Syntheses of Novel Galactosyl Ligands for Liposomes and the Influence of the Spacer on Accumulation in the Rat Liver

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We modified the surface of liposomes with galactosyl ligands. At first we determined whether or not the galactosyl moiety was exposed on the liposomes. We then investigated the effect of the ligands on the hepatic accumulation of liposomes in rats.

We introduced an oligoethylene glycol moiety as a spacer. Among the various ligands tested, those with a tri- or tetraethylene glycol moiety as a spacer caused the greatest accumulation of liposomes in the liver.

Liposomes bearing ligands with a tri- or tetraethylene glycol moiety as a spacer, were aggregated by *Ricinus communis* agglutinin. On the other hand, those modified with ligands with a mono- or diethylene glycol spacer did not clearly agglutinate.

These results show the importance of a spacer between the homing device and the ligand anchor.

Key words neogalactolipid; liposome; targeting; ethylene glycol; spacer; branched anchor

Many trials have been performed in an attempt to make liposomes selective carriers of entrapped molecules. The liver is one of the target organs.[1-3] A Gal/GalNAc lectin is present at the surface of mammalian hepatocytes,[4] and it has been considered as a target for directing liposomes to the liver.[5]

Glycolipids of animal origin have many important functions based on molecular recognition *in vivo*. Consequently, the design and synthesis of glycolipid analogs, as well as their naturally occurring counterparts, are of importance in the study of molecular recognition, the creation of novel systems for liposome formation and drug delivery systems based upon carbohydrate-protein recognition. Various glycolipids have been synthesized to modify liposomes.[6]

However, the optimal ligand conditions for the recognition of galactosyl residues remain unclear.

We previously synthesized various ligands for liposome modification using galactose as a recognition element and evaluated the relationship between the glycolipid structure and accumulation in the liver.[7] The galactose derivatives with a palmitoyl anchor bound to serum albumin and were released from liposomes.[8] The branched structure of a fatty acid may be appropriate as an anchor in glycolipid derivatives for the modification of liposomes.

In this study, we synthesized a series of galactosylated ligands to examine the influence of the spacer.

MATERIALS AND METHODS

Penta-*O*-acetyl-β-D-galactoside, *N*-(palmitoyloxy)succinimide, L-α-phosphatidylcholine dipalmitoyl (DPPC), dicetyl phosphate (DCP), cholesterol (CH), and inulin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 2-(Benzyloxy)ethanol, diethylene glycol, and tetraethylene glycol were from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 2-Hexadecyloctadecanoic acid and a phospholipid test kit were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Phosphate buffered saline (PBS) was from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). [^{3}H]Inulin was from DuPont-NEN Research Products (Boston, MA, U.S.A.). *Ricinus communis* agglutinin (RCA_{120}) was obtained from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). All other chemicals were of reagent grade or better.

Chromatography was performed using Art 9385 silica gel (Merck Co., Darmstadt, Germany). Gel filtration proceeded on a Sephadex LH-20 (Pharmacia LKB Biotechnology AB, Uppsala, Sweden).

(8-Hexadecanoylamido-3,6-dioxaoctyl)-β-D-galactoside (Gal-t-pa) and [8-(2-hexadecyloctadecanoylamido)-3,6-dioxaoctyl]-β-D-galactoside (Gal-t-psa) were prepared as described previously.[7]

Instrumentation NMR spectra were measured at 500 MHz with a Varian VXR-500S spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as an internal standard, and these data are shown in Table 1. Fast atom bombardment (FAB) mass spectra were obtained with a JEOL HX-100 spectrometer. Optical rotations were determined with a Perkin-Elmer 430 polarimeter.

Syntheses of Galactose and the Ethylene Glycol Moiety (Chart 1) 2,3,4,6-Tetraacetyl-[2-(2-chloroethoxy)ethyl]-β-D-galactoside (**5**): Penta-*O*-acetyl-β-D-galactoside (**1**) (5.030 g, 12.9 mmol) and 2-(2-chloroethoxy)ethanol (2.197 g, 17.6 mmol) were dissolved in 50 ml of dichloromethane, then boron trifluoride etherate (6.34 ml, 51.6 mmol) was added at 0 °C. The solution was stirred at room temperature for 11 h. The mixture was washed 4 times with brine, dried over magnesium sulfate and the solvent was evaporated. The residue was purified on a column of silica gel, which was eluted with *n*-hexane–ethyl acetate (3 : 2, v/v). Compound **5** was obtained as a colorless viscous oil: yield 3.810 g (60%). $[\alpha]_D^{20} = -10.6°$ ($c = 1.03$, $CHCl_3$).

2,3,4,6-Tetraacetyl-[2-(2-azidoethoxy)ethyl]-β-D-galactoside (**6**): Sodium azide (1.035 g, 15.9 mmol) was added to a solution of **5** (3.619 g, 7.49 mmol) in 50 ml of *N*,*N*-dimethylformamide (DMF). This mixture was heated at 60 °C for 16 h. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The

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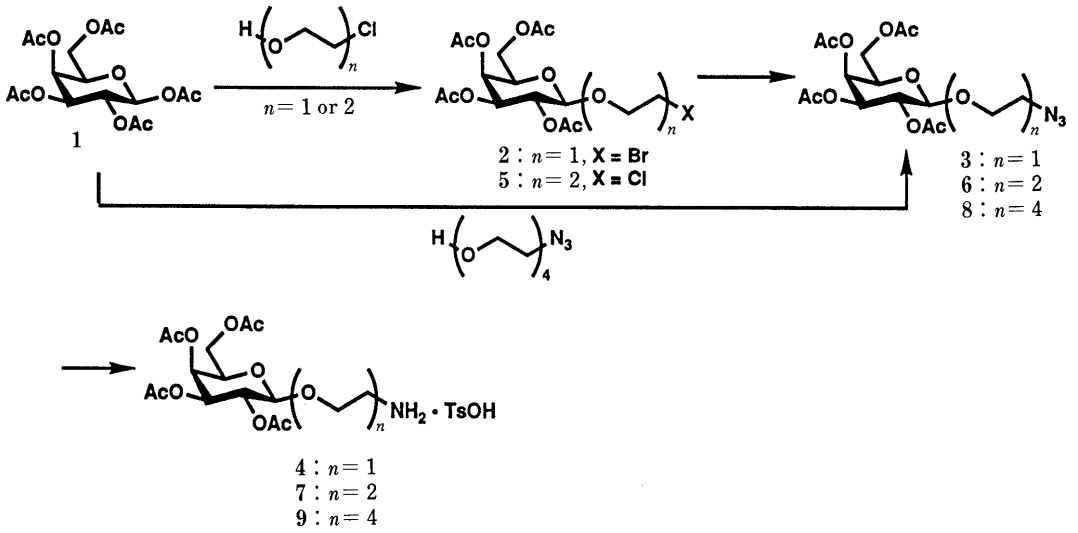

