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Synthesis and Biological Evaluation of a Chitobiose-Based Peptide *N*-Glycanase Inhibitor Library

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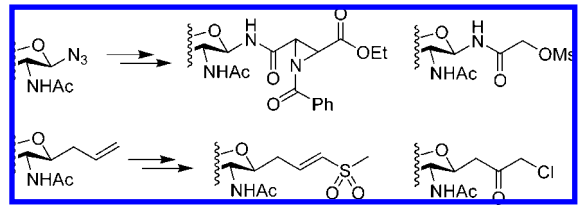

Peptide *N*-glycanase (PNGase), the enzyme responsible for the deglycosylation of *N*-linked glycoproteins, has an active site related to that of cysteine proteases. Chitiobiose was equipped with electrophilic traps often used in cysteine protease inhibitors, and the resulting compounds were evaluated as PNGase inhibitors. We found that the electrophilic trap of the inhibitor has a great influence on the potency of the compounds with the chloromethyl ketone inhibitor being the first potent *C*-glycoside-based PNGase inhibitor.

Introduction

Nascent proteins that are secreted into the endoplasmic reticulum during ribosomal protein synthesis receive one or more *N*-linked glycans in a cotranslational process. These *N*-glycans assist in folding of the protein and serve as a signal molecule for localization and functioning of the protein once the folding process is completed successfully.[1] Current estimations are that about 10% of the thus-generated *N*-glycan proteins do not have the appropriate fold, and these are subsequently degraded by the endoplasmatic reticulum associated degradation pathway.[2] During degradation, peptide *N*-glycanase (PNGase) is responsible for the removal of *N*-linked glycans in the cytosol. The carbohydrate core of the *N*-glycan binds to the C-terminal domain of PNGase after which the glycosyl amide bond is cleaved by the thiol residue of the catalytic cysteine, histidine, aspartic acid triad.[3] Deglycosylated proteins are then further processed by the proteasome and downstream aminopeptidases. Some oligopeptides escape via the transporter associated with antigen presentation (TAP) back to the ER to become part of the major histocompatibilty complex class I (MHCI) and are thus presented to the cell surface immune surveillance system.[4] The importance of deglycosylation by PNGase in epitope formation was demonstrated in the following. The epitope of tyrosinase (369−377) is glycosylated at Asn(369). This *N*-glycan is removed by PNGase, thereby converting asparagine to aspartic acid prior to expression by MHC class 1 molecules.[5] In addition to this, it was shown that *N*-linked glycans near epitopes also influence the epitope formation. Although fully glycosylated glycoproteins can still be partially processed by the proteasome, the amount of cleavage sites is restricted, thereby limiting the formation of MHC class I peptides.[6]

The first small molecule found to inhibit PNGase is the peptide fluoromethylketone ZVAD-Fmk. This inhibitor, identified from a generic compound library by Ploegh and co-workers, is by no means specific for PNGase.[7] Rather, the peptide-based

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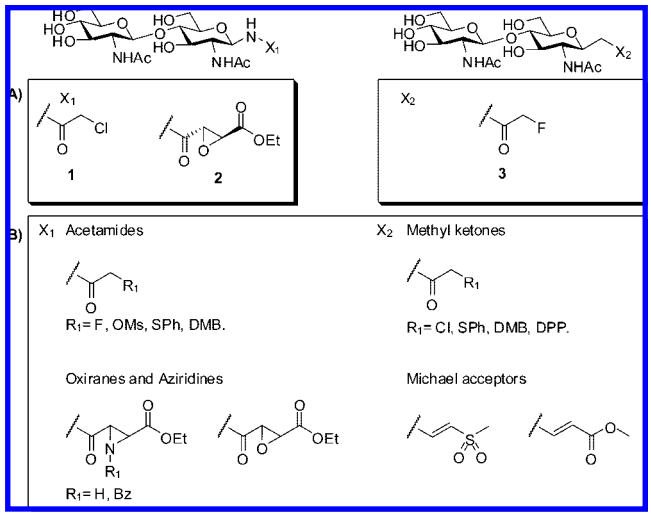

FIGURE 1. (A) Known inhibitors of PNGase. (B) Electrophilic traps that are the subject of this paper.

inhibitor is a broad-spectrum caspase inhibitor and as such widely used in studies on caspase activity and their role in apoptosis events.[8] Ito and co-workers were the first to address the subject of selective PNGase inhibitors in their studies on the rational design of chitobiose-based inhibitor **1** (Figure 1A).[9] We recently synthesized chitobiose-based epoxysuccinate **2** and fluoromethyl ketone **3** and revealed that these, too, inhibit the enzyme.[10] Interestingly, the nature of the electrophilic trap appeared to have a large effect on the biological activity. Whereas chitobiose derivatives **1** and **2** are potent inhibitors, fluoromethyl ketone **3** is a poor inhibitor of PNGase. We reasoned that chitobiose equipped with a variety of traps would be helpful to get insight in the influence of the electrophilic trap on the potency of the inhibitor. Since the active site of PNGase shares great similarities to the active site of cysteine proteases, the nature of the known cysteine protease inhibitors provide a guidline for the design of these inhibitors.

The synthesis of selective and covalent inhibitors of cysteine proteases has received considerable attention over the past decades, and various electrophilic traps have been evaluated.[11] Three of the major classes that are used as warheads nowadays are as follows: (A) the activated methylketones and their acetamide analogues such as halomethyl ketones,[12] acyloxymethyl ketones,[13] diphenylphosphinyloxymethyl ketones,[14] and thiomethyl ketones,[15] (B) the oxiranes[16] and aziridines,[17] and (C) Michael acceptors such as vinyl sulfones[18] and α,β-unsaturated esters.[19] Based on these known cysteine protease inhibitors, we synthesized a library of potential PNGase inhibitors (Figure 1B) and evaluated their biological activity.

Results and Discussion

Synthesis of Acetamide Inhibitors. Potential acetamide inhibitors **10**−**14** were synthesized as depicted in Scheme 1. Known azide **4**[20] was reduced by Lindlars' catalyst, and subsequent condensation of the resulting amine with acids **5**−**9**[21] gave the fully protected inhibitors. Global deprotection was accomplished by either hydrogenation in the presence of $Pd(OH)_2$ in trifluoroethanol(TFE)/MeOH (for **10**, **12**−**14**) or selective removal of the benzylidene using 10% TFA in CH_2Cl_2 followed by hydrogenation in the presence $Pd(OH)_2$ in MeOH (for **11**).

Martichonok et al. revealed that the potency of the aziridine warhead depended on the pH, with pH 4 being the optimum for such inhibitors.[22] The pH dependency could be reduced by *N*-acylation of the aziridine.[23] Since cytosolic PNGase is active at neutral pH, it is likely that compounds **13** and **14** will not be very potent at this pH. Therefore, aziridines **15** and **16**, intermediates of the synthesis of **13** and **14**, were benzoylated under the agency of benzoyl chloride in pyridine/CH_2Cl_2 affording **17** and **18**. Removal of the protective groups gave acylated aziridine inhibitors **19** and **20** (Scheme 2).

The synthesis of thioacetamide **25** was first attempted following the route detailed in Scheme 1. Although inhibitor **25** in its protected form was obtained in reasonable to good yield, deprotection with $Pd(OH)_2$ proved ineffective. We reasoned that introduction of the thioether after global deprotection would not only avoid this problem (Scheme 3) but in addition would provide us mesyl derivative **24** as a potential inhibitor. Therefore, the azide function in **4** was reduced and coupled with glycolic acid to furnish hydroxyacetamide **21**. The hydroxyl in **21** was mesylated under the agency of methanesulfonic anhydride (Ms_2O) and Et_3N in dioxane/DMF. During synthesis, it appeared that mesylate **22** is extremely labile. Conversion of mesylate **22** to the corresponding chloride **23** was observed by LC/MS when the reaction was performed in solvents containing traces of chloride ions, such as CH_2Cl_2 and after purification over silica column chromatography, probably due to traces of $CaCl_2$.[24] Removal of the protective groups in

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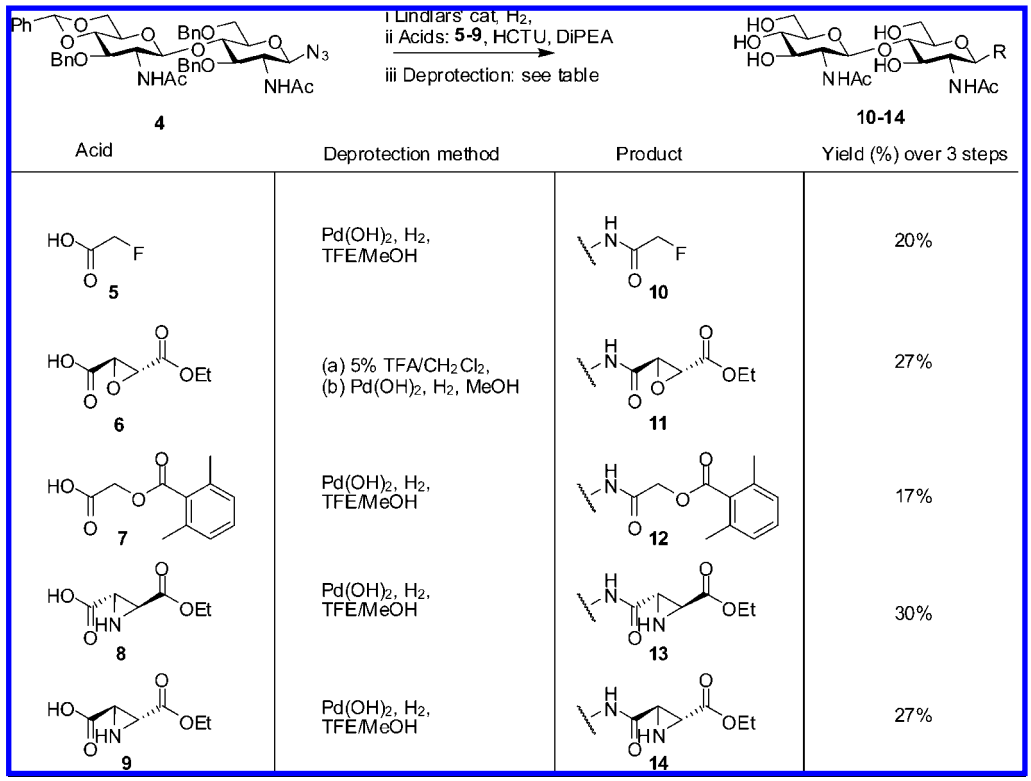
SCHEME 1



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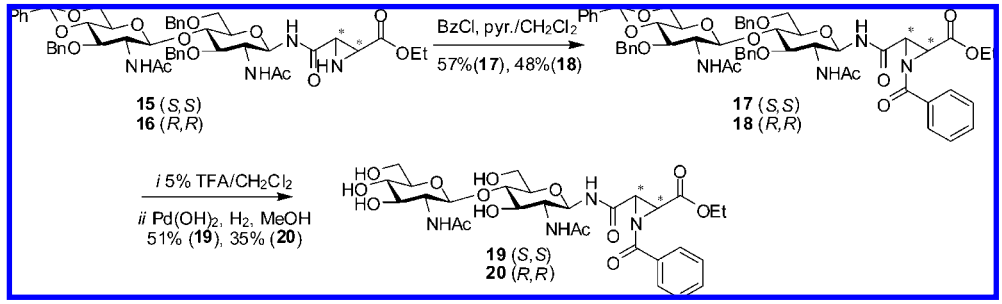

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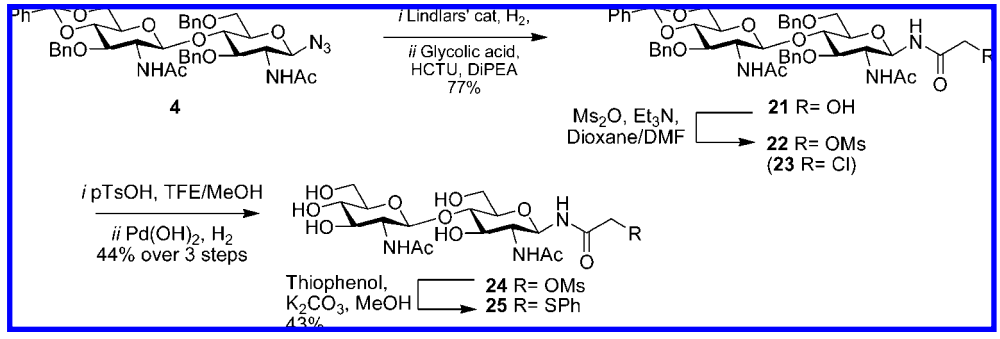

crude **22** followed by HPLC purification prevented the formation of chloroacetamide **23** giving **24** in 44% yield over three steps. The mesylate in **24** was converted to the corresponding thioether **25** by reaction with thiophenol and K_2CO_3 in methanol.

