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Synthesis of linear-type chondroitin clusters having a C_8 spacer between disaccharide moieties and enzymatic transfer of D-glucuronic acid to the artificial glycans

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Abstract

Newly designed linear-type glycoclusters were synthesized which involve a chondroitin repeating disaccharide ligand and a hydrophobic octyl ether spacer. The spacer mimics the corresponding disaccharide unit. Repeating elongation of the pseudo-tetrasaccharide that was derived from the common cluster unit [→8)-octyl-(1→3)-β-D-GalNAc-(1→4)-β-D-GlcA-(1→] allowed the syntheses of up to the pseudo-decasaccharide analog of chondroitin. An enzymatic D-GlcA transfer at the non-reducing end of the synthesized artificial glycans by GlcATase II was observed.

Keywords: Chondroitin; Glycocluster; Linear-type glycocluster; Spacers; Glycosyl transfer

1. Introduction

Many artificial carbohydrate clusters (glycoclusters) have already been synthesized, involving different shapes, spacer lengths and saccharide moieties.[1,2] These efforts have proved valuable in the identification of the so-called 'cluster effect' involving aggregated ligands and flexible spacers in the modulation of the interaction between carbohydrates and proteins.[1] By using simplified glycoclusters, it is anticipated that carbohydrate-based drugs that inhibit bacterial and viral infections can be developed. Thus, structurally defined glycoclusters are required in order to understand the carbohydrate–protein interactions at the molecular level.

Major glycoclusters can be classified into two categories based on their shapes: the dendrimer (A) and pendant (B) types as shown in Scheme 1. Commonly, these ligands locate at the end of spacers extending from the core.

We now report the synthesis of novel linear-type glycoclusters mimicking chondroitin sulfate that is a member of the GAGs. These glycoclusters include a hydrophobic spacer between the carbohydrate ligand as depicted in Scheme 1(C). There is a decisive difference from the above dendrimer- and pendant-type glycoclusters. Two target linear-type glycoclusters (**1** and **2**) mimicking the hexa- and decasaccharides of chondroitin, respectively,

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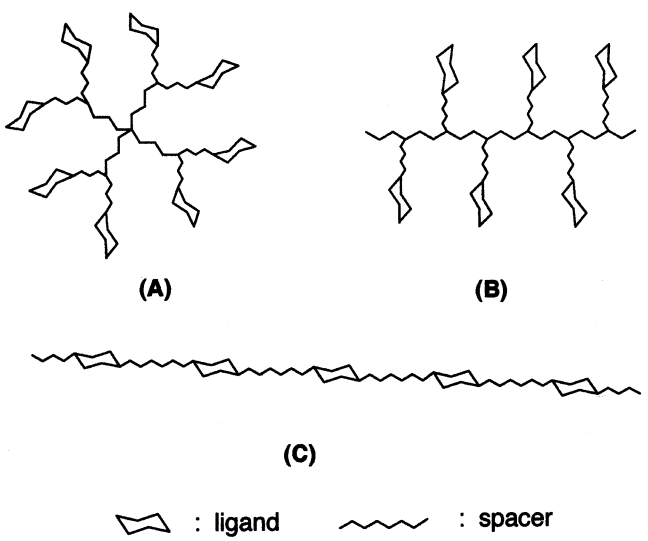
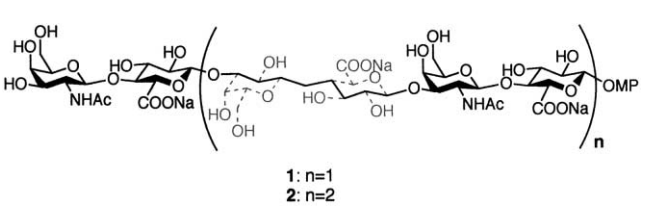
are shown in Scheme 2. To more easily synthesize these cluster molecules, we first planned to avoid any chemical sulfation.

Some linear-type glycoclusters have already been synthesized. In 1984, Jegge and Lehmann reported the synthesis of a pseudo-maltotrioside.[3] They replaced the middle D-glucose moiety of a D-maltotrioside with an acyclic hydrophilic spacer. This artificial pseudo-maltotrioside competitively inhibited the hydrolysis of *p*-nitrophenyl α-D-maltotrioside by porcine α-amylase. In 1990, Lehmann and Petry synthesized a spacer-modified glycoconjugate having an aliphatic C_{10} spacer between the β-D-GlcNAc moieties that were part of the biantennary core of the N-linked glycoprotein.[4] This so-called head-on-type glycocluster, which contains ligands (β-D-GlcNAc) glycosylated with 1,10-decanediol, acted as a substrate for galactosyltransferase from bovine milk. Recently, the Kusumoto's group published the synthesis of glycoclusters with a bis(disaccharide) structure linked through an amide-type spacer.[5] Contrary to these linear-type glycoclusters, our glycocluster is suitably designed for elongation like natural polysaccharides. Thus, higher biological activities are expected by increasing the number of ligands.



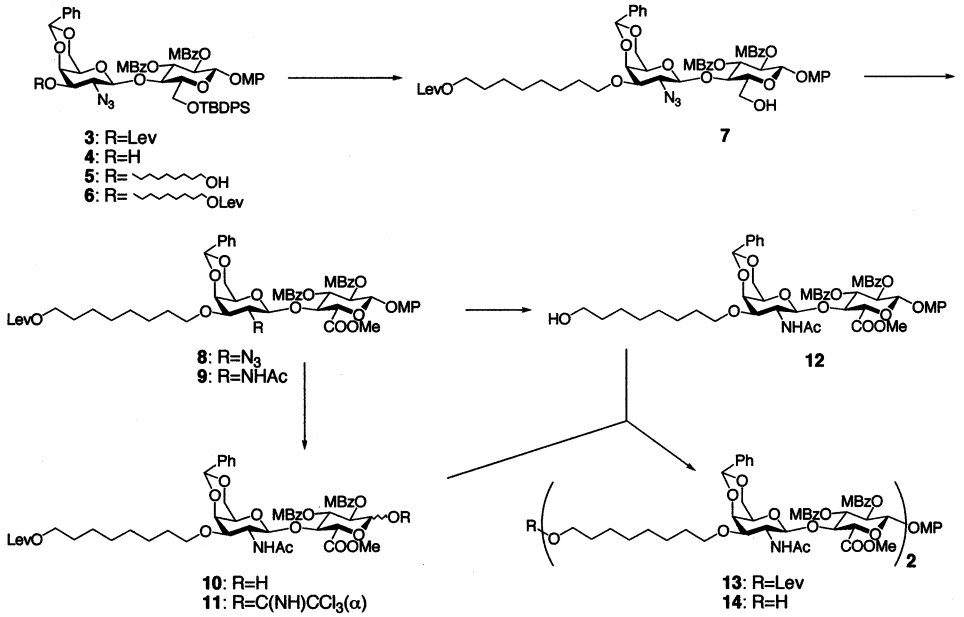
Scheme 1. Some typical artificial glycoclusters: dendrimer-type (A) and pendant-type (B) glycoclusters have ligands at the end of spacers. The linear-type glycocluster that we propose incorporate ligands between the spacers as in (C).



Scheme 2.