Chart 1. Synthesis of Galactose and the Spacer Moiety

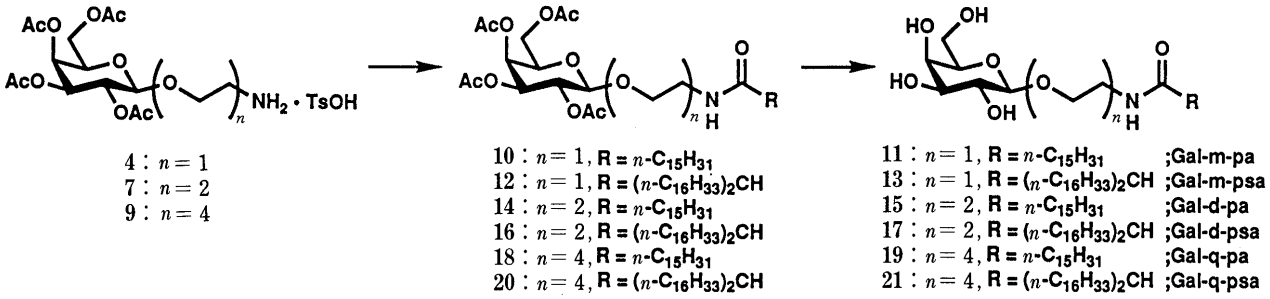

Chart 2. Synthesis of Neogalactolipids

residue was dissolved in ethyl acetate and washed with brine, dried over magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was purified on a column of silica gel, which was eluted with *n*-hexane–ethyl acetate (2:3, v/v). Compound **6** was obtained as a colorless viscous oil: yield 3.479 g (95%). $[\alpha]_D^{27} = -11.5°$ ($c = 1.00$, $CHCl_3$).

2,3,4,6-Tetraacetyl-[2-(2-aminoethoxy)ethyl]-β-D-galactoside *p*-Toluenesulfonate (**7**): To a solution of **6** (0.866 g, 1.77 mmol) in 90 ml of ethyl acetate was added *p*-toluenesulfonic acid monohydrate (0.337 g, 1.77 mmol), and the mixture was hydrogenated over Lindlar's catalyst (0.483 g) at 345 kPa for 3 h. More Lindlar's catalyst (0.480 g) was added to the reaction mixture, and hydrogenation was continued for an additional 5.5 h. The catalyst was filtered off and the solvent was removed under reduced pressure. Compound **7** was obtained as a colorless viscous oil; yield 1.08 g (96%). This compound was used without further purification.

2,3,4,6-Tetraacetyl-(2-aminoethyl)-β-D-galactoside (**4**) was prepared as described for **7**. Firstly, **2** was obtained by introducing a 2-bromoethyl moiety to **1** using 2-bromoethanol and boron trifluoride etherate.[9] Compound **3**[10] was obtained by the reaction of **2** and sodium azide in DMF. Compound **4** was obtained as a colorless amorphous solid (yield 95% from **3**) by the hydrogenation of **3** over Lindlar's catalyst in ethyl acetate, and was used without further purification.

2,3,4,6-Tetraacetyl-(11-azido-3,6,9-trioxaundecyl)-β-D-galactoside (**8**): 11-Azido-3,6,9-trioxa-1-undecanol was prepared according to the method of C. R. Bertozzi *et al.*[11] Penta-*O*-acetyl-β-D-galactoside (**1**) (1.980 g, 5.07 mmol) and 11-azido-3,6,9-trioxa-1-undecanol (3.525 g, 16.1 mmol) were dissolved in 50 ml of dichloromethane, then boron trifluoride etherate (4.44 ml, 36.1 mmol) was added at 0 °C. The solution was stirred at room temperature for 17.5 h. The mixture was washed 5 times with brine, dried over magnesium sulfate, and the solvent was evaporated *in vacuo*. The residue was purified on a column of silica gel, which was eluted with *n*-hexane–ethyl acetate (1 : 5, v/v). Compound **8** was obtained as a colorless viscous oil: yield 1.238 g (46%). $[\alpha]_D^{23} = -5.4°$ ($c = 1.02$, $CHCl_3$).

2,3,4,6-Tetraacetyl-(11-amino-3,6,9-trioxaundecyl)-β-D-galactoside *p*-Toluenesulfonate (**9**): To a solution of **8** (1.129 g, 2.12 mmol) in 120 ml of ethyl acetate was added *p*-toluenesulfonic acid monohydrate (0.404 g, 2.12 mmol), and the mixture was hydrogenated over Lindlar's catalyst (0.570 g) at 345 kPa for 5.5 h. More Lindlar's catalyst (0.564 g) was added to the reaction mixture, and hydrogenation was continued for an additional 5 h. The catalyst was filtered off and the solvent was removed under reduced pressure. Compound **9** was obtained as a pale brown viscous oil; yield 1.172 g (81%). This compound was used without further purification.

Synthesis of Neogalactolipids (Chart 2) 2,3,4,6-Tetraacetyl-(5-hexadecanoylamido-3-oxapentyl)-β-D-galactoside (**14**): To a solution of **7** (0.76 g, 1.25 mmol) in 10 ml of dichloromethane was added triethylamine (174 μl, 1.25 mmol) and *N*-(palmitoyloxy)succinimide (0.882 g,

2.50 mmol) at 0 °C. The mixture was stirred for 21 h at room temperature, then washed successively with 10% citric acid, 10% sodium carbonate and brine, and finally dried over magnesium sulfate. The solvent was evaporated *in vacuo*, and the residue was purified on a column of silica gel, which was eluted with *n*-hexane–ethyl acetate (1 : 3, v/v). Compound **14** was obtained as a colorless amorphous solid: yield 0.70 g (83%). $[\alpha]_D^{27} = -14.9°$ ($c = 1.00$, $CHCl_3$).

(5-Hexadecanoylamido-3-oxapentyl)-β-D-galactoside (**15**) Gal-d-pa: To **14** (0.609 g, 0.87 mmol) was added 15 ml of benzene and 7.5 ml of methanol. Sodium methoxide, 28% in methanol, 50 μl was added to this solution until the pH reached 11.0. The mixture was stirred for 2.5 h, and neutralized with Dowex 50W X-8 (H^+ form). The resin was filtered off and the filtrate was evaporated to dryness under reduced pressure. The residue was purified on a column of Sephadex LH-20, which was eluted with chloroform–methanol (1 : 1, v/v). Compound **15** was obtained as a colorless amorphous solid: yield 0.440 g (96%). $[\alpha]_D^{25} = -2.9°$ ($c = 1.01$, $CHCl_3$–MeOH (1 : 1, v/v)). FAB-MS *m/z*: 506 ($[M^{\cdot} + H]^+$).

2,3,4,6-Tetraacetyl-[5-(2-hexadecyloctadecanoylamido)-3-oxapentyl]-β-D-galactoside (**16**): To 2-hexadecyloctadecanoic acid (0.956 g, 1.88 mmol) was added 4 ml of thionyl chloride, and the mixture was heated at 80 °C for 3 h. Thionyl chloride was removed by distillation under reduced pressure. The residue was dissolved in benzene and evaporated twice. The crude acid chloride was dissolved in 5 ml of dichloromethane and used without further purification.

