# Synthesis and Evaluation of Potential Inhibitors of Chondroitin AC Lyase from *Flavobacterium heparinum*

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Chondroitin AC lyase from *Flavobacterium heparinum* degrades chondroitin sulfate glycosaminoglycans via an elimination mechanism, resulting in disaccharides or oligosaccharides with Δ4,5-unsaturated uronic acid residues at their nonreducing end. The syntheses and testing of two potential inhibitors of this lyase are described. Methyl *O*-(2-acetamido-2-deoxy-*β*-D-galactopyranosyl)-(1→4)-α-L-*threo*-hex-4-enopyranoside, **1**, has the trigonal geometry at C5 of the uronic acid moiety expected at the transition state, yet retains the "leaving group" sugar moiety. Surprisingly, compound **1** showed no inhibition of the enzyme. The novel 5-nitro sugar, phenyl (5*S*)-5-nitro-*β*-D-xylopyranoside, **2**, is a monosaccharide nitro analogue of the natural substrate, with C5 being a carbon acid of p$K_a$ 8.8. The rate of reprotonation of the anion generated at this center is sufficiently low that the anion of **2** can be used directly in initial steady-state velocity measurements without significant interference from the conjugate carbon acid. The anion of compound **2** was found to be a competitive inhibitor with a $K_i$ value of 5 mM, whereas the conjugate acid had a $K_i$ value of 35 mM.

## Introduction

Glycosaminoglycans (GAGs) are highly sulfated, unbranched oligosaccharide chains built of a sequence of repeating disaccharide units consisting of hexosamine and uronic acid residues. These important constituents of the extracellular matrix serve four main roles in the body: lubrication and cushioning of joints, as barriers to diffusion across basement membranes, as anticoagulant coatings of blood vessels, and as reservoirs for specific binding of proteins in order to regulate or stabilize their activity.[1] GAGs may be divided up into four main classes: chondroitin sulfate and dermatan sulfate, hyaluronic acid, heparin and heparan sulfate, and keratan sulfate. With the exception of hyaluronic acid, all GAGs are linked covalently to a protein core, forming a proteoglycan. Of these proteoglycans, chondroitin sulfates are the most common type of GAG chain, consisting of three major classes: chondroitin sulfate A (chondroitin 4-sulfate), chondroitin sulfate B (dermatan sulfate), and chondroitin sulfate C (chondroitin 6-sulfate). The chondroitin sulfates consist of an *N*-acetyl-D-galactosamine residue (usually *O*-sulfated at C4 or C6) attached through a *β*-(1,4) linkage to D-glucuronic acid or L-iduronic acid (dermatan sulfate) that is in turn attached via a *β*-(1,3) linkage (α-(1,3) for dermatan sulfate) to the next hexosamine residue.

Two classes of enzymes that act on GAGs are polysaccharide hydrolases (eukaryotic) and lyases (prokaryotic). The hydrolases cleave the anomeric carbon−oxygen bond with either retention or inversion of configuraion. The lyases act via an elimination mechanism, resulting in disaccharides or oligosaccharides with Δ4,5-unsaturated uronic acid residues at their nonreducing end (Figure 1). Although the substrate specificity and product compositions have been characterized,[1−5] little is known about the lyase mechanism at the molecular level. Inhibitors of these enzymes have potential as antibiotics, since these enzymes are only known to be present in bacterial sources, not in mammalian cells.

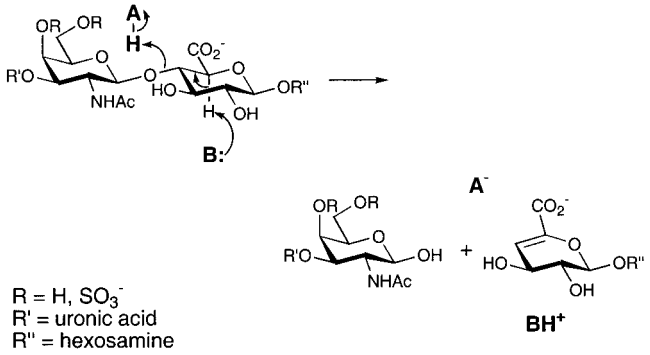


**Figure 1.** Proposed mechanism of polysaccharide lyases.

Chondroitin AC lyase from the Gram-negative soil bacterium *Flavobacterium heparinum* (EC 4.2.2.5) cleaves GAG chains in a random endolytic fashion. It cleaves a variety of GAGs, including chondroitin sulfates A (chondroitin 4-sulfate) and C (chondroitin 6-sulfate), as well as the unsulfated chondroitin and hyaluronic acid. The proposed mechanism involves acid/base catalysis (Figure

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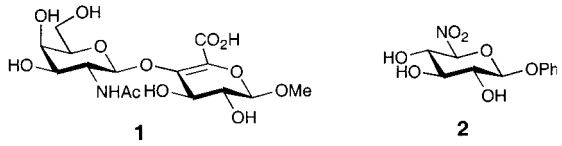
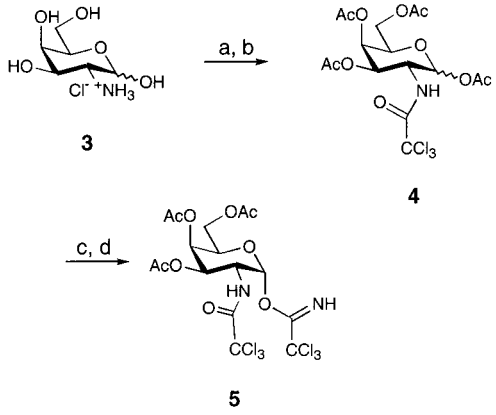


**Figure 2.** Potential inhibitors **1** and **2**.

1), in which the developing anion at C5 is resonance stabilized into the carboxylate, which itself interacts with a cationic center on the protein. The identity of the catalytic residues remains elusive, even though the high-resolution X-ray crystal structure of the free enzyme has been solved[6] as well as structures of various enzyme−oligosaccharide complexes.[7] The development of tight binding inhibitors would be extremely helpful to these structural studies, facilitating the identification of key residues involved in binding and catalysis. At the present time there are no known specific inhibitors of polysaccharide lyase enzymes.

