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. 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. 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. 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 histocompatibility complex class I (MHC-I) and are thus presented to the cell surface immune surveillance system. 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. 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.

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. Rather, the peptide-based...
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 guideline 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 acylating equivalents, (B) Michael acceptors such as vinyl sulfones, and (C) Michael acceptors such as vinyl sulfones and unsaturated esters. 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 Lindlar's catalyst, and subsequent condensation of the resulting amine with acids 5–9\(^11\) gave the fully protected inhibitors. Global deprotection was accomplished by either hydrogenation in the presence of Pd(OH)\(_2\) in trifluoroethanol (TFA)/MeOH (for 10, 12–14) or selective removal of the benzylidene using 10% TFA in CH\(_2\)Cl\(_2\) 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-acetylation of the aziridine.\(^23\) Since cysteolic 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\(_2\)Cl\(_2\), 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 thiourea 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 hydroxyacacetamide 21. The hydroxyl in 21 was mesylated under the agency of methane-sulfonic anhydride (MS\(_2\)O) and Et\(_3\)N 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\(_2\)Cl\(_2\) 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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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 K₂CO₃ 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 Ph₃SO/Tf₂O-mediated condensation of donor 27 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

28 giving disaccharide 29 in 97% yield. 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.

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. 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/H₂O/Et₃N 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.

The migration under alkaline conditions was proven by treating 34 with DBU in CH₂Cl₂. 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 hydroxymethyl 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 OsO₄, 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, giving key intermediate 41 in 50% yield over four steps. Mesylation of hydroxymethyl ketone 41 and in situ conversion to the corre-

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(25) Structure 27:


Chitobiose-Based Peptide N-Glycanase Inhibitor Library

Inhibition Studies. With the inhibitors in hand, we evaluated their biological activity as described. 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). 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 nucleophilic 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, IC₅₀ 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 IC₅₀ 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 IC₅₀ values allowed us to compare the different inhibitors (Table 1).

The IC₅₀ 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 diphenylphosphinomethyl 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 nucleophiles, 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 azidine (8.77 μM for 19 compared to >1000 μM for 13) is beneficial for the IC₅₀ 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. 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. 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 [³⁵S]-cysteine/methionine. The cells were lysed in the presence of detergent, and the HCs of US11 were incubated with a serial dilution of the inhibitors 1–11 and 19–49. After quantification of the intensities of the bond, the relative IC₅₀ 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 IC₅₀ values allowed us to compare the different inhibitors (Table 1).

(32) Structure/β-VAD-Fmk:

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 ZL3VS, 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 ZL3VS (lane 4). To test our compounds, US11 cells were preincubated with epoxides 2 and 49 in the presence of ZL3VS, followed by labeling of the proteins with [35S]-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 ZL3VS 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 ZL3VS 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 IC50 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 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.

Experimental Section

General Procedure for the Synthesis of Acetamide Inhibitors. Disacharride 4 was dissolved in DMF (10 mL/mmol). Lindlar’s catalyst was added, and the solution was stirred under H2 atmosphere for 3 h. The solution was purged with argon after which acid (3 equiv), 2-(6-chloro-1H-benzotriazol-1-yl)-1,1,3,3-tetramethylammonium 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 (CH2Cl2–MeOH/CHCl3) 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 H2 atmosphere until TLC revealed complete conversion to a lower 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 CH2Cl2 (2 mL) and cooled to 0 °C before trifluoroacetic acid (100 µL) and H2O (10 µL) were added. The mixture was stirred until TLC showed complete conversion to a lower running product. The reaction was quenched with NaHCO3 (satd aq) and diluted with CH2Cl2. The organic layer was washed with Na2SO4 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 H2 atmosphere until TLC showed complete conversion. Subsequently, the reaction was purged with argon, filtered, concentrated, and purified by reversed-phase HPLC.

