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Enzymatic glycosidation of sugar oxazolines having a carboxylate group catalyzed by chitinase

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Abstract—Enzymatic glycosidation using sugar oxazolines **1**–**3** having a carboxylate group as glycosyl donors and compounds **4**–**6** as glycosyl acceptors was performed by employing a chitinase from *Bacillus* sp. as catalyst. All the glycosidations proceeded with full control in stereochemistry at the anomeric carbon of the donor and regio-selectivity of the acceptor. The *N*,*N*′-diacetyl-6′-*O*-carboxymethylchitobiose oxazoline derivative **1** was effectively glycosidated, under catalysis by the enzyme, with methyl *N*,*N*′-diacetyl-β-chitobioside (**4**), pent-4-enyl *N*-acetyl-β-D-glucosaminide (**5**), and methyl *N*-acetyl-β-D-glucosaminide (**6**), affording in good yields the corresponding oligosaccharide derivatives having 6-*O*-carboxymethyl group at the nonreducing GlcNAc residue. The *N*,*N*′-diacetyl-6-*O*-carboxymethylchitobiose oxazoline derivative **2** was subjected to catalysis by the enzyme catalysis; however, no glycosidated products were produced through the reactions with **4**, **5**, and **6**. Glycosidation reactions of the β-D-glucosyluronic-(1→4)-*N*-acetyl-D-glucosamine oxazoline derivative **3** proceeded with each of the glycosyl acceptors, giving rise to the corresponding oligosaccharide derivative having a GlcA residue at their nonreducing termini in good yields.

Keywords: Enzymatic glycosidation; Functionalized sugar oxazolines; Chitinase from *Bacillus* sp.; End functionalization

1. Introduction

Carbohydrates exist in all living cells as components of such glycoconjugates as glycoproteins, glycolipids, and proteoglycans. They play critical roles for vital activities of living cells; adhesion,[1] development and proliferation of cells,[2] transformation and metastasis of tumors,[3] infection of microorganisms,[4] and parasites,[5] and such autoimmune diseases as rheumatoid arthritis.[6] Chemical synthesis provides structurally well-defined carbohydrate samples for studying these processes, via organo-chemical or enzyme-catalyzed methods. These methodologies require a glycosyl donor and a glycosyl acceptor. Various glycosyl donors have been found effective;[7–9] but, control of anomeric stereo- and regio-chemistry is often difficult.[10,11]

Enzyme-catalyzed synthesis of carbohydrates may be performed with a nonprotected glycosyl donor and acceptor with catalysis by a glycosyltransferase or glycoside hydrolase.[12] The former enzyme catalyzes glycosidic bond formations exclusively, whereas the latter, catalyzing hydrolytic bond-cleavage, permits bond formation under selected reaction conditions in vitro. Such in vitro catalyses may be utilized for constructing structurally complex carbohydrates, because of the high regio- and stereo-selectivity of the reactions. Glycoside hydrolases are readily available, are more stable, and less expensive than glycosyltransferases, and have frequently been used for glycosidation reactions, including polymerizations requiring a repeated glycosidation.[13,14]

We previously reported the synthesis of *N*,*N*′-diacetylchitobiose [β-GlcNAc-(1→4)-GlcNAc]; via enzymatic glycosidation of an activated form of an *N*-acetyl-D-glucosamine (GlcNAc) oxazoline derivative as the glycosyl donor, with GlcNAc as the glycosyl acceptor.[15] The reaction was catalyzed by a chitinase from *Bacillus* sp. (EC 3.2.1.14), one of the *endo*-glycanases responsible for chitin hydrolysis, belonging to the glycoside hydrolase family 18.[16] In this glycosidation, the oxazoline part

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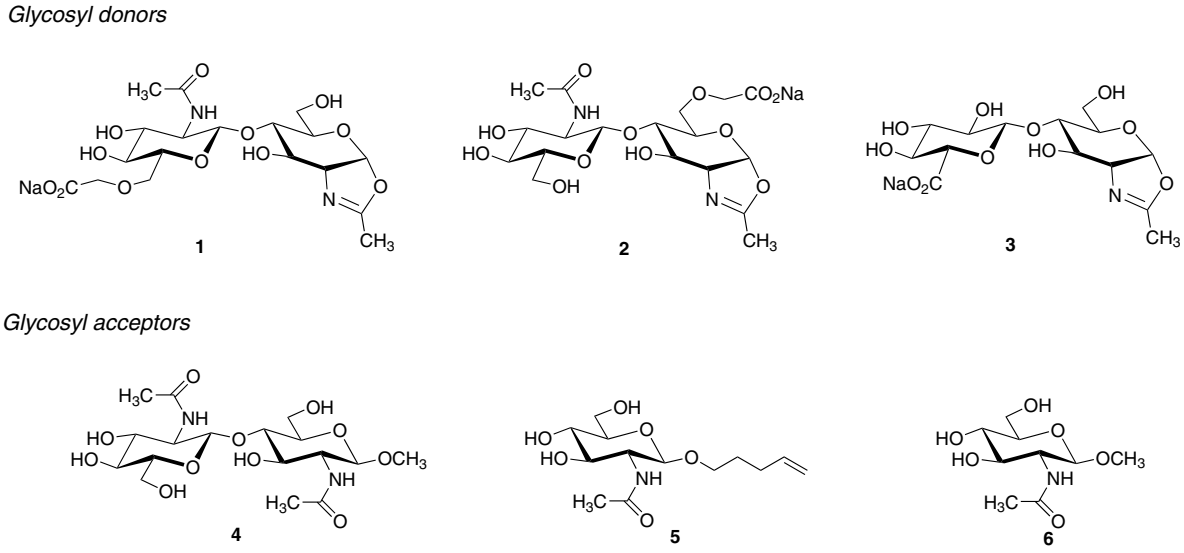


Figure 1. Sugar oxazoline derivatives having a carboxylate group, designed as glycosyl donors (**1**–**3**), and glycosyl acceptors (**4**–**6**).

on the glycosyl donor is key for the reaction; the transition state of the enzymatic hydrolysis involves an oxazolinium ion species as a high-energy form structurally analogous to the oxazoline.[17] The GlcNAc oxazoline derivative is, therefore, immediately recognized and catalyzed by the chitinase as a 'transition-state analogue substrate', serving as an activated glycosyl donor. This concept has been applied to the synthesis of natural heteropolysaccharides of hyaluronan[18] and chondroitin.[19]

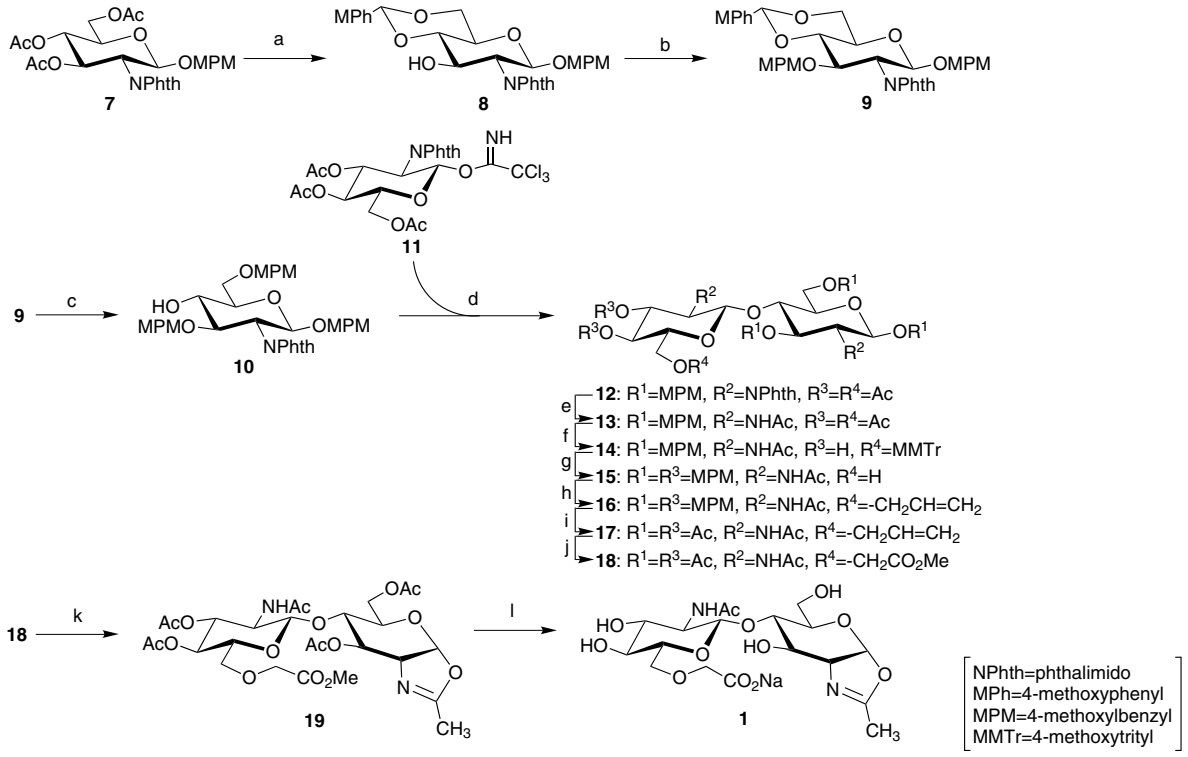
Few reports describe the synthesis of functionalized oligosaccharides via enzymatic glycosidation using functionalized glycosyl donors.[20] The functionalization of oligosaccharides through enzymatic glycosidation is useful for facile and rapid production of such artificial glycoconjugates as neoglycoconjugates,[21] which are frequently used as tools for investigating carbohydrate functions in vivo. Here we report glycosidation reactions catalyzed by a chitinase from *Bacillus* sp., using new sugar oxazoline derivatives (**1**–**3**) having a carboxylate functional group as glycosyl donors and GlcNAc derivatives (**4**–**6**) as glycosyl acceptors. The functional group may serve for further chemical modifications of the products (Fig. 1).

2. Results and discussion

2.1. Synthesis of disaccharide oxazoline derivatives having a carboxylate group

The newly designed oxazoline derivative (**1**) was prepared as outlined in Scheme 1. Compound **10**, synthesized via a four-step reactions from **7**[22] was glycosylated with a readily accessible glycosyl trichloroacetimidate **11** to afford the disaccharide derivative **12**. Compound **13** was prepared by removal of the phthalimido group of **12** by hydrazine monohydrate followed by acetylation. The *O*-acetyl groups of **13** were all removed by sodium methoxide, followed by selective protection of the 6′-hydroxyl group by the 4-methoxytrityl (MMTr) group to provide **14**. All of the hydroxyl groups of **14** were protected by 4-methoxybenzyl (MPM) groups and then MMTr group was hydrolyzed by aqueous acetic acid, giving rise to **15**. Allyl bromide was allowed to react with the 6′-hydroxyl group of **15**, converting it into **16**. The MPM groups of **16** were all removed by the oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), with subsequent acetylation of the hydroxyl groups to produce **17**. The alkenyl group of **17** was oxidized to the carboxylic acid group by ozonolysis and then methylated by Dowex 50W-X4 (H^+ form) in methanol to give **18**. Compound **18** was treated with trimethylsilyl triflate (Me_3SiOTf) to form the oxazoline derivative **19**. The *O*-acetyl groups of **19** were removed by sodium methoxide, and the methyl ester was hydrolyzed in a carbonate buffer to provide the 6′-*O*-carboxymethylated oxazoline **1**.

The novel oxazoline derivative **2** was also synthesized chemically (Scheme 2). Compound **11** was glycosidated with **21** prepared via two-step reactions from **9** to afford **22**. The phthalimido group of **22** was converted to an amino group by treatment with hydrazine monohydrate, followed by acetylation to give **23**. All of the *O*-acetyl groups of **23** were removed by the action of sodium methoxide in methanol, and the resulting hydroxyl groups were protected by the MPM group to provide **24**. The *tert*-butyldimethylsilyl (TBDMS) group of **24** was removed by tetra-*n*-butylammonium fluoride in tetrahydrofuran (THF), giving rise to **25**. The hydroxyl group of **25** reacted with allyl bromide in *N*,*N*-dimethylformamide (DMF) to produce **26**. Compound **27** was obtained through the complete removal of the MPM groups from **26** by DDQ oxidation followed by acetyl-



Scheme 1. Reagents and conditions: (a) (i) NaOMe/MeOH, rt, 2 h, (ii) 4-$MeOC_6H_4CH(OMe)_2$, CSA/DMF, 30 °C, 4 h, 80% (two steps); (b) MPMCl, NaH, *n*-Bu_4NI/DMF, rt, overnight, 51%; (c) $NaCNBH_3$, CF_3CO_2H, MS3A/DMF, rt, 48 h, quant.; (d) $Me_3SiOSO_2CF_3$, MS4A/CH_2Cl_2, −20 °C, 1 h, 91%; (e) (i) NH_2NH_2 monohydrate/EtOH, 90 °C, 5 h, (ii) Ac_2O-pyridine, rt, 3 h, 88% (two steps); (f) (i) NaOMe/MeOH, rt, 1 h, quant, (ii) MMTrCl/pyridine, rt, 7 h, 96%; (g) MPMCl, NaOH/DMF, rt, overnight, then 70% aq AcOH, 50 °C, 4 h, 71% (two steps); (h) CH_2=$CHCH_2Br$, NaOH/DMF, rt, 3 h, 92%; (i) (i) DDQ/CH_2Cl_2–H_2O, rt, 4 h, (ii) Ac_2O–pyridine, rt, 4 h, 90% (two steps); (j) (i) O_3/CH_2Cl_2–MeOH, −78 °C, 0.5 h, (ii) $NaClO_2$, NaH_2PO_4, Me_2C=CHMe/*tert*-BuOH–H_2O, rt, 2 h, (iii) Dowex 50W-X4 (H^+ form)/MeOH, 40 °C, 4.5 h, 74% (three steps); (k) $Me_3SiOSO_2CF_3$/CH_2ClCH_2Cl,40 °C, 12 h, 87%; (l) NaOMe/MeOH, rt, 2 h, then carbonate buffer (25 mM, pH 12), rt, 2 h.

ation. The alkenyl group of **27** was oxidized to the carboxylic acid form of **28** by ozonolysis followed by methylation in methanol with Dowex 50W-X4 (H^+ form) as catalyst. Formation of the oxazoline ring to furnish **29** was performed in 1,2-dichloroethane by using Me_3-SiOTf. The *O*-acetyl groups of **29** were all removed by sodium methoxide in methanol, followed by hydrolysis of the methyl ester in a carbonate buffer, giving rise to the 6-*O*-carboxymethylated oxazoline derivative **2**.

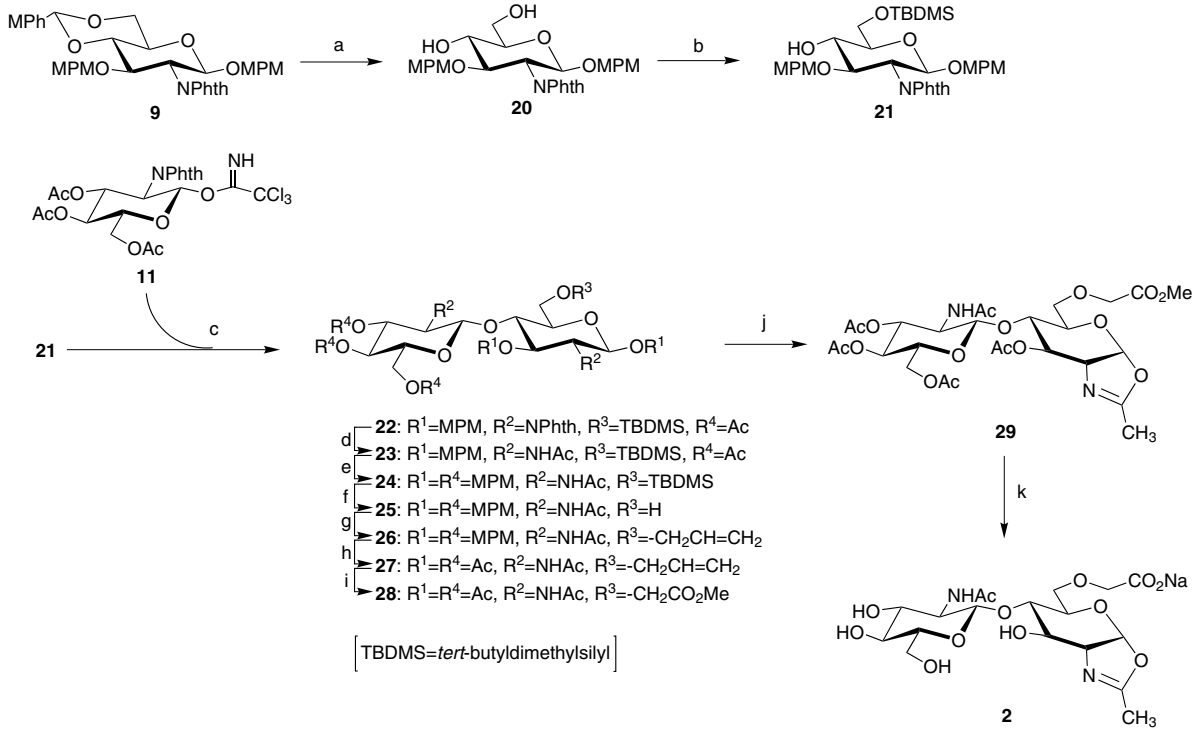
The new oxazoline derivative **3** was synthesized as a potential glycosyl donor for the chitinase (Scheme 3). The acetylated methyl glucuronate trichloroacetimidate **30**[23] was glycosidated with the readily accessible compound **31**[24] to give the disaccharide **32**. The oxazoline derivative **33** was produced by the treatment of **32** with Me_3SiOTf in 1,2-dichloroethane. Compound **33** was *O*-deacylated by sodium methoxide in methanol, and the methyl ester was hydrolyzed in carbonate buffer to yield the target compound **3**.