Two separate concepts for inhibitor design were explored on the basis of mimics of reaction intermediates or transition states. The formation of the proposed *aci*-carboxylate intermediate yields $sp^2$ hybridization at C5 of the uronic acid moiety. The potential inhibitor **1** (Figure 2) attempts to take advantage of this tetrahedral $sp^3$ to planar $sp^2$ hybridization transformation that takes place between substrate and intermediate and/or product. Compound **1** cannot be cleaved by the enzyme due to the lack of a proton at C5, satisfies the trigonal geometric requirements of the carbanion intermediate and/or transition state, and contains the hexosamine "leaving group" sugar moiety from which it can derive binding interactions. The second approach to an inhibitor was the synthesis of the novel 5-nitro sugar **2** (Figure 2). This design was based on the remarkable stability of the anion formed by the loss of a proton from the carbon acid (C5) adjacent to a nitro group. It was hoped that the anion of **2** would mimic the anionic transition state and thus bind tightly to the enzyme. The nitronate anion thus formed is a mimic of the *aci*-carboxylate anion, due to the fact that $sp^2$ hybridization of C5 has already occurred as a result of the delocalization of electrons from the carbanion to the oxygens of the nitro group (Figure 3). Nitro analogues of the substrates of several non-carbohydrate lyases[8−11] have been shown to bind more tightly to these enzymes than do the corresponding substrates, acting as potent inhibitors. Invariably, it has been found that the inhibitory species was the ionized form of these nitroalkanes ($pK_a$ values from 9 to 11). Proton transfers to and from carbon acids are usually much slower than those to and from oxygen, nitrogen, and sulfur.[12] This results from the need for structural reorganization accompanying the delocalization of the negative charge, solvent reorganization, and from the poor hydrogen-bonding capability of carbon acids and of the carbanionic carbon.[13] This interesting phenomenon allows the use of a preformed carbanion in initial steady-state velocity measurements without significant interference from the conjugate carbon acid.

**Scheme 1**



(a) $CCl_3COCl$, $NaHCO_3$(aq); (b) $Ac_2O$, pyridine; (c) hydrazine acetate, DMF; (d) $CCl_3CN$, DBU, $CH_2Cl_2$.

## Results and Discussion

**I. Synthesis of Methyl *O*-(2-Acetamido-2-deoxy-*β*-D-galactopyranosyl)-(1→4)-α-L-*threo*-hex-4-enopyranoside, 1.** Commercially available D-galactosamine hydrochloride, **3**, was converted first to **4**[14] (32%), following the literature procedure for the glucopyranoside analogue,[15] and then to the donor **5** (57%) according to published procedures[14] (Scheme 1).

The acceptor **9** was prepared in five steps as follows (Scheme 2). Commercially available methyl *β*-D-glucopyranoside was reacted with *p*-anisaldehyde dimethyl acetal and catalytic *p*-toluenesulfonic acid in DMF at 50 °C under aspirator pressure,[16] and the resulting crude material was treated with pyridine and acetic anhydride to give **6** as a white crystalline solid (80%). The benzylidene protecting group was removed using a 1% (w/v) solution of iodine in methanol at reflux[17] to afford **7**[18] (87%). Selective oxidation of the primary hydroxyl group was accomplished using 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and NaOCl under phase-transfer conditions with tetrabutylammonium bromide (TBAB) in $NaHCO_3$(aq) and EtOAc.[19] Formation of the methyl ester using acidic ion-exchange resin in methanol yielded the acceptor **9** (25% from **7**).

Glycosylation of **9** with **5** was accomplished[15] using a catalytic amount of trimethylsilyl triflate (TMSOTf) in 1,2-dichloroethane at 0 °C, affording the disaccharide **10**

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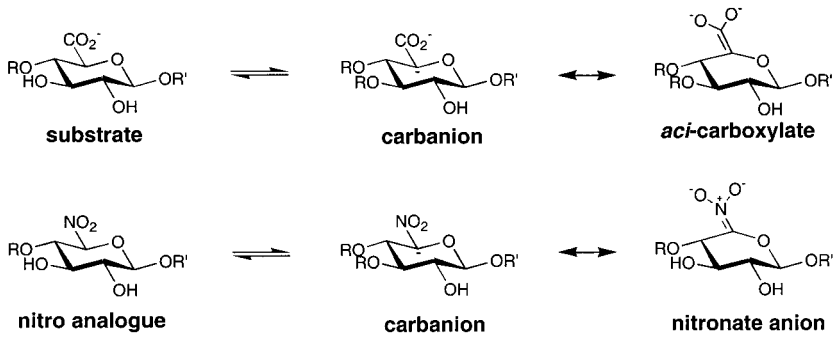
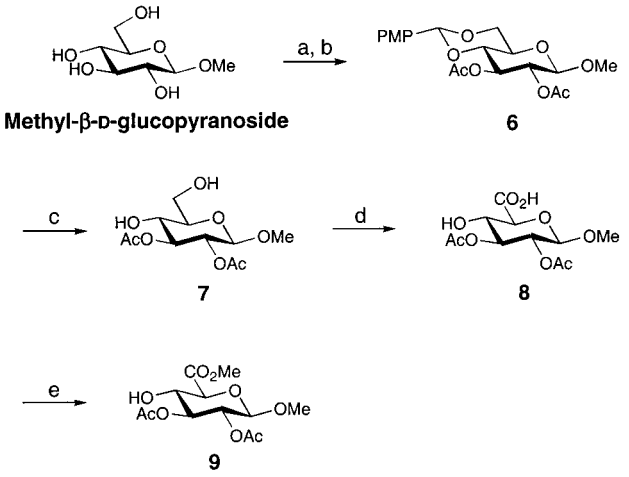