Synthesis of C-Glycoside Inhibitors. The synthesis of the Michael acceptors **33** and **38** commences with the synthesis of alkene **31** (Scheme 4). Known 3-*C*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-1-propene was transformed to acceptor **26** by reductive opening of the benzylidine group.[10] Ph_2SO/Tf_2O-mediated condensation of donor **27**[25] with acceptor **26** gave disaccharide **29** in a low (45%) yield.[26] Furthermore, activation of donor **27** by treatment with *N*-iodosuccinimide/TMSOTf did not result in productive coupling. Therefore, acceptor **26** was reacted with known imidate

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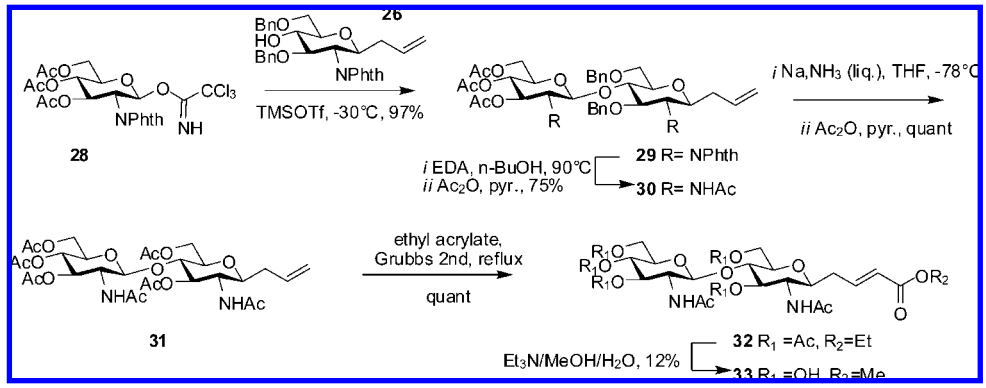
SCHEME 4



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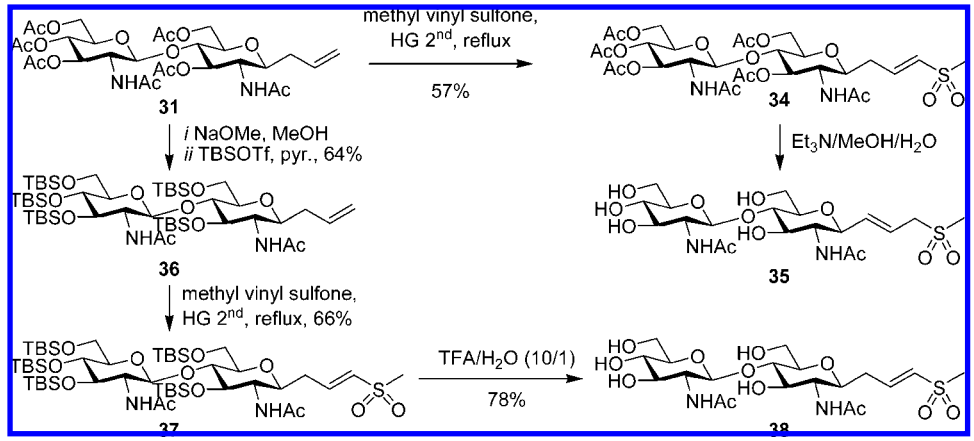
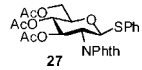


28 giving disaccharide **29** in 97% yield.[27] Removal of the phthaloyl groups followed by acetylation of the resulting amine, debenzylation under Birch conditions, and ensuing acetylation of the hydroxyls furnished fully protected alkene **31**. Vinyl ester **32** was synthesized by cross-metathesis (CM) of alkene **31** with ethyl acrylate in the presence of Grubbs second-generation catalyst as was previously described for monosaccharides.[28] Upon ensuing deacetylation, the ethyl ester was transesterified with methanol to give methyl vinyl ester **33**.

Although alkene **31** was easily converted to vinyl ester **32** by reaction with Grubbs second-generation catalyst, this catalyst was ineffective for the synthesis of electron-deficient vinyl sulfone **34** (Scheme 5). We therefore switched to Hoveyda−Grubbs second-generation catalyst, which is more reactive toward electron-deficient alkenes.[29] After 2 days of refluxing in the presence of 20% of Hoveyda−Grubbs second-generation catalyst, alkene **31** was converted to the corresponding vinyl sulfone **34** in 57%. Removal of the acetyl protective groups with MeOH/H_2O/Et_3N resulted in a mixture of compounds. NMR analysis showed that the double bond in α,β-unsaturated sulfone **38** was migrated, forming β,γ-unsaturated sulfone **35**.[30] The migration under alkaline conditions was proven by treating **34** with DBU in CH_2Cl_2. After overnight stirring, complete migration of the double bond was observed as judged by NMR analysis. To prevent alkene migration during deprotection, disaccharide **31** was equipped with acid-labile protective groups (Scheme 5). To this end, **31** was deacetylated under Zemplen conditions and subsequently silylated with *tert*-butyldimethylsilyl trifluoromethanesulfonate in pyridine affording **36**. Cross-metathesis with methyl vinyl sulfone under the agency of Hoveyda−Grubbs second-generation catalyst afforded fully protected vinyl sulfone **37** in 66% yield. Cleavage of the silyl ethers in **37** under acidic conditions proceeded smoothly to give vinyl sulfone **38**.

The various methyl ketone based inhibitors **44**, **45**, **47**, and **48** were synthesized from general intermediate hydroxylmethyl ketone **41** (Scheme 6). The synthesis of this key intermediate started with the condensation of acceptor **26** with imidate **39** giving disaccharide **40** in excellent yield. Conversion of **40** to **41** was achieved in four successive steps: dihydroxylation of the double bond in **40** with OsO_4, removal of the phthaloyl group by treatment with ethylenediamine (EDA), selective acetylation of the resulting amines, and oxidation of the secondary alcohol under the agency of dibutyltin oxide and NBS,[31] giving key intermediate **41** in 50% yield over four steps. Mesylation of hydroxymethyl ketone **41** and in situ conversion to the corre-

(25) Structure **27**:



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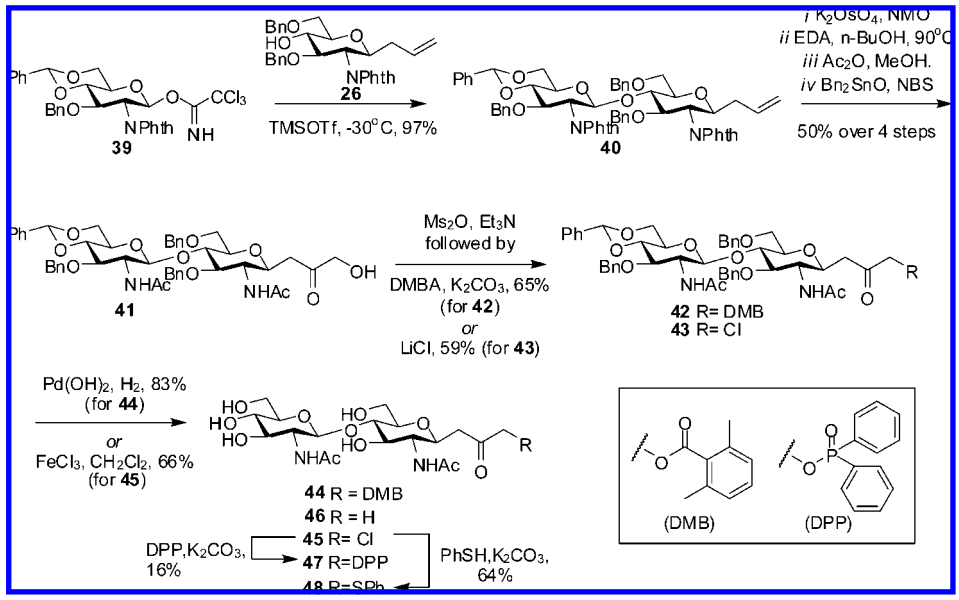
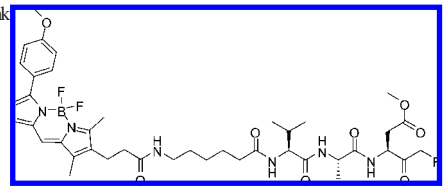
SCHEME 6



sponding acyloxymethyl and chloromethyl ketones using dimethylbenzoic acid (DBA) or lithium chloride furnished **42** and **43**. Global deprotection of **42** under reductive conditions proceeded smoothly giving acyloxymethyl ketone **44** in 83% yield. For the synthesis of chloromethyl ketone **45**, however, hydrogenolysis was not suitable. Due to reductive dehalogenation, ketone **46** was the only product observed by LC/MS after hydrogenolysis. The benzyl/benzylidene protective groups could, however, be efficiently removed under the agency of $FeCl_3$ in CH_2Cl_2 furnishing chloromethyl ketone **45**. Treatment of fully deprotected **45** with diphenylphosphinic acid (DPP) and K_2CO_3 or thiophenol and K_2CO_3 afforded compounds **47** and **48**.

Inhibition Studies. With the inhibitors in hand, we evaluated their biological activity as described.[10] Purified yeast peptide *N*-glycanase was incubated with a serial dilution of the chitobiose-based inhibitors followed by treatment with the fluorescent active-site label, β-VAD-Fmk (0.5 μM).[32] Although the chitobiose-based inhibitors bind to the carbohydrate binding subsite and β-VAD-Fmk binds in the peptide binding subsite, both covalently modify the nulceophilic thiol of cysteine 191. Therefore, an increasing concentration of a potent inhibitor will result in a drop of β-VAD-Fmk binding which is evidenced by reduced fluorescence. However, we realize that both the β-VAD-Fmk and the chitobiose based inhibitors bind in a time-dependent fashion, and therefore, IC_{50} values will have to be interpreted as relative values. Furthermore, we cannot exclude that weaker inhibitors may bind to the active site of yeast PNGase and induce the binding of β-VAD-Fmk via an induced fit. After quantification of the intensities of the bond, the relative IC_{50} values were determined of both known inhibitor **1**, as a reference, and the newly synthesized inhibitors **10**−**14**, **19**, **20**, **24**, **25**, **33**, **38**, **44**, **45**, **47** and **48**. The apparent IC_{50} values allowed us to compare the different inhibitors (Table 1).

(32) Structure β-VAD-Fmk



The IC_{50} values of the synthesized inhibitors reveal several trends. First, the nature of the leaving group of the acetamides **1**, **10**, **12**, **24**, and **25** and methyl ketones **3**, **44**, **45**, **47**, and **48** has a great influence on the inhibitory potential of the compound. A good leaving group is required for strong irreversible inhibition of purified yeast PNGase. Fluoromethyl ketone, acyloxymethyl ketone, and diphenylphosphinyloxymethyl ketone warheads, which were originally designed to react selectively with the thiol of the catalytic triad and to be quiescent in the presence of other bionucleophiles, were inefficient inhibitors of yeast PNGase. The stereochemistry of the epoxide and aziridine electrophilic trap is of great importance. Inhibitors with the *S*,*S*-configuration (**2** and **19**) showed an approximately 100-fold higher activity in comparison with the *R*,*R*-configured **11** and **20**. In addition to this stereoselectivity, *N*-acylation of the aziridine (8.77 μM for **19** compared to >1000 μM for **13**) is beneficial for the IC_{50} value at pH 7.4. Somewhat surprisingly, the Michael acceptor containing compounds **33** and **38** did not reveal inhibitory activity at all. The lack of activity might be due to improper alignment of *E*-vinylsulfone **38** and *E*-vinylester **33** with the active site cysteine, thereby preventing nucleophilic attack on the Michael acceptor. The corresponding Z-vinylsulfones and Z-vinylesters might align properly in the active site of yeast PNGase and therefore might be inhibitors of PNGase.

