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Synthesis of chondroitin sulfate E octasaccharide in a repeating region involving an acetamide auxiliary

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Abstract—Chondroitin sulfate E repeating octasaccharide was effectively synthesized in a stereocontrolled manner by adopting an acetamide-type disaccharide unit. In the tetrasaccharide synthesis we isolated a characteristic glycosyl imidate as a reactive intermediate. An acetamide auxiliary involves the glycosylation mechanism.

Keywords: Glycosylation; Oligosaccharide; Chondroitin sulfate

1. Introduction

Chondroitin sulfate (CS) is one of the several classes of glycosaminoglycans (GAGs) that widely exists in animal tissues. The nonreducing part of CS contains a repetitive disaccharide region composed of β-D-glucuronic acid (GlcA) and *N*-acetylgalactosamine (GalNAc). Recent reports have described the biological properties of CSs in the repeating disaccharide region, especially at the oligosaccharide level.[1–3] However, little is known about the optimum structures of the CS oligosaccharides, such as the length of the glycans for respective biological activities. These facts prompted us to work toward achieving the systematic and effective syntheses of CS oligosaccharides. In the synthesis of CS repeating oligosaccharides, we optimized the glycan-elongation reaction by adopting the trichloroacetimidate of 2-*O*-(4-methylbenzoyl)GlcA and the 3-OH of GalNAc, and finally succeeded in obtaining the CS repeating octasaccharide (**1**).

2. Results and discussion

We used an acetamide-type (β-GalNAc-GlcA) of disaccharide units **3**[4] by the reduction of azide of **2**[5] for the synthesis of the CS oligosaccharides (Scheme 1). The azide group was hydrogenolyzed in the presence of Pd/C and AcOH, then acetylated with Ac_2O to give **3** in 91% yield (in two steps). Byproduct **4** was generated in the absence of AcOH. In fact, Lindlar-catalyzed reduction of **2** for 13 days resulted in a complete migration of the luvulinoyl group. Subsequent acetylation afforded **4** in 75% yield (in two steps). The 4-methoxyphenyl (MP) group of **3** was removed, and the resulting hemiacetal was converted to the corresponding trichloroacetimidate **5**.[6] As described previously,[6] glycosylation with the acetamide-type substrates **5** + **8**[5] as well as **5** + **12**[6] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.7 equiv of donor) and AW300 molecular sieves in CH_2Cl_2 at −20 °C to room temperature yielded **11** and **22** in 71% and 66%, respectively.

In the synthesis of tetrasaccharide **11**, we observed dynamic changes on TLC analysis during the coupling reaction as follows: (1) **8** was trimethylsilylated at the beginning; (2) the silylated product decreased and a new spot appeared within a few hours; then (3) the

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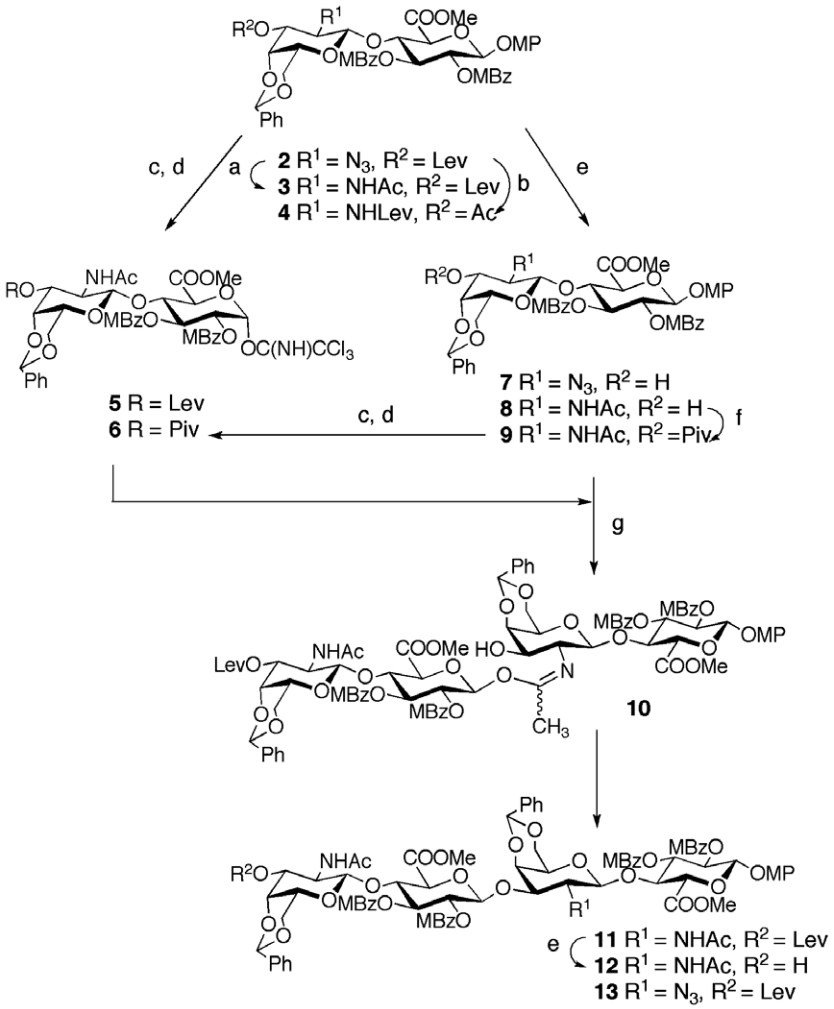


Scheme 1. Synthetic route to the tetrasacharides. Reagents and conditions: (a) Lindlar catalyst, H_2, AcOH/EtOAc; (b) Lindlar catalyst, H_2/EtOAc; (c) CAN/CH_3CN–H_2O; (d) CCl_3CN, DBU/CH_2Cl_2; (e) H_2NNH_2·AcOH/toluene–EtOH; (f) PivCl, DMAP/CH_2Cl_2; (g) TMSOTf, MS AW300/CH_2Cl_2, −20 °C to room temperature, overnight. *Abbreviations*: Lev, $MeCO(CH_2)_2CO$–; MBz, *p*-MeC_6H_4CO–; MP, *p*-$MeOC_6H_4$–, Piv, Me_3CCO–.

TLC pattern became complicated, but (4) the spots finally focused on the desired product **11**. On the other hand, the coupling of the corresponding azide-type substrate did not show such complicated TLC patterns. A spot that transiently appeared in the acetamide-type system may indicate a rather pivotal intermediate that certainly involved the acetamide.