**Figure 3.** Illustration of how a nitro group mimics the normal carboxylate of the substrate.

**Scheme 2**




(a) *p*-anisaldehyde dimethyl acetal, *p*-TsOH, DMF, 50 °C; (b) $Ac_2O$, pyridine; (c) $I_2$, MeOH, reflux; (d) TEMPO, NaOCl, NaBr, TBAB, $NaHCO_3$(aq), EtOAc, 0 °C; (e) $H^+$ resin, MeOH.

as a white foam (78%) (Scheme 3). The $^1$H NMR spectrum of **10** showed a doublet with a coupling constant of 8.4 Hz for the anomeric proton (H1′), confirming that the newly formed linkage was of the $\beta$-configuration. Conversion of the *N*-trichloroacetate to an *N*-acetate was effected by treating **10** with tributyltin hydride (TBTH) and AIBN in refluxing benzene[15] to give **11** as a white crystalline solid (78%). Since the next key step involved a radical bromination, which is not compatible with an acetamide, it was necessary to introduce a second acetyl group onto the nitrogen. This second acetate group was easily introduced onto the nitrogen center by treating **11** with isopropenyl acetate and catalytic *p*-toluenesulfonic acid[20] at 65 °C to afford the *N*,*N*-diacetate compound **12** (98%). Directed by the adjacent carbonyl functionality, bromination[21] at C5 of the glucopyranosiduronate moiety using NBS and light in refluxing $CCl_4$ proceeded smoothly to give **13** as a colorless foam (64%). The alkene functionality was then introduced by the elimination of HBr with DBU in DMF[22] to afford **14** as a white foam (75%). Global deprotection of **14** using NaOH in 5:1 MeOH:$H_2O$[23] afforded the desired methyl *O*-(2-acetamido-2-deoxy-$\beta$-D-galactopyranosyl)-(1→4)-α-L-*threo*-hex-4-enopyranoside **1** (48%). Purification of **1** proved to be somewhat problematic; successive separations by silica gel and size exclusion chromatography were necessary to obtain a pure product.

**II. Synthesis of Phenyl (5*S*)-5-Nitro-$\beta$-D-xylopyranoside, 2.** Phenyl 2,3,4-tri-*O*-acetyl-$\beta$-D-xylopyranoside, **16**,[24] was prepared from 1,2,3,4-tetra-*O*-acetyl-$\beta$-D-xylopyranose, **15**, (Scheme 4) by first making the α-bromide with HBr/AcOH, followed by glycosylation using phase-transfer catalysis with phenol and tetrabutylammonium sulfate (TBAHS) in a vigorously stirred mixture of 1 M NaOH and $CH_2Cl_2$ (42%). Bromination[25] at C5 using NBS and light in refluxing $CCl_4$ gave **17** as a pale yellow solid (25%). A coupling constant of 4.0 Hz was measured for the H4−H5 protons, indicating that the bromine was in the axial orientation. Extended reaction times not only resulted in bromination of one or more of the acetate groups but also gave a *p*-bromophenyl derivative that was extremely difficult to separate from the desired compound. Displacement of the bromide with sodium azide in DMF[25] gave the product of inverted stereochemistry, **18**, as a crystalline solid (45%). The $^1$H NMR spectrum of **18** showed the expected upfield shift of H5 relative to that in **17**, and the $^3J_{5,4}$ coupling constant was measured to be 7.6 Hz, as compared to 4.0 Hz for **17**, indicating the equatorial stereochemistry of the C5-azido group. The transformation of the azide into the nitro functionality was accomplished via the reduction of the azide with hydrogen over Adam's catalyst ($PtO_2$) in ethanol/EtOAc, followed by the immediate oxidation with freshly prepared dimethyldioxirane (DMDO)[26] in acetone to afford the crystalline 5-nitro compound **19** (42%). The oxidation with DMDO was performed without purification of the intermediate amine to avoid epimerization and hydrolysis of this newly formed glycosylamine. From the standard 1D $^1$H NMR data, the stereochemistry of the nitro group at C5 and assignment of each signal was not obvious. Selective NOE and COSY NMR experiments ($CDCl_3$) allowed the assignment and indicated that the sugar ring was not in the normal $^4C_1$ chair conformation. The X-ray crystal structure of **19** was obtained (crystallized from ethanol), confirming the structure of the sugar and revealing a twist boat conformation ($^4S_0$), with C1, C2, C3, and C5 defining a plane (Figure 4). The coupling constants in the $^1$H NMR spectrum correlated nicely with the X-ray structure, indicating that the conformation in solution was nearly the same as that in the crystal.