To a solution of **7** (0.887 g, 1.46 mmol) in 10 ml of dichloromethane at 0 °C was added triethylamine (465 μl, 3.34 mmol) and the acid chloride solution obtained above. The solution was stirred for 12 h and the solvent was evaporated. The residue was loaded on a column of silica gel, which was eluted with *n*-hexane–ethyl acetate (1 : 1, v/v). Compound **16** was obtained as a colorless amorphous solid: yield 0.935 g (70%). $[\alpha]_D^{26} = -10.0°$ ($c = 1.01$, $CHCl_3$).

[5-(2-Hexadecyloctadecanoylamido)-3-oxapentyl]-β-D-galactoside (**17**) Gal-d-psa: To **16** (0.865 g, 0.91 mmol) was added 15 ml of benzene and 7.5 ml of methanol. Sodium methoxide, about 28% in methanol, 50 μl, was added to the reaction mixture until the pH reached 11.0. The solution was stirred at room temperature for 75 min, and neutralized with Dowex 50W X-8 (H^+ form). The resin was removed by filtration and the filtrate was evaporated to dryness. The residue was purified on a column of Sephadex LH-20, which was eluted with chloroform–methanol (1 : 1 v/v). Compound **17** was obtained as a colorless amorphous solid: yield 0.672 g (94%). $[\alpha]_D^{28} = -1.9°$ ($c = 1.00$, $CHCl_3$–MeOH (1 : 1, v/v)). FAB-MS *m/z*: 758 ($[M^{\cdot} + H]^+$).

[2-(Hexadecanoylamido)ethyl]-β-D-galactoside (**11**) Gal-m-pa, [2-(2-hexadecyloctadecanoylamido)ethyl]-β-D-galactoside (**13**) Gal-m-psa, (11-hexadecanoylamido-3,6,9-trioxaundecyl)-β-D-galactoside (**19**) Gal-q-pa, and [11-(2-hexadecyloctadecanoylamido)-3,6,9-trioxaundecyl]-β-D-galactoside (**21**) Gal-q-psa were prepared as compounds Gal-d-pa (**15**) or Gal-d-psa (**17**). The acyl moiety was introduced to **4** or **9** using *N*-(palmitoyloxy) succinimide or the acid chloride of 2-hexadecyloctadecanoic acid.

Then, **10**, **12**, **18**, and **20** were deprotected with sodium methoxide in methanol–benzene.

The analytical data of compounds **10**—**13** and **18**—**21** are shown below.

2,3,4,6-Tetraacetyl-[2-(hexadecanoylamido)ethyl]-β-D-galactoside (**10**): Colorless amorphous, $[\alpha]_D^{25} = +0.1°$ ($c = 1.014$, $CHCl_3$–MeOH (1 : 1, v/v)).

[2-(Hexadecanoylamido)ethyl]-β-D-galactoside (**11**) Gal-m-pa: Colorless amorphous, $[\alpha]_D^{25} = +2.6°$ ($c = 1.005$, $CHCl_3$–MeOH–H_2O (10 : 10 : 3, v/v)). FAB-MS *m/z*: 462 ($[M^{\cdot} + H]^+$).

2,3,4,6-Tetraacetyl-[2-(2-hexadecyloctadecanoylamido)ethyl]-β-D-galactoside (**12**): Colorless amorphous, $[\alpha]_D^{18} = -0.4°$ ($c = 1.012$, $CHCl_3$).

[2-(2-Hexadecyloctadecanoylamido)ethyl]-β-D-galactoside (**13**) Gal-m-psa: Colorless amorphous, $[\alpha]_D^{21} = +1.5°$ ($c = 1.060$, $CHCl_3$–MeOH (9 : 1, v/v)). FAB-MS *m/z*: 714 ($[M^{\cdot} + H]^+$).

2,3,4,6-Tetraacetyl-(11-hexadecanoylamido-3,6,9-trioxaundecyl)-β-D-galactoside (**18**): Colorless amorphous, $[\alpha]_D^{254} = -1.9°$ ($c = 0.99$, $CHCl_3$–MeOH (1 : 1, v/v)).

(11-Hexadecanoylamido-3,6,9-trioxaundecyl)-β-D-galactoside (**19**) Gal-q-pa: Colorless amorphous, $[\alpha]_D^{26} = -2.3°$ ($c = 0.99$, $CHCl_3$–MeOH (1 : 1, v/v)). FAB-MS *m/z*: 594 ($[M^{\cdot} + H]^+$).

2,3,4,6-Tetraacetyl-[11-(2-hexadecyloctadecanoylamido)-3,6,9-trioxaundecyl]-β-D-galactoside (**20**): Colorless amorphous, $[\alpha]_D^{23} = -2.5°$ ($c = 1.00$, $CHCl_3$–MeOH (1 : 1, v/v)).

[11-(2-Hexadecyloctadecanoylamido)-3,6,9-trioxapentyl]-β-D-galactoside (**21**) Gal-q-psa: Colorless amorphous, $[\alpha]_D^{28} = -3.6°$ ($c = 1.00$, $CHCl_3$–MeOH (1 : 1, v/v)). FAB-MS *m/z*: 846 ($[M^{\cdot} + H]^+$).

Preparation of the Galactosylated Liposomes Multilamellar vesicles (MLVs) were prepared according to the method of Bangham *et al.*,[12] with some modification as follows. The control liposomes contained 80 mM DPPC, 80 mM CH, and 8 mM DCP. Galactosylated liposomes were constructed using 16 mM neogalactolipid, together with DPPC, CH and DCP, which were used as the control liposomes. These lipids were dissolved in 10 ml of chloroform–methanol (1 : 1, v/v). The solvent was removed by distillation under a stream of nitrogen, followed by drying under reduced pressure. The residues were hydrated with 8 ml of PBS containing cold 0.5% (w/v) inulin and 5.92 MBq [^{3}H]inulin as an aqueous marker. The suspensions were vortexed mechanically and sonicated for 2 min with a bath type sonicator (Branson B1200, Emerson-Japan, Tokyo, Japan). The liposomes were extruded through polycarbonate membranes (Nuclepore Co., MA, U.S.A.) with successive pore sizes of 0.2, 0.1 and 0.08 nm. These procedures were done at a temperature above the gel-liquid crystalline phase transition temperature (T_c) of the lipid materials. Non-encapsulated [^{3}H]-inulin was removed with an ultracentrifuge (Himac, Hitachi Koki Co., Ltd., Tokyo, Japan) at 60000 rpm for 15, 2 and 2 h, successively. The phospholipid concentration was measured by a phospholipid test kit and the

