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TOTAL SYNTHESIS OF A HEXAOSYL CERAMIDE GLYCOLIPID ACTING AS A RECEPTOR FOR MACROPHAGE MIGRATION INHIBITION-FACTOR[1]

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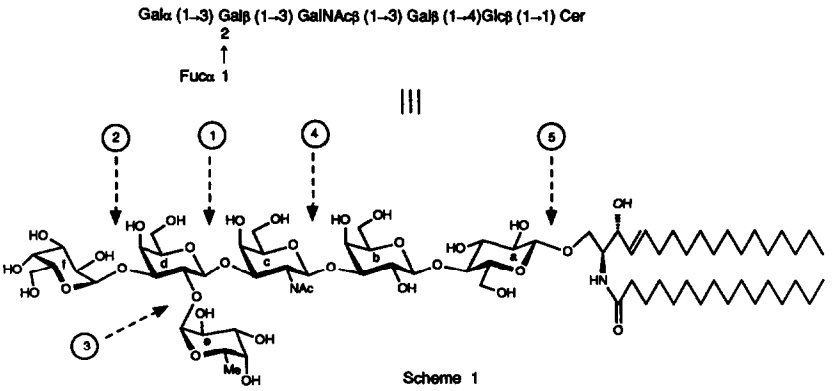
Abstract: A synthesis of the glycosphingolipid **1** constituting a macrophage receptor for MIF is described. It is based on trichloroacetimidate glycosyl donors and on the azidosphingosine glycosylation method. Regioselective O-glycosylation of a partially O-protected acceptor and direct transformation of the sphingosine azido group into a fatty acyl amido function were successfully applied in the execution of the synthesis.

Sugar residues play an important role as structure specific epitopes in biological recognition[2-4]. They also participate in the recognition process of macrophages[5,6]. Thus, studies concerning the interaction of the MIF (migration inhibition factor) with rat macrophages exhibited that MIF activity was specifically blocked by glycosphingolipid **1**[6] which was detected as a major constituent of the glycosphingolipids in rat peritoneal macrophages and structurally elucidated[7,8].