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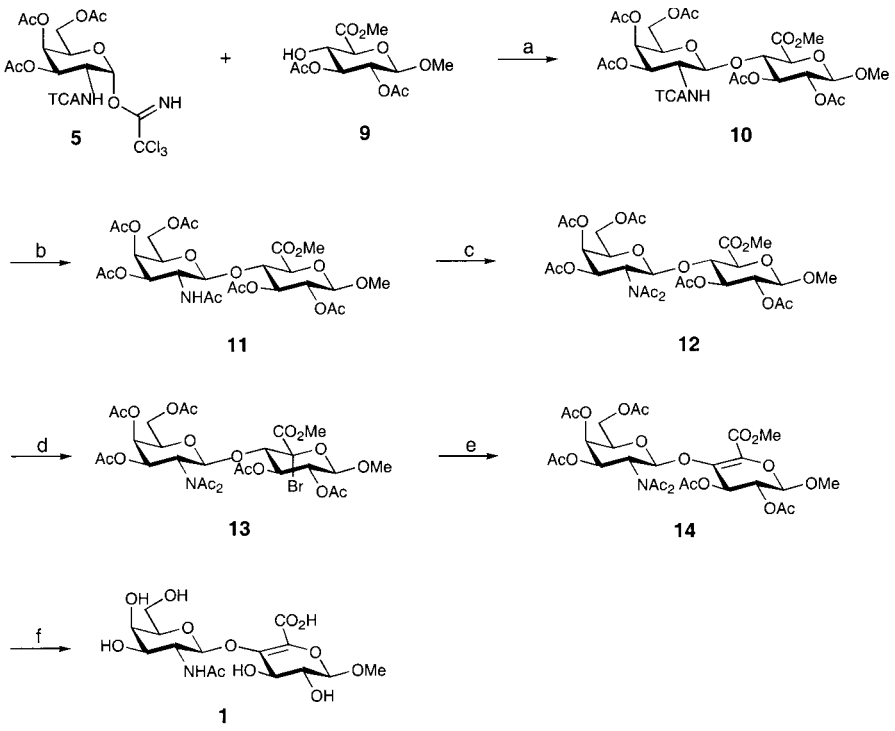
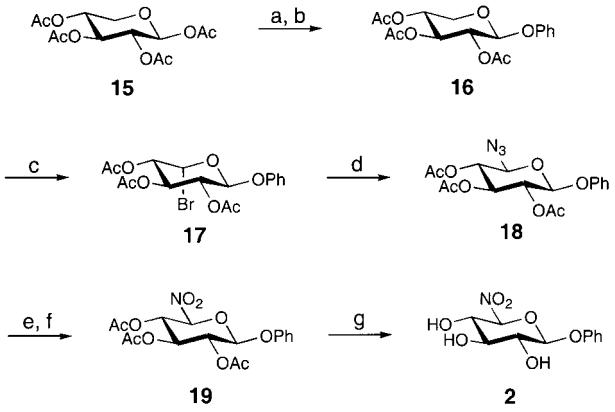
**Scheme 3**



(a) TMSOTf, 1,2-dichloroethane, 0 °C; (b) TBTH, AIBN, benzene, reflux; (c) *p*-TsOH, isopropenyl acetate, 65 °C; (d) NBS, $CCl_4$, light, reflux; (e) DBU, DMF; (f) NaOH, MeOH/$H_2O$.

**Scheme 4**



(a) HBr/AcOH; (b) PhOH, TBAHS, 1 M NaOH, $CH_2Cl_2$; (c) NBS, $CCl_4$, light, reflux; (d) $NaN_3$, DMF; (e) $PtO_2$, $H_2$, EtOAc/EtOH; (f) DMDO, acetone; (g) AcCl, MeOH.

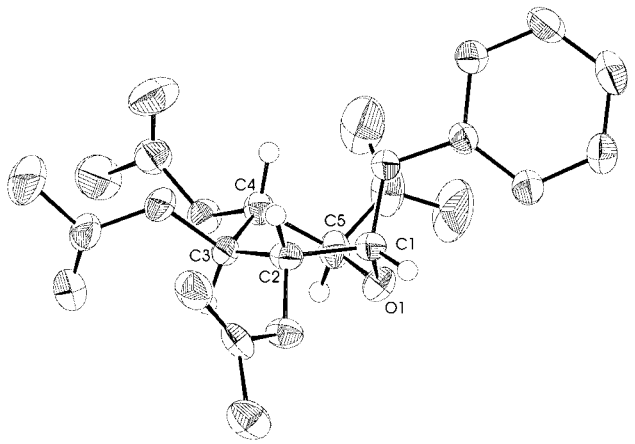



**Figure 4.** X-ray crystal structure of **19** showing the $^4S_0$ conformation.

Deprotection of the acetates with HCl/MeOH provided the desired phenyl (5*S*)-5-nitro-$\beta$-D-xylopyranoside**, 2**, as a white solid (78%). Selective NOE and COSY NMR experiments (methanol) facilitated the assignment of the $^1$H spectrum and showed that the conformation of **2** was a normal $^4C_1$ chair.

**III. Inhibition of Chondroitin AC Lyase by 1 and 2.** Transition state theory[27] predicts that an enzyme should bind to the transition state several orders of magnitude more strongly than to the ground state via protein−substrate interactions that are optimized only at the transition state. The short-lived nature of the transition state with partially formed and/or broken bonds presents an insurmountable barrier to the synthetic organic chemist who cannot recreate perfectly these nonequilibrium bond lengths with stable compounds. Thus, transition state analogue designs, intended as enzyme inhibitors, take advantage of the differences in electronic and geometric characteristics of the ground and transition states in order to capture a fraction of the immense binding energy for the transition state species.