N-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl fluorooacetamide (10). Disacharride 4 (76 mg, 94 µmol) was converted to the protected fluorooacetamide as described in the general procedure. Silica gel chromatography (CHCl3–4% MeOH/CHCl3) gave the protected fluorooacetamide: LC/MS rt 9.43 min; linear gradient 0–90% B in 13.5 min; ESI/MS m/z = 482.27 (M + H)+. Deprotection according to general procedure 1 followed by preparative RP-HPLC (linear gradient 0–50% B in 10 min) afforded title compound 10 (20% over three steps, 9.14 mg, 18.9 µmol): 1H NMR (600 MHz, D2O) δ ppm 5.12 (d, J = 9.6 Hz, 1H), 4.51 (d, J = 8.4 Hz, 1H), 4.21 (q, J = 7.2, 7.1, 1.1 Hz, 2H), 3.84 (d, J = 12.1 Hz, 1H), 3.81 (d, 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, 7.3 Hz, 2H). 13C NMR (150 MHz, D2O) δ ppm 174.2, 174.0, 170.7, 169.6, 135.0, 131.1, 130.0, 104.3, 104.1, 101.2; LC/MS rt 1.28 min; linear gradient 0–20% B in 13.5 min; ESI/MS m/z = 484.19371, found 484.19344; mp 203 °C (start of decompostion).

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2-Benzoyl-2,3,5-tri-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 mg, 42 mg) was deprotected as described in general procedure 2. Semipreparative RP-HPLC (linear gradient 20%–95% MeOH in 7 min) furnished title compound (19) (51%, 13.8 mg, 21 µmol); HRMS (400 MHz, CDCl3/MeOD) δ ppm 794.4 (d, J = 9.7 Hz, H2), 7.71 (t, J = 7.4 Hz, 1H), 7.57 (t, J = 7.7 Hz, 2H), 4.95 (d, J = 9.7 Hz, 1H), 4.58 (d, J = 12.2 Hz, 1H), 4.87 (1H), 4.15 (q, J = 7.1 Hz, 2H), 3.78 (d, J = 12.1 Hz, 1H), 3.78 (d, J = 2.3 Hz, 1H), 3.77–3.80 (m, 2H), 3.79 (t, J = 9.0 Hz, 1H), 3.65 (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 Hz, 3H); 13C NMR (400 MHz, CDCl3/MeOD) δ ppm 177.6, 174.7, 174.6, 167.8, 167.5, 134.3, 131.6, 129.2, 128.3, 103.1, 78.7, 75.6, 75.9, 73.5, 72.6, 69.7, 63.6, 60.6, 59.9, 55.6, 53.6, 42.3, 41.3, 22.2, 21.1, 13.1; FT-IR v_max (neat/cm–1) 3290.2, 1668.0, 1651.7, 1645.7, 1563.5, 1557.7, 1537.8, 1453.5, 1376.7, 1313.4, 1210.2, 1113.6, 1067.2, 1026.4, LCMS t_R 97.5 min; linear gradient 10–99% B in 13.5 min; ESI/MS m/z = 669.07 (M+H)+; HRMS (M+H)+; calcd for C29H41N4O14 669.26138, found 669.26145; mp 150°C (start of decomposition).

