A Convenient Synthesis of Galactocerebroside Using D-Glucosamine as a Chiral Source of the Ceramide Moiety

Teiichi Murakami,* Hiroyuki Minamikawa and Masakatsu Hato
Research Institute for Polymers and Textiles, 1-1-4 Higashi, Tsukuba, Ibaraki 305, Japan

Galactocerebroside has been efficiently synthesized in 11 steps starting from *N*-benzoyl-D-glucosamine *via* 3-*O*-benzoylceramide with minimal manipulations of protecting groups.

Glycosphingolipids, a class of membrane constituents found mainly in the external surface of eukaryotic cells, are assumed to participate in many vital functions, such as intercellular specific recognition and immunological interactions.[1] These lipids consist of hydrophilic carbohydrate moieties and hydrophobic ceramide moieties, in which long-chain fatty acids are attached to long-chain amino alcohols named spingosines *via* amide linkages. Their increasingly recognized importance in biomedical research and their inaccessibility in homogeneous form from natural sources have promoted the development of efficient synthetic methods for glycosphingolipids,[2] especially for D-*erythro*-sphingosine [(2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol],[3] the most widely occurring of the sphingoid bases.

In searching for a more convenient route, we supposed that D-glucosamine (2-amino-2-deoxy-D-glucose) would be a suitable chiral starting material for the synthesis of D-*erythro*-sphingosine since both compounds have an amino group at the C-2 position with the same absolute configuration, as well as oxygen functionality at C-1 and C-3. The only reported approach[4] starting from D-glucosamine, however, required 15 steps to sphingosine resulting in low overall yield.

Here, we report a practical and short route to galactocerebroside (1-*O*-β-D-galactopyranosylceramide), the simplest but biologically important glycolipid,[1,5] utilizing D-glucosamine as a chiral source of the sphingosine moiety (Scheme 1). Thus, readily available *N*-benzoyl-D-glucosamine **1**, which was also used for the synthesis of phytosphingosine,[6] was treated with paraldehyde in the presence of conc. sulfuric acid[7] to give the 4,6-*O*-ethylidene derivative **2** [m.p. 225–228 °C (decomp.)]† in high yield. The acetal **2** was treated with trifluoromethanesulfonic anhydride in the presence of pyridine at −20 °C, and the course of the reaction was followed by TLC. Below 0 °C, **2** was smoothly converted into a major product, presumably the mono-*O*-triflate, which gradually decomposed to several products on warming to room temperature. The structure of the main product was determined to be 2-phenyl-(2,3-dideoxy-4,6-*O*-ethylidene-D-allopyranoso)[2,3-*d*]-4,5-dihydrooxazole **3**, a desired key intermediate, by its physical data and confirmed by further conversion. Although the yield of **3** was modest (41%), both the inversion of configuration and the protection of the C-3 hydroxy group was achieved in one step without C-1 protection. Subsequent reduction with $NaBH_4$ afforded the 1,5-diol **4** in 92% yield. Acid hydrolysis of **4** to remove the ethylidene acetal group caused the fission of the dihydrooxazole ring predominantly. However, the acetal was selectively cleaved by boron trifluoride–diethyl ether and thiophenol in dichloromethane[8] at 0 °C to give a water-soluble tetraol, which was treated with $NaIO_4$ in aqueous MeOH to give the lactol **5**‡ (3-amino-3-deoxy-L-erythrose derivative) in 77% yield from **4**. Treatment of **5** with tetradecylidenetriphenylphosphorane, derived from the corresponding phosphonium bromide and butyllithium, in tetrahydrofuran (THF)–toluene (1:1) gave 2-*N*-, 3-*O*-protected sphingosine **6** as a 1:9 mixture of *E*- and *Z*-olefins,§ from which the *E*-isomer could not be isolated. The *E*/*Z* mixture was treated with aqueous HCl–MeOH to give 3-*O*-benzoylsphingosine hydrochloride, which, without isolation, was treated with palmitoyl chloride in the presence of sodium acetate[9] to give 3-*O*-benzoyl-ceramide **7** (*E*:*Z* = 1:9) in 82% yield. Photoisomerization of **7** using a high pressure mercury lamp in the presence of diphenyl disulfide[10] afforded a 4:1 equilibrium mixture of *E*-**7a** and *Z*-**7b** olefins, which could be successfully separated by silica gel column chromatography eluting with CH_2Cl_2–AcOEt (6:1).¶ Glycosylation of **7a** with tetraacetylgalactopyranosyl trichloroacetimidate according to the method of Schmidt *et al.*[11] gave the corresponding β-glycoside stereoselectively in 72% yield. Finally, deprotection with an excess of NaOMe in MeOH afforded the desired cerebroside **8a** {m.p. 146–148 °C (transition temperature to liquid crystal);‖ $[\alpha]_D^{22} < \pm 0.5$ (*c* 0.80, $CHCl_3$–MeOH = 1:1)}. In a similar manner, unnatural galactosyl-*Z*-ceramide **8b** {m.p. 135–137 °C (liquid crystal);‖ $[\alpha]_D^{22}$ −11.9 (*c* 0.46, $CHCl_3$–MeOH = 1:1)} was also obtained in 68% yield from **7b**.