Table 1. ^{1}H-NMR Spectral Data

No.	Solvent[a)]	^{1}H-NMR chemical shifts (ppm)
3	A	1.99 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.07 (3H, s, acetyl), 2.16 (3H, s, acetyl), 3.30 (1H, ddd, J=3.5, 4.5, 13.0 Hz, C$\underline{H}_2$N$_3$), 3.51 (1H, ddd, J=3.5, 8.5, 13.0 Hz, C$\underline{H}_2$N$_3$), 3.70 (1H, ddd, J=3.5, 8.5, 11.0 Hz, OC$\underline{H}_2$CH$_2$N$_3$), 3.93 (1H, dt, J=1.0, 6.5 Hz, Gal 5-H), 4.05 (1H, ddd, J=3.5, 4.5, 11.0 Hz, OC$\underline{H}_2$CH$_2$N$_3$), 4.13 (1H, dd, J=6.5, 11.0 Hz, Gal 6-H$_a$), 4.19 (1H, dd, J=6.5, 11.0 Hz, Gal 6-N$_b$), 4.56 (1H, d, J=8.0 Hz, Gal 1-H), 5.02 (1H, dd, J=3.5, 10.5 Hz, Gal 3-H), 5.25 (1H, dd, J=10.5, 8.0 Hz, Gal 2-H), 5.39 (1H, d, J=3.5 Hz, Gal 4-H)
5	A	1.99 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.07 (3H, s, acetyl), 2.15 (3H, s, acetyl), 3.61—3.63 (2H, m, ethylene glycol moiety), 3.67—3.70 (2H, m, ethylene glycol moiety), 3.73—3.79 (3H, m, ethylene glycol moiety), 3.90—3.93 (1H, m, Gal 5-H), 3.95—3.99 (1H, m, Gal 1β-OCH$_2$), 4.13 (1H, dd, J=6.8, 11.2 Hz, Gal 6-H$_a$), 4.18 (1H, dd, J=6.6, 11.2 Hz, Gal 6-H$_b$), 4.58 (1H, d, J=8.0 Hz, Gal 1-H), 5.02 (1H, dd, J=3.4, 10.5 Hz, Gal 3-H), 5.22 (1H, dd, J=8.0, 10.5 Hz, Gal 2-H), 5.39 (1H, dd, J=1.0, 3.4 Hz, Gal 4-H)
6	A	1.99 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.07 (3H, s, acetyl), 2.15 (3H, s, acetyl), 3.33—3.42 (2H, m, CH$_2$N$_3$), 3.65—3.68 (4H, m, ethylene glycol moiety), 3.75—3.79 (1H, m, Gal 1β-OCH$_2$), 3.90—3.93 (1H, m, Gal 5-H), 3.97 (1H, dt, J=4.0, 7.2 Hz, Gal 1β-OCH$_2$), 4.13 (1H, dd, J=6.8, 11.2 Hz, Gal 6-H$_a$), 4.18 (1H, dd, J=6.3, 11.2 Hz, Gal 6-H$_b$), 4.59 (1H, d, J=7.8 Hz, Gal 1-H), 5.03 (1H, dd, J=3.4, 10.5 Hz, Gal 3-H), 5.22 (1H, dd, J=7.8, 10.5 Hz, Gal 2-H), 5.39 (1H, dd, J=1.0, 3.4 Hz, Gal 4-H)
8	A	1.99 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.06 (3H, s, acetyl), 2.15 (3H, s, acetyl), 3.40 (2H, t, J=5.0 Hz, CH$_2$N$_3$), 3.62—3.69 (12H, m, ethylene glycol moiety), 3.75 (1H, ddd, J=3.7, 7.4, 11.1 Hz, Gal 1β-OCH$_2$), 3.90—3.93 (1H, m, Gal 5-H), 3.94—3.98 (1H, dt, J=4.3, 11.1 Hz, Gal 1β-OCH$_2$), 4.13 (1H, dd, J=6.8, 11.2 Hz, Gal 6-H$_a$), 4.18 (1H, dd, J=6.6, 11.2 Hz, Gal 6-H$_b$), 4.57 (1H, d, J=8.1 Hz, Gal 1-H), 5.02 (1H, dd, J=3.4, 10.5 Hz, Gal 3-H), 5.21 (1H, dd, J=8.1, 10.5 Hz, Gal 2-H), 5.39 (1H, dd, J=1.0, 3.4 Hz, Gal 4-H)
10	A	0.88 (3H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{12}$CH$_2$CH$_2$CO), 1.22—1.34 (24H, m, CH$_3$(C$\underline{H}_2$)$_{12}$CH$_2$CH$_2$CO), 1.59—1.67 (2H, m, CH$_3$(CH$_2$)$_{12}$C$\underline{H}_2$CH$_2$CO), 1.99 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.07 (3H, s, acetyl), 2.16 (3H, s, acetyl), 2.16 (2H, t, J=7.6 Hz, CH$_3$(CH$_2$)$_{12}$CH$_2$C$\underline{H}_2$CO), 3.39—3.46 (1H, m, OCH$_2$C$\underline{H}_2$NHCO), 3.48—3.54 (1H, m, OCH$_2$C$\underline{H}_2$NHCO), 3.66—3.70 (1H, m, OC$\underline{H}_2$CH$_2$NHCO), 3.85—3.93 (1H, m, OC$\underline{H}_2$CH$_2$NHCO), 3.91—3.93 (1H, m, Gal 5-H), 4.14 (1H, dd, J=5.5, 10.5 Hz, Gal 6-H$_a$), 4.17 (1H, dd, J=6.0, 10.5 Hz, Gal 6-H$_b$), 4.47 (1H, d, J=8.0 Hz, Gal 1-H), 5.02 (1H, dd, J=3.4, 10.4 Hz, Gal 3-H), 5.19 (1H, dd, J=8.0, 10.4 Hz, Gal 2-H), 5.40 (1H, dd, J=1.0, 3.4 Hz, Gal 4-H), 5.84 (1H, br t, CONH)
11	B	0.88 (3H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{12}$CH$_2$CH$_2$CO), 1.16—1.33 (24H, m, CH$_3$(C$\underline{H}_2$)$_{12}$CH$_2$CH$_2$CO), 1.78 (2H, quintet, J=7.6 Hz, CH$_3$(CH$_2$)$_{12}$C$\underline{H}_2$CH$_2$CO), 2.40 (2H, t, CH$_3$(CH$_2$)$_{12}$CH$_2$C$\underline{H}_2$CO), 3.71—3.82 (2H, m, OCH$_2$C$\underline{H}_2$NHCO), 3.97—4.01 (1H, m, OC$\underline{H}_2$CH$_2$NHCO), 4.03 (1H, br t, Gal 5-H), 4.13 (1H, dd, J=3.3, 9.6 Hz, Gal 3-H), 4.20—4.25 (1H, m, OC$\underline{H}_2$CH$_2$NHCO), 4.37 (1H, dd, J=5.5, 11.1 Hz, Gal 6-H$_a$), 4.41 (1H, dd, J=6.5, 11.1 Hz, Gal 6-H$_b$), 4.43 (1H, br t, Gal 2-H), 4.50 (1H, br d, Gal 4-H), 4.77 (1H, d, J=7.8 Hz, Gal 1-H), 8.61 (1H, br t, CONH)
12	A	0.88 (6H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{15}$), 1.21—1.34 (56H, m, CH$_3$(C$\underline{H}_2$)$_{14}$CH$_2$), 1.35—1.44 (2H, m, CH$_3$(CH$_2$)$_{14}$C$\underline{H}_2$), 1.53—1.61 (2H, m, CH$_3$(CH$_2$)$_{14}$C$\underline{H}_2$), 1.82—1.88 (1H, m, (n-C$_{16}$H$_{33}$)$_2$C$\underline{H}$CO), 2.00 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.16 (3H, s, acetyl), 3.41—3.47 (1H, m, OCH$_2$C$\underline{H}_2$NH), 3.50—3.55 (1H, m, OCH$_2$C$\underline{H}_2$NH), 3.56—3.68 (1H, m, OC$\underline{H}_2$CH$_2$NH), 3.87—3.93 (2H, m, OC$\underline{H}_2$CH$_2$NH and Gal 5-H), 4.13—4.16 (2H, m, Gal 6-H), 4.48 (1H, d, J=7.9 Hz, Gal 1-H), 5.02 (1H, dd, J=3.4, 10.5 Hz, Gal 3-H), 5.20 (1H, dd, J=7.9, 10.5 Hz, Gal 2-H), 5.40 (1H, dd, J=1.0, 3.4 Hz, Gal 4-H), 5.82 (1H, br t, CONH)
