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Synthesis of 8-aminooctyl glycopyranosides and of their conjugates with poly(L-glutamic acid) having a 2-(4-hydroxyphenyl)ethylamino group for radiolabeling

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ABSTRACT

8-Aminooctyl glycopyranosides of β-D-galactose, β-L-fucose, α- and β-D-xyloses, α- and β-D-mannoses, 2-acetamido-2-deoxy-β-D-mannose, and 2-acetamido-2-deoxy-α-L-fucose were synthesized under Koenigs–Knorr type glycosylation reaction conditions using the corresponding glycopyranosyl halides or 2-azido-2-deoxy-glycopyranosyl halides and *N*-(8-hydroxyoctyl)phthalimide. Condensation of 8-aminooctyl glycopyranosides of β-D-galactose, β-L-fucose, α-D-xylose, and α- and β-D-mannoses with poly(L-glutamic acid) in the presence of 4-(2-aminoethyl)phenol and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride or 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline as a condensation reagent, gave [8-(β-D-galactopyranosyloxy)octylamine]$_{36}$-[2-(4-hydroxyphenyl)ethylamine]$_3$-poly(L-glutamic acid) conjugate (**41**), [8-(β-L-fucopyranosyloxy)octylamine]$_{16}$-[2-(4-hydroxyphenyl)ethylamine]$_4$-poly(L-glutamic acid) conjugate (**42**), [8-(α-D-xylopyranosyloxy)octylamine]$_{12}$-[2-(4-hydroxyphenyl)ethylamine]$_2$-poly(L-glutamic acid) conjugate (**43**), [8-(α-D-mannopyranosyloxy)octylamine]$_{18}$-[2-(4-hydroxyphenyl)ethylamine]$_5$-poly(L-glutamic acid) conjugate (**44**), and [8-(β-D-mannopyranosyloxy)octylamine]$_{27}$-[2-(4-hydroxyphenyl)ethylamine]$_2$-poly(L-glutamic acid) conjugate (**45**), respectively.

The plasma elimination rates of [^{125}I]-labeled carbohydrate–poly(L-glutamic acid) conjugates bearing 8-(β-D-galactopyranosyloxy)octylamino and 8-(α-D-mannopyranosyloxy)octylamino residues (**41** and **45**) after intravenous administration to rats were more rapid than that of [^{125}I]-labeled [2-(4-hydroxyphenyl)ethylamine]$_5$-poly(L-glutamic acid) conjugate (**40**).

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INTRODUCTION

The carbohydrate sequence of glycolipids and glycoproteins occurring on the surface of cells or in the soluble form in body fluids are important in many biological recognition processes. Since the initial observation by Ashwell and Morell[1] that mammalian hepatocytes possess carbohydrate-binding proteins on their cell surface that mediate clearance of galactose-terminated glycoproteins in circulation, an increasing number of cell-surface carbohydrate-binding proteins[2], such as fucose-binding protein on Kupffer cells[3], mannose-binding protein in serum and liver[4], and mannose 6-phosphate-binding protein in bovine testes and liver[5], have been recognized and characterized. The interaction between the carbohydrate-binding proteins and the ligands depends on the degree of branching of the oligosaccharide structures and is enhanced greatly by a clustering of determinants having a favored conformation[6].

A variety of naturally occurring and synthetic oligosaccharide derivatives have been attached to larger molecules such as proteins[7], poly(L-lysine)[8], and polyacrylamide[9], or incorporated into liposomes[10] for use either as synthetic antigens or as homing devices for the delivery of polymeric drugs to a selected site.

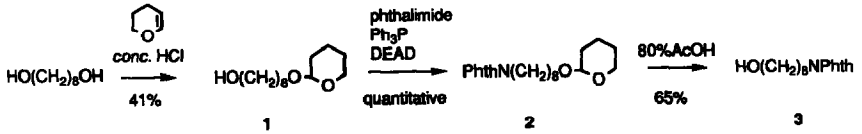
Of macromolecular systems that have been proposed as drug carriers[11], we concentrated on the use of an anionic and biodegradable homopolymer, poly(L-glutamic acid), as a macromolecular backbone for coupling through an amide bond to both carbohydrate moieties and drugs because the molecule might be relatively stable in blood, be soluble in water, and be readily available in different molecular-weight fractions.

As part of our project of developing carbohydrate sensors as a homing device for cell-specific targeted drug delivery, we synthesized 8-aminooctyl glycopyranosides and coupled them to poly(L-glutamic acid) via a C_8 carbon-chain spacer-arm to obtain carbohydrate-poly(L-glutamic acid) conjugates having a 2-(4-hydroxyphenyl)ethylamino group in order to evaluate the efficiency of carbohydrate sensors attached to the poly(L-glutamic acid) backbone.

We describe herein the details of synthesis of monosaccharides having a C_8 carbon-chain spacer-arm and of their conjugates with poly(L-glutamic acid) (mol wt 13 000 for the sodium salt, dp 70) having a 2-(4-hydroxyphenyl)ethylamino group for radiolabeling.

RESULTS AND DISCUSSION

Treatment of 1,8-octanediol with 3,4-dihydro-2*H*-pyran in the presence of a catalytic amount of concd hydrochloric acid in ethylene glycol dimethyl ether for 2 h at room temperature, and chromatography of the product on a column of silica gel gave 8-[(tetrahydropyran-2-yl)oxy]octan-1-ol (**1**) in 41% yield. Substitution of the hydroxyl group of **1** with a phthalimido group by treatment with phthalimide in the presence of diethyl azodicarboxylate and triphenylphosphine in tetrahydrofu-



Scheme 1.

ran for 4 h at room temperature followed by chromatography of the product on a column of silica gel gave *N*-{[8-(tetrahydropyran-2-yl)oxy]octy}phthalimide (**2**) in quantitative yield. Acid hydrolysis of the tetrahydropyranyl group in **2** by treatment with 80% acetic acid for 3 h at 80°C and chromatography of the product on a column of silica gel gave the desired *N*-(8-hydroxyoctyl)phthalimide (**3**) in 65% yield.

Condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[12] (**4**), 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide[13] (**8**), and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide[14] (**12**) with **3** under Koenigs–Knorr type glycosylation reaction conditions (see Table I) gave 8-(phthalimido)octyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**5**), 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside (**9**), and 2,3,4-tri-*O*-acetyl-β- (**13**) and -α-D-xylopyranosides (**16**). Stereochemistry of the glycosidic bond of these condensation products was confirmed by ^{1}H NMR spectrum as shown in Table I.

Treatment of **5**, **9**, **13**, and **16** with sodium methoxide in methanol gave, after chromatography on a column of silica gel, the *O*-deacetylated products **6**, **10**, **14**, and **17** in 59, 54, 60, and 84.4% yield, respectively. Dephthaloylation of **6**, **10**, **14**, and **17** by treatment with hydrazine hydrate in boiling ethanol for 2 h and chromatography of the product on a column of Amberlite IRC-50 (NH_4^+) resin gave the fully deprotected products, 8-aminooctyl β-D-galactopyranoside (**7**), β-L-fucopyranoside (**11**), and β-D- (**15**), and α-D-xylopyranosides (**18**) in 87.1, 91.3, 91, and 77.8% yields, respectively.

To obtain the anomeric pairs of 8-aminooctyl D-mannopyranosides, 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[15] (**19**) and 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl chloride[16] (**20**) were coupled with **3** under silver triflate-promoted or silver silicate-promoted[17] glycosylation reaction conditions (see Table I), giving 8-(phthalimido)octyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**21**) 8-(phthalimido)octyl 2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranoside (**26**), and its α anomer (**23**), respectively. Stereochemistry of the glycosidic bond of these condensation products was confirmed by comparison of the ^{1}H NMR chemical shift of the anomeric proton and the specific rotation of both anomers as shown in Table I.

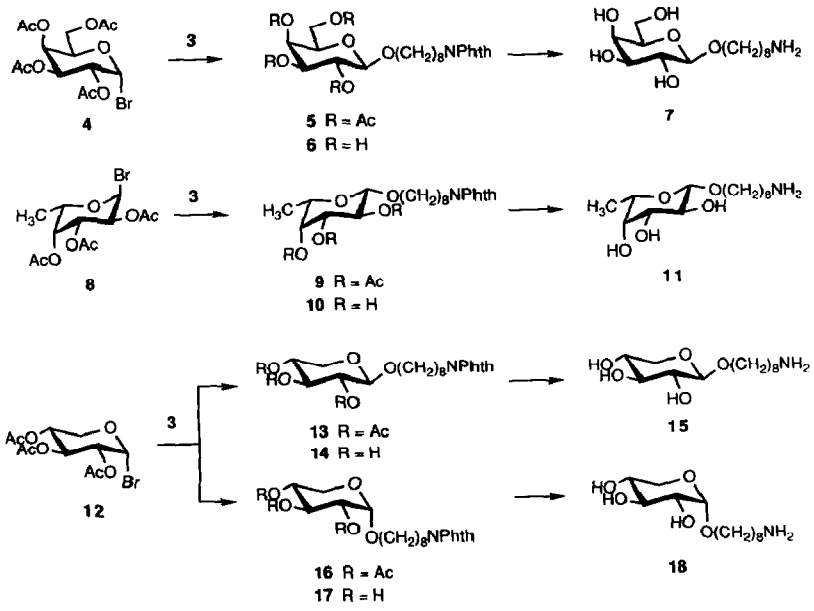
Dephthaloylation of *O*-deacetylated product (**22**) of **21** and **26** by treatment with hydrazine hydrate in boiling ethanol under conditions similar to those for **7** and chromatography of the product on a column of silica gel gave 8-aminooctyl 3,4,6-tri-*O*-benzyl-α- (**24**) and 2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosides (**27**) in 85 and 95% yields, respectively. Removal of the benzyl groups in **24** and **27** by

TABLE I

Synthesis of 8-(phthalimido)octyl glycopyranosides **5**, **9**, **13**, **16**, **21**, **23**, **26**, **30**, **34**, **36**, and **39** and selected ^{1}H NMR chemical shifts (ppm) and optical rotations [a]