Despite possessing the leaving group hexosamine sugar and having $sp^2$ hybridization at C5 of the uronic acid moiety, no inhibition was observed with **1** at concentrations up to 25 mM. The lack of anionic character in this compound may provide a rationale for the surprising lack of inhibition observed. Alternatively, the preformed C4−C5 alkene structure may be too product-like to be tightly bound by the enzyme, or perhaps a $\beta$-1,3 linked hexosamine residue is required at the reducing end of the chain to afford proper recognition and binding. This latter point is emphasized by the fact that chondroitinases do not

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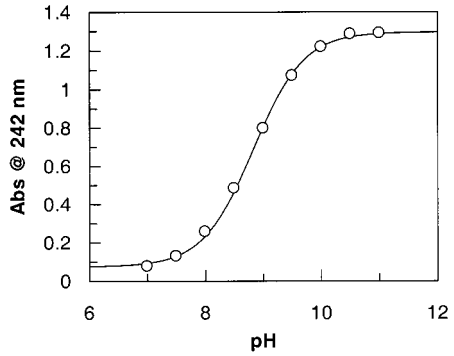


**Figure 5.** Spectrophotometric titration of **2** measuring the p$K_a$ of the carbon acid (H5) to be 8.8. For each point, an aliquot of a solution of **2** was equilibrated at the desired pH (buffered) at 30 °C for 75 min. Corrections were made for buffer effects caused by the different buffers used at each pH.

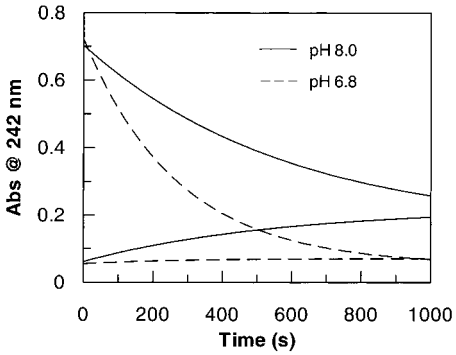


**Figure 6.** Rates of protonation (descending curves) of the preformed carbanion of **2** and deprotonation (ascending curves) of **2** at pH 8.0 and 6.8. The reactions were carried out at 30 °C in 50 mM sodium phosphate (pH 6.8) or Tris (pH 8.0) buffers and 100 mM NaCl.

cleave heparin or heparan sulfate, which are entirely 1,4-linked, suggesting that the 1,3-linkage may be a strong determinant for enzymatic activity.[1] However, synthetic monosaccharide substrates have been developed that are turned over by this enzyme;[28] thus, some inhibition might have been expected.

The novel 5-nitro sugar **2** was synthesized with the hope that the carbon acid would have a p$K_a$ sufficiently low, and the anion be sufficiently stable, to be used in inhibition studies. As a first step it was necessary to measure the p$K_a$ of this molecule. Fortunately, this is easily achieved, since the formation of the anion of the carbon acid **2** can be monitored by the absorbance of this species at 242 nm. In this way, the p$K_a$ of the carbon acid **2** (H5) was spectrophotometrically measured to be 8.8 (Figure 5), which is well within the expected range. A second consideration in using **2** as an inhibitor is the kinetic stability of the anion. Rates of deprotonation and reprotonation were determined simply by monitoring changes in absorbance at 242 nm after altering the solution pH. Figure 6 shows such data for the deprotonation of **2** and the protonation of its conjugate base at pH 6.8 and 8.0 in 50 mM buffer and 100 mM NaCl at 30 °C. At pH 6.8, $k_{prot} = 3.7 \times 10^{-3}$ s$^{-1}$ ($t_{1/2} = 4$ min) and at pH 8.0, $k_{prot} = 1.7 \times 10^{-3}$ s$^{-1}$ ($t_{1/2} = 15$ min). The deprotonation and reprotonation rates were found to be dependent on the concentration of the buffer. A change

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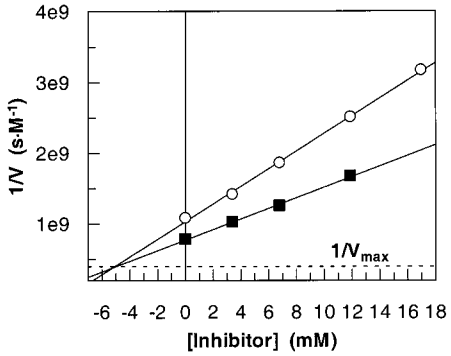


**Figure 7.** Dixon plot showing inhibition by the anion of compound **2**. Kinetics were carried out in 200 mM Tris buffer and 100 mM NaCl, pH 8.0, 30 °C, using a fluorescent substrate[28] at concentrations of 0.75$K_m$ and 1.5$K_m$.

to 200 mM buffer and 100 mM NaCl at pH 8 reduced the half-life of the anion to 7 min. These kinetic results show that the anion of **2** is sufficiently stable at pH 8.0 to be used directly as a preformed carbanion in initial steady-state velocity measurements (performed within a few minutes) without significant interference from its conjugate carbon acid.

Inhibition studies showed the anion of **2** to be a competitive inhibitor with a $K_i$ of 5 mM at pH 8, as shown by the Dixon plot in Figure 7. The conjugate acid, **2**, was also found to inhibit the enzyme (at pH 6.8) but with a $K_i$ of approximately 35 mM. Disappointingly, the inhibition shown by the anion of **2** is not representative of a transition state analogue, which would be expected to bind much more tightly to the enzyme. Possibly this is a consequence of the inhibitor not occupying other binding sites on the enzyme. Inhibition might therefore be increased dramatically by combining the inhibitory strategy of compound **1** in which the hexosamine "leaving group" is present, as well as more saccharide units at the reducing end with the hope of increasing the binding to an enzyme that normally prefers a polymeric substrate.

## Experimental Section

**General Methods.** Unless otherwise stated, all reagents were obtained from commercial suppliers and were used without further purification. Column chromatography was performed with silica gel (230−400 mesh). TLC was performed on precoated silica plates and visualized using UV light and/or by applying a solution of 10% ammonium molybdate in 2 M $H_2SO_4$ followed by heating. $CH_2Cl_2$, $CCl_4$, pyridine, and benzene were distilled over $CaH_2$. Methanol was distilled over magnesium and iodine. DMF was dried successively over 4 Å molecular sieves (3×). Compounds **4**[14,15] and **5**[14] were prepared as described in the literature.