2. Results and discussion

The targeted glycoclusters, **1** and **2** (Scheme 2), were synthesized as follows. To effectively elongate the cluster molecules, we designed a common disaccharide unit **9** having an 8-levulinoyloxyoctyl ether at O-3 of the D-GalNAc residue as shown in Scheme 3. The levulinoyl protecting group of the known disaccharide **3**[6] was removed with hydrazine acetate to give **4** in 84% yield, thus allowing the introduction of a C_8 moiety at the liberated hydroxyl group of **4**. In 1999, Neda et al. reported a similar synthesis of a glycocluster having a C_3 spacer between the monosaccharide moieties by employing 2-(3-bromopropoxy)tetrahydro-2*H*-pyran.[7] The alkylation with 8-bromooctanol (1.5 equiv) and sodium hydride (1.5 equiv) at $-50 \rightarrow 0$ °C for 5 h afforded the desired compound **5** without affecting the acyl and silyl groups. The residual **4** was recovered in 38% yield. A higher temperature and excess amount of sodium hydride decreased the yield of **5** and the recovery of the starting **4**. The hydroxyl group of the octyl moiety was then quantitatively protected as a levulinoyl ester. The silyl group of **6** was also quantitatively removed by using tetra-*n*-butylammonium fluoride and acetic acid as previously reported.[6] The resulting C-6 alcohol **7** was converted to a carboxylic acid via a Swern oxidation followed by further oxidation mediated with sodium chlorite. The carboxylic acid was finally esterified with (trimethylsilyl)diazomethane to yield the methyl ester **8**. The total yield was 70% in these three steps. The azide of **8** was reduced by the Lindlar catalyzed hydrogenolysis and the following conventional acetylation gave the acetamide **9** in 96% yield (two steps). Having in hand the key disaccharide **9**, we converted it to the corresponding glycosyl donor **11** and acceptor **12**. Protecting groups capping the head and tail of **9** (MP and Lev) were chemoselectively

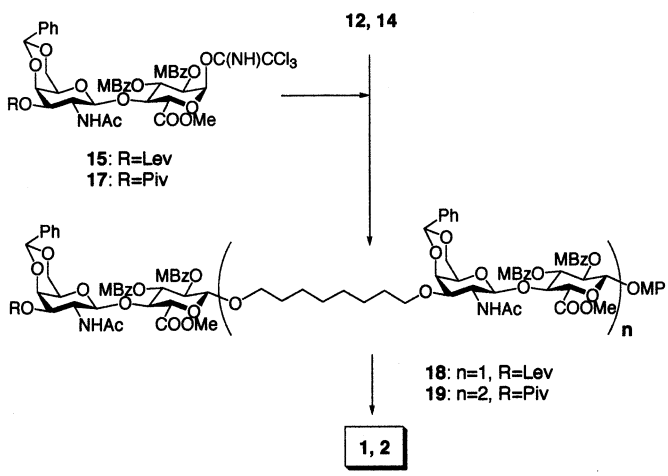


Scheme 3. Abbreviations: MP, 4-MeOC$_6$H$_4$; MBz, 4-MeC$_6$H$_4$CO; TBDPS, *t*-BuPh$_2$Si; Lev, MeCO(CH$_2$)$_2$CO; Piv, *t*-BuCO.

removed to give **10** and **12**, respectively, both in 95% yields. The hemiacetal **10** was then converted to the corresponding imidate **11** in 88% yield. These disaccharide moieties were coupled by employing trimethylsilyl trifluoromethanesulfonate in dichloromethane to give the tetrasaccharide **13** in 69% yield. It is noteworthy that the 4-methylbenzoyl group completely controlled the newly generated anomeric configuration of **13** as β during the coupling reaction. The levulinoyl group of **13** was then removed as above in 70% yield. Thus, we have regio- and stereoselectively prepared the di- and tetrasaccharide acceptors, **12** and **14**.

In order to protect the cluster-end, we coupled the corresponding disaccharide donors **15**[6] and **17** with the acceptors **12** and **14**, respectively, as shown in Scheme 4. As for the synthesis of **13**, the protected pseudo-hexa- and decasaccharides **18** and **19** were stereoselectively obtained in 75 and 48% yields, respectively. Compared to the elongation results for the synthesis of the natural-type chondroitin oligosaccharide, the coupling yields substantially increased.[8†] Finally, all the protecting groups of **18** and **19** were removed as follows: (i) The levulinoyl group of **18** was removed prior to the other deprotections; (ii) benzylidene acetals were hydrolyzed in the presence of camphorsulfonic acid; (iii) methyl uronates were then exchanged with lithium salts using aqueous lithium hydroxide and the remaining acyl groups were removed by hydrolysis with aqueous sodium hydroxide. Thus, the targeted compounds (**1** and **2**) were obtained in 29 and 56% yields as sodium salts, respectively (four and three steps, respectively).

We also examined the enzymatic incorporation of a D-GlcA residue into the glycoclus-



Scheme 4.

† Coupling of the corresponding donor (disaccharide) and acceptor (tetrasaccharide) afforded the hexasaccharide in 41% yield. These saccharides possess azide and TBDMS groups instead of acetamide and MP, respectively.

Table 1
Incorporation of D-[^{14}C]GlcA into various acceptors by glucuronosyltransferase II

Acceptor	D-[^{14}C]GlcA (dpm)
1: D-GalNAc-D-GlcA-(C$_8$-D-GalNAc-D-GlcA)-MP	120
2: D-GalNAc-D-GlcA-(C$_8$-D-GalNAc-D-GlcA)$_2$-MP	50
D-GalNAc-(D-GlcA-D-GalNAc)	200
D-GalNAc-(D-GlcA-D-GalNAc)$_2$	14,000
D-GalNAc-(D-GlcA-D-GalNAc)$_5$	98,000

ters, **1** and **2**, using GlcATase II[9] partially purified from fetal bovine serum. This enzyme specifically transfers the D-GlcA residue to O-3 of β-D-GalNAc in the repeating disaccharide region. These results were compared with those of the chondroitin oligosaccharides as shown in Table 1. It proved that **1** and **2** were able to accept D-GlcA with a similar specificity as that found for the shorter chondroitin repeating oligosaccharide units.

In conclusion, we have synthesized newly designed linear-type glycoclusters, **1** and **2**, having a hydrophobic spacer between the disaccharide moieties. We replaced the chondroitin repeating disaccharide with a spacer involving the hydrophobic octyl ether. The repeating elongation of the cluster unit allowed the syntheses of a pseudo-decasaccharide analog of chondroitin showing the possible synthesis of longer linear-type glycoclusters. In addition, it proved that the glycoclusters, **1** and **2**, were able to enzymatically accept a D-GlcA residue at the non reducing-end.

3. Experimental

General methods.—Optical rotations were measured at 22 ± 3 °C with a HORIBA polarimeter. ^{1}H NMR assignments were confirmed by two-dimensional HH COSY experiments with a JEOL LA 400 MHz spectrometer. The FAB mass spectra were measured with a triple-stage quadrupole mass spectrometer (Finnigan MAT TSQ 700) equipped with a FAB ion source. Silica-gel chromatography, analytical TLC and preparative TLC (PTLC) were done on a column of Silica Gel 60 (E. Merck) or glass plates coated with Silica Gel F_{254} (E. Merck), respectively. Gels for size-exclusion chromatography (Sephadex LH-20, LH-60 and Biobeads S-X1) were purchased from Pharmacia and BIORAD, respectively. Molecular sieves (MS) were purchased from GL Science, Inc. and activated at 180 °C under diminished pressure prior to use. All reactions in organic solvents were performed under dry Ar atmosphere. As a usual work-up, the organic phase of the reaction mixture was washed with aq $NaHCO_3$, brine and dried over anhyd $MgSO_4$.

