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# Synthesis of multivalent β-lactosyl clusters as potential tumor metastasis inhibitors

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## ABSTRACT

A β-lactosyl residue was linked to the amino groups of L-lysyl-L-lysine through spacer arms of three different lengths ($C_2$, $C_4$, and $C_9$) to give trivalent β-lactosyl clusters in order to increase the inhibitory activity of the β-lactosyl group against tumor cell colonization. Thus, *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-glucopyranosyl trichloroacetimidate was treated with methyl or benzyl hydroxyethanoate, methyl or benzyl 4-hydroxybutanoate, and methyl 9-hydroxynonanoate, respectively, in the presence of trimethylsilyl trifluoromethanesulfonate to give the corresponding β-lactosides. These were coupled to L-lysyl–L-lysine, after conversion to the *N*-hydroxysuccinimide esters, to yield the corresponding trivalent β-lactosyl-L-lysyl-L-lysine conjugates in good yields. The β-lactosyl group with a $C_4$ spacer arm was also coupled similarly to poly(L-lysine) ($M_r$ 3800) to form a polyvalent β-lactosyl cluster. Coinjection of the trivalent (with $C_2$ and $C_4$ spacer arms) and polyvalent β-lactosyl clusters with the highly metastatic B16 murine melanoma cells inhibited the formation of lung colonies in C57/BL mice, whereas the trivalent cluster with a $C_9$ spacer arm displayed no activity.

## INTRODUCTION

Metastasis is defined as the colonization of cancerous cells at distant sites and is the major cause of cancer mortality. The metastatic process consists of a complex series of events involving detachment of tumor cells from the primary sites, their invasion into surrounding tissues, and settlement at distant secondary sites. Controlling metastasis is of the utmost importance as it would lead to an effective cancer treatment.

In 1973, it was concluded from pathological observations in humans that almost all metastatic growths in the lungs arise from malignant emboli carried in the circulatory system[1]. Subsequently, Raz and Lotan[2] demonstrated the presence of lactose-binding lectins at the tumor cell surface and proposed that lectin-mediated

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intercellular adhesion was responsible for homotypic and heterotypic cell aggregation and embolization. Furthermore, their extensive studies revealed the positive relationship between endogenous-lectin expression and the acquisition of metastatic properties[3,4]. These findings stimulated us to explore the possible prevention of metastatic spread by blocking the cognitive interactions among tumor cells and between tumor and host cells with competitive lactose derivatives. Inhibition of liver tumor cell colonization in mice by blocking hepatic lectin with D-galactose or arabinogalactan has been reported[5].

Recently, we reported that methyl β-lactoside dramatically reduces the formation of lung colonies in mice injected with mouse B16 melanoma cells, and that the trivalent β-lactosyl–L-lysyl-L-lysine conjugate with a $C_9$ spacer arm showed rather a stimulative effect on metastatic colonization[6]. The latter finding was unexpected as the carbohydrate–lectin interaction seems to be strengthened by multivalency or clustering of carbohydrate residues[7]. This discrepancy may be due to the long spacer arm ($C_9$) used for linking a β-lactosyl group to L-lysyl-L-lysine, resulting in inappropriate spacial arrangement of the β-lactosyl groups[8]. We have, therefore, synthesized trivalent β-lactosyl–L-lysyl-L-lysine conjugates with shorter spacer arms ($C_2$ and $C_4$) together with a polyvalent β-lactosyl–poly(L-lysine) ($M_r$ 3800) conjugate through a $C_4$ spacer arm. We describe herein the synthesis of these conjugates and their in vivo activities.

## RESULTS AND DISCUSSION

Three spacer arms differing in chain lengths ($C_2$, $C_4$, and $C_9$) were chosen to link β-lactosyl groups to the amino groups of L-lysyl-L-lysine[9]. The $C_9$ spacer arm, methyl 9-hydroxynonanoate (**5**), developed by Lemieux et al.[10], has been frequently used to prepare artificial carbohydrate antigens via covalent attachment to proteins. During the course of this study, it became necessary to synthesize the acetylated derivatives of the carboxymethyl (**19**) and 3-carboxypropyl β-lactoside (**20**) to increase their solubility in nonaqueous solvents. Therefore, the benzyl ester derivatives of $C_2$ (**2**) and $C_4$ (**4**) spacer arms were prepared in addition to the corresponding methyl analogues **1** and **3**, since the benzyl group is selectively removed by catalytic hydrogenation leaving the acetylated lactosyl group intact. The spacer arms, **3**[11] and **5**, were obtained from monomethyl succinate and azelaic acid monomethyl ester, respectively, by selective reduction of the carboxyl group with borane–oxolane complex[10,12]. The spacer arm **1** is commercially available. The benzyl analogues, **2**[13,14] and **4**[14], were readily prepared by base-catalyzed transesterification of **1** and 1,4-butyrolactone, respectively, with benzyl alcohol.

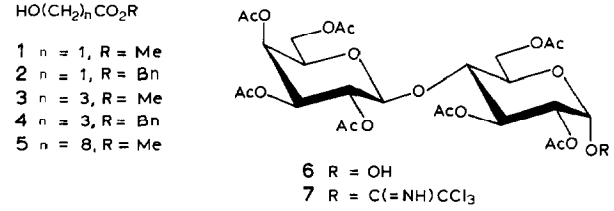
It was reported that condensation of **5** with a lactosyl halide under the standard Koenigs–Knorr or Helfrich conditions yields a β-lactoside (**10**) in a poor yield[15]. The yield was later improved either by use of silver trifluoromethanesulfonate–tetramethylurea-promoted Koenigs–Knorr reaction[16] or by Lewis acid-catalyzed glycosidation starting from lactose octaacetate[17]. The trichloroacetimidate method,

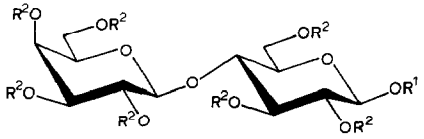
introduced by Schmidt et al.[18], has been employed to synthesize a wide range of complex glycosides, including oligosaccharides[19], glycolipids[20], glycopeptides[21], and glycosyl phosphates[22]. This method involves the activation of a stable intermediate, *O*-glycosyl imidate, to give a highly reactive glycosyl donor upon treatment with acid. Since the formation of an orthoester was responsible for the poor yield under the Koenigs–Knorr condition[15,16], the acidic medium used in the trichloroacetimidate process was expected to avoid the formation of an orthoester, thus leading to a better yield of glycoside.

The thermodynamically more stable *O*-α-lactosyl trichloroacetimidate[23] (**7**) was obtained exclusively from *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-D-glucopyranose (**6**) by treatment with trichloroacetonitrile and potassium carbonate in dichloromethane for 18 h. As expected, glycosylation of **1**, **3**, and **5** with **7** in the presence of trimethylsilyl trifluoromethanesulfonate as an acid catalyst gave high yields of the β-lactosides **8** (65%), **9** (73%), and **10** (72%), respectively. Under similar conditions, the benzyl derivatives **2** and **4** gave the corresponding glycosides **11** (71%) and **12** (67%). The anomeric configuration of the lactosides was confirmed by $^1$H NMR spectra in which the corresponding H-1 protons appeared as a doublet ($J$ 7.8–8.0 Hz) at δ 4.41–4.63, indicative of 1,2-*trans*-glycosides. The exclusive formation of β-lactosides is explained on the basis of neighboring-group participation. The lactosides, **8**, **9**, and **10** were *O*-deacetylated to give methoxycarbonyl lactosides, **13**, **14**, and **15**, respectively.

The azide procedure was first attempted to couple these lactosides to the hydrochloride of L-lysl-L-lysine (**29**). Compound **15** was hydrazinolyzed to give the hydrazino derivative **23**. Conversion of the hydrazide to an acyl azide group, followed by coupling to **29** was conducted according to a standard procedure[10,24]. TLC analysis of the reaction mixture, however, indicated the presence of many products, from which the desired conjugate could not be obtained. Consequently, the active ester method was next employed for the coupling. Compounds **13**, **14**, and **15** were hydrolyzed to provide the corresponding acid derivatives, **16**, **17**, and **18**. Treatment of **18** with *N*-hydroxysuccinimide (NHS) and *N*,*N*′-dicyclohexylcarbodiimide in dry *N*,*N*-dimethylformamide (DMF) yielded the active ester **24**. Subsequent coupling with the hydrochloride of **29** in aqueous DMF produced the desired conjugate **35** in high yield. On the other hand, attempts to prepare the active esters of **16** and **17** were not successful as these acid derivatives were only slightly soluble in nonaqueous solvents.