To evaluate the inhibitory potential in a cell culture model, epoxide **2** and its peracetylated analogue **49** were tested in a model involving the deglycosylation and degradation of MHC class I heavy chains in the context of a viral protein, US11.[33] Human cytomegalovirus encodes two glycoproteins, US2 and US11, each of which is sufficient to catalyze the dislocation of MHC class I heavy chains (HCs) from the ER into the cytosol, where they become ubiquitinated, deglycosylated, and degraded by the proteasome.[33,34] Here, U373 cells stably expressing US11 were used to monitor the effects of our compounds on HC deglycosylation by PNGase. Control cells and US11-expressing cells were metabolically labeled with [^{35}S]-cysteine/methonine. The cells were lysed in the presence of detergent, and the HCs

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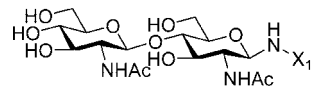
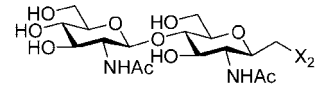
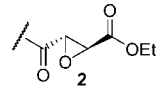
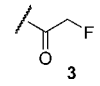
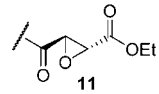
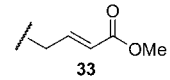
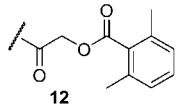
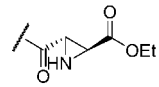
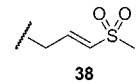
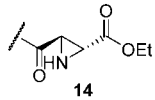
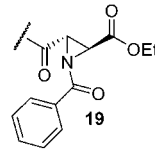
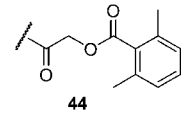
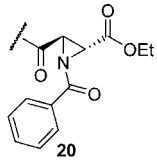
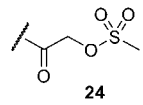
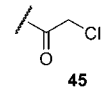
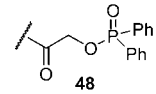
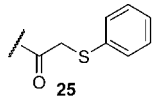
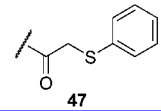
TABLE 1. Apparent IC_{50} Values of the Inhibitors





X_1	IC_{50} (μM)	X_1	IC_{50} (μM)	X_2	IC_{50} (μM)
 **1**	1.6 ± 0.5	 **2**	1.6 ± 0.5	 **3**	>1000
 **10**	563 ± 50	 **11**	253 ± 15	 **33**	>1000
 **12**	>1000	 **13**	>1000	 **38**	>1000
 **14**	>1000	 **19**	8.77 ± 0.6	 **44**	>1000
 **20**	618 ± 25	 **24**	1.2 ± 0.1	 **45**	0.74 ± 0.1
				 **48**	563 ± 35
 **25**	>1000			 **47**	>1000

were isolated by immunoprecipitation. Whereas the HCs were readily detectable in control U373 cells (Figure 2, lane 1), the HCs were completely degraded in U373 cells expressing US11 (lane 2). However, in the presence of the proteasome inhibitor

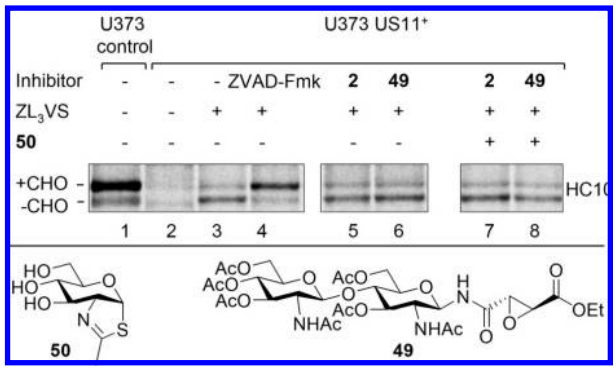


FIGURE 2. Inhibition of human PNGase in U373 cells. U373 control cells and US11-expressing U373 cells were incubated with the inhibitors indicated (final concentrations: ZVAD-Fmk, 30 μM; **2** and **49**, 100 μM; ZL_3VS, 1.25 μM; and NGT, 50 μM) in starvation medium for 45 min, followed by metabolic labeling for 30 min at 37 °C with [^{35}S]-labeled cysteine and methionine, again in the presence of the compounds indicated. The cells were lysed in the presence of NP40 lysis buffer, and the MHC class I HCs were immunoprecipitated using the monoclonal antibody HC10. The samples were separated by SDS-PAGE and visualized by phosphoimaging. The presence of the single *N*-linked glycan on the HCs is indicated (+ and − CHO).

ZL_3VS, a deglycosylated form of the HCs was observed (lane 3). Fully glycosylated HCs were detected when the cells were incubated with the PNGase-inhibitor ZVAD-Fmk and ZL_3VS (lane 4). To test our compounds, US11 cells were preincubated with epoxides **2** and **49** in the presence of ZL_3VS, followed by labeling of the proteins with [^{35}S]-labeled cysteine and methionine (lanes 5 and 6). In the presence of 100 μM of epoxides **2** and **49**, the HCs were deglycosylated with similar efficiency as in cells treated with ZL_3VS alone (compare lanes 3, 5, and 6). We reasoned that the lack of activity of the inhibitors could be caused by hydrolysis of the glycosidic bond in **2** and **49** by hexosaminidase present inside the cell. To investigate this possibility, the cells were treated with **2** and **49** in the presence of ZL_3VS and the broad spectrum hexosaminidase inhibitor NGT **50**. The addition of **50** had, however, no effect on the state of glycosylation of the HCs (lanes 7 and 8). An explanation for this fact could be that epoxides **2** and **49** cannot penetrate the cell membrane. Alternatively, the epoxides may enter the cell but the electrophilic group is hydrolyzed inside the cell. It is also possible that the acetates in **49** are stable to esterases present in the U373 cells.

In summary, a library of modified chitobiose derivatives as potential PNGase inhibitors was synthesized. The IC_{50} value of the newly synthesized inhibitors was determined by incubating them with purified yeast PNGase followed by visualizing the remaining activity by activity-based enzyme profiling. The results of this assay demonstrate that the nature of electrophilic

trap has significant influence on the potency of the inhibitor. To study the selectivity, the library will be tested for their inhibitory activity of human chitinases[35] and the inhibitors will be converted to probes by the introduction of an azidoacetyl or by equipping them with a fluorophore (e.g., BodipyFl). The resulting probes may find use in the study of the selectivity of the inhibitors in cell lysates as well as whole cells. Furthermore, the fluorescent probes can be used to study their cell-permeability by microscopy as was reported by Ito and co-workers.[36]

Experimental Section

General Procedure for the Synthesis of Acetamide Inhibitors. Disacharride **4** was dissolved in DMF (10 mL/mmol). Lindlars' catalyst was added, and the solution was stirred under H_2 atmosphere for 3 h. The solution was purged with argon after which acid (3 equiv), 2-(6-chloro-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) (3 equiv), and DiPEA (6 equiv) were added. The resulting mixture was stirred overnight, concentrated under reduced pressure, and purified over silica gel chromatography (CH_2Cl_2→MeOH/CH_2Cl_2) to give the fully protected inhibitors.

General Procedure 1 for Global Deprotection. The protected inhibitors were directly deprotected. Hence, they were dissolved in 2,2,2-trifluoroethanol/methanol (10 mL/mmol). Subsequently, 20% $Pd(OH)_2$ on activated charcoal (20 mg) was added. The reaction mixture was stirred under H_2 atmosphere until TLC revealed complete conversion to a very polar product. The mixture was purged with argon, filtered, concentrated, and purified over reversed-phase HPLC.

General Procedure 2 for Global Deprotection. The inhibitor was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C before trifluoroacetic acid (100 μL) and H_2O (10 μL) were added. The mixture was stirred until TLC showed complete conversion to a lower running product. The reaction was quenched with $NaHCO_3$ (satd aq) and diluted with CH_2Cl_2. The organic layer was washed with $NaHCO_3$ (satd aq), dried over Na_2SO_4, and concentrated under reduced pressure. The resulting disaccharide was redissolved in MeOH (2 mL) and 20% $Pd(OH)_2$ on activated charcoal (20 mg) was added after which the reaction was stirred under H_2 atmosphere until TLC showed complete conversion. Subsequently, the reaction was purged with argon, filtered, concentrated, and purified by reversed-phase HPLC.

***N*-(*O*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl) fluoroacetamide (10).** Disacharride **4** (76 mg, 94 μmol) was converted to the protected fluoroacetamide as described in the general procedure. Silica gel chromatography (CH_2Cl_2→4% MeOH/CH_2Cl_2) gave the protected fluoroacetamide: LC/MS t_R 9.43 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 842.27 $(M + H)^+$. Deprotection according to general procedure 1 followed by preparative RP-HPLC (linear gradient 0→8% B in 10 min) furnished title compound **10** (20% over three steps, 9.14 mg, 18.9 μmol): ^{1}H NMR (600 MHz, D_2O) δ ppm 5.12 (d, J = 9.7 Hz, 1H), 4.89 (dd, J = 46.2, 14.8 Hz, 1H), 4.87 (dd, J = 46.2 Hz, 14.7 Hz, 1H), 4.58 (d, J = 8.4 Hz, 1H), 3.90 (t, J = 9.7, 9.7 Hz, 1H), 3.90 (dd, J = 12.5, 1.2 Hz, 1H), 3.81 (d, J = 11.7 Hz, 1H), 3.77 (dd, J = 9.8, 9.1 Hz, 1H), 3.75−3.71 (m, 2H), 3.66 (t, J = 8.3, 8.3 Hz, 1H), 3.63 (dd, J = 11.2, 3.4 Hz, 1H), 3.59 (dd, J = 9.6, 3.5 Hz, 1H), 3.55 (dd, J = 10.0, 8.8 Hz, 1H), 3.50−3.42 (m, 2H), 2.04 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (150 MHz, D_2O) δ ppm 174.4, 174.1, 171.0, 170.9, 100.8, 79.6, 78.4, 78.2, 77.5, 75.7, 75.3, 72.8, 72.0, 69.1, 59.9, 59.3, 55.0, 53.1, 21.5, 21.3; FT-IR υ_{max} (neat)/cm^{-1}3280.8, 1661.4, 1651.8, 1562.8, 1557.7, 1538.4, 1435.8, 1373.4, 1319.7, 1200.7, 1103.2, 1043.1, 1012.4; LC/MS t_R 1.28 min; linear gradient 0→20% B in 13.5 min; ESI/MS m/z = 484.00 $(M + H)^+$; HRMS $(M + H^+)$ calcd for $C_{18}H_{31}FN_3O_{11}$ 484.19371, found 484.19344; mp 203 °C (start of decomposition).

(2*R*,3*R*)-3-*N*-(*O*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosylcarbamoyl)oxirane-2-carboxylic Acid Ethyl Ester (11). Azide **4** (100 mg, 0.124 mmol) was reduced, and the resulting amine was condensed with (*R*,*R*) epoxysuccinate monoethyl ester as described in the general procedure. Silica gel column chromatography (CH_2Cl_2→6% MeOH/CH_2Cl_2) yielded the protected epoxide. The resulting epoxide was deprotected according procedure 2. RP-HPLC purification (linear gradient 3→10.5% B in 15 min) afforded epoxide **11** (27% over three steps, 19.20 mg, 34 μmol): ^{1}H NMR (400 MHz, D_2O) δ ppm 5.01 (d, J = 9.6 Hz, 1H), 4.51 (d, J = 8.4 Hz, 1H), 4.21 (q, J = 7.2, 7.1, 7.1 Hz, 2H), 3.84 (d, J = 12.1 Hz, 1H), 3.81 (dd, J = 10.0, 9.6 Hz, 1H), 3.75 (d, J = 11.7 Hz, 1H), 3.72−3.35 (m, 11H), 1.98 (s, 3H), 1.93 (s, 3H), 1.21 (t, J = 7.2, 7.2 Hz, 3H); ^{13}C NMR (150 MHz, D_2O) δ ppm 174.9, 174.6, 168.8, 168.6, 101.4, 78.8, 78.4, 76.4, 75.9, 73.4, 72.6, 69.7, 63.5, 60.5, 59.9, 55.6, 53.7, 53.3, 53.2, 52.4, 29.6, 22.1, 22.0, 13.2; FT-IR υ_{max} (neat)/cm^{-1} 3270.2, 1651.8, 1563.5, 1557.7, 1373.7, 1312.0, 1202.3, 1025.7. LC/MS t_R 6.02 min; linear gradient 0→20% B in 13.5 min; ESI/MS m/z = 566.00 $(M + H)^+$; HRMS $(M + H^+)$ calcd for $C_{22}H_{36}N_3O_{14}$ 566.21918, found 566.21894; mp 201 °C (start of decomposition).