Glycosyl donor	Promoter	Solvent	1,2-*trans*-linked				1,2-*cis*-linked			
			Product	Yield (%)	$\delta_{H\text{-}1}$ ($J_{1,2}$ in Hz)	$[\alpha]_D$	Product	Yield (%)	$\delta_{H\text{-}1}$ ($J_{1,2}$ in Hz)	$[\alpha]_D$
4	Ag_2CO_3	$ClCH_2CH_2Cl$	**5**	54.8	4.43 (7.8)	−8.0°				
8	Ag_2CO_3	$ClCH_2CH_2Cl$	**9**	48.6	4.42 (7.8)	+5.6°				
12	Ag_2CO_3	$ClCH_2CH_2Cl$	**13**	48.8	4.45 (6.8)	−27.9°				
12	$Hg(CN)_2$	benzene	**13** [b]	10.9			**16** [b]	35.6	4.97 (3.7)	+81.5°
19	CF_3SO_3Ag	$ClCH_2CH_2Cl$	**21**	52	4.80 (1.5)	+21.9°				
20	Ag-silicate [c]	toluene	**23**	7.8	4.62 (s)	+26.2°	**26**	65.4	4.37 (s)	−39.2°
20	Ag_2CO_3	CH_2Cl_2					**26**	65.7		
29	Ag-silicate	toluene	**34**	12.4	4.81 (1.5)	+47.7°	**30**	35.6	4.64 (1.5)	−49°
35	$Hg(CN)_2$	benzene	**39**	42.4	4.31 (7.8)	+9.1°	**36**	21.4	4.93 (3.4)	−110.4°

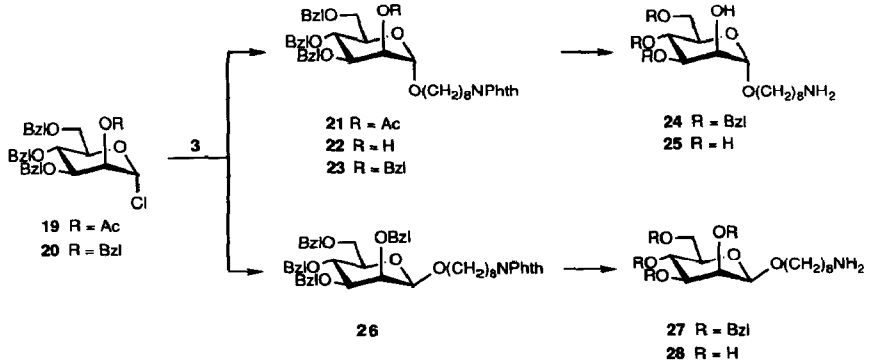
[a] Measured in $CHCl_3$. [b] This compound was obtained after acetylation of the condensation products. [c] Ref. 17.



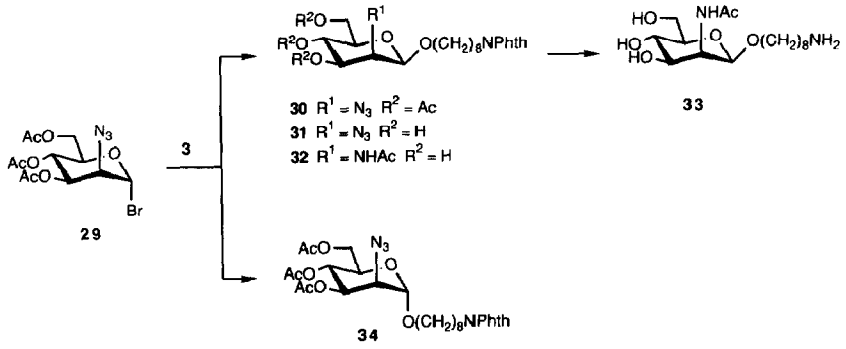
Scheme 2.

catalytic hydrogenolysis over 10% palladium-on-carbon in the presence of an equimolar amount of hydrochloric acid gave the hydrochlorides of 8-aminooctyl α-(**25**) and β-D-mannopyranosides (**28**) in 75 and 94.7% yields, respectively, which were converted into the free bases by passage through a column of Amberlite IR-400 (OH^-) resin. The ^1H NMR spectrum of the free base showed a doublet for H-1 at δ 4.69 ($J_{1,2}$ 1.5 Hz) in **25** and at δ 4.66 ($J_{1,2}$ 1 Hz) in **28**, according to the stereochemistry assigned.

To obtain 8-aminooctyl glycopyranosides of 2-acetamido-2-deoxy-D-mannose and 2-acetamido-2-deoxy-L-fucose, 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl bromide[18] (**29**) and 3,4-di-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl



Scheme 3.



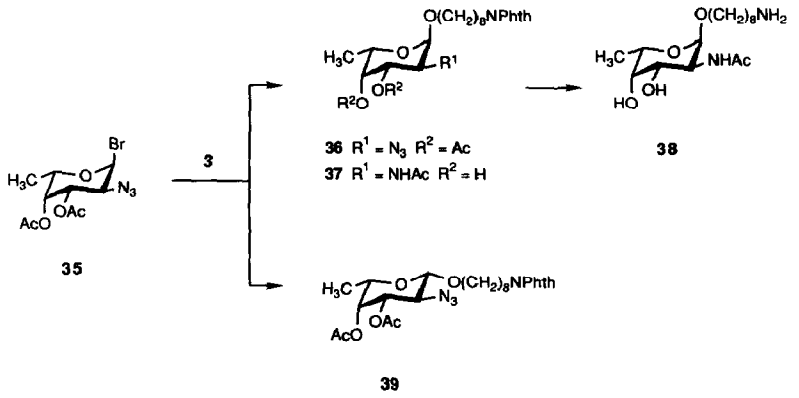
Scheme 4.

bromide[19] (**35**) were condensed with **3** under Koenigs–Knorr type glycosylation reaction conditions (see Table I), giving the anomeric pairs of the condensation products 8-(phthalimido)octyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β- (**30**) and -α-D-mannopyranosides (**34**) and 8-(phthalimido)octyl 3,4-di-*O*-acetyl-2-azido-2-deoxy-α- (**36**), and -β-L-fucopyranosides (**39**), respectively. The stereochemistry of the glycosidic bond of these products was confirmed by the ^{1}H NMR spectrum and the specific rotation.

Treatment of **30** with sodium methoxide in methanol and chromatography of the product on a column of silica gel gave the *O*-deacetylated product **31** in 58% yield. Conversion of the azido group into the acetamido group in **31** by treatment with sodium borohydride in the presence of a catalytic amount of nickel chloride[20] in ethanol, followed by *N*-acetylation with acetic anhydride, gave 8-(phthalimido)octyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**32**) in 34% yield. The ^{1}H NMR spectrum contained a H-1 doublet at δ 4.58 ($J_{1,2}$ 1.5 Hz) and a singlet for the *N*-acetyl group at δ 2.00. Dephthaloylation of **32** by treatment with hydrazine hydrate in boiling ethanol under conditions similar to those for **7** and chromatography of the product on a column of Amberlite IRC-50 (NH_4^+) resin gave 8-aminooctyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**33**) in 34% yield, together with recovered **32** (21%). The ^{1}H NMR spectrum of **33** showed a doublet for H-1 at δ 4.47 ($J_{1,2}$ 1.5 Hz) and a singlet for the *N*-acetyl group at δ 1.98.

Similarly, conversion of **36** into the acetamido derivative (**37**) as described for the preparation of **32**, led to a yield of 85%. Dephthaloylation of **37** by treatment with hydrazine hydrate in boiling ethanol under conditions similar to those for **7** and chromatography of the product on a column of silica gel, and then on a column of Amberlite IRC-50 (NH_4^+) resin gave 8-aminooctyl 2-acetamido-2-deoxy-α-L-fucopyranoside (**38**) in 26.7% yield. The ^{1}H NMR spectrum showed a singlet for the *N*-acetyl group at δ 2.05 and a doublet for H-1 at δ 4.73 ($J_{1,2}$ 3.5 Hz), according to the stereochemistry assigned.

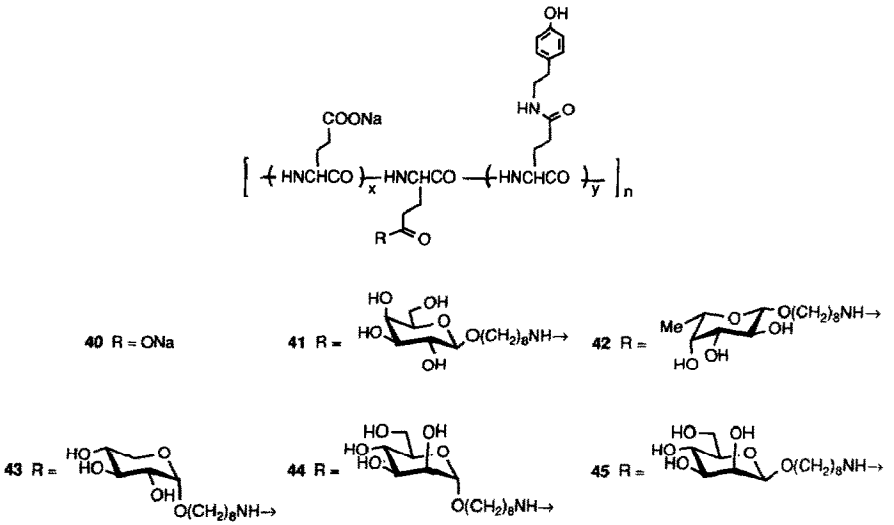
Having prepared 8-aminooctyl glycopyranosides, we then attached them to poly(L-glutamic acid) (purchased from Sigma, mol wt 13 000 for the sodium salt, dp 70) with a 2-(4-hydroxyphenyl)ethylamino group for radiolabeling.



Scheme 5.

Condensation of 4-(2-aminoethyl)phenol with poly(L-glutamic acid), which was prepared from the sodium salt of poly(L-glutamic acid) by treatment with cold M hydrochloric acid, in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in *N*,*N*-dimethylformamide for 18 h at room temperature, and dialysis of the mixture against deionized water, followed by gel filtration on a column of Sephadex G-100, gave [2-(4-hydroxyphenyl)ethylamine]$_5$-poly(L-glutamic acid) conjugate (**40**) in 75% yield. The ratio of the number of 2-(4-hydroxyphenyl)ethylamino residues incorporated per mol of poly(L-glutamic acid) in **40** was determined by spectrophotometry using absorption at 275 nm and was found to be 7% of 2-(4-hydroxyphenyl)ethylamino residues introduced in the total number of the carboxyl groups.

