Synthetic Studies on Glycosphingolipids from Protostomia Phyla: Synthesis of Amphoteric Glycolipid Analogues Containing a Phosphocholine Residue from the Earthworm Pheretima hilgendorfi

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Two kinds of amphoteric glycosphingolipid analogues from the earthworm Pheretima hilgendorfi were synthesized as follows: The key reaction is a coupling of a phosphocholine group at the position C-6 of 1 and 6 which was attempted using 2-chloro-2-oxo-1,3,2-dioxaphospholane, followed by reaction of the resulting cyclic phosphate intermediate with anhydrous trimethylamine to give 2 and 7. Subsequent debenzylation afforded target compounds 3, 8. Their ability to inhibit the histamine release in vitro was examined.

Key words amphoteric glycosphingolipid; Pheretima hilgendorfi; chemical synthesis; phosphocholine

We have been interested in structure–activity relationships of glycosphingolipids derived from invertebrate animal species and have synthesized oligosaccharides from various protostomia phyla.1 Sugita et al.2 found and characterized a new neogala series of glycosphingolipids, of which core structure was a β-D-Galp-(1→6)-β-D-Galp- containing a mannosine, glucose and phosphocholine residue from the earthworm Pheretima (P) hilgendorfi. In the previous paper3 we reported the synthesis of neogala series glycosphingolipids containing a mannosine residue from P. hilgendorfi. In the present study, we attempted the synthesis of the novel neogala series of glycosphingolipids containing the phosphocholine residue. These compounds have a phosphocholine group at the C-6 position of the galactose moiety to form a zwitterionic structure A and B (Fig. 1). Furthermore Noda and his co-workers also isolated similar compounds and examined the structure–bioactivity relationships about fruiting-inducing effect.4 As part of our program of oligosaccharide synthesis, the preparation of neogala series containing a phosphocholine group at the C-6 position of the galactose moiety, from P. hilgendorfi, became of interest. Toward that end, the key reaction is a coupling of a phosphocholine group at the C-6 position of the galactose moiety. Concerning that, another novel phosphocholine-containing glycosphingolipids called GGPL-I and GGPL-III were found from the main cell membrane lipid components of Mycoplasma fermentans and these compounds were synthesized by Kobayashi and his co-workers.5 They used a phosphorodiamidate method at the C-6 position of glucose unit, p-nitrophenyl (pNP) 6-O-phosphocholine α-D-glucopyranoside. Up to the present, coupling incorporation of phosphocholine was performed as follows: A) phosphorylation with 2-bromoethyl phosphoryl dichloride and trimethylamine;6 B) a phosphorodiamidate method5; C) phosphorylation with 2-chloro-2-oxo-1,3,2-dioxaphospholane5 in place of 2-bromoethyl phosphoryl dichloride. However, for the phosphorylation of carbohydrate hydroxy group, either A) or B)5,6 was used. In the present study, we investigated mono- and disaccharides phosphorylation by means of method C) using 2-chloro-2-oxo-1,3,2-dioxaphospholane and trimethylamine and attempted the synthesis of these novel amphoteric glycosphingolipid analogues containing fatty alkyl residue in place of ceramide. Furthermore, final compounds were examined for their ability to inhibit the histamine release in vitro.

Results and Discussion

Synthesis of Monosaccharide Containing Phosphocholine Octyl 2,3,4-tri-O-benzyl-β-D-galactopyranoside (1)9 was chosen as an acceptor prepared from octyl β-D-galactopyranoside. Introduction of a phosphocholine group at the position C-6 of 1 was attempted using 2-chloro-2-oxo-1,3,2-dioxaphospholane, followed by reaction of the resulting cyclic phosphate intermediate with anhydrous trimethylamine at 65 °C in a sealed vessel to give 2 in 68% yield for two steps. Compound 2 revealed an [M+H]+ ion peak at m/z 728 in the time of flight mass spectrometer (TOF-MS) spectrum. Removal of the benzyl groups from 2 by catalytic hydrogenolysis over 10% Pd-C gave the target compound 3 (Chart 1).

Synthesis of Disaccharide Containing Phosphocholine The disaccharide derivative 5 was obtained by condensation of 1 with phenyl 1-thio-2,3,4-tri-O-benzyl-6-O-tert-butyldimethylsilyl-β-D-galactopyranoside (4) in the presence of N-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH).9 Stereochemical control was achieved by using the solvent effect of nitrile10 to give the desired β-c-glycoside 5 in 91% yield and the α-glycoside was not detected. The anomeric hydrogen atom of the nonreducing-end galactose unit appeared as a signal at δ 4.22 (d, J = 7.9 Hz). The β-D-
configuration of the newly formed glycosidic bond was also supported by the $J_{	ext{C,H}}$ value of 159.7 Hz in the $^{13}$C-NMR spectrum. Removal of the tert-butyldimethylsilyl (TBDMS) group from 5 by Bu$_4$NF gave the disaccharide intermediate 6 in 86% yield. Coupling of a phosphocholine group with 6 as described for 2 gave the desired compound 7 (91%), and removal of the benzyl groups from 7 by catalytic hydrogenolysis over 10% Pd–C gave the target compound 8 (Chart 2).