In conclusion, 1-*O*-β-D-galactopyranosyl-2-*N*-palmitoyl-D-*erythro*-C_{18}-sphingosine **8a** was synthesized in only 11 steps in *ca.* 10% overall yield from **1** with minimal manipulations of protecting groups. More importantly, this novel route conveniently provides a glycosyl acceptor, 1-*O*-unprotected-3-*O*-

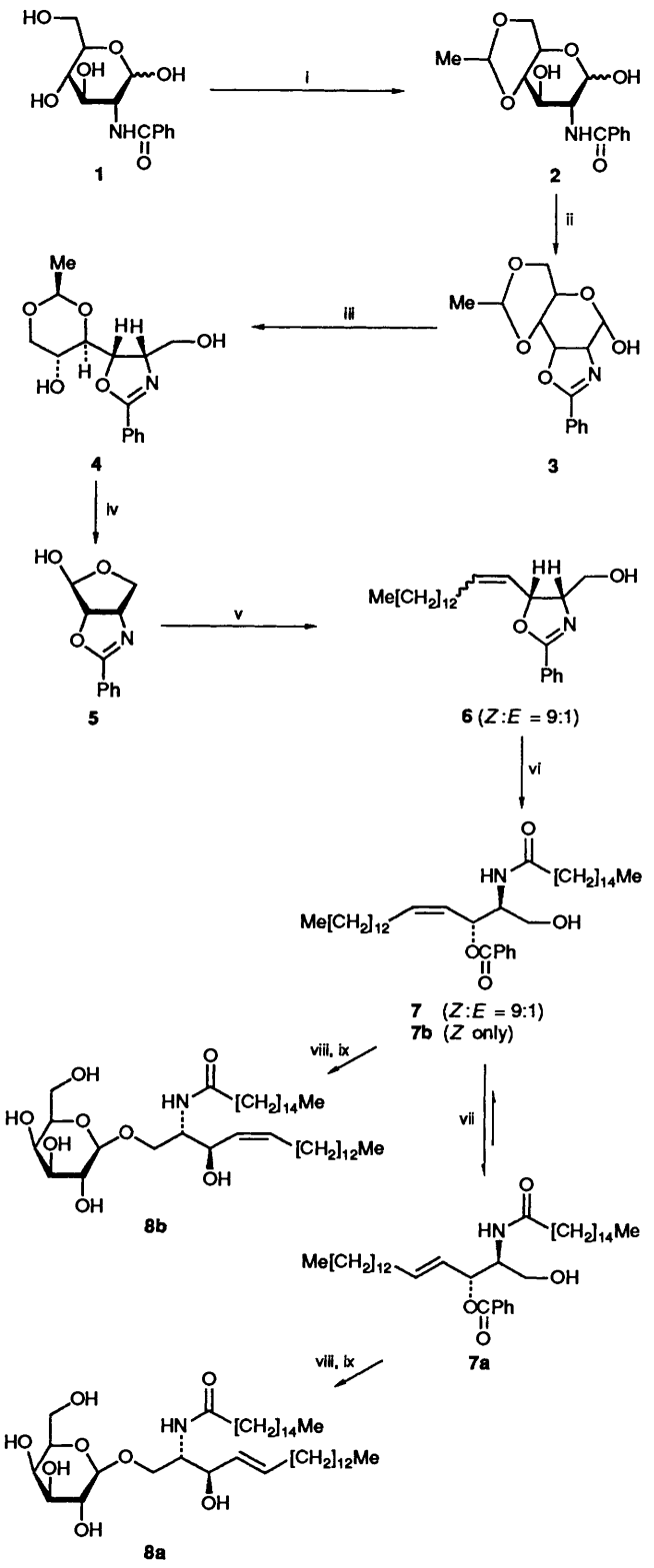
† Satisfactory spectroscopic and analytical data were obtained for all new compounds reported.