For preparation of a carbohydrate-poly(L-glutamic acid) conjugate having the 2-(4-hydroxyphenyl)ethylamino group, both **7** and 4-(2-aminoethyl)phenol were condensed with poly(L-glutamic acid) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and *N*-hydroxysuccinimide in *N*,*N*-dimethylformamide for 18 h at room temperature. The product was isolated by dialysis against deionized water, followed by gel filtration on a column of Sephadex G-100 (50 × 2.5 cm), giving [8-(β-D-galactopyranosyloxy)octylamine]$_{36}$-[2-(4-hydroxyphenyl)ethylamine]$_3$-poly(L-glutamic acid) conjugate (**41**) in 89% yield. The ratio of the numbers of sugar and 2-(4-hydroxyphenyl)ethylamino residues incorporated per mol of poly(L-glutamic acid) in **41** was determined by the phenol–sulfuric acid method[21] and also by spectophotometry, and found to be 51% of galactose and 4% of 2-(4-hydroxyphenyl)ethylamino residues introduced in the total number of carboxyl groups. Similarly, coupling of **11**, **18**, **25**, and **28** with poly(L-glutamic acid) in the presence of 4-(2-aminoethyl)phenol and EEDQ in *N*,*N*-dimethylformamide and isolation of the conjugates by dialysis against deionized water, followed by gel filtration on a column of Sephadex G-100, gave [8-(β-L-fucopyranosyloxy)octylamine]$_{16}$-[2-(4-hydroxyphenyl)ethylamine]$_4$-poly(L-glutamic acid) conjugate (**42**), [8-(α-D-xylopyranosyloxy)octylamine]$_{12}$-[2-(4-hydroxy-



Scheme 6.

phenyl)ethylamine]$_2$-poly(L-glutamic acid) conjugate (**43**), [8-(α-D-mannopyranosyloxy)octylamine]$_{18}$-[2-(4-hydroxyphenyl)ethylamine]$_5$-poly(L-glutamic acid) conjugate (**44**), and [8-(β-D-mannopyranosyloxy)octylamine]$_{27}$-[2-(4-hydroxyphenyl)ethylamine]$_2$-poly(L-glutamic acid) conjugate (**45**) in 90, 94, 90, and 90% yields, respectively. Analysis of the content of sugar and 2-(4-hydroxyphenyl)ethylamino residues in the conjugates as just described showed, for sugar and 2-(4-hydroxyphenyl)ethylamino residues introduced in the total number of the carboxyl groups, 23 and 6% in **42**, 17 and 3% in **43**, 26 and 7% in **44**, and 38 and 3% in **45**, respectively.

Finally, the plasma elimination rates in rats after intravenous administration of [^{125}I]-labeled conjugates **40**, **41** and **45** are shown in Fig. 1. The [^{125}I]-labeled carbohydrate-poly(L-glutamic acid) conjugates bearing β-D-galactopyranosyl (**41**) and β-D-mannopyranosyl residues (**45**) showed faster clearance than the non-glycosylated poly(L-glutamic acid) (**40**).

EXPERIMENTAL

General methods.—Melting points were measured with a Yanagimoto micro melting-point apparatus and are not corrected. Evaporations were conducted under diminished pressure. Column chromatography was performed on columns of silica gel (Merck, 230–240 mesh). HPLC was performed on a Shimazu Model C-R4A equipped with a column of (7.5 × 300 mm) TSKgel G3000PW$_{XL}$ (Tosoh Co., Ltd., Tokyo, Japan) and developed with 0.2 M NaCl at a flow rate of 0.5 mL min^{-1}. Optical rotations were measured in $CHCl_3$, with a Perkin–Elmer Model 141 polarimeter, unless otherwise noted. IR spectra were recorded with a Hitachi 215 spectrometer. ^{1}H NMR spectra were recorded with a Varian VXR-200 or VXR-500 FT NMR spectrometer, for solutions in $CDCl_3$, unless otherwise noted.

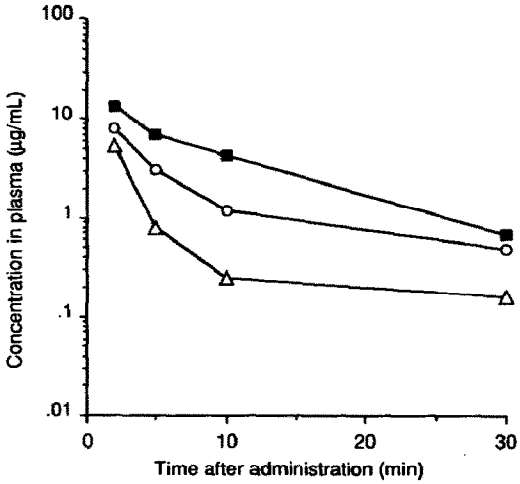


Fig. 1. Plasma concentration of [^{125}I]-labeled poly(L-glutamic acid) (**40**, ■———■), galactosyl (**41**, △———△), and mannosyl poly(L-glutamic acid) conjugates (**45**, ○———○) in rats after intravenous administration (1 mg/Kg).

The values of δ_H are expressed in ppm downfield from the signal for internal Me_4Si, unless otherwise noted. Secondary-ion mass spectra (SIMS), high resolution liquid secondary-ion mass spectra (HRLSIMS), and fast-atom bombardment-mass spectra (FABMS) were measured with a Hitachi M-90 mass spectrameter, with Xe as the the primary ion gas, using *m*-nitrobenzyl alcohol or glycerol as the matrix and poly(ethylene glycol) 300 with or without KI or NaI as internal standard.

8-[(Tetrahydropyran-2-yl)oxy]octan-1-ol (**1**).—3,4-Dihydro-2*H*-pyran (36 mL, 392 mL) was added dropwise to a mixture of 1,8-octanediol (50 g, 342 mmol) and concd HCl (0.5 mL, 6 mmol) in ethylene glycol dimethyl ether (400 mL), with stirring. Stirring was continued for 2 h at room temperature. The mixture was neutralized with Et_3N (1 mL) and the salt which appeared was filtered off. Evaporation of the solvent left a syrup, which was purified on a column of SiO_2 with 2:1 toluene–EtOAc, giving **1** (32.1 g, 41%) as a syrup; $bp_{1.5\ mmHg}$ 140–150°C, $\nu_{max}^{CHCl_3}$: 3400 (OH), 1460, 1355, and 1015 cm^{-1}; NMR data: δ_H 4.57 (dd, *J* 3 and 4.2 Hz, OC*H*O), 3.95–3.3 (m, 6 H, CH_2O), and 2.0–1.2 (m, 18 H, –CH_2–). Anal. Calcd for $C_{13}H_{26}O_3$: C, 67.78; H, 11.38. Found: C, 67.66; H, 11.29.

N-*{[8-(Tetrahydropyran-2-yl)oxy]octyl}phthalimide* (**2**).—Diethyl azodicarboxylate (11.2 mL, 71.2 mmol) was added dropwise to a stirred mixture of **1** (14.9 g, 64.7 mmol), Ph_3P (18.67 mmol), and phthalimide (10.47 g, 71.2 mmol) in anhyd tetrahydrofuran (300 mL) under an atmosphere of N_2. Stirring was continued for 4 h at room temperature. Evaporation of the solvent left a syrup, which was purified on a column of SiO_2 with 3:1 hexane–EtOAc, giving **2** (23 g, quantitatively) as a syrup, $\nu_{max}^{CHCl_3}$ 1773, 1711, 1400 cm^{-1}; NMR data: δ_H 7.9–7.5 (m, 4 H, aromatic H), 4.56 (dd, 1 H, *J* 2.4 and 4.1 Hz, OC*H*O), 3.94–3.44 (m, 3 H, CH_2O), 3.68 (t, 2 H, *J*

7 Hz, CH_2N), 3.75 (dt, 1 H, *J* 6.6 and 9.6 Hz, CH_2O), and 1.9–1.2 (m, 18 H, $-CH_2-$). Anal. Calcd for $C_{21}H_{29}NO_4$: C, 70.17; H, 8.13; N, 3.90. Found: C, 69.99; H, 8.08; N, 3.95.

N-*(8-Hydroxyoctyl)phthalimide* (**3**).—A mixture of **2** (47 g, 137 mmol) in 80% acetic acid was stirred for 3 h at 80°C. After being cooled to room temperature, the solvent was removed by evaporation. The residue was partitioned between EtOAc and water. The organic phase was successively washed with aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. Chromatography of the residue on a column of SiO_2 with 2:1 hexane–EtOAc gave **3** (24.3 g, 65%), which crystallized from ether–hexane; mp 64.5–65°C; $\nu_{max}^{CHCl_3}$: 3630 (OH), 1773, 1712, and 1390 cm^{-1}; NMR data: δ_H 7.9–7.65 (m, 4 H, aromatic H), 3.8–3.6 (m, 4 H, CH_2N and CH_2O), and 1.8–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.78; H, 7.67; N, 5.13.

*8-(Phthalimido)octyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranoside* (**5**).—A mixture of **3** (2.82 g, 10.24 mmol), tetra-*O*-acetyl-α-D-galactopyranosyl bromide[12] (**4**, 4.21 g, 10.24 mmol), Ag_2CO_3 (2.8 g), and 4A molecular sieves (5 g) in 1,2-dichloroethane (30 mL) was stirred at 0°C for 16 h under N_2. After filtration through a bed of Celite, the filtrate was concentrated. Chromatography of the residue on a column of SiO_2 with 5:1 toluene–EtOAc gave **5** (3.34 g, 54.8%) as a syrup; $[\alpha]_D^{24}$ −8.0° (*c* 1.01); $\nu_{max}^{CHCl_3}$: 1750 and 1710 cm^{-1}; NMR data: δ_H 7.80–7.20 (m, 4 H, aromatic H), 5.36 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 5.18 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 4.99 (dd, 1 H, H-3), 4.43 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.20–4.05 (m, 1 H, CH_2O), 3.82 (dt, 1 H, *J* 6.6 and 9.2 Hz, CH_2O), 3.65 (t, 2 H, *J* 7.6 Hz, CH_2N), 3.45 (m, 1 H, H-5), 2.12 (s, 3 H, OAc), 2.03 (s, 6 H, 2 × OAc), 1.96 (s, 3 H, OAc), and 1.70–1.20 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{30}H_{39}NO_{12}$: C, 59.49; H, 6.49; N, 2.31. Found: C, 55.49; H, 6.06; N, 2.41.