4-Methoxyphenyl O-*(2-azido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-6-*O*-tert-butyldiphenylsilyl-2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranoside* (**4**).—To a solution of 4-methoxyphenyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-D-galactopyranosyl)-(1→4)-6-*O*-*tert*-butyldiphenylsilyl-2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranoside (**3**)[6] (705.4 mg, 621.9 μmol) in toluene (1 mL) and EtOH (5 mL) was added H_2NNH_2·AcOH (570 mg, 6.19 mmol) with stirring for 70 min. The volatiles were removed under diminished pressure and the residue was eluted from a column of gel permeation (LH-20, 1:1 $CHCl_3$–MeOH) to give **4** (571.3 mg) in 89% yield as a syrup: $[\alpha]_D$ +9.8° (*c* 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.93–6.72 (m, 27 H, Ph), 5.76 (dd, 1 H, $J_{2,3}$ 9.76, $J_{3,4}$ 9.51 Hz, H-3^I), 5.61 (dd, 1 H, $J_{1,2}$ 7.81 Hz, H-2^I), 5.32 (s, 1 H, PhC*H*), 5.14 (d, 1 H, H-1^I), 4.55 (d, 1 H, $J_{1,2}$ 8.05 Hz, H-1^{II}), 4.41 (t, 1 H, $J_{4,5}$ 9.51 Hz, H-4^I), 4.24 (dd, 1 H, $J_{5,6a}$ 2.93, J_{gem} 11.96 Hz, H-6a^I), 4.14 (dd, 1 H, H-6b^I), 3.95 (d, 1 H, $J_{3,4}$ 3.66 Hz, H-4^{II}), 3.89 (d, 1 H, H-6a^{II}), 3.73–3.72 (m, 5 H, H-5^I, H-6b^{II}, OMe), 3.49 (dd, 1 H, $J_{2,3}$ 10.00 Hz, H-2^{II}), 3.41–3.39 (m, 1 H, H-3^{II}), 3.09 (bs, 1 H, H-5^{II}), 2.48 (d, 1 H, $J_{3,OH}$ 9.76 Hz, OH), 2.36, 2.19 (2 s, 3 H × 2, 2 Ph*Me*), 1.09 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{58}H_{61}N_3O_{13}Si$: C, 67.23; H, 5.93; N, 4.06. Found: C, 67.46; H, 6.18; N, 3.94.

4-Methoxyphenyl O-*[2-azido-4,6*-O-*benzylidene-2-deoxy-3*-O-*(8-hydroxyoctyl)-β*-D-*galactopyranosyl]-(1→4)-6*-O-tert-*butyldiphenylsilyl-2,3-di*-O-*(4-methylbenzoyl)-β*-D-*glucopyranoside* (**5**).—To a solution of **4** (2.36 g, 2.28 mmol) in DMF (20 mL) cooled to −50 °C, NaH (50%, 140 mg, 3.5 mmol) was added with stirring. 8-Bromooctanol (0.6 mL, 3.5 mmol) was added to the reaction mixture 1 h later, and then the temperature was raised gradually up to 0 °C within 5 h. The reaction was quenched with ice and extracted with EtOAc. The organic phase was treated as described in general methods. The volatiles were removed under diminished pressure and the residue was eluted from a column of silica gel (50:1–5:1 toluene–toluene–EtOAc) to give **5** (1.19 g, 53%) as a syrup together with residual **4** (865.5 mg, 38%): $[\alpha]_D$ +19.8° (*c* 1.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.88–6.71 (m, 27 H, Ph), 5.75 (dd, 1 H, $J_{2,3}$ 9.76, $J_{3,4}$ 9.51 Hz, H-3^I), 5.59 (dd, 1 H, $J_{1,2}$ 7.81 Hz, H-2^I), 5.30 (s, 1 H, PhC*H*), 5.13 (d, 1 H, H-1^I), 4.49 (d, 1 H, $J_{1,2}$ 8.29 Hz, H-1^{II}), 4.35 (t, 1 H, $J_{4,5}$ 9.51 Hz, H-4^I), 4.28 (dd, 1 H, $J_{5,6a}$ 3.17, J_{gem} 11.71 Hz, H-6a^I), 4.15 (d, 1 H, H-6b^I), 4.01 (d, 1 H, $J_{3,4}$ 3.42 Hz, H-4^{II}), 3.86 (d, 1 H, J_{gem} 10.25 Hz, H-6a^{II}), 3.76–3.57 (m, 5 H, H-5^I, H-6b^{II}, 3/2 CH_2O), 3.75 (s, 3 H, OMe), 3.61 (dd, 1 H, H-2^{II}), 3.45–3.36 (m, 2 H, 1/2 CH_2O, OH), 3.10 (dd, 1 H, $J_{2,3}$ 10.25 Hz, H-3^{II}), 2.95 (bs, 1 H, H-5^{II}), 2.36, 2.21 (2 s, 3 H × 2, 2 Ph*Me*), 1.58–1.25 (m, 12 H, 6 CH_2), 1.11 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{66}H_{77}N_3O_{14}Si$: C, 68.07; H, 6.68; N, 3.61. Found: C, 68.13; H, 7.01; N, 3.28.

4-Methoxyphenyl O-*[2-azido-4,6*-O-*benzylidene-2-deoxy-3*-O-*(8-levulinoyloxyoctyl)-β*-D-*galactopyranosyl]-(1→4)-6*-O-tert-*butyldiphenylsilyl-2,3-di*-O-*(4-methylbenzoyl)-β*-D-*glucopyranoside* (**6**).—To a solution of **5** (452.2 mg, 388.3 μmol) in Py (5 mL) were added a solution of 1 M levulinic anhydride in 1,2-$(CH_2Cl)_2$ (2.0 mL) and a catalytic amount of DMAP with stirring. After 4 h, an excess of ice was added to the reaction mixture. The reaction mixture was diluted with $CHCl_3$. The organic phase was treated as described in general methods. The residue obtained was eluted from a column of silica gel (25:1–10:1 toluene–EtOAc) to give **6** (470.9 mg, 96%) as a syrup: $[\alpha]_D$ +20.5° (*c* 1.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.88–6.71 (m, 27 H, Ph), 5.76 (dd, 1 H, $J_{2,3}$ 9.76, $J_{3,4}$ 9.51 Hz, H-3^I), 5.59 (dd, 1 H, $J_{1,2}$ 7.81 Hz, H-2^I), 5.30 (s, 1 H, PhC*H*), 5.13 (d, 1 H, H-1^I), 4.49 (d, 1 H, $J_{1,2}$ 8.29 Hz, H-1^{II}), 4.35 (t, 1 H, $J_{4,5}$ 9.51 Hz, H-4^I), 4.28 (dd, 1 H, $J_{5,6a}$ 3.15, J_{gem} 11.71 Hz, H-6a^I), 4.15 (d, 1 H, H-6b^I), 4.08–4.01 (m, 3 H, H-4^{II}, CH_2O), 3.86 (d, 1 H, J_{gem} 11.47 Hz, H-6a^{II}), 3.78–3.53 (m, 3 H, H-5^I, H-6b^{II}, 1/2 CH_2O), 3.75 (s, 3 H, OMe), 3.65 (dd, 1 H, H-2^{II}), 3.45–3.37 (m, 1 H, 1/2 CH_2O), 3.10 (dd, 1 H, $J_{2,3}$ 10.25, $J_{3,4}$ 3.42 Hz, H-3^{II}), 2.95 (bs, 1 H, H-5^{II}), 2.76–2.54 (m, 4 H, 2 CH_2), 2.35, 2.28, (2 s, 3 H × 2, 3 Ph*Me*), 2.19 (s, 3 H, COMe), 1.60–1.25 (m, 12 H, 6 CH_2), 1.09 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{71}H_{83}N_3O_{16}Si$: C, 67.53; H, 6.64. Found: C, 67.23; H, 7.01.