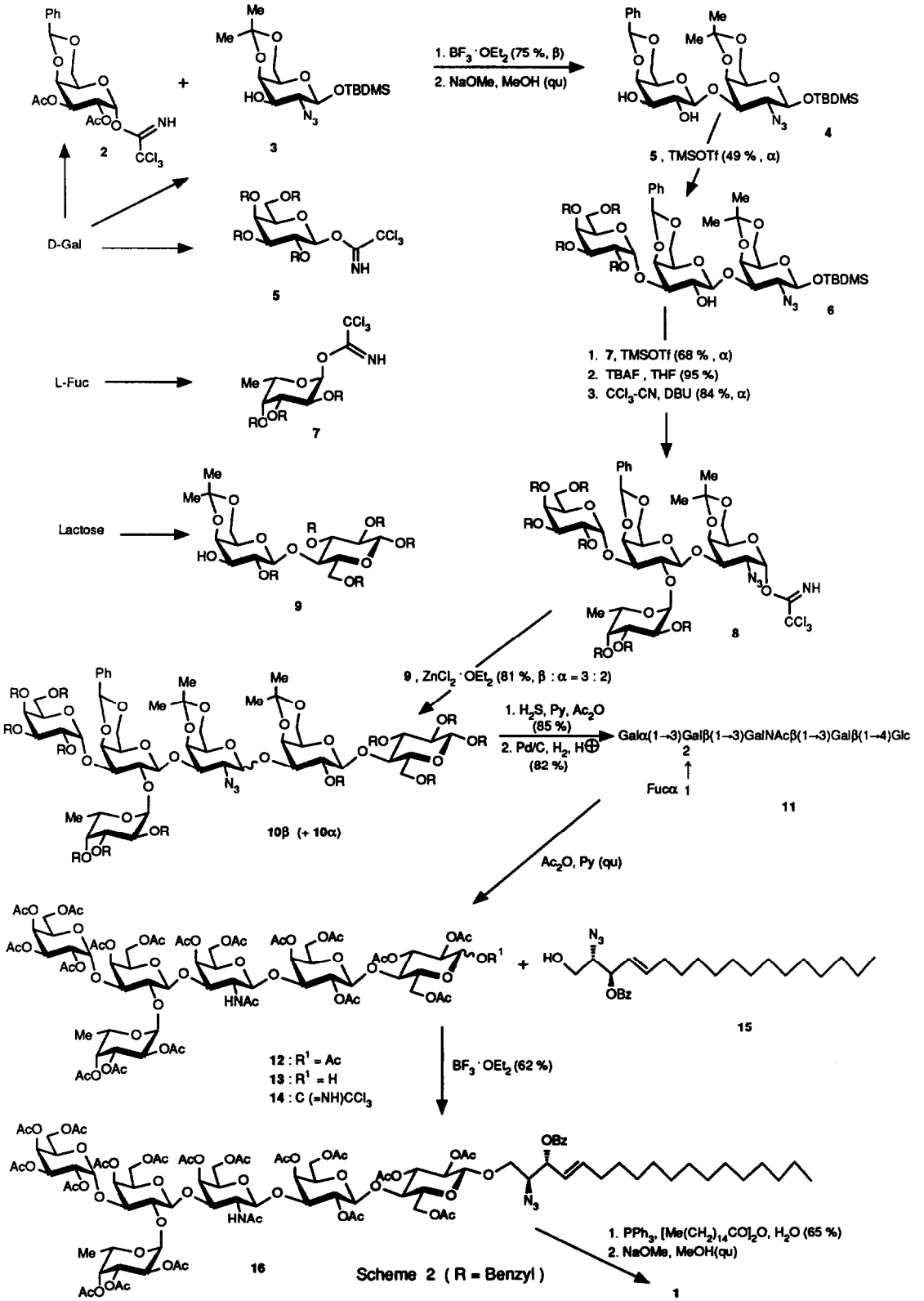
Scheme 1

This compound exhibits a unique structural feature: galactose moiety **d** (see Scheme 1) is consecutively connected to sugar residues (at position 1 β-connection to GalNAc, at position 2 α-fucosylation, and at position 3 α-galactosylation). Besides the possible sterical crowding, the biological importance is a challenge for the synthesis of **1**.

In the synthetic strategy galactose moiety **d** played an important role because chemoselective glycosylation of either HO-3 or HO-2, respectively, would require only a partially protected readily accessible building block, thus providing an efficient means for constructing the tetrasaccharide fragment **c** - **f** in the order **d** + **c** → **cd**, + **f** → **cdf**, + **e** → **c** - **f** (glycosylation reactions ① - ③). Connection of this fragment with a HO - 3b unprotected lactose building block **ab** (glycosylation ④), then application of the versatile and efficient azido sphingosine glycosylation method[9,10] (glycosylation ⑤), subsequently, transformation into a ceramide, and finally complete deprotection should successfully conclude the synthesis of glycosphingolipid **1**.