We were able to isolate the product of the coupling reaction of **5** + **8** after 3 h in 50% yield by silica gel column chromatography. This compound was stable only for a few days as a solution in chloroform. The fully characterized ^{1}H NMR spectrum showed H-1^{III} at 6.12 ppm with $J_{1,2} = 7.8$ Hz. The chemical shift of H-2^{III} was not affected, which means the newly formed glycoside did not link through an orthoester-type linkage. The newly generated stereochemistry at C-1^{III} of GlcA was determined as β (no α-isomer was detected), perhaps due to the neighboring-group-effect of GlcA. In addition, the NH^{II} peak disappeared. The ^{13}C NMR spectrum showed two characteristic peaks at 161.41 and 15.99 ppm that can be assigned as N=*C* and N=C–*Me*, respectively. These values were in good agreement with those previously reported by Liao and Auzanneau.[7] We determined the structure of temporary product **10** as depicted in Scheme 1. The MALDITOF MS data also supported the structure.

Imidate **10**, which is of limited stability, seems kinetically formed by a nucleophilic attack of the acetamide oxygen that competes with the vicinal hydroxyl group. Similar glycosyl imidates with an acetamido group have already been reported. In 1976 Pougny and Sinaÿ first isolated the glycosyl imidate formed with fucosyl chloride and fully protected GlcNAc in a Koenigs–Knorr-type condensation.[8] In 1982 Hindsgaul et al. proposed a similar glycosyl imidate formation by circumstantial evidence.[9] In 2003 Liao and Auzanneau isolated the 1,2-*trans* glycosyl imidate by the coupling of rhamosyl trichloroacetimidate with the 4-OH of LacNAc.[7] The

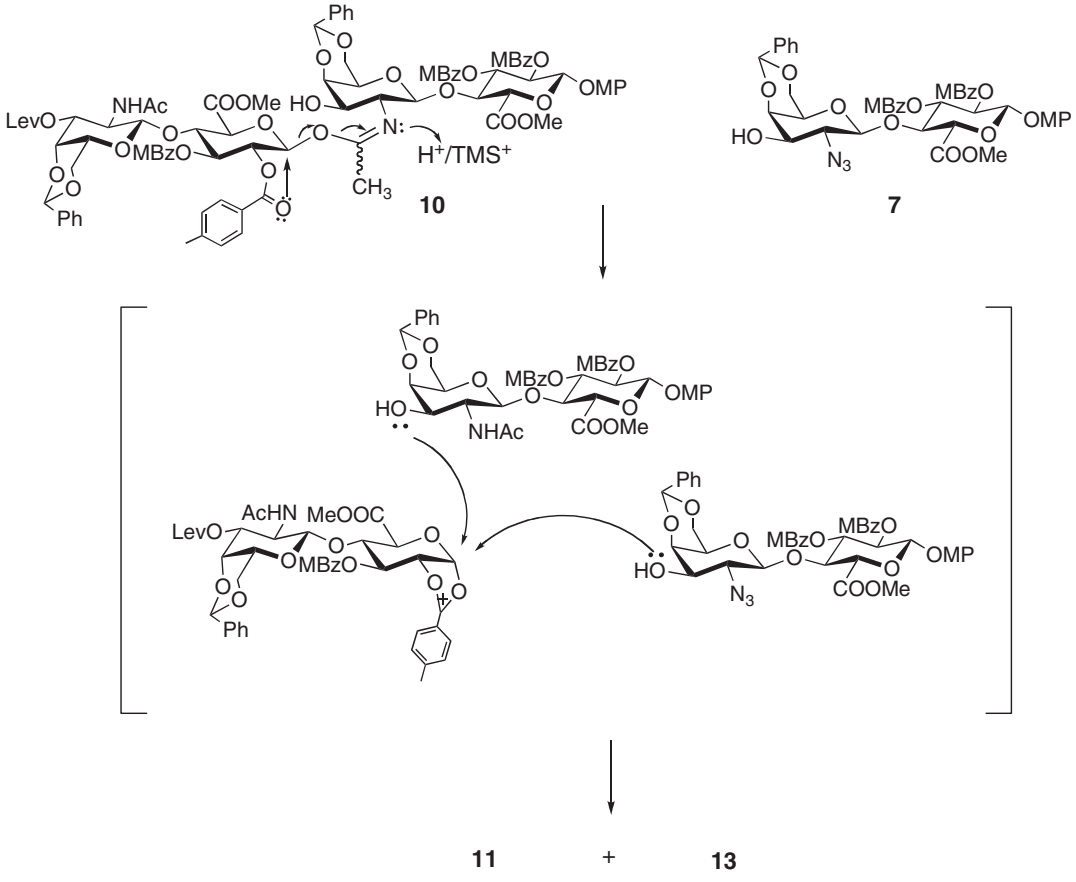
authors reported that the glycosyl imidate independently rearranged to the desired (1→4)-linkage. However, a very recent report by Martin-Lomas and co-workers suggested that the isolated 1,2-*cis* glycosyl imidate was not converted to the final glycoside.[10] Our results showed, at least, that the 3-OH of GalNAc had low reactivity in the glycosylation comparable to that of 4-OH of GlcNAc, which was notoriously difficult to glycosylate.

To investigate the glycosylation mechanism and the role of **10** in detail, we mixed **10** and azide-type acceptor **7**[4,11] in the presence of TMSOTf and AW300 molecular sieves in CH_2Cl_2 at 0 °C to room temperature. The cross reaction gradually proceeded and was completed after 6.5 h to give **11** and **13** in 36% and 18% yields, respectively (Table 1, entry1). The decreased concentration of the reaction mixture gave little change in the ratio of **11/13** (entry 2), while the concentration of **7** affected the ratio (entry 3). These facts suggest that **10** was not converted to **11** via an intramolecular attack including anomerization of **10**, but the reaction proceeded via an SN1 type reaction (Scheme 2).