*8-(Phthalimido)octyl β-*D*-galactopyranoside* (**6**).—A solution of **5** (2.41 g, 4.04 mmol) in MeOH (50 mL) containing M NaOMe in MeOH (1.2 mL) was kept for 2 h at room temperature. The solution was neutralized with Amberlite IR-120B (H^+) resin. The resin was filtered off and washed with MeOH. The filtrate and washings were combined, and the solvent was removed by evaporation. Chromatography of the residue on a column of SiO_2 with 10:1 $CHCl_3$–MeOH gave **6** (1.02 g, 59%), which crystallized from ether-petroleum ether; mp 123–125°C, $[\alpha]_D^{24}$ −9.9° (*c* 1); NMR data: δ_H (CD_3OD); 7.9–7.75 (m, 4 H, aromatic H), 4.20 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.88 (dt, 1 H, *J* 6.6 and 9.2 Hz, CH_2O), 3.80 (dd, 1 H, $J_{3,4}$ 2.4 Hz, $J_{4,5}$ 1 Hz, H-4), 3.62 (t, *J* 7.3 Hz, CH_2N), and 1.70–1.20 (m, 12 H, $-(CH_2)_6-$); FABMS: *m/z* 460 $[M + Na]^+$ and 438 $[M + H]^+$. Anal. Calcd for $C_{22}H_{31}NO_8 \cdot 0.1H_2O$: C, 60.14; H, 7.16; N, 3.19. Found: C, 59.90; H, 7.12; N, 3.20.

*8-Aminooctyl β-*D*-galactopyranoside* (**7**).—A mixture of **6** (970 mg, 2.22 mmol) and hydrazine hydrate (0.5 mL, 15.3 mmol) in EtOH (30 mL) was refluxed for 2 h under N_2. After being cooled to room temperature, the precipitate was removed by filtration. The filtrate was concentrated. A solution of the residue in water (30 mL) was neutralized with dil HCl, adsorbed on a column of Amberlite IRC-50

(NH_4^+) resin, which was eluted with 2 M NH_4OH, giving **7** (594 mg, 87.1%), which crystallized from isopropyl ether; mp 146–148°C; $[\alpha]_D^{22}$ −16.3° (*c* 1.01, MeOH); NMR data: δ_H (CD_3OD); 4.19 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.89 (dt, 1 H, *J* 6.8 and 9.6 Hz, CH_2O), 3.82 (dd, 1 H, $J_{3,4}$ 3 Hz, $J_{4,5}$ 1 Hz, H-4), 2.63 (t, 2 H, *J* 7.1 Hz, CH_2N), and 1.65–1.33 (m, 12 H, –(CH_2)$_6$–); FABMS: *m/z* 308 $[M + H]^+$. Anal. Calcd for $C_{14}H_{29}NO_6 \cdot 0.3H_2O$: C, 53.76; H, 9.54; N, 4.48. Found: C, 53.71; H, 9.38; N, 4.47.

*8-(Phthalimido)octyl 2,3,4-tri-*O*-acetyl-β-*L*-fucopyranoside* (**9**).—Condensation of tri-*O*-acetyl-α-L-fucopyranosyl bromide[13] (**8**, 2.65 g, 7.52 mmol) with **3** (2.07 g, 7.52 mmol) in the presence of Ag_2CO_3 (2.07 g) and 4A molecular sieves in 1,2-dichloroethane (40 mL) as described for **5**, and chromatography of the product on a column of SiO_2 with 7:1 hexane–EtOAc gave **9** (2 g, 48.6%) as a syrup; $[\alpha]_D^{23}$ +5.6° (*c* 1.07); $\nu_{max}^{CHCl_3}$: 1750, 1711, 1398, 1370, and 1172 cm^{-1}; NMR data: δ_H 7.80–7.20 (m, 4 H, aromatic H), 5.23 (dd, 1 H, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 1 Hz, H-4), 5.18 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 5.01 (dd, 1 H, H-3), 4.42 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.88 (dt, 1 H, *J* 6.6 and 9.8 Hz, CH_2O), 3.79 (dq, 1 H, $J_{4,5}$ 1 Hz, $J_{5,6}$ 6.6 Hz, H-5), 3.67 (t, 2 H, *J* 7.2 Hz, CH_2N), 3.44 (dt, 1 H, *J* 6.6 and 9.8 Hz, CH_2O), 2.17 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 1.98 (s, 3 H, OAc), 1.7–1.2 (m, 12 H, –(CH_2)$_6$–), and 1.22 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6). Anal. Calcd for $C_{28}H_{37}NO_{10}$: C, 61.41; H, 6.81; N, 2.56. Found: C, 59.67; H, 6.66; N, 2.54.

*8-(Phthalimido)octyl β-*L*-fucopyranoside* (**10**).—Compound **9** (2 g, 3.65 mmol) was *O*-deacetylated with M NaOMe in MeOH (1 mL) as described for **6**, and chromatography of the product on a column of SiO_2 with 15:1 $CHCl_3$–MeOH gave **10** (830 mg, 54%), which crystallized from ether; mp 140–141°C; $[\alpha]_D^{24}$ +11.5° (*c* 1.01, MeOH); ν_{max}^{KBr}: 1765, 1689, 1403, 1368, 1088, and 1055 cm^{-1}; NMR data: δ_H (CD_3OD); 7.8–7.9 (m, 4 H, aromatic H), 4.16 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.85 (dt, *J* 6.6 and 9.8 Hz, 1 H, CH_2O), 3.66 (t, 2 H, *J* 7.1 Hz, CH_2N), 3.6–3.5 (m, 2 H, H-4 and 5), 3.5–3.4 (m, 3 H, H-2,3, and CH_2O), 1.7–1.3 (m, 12 H, –(CH_2)$_6$–), and 1.25 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6). Anal. Calcd for $C_{22}H_{31}NO_7$: C, 62.69; H, 7.41; N, 3.32. Found: C, 62.42; H, 7.49; N, 3.25.

*8-Aminooctyl β-*L*-fucopyranoside* (**11**).—Compound **10** (800 mg, 1.9 mmol) was dephthaloylated with hydrazine hydrate (952 mg, 19 mmol) in boiling EtOH (10 mL) as described for **7**, and chromatography of the product on a column of Amberlite IRC-50 (NH_4^+) resin with 2 M NH_4OH, giving **11** (505 mg, 91.3%) as an amorphous powder; $[\alpha]_D^{22}$ +10.6° (*c* 0.55, H_2O); ν_{max}^{KBr}: 1610, 1380, and 1080 cm^{-1}; NMR data: δ_H (D_2O); 4.36 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 3.88 (dt, 1 H, *J* 6.8 and 10 Hz, CH_2O), 3.8 (q, 1 H, $J_{5,6}$ 6.8 Hz, H-5), 3.74 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 3.65 (dt, 1 H, *J* 6.8 and 10 Hz, CH_2O), 3.63 (dd, 1 H, $J_{2,3}$ 10 Hz, H-3), 3.45 (dd, 1 H, H-2), 2.98 (t, 2 H, *J* 7.8 Hz, CH_2N), 1.7–1.4 (m, 12 H, –(CH_2)$_6$–), and 1.25 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6). Anal. Calcd for $C_{14}H_{29}NO_5 \cdot 0.5H_2O$: C, 55.97; H, 10.07; N, 4.66. Found: C, 55.88; H, 10.34; N, 4.55.

*8-(Phthalimido)octyl 2,3,4-tri-*O*-acetyl-β-* (**13**) *and -α-*D*-xylopyranosides* (**16**).—*(A)*. Condensation of tri-*O*-acetyl-α-D-xylopyranosyl bromide[14] (**12**, 2.6 g, 7.85

mmol) with **3** (2.16 g, 7.85 mmol) in the presence of Ag_2CO_3 (2.16 g, 7.85 mmol) and 4A molecular sieves (5 g) in 1,2-dichloroethane (40 mL) as described for **5**, and chromatography of the product on a column of SiO_2 with 7:1 toluene–EtOAc gave **13** (2.04 g, 48.8%) as a syrup, $[\alpha]_D^{23}$ −27.9° (*c* 1.05); $\nu_{max}^{CHCl_3}$: 1750, 1710, 1390, 1370, and 1050 cm^{-1}; NMR data: δ_H 7.85–7.70 (m, 4 H, aromatic H), 5.12 (t, 1 H, $J_{2,3} = J_{3,4} = 8.6$ Hz, H-3), 4.95 (m, 1 H, H-4), 4.90 (dd, 1 H, $J_{2,3}$ 8.6 Hz, H-2), 4.45 (d, 1 H, $J_{1,2}$ 6.8 Hz, H-1), 4.11 (dd, 1 H, $J_{4,5e}$ 5.1 Hz, $J_{5a,5e}$, 11.8 Hz, H-5e), 3.75 (dt, 1 H, *J* 6.8 and 9.6 Hz, CH_2O), 3.67 (t, 2 H, *J* 7.1 Hz, CH_2N), 3.45 (dt, 1 H, *J* 6.8 and 9.6 Hz, CH_2O), 3.35 (dd, 1 H, $J_{4,5a}$ 8.8 Hz, H-5*a*), 2.05 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{27}H_{35}NO_{10}$: C, 60.78; H, 6.61; N, 2.63. Found: C, 60.62; H, 6.58; N, 2.62.