***N*-(*O*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl) 2,6-Dimethylbenzoyloxyacetamide (12).** Disacharride **4** (78 mg, 98 μmol) was transformed to the acyloxyamide as depicted in the general procedure. Purification over silica gel chromatography (CH_2Cl_2→4% MeOH/CH_2Cl_2) gave the protected acyloxyacetamide: LC/MS t_R 10.76 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 972.27 $(M + H)^+$. Deprotection was performed as described in general procedure 1. Preparative RP-HPLC (linear gradient 0→50% B in 10 min) afforded title compound **12** (17% over three steps, 10.18 mg, 16.6 μmol): ^{1}H NMR (600 MHz, D_2O) δ ppm 7.16 (d, J = 7.7 Hz, 1H), 5.11 (d, J = 9.7 Hz, 1H), 4.88 (d, J = 15.1 Hz, 1H), 4.84 (d, J = 15.1 Hz, 1H), 4.57 (d, J = 8.5 Hz, 1H), 3.90 (d, J = 12.6 Hz, 1H), 3.86 (t, J = 9.7, 9.7 Hz, 1H), 3.81 (d, J = 11.8 Hz, 1H), 3.77−3.71 (m, 3H), 3.65 (t, J = 9.5, 9.5 Hz, 1H), 3.63 (dd, J = 12.6, 4.7 Hz, 1H), 3.60−3.57 (m, 1H), 3.55 (dd, J = 10.0, 9.0 Hz, 1H), 3.50−3.42 (m, 2H), 2.29 (s, 1H), 2.04 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (150 MHz, D_2O) δ ppm 174.2, 174.0, 170.7, 169.6, 135.0, 131.1, 130.0, 127.2, 100.8, 78.2, 77.7, 75.8, 75.3, 72.9, 72.1, 69.1, 62.6, 59.9, 59.3, 55.0, 53.2, 21.5, 21.4, 18.1; FT-IR υ_{max} (neat)/cm^{-1}3273.9, 1668.1, 1651.8, 1645.8, 1564.0, 1557.7, 1435.8, 1373.8, 1115.9, 1075.9; LC/MS t_R 6.43 min; linear gradient 10→50% B in 13.5 min; ESI/MS m/z = 614.00 $(M + H)^+$. HRMS $(M + H^+)$ calcd for $C_{27}H_{40}N_3O_{13}$ 614.25556, found 614.25546; mp 228 °C (start of decomposition).

(2*S*,3*S*)-3-*N*-(*O*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosylcarbamoyl)aziridine-2-carboxylic Acid Ethyl Ester (13). Protected aziridine **15** (50 mg, 62 μmol) was synthesized as described in the general procedure. Silica gel chromatography (CH_2Cl_2→3% MeOH/CH_2Cl_2) furnished protected aziridine **15** (72%, 41 mg, 44.4 μmol): LC/MS t_R 9.46 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 923.27 $(M + H)^+$. The resulting aziridine was converted to the title compound as depicted in general procedure 1. Preparative RP-HPLC (linear gradient 0→10% B in 14.5 min) furnished title compound **13** (30% over three steps, 10.46 mg, 18.5 μmol): ^{1}H NMR (600 MHz, D_2O) δ ppm 5.05 (d, J = 9.8 Hz, 1H), 4.57 (d, J = 8.4 Hz, 1H), 4.21 (q, J = 7.1, 7.1, 7.1 Hz, 2H), 3.89 (d, J = 11.0 Hz, 1H), 3.87 (dd, J = 10.0, 9.5 Hz, 1H), 3.80 (d, J = 11.4

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Hz, 1H), 3.76−3.70 (m, 3H), 3.64 (dd, J = 9.5, 8.8 Hz, 1H), 3.62 (dd, J = 12.1, 4.7 Hz, 1H), 3.58−3.52 (m, 2H), 3.50−3.42 (m, 2H), 2.91 (s, 1H), 2.81 (s, 1H), 2.03 (s, 3H), 1.99 (s, 3H), 1.23 (t, J = 7.2, 7.2 Hz, 3H); ^{13}C NMR (150 MHz, D_2O) δ ppm 174.2, 174.0, 170.3, 170.0, 100.8, 78.2, 77.9, 75.7, 75.3, 72.8, 72.1, 69.1, 62.4, 59.9, 59.3, 55.0, 53.1, 35.9, 34.9, 21.5, 21.4, 12.6; FT-IR υ_{max} (neat)/cm^{-1} 3271.2, 1667.9, 1651.7, 1645.7, 1634.2, 1563.5, 1557.7, 1538.7, 1435.8, 1373.9, 1200.2, 1049.7; LC/MS t_R 5.58 min; linear gradient 0→20% B in 13.5 min; ESI/MS m/z = 565.20 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{22}H_{37}N_4O_{13}$ 565.23516, found 565.23497; mp 177 °C (start of decomposition).

(2*R*,3*R*)-3-*N*-(*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosylcarbamoyl)aziridine-2-carboxylic Acid Ethyl Ester (14). Disacharride **4** (80 mg, 99 μmol) was transformed to **16** according the general procedure. Subsequent purification by silica gel chromatography gave protected **16** (94%, 86 mg, 93 μmol): LC/MS t_R 9.40 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 923.33 (M + H)$^+$. Fully protected aziridine **16** was deprotected as given in general procedure 1. Ensuing preparative RP-HPLC (linear gradient 0→10% B in 14.5 min) furnished title compound **14** (27% over three steps, 15.47 mg, 27.4 μmol): ^{1}H NMR (400 MHz, D_2O) δ ppm 5.11 (d, J = 9.8 Hz, 1H), 4.62 (d, J = 8.3 Hz, 1H), 4.28 (q, J = 7.2, 7.2, 7.1 Hz, 2H), 3.98−3.45 (m, 12H), 3.15 (s, 1H), 3.09 (s, 1H), 2.08 (s, 3H), 2.03 (s, 3H), 1.30 (t, J = 7.1, 7.1 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ ppm 174.8, 174.7, 101.5, 78.9, 78.7, 76.4, 76.0, 73.5, 72.7, 69.8, 63.4, 60.6, 60.0, 55.7, 53.9, 36.7, 35.6, 22.2, 22.1, 13.3; FT-IR υ_{max} (neat)/cm^{-1} 3261.5, 1668.3, 1650.7, 1557.6, 1435.3, 1376.7, 1313.4, 1201.2, 1113.6, 1067.2, 1026.4; LC/MS t_R 5.57 min; linear gradient 0→20% B in 13.5 min; ESI/MS m/z = 565.53 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{22}H_{37}N_4O_{13}$ 565.23516, found 565.23500; mp 177 °C (start of decomposition).

1-Benzoyl-(2*S*,3*S*)-3-*N*-(*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosylcarbamoyl)aziridine-2-carboxylic Acid Ethyl Ester (17). Fully protected aziridine **15** (66 mg, 72 μmol) was dissolved in CH_2Cl_2/pyridine (2 mL, 1/1 v/v). The solution was cooled to 0 °C before benzoyl chloride (17 μL, 0.143 mmol) was added. Additional benzoyl chloride (130 μL) was added after 3 h of stirring. The reaction was stirred until TLC showed complete consumption of starting material after which the reaction was diluted with CH_2Cl_2 and washed with $NaHCO_3$ (satd aq) and 10% citric acid. The organic layer was dried over Na_2SO_4, concentrated in vacuo, and purified by column chromatography (CH_2Cl_2→3% MeOH/CH_2Cl_2) affording title compound **17** (57%, 42 mg, 41 μmol): ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ ppm 7.93−7.90 (m, 2H), 7.57−7.51 (m, 1H), 7.47−7.19 (m, 22H), 5.47 (s, 1H), 4.87 (d, J = 11.3 Hz, 1H), 4.85 (d, J = 9.9 Hz, 1H), 4.82 (d, J = 12.4 Hz, 1H), 4.65 (d, J = 11.7 Hz, 1H), 4.62 (d, J = 7.7 Hz, 1H), 4.49 (d, J = 11.7 Hz, 1H), 4.10−3.92 (m, 5H), 3.79 (t, J = 9.0, 9.0 Hz, 1H), 3.71−3.67 (m, 2H), 3.63 (dd, J = 12.7, 9.2 Hz, 1H), 3.58 (d, J = 2.4 Hz, 1H), 3.52 (dd, J = 7.9, 7.3 Hz, 1H), 3.49 (d, J = 2.4 Hz, 1H), 3.49−3.46 (m, 1H), 3.40 (t, J = 10.3, 10.3 Hz, 1H), 3.19 (dt, J = 9.8, 9.6, 4.9 Hz, 1H), 1.86 (s, 3H), 1.85 (s, 3H), 1.10 (t, J = 7.1, 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ ppm 175.5, 173.3, 172.5, 167.2, 167.1, 139.4, 139.0, 138.6, 137.9, 133.8, 133.2, 129.6, 129.2, 129.1, 129.0, 128.9, 128.83, 128.77, 128.62, 128.58, 128.4, 128.3, 128.1, 126.6, 101.8, 101.2, 82.8, 81.3, 79.7, 78.7, 77.3, 75.9, 74.9, 74.8, 74.0, 69.1, 68.7, 66.4, 63.0, 56.6, 53.9, 42.4, 42.0, 23.2, 23.0, 14.1; FT-IR υ_{max} (neat)/cm^{-1}3276.4, 1738.9, 1668.0, 1651.7, 1645.7, 1562.9, 1557.6, 1452.1, 1371.8, 1317.9, 1204.3, 1174.6, 1069.9; LC/MS t_R 10.38 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 1027.27 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{57}H_{62}N_4O_{14}$ 1027.43353, found 1027.43497; mp 250 °C (start of decomposition).

1-Benzoyl-(2*R*,3*R*)-3-*N*-(*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosylcarbamoyl)aziridine-2-carboxylic Acid Ethyl Ester (18). Protected aziridine **16** (100 mg, 109 μmol) was dissolved in CH_2Cl_2/pyridine (2 mL, 1/1 v/v) before being cooled to 0 °C. Benzoyl chloride (25 μL, 0.22 mmol) was added. An additional amount of benzoyl chloride (300 μL) was added after 1 h of stirring, and the reaction was stirred until TLC showed complete conversion of the starting material. The solution was diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq) and 10% citric acid, dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography (CH_2Cl_2→3% MeOH/CH_2Cl_2) gave title compound **18** (48%, 52 mg, 51 μmol): ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ ppm 7.91−7.88 (m, 2H), 7.53−7.21 (m, 23H), 5.47 (s, 1H), 4.84 (d, J = 7.5 Hz, 1H), 4.82 (d, J = 6.6 Hz, 1H), 4.82 (d, J = 7.4 Hz, 1H), 4.65 (d, J = 11.7 Hz, 1H), 4.61 (d, J = 7.2 Hz, 1H), 4.58 (d, J = 6.8 Hz, 1H), 4.54 (d, J = 8.3 Hz, 1H), 4.45 (d, J = 11.7 Hz, 1H), 4.10 (dd, J = 10.5, 5.0 Hz, 1H), 4.07−3.93 (m, 4H), 3.78 (t, J = 8.7, 8.7 Hz, 1H), 3.65−3.57 (m, 4H), 3.56 (d, J = 2.4 Hz, 1H), 3.55 (d, J = 2.4 Hz, 1H), 3.50 (dd, J = 9.0, 8.5 Hz, 1H), 3.47−3.42 (m, 2H), 3.39 (d, J = 10.3 Hz, 1H), 3.19 (td, J = 14.6, 5.1, 5.1 Hz, 1H), 1.85 (s, 3H), 1.84 (s, 3H), 1.08 (t, J = 7.1, 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ ppm 138.3, 137.9, 137.5, 136.8, 132.8, 132.0, 128.6, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.5, 127.3, 127.1, 125.6, 100.8, 100.1, 81.8, 80.1, 78.7, 77.5, 76.2, 74.8, 73.7, 73.1, 68.1, 67.5, 65.3, 62.0, 55.4, 52.7, 41.4, 40.8, 22.3, 21.9, 13.1; FT-IR υ_{max} (neat)/cm^{-1}3277.1, 2873.3, 1739.0, 1656.9, 1651.7, 1562.9, 1557.7, 1538.4, 1452.0, 1370.9, 1318.1, 1202.2, 1174.1, 1069.2; LC/MS t_R 9.75 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 1027.5 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{57}H_{62}N_4O_{14}$ 1027.43353, found 1027.43508; mp 252 °C (start of decomposition).

1-Benzoyl-(2*S*,3*S*)-3-*N*-(*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosylcarbamoyl)aziridine-2-carboxylic Acid Ethyl Ester (19). Fully protected benzoylated aziridine **17** (42 mg, 41 μmol) was deprotected as described in general procedure 2. Semipreparative RP-HPLC (linear gradient 20%→29.375% B in 12.5 min) furnished title compound **19** (51%, 13.8 mg, 21 μmol): ^{1}H NMR (400 MHz, D_2O) δ ppm 7.94 (d, J = 7.8 Hz, 2H), 7.71 (t, J = 7.4, 7.4 Hz, 1H), 7.57 (t, J = 7.7, 7.7 Hz, 2H), 4.95 (d, J = 9.7 Hz, 1H), 4.58 (d, J = 8.4 Hz, 1H), 4.15 (q, J = 7.1, 7.1, 7.1 Hz, 2H), 3.92 (d, J = 12.2 Hz, 1H), 3.87 (t, J = 10.0, 10.0 Hz, 1H), 3.81 (dd, J = 12.1, 1.3 Hz, 1H), 3.78 (d, J = 2.3 Hz, 1H), 3.77−3.71 (m, 3H), 3.69 (d, J = 2.2 Hz, 1H), 3.65 (dd, J = 9.5, 8.7 Hz, 1H), 3.63 (d, J = 12.4 Hz, 1H), 3.60−3.42 (m, 4H), 2.06 (s, 3H), 1.98 (s, 3H), 1.15 (t, J = 7.1, 7.1 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ ppm 177.6, 174.7, 174.6, 167.8, 167.5, 134.3, 131.6, 129.2, 128.3, 101.4, 78.7, 78.5, 76.3, 75.9, 73.5, 72.6, 69.7, 63.6, 60.6, 59.9, 55.6, 53.6, 42.3, 41.3, 22.2, 22.1, 13.1; FT-IR υ_{max} (neat)/cm^{-1} 3290.2, 1668.0, 1651.7, 1645.7, 1563.5, 1557.7, 1538.4, 1435.8, 1373.6, 1318.4, 1200.9, 1050.1. LC/MS t_R 7.45 min; linear gradient 10→50% B in 13.5 min; ESI/MS m/z = 669.07 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{29}H_{41}N_4O_{14}$ 669.26138, found 669.26145; mp 150 °C (start of decomposition).