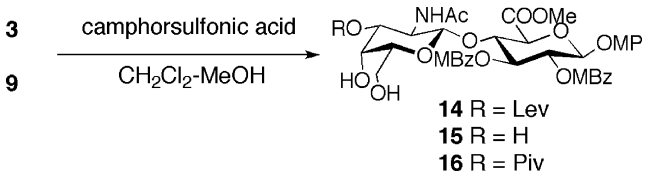
In a previous report[6] on the synthesis of chondroitin sulfate E hexasaccharide, we also described the successful pivaloylation at the nonreducing terminal for the hexasaccharide in a final step. However, the pivaloylation for the hexasaccharide proved to be tricky and sometimes generated overacylated products containing –NAcPiv or –N=C(OPiv)Me type structure in moderate yields (data not shown). This unfavorable reaction happened due to the high reactivity of the acetamides of **19**. In the synthesis of the chondroitin sulfate E repeating oligosaccharide, the protection of the hydroxyl group at the nonreducing end is critical. We realized that the levulinoyl group was not suitable for the reaction by the following reaction. Deprotection of the benzylidene acetal of **3** with camphorsulfonic acid for 5 d generated **14** together with a triol **15** in 54% and 24% yield, respectively. In contrast, the pivaloyl group was not affected under the same reaction conditions

Table 1. Cross reaction of **10** and **7**

Entry	**10** (μmol)	**7** (μmol)	Ratio **10/7**	Solvent (mL)	Yield **11** (%)	Yield **13** (%)	Ratio **11/13**
1	17	21	0.81	2	36	18	2.0
2	22	27	0.81	20	29	12	2.4
3	20	121	0.17	2	13	31	0.4



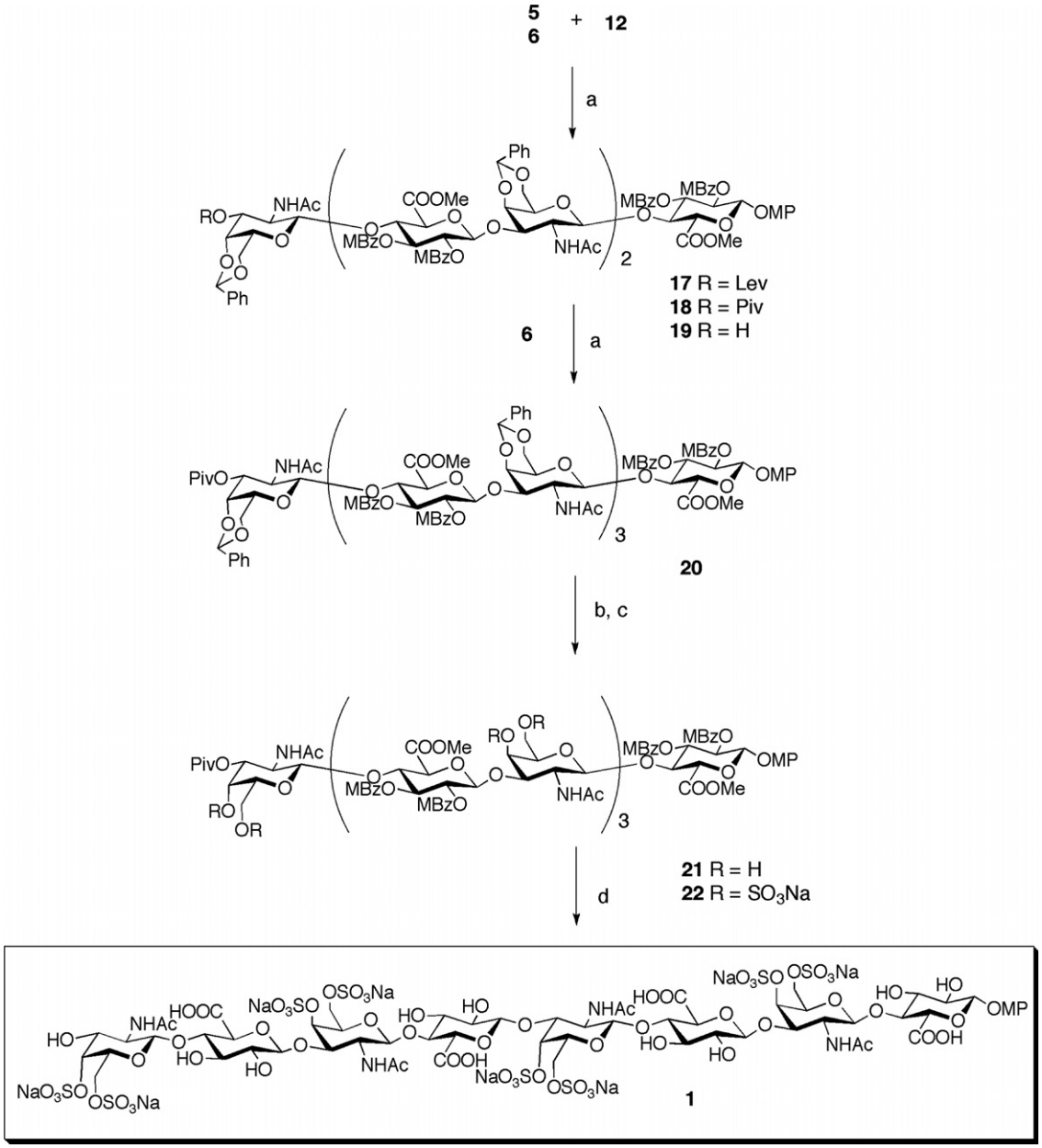
Scheme 2. Postulated reaction pathway via interglycosidic imidate **10**.



Scheme 3. Removal of the benzylidene acetal in an acidic condition.

(Scheme 3). The corresponding pivaloate **9** afforded the 4,6-diol **16** quantitatively.[5] These facts encouraged us to use a pivaloated donor for end-capping.

We examined the glycan elongation with the pivaloated donor **6**, which was obtained from **9** in 71% yield (in two steps). Surprisingly, the coupling of **6** and the tetrasaccharide acceptor **12** in the same manner as above afforded hexasaccharide **18** in 95% yield (Scheme 4). Corresponding levulinated donor **5** afforded **17** in 66% yield.[6] Further elongation on **19**[6] with **6** gave octasaccharide **20** in 58% yield. The four benzylidene acetals of **20** were completely removed with camphorsulfonic acid to afford **21** in 61% yield without affecting the pivaloate, then **21** was sulfated with $SO_3{\cdot}Me_3N$ to give **22** in 95% yield. Finally, all the protecting groups of **22** were removed under basic conditions to give **1** in 70% yield. The ^1H NMR and ESI mass spectra showed satisfactory results.



Scheme 4. Synthesis of targeting chondroitin sulfate E repeating octasaccharide **1**. Reagents and conditions: (a) TMSOTf, MS AW300/CH_2Cl_2, −20 °C to room temperature, overnight; (b) camphorsulfonic acid/CH_2Cl_2–MeOH; (c) $SO_3{\cdot}Me_3N$/DMF; (d) LiOH/aq THF, then NaOH/aq MeOH.