4-Methoxyphenyl O-*[2-azido-4,6*-O-*benzylidene-2-deoxy-3*-O-*(8-levulinoyloxyoctyl)-β*-D-*galactopyranosyl]-(1→4)-2,3-di*-O-*(4-methylbenzoyl)-β*-D-*glucopyranoside* (**7**).—To a solution of **6** (2.902 g, 2.299 mmol) in THF (30 mL) were added a 1 M solution of TBAF in THF (11.0 mL, 11.0 mmol) and HOAc (1.3 mL, 23 mmol) with stirring. After 4 days, the volatiles were removed and the residue was diluted with $CHCl_3$ and brine. The organic phase was treated as described in general methods and the residue was eluted from a column of silica gel (10:1–1:1 toluene–EtOAc) to give **7** (1.847 g, 80%) as a syrup: $[\alpha]_D$ +38° (*c* 0.98, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.86–6.76 (m, 17 H, Ph), 5.79 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.51 Hz, H-3^I), 5.54 (dd, 1 H, $J_{1,2}$ 7.81 Hz, H-2^I), 5.31 (s, 1 H, PhC*H*), 5.18 (d, 1 H, H-1^I), 4.33 (d, 1 H, $J_{1,2}$ 8.29 Hz, H-1^{II}), 4.27 (t, 1 H, $J_{4,5}$ 9.51 Hz, H-4^I), 4.09 (d, 1 H, $J_{3,4}$ 2.93 Hz, H-4^{II}), 4.05–4.01 (m, 4 H, H-6ab^I, CH_2O), 3.79 (dt, 1 H, $J_{5,6a}$ 4.39, $J_{5,6b}$ 2.39 Hz, H-5^I), 3.75 (s, 3 H, OMe), 3.70 (dd, 1 H, H-2^{II}), 3.68–3.37 (m, 5 H, H-6ab^{II}, CH_2O, OH), 3.25 (dd, 1 H, $J_{2,3}$ 10.25 Hz, H-3^{II}), 2.93 (bs, 1 H, H-5^{II}), 2.76–2.55 (m, 4 H, 2 CH_2), 2.35, 2.28, (2 s, 2 H × 3, 2 Ph*Me*), 2.19 (s, 3 H, COMe), 1.55–1.25 (m, 12 H, 6 CH_2). Anal. Calcd for $C_{55}H_{65}N_3O_{16}$: C, 64.50; H, 6.40; N, 4.10. Found: C, 64.71; H, 6.56; N, 3.90.

Methyl [2-azido-4,6-O-*benzylidene-2-deoxy-3*-O-*(8-levulinoyloxyoctyl)-β*-D-*galactopyran-*

osyl]-(1→4)-[4-methoxyphenyl 2,3-di-O-*(4-methylbenzoyl)-β*-D-*glucopyranosid] uronate* (**8**).—Dimethyl sulfoxide (1.3 mL, 18.3 mmol) was added dropwise within 3 min to a 1 M solution of $(COCl)_2$ in CH_2Cl_2 (9.2 mL) at −78 °C with stirring. After 5 min, a solution of **7** (1.892 g, 1.847 mmol) in CH_2Cl_2 (20 mL) was added within 3 min and stirring was continued for 30 min. Then, $Et(i\text{-}Pr)_2N$ (6.6 mL, 36.8 mmol) diluted with the same volume of CH_2Cl_2 was added to the reaction mixture and the cooling bath was removed 5 min later. The reaction mixture was stirred for more 25 min and diluted with CH_2Cl_2, washed with 1 M HCl, brine, aq $NaHCO_3$ and brine again. The volatiles were removed under diminished pressure and the residue was diluted in *tert*-BuOH (25 mL). To the solution were added 2-methyl-2-butene (10 mL), water (25 mL), $NaHPO_4 \cdot 2\ H_2O$ (2.5 g), and $NaClO_2$ (2.5 g). The mixture was stirred overnight and the volatiles were removed under diminished pressure. The residue was dissolved in toluene (9 mL) and MeOH (3 mL), and an excess of Me_3SiCHN_2 (*n*-hexane solution) was added to the solution. The reaction mixture was concentrated and the residue was eluted from a column of silica gel (5:1–1:1 toluene–EtOAc) to give **8** (1.498 g, 77%) as a syrup: $[\alpha]_D$ +25.3° (*c* 1.20, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.86–6.75 (m, 17 H, Ph), 5.79 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.03 Hz, H-3^I), 5.59 (dd, 1 H, $J_{1,2}$ 7.32 Hz, H-2^I), 5.30 (s, 1 H, PhC*H*), 5.23 (d, 1 H, H-1^I), 4.51 (t, 1 H, $J_{4,5}$ 9.03 Hz, H-4^I), 4.35 (d, 1 H, H-5^I), 4.33 (d, 1 H, $J_{1,2}$ 8.05 Hz, H-1^{II}), 4.09–4.03 (m, 3 H, H-4^{II}, CH_2O), 3.83, 3.75 (2 s, 3 H × 2, 2 OMe), 3.74–3.55 (m, 3 H, H-6ab^{II}, 1/2 CH_2O), 3.36 (dd, 1 H, H-2^{II}), 3.44–3.38 (m, 1 H, 1/2 CH_2O), 3.15 (dd, 1 H, $J_{2,3}$ 10.49, $J_{3,4}$ 3.66 Hz, H-3^{II}), 3.00 (bs, 1 H, H-5^{II}), 2.75–2.54 (m, 4 H, 2 CH_2), 2.35, 2.28, (2 s, 3 H × 2, 2 Ph*Me*), 2.19 (s, 3 H, COMe), 1.59–1.26 (m, 12 H, 6 CH_2). Anal. Calcd for $C_{56}H_{65}N_3O_{17} \cdot 0.8\ H_2O$: C, 63.05; H, 6.31; N, 3.94. Found: C, 63.19; H, 6.34; N, 3.64.

Methyl [2-acetamido-4,6-O-*benzylidene-2-deoxy-3*-O-*(8-levulinoyloxyoctyl)-β*-D-*galacto-pyranosyl]-(1→4)-[4-methoxyphenyl 2,3-di*-O-*(4-methylbenzoyl)-β*-D-*glucopyranosid] uronate* (**9**).—The Lindlar catalyst (100 mg) was added to a solution of **8** (24.3 mg, 23.1 μmol) in EtOAc (1 mL) and the mixture was stirred under H_2 atmosphere overnight. Insoluble materials were removed on Celite and the filtrate was concentrated under diminished pressure. The residue was acetylated (Ac_2O-Py) and the crude materials was purified on a column of silica gel (toluene ~ 5:1 ~ 1:5 toluene–EtOAc) to give **9** (23.6 mg, 96%) as a syrup: $[\alpha]_D$ +42.2° (*c* 1.36, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.88–6.75 (m, 17 H, Ph), 5.77 (t, 1 H, $J_{2,3} = J_{3,4}$ 8.78 Hz, H-3^I), 5.64 (d, 1 H, *J* 6.83 Hz, NH), 5.53 (dd, 1 H, $J_{1,2}$ 7.32 Hz, H-2^I), 5.33 (s, 1 H, PhC*H*), 5.23 (d, 1 H, $J_{1,2}$ 8.05 Hz, H-1^{II}), 5.21 (d, 1 H, H-1^I), 4.56 (t, 1 H, $J_{4,5}$ 9.03 Hz, H-4^I), 4.28 (d, 1 H, H-5^I), 4.22 (dd, 1 H, $J_{2,3}$ 8.78 Hz, H-3^{II}), 4.05 (d, 1 H, $J_{3,4}$ 5.37 Hz, H-4^{II}), 4.04–4.01 (m, 2 H, CH_2O), 3.86 (d, 1 H, J_{gem} 12.20 Hz, H-6a^{II}), 3.78, 3.74 (2 s, 3 H × 2, 2 OMe), 3.64 (dd, 1 H, H-6b^{II}), 3.57–3.24 (m, 3 H, H-2^{II}, CH_2O), 2.96 (bs, 1 H, H-5^{II}), 2.75–2.54 (m, 4 H, 2 CH_2), 2.34, 2.29, (2 s, 3 H × 2, 2 Ph*Me*), 2.18 (s, 3 H, COMe), 1.97 (s, 3 H, NAc), 1.60–1.25 (m, 12 H, 6 CH_2). Anal. Calcd for $C_{58}H_{69}NO_{18} \cdot 0.5\ H_2O$: C, 64.66; H, 6.56; N, 1.30. Found: C, 64.69; H, 6.56; N, 1.30.