The acetylated derivatives, **19** and **20**, were prepared by catalytic hydrogenolysis of **11** and **12**, respectively, over 10% Pd–C. Compound **20** was also obtained by treatment of **17** with acetic anhydride and pyridine. This acetylation, however, gave **20** in yields < 50% because of the formation of an ethyl ester **26**. It is noteworthy that similar acetylation of **16** yielded **27** along with **25**, instead of **19**, which lacks a 2-*O*-acetyl group. The formation of these ethyl ester could be envisaged in terms of mixed-anhydride formation between acetic anhydride and the carboxyl groups in **16** and **17**. For **17** the mixed anhydride reacted with ethanol, which was used



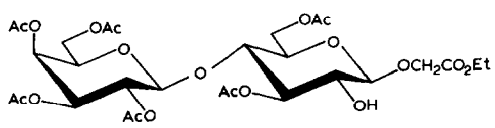




| | $R^1$ | $R^2$ |
|---|---|---|
| 8 | $CH_2CO_2Me$ | Ac |
| 9 | $(CH_2)_3CO_2Me$ | Ac |
| 10 | $(CH_2)_8CO_2Me$ | Ac |
| 11 | $CH_2CO_2Bn$ | Ac |
| 12 | $(CH_2)_3CO_2Bn$ | Ac |
| 13 | $CH_2CO_2Me$ | H |
| 14 | $(CH_2)_3CO_2Me$ | H |
| 15 | $(CH_2)_8CO_2Me$ | H |
| 16 | $CH_2CO_2H$ | H |
| 17 | $(CH_2)_3CO_2H$ | H |
| 18 | $(CH_2)_8CO_2H$ | H |
| 19 | $CH_2CO_2H$ | Ac |
| 20 | $(CH_2)_3CO_2H$ | Ac |
| 21 | $CH_2CO_2Su$ | Ac |
| 22 | $(CH_2)_3CO_2Su$ | Ac |
| 23 | $(CH_2)_8CONHNH_2$ | H |
| 24 | $(CH_2)_8CO_2Su$ | H |
| 25 | $CH_2CO_2Et$ | Ac |
| 26 | $(CH_2)_3CO_2Et$ | Ac |

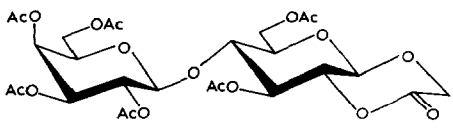
Bn = benzyl
Su = succinimido

Scheme 1.

during the workup to remove the excess acetic anhydride, to give **26**, whereas for **16** the formation of a mixed anhydride was followed either by ring closure with the OH-2 to give a 1,5-lactone **28**, which was then opened by reaction with ethanol during the workup to give **27**, or by ethanolysis of the mixed anhydride to give **26**. The $^1H$ NMR spectrum of **27** showed the presence of six acetyl groups and a triplet ($J$ 9.0 Hz) at $\delta$ 3.55 for H-2, in agreement with the presence of OH-2.

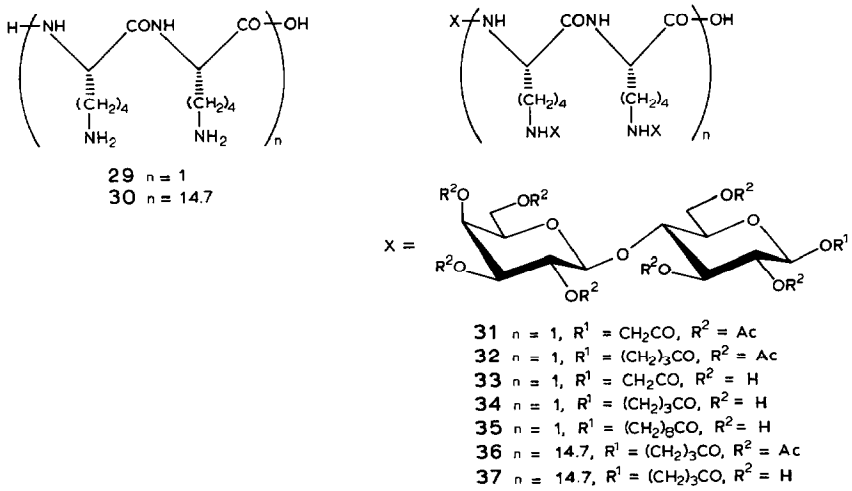



27




28

Scheme 2.




Scheme 3.

The active esters **21** and **22** were prepared in good yields from **19** and **20**, respectively, by treatment with NHS and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) in dry dichloromethane. The coupling of **21** and **22** with the hydrochloride of **29** gave high yields of the conjugates **31** and **32**, respectively, which were *O*-deacetylated to yield **33** and **34**, respectively. A similar coupling reaction between **22** and poly(L-lysine), followed by *O*-deacetylation, led to the formation of polyvalent cluster, **37** (through **36**). The $^1$H NMR spectra of trivalent conjugates, **33–35**, confirmed the presence of the lactosyl and lysyllysine residues in a 3-to-1 ratio with high purity. It should be mentioned that the conjugation of a $\beta$-lactosyl to a lysyllysine residue and poly(lysine) backbone did not cause any significant conformational changes in the lactosyl residue, since the spectral patterns of the lactosyl residues in each conjugate were almost identical to that of methyl $\beta$-lactoside[6].

*In vivo assay.*—The synthesized $\beta$-lactosyl clusters were tested for their ability to inhibit lung colonization (i.e., metastatic deposition) of the highly metastatic B16 mouse melanoma cells in C57/BL mice (Fig. 1). The concentrations of each cluster were adjusted to obtain, in the solutions, the same concentration (0.1 M) of lactosyl residue, at which methyl $\beta$-lactoside exhibits the maximum inhibitory activity[6]. The trivalent clusters, **33** and **34**, and the polyvalent cluster, **37**, reduced the number of metastatic lung colonies by 61, 42, and 63%, respectively, as compared to the control, when administered with melanoma cells by tail vein injection into mice. On the other hand, the trivalent cluster with a $C_9$ spacer arm, **35**, exhibited neither stimulatory[6] nor inhibitory activities. Although **33** and **37** were as effective as methyl $\beta$-lactoside, the cluster formation did not enhance the inhibitory activity of the monomer. It should be noticed that the inhibitory activity of the trivalent clusters decreased with lengthening of the spacer arm, thus

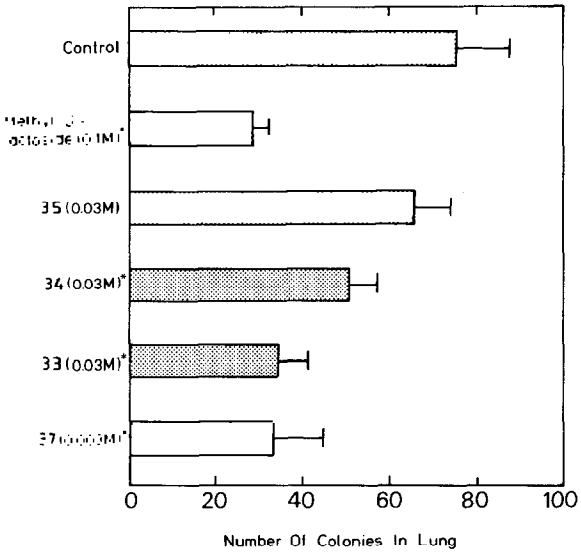



Fig. 1. Effect of synthetic β-lacatosyl clusters on inhibition of lung tumor colonization. The assay was carried out as described in the Experimental section. Bar represents standard error of the mean ($n = 6$) and * indicates a significant difference ($p < 0.05$) as compared to the control.

indicating that the hydrophobic nature of the longer alkyl chain could interfere with the binding by a β-lactosyl residue.

## EXPERIMENTAL

*General methods.*—Melting points were measured with a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were determined at 23°C with a Perkin–Elmer 241MC polarimeter. $^1$H NMR spectra were recorded on a Bruker WM-500 spectrometer in $CDCl_3$, DMF-$d_6$, or in $D_2O$. Chemical shift standards were $Me_4Si$ for $CDCl_3$ and DMF-$d_6$, and sodium 4,4-dimethyl-4-silapentanesulfonate for solutions in $D_2O$. TLC was performed on precoated Silica gel $60F_{254}$ plates (Merck, Darmstadt; 0.25-mm thickness) and the spots were detected by spraying with 0.5% orcinol in 10% aq $H_2SO_4$ or with 0.2% ninhydrin in EtOH, and subsequent heating. Silica gel used for flash column chromatography[26] was purchased from EM Science (Gibbstown, NJ; particle mesh size 0.040–0.063 mm). Solutions were concentrated under reduced pressure at 40°C unless otherwise stated. FAB and EI mass spectra, including exact mass measurements, were obtained with VG Instruments, Inc., Savannah Labs., Savannah, GA. Some of the spectra were recorded by Mary Ellen Salyan of this Institute using a Jeol JMS-HX 110 mass spectrometer *

*Methyl 4-hydroxybutanoate* (**3**) *and methyl 9-hydroxynonanoate* (**5**).—These com-

* The identity of the compounds described herein was verified by high resolution mass spectrometry but their purity was controlled by $^1$H NMR spectroscopy, not by elemental analysis (*the Editor*).

pounds were prepared from monomethyl succinate and azelaic acid monomethyl ester (both from Aldrich, Milwaukee, WI), respectively, according to the reported method for ethyl 9-hydroxynonanoate[10].

*Compound* **3**. Colorless oil (55%); $^1$H NMR ($CDCl_3$): $\delta$ 1.89 (quint. 2 H, $J$ 6.6 Hz, H-3a, 3b), 2.03 (br s, 1 H, OH), 2.45 (t, 2 H, $J$ 6.6 Hz, H-2a, 2b), 3.69 (s, 3 H, $OCH_3$), and 3.69 (t, 2 H, $J$ 6.6 Hz, H-4a, 4b); EIMS: $m/z$ 118 $[M]^+$.

*Compound* **5**. Colorless oil (80%); $^1$H NMR ($CDCl_3$): $\delta$ 1.32 (br s, 8 H, 4 $CH_2$), 1.50–1.68 (m, 5 H, H-3a, 3b, 8a, 8b, and OH), 2.31 (t, 2 H, $J$ 7.5 Hz, H-2a, 2b), 3.64 (t, 2 H, $J$ 6.7 Hz, H-9a, 9b),. and 3.67 (s, 3 H, $OCH_3$). FABMS: $m/z$ 189 $[M + H]^+$, 171 $[M + H - H_2O]^+$, and 157 $[M + H - CH_3OH]^+$.