2.2. Chitinase-catalyzed hydrolysis of the oxazoline derivatives

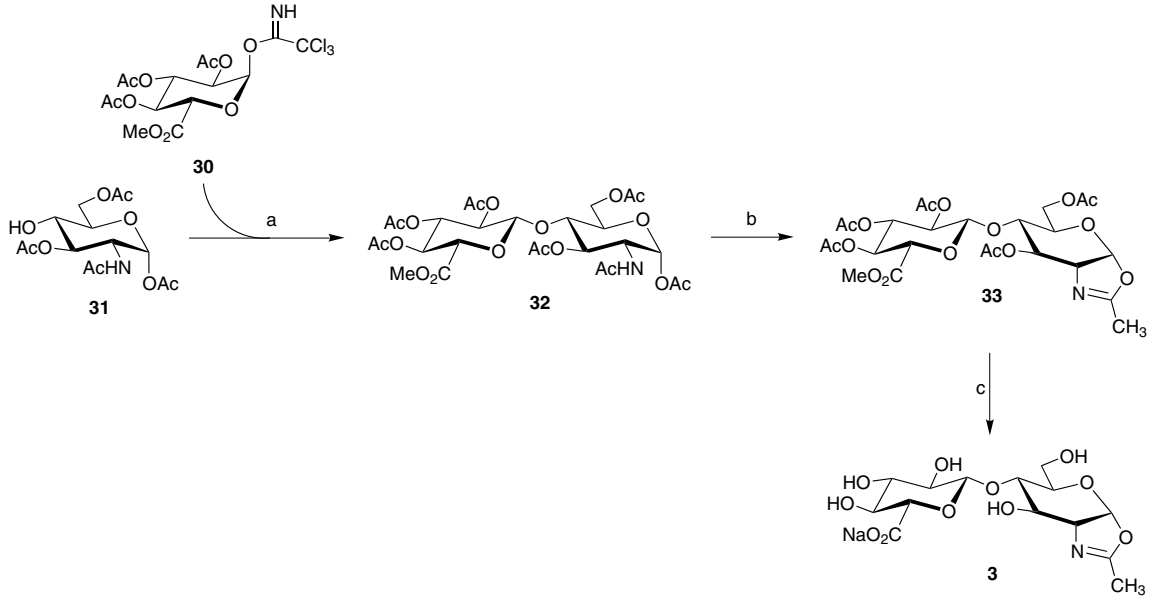
Compounds **1**, **2**, and **3** were subjected as substrates to the enzymatic hydrolysis catalyzed by the chitinase from *Bacillus* sp. without any glycosyl acceptor in a carbonate buffered D_2O (50 mM, pD 9.0) at 30 °C. The initial concentration of the substrate was 0.1 M and the amount of enzyme was 10 wt% for the substrate. The reaction was monitored by ^1H NMR spectroscopy (Fig. 2). Without the enzyme the substrates were not consumed, whereas with addition of the enzyme the substrates were completely consumed after 4, 6, and 15 h for **1**, **3**, and **2**, respectively. These observations indicate that these substrates were recognized and activated by enzyme–substrate complex formation. The reactivity order in hydrolysis, **1** > **3** > **2**, reflects the ability of these substrates to be recognized at the donor site of the enzyme. The products were confirmed as being **34**, **35**, and **36**, derived by hydrolysis via oxazoline ring opening from **1**, **2**, and **3**, respectively (Scheme 4). Analytical data for **34**, **35**, and **36** are given in Section 4.

2.3. Glycosidation reactions of 1 catalyzed by chitinase

The oxazoline derivative **1** as a glycosyl donor was allowed to react with each glycosyl acceptor, methyl-*N*,*N*′-diacetyl-β-chitobioside (**4**), pent-4-enyl *N*-acetyl-β-D-glucosaminide (**5**), or methyl *N*-acetyl-β-D-glucosaminide (**6**). Compound **5** was designed as glycosyl



Scheme 2. Reagents and conditions: (a) 70% aqAcOH, 70 °C, 1 h, 83%; (b) TBDMSCl/pyridine, rt, 1 h, 97%; (c) BF_3 etherate, MS4A/CH_2Cl_2, −20 °C, 1 h, 87%; (d) (i) NH_2NH_2 monohydrate/EtOH, 90 °C, 5 h, (ii) Ac_2O–pyridine, rt, 5 h, 78% (two steps); (e) (i) NaOMe/MeOH, rt, 3 h, quant, (ii) NaOH, MPMCl/DMF, rt, overnight, 47%; (f) *n*-Bu_4F/THF, rt, 1 h, 99%; (g) NaOH, CH_2=$CHCH_2Br$/DMF, rt, 5 h, 81%; (h) (i) DDQ/CH_2Cl_2–H_2O, rt, overnight, (ii) Ac_2O–pyridine, 0 °C, 5 h, 87% (two steps); (i) (i) O_3/CH_2Cl_2–MeOH, −78 °C, 0.5 h, (ii) $NaClO_2$, NaH_2PO_4, $(CH_3)_2C$=$CHCH_3$/CH_2Cl_2-*tert*-BuOH–H_2O, rt, 2 h, (iii) Dowex 50W-X4 (H^+ form)/CH_2Cl_2–MeOH, 40 °C, 5 h, 91% (three steps); (j) $Me_4SiOSO_2CF_3$/CH_2ClCH_2Cl, 40 °C, 5 h, 56%; (k) (i) NaOMe/MeOH, rt, 2 h, (ii) carbonate buffer (20 mM, pH 12), rt, 2 h.



Scheme 3. Reagents and conditions: (a) BF_3 etherate, MS4A/CH_2Cl_2, −20 °C, 5 h, 51%; (b) $Me_3SiOSO_2CF_3$/CH_2ClCH_2Cl, 50 °C, 5 h, 77%; (c) NaOMe/MeOH, rt, 1.5 h, then carbonate buffer (25 mM, pH 12), rt, 1.5 h.

acceptor because the pentenyl group serves as a reactive alkene or a good leaving group for further reactions of the glycosylated product.[25]

Enzymatic glycosidation of **1** with **4** was performed under various reaction conditions (Scheme 5, Table 1).

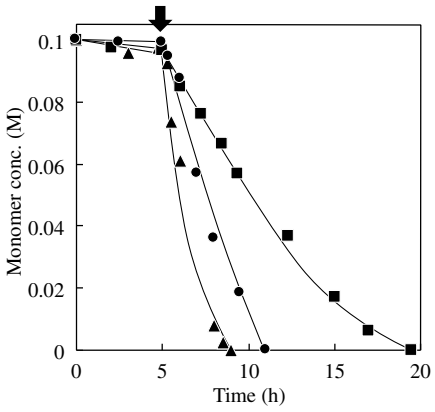


Figure 2. Reaction–time courses of **1** (–▲–), **2** (–■–), and **3** (–●–). The arrow shows the time of enzyme addition.

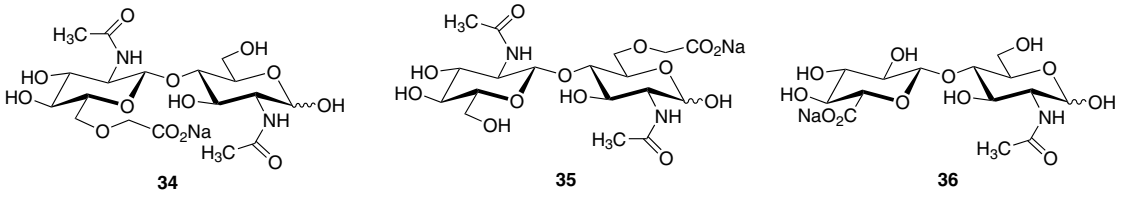
In the reaction of **1** (0.01 M) and **4** (0.03 M) carried out at a pH lower than 9.0 and at 30 °C, **1** was rapidly consumed within 1.5 h, affording the product tetrasaccharide **37** in lower yields (entries 1–3). The other compounds detected in the mixture were hydrolysis product **34** and unchanged **4**. These observations show that the enzymatic and nonenzymatic hydrolysis of **1** is faster than the enzymatic glycosidation of **1** with **4**, although the consumption of **1** was fast under these reaction conditions. The reaction at pH 9.5 progressed more slowly, yet the yield of **37** was much improved (to 57%, entry 4). The optimal yield was obtained at pH 10.0 (74%, entry 5). At pH 10.5, the yield was slightly decreased to 61% (entry 6). It was drastically decreased (to 12%) at the higher pH of 11.0 (entry 7). Thus, the reactions at a pH ranging from 9.5 to 10.5 gave the product tetrasaccharide **37** in high yields. The reaction temperature had little effect on the yield of **37**, at 30, 20, and 40 °C (entries 5, 8, and 9). The yield of **37** increased with increasing concentration of **4**, and reached a maximum of 74% in 0.03 M of **4** (entries 5, 10, and 11). The glycosidation of **1** with an increased amount of **4** (10 or 30 mol equiv) gave somewhat lower yields (entries 12 and 13). A small amount of the enzyme was sufficient for the reaction (entries 14–17 and also entries 18–20 in large amount for comparison). The reaction in entry 5 gave **37** in 45% yield.

Enzymatic glycosidation of **1** with **5** was carried out under the optimal conditions established for the reaction (Scheme 6). The reaction was complete after 12 h, giving the product trisaccharide **38** in 37% yield. Compound **1** also reacted with **6** by enzymatic catalysis under similar conditions (Scheme 7); **1** was completely consumed after 9 h, affording the trisaccharide **39** as the sole glycosidated product in 45% isolated yield.

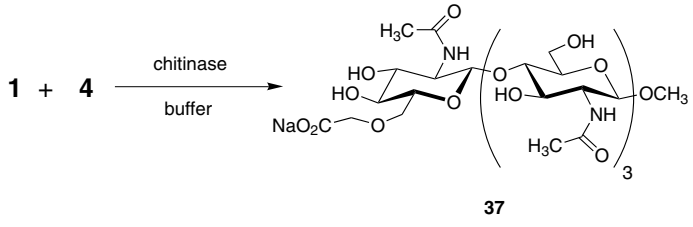
All three acceptors can thus be glycosylated regio- and stereo-specifically by donor **1** to give the corresponding tetra- and trisaccharides in good yields. Other compounds in the reaction mixture were the remaining acceptor and **34**.

2.4. Enzymatic reactions of 2 catalyzed by chitinase

The oxazoline derivative **2** was subjected to the chitinase enzymatic reaction with glycosyl acceptor **4**, **5**, or **6**, but it did not react with these glycosyl acceptors, whereas it was effectively hydrolyzed by the enzyme (Fig. 2), and the compounds detected after the reaction were **35** (the hydrolyzed product from **2**), together with the unchanged acceptors **4**, **5**, or **6**. These observations can be explained as illustrated in Figure 3. Compound **2** is readily recognized and protonated at the donor site to form the oxazolinium ion. Because of the steric



Scheme 4.

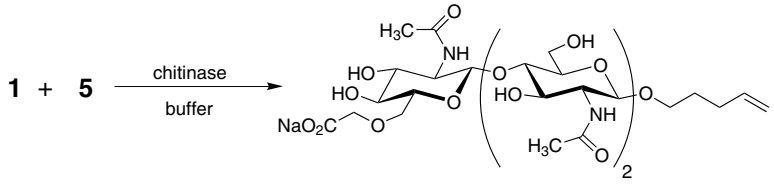


Scheme 5.

Table 1. Results of the enzymatic glycosidation of **1** with **4** under various conditions[a]

Entry	[**1**]/M	[**4**]/M	Amount of enzyme/wt% for **1**	pH	Temperature/°C	Time/h	Yield of **37**[d]/%
1	0.01	0.03	10	7.0	30	0.67[b]	10
2	0.01	0.03	10	8.0	30	1.0[b]	21
3	0.01	0.03	10	9.0	30	1.5[b]	23
4	0.01	0.03	10	9.5	30	12[b]	57
5	0.01	0.03	10	10.0	30	12[b]	74
6	0.01	0.03	10	10.5	30	18[b]	61
7	0.01	0.03	10	11.0	30	24[b]	12
8	0.01	0.03	10	10.0	20	12[c]	70
9	0.01	0.03	10	10.0	40	12[c]	60
10	0.01	0.01	10	10.0	30	12[c]	48
11	0.01	0.02	10	10.0	30	12[c]	58
12	0.01	0.10	10	10.0	30	12[c]	68
13	0.01	0.30	10	10.0	30	12[c]	66
14	0.01	0.03	1	10.0	30	12[c]	30
15	0.01	0.03	2	10.0	30	12[c]	45
16	0.01	0.03	3	10.0	30	12[c]	56
17	0.01	0.03	5	10.0	30	12[c]	62
18	0.01	0.03	20	10.0	20	12[c]	73
19	0.05	0.15	10	10.0	30	12[c]	72
20	0.10	0.30	20	10.0	30	12[c]	69

[a] In a carbonate buffer (50 mM, 100 μL).
[b] The time for complete consumption of **1**.
[c] The reaction was terminated at the indicated time.
[d] Determined by HPLC based on **1**.



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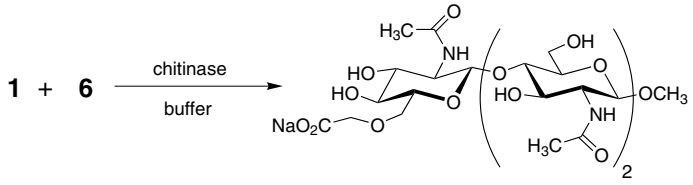
Scheme 6.



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Scheme 7.

hindrance of the 6-*O*-CM group at the donor site, as shown in A, the glycosyl acceptor cannot access the acceptor site and even if it would do so, nucleophilic attack of the 4-hydroxyl group of the GlcNAc residue situated in the acceptor site cannot occur. The water molecule is smaller than the CM group, and it can easily locate at the acceptor site and attack the anomeric carbon of the oxazolinium intermediate in preference to the 4-hydroxyl group of the glycosyl acceptor, as illustrated in B and C. Therefore, only hydrolysis of **2** occurred under enzymatic catalysis, that is, **2** is able to act as a good donor molecule but reacts with difficulty with the glycosyl acceptor.

2.5. Glycosidation reaction of 3 catalyzed by chitinase

The oxazoline derivative **3** was allowed to react enzymatically with **6** (Scheme 8).

Table 2 shows the results of two series of the reactions (entries 1–7 and 8–18). The enzymes used for these two

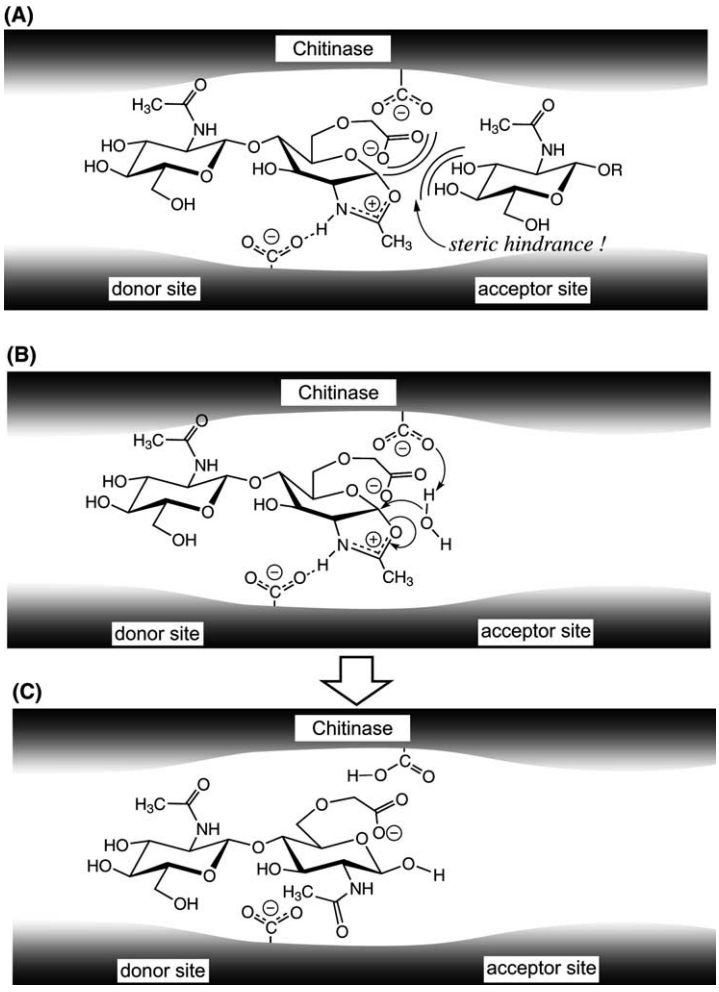
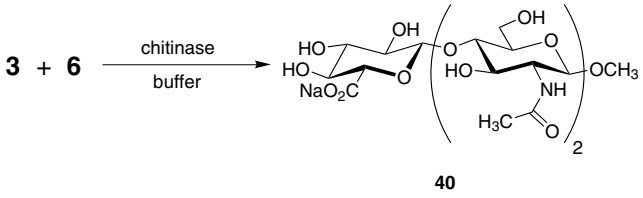


Figure 3. Illustration of the reaction mechanism of **2**.