(B). A mixture of **12** (1.85 g, 5.45 mmol), **3** (1.5 g, 5.45 mmol), $Hg(CN)_2$ (1.38 g, 5.45 mmol), and powdered 4A molecular sieves (3.5 g) in benzene (30 mL) was stirred for 18 h at room temperature under Ar. The mixture was filtered through a bed of Celite. The filtrate was partitioned between EtOAc and water. The organic phase was washed with water, dried ($MgSO_4$), and concentrated. The residue was dissolved in pyridine (5 mL) and Ac_2O (2 mL) was added. The mixture was kept for 16 h at room temperature. The mixture was partitioned between EtOAc and water. The organic phase was washed successively with dil HCl, aq $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. Chromatography of the residue on a column of SiO_2 with 10:1 toluene–EtOAc gave **13** (313 mg, 10.9%) and **16** (1.07 g, 35.6%).

Compound **16** had: $[\alpha]_D^{23}$ +81.5° (*c* 1); $\nu_{max}^{CHCl_3}$: 1760, 1710, 1230, and 1050 cm^{-1}; NMR data: δ_H 7.85–7.70 (m, 4 H, aromatic H), 5.47 (t, 1 H, $J_{2,3} = J_{3,4} = 10.2$ Hz, H-3), 4.97 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.95 (m, 1 H, H-4), 4.78 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 3.76 (dd, 1 H, $J_{4,5e}$ 6.1 Hz, $J_{5a,5e}$ 11 Hz, H-5*e*), 3.8–3.6 (m, 4 H, H-5*a*, CH_2O and CH_2N), 3.55 (dt, 1 H, *J* 6.8 and 9.6 Hz, CH_2O), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{27}H_{35}NO_{10}$: C, 60.78; H, 6.61; N, 2.63. Found: C, 60.58; H, 6.88; N, 2.69.

*8-(Phthalimido)octyl β-*D*-xylopyranoside* (**14**).—Compound **13** (680 mg, 1.27 mmol) was *O*-deacetylated with M NaOMe in MeOH (0.38 mL) as described for **6**, and chromatography of the product on a column of SiO_2 with 15:1 $CHCl_3$–MeOH gave **14** (310 mg, 60%), which crystallized from ether–petroleum ether; mp 119–120°C; $[\alpha]_D^{24}$ −28.8° (*c* 1, MeOH), ν_{max}^{KBr}: 1775, 1720, 1465, 1435, 1398, 1365, and 1160 cm^{-1}; NMR data: δ_H (CD_3OD); 7.95–7.7 (m, 4 H, aromatic H), 4.17 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 3.84 (dd, 1 H, $J_{4,5e}$ 5, $J_{5a,5e}$ 11.2 Hz, H-5*e*), 3.79 (dt, 1 H, *J* 6.5 and 9.6 Hz, CH_2O), 3.65 (t, 2 H, *J* 7.4 Hz, CH_2N), and 1.8–1.25 (m, 12 H, $-(CH_2)_6-$). *Anal.* Calcd for $C_{21}H_{29}NO_7$: C, 61.90; H, 7.17; N, 3.44. Found: C, 61.79; H, 7.20; N, 3.37.

*8-Aminooctyl β-*D*-xylopyranoside* (**15**).—Compound **14** (170 mg, 0.417 mmol) was dephthaloylated with hydrazine hydrate (0.2 mL) in boiling EtOH (3 mL) as described for **7**, and chromatography of the product on a column of Amberlite IRC-50 (NH_4^+) resin with 2 M NH_4OH gave **15** (105 mg, 91%), which crystallized

from MeOH and ether; mp 98–100°C; $[\alpha]_D^{23}$ −42.6° (*c* 1, MeOH); ν_{max}^{KBr}: 1732, 1710, 1398, 1370, and 1050 cm^{-1}; NMR data: δ_H (CD_3OD); 4.17 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 3.84 (dd, 1 H, $J_{4,5a}$ 5 Hz, $J_{5a,5e}$ 11.4 Hz, H-5*a*), 3.78 (t, 1 H, $J_{2,3} = J_{3,4} = 7$ Hz, H-3), 3.58–3.38 (m, 2 H, CH_2O), 2.63 (t, 2 H, *J* 7 Hz, CH_2N), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{13}H_{27}NO_5 \cdot 0.3H_2O$: C, 55.22; H, 9.84; N, 4.95. Found: C, 55.12; H, 9.71; N, 5.16.

8-(Phthalimido)octyl α-D-xylopyranoside (**17**).—Compound **16** (700 mg, 1.31 mmol) was *O*-deacetylated with M NaOMe in MeOH (0.4 mL) as described for **6**, and chromatography of the product on a column of SiO_2 with 15:1 $CHCl_3$–MeOH, gave **17** (451 mg, 84.4%), which crystallized from MeOH–ether; mp 88–89°C; $[\alpha]_D^{22}$ +73.8° (*c* 1, MeOH); ν_{max}^{KBr}: 1775, 1708, 1398, and 1031 cm^{-1}; NMR data: δ_H (CD_3OD); 7.9–7.75 (m, 4 H, aromatic H), 4.69 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.66 (t, 2 H, *J* 7.4 Hz, CH_2N), and 1.8–1.25 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{21}H_{29}NO_7$: C, 61.90; H, 7.17; N, 3.44. Found: C, 61.87; H, 7.09; N, 3.47.

8-Aminooctyl α-D-xylopyranoside (**18**).—Compound **17** (400 mg, 0.98 mmol) was dephthaloylated with hydrazine hydrate (636 mg, 12.7 mmol) in boiling EtOH (2 mL) as described for **7**, and chromatography of the product on a column of Amberlite IRC-50 (NH_4^+) resin with 2 M NH_4OH, gave **18** (274 mg, 77.8%) as an amorphous powder; $[\alpha]_D^{23}$ +72.5° (*c* 1.03, H_2O); ν_{max}^{KBr}: 1580 and 1030 cm^{-1}; NMR data: δ_H (D_2O); 4.88 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 3.7–3.5 (m, 7 H, H-2,3,4,5, and CH_2O), 2.98 (t, 2 H, *J* 7.8 Hz, CH_2N), and 1.7–1.3 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{13}H_{27}NO_5 \cdot 0.3H_2O$: C, 55.22; H, 9.84; N, 4.95. Found: C, 55.34; H, 9.76; N, 5.06.

8-(Phthalimido)octyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**21**).—A solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[16] (**19**, 1.6 g, 31.58 mmol) in 1,2-dichloroethane (30 mL) was added, with stirring, to a cooled (−40°C) solution of silver triflate (8.93 g, 34.73 mmol), **3** (8.7 g, 31.58 mmol), and 1,1,3,3-tetramethylurea (17 mL, 126.3 mmol) in 1,2-dichloroethane (200 mL) under N_2. The mixture was stirred for 30 min. The cooling bath was removed and the temperature was allowed to rise gently to room temperature. After stirring for 16 h at ambient temperature, the mixture was filtered through a bed of Celite. The filtrate was washed successively with aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. Chromatography of the residue on a column of SiO_2 with 3:1 hexane–EtOAc gave **21** (12.3 g, 52%) as a syrup; $[\alpha]_D^{24}$ +21.9° (*c* 1.11); $\nu_{max}^{CHCl_3}$: 1770, 1740, 1710, 1355, 1380, and 1085 cm^{-1}; NMR data: δ_H 7.82–7.60 (m, 4 H, aromatic H), 7.4–7.2 (m, 15 H, Ph), 5.33 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 4.80 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.96 (dd, 1 H, $J_{3,4}$ 9 Hz, H-3), 3.86 (t, 1 H, $J_{3,4} = J_{4,5} = 9$ Hz, H-4), 3.67 (t, 2 H, *J* 7 Hz, CH_2N), 3.38 (dt, 1 H, *J* 6.2 and 9.4 Hz, CH_2O), 2.13 (s, 3 H, OAc), and 1.75–1.20 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{45}H_{51}NO_9$: C, 72.07; H, 6.86; N, 1.87. Found: C, 71.78; H, 6.85; N, 2.13.

8-(Phthalimido)octyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (**22**).—Compound **21** (12.3 g, 16.4 mmol) was *O*-deacetylated with M NaOMe in MeOH as described for **6**, and chromatography of the residue on a column of SiO_2 with 2:1 hexane–

AcOEt gave **22** (8.5 g, 73.3%) as a syrup; $[\alpha]_D^{24}$ +35.7° (*c* 1.12); ν_{max}^{film} 1772, 1712, 1395, and 1365 cm^{-1}; NMR data: δ_H 7.90–7.65 (m, 4 H, aromatic H), 7.4–7.1 (m, 15 H, Ph), 4.88 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.39 (dt, 1 H, *J* 6.4 and 9.8 Hz, CH_2O), and 1.75–1.20 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{43}H_{49}NO_8$: C, 73.00; H, 6.98; N, 1.98. Found: C, 72.68; H, 6.98; N, 2.09.

*8-(Phthalimido)octyl 2,3,4,6-tetra-*O*-benzyl-α-* (**23**) *and -β-*D*-mannopyranosides* (**26**).—*(A).* A solution of tetra-*O*-benzyl-α-D-mannopyranosyl chloride[16] (**20**, 1.7 g, 2.89 mmol) in toluene (10 mL) was added dropwise to a stirred mixture of **3** (795 mg, 2.89 mmol), silver silicate (2 g), and 4A molecular sieves in toluene (20 mL) at −18°C under N_2. The cooling bath was removed and the temperature was allowed to rise gently to room temperature. After stirring for 18 h, the mixture was filtered through a bed of Celite. The filtrate was concentrated. Chromatography of the residue on a column of SiO_2 with 4:1 hexane–EtOAc gave **23** (180 mg, 7.8%) and **26** (1.51 g, 65.4%).

Compound **23** had: $[\alpha]_D^{26}$ +26.2° (*c* 0.13); ν_{max}^{film}: 1770, 1712, 1496, 1467, 1105, and 1068 cm^{-1}; NMR data: δ_H 7.80–7.2 (m, 24 H, aromatic H), 4.62 (s, 1 H, H-1), 3.97 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.80 (dd, 1 H, $J_{2,3}$ 3 Hz, $J_{3,4}$ 9.5 Hz, H-3), 3.66 (m, 1 H, CH_2N), 3.63 (dt, 1 H, *J* 6.8 and 9.5 Hz, CH_2O), 3.33 (dt, 1 H, *J* 6.8 and 9.5 Hz, CH_2O), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{50}H_{55}NO_8$: C, 75.26; H, 6.95; N, 1.76. Found: C, 75.46; H, 6.75; N, 1.56.