Methyl [2-acetamido-4,6-O-*benzylidene-2-deoxy-3*-O-*(8-levulinoyloxyoctyl)-β*-D-*galacto-pyranosyl]-(1→4)-2,3-di*-O-*(4-methylbenzoyl)-*D-*glucopyranuronate* (**10**).—To a solution of **9** (420.4 mg, 393.5 μmol) in MeCN (10 mL) and water (2 mL) was added cerium (IV) ammonium nitrate (650 mg, 1.19 mmol) with stirring at 0 °C. After 1.5 h, $CHCl_3$ was added to the reaction mixture. The organic phase was washed with brine and dried over $HgSO_4$. The volatiles were removed under diminished pressure and the residue was eluted from a column of silica gel (10:1–1:2 toluene–EtOAc) to give **10** (290.1 mg, 77%) as a syrup: ^1H NMR ($CDCl_3$): δ 7.91–7.08 (m, 13 H, Ph), 6.05 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.03 Hz, H-3^I), 5.72 (d, 1 H, *J* 6.58 Hz, NH), 5.68 (m, 1 H, H-1^I), 5.34 (s, 1 H, PhC*H*), 5.25 (d, 1 H, $J_{1,2}$ 8.05 Hz, H-1^{II}), 5.10 (dd, 1 H, $J_{1,2} < 1.0$ Hz, H-2^I), 4.68 (d, 1 H, $J_{4,5}$ 9.27 Hz, H-5^I), 4.42 (dd, 1 H, H-4^I), 4.24 (m, 1 H, H-3^{II}), 4.06–4.01 (m, 3 H, H-4^{II}, CH_2O), 3.92 (d, 1 H, J_{gem} 12.20 Hz, H-6a^{II}), 3.81 (s, 3 H, COOMe), 3.77–3.42 (m, 2 H, H-6b^{II}, 1/2 CH_2O), 3.34–3.17 (m, 2 H, H-2^{II}, 1/2 CH_2O), 2.96 (s, 1 H, H-5^{II}), 2.75–2.54 (m,

4 H, 2 CH_2), 2.33, 2.28, (2 s, 3 H × 2, 2 Ph*Me*), 2.18, (s, 3 H, COMe), 1.97 (s, 3 H, NAc), 1.61–1.25 (m, 12 H, 6 CH_2). Anal. Calcd for $C_{51}H_{63}NO_{17}\cdot H_2O$: C, 62.49; H, 6.70; N, 1.43. Found: C, 62.28; H, 6.63; N, 1.73.

*Methyl [2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(8-levulinoyloxyoctyl)-β-*D*-galacto-pyranosyl]-(1→4)-[2,3-di-*O*-(4-methylbenzoyl)-α-*D*-glucopyranosyl trichloroacetimidate] uronate* (**11**).—To a solution of **10** (226.7 mg, 235.6 μmol) in CH_2Cl_2 (5 mL) were added CCl_3CN (120 μL) and DBU (18 μL) with stirring at 0 °C. After 1 h, the reaction mixture was purified on a column of silica gel (5:1–1:3 toluene–EtOAc) to give **11** (228.7 mg, 88%) as a syrup. A small amount of the product was further purified over PTLC (1:5 toluene–EtOAc): $[\alpha]_D$ +87.4° (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.59 (s, 1 H, NH), 7.90–7.80 (m, 4 H, Ph), 7.33–7.08 (m, 9 H, Ph), 6.75 (d, 1 H, $J_{1,2}$ 3.66 Hz, H-1^I), 6.13 (brt, 1 H, *J* 9.52 Hz, H-3^I), 5.63 (d, 1 H, *J* 7.07 Hz, NH), 5.38 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2^I), 5.33 (s, 1 H, PhC*H*), 5.29 (d, 1 H, $J_{1,2}$ 8.29 Hz, H-1^{II}), 4.60 (d, 1 H, $J_{4,5}$ 10.25 Hz, H-5^I), 4.48 (dd, 1 H, $J_{3,4}$ 9.42 Hz, H-4^I), 4.25 (dd, 1 H, $J_{2,3}$ 10.49, $J_{3,4}$ 3.91 Hz, H-3^{II}), 4.05–4.01 (m, 3 H, H-4^{II}, CH_2O), 3.86–3.76 (m, 1 H, H-6a^{II}), 3.82 (s, 3 H, COOMe), 3.61–3.53 (m, 2 H, H-6b^{II}, 1/2 CH_2O), 3.33 (m, 1 H, H-2^{II}), 3.31–3.17 (m, 1 H, 1/2 CH_2O), 2.86 (s, 1 H, H-5^{II}), 2.75–2.54 (m, 4 H, 2 CH_2), 2.33, 2.28, (2 s, 3 H × 2, 2 Ph*Me*), 2.19, (s, 3 H, COMe), 2.00 (s, 3 H, NAc), 1.58–1.25 (m, 12 H, 6 CH_2). This compound was used for glycosylation without further purification.

*Methyl [2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(8-hydroxyoctyl)-β-*D*-galacto-pyranosyl]-(1→4)-[4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosid]uronate* (**12**).—To a solution of **9** (136.4 mg, 127.7 μmol) in EtOH (10 mL) and toluene (2 mL) was added $H_2NNH_2\cdot$AcOH (120 mg, 1.30 mmol) with stirring for 3 h. The volatiles were removed under diminished pressure and the residue was eluted from a column of gel permeation (LH-20, 1:1 $CHCl_3$–MeOH) to give **12** (117.6 mg) in 95% yield as a syrup: $[\alpha]_D$ +43° (*c* 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.88–6.74 (m, 17 H, Ph), 5.78 (t, 1 H, $J_{2,3} = J_{3,4}$ 8.78 Hz, H-3^I), 5.69 (d, 1 H, *J* 6.83 Hz, NH), 5.53 (dd, 1 H, $J_{1,2}$ 7.08 Hz, H-2^I), 5.33 (s, 1 H, PhC*H*), 5.23 (d, 1 H, $J_{1,2}$ 8.05 Hz, H-1^{II}), 5.21 (d, 1 H, H-1^I), 4.56 (dd, 1 H, $J_{4,5}$ 9.03 Hz, H-4^I), 4.30 (d, 1 H, H-5^I), 4.22 (dd, 1 H, $J_{2,3}$ 10.98, $J_{3,4}$ 3.17 Hz, H-3^{II}), 4.05 (d, 1 H, H-4^{II}), 3.86 (d, 1 H, J_{gem} 12.20 Hz, H-6a^{II}), 3.78, 3.73 (2 s, 3 H × 2, 2 OMe), 3.69–3.23 (m, 5 H, H-6b^{II}, 2 CH_2O, OH), 2.97 (bs, 1 H, H-5^{II}), 2.33, 2.29, (2 s, 3 H × 2, 2 Ph*Me*), 1.95 (s, 3 H, NAc), 1.65–1.26 (m, 12 H, 6 CH_2). Anal. Calcd for $C_{53}H_{63}NO_{16}\cdot H_2O$: C, 64.41; H, 6.64. Found: C, 64.09; H, 6.44.