1-Benzoyl-(2*R*,3*R*)-3-*N*-(*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosylcarbamoyl)aziridine-2-carboxylic Acid Ethyl Ester (20). Benzoylated **18** (52 mg, 51 μmol) was deprotected as described in general procedure 2. Semipreparative RP-HPLC (linear gradient 20%→29.375% B in 12.5 min) gave title compound **20** (35%, 11.9 mg, 18 μmol): ^{1}H NMR (400 MHz, D_2O) δ ppm 7.95 (d, J = 7.9 Hz, 2H), 7.71 (t, J = 7.5, 7.5 Hz, 1H), 7.57 (t, J = 7.8, 7.8 Hz, 2H), 4.98 (d, J = 9.6 Hz, 1H), 4.59 (d, J = 8.4 Hz, 1H), 4.15 (dq, J = 7.1, 7.0, 7.0, 2.9 Hz, 2H), 3.92 (dd, J = 12.3, 1.6 Hz, 1H), 3.86 (t, J = 9.9, 9.9 Hz, 1H), 3.79−3.70 (m, 6H), 3.66 (dd, J = 9.5, 8.9 Hz, 1H), 3.60 (dd, J = 17.8, 5.8 Hz, 1H), 3.57 (dd, J = 9.8, 8.9 Hz, 1H), 3.53−3.44 (m, 2H), 2.06 (s, 3H), 1.99 (s, 3H), 1.14 (t, J = 7.1, 7.1 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ ppm 177.4, 174.8, 174.6, 167.7, 167.4, 134.3, 131.4, 129.2, 128.3, 101.4, 78.7, 78.6, 76.2, 75.9, 73.5, 72.6, 69.7, 63.7, 60.6, 59.8, 55.6, 53.8, 42.2, 41.1, 22.1, 22.0, 13.1; FT-IR υ_{max} (neat)/cm^{-1}3273.5, 1661.5, 1651.7, 1645.7,

1563.0, 1557.7, 1373.7, 1305.4, 1199.9, 1117.0, 1023.9; LC/MS t_R 5.67 min; linear gradient 0→90% B in 13.5 min; ESI/MS m/z = 669.07 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{29}H_{41}N_4O_{14}$ 669.26138, found 669.26145; mp 164 °C (start of decomposition).

***N*-(*O*-(2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)hydroxyacetamide (21).** Glycolic acid (14 mg, 0.186 mmol) was condensed with azide **4** (100 mg, 0.124 mmol) in the presence of HCTU (77 mg, 0.186 mmol) and DiPEA (65 μL, 0.371 mmol) as described in the general procedure. The resulting hydroxyacetamide was purified (CH_2Cl_2→5% MeOH/CH_2Cl_2) affording **21** (77%, 80 mg, 95 μmol) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ ppm 8.01 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 7.52−7.21 (m, 20H), 6.62 (d, J = 9.2 Hz, 1H), 5.50 (s, 1H), 4.91 (dd, J = 9.4, 6.3 Hz, 1H), 4.86 (d, J = 12.4 Hz, 2H), 4.71 (d, J = 11.7 Hz, 1H), 4.64 (d, J = 12.0 Hz, 1H), 4.64 (d, J = 11.4 Hz, 1H), 4.55 (d, J = 8.3 Hz, 1H), 4.46 (d, J = 11.7 Hz, 1H), 4.16−4.11 (m, 1H), 4.09−4.00 (m, 2H), 3.99 (s, 2H), 3.83 (t, J = 8.8, 8.8 Hz, 1H), 3.71−3.50 (m, 6H), 3.44 (t, J = 10.3, 10.3 Hz, 1H), 3.24 (dt, J = 9.6, 9.5, 5.0 Hz, 1H), 1.87 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ ppm 174.1, 172.2, 171.3, 138.4, 138.0, 137.6, 136.9, 128.6, 128.1, 127.9, 127.8, 127.8, 127.6, 127.4, 127.3, 127.1, 125.6, 100.8, 100.2, 81.8, 80.1, 78.4, 76.2, 74.9, 73.7, 73.6, 73.1, 68.1, 67.7, 65.3, 61.1, 55.5, 52.8, 22.3, 21.8; FT-IR υ_{max} (neat)/cm^{-1} 3273.7, 1652.0, 1537.8, 1452.6, 1371.2, 1315.8, 1066.7; LC/MS t_R 8.26 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 840.4 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{46}H_{53}N_3O_{12}$ 839.37020, found 839.37101; mp 159 °C (start of decomposition).

***N*-(*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)mesyloxyacetamide (24).** Hydroxyacetamide **21** (80 mg, 95 μmol) was dissolved in dioxane/DMF (4/1, v/v, 1.5 mL). Ms_2O (60 mg, 0.34 mmol) and Et_3N (53 μL, 0.38 mmol) were added. After 2 h, an additional amount of Ms_2O (15 mg, 86 μmo l) and Et_3N (26 μL, 0.188 mmol) was added. The reaction was quenched after additional stirring with MeOH and subsequently concentrated under reduced pressure: LC/MS t_R 9.44 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 918.5 (M + H)$^+$. Crude mesylate **22** was dissolved in MeOH/TFE. *p*-Toluenesulfonic acid (25 mg) was added after which the reaction was stirred at 50 °C. After 1 h, the solution was cooled to room temperature, $Pd(OH)_2$ on charcoal was added, and the mixture was stirred under H_2 atm. TLC showed complete conversion to a lower running spot after 30 min. The mixture was filtered, concentrated in vacuo and purfied by semipreparative RP-HPLC (linear gradient 0→6% B in 15.25 min) furnishing mesylate **24** (44%, 23.14 mg, 41.4 μmol): ^{1}H NMR (600 MHz, D_2O) δ ppm 5.11 (d, J = 9.8 Hz, 1H), 4.58 (d, J = 8.5 Hz, 1H), 3.91 (dd, J = 11.8, 2.8 Hz, 1H), 3.90 (t, J = 10.8, 10.8 Hz, 1H), 3.82 (dd, J = 12.2, 1.4 Hz, 1H), 3.79−3.72 (m, 3H), 3.66 (dd, J = 9.3, 8.8 Hz, 1H), 3.64 (dd, J = 12.1, 4.5 Hz, 1H), 3.59 (ddd, J = 10.0, 4.6, 1.7 Hz, 1H), 3.55 (dd, J = 10.3, 8.6 Hz, 1H), 3.49 (ddd, J = 10.2, 5.6, 1.7 Hz, 1H), 3.46 (dd, J = 9.6, 8.7 Hz, 1H), 3.27 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (150 MHz, D_2O) δ ppm 174.3, 174.1, 168.6, 100.8, 78.2, 77.7, 75.8, 75.3, 72.9, 72.1, 69.1, 65.8, 60.0, 59.3, 55.0, 53.0, 21.5, 21.4; FT-IR υ_{max} (neat)/cm^{-1} 3269.8, 1681.6, 1651.4, 1544.9, 1431.7, 1351.6, 1201.0, 1165.4, 1108.7, 1041.5; LC/MS t_R 2.77 min; linear gradient 0→20% B in 13.5 min; ESI/MS m/z = 560.00 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{19}H_{34}N_3O_{14}S$ 560.17560, found 560.17540; mp 181 °C (start of decomposition).

***N*-(*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)thiophenylacetamide (25).** Mesylate **24** (44 mg, 78 μmol) was dissolved in MeOH (1 mL). Thiophenol (16.5 μL, 0.16 mmol) and K_2CO_3 (22 mg, 0.16 mmol) were added. The solution was stirred for 16 h before removal of the volatiles and extraction with H_2O/Et_2O. The aqueous layer was concentrated in vacuo and purified by RP-HPLC (linear gradient 10→30% B in 10 min) giving title compound **25** (43%, 19.12 mg, 33.3 μmol): ^{1}H NMR (400 MHz, MeOD/D_2O) δ ppm 7.43−7.22 (m, 5H), 4.96 (d, J = 9.7 Hz, 1H), 4.55 (d, J = 8.4 Hz, 1H), 3.90 (dd, J = 12.1, 1.5 Hz, 1H), 3.81 (t, J = 9.3, 9.3 Hz, 1H), 3.80 (dd, J = 12.0, 1.5 Hz, 1H), 3.76−3.39 (m, 10H), 2.05 (s, 3H), 1.73 (s, 3H); ^{13}C NMR (100 MHz, MeOD/D_2O) δ ppm 175.3, 175.2, 173.8, 135.3, 130.4, 129.5, 128.0, 102.5, 80.0, 79.9, 77.3, 77.1, 74.6, 73.8, 70.9, 61.7, 61.0, 56.7, 54.5, 37.8, 23.1, 22.8; FT-IR υ_{max} (neat)/cm^{-1} 3272.1, 1651.1, 1539.7, 1435.8, 1373.7, 1310.0, 1026.9; LC/MS t_R 6.52 min; linear gradient 0→50% B in 13.5 min; ESI/MS m/z = 573.93 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{24}H_{36}N_3O_{11}S$ 574.20651, found 574.20644; mp 230 °C (start of decomposition).

3-*C*-(*O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)propene (29). Imidate **28** (2.53 g, 4.36 mmol) and acceptor **26** (1.54 g, 3 mmol) were coevaporated three times with anhydrous Tol and dissolved in CH_2Cl_2 (5 mL/mmol) after which 3 Å moleculare sieves (MS) were added. The mixture was cooled to −30 °C and stirred for 30 min before TMSOTf (cat.) was added. After 2 h, the reaction was quenched with Et_3N (1 mL), diluted with CH_2Cl_2, extracted with $NaHCO_3$ (satd aq), dried (Na_2SO_4), and concentrated. Purification by Sephadex LH-20 column (50 mmD × 1500 mmL) gave disaccharide **29** (97%, 2.719 g, 2.9 mmol) as a thick syrup: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.96−7.84 (m, 2H), 7.79−7.71 (m, 3H), 7.69−7.62 (m, 2H), 7.57 (d, J = 6.4 Hz, 1H), 7.39−7.15 (m, 5H), 7.06−6.96 (m, 2H), 6.87−6.82 (m, 3H), 5.81 (t, J = 9.9, 9.9 Hz, 1H), 5.70−5.58 (m, 1H), 5.54 (d, J = 8.3 Hz, 1H), 5.13 (t, J = 9.6, 9.6 Hz, 1H), 4.90−4.76 (m, 3H), 4.59 (d, J = 12.1 Hz, 1H), 4.53 (d, J = 12.1 Hz, 1H), 4.46 (d, J = 12.5 Hz, 1H), 4.34 (dd, J = 10.4, 8.5 Hz, 1H), 4.26−4.18 (m, 3H), 4.06−4.00 (m, 2H), 3.95 (d, J = 12.7 Hz, 1H), 3.56 (d, J = 11.1 Hz, 1H), 3.53−3.47 (m, 1H), 3.45 (dd, J = 11.4, 3.0 Hz, 1H), 3.33−3.28 (m, 1H), 2.18−2.06 (m, 2H), 2.00 (s, 3H), 1.96 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 170.6, 170.0, 169.4, 168.0, 167.7, 138.7, 138.5, 134.4, 133.7, 133.6, 133.4, 131.5, 131.4, 128.7, 128.2, 127.8, 127.7, 127.4, 127.3, 126.9, 123.5, 123.2, 123.1, 116.9, 96.8, 78.3, 77.8, 76.4, 74.5, 74.2, 72.6, 71.4, 70.6, 68.8, 68.2, 61.5, 55.7, 55.3, 36.9; FT-IR υ_{max} (neat)/cm^{-1}2945.5, 1747.8, 1713.8, 1469.3, 1455.3, 1385.6, 1222.8, 1081.2, 1036.1; HRMS (M + Na$^+$) calcd for $C_{51}H_{50}N_2O_{15}Na$ 953.31034, found 953.31077.