Scheme 8.

series were from the same lot number but in the different bottles. In the first series, the product **40** was obtained under the pH range from 7.0 to 10.5 (entries 1–6). In the reactions at pH 7.0 and 8.0, **3** was rapidly consumed within 7 h, giving **40** in slightly lower yields (entries 1 and 2). Reactions at pH 9.0–10.0 yielded **40** in good yields with longer reaction time for the disappearance of **3** (entries 3–5). At pH 10.5, yield decreased drastically to 18% (entry 6), and at pH 11.0 the reaction did not take place (entry 7). The best yield of **40** (58%) was obtained at pH 10.0. Other compounds detected after the reaction were **36**, the hydrolysis product from **3**, and the unchanged remaining **6**.

In the second series of enzymatic reactions (entries 8–18), the effects of enzyme amount (entries 8–11), the reaction temperature (entries 12 and 13), and concentration of the acceptor and donor (entries 14–18) were investigated. The yield of **40** increased with increasing amount of enzyme (entries 8–10), and the maximum yield was 56% on using 20 wt% of the enzyme for **3** (entry 10). When the enzyme was used in 30 wt% for **3**, the yield decreased a little (entry 11), and therefore, the enzyme amount was fixed to 20 wt% for **3** in the following reactions. The reactions at 20 °C gave **40** in 33% yield (entry 12), increasing at 40 °C to 42% (entry 13). Next, the concentration of donor **3** and acceptor **6** in a fixed ratio (1:3) was varied to 0.10 and 0.30 M (entry 14), and 0.01 and 0.03 M (entry 15), respectively. Higher concentrations of the substrates gave results similar to entry 10, showing a little concentration effects on the yield of **40** (entry 14). At 0.05 M concentration of **3**, the reaction took place most efficiently. Effects of the concentration of **6** were also investigated (entries 16–18); but, the yield of **40** was decreased as compared with that of the reaction using 0.15 M of **6** (entry 10). These

Table 2. Results of the enzymatic glycosidation of **3** with **6** under various conditions[a]

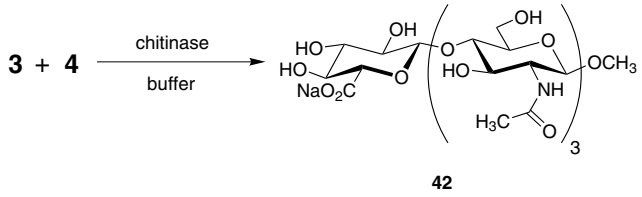
Entry	[3]/M	[6]/M	Amount of enzyme/wt% for **3**	pH	Temperature/°C	Time/h	Yield of **40**[d]/%
1	0.05	0.15	10	7.0	30	5.5[b]	24
2	0.05	0.15	10	8.0	30	7.0[b]	32
3	0.05	0.15	10	9.0	30	18[b]	38
4	0.05	0.15	10	9.5	30	56[b]	41
5	0.05	0.15	10	10.0	30	78[b]	58
6	0.05	0.15	10	10.5	30	168[b]	18
7	0.05	0.15	10	11.0	30	192[b]	0
8	0.05	0.15	1	10.0	30	78[c]	31
9	0.05	0.15	10	10.0	30	78[c]	42
10	0.05	0.15	20	10.0	30	78[c]	56
11	0.05	0.15	30	10.0	30	72[b]	24
12	0.05	0.15	20	10.0	20	78[c]	33
13	0.05	0.15	20	10.0	40	60[b]	42
14	0.10	0.30	20	10.0	30	78[c]	52
15	0.01	0.03	20	10.0	30	60[b]	37
16	0.05	0.05	20	10.0	30	78[c]	24
17	0.05	0.50	20	10.0	30	78[c]	44
18	0.05	1.50	20	10.0	30	78[c]	35

[a] In a carbonate buffer (50 mM, 100 μL).
[b] The time for complete consumption of **3**.
[c] The reaction was terminated at the indicated time.
[d] Determined by HPLC based on **3**.

results imply that **3** reacted with water rather than with **6** at lower concentration, and a higher concentration of **6** inhibited reaction, resulting in lower yields.

Enzymatic glycosidation of **3** with **5** was carried out under the optimal conditions of the foregoing reaction (Scheme 9), and was complete after 168 h. The isolated yield of **41** was 38%. The glycosyl donor **3** was also reacted with **4** under catalysis by the enzyme under similar conditions (Scheme 10). The reaction was slowed, giving rise to **42** in 36% yields after 168 h.

Thus, **3** reacted with all of the acceptors, affording the corresponding glycosidated products in satisfactory yields. Other compounds in the reaction mixtures were the hydrolyzed compound **36** and the remaining acceptor.

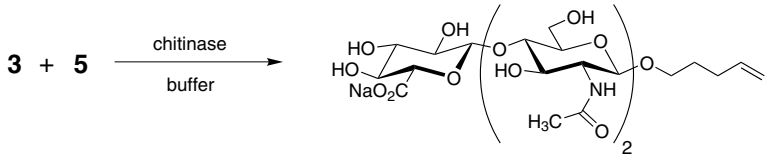


42

Scheme 10.

3. Conclusion

This study shows that chitinase from *Bacillus* sp. catalyzes the glycosidation reactions of carboxylate-functionalized sugar oxazolines serving as glycosyl donors. All the glycosidated products, **37**–**42**, were formed with complete stereo-chemical control (β configuration) at C-1 carbon of donors **1** and **3**, and regio-selectivity at 4-OH of acceptors **4**–**6**, during the reaction. This is the first example of chitinase-catalyzed terminal functionalization of chitooligosaccharide derivatives by using glycosyl donors having a carboxylate group. These combinations of enzyme and the glycosyl donors should permit synthesis of carboxylate end-functionalized carbohydrate derivatives, providing tools for synthesis of neoglycoconjugates and for other modifications of carbohydrate-based materials.



41

Scheme 9.

4. Experimental

4.1. General methods

NMR spectra were recorded with a Bruker DPX400 spectrometer. For solutions in D_2O, acetone served as the reference 2.24 (^1H) and 30.91 ppm (^{13}C). All assignments were based on COSY, DEPT, and HMQC experiments. High-resolution fast-atom-bombardment mass (HRFAB/MS) spectra were recorded on a Jeol XPS spectrometer using 2,4-dinitrobenzyl alcohol or dithiothreitol–thioglycerol (1:1, v/v) as the matrix. Optical rotations were determined with a Jasco P-1010 polarimeter. Melting points were determined by a Yamato MP-21 apparatus. High-performance liquid chromatography (HPLC) was performed on a Tosoh LC-8020 system with a Shodex Asahipak NH2P-50 4E column. Size-exclusion chromatography (SEC) was carried out on a Tosoh GPC-8020 system with a Shodex Ohpak SB-802HQ column, eluting with 0.1 M aqueous sodium nitrate (flow rate: 0.5 mL/min). MALDI-TOF/MS spectra were recorded on a Jeol JMS-ELITE spectrometer by using 2,5-dihydroxybenzoic acid as the matrix on Nafion-coated plate in the negative ion mode.[26]

1,2-Dichloroethane was distilled from diphosphorus pentoxide and stored over activated 4 Å molecular sieves (MS4A) under argon before use. Other chemicals were of reagent grade and used without further purification. Chitinase from *Bacillus* sp. (Lot No LDH7064, EC 3.2.1.14, 0.04 unit/mg) was purchased from Wako Pure Chemicals, Inc. (Tokyo, Japan), and used without further purification.

4.2. Synthesis

4.2.1. 4-Methoxybenzyl 2-deoxy-4,6-*O*-(4-methoxybenzylidene)-2-phthalimido-β-D-glucopyranoside (8). 4-Methoxybenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**7**)[25] (2.20 g, 4.0 mmol) was dissolved in 4:1 MeOH–CH_2Cl_2 (25 mL), and then NaOMe in MeOH (28 wt%, 0.1 mL) was added dropwise. After stirring for 1 h, additional NaOMe in MeOH (28 wt%, 0.1 mL) was added. The mixture was stirred for 1 h at room temperature, neutralized with Dowex 50W-X4 (H^+ form), filtered through cotton and the filtrate concentrated to dryness under diminished pressure. The residue was dissolved in DMF (10 mL) followed by the addition of 4-methoxybenzaldehyde dimethyl acetal (1.5 mL, 8.7 mmol) and (±)-camphor-10-sulfonic acid (134 mg, 0.58 mmol). The reaction mixture was stirred at room temperature for 3 h, and then at 30 °C under diminished pressure for 1 h. The mixture was concentrated, and the residue was purified by silica gel column chromatography (5:1–1:1 *n*-hexane–EtOAc) to afford **8** (2.52 g, 80%) as a white amorphous powder: R_f 0.39 (1:1 *n*-hexane–EtOAc); $[\alpha]_D^{25}$ −68.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.00–6.52 (m, 12H, Ar), 5.54 (s, 1H, C*H*Ph of benzylidene), 5.25 (d, 1H, $J_{1,2}$ 8.54 Hz, H-1), 4.77 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.63 (m, 1H, H-3), 4.45 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.41 (dd, 1H, $J_{6a,6b}$ 10.29, $J_{5,6a}$ 4.77 Hz, H-6a), 4.26 (dd, 1H, $J_{2,3}$ 10.54 Hz, H-2), 3.87–3.80 (m, 4H, H-6b, OCH_3), 3.68 (s, 3H, OCH_3), 3.66–3.58 (m, 2H, H-4, H-5), 2.44 (d, 1H, $J_{3,3\text{-OH}}$ 3.51 Hz, 3-O*H*); ^{13}C NMR ($CDCl_3$): δ 168.30, 163.01 (*C*O of phthalimido), 160.20–113.49 (C_{Ar}), 101.79 (*C*HPh of benzylidene), 97.63 (C-1), 82.03 (C-4), 71.04 (*C*H_2Ph), 68.61 (C-6), 68.35 (C-3), 66.02 (C-5), 56.59 (C-2), 55.25, 54.98 (O*C*H_3); HRMS (FAB^+) calcd for $C_{30}H_{30}NO_9$ $[M+H]^+$ 548.1921, found 548.1931.

4.2.2. 4-Methoxybenzyl 2-deoxy-3-*O*-(4-methoxybenzyl)-4,6-*O*-(4-methoxybenzylidene)-2-phthalimido-β-D-glucopyranoside (9). To a solution of compound **8** (300 mg, 0.55 mmol) in dry DMF (1 mL) was added NaH (60% oil dispersion, 32 mg, 0.80 mmol). The reaction mixture was stirred at room temperature for 15 min followed by the addition of 4-methoxybenzyl chloride (0.11 mL, 0.82 mmol). The mixture was stirred at room temperature for 1 h, and then tetrabutylammonium iodide (100 mg, 0.27 mmol) was added. After stirring overnight, additional NaH (60% oil dispersion, 21 mg, 0.53 mmol) and 4-methoxybenzyl chloride (73 μL, 0.54 mmol) was added to the mixture. Methanol (0.5 mL) was added after 4 h to terminate the reaction. The mixture was concentrated under diminished pressure, diluted with EtOAc, and washed with satd aq $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), and concentrated. The residue was purified by silica gel column chromatography (5:1–2:1 *n*-hexane–EtOAc) to afford **9** (185 mg, 51%) as a white amorphous powder: R_f 0.47 (1:1 *n*-hexane–EtOAc); $[\alpha]_D^{25}$ +7.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.79–6.33 (m, 16H, Ar), 5.58 (s, 1H, C*H*Ph of benzylidene), 5.16 (d, 1H, $J_{1,2}$ 8.53 Hz, H-1), 4.71 (d, 1H, *J* 12.55 Hz, CH_2Ph), 4.67 (d, 1H, *J* 12.55 Hz, CH_2Ph), 4.42–4.34 (m, 4H, H-3, H-6a, CH_2Ph), 4.17 (dd, 1H, $J_{2,3}$ 10.29 Hz, H-2), 3.88–3.75 (m, 5H, H-4, H-6b, OCH_3), 3.65–3.59 (m, 7H, H-5, OCH_3); ^{13}C NMR ($CDCl_3$): δ 167.38 (*C*O of phthalimido), 159.97–113.21 (C_{Ar}), 101.22 (*C*HPh of benzylidene), 97.55 (C-1), 82.85 (C-4), 73.87 (C-3), 73.52 (*C*H_2Ph), 70.94 (*C*H_2Ph), 68.70 (C-6), 65.97 (C-5), 55.80 (C-2), 55.24–54.80 (O*C*H_3); HRMS (FAB^+) calcd for $C_{38}H_{38}NO_{10}$ $[M+H]^+$ 668.2496, found 668.2495.

4.2.3. 4-Methoxybenzyl 2-deoxy-3,6-di-*O*-(4-methoxybenzyl)-2-phthalimido-β-D-glucopyranoside (10). Compound **9** (400 mg, 0.60 mmol) was added to a solution of sodium cyanoborohydride (197 mg, 3.2 mmol) and activated 3 Å molecular sieves (MS3A, 5.0 g) in DMF (35 mL). The mixture was stirred at 0 °C under argon, and then trifluoroacetic acid (TFA, 0.47 mL, 6.0 mmol) in DMF (1.8 mL) was added dropwise. The mixture

was stirred at room temperature for 24 h under argon followed by further addition of TFA (0.45 mL) in DMF (1.8 mL) at 0 °C. After stirring for 24 h at room temperature, the mixture was filtered through Celite, poured into satd aq $NaHCO_3$, and extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The residue was subjected to silica gel column chromatography (5:1–1:1 *n*-hexane–EtOAc), affording **10** (400 mg, quant.) as a colorless syrup: R_f 0.32 (1:1 *n*-hexane–EtOAc); $[\alpha]_D^{27}$ −10.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.77–6.42 (m, 16H, Ar), 5.12 (d, 1H, $J_{1,2}$ 8.03 Hz, H-1), 4.69 (d, 1H, *J* 12.05 Hz, C*H*$_2$Ph), 4.64–4.58 (m, 2H, C*H*$_2$Ph), 4.53 (d, 1H, *J* 11.54 Hz, C*H*$_2$Ph), 4.43 (d, 1H, *J* 12.04 Hz, C*H*$_2$Ph), 4.39 (d, 1H, *J* 12.04 Hz, C*H*$_2$Ph), 4.21–4.10 (m, 2H, H-2, H-3), 3.82–3.72 (m, 6H, H-4, H-6a, H-6b, OC*H*$_3$), 3.68 (s, 3H, OC*H*$_3$), 3.65–3.60 (m, 4H, H-5, OC*H*$_3$), 2.94 (s, 1H, 4-O*H*); ^{13}C NMR ($CDCl_3$): δ 168.01 (*C*O of phthalimido), 159.35–113.38 (C_{Ar}), 97.11 (C-1), 78.04 (C-3), 74.59 (C-4), 73.79 (*C*H$_2$Ph), 73.41 (*C*H$_2$Ph), 73.31 (C-5), 70.61 (*C*H$_2$Ph), 70.48 (C-6), 55.40 (C-2), 55.27, 55.01, 54.83 (O*C*H$_3$); HRMS (FAB^+) calcd for $C_{38}H_{40}NO_{10}$ $[M+H]^+$ 670.2652, found 670.2647.