*Benzyl hydroxyethanoate* (**2**).—Methyl glycolate (**1**) (Aldrich, Milwaukee, WI) (1.0 g, 10.9 mmol) was dissolved in 0.1 M sodium benzyloxide in anhyd benzyl alcohol (50 mL), and the mixture was stirred under dry $N_2$ at room temperature overnight. The base was neutralized with Amberlite IR-120 ($H^+$) cation-exchange resin and a large portion of benzyl alcohol was removed by evaporation at 70°C. The oily residue was applied to a column of silica gel and eluted with 2:1 hexane–EtOAc to give **2** (1.0 g, 55%) as a colorless oil; $R_f$ 0.20 (2:1 hexane–EtOAc); $^1$H NMR ($CDCl_3$): $\delta$ 4.20 (d, 2 H, $J$ 5.5 Hz, H-2a, 2b), 5.22 (s, 2 H, $CH_2$Ph), and 7.32–7.40 (m, 5 H, Ph); EIMS: Calcd for $C_9H_{10}O_3$ $[M]^+$, 166.0630. Found, 166.0630.

*Benzyl 4-hydroxybutanoate* (**4**).—1,4-Butyrolactone (Aldrich, Milwaukee, WI) (1.0 g, 11.5 mmol) was treated with 0.1 M sodium benzyloxide in anhyd benzyl alcohol (50 mL) as in the prepartion of **2**. Column chromatography on silica gel (1:1 hexane–EtOAc) yielded **4** (1.18 g, 53%) as a colorless oil; $R_f$ 0.13 (2:1 hexane–EtOAc); $^1$H NMR ($CDCl_3$): $\delta$ 1.92 (quint. 2 H, $J$ 6.6 Hz, H-3a, 3b), 2.49 (t, 2 H, $J$ 6.6 Hz, H-2a, 2b), 3.69 (m, 2 H, H-4a, 4b), 5.12 (s, 2 H, $CH_2$Ph), and 7.29–7.38 (m, 5 H, Ph). FABMS: Calcd for $C_{11}H_{15}O_3$ $[M + H]^+$, 195.1021. Found, 195.1027.

*O-[O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl] trichloroacetimidate* (**7**).—This compound was prepared as described for the maltose derivative[23,27], except for the use of $K_2CO_3$ as base and the reaction time of 18 h; colorless amorphous solid (78%), $R_f$ 0.63 (3:1 $CHCl_3$–$Me_2CO$), $[\alpha]_D$ +48° ($c$ 4.2, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 1.97 (s, 3 H), 2.01 (s, 3 H), 2.04 (s, 3 H), 2.06 (br s, 6 H), 2.11 (s, 3 H), and 2.15 (s, 3H) (7 OAc), 3.83–3.92 (m, 2 H, H-4,5′), 4.07–4.19 (m, 4 H, H-5,6a,6b,6′a), 4.48 (d, 1 H, $J$ 10.3 Hz, H-6′b), 4.52 (d, 1 H, $J$ 7.8 Hz, H-1′), 4.97 (dd, 1 H, $J$ 10.3, 3.2 Hz, H-3′), 5.07 (dd, 1 H, $J$ 10.2, 3.7 Hz, H-2), 5.12 (dd, 1 H, $J$ 10.3, 7.8 Hz, H-2′), 5.36 (d, 1 H, $J$ 3.2 Hz, H-4′), 5.55 (dd, 1 H, $J$ 10.2, 10.2 Hz, H-3), and 6.49 (d, 1 H, $J$ 3.7 Hz, H-1). FABMS: Calcd for $C_{28}H_{37}Cl_3NO_{18}$ $[M + H]^+$, 780.1076. Found, 780.1081.

*Methoxycarbonylmethyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**8**).—A 0.22 M solution of trimethylsilyl trifluoromethanesulfonate in dry $CH_2Cl_2$ (1.1 mL) was added dropwise to a mixture of **7** (3.5 g, 4.49 mmol) and **1** (0.61 g, 6.8 mmol) in dry $CH_2Cl_2$ (200 mL)

vigorously stirred at −15°C under dry $N_2$. After 30 min, the mixture was washed successively with ice-cold satd $NaHCO_3$ and water, and dried ($Na_2SO_4$). Evaporation, followed by silica gel column chromatography (5:1 $CHCl_3$–$Me_2CO$) yielded **8** (2.06 g, 65%) as colorless crystals; $R_f$ 0.40 (5:1 $CHCl_3$–$Me_2CO$); mp 172–173°C; $[\alpha]_D$ −22.5° (*c* 4.8, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 1.96 (s, 3 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 2.06 (s, 3 H), 2.08 (s, 3 H), 2.12 (s, 3 H), and 2.15 (s, 3 H) (7 OAc), 3.61 (ddd, 1 H, *J* 9.5, 5.1, 2.1 Hz, H-5), 3.74 (s, 3 H, $OCH_3$), 3.82 (dd, 1 H, *J* 9.5, 9.5 Hz, H-4), 3.88 (t, 1 H, *J* 6.9 Hz, H-5′), 4.07–4.15 (m, 3 H, H-6a,6′a,6′b), 4.25 (s, 2 H, $OCH_2CO$), 4.49 (d, 1 H, *J* 7.8 Hz, H-1′), 4.50 (dd, 1 H, *J* 12.2, 1.9 Hz, H-6b), 4.63 (d, 1 H, *J* 7.8 Hz, H-1), 4.95 (dd, 1 H, *J* 9.3, 7.8 Hz, H-2), 4.96 (dd, 1 H, *J* 10.4, 3.4 Hz, H-3′), 5.11 (dd, 1 H, *J* 10.4, 7.8 Hz, H-2′), 5.23 (dd, 1 H, *J* 9.3, 9.3 Hz, H-3), and 5.35 (d, 1 H, *J* 3.4 Hz, H-4′). FABMS: Calcd for $C_{29}H_{41}O_{20}$ $[M+H]^+$, 709.2191. Found, 709.2169.

*3-Methoxycarbonylpropyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**9**).—Glycosylation of **3** (0.4 g, 3.39 mmol) with **7** (1.3 g, 1.67 mmol) similarly to the preparation of **8** gave **9** (0.9 g, 73%) as a colorless syrup; $R_f$ 0.38 (5:1 $CHCl_3$–$Me_2CO$); $[\alpha]_D$ −5.6° (*c* 8.1, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 1.85–1.92 (m, 2 H, $OCH_2CH_2CH_2CO$), 1.96 (s, 3 H), 2.04 (s, 9 H), 2.06 (s, 3 H), 2.11 (s, 3 H), and 2.14 (s, 3 H) (7 OAc), 2.36 (t, 2 H, *J* 7.3 Hz, $OCH_2CH_2CH_2CO$), 3.52 (dt, 1 H, *J* 9.8, 6.4 Hz, $OCHHCH_2CH_2CO$), 3.59 (ddd, 1 H, *J* 9.4, 5.0, 2.1 Hz, H-5), 3.66 (s, 3 H, $OCH_3$), 3.79 (dd, 1 H, *J* 9.4, 9.4 Hz, H-4), 3.83–3.88 (m, 1 H, $OCHHCH_2CH_2CO$), 3.86 (t, 1 H, *J* 6.2 Hz, H-5′), 4.06–4.15 (m, 3 H, H-6a,6′a,6′b), 4.45 (d, 1 H, *J* 7.9 Hz, H-1), 4.48 (dd 1 H, *J* 11.8, 2.1 Hz, H-6b), 4.49 (d, 1 H, *J* 7.7 Hz, H-1′), 4.87 (dd, 1 H, *J* 9.4, 7.9 Hz, H-2), 4.96 (dd, 1 H, *J* 10.4, 3.2 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.4, 7.7 Hz, H-2′), 5.18 (dd, 1 H, *J* 9.4, 9.4 Hz, H-3), and 5.34 (d, 1 H, *J* 3.2 Hz, H-4′). FABMS: Calcd for $C_{31}H_{45}O_{20}$ $[M+H]^+$, 737.2504. Found, 737.2481.

*8-Methoxycarbonyloctyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**10**).—Glycosylation of **5** (0.58 g, 3.09 mmol) with **7** (1.2 g, 1.54 mmol) was carried out as described for **8**, yielding **10** (0.89 g, 72%) as a colorless syrup; $R_f$ 0.44 (5:1 $CHCl_3$–$Me_2CO$); $[\alpha]_D$ −11° (*c* 5.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 1.26–1.42 (m, 8 H, 4 spacer $CH_2$), 1.54–1.60 (m, 4 H, 2 spacer $CH_2$), 1.97 (s, 3 H), 2.04 (s, 3 H), 2.05 (s, 6 H), 2.07 (s, 3 H), 2.12 (s, 3 H), and 2.16 (s, 3 H) (7 OAc), 2.30 (t, 2 H, *J* 7.2 Hz, spacer $CH_2CO$), 3.45 (dt, 1 H, *J* 9.7, 6.0 Hz, spacer $OCHHCH_2$), 3.60 (ddd, 1 H, *J* 10.6, 5.6, 2.6 Hz, H-5), 3.67 (s, 3 H, $OCH_3$), 3.78 (dd, 1 H, *J* 10.6, 10.6 Hz, H-4), 3.82 (dt, 1 H, *J* 9.7, 6.5 Hz, spacer $OCHHCH_2$), 3.87 (t, 1 H, *J* 7.8 Hz, H-5′), 4.09 (dd, 1 H, *J* 10.5, 7.8 Hz, H-6′a), 4.11 (dd, 1 H, *J* 12.0, 5.6 Hz, H-6a), 4.14 (dd, 1 H, *J* 10.5, 7.8 Hz, H-6′b), 4.46 (d, 1 H, *J* 8.0 Hz, H-1), 4.49 (dd, 1 H, *J* 12.0, 2.6 Hz, H-6b), 4.50 (d, 1 H, *J* 7.6 Hz, H-1′), 4.88 (dd, 1 H, *J* 10.0, 8.0 Hz, H-2), 4.97 (dd, 1 H, *J* 10.5, 3.6 Hz, H-3′), 5.11 (dd, 1 H, *J* 10.5, 7.6 Hz, H-2′), 5.20 (dd, 1 H, *J* 10.0, 10.0 Hz, H-3), and 5.35 (d, 1 H, *J* 3.6 Hz, H-4′). FABMS: Calcd for $C_{36}H_{55}O_{20}$ $[M+H]^+$, 807.3287. Found, 807.3309.