To this aim, as **d** building block the 2,3-di-O-acetyl-4,6-O-benzylidene protected O-(α-galactosyl)trichloroacetimidate **2** (Scheme 2) was prepared from galactose in a convenient four step route: transformation of galactose into the 4,6-O-benzylidene derivative[11], per O-acetylation of the hydroxylic groups[12], selective 1-O-deacetylation with hydrazinium acetate[13], and reaction with trichloroacetonitrile in presence of sodium hydride afforded donor **2**[14] in high overall yield. The acceptor **3** is readily obtained, as earlier described[15], from tri-O-acetyl-galactal. With $BF_3 \cdot OEt_2$ as the catalyst, **2** and **3** gave exclusively the β-connected disaccharide which furnished after NaOMe/MeOH treatment the O-2d,3d-deprotected compound **4**[14]. The galactosyl donor **5**[16] afforded with trimethylsilyl triflate (TMSOTf) as the catalyst, favoring formation of the thermodynamically more stable product[4], at -30° C regio- and stereoselectively the α-product at the less hindered O-3d position; thus, the desired trisaccharide **6**[14] was obtaind. Subsequent α-fucosylation with the readily available fucosyl donor **7**[17] in presence of the same catalyst furnished exclusively the α-connected tetrasaccharide, thus exhibiting the accessibility of the 2d-OH group in spite of the expected sterical crowding. Tetrabutylammonium fluoride (TBAF) promoted desilylation and then treatment with CCl_3-CN in presence of DBU afforded the donor **8**[14] in high yield. Reaction with the lactosyl acceptor **9**, readily obtained from lactose[18], displayed comparatively high donor reactivity necessitating the use of a milder catalyst. $ZnCl_2 \cdot OEt_2$ then furnished tetraosylation of the 3b-OH group in high yield (81 %); however, besides the desired β-isomer **10β**[14], also the α-isomer **10α**[14] (3:2-ratio) was obtained. Separation, azido group reduction with H_2S/pyridine, N-acetylation with acetic anhydride, subsequent hydrogenolytic debenzylation, and mild acidic deisopropylidenation afforded the deprotected hexasaccharide **11** as an anomeric mixture.

The required hexaosyl donor for the glycosphingolipid synthesis was generated from **11** via per-O-acetylation (→ **12**), selective 1-O-deacetylation with hydrazinium acetate[13] (→ **13**), and then treatment with trichloroacetonitrile in presence of NaH furnishing trichloroacetimidate **14** (α/β-mixture)[19] in high overall yield. Reaction with the 3-O-benzoyl protected azidosphingosine **15**[9] in presence of $BF_3 \cdot OEt_2$ as the catalyst provided due to neighboring group participation the desired hexaosyl β-glycoside **16** in 62 % yield, thus displaying the usefulness of the azidosphingosine glycosylation method. Azide group reduction and N-palmitoylation could be performed as a one pot procedure[20] by subsequently adding triphenylphosphine and then palmitic anhydride. Finally, complete de-O-acylation with NaOMe/MeOH provided the MIF receptor **1**. The ^{1}H-NMR data[14] for compounds **2**, **4**, **6**, **8**, **10α,β** and **1** are compatible with the assigned structures.



Scheme 2 (R = Benzyl)

Thus, an efficient, mainly highly stereoselective total synthesis of 1 solely based on trichloroacetimidate donors and on hexaosylation of azidosphingosine could be performed. The building blocks were readily obtained from D-galactose, L-fucose, and lactose. In an important step only partial O-protection was required.