‡ Physical data for **5**: m.p. 155–157 °C; $[\alpha]_D^{22}$ +68.5 (*c* 0.42, $CHCl_3$) Found: C, 64.4; H, 5.4; N, 6.8. $C_{11}H_{11}NO_3$ requires C, 64.4; H, 5.4; N, 6.8); ν_{max}(KBr)/cm^{-1} 3250, 2950 and 1645; δ_H(360 MHz; $CDCl_3$) 4.12 (1 H, d, *J* 9.5, 1-H_a), 4.29 (1 H, dd, *J* 5.3 and 9.5, 1-H_b), 4.90 (1 H, dd, *J* 5.3 and 7.4, 2-H), 5.01 (1 H, d, *J* 7.4, 3-H), 5.59 (1 H, s, 4-H), 7.41 (2 H, m, Ph), 7.48 (1 H, m, Ph) and 7.91 (2 H, m, Ph).

§ The *E*/*Z* ratio, determined by ^{1}H NMR spectroscopy, varied slightly depending on the reaction solvents, between 1:5 (in toluene only) and 1:13 (in THF only). The *Z*-isomer of **6** could be isolated by recrystallization from hexane–AcOEt; m.p. 101–103 °C; $[\alpha]_D^{22}$ −57.8 (*c* 0.45, $CHCl_3$); δ_H(360 MHz; $CDCl_3$) 0.88 (3 H, t, *J* 6.7, 18-Me), 1.26 (20 H, s-like, 8 to 17-CH_2), 1.42 (2 H, m, 7-CH_2), 2.13 (2 H, m, 6-CH_2), 2.70 (1 H, br s, OH), 3.67 (1 H, dd, *J* 5.6 and 11.6, 1-H_a), 3.79 (1 H, dd, *J* 4.1 and 11.6, 1-H_b), 4.39 (1 H, ddd, *J* 4.1, 5.6 and 9.8, 2-H), 5.52 (1 H, dd, *J* 8.6 and 9.8, 3-H), 5.70 (1 H, dd, *J* 8.6 and 10.9, 4-H), 5.77 (1 H, dt, *J* 7.0 and 10.9, 5-H), 7.39 (2 H, m, Ph), 7.47 (1 H, m, Ph) and 7.94 (2 H, m, Ph). (The numbering corresponds to that of sphingosine.)

¶ **7a**: R_f 0.24; m.p. 90–92 °C; $[\alpha]_D^{22}$ +17.8 (*c* 0.56, $CHCl_3$); ^{1}H NMR ($CDCl_3$) $J_{4,5}$ 15.0 {lit.,[13a] m.p. 88–90 °C; $[\alpha]_D^{25}$ +13.0 (*c* 1.05, $CHCl_3$)}. **7b**: R_f 0.32; m.p. 115–117 °C: $[\alpha]_D^{22}$ +37.3 (*c* 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$) $J_{4,5}$ 10.7.

‖ Both lipids **8a** and **8b** showed a further phase transition from liquid crystal to isotropic liquid at a temperature *ca.* 40 °C higher than its m.p. This phase behaviour was identical with natural galactocerebroside purchased from Sigma; for a related report, see ref. 12.



