SYNTHESES OF BIOLOGICALLY ACTIVE SIALOSYLGLYCEROL DERIVATIVES

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New sialosylglycerol derivatives were synthesized and found to inhibit the phospholipase A_2 and C activities

KEYWORDS sialosylglycerol derivative; phospholipase A_2 inhibitor; phospholipase C inhibitor; polysaccharide; hexadecanoic acid

A constituent of bacterial cell wall has various biological activities such as immnological response, phage receptor, endotoxin etc.. In 1981, the capsular polysaccharide of *Neisseria meningitidis*, which plays the main antigen role, was structurally defined (Fig 1).[1] The polysaccharide consists of a polymer of (α2→9) sialic acid, which has many important functions as constituents of glycoconjugate, and phosphoglycerolipid. Recently we have synthesized a series of biologically active compounds designed on the basis of the chemical structure of bacterial cell wall.[2] Here, we describe the synthesis of the sialosylglycerolipids ($1\alpha_{a-d}$, $1\beta_{a-d}$, $2\alpha_a$, and $2\beta_a$) which imitate the partial structure of the capsular polysaccharide. The synthetic design of these compounds was determined as follows. The absolute configuration of the glycerol C-2 (S) was the same as that of the natural product and the 2-hydroxyl glycerolipid (lyso type) was expected to inhibit phospholipase A_2 by feedback regulation. Four kinds of fatty acid (a-d) were studied to determine the differences among their biological activities due to the fatty acid type. Similarly, sialosyl-(R)-glycerol derivatives were studied with regard to their palmitoyl type.

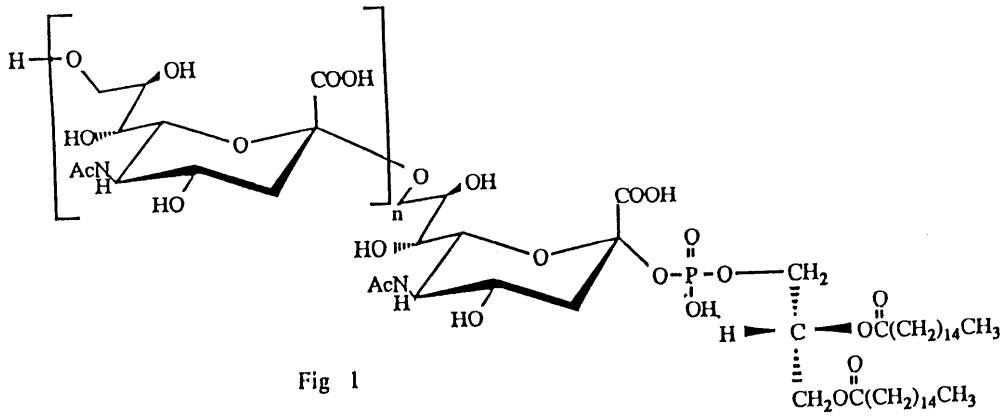

Fig 1

Chart 1 shows the synthetic route of the sialosyl-(S)-glycerol derivatives. (S)-1-O-Acetyl-2-O-benzylglycerol (3),[3] the chiral starting material was treated with trityl chloride and pyridine to give the 3-tritylated compound (4; 72.5%, oil, $[\alpha]_D$ +11.3°). Alkaline hydrolysis of the acetate (4) led to the (R)-1-O-trityl-2-O-benzylglycerol derivatives (5; 77.4%, mp 59-62 C°, $[\alpha]_D$ +22.5°). The 3-hydroxyl compound (5) was acylated with acyl chlorides and triethylamine to yield 6_{a-d} (6_a; 85.0%, oil,