4.2.4. 4-Methoxybenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-(4-methoxybenzyl)-2-phthalimido-β-D-glucopyranoside (12). To a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**11**, 172 mg, 0.30 mmol) and compound **10** (107 mg, 0.16 mmol) in dry CH_2Cl_2 (2.1 mL) over activated MS4A (326 mg) was added trimethylsilyl triflate (Me_3-SiOTf; 5 μL, 28 μmol) in dry CH_2Cl_2 (0.2 mL). The reaction mixture was stirred at −20 °C for 1 h under argon and then filtered through Celite, and diluted with $CHCl_3$. The solution was washed with satd aq $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) and concentrated. The residual mixture was purified by silica gel column chromatography (3:1–1:1 *n*-hexane–EtOAc) to provide **12** (159 mg, 91%) as a white amorphous powder: R_f 0.27 (1:1 *n*-hexane–EtOAc); $[\alpha]_D^{25}$ −8.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.93–6.30 (m, 20H, Ar), 5.80 (t, 1H, $J_{3',4'}$ 9.79 Hz, H-3′), 5.48 (d, 1H, $J_{1',2'}$ 8.03 Hz, H-1′), 5.13 (t, 1H, $J_{4',5'}$ 9.54 Hz, H-4′), 4.91 (d, 1H, $J_{1,2}$ 8.03 Hz, H-1), 4.69 (d, 1H, *J* 12.54 Hz, C*H*$_2$Ph), 4.61 (d, 1H, *J* 12.04 Hz, C*H*$_2$Ph), 4.50 (s, 2H, C*H*$_2$Ph), 4.41 (d, 1H, *J* 12.55 Hz, C*H*$_2$Ph), 4.34–4.19 (m, 3H, H-4, H-2′, H-6a′), 4.29 (d, 1H, *J* 12.55 Hz, C*H*$_2$Ph), 4.10–4.07 (m, 2H, H-2, H-3), 3.97 (d, 1H, $J_{6a',6b'}$ 10.04 Hz, H-6b′), 3.84 (s, 3H, OC*H*$_3$), 3.65 (s, 3H, OC*H*$_3$), 3.57 (s, 3H, OC*H*$_3$), 3.57 (m, 1H, H-6a), 3.47 (dd, 1H, $J_{6a,6b}$ 11.29, $J_{5,6b}$ 3.76 Hz, H-6b), 3.42 (m, 1H, H-5′), 3.30 (m, 1H, H-5), 2.02, 2.00, 1.85 (3s, 9H, COC*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 170.68, 170.08, 169.52 (*C*OCH$_3$), 167.46 (*C*O of phthalimido), 159.09–113.12 (C_{Ar}), 96.74 (C-1), 96.62 (C-1′), 76.48 (C-4), 76.39 (C-3), 74.72 (C-5), 74.00 (*C*H$_2$Ph), 72.84 (*C*H$_2$Ph), 71.82 (C-5′), 71.00 (C-3′), 70.71 (*C*H$_2$Ph), 69.27 (C-4′), 68.32 (C-6), 61.96 (C-6′), 56.06 (C-2), 55.67 (C-2′, O*C*H$_3$), 55.42, 55.16 (O*C*H$_3$), 20.64, 20.59, 20.42 (CO*C*H$_3$); HRMS (FAB^+) calcd for $C_{58}H_{58}N_2O_{19}Na$ $[M+Na]^+$ 1109.3532, found 1109.3542.

4.2.5. 4-Methoxybenzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (13). Compound **12** (2.00 g, 1.8 mmol) was dissolved in a 10:1 mixture (v/v) of EtOH and H_2O (22 mL), and hydrazine monohydrate (0.5 mL, 10 mmol) was added. The mixture was stirred at 90 °C for 2 h followed by the further addition of hydrazine monohydrate (0.5 mL, 10 mmol). After stirring for 3 h at 90 °C, the mixture was evaporated under diminished pressure, and Ac_2O (10 mL) was added to the residue suspended in pyridine (30 mL) under a dry atmosphere. The mixture was stirred at room temperature for 3 h. After the reaction was complete, MeOH (10 mL) was added to quench the excess of reagent, and the mixture was concentrated under diminished pressure. The residue was diluted with $CHCl_3$, washed with 1 M aq HCl, satd aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and concentrated. Silica gel column chromatography (2:1–1:1 toluene–EtOAc, then 3:1–2:1 $CHCl_3$–acetone) purified the residue, giving rise to **13** (1.47 g, 88%) as a white solid: R_f 0.30 (3:1 $CHCl_3$–acetone); mp 204–205 °C; $[\alpha]_D^{25}$ −95.0 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.25–6.79 (m, 12H, Ar), 6.10 (d, 1H, $J_{2,NH}$ 9.03 Hz, N*H*), 5.36 (d, 1H, $J_{2',N'H}$ 9.54 Hz, N′*H*), 5.06 (t, 1H, $J_{4',5'}$ 9.54 Hz, H-4′), 4.92 (t, 1H, $J_{3',4'}$ 9.54 Hz, H-3′), 4.76 (d, 1H, *J* 11.55 Hz, C*H*$_2$Ph), 4.63–4.61 (m, 3H, H-1, C*H*$_2$Ph), 4.55 (d, 1H, *J* 11.55 Hz, C*H*$_2$Ph), 4.45 (d, 1H, *J* 11.54 Hz, C*H*$_2$Ph), 4.38 (d, 1H, $J_{1',2'}$ 8.54 Hz, H-1′), 4.37 (d, 1H, *J* 11.55 Hz, C*H*$_2$Ph), 4.24 (dd, 1H, $J_{6a',6b'}$ 12.55, $J_{5',6a'}$ 4.51 Hz, H-6a′), 4.08–3.96 (m, 3H, H-2, H-2′, H-6b′), 3.93 (t, 1H, $J_{3,4}$ 5.52 Hz, H-3), 3.82–3.72 (m, 3H, H-4, H-6a, H-6b), 3.82, 3.80, 3.78 (3s, 9H, OC*H*$_3$), 3.60 (m, 1H, H-5), 3.45 (ddd, 1H, $J_{5',6b'}$ 2.01 Hz, H-5′), 2.03, 2.03, 2.02, 1.93, 1.78 (5s, 15H, COC*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 171.28, 170.69, 170.57, 170.19, 169.27 (*C*OCH$_3$), 159.53–113.58 (C_{Ar}), 100.17 (C-1′), 99.45 (C-1), 77.20 (C-4), 75.21 (C-3), 74.36 (C-5), 73.32 (*C*H$_2$Ph), 72.56 (C-3′), 72.26 (*C*H$_2$Ph), 71.67 (C-5′), 70.01 (*C*H$_2$Ph), 69.24 (C-6), 68.09 (C-4′), 61.76 (C-6′), 55.27, 55.23, 55.20 (O*C*H$_3$), 54.14 (C-2), 51.66 (C-2′) 23.22, 23.19, 20.71, 20.65, 20.59 (CO*C*H$_3$); HRMS (FAB^+) calcd for $C_{46}H_{59}N_2O_{17}$ $[M+H]^+$ 911.3814, found 911.3818.

4.2.6. 4-Methoxybenzyl 2-acetamido-2-deoxy-6-*O*-(4-methoxytrityl)-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (14). Compound **13** (520 mg, 0.57 mmol) was dissolved

in 7:15 (v/v) $CHCl_3$–MeOH (22 mL), and NaOMe in MeOH (28 wt%, 0.2 mL) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. The mixture was neutralized with Dowex 50W-X4 (H^+ form), filtered through cotton, and evaporated. To the residue dissolved in pyridine (10 mL) was added 4-methoxytrityl chloride (350 mg, 1.1 mmol), and the reaction mixture was stirred at room temperature for 7 h. Methanol (5 mL) was then added to quench the excess reagent. The mixture was then concentrated, poured into satd aq $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (10:1 EtOAc–MeOH) to give **14** (579 mg, 96%) as a white amorphous powder: R_f 0.43 (8:1 $CHCl_3$–MeOH); $[\alpha]_D^{26}$ −45.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_3OD): δ 7.45–6.62 (m, 26H, Ar), 4.78 (d, 1H, *J* 11.54 Hz, CH_2Ph), 4.74 (d, 1H, *J* 11.55 Hz, CH_2Ph), 4.65 (d, 1H, $J_{1',2'}$ 8.53 Hz, H-1′), 4.60 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.58 (d, 1H, *J* 11.04 Hz, CH_2Ph), 4.51–4.47 (m, 3H, H-1, CH_2Ph), 4.27 (t, 1H, $J_{4,5}$ 8.29 Hz, H-4), 3.95 (t, 1H, $J_{2,3}$ 8.54 Hz, H-2), 3.82 (m, 2H, H-6a, H-6b), 3.75 (s, 3H, OCH_3), 3.70 (m, 1H, H-2′), 3.70, 3.68, 3.65 (3s, 9H, OCH_3), 3.60 (t, 1H, $J_{3,4}$ 8.79 Hz, H-3), 3.51–3.39 (m, 3H, H-5, H-3′, H-4′), 3.35 (d, 1H, *J* 7.53 Hz, H-6a′), 3.21 (m, 1H, H-5′), 3.14 (m, 1H, H-6b′), 1.97, 1.81 (2s, 6H, COCH_3); ^{13}C NMR (CD_3OD): δ 174.22, 173.43 (*C*OCH$_3$), 161.19–114.46 (C_{Ar}), 101.89 (C-1), 101.56 (C-1′), 87.73 (*C*(Ph)$_3$), 81.45 (C-3), 77.29 (C-5′), 76.94 (C-5), 76.41 (C-3′), 75.94 (C-4), 74.59 (*C*H$_2$Ph), 74.44 (*C*H$_2$Ph), 72.82 (C-4′), 71.72 (*C*H$_2$Ph), 70.26 (C-6), 64.33 (C-6′), 58.68 (C-2′), 56.10, 56.06, 56.04 (*C*H$_3$O), 55.99 (C-2), 23.55, 23.43 (CO*C*H$_3$); HRMS (FAB^+) calcd for $C_{60}H_{69}N_2O_{15}$ $[M+H]^+$ 1057.4698, found 1057.4664.

4.2.7. 4-Methoxybenzyl 2-acetamido-2-deoxy-3,4-di-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (15). To a solution of compound **14** (4.31 g, 4.1 mmol) in dry DMF (20 mL) was added powdered NaOH (490 mg, 12 mmol), then the mixture was stirred at room temperature. After 20 min, 4-methoxybenzyl chloride (1.7 mL, 12 mmol) was added. To the mixture was added powdered NaOH (160 mg, 4.0 mmol) twice at 4.5 and 16.5 h during the reaction for 20.5 h. Methanol (10 mL) was added to quench the excess of reagent, and the reaction mixture was concentrated, poured into satd aq $NaHCO_3$, extracted with $CHCl_3$, and washed with brine. The dried ($MgSO_4$) extract was concentrated and the residue roughly purified by silica gel column chromatography (2:1–1:2 toluene–EtOAc, then EtOAc) to provide crude 4-methoxybenzyl 2-acetamido-2-deoxy-3,4-di-*O*-(4-methoxybenzyl)-6-*O*-(4-methoxytrityl)-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-(4-methoxybenzyl)-β-D-glucopyranoside as a yellowish syrup.

To the crude product was added 70% aq AcOH (40 mL), and the mixture was stirred at 50 °C for 4 h. The reaction mixture was then poured into satd aq $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with satd aq $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (1:1 $CHCl_3$–EtOAc, then 100:1–30:1 $CHCl_3$–MeOH) to afford **15** (2.96 g, 71% from **14**) as a white solid: R_f 0.44 (50:3 $CHCl_3$–MeOH); mp 208-209 °C; $[\alpha]_D^{26}$ −17.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.26–6.79 (m, 20H, Ar), 6.14 (d, 1H, $J_{2',N'H}$ 8.53 Hz, N′*H*), 4.77–4.69 (m, 5H, N*H*, CH_2Ph), 4.63 (d, 1H, $J_{1',2'}$ 6.03 Hz, H-1′), 4.59–4.51 (m, 4H, CH_2Ph), 4.43 (d, 1H, *J* 11.54 Hz, CH_2Ph), 4.34 (d, 1H, *J* 11.55 Hz, CH_2Ph), 4.23 (d, 1H, $J_{1,2}$ 8.03 Hz, H-1), 3.94 (m, 1H, H-2′), 3.88–3.67 (m, 6H, H-2, H-6a, H-6b, H-3′, H-5′, H-6a′), 3.81, 3.79, 3.78, 3.76 (4s, 15H, OCH_3), 3.57 (m, 1H, H-4′), 3.54–3.47 (m, 2H, H-4, H-6b′), 3.35 (t, 1H, $J_{3,4}$ 9.54 Hz, H-3), 3.19 (m, 1H, H-5), 2.00 (t, 1H, $J_{6',6'\text{-OH}}$ 6.78 Hz, 6′-O*H*), 1.93, 1.71 (2s, 6H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 170.31, 170.25 (*C*OCH$_3$), 159.44–113.61 (C_{Ar}), 99.98 (C-1′), 99.29 (C-1), 80.86 (C-3), 78.03 (C-4), 77.20 (C-3′), 75.07 (C-5), 74.82 (C-5′), 74.54 (*C*H$_2$Ph), 74.38 (C-4′), 74.10, 73.19, 72.39, 70.01 (*C*H$_2$Ph), 69.17 (C-6), 61.84 (C-6′), 55.28, 55.26, 55.24, 55.22, 55.20 (O*C*H$_3$), 55.12 (C-2), 52.06 (C-2′), 23.45, 23.31 (CO*C*H$_3$); HRMS (FAB^+) calcd for $C_{56}H_{69}N_2O_{16}$ $[M+H]^+$ 1025.4647, found 1025.4657.

4.2.8. 4-Methoxybenzyl 2-acetamido-6-*O*-allyl-2-deoxy-3,4-di-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (16). Compound **15** (2.96 g, 2.9 mmol) was dissolved in dry DMF (20 mL), and powdered NaOH (173 mg, 4.33 mmol) was added under a dry atmosphere. The mixture was stirred at room temperature and after 20 min, allyl bromide (0.37 mL, 4.3 mmol) was added, followed by powdered NaOH (58 mg, 1.5 mmol) after 12 h during the reaction for 17 h. Methanol (10 mL) was added to quench the excess of reagent and the mixture was concentrated, diluted with $CHCl_3$ and washed with satd aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and concentrated. Silica gel column chromatography (3:1 $CHCl_3$–EtOAc, then 3:1 $CHCl_3$–acetone) of the residue afforded **16** (2.82 g, 92%) as a white solid: R_f 0.35 (3:1 $CHCl_3$–acetone); mp 174–175 °C; $[\alpha]_D^{25}$ +42.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.24–6.76 (m, 20H, Ar), 6.49 (d, 1H, $J_{2',N'H}$ 9.54 Hz, N′*H*), 5.87 (m, 1H, C*H*=CH$_2$), 5.27 (dd, 1H, *J* 17.32, 1.76 Hz, CH=CH_2), 5.16 (dd, 1H, *J* 10.29, 1.26 Hz, CH=CH_2), 4.78 (d, 1H, $J_{2,NH}$ 9.03 Hz, N*H*), 4.75 (m, 3H, CH_2Ph), 4.65 (d, 1H, *J* 11.05 Hz, CH_2Ph), 4.59 (d, 1H, *J* 10.54 Hz, CH_2Ph), 4.58 (d, 1H, $J_{1',2'}$ 4.52 Hz, H-1′), 4.53 (d, 1H, *J* 11.54 Hz, CH_2Ph), 4.52

(d, 1H, *J* 11.54 Hz, CH_2Ph), 4.42 (d, 2H, *J* 11.04 Hz, CH_2Ph), 4.34 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.21–4.18 (m, 2H, H-1, H-2′), 4.01–3.99 (m, 2H, CH_2CH=CH$_2$), 3.91 (t, 1H, *J* 4.02 Hz, H-4′), 3.82–3.61 (m, 8H, H-2, H-4, H-6a, H-6b, H-3′, H-5′, H-6a′, H-6b′), 3.81, 3.79, 3.78, 3.76 (4s, 15H, OCH_3), 3.38 (t, 1H, $J_{3,4}$ 9.54 Hz, H-3), 3.28 (m, 1H, H-5), 1.97, 1.69 (2s, 6H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 170.55, 170.19 (*C*OCH$_3$), 159.34–158.84 (C$_{Ar}$), 134.53 (*C*H=CH$_2$), 130.61–129.02 (C$_{Ar}$), 117.04 (CH=*C*H$_2$), 113.90–113.47 (C$_{Ar}$), 99.96 (C-1), 99.49 (C-1′), 80.40 (C-3), 78.02 (C-4), 76.41 (C-3′), 74.90 (C-5), 74.49 (C-5′), 74.44, 73.78 (*C*H$_2$Ph), 73.53 (C-4′), 73.07 (*C*H$_2$Ph), 72.36 (*C*H$_2$CH=CH$_2$), 71.47 (*C*H$_2$Ph), 69.79 (C-6), 69.74 (*C*H$_2$Ph), 68.54 (C-6′), 52.22, 55.16, 55.12 (O*C*H$_3$), 54.94 (C-2), 49.93 (C-2′), 23.40, 23.12 (CO*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{59}H_{73}N_2O_{16}$ [M+H]$^+$ 1065.4960, found 1065.4960.