1-Benzyl(2S,3S)-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 mg, 42 mg) was deprotected as described in general procedure 2. Semipreparative RP-HPLC (linear gradient 20%–99.75% B in 12.5 min) furnished title compound (19) (51%, 13.8 mg, 21 µmol); HRMS (400 MHz, CDCl3/MeOD) δ ppm 794.4 (d, J = 9.7 Hz, H2), 7.71 (t, J = 7.4 Hz, 1H), 7.57 (t, J = 7.7 Hz, 2H), 4.95 (d, J = 9.7 Hz, 1H), 4.58 (d, J = 12.2 Hz, 1H), 4.87 (1H), 4.15 (q, J = 7.1 Hz, 2H), 3.78 (d, J = 12.1 Hz, 1H), 3.78 (d, J = 2.3 Hz, 1H), 3.77–3.80 (m, 2H), 3.79 (t, J = 9.0 Hz, 1H), 3.65 (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 Hz, 3H); 13C NMR (400 MHz, CDCl3/MeOD) δ ppm 177.6, 174.7, 174.6, 167.8, 167.5, 134.3, 131.6, 129.2, 128.3, 103.1, 78.7, 75.6, 75.9, 73.5, 72.6, 69.7, 63.6, 60.6, 59.9, 55.6, 53.6, 42.3, 41.3, 22.2, 21.1, 13.1; FT-IR v_max (neat/cm–1) 3290.2, 1668.0, 1651.7, 1645.7, 1563.5, 1557.7, 1537.8, 1453.5, 1376.7, 1313.4, 1210.2, 1113.6, 1067.2, 1026.4, LCMS t_R 97.5 min; linear gradient 10–99% B in 13.5 min; ESI/MS m/z = 669.07 (M+H)+; HRMS (M+H)+; calcd for C29H41N4O14 669.26138, found 669.26145; mp 150°C (start of decomposition).

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5.49 (d, J = 9.2 Hz, 1H), 4.59 (d, J = 12.1 Hz, 1H), 4.33 (d, J = 10.4 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, 3H), 3.47 (d, J = 11.4, 3.0 Hz, 1H), 3.33–3.28 (m, 1H), 2.18–2.06 (m, 2H), 2.03 (s, 3H), 1.96 (s, 3H), 1.84 (s, 3H); 13C NMR (100 MHz, CDCl3) δ ppm 170.8, 169.4, 168.0, 167.7, 137.8, 138.5, 143.4, 133.7, 133.6, 133.4, 131.5, 131.4, 128.7, 128.2, 127.8, 127.4, 127.3, 126.9, 125.3, 123.5, 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, 53.3, 36.9; FT-IR νmax (neat/cm⁻¹) 2949.5, 1747.8, 1713.8, 1469.3, 1455.3, 1385.6, 1222.8, 1081.2, 1036.1; HRMS (M + Na⁺) calcd for C38H38N4O15Na 593.30134, found 593.30177.