**Inhibition Assays. I. Inhibition by Compounds 1 and 2.** Assays were carried out in glass vials, total solution volume 300 $\mu$L. Mixtures containing buffer (50 mM sodium phosphate, 100 mM NaCl, pH 6.8) and the desired amounts of substrate (benzyl 4-deoxy-4-fluoro-$\beta$-D-glucopyranosiduronic acid)[28] and inhibitor were incubated at 30 °C for at least 15 min to thermally equilibrate them. Compound **2** was equilibrated in buffer for at least 1.5 h prior to testing to ensure that a consistent ionization state of the carbon acid had been reached. Enzyme (30 $\mu$L of $1.0 \times 10^{-4}$ M chondroitin AC lyase in buffer) was added and the change in fluoride ion concentration was monitored at 30 °C with a fluoride ion-selective electrode interfaced with a computer. The rates were constant over a period of at least 10 min. The pH of the solutions was measured both at the beginning and at the end of the reaction.

**II. Inhibition by the Anion of Compound 2.** Assays were carried out in fluorescence cuvettes (1 cm path length), total solution volume 500 $\mu$L. Mixtures containing buffer (200 mM Tris, 100 mM NaCl, pH 8.0) and the desired amount of substrate (phenyl 4-methylumbelliferyl-$\beta$-D-glucopyranosiduronic acid)[28] were incubated at 30 °C for at least 15 min to thermally equilibrate them. The anion of **2** was prepared by adding 1 equiv of NaOH and equilibrating for at least 1.5 h. Desired amounts of the anion of **2** and enzyme (25 $\mu$L of 3.5 × $10^{-5}$ M chondroitin AC lyase in buffer) were then added, and the change in fluorescence intensity was monitored at 30 °C over 5 min ($\lambda_{excitation}$ 360 nm, $\lambda_{emission}$ 450 nm) using a fluorescence spectrometer equipped with a temperature-controlled cuvette holder. Rates were calculated using a standard curve of 7-hydroxy-4-methylcoumarin (4-methylumbelliferone). The pH of the solutions was measured both at the beginning and at the end of the reaction.

**Methyl 2,3-Di-*O*-acetyl-4,6-*O*-(4-methoxybenzylidene)-$\beta$-D-glucopyranoside (6).** A solution of methyl $\beta$-D-glucopyranoside (10.0 g, 51.5 mmol), 4-methoxybenzaldehyde dimethyl acetal (14.0 g, 76.8 mmol, 1.5 equiv), and *p*-toluenesulfonic acid monohydrate (200 mg, 1.0 mmol, 0.02 equiv) in dry DMF (50 mL) was rotated under aspirator pressure at 50 °C for 4.5 h. The temperature was raised to 70 °C and the reaction mixture concentrated until a solid began to form. The mixture was poured into a stirred mixture of saturated $NaHCO_3$(aq) (50 mL), diethyl ether (50 mL), and ice. The resulting solid was filtered off, washed successively with petroleum ether (PE) (bp 35−60 °C), and water, and dried over $P_2O_5$ in vacuo. The solid was dissolved in pyridine (50 mL) and acetic anhydride (45 mL) and stirred at room temperature for 1.5 h. The reaction mixture was diluted with $CH_2Cl_2$, washed with $H_2O$ (1×), cold 1 M HCl (4×), saturated $NaHCO_3$ (5×), $H_2O$ (1×), dried over $MgSO_4$, and concentrated under vacuum, leaving **6** (17.2 g, 80%) as a white solid: mp 190−191 °C; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.33. (2 H, d, $J$ = 8.8 Hz), 6.85 (2 H, d, $J$ = 8.8 Hz), 5.44 (1 H, s), 5.29 (1 H, dd, $J$ = 9.3, 9.3 Hz), 4.96 (1 H, dd, $J$ = 9.3, 7.8 Hz), 4.49 (1 H, d, $J$ = 7.8 Hz), 4.35 (1 H, dd, $J$ = 10.4, 4.8 Hz), 3.84−3.72 (4 H, m) 3.67 (1 H, dd, $J$ = 9.3, 9.3 Hz), 3.50 (3 H, s), 3.49 (1 H, ddd, $J$ = 9.3, 9.3, 4.8 Hz), 2.04, 2.01 (6 H, 2 s).

**Methyl 2,3-Di-*O*-acetyl-$\beta$-D-glucopyranoside (7).**[18] A solution of **6** in 1% (w/v) of iodine in methanol (200 mL) was heated at reflux overnight. The solvent was removed in vacuo and the residue dissolved in ethyl acetate (EtOAc) and washed with saturated $Na_2S_2O_3$ (1×). The aqueous phase was extracted with EtOAc (5×), and the combined organic phases were dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (PE:EtOAc 1:4 to 1:5) to yield **7** (3.40 g, 87%) as an oil which crystallized over time: $^1$H NMR (200 MHz, $CD_3OD$) $\delta$ 5.04 (1 H, dd, $J$ = 9.5, 9.0 Hz), 4.74 (1 H, dd, $J$ = 9.5, 7.8 Hz), 4.47 (1 H, d, $J$ = 7.8 Hz), 3.88 (1 H, dd, $J$ = 12.0, 2.4 Hz), 3.71 (1 H, dd, $J$ = 12.0, 5.1 Hz), 3.56 (1 H, dd, $J$ = 9.0, 9.0 Hz), 3.48 (3 H, s), 3.37 (1 H, ddd, $J$ = 9.0, 5.1, 2.4 Hz), 2.02, 1.99 (6 H, 2 s).