REFERENCES AND FOOTNOTES

1. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.- Glycosylimidates, Part 46. For Part 45, see R.R. Schmidt, H. Gaden, H. Jatzke, *Tetrahedron Lett.*, in print.
2. S. Hakomori, *Chem. Phys. Lipids* **42** (1986) 209, T. Feizi, *Nature (London)* **314** (1986) 209; J. Koscielak, *Glycoconjugate J.* **3** (1986) 85.
3. H. Paulsen, *Angew. Chem.* **94** (1982) 184; *Angew. Chem. Int. Ed. Engl.* **21** (1982) 155.
4. R. R. Schmidt, *Angew. Chem.* **98** (1986) 213; *Angew. Chem. Int. Ed. Engl.* **25** (1986) 212; *Pure Appl. Chem.* **61** (1989) 1257.
5. D. L. Coleman, D. C. Morrison, J. L. Ryan, *Cell Immunol.* **100** (1986) 288; D. Y. Liu, S.-F. Yu, H.G. Remold, J. R. David, *ibid.* **90** (1985) 605.
6. T. Miura, S. Handa, T. Yamakawa, *J. Biochem.* **86** (1979) 773.
7. E. Hanada, S. Handa, K. Konno, T. Yamakawa, *ibid.* **83** (1978) 85.
8. Because of the structural similarity of **1** to the frequently occuring *ganglio* series, which possesses a GalNAcβ(1→4)Gal instead of a GalNAcβ(1→3)Gal linkage, this compound belongs to an "*isoganglio*" type.
9. R. R. Schmidt, P. Zimmermann, *Tetrahedron Lett.* **27** (1986) 481; *Angew. Chem.* **98** (1986) 722; *Angew. Chem. Int. Ed. Engl.* **25** (1986) 725; P. Zimmermann, R. Bommer, T. Bär, R. R. Schmidt, *J. Carbohydr. Chem.* **7** (1988) 435.
10. This aspect was recently also stressed by K. C. Nicolaou, T. Caulfield, H. Kataoka, T. Kumazawa, *J. Am. Chem. Soc.* **110** (1988) 7910.
11. E. G. Gros, V. Deulofeu, *J. Org. Chem.* **29** (1964) 3647.
12. J. Thiem, H.-P. Wessel, *Liebigs Ann. Chem.* **1981**, 2216.
13. G. Excoffier, D. Gagnaire, J.-P. Utille, *Carbohydr. Res.* **39** (1985) 368.
14. Values of $[\alpha]_D$ and δ_H were measured for solutions in $CHCl_3$ and $CDCl_3$ at 22° C, unless noted otherwise: **2**: $[\alpha]_D$ +152.5 (c, 1.5); δ_H (250 MHz), 6.71 (d, J = 3.4 Hz, H-1), 5.57 (dd, J = 3.4, 11 Hz, H-2), 5.39 (dd, J = 3.3, 11 Hz, H-3), 4.60 (d, J = 3.3 Hz, H-4); **4**: δ_H (250 MHz), 4.54 (d, 7.3 Hz, H-1), 4.51 (d, 7.6 Hz, H-1); **6**: $[\alpha]_D$ +44.5 (c 1); δ_H (250 MHz), 5.20 (d, J = 3.0 Hz, H-1f); **8**: $[\alpha]_D$ +40.0 (c 1); δ_H (250 MHz), 6.57 (d, J = 3.6 Hz, H-1c), 5.60 (d, J = 4.0 Hz, H-1e), 5.31 (d, J = 3.6 Hz, H-1f); **10α**: δ_H (250 MHz), 5.59 (d, J = 3.4 Hz, H-1e), 5.31 (d, J = 3.4 Hz, H-1f), 5.15 (d, J = 3.6 Hz, H-1c); **10β**: $[\alpha]_D$ +11.5 (c 1); δ_H (250 MHz), 5.59 (d, J = 3.3 Hz, H-1e), 5.32 (d, J = 3.4 Hz, H-1f); **1**: $[\alpha]_D$ +8.6 (c 0.2, pyridine); δ_H (400 MHz, D_6-DMSO/D_2O), 5.53 (ddd, J = 15.8, 6.9, 6.9 Hz, H-5cer), 5.31 (dd, J = 15.8, 6.6 Hz, H-4cer), 5.02, 4.95 (2d, J = 3.6, 3.6 Hz, H-1e, H-1f), 4.48, 4.45, 4.24, 4.16 (4d, J = 7.8, 7.8 8.0, 8.0 Hz, H-1a, H-1b, H-1c, H-1d), 1.82 (s, NAc), 1.06 (d, J = 6.7 Hz, 1 Me-Fuc), 0.83 (t, J = 6.6 Hz, 2 Me).
15. W. Kinzy, R. R. Schmidt *Carbohydr. Res.* **166** (1987) 265.
16. Obtained in four steps from D-Gal: R. R. Schmidt, J. Michel, M. Roos, *Liebigs Ann. Chem.* **1984**, 1343.
17. B. Wegmann, R. R. Schmidt, *Carbohydr. Res.* **184** (1988) 225.
18. R. Bommer, R. R. Schmidt, *Liebigs Ann. Chem.* **1989**, 1107
19. Due to neighboring group participation a single isomer was not required[4].
20. J. Hiebl, E. Zbiral, *Liebigs Ann. Chem.* **1988**, 765.

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