In summary, we optimized the superior glycan-elongation system of the glycosyl donor equipped with 2-*O*-(4-methylbenzoyl)GlcA with the 3-OH of the GalNAc acceptor in the presence of TMSOTf. On the way to the tetrasaccharide we isolated a pivotal tetraosyl intermediate composed of an interglycosidic imidate. Prolonged reaction converted the intermediate to the desired glycoside. Finally we attained a synthesis of chondroitin sulfate E octasaccharide. The reactive and β-selective coupling reaction provided an effective strategy for the synthesis of longer CS oligosaccharides.

3. Experimental

3.1. General methods

Optical rotation values were obtained at 22 ± 3 °C with a Horiba SEPA-200 polarimeter. ^{1}H and ^{13}C NMR assignments were confirmed by 2D HH COSY and HMQC experiments, respectively, with a JEOL ECP 500 MHz spectrometer. Mass spectra were recorded on an Autoflex III MALDITOF mass spectrometer (Bruker Daltonics) and a Q-TOF2 ESI mass spectrometer (Micromass). Signal assignments such as H-1^{III} stand for a proton at C-1 of sugar residue 3. Silica gel chromatography and analytical TLC were, respectively, conducted in a column of Silica Gel 60 (E. Merck), a column of Silica Gel 60N (spherical neutral; Kanto Kagaku), and on glass plates coated with Silica Gel F_{254} (E. Merck). The gel for size-exclusion chromatography (Sephadex LH-20) was purchased from GE Healthcare Bio-Sciences. Molecular sieves (MS) 4A and AW300 were purchased from GL Science and activated at 200 °C under reduced pressure prior to use. All reactions in organic solvents were performed under a dry Ar atmosphere. The usual workup involved the organic phase of the reaction mixture being successively washed with aq $NaHCO_3$ and brine, and dried over anhyd $MgSO_4$.

3.2. Preparation of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-*O*-{4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid}uronate (3)[4]

To a solution of **2**[4] (301.6 mg, 331.4 μmol) in EtOAC (13 mL) were added Lindlar catalyst (618 mg) and HOAc (250 μL). The mixture was vigorously stirred under a hydrogen atmosphere for 1 day. The reaction mixture was passed through Celite, and the volatiles of the filtrate were removed under diminished pressure. To the solution of the residue in EtOAC (13 mL) were added Lindlar catalyst (606 mg) and HOAc (180 μL) again, and the mixture was vigorously stirred overnight under a hydrogen atmosphere. The insoluble materials were removed as above, and the volatiles were removed under diminished pressure. The residue was diluted with pyridine (3 mL). Ac_2O (3 mL) was added to the solution. Two hours later a portion of ice was added to the reaction mixture with stirring overnight. After usual workup the crude materials obtained were passed through a silica gel column chromatography eluted with 5:1→1:1 toluene–EtOAc to give **3** (279.0 mg, 91%) as a syrup. ^{1}H NMR data were in good agreement with those already reported.[4]

3.3. Methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-levulinamido-β-D-galactopyranosyl-(1→4)-*O*-{4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid}uronate (4)

To a solution of **2** (186.0 mg, 201.0 μmol) in EtOAC (10 mL) was added Lindlar catalyst (514 mg). The mixture was vigorously stirred under a hydrogen atmosphere for 3 days. The reaction mixture was passed through a Celite, and the volatiles were removed under diminished pressure. To a solution of the residue in EtOAC (10 mL) was again added Lindlar catalyst (503 mg) and the mixture was vigorously stirred for 10 days under a hydrogen atmosphere. Insoluble materials were removed as above, and the volatiles were removed under diminished pressure. The residue was diluted with MeOH (3 mL), and Ac_2O (3 mL) was added to the solution with stirring overnight. A portion of ice was then added to the reaction mixture. After usual workup the crude materials obtained were passed through a silica gel column and eluted with 10:1→1:10 toluene–EtOAc to give **4** (142.3 mg, 75%) as a syrup: R_f 0.45 (40:1 EtOAc–MeOH); $[\alpha]_D$ +98 (*c* 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.86–7.79 (m, 4H, Ph), 7.49–7.48 (m, 2H, Ph), 7.35–7.31 (m, 3H, Ph), 7.18–7.15 (m, 4H, Ph), 6.92–6.89 (m, 2H, *Ph*OMe), 6.79–6.76 (m, 2H, *Ph*OMe), 5.77 (t, 1H, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3^I), 5.71 (dd, 1H, $J_{1,2} = 8.7$ Hz, H-2^I), 5.48 (s, 1H, PhC*H*), 5.36 (d, 1H, $J_{NH,2} = 10.1$ Hz, NH^{II}), 5.21 (d, 1H, H-1^I), 5.20 (m, 1H, H-1^{II}), 5.06 (dd, 1H, $J_{2,3} = 11.5$ Hz, $J_{3,4} = 1.8$ Hz, H-3^{II}), 4.65 (m, 1H, H-2^{II}), 4.60 (t, 1H, H-4^I), 4.24 (d, 1H, H-5^I), 4.24 (m, 1H, H-6b^{II}), 4.23 (d, 1H, H-4^{II}), 3.99 (d, 1H, $J_{6a,6b} = 12.6$ Hz, H-6a^{II}), 3.75 (2s, 3H × 2, 2OMe), 3.60 (s, 1H, H-5^{II}), 2.76–2.50 (m, 4H, 2CH_2), 2.35 (s, 3H × 2, 2Ph*Me*), 2.05 (s, 3H, $COCH_3$), 1.60 (s, 3H, NAc). Anal. Calcd for $C_{50}H_{53}NO_{17}\cdot 0.5H_2O$: C, 63.27; H, 5.75; N, 1.48. Found: C, 63.42; H, 5.71; N, 1.13.