13	B	0.88 (6H, t, J=6.8 Hz, C$\underline{H}_3$(CH$_2$)$_{15}$), 1.20—1.38 (52H, m, CH$_3$(C$\underline{H}_2$)$_{13}$CH$_2$CH$_2$), 1.42—1.62 (6H, m, CH$_3$(CH$_2$)$_{13}$C$\underline{H}_2$-CH$_2$ and CH$_3$(CH$_2$)$_{13}$CH$_2$C$\underline{H}_2$), 1.84—1.91 (2H, m, CH$_3$(CH$_2$)$_{13}$CH$_2$C$\underline{H}_2$), 2.47—2.52 (1H, m, (n-C$_{16}$H$_{33}$)$_2$C$\underline{H}$CO), 3.70—3.76 (1H, m, OCH$_2$C$\underline{H}_2$NHCO), 3.79—3.85 (1H, m, OCH$_2$C$\underline{H}_2$NHCO), 3.94—4.00 (2H, m, OC$\underline{H}_2$CH$_2$NHCO and Gal 5-H), 4.04 (1H, dd, J=3.4, 9.5 Hz, Gal 3-H), 4.17—4.21 (1H, m, OC$\underline{H}_2$CH$_2$NHCO), 4.28—4.36 (3H, m, Gal 2-H and 6-H), 4.43 (1H, dd, J=0.9, 3.4 Hz, Gal 4-H), 4.71 (1H, d, J=7.6 Hz, Gal 1-H), 8.63 (1H, br t, CONH)
14	A	0.88 (3H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{14}$CO), 1.22—1.33 (24H, m, CH$_3$(C$\underline{H}_2$)$_{12}$CH$_2$CH$_2$CO), 1.61—1.67 (2H, m, CH$_3$(CH$_2$)$_{12}$C$\underline{H}_2$CH$_2$CO), 2.00 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.06 (3H, s, acetyl), 2.15 (3H, s, acetyl), 2.17 (2H, t, J=7.6 Hz, CH$_3$(CH$_2$)$_{12}$CH$_2$C$\underline{H}_2$CO), 3.34—3.40 (1H, m, OCH$_2$C$\underline{H}_2$NHCO), 3.47—3.57 (3H, m, OC$\underline{H}_2$CH$_2$NHCO and OCH$_2$C$\underline{H}_2$NHCO), 3.58—3.65 (2H, m, Gal 1β-OCH$_2$C$\underline{H}_2$O), 3.68—3.72 (1H, m, Gal 1β-OC$\underline{H}_2$CH$_2$O), 3.90—3.93 (1H, m, Gal 5-H), 3.96—4.00 (1H, m, Gal 1β-OC$\underline{H}_2$CH$_2$O), 4.13 (1H, dd, J=7.1, 11.3 Hz, Gal 6-H$_a$), 4.19 (1H, dd, J=6.5, 11.3 Hz, Gal 6-H$_b$), 4.52 (1H, d, J=7.8 Hz, Gal 1-H), 5.04 (1H, dd, J=3.4, 10.5 Hz, Gal 3-H), 5.22 (1H, dd, J=7.8, 10.5 Hz, Gal 2-H), 5.39 (1H, dd, J=1.0, 3.4 Hz, Gal 4-H), 6.06 (1H, br s, CONH)
15	B	0.87 (3H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{14}$CO), 1.21—1.36 (24H, m, CH$_3$(C$\underline{H}_2$)$_{12}$CH$_2$CH$_2$CO), 1.79 (2H, quintet, J=7.6 Hz, CH$_3$(CH$_2$)$_{12}$C$\underline{H}_2$CH$_2$CO), 2.41 (2H, t, J=7.6 Hz, CH$_3$(CH$_2$)$_{12}$CH$_2$C$\underline{H}_2$CO), 3.59—3.71 (6H, m, OCH$_2$C$\underline{H}_2$OC$\underline{H}_2$C$\underline{H}_2$-NHCO), 3.88 (1H, dt, J=5.3, 10.8 Hz, OC$\underline{H}_2$CH$_2$OCH$_2$CH$_2$NHCO), 4.00—4.03 (1H, m, Gal 5-H), 4.11 (1H, dd, J=3.4, 9.5 Hz, Gal 3-H), 4.20 (1H, dt, J=4.6, 10.8 Hz, OC$\underline{H}_2$CH$_2$OCH$_2$CH$_2$NHCO), 4.39—4.42 (3H, m, Gal 2-H and Gal 6-H), 4.52 (1H, br d, Gal 4-H), 4.77 (1H, d, J=7.6 Hz, Gal 1-H), 8.54 (1H, br s, CONH)
16	A	0.88 (6H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{15}$), 1.21—1.34 (56H, m, CH$_3$(C$\underline{H}_2$)$_{14}$CH$_2$), 1.35—1.44, (2H, m, CH$_3$(CH$_2$)$_{14}$C$\underline{H}_2$), 1.53—1.63 (2H, m, CH$_3$(CH$_2$)$_{14}$C$\underline{H}_2$), 2.04—2.08 (1H, m, (n-C$_{16}$H$_{33}$)$_2$C$\underline{H}$CO), 1.99 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.06 (3H, s, acetyl), 2.15 (3H, s, acetyl), 3.36—3.43 (1H, m, OCH$_2$C$\underline{H}_2$NH), 3.48—3.54 (3H, m, OCH$_2$C$\underline{H}_2$NH and OC$\underline{H}_2$CH$_2$NH), 3.60 (2H, br t, Gal 1β-OCH$_2$C$\underline{H}_2$O), 3.70 (1H, dt, J=5.3, 10.6 Hz, Gal 1β-OC$\underline{H}_2$CH$_2$O), 3.90—3.93 (1H, m, Gal 5-H), 3.98 (1H, dt, J=4.2, 10.6 Hz, Gal 1β-OC$\underline{H}_2$CH$_2$O), 4.13 (1H, dd, J=7.0, 11.2 Hz, Gal 6-H$_a$), 4.19 (1H, dd, J=6.3, 11.2 Hz, Gal 6-H$_b$), 4.53 (1H, d, J=8.0 Hz, Gal 1-H), 5.04 (1H, dd, J=3.3, 10.5 Hz, Gal 3-H), 5.22 (1H, dd, J=8.0, 10.5 Hz, Gal 2-H), 5.40 (1H, dd, J=0.7, 3.3 Hz, Gal 4-H), 5.97 (1H, t, J=5.2 Hz, CONH)
17	B	0.88 (6H, t, J=6.8 Hz, C$\underline{H}_3$(CH$_2$)$_{15}$), 1.20—1.38 (52H, m, CH$_3$(C$\underline{H}_2$)$_{13}$CH$_2$CH$_2$), 1.42—1.60 (6H, m, CH$_3$(CH$_2$)$_{13}$C$\underline{H}_2$-CH$_2$ and CH$_3$(CH$_2$)$_{13}$CH$_2$C$\underline{H}_2$), 1.90—1.98 (2H, m, CH$_3$(CH$_2$)$_{13}$CH$_2$C$\underline{H}_2$), 2.51—2.57 (1H, m, (n-C$_{16}$H$_{33}$)$_2$C$\underline{H}$CO), 3.68—3.78 (6H, m, OCH$_2$C$\underline{H}_2$OC$\underline{H}_2$NHCO), 3.92 (1H, dt, J=5.3, 10.8 Hz, OC$\underline{H}_2$CH$_2$OCH$_2$CH$_2$NHCO), 4.01—4.04 (1H, m, Gal 5-H), 4.13 (1H, dd, J=3.4, 9.5 Hz, Gal 3-H), 4.24 (1H, dt, J=4.8, 10.8 Hz, OC$\underline{H}_2$CH$_2$OCH$_2$CH$_2$NHCO), 4.39—4.43 (3H, m, Gal 2-H and Gal 6-H), 4.53 (1H, br d, Gal 4-H), 4.78 (1H, d, J=7.8 Hz, Gal 1-H), 8.76 (1H, br s, CONH)