**Methyl 2,3-Di-*O*-acetyl-$\beta$-D-glucopyranosiduronic Acid (8).** A solution of commercial bleach (30 mL, 6% w/v NaOCl, 23 mmol), saturated $NaHCO_3$ (15 mL), and saturated NaCl (30 mL) at 0 °C was added dropwise to a cooled solution of **7** (1.7 g, 6.1 mmol), sodium bromide (76 mg, 0.74 mmol, 0.12 equiv), tetrabutylammonium bromide (135 mg, 0.42 mmol, 0.068 equiv), and TEMPO (24 mg) in EtOAc (30 mL) and saturated $NaHCO_3$ (17 mL). The reaction was stirred for 2.5 h at 0 °C and diluted with EtOAc and water. The organic layer was extracted with half-saturated $NaHCO_3$ (3×). The combined aqueous phases were acidified to pH 2 with 2 M HCl and then extracted with EtOAc (5×) and $CH_2Cl_2$ (3×), dried over $MgSO_4$, and concentrated to give **8** (1.25 g, 70%) as a white solid: $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 5.14 (1 H, dd, $J$ = 9.5, 9.5 Hz), 4.93 (1 H, dd, $J$ = 9.5, 7.6 Hz), 4.51 (1 H, d, $J$ = 7.6 Hz), 4.06−3.85 (2 H, m), 3.50 (3 H, s), 2.07, 2.04 (6 H, 2 s).

**Methyl (Methyl 2,3-Di-*O*-acetyl-$\beta$-D-glucopyranosid)-uronate (9).** A solution of **8** (1.48 g, 5.06 mmol) in methanol (25 mL) was stirred with acid resin (200−400 mesh) for 3.5 h. The resin was removed by filtration and the reaction mixture was concentrated. The residue was dissolved in EtOAc, washed with saturated $NaHCO_3$ (2×) and $H_2O$ (1×), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (PE:EtOAc 1:1), giving **9** (587 mg, 36%) as a colorless oil which crystallized over time: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.08 (1 H, ddd, $J$ = 9.5, 7.4, J 1.6 Hz), 4.89 (1 H, dd, $J$ = 9.5, 7.7 Hz), 4.44 (1 H, d, $J$ = 7.7 Hz), 3.97−3.88 (2 H, m), 3.81 (3 H, s), 3.48 (3 H, s), 2.04, 2.01 (6 H, 2 s).

**Methyl [Methyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-$\beta$-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-$\beta$-D-glucopyranosid]uronate (10).** A solution of **9** (578 mg, 1.89 mmol) and **5** (1.46 g, 2.45 mmol, 1.3 equiv) in dry 1,2-dichloroethane (30 mL) was stirred over powdered 4 Å molecular sieves for 1.5 h before cooling to 0 °C and adding trimethylsilyl trifluoromethanesulfonate (68 $\mu$L, 0.38 mmol, 0.2 equiv). After 3 h, an additional 100 mg of **5** was added, and the reaction mixture was stirred at 0 °C for another 45 min before triethylamine (0.37 mL) was added to quench the reaction. The reaction mixture was diluted with $CH_2Cl_2$, filtered, and concentrated. The residue was purified by column chromatography (PE:EtOAc 5:4 to 2:3) to give **10** (1.02 g, 73%) as a white foam: $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 6.74 (1 H, d, $J$ = 8.8 Hz), 5.32 (1 H, d, J 2.5 Hz), 5.22−5.10 (2 H, m), 4.93 (1 H, d, $J$ = 8.4 Hz), 4.88 (1 H, dd, $J$ = 9.6, 7.1 Hz), 4.44 (1 H, d, $J$ = 7.1 Hz), 4.15−3.88 (6 H, m), 3.82 (3 H, s), 3.48 (3 H, s), 2.14, 2.05, 2.03, 2.01, 1.95 (15 H, 5 s); ESI-MS $m/z$ 761.0 $[M + Na]^+$.

**Methyl [Methyl *O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-$\beta$-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-$\beta$-D-glucopyranosid]uronate (11).** A solution of **10** (1.01 g, 1.37 mmol), tributyltin hydride (1.73 mL, 6.42 mmol, 4.7 equiv), and 2,2′-azobisisobutyronitrile (AIBN, 36 mg, 0.22 mmol, 0.17 equiv) in dry benzene (45 mL) was stirred at room temperature for 15 min and then heated to reflux for 40 min. The reaction mixture was then cooled and concentrated in vacuo. The residue was dissolved in acetonitrile and washed with hexanes (5×) and concentrated, leaving a white solid that was recrystallized from EtOAc/hexanes to yield **11** (677 mg, 78%) as fine colorless needles: mp ∼195 °C dec; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.42 (1 H, d, $J$ = 8.6 Hz), 5.28 (1 H, d, $J$ = 3.4), 5.18 (1 H, dd, $J$ = 11.8, 3.4 Hz), 5.17 (1 H, dd, $J$ = 9.2, 9.2 Hz), 4.89 (1 H, dd, $J$ = 7.5, 9.2 Hz), 4.69 (1 H, d, $J$ = 8.4 Hz), 4.43 (1 H, d, $J$ = 7.5 Hz), 4.11−4.03 (3 H, m), 3.97 (1 H, d, $J$ = 9.6 Hz), 3.90−3.77 (2 H, m), 3.81 (3 H, s), 3.42 (3 H, s), 2.12, 2.04, 2.03, 2.02, 1.96, 1.90 (18 H, 6 s); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 180.34, 180.23, 180.10, 179.81, 179.48, 178.60, 102.04, 100.69, 76.14, 72.42, 71.39, 70.72, 70.05, 66.50, 61.17, 57.21, 53.03, 51.65, 23.26, 20.72, 20.59; ESI-MS $m/z$ 635.8 $[M]^+$. Anal. Calcd for $C_{26}H_{37}NO_{18}$: C, 49.13; H, 5.87; N, 2.20. Found: C, 48.87; H, 5.98; N, 2.26.