3.4. Methyl 2-*N*-[(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-D-galactopyranosyl)-(1→4)-*O*-{methyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranuronosyl}]acetimido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl-(1→4)-*O*-{4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid}uronate (10)

To a solution of **5**[6] (87.8 mg, 87.9 μmol, 1.46 equiv) and **8**[5] (51.7 mg, 61.4 μmol) in CH_2Cl_2 (3 mL) was added

MS AW300 (500 mg). The mixture was stirred for 1 h at room temperature and then cooled to −20 °C. TMSOTf (11 μL, 61 μmol) was added while continuing the stirring for 3 h, gradually increasing the temperature to 3 °C. Et_3N (18 μL, 0.13 mmol) was added, the mixture was filtered through cotton, and the volatiles were removed under diminished pressure. The crude materials obtained were passed through a column of silica gel (2:1→1:3 toluene–EtOAc containing 0.1% Et_3N) to give **10** (51.3 mg, 50%) as a syrup: R_f 0.55 (40:1 EtOAc–MeOH); ^{1}H NMR ($CDCl_3$): δ 7.89–7.78 (m, 8H, Ph), 7.33–7.22 (m, 10H, Ph), 7.16–7.07 (m, 8H, Ph), 6.96–6.95 (m, 2H, *Ph*OMe), 6.81–6.79 (m, 2H, *Ph*OMe), 6.12 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1^{III}), 5.76 (t, 1H, $J_{2,3} = J_{3,4} = 8.9$ Hz, H-3^{III}), 5.61 (t, 1H, $J_{2,3} = J_{3,4} = 7.8$ Hz, H-3^{I}), 5.48 (m, 2H, H-2^{III}, NH^{IV}), 5.47 (m, 1H, H-2^{I}), 5.34 (s, 1H, PhC*H*), 5.26 (s, 1H, PhC*H*), 5.17 (d, 1H, $J_{1,2} = 6.2$ Hz, H-1^{I}), 5.12 (dd, 1H, $J_{2,3} = 11.2$ Hz, $J_{3,4} = 3.4$ Hz, H-3^{IV}), 4.87 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1^{IV}), 4.60 (t, 1H, H-4^{I}), 4,54 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1^{II}), 4.44 (br t, 1H, $J = 9.2$ Hz, H-4^{III}), 4.30 (d, 1H, $J_{4,5} = 9.6$ Hz, H-5^{III}), 4.20 (d, 1H, H-5^{I}), 4.14 (d, 1H, $J_{6a,6b} = 11.5$ Hz, H-6a^{II}), 3.97 (d, 1H, H-4^{IV}), 3.88 (m, 1H, H-2^{IV}), 3.85 (m, 1H, H-6b^{II}), 3.84 (m, 1H, H-4^{II}), 3.78 (m, 1H, H-6a^{IV}), 3.78, 3.73, 3.72 (3s, 3H × 3, 3OMe), 3.57 (dd, 1H, $J_{2,3} = 9.2$ Hz, $J_{3,4} = 3.2$ Hz, H-3^{II}), 3.54 (d, $J_{6a,6b} = 12.4$ Hz, 1H, H-6b^{IV}), 3.41 (dd, 1H, H-2^{II}), 3.24 (s, 1H, H-5^{II}), 2.94 (s, 1H, H-5^{IV}), 2.74–2.35 (m, 4H, 2CH_2), 2.30, 2.29, 2.26, 2.24 (4s, 3H × 4, 4Ph*Me*), 2.04 (s, 3H, $COCH_3$), 1.91 (s, 3H, NAc), 1.66 (s, 3H, N=C*Me*); ^{13}C NMR (125 MHz, $CDCl_3$): δ 206.62 (C=O, ketone), 172.11, 170.53, 168.57, 168.36, 165.47, 165.28, 165.18, 164.96 (OC=O, NHC=O), 161.41 (N=*C*Me), 155.99–126.42 (aromatic), 118.56, 114.48 (*o*-, *m*-C of MP), 103.00 (C-1^{II}), 100.95, 100.60 (Ph*C*H × 2), 100.22 (C-1^{IV}), 100.05 (C-1^{I}), 91.92 (C-1^{III}), 77.00 (C-4^{III}), 75.38 (C-4^{II}), 74.66 (C-5^{I}), 74.48 (C-4^{I}), 74.31 (C-5^{III}), 73.09 (C-3^{II}), 73.06 (C-3^{III}), 72.72 (C-4^{IV}), 71.54 (C-2^{I}), 71.46 (C-3^{I}), 71.04 (C-2^{III}), 70.72 (C-3^{IV}), 68.95 (C-6^{II}), 68.19 (C-6^{IV}), 66.82 (C-5^{II}), 66.17 (C-5^{IV}), 61.04 (C-2^{II}), 55.54, 52.88, 52.62 (OMe × 3), 51.47 (C-2^{IV}), 37.75 (CH_2), 29.60 (Me of Lev), 28.13 (CH_2), 23.46 (NCO*Me*), 21.56 (Ph*Me*), 15.99 (N=*C*Me); MALDITOF MS: $[M+Na]^+$ calcd for $C_{88}H_{92}N_2O_{30}$, 1679.56; found, 1679.56.

3.5. Methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-D-galactopyranosyl)-(1→4)-*O*-{methyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosyluronate}-(1→3)-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-{4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid}uronate (13)

To a solution of disaccharide **5** (165.8 mg, 169.5 μmol, 1.3 equiv) and disaccharide **7**[4,11] (107.6 mg, 130.3 μmol) in CH_2Cl_2 (6 mL) was added MS AW300 (800 mg). The mixture was stirred for 1 h at room temperature and then cooled to −20 °C. TMSOTf (22 μL, 0.12 mmol, 0.9 equiv) was added while continuing the stirring overnight, gradually increasing the temperature to room temperature. After similar workup the crude materials obtained were passed through a gel-permeation column (LH-20, 1:1 $CHCl_3$–MeOH) to give tetrasaccharide **13** (154.9 mg, 72%) as a syrup: R_f 0.46 (40:1 EtOAc–MeOH); $[\alpha]_D$ +23 (*c* 0.86, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.96 (d, 2H, $J = 8.0$ Hz, Ph), 7.90–7.78 (m, 6H, Ph), 7.58 (d, 2H, $J = 6.7$ Hz, Ph), 7.41–7.28 (m, 9H, Ph), 7.23–7.09 (m, 5H, Ph), 7.03 (d, 2H, $J = 8.0$ Hz, Ph), 6.92–6.89 (m, 2H, *Ph*OMe), 6.77–6.74 (m, 2H, *Ph*OMe), 5.75 (t, 1H, $J_{2,3} = J_{3,4} = 8.9$ Hz, H-3^{I}), 5.67 (s, 1H, PhC*H*), 5.61 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 7.6$ Hz, H-3^{III}), 5.56 (dd, 1H, $J_{1,2} = 7.3$ Hz, H-2^{I}), 5.34–5.28 (m, 3H, H-1^{III}, 2^{III}, NH^{IV}), 5.26 (s, 1H, PhC*H*), 5.21 (d, 1H, H-1^{I}), 4.81 (dd, 1H, $J_{4,5} = 10.5$ Hz, H-4^{III}), 4.50 (t, 1H, H-4^{I}), 4.48 (d, 1H, $J_{3,4} = 3.4$ Hz, H-4^{II}), 4.43 (m, 1H, H-3^{IV}), 4.38 (d, 1H, H-5^{III}), 4,35 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1^{II}), 4.30 (d, 1H, H-5^{I}), 4.21 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1^{IV}), 4.05 (m, 1H, H-2^{IV}), 3.90 (d, 1H, $J_{3,4} = 3.2$ Hz, H-4^{IV}), 3.86 (s, 3H, OMe), 3.85 (m, 1H, H-6a^{IV}), 3.75–3.74 (2s, 3H × 2, 2OMe), 3.69 (m, 2H, H-2^{II},6a^{II}), 3.56 (d, 1H, $J_{6a,6b} = 12.8$ Hz, H-6b^{II}), 3.49 (d, 1H, $J_{6a,6b} = 12.2$ Hz, H-6b^{IV}), 3.47 (dd, 1H, $J_{2,3} = 10.3$ Hz, H-3^{II}), 3.06 (s, 1H, H-5^{II}), 2.75–2.40 (m, 4H, 2CH_2), 2.59 (s, 1H, H-5^{IV}), 2.45, 2.39, 2.34, 2.30 (4s, 3H × 4, 4Ph*Me*), 2.05 (s, 3H, $COCH_3$), 1.78 (s, 3H, NAc). Anal. Calcd for $C_{86}H_{88}N_4O_{29}\cdot 3.3H_2O$: C, 60.71; H, 5.62; N, 3.29. Found: C, 61.02; H, 5.32; N, 2.89.