Table 1. (continued)

No.	Solvent[a]	^{1}H-NMR chemical shifts (ppm)
18	A	0.88 (3H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{14}$CO), 1.21—1.32 (24H, m, CH$_3$(C$\underline{H}_2$)$_{12}$CH$_2$CH$_2$CO), 1.59—1.65 (2H, m, CH$_3$(CH$_2$)$_{12}$C$\underline{H}_2$CH$_2$CO), 1.99 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.06 (3H, s, acetyl), 2.15 (3H, s, acetyl), 2.17 (2H, t, J=7.6 Hz, CH$_3$(CH$_2$)$_{12}$CH$_2$C$\underline{H}_2$CO), 3.44—3.47 (2H, m, OCH$_2$C$\underline{H}_2$NHCO), 3.56 (2H, t, J=5.1 Hz, OC$\underline{H}_2$CH$_2$NHCO), 3.60—3.68 (10H, m, ethylene glycol moiety), 3.75 (1H, ddd, J=4.3, 6.7, 11.0 Hz, Gal 1β-OC$\underline{H}_2$CH$_2$O), 3.90—3.93 (1H, m, Gal 5-H), 3.97 (1H, dt, J=4.3, 11.0 Hz, Gal 1β-OC$\underline{H}_2$CH$_2$O), 4.13 (1H, dd, J=7.1, 11.2 Hz, Gal 6-H$_a$), 4.18 (1H, dd, J=6.6, 11.2 Hz, Gal 6-H$_b$), 4.57 (1H, d, J=8.1 Hz, Gal 1-H), 5.02 (1H, dd, J=3.4, 10.5 Hz, Gal 3-H), 5.21 (1H, dd, J=8.1, 10.5 Hz, Gal 2-H), 5.39 (1H, dd, J=1.0, 3.4 Hz, Gal 4-H), 6.05 (1H, br s, CON$\underline{H}$)
19	B	0.87 (3H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{14}$CO), 1.19—1.38 (24H, m, CH$_3$(C$\underline{H}_2$)$_{12}$CH$_2$CH$_2$CO), 1.81 (2H, quintet, J=7.6 Hz, CH$_3$(CH$_2$)$_{12}$C$\underline{H}_2$CH$_2$CO), 2.42 (2H, t, J=7.6 Hz, CH$_3$(CH$_2$)$_{12}$CH$_2$C$\underline{H}_2$CO), 3.59—3.73 (14H, m, ethylene glycol moiety), 3.91 (1H, dt, J=5.3, 10.5 Hz, Gal 1β-OC$\underline{H}_2$), 4.02—4.04 (1H, m, Gal 5-H), 4.13 (1H, dd, J=3.4, 9.5 Hz, Gal 3-H), 4.24 (1H, dt, J=4.7, 10.5 Hz, Gal 1β-OC$\underline{H}_2$), 4.40—4.44 (3H, m, Gal 2-H and Gal 6-H), 4.53 (1H, br d, Gal 4-H), 4.77 (1H, d, J=7.6 Hz, Gal 1-H), 8.62 (1H, br t, CON$\underline{H}$)
20	A	0.88 (6H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{15}$), 1.19—1.33 (56H, m, CH$_3$(C$\underline{H}_2$)$_{14}$CH$_2$), 1.35—1.42 (2H, m, CH$_3$(CH$_2$)$_{14}$C$\underline{H}_2$), 1.53—1.62 (2H, m, CH$_3$(CH$_2$)$_{14}$C$\underline{H}_2$), 1.97—2.03 (1H, m, (n-C$_{16}$H$_{33}$)$_2$C$\underline{H}$CO), 1.99 (3H, s, acetyl), 2.05 (3H, s, acetyl), 2.06 (3H, s, acetyl), 2.15 (3H, s, acetyl), 3.45—3.48 (2H, m, OCH$_2$C$\underline{H}_2$NH), 3.48—3.54 (2H, t, J=5.0 Hz, OC$\underline{H}_2$CH$_2$NH), 3.61—3.68 (10H, m, ethylene glycol moiety), 3.75 (1H, ddd, J=3.7, 7.3, 11.0 Hz, Gal 1β-OC$\underline{H}_2$CH$_2$O), 3.90—3.94 (1H, m, Gal 5-H), 3.96 (1H, dt, J=4.4, 11.0 Hz, Gal 1β-OC$\underline{H}_2$CH$_2$O), 4.13 (1H, dd, J=7.1, 11.2 Hz, Gal 6-H$_a$), 4.17 (1H, dd, J=6.6, 11.2 Hz, Gal 6-H$_b$), 4.56 (1H, d, J=8.1 Hz, Gal 1-H), 5.02 (1H, dd, J=3.5, 10.5 Hz, Gal 3-H), 5.21 (1H, dd, J=8.1, 10.5 Hz, Gal 2-H), 5.39 (1H, dd, J=1.0, 3.5 Hz, Gal 4-H), 5.96 (1H, t, J=5.6 Hz, CON$\underline{H}$)
21	B	0.88 (6H, t, J=7.0 Hz, C$\underline{H}_3$(CH$_2$)$_{15}$), 1.20—1.39 (52H, m, CH$_3$(C$\underline{H}_2$)$_{13}$CH$_2$CH$_2$), 1.44—1.62 (6H, m, CH$_3$(CH$_2$)$_{13}$C$\underline{H}_2$-CH$_2$ and CH$_3$(CH$_2$)$_{13}$CH$_2$C$\underline{H}_2$), 1.92—2.00 (2H, m, CH$_3$(CH$_2$)$_{13}$CH$_2$C$\underline{H}_2$), 2.51—2.57 (1H, m, (n-C$_{16}$H$_{33}$)$_2$C$\underline{H}$CO), 3.63—3.77 (14H, m, ethylene glycol moiety), 3.93 (1H, dt, J=5.3, 10.7 Hz, Gal 1β-OC$\underline{H}_2$), 4.02—4.05 (1H, m, Gal 5-H), 4.14 (1H, dd, J=3.4, 9.5 Hz, Gal 3-H), 4.26 (1H, dt, J=4.9, 10.7 Hz, Gal 1β-OC$\underline{H}_2$), 4.40—4.44 (3H, m, Gal 2-H and Gal 6-H), 4.54 (1H, br d, Gal 4-H), 4.78 (1H, d, J=7.6 Hz, Gal 1-H), 8.78 (1H, br t, CON$\underline{H}$)