**Biological Activities** The biological activity of compound 3, 8 and phosphocholine free compound 9 were assayed in vitro by histamine release-inhibition test. The method using rat basophilic leukemia cells (RBL-2H3) was reported by Teshima et al. As shown in Fig. 2, compounds 3 and 8 showed a better activity for histamine release-inhibition from RBL-2H3 cell than compound 9. The IC$_{50}$ values were 2.6 mm (3), 2.9 mm (8) and >10 mm (9). On the other hand, octyl $\beta$-D-galactopyranoside could not assay in order to show a potent cytotoxicity.
In summary, we have synthesized for the first time the oligosaccharides including phosphocholine using 2-chloro-2-oxo-1,3,2-dioxaphospholane in good yield and a phosphocholine group seemed to act as an inhibitor to a histamine release.

Experimental

Optical rotations were determined with a JASCO digital polarimeter. $^1$H- and $^13$C-NMR spectra were recorded on a JNM A 500 FT NMR spectrometer in CDCl$_3$ with Me$_4$Si as the internal standard. Matrix-assisted laser desorption ionization (MALDI-TOF-MS) was recorded on a Perceptive Voyager RP mass spectrometer. TLC was performed on Silica gel 60 F$_{254}$ (E. Merck) with detection by quenching of UV fluorescence and by spraying with 10% H$_2$SO$_4$. Column chromatography was carried out on Silica Gel 60 (E. Merck).

**Octyl 2,3,4-Tri-o-benzyl-$\beta$-galactopyranoside (1)** was prepared by a literature method.8

**Octyl 2,3,4-Tri-o-benzyl-$\beta$-galactopyranoside-6-$\beta$-phosphocholine (2)** To a solution of 1 (102 mg, 0.18 mmol) and triethylamine (21 µl, 0.15 mmol) in dry benzene (5 ml) was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (50 µl, 0.54 mmol) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was filtered to remove the precipitated salts. The filtrate and washings were combined and concentrated. This compound was transferred into a sealed vessel as a solution in 10 ml of dry acetonitrile. To the solution was added 100 µl of trimethylamine and the bottle was sealed and then heated in an oil bath at 65 °C for 48 h. Resulting mixture was concentrated and the residue was purified by fatchromatography, eluting with CHCl$_3$-MeOH-H$_2$O (8 : 4 : 1) to give the desired compound (2) (89.1 mg, 67.5%). $[^{13}C]_{\mathrm{NMR}}$ (CDCl$_3$): $\delta$ 4.34 (1H, d, $J$ = 7.9 Hz, H-1), 4.22 (2H, m, CH$_2$CH$_3$N), 3.64 (2H, m, CH$_2$CH$_3$N), 3.16 (9H, s, N(CH$_3$)$_3$). MALDI-TOF-MS: Calculated for C$_{25}$H$_{50}$NO$_{14}$P $m/z$ 547. Found: $m/z$ 528 [M+H$^+$].

**Octyl 6-O-Phosphocholine-$\beta$-galactopyranoside (3)** A solution of 2 (81.5 mg, 0.11 mmol) in MeOH (4 ml) containing conc. HCl (0.1 ml) was hydrogenated over 10% Pd-C (54 mg) at 12 h room temperature, then filtered through Celite and the filter cake was washed with methanol. Combined filtrate and washings were concentrated. Column chromatography (MeOH:CH$_2$Cl$_2$ = 1 : 1) of the residue on Sephadex LH-20 gave 8 (36.4 mg, 92%). $[^{13}C]_{\mathrm{NMR}}$ (CDCl$_3$): $\delta$ 4.69 (1H, d, $J$ = 8.6 Hz, H-1), 4.14 (2H, m, OCH$_2$CH$_3$), 3.49 (2H, m, CH$_2$CH$_3$N), 3.05 (9H, s, N(CH$_3$)$_3$). MALDI-TOF-MS: Calculated for C$_{25}$H$_{49}$NO$_8$P $m/z$ 529. Found $m/z$ 527 [M+H$^+$].

**Octyl 6-O-Phosphocholine-$\beta$-galactopyranosyl-(1 $\rightarrow$ 6)-$\beta$-galactopyranosyl (4)** A solution of 8 (74.3 mg, 0.06 mmol) in MeOH (4 ml) containing conc. HCl (0.1 ml) was hydrogenated over 10% Pd-C (54 mg) for 12 h at room temperature, then filtered through Celite and the filter cake was washed with methanol. Combined filtrate and washings were concentrated. Column chromatography (MeOH:CH$_2$Cl$_2$ = 1 : 1) of the residue on Sephadex LH-20 gave 9 (67.7 mg, 89%). $[^{13}C]_{\mathrm{NMR}}$ (CDCl$_3$): $\delta$ 4.30 (1H, d, $J$ = 8.0 Hz, H-1), 4.19 (1H, d, $J$ = 7.5 Hz, H-1). MOLDI-TOF-MS: Calculated for C$_{62}$H$_{74}$O$_{11}$N$_3$ $m/z$ 1019. Found: $m/z$ 1018 [M+H$^+$].

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