3-*C*-(*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)propene (30). After coevaporation with toluene, disaccharide **29** (3.35 g, 3.6 mmol) was dissolved in *n*-BuOH/ethylenediamine (50 mL, 9/1 v/v), 4 Å MS were added, and the solution was subsequently stirred overnight at 90 °C. The solution was filtered, concentrated in vacuo, coevaporated with toluene (3×), redissolved in pyridine (50 mL), and cooled to 0 °C before Ac_2O (30 mL) was added. The reaction mixture was stirred overnight, after which TLC analysis showed complete conversion of the starting material. Concentration in vacuo, redissolving in CH_2Cl_2, extraction with 1 M HCl, drying (Na_2SO_4), and concentrating gave the crude title compound **30**. Purification by silica gel chromatography (CH_2Cl_2→2% MeOH/CH_2Cl_2) furnished **30** (75%, 2.048 g, 2.71 mmol) as an off-white solid: ^{1}H NMR (400 MHz, DMSO) δ ppm 8.05 (d, J = 9.2 Hz, 1H), 7.86 (d, J = 9.3 Hz, 1H), 7.37−7.21 (m, 10H), 5.88−5.76 (m, 1H), 5.15−4.96 (m, 3H), 4.89−4.78 (m, 3H), 4.63−4.54 (m, 3H), 4.02 (dd, J = 12.2, 4.0 Hz, 1H), 3.81 (d, J = 12.1 Hz, 1H), 3.79−3.67 (m, 3H), 3.66−3.58 (m, 2H), 3.51 (t, J = 9.4, 9.4 Hz, 1H), 3.48−3.43 (m, 1H), 3.37−3.33 (m, 1H), 3.26 (m, 1H) 2.26 (dd, J = 14.2, 7.6 Hz, 1H), 2.04 (td, J = 14.4, 6.9, 6.9 Hz, 1H), 1.94 (s, 3H), 1.92 (s, 3H), 1.86 (s, 3H), 1.77 (s, 6H); ^{13}C NMR (100 MHz, DMSO) δ ppm 169.8, 169.6, 169.4, 169.3, 169.1, 139.4, 138.8, 135.0, 128.2, 127.9, 127.2, 127.0, 126.9, 126.9, 116.6, 99.7, 82.2, 78.2, 77.6, 76.0, 72.7, 72.3, 71.9, 70.7, 68.8, 68.5, 61.7, 54.1, 53.6, 35.9, 22.9, 22.7, 20.4, 20.3; FT-IR υ_{max} (neat)/cm^{-1} 3297.9, 2940.5, 1740.4, 1652.0, 1557.8, 1538.9, 1455.7, 1373.3, 1227.3, 1118.2, 1045.5; LC/MS t_R 7.96 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 755.00 (M +

H)$^+$; HRMS (M + H$^+$) calcd for $C_{39}H_{51}N_2O_{13}$ 755.33857, found 755.33881; mp 224 °C.

3-*C*-(*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)propene (31). Disaccharide **30** (154 mg, 0.2 mmol) was suspended in THF (5 mL) and added to a solution of NH_3 (5 mL) and sodium (100 mg) at −78 °C. The mixture was stirred at −78 °C for 2 h after which it was quenched with MeOH. The temperature was raised to room temperature. Residual NH_3 was removed by a flow of argon. Subsequently, the solution was neutralized with AcOH, concentrated, redissolved in pyridine/Ac_2O, and stirred overnight. The reaction mixture was concentrated in vacuo, redissolved in CH_2Cl_2, extracted with 1 M HCl and $NaHCO_3$ (satd aq), dried (Na_2SO_4), and concentrated under reduced pressure. Silica gel column chromatography ($CHCl_3$→2% MeOH/$CHCl_3$) afforded **31** (quant, 131 mg, 0.2 mmol) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ ppm 5.84−5.72 (m, 1H), 5.25 (t, *J* = 9.9, 9.9 Hz, 1H), 5.05−4.94 (m, 4H), 4.69 (d, *J* = 8.3 Hz, 1H), 4.40 (d, *J* = 11.8 Hz, 1H), 4.39 (dd, *J* = 12.3, 4.0 Hz, 1H), 4.04 (dd, *J* = 11.8, 6.1 Hz, 1H), 3.99 (dd, *J* = 12.4, 1.8 Hz, 1H), 3.82 (t, *J* = 10.2, 10.2 Hz, 1H), 3.75−3.65 (m, 3H), 3.54−3.49 (m, 1H), 3.37 (ddd, *J* = 10.3, 7.6, 3.0 Hz, 1H), 2.35−2.10 (m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.88 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ ppm 172.7, 172.4, 172.0, 171.68, 171.65, 171.4, 170.6, 134.3, 117.5, 101.4, 78.2, 77.3, 77.1, 75.8, 73.1, 72.1, 69.2, 63.6, 62.5, 55.53, 55.45, 54.0, 36.6, 22.9, 22.8, 21.1, 21.0, 20.84, 20.77, 20.7; FT-IR υ_{max} (neat)/cm^{-1}3293.9, 1739.7, 1699.9, 1660.9, 1652.0, 1538.1, 1435.8, 1373.5, 1223.9, 1136.9, 1042.6; LC/MS t_R 5.58 min; linear gradient 10→90% B in 13.5 min; ESI/MS *m*/*z* = 659.00 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{29}H_{43}N_2O_{15}$ 659.26579, found 659.26579; mp 235 °C (start of decomposition).

(*E*)-Ethyl 4-*C*-(*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)but-2-enoate (32). Allyl *C*-glycoside **31** (131 mg, 0.2 mmol) was coevaporated with toluene and dissolved in CH_2Cl_2 (6 mL). Grubbs second-generation catalyst (8 mg) and ethyl acrylate (92 μL, 1 mmol) were added. The reaction mixture was refluxed overnight, concentrated, and applied to silica gel chromatography (CH_2Cl_2→5% MeOH/CH_2Cl_2), furnishing title compound **32** as an off-white solid (quant, 146 mg, 0.2 mmol): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.92 (dt, *J* = 15.2, 6.7 Hz, 1H), 6.40 (d, *J* = 8.8 Hz, 1H), 6.15 (d, *J* = 9.5 Hz, 1H), 5.88 (d, *J* = 15.6 Hz, 1H), 5.27 (t, *J* = 9.8, 9.8 Hz, 1H), 5.06 (t, *J* = 9.6, 9.6 Hz, 1H), 4.99 (t, *J* = 9.6, 9.6 Hz, 1H), 4.67 (d, *J* = 8.2 Hz, 1H), 4.39 (dd, *J* = 12.5, 3.8 Hz, 1H), 4.36 (d, *J* = 12.0 Hz, 1H), 4.22 (dd, *J* = 12.1, 6.0 Hz, 1H), 4.17 (q, *J* = 6.9 Hz, 2H), 4.06−3.98 (m, 2H), 3.81 (q, *J* = 9.4, 9.1, 9.1 Hz, 1H), 3.74−3.64 (m, 2H), 3.57−3.52 (m, 1H), 3.36 (dt, *J* = 10.2, 9.9, 2.9 Hz, 1H), 2.49 (dd, *J* = 13.6, 6.8 Hz, 1H), 2.40 (td, *J* = 15.6, 7.2, 7.2 Hz, 1H), 2.13 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.00 (s, 6H), 1.94 (s, 6H), 1.27 (t, *J* = 7.1, 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 171.3, 171.1, 170.7, 170.6, 170.5, 170.4, 169.4, 166.3, 144.2, 123.3, 101.1, 101.0, 77.7, 76.7, 76.5, 74.3, 72.4, 71.7, 68.3, 62.6, 61.9, 60.2, 54.9, 53.6, 34.4, 23.1, 20.9, 20.64, 20.57, 20.56, 14.2; FT-IR υ_{max} (neat)/cm^{-1}3286.0, 1738.6, 1656.7, 1651.8, 1562.7, 1538.0, 1435.7, 1373.1, 1221.9, 1179.5, 1032.5, 979.7, 905.9; LC/MS t_R 5.43 min; linear gradient 10→90% B in 13.5 min; ESI/MS *m*/*z* = 731.5(M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{32}H_{47}N_2O_{17}$ 731.28692, found 731.28713; mp 225 °C.

(*E*)-Methyl 4-*C*-(*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)but-2-enoate (33). Vinyl ester **32** (73 mg, 0.1 mmol) was dissolved in methanol/H_2O (1/1 v/v, 4 mL). Et_3N (1 mL) was added, and the resulting mixture was stirred for 48 h. Concentration under reduced pressure followed by preparative RP-HPLC purification (linear gradient 0→40% B in 10 min) gave title compound **33** (12%, 6.01 mg, 11.9 μmol) as a white solid: ^{1}H NMR (500 MHz, D_2O) δ ppm 7.01 (td, *J* = 14.6, 7.0, 7.0 Hz, 1H), 5.95 (d, *J* = 15.8 Hz, 1H), 4.59 (d, *J* = 8.5 Hz, 1H), 3.92 (dd, *J* = 12.5, 0.9 Hz, 1H), 3.83 (dd, *J* = 12.0, 1.3 Hz, 1H), 3.78−3.72 (m, 5H), 3.71−3.53 (m, 6H), 3.53−3.43 (m, 3H), 2.56 (ddd, *J* = 15.6, 6.3, 5.1 Hz, 1H), 2.48 (td, *J* = 14.5, 6.6, 6.6 Hz, 1H), 2.07 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (125 MHz, D_2O) δ ppm 174.6, 174.3, 169.2, 146.1, 122.0, 101.4, 79.7, 78.1, 76.5, 75.9, 73.8, 73.5, 69.7, 60.5, 60.3, 55.6, 54.5, 52.1, 34.4, 22.10, 22.07; FT-IR υ_{max} (neat)/cm^{-1}3268.9, 1627.8, 1562.6, 1557.7, 1436.0, 1377.2, 1304.3, 1199.8, 1079.8, 1044.4; LC/MS t_R 4.583 min; linear gradient 0→20% B in 13.5 min; ESI/MS *m*/*z* = 507.00 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{21}H_{35}N_2O_{12}$ 507.21845, found 507.21818; mp 150 °C (start of decomposition).

3-*C*-(*O*-(2-Acetamido-3,4,6-tri-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl)propene (36). Peracetylated **31** (255 mg, 387 μmol) was dissolved in MeOH (10 mL) before NaOMe in MeOH (3M, 0.3 mL) was added. TLC analysis showed complete conversion to a very polar product after 2 h. The mixture was neutralized with Amberlite IR-120 H$^+$, filtered, concentrated, and redissolved in pyridine. The solution was cooled, after which *tert*-butyldimethylsilyl triflate (TBSOTf) (0.533 mL, 2.32 mmol) was added. Additional amounts of TBSOTf (0.15 mL, 0.653 mmol) were added after 24 and 72 h. The reaction was quenched by the addition of MeOH (1 mL) and concentrated under reduced pressure. Residual amounts of pyridine were removed by coevaporation with toluene. Silica gel column chromatography (CH_2Cl_2 → 2% MeOH/CH_2Cl_2) afforded title compound **36** (64%, 251 mg, 0.246 mmol) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.25 (d, *J* = 9.7 Hz, 1H), 5.90−5.79 (m, 1H), 5.77 (d, *J* = 10.3 Hz, 1H), 5.06 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.00 (dd, *J* = 10.2, 1.8 Hz, 1H), 4.52 (d, *J* = 6.9 Hz, 1H), 4.14−4.08 (m, 1H), 3.97 (d, *J* = 4.2 Hz, 1H), 3.94 (dd, *J* = 4.8, 2.7 Hz, 1H), 3.89 (dd, *J* = 9.3, 2.9 Hz, 1H), 3.80−3.63 (m, 7H), 3.51 (ddd, *J* = 6.6, 6.0, 2.8 Hz, 1H), 3.09 (dt, *J* = 8.6, 8.5, 3.6 Hz, 1H), 2.32 (ddd, *J* = 10.3, 6.7, 3.2 Hz, 1H), 2.23 (td, *J* = 14.9, 7.3, 7.3 Hz, 1H), 1.98 (s, 3H), 1.93 (s, 3H), 0.93 (s, 9H), 0.89 (s, 9H), 0.89 (s, 18H), 0.87 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), 0.12 (s, 6H), 0.11 (s, 3H), 0.08 (s, 3H), 0.05 (s, 9H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 169.4, 168.8, 135.0, 116.4, 99.0, 81.2, 78.9, 77.0, 76.4, 73.9, 73.2, 70.0, 63.8, 63.3, 55.4, 54.3, 38.3, 25.94, 25.92, 25.87, 25.84, 25.77, 25.67, 23.4, 23.2, 18.3, 18.2, 17.82, 17.79, −4.0, −4.4, −4.6, −4.7, −4.8, −5.1, −5.2, −5.4; FT-IR υ_{max} (neat)/cm^{-1}2930.3, 2857.5, 1651.9, 1558.6, 1473.7, 1374.0, 1308.3, 1252.1, 1102.6, 1005.9; HRMS (M + H$^+$) calcd for $C_{49}H_{102}N_2O_{10}Si_5$ 1019.64536, found 1019.64668; mp 193.2−194.7 °C.