3.6. Methyl 2-acetamido-2-deoxy-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-*O*-{4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid}uronate (14) and methyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-*O*-{4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid}uronate (15)

To a solution of **3** (75.5 mg, 80.3 μmol) in CH_2Cl_2 (4.4 mL) and MeOH (4.4 mL) was added camphorsulfonic acid (95.4 mg, 411 μmol) with stirring at room temperature. After 5 days, an excess amount of Et_3N was added to the reaction mixture, and the volatiles were removed under diminished pressure. The crude materials obtained were passed through a gel-permeation column (LH-20, 1:1 $CHCl_3$–MeOH) and a silica gel column eluting with 1:3→1:5 toluene–EtOAc followed by 100:1→10:1 EtOAc–MeOH to give **14** (37.0 mg) and **15** (14.8 mg) in 54 and 24% yield, respectively.

3.6.1. Data for 14. R_f 0.55 (5:1 EtOAc–MeOH); $[\alpha]_D$ +48.0 (*c* 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.91 (d, 2H, $J = 8.0$ Hz, Ph), 7.85 (d, 2H, $J = 8.0$ Hz, Ph), 7.22 (d, 2H, $J = 8.3$ Hz, Ph), 7.17 (d, 2H, $J = 8.3$ Hz, Ph),

6.93–6.91 (m, 2H, Ph), 6.78–6.76 (m, 2H, Ph), 5.73 (t, 1H, $J_{2,3} = J_{3,4} = 8.7$ Hz, H-3^{I}), 5.58 (m, 2H, H-2^{II}, NH), 5.25 (d, 1H, $J_{1,2} = 7.1$ Hz, H-1^{I}), 4.98 (dd, 1H, $J_{2,3} = 8.0$ Hz, $J_{3,4} = 3.0$ Hz, H-3^{II}), 4.85 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1^{II}), 4.47 (dd, 1H, $J_{4,5} = 8.9$ Hz, H-4^{I}), 4.33 (d, 1H, H-5^{I}), 3.98 (m, 1H, H-2^{II}), 3.89 (br s, 1H, H-4^{II}), 3.78, 3.75 (2s, 3H × 2, 2OMe), 3.35 (br s, 1H, H-5^{II}), 3.32 (m, 2H, H-6^{II}ab), 2.89 (d, 1H, $J_{4,OH} = 4.4$ Hz, OH-4^{II}), 2.82–2.48 (m, 4H, 2CH_2), 2.38, 2.36 (2s, 3H × 2, PhMe), 2.17 (s, 3H, $COCH_3$), 1.95 (m, 1H, OH-6^{II}), 1.86 (s, 3H, NAc). Anal. Calcd for $C_{43}H_{49}NO_{17}·H_2O$: C, 59.36; H, 5.92; N, 1.61. Found: C, 59.53; H, 5.61; N, 1.38.

3.6.2. Data for 15. R_f 0.25 (5:1 EtOAc–MeOH); $[\alpha]_D$ +23.8 (*c* 1.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.88 (d, 2H, $J = 8.0$ Hz, Ph), 7.84 (d, 2H, $J = 8.3$ Hz, Ph), 7.22 (d, 2H, $J = 8.3$ Hz, Ph), 7.18 (d, 2H, $J = 8.3$ Hz, Ph), 6.95–6.93 (m, 2H, Ph), 6.80–6.78 (m, 2H, Ph), 6.23 (s, 1H, NH), 5.69 (br t, 1H, $J = 8.3$ Hz, H-3^{I}), 5.63 (dd, 1H, $J_{1,2} = 6.9$ Hz, $J_{2,3} = 9.2$ Hz, H-2^{I}), 5.20 (d, 1H, H-1^{I}), 4.39 (br d, 1H, $J = 9.6$ Hz, H-5^{I}), 4.39 (br d, 1H, $J = 7.1$ Hz, H-1^{II}), 4.37 (br t, 1H, $J = 8.0$ Hz, H-4^{I}), 3.87, 3.76 (2s, 3H × 2, 2OMe), 3.67 (m, 1H, H-2^{II}), 3.66 (m, 1H, H-4^{II}), 3.57 (m, 1H, H-3^{II}), 3.31 (m, 1H, H-6a^{II}), 3.18 (m, 1H, H-6b^{II}), 2.79 (s, 1H, H-5^{II}), 2.38, 2.36 (2s, 3H × 2, 2PhMe), 2.03 (s, 3H, NAc). Anal. Calcd for $C_{38}H_{43}NO_{15}·H_2O$: C, 59.13; H, 5.89; N, 1.82. Found: C, 59.03; H, 5.51; N, 1.56.