3-C(0-β-2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1-4-4-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)propane (30). After coevaporation with toluene, discaridade (29, 3.35 g, 3.6 mmol) was dissolved in n-BuOH/ ethylene diamine (50 mL, 9/1 v/v), 4 Å MS were added, and the solution was subsequently stirred overnight at 90 °C. The solution was concentrated, and the crude mixture was redissolved in pyridine (50 mL), and cooled to 0 °C before Ac₂O (30 mL) was added. The reaction mixture was stirred overnight, after which TLC analysis showed complete conversion of the starting material. Concentration in vacuo, followed by CH₂Cl₂, extraction with NaHCO₃, and concentration gave the crude title compound 30. Purification by silica gel chromatography (CH₂Cl₂/MeOH) furnished 30 (75%, 2.048 g, 2.71 mmol) as an off-white solid: 1H NMR (400 MHz, DMSO-D₆) 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 (d, 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 (d, J = 14.2, 7.6 Hz, 1H), 2.04 (d, J = 14.4, 6.9 Hz, 1H), 1.94 (s, 3H), 1.92 (s, 3H), 1.86 (s, 3H), 1.77 (s, 6H); 13C NMR (100 MHz, DMSO-D₆) 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 νmax (neat/cm⁻¹) 3297.9, 2940.5, 1740.4, 1652.0, 1557.8, 1538.9, 1545.7, 1537.3, 1273.7, 1118.2, 1045.5; LC/MS 9.76 min; linear gradient 10–90% B in 13.5 min; ESI/MS m/z 574.20651, found 574.20644; mp 230 °C (start of decomposition).
(q, methanol/H2O (1/1 v/v, 4 mL). Et3N (1 mL) was added, and the reaction mixture was concentrated in vacuo, redissolved in CH2Cl2, extracted with 1 M HCl and NaHCO3 (saturated aq), dried (Na2SO4), and concentrated under reduced pressure. Silica gel column chromatography (CHCl3/MeOH = 99:1) afforded 31 (quant., 131 mg, 0.2 mmol) as a white solid: 1H NMR (400 MHz, CDCl3/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); 13C NMR (100 MHz, CDCl3/MeOD) δ ppm 172.7, 172.4, 172.0, 171.68, 171.65, 171.4, 170.6, 134.3, 113.5, 72.4, 71.7, 78.2, 77.3, 77.1, 75.8, 73.1, 62.1, 63.6, 62.5, 55.5, 55.45, 54.0, 36.6, 22.9, 22.8, 21.1, 21.0, 20.84, 20.77, 20.7; FT-IR νmax (neat/cm–1) 3293.9, 1739.7, 1699.9, 1660.9, 1652.0, 1538.1, 1435.8, 1373.5, 1232.9, 1139.6, 1042.6; LC/MS tR 5.58 min; linear gradient 0–90% B in 13.5 min; ESI/MS m/z = 659.0 (M + H+); HRMS (M + H+) calcd for C29H43N2O15 659.2682, found 659.2687; 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 CH2Cl2 (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 (CHCl3/MeOH = 5:1), furnishing title compound 32 as off-white solid (quant. 146 mg, 0.2 mmol): 1H NMR (400 MHz, CDCl3) δ ppm 6.92 (d, 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 Hz, 1H), 3.74–3.64 (m, 2H), 3.57–3.52 (m, 1H), 3.36 (dt, J = 12.2, 9.9 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); 13C NMR (100 MHz, CDCl3) δ ppm 171.3, 171.1, 169.7, 170.6, 170.5, 170.4, 169.4, 166.3, 144.2, 123.3, 101.1, 101.0, 77.7, 76.5, 74.3, 72.4, 71.7, 68.3, 62.6, 61.9, 60.2, 54.9, 53.6, 34.4, 23.1, 11.7, 40.6; FT-IR νmax (neat/cm–1) 1738.6, 1656.7, 1651.8, 1582.7, 1435.7, 1373.1, 1221.9, 1197.3, 1035.2, 979.7, 905.9; LC/MS tR 5.43 min; linear gradient 0–90% B in 13.5 min; ESI/MS m/z = 731.5 (M + H+); HRMS (M + H+) calcd for C32H43N2O17 731.2867, found 731.2871; 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, 0.11 mmol) was dissolved in methanol/H2O (1/1 v/v, 4 mL). EtN (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: 1H NMR (500 MHz, D2O) 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, 4H), 3.53–3.43 (m, 3H), 2.56 (d, J = 15.6, 6.3, 5.1 Hz, 1H), 2.48 (td, J = 14.5, 6.6, 6.6 Hz, 1H), 2.07 (s, J = 3.8 Hz), 1.98 (s, 3H); 13C NMR (125 MHz, D2O) δ ppm 174.6, 174.3, 169.2, 146.1, 142.2, 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 νmax (neat/cm–1) 3268.9, 1627.8, 1562.6, 1557.7, 1436.0, 1372.7, 1304.3, 1199.8, 1079.8, 1044.4; LC/MS tR 4.583 min; linear gradient 0–20% B in 13.5 min; ESI/MS m/z = 507.0 (M + H+); HRMS (M + H+) calcd for C24H28N2O12 507.21845, found 507.21818; mp 150 °C (start of decomposition).
residual traces of n-BuOH/ethylendiamine. The resulting amine was dissolved in MeOH (50 mL) and cooled to 0 °C before addition of $\text{Ac}_2\text{O}$ (5 mL) and Et$_3$N (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-bromosuccinimide (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$_3$Cl$_2$–6% MeOH/CHCl$_3$) afforded hydroxymethyl ketone 41 (50% over four steps, 1.35 g, 1.6 mmol): $\text{H}$ 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), 4.46 (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, $J = 10.7$ Hz, 1H), 4.21 (2H), 4.15 (s, $J = 10.8$ Hz, 1H), 3.94 (t, $J = 8.1$, 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 (dd, $J = 9.3$, 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); $\text{C}$ 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, 125.4, 124.6, 128.4, 128.2, 128.0, 127.7, 127.6, 126.2, 111.4, 108.4, 81.5, 78.9, 78.1, 76.0, 75.0, 74.3, 74.1, 73.9, 69.1, 68.7, 68.3, 65.9, 55.9, 53.9, 49.9, 21.3, 22.8; FT-IR $\nu_{\text{max}}$ (neat/cm$^{-1}$) 1327.82, 2870.1, 1717.7, 1658.1, 1651.8, 1557.7, 1538.5, 1497.4, 1455.5, 1373.1, 1321.7, 1210.2, 1173.8. 1070.2, 1027.9, 1011.9; LC/MS $m/z$ 896.8; linear gradient 19–90% B in 13.5 min; ESI/MS $m/z$ = 839.60 (M + H$^+$); HRMS (M + H$^+$) calc. for C$_{27}$H$_{42}$N$_5$O$_3$, 839.37495, found 839.37581; mp 159 °C (start of decomposition).