(*E*)-3-*C*-(*O*-(2-Acetamido-3,4,6-tri-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl)-1-methanesulfonylpropene (37). Alkene **36** (240 mg, 0.235 mmol) was dissolved in freshly distilled CH_2Cl_2 under argon atmosphere. Methyl vinylsulfone (206 μL, 2.35 mmol) and Hoveyda−Grubbs second-generation catalyst (15 mg, 23.5 μmol) were added. The reaction was refluxed overnight before an additional amount of catalyst and vinylsulfone was added. The resulting mixture was refluxed until TLC analysis showed complete conversion to a more polar product. Concentration under reduced pressure followed by purification over silica gel chromatography (CH_2Cl_2→3% MeOH/CH_2Cl_2) afforded vinyl sulfone **37** (66%, 171 mg, 0.156 mmol) as a brown oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.93 (td, *J* = 14.3, 6.9, 6.9 Hz, 1H), 6.43 (d, *J* = 14.9 Hz, 2H), 5.81 (d, *J* = 10.2 Hz, 1H), 4.51 (d, *J* = 6.8 Hz, 1H), 4.08 (dd, *J* = 9.5, 7.3 Hz, 1H), 4.00−3.95 (m, 2H), 3.90 (dt, *J* = 9.1, 9.0, 1.8 Hz, 1H), 3.82 (dd, *J* = 4.4, 2.8 Hz, 1H), 3.78−3.63 (m, 6H), 3.54 (dt, *J* = 6.5, 6.2, 1.9 Hz, 1H), 3.22 (dt, *J* = 8.7, 8.6, 3.4 Hz, 1H), 2.92 (s, 3H), 2.54 (ddd, *J* = 15.1, 7.2, 2.9 Hz, 1H), 2.45 (dd, *J* = 15.3, 7.6 Hz, 1H), 2.00 (s, 3H), 1.95 (s, 3H), 0.94 (s, 9H), 0.89 (s, 18H), 0.89 (s, 9H), 0.87 (s, 9H), 0.13 (s, 6H), 0.12 (s, 6H), 0.11 (s, 3H), 0.08 (s, 3H), 0.05 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 169.8, 169.1, 144.7, 131.1, 98.9, 81.3, 78.9, 76.3, 75.4, 73.6, 72.7, 70.0, 63.8, 63.2, 55.7,

54.2, 42.8, 36.2, 26.0, 25.9, 25.83, 25.79, 23.5, 23.2, 18.4, 18.23, 18.19, 17.9, −4.3, −4.4, −4.6, −4.66, −4.73, −5.0, −5.2, −5.3, −5.4; FT-IR: υ_{max} (neat)/cm^{-1}2931.6, 2857.8, 1667.3, 1469.6, 1302.1, 1253.1, 1092.8; HRMS (M + H$^+$) calcd for $C_{50}H_{104}N_2O_{12}SSi_5$ 1097.62291, found 1097.62394.

(*E*)-3-*C*-(*O*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-1-methanesulfonylpropene (38). Vinyl sulfone **37** (89 mg, 74.7 μmol) was cooled to 0 °C before being dissolved in trifluoroacetic acid (2 mL) in the presence of H_2O (200 μL). The reaction was allowed to come to room temperature. LC/MS analysis showed complete conversion after 4 h. The solution was diluted with Tol/H_2O and concentrated under reduced pressure. The resulting solids were redissolved in H_2O, washed with EtOAc (3×), and concentrated. Semipreparative reversed-phase HPLC purification (linear gradient in 0→6% B in 14.00 min) afforded deprotected vinyl sulfone **38** (78%, 30.63 mg, 58.2 μmol) as a white solid: ^{1}H NMR (400 MHz, D_2O) δ ppm 6.96 (td, *J* = 14.8, 6.8, 6.8 Hz, 1H), 6.66 (d, *J* = 15.3 Hz, 1H), 4.57 (d, *J* = 8.4 Hz, 1H), 3.91 (dd, *J* = 12.4, 1.3 Hz, 1H), 3.82 (dd, *J* = 11.9, 1.6 Hz, 1H), 3.77−3.52 (m, 8H), 3.52−3.40 (m, 3H), 3.09 (s, 3H), 2.68−2.59 (m, 1H), 2.51 (td, *J* = 15.6, 6.7, 6.7 Hz, 1H), 2.06 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ ppm 174.6, 174.4, 145.1, 130.0, 101.5, 79.8, 78.1, 76.1, 76.0, 73.8, 73.5, 69.7, 60.6, 60.4, 55.6, 54.5, 41.8, 33.4, 22.2, 22.2; FT-IR υ_{max} (neat)/cm^{-1}3287.6, 1636.5, 1557.7, 1420.6, 1374.6, 1277.4, 1202.8, 1124.8, 1026.6; LC/MS t_R 3.767 min; linear gradient 10→90% B in 13.5 min; ESI/MS *m*/*z* = 527.5 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{20}H_{34}N_2O_{12}S$ 527.19052, found 527.19018; mp 136 °C (start of decomposition).

3-*C*-(*O*-(3-*O*-Benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)propene (40). Imidate **39** (2.8 g, 3.7 mmol) and acceptor **26** (1.9 g, 3.5 mmol) were coevaporated three times with toluene before being dissolved in freshly distilled CH_2Cl_2 (30 mL). Activated molecular sieves 4 Å were added, after which the solution was cooled to −30 °C. TMSOTf (73 μL) was added followed by stirring until TLC showed complete conversion. The reaction was quenched with Et_3N, diluted with EtOAc, extracted with $NaHCO_3$ (satd aq) and brine, dried (Na_2SO_4), and concentrated. Silica gel column chromatography (10% EA/PE→30% EA/PE) furnished disaccharide **40** (97%, 3.35 g, 3.41 mmol) as a thick syrup: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.93−7.83 (m, 1H), 7.77−7.55 (m, 7H), 7.53−7.48 (m, 2H), 7.42−7.23 (m, 8H), 7.07−7.02 (m, 2H), 7.00−6.96 (m, 2H), 6.95−6.85 (m, 6H), 5.64 (tdd, *J* = 17.0, 10.0, 6.9, 6.9 Hz, 1H), 5.52 (s, 1H), 5.43 (d, *J* = 8.3 Hz, 1H), 4.88−4.76 (m, 4H), 4.55−4.40 (m, 5H), 4.29−4.15 (m, 4H), 4.07−4.01 (m, 2H), 3.73 (t, *J* = 9.1, 9.1 Hz, 1H), 3.56 (t, *J* = 10.2, 10.2 Hz, 1H), 3.49 (d, *J* = 10.9 Hz, 1H), 3.46−3.38 (m, 1H), 3.34 (dd, *J* = 11.1, 3.0 Hz, 1H), 3.28 (dd, *J* = 9.3, 2.7 Hz, 1H), 2.21−2.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 167.8, 167.6, 138.3, 138.1, 137.6, 137.1, 133.83, 133.82, 133.64, 133.56, 133.0, 131.2, 128.8−125.8, 123.1, 123.0, 116.8, 100.9, 97.5, 82.9, 78.2, 77.5, 76.2, 74.24, 74.17, 74.1, 73.8, 72.4, 68.4, 67.9, 65.5, 56.3, 55.5, 36.6; FT-IR υ_{max} (neat)/cm^{-1}3034.0, 2858.4, 1775.6, 1710.9, 1613.0, 1496.0, 1453.1, 1383.6, 1310.6, 1174.1, 1075.6, 1026.5; HRMS (M + Na$^+$) calcd for $C_{59}H_{54}N_2O_{12}Na$ 1005.35690, found 1005.35776.

3-*C*-(*O*-(2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-1-hydroxypropan-2-one (41). Disaccharide **40** (3.33 g, 3.38 mmol) was dissolved in THF/H_2O (30 mL, 6/1 v/v). K_2OsO_4 (25 mg, 68 μmol) and morpholine *N*-oxide (0.99 g, 8.46 mmol) were added. After being stirred overnight, the reaction was quenched with 1 M HCl, extracted with EtOAc, washed with $Na_2S_2O_3$ (aq), $NaHCO_3$ (satd aq), and brine, dried (Na_2SO_4), and concentrated. The resulting diol was redissolved in *n*-BuOH/ethylenediamine (50 mL, 9/1 v/v) and activated 4 Å MS were added after which the reaction was stirred overnight at 90 °C. The reaction was filtered, concentrated, and coevaporated with toluene to remove residual traces of *n*-BuOH/ethylenediamine. The resulting amine was dissolved in MeOH (50 mL) and cooled to 0 °C before Ac_2O (5 mL) and Et_3N (7 mL) were added. The reaction was stirred overnight and crystallized. The diol was dissolved in $CHCl_3$/MeOH (30 mL, 1/1 v/v). Dibutyltin oxide (0.883 g, 3.5 mmol) was added, and the reaction was refluxed for 2 h. The resulting clear solution was concentrated and dissolved in $CHCl_3$ (20 mL), and *N*-bromosuccimide (0.627 g, 3.52 mmol) was added. After 2 h of stirring, Celite was added, and the reaction mixture was concentrated in vacuo. Purification by silica gel chromatography (CH_2Cl_2→6% MeOH/CH_2Cl_2) afforded hydroxymethyl ketone **41** (50% over four steps, 1.35 g, 1.6 mmol): ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ ppm 7.56−7.18 (m, 20H), 5.49 (s, 1H), 4.86 (d, *J* = 11.9 Hz, 1H), 4.83 (d, *J* = 11.1 Hz, 1H), 4.67 (d, *J* = 10.4 Hz, 1H), 4.64 (d, *J* = 10.5 Hz, 1H), 4.62 (d, *J* = 11.5 Hz, 1H), 4.52 (d, *J* = 8.3 Hz, 1H), 4.45 (d, *J* = 11.7 Hz, 1H), 4.21 (s, 2H), 4.13 (dd, *J* = 10.8, 5.1 Hz, 1H), 3.94 (t, *J* = 8.1, 8.1 Hz, 1H), 3.90 (dd, *J* = 9.5, 8.9 Hz, 1H), 3.84 (t, *J* = 8.8, 8.8 Hz, 1H), 3.75−3.68 (m, 1H), 3.68−3.54 (m, 3H), 3.50 (t, *J* = 8.4, 8.4 Hz, 1H), 3.47−3.37 (m, 2H), 3.23 (dt, *J* = 9.3, 9.2, 4.8 Hz, 1H), 2.69 (dd, *J* = 16.1, 8.9 Hz, 1H), 2.54 (dd, *J* = 16.0, 2.4 Hz, 1H), 1.87 (s, 3H), 1.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ ppm 209.0, 171.9, 171.8, 139.1, 138.6, 138.1, 137.5, 129.2, 128.8, 128.54, 128.46, 128.4, 128.2, 128.0, 127.72, 127.67, 126.2, 101.4, 101.0, 82.4, 81.5, 78.9, 78.1, 76.0, 75.0, 74.3, 74.1, 73.9, 69.1, 68.73, 68.65, 65.9, 55.9, 53.9, 41.9, 23.1, 22.8; FT-IR υ_{max} (neat)/cm^{-1}3278.2, 2870.1, 1717.7, 1658.1, 1651.8, 1557.7, 1538.5, 1497.4, 1455.5, 1373.1, 1321.7, 1210.2, 1173.8, 1072.0, 1027.9, 1011.9; LC/MS t_R 8.96 min; linear gradient 10→90% B in 13.5 min; ESI/MS *m*/*z* = 839.60 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{47}H_{55}N_2O_{12}$ 839.37495, found 839.37581; mp 159 °C (start of decomposition).