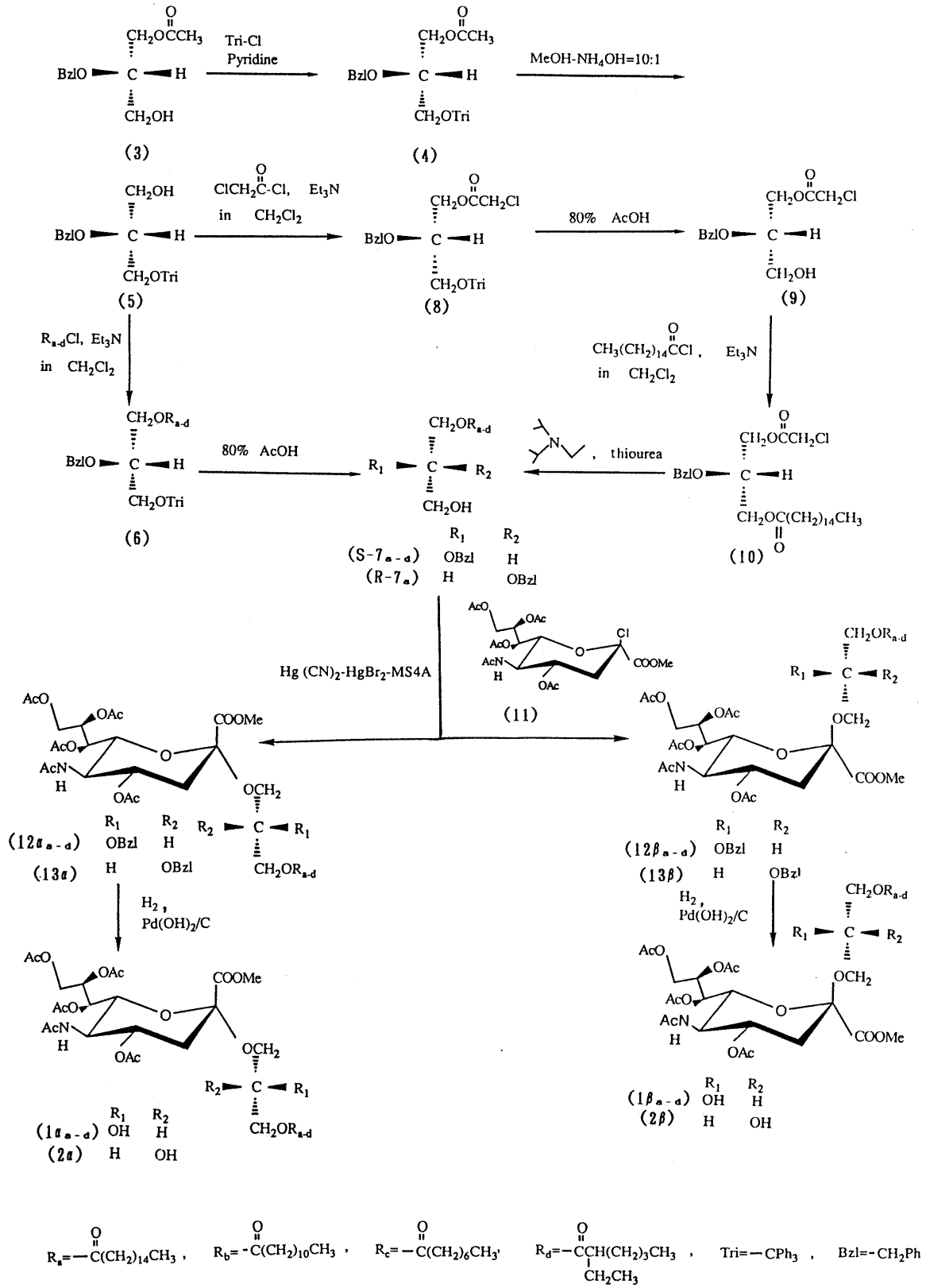

Chart 1

$[\alpha]_D$ +12.6°, 6_b; 93.7%, oil, $[\alpha]_D$ +11.6°, 6_c; 86.0%, oil, $[\alpha]_D$ +4.7°, 6_d; 88.8%, oil, $[\alpha]_D$ +9.3°). The trityl group of 6_{a-d} was removed by hydrolysis with 80% aqueous acetic acid at 80°C to afford (S)-1-O-acyl-2-O-benzylglycerols (S-7_{a-d}, S-7_a; 74.6%, oil, $[\alpha]_D$ -4.8°, S-7_b; 80.3%, oil, $[\alpha]_D$ -5.7°, S-7_c; 73.9%, oil, $[\alpha]_D$ -6.4°, S-7_d; 85.8%, oil, $[\alpha]_D$ -8.0°). These were used as the glycosyl acceptor. (R)-Glycerol derivative (R-7_a) was synthesized in the following way. The 1-O-hydroxyl compound (5) was chloroacetylated to give 8 (91.5%, oil, $[\alpha]_D$ +10.7°). Detritylation of the chloroacetyl compound (8) gave the 3-hydroxyl compound (9, 69.0%, oil, $[\alpha]_D$ -3.1°). The compound (10, 70.1%, oil, $[\alpha]_D$ +2.8°) was obtained by treating 9 with palmitoyl chloride and triethylamine. Dechloroacetylation of 10 was achieved with diisopropylethylamine and thiourea to yield the (R)-glycosyl acceptor (R-7_a, 92.7%, oil, $[\alpha]_D$ +4.7°). S-7_{a-d} was glycosylated with the glycosyl donor (11) in the presence of $Hg(CN)_2$, $HgBr_2$ and molecular sieves 4A to give $12\alpha_{a-d}$ and $12\beta_{a-d}$, respectively. The resulting anomeric mixture was separated by preparative TLC ($CHCl_3$-MeOH = 20:1) and their structures were confirmed on the basis of the chemical shift of the H-3eq atom of $12\alpha_{a-d}$ and $12\beta_{a-d}$ in the ^{1}H-NMR spectrum: the H-3eq chemical shift of α glycoside was lower than that of β glycoside ($12\alpha_a$; 26.9%, amorphous, $[\alpha]_D$ -8.5°, $12\beta_a$; 32.4%, amorphous, $[\alpha]_D$ -5.5°, $12\alpha_b$; 10.8%, amorphous, $[\alpha]_D$ -3.7°, $12\beta_b$; 11.5%, amorphous, $[\alpha]_D$ -6.6°, $12\alpha_c$; 15.6%, amorphous, $[\alpha]_D$ -18.2°, $12\beta_c$; 27.0%, amorphous, $[\alpha]_D$ -10.7°, $12\alpha_d$; 17.1%, amorphous, $[\alpha]_D$ -16.7°, $12\beta_d$; 23.5%, amorphous, $[\alpha]_D$ -8.2°). $12\alpha_{a-d}$ and $12\beta_{a-d}$ were hydrogenolyzed with $Pd(OH)_2$/C in methanol to give the sialosyl-(S)-glycerol derivatives ($1\alpha_{a-d}$ and $1\beta_{a-d}$, $1\alpha_a$; 71.2%, amorphous, $[\alpha]_D$ -11.3°, $1\beta_a$; 74.1%, amorphous, $[\alpha]_D$ -12.6°, $1\alpha_b$; 95%, amorphous, $[\alpha]_D$ -8.0°, $1\beta_b$; 87%, amorphous, $[\alpha]_D$ -10.0°, $1\alpha_c$; 95%, amorphous, $[\alpha]_D$ -7.8°, $1\beta_c$; 96%, amorphous, $[\alpha]_D$ -5.1°, $1\alpha_d$; 82%, amorphous, $[\alpha]_D$ -4.9°, $1\beta_d$; 85%, amorphous, $[\alpha]_D$ -6.0°).[4-5]

Sialosyl-(R)-glycerol derivatives (2α and 2β) were synthesized in the same manner. The (R)-1-O-hexadecanoyl-2-O-benzylglycerol (R-7_a) was glycosylated with 11 in the presence of $Hg(CN)_2$, $HgBr_2$, and molecular sieves 4A to give an anomeric mixture of sialosylglycerol compounds (13α and 13β, 13α; 24.6%, amorphous, $[\alpha]_D$ -10.8°, 13β; 20.9%, amorphous, $[\alpha]_D$ -7.0°). 13α and 13β were each hydrogenolyzed with $Pd(OH)_2$/C to yield the sialosyl-(R)-glycerol derivatives (2α and 2β, 2α; 86.2%, amorphous, $[\alpha]_D$ -3.6°, 2β; 84.8%, amorphous, $[\alpha]_D$ -4.8°).

Preliminary examination of the biological activities revealed that the lyso-sialosylpalmitoylglycerol derivatives ($1\alpha_a$, $1\beta_a$, $2\alpha_a$, and $2\beta_a$) have the most powerful phospholipase A_2 and phospholipase C inhibitory activities among the investigated sialosyl derivatives.[6-7]

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4. ^{1}H-NMR of H-3eq (ppm, J=Hz): $12\alpha_a$; 2.59 (1H, dd, J=4.9, 12.9), $12\alpha_b$; 2.61 (1H, dd, J=4.4, 12.4), $12\alpha_c$; 2.61 (1H, dd, J-4.9, 12.4), $12\alpha_d$; 2.61 (1H, dd, J=4.6, 12.4), 13α; 2.61 (1H, dd, J=4.6, 12.7): Because H-3eq signals of $12\beta_{a-d}$ and 13β were overlapped by the methylene protons of fatty acid, the evidence of β linkage was the fact that H-3eq signals were not found at downfield less than 2.49 ppm.

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