Scheme 1 *Reagents and conditions:* i, $(CH_3CHO)_3$, conc. H_2SO_4 (0.05 equiv.), room temp., 12 h, 95%; ii, $(CF_3SO_2)_2O$ (1.05 equiv.), pyridine, CH_2Cl_2, −20 to 0 °C to room temp., 41%; iii, $NaBH_4$, MeOH, 0 °C, 1 h, 92%; iv, BF_3·OEt_2 (4 equiv.), PhSH (8 equiv.), CH_2Cl_2, 0 °C, 10 h; then $NaIO_4$ (2.5 equiv.), $NaHCO_3$ (pH 7.5–8.0), aq. MeOH, 0 °C to room temp., 24 h, 77%; v, $Ph_3P^+(CH_2)_{13}CH_3$·Br^- (1.7 equiv.), BuLi (2.0 equiv.), THF–toluene (1:1), −30 to 0 °C, 3 h to room temp., 91%; vi, 2 mol dm^{-3} aq. HCl–MeOH (1:9), room temp., 24 h; then $C_{15}H_{31}COCl$ in CH_2Cl_2 and NaOAc in H_2O, 0 °C, 2 h, 82%; vii, *hv*, PhSSPh, cyclohexane–dioxane (10:1), room temp., 3 h, 73%; viii, *O*-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)trichloroacetimidate (2 equiv.), BF_3·OEt_2 (4 equiv.), molecular sieves 4 Å, CH_2Cl_2, −20 to 0 °C, 72%; ix, NaOMe (2 equiv.), MeOH, 0 °C to room temp., 3 h, 95%

benzoyl-ceramide **7a**, which can be used not only for the synthesis of more complex glycosphingolipids[13] but also for the synthesis of sphingomyelins[14] in optically active form.

Experimental

J values are given in Hz and $[\alpha]_D$ values in 10^{-1} deg cm^2 g^{-1} throughout.

2-*Phenyl*-(2,3-*dideoxy*-4,6-O-*ethylidene*-D-*allopyranoso*)[2,3-d]-4,5-*dihydrooxazole* **3**.—To a cooled (−20 °C) solution of *N*-benzoyl-4,6-*O*-ethylidene-D-glucosamine **2** (3.09 g, 10 mmol) in dichloromethane (30 cm^3) and pyridine (8 cm^3) was added trifluoromethanesulfonic anhydride (2.95 g, 10.5 mmol) in dichloromethane (8 cm^3) over 20 min under nitrogen. The reaction mixture was stirred at 0 °C for 1 h and allowed to warm to room temp., and the stirring was continued for 3 h. To this was added saturated aqueous $NaHCO_3$ (20 cm^3), and the aqueous phase was extracted with dichloromethane. The combined organic layer was washed successively with aqueous $NaHCO_3$, water, brine and dried (Na_2SO_4). Evaporation of the solvent gave an orange syrup, to which was added AcOEt (*ca.* 5 cm^3) to deposit the title compound **3** as colourless crystals (960 mg). The filtrate was chromatographed on silica gel (hexane–AcOEt, 1:1) to give additional compound **3** (234 mg) (total 1.194 g, 41%); m.p. 162–165 °C; $[\alpha]_D^{22}$ +33.8 (*c* 1.0, $CHCl_3$) (Found: C, 61.5; H, 5.9; N, 4.65. $C_{15}H_{17}NO_5$ requires C, 61.85; H, 5.9; N, 4.8%); ν_{max}(KBr)/cm^{-1} 3180, 2930 and 1645 (C=N); δ_H(360 MHz; $CDCl_3$) (α-anomer predominant) 1.41 (3 H, d, *J* 5.0, Me), 3.45 (1 H, t, *J* 10.3, 6-Hax), 3.73 (1 H, dt, *J* 5.0 and 10.1, 5-H), 4.09 (1 H, dd, *J* 5.0 and 10.3, 6-Heq), 4.37 (1 H, dd, *J* 3.2 and 10.1, 4-H), 4.57 (1 H, dd, *J* 2.3 and 9.6, 2-H), 4.79 (1 H, q, *J* 5.0, MeC*H*), 5.04 (1 H, dd, *J* 3.2 and 9.6, 3-H), 5.26 (1 H, d, *J* 2.3, 1-H), 7.42 (2 H, m, Ph), 7.52 (1 H, m, Ph) and 8.02 (2 H, m, Ph).

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