4.2.9. 2-Acetamido-3,4-di-*O*-acetyl-6-*O*-allyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl acetate (17). Compound **16** (300 mg, 0.28 mmol) was suspended in 18:1 (v/v) CH_2Cl_2–H_2O (19 mL) and then 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 350 mg, 1.5 mmol) was added. The mixture was stirred at room temperature overnight followed by evaporation. The residue was suspended in pyridine (10 mL) at 0 °C, and Ac_2O (5 mL) was added. After stirring at 0 °C for 5 h, MeOH (5 mL) was added to quench the excess of reagent. The mixture was concentrated, diluted with $CHCl_3$, and washed with 1 M aq HCl, satd aq $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$)and evaporated, and the residue was re-suspended in 18:1 CH_2Cl_2–H_2O (19 mL) and DDQ (190 mg, 0.837 mmol) was added. The mixture was stirred at room temperature overnight, and then evaporated. The residue was suspended in pyridine (10 mL) and Ac_2O (5 mL) was added at 0 °C. After stirring at 0 °C for 5 h, MeOH (5 mL) was added to terminate the reaction. The mixture was concentrated, diluted with $CHCl_3$, and washed with 1 M aq HCl, satd aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$), evaporated, and the residue purified by silica gel column chromatography (2:1 toluene–EtOAc, then 2:1 $CHCl_3$–acetone) to give **17** (171 mg, 90%) as a white solid: R_f 0.19 (2:1 $CHCl_3$–acetone); mp 279–280 °C (dec.); $[\alpha]_D^{24}$ +28.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.10 (d, 1H, $J_{1,2}$ 3.51 Hz, H-1), 5.87 (d, 1H, $J_{2',N'H}$ 9.53 Hz, N′*H*), 5.84 (m, 1H, C*H*=CH$_2$), 5.61 (d, 1H, $J_{2,NH}$ 9.04 Hz, N*H*), 5.28–5.18 (m, 3H, H-3, CH=CH_2), 5.12 (t, 1H, $J_{3',4'}$ 9.79 Hz, H-3′), 5.05 (t, 1H, $J_{4',5'}$ 9.54 Hz, H-4′), 4.47–4.44 (m, 2H, H-6a, H-1′), 4.36 (m, 1H, H-2), 4.18 (d, 1H, $J_{6a,6b}$ 12.05 Hz, H-6b), 3.95-3.88 (m, 4H, H-5, H-2′, CH_2CH=CH$_2$), 3.76 (t, 1H, $J_{4,5}$ 9.53 Hz, H-4), 3.56–3.46 (m, 3H, H-5′, H-6a′, H-6b′), 2.19, 2.15, 2.09, 2.02, 2.01, 1.96, 1.93 (7s, 21H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 171.68, 171.25, 170.89, 170.33, 170.13, 169.40, 168.92 (*C*OCH$_3$), 133.91 (*C*H=CH$_2$), 117.74 (CH=*C*H$_2$), 101.55 (C-1′), 90.44 (C-1), 75.67 (C-4), 73.01 (C-5′), 72.69 (C-3′), 72.33 (*C*H$_2$CH=CH$_2$), 70.68 (C-5), 70.57 (C-3), 69.11 (C-4′), 68.76 (C-6′), 61.49 (C-6), 54.51 (C-2′), 51.10 (C-2), 23.17, 23.02, 20.99, 20.96, 20.71, 20.68, 20.62 (CO*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{29}H_{43}N_2O_{16}$ [M+H]$^+$ 675.2613, found 675.2631.

4.2.10. 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-methoxycarbonylmethyl-β-D-glucopyranosyl-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (18). A solution of compound **17** (119 mg, 0.18 mmol) in 4:1 (v/v) CH_2Cl_2–MeOH (25 mL) was cooled to −78 °C, purged with oxygen for 15 min, and treated with O_3 by bubbling for 30 min. The O_3 was evacuated with oxygen for 10 min, and then triphenylphosphine (120 mg, 0.46 mmol) was added. The mixture was stirred at room temperature overnight, evaporated, and the residue was subjected to silica gel column chromatography (1:3 toluene–EtOAc, then 2:1–1:1 $CHCl_3$–acetone) to provide crude 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-formylmethyl-β-D-glucopyranosyl-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose as a white solid.

The crude product was dissolved in 2:12:3 (v/v/v) CH_2Cl_2–*tert*-BuOH–H_2O (17 mL) followed by the addition of NaH_2PO_4 (31 mg, 0.26 mmol), $NaClO_2$ (58 mg, 0.64 mmol) and 2-methyl-2-butene (83 μL, 0.78 mmol). The mixture was stirred at room temperature for 2 h, and 1 M aq HCl was added to adjust the pH to 1.0. The mixture was diluted with brine and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in 10:3 (v/v) MeOH–CH_2Cl_2 (13 mL) followed by the addition of Dowex 50W-X4 (H^+ form) (100 mg). The mixture was stirred at 40 °C for 4.5 h, then filtered and evaporated. The residue was dissolved in pyridine (10 mL) and Ac_2O (5 mL) added. After stirring for 5 h, MeOH (5 mL) was added to terminate the reaction. The mixture was then concentrated, diluted with $CHCl_3$, washed with 1 M aq HCl, satd aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (2:1 toluene–EtOAc, then 1:1 $CHCl_3$–acetone) to afford **18** (92 mg, 74%) as a white solid: R_f 0.24 (1:1 $CHCl_3$–acetone); mp 249–250 °C (dec.); $[\alpha]_D^{24}$ +26.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.10 (d, 1H, $J_{1,2}$ 3.51 Hz, H-1), 5.97 (d, 1H, $J_{2',N'H}$ 9.54 Hz, N′*H*), 5.69 (d, 1H, $J_{2,NH}$ 9.03 Hz, N*H*), 5.24 (dd, 1H, $J_{2,3}$ 10.54, $J_{3,4}$ 9.04 Hz, H-3), 5.14 (t, 1H, $J_{3',4'}$ 9.03 Hz, H-3′), 5.37 (t, 1H, $J_{4',5'}$ 9.04 Hz, H-4′), 4.50 (d, 1H, $J_{1',2'}$ 8.54 Hz, H-1′), 4.43 (dd, 1H, $J_{6a,6b}$ 12.05, $J_{5,6a}$ 3.51 Hz, H-6a), 4.35 (m, 1H, H-2), 4.20 (m, 1H, H-6b), 4.08 (d, 1H, *J* 1.01 Hz, OCH_2), 3.96–3.89 (m, 2H, H-5, H-2′), 3.77 (m, 1H, H-4), 3.74 (s, 3H, OCH_3), 3.67–3.61 (m, 3H, H-5′, H-6a′, H-6b′), 2.19, 2.16, 2.10, 2.05, 2.02, 1.96, 1.93 (7s, 21H,

COCH_3); ^{13}C NMR ($CDCl_3$): δ 171.59, 171.22, 170.83, 170.39, 170.28, 170.19, 169.58, 168.93 (*C*OOCH$_3$, *C*OCH$_3$), 101.30 (C-1′), 90.38 (C-1), 75.45 (C-4), 73.12 (C-5′), 72.64 (C-3′), 70.71 (C-3), 70.63 (C-5), 70.21 (C-6′), 68.78 (C-4′), 68.39 (O*C*H$_2$), 61.59 (C-6), 54.47 (C-2′), 51.75 (O*C*H$_3$), 51.13 (C-2), 23.11, 22.96, 20.93, 20.91, 20.78, 20.65, 20.59 (CO*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{29}H_{43}O_{18}N_2$ $[M+H]^+$ = 707.2511, found 707.2541.

4.2.11. 2-Methyl-[2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-methoxycarbonylmethyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-acetyl-1,2-di-deoxy-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (19). To a solution of compound **18** (90 mg, 0.13 mmol) in dry 1,2-dichloroethane (0.4 mL) was added Me_3SiOTf (50 μL, 0.28 mmol) in dry MeCN (0.2 mL) under argon. The reaction mixture was stirred at 40 °C for 12 h and cooled down to 0 °C. Triethylamine (0.1 mL) was then added at 0 °C to terminate the reaction. The mixture was concentrated, and the residue was purified by silica gel column chromatography (2:1 $CHCl_3$–EtOAc) and size-exclusion chromatography on a Sephadex LH-20 column using MeOH as eluent to afford **19** (72 mg, 87%) as a white amorphous powder: R_f 0.49 (10:1 $CHCl_3$–MeOH); $[\alpha]_D^{24}$ +4.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.91 (d, 1H, $J_{2,NH}$ 9.04 Hz, *N*H), 5.90 (d, 1H, $J_{1,2}$ 7.53 Hz, H-1), 5.63 (d, 1H, $J_{2,3}$ 1.51 Hz, H-3), 5.18 (dd, 1H, $J_{2,3}$ 10.54, $J_{3,4}$ 9.53 Hz, H-3′), 5.02 (t, 1H, H-4′), 4.71 (d, 1H, $J_{1',2'}$ 8.53 Hz, H-1′), 4.35 (dd, 1H, $J_{6a,6b}$ 12.05, $J_{5,6a}$ 4.52 Hz, H-6a), 4.18–4.08 (m, 4H, H-2, H-6b, OCH_2), 3.96 (m, 1H, H-2′), 3.74 (s, 3H, OCH_3), 3.73 (m, 1H, H-5′), 3.66–3.60 (m, 3H, H-4, H-6a′, H-6b′), 3.48 (ddd, 1H, $J_{4,5}$ 9.87, $J_{5,6b}$ 1.89 Hz, H-5), 2.14 (s, 3H, COCH_3), 2.09 (d, 3H, *J* 2.01 Hz, CH_3C of oxazoline), 2.08, 2.03, 2.02, 1.94 (4s, 12H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 171.15, 170.92, 170.56, 170.37, 169.61, 169.30 (*C*OOCH$_3$, *C*OCH$_3$), 166.79 (O*C*=N), 102.00 (C-1′), 99.11 (C-1), 77.25 (C-4), 73.28 (C-5′), 72.88 (C-3′), 70.58 (C-3, C-6′), 69.10 (C-4′), 68.74 (O*C*H$_2$), 67.62 (C-5), 65.03 (C-2), 62.96 (C-6), 54.29 (C-2′), 51.74 (O*C*H$_3$), 23.15, 22.62, 21.00, 20.87, 20.68 (CO*C*H$_3$), 13.94 (*C*H$_3$ of oxazoline); HRMS (FAB$^+$) calcd for $C_{27}H_{39}O_{16}N_2$ $[M+H]^+$ 647.2300, found 647.2297.

4.2.12. 2-Methyl-[2-acetamido-6-*O*-carboxymethyl-2-deoxy-β-D-glucopyranosyl-(1→4)-1,2-di-deoxy-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline sodium salt (1). To a MeOH solution (10 mL) of compound **19** (40 mg, 62 μmol) was added NaOMe in MeOH (28 wt%, 20 μL). After stirring at room temperature for 2 h, the mixture was evaporated and the residue dissolved in a carbonate buffer (25 mM, pH 12.0, 1238 μL), and stirred at room temperature for 2 h. The mixture was then lyophilized to give **1**: ^{1}H NMR (D_2O): δ 6.09 (d, 1H, $J_{1,2}$ 7.03 Hz, H-1), 4.56 (d, 1H, $J_{1',2'}$ 8.03 Hz, H-1′), 4.41 (s, 1H, H-3), 4.21 (m, 1H, H-2), 4.05 (m, 1H, OCH_2), 4.00 (m, 1H, OCH_2), 3.91 (d, 1H, *J* 11.54 Hz, H-6a′), 3.79 (m, 1H, H-6b′), 3.74–3.53 (m, 7H, H-4, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′), 3.30 (m, 1H, H-5), 2.07, 2.05 (2s, 6H, CH_3C of oxazoline, COCH_3); ^{13}C NMR (D_2O): δ 178.79 (*C*OONa), 175.04 (*C*OCH$_3$), 168.94 (O*C*=N), 103.86 (C-1′), 100.39 (C-1), 79.48 (C-4), 75.50 (C-5′), 73.90 (C-3′), 71.63 (C-5), 70.77 (O*C*H$_2$), 70.47 (C-4′), 70.23 (C-6′), 69.46 (C-3), 65.80 (C-2), 62.42 (C-6), 56.41 (C-2′), 22.70 (CO*C*H$_3$), 13.51 (*C*H$_3$C of oxazoline); HRMS (FAB$^+$) calcd for $C_{18}H_{27}O_{12}N_2Na_2$ $[M+Na]^+$ 509.1359, found 509.1364.

4.2.13. 4-Methoxybenzyl 2-deoxy-3-*O*-(4-methoxybenzyl)-2-phthalimido-β-D-glucopyranoside (20). Compound **9** (185 mg, 0.28 mmol) was dissolved in 70% aq AcOH (20 mL), and the mixture stirred at 70 °C for 1 h, and then concentrated, diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (2:1–1:5 *n*-hexane–EtOAc) to afford **20** (127 mg, 83%) as a white amorphous powder: R_f 0.36 (1:5 *n*-hexane–EtOAc); $[\alpha]_D^{25}$ +1.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.80–6.50 (m, 16H, Ar), 5.16 (d, 1H, $J_{1,2}$ 8.54 Hz, H-1), 4.69 (d, 1H, *J* 12.04 Hz, CH_2Ph), 4.54 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.43 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.43 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.23 (dd, 1H, $J_{3,4}$ 8.54 Hz, H-3), 4.13 (dd, 1H, $J_{2,3}$ 10.79 Hz, H-2), 3.96 (m, 1H, H-6a), 3.85 (m, 1H, H-6b), 3.74 (m, 1H, H-4), 3.69 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 3.52 (m, 1H, H-5), 2.40 (d, 1H, $J_{4,4\text{-OH}}$ 3.48 Hz, 4-O*H*), 2.06 (t, 1H, $J_{6,6-OH}$ 6.78 Hz, 6-O*H*); ^{13}C NMR ($CDCl_3$): δ 167.62 (*C*O of phthalimido) 158.81–113.16 (C_{Ar}), 97.11 (C-1), 78.23 (C-3), 75.35 (C-5), 73.92 (*C*H$_2$Ph), 71.76 (C-6), 70.78 (*C*H$_2$Ph), 61.81 (C-6), 55.55 (C-2), 54.85 (O*C*H$_3$), 54.62 (O*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{30}H_{31}NNaO_9$ $[M+Na]^+$ 572.1897, found 572.1871.

4.2.14. 4-Methoxybenzyl 6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-3-*O*-(4-methoxybenzyl)-2-phthalimido-β-D-glucopyranoside (21). Compound **20** (7.43 g, 13.5 mmol) was dissolved in pyridine (50 mL), and *tert*-butyldimethylchlorosilane (4.07 g, 27.0 mmol) was added. The mixture stirred at room temperature for 1 h, then poured into satd aq $NaHCO_3$, extracted with $CHCl_3$, and washed with brine. The organic layer was dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (5:1–2:1 *n*-hexane–EtOAc) to give **21** (8.72 g, 97%) as a white amorphous powder: R_f 0.55 (1:1 *n*-hexane–EtOAc); $[\alpha]_D^{28}$ +1.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.77–6.39 (m, 12H, Ar), 5.13 (d, 1H, $J_{1,2}$ 8.54 Hz, H-1), 4.70 (d, 1H, *J* 12.04 Hz, CH_2Ph), 4.68 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.45 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.38 (d, 1H, *J* 12.05 Hz, CH_2Ph), 4.20 (dd, 1H, $J_{3,4}$ 8.53 Hz, H-3), 4.10 (dd, 1H, $J_{2,3}$ 10.54 Hz, H-2), 4.01

(dd, 1H, $J_{6a,6b}$ 10.29, $J_{5,6a}$ 4.77 Hz, H-6a), 3.90 (dd, 1H, $J_{5,6b}$ 6.52 Hz, H-6b), 3.81 (t, 1H, $J_{4,5}$ 8.79 Hz, H-4), 3.67, 3.59 (2s, 6H, OCH_3), 3.41 (s, 1H, 4-O*H*), 0.94 (s, 9H, C(CH_3)$_3$), 0.15 (s, 6H, SiCH_3); ^{13}C NMR ($CDCl_3$): δ 167.72, 167.56 (*C*O of phthalimido), 158.92–113.25 (C_{Ar}), 96.86 (C-1), 77.97 (C-3), 75.66 (C-4), 73.77 (*C*H_2Ph), 73.49 (C-5), 70.44 (*C*H_2Ph), 65.19 (C-6), 55.28 (C-2), 54.98 (O*C*H_3), 54.76 (O*C*H_3), 25.82 (C(*C*H_3)$_3$), 18.21 (*C*(CH$_3$)$_3$), −5.51 (Si*C*H_3); HRMS (FAB$^+$) calcd for $C_{36}H_{46}NO_9Si$ [M+H]$^+$ 664.2942, found 664.2940.