a) A, $CDCl_3$; B, pyridine d_5–D_2O (100 : 1).

phospholipid concentration of the liposomes was adjusted to 10 mmol/ml. The size of liposomes was determined using a submicron particle analyzer (NICOMP 370, Pacific Scientific, MD, U.S.A.). The mean diameter of liposomes ranged from 120—150 nm and did not change after the ultracentrifugation. The trapping efficiency of [^{3}H]inulin ranged from 1.5—2.0%. The liposome showed a multilamellar structure by negative-stain electron micrographs.

Lectin-Induced Agglutination of Galactosylated Liposomes One hundred microliters of galactosylated liposomes (25 mmol phospholipid) were incubated with 100 μl of RCA_{120} (1 mg/ml) and 800 μl of PBS in a cuvette. After rapid mixing, agglutination of the liposomes was estimated at room temperature by the time dependent increase in turbidity, as measured by the absorbance at 460 nm with a UV-3100 spectrometer (Shimadzu, Kyoto, Japan). The reversibility of the agglutination was assessed by the addition of 100 μl (10 mg/ml) of free galactose.

Animal Experiment Male Sprague-Dawley rats (180—230 g body weight) were given a single intravenous injection of 2.5 ml/kg of a liposome (25 mmol phospholipid) suspension, containing about 1×10^6 dpm per dose, into the jugular vein under ether anesthesia. Blood samples were drawn from the contralateral jugular vein with heparinized syringes at 15 and 30 min, and 1, 2, 4, and 6 h after injection. The plasma was separated by centrifugation within 10 min. Immediately after the last sampling of blood, the rat was sacrificed under ether anesthesia and the liver was removed and combusted using an automatic sample combustion system (ASC-113, Aloka, Tokyo, Japan). The ^{3}H was collected as ^3H_2O. After adding a scintillator (Aquasol II, DuPont-NEN Research Products, Boston, MA, U.S.A.), the radioactivity was measured in a liquid scintillation counter (LSC-3600, Aloka, Tokyo, Japan).

RESULTS AND DISCUSSION

To examine the influence of the spacer on the recognition of the galactosyl residue, we synthesized a series of galactosyl ligands and incorporated them into liposomes. We then introduced an oligoethylene glycol moiety as a spacer.

Synthesis of Neogalactolipids As has been reported, a hexamethylene spacer can recognize the galactosyl residue on hepatocytes because of the cluster effect.[13] However, the hexamethylene moiety is too hydrophobic to modify liposomes, so we introduced an oligoethylene glycol to improve ligand solubility. To determine the most suitable length and polarity of the spacer for recognition of the galactosyl moiety, we prepared a series of ligands with various spacers.

We synthesized galactosyl ligands as we reported.[7] Various alcohols were coupled with penta-*O*-acetyl-β-D-galactoside to yield glycoside according to the method of Dahmén *et al.*[9] The β configuration of the glycosidic bond was established by ^{1}H-NMR spectroscopy. For tetraethylene glycol spacers, azido alcohol was used in glycosylation. Otherwise, a chloro or a bromo group was substituted with azido using sodium azide after glycosylation. The azido group was hydrogenated over the Lindlar's catalyst to yield the amino derivatives.

The anchor was then introduced to these amines by acylation, and the protected products **10**, **12**, **14**, **16**, **18**, and **20** were obtained. Removal of the acetyl groups with

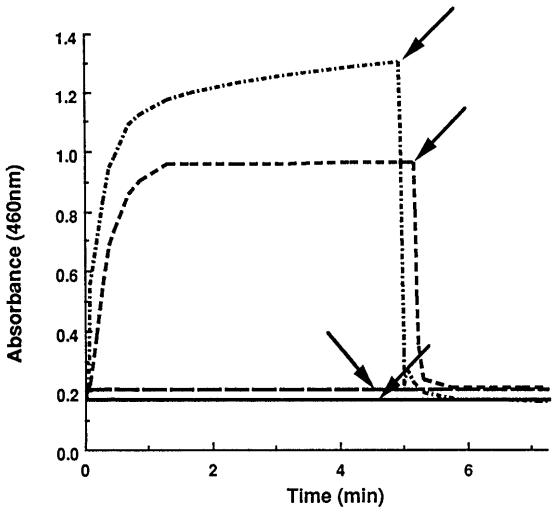

Fig. 1. Agglutination of the Liposomes by *Ricinus communis* Lectin, when Modified with the Galactosyl Ligand of a Palmitoyl Anchor

The arrows indicate D-galactose addition. Liposomes containing ——, Gal-m-pa; ——, Gal-d-pa; – – –, Gal-t-pa; –·–·, Gal-q-pa.

