3600 mCi/mmol.

Reduction of the azido group of compounds 7A,B and 8A,B. General procedure.

To a solution of the corresponding labelled compounds 7 or 8 in 1500 µl pure tetrahydrofuran and water (5:1) were added 1.5 equivalents triphenylphosphine at room temperature. Reaction control was made by thin-layer chromatography. When the reaction was completed after few days, purification was accomplished directly by preparative TLC (silica gel; chloroform/methanol/2N aqueous NH_3, 40:10:1). The product was then extracted from silica gel with methanol. The solution was filtered through reversed phase silica gel (RP-18).

The purity of the compounds was checked by TLC and by radiochromatogram scanning.

All labelled products were TLC identical to authentic unlabelled material.

(2S,3R,4E)-[3-^{3}H]-4-Amino-4-dodecen-1,3-diol (9A)

TLC (silica gel; chloroform/methanol/2N aqueous NH_3, 40:10:1): R_F = 0.25; specific radioactivity: 505 mCi/mmol.

(2S,3S,4E)-[3-^{3}H]-4-Amino-4-dodecen-1,3-diol (10A)

TLC (silica gel; chloroform/methanol/2N aqueous NH_3, 40:10:1): R_F = 0.21; specific radioactivity: 932 mCi/mmol.

(2S,3R,4E)-[3-^{3}H]-4-Amino-4-octadecen-1,3-diol (9B)

TLC (silica gel; chloroform/methanol/2N aqueous NH_3, 40:10:1): R_F = 0.29; specific radioactivity : 531 mCi/mmol.

2S,3S,4E)-[3-^{3}H]-4-Amino-4-octadecen-1,3-diol (10B)

TLC (silica gel; chloroform/methanol/2N aqueous NH_3, 40:10:1): R_F = 0.17; specific radioactivity: 966 mCi/mmol.

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REFERENCES

1. Kishimoto Y. - The Enzymes: Sphingolipids Formation (ed. P.D. Boyer), Vol. XVI, Academic Press, New York 1983
2. Wiegandt H. - Glycolipids, Elsevier, Amsterdam 1984
3. Sandhoff K. - Funkt. Biol. Med. 3: 141-151 (1984)
4. Hakomori S. - Trends Biochem. Sci. 9: 453-459 (1984)
5. Hakomori S. - Ann. Rev. Biochem. 50: 733-764 (1981)
6. Ando S. - Neurochem. Intern. 5: 507-537 (1983)

7. Hannun Y.A. and Bell R.M. - Science 243: 500-507 (1989)

8. van Echten G., Birk R., Brenner-Weiß G., Schmidt R.R. and Sandhoff K.- J. Biol. Chem. 265: 9333-9339 (1990)

9. Schwarzmann G. - Biochim. Biophys. Acta. 529: 106-114 (1978)

10. Stoffel W. and Sticht G. - Hoppe Seylers's Z. Physiol. Chem. 348: 1561-1569 (1967)

11. Ghidoni R., Sonnino S., Masserini M., Orlando P. and Tettamanti G. - J. Lipid. Res. 22: 1286-1295 (1981)

12. Kishimoto Y. and Mitry M.T. - Arch. Biochem. Biophys. 161: 426-434 (1974)

13. Iwamori M., Moser H.W. and Kishimoto Y. - J. Lipid. Res. 16: 332-336 (1975)

14. Iwamori M., Moser H.W., McCluser R.H. and Kishimoto Y. - Biochim. Biophys. Acta. 380: 308-310 (1975)

15. Schmidt R.R. and Zimmermann P. - Tetrahedron Lett. 27: 481-484 (1986)

16. Schmidt R.R. and Zimmermann P. - Liebigs Ann. Chem. 1988: 663-667 and references therein

17. Birk R. - Thesis, Universität Konstanz, to be submitted

18. Walker D. and Hiebert J.D. - Chem. Rev. 67: 153-195 (1967)

19. Turner A.B. - Synthetic Reagents (ed. J.S. Ellis), Vol. 3, pp. 193-225, Pizey-Horwood, Chichester, England 1977

20. a) Gololobov Y.G, Zhnmurova I.N. and Kasukhin L.F. - Tetrahedron 102: 437-472 (1981). b) Staudinger H. and Meyer J. - Helv. Chim. Acta. 2: 635-646 (1919)

21. a) Vaultier M., Knouzi N. and Carrié R. - Tetrahedron Lett. 1983: 763-764. b) Knouzi N., Vaultier M. and Carrié R. - Bull. Soc. Chim. Fr. 1985: 815-819