Compound **26** had: $[\alpha]_D^{26}$ −39.2° (*c* 0.13); ν_{max}^{film}: 1770, 1712, 1496, 1467, 1105, and 1068 cm^{-1}; NMR data: δ_H 7.80–7.2 (m, 24 H, aromatic H), 4.37 (s, 1 H, H-1), 3.96 (dt, 1 H, *J* 6.6 and 9.2 Hz, CH_2O), 3.90 (dd, 1 H, $J_{2,3}$ 3 Hz, H-2), 3.86 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.81 (dd, 1 H, $J_{5,6a}$ 2, $J_{6a,6b}$ 11 Hz, H-6a), 3.74 (dd, 1 H, $J_{5,6b}$ 6.5 Hz, H-6b), 3.67 (t, 2 H, *J* 7.6 Hz, CH_2N), 3.50 (dd, 1 H, $J_{2,3}$ 3 Hz, H-3), 3.45 (ddd, 1 H, H-5), 3.40 (dt, 1 H, *J* 6.6 and 9.2 Hz, CH_2O), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{50}H_{55}NO_8$: C, 75.26; H, 6.95; N, 1.76. Found: C, 75.55; H, 6.85; N, 2.01.

(B). Condensation of **20** (8.9 g, 14.5 mmol) with **3** (10 g, 36.25 mmol) in the presence of Ag_2CO_3 and Drierite (7.8 g) in CH_2Cl_2 as described for **5**, and chromatography of the product on a column of SiO_2 with 4:1 hexane–EtOAc gave **26** (7.49 g, 65.7%) as a syrup, which was identical with **26** obtained above by comparison of specific rotation, TLC, and ^1H NMR data.

*8-Aminooctyl 3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**24**).—A mixture of **22** (8.5 g, 12 mmol) and hydrazine hydrate (3 mL) in EtOH (60 mL) was refluxed for 1 h under N_2. After being cooled to room temperature, the precipitate was removed by filtration. The filtrate was partitioned between EtOAc and water. The organic phase was washed with water, dried ($MgSO_4$), and concentrated. Chromatography of the residue on a column of SiO_2 with 10:1:0.1 $CHCl_3$–MeOH–NH_4OH gave **24** (6 g, 85%) as a syrup; $[\alpha]_D^{23}$ +43.1° (*c* 1.08, MeOH); ν_{max}^{film}: 1496, 1454, 1363, 1103, and 1058 cm^{-1}; NMR data: δ_H 7.4–7.1 (m, 15 H, Ph), 4.75 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.00 (dd, $J_{2,3}$ 3.5 Hz, H-2), 2.62 (t, 2 H, *J* 8 Hz, CH_2N), and 1.6–1.25 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{35}H_{47}NO_6$: C, 72.76; H, 8.20; N, 2.42. Found: C, 72.61; H, 8.32; N, 2.42.

8-Aminooctyl α-D-mannopyranoside (**25**).—A solution of **24** (6.9 g, 12 mmol) in MeOH (200 mL) containing M HCl (12 mL) was hydrogenated over 10% Pd–C (1 g) for 16 h at room temperature. After removal of the catalyst by filtration, the solvent was removed by evaporation, giving the hydrochloride of **25** (2.99 g, 75%) as a hygroscopic powder; $[\alpha]_D^{26}$ +42.9° (*c* 1.13, MeOH); ν_{max}^{KBr}: 1620, 1506, 1460, 1103, and 1377 cm^{-1}; NMR data: δ_H (CD_3OD); 4.72 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 3.81 (dd, 1 H, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.76 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 3.59 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.51 (ddd, 1 H, H-5), 2.90 (t, 2 H, *J* 8 Hz, CH_2N), and 1.7–1.35 (m, 12 H, $-(CH_2)_6-$).

The hydrochloride of **25** (2.64 g) was dissolved in 50% aq MeOH (20 mL) and passed through a column of Amberlite IRA-400 (OH^-) resin. The resin was washed with 50% aq MeOH (500 mL). The eluant and washings were combined, and then evaporated, giving **25** (2.31 g) as an amorphous powder; $[\alpha]_D^{22}$ +54° (*c* 1.02, MeOH), NMR data: δ_H (CD_3OD); 4.69 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.78 (dd, 1 H, $J_{5,6a}$ 2.5, $J_{6a,6b}$ 12 Hz, H-6a), 3.74 (dd, 1 H, $J_{2,3}$ 3 Hz, H-2), 3.57 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.49 (ddd, 1 H, H-5), 2.69 (t, 2 H, *J* 7.5 Hz, CH_2N), and 1.6–1.25 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{14}H_{29}NO_6 \cdot H_2O$: C, 51.51; H, 9.57; N, 4.29. Found: C, 51.63; H, 9.85; N, 4.55.

8-Aminooctyl 2,3,4,6-tetra-O-benzyl-β-D-mannopyranoside (**27**).—A solution of **26** (1.39 g, 1.74 mmol) and hydrazine acetate (8 g, 87 mmol) in MeOH (90 mL) was boiled under reflux for 4 h. After being cooled to room temperature, the mixture was partitioned between EtOAc and water. The organic phase was washed with water, dried ($MgSO_4$), and evaporated, giving **27** (1.1 g, 95%), which crystallized from ether; mp 89–90°C; $[\alpha]_D^{23}$ −42.2° (*c* 0.49, MeOH); ν_{max}^{KBr}: 1661, 1603, 1497, 1454, 1301, and 1059 cm^{-1}; NMR data: δ_H 7.5–7.1 (m, 20 H, Ph), 4.37 (d, 1 H, $J_{1,2}$ 1 Hz, H-1), 3.98 (dt, 1 H, *J* 6.6 and 9 Hz, CH_2O), 3.90 (dd, 1 H, $J_{2,3}$ 2.5 Hz, H-2), 3.85 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.50 (dd, 1 H, H-3), 3.46 (ddd, 1 H, H-5), 3.41 (dt, 1 H, *J* 6.6 and 9 Hz, CH_2O), 2.66 (t, 2 H, *J* 7.5 Hz, CH_2N), and 1.6–1.25 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{42}H_{53}NO_6$: C, 75.53; H, 8.00; N, 2.10. Found: C, 75.56; H, 8.44; N, 1.98.

8-Aminooctyl β-D-mannopyranoside (**28**).—A solution of **27** (400 mg, 0.6 mmol) in MeOH (30 mL) containing M HCl (0.6 mL) was hydrogenated over 10% Pd–C (100 mg) as described for **25**, giving the hydrochloride of **28** (195 mg, 94.7%) as an amorphous powder; $[\alpha]_D^{24}$ −10.3° (*c* 0.62, MeOH); NMR data: δ_H 4.49 (s, 1 H, H-1), 3.44 (dd, 1 H, $J_{5,6a}$ 3.5, $J_{6a,6b}$ 12 Hz, H-6a), 3.19 (ddd, 1 H, H-5), 2.90 (t, 2 H, *J* 7.5 Hz, CH_2N), and 1.7–1.3 (m, 12 H, $-(CH_2)_6-$).

The hydrochloride of **28** (195 mg) was dissolved in 50% aq MeOH (15 mL) and adsorbed on a column of Amberlite IRC-50 (NH_4^+ form) resin, and was eluted with 6% NH_4OH giving **28** (152 mg, 80%) as an amorphous powder; $[\alpha]_D^{21}$ −38.9° (*c* 1.01, MeOH); NMR data: δ_H (D_2O); 4.66 (d, 1 H, $J_{1,2}$ 1 Hz, H-1), 3.97 (dd, 1 H, $J_{2,3}$ 3 Hz, H-2); 3.92 (dd, 1 H, $J_{5,6a}$ 2.2 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 3.87 (dt, 1 H, *J* 6.4 and 10 Hz, CH_2O), 3.72 (dd, 1 H, $J_{5,6b}$ 6.6 Hz, H-6b), 3.65 (dt, 1 H, *J* 6.4 and 10 Hz, CH_2O), 3.63 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 3.56 (t, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4),

3.35 (ddd, 1 H, H-5), 2.68 (t, 2 H, *J* 7 Hz, CH_2N), and 1.7–1.3 (m, 12 H, $-(CH_2)_6-$); FABMS: *m/z* 308 $[M+H]^+$. Anal. Calcd for $C_{14}H_{29}NO_6 \cdot H_2O$: C, 51.51; H, 9.57; N, 4.29. Found: C, 51.55; H, 9.35; N, 4.19.

8-(Phthalimido)octyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-β- (**30**) *and α-D-mannopyranosides* (**34**).—Condensation of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl bromide[18] (**29**, 9.5 g, 24.1 mmol) with **3** (6.63 g, 24.09 mmol) in the presence of silver silicate (10 g) and 4A molecular sieves (10 g) in toluene (160 mL) as described for **23** and **26**, and chromatography of the product on a column of SiO_2 with 2:1 hexane–EtOAc, gave **30** (5.01 g, 35.6%) and **34** (1.84 g, 12.4%).

Compound **30** had: $[\alpha]_D^{23}$ −49° (*c* 0.5), $\nu_{max}^{CHCl_3}$: 2250, 1750, 1715, 1398, and 1370 cm^{-1}; NMR data: δ_H 7.8–7.65 (m, 4 H, aromatic H), 5.23 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.96 (dd, 1 H, $J_{2,3}$ 4 Hz, H-3), 4.64 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.25 (dd, 1 H, $J_{5,6a}$ 5, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.11 (dd, 1 H, $J_{5,6b}$ 2.5 Hz, H-6b), 4.07 (dd, 1 H, H-2), 3.89 (dt, 1 H, *J* 6.6 and 9.6 Hz, CH_2O), 3.65 (t, 2 H, *J* 7.5 CH_2N), 3.58 (ddd, 1 H, H-5), 2.09 (s, 3 H, OAc), 2.07 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{28}H_{36}N_4O_{10}$: C, 57.13; H, 6.17; N, 9.52. Found: C, 57.00; H, 6.35; N, 9.46.