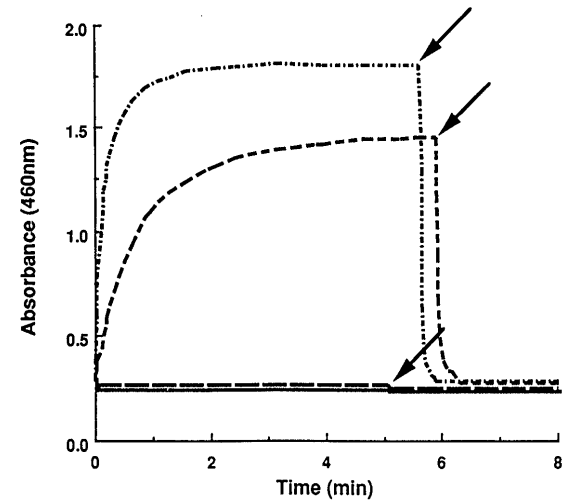

Fig. 2. Agglutination of the Liposomes by *Ricinus communis* Lectin, When Modified with the Galactosyl Ligand of a 2-Hexadecyloctadecanoyl Anchor

The arrows indicate D-galactose addition. Liposomes containing ——, Gal-m-psa; ——, Gal-d-psa; – – –, Gal-t-psa; –·–·, Gal-q-psa.

sodium methoxide, followed by gel filtration on Sephadex LH-20 gave the desired neogalactolipids **11** (Gal-m-pa), **13** (Gal-m-psa), **15** (Gal-d-pa), **17** (Gal-d-psa), **19** (Gal-q-pa), and **21** (Gal-q-psa), respectively.

Lectin-Induced Agglutination of Galactosylated Liposomes Liposomes containing compounds Gal-m-pa, Gal-m-psa, Gal-d-pa, Gal-d-psa, Gal-q-pa, or Gal-q-psa were prepared as described above. Compounds Gal-t-pa and Gal-t-psa were synthesized as we have reported.[7]

As a measure of galactose exposure, we determined the amount of agglutination caused by the lectin from *Ricinus communis* (RCA_{120}).[14] The agglutination was monitored by means of the absorbance at 460 nm as a function of time. Galactosylated liposomes containing compound Gal-q-pa, Gal-q-psa, Gal-t-pa, or Gal-t-psa were aggregated by RCA_{120} as shown in Figs. 1 and 2. This effect was reversed with free D-galactose. These results showed that the agglutination depended on the galactosyl residue

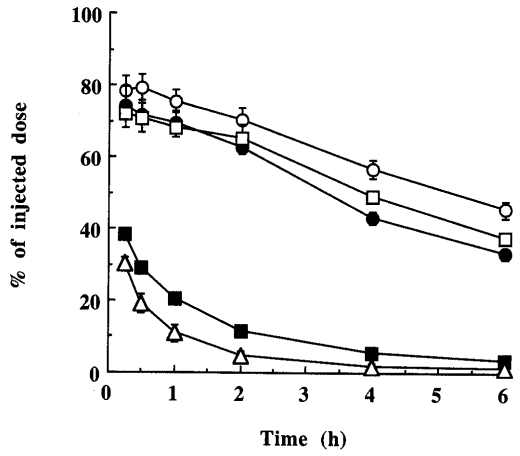

Fig. 3. Elimination of [^{3}H]Inulin Encapsulated Liposomes from Plasma after Intravenous Injection into the Jugular Vein of Rats

Each column represents the mean ± S.D. from three animals. Liposomes containing ○, control; ●, Gal-m-psa; □, Gal-d-psa; ■, Gal-t-psa; △, Gal-q-psa.

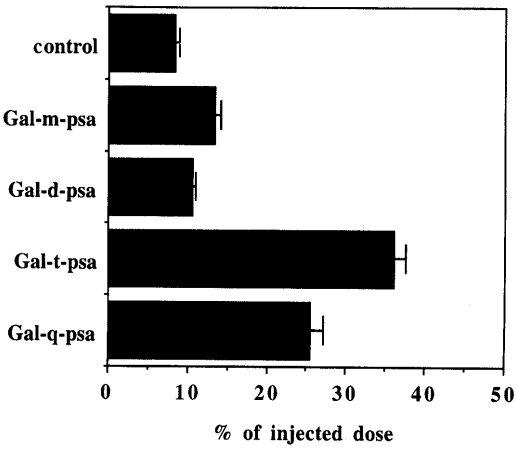

Fig. 4. Hepatic Accumulation of [^{3}H]Inulin Encapsulated Liposomes after Intravenous Injection into the Jugular Vein in Rats

Each column represents the mean ± S.D. from three animals.

of the ligands on the liposomes. However, liposomes bearing compounds Gal-m-pa, Gal-m-psa, Gal-d-pa, or Gal-d-psa did not agglutinate.

Liposomes bearing ligands with only a tri- or tetraethylene glycol group as a spacer caused agglutination.

These results showed that triethylene glycol or a longer spacer is necessary for recognition of the galactosyl residues.

There was no significant difference in the agglutination between ligands with branched and non-branched anchors. This suggested that the branch of the anchors did not influence the exposure of the galactosyl moiety on the surface of the liposomes in these experiments, which corresponds with our previous results.[7] Dihexadecyl and pentadecyl moieties served equally well as anchors in the agglutination assay.

These results showed that the length of the spacer is important for the recognition of a "homing device" in *in vitro* assays.

Uptake of the Galactosylated Liposomes to the Liver Figure 3 shows the elimination of liposomes from plasma and Fig. 4 shows the hepatic accumulation of liposomes modified with galactose derivatives. The blood volume

was considered to be 6.5 ml/100 g body weight, and the hematocrit level to be 45%.[15] Liposomes bearing compounds Gal-t-psa or Gal-q-psa were rapidly cleared from circulation and accumulated in high levels in the liver. Liposomes bearing compounds Gal-m-psa or Gal-d-psa only weakly accumulated in the liver. These results were consistent with the results of the lectin-induced agglutination of liposomes.

We found that compound Gal-t-pa is removed from liposomes when they are incubated with rat plasma. This suggested that the non-branched anchor is not well-rooted and that compound Gal-t-pa is rapidly removed. Serum albumin interacts with the ligands, and removes them from the liposomes.[8] Thus, we did not perform *in vivo* evaluation of the liposomes bearing compounds Gal-m-pa, Gal-d-pa, Gal-t-pa and Gal-q-pa.

Conclusion We found that compounds Gal-t-psa and Gal-q-psa enhanced the uptake of liposomes. Our neogalactolipids have simple structures, and were easily prepared. These neoglycolipids may be useful as ligands with which to modify the surface of liposomes.

Though the compounds we prepared were equally incorporated into liposomes, compound Gal-t-pa caused only weak uptake by the liver compared to Gal-t-psa or Gal-q-psa. There may be several reasons for this.

Our results indicated that the hepatic uptake of liposomes is best achieved by liposomes bearing galactosyl ligands with a spacer of suitable length. A branched anchor of suitable length may prohibit removal of the ligand.

The ethylene glycol moiety should be longer than diethylene glycol in order to expose the galactosyl moiety.

We synthesized a series of galactosyl ligands of various spacers and anchors. This methodology is applicable to other glycosides. Moreover, according to this method, the length of the spacer can be controlled.

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