3-C-(O-3-Benzyloxy-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-(4)-4,3,6-di-0-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)propane (40). Imitade 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$_2$Cl$_2$ (30 mL). Activated molecular sieves 4 Å were added, after which the solution was cooled to −30 °C. TMSOTf (73 µL) was added was followed by stirring until TLC showed complete conversion. The reaction was quenched with Et$_3$N, diluted with EtOAc, extracted with NaHCO$_3$ (satd aq) and brine, dried (Na$_2$SO$_4$), 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: $\text{H}$ 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 (tt, $J = 17.0, 10.0, 6.9, 6.9$ 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, $J = 9.1$, 1H), 3.56 (t, $J = 10.2$, 10.2 Hz, 1H), 3.49 (d, $J = 10.9$, 1H), 3.46–3.38 (m, 3H), 3.34 (dd, $J = 11.1$, 3.0 Hz, 1H), 3.28 (dd, $J = 9.3$, 2.7 Hz, 1H), 2.12–2.05 (m, 2H); $\text{C}$ NMR (100 MHz, CDCl$_3$) δ ppm 167.8, 167.6, 138.3, 138.1, 137.6, 137.1, 133.8, 133.8, 133.6, 133.56, 133.0, 131.3, 128.8–125.8, 123.1, 123.0, 116.8, 109.0, 99.7, 82.9, 78.2, 77.5, 76.2, 74.2, 74.1, 74.1, 73.8, 72.4, 68.4, 67.9, 65.5, 56.3, 55.5, 36.6; FT-IR $\nu_{\text{max}}$ (neat/cm$^{-1}$) 3030.4, 2854.8, 1775.9, 1710.9, 1701.6, 1696.6, 1543.1, 1383.6, 1310.6, 1167.1, 1075.6; HRMS (M + Na$^+$) calc. for C$_{26}$H$_{42}$O$_7$Na$_2$ 1005.36590, found 1005.35776.