4.2.15. 4-Methoxybenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-(4-methoxybenzyl)-2-phthalimido-β-D-glucopyranoside (22). Compounds **11** (5.30 g, 9.1 mmol) and **21** (3.03 g, 4.5 mmol) were dissolved together in dry CH_2Cl_2 (83 mL) with activated MS4A (10.0 g). The mixture was cooled to −20 °C and then BF_3 etherate (0.23 mL, 1.8 mmol) in dry CH_2Cl_2 (1 mL) was added. After stirring at −20 °C for 1 h, the mixture was filtered through Celite, diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and evaporated, and the residue was purified by silica gel column chromatography (3:1–2:1 *n*-hexane–EtOAc) to provide **22** (4.32 g, 87%) as a white amorphous powder: R_f 0.31 (1:1 *n*-hexane–EtOAc); $[\alpha]_D^{27}$ +25.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.91–6.30 (m, 16H, Ar), 5.88 (dd, 1H, $J_{2',3'}$ 10.54, $J_{3',4'}$ 9.04 Hz, H-3′), 5.58 (d, 1H, $J_{1',2'}$ 8.03 Hz, H-1′), 5.17 (t, 1H, H-4′), 4.93 (d, 1H, $J_{1,2}$ 8.53 Hz, H-1), 4.71 (d, 1H, *J* 12.55 Hz, C*H*$_2$Ph), 4.57 (d, 1H, *J* 11.55 Hz, C*H*$_2$Ph), 4.43 (d, 1H, *J* 12.55 Hz, C*H*$_2$Ph), 4.42 (dd, 1H, $J_{6a',6b'}$ 12.05, $J_{5,6a'}$ 4.52 Hz, H-6a′), 4.34 (dd, 1H, H-2′), 4.28 (d, 1H, *J* 12.04 Hz, C*H*$_2$Ph), 4.13–4.10 (m, 3H, H-3, H-4, H-6b′), 4.00 (t, 1H, $J_{2,3}$ 9.54 Hz, H-2), 3.84 (m, 1H, H-5′), 3.69–3.66 (m, 4H, H-6a, OCH_3), 3.57 (s, 3H, OCH_3), 3.46 (dd, 1H, $J_{6a,6b}$ 12.04, $J_{5,6b'}$ 3.51 Hz, H-6b), 3.14 (d, 1H, H-5), 2.03, 2.03, 1.85 (3s, 9H, COCH_3), 0.96 (s, 9H, C(CH_3)$_3$), 0.13, 0.10 (2s, 6H, SiCH_3); ^{13}C NMR ($CDCl_3$): δ 170.14, 170.48, 170.00(*C*OCH$_3$), 167.94 (*C*O of phthalimido), 159.36–113.54 (C_{Ar}), 97.28 (C-1′), 96.70 (C-1), 76.49 (C-4), 76.24 (C-3), 75.36 (C-5), 74.12 (*C*H_2Ph), 71.94 (C-5′), 71.04 (C-3′), 70.10 (*C*H_2Ph), 69.47 (C-4′), 62.24 (C-6′), 61.68 (C-6), 56.14 (C-2), 55.74 (C-2′), 55.45 (O*C*H_3), 55.14 (O*C*H_3), 26.30 (C(*C*H_3)$_3$), 21.09, 21.04, 20.85 (CO*C*H_3), 18.75 (*C*(CH$_3$)$_3$), −4.68, −4.90 (Si*C*H_3); HRMS (FAB$^+$) calcd for $C_{56}H_{64}N_2O_{18}SiNa$ [M+Na]$^+$ 1103.3821, found 1103.3839.

4.2.16. 4-Methoxybenzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (23). The phthalimido group of compound **22** (4.24 g, 3.9 mmol) was converted into the acetamido group by treatment with hydrazine monohydrate (1.00 mL, 20.6 mmol) in 10:1 (v/v) EtOH–H_2O (55 mL) followed by acetylation with Ac_2O (10 mL) in pyridine (30 mL) as described in the preparation of compound **13**. The mixture was subjected to silica gel column chromatography (2:1 toluene–EtOAc, then EtOAc) to isolate **23** (2.78 g, 78%) as a white amorphous powder: R_f 0.48 (EtOAc); $[\alpha]_D^{26}$ −73.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.27–6.75 (m, 8H, Ar), 6.44 (d, 1H, $J_{2,NH}$ 9.54 Hz, N*H*), 5.65 (d, 1H, $J_{2',N'H}$ 9.04 Hz, N′*H*), 5.12 (t, 1H, $J_{4',5'}$ 9.54 Hz, H-4′), 5.02 (t, 1H, $J_{3',4'}$ 10.04 Hz, H-3′), 4.73 (d, 1H, *J* 11.55 Hz, C*H*$_2$Ph), 4.68 (d, 1H, *J* 11.05 Hz, C*H*$_2$Ph), 4.51 (d, 1H, $J_{1,2}$ 4.51 Hz, H-1), 4.52 (d, 1H, *J* 11.04 Hz, C*H*$_2$Ph), 4.43 (d, 1H, *J* 11.54 Hz, C*H*$_2$Ph), 4.41 (d, 1H, $J_{1',2'}$ 8.03 Hz, H-1′), 4.29(dd, 1H, $J_{6a',6b'}$ 12.05, $J_{5',6a'}$ 4.52 Hz, H-6a′), 4.24 (m, 1H, H-2), 4.15–3.98 (m, 4H, H-3, H-6a, H-2′, H-6b′), 3.80 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.74–3.70 (m, 2H, H-4, H-6b), 3.58 (m, 1H, H-5), 3.53 (ddd, 1H, $J_{5',6b'}$ 2.00 Hz, H-5′), 2.06, 2.04, 2.03, 2.02, 1.89 (5s, 15H, COCH_3), 0.87 (s, 9H, C(CH_3)$_3$), 0.00, −0.04 (2s, 6H, SiCH_3); ^{13}C NMR ($CDCl_3$): δ 171.47, 170.84, 170.59, 170.31, 169.19 (*C*OCH$_3$), 158.91–113.43 (C_{Ar}), 99.99 (C-1′), 99.14 (C-1), 76.47 (C-4), 75.81 (C-5), 73.02 (C-3), 72.27 (C-3′), 71.94 (C-5′), 71.40, 69.73 (*C*H_2Ph), 67.92 (C-4′), 62.51 (C-6), 61.79 (C-6′), 55.17, 55.14 (O*C*H_3), 54.11 (C-2′), 49.53 (C-2), 25.82 (C(*C*H_3)$_3$), 23.27, 23.07, 20.67, 20.61, 20.53 (CO*C*H_3), 18.07 (*C*(CH$_3$)$_3$), −5.35, −5.41 (Si*C*H_3); HRMS (FAB$^+$) calcd for $C_{44}H_{65}N_2O_{16}Si$ [M+H]$^+$ 905.4103, found 905.4110.

4.2.17. 4-Methoxybenzyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (24). Sodium methoxide in MeOH (28 wt%, 0.2 mL) was added dropwise to compound **23** (1.22 g, 1.3 mmol) in MeOH (10 mL). After stirring at room temperature for 3 h, the mixture was evaporated, and the residue was dissolved in dry DMF (10 mL) and then powdered NaOH (330 mg, 8.3 mmol) and 4-methoxybenzyl chloride (1.1 mL, 8.2 mmol) were added. The mixture stirred at room temperature overnight and then MeOH (5 mL) was added to quench the excess of the reagent, and the mixture was evaporated. The residue was diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$), evaporated, and the residue purified by silica gel column chromatography (2:1 $CHCl_3$–EtOAc) to give **24** (715 mg, 47%) as a white amorphous powder: R_f 0.41 (3:1 $CHCl_3$–acetone); $[\alpha]_D^{28}$ −58.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.25–6.72 (m, 20H, Ar), 6.62 (d, 1H, $J_{2',N'H}$ 9.04 Hz, N′*H*), 4.87 (d, 1H, $J_{2,NH}$ 8.53 Hz, N*H*), 4.79–4.68 (m, 4H, C*H*$_2$Ph), 4.48 (m, 1H, H-1′, C*H*$_2$Ph), 4.29 (m, 1H, H-2′), 4.22 (d, 1H, $J_{1,2}$ 8.03 Hz, H-1), 4.02–3.98 (m, 2H, H-6a, H-4′),

3.86–3.54 (m, 7H, H-2, H-4, H-6b, H-3′, H-5′, H-6a′, H-6b′), 3.81, 3.79, 3.79, 3.76, 3.75 (5s, 15H, OCH_3), 3.43 (t, 1H, $J_{3,4}$ 9.53 Hz, H-3), 3.30 (d, 1H, $J_{4,5}$ 9.54 Hz H-5), 2.01, 1.71 (2s, 6H, COCH_3), 0.82 (s, 9H, C(CH_3)$_3$), −0.04, −0.08 (2s, 6H, SiCH_3); ^{13}C NMR ($CDCl_3$): δ 170.57, 170.27 (*C*OCH$_3$), 159.45–113.42 (C_{Ar}), 99.93 (C-1), 99.41 (C-1′), 80.05 (C-3), 78.05 (C-4), 76.91 (C-3′), 76.24 (C-5′), 74.96 (C-5), 74.53, 73.56, 73.12 (*C*H$_2$Ph), 72.12 (C-4′), 71.18, 69.73 (*C*H$_2$Ph), 67.78 (C-6′), 62.80 (C-6), 55.28, 55.24, 55.51, 55.17, 55.14 (O*C*H$_3$), 54.75 (C-2), 49.29(C-2′), 25.85 (C(*C*H$_3$)$_3$), 23.48, 23.14 (CO*C*H$_3$), 18.12 (*C*(CH$_3$)$_3$), −5.40 (Si*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{62}H_{83}N_2O_{16}Si$ [M+H]$^+$ 1139.5512, found 1139.5511.

4.2.18. 4-Methoxybenzyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (25). To a solution of compound **24** (1.61 g, 1.4 mmol) in dry THF (20 mL) was added tetrabutylammonium fluoride (1.0 M in THF, 2.8 mL). The mixture stirred at room temperature for 1 h, then poured into satd aq $(NH_4)_2SO_4$, extracted with $CHCl_3$, and washed with brine. The organic layer was dried ($MgSO_4$), evaporated, and the residue purified by silica gel column chromatography (1:1 $CHCl_3$–EtOAc, 5:1–3:1 $CHCl_3$–acetone, then 10:1 $CHCl_3$–MeOH) to provide **25** (1.43 g, 99%) as a white solid: R_f 0.29 (10:1 $CHCl_3$–MeOH); mp 212–213 °C; $[\alpha]_D^{28}$ −35.6 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.22–6.76 (m, 20H, Ar), 6.15 (d, 1H, $J_{2',N'H}$ 8.54 Hz, N′*H*), 5.15 (d, 1H, $J_{2,NH}$ 8.03 Hz, N*H*), 4.76–4.39 (m, 12H, H-1, H-1′, CH_2Ph), 4.02 (m, 1H, H-2′), 3.89 (t, 1H, $J_{3',4'}$ 5.52 Hz, H-4′), 3.82–3.69 (m, 4H, H-2, H-6a, H-6b, H-3′), 3.81, 3.79, 3.79, 3.75 (15H, 4s, OCH_3), 3.65–3.55 (m, 5H, H-3, H-4, H-5′, H-6a′, H-6b′), 3.37 (m, 1H, H-5), 2.34 (t, 1H, $J_{6,6-OH}$ 6.52 Hz, 6-O*H*), 1.91, 1.73 (2s, 6H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 170.75, 170.27 (*C*OCH$_3$), 159.34–113.63 (C_{Ar}), 100.37 (C-1), 97.74 (C-1′), 80.27 (C-3), 78.01 (C-4), 77.82 (C-3′), 76.06 (C-5′), 74.79 (C-5), 74.49 (C-4′), 74.33, 73.86, 73.03, 72.14, 70.30 (*C*H$_2$Ph), 68.20 (C-6′), 62.29 (C-6), 55.57 (C-2), 55.25, 55.21, 55.16 (O*C*H$_3$), 52.34 (C-2′), 23.44, 23.23 (CO*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{56}H_{69}N_2O_{16}$ [M+H]$^+$ 1025.4647, found 1025.4640.

4.2.19. 4-Methoxybenzyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-allyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (26). Compound **25** (1.43 g, 1.4 mmol) in dry DMF (20.0 mL) was treated with allyl bromide (0.180 mL, 2.13 mmol) and powdered NaOH (84.0 mg, 2.10 mmol) as described in the preparation of compound **16**. Silica gel column chromatography (3:1–2:1 $CHCl_3$–acetone) of the residue afforded **26** (1.22 g, 81%) as a white solid: R_f 0.47 (2:1 $CHCl_3$–acetone); mp 201–202 °C; $[\alpha]_D^{27}$ −51.0 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.24–6.73 (m, 20H, Ar), 6.60 (d, 1H, $J_{2',N'H}$ 9.04 Hz, N′*H*), 5.81 (m, 1H, C*H*=CH$_2$), 5.16 (dd, 1H, *J* 17.56, 1.50 Hz, CH=CH_2), 5.09 (d, 1H, *J* 10.04 Hz, CH=CH_2), 4.88 (d, 1H, $J_{2,NH}$ 8.54 Hz, N*H*), 4.78–4.68 (m, 4H, CH_2Ph), 4.59–4.41 (m, 7H, H-1′, CH_2Ph), 4.29–4.25 (m, 2H, H-1, H-2′), 3.93 (m, 1H, H-4′), 3.89 (d, 2H, *J* 5.52 Hz, CH_2CH=CH$_2$), 3.81–3.68 (m, 5H, H-2, H-6a, H-6b, H-3′, H-5′), 3.81, 3.80, 3.79, 3.76 (4s, 15H, OCH_3), 3.66–3.64 (m, 3H, H-4, H-6a′, H-6b′), 3.47 (dd, 1H, $J_{2,3}$ 10.04, $J_{3,4}$ 9.03 Hz, H-3), 3.35 (m, 1H, H-5), 1.99, 1.72 (2s, 6H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 170.70, 170.24 (*C*OCH$_3$), 159.43–158.84 (C_{Ar}), 134.49 (*C*H=CH$_2$), 130.55–129.04 (C_{Ar}), 117.19 (CH=*C*H$_2$), 113.96–113.45 (C_{Ar}), 99.96 (C-1), 99.46 (C-1′), 79.85 (C-3), 78.17 (C-4), 76.31 (C-3′), 74.99 (C-5), 74.47 (C-5′, *C*H$_2$Ph), 73.67 (*C*H$_2$Ph), 73.24 (C-4′), 73.10 (*C*H$_2$Ph), 72.19 (*C*H$_2$CH=CH$_2$), 71.35 (*C*H$_2$Ph), 70.18 (C-6), 69.72 (*C*H$_2$Ph), 68.07 (C-6′), 55.25, 55.22, 55.18, 55.15, 55.13 (O*C*H$_3$), 55.02 (C-2), 49.33 (C-2′), 23.49, 23.14 (CO*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{59}H_{73}N_2O_{16}$ [M+H]$^+$ 1065.4960, found 1065.4984.

4.2.20. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-1,3-di-*O*-acetyl-6-*O*-allyl-2-deoxy-α-D-glucopyranose (27). The 4-methoxybenzyl groups of compound **26** (57.0 mg, 54 μmol) were removed by treatment with DDQ (67.0 mg, 0.30 mmol) in 18:1 (v/v) CH_2Cl_2–H_2O (3.8 mL) followed by acetylation with Ac_2O (2 mL) in pyridine (4 mL) as described in the preparation of compound **17**. The residue was purified by silica gel column chromatography (3:1 $CHCl_3$–acetone) to provide **27** (32.0 mg, 87%) as a white solid: R_f 0.31 (1:1 $CHCl_3$–acetone); mp 255–256 °C (dec.); $[\alpha]_D^{28}$ +36.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.11 (d, 1H, $J_{1,2}$ 4.02 Hz, H-1), 5.95 (m, 1H, C*H*=CH$_2$), 5.68 (d, 1H, $J_{2',N'H}$ 9.04 Hz, N′*H*), 5.63 (d, 1H, $J_{2,NH}$ 9.03 Hz, N*H*), 5.34 (dd, 1H, *J* 17.07, 1.51 Hz, CH=CH_2), 5.29 (dd, 1H, *J* 10.54, 1.50 Hz, CH=CH_2), 5.21 (dd, 1H, $J_{2,3}$ 9.54, $J_{3,4}$ 4.01 Hz, H-3), 5.18 (dd, 1H, $J_{2',3'}$ 4.52, $J_{3',4'}$ 9.54 Hz, H-3′), 5.06 (t, 1H, $J_{4',5'}$ 9.79 Hz, H-4′), 4.73 (d, 1H, $J_{1',2'}$ 8.53 Hz, H-1′), 4.41–4.33 (m, 2H, H-2, H-6a′), 4.18 (m, 1H, CH_2CH=CH$_2$), 4.06–3.99 (m, 3H, H-4, H-6b′, CH_2CH=CH$_2$), 3.86–3.71 (m, 3H, H-5, H-6a, H-2′), 3.65 (ddd, 1H, $J_{5',6a'}$ 4.39, $J_{5',6b'}$ 2.51 Hz, H-5′), 3.57 (dd, 1H, $J_{6a,6b}$ 11.55, $J_{5,6b}$ 2.01 Hz, H-6b), 2.18, 2.09, 2.07, 2.03, 2.02, 1.93 (6s, 21H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 171.66, 170.91, 170.54, 170.09, 170.07, 169.35, 168.99 (*C*OCH$_3$), 134.36 (*C*H=CH$_2$), 117.85 (CH=*C*H$_2$), 100.62 (C-1′), 90.67 (C-1), 74.84 (C-4), 72.60 (*C*H$_2$CH=CH$_2$), 72.48 (C-3′), 72.36 (C-5), 71.82 (C-5′), 70.73 (C-3), 67.99 (C-4′), 67.49 (C-6), 61.76 (C-6′), 54.94 (C-2′), 51.17 (C-2), 23.29, 23.02, 21.00, 20.65, 20.61, 20.57 (CO*C*H$_3$); HRMS (FAB$^+$)

calcd for $C_{29}H_{43}N_2O_{16}$ $[M+H]^+$ 675.2613, found 675.2638.