Compound **34** had: $[\alpha]_D^{22}$ +47.7° (*c* 0.56); $\nu_{max}^{CHCl_3}$: 2250, 1750, 1715, 1398, and 1370 cm^{-1}; NMR data: δ_H 7.85–7.65 (m, 4 H, aromatic H), 5.38 (dd, $J_{2,3}$ 4, $J_{3,4}$ 9.5 Hz, H-3), 5.30 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.81 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.24 (dd, 1 H, $J_{5,6a}$ 5, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.07 (dd, 1 H, $J_{5,6b}$ 2.5 Hz, H-6b), 4.00 (dd, 1 H, H-2), 3.72–3.62 (m, 3 H, CH_2O and CH_2N), 3.58 (ddd, 1 H, H-5), 3.41 (dt, 1H, *J* 6.6 and 9.6 Hz, CH_2O), 2.09 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{28}H_{36}N_4O_{10}$: C, 57.13; H, 6.17; N, 9.52. Found: C, 57.30; H, 6.40; N, 9.35.

8-(Phthalimido)octyl 2-azido-2-deoxy-β-D-mannopyranoside (**31**).—Compound **30** (4 g, 6.795 mmol) was *O*-deacetylated with NaOMe in MeOH as described for **6**, and chromatography of the product on a column of SiO_2 with 10:1 $CHCl_3$–MeOH, gave **31** (1.82 g, 58%) as a syrup; $[\alpha]_D^{28}$ −72.5° (*c* 1.03); $\nu_{max}^{CHCl_3}$: 2114, 1772, 1711, 1398, and 1372 cm^{-1}; NMR data: δ_H 7.8–7.65 (m, 4 H, aromatic H), 4.64 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.89 (dt, 1 H, *J* 6.6 and 9.6 Hz, CH_2O), 3.56 (dd, 1 H, $J_{2,3}$ 4, $J_{3,4}$ 9.5 Hz, H-3), 3.50 (dt, 1 H, *J* 6.6 and 9.6 Hz, CH_2O), 3.39 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.16 (ddd, 1 H, $J_{5,6a}$ 2.5 Hz, $J_{5,6b}$ 6.3 Hz, H-5), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{22}H_{30}N_4O_7$: C, 57.13; H, 6.54; N, 12.12. Found: C, 57.24; H, 6.64; N, 12.30.

8-(Phthalimido)octyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**32**).—A solution of $NaBH_4$ (412 mg, 10.89 mmol) in EtOH (40 mL) was added dropwise to a solution of **31** (1.68 g, 3.63 mmol) in EtOH (10 mL) containing $NiCl_2 \cdot 6H_2O$ (ref. 20) (380 mg, 1.6 mmol), with stirring. The mixture was stirred for 30 min at room temperature. After neutralization of the mixture with a small amount of AcOH, Ac_2O (2 mL) was added. The mixture was kept for 18 h at room temperature and then concentrated. Chromatography of the residue on a column of SiO_2 with 7:1 $CHCl_3$–MeOH gave **32** (587 mg, 34%) as an amorphous powder; $[\alpha]_D^{26.5}$ −28.2° (*c*

1.42, MeOH); ν_{max}^{KBr}: 1672 and 1060 cm^{-1}; NMR data: δ_H (CD_3OD); 7.7–7.5 (m, 4 H, aromatic H), 4.58 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.36 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 3.48 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 2.00 (s, 3 H, NAc), and 1.7–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{24}H_{34}N_2O_8 \cdot H_2O$: C, 58.05; H, 7.31; N, 5.64. Found: C, 57.85; H, 7.61; N, 5.54.

8-Aminooctyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**33**).—Compound **32** (580 mg, 1.212 mmol) was dephthaloylated with hydrazine hydrate (2 mL) in boiling EtOH (10 mL) as described for **7**, and chromatography of the product on a column of Amberlite IRC-50 (NH_4^+) resin with 2 M NH_4OH gave **33** (140 mg, 34%) as an amorphous powder, together with recovered **32** (121 mg, 21%).

Compound **33** had: $[\alpha]_D^{21}$ −35.8° (*c* 0.45, MeOH); ν_{max}^{KBr}: 1715, 1660, 1545, 1370, and 1070 cm^{-1}; NMR data: δ_H (CD_3OD); 4.56 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 4.47 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 1.98 (s, 3 H, NAc), and 1.6–1.2 (m, 12 H, $-(CH_2)_6-$). Anal. Calcd for $C_{16}H_{32}N_2O_6 \cdot H_2O$: C, 52.44; H, 9.35; N, 7.64. Found: C, 52.14; H, 9.25; N, 7.65.

8-(Phthalimido)octyl 3,4-di-O-acetyl-2-azido-2-deoxy-α- (**36**) *and -β-L-fucopyranoside* (**39**).—A solution of 3,4-di-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl bromide[19] (**35**, 911 mg, 2.71 mmol) in anhyd benzene (10 mL) was added dropwise to a stirred mixture of **3** (746 mg, 2.71 mmol), $Hg(CN)_2$ (685 mg, 2.71 mmol), and 4A molecular sieves (1 g) under Ar. After stirring for 16 h at room temperature, the mixture was filtered through a bed of Celite. The filtrate was partitioned between EtOAc and water. The organic phase was washed with water, dried ($MgSO_4$), and then concentrated. Chromatography of the residue on a column of SiO_2 with 4:1 hexane–EtOAc gave **36** (308 mg, 21.4%) and **39** (610 mg, 42.4%).

Compound **36** had: $[\alpha]_D^{22}$ −110.4° (*c* 1.0); ν_{max}^{film}: 2110, 1750, and 1234 cm^{-1}; NMR data: δ_H 7.80–7.20 (m, 4 H, aromatic H), 5.36 (dd, 1 H, $J_{2,3}$ 11.2, $J_{3,4}$ 3.2 Hz, H-3), 5.30 (d, 1 H, H-4), 4.93 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.14 (q, 1 H, $J_{5,6}$ 6.6 Hz, H-5), 3.6–3.7 (m, 3 H, CH_2O and CH_2N), 3.57 (dd, 1 H, H-2), 3.45 (dt, 1 H, *J* 6.6 and 9.8 Hz, CH_2O), 2.05 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 1.7–1.2 (m, 12 H, $-(CH_2)_6-$), and 1.13 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6). Anal. Calcd for $C_{26}H_{34}N_4O_8$: C, 58.85; H, 6.46; N, 10.56. Found: C, 58.97; H, 6.77; N, 10.76.

Compound **39** had: $[\alpha]_D^{22}$ +9.1° (*c* 1.0); ν_{max}^{film}: 2110, 1750, and 1242 cm^{-1}; NMR data: δ_H 7.80–7.20 (m, 4 H, aromatic H), 5.17 (dd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ 1 Hz, H-4), 4.75 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-3), 4.31 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.95 (dt, 1 H, *J* 6.6 and 9.8 Hz, CH_2O), 3.73 (dq, 1 H, $J_{4,5}$ 1, $J_{5,6}$ 6.6 Hz, H-5), 3.6–3.7 (m, 3 H, H-2 and CH_2N), 3.55 (dt, 1 H, *J* 6.6 and 9.8 Hz, CH_2O), 2.05 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 1.7–1.2 (m, 12 H, $-(CH_2)_6-$), and 1.20 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6). Anal. Calcd for $C_{26}H_{34}N_4O_8$: C, 58.85; H, 6.46; N, 10.56. Found: C, 58.55; H, 6.34; N, 10.27.

8-Aminooctyl 2-acetamido-2-deoxy-α-L-fucopyranoside (**38**) *via 8-(phthalimido)octyl 2-acetamido-2-deoxy-α-L-fucopyranoside* (**37**).—A solution of **36** (300 mg, 0.565 mmol) in MeOH containing 28% NaOMe in MeOH (0.08 mL) was kept for 24 h at room temperature. The solution was neutralized with Amberlite

IR-120B (H^+) resin. The resin was filtered off and washed with water. The filtrate and washings were combined, and then concentrated. To a solution of the residue in EtOH (15 mL) was added $NaBH_4$ (64 mg, 1.7 mmol), followed by a solution of $NiCl_2 \cdot 6H_2O$ (40 mg) in EtOH (0.5 mL). After stirring for 2 h at room temperature, the mixture was neutralized with 6 M HCl. After removal of the precipitate, the filtrate was concentrated. The residue was dissolved in MeOH (5 mL) and Ac_2O (1 mL) was added. After being kept for 3 h at room temperature, the mixture was concentrated. Chromatography of the residue on a column of SiO_2 with 30:1 $CHCl_3$–MeOH gave 8-(phthalimido)octyl 2-azido-2-deoxy-α-L-fucopyranoside (**37**, 215 mg, 85%) as an amorphous powder; NMR data: δ_H (CD_3OD); 7.80–7.60 (m, 4 H, aromatic H), 4.73 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.19 (dd, 1 H, $J_{2,3}$ 11 Hz, H-2), 1.97 (s, 3 H, NAc), 1.75–1.2 (m, 12 H, $-(CH_2)_6-$), and 1.21 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6).

A solution of **37** (215 mg, 0.46 mmol) in EtOH (5 mL) containing hydrazine hydrate (141 mg, 2.83 mmol) was boiled under reflux for 2 h. After being cooled to room temperature, the precipitate was removed by filtration. The filtrate was concentrated. Chromatography of the residue on a column of SiO_2 with 30:10:1 $CHCl_3$–MeOH–NH_4OH, and then on a column of Amberlite IRC-50 (NH_4^+) resin with 2 M NH_4OH gave **38** (41 mg, 26.7%) as an amorphous powder; $[\alpha]_D^{22}$ −74.8° (*c* 1.0 H_2O), ν_{max}^{film}: 1640 and 1030 cm^{-1}; NMR data: δ_H (D_2O); 4.73 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.1–4.2 (m, 2 H, H-2 and 5), 3.92 (dd, 1 H, $J_{2,3}$ 11.2, $J_{3,4}$ 3.2 Hz, H-3), 3.82 (dd, 1 H, $J_{4,5}$ 1 Hz, H-4), 3.65 (dt, 1 H, *J* 6.6 and 9.8 Hz, CH_2O), 3.45 (dt, 1 H, *J* 6.6 and 9.8 Hz, CH_2O), 3.00 (t, 2 H, *J* 7.6 Hz, CH_2N), 2.05 (s, 3H, NAc), 1.7–1.2 (m, 12 H, $-(CH_2)_6-$), and 1.24 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6). Anal. Calcd for $C_{16}H_{32}N_2O_5 \cdot H_2O$: C, 54.83; H, 9.78; N, 7.99. Found: C, 54.53; H, 9.65; N, 7.64.