3-C-(O-2-Acetamido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-1-(4)-4,3,6-di-0-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)hydroxypropan-2-one (41). Disaccharide 30 (3.33 g, 3.38 mmol) was dissolved in THF/H$_2$O (30 mL, 6/1 v/v). K$_2$CO$_3$ (25 mg, 0.68 mmol) and morpholine-N-oxide (0.99 g, 8.46 mmol) were added. After being stirred overnight, the mixture was quenched with 1 M HCl, extracted with EtOAc, washed with Na$_2$SO$_4$ (aq), NaHCO$_3$ (satd aq), and brine, dried (Na$_2$SO$_4$), 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...
reaction was diluted with CHCl₃, washed with 10% (aq) citric acid, dried (Na₂SO₄), and concentrated. Silica column chromatography (CH₂Cl₂ → 4% MeOH/CH₂Cl₂) afforded fully protected chloromethyl ketone 43 (59%, 111 mg, 129 μmol) as a white solid: ¹H NMR (400 MHz, CDCl₃/MeOD) δ ppm 7.50−7.27 (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); ¹³C NMR (100 MHz, CDCl₃/MeOD) δ ppm 200.4, 171.5, 171.4, 138.5, 137.9, 137.5, 136.8, 128.6, 128.1, 128.74, 127.6, 127.6, 127.4, 127.3, 127.0, 125.6, 100.8, 100.1, 81.7, 81.0, 78.3, 77.6, 75.5, 74.6, 73.6, 73.0, 68.1, 68.0, 65.3, 55.4, 53.4, 48.9, 42.5, 22.3, 22.0, FT-IR νmax (neat/cm⁻¹) 3287.5, 2865.9, 1727.6, 1644.5, 1557.7, 1454.9, 1368.3, 1319.8, 1175.0, 1071.4; LC/MS tR 9.85 min; gradient linear 10→90% B in 13.5 min; ESI/MS m/z 587.08 (M + H⁺); HRMS (M + H⁺) calcld for C₄₇H₅₄ClN₂O₁₁ 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-dimethylenzoxloyloxy)propan-2-one (44). Reductive removal of the protective groups of disaccharide 42 (70 mg, 72 μmol) was performed as described in general procedure 7. Semiempirical 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: ¹H NMR (400 MHz, D₂O) δ ppm 7.38 (t, J = 7.6, 7.6 Hz, 1H), 7.22 (d, J = 7.6 Hz, 2H), 5.23 (s, 2H), 4.78 (d, J = 8.4 Hz, 1H), 4.04 (d, J = 12.2 Hz, 1H), 3.99 (dd, J = 17.5, 8.6 Hz), 3.93−3.89 (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); ¹³C NMR (100 MHz, D₂O) δ ppm 204.9, 175.2, 175.1, 171.4, 136.7, 133.7, 131.5, 129.1, 102.8, 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, 23.3, 20.3; FT-IR νmax (neat/cm⁻¹) 3271.8, 2706.1, 2368.4, 1657.8, 1562.9, 1557.7, 1432.9, 1373.9, 1269.0, 1203.0, 1165.7, 1075.7, 1049.0; LC/MS tR 8.46 min; linear gradient 0→50% B in 13.5 min; ESI/MS m/z 613.07 (M + H⁺); HRMS (M + H⁺) calcld for C₃₃H₃₃ClN₁₄O₁₄, 613.26302, 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→2-thiophenylpropan-2-one (48). Chloromethyl ketone 45 (31.5 mg, 66 μmol) was dissolved in MeOH (2 mL) before thionophenol (20 mL, 150 μmol) and K₂CO₃ (18 mg, 132 μmol) were added. After 16 h of stirring, the volatiles were removed; the reaction was diluted in H₂O/Et₂O, and the layers were separated. Concentration in vacuo followed by semiempirical RP-HPLC (linear gradient 17.5→26.5% B in 14.0 min) gave 48 (64%, 24.23 mg, 42.3 μmol) as a white solid: ¹H NMR (400 MHz, D₂O/MeOD) δ ppm 7.54 (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 (d, J = 16.8 Hz, 8.8 Hz, 1H), 2.73 (dd, J = 16.8, 2.7 Hz, 1H), 2.03 (s, 3H), 1.94 (s, 3H); ¹³C NMR (101 MHz, D₂O/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 νmax (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 tR 4.52 min; linear gradient 10→90% B in 13.5 min; ESI/MS m/z 573.00 (M + H⁺); HRMS (M + H⁺) calcld for C₅₁H₄₃ClN₁₂O₇, 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. ¹H NMR and ¹³C NMR spectra of all new compounds. LC/MS spectra of 10−14, 17−21, 24, 25, 30−33, 38, 41−45, and 48. This material is available free of charge via the Internet at http://pubs.acs.org.