4.2.21. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-1,3-di-*O*-acetyl-2-deoxy-6-*O*-methoxycarbonylmethyl-α-D-glucopyranose (28). Compound **27** (271 mg, 0.40 mmol) in 4:1 (v/v) CH_2Cl_2–MeOH (30 mL) was ozonized as described in the preparation of compound **18**. Silica gel column chromatography (2:1 *n*-hexane–EtOAc, then 1:1 $CHCl_3$–acetone) of the residue afforded crude 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-1,3-di-*O*-acetyl-2-deoxy-6-*O*-formylmethyl-α-D-glucopyranose as a white solid.

The crude product was oxidized with NaH_2PO_4 (31 mg, 0.26 mmol)–$NaClO_2$ (58 mg, 0.64 mmol) in 2:12:3 (v/v/v) CH_2Cl_2–*tert*-BuOH–H_2O (51 mL) containing 2-methyl-2-butene (0.24 mL, 2.3 mmol) followed by treatment with Dowex 50W-X4 (H^+ form) (300 mg) in 4:1 (v/v) MeOH–CH_2Cl_2 (25 mL). Silica gel column chromatography (2:1 *n*-hexane–EtOAc, then 2:1–1:1 $CHCl_3$–acetone) gave **28** (259 mg, 91%) as a white solid: R_f 0.29 (1:1 $CHCl_3$–acetone); mp 250–251 °C (dec.); $[\alpha]_D^{28}$ +44.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.89 (d, 1H, $J_{2',N'H}$ 10.03 Hz, N′*H*), 6.10 (d, 1H, $J_{1,2}$ 3.51 Hz, H-1), 5.66 (d, 1H, $J_{2,NH}$ 9.04 Hz, N*H*), 5.17 (dd, 1H, $J_{2,3}$ 10.79, $J_{3,4}$ 9.28 Hz, H-3), 5.09–5.07 (m, 2H, H-3′, H-4′), 4.86 (d, 1H, $J_{1',2'}$ 9.04 Hz, H-1′), 4.49 (d, 1H, *J* 17.57 Hz, OC*H*$_2$), 4.38 (dd, 1H, *J* 12.55, 4.01 Hz, H-6a′), 4.34 (m, 1H, H-2), 4.29 (dd, 1H, $J_{6a,6b}$ 10.29, $J_{5,6a}$ 2.26 Hz, H-6a), 4.22 (m, 1H, H-2′), 4.06–3.98 (m, 3H, H-4, H-6b′, OC*H*$_2$), 3.84 (s, 3H, OC*H*$_3$), 3.77 (d, 1H, H-5), 3.68 (m, 1H, H-5′), 3.38 (d, 1H, H-6b), 2.18, 2.09, 2.06, 2.02, 2.01, 1.92 (6s, 21H, COC*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 172.43, 171.77, 170.89, 170.66, 170.10, 170.06, 169.34, 169.14 (*C*OOCH$_3$, *C*OCH$_3$), 101.44 (C-1′), 90.75 (C-1), 74.93 (C-4), 73.43 (C-3′), 71.65 (C-5, C-5′), 70.34 (C-3), 68.97 (C-6), 68.45 (O*C*H$_2$), 68.07 (C-4′), 61.88 (C-6′), 53.41 (C-2′), 52.38 (O*C*H$_3$), 51.30 (C-2), 23.13, 22.97, 21.01, 20.68, 20.62, 20.58, 20.50 (CO*C*H$_3$); HRMS (FAB^+) calcd for $C_{29}H_{43}O_{18}N_2$ $[M+H]^+$ 707.2511, found 707.2479.

4.2.22. 2-Methyl-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-1,2-di-deoxy-6-*O*-methoxycarbonylmethyl-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (29). Compound **28** (118 mg, 0.17 mmol) in dry 1,2-dichloroethane (10 mL) was treated with Me_3-SiOTf (33 μL, 0.18 mmol) as described in the production of compound **19**. Compound **29** (60 mg, 56%) was isolated as a white amorphous powder through column chromatography on silica gel (2:1 $CHCl_3$–acetone) and then a Sephadex LH-20 column (MeOH as eluent): R_f 0.53 (10:1 $CHCl_3$–MeOH); $[\alpha]_D^{28}$ +8.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.48 (d, 1H, $J_{2,NH}$ 9.04 Hz, N*H*), 5.93 (d, 1H, $J_{1,2}$ 7.53 Hz, H-1), 5.59 (s, 1H, H-3), 5.13–5.10 (m, 2H, H-3′, H-4′), 4.79 (d, 1H, $J_{1',2'}$ 8.54 Hz, H-1′), 4.35 (d, 1H, *J* 17.07 Hz, OC*H*$_2$), 4.29 (dd, 1H, $J_{6a',6b'}$ 12.05, $J_{5,6a'}$ 4.01 Hz, H-6a′), 4.19–4.03 (m, 4H, H-2, H-6a, H-2′, H-6b′), 4.05 (d, 1H, *J* 17.07 Hz, OC*H*$_2$), 3.88–3.74 (m, 2H, H-4, H-5′), 3.79 (s, 3H, OC*H*$_3$), 3.56 (dd, 1H, $J_{4,5}$ 10.04 Hz, $J_{5,6a}$ 2.51 Hz, H-5), 3.41 (d, 1H, $J_{6a,6b}$ 9.03 Hz, H-6b), 2.09, 2.08, 2.03, 2.01, 1.88 (5s, 18H, C*H*$_3$C of oxazoline, COC*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 171.67, 171.00, 170.90, 170.01, 169.42, 169.31 (*C*OOCH$_3$, *C*OCH$_3$), 166.61 (O*C*=N), 102.74 (C-1′), 99.74 (C-1), 77.20 (C-4), 73.57 (C-3′), 71.90 (C-5′), 71.35 (C-3), 69.71 (C-6), 68.69 (C-5), 68.26 (C-4′), 67.81 (O*C*H$_2$), 65.09 (C-2), 61.95 (C-6′), 53.72 (C-2′), 52.17 (O*C*H$_3$), 23.03, 22.06, 21.01, 20.73, 20.70, 20.61 (CO*C*H$_3$), 14.00 (*C*H$_3$ of oxazoline); HRMS (FAB^+) calcd for $C_{27}H_{39}O_{16}N_2$ $[M+H]^+$ 647.2300, found 647.2309.

4.2.23. 2-Methyl-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-6-*O*-carboxymethyl-1,2-di-deoxy-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline sodium salt (2). Compound **29** (13 mg, 20 μmol) was treated with NaOMe in MeOH (0.1 M, 80 μL) as described in the synthesis of compound **1**. The remaining methyl ester was hydrolyzed in a carbonate buffer (25 mM, pH 12.0, 402 μL) to afford **2**: ^{1}H NMR (D_2O): δ 6.11 (d, 1H, $J_{1,2}$ 7.02 Hz, H-1), 4.62 (d, 1H, $J_{1',2'}$ 8.53 Hz, H-1′), 4.43 (s, 1H, H-3), 4.22 (m, 1H, H-2), 4.00–3.93 (m, 3H, H-6a′, H-6b′, OC*H*$_2$), 3.80–3.69 (m, 3H, H-4, H-2′, OC*H*$_2$), 3.64–3.44 (m, 6H, H-5, H-6a, H-6b, H-3′, H-4′, H-5′), 2.09, 2.07 (2s, 6H, C*H*$_3$C of oxazoline, COC*H*$_3$); ^{13}C NMR (D_2O): δ 178.12 (*C*OONa), 174.93 (*C*OCH$_3$), 168.76 (O*C*=N), 103.36 (C-1), 100.23 (C-1′), 79.09 (C-4), 76.36 (C-5′), 74.16 (C-3′), 70.96 (C-6), 70.62 (O*C*H$_2$), 70.23 (C-4′), 70.00 (C-5), 69.22 (C-3), 65.51 (C-2), 61.10 (C-6′), 56.22 (C-2′), 22.67 (CO*C*H$_3$), 13.37 (*C*H$_3$ of oxazoline); HRMS (FAB^+) calcd for $C_{18}H_{27}O_{12}N_2Na_2$ $[M+Na]^+$ 509.1359, found 509.1373.

4.2.24. (Methyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (32). Methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate)uronate **30**[23] (570 mg, 1.2 mmol) and 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (**31**,[24] 276 mg, 0.80 mmol) were dissolved together in dry CH_2Cl_2 (8 mL), and BF_3 etherate (0.15 mL, 1.2 mmol) in dry CH_2Cl_2 (0.35 mL) was added in the presence of activated MS4A (1.0 g) at −20 °C under argon. After stirring at −20 °C for 5 h, the mixture filtered through Celite, diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and evaporated, and the residue purified by silica gel column chromatography (2:1 *n*-hexane–EtOAc, then EtOAc) and then size-exclusion chromatography on a Sephadex LH-20 column eluted with MeOH to provide **32** (268 mg, 51%) as a white solid:

R_f 0.38 (10:1 $CHCl_3$–MeOH); mp 249–250 °C (dec.); $[\alpha]_D^{25}$ +43.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.09 (d, 1H, $J_{1,2}$ 3.52 Hz, H-1), 5.69 (d, 1H, $J_{2,NH}$ 9.04 Hz, N*H*), 5.26–5.14 (m, 3H, H-3, H-3′, H-4′), 4.96 (t, 1H, $J_{2',3'}$ 8.29 Hz, H-2′), 4.60 (d, 1H, $J_{1',2'}$ 8.03 Hz, H-1′), 4.42 (d, 1H, $J_{6a,6b}$ 12.04 Hz, H-6a), 4.38 (m, 1H, H-2), 4.10 (dd, 1H, $J_{5,6b}$ 2.77 Hz, H-6b), 3.99 (d, 1H, $J_{4',5'}$ 9.54 Hz, H-5′), 3.88–3.87 (m, 2H, H-4, H-5), 3.75 (s, 3H, CH_3O), 2.19, 2.13, 2.08, 2.05, 2.02, 2.01, 1.93 (7s, 21H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 171.64, 170.31, 170.10, 169.98, 169.29, 169.17, 168.78, 166.66 (*C*OCH_3, *C*OOCH_3), 100.85 (C-1), 90.43 (C-1′), 76.11 (C-4), 72.69 (C-5′), 72.00 (C-3′), 71.33 (C-2′), 70.51 (C-3), 70.24 (C-5), 69.21 (C-4′), 61.36 (C-6), 52.83 (*C*H_3O), 51.03 (C-2), 22.99, 20.94, 20.79, 20.63, 20.53, 20.50, 20.40 (*C*H_3CO); HRMS (FAB^+) calcd for $C_{27}H_{38}NO_{18}$ $[M+H]^+$ 664.2089, found 664.2086.

4.2.25. 2-Methyl-[3,6-di-*O*-acetyl-1,2-di-deoxy-4-*O*-(methyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (33). A solution of compound **32** (33.0 mg, 50 μmol) in dry 1,2-dichloroethane (500 μL) was treated with Me_3SiOTf (18 μL, 99 μmol) as described in the preparation of compound **19**. The residue was purified on a silica gel column (1:2 *n*-hexane–EtOAc) and then a Sephadex LH-20 column (MeOH as eluent) to give **33** (23.0 mg, 77%) as a white amorphous powder: R_f 0.45 (15:1 $CHCl_3$–MeOH); $[\alpha]_D^{27}$ +6.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.91 (d, 1H, $J_{1,2}$ 7.53 Hz, H-1), 5.61 (m, 1H, H-3), 5.27–5.21 (m, 2H, H-3′, H-4′), 5.01 (m, 1H, H-2′), 4.79 (d, 1H, $J_{1',2'}$ 8.03 Hz, H-1′), 4.22 (dd, 1H, $J_{6a,6b}$ 12.05 Hz, $J_{5,6a}$ 2.51 Hz, H-6a), 4.13–4.11 (m, 2H, H-2, H-5′), 4.04 (dd, 1H, $J_{5,6b}$ 5.52 Hz, H-6b), 3.76 (s, 3H, COOCH_3), 3.68 (d, 1H, $J_{4,5}$ 9.54 Hz, H-4), 3.50 (m, 1H, H-5), 2.11, 2.09 (2s, 6H, COCH_3), 2.09 (d, 3H, *J* 1.51 Hz, CH_3C of oxazoline), 2.04, 2.02, 2.01 (3s, 9H, COCH_3); ^{13}C NMR ($CDCl_3$): δ 170.56, 170.15, 169.40, 169.32, 169.28, 166.78, 166.67 (*C*OCH_3, *C*OOCH_3, O*C*=N), 101.65 (C-1′), 99.02 (C-1), 78.12 (C-4), 72.55 (C-5′), 72.30 (C-3′), 70.96 (C-2′), 70.51 (C-3), 69.08 (C-4′), 67.35 (C-5), 65.09 (C-2), 63.32 (C-6), 52.88 (O*C*H_3), 20.97, 20.79, 20.58, 20.50 (CO*C*H_3), 13.93 (*C*H_3 of oxazoline); HRMS (FAB^+) calcd for $C_{25}H_{34}O_{16}N$ $[M+H]^+$ 604.1878, found 604.1862.

4.2.26. 2-Methyl-[1,2-di-deoxy-4-*O*-(sodium β-D-glucopyranosyluronate)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (3). Compound **33** (23.0 mg, 38 μmol) was treated with NaOMe in MeOH (28 wt%, 20 μL) as described for the synthesis of compound **1**. The methyl ester was hydrolyzed in a carbonate buffer (25 mM, pH 12.0, 600 μL) to provide **3**: ^{1}H NMR (D_2O): δ 6.10 (d, 1H, $J_{1,2}$ 7.03 Hz, H-1), 4.50 (d, 1H, $J_{1',2'}$ 8.03 Hz, H-1′), 4.39 (s, 1H, H-3), 4.19 (m, 1H, H-2), 3.87–3.61 (m, 4H, H-4, H-6a, H-6b, H-5′), 3.46–3.39 (m, 3H, H-5, H-3′, H-4′), 3.34 (t, 1H, $J_{2',3'}$ 7.28 Hz, H-2′), 2.08 (s, 3H, CH_3C of oxazoline); ^{13}C NMR (D_2O): δ 176.53 (*C*OONa), 168.88 (O*C*=N), 104.48 (C-1′), 100.43 (C-1), 78.90 (C-4), 76.60 (C-5′), 75.94 (C-3′), 73.59 (C-2′), 72.38 (C-4′), 71.55 (C-5), 69.70 (C-3), 65.79 (C-2), 62.23 (C-6), 13.58 (*C*H_3 of oxazoline); HRMS (FAB^+) calcd for $C_{14}H_{20}O_{11}NNa_2$ $[M+Na]^+$ 424.0832, found 424.0851.

4.3. Chitinase-catalyzed hydrolysis of the oxazoline derivatives

4.3.1. Hydrolysis of compound 1. A solution of compound **1** (12.8 mg, 26.3 μmol) in carbonate buffered deuterium oxide solution (50 mM, pD 9.0, 263 μL) was kept in an NMR probe tube at 30 °C for 5 h. Chitinase from *Bacillus* sp. (1.3 mg) was added, and the mixture was kept standing at 30 °C. The concentration of **1** was calculated from the integrated values of the ^{1}H NMR signals from H-1 proton and the methyl protons as internal standard. After complete consumption of **1**, the enzyme was thermally inactivated at 90 °C for 20 min. An aliquot of the mixture was subjected to HPLC and MALDI-TOF/MS. The mixture was purified through a Sephadex G-10 column running with distilled H_2O to give 2-acetamido-6-*O*-carboxymethyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose sodium salt (**34**) (9.0 mg, 68%) as a white amorphous powder: ^{1}H NMR (D_2O): δ 5.20 (d, 0.57H, $J_{1\alpha,2\alpha}$ 3.01 Hz, H-1α), 4.72–4.70 (m, 0.43H, H-1β), 4.61 (d, 1H, $J_{1',2'}$ 8.54 Hz, H-1′), 2.08, 2.05 (2s, 6H, COCH_3); ^{13}C NMR (D_2O): δ 178.73 (*C*OONa), 175.39, 175.21, 175.12 (*C*OCH_3), 102.22, 102.18 (C-1′α, C-1′β), 95.47 (C-1β), 91.02 (C-1α), 22.84–22.53 (CO*C*H_3); HRMS (FAB^+) calcd for $C_{18}H_{29}O_{13}N_2Na_2$ $[M+Na]^+$, 527.1465, found 527.1453.