*Methoxycarbonylmethyl* O-β-D-*galactopyranosyl-(1 → 4)-β-D-glucopyranoside* (**13**).—Compound **8** (3.7 g, 5.2 mmol) was treated with 0.01 M methanolic NaOMe (100 mL) at room temperature overnight. After neutralization with Amberlite IR-120 ($H^+$) cation-exchange resin, the filtrate was concentrated to give **13** (1.8 g, 83%) as colorless crystals: mp 194–196°C; $R_f$ 0.44 (5:4:1 $CHCl_3$–MeOH–$H_2O$); $[\alpha]_D$ −8° (*c* 1.4, $H_2O$); $^1H$ NMR ($D_2O$): δ 3.37 (t, 1 H, *J* 8.3 Hz, H-2), 3.51 (dd, 1 H, *J* 10.0, 7.9 Hz, H-2′), 3.53 (m, 1 H, H-5), 3.75 (s, 3 H, $OCH_3$), 3.89 (d, 1 H, *J* 3.3 Hz, H-4′), 3.93 (dd, 1 H, *J* 12.4, 2.2 Hz, H-6b), 4.42 (d, 1 H, *J* 7.9 Hz, H-1′), 4.41 and 4.47 (ABq, 2 H, *J* 16.5 Hz, $OCH_2CO$), and 4.51 (d, 1 H, *J* 8.3 Hz, H-1). FABMS: Calcd for $C_{15}H_{27}O_{13}$ $[M + H]^+$, 415.1452. Found, 415.1440.

*3-Methoxycarbonylpropyl* O-β-D-*galactopyranosyl-(1 → 4)-β-D-glucopyranoside* (**14**).—Treatment of **9** (1.1 g, 1.5 mmol) with 0.01 M methanolic NaOMe (30 mL), as described for the preparation of **13**, afforded **14** (0.53 g, 80%) as colorless crystals; mp 153°C; $R_f$ 0.54 (5:4:1 $CHCl_3$–MeOH–$H_2O$); $[\alpha]_D$ −2° (*c* 1.0, $H_2O$); $^1H$ NMR ($D_2O$): δ 1.89 (quint. 2 H, *J* 6.4 Hz, $OCH_2CH_2CH_2CO$), 2.46 (t, 2 H, *J* 6.4 Hz, $OCH_2CH_2CH_2CO$), 3.27 (dd, 1 H, *J* 8.0, 8.0 Hz, H-2), 3.51 (dd, 1H, *J* 10.0, 7.8 Hz, H-2′), 3.54–3.56 (m, 1 H, H-5), 3.67 (s, 3 H, $OCH_3$), 3.89 (d, 1 H, *J* 3.2 Hz, H-4′), 3.93 (dd, 1 H, *J* 12.2, 2.2 Hz, H-6b), 4.41 (d, 1 H, *J* 7.8 Hz, H-1′), and 4.43 (d, 1 H, *J* 8.0 Hz, H-1). FABMS: Calcd for $C_{17}H_{31}O_{13}$ $[M + H]^+$, 443.1765. Found, 443.1761.

*8-Methoxycarbonyloctyl* O-β-D-*galactopyranosyl-(1 → 4)-β-D-glucopyranoside* (**15**).—Treatment of **10** (2.5 g, 3.1 mmol) with 0.01 M methanolic NaOMe (70 mL), as for the preparation of **13**, yielded **15** (1.5 g, 95%) as colorless crystals; mp 162–163°C (lit.[17] 158–159°C); $R_f$ 0.52 (2:1:1 butanol–AcOH–$H_2O$); $[\alpha]_D$ +3° (*c* 1.0, MeOH) (lit.[17] $[\alpha]_D$ +3.1° (*c* 1.1, $CHCl_3$)); $^1H$ NMR ($D_2O$): δ 1.24–1.35 (m, 8 H, 4 spacer $CH_2$), 1.52–1.62 (m, 4 H, 2 spacer $CH_2$), 2.34 (t, 2 H, *J* 7.3 Hz, spacer $CH_2CO$), 3.26 (dd, 1 H, *J* 8.3, 8.3 Hz, H-2), 3.50 (dd, 1 H, *J* 9.3, 7.5 Hz, H-2′), 3.52–3.57 (m, 1 H, H-5), 3.64 (s, 3 H, $OCH_3$), 3.88 (d, 1 H, *J* 3.2 Hz, H-4′), 3.93 (dd, 1 H, *J* 12.1, 2.8 Hz, H-6b), 4.41 (d, 1 H, *J* 7.5 Hz, H-1′), and 4.43 (d, 1 H, *J* 8.3 Hz, H-1). FABMS: Calcd for $C_{22}H_{41}O_{13}$ $[M + H]^+$, 513.2547. Found, 513.2510.

*Carboxymethyl* O-β-D-*galactopyranosyl-(1 → 4)-β-D-glucopyranoside* (**16**).—A precooled solution of LiOH (1.1 g, 45.9 mmol) in water (40 mL) was added to a precooled solution of **13** (3.8 g, 9.2 mmol) in MeOH (120 mL). The mixture was stirred in a cold room overnight. After treatment with Amberlite IR-120 ($H^+$) cation-exchange resin, the filtrate was evaporated to give **16** (2.8 g, 76%) as a colorless syrup; $R_f$ 0.21 (2:1:1 butanol–AcOH–$H_2O$); $[\alpha]_D$ −6.5° (*c* 1.8, $H_2O$); $^1H$ NMR ($D_2O$): δ 3.32 (dd, 1 H, *J* 8.4, 8.4 Hz, H-2), 3.44 (dd, 1 H, *J* 9.8, 7.9 Hz, H-2′), 3.47–3.52 (m, 1 H, H-5), 3.83 (d, 1 H, *J* 3.4 Hz, H-4′), 3.87 (dd, 1 H, *J* 12.4, 1.9 Hz, H-6a), 4.36 (d, 1 H, *J* 7.9 Hz, H-1′), 4.29 and 4.38 (ABq, 2 H, *J* 16.7 Hz, $OCH_2CO$), and 4.46 (d, 1 H, *J* 8.4 Hz, H-1). FABMS: Calcd for $C_{14}H_{25}O_{13}$ $[M + H]^+$, 401.1295. Found, 401.1284.

Compound **16** was also obtained from **8** by treatment with aq methanolic NaOMe.

*3-Carboxypropyl O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside* (**17**).—A solution of **14** (1.0 g, 2.3 mmol) in MeOH (30 mL) was treated with LiOH (0.2 g, 8.4 mmol) in water (10 mL) in the same manner as described for the preparation of **16** to give **17** (0.8 g, 83%) as a colorless syrup; $R_f$ 0.22 (2:1:1 butanol–AcOH–$H_2O$); $[\alpha]_D$ −2.0° (*c* 1.5, $H_2O$); $^1$H NMR ($D_2O$): δ 1.83 (quint. 2 H, *J* 6.9 Hz, $OCH_2CH_2CH_2CO$), 2.40 (t, 2 H, *J* 6.9 Hz, $OCH_2CH_2CH_2CO$), 3.22 (dd, 1 H, *J* 8.0, 8.0 Hz, H-2), 3.45 (dd, 1 H, *J* 10.0, 7.8 Hz, H-2′), 3.48–3.52 (m, 1 H, H-5) 3.83 (d, 1 H, *J* 3.6 Hz, H-4′), 3.88 (dd, 1 H, *J* 10.9, 2.2 Hz, H-6a), 4.36 (d, 1 H, *J* 7.8 Hz, H-1′), and 4.38 (d, 1 H, *J* 8.0 Hz, H-1). FABMS Calcd for $C_{16}H_{29}O_{13}$ $[M + H]^+$, 429.1608. Found, 429.1598.

Compound **17** was also prepared from **9** by treatment with aq methanolic NaOMe.

*8-Carboxyoctyl O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside* (**18**).—A solution of **15** (1.6 g, 3.1 mmol) in MeOH (90 mL) was treated with LiOH (0.4 g, 16.7 mmol) in water (30 mL), as for the preparation of **16**, to yield **18** (1.4 g, 90%) as a colorless solid; $R_f$ 0.52 (2:1:1 butanol–AcOH–$H_2O$); $[\alpha]_D$ −6.5° (*c* 0.9, $H_2O$); $^1$H NMR ($D_2O$): δ 1.29 (br s, 8 H, 4 spacer $CH_2$), 1.46–1.62 (m, 4 H, 2 spacer $CH_2$), 2.33 (t, 2 H, *J* 7.9 Hz, spacer $CH_2CO$), 3.26 (dd, 1 H, *J* 8.5, 8.5 Hz, H-2), 3.50 (dd, 1 H, *J* 9.3, 7.8 Hz, H-2′), 3.55 (t, 2 H, *J* 6.0 Hz, spacer $OCH_2CH_2$), 3.89 (d, 1 H, *J* 3.8 Hz, H-4′), 3.93 (dd, 1 H, *J* 7.1, 2.0 Hz, H-6a), 4.41 (d, 1 H, *J* 8.5 Hz, H-1), and 4.43 (d, 1 H, *J* 7.8 Hz, H-1′). FABMS: Calcd for $C_{21}H_{39}O_{13}$ $[M + H]^+$, 499.2391. Found, 499.2360.

Compound **18** was also prepared from **10** by treatment with aq methanolic NaOMe.

*Benzyloxycarbonylmethyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**11**).—A 0.22 M solution of trimethyl trifluoromethanesulfonate in dry $CH_2Cl_2$ (1.6 mL) was added dropwise to a mixture of **7** (5.6 g, 7.2 mmol) and **2** (2.4 g, 14.5 mmol) in dry $CH_2Cl_2$ (200 mL) vigorously stirred at −15°C under dry $N_2$. After 30 min, the mixture was washed successively with ice-cold satd $NaHCO_3$ and water, and dried ($Na_2SO_4$). Evaporation followed by silica gel column chromatography (1:1 hexane–EtOAc) afforded **11** (4.0 g, 71%) as a colorless syrup; $R_f$ 0.32 (1:1 hexane–EtOAc); $[\alpha]_D$ −17° (*c* 4.4, $CHCl_3$); $^1$H NMR ($CDCl_3$): δ 1.92 (s, 3 H), 2.01 (s, 3 H), 2.03 (s, 3 H), 2.04 (s, 3 H), 2.06 (s, 3 H), 2.10 (s, 3 H), and 2.14 (s, 3 H) (7 OAc), 3.59 (ddd, 1 H, *J* 9.7, 4.9, 2.0 Hz, H-5), 3.81 (dd, 1 H, *J* 9.7, 9.7 Hz, H-4), 3.86 (t, 1 H, *J* 7.0 Hz, H-5′), 4.06–4.15 (m, 3 H, H-6a,6′a,6′b), 4.26 and 4.30 (ABq, *J* 16.6 Hz, spacer $OCH_2CO$), 4.47 (dd, 1 H, *J* 12, 2.0 Hz, H-6b), 4.48 (d, 1 H, *J* 7.9 Hz, H-1′), 4.63 (d, 1 H, *J* 7.9 Hz, H-1), 4.95 (dd, 1 H, *J* 9.7, 7.2 Hz, H-2), 4.96 (dd, 1 H, *J* 10.9, 3.6 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.9, 7.9 Hz, H-2′), 5.18 (s, 2 H, $CH_2Ph$), 5.21 (dd, 1 H, *J* 9.7, 9.7 Hz, H-3), and 5.34 (d, 1 H, *J* 3.6 Hz, H-4′). FABMS: Calcd for $C_{35}H_{44}NaO_{20}$ $[M + Na]^+$, 807.2324. Found, 807.2330.

*3-Benzyloxycarbonylpropyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**12**).—Glycosylation of **4** (0.5 g, 2.6 mmol)

with **7** (1.0 g, 1.3 mmol) was carried out as described for **11** to give **12** (0.7 g, 67%) as a colorless syrup; $R_f$ 0.50 (3:1 $CHCl_3$–$Me_2CO$); $[\alpha]_D$ −12.5° (*c* 1.9, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 1.86–1.94 (m, 2 H, $OCH_2CH_2CH_2CO$), 1.95 (s, 3 H), 2.00 (s, 3 H), 2.03 (s, 3 H), 2.05 (s, 3 H), 2.09 (s, 3 H), 2.14 (s, 3 H), and 2.15 (s, 3 H) (7 OAc), 2.40 (t, 2 H, *J* 7.4 Hz, $OCH_2CH_2CH_2CO$), 3.51 (dt, 1 H, *J* 9.9, 6.2 Hz, $OCHHCH_2CH_2CO$), 3.56 (ddd, 1 H, *J* 11.6, 5.6, 2.4 Hz, H-5), 3.77 (dd, 1 H, *J* 9.3, 9.3 Hz, H-4), 3.82–3.88 (m, 1 H, $OCHHCH_2CH_2CO$), 3.86 (t, 1 H, *J* 6.5 Hz, H-5′), 4.05–4.15 (m, 3 H, H-6a,6′a,6′b), 4.41 (d, 1 H, *J* 7.9 Hz, H-1), 4.46 (dd, 1 H, *J* 11.6, 2.4 Hz, H-6b), 4.48 (d, 1 H, *J* 7.7 Hz, H-1′), 4.86 (dd, 1 H, *J* 9.3, 7.9 Hz, H-2), 4.95 (dd, 1 H, *J* 10.3, 3.3 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.3, 7.7 Hz, H-2′), 5.09 and 5.13 (ABq, 2 H, *J* 11.0 Hz, $CH_2Ph$), 5.17 (dd, 1 H, *J* 9.3, 9.3 Hz, H-3), and 5.34 (d, 1 H, *J* 3.3 Hz, H-4′). FABMS: Calcd for $C_{37}H_{49}O_{20}$ $[M+H]^+$, 813.2817. Found, 813.2828.

*Carboxymethyl* O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**19**).—A solution of **11** (1.4 g, 1.8 mmol) in EtOH (20 mL) was treated with $H_2$ (0.1 MPa) in the presence of 10% Pd–C (0.1 g) at room temperature for 2 h. The mixture was filtered and concentrated to give **19** (1.2 g, 97%) as a colorless solid; $R_f$ 0.28 (10:1 $CHCl_3$–MeOH); $[\alpha]_D$ −2.5° (*c* 3.2, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 1.96 (s, 3 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 2.06 (s, 3 H), 2.07 (s, 3 H), 2.12 (s, 3 H), and 2.15 (s, 3 H) (7 OAc), 3.63–3.69 (m, 1 H, H-5), 3.83 (dd, 1 H, *J* 8.8, 8.8 Hz, H-4), 3.89 (t, 1 H, *J* 6.8 Hz, H-5′), 4.06–4.18 (m, 3 H, H-6a,6′a,6′b), 4.25 (br s, 2 H, $OCH_2CO$), 4.48 (br d, 1 H, *J* 12 Hz, H-6b), 4.51 (d, 1 H, *J* 8.2 Hz, H-1), 4.61 (d, 1 H, *J* 7.7 Hz, H-1′), 4.95 (dd, 1 H, *J* 8.8, 8.8 Hz, H-2), 4.98 (dd, 1 H, *J* 3.3, 10.6 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.6, 7.7 Hz, H-2′), 5.23 (dd, 1 H, *J* 8.8, 8.8 Hz, H-3), and 5.35 (d, 1 H, *J* 3.3 Hz, H-4′). FABMS: Calcd for $C_{28}H_{39}O_{20}$ $[M+H]^+$, 695.2035. Found, 695.2013.

*3-Carboxypropyl* O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**20**).—Hydrogenolysis of **12** (1.3 g, 1.6 mmol) with 10% Pd–C (0.1 mg) in EtOH (20 mL) as described for the preparation of **19** afforded, after silica gel column chromatography (12:1 $CHCl_3$–MeOH), **20** (0.9 g, 78%) as a colorless syrup; $R_f$ 0.31 (10:1 $CHCl_3$–MeOH); $[\alpha]_D$ −8.5° (*c* 1.4, MeOH); $^1H$ NMR ($CDCl_3$): δ 1.85–1.93 (m, 2 H, $OCH_2CH_2CH_2CO$), 1.95 (s, 3 H), 2.04 (s, 9 H), 2.05 (s, 3 H), 2.11 (s, 3 H), and 2.14 (s, 3 H) (7 OAc), 2.41 (t, 2 H, *J* 7.2 Hz, $OCH_2CH_2CH_2CO$), 3.53–3.62 (m, 2 H, H-5 and $OCHHCH_2CH_2CO$), 3.79 (t, 1 H, *J* 9.4 Hz, H-4), 3.84–3.90 (m, 2 H, H-5′ and $OCHHCH_2CH_2CO$), 4.05–4.16 (m, 3 H, H-6a,6′a,6′b), 4.45 (d, 1 H, *J* 7.9 Hz, H-1), 4.49 (d, 1 H, *J* 7.8 Hz, H-1′), 4.50 (m, 1 H, H-6b), 4.87 (dd, 1 H, *J* 9.4, 7.9 Hz, H-2), 4.96 (dd, 1 H, *J* 10.4, 3.4 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.4, 7.8 Hz, H-2′), 5.18 (dd, 1 H, *J* 9.4, 9.4 Hz, H-3), and 5.34 (d, 1 H, *J* 3.4 Hz, H-4′). FABMS: Calcd for $C_{30}H_{43}O_{20}$ $[M+H]^+$, 723.2348. Found, 723.2320.

*Succinimidoxycarbonylmethyl* O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**21**).—1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (0.45 g, 2.3 mmol) was added portionwise

to a mixture of **19** (1.2 g, 1.7 mmol) and *N*-hydroxysuccinimide (NHS) (0.29 g, 2.5 mmol) in dry $CH_2Cl_2$ (100 mL) vigorously stirred in an ice bath. The mixture was stirred at room temperature for 8 h and thoroughly washed with water. The organic layer was dried ($Na_2SO_4$) and concentrated to give **21** (1.3 g, 95%) as a colorless solid; $R_f$ 0.61 (20:1 $CHCl_3$–EtOH); $^1$H NMR ($CDCl_3$): $\delta$ 1.95 (s, 3 H), 2.04 (s, 6 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 2.12 (s, 3 H), and 2.14 (s, 3 H) (7 OAc), 2.85 (br s, 4 H, succinimido $CH_2CH_2$), 3.66 (ddd, 1 H, *J* 9.0, 5.5, 2.2 Hz, H-5), 3.84 (dd, 1 H, *J* 9.0, 9.0 Hz, H-4), 3.87 (t, 1 H, *J* 6.6 Hz, H-5′), 4.05–4.15 (m, 3 H, H-6a,6′a,6′b), 4.49 (d, 1 H, *J* 6.8 Hz, H-1′), 4.51 (dd, 1 H, *J* 12.8, 2.2 Hz, H-6b), 4.56 and 4.60 (ABq, 2 H, *J* 17.6 Hz, $OCH_2CO$), 4.67 (d, 1 H, *J* 8.5 Hz, H-1), 4.95 (dd, 1 H, *J* 11.0, 3.7 Hz, H-3′), 4.96 (dd, 1 H, *J* 8.5, 8.5 Hz, H-2), 5.09 (dd, 1 H, *J* 11.0, 6.8 Hz, H-2′), 5.21 (dd, 1 H, *J* 8.5, 8.5 Hz, H-3), and 5.34 (d, 1 H, *J* 3.7 Hz, H-4′). FABMS: *m/z* 792 $[M+H]^+$, 677 $[M-Osuccinimido]^+$, and 619 $[M-OCH_2CO_2succinimido]^+$.

*3-Succinimidoxycarbonylpropyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**22**).—Treatment of **20** (0.8 g, 1.1 mmol) and NHS (0.15 g, 1.3 mmol) in dry $CH_2Cl_2$ (80 mL) with EDC (0.23 g, 1.2 mmol), as in the preparation of **21**, yielded **22** (0.83 g, 91%) as a homogeneous solid; $R_f$ 0.85 (10:1 $CHCl_3$–MeOH); $[\alpha]_D$ −10° (*c* 1.4, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 1.96 (s, 3 H), 2.03 (s, 6 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 2.11 (s, 3 H), and 2.14 (s, 3 H) (7 OAc), 1.97–2.05 (m, 2 H, $OCH_2CH_2CH_2CO$), 2.68 (t, 2 H, *J* 7.1 Hz, $OCH_2CH_2CH_2CO$), 2.83 (br s, 4 H, succinimido $CH_2CH_2$), 3.58–3.65 (m, 2 H, H-5 and $OCHHCH_2CH_2CO$), 3.80 (dd, 1 H, *J* 9.4, 9.4 Hz, H-4), 3.87 (t, 1 H, *J* 7.1 Hz, H-5′), 3.88–3.94 (dt, 1 H, *J* 10.1, 5.3 Hz, $OCHHCH_2CH_2CO$), 4.05–4.15 (m, 3 H, H-6a,6′a,6′b), 4.46–4.49 (m, 1 H, H-6b), 4.49 (br d, 2 H, *J* 8 Hz, H-1, 1′), 4.88 (dd, 1 H, *J* 9.4, 8.1 Hz, H-2), 4.96 (dd, 1 H, *J* 10.3, 3.6 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.3, 7.8 Hz, H-2′), 5.18 (dd, 1 H, *J* 9.4, 9.4 Hz, H-3), and 5.34 (d, 1 H, *J* 3.6 Hz, H-4′). FABMS: Calcd for $C_{34}H_{46}NO_{22}$ $[M+H]^+$, 820.2511. Found, 820.2500.

*8-Hydrazinocarbonyloctyl O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside* (**23**).—A suspension of **15** (2 g, 3.9 mmol) in EtOH (20 mL) was treated with 80% aq hydrazine (4 mL) according to the reported procedure[19]. A white precipitate was collected and crystallized from EtOH to give **23** (1.86 g, 93%) as colorless crystals; mp, 193–195°C (lit.[17] 190–192°C); $[\alpha]_D$ −1.5° (*c* 1.0, $H_2O$) (lit.[17] −1.9° (*c* 1.0, $H_2O$)); FABMS: *m/z* 511 $[M-H]^-$.

*8-Succinimidoxycarbonyloctyl O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside* (**24**).—*N,N′*-Dicyclohexylcarbodiimide (3.2 g, 15.5 mmol) was added portionwise to a mixture of **18** (5.2 g, 10.4 mmol) and NHS (1.5 g, 13 mmol) in dry DMF (50 mL) vigorously stirred in an icebath. The mixture was stirred at room temperature for 12 h and filtered to remove the precipitated urea. The filtrate was concentrated (< 35°C) to one-third of its original volume, poured into precooled EtOAc (200 mL), and kept in a cold room overnight to give **24** (4.54 g, 73%) as colorless crystal; mp 154–156°C; $R_f$ 0.46 (5:4:1 $CHCl_3$–MeOH–$H_2O$); $[\alpha]_D$ −7° (*c* 5.0, DMF); $^1$H NMR (DMF-$d_6$): $\delta$ 1.21–1.75 (m, 12 H, 6 spacer $CH_2$), 2.29 (dt,

2 H, *J* 18, 7.1 Hz, spacer $CH_2CO$), 2.90 (m, 4 H, succinimido $CH_2CH_2$), 3.17 (dd, 1 H, *J* 7.7, 7.7 Hz, H-2(, 4.28 (d, 1 H, *J* 7.7 Hz, H-1), and 4.37 (d, 1 H, *J* 7.5 Hz, H-1′): FABMS: Calcd for $C_{25}H_{42}NO_{15}$ $[M + H]^+$ 596.2554. Found 596.2542 (high resolution f.a.b.-m.s.).

*$N^2$-{{$N^2$,$N^6$-Bis{2-[O-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyloxy]acetyl}-*L*-lysyl}}-$N^6$-{2-[O-(-[2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyloxy]-acetyl*L*-lysine* (**31**).—A solution of L-lysyl-L-lysine (**29**) dihydrochloride (BACHEM Bioscience, Philadelphia, PA) (0.102 g, 0.29 mmol) in water (8 mL) containing $Et_3N$ (0.13 mL, 0.93 mmol) was added dropwise to a solution of **21** (0.9 g, 1.0 mmol) in DMF (45 mL) vigorously stirred in an ice bath. The mixture was stirred at room temperature for 1 h, the base neutralized with Amberlite IR-120 ($H^+$) cation-exchange resin, and evaporated to dryness. The residue was purified by Sephadex LH-20 column chromatography (i.d. 1.5 cm, length 120 cm) with $Me_2CO$ as the eluent to give **31** (0.47 g, 69% based on **29**) as a colorless solid; $R_f$ 0.79 (2:1:1 butanol–AcOH–$H_2O$); $[\alpha]_D$ −13.5° (*c* 2.4, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 1.35–1.59 (m, lysine $CH_2$), 1.96–2.18 (m, 63 H, 21 OAc), 3.21–3.29 (m, 2 H) and 3.29–3.38 (m, 2 H) (2 lysine H-6a,6b), 3.63–3.71 (m, 3 H, 3 H-5), 3.77–3.86 (m, 3 H, 3 H-4), 3.87–3.93 (m, 3 H, 3 H-5′), 4.41–4.61 (m, 9 H, 3 H-1, 1′, 6b), 4.93–5.01 (m, 6 H, 3 H-2, 3′), 5.10 (dd, 3 H, *J* 10.1, 8.0 Hz, 3 H-2′), 5.23 (br q, 3 H, *J* 9 Hz, 3 H-3), 5.35 (d, 3 H, *J* 4.0 Hz, 3 H-4′), 6.55 (t, 1 H, *J* 5.6 Hz), and 6.59 (t, 1 H, *J* 5.9 Hz) (2 lysine $N^6H$), 6.97 (d, 1 H, *J* 8.0 Hz), and 7.01 (d, 1 H, *J* 7.2 Hz) (2 lysine N-2 *H*). FABMS: Calcd for $C_{96}H_{134}N_4O_{60}Na$ $[M + Na]^+$, 2325.7452. Found, 2325.7545.

*$N^2$-{{$N^2$,$N^6$-Bis{4-[O-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyloxy]butyryl-*L*-lysyl}}$N^6$-{4-[O-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyloxy]butyryl}-*L*-lysine* (**32**).—A solution of **29** dihydrochloride (0.068 g, 0.20 mmol) in water (7 mL) containing $Et_3N$ (0.085 mL, 0.61 mmol) was added dropwise to a solution of **22** (0.62 g, 0.76 mmol) in DMF (30 mL) vigorously stirred in an ice bath. The mixture was stirred at room temperature for 1 h, the base neutralized with Amberlite IR-120 ($H^+$) cation-exchange resin, and evaporated to dryness. The residue was chromatographed on Sephadex LH-20 (i.d., 1.5 cm, length 120 cm) with $Me_2CO$ as the eluent to yield **32** (0.34 g, 73% based on **29**) as a colorless solid; $R_f$ 0.69 (2:1:2 butanol–AcOH–$H_2O$); $[\alpha]_D$ −11.5° (*c* 1.2, $Me_2CO$); $^1H$ NMR ($CDCl_3$): δ 1.35–1.55 (m, lysine $CH_2$), 1.80–1.92 (m, 6 H, 3 $OCH_2CH_2CH_2CO$), 1.95 (s, 9 H), 2.04 (s, 27 H), 2.05 (s, 9 H), 2.11 (s, 9 H), and 2.14 (s, 9 H) (21 OAc), 2.14–2.27 (m, 6 H, 3 $OCH_2CH_2CH_2CO$), 3.10–3.36 (m, 4 H, 2 lysine H-6a, 6b), 3.51–3.65 (m, 6 H, 3 H-5 and 3 $OCHHCH_2CH_2CO$), 3.74–3.84 (m, 6 H, 3 H-4 and 3 $OCHHCH_2CH_2CO$) 3.89 (br t, 3 H, *J* 6 Hz, 3 H-5′), 4.05–4.16 (m, 9 H, 3 H-6a,6′a, 6′b), 4.40–4.56 (m, 11 H, 3 H-1, 1′, 6b, and 2 lysine H-2), 4.81–4.90 (m, 3 H, 3 H-2), 4.94–5.00 (m, 3 H, 3 H-3′), 5.09 (dd, 3 H, *J* 10.3, 8.0 Hz, 3 H-2′), 5.15–5.22 (m, 3 H, 3 H-3), 5.34 (d, 3 H, *J* 3.5 Hz, 3 H-4′).

FABMS: Calcd for $C_{102}H_{146}N_4O_{60}Na$ $[M + Na]^+$ 2409.8394. Found 2409.8503.

