

SYNTHESES OF BIOLOGICALLY ACTIVE SIALOSYLGLYCEROL DERIVATIVES

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New sialosylglycerol derivatives were synthesized and found to inhibit the phospholipase A₂ and C activities

KEYWORDS sialosylglycerol derivative; phospholipase A₂ inhibitor; phospholipase C inhibitor; polysaccharide; hexadecanoic acid

A constituent of bacterial cell wall has various biological activities such as immunological 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 (α 2 $\rightarrow$ 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 α -d, 1 β -d, 2 α , and 2 β) 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 A₂ 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.

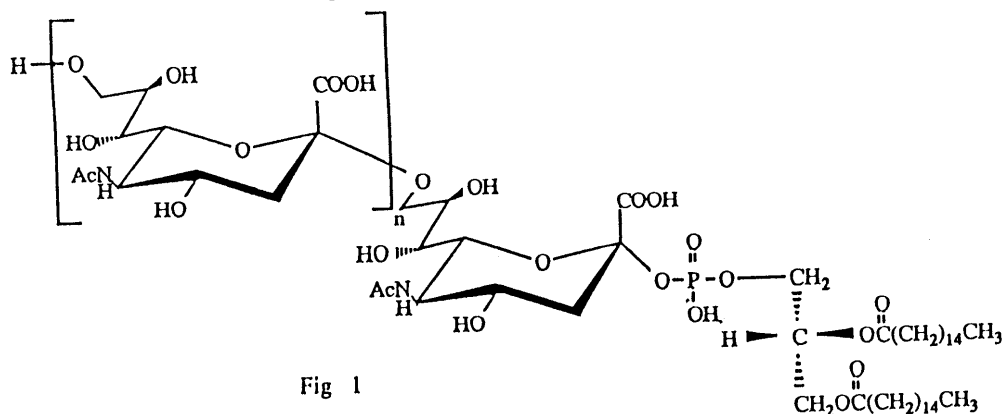
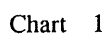


Fig 1

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, $[\alpha]_D +11.3^\circ$). Alkaline hydrolysis of the acetate (4) led to the (R)-1-O-trityl-2-O-benzylglycerol derivatives (5; 77.4%, mp 59–62 C°, $[\alpha]_D +22.5^\circ$). The 3-hydroxyl compound (5) was acylated with acyl chlorides and triethylamine to yield 6 α -d (6 α ; 85.0%, oil,



$[\alpha]_D +12.6^\circ$, 6_b ; 93.7%, oil, $[\alpha]_D +11.6^\circ$, 6_c ; 86.0%, oil, $[\alpha]_D +4.7^\circ$, 6_d ; 88.8%, oil, $[\alpha]_D +9.3^\circ$). 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, $[\alpha]_D -4.8^\circ$, S-7 $_b$; 80.3%, oil, $[\alpha]_D -5.7^\circ$, S-7 $_c$; 73.9%, oil, $[\alpha]_D -6.4^\circ$, S-7 $_d$; 85.8%, oil, $[\alpha]_D -8.0^\circ$). 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, $[\alpha]_D +10.7^\circ$). Detritylation of the chloroacetyl compound (8) gave the 3-hydroxyl compound (9, 69.0%, oil, $[\alpha]_D -3.1^\circ$). The compound (10, 70.1%, oil, $[\alpha]_D +2.8^\circ$) 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, $[\alpha]_D +4.7^\circ$). S-7 $_{a-d}$ was glycosylated with the glycosyl donor (11) in the presence of $\text{Hg}(\text{CN})_2$, HgBr_2 and molecular sieves 4A to give 12 α_{a-d} and 12 β_{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 α_{a-d} and 12 β_{a-d} in the ^1H -NMR spectrum: the H-3eq chemical shift of α glycoside was lower than that of β glycoside (12 α_a ; 26.9%, amorphous, $[\alpha]_D -8.5^\circ$, 12 β_a ; 32.4%, amorphous, $[\alpha]_D -5.5^\circ$, 12 α_b ; 10.8%, amorphous, $[\alpha]_D -3.7^\circ$, 12 β_b ; 11.5%, amorphous, $[\alpha]_D -6.6^\circ$, 12 α_c ; 15.6%, amorphous, $[\alpha]_D -18.2^\circ$, 12 β_c ; 27.0%, amorphous, $[\alpha]_D -10.7^\circ$, 12 α_d ; 17.1%, amorphous, $[\alpha]_D -16.7^\circ$, 12 β_d ; 23.5%, amorphous, $[\alpha]_D -8.2^\circ$). 12 α_{a-d} and 12 β_{a-d} were hydrogenolyzed with $\text{Pd}(\text{OH})_2/\text{C}$ in methanol to give the sialosyl-(S)-glycerol derivatives (1 α_{a-d} and 1 β_{a-d} , 1 α_a ; 71.2%, amorphous, $[\alpha]_D -11.3^\circ$, 1 β_a ; 74.1%, amorphous, $[\alpha]_D -12.6^\circ$, 1 α_b ; 95%, amorphous, $[\alpha]_D -8.0^\circ$, 1 β_b ; 87%, amorphous, $[\alpha]_D -10.0^\circ$, 1 α_c ; 95%, amorphous, $[\alpha]_D -7.8^\circ$, 1 β_c ; 96%, amorphous, $[\alpha]_D -5.1^\circ$, 1 α_d ; 82%, amorphous, $[\alpha]_D -4.9^\circ$, 1 β_d ; 85%, amorphous, $[\alpha]_D -6.0^\circ$).⁴⁻⁵⁾

Sialosyl-(R)-glycerol derivatives (2 α and 2 β) 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 $\text{Hg}(\text{CN})_2$, HgBr_2 , and molecular sieves 4A to give an anomeric mixture of sialosylglycerol compounds (13 α and 13 β , 13 α ; 24.6%, amorphous, $[\alpha]_D -10.8^\circ$, 13 β ; 20.9%, amorphous, $[\alpha]_D -7.0^\circ$). 13 α and 13 β were each hydrogenolyzed with $\text{Pd}(\text{OH})_2/\text{C}$ to yield the sialosyl-(R)-glycerol derivatives (2 α and 2 β , 2 α ; 86.2%, amorphous, $[\alpha]_D -3.6^\circ$, 2 β ; 84.8%, amorphous, $[\alpha]_D -4.8^\circ$).

Preliminary examination of the biological activities revealed that the lyso-sialosylpalmitoylglycerol derivatives (1 α_a , 1 β_a , 2 α_a , and 2 β_a) have the most powerful phospholipase A₂ and phospholipase C inhibitory activities among the investigated sialosyl derivatives.⁶⁻⁷⁾

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4. ^1H -NMR of H-3eq (ppm, J=Hz): 12 α_a ; 2.59 (1H, dd, J=4.9, 12.9), 12 α_b ; 2.61 (1H, dd, J=4.4, 12.4), 12 α_c ; 2.61 (1H, dd, J=4.9, 12.4), 12 α_d ; 2.61 (1H, dd, J=4.6, 12.4), 13 α ; 2.61 (1H, dd, J=4.6, 12.7): Because H-3eq signals of 12 β_{a-d} and 13 β 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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