*Methyl [2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(8-levulinoyloxyoctyl)-β-*D*-galacto-pyranosyl]-(1→4)-[methyl 2,3-di-*O*-(4-methyl-benzoyl)-β-*D*-glucopyranosyluronate]-(1→8)-octyl-(1→3)-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl]-(1→4)-[4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosid] uronate* (**13**).—To a solution of **11** (72.4 mg, 65.4 μmol) and **12** (49.4 mg, 50.9 μmol) in CH_2Cl_2 (3 mL) was added MS AW300 (100 mg). This mixture was stirred for 30 min at rt, and then cooled to −20 °C. To this solution was added a solution of 0.15 M Me_3SiOTf in CH_2Cl_2 (1.0 mL, 0.15 mmol) with stirring and the reaction temperature was gradually raised to rt. After stirring overnight, an excess of Et_3N, CH_2Cl_2, and brine were added. Insoluble materials were filtered on Celite. The organic phase was treated as described in general methods. The crude materials were subjected to a gel permeation chromatography (LH-60, 1:1 $CHCl_3$–MeOH) to give **13** (67.3 mg) in 68% yield as a syrup: $[\alpha]_D$ +72.0° (*c* 0.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.88–6.75 (m, 30 H, Ph), 5.80 (dd, 1 H, $J_{2,3}$ 8.54, $J_{3,4}$ 9.03 Hz, H-3^I), 5.70 (dd, 1 H, $J_{2,3}$ 9.27, $J_{3,4}$ 9.03 Hz, H-3^{III}), 5.61 (d, 1 H, *J* 6.83 Hz, NH), 5.59 (d, 1 H, *J* 7.07 Hz, NH), 5.53 (dd, 1 H, $J_{1,2}$ 7.32 Hz, H-2^I), 5.31, 5.31 (2 s, 1 H × 2, 2 PhC*H*), 5.28 (t, 1 H, $J_{1,2}$ 7.32 Hz, H-2^{III}), 5.22 (d, 1 H, $J_{1,2}$ 8.54 Hz, H-1^{IV}), 5.21 (d, 1 H, H-1^I), 5.17 (d, 1 H, $J_{1,2}$ 8.29 Hz, H-1^{II}), 4.69 (d, 1 H, H-1^{III}), 4.56 (t, 1 H, $J_{4,5}$ 8.57 Hz, H-4^I), 4.42 (t, 1 H, $J_{4,5}$ 9.03 Hz, H-4^{III}), 4.28 (d, 1 H, H-5^I), 4.18–4.16 (m, 2 H, H-3^{II}, 3^{IV}), 4.14 (d, 1 H, H-5^{III}), 4.08–4.01 (m, 6 H, H-4^{II}, 4^{IV}, 2 CH_2O), 3.86–3.24 (m, 10 H, H-2^{II}, 2^{IV}, 6ab^{II}, 6ab^{IV}, 2 CH_2O), 3.82, 3.78, 3.74 (3 s, 3 H × 2, 3 OMe), 2.95 (bs, 1 H,

H-5^{IV}), 2.89 (bs, 1 H, H-5^{II}), 2.74–2.54 (m, 4 H, 2 CH_2), 2.34, 2.31, 2.28, 2.27 (4 s, 3 H × 4, 4 Ph*Me*), 2.22, (s, 3 H, COMe), 1.98, 1.95 (2 s, 3 H × 2, 2 NAc), 1.47–0.96 (m, 12 H, 6 CH_2). Anal. Calcd for $C_{104}H_{124}N_2O_{30}$: C, 65.25; H, 6.54; N, 1.46. Found: C, 65.08; H, 6.73; N, 1.88.

*Methyl [2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(8-hydroxyloctyl)-β-*D*-galacto-pyranosyl]-(1→4)-[methyl 2,3-di-*O*-(4-methyl-benzoyl)-β-*D*-glucopyranosyluronate]-(1→8)-octyl-(1→3)-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl]-(1→4)-[4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosid] uronate* (**14**).—To a solution of **13** (122.9 mg, 64.20 μmol) in EtOH (5mL) and toluene (1 mL) was added H_2NNH_2·AcOH (60.0 mg, 651 μmol) with stirring for 2.5 h. The volatiles were removed under diminished pressure and the residue was diluted with EtOAc and brine. The organic phase was washed first with cold 1 M HCl, and was treated as described in general methods. The crude materials were eluted from a column of silica gel (1:1–1:10 toluene–EtOAc) to give **14** (81.1 mg) in 70% yield as a syrup: $[\alpha]_D$ +58° (*c* 0.34, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.88–6.74 (m, 30 H, Ph), 5.77 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.03 Hz, H-3^I), 5.70 (dd, 1 H, $J_{2,3}$ 9.03, $J_{3,4}$ 9.51 Hz, H-3^{III}), 5.63 (d, 1 H, *J* 6.59 Hz, NH), 5.61 (d, 1 H, *J* 6.59 Hz, NH), 5.53 (dd, 1 H, $J_{1,2}$ 7.07 Hz, H-2^I), 5.31, 5.31 (2 s, 1 H × 2, 2 PhC*H*), 5.28 (dd, 1 H, $J_{1,2}$ 7.32 Hz, H-2^{III}), 5.22 (d, 1 H, $J_{1,2}$ 8.54 Hz, H-1^{IV}), 5.21 (d, 1 H, H-1^I), 5.16 (d, 1 H, $J_{1,2}$ 8.30 Hz, H-1^{II}), 4.69 (d, 1 H, H-1^{III}), 4.56 (dd, 1 H, $J_{4,5}$ 8.54 Hz, H-4^I), 4.42 (dd, 1 H, $J_{4,5}$ 9.03 Hz, H-4^{III}), 4.29 (d, 1 H, H-5^I), 4.20–4.16 (m, 2 H, H-3^{II}, 3^{IV}), 4.15 (d, 1 H, H-5^{III}), 4.03, 4.03 (2d, 2 H, H-4^{II}, 4^{IV}) 3.82–3.26 (m, 17 H, H-2^{II}, 2^{IV}, H-6ab^{II}, H-6ab^{IV}, OH, 4 CH_2O), 3.82, 3.78, 3.74 (3 s, 3 H × 3, 3 OMe), 2.95 (bs, 1 H, H-5^{IV}), 2.89 (bs, 1 H, H-5^{II}), 2.34, 2.31, 2.28, 2.27 (4 s, 3 H × 4, 4 Ph*Me*), 1.98, 1.95 (2 s, 3 H × 2, 2 NAc), 1.68–0.87 (m, 12 H, 6 CH_2). Anal. Calcd for $C_{99}H_{118}N_2O_{30}$·3 H_2O: C, 63.57; H, 6.69. Found: C, 63.50; H, 6.40.

*Methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1→4)-[2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosyl trichloroacetimidate] uronate* (**17**).—To a solution of 4-methoxyphenyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1→4)-[methyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosid] uronate (**16**)[10] (499.7 mg, 539.7 μmol) in MeCN (10 mL) and water (2 mL) was added cerium(IV) ammonium nitrate (900 mg, 1.64 mmol) with stirring at 0 °C. After 3 h, the reaction mixture was treated as described for the synthesis of **10** and was eluted from a column of silica gel (3:1–1:4 toluene–EtOAc) to give the corresponding hemiacetal (326.7 mg, 74%). This hemiacetal was diluted with CH_2Cl_2 (5 mL) and CCl_3CN (0.2 mL). To this solution was added DBU (30 μL) at 0 °C with stirring. After 1 h, the reaction mixture was directly chromatographed on a column of silica gel (5:1–4:3 toluene–EtOAc) to give **17** (305.3 mg, 79%) as a syrup. A small amount of the product was purified over PTLC (1:5 toluene–EtOAc): $[\alpha]_D$ +96.6° (*c* 1.31, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.53 (s, 1 H, NH), 7.83–7.05 (m, 13 H, Ph), 6.66 (d, 1 H, $J_{1,2}$ 3.66 Hz, H-1^I), 6.06 (t, 1 H, *J* 9.76 Hz, H-3^I), 5.30 (m, 1 H, NH), 5.30 (dd, 1 H, H-2^I), 5.24 (s, 1 H, PhC*H*), 5.10 (dd, 1 H, $J_{2,3}$ 10.74, $J_{3,4}$ 3.76 Hz, H-3^{II}), 4.89 (d, 1 H, $J_{1,2}$ 8.30 Hz, H-1^{II}), 4.53 (d, 1 H, $J_{4,5}$ 9.76 Hz, H-5^I), 4.40 (t, 1 H, H-4^I), 4.05 (d, 1 H, H-4^{II}), 3.80 (m, 1 H, H-2^{II}), 3.77 (m, 1 H, H-6a^{II}), 3.76 (s, 3 H, COOMe), 3.50 (dd, 1 H, $J_{5,6b}$ 1.71, J_{gem} 12.44 Hz, H-6b^{II}), 2.82 (s, 1 H, H-5^{II}), 2.26, 2.25 (2 s, 3 H × 2, 2 Ph*Me*), 1.87 (s, 3 H, NAc), 1.07 (s, 9 H, *tert*-Bu). This compound was used for glycosylation without further purification.

*Methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-*D*-galactopyranosyl)-(1→4)-[methyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosyluronate]-(1→8)-octyl-(1→3)-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl]-(1→4)-[4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosid] uronate* (**18**).—To a solution of methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-*D*-galactopyranosyl)-(1→4)-[2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosyl trichloroacetimidate] uronate (**15**,[6] 144.4 mg, 147.6 μmol) and **12** (117.6 mg, 121.2 μmol) in CH_2Cl_2 (5 mL) was added MS AW300 (400 mg). This mixture was stirred for 1 h at rt,

and then cooled to −20 °C. To this was added a solution of Me_3SiOTf (22 μL, 121.6 μmol) in CH_2Cl_2 (1 mL) with stirring and the reaction temperature was gradually raised to rt. After 24 h, the reaction mixture was worked up as described in the synthesis of **13**. The crude materials were purified by gel permeation (LH-60, 1:1 $CHCl_3$–MeOH) and silica-gel chromatography (3:1–1:4 toluene–EtOAc, 1:1 EtOAc–MeOH) to give **18** (161.6 mg) in 75% yield as a syrup: $[\alpha]_D$ +62.0° (*c* 0.20, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.87–6.75 (m, 30 H, Ph), 5.78 (dd, 1 H, $J_{2,3}$ 8.78, $J_{3,4}$ 8.54 Hz, H-3^I), 5.72 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.03 Hz, H-3^{III}), 5.58 (d, 1 H, NH), 5.53 (dd, 1 H, $J_{1,2}$ 7.07 Hz, H-2^I), 5.47 (d, 1 H, NH), 5.29, 5.27 (2 s, 1 H × 2, 2 PhC*H*), 5.23 (dd, 1 H, $J_{1,2}$ 7.56 Hz, H-2^{III}), 5.21 (d, 1 H, $J_{1,2}$ 8.05 Hz, H-1^{II}), 5.20 (d, 1 H, H-1^I), 5.12 (dd, 1 H, $J_{2,3}$ 8.05 Hz, H-3^{IV}), 4.88 (d, 1 H, $J_{1,2}$ 8.05 Hz, H-1^{IV}), 4.69 (d, 1 H, H-1^{III}), 4.56 (t, 1 H, $J_{4,5}$ 8.54 Hz, H-4^I), 4.40 (dd, 1 H, $J_{3,4}$ 9.03, $J_{4,5}$ 9.27 Hz, H-4^{III}), 4.29 (d, 1 H, H-5^I), 4.17–4.01 (m, 4 H, H-3^{II}, 4^{II}, 4^{IV}, 5^{III}), 3.86–3.27 (m, 10 H, H-2^{II}, 2^{IV}, 6ab^{II}, 6ab^{IV}, 2 CH_2O), 3.83, 3.78, 3.74 (3 s, 3 H × 3, 3 OMe), 2.94 (bs, 1 H, H-5^{IV}), 2.89 (bs, 1 H, H-5^{II}), 2.68–2.50 (m, 4 H, 2 CH_2), 2.35, 2.31, 2.28 (3 s, 3 H × 4, 4 Ph*Me*), 2.04 (s, 3 H, COMe), 1.96, 1.94 (2 s, 3 H × 2, 2 NAc), 1.68–1.26 (m, 12 H, 6 CH_2). Anal. Calcd for $C_{96}H_{108}N_2O_{31}\cdot 2\ H_2O$: C, 63.21; H, 6.21; N, 1.54. Found: C, 62.91; H, 5.76; N, 1.95.

*Sodium (2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-(sodium β-*D*-glucopyranosyluronate)-(1→8)-octyl-(1→3)-[2-acetamido-2-deoxy-β-*D*-galactopyranosyl]-(1→4)-(4-methoxyphenyl β-*D*-glucopyranosid] uronate* (**1**).—To a solution of **18** (34.2 mg, 19.2 μmol) in EtOH (5 mL) and toluene (1 mL) was added $H_2NNH_2\cdot$AcOH (18 mg, 0.20 mmol) with stirring. The volatiles were removed under diminished pressure 1 h later and the residue was eluted from a column of gel permeation (LH-60, 1:1 $CHCl_3$–MeOH). Fractions containing the pseudo-hexasaccharide were concentrated and diluted with 1:1 CH_2Cl_2–MeOH (1 mL). Camphorsulfonic acid (22 mg, 95 μmol) was added and the solution was stirred for 24 h. Then an excess of Et_3N was added and the reaction mixture was concentrated to dryness. The residue was purified on a column of gel permeation (LH-20, 1:1 $CHCl_3$–MeOH) and the fractions containing the pseudo-hexasaccharide were concentrated and diluted with THF (5 mL). To the stirred solution was added 1.25 M LiOH (0.35 mL) at 0 °C. The volatiles were removed after 2 h. The residue was diluted with 1:3 CH_2Cl_2–MeOH (4 mL), and 0.5 M NaOH (2 mL) was added. The reaction mixture was stirred overnight and neutralized with HOAc. All the solvents were removed and the residue was purified on a column of gel permeation (LH-20, water) to give **1** as an amorphous hygroscopic powder (7.6 mg, 29% from **18**): $[\alpha]_D$ −16.7° (*c* 0.18, water); ^1H NMR (D_2O): δ 6.98–6.84 (m, 4 H, Ph), 4.87 (d, 1 H, $J_{1,2}$ 7.81 Hz, H-1^I), 4.37 (d, 1 H, $J_{1,2}$ 8.30 Hz, H-1^{II}), 4.35 (d, 1 H, $J_{1,2}$ 8.30 Hz, H-1^{IV}), 4.31 (d, 1 H, $J_{1,2}$ 7.82 Hz, H-1^{III}), 3.99 (d, 1 H, $J_{3,4}$ 2.93 Hz, H-4^{II}), 3.79 (d, 1 H, $J_{3,4}$ 2.93 Hz, H-4^{IV}), 3.78–3.73 (m, 1 H, 1/2 CH_2O), 3.77 (dd, 1 H, H-2^{II}), 3.75 (dd, 1 H, H-2^{IV}), 3.72–3.67 (m, 4 H, H-6^{II,IV}), 3.71, 3.69 (2 d, 2 H, H-5^{I,III}), 3.68 (s, 3 H, OMe), 3.64 (dd, 1 H, H-4^I), 3.63, 3.63 (2 bs, 2 H, H-5^{II,IV}), 3.62 (dd, 1 H, H-4^{III}), 3.60 (m, 2 H, CH_2O), 3.58 (dd, 1 H, H-3^I), 3.56 (dd, 1 H, H-3^{IV}), 3.48 (t, 1 H, $J_{2,3}$ 7.81 Hz, H-2^I), 3.45 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.27 Hz, H-3^{III}), 3.34 (dd, 1 H, H-3^{II}), 3.31–3.24 (m, 1 H, 1/2 CH_2O), 3.19 (dd, 1 H, H-2^{III}), 1.91, 1.91 (2 s, 3 H × 2, 2 NAc), 1.49–1.17 (m, 12 H, 6 CH_2); FAB$^-$MS: *m/z* 1009.5, $[M-H]^-$; FAB$^+$MS: *m/z* 1033.5, $[M+Na]^+$, 1077.4, $[M+3Na-2H]^+$.

*Methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1→4)-[methyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosyluronate]-(1→8)-octyl-(1→3)-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl]-(1→4)-[methyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosyluronate]-(1→8)-octyl-(1→3)-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl]-(1→4)-[4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-*D*-glucopyranosid] uronate* (**19**).—To a solution of **17** (66.6 mg, 37.9 μmol) and **14** (68.8 mg, 37.9 μmol) in CH_2Cl_2 (3 mL) was added MS AW300 (100 mg). This mixture was stirred for 30 min at rt, and then cooled to −20 °C. To this was added a solution of Me_3SiOTf (6.0 μL, 33 μmol) in CH_2Cl_2 (1 mL)

with stirring and the reaction temperature was gradually raised to rt. After 24 h, the reaction mixture was worked up as described in the synthesis of **13**. The crude materials were purified on a column of gel permeation (LH-60, 1:1 $CHCl_3$–MeOH) and then by silica-gel chromatography (2:1–1:4 toluene–EtOAc) to give **19** (42.9 mg) in 43% yield as a white powder: $[\alpha]_D$ +63.6° (*c* 0.28, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.88–6.74 (m, 43 H, Ph), 5.77 (t, 1 H, $J_{2,3} = J_{3,4}$ 8.78 Hz, H-3^I), 5.72 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.03 Hz, H-3^{III}), 5.70 (dd, 1 H, $J_{2,3}$ 8.72, $J_{3,4}$ 9.27 Hz, H-3^V), 5.59, 5.59 (m, 2 H, 2 NH), 5.53 (dd, 1 H, $J_{1,2}$ 7.32 Hz, H-2^I), 5.31 (d, 1 H, *J* 8.78 Hz, NH), 5.31, 5.30, 5.29 (3 s, 1 H × 3, 3 PhC*H*), 5.27–5.23 (m, 2 H, H-2^{III}, H-2^V), 5.22 (d, 1 H, $J_{1,2}$ 8.54 Hz, H-1^{IV}), 5.21 (d, 1 H, H-1^I), 5.16 (d, 1 H, $J_{1,2}$ 8.29 Hz, H-1^{II}), 5.04 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 3.42 Hz, H-3^{VI}), 4.81 (d, 1 H, $J_{1,2}$ 8.05 Hz, H-1^{VI}), 4.69 (d, 1 H, $J_{1,2}$ 7.56 Hz, H-1^{III}), 4.69 (d, 1 H, $J_{1,2}$ 7.56 Hz, H-1^V), 4.56 (t, 1 H, $J_{4,5}$ 9.03 Hz, H-4^I), 4.42 (t, 1 H, $J_{4,5}$ 8.78 Hz, H-4^{III}), 4.39 (t, 1 H, $J_{4,5}$ 9.51 Hz, H-4^V), 4.28 (d, 1 H, H-5^I), 4.19–3.86 (m, 5 H, H-2^{VI}, 3^{II}, 3^{IV}, 5^{III}, 5^V), 4.08 (d, 1 H, H-4^{VI}), 4.03 (d, 1 H, $J_{3,4}$ 2.93 Hz, H-4^{IV}), 4.00 (d, 1 H, $J_{3,4}$ 2.93 Hz, H-4^{II}), 3.86–3.23 (m, 16 H, H-2^{II}, 2^{IV}, $6ab^{II}$, $6ab^{IV}$, $6ab^{VI}$, 4 CH_2O), 3.82, 3.82, 3.78, 3.74 (4 s, 3 H × 4, 4 OMe), 2.94 (bs, 1 H, H-5^{IV}), 2.90 (bs, 1 H, H-5^{II}), 2.87 (bs, 1 H, H-5^{VI}), 2.34, 2.31, 2.31, 2.31, 2.28, 2.27 (6 s, 3 H × 6, 6 Ph*Me*), 1.96, 1.96, 1.92 (3 s, 3 H × 3, 3 NAc), 1.39–0.85 (m, 12 H, 6 CH_2), 1.09 (s, 9 H, *tert*-Bu).

*Sodium (2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-(sodium β-*D*-glucopyranosyluronate)-(1→8)-octyl-(1→3)-(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-(sodium β-*D*-glucopyranosyluronate)-(1→8)-octyl-(1→3)-[2-acetamido-2-deoxy-β-*D*-galactopyranosyl]-(1→4)-(4-methoxyphenyl β-*D*-glucopyranosid] uronate* (**2**).—To a solution of **19** (20.3 mg, 7.76 μmol) in 1:1 CH_2Cl_2–MeOH (1 mL) was added camphorsulfonic acid (18 mg, 77 μmol) followed by stirring for 29 h. Then, an excess of Et_3N was added and the reaction mixture was concentrated to dryness. The residue was chromatographed on a column of gel permeation (LH-20, 1:1 $CHCl_3$–MeOH) and the fractions containing the pseudo-decasaccharide were concentrated and diluted with THF (2 mL). To the stirred solution was added 1.25 M LiOH (0.3 mL) at 0 °C. The volatiles were removed after 1 h. The residue was diluted with 1:3 CH_2Cl_2–MeOH (2 mL), and 0.5 M NaOH (0.3 mL) was added. The reaction mixture was stirred overnight and neutralized with HOAc. Following the purification process described for the synthesis of **1**, the pseudo-decasaccharide **2** was obtained as an amorphous hygroscopic powder (7.2 mg, 56% from **19**): $[\alpha]_D$ −9 ° (*c* 0.09, water); selected ^1H NMR data (D_2O): δ 6.99–6.84 (m, 4 H, Ph), 4.92 (d, 1 H, $J_{1,2}$ 8.54 Hz, H-1^I), 4.41–4.35 (m, 5 H, H-$1^{II,III,IV,V,VI}$), 4.00, 3.98 (m, 2 H, H-$4^{II,IV}$), 3.68 (s, 3 H, OMe), 1.91, 1.90, 1.87 (3 s, 3 H × 3, 3 NAc), 1.49–1.13 (m, 24 H, 12 CH_2); FAB$^-$MS: *m*/*z* 1516.7, $[M-H]^-$; FAB$^+$MS: *m*/*z* 1540.5, $[M+Na]^+$, 1606.4, $[M+4Na-3H]^+$.

Enzymatic transfer of GlcA.—Incubation mixtures contained the following constituents in a total volume of 20 μL: 3 nmol of **1** or **2**, 14.3 μM UDP-D-[^{14}C]GlcA [150 nCi (240,000 dpm)], 171 μM ATP-2Na, 50 mmol NaOAc buffer, pH 5.6, 10 mM $MnCl_2$ and 12 μL of enzyme solution. They were incubated at 37 °C for 12 h. The products were fractionated by gel filtration chromatography on a column (1 × 30 cm) of Superdex Peptide® equilibrated and eluted with 0.25 M NH_4HCO_3, 7% *n*-PrOH. Fractions (0.5 mL each) were collected at a rate of 0.4 mL/min and analyzed for radioactivity. The results are summarized in Table 1.

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