*$N^2$-{{$N^2$,$N^6$-Bis{2-[Oβ-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]acetyl}-L-lysyl}}-$N^6$-{2-[Oβ-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]acetyl}-L-lysine* (**33**).—A solution of **31** (0.12 g, 53μmol) in 10:6:1 MeOH–$H_2O$–$Et_3N$ (15 mL) was stirred at room temperature for 3 h. After neutralization with Amberlite IR-120 ($H^+$) cation-exchange resin, the filtrate was evaporated to give a pale-yellow syrup. Purification by Bio-Gel P-2 column chromatography (i.d., 1.5 cm, length 180 cm) with water as the eluent yielded, after lyophilization, **33** (73 mg, 97%) as a colorless solid; $R_f$ 0.21 (2:1:1 propanol–AcOH–$H_2O$); $[\alpha]_D$ −3.5° (*c* 2.5, $H_2O$); $^1H$ NMR ($D_2O$): δ 1.30–1.44 (m, 4 H, 2 lysine H-4a,4b), 1.49–1.59 (m, 4 H, 2 lysine H-5a,5b), 1.65–1.89 (m, 4 H, 2 lysine H-3a, 3b), 3.20–3.27 (m, 4 H, 2 lysine H-6a,6b), 3.37–3.44 (m, 3 H, 3 H-2), 3.52 (dd, 3 H, *J* 9.8, 8.0 Hz, 3 H-2′), 3.55–3.61 (m, 3 H, 3 H-5), 3.90 (d, 3 H, *J* 3.3 Hz, 3 H-4′), 3.93 (dd, 2 H, *J* 12.3, 2.0 Hz, 2 H-6b), 3.94(dd, 1 H, *J* 12.3, 2.1 Hz, H-6b), 4.17–4.41 (m, 8 H, 3 $OCH_2CO$ and 2 lysine H-2), 4.43 (d, 3 H, *J* 8.0 Hz, 3 H-1′), 4.49 (d, 2 H, *J* 8.1 Hz, 2 H-1), and 4.55 (d, 1 H, *J* 8.0 Hz, H-1). FABMS: Calcd for $C_{54}H_{93}N_4O_{39}$ $[M + H]^+$, 1421.5416. Found, 1421.5474.

*$N^2$-{{$N^2$,$N^6$-Bis{4-[O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]butyryl}-L-lysyl}}-$N^6$-{4-4-[O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]-butyryl}-L-lysine* (**34**).—Compound **32** (0.13 g, 54.5 μmol) was *O*-deacetylated as described for the preparation of **33**. Column chromatography on Bio-Gel P-2 (i.d., 1.5 cm, length 180 cm) with water as the eluent afforded, after lyophilization, **34** (78 mg, 95%) as a colorless solid; $R_f$ 0.22 (2:1:1 propanol–AcOH–$H_2O$); $[\alpha]_D$ −5.5° (*c* 1.3, $H_2O$); $^1H$ NMR ($D_2O$): δ 1.27–1.44 (m, 4 H, 2 lysine H-4a,4b), 1.45–1.55 (m, 4 H, 2 lysine H-5a,5b), 1.62–1.72 (m, 2 H) and 1.74–1.83 (m, 2 H) (2 lysine H-3a,3b), 1.84–1.93 (m, 6 H, 3 $OCH_2CH_2CH_2CO$), 2.30 (t, 4 H, *J* 7.4 Hz, 2 $CH_2CH_2CH_2CO$), 2.38 (t, 2 H, *J* 7.4 Hz, $OCH_2CH_2CH_2CO$), 3.14 (q, 4 H, *J* 6.9 Hz, 2 lysine H-6a,6b), 3.28 (br dd, 3 H, *J* 8, 8 Hz, 3 H-2), 3.52 (br dd, *J* 9, 9 Hz, 3 H-2′), 3.54–3.59 (m, 3 H, 3 H-5), 3.90 (d, 3 H, *J* 2.7 Hz, 3 H-4′), 3.94 (br d, 3 H, *J* 12 Hz, 3 H-6b), 4.16 (dd, 1 H, *J* 8.1, 5.1 Hz) and 4.26 (dd, 1 H, *J* 8.5, 6.0 Hz) (2 lysine H-2), 4.42 (d, 3 H, *J* 7.5 Hz, 3 H-1′), and 4.44 (d, 3 H, *J* 7.6 Hz, 3 H-1). FABMS: Calcd for $C_{60}H_{105}N_4O_{39}$ $[M + H]^+$, 1505.6355. Found, 1505.6276.

*$N^2$-{{$N^2$,$N^6$-Bis{9-[O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]nonanoyl}-L-lysyl}}-$N^6$-{9-[O-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyloxy]-nonanoyl}-L-lysine* (**35**).—A solution of **24** (2.4 g, 4.0 mmol) in DMF (37 mL) was added dropwise to a solution of **29** dihydrochloride (0.35 g, 1.0 mmol) in water (15 mL) containing $NaHCO_3$ (0.51 g, 6.1 mmol) vigorously stirred in an ice bath. The mixture was stirred at room temperature for 2 h and concentrated to dryness. The residue was purified by Bio-Gel P-2 column chromatography (i.d., 2.5 cm, length 130 cm) with water as the eluent to give, after lyophilization, **35** (1.6 g, 93% based on **29**) as a colorless solid; $R_f$ 0.33 (2:1:1 butanol–AcOH–$H_2O$); $[\alpha]_D$ −5.5° (*c* 3.0, $H_2O$); $^1H$ NMR ($D_2O$): δ 1.28 (br s, 24 H, 12 spacer $CH_2$), 1.43–1.52 (m, 4 H, 2 lysine H-4a, 4b), 1.52–1.62 (m, 16 H, 6 spacer $CH_2$ and 2 lysine H-5a, 5b),

1.62–1.70 (m, 2 H) and 1.75–1.84 (m, 2 H) (2 lysine H-3a, 3b), 2.19 (t, 4 H, *J* 7.3 Hz, 2 spacer $CH_2CH_2CO$), 2.26 (t, 2 H, *J* 7.3 Hz, spacer $CH_2CH_2CO$), 3.08–3.18 (m, 4 H, 2 lysine H-6a, 6b), 3.27 (br dd, 3 H, *J* 9, 9 Hz, 3 H-2), 3.51 (dd, 3 H, *J* 9.7, 7.8 Hz, 3 H-2′), 3.52–3.58 (m, 3 H, 3 H-5), 3.90 (d, 3 H, *J* 3.4 Hz, 3 H-4′), 3.94 (dd, 3 H, *J* 12.4, 1.9 Hz, 3 H-6b), 4.14 (dd, 1 H, *J* 8.2, 4.5 Hz) and 4.25 (dd, 1 H, *J* 9.2, 5.1 Hz) (2 lysine H-2), 4.42 (d, 3 H, *J* 8.2 Hz, 3 H-1), and 4.43 (d, 3 H, *J* 7.8 Hz, 3 H-1′). FABMS: Calcd for $C_{75}H_{134}N_4O_{39}Na$ $[M + Na]^+$, 1737.8523. Found, 1737.8572.

*Polyvalent β-lactosyl-poly(L-lysine) cluster* (**37**).—A solution of poly(L-lysine) (**30**) ($M_r$ 3800; Sigma, St. Louis, MO) hydrobromide (20 mg, ~3 μmol) in water (0.5 mL) containing $Et_3N$ (15 μL, 107 μmol) was added dropwise to a solution of **22** (95 mg, 116 μmol) in DMF (2 mL) vigorously stirred in an ice bath. The mixture was brought to room temperature and stirred for 3 h. After treatment with Amberlite IR-120 ($H^+$) cation-exchange resin, the mixture was concentrated to dryness. Chromatography of the residue on Sephadex LH-20 (i.d., 1.5 cm, length 90 cm) with $Me_2CO$ as the eluent yielded **36** (51 mg, ~60% based on **30**) as a colorless solid; ninhydrin negative; $^1H$ NMR ($CDCl_3$): δ 1.30–1.60 (m, lysine $CH_2$), 1.75–1.90 (m, $OCH_2CH_2CH_2CO$), 1.95–2.15 (m, OAc), 2.15–2.30 (m, $OCH_2CH_2CH_2CO$), 3.05–3.25 (m, lysine H-6a,6b), 3.50–3.60 (m, H-5 and $OCHHCH_2CH_2CO$), 3.72–3.82 (m, H-4 and $OCHHCH_2CH_2CO$), 3.88 (br t, *J* 6 Hz, H-5′), 4.04–4.16 (m, H-6a,6′a,6′b), 4.43–4.63 (m, H-1,1′,6, and lysine H-2), 4.84 (dd, *J* 8.5, 8.5 Hz, H-2), 4.97 (br dd, *J* 9.5, 3.5 Hz, H-3′), 5.11 (dd, *J* 9.5, 8.0 Hz, H-2′), 5.20 (br t, *J* 8.5 Hz, H-3), and 5.38 (br d, *J* 3.5 Hz, H-4′).

Compound **36** (50 mg, ~2 μmol) was dissolved in MeOH (4 mL) and treated with precooled 1 M NaOH (0.4 mL) for 20 min. The mixture was then diluted with water (20 mL) and the base neutralized with IR-120 ($H^+$) cation-exchange resin. Concentration followed by purification by Bio-Gel P4 column chromatography (i.d., 1.0 cm, length 70 cm) with water as the eluent gave, after lyophilization, **37** (29 mg, ~90%) as a colorless solid; $^1H$ NMR ($D_2O$) δ 1.26–1.44 (m, lysine H-4a, 4b), 1.45–1.57 (m, lysine H-5a,5b), 1.63–1.94 (m, lysine H-3a, 3b, and $OCH_2CH_2CH_2CO$), 2.28 (t, *J* 7.3 Hz, $OCH_2CH_2CH_2CO$), 3.12 (m, lysine H-6a, 6b), 3.28 (br dd, *J* 8.0, 8.0 Hz, H-2), 3.51 (br d, *J* 9 Hz, H-2′), 3.54–3.58 (m, H-5), 3.90 (d, *J* 3.0 Hz, H-4′), 3.94 (br d, *J* 11 Hz, H-6b), 4.17–4.29 (m, lysine H-2), 4.41 (d, *J* 8.5 Hz), and 4.43 (d, *J* 8.0 Hz) (H-1, 1′).