3.7. Preparation of 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→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→}$_2$4)-[4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid]uronate (18)[6]

3.7.1. Methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-pivaloyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-(4-methylbenzoyl)-l-*O*-trichloroacetimidoyl-α-D-glucopyranuronate (6). To a solution of **9**[5] (1.72 g, 1.86 mmol) in CH_3CN (80 mL) and H_2O (20 mL) was added cerium(IV) ammonium nitrate (5.08 g, 9.27 mmol) with stirring. After 1 h the reaction mixture was extracted with $CHCl_3$. The organic phase was washed with brine and dried over anhyd $MgSO_4$. The crude materials were eluted through a silica gel column (5:1→1:5 toluene–EtOAc). The hemiacetal thus obtained (1.27 g, 1.55 mmol, 84% yield) was diluted with CH_2Cl_2 (40 mL) and CCl_3CN (6.0 mL, 60 mmol). To the solution was added DBU (60 μL, 0.60 μmol) at 0 °C with stirring for 1 h. The reaction mixture was directly eluted through a silica gel column (5:1→2:1 toluene–EtOAc) to give **6** quantitatively as a syrup that was used for the next reaction without further purification. R_f 0.60 (1:3 toluene–EtOAc); ^{1}H NMR ($CDCl_3$): δ 8.64 (s, 1H, NH), 7.89 (d, 2H, $J = 8.3$ Hz, Ph), 7.81 (d, 2H, $J = 8.3$ Hz, Ph), 7.72–7.16 (m, 5H, Ph), 7.14–7.12 (m, 4H, Ph), 6.73 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1^{I}), 6.13 (t, 1H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3^{I}), 5.37 (dd, 1H, H-2^{I}), 5.36 (d, 1H, $J_{2,NH} = 7.3$ Hz, NH^{II}), 5.31 (s, 1H, PhCH), 5.16 (dd, 1H, $J_{2,3} = 11.5$ Hz, $J_{3,4} = 3.4$ Hz, H-3^{II}), 4.96 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1^{II}), 4.60 (d, 1H, $J_{4,5} = 10.1$ Hz, H-5^{I}), 4.46 (t, 1H, H-4^{I}), 4.11 (d, 1H, H-4^{II}), 3.88 (m, 2H, H-6a^{II}, 2^{II}), 3.82 (s, 3H, OMe), 3.56 (dd, 1H, $J_{5,6a} = 1.6$ Hz, $J_{gem} = 12.4$ Hz, H-6b^{II}), 2.88 (s, 1H, H-5^{II}), 2.33, 2.32 (2s, 3H × 2, 2PhMe), 1.94 (s, 3H, NAc), 1.13 (s, 9H, *tert*-Bu).

3.7.2. 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→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→}$_2$4)-[4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid]uronate (18).[6] To a solution of **6** (6.28 g, 6.51 mmol) and **12** (5.08 g, 3.26 mmol) in CH_2Cl_2 (255 mL) was added MS AW300 (15.4 g). The mixture was stirred for 1 h at room temperature and then cooled to −20 °C. TMSOTf (0.88 mL, 4.9 mmol) was added while continuing the stirring for 4 h until a temperature of 0 °C was reached. Additional TMSOTf (0.38 mL, 2.1 mmol) was then added with a gradual increase to room temperature and stirring overnight. The reaction was worked up by the addition of excess amount of Et_3N (1.8 mL) and aq $NaHCO_3$. $CHCl_3$ was added to the mixture, and the insoluble materials were removed by filtering through Celite. The organic phase was washed with aq $NaHCO_3$ and brine, and dried over anhyd $MgSO_4$. The crude materials thus obtained were passed through a gel-permeation column (LH-20, 1:1 $CHCl_3$–MeOH) and a silica gel column (2:1→1:15 toluene–EtOAc followed by 50:1→5:1 EtOAc–MeOH) to give **18** (7.32 g) in 95% yield. Physical data were in good agreement with those already reported.[6]

3.8. 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→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→}$_3$4)-[4-methoxyphenyl 2,3-di-*O*-(4-methylbenzoyl)-β-D-glucopyranosid]uronate (20)

To a solution of **6** (233.2 mg, 0.242 mmol) and **19** (277.2 mg, 0.122 mmol) in CH_2Cl_2 (10 mL) was added MS AW300 (580 mg). The mixture was stirred for 1 h at room temperature and then cooled to −20 °C. TMSOTf (44 μL, 0.24 mmol) was added while continuing the stirring for 1 day and gradually increasing the temperature to room temperature. The reaction was worked up by the addition of an excess amount of

Et_3N (67 μL) and aq $NaHCO_3$. $CHCl_3$ was added to the mixture, and the insoluble materials were removed by filtering through Celite. The organic phase was washed with aq $NaHCO_3$ and brine, and dried over anhyd $MgSO_4$. The crude materials obtained were passed through a gel-permeation column (LH-20, 1:1 $CHCl_3$–MeOH) to give **20** (219.0 mg) in 58% yield: R_f 0.73 (40:1 EtOAc–MeOH); $[\alpha]_D$ +11.6 (*c* 0.88, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.97 (d, 2H, $J = 8.2$ Hz, Ph), 7.92 (m, 4H, Ph), 7.87–7.79 (m, 12H, Ph), 7.66 (d, 2H, $J = 7.6$ Hz, Ph), 7.56 (m, 2H, Ph), 7.45 (m, 2H, Ph), 7.40–7.35 (m, 3H, Ph), 7.32–7.24 (m, 11H, Ph), 7.21–7.12 (m, 12H, Ph), 7.06 (d, 2H, $J = 8.3$ Hz, Ph), 6.89–6.87 (m, 2H, Ph), 6.76–6.73 (m, 2H, Ph), 5.72 (t, 1H, $J_{2,3} = J_{3,4} = 8.9$ Hz, H-3^{I}), 5.70 (s, 1H, Ph*CH*), 5.58 (m, 4H, H-3^{III}, 3^{V}, 3^{VII}, Ph*CH*), 5.47 dd, (1H, $J_{1,2} = 7.1$ Hz, H-2^{I}), 5.45 (s, 1H, Ph*CH*), 5.42 (d, 1H, $J_{2,NH} = 6.9$ Hz, NH^{VIII}), 5.29 (s, 1H, Ph*CH*), 5.17 (m, 3H, H-1^{I}, $2^{III/V/VII}$, 1^{VIII}), 5.04 (br t, 1H, $J = 4.0$ Hz, H-$2^{III/V/VII}$), 4.96 (m, 4H, H-1^{III}, 1^{V}, 1^{VII}, $2^{III/V/VII}$), 4.86 (dd, 1H, $J_{3,4} = 7.3$ Hz, $J_{4,5} = 10.8$ Hz, H-4^{VII}), 4.73 (m, 2H, H-$4^{III/V}$, $NH^{2/4/6}$), 4.67–4.58 (m, 3H, H-$1^{II/IV/VI}$, $4^{V/III}$, 3^{VIII}), 4.50 (t, 1H, H-4^{I}), 4.49 (d, 1H, $J_{3,4} = 4.1$ Hz, H-$4^{II/IV/VI}$), 4.40 (d, 1H, $J_{3,4} = 3.2$ Hz, H-$4^{II/IV/VI}$), 4.38 (d, 1H, $J_{2,NH} = 8.7$ Hz, $NH^{II/IV/VI}$), 4.32 (d, 1H, H-5^{VII}), 4.30 (d, 1H, $J_{3,4} = 3.4$ Hz, H-4^{VIII}), 4.27 (d, 1H, $J_{4,5} = 10.3$ Hz, H-$5^{III/V}$), 4.19 (br d, 3H, $J = 9.1$ Hz, H-5^{I}, $5^{V/III}$, $1^{II/IV/VI}$), 4.12 (br d, 3H, $J = 4.4$ Hz, H-$1^{II/IV/VI}$ $4^{II/IV/VI}$, $NH^{II/IV/VI}$), 3.99–3.78 (m, 7H, H-$2^{II/IV/VI} \times 2$, $3^{II/IV/VI} \times 2$, $6ab^{II/IV/VI}$, $6a^{VIII}$), 3.75–3.49 (m, 7H, H-$2^{II/IV/VI}$, $3^{II/IV/VI}$, $6ab^{II/IV/VI} \times 2$, $6b^{VIII}$), 3.73, 3.70, 3.68, 3.64, 3.64 (5s, $3H \times 5$, 5OMe), 3.22 (m, 1H, H-2^{VIII}), 2.97 (s, 1H, H-5^{VIII}), 2.78, 2.68, 2.49 (3s, $1H \times 3$, H-5^{II}, 5^{IV}, 5^{VI}), 2.44, 2.41, 2.40, 2.39, 2.36, 2.34, 2.30 (7s, 24H, 8*Me*Ph), 1.75, 1.68, 1.67, 1.63 (4s, $3H \times 4$, 4NAc), 1.07 (s, 9H, *tert*-Bu). Anal. Calcd for $C_{164}H_{172}N_4O_{55}\cdot 4H_2O$: C, 62.50; H, 5.77; N, 1.77. Found: C, 62.42; H, 5.48; N, 1.44.