**Methyl [Methyl *O*-(3,4,6-Tri-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-$\beta$-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-$\beta$-D-glucopyranosid]uronate (12).** A suspension of **11** (595 mg, 0.94 mmol) and *p*-toluenesulfonic acid monohydrate (84 mg, 0.44 mmol, 0.47 equiv) in isopropenyl acetate (12 mL) was heated to 65 °C under argon for 3.5 h (**11** has dissolved after ∼1 h). Triethylamine (1.5 mL) was added to quench the reaction, and the solvent was removed in vacuo. The residue was purified by column chromatography (PE:EtOAc 2:3), giving **12** (622 mg, 98%) as a white foam: $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 5.75 (1 H, dd, $J$ = 11.0, 3.7 Hz), 5.38 (1 H d, $J$ = 3.7 Hz), 5.27 (1 H, d, $J$ = 7.6 Hz), 5.12 (1 H, dd, $J$ = 9.5, 9.5 Hz), 4.94 (1 H, dd, $J$ = 9.5, 7.5 Hz), 4.40 (1 H, d, $J$ = 7.5 Hz), 4.18−4.08 (3 H, m), 3.95 (1 H, dd, $J$ = 7.3, 7.3 Hz), 3.86 (1 H, d, $J$ = 9.8), 3.80 (3 H, s), 3.46 (3 H, s), 2.49, 2.29, 2.12 (9 H, 3s), 2.06 (6H, s), 2.04, 1.93 (6 H, 2 s); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 184.38, 184.08, 180.34, 180.20, 179.63, 179.32, 179.10, 177.50, 102.08, 97.66, 74.77, 74.26, 72.20, 70.80, 70.50, 67.32, 67.04, 60.79, 58.93, 57.24, 53.14, 27.71, 24.93, 20.90, 20.68, 20.62, 20.46, 20.36. Anal. Calcd for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07. Found: C, 50.02; H, 5.81; N, 2.20.

**Methyl [Methyl *O*-(3,4,6-Tri-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-$\beta$-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-5-bromo-$\beta$-D-glucopyranosid]uronate (13).** NBS (309 mg, 1.74 mmol, 2 equiv) was added to a solution of **12** (588 mg,

0.87 mmol) in dry $CCl_4$ (15 mL) and irradiated with two 200 W light bulbs (reflux). After 45 min, a further 140 mg of NBS was added and the reaction mixture irradiated for another 25 min. The mixture was cooled, filtered, and concentrated and the residue was dissolved in $CH_2Cl_2$, washed with saturated $NaHCO_3$ (1×) and $H_2O$ (1×), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (PE:EtOAc 1:1), giving **13** (422 mg, 64%) as a white foam: $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 5.86 (1 H, dd, $J$ = 11.0, 3.9 Hz), 5.56 (1 H, d, $J$ = 7.9 Hz), 5.46−5.33 (2 H, m), 5.06 (1 H, dd, $J$ = 9.8, 8.1 Hz), 4.91 (1 H, d, $J$ = 8.3 Hz), 4.20−4.06 (3 H, m), 4.20−3.71 (2 H, m), 3.88 (3 H, s), 3.50 (3 H, s), 2.36, 2.33, 2.12, 2.06, 2.05, 1.92 (21 H, 6 s); ESI-MS $m/z$ 756.2 $[M]^+$.

**Methyl [Methyl *O*-(3,4,6-Tri-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-α-L-*threo*-hex-4-enopyranosid]uronate (14).** DBU (9 μL, 0.06 mmol, 1.5 equiv) was added to a solution of **13** (30 mg, 0.04 mmol) in dry DMF (0.8 mL) at 0 °C. The reaction was then allowed to attain ambient temperature and stirred for 2.5 h. The reaction mixture was diluted with $CH_2Cl_2$, washed with $H_2O$ (1×), 1 M HCl (2×), and $H_2O$ (1×), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (PE:EtOAc 2:3), giving **14** (20 mg, 75%) as a white foam: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.78 (1 H, dd, $J$ = 11.0, 3.5 Hz), 5.68 (1 H, d, $J$ = 7.6 Hz), 5.49 (1 H, d, $J$ = 1.8 Hz), 5.40 (1 H, d, $J$ = 3.5 Hz), 4.98 (1 H, dd, $J$ = 2.4, 2.4 Hz), 4.92 (1 H, d, $J$ = 2.4 Hz), 4.18−4.08 (2 H, m), 4.04 (1 H, dd, $J$ = 11.3, 6.5), 3.93 (1 H, ddd, $J$ = 6.5, 6.5, 0.7 Hz), 3.78 (3 H, s), 3.46 (3 H, s), 2.42, 2.37, 2.13, 2.11, 2.06, 2.02, 1.94 (21 H, 7 s).

**Methyl *O*-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-α-L-*threo*-hex-4-enopyranoside (1).** NaOH (3 M, 3.4 mL) was added dropwise to stirred solution of **14** (250 mg, 0.37 mmol) in 5:1 MeOH:$H_2O$ (7 mL). The reaction mixture was stirred at room temperature for 4 h, neutralized with dilute acetic acid, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:MeOH:$H_2O$ 10:8:2 + 0.4% acetic acid to 5:4:2 + 0.4% acetic acid), concentrated to remove EtOAc and MeOH, and then freeze-dried to yield 785 mg white solid (mostly salts). The solid was then dissolved in water (2 mL) and passed down a size exclusion column (Sephadex G-10, 1.6 × 60 cm). Fractions containing the desired product were pooled, concentrated, and repurified by column chromatography (EtOAc:MeOH:$H_2O$ 5:4:1 