4.3.2. Hydrolysis of compound 2. The hydrolysis reaction of **2** (22.0 mg, 45.2 μmol) was performed with chitinase from *Bacillus* sp. (2.2 mg) in a carbonate-buffered deuterium oxide solution (50 mM, pD 9.0, 452 μL) according to the procedure for hydrolysis of **1**. Isolation of the product was also carried out as already described to provide 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-carboxymethyl-D-glucopyranose sodium salt (**35**) (11.2 mg, 49%) as a white amorphous powder: ^{1}H NMR (D_2O): δ 5.20 (d, 0.5H, $J_{1\alpha,2\alpha}$ 3.01 Hz, H-1α), 4.73–4.68 (m, 1.5H, H-1β, H-1′α, H-1′β), 2.07, 2.05 (2s, 6H, COCH_3); ^{13}C NMR (D_2O): δ 178.37 (*C*OONa), 175.36, 175.13, 175.05 (*C*OCH_3), 101.81 (C-1′), 95.39 (C-1β), 91.01 (C-1α), 22.89–22.54 (CO*C*H_3); HRMS (FAB^+) calcd for $C_{18}H_{29}O_{13}N_2Na_2$ $[M+Na]^+$, 527.1465, found 527.1484.

4.3.3. Hydrolysis of compound 3. Compound **3** (10.9 mg, 27.2 μmol) was enzymatically hydrolyzed with chitinase from *Bacillus* sp. (1.1 mg) in a carbonate-buffered

deuterium oxide solution (50 mM, pD 9.0, 272 μL) according to the procedure for hydrolysis of **1**. The mixture was similarly purified through a Sephadex G-10 column to afford (sodium β-D-glucopyranosyluronate)-(1→4)-2-acetamido-2-deoxy-4-*O*-D-glucopyranose (**36**) (9.7 mg, 85%) as a white amorphous powder: ^{1}H NMR (D_2O): δ 5.22 (d, 0.65H, $J_{1\alpha,2\alpha}$ 3.01 Hz, H-1α), 4.74–4.72 (m, 0.35H, H-1β), 4.55 (d, 1H, $J_{1',2'}$ 8.03 Hz, H-1′), 2.05 (s, 3H, COCH_3); ^{13}C NMR (D_2O): δ 176.20 (*C*OONa), 175.37, 175.09 (*C*OCH$_3$), 102.91, 102.86 (C-1′α, C-1′β), 95.49 (C-1β), 91.09 (C-1α), 22.84, 22.54 (CO*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{14}H_{23}O_{12}NNa$ [M+H]$^+$, 420.1118, found 420.1122.

4.4. Enzymatic glycosidation

4.4.1. Methyl 2-acetamido-6-*O*-carboxymethyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside sodium salt (37). Compound **4** (52.0 mg, 120 μmol) in a carbonate buffer (50 mM, pH 10.0, 400 μL) containing chitinase from *Bacillus* sp. (2.0 mg) was added to a solution of **1** (19.5 mg, 40 μmol) in a carbonate buffer (50 mM, pH 10.0, 400 μL). The mixture was kept at 30 °C in an NMR probe tube. The concentration of **1** was calculated from the integrated ^{1}H NMR values of the H-1 signal and the methyl protons as the internal standard. After complete consumption of **1**, the reaction was terminated by thermal inactivation of the enzyme at 90 °C for 20 min. The mixture was lyophilized and the residue purified through a BioGel P-2 column using 0.1 M aq NaCl as eluent. The combined fractions were lyophilized and then desalted through a BioGel P-2 column eluting with distilled H_2O to give **37** (18.1 mg, 49%) as a white amorphous powder: $[\alpha]_D^{26}$ −53.0 (*c* 0.1, H_2O); ^{1}H NMR (D_2O): δ 4.61–4.57 (m, 2H, H-1′, H-1″, H-1‴), 4.44 (d, 1H, $J_{1,2}$ 7.53 Hz, H-1), 3.98 (d, 2H, *J* 4.52 Hz, CH_2COONa), 3.88–3.50 (m, 24H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-2″, H-3″, H-4″, H-5″, H-6a″, H-6b″, H-2‴, H-3‴, H-4‴, H-5‴, H-6a‴, H-6b‴), 3.50 (s, 3H, OCH_3), 2.07, 2.07, 2.06, 2.04 (4s, 12H, COCH_3); ^{13}C NMR (D_2O): δ 178.41 (*C*OONa), 174.96 (*C*OCH$_3$), 102.28 (C-1), 101.96, 101.65 (C-1′, C-1″, C-1‴), 79.78, 79.67, 79.43 (C-4, C-4′, C-4″), 74.95 (C-5, C-5′, C-5″), 73.63, 72.96, 72.59, 72.48 (C-3, C-3′, C-3″, C-3‴), 70.60 (*C*H$_2$COONa), 70.15 (C-4‴), 69.90 (C-6‴), 60.50 (C-6, C-6′, C-6″), 57.67 (O*C*H$_3$), 56.10, 55.53, 55.31 (C-2, C-2′, C-2″, C-2‴), 22.73 (CO*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{35}H_{57}O_{23}N_4Na_2$ [M+Na]$^+$ 947.3209, found 947.3212.

4.4.2. Pent-4-enyl 2-acetamido-6-*O*-carboxymethyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside sodium salt (38). A solution of compound **1** (39.0 mg, 80 μmol) with **5** (69.0 mg, 240 μmol) in a carbonate buffer (50 mM, pH 10.0, 1.6 mL) was incubated with chitinase from *Bacillus* sp. (3.9 mg) at 30 °C as described in the preparation of **37**. The residue was purified by HPLC through a Shodex Asahipak NH2P-50 4E column eluting with 17:33 (v/v) phosphate buffer (10 mM, pH 7.0)–MeCN. The combined fractions were lyophilized and then desalted through a BioGel P-2 column eluting with distilled H_2O to afford (**38**) (23.0 mg, 37%) as a white amorphous powder: $[\alpha]_D^{26}$ −19.0 (*c* 0.1, H_2O); ^{1}H NMR (D_2O): δ 5.88 (m, 1H, C*H*=CH$_2$), 5.09–5.01 (m, 2H, CH=CH_2), 4.61–4.57 (m, 2H, H-1′, H-1″), 4.49 (d, 1H, $J_{1,2}$ 8.03 Hz, H-1), 3.98–3.97 (d, 2H, *J* 4.02 Hz, CH_2COONa), 3.93–3.47 (m, 20H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-2″, H-3″, H-4″, H-5″, H-6a″, H-6b″, OCH_2CH$_2$), 2.11–2.04 (m, 2H, CH_2CH=CH$_2$), 2.07, 2.07, 2.04 (3s, 9H, COCH_3), 1.68–1.61 (m, 2H, OCH$_2$CH_2); ^{13}C NMR (D_2O): δ 178.42 (*C*OONa), 174.99, 174.81 (*C*OCH$_3$), 139.10 (*C*H=CH$_2$), 115.52 (CH=*C*H$_2$), 102.00, 101.72 (C-1′, C-1″), 101.45 (C-1), 79.82, 79.68 (C-4, C-4′), 75.02, 74.93 (C-5, C-5′, C-5″), 73.60, 72.83, 72.60 (C-3, C-3′, C-3″), 70.62 (*C*H$_2$COONa), 70.18 (C-4″), 70.13 (O*C*H$_2$CH$_2$), 69.95 (C-6″), 60.49 (C-6, C-6′), 56.09, 55.54, 55.48 (C-2, C-2′, C-2″), 29.77 (*C*H$_2$CH=CH$_2$), 28.32 (OCH$_2$CH_2), 22.82, 22.74 (CO*C*H$_3$); HRMS (FAB$^+$) calcd for $C_{31}H_{51}O_{18}N_3Na$ [M+H]$^+$ 776.3065, found 776.3049.

4.4.3. Methyl 2-acetamido-6-*O*-carboxymethyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside sodium salt (39). A mixture of compounds **1** (19.5 mg, 40 μmol) and **6** (28.0 mg, 120 μmol) in a carbonate buffer (50 mM, pH 10.0, 800 μL) was incubated with chitinase from *Bacillus* sp. (2.0 mg) at 30 °C as described in the production of **37**. The residue was purified by HPLC through a Shodex Asahipak NH2P-50 4E column using 17:33 (v/v) phosphate buffer (10 mM, pH 7.0)–MeCN as eluent. The combined fractions were lyophilized and then desalted through a BioGel P-2 column eluting with distilled H_2O to provide **39** (12.6 mg, 45%) as a white amorphous powder: $[\alpha]_D^{26}$ −17.0 (*c* 0.1, H_2O); ^{1}H NMR (D_2O): δ 4.65–4.56 (m, 2H, H-1′, H-1″), 4.43 (d, 1H, $J_{1,2}$ 7.53 Hz, H-1), 4.06–3.95 (m, 2H, CH_2COONa), 3.92–3.45 (m, 18H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-2″, H-3″, H-4″, H-5″, H-6a″, H-6b″), 3.50 (s, 3H, OCH_3), 2.07, 2.04 (2s, 9H, COCH_3); ^{13}C NMR (D_2O): δ 178.67 (*C*OONa), 175.27, 175.21, 175.18 (*C*OCH$_3$), 102.48 (C-1), 102.18, 101.88 (C-1′, C-1″), 80.01, 79.81 (C-4, C-4′), 75.21, 75.16, 75.09 (C-5, C-5′, C-5″), 73.83, 73.14, 72.80 (C-3, C-3′, C-3″), 70.71 (*C*H$_2$COONa), 70.33 (C-4″), 70.08 (C-6″), 60.68, 60.63 (C-6, C-6′), 57.76 (O*C*H$_3$), 56.27, 55.69, 55.48 (C-2, C-2′, C-2″), 22.81, 22.77 (CO*C*H$_3$); HRMS (FAB$^+$)

calcd for $C_{27}H_{45}O_{18}N_3Na$ $[M+H]^+$ 722.2596, found 722.2596.

4.4.4. Methyl (sodium β-D-glucopyranosyluronate)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (40). Compound **3** (28.0 mg, 70 μmol) was incubated with **6** (47.3 mg, 209 μmol) in a carbonate buffer (50 mM, pH 10.0, 1.4 mL) containing chitinase from *Bacillus* sp. (2.0 mg) at 30 °C as described in the preparation of **37**. The residue was purified through a Shodex Asahipak NH2P-50 4E column eluting with 3:2 (v/v) phosphate buffer (10 mM, pH 7.0)–MeCN. The fractions were combined, lyophilized and desalted through a BioGel P-2 column running with distilled H_2O to give **40** (16.0 mg, 36%) as a white amorphous powder: $[\alpha]_D^{26}$ −15.0 (*c* 0.1, H_2O); ^1H NMR (D_2O): δ 4.61 (d, 1H, $J_{1',2'}$ 7.53 Hz, H-1′), 4.54 (d, 1H, $J_{1'',2''}$ 7.53 Hz, H-1″), 4.44 (d, 1H, $J_{1,2}$ 8.03 Hz, H-1), 3.99–3.51 (m, 15H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-3″, H-4″, H-5″), 3.51 (s, 3H, OCH_3), 3.37 (m, 1H, H-2″), 2.08, 2.04 (2s, 6H, COCH_3); ^{13}C NMR (D_2O): δ 176.22 (*C*OONa), 175.33, 175.24 (*C*OCH$_3$), 102.86 (C-1″), 102.48 (C-1), 101.90 (C-1′), 79.83, 79.17 (C-4, C-4′), 76.44 (C-5″), 75.86 (C-3″), 75.37, 75.12 (C-5, C-5′), 73.52 (C-2″), 73.11, 72.56 (C-3, C-3′), 72.32 (C-4″), 60.66, 60.46 (C-6, C-6′), 57.79 (O*C*H$_3$), 55.80 (C-2), 55.48 (C-2′), 22.82 (CO*C*H$_3$); HRMS (FAB^+) calcd for $C_{23}H_{38}O_{17}N_2Na$ $[M+H]^+$ 637.2068, found 637.2062.

4.4.5. Pent-4-enyl (sodium β-D-glucopyranosyluronate)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (41). Compound **3** (24.1 mg, 60 μmol) was dissolved together with **5** (54.0 mg, 190 μmol) in a carbonate buffer (50 mM, pH 10.0, 1.2 mL) containing chitinase from *Bacillus* sp. (2.4 mg) at 30 °C. The mixture was treated according to the procedure for the production of **37**. The residue was purified by HPLC through a Shodex Asahipak NH2P-50 4E column using 1:1 (v/v) phosphate buffer (10 mM, pH 7.0)–MeCN as eluent. The combined fractions were lyophilized and desalted through a BioGel P-2 column running with distilled H_2O to afford **41** (15.8 mg, 38%) as a white amorphous powder: $[\alpha]_D^{26}$ −16.0 (*c* 0.1, H_2O); ^1H NMR (D_2O): δ 5.89 (m, 1H, C*H*=CH$_2$), 5.09–5.01 (m, 2H, CH=CH_2), 4.61 (d, 1H, $J_{1',2'}$ 7.53 Hz, H-1′), 4.54 (d, 1H, $J_{1'',2''}$ 8.03 Hz, H-1″), 4.50 (d, 1H, $J_{1,2}$ 7.53 Hz, H-1), 3.99–3.49 (m, 17H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-3″, H-4″, H-5″, OCH_2CH$_2$), 3.36 (m, 1H, H-2″), 2.11–2.04 (m, 2H, CH_2CH=CH$_2$), 2.07, 2.04 (2s, 6H, COCH_3), 1.68–1.62 (m, 2H, OCH$_2$CH_2); ^{13}C NMR (D_2O): δ 176.16 (*C*OONa), 175.17, 175.03 (*C*OCH$_3$), 139.23 (*C*H=CH$_2$), 115.52 (CH=*C*H$_2$), 102.83 (C-1″), 101.88 (C-1′), 101.58 (C-1), 79.83, 79.13 (C-4, C-4′), 76.36 (C-5″), 75.83 (C-3″), 75.33, 75.05 (C-5, C-5′), 73.49 (C-2″), 72.96, 72.54 (C-3, C-3′), 72.27 (C-4″), 70.27 (O*C*H$_2$CH$_2$), 60.61, 60.44 (C-6, C-6′), 55.75 (C-2′), 55.58 (C-2), 29.85 (*C*H$_2$CH=CH$_2$), 28.41 (OCH$_2$*C*H$_2$), 22.84, 22.78 (CO*C*H$_3$); HRMS (FAB^+) calcd for $C_{27}H_{43}O_{17}N_2Na_2$ $[M+Na]^+$ 713.2357, found 713.2371.

4.4.6. Methyl (sodium β-D-glucopyranosyluronate)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (42). Glycosyl donor **3** (8.0 mg, 20 μmol) and glycosyl acceptor **4** (26.0 mg, 60 μmol) in a carbonate buffer (50 mM, pH 10.0, 400 μL) were incubated with chitinase from *Bacillus* sp. (0.8 mg) at 30 °C as described for the preparation of compound **37**. The residue was purified by HPLC through a Shodex Asahipak NH2P-50 4E column eluting with 2:3 (v/v) phosphate buffer (10 mM, pH 7.0)–MeCN. The combined fractions were lyophilized and desalted through a Bio Gel P-2 column running with distilled H_2O to give **42** (6.0 mg, 36%) as a white amorphous powder: $[\alpha]_D^{26}$ −27.0 (*c* 0.1, H_2O); ^1H NMR (D_2O): δ 4.61–4.57 (m, 2H, H-1′, H-1″), 4.54 (d, 1H, *J* 8.03 Hz, H-1‴), 4.44 (d, 1H, $J_{1,2}$ 8.03 Hz, H-1), 4.00–3.50 (m, 21H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-2″, H-3″, H-4″, H-5″, H-6a″, H-6b″, H-3‴, H-4‴, H-5‴), 3.50 (s, 3H, OCH_3), 3.36 (m, 1H, H-2‴), 2.07, 2.04 (2s, 9H, COCH_3); ^{13}C NMR (D_2O): δ 176.11 (*C*OONa), 175.19, 175.12 (*C*OCH$_3$), 102.83 (C-1‴), 102.40 (C-1), 101.85, 101.81 (C-1′, C-1″), 79.76, 79.54, 79.14 (C-4, C-4′, C-4″), 76.32 (C-5‴), 75.83 (C-3‴), 75.32, 75.07 (C-5, C-5′, C-5″), 73.48 (C-2‴), 73.06, 72.59, 72.51 (C-3, C-3′, C-3″), 72.26 (C-4‴), 60.60, 60.48 (C-6, C-6′, C-6″), 57.73 (O*C*H$_3$), 55.74, 55.60 (C-2′, C-2″), 55.43 (C-2), 22.79, 22.76 (CO*C*H$_3$); HRMS (FAB^+) calcd for $C_{31}H_{51}O_{22}N_3Na$ $[M+H]^+$ 840.2862, found 840.2864.

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