3.9. Disodium 2-acetamido-2-deoxy-4,6-di-*O*-sulfonate-β-D-galactopyranosyl-(1→[4)-β-D-glucopyranosyluronic acid-(1→3)-(disodium 2-acetamido-2-deoxy-4,6-di-*O*-sulfonate-β-D-galactopyranosyl)-(1→]$_3$4)-β-D-glucopyranosiduronic acid (1)

Camphorsulfonic acid (28.2 mg, 121 μmol) was added to a solution of **20** (55.6 mg, 18.0 μmol) in CH_2Cl_2 (4.2 mL) and MeOH (4.2 mL) while stirring. After stirring for 22 h an excess amount of Et_3N was added to the reaction mixture, and the volatiles were removed under reduced pressure. The residue was subjected to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) to give **21** (30.3 mg) in 61% yield. ^1H NMR measurement indicated complete disappearance of the four benzylidene acetals. To a solution of **21** (30.3 mg, 11.1 μmol) in DMF (2 mL) was added $SO_3\cdot Me_3N$ (246 mg, 1.77 mmol) while stirring at 60 °C for 3 days. The reaction mixture was then cooled to room temperature and subjected directly to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) and an ion-exchange resin column [Dowex AG50W-X8 (Na^+), 8:1 MeOH–H_2O] to give **22** (37.4 mg) in 95% yield. This compound was used for the next reaction without further purification. To a solution of **22** (37.4 mg, 10.5 μmol) in THF (2.2 mL) and H_2O (0.3 mL) was added 1.25 M LiOH (0.6 mL) at 0 °C. After stirring overnight, all the volatiles were removed in vacuo. The residue was dissolved in MeOH (1.6 mL) and CH_2Cl_2 (0.3 mL), to which was added 0.5 M NaOH (1 mL) dropwise while stirring at room temperature. After 18 h, the reaction was quenched with 50% AcOH. All the volatiles were removed under reduced pressure, and the residue was subjected to gel-permeation chromatography (LH-20, 1% AcOH) to afford **1** (18.0 mg) in 70% yield: R_f 0.32 (RP-TLC, 82:18 CH_3CN–H_2O); $[\alpha]_D$ +4.9 (*c* 0.61, H_2O); ^1H NMR (D_2O): δ 7.13–7.09 (m, 2H, Ph), 6.99–6.97 (m, 2H, Ph), 5.09 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1^{I}), 4.79 [3s, 3H, H-4 (GalNAc × 3)], 4.72 [s, 1H, H-4 (GalNAc)], 4.68 [d, 1H, $J_{1,2} = 7.1$ Hz, H-1 (GalNAc)], 4.66 [d, 1H, $J_{1,2} = 6.9$ Hz, H-1(GalNAc)], 4.62–6.55 [m, 5H, H-1 (GlcA × 3, GalNAc × 2)], 4.30–4.19 [m, 8H, H-6ab (GalNAc × 4)], 4.16 (d, 1H, $J_{4,5} = 9.6$ Hz, H-5^{I}), 4.11–4.07 [m, 4H, H-5 (GalNAc × 4)], 4.09–4.06 [m, 3H, H-3 (GalNAc × 3)], 4.03–3.97 [m, 6H, H-5 (GlcA × 3), H-2 (GalNAc × 3)], 3.94 (t, 1H, H-4^{I}), 3.89 [m, 2H, H-2, 3 (GalNAc)], 3.89–3.83 [m, 3H, H-4 (GlcA × 3)], 3.81 (s, 3H, OMe), 3.79 (m, 1H, H-3^{I}), 3.68–3.64 [m, 3H, H-3 (GlcA × 3)], 3.64 (m, 1H, H-2^{I}), 3.44–3.40 [m, 3H, H-2 (GlcA × 3)], 2.02, 2.00, 1.99, 1.99 (4s, $4H \times 3$, 4NAc). ESIMS: calcd for $C_{63}H_{81}N_4O_{70}S_8Na_8$ $(M-3H)^{3-}$, 817.66; found, 817.65; calcd for $C_{63}H_{82}N_4O_{70}S_8Na_7$ $(M-Na-2H)^{3-}$, 810.33; found, 810.32; calcd for $C_{63}H_{83}N_4O_{70}S_8Na_6$ $(M-2Na-H)^{3-}$, 803.01; found, 803.00; calcd for $C_{63}H_{84}N_4O_{70}S_8Na_5$ $(M-3Na)^{3-}$, 795.68; found, 795.67.

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Supplementary data

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