[2-(4-Hydroxyphenyl)ethylamine]$_5$-poly(L-glutamic acid) conjugate (**40**).—M HCl (51 mL) was added dropwise to an ice-cooled solution of poly(L-glutamic acid) sodium salt (10 g, mol wt 13000, dp 70, purchased from Sigma, St. Louis, USA) in water (200 mL) with stirring. The precipitate was collected by centrifugation and washed throughly by several additions of cold water and repeated centrifugation. Lyophilisation of a suspension of the precipitate in water gave poly(L-glutamic acid) (6.5 g) as an amorphous powder; $[\alpha]_D^{24}$ +5.4° (*c* 0.68, DMF); ν_{max}^{KBr}: 3342, 1736, 1643, 1608, 1520, 1408, and 1169 cm^{-1}.

A mixture of poly(L-glutamic acid) (100 mg, 0.0087 mmol), 4-(2-aminoethyl)phenol (10 mg, 0.072 mmol), and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ, 19 mg, 0.077 mmol) in *N*,*N*-dimethylformamide (3.5 mL) was stirred for 18 h at room temperature. The mixture was diluted with 0.01 M sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl (10 mL), applied on a column (50 × 2.5 cm) of Sephadex G-100 equibrated with 0.01 M sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl (pH 7.4), and eluted with the same medium. Each fraction was 5 mL/5 min. The collected fractions were analyzed by HPLC on TSKgel $G3000PW_{XL}$ in 0.2 M NaCl using UV spectrophotometry at 275 nm and a differential refractometer. The higher molecular weight fraction (Fractions 15–27)

was pooled, desalted by dialysis against deionized water using ~3500 mol wt cutoff tubing (Spectra/Por 3, Spectrum), and then lyophilized, giving **40** (75 mg) as an amorphous powder; $[\alpha]_D^{24}$ −70.4° (*c* 0.27, H_2O); ν_{max}^{KBr}: 3300, 1711, 1655, 1542, and 1252 cm^{-1}. The ratio of the number of 2-(4-hydroxyphenyl)ethylamino residues incorporated per mol of poly(L-glutamic acid) was determined by spectrophotometry using absorption at 275 nm and showed that 7% of 2-(4-hydroxyphenyl)ethylamino residue had been introduced in the total number of the carboxyl groups.

[8-(β-D-Galactopyranosyloxy)octylamine]$_{36}$-[2-(4-hydroxyphenyl)ethylamine]$_3$-poly(L-glutamic acid) conjugate (**41**).—Compound **7** (180 mg, 0.586 mmol) and 4-(2-aminoethyl)phenol (7.2 mg, 0.055 mmol) were condensed with poly(L-glutamic acid) (75 mg, 0.0064 mmol) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (135 mg, 0.01 mmol) and *N*-hydroxysuccinimide (80.5 mg, 0.7 mmol) in *N*,*N*-dimethylformamide (13 mL) as described for **40**. The mixture was diluted with 0.01 M sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl (30 mL), dialysed against deionized water using ~3500 mol wt cutoff tubing (Spectra/Por 3, Spectrum), and lyophilized. The residue was dissolved in water (10 mL), and applied on a column (50 × 2.5 cm) of Sephadex G-100 equilibrated with 0.01 M sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl (pH 7.4), and eluted with the same medium. The higher molecular weight fraction (Fractions 35–65) was pooled, desalted by dialysis against deionized water using ~3500 mol wt cutoff tubing (Spectra/Por 3, Spectrum), and then lyophilized as described for **40**, giving **41** (67 mg, 89%) as an amorphous powder; $[\alpha]_D^{24}$ −15.4° (*c* 0.37, H_2O); ν_{max}^{KBr}: 3300 and 1650 cm^{-1}. Analysis of the conjugate to determine the ratio of the number of galactose residues by the phenol–H_2SO_4 method[21] and 2-(4-hydroxyphenyl)ethylamino residues by spectophotometry showed that 51% of galactose and 4% of 2-(4-hydroxyphenyl)ethylamino residues had been introduced in the total number of carboxyl groups, respectively.

[8-(β-L-Fucopyranosyloxy)octylamine]$_{16}$-[2-(4-hydroxyphenyl)ethylamine]$_4$-poly(L-glutamic acid) conjugate (**42**).—Compound **11** (190 mg, 0.56 mmol) and 4-(2-aminoethyl)phenol (5 mg, 0.036 mmol) were condensed with poly(L-glutamic acid) (50 mg, 0.0037 mmol) in the presence of EEDQ (152 mg, 0.615 mmol) in *N*,*N*-dimethylformamide (16 mL) as described for **40**, and isolation of the product by dialysis of the mixture against deionized water, followed by gel filtration on a column of Sephadex G-100 as described for **41**, gave **42** (45 mg, 90%) as an amorphous powder; $[\alpha]_D^{24}$ −31.1° (*c* 0.73, H_2O); ν_{max}^{KBr}: 3430, 1650, 1160, and 1070 cm^{-1}. Determination of the sugar content and 2-(4-hydroxyphenyl)ethylamino residues as described above showed that 23% of fucose and 4% of 2-(4-hydroxyphenyl)ethylamino residues had been introduced in the total number of carboxyl groups.

[8-(α-D-Xylopyranosyloxy)octylamine]$_{12}$-[2-(4-hydroxyphenyl)ethylamine]$_2$-poly(L-glutamic acid) conjugate (**43**).—Compound **18** (190 mg, 0.56 mmol) and 4-(2-aminoethyl)phenol (5 mg, 0.036 mmol) were condensed with poly(L-glutamic acid) (50 mg, 0.0037 mmol) in the presence of EEDQ (152 mg, 0.615 mmol) in

N,*N*-dimethylformamide (16 mL) as described for the preparation of **40**, and isolation of the product by dialysis of the mixture against deionized water, followed by gel filtration on a column of Sephadex G-100 as described for **41**, gave **43** (47 mg, 94%) as an amorphous powder; $[\alpha]_D^{24}$ −21.5° (*c* 1.04, H_2O); ν_{max}^{KBr}: 3430, 1650, 1160, and 1070 cm^{-1}. Determination of the sugar content and 2-(4-hydroxyphenyl)ethylamino residues as described above showed 17% of xylose and 3% of 2-(4-hydroxyphenyl)ethylamino residues had been introduced in the total number of carboxyl groups.

[8-(α-D-Mannopyranosyloxy)octylamine]$_{18}$-[2-(4-hydroxyphenyl)ethylamine]$_5$-poly(L-glutamic acid) conjugate (**44**).—Compound **25** (180 mg, 0.586 mmol) and 4-(2-aminoethyl)phenol (7.2 mg, 0.055 mmol) were condensed with poly(L-glutamic acid) (75 mg, 0.0064 mmol) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (135 mg, 0.01 mmol) and *N*-hydroxysuccinimide (80.5 mg, 0.7 mmol) in *N*,*N*-dimethylformamide (13 mL) as described for **40**, and isolation of the product by dialysis of the mixture against deionized water, followed by gel filtration on a column of Sephadex G-100 as described for **41**, gave **44** (67 mg, 90%) as an amorphous powder; $[\alpha]_D^{24}$ +20.2° (*c* 0.11, H_2O); ν_{max}^{KBr}: 3315, 1651, 1540, and 1043 cm^{-1}. Determination of the sugar content and 2-(4-hydroxyphenyl)ethylamino residues as already described showed that 26% of mannose and 7% of 2-(4-hydroxyphenyl)ethylamino residues had been introduced in the total number of carboxyl groups.

[8-(β-D-Mannopyranosyloxy)octylamine]$_{27}$-[2-(4-hydroxyphenyl)ethylamine]$_2$-poly(L-glutamic acid) conjugate (**45**).—Compound **28** (190 mg, 0.56 mmol) and 4-(2-aminoethyl)phenol (5 mg, 0.036 mmol) were condensed with poly(L-glutamic acid) (50 mg, 0.0037 mmol) in the presence of EEDQ (152 mg, 0.615 mmol) in *N*,*N*-dimethylformamide (160 mL) as described for the preparation of **40**, and isolation of the product by dialysis of the mixture against deionized water, followed by gel filtration on a column of Sephadex G-100 as described for **41**, gave **45** (45 mg, 90%) as an amorphous powder; $[\alpha]_D^{24}$ +21.6° (*c* 0.5, H_2O); ν_{max}^{KBr}: 3400, 1657, 1560, 1488, and 1015 cm^{-1}. Determination of the sugar content and 2-(4-hydroxyphenyl)ethylamino residues as described above showed that 38% of mannose and 3% of 2-(4-hydroxyphenyl)ethylamino residues had been introduced in the total number of carboxyl groups.

Measurement of plasma elimination rates of carbohydrate-poly(L-glutamic acid) conjugates.—The conjugates were iodinated with $Na^{125}I$ (Amersham, Tokyo, Japan) using the Chloramine T method[22]. The [^{125}I]-labeled conjugates were isolated by chromatography on a column of Sephadex G-25 with isotonic NaCl solution and used for injection after adjustment with the unlabeled conjugate to the appropriate concentration (1 mg/mL).

Male SD rats (200–220 g, 7 weeks old) were anesthetized lightly with ether and [^{125}I]-labeled conjugates (1 mg/kg) were administered intravenously into the femoral vein. Blood samples (200 μL) were taken at regular intervals over a 1 h period from the jugular vein and were centrifuged for 5 min at 5000 rpm to

separate the plasma. Trichloroacetic acid (15%, 400 μL) was added to a sample (100 μL) of the plasma and the resulting precipitate was collected by centifugation for 5 min at 5000 rpm. The total radioactivity of the precipitate was measured by a gamma-counter (Aloka ARC-301B, Tokyo, Japan), and the concentration of the conjugate in the plasma was calculated.

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