3-*C*-(*O*-(2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-1-(2,6-dimethylbenzoyloxy)propan-2-one (42). Hydroxy methylketone **41** (20 mg, 24 μmol) was dissolved in CH_2Cl_2/DMF (0.5 mL, 3/1 v/v). Methanesulfonic anhydride (9 mg, 50 μmol) and Et_3N (14 μL, 0.1 mmol) were added. After 3 h, the solution was concentrated. DMF (0.4 mL), K_2CO_3 (20 mg, 0.1 mmol), and 2,6-dimethylbenzoic acid (20 mg, 0.1 mmol) were added. The reaction was stirred overnight, diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq), dried, and concentrated. Silica gel column chromatography (CH_2Cl_2→3% MeOH/CH_2Cl_2) gave acyloxy methylketone **42** (65%, 15 mg, 15 μmol): ^{1}H NMR (400 MHz, DMSO) δ ppm 8.10 (d, *J* = 8.5 Hz, 1H), 7.99 (d, *J* = 8.9 Hz, 1H), 7.44−7.21 (m, 21H), 7.10 (d, *J* = 7.6 Hz, 2H), 5.67 (s, 1H), 5.09 (d, *J* = 17.2 Hz, 1H), 5.03 (d, *J* = 17.2 Hz, 1H), 4.84 (d, *J* = 11.1 Hz, 1H), 4.75−4.69 (m, 2H), 4.61−4.51 (m, 4H), 4.04−3.99 (m, 1H), 3.81−3.48 (m, 11H), 3.39 (ddd, *J* = 9.9, 4.8, 1.3 Hz, 1H), 3.17−3.10 (m, 1H), 2.66 (dd, *J* = 16.4, 7.2 Hz, 1H), 2.61 (dd, *J* = 16.2, 3.7 Hz, 1H), 2.29 (s, 6H), 1.84 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ ppm 201.4, 169.4, 169.3, 168.0, 139.1, 138.7, 138.6, 137.5, 134.6, 132.9, 129.5, 128.7, 128.6, 128.1, 128.01, 127.99, 127.9, 127.4, 127.3, 127.2, 127.1, 126.9, 125.9, 100.7, 99.9, 81.5, 80.9, 78.3, 76.0, 74.2, 73.3, 73.2, 71.8, 68.7, 68.6, 67.7, 65.5, 55.3, 53.7, 53.6, 41.7, 22.9, 22.82, 22.75, 19.2; FT-IR υ_{max} (neat)/cm^{-1}3266.0, 2865.8, 1728.0, 1651.7, 1538.0, 1452.3, 1370.5, 1264.7, 1086.8, 1060.9; LC/MS t_R 10.76 min; linear gradient 10→90% B in 13.5 min; ESI/MS *m*/*z* = 971.27 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{56}H_{63}N_2O_{13}$ 971.43247, found 971.43365; mp 211 °C (start of decomposition).

3-*C*-(*O*-(2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-1-chloropropan-2-one (43). Hydroxymethyl ketone **41** (183 mg, 218 μmol) was dissolved in DMF (0.5 mL) and dioxane (5 mL). Methanesulfonic anhydride (180 mg, 1.03 mmol) and Et_3N (280 μL, 2.0 mmol) were added. TLC analysis showed complete conversion to a higher running spot after 3 h. The solution was concentrated and redissolved in DMF (5 mL), and LiCl (400 mg) was added followed by stirring overnight. The

reaction was diluted with CH_2Cl_2, washed with 10% (aq) citric acid, dried (Na_2SO_4), and concentrated. Silica column chromatography (CH_2Cl_2 → 4% MeOH/CH_2Cl_2) afforded fully protected chloromethyl ketone **43** (59%, 111 mg, 129 μmol) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ ppm 7.50−7.25 (m, 20H), 5.51 (s, 1H), 4.87 (d, J = 11.5 Hz, 1H), 4.86 (d, J = 11.9 Hz, 1H), 4.65 (d, J = 11.9 Hz, 1H), 4.65 (d, J = 11.0 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.59 (d, J = 8.1 Hz, 1H), 4.52−4.49 (m, 1H), 4.24 (s, 2H), 4.11 (dd, J = 10.4, 5.0 Hz, 1H), 3.93 (dd, J = 8.7, 8.3 Hz, 1H), 3.90 (dd, J = 18.5, 9.2 Hz, 1H), 3.86−3.80 (m, 1H), 3.75−3.69 (m, 1H), 3.68−3.61 (m, 3H), 3.51 (t, J = 8.7, 8.7 Hz, 1H), 3.47−3.38 (m, 2H), 3.24 (dt, J = 9.5, 9.3, 4.2 Hz, 1H), 2.83 (dd, J = 16.2, 8.9 Hz, 1H), 2.66 (dd, J = 16.2, 3.1 Hz, 1H), 1.90 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ ppm 200.4, 171.5, 171.4, 138.5, 137.9, 137.5, 136.8, 128.6, 128.1, 127.84, 127.76, 127.6, 127.4, 127.3, 127.0, 125.6, 100.8, 100.4, 81.7, 81.0, 78.3, 77.6, 75.5, 74.6, 73.7, 73.6, 73.0, 68.1, 68.0, 65.3, 55.4, 53.4, 48.9, 42.5, 22.3, 22.0; FT-IR υ_{max} (neat)/cm^{-1}3287.5, 2865.9, 1727.6, 1645.4, 1557.7, 1454.9, 1368.3, 1319.8, 1175.0, 1071.4; LC/MS t_R 9.85 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 857.07 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{47}H_{54}ClN_2O_{11}$ 857.34106, found 857.34206; mp 194 °C (start of decomposition).

3-*C*-(*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-(2,6-dimethylbenzoyloxy)propan-2-one (44). Reductive removal of the protective groups of disaccharide **42** (70 mg, 72 μmol) was performed as described in general procedure 1. Semipreparative RP-HPLC (linear gradient 25%→34.375% B in 12.5 min) afforded title compound **44** (83%, 36.7 mg, 60 μmol) as a white solid: ^{1}H NMR (400 MHz, D_2O) δ ppm 7.38 (t, J = 7.6, 7.6 Hz, 1H), 7.22 (d, J = 7.6 Hz, 2H), 5.23 (s, 2H), 4.72 (d, J = 8.4 Hz, 1H), 4.04 (d, J = 12.2 Hz, 1H), 3.99 (dd, J = 17.5, 8.6 Hz, 1H), 3.93−3.81 (m, 5H), 3.80−3.66 (m, 3H), 3.65−3.49 (m, 3H), 2.95 (dd, J = 16.1, 9.1 Hz, 1H), 2.85 (dd, J = 16.2, 2.1 Hz, 1H), 2.43 (s, 6H), 2.16 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ ppm 204.9, 175.2, 175.1, 171.4, 136.7, 133.7, 131.5, 129.1, 102.9, 81.1, 79.5, 77.5, 75.6, 75.0, 74.8, 71.3, 70.5, 62.1, 61.5, 57.0, 56.0, 42.8, 31.3, 23.6, 23.5, 20.3; FT-IR υ_{max} (neat)/cm^{-1}3271.6, 1728.5, 1651.8, 1562.9, 1557.7, 1423.9, 1373.9, 1269.0, 1203.0, 1165.7, 1106.4, 1077.5, 1049.0; LC/MS t_R 8.46 min; linear gradient 0→50% B in 13.5 min; ESI/MS m/z = 613.07 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{28}H_{41}N_2O_{13}$ 613.26032, found 613.26018; mp 190 °C (start of decomposition).

3-*C*-(*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-chloropropan-2-one (45). Chloromethyl ketone **43** (80 mg, 95 μmol) was coevaporated three times with toluene before being suspended in freshly distilled CH_2Cl_2. The suspension was cooled to 0 °C after which $FeCl_3$ (250 mg, 0.152 mmol) was added. TLC analysis showed conversion to a very polar product after 5 h. The reaction was quenched by the addition of H_2O and washed with CH_2Cl_2. The aqueous layer was concentrated to give the crude title compound. Semipreprative RP-HPLC purification (linear gradient 0→12% B in 15.7 min) afforded pure title compound **45** (66%, 31.46 mg, 63 μmol): ^{1}H NMR (400 MHz, D_2O) δ ppm 4.59 (d, J = 8.4 Hz, 1H), 4.47 (s, 2H), 3.93 (dd, J = 12.3, 1.7 Hz, 1H), 3.89 (ddd, J = 7.4, 4.8, 2.1 Hz, 1H), 3.80 (dd, J = 12.4, 2.2 Hz, 1H), 3.79−3.66 (m, 5H), 3.65−3.55 (m, 3H), 3.54−3.43 (m, 3H), 2.88 (dd, J = 15.3, 5.7 Hz, 1H), 2.83 (dd, J = 12.5, 1.0 Hz, 1H), 2.08 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ ppm 203.9, 174.6, 101.5, 79.5, 78.1, 75.9, 74.0, 73.6, 73.5, 69.7, 60.5, 60.2, 55.6, 54.5, 49.5, 42.3, 22.1; FT-IR υ_{max} (neat)/cm^{-1}3289.0, 1651.7, 1634.2, 1557.8, 1373.8, 1033.8; LC/MS t_R 3.16 min; linear gradient 0→50% B in 13.5 min; ESI/MS m/z = 498.93 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{19}H_{32}ClN_2O_{11}$ 499.16891, found 499.16869; mp 140 °C (start of decomposition).

3-*C*-(*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-(diphenylphosphinoyloxy)propan-2-one (47). Chloromethyl ketone **45** (6.33 mg, 12.7 μmol) was dissolved in DMF (0.5 mL). Diphenylphosphinic acid (5.6 mg, 25 μmol), K_2CO_3 (3.5 mg, 25 μmol), and potassium iodide (cat.) were added after which the solution was stirred at 50 °C for 16 h. The volatiles were removed, and subsequently, the mixture was purified. Semipreparative RP-HPLC (linear gradient 11%→26% B in 14.00 min) afforded title compound **47** (16%, 1.394 mg, 2.05 μmol) as a white solid: ^{1}H NMR (400 MHz, D_2O) δ ppm 7.84 (tdd, J = 12.8, 8.4, 1.4, 1.4 Hz, 4H), 7.76−7.71 (m, 2H), 7.62 (dt, J = 7.6, 7.4, 3.9 Hz, 4H), 4.82 (d, J = 8.7 Hz, 2H), 4.56 (d, J = 8.4 Hz, 1H), 3.93 (dd, J = 12.4, 1.5 Hz, 1H), 3.81−3.70 (m, 3H), 3.68−3.62 (m, 2H), 3.62−3.54 (m, 3H), 3.54−3.45 (m, 3H), 3.33 (ddd, J = 9.6, 4.6, 2.1 Hz, 1H), 2.75 (dd, J = 16.2, 7.8 Hz, 1H), 2.70 (dd, J = 16.1, 3.9 Hz, 1H), 2.08 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ ppm 205.34, 205.28, 174.5, 133.8, 132.0, 131.6, 131.53, 131.47, 131.4, 129.2, 129.1, 129.0, 127.14, 127.05, 101.4, 79.3, 77.9, 75.9, 73.9, 73.4, 69.7, 69.1, 69.1, 60.5, 60.0, 55.6, 54.4, 41.0, 29.6, 22.1, 22.0; FT-IR υ_{max} (neat)/cm^{-1}3289.9, 1668.0, 1651.7, 1557.7, 1435.9, 1374.0, 1202.2, 1131.0, 1028.1; LC/MS t_R 4.050 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 681.3 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{31}H_{42}N_2O_{13}P$ 681.24190, found 681.24198; mp 84 °C (start of decomposition).

3-*C*-(*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranyl)-1-thiophenylpropan-2-one (48). Chloromethyl ketone **45** (31.5 mg, 66 μmol) was dissolved in MeOH (2 mL) before thiophenol (20 μL, 150 μmol) and K_2CO_3 (18 mg, 132 μmol) were added. After 16 h of stirring, the volatiles were removed; the reaction was diluted in H_2O/Et_2O, and the layers were separated. Concentration in vacuo followed by semipreparative RP-HPLC (linear gradient 17.5 → 26.5% B in 14.00 min) gave **48** (64%, 24.23 mg, 42.3 μmol) as a white solid: ^{1}H NMR (400 MHz, D_2O/MeOD) δ ppm 7.34 (d, J = 3.9 Hz, 4H), 7.32−7.24 (m, 1H), 4.53 (d, J = 8.4 Hz, 1H), 3.91 (s, 1H), 3.88 (dd, J = 12.4, 1.0 Hz, 1H), 3.80−3.37 (m, 12H), 3.34−3.23 (m, 1H), 2.86 (dd, J = 16.8, 8.8 Hz, 1H), 2.73 (dd, J = 16.8, 2.7 Hz, 1H), 2.03 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (101 MHz, D_2O/MeOD) δ ppm 207.9, 175.4, 175.3, 134.8, 130.6, 130.4, 128.3, 102.4, 80.4, 78.9, 77.0, 75.1, 74.6, 74.5, 70.8, 61.6, 61.0, 56.6, 55.4, 44.7, 23.10, 23.06; FT-IR υ_{max} (neat)/cm^{-1}3275.0, 1713.4, 1636.0, 1555.6, 1435.9, 1373.3, 1319.6, 1203.1, 1164.4, 1028.6; LC/MS t_R 4.52 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z = 573.00 (M + H)$^+$; HRMS (M + H$^+$) calcd for $C_{25}H_{36}N_2O_{11}S$ 573.21126, found 573.21112; mp 229 °C (start of decomposition).

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Supporting Information Available: General experimental procedures. Synthesis and characterization of compounds **5**, **7**, **26**, **39**, and **49**. Procedures for in vitro and cell culture inhibitory studies. ^{1}H NMR and ^{13}C NMR spectra of all new compounds. LC/MS spectra of **10**−**14**, **17**−**21**, **24**, **25**, **30**−**33**, **38**, **41**−**45**, **47**, and **48**. This material is available free of charge via the Internet at http://pubs.acs.org.

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