*Attempted preparation of* **35** *by the azide method.*—A 3.3 M solution of HCl in 1,4-dioxane (0.5 mL) and 90% *tert*-butyl nitrite (59 mg, 0.51 mmol) in DMF (0.2 mL) was added sequentially to a precooled (−25°C) solution of **23** (0.19 g, 0.37 mmol) in DMF (5 mL). After 30 min, sulfamic acid (33 mg) was added and the stirring was continued for 15 min. This acyl azide preparation was added dropwise to a precooled (0°C) solution of the dihydrochloride of **29** (25 mg, 0.07 mmol) in a buffer solution (8 mL, pH 9.3), which was prepared by mixing 0.08 M $Na_2B_4O_7$ and 0.35 M $KHCO_3$. During the addition, the pH of the solution was kept between 9.1–9.3. After 30 min, TLC (2:1:1 butanol–AcOH–$H_2O$) indicated the presence

of several spots. Repeated column chromatography on Bio-Gel P-2 (i.d., 1.5 cm, length 120 cm) with water as the eluent provided, after lyophilization, a colorless solid (19 mg) which on TLC appeared to be the conjugate **35**. Examination by $^1$H NMR showed, however, contamination with unknown byproducts.

*Attempted preparation of* **19** *from* **16**.—Compound **16** (0.1 g, 0.25 mmol) was treated with $Ac_2O$ (5 mL) and pyridine (6 mL) at room temperature overnight. The reaction was quenched by addition of EtOH (3 mL) and the solvent was evaporated and coevaporated with toluene several times at 50°C to give a pale-yellow syrup, which was chromatographed on a silica gel column with 5:2 $CHCl_3$–$Me_2CO$. The first fraction yielded ethyloxycarbonylmethyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**25**) (23 mg, 13%) as a colorless solid; $R_f$ 0.72 (5:2 $CHCl_3$–$Me_2CO$); $[\alpha]_D$ −22° (*c* 3.1, $CHCl_3$); $^1$H NMR ($CDCl_3$): δ 1.28 (t, 3 H, *J* 7.1 Hz, $OCH_2CH_3$), 1.96 (s, 3 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 2.06 (s, 3 H), 2.08 (s, 3 H), 2.12 (s, 3 H), and 2.15 (s, 3 H) (7 OAc), 3.61 (ddd, 1 H, *J* 9.3, 5.0, 2.1 Hz, H-5), 3.82 (dd, 1 H, *J* 9.3, 9.3 Hz, H-4), 3.87 (br t, 1 H, *J* 6.0 Hz, H-5′), 4.08 (dd, 1 H, *J* 11.3, 7.1 Hz) and 4.13 (dd, 1 H, *J* 11.3, 5.0 Hz) (H-6′a, 6′b), 4.17–4.22 (m, 3 H, H-6a and $OCH_2CH_3$), 4.23 (s, 2 H, $OCH_2CO$), 4.49 (d, 1 H, *J* 7.9 Hz, H-1′), 4.49 (dd, 1 H, *J* 11.6, 2.1 Hz, H-6b), 4.63 (d, 1 H, *J* 7.9 Hz, H-1), 4.95 (dd, 1 H, *J* 9.3, 7.9 Hz, H-2), 4.96 (dd, 1 H, *J* 10.3, 3.5 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.3, 7.9 Hz, H-2′), 5.22 (dd, 1 H, *J* 9.3, 9.3 Hz, H-3), and 5.35 (br d, 1 H, *J* 3.5 Hz, H-4′). FABMS: Calcd for $C_{30}H_{43}O_{20}$ $[M + H]^+$, 723.2348. Found, 723.2329.

The second fraction gave ethoxycarbonylmethyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-acetyl-β-D-glucopyranoside (**27**) (90 mg, 53%) as a colorless solid; $R_f$ 0.40 (5:2 $CHCl_3$–$Me_2CO$); $[\alpha]_D$ −9.5° (*c* 2.6, $CHCl_3$); $^1$H NMR ($CDCl_3$): δ 1.80 (t, 3 H, *J* 7.1 Hz, $OCH_2CH_3$), 1.96 (s, 3 H), 2.03 (s, 3 H), 2.06 (s, 3 H), 2.11 (s, 3 H), 2.13 (s, 3 H), and 2.15 (s, 3 H) (6 OAc), 3.50 (br s, 1 H, OH), 3.55 (br dd, 1 H, *J* 9.0, 9.0 Hz, H-2), 3.61 (ddd, 1 H, *J* 9.8, 5.1, 1.9 Hz, H-5), 3.73 (dd, 1 H, *J* 9.8, 9.8 Hz, H-4), 3.87 (br t, 1 H, *J* 7.0 Hz, H-5′), 4.08 (dd, 1 H, *J* 11.1, 7.3 Hz, H-6′a), 4.11 (dd, 1 H, *J* 11.9, 5.1 Hz, H-6a), 4.17 (dd, 1 H, *J* 11.1, 6.5 Hz, H-6′b), 4.24 and 4.38 (ABq, 2 H, *J* 16.9 Hz, $OCH_2CO$), 4.24 (q, 2 H, *J* 7.1 Hz, $OCH_2CH_3$), 4.40 (d, 1 H, *J* 8.0 Hz, H-1), 4.46 (dd, 1 H, *J* 11.9, 1.9 Hz, H-6b), 4.49 (d, 1 H, *J* 7.8 Hz, H-1′), 4.96 (dd, 1 H, *J* 10.4, 3.5 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.4, 7.8 Hz, H-2′), 5.14 (dd, 1 H, *J* 9.4, 9.4 Hz, H-3), and 5.35 (d, 1 H, *J* 3.5 Hz, H-4′). FABMS: Calcd for $C_{28}H_{41}O_{19}$ $[M + H]^+$, 681.2242. Found, 681.2226.

The acetylation of **27** gave **25** quantitatively.

*Attempted preparation of* **20** *from* **17**.—Compound **17** (0.15 g, 0.35 mmol) was treated with $Ac_2O$ (5 mL) and pyridine (6 mL) as for the acetylation of **16** to give a pale-yellow syrup, which was subjected to silica gel column chromatography. The first eluent (5:1 $CHCl_3$–$Me_2CO$) gave 3-ethoxycarbonylpropyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**26**) (89 mg, 34%) as a colorless solid; $R_f$ 0.40 (5:1 $CHCl_3$–$Me_2CO$); $[\alpha]_D$ −12.5° (*c* 2.4, $CHCl_3$); $^1$H NMR ($CDCl_3$): δ 1.25 (t, 3 H, *J* 6.9 Hz, $OCH_2CH_3$), 1.88 (br quint., 2

H, *J* 7.0 Hz, $OCH_2CH_2CH_2CO$), 1.96 (s, 3 H), 2.04 (s, 9 H), 2.06 (s, 3 H), 2.12 (s, 3 H), and 2.14 (s, 3 H) (7 OAc), 2.34 (t, 2 H, *J* 7.3 Hz, $OCH_2CH_2CH_2CO$), 3.52 (dt, 1 H, *J* 9.4, 6.2 Hz, $OCHHCH_2CH_2CO$), 3.57–3.62 (m, 1 H, H-5), 3.79 (dd, 1 H, *J* 9.3, 9.3 Hz, H-4), 3.82–3.88 (m, 1 H, $OCHHCH_2CH_2CO$), 3.87 (t, 1 H, *J* 6.0 Hz, H-5′), 4.06–4.15 (m, 3 H, H-6a, 6′a, 6′b), 4.45 (d, 1 H, *J* 7.9 Hz, H-1), 4.46–4.50 (m, 1 H, H-6b), 4.48 (d, 1 H, *J* 7.8 Hz, H-1′), 4.88 (dd, 1 H, *J* 9.3, 7.9 Hz, H-2), 4.95 (dd, 1 H, *J* 10.6, 3.3 Hz, H-3′), 5.10 (dd, 1 H, *J* 10.6, 7.8 Hz, H-2′), 5.19 (dd, 1 H, *J* 9.3, 9.3 Hz, H-3), 5.34 (d, 1 H, *J* 3.3 Hz, H-4′). FABMS: Calcd for $C_{32}H_{47}O_{20}$ $[M + H]^+$, 751.2661. Found, 751.2679.

The second eluent (10:1 $CHCl_3$–MeOH) yielded **20** (95 mg, 38%) as a colorless syrup.

*In vivo assay*.—The lung tumor colonization assay was performed as previously described[6]. The suspension of BL6 cells ($2 \times 10^4$) in RPMI-1640 medium (0.15 mL) was mixed with either 0.1 M methyl β-lactoside, 0.03 M trivalent clusters (**33**, **34** or **35**) or 3 mM of polyvalent cluster (**37**). After incubation at 37°C for 10 min, the mixture was injected into the tail vein of syngeneic C57/BL female mice at 8 weeks of age. Control mice received the same amount of cells and RPMI-1640 without the lactoside derivatives. Each treatment and control group consisted of six mice. Eighteen days after the injections, the mice were killed and the lungs were fixed with 10% formaldehyde in PBS (pH 7.4). The number of pulmonary tumors on the surface of the lungs was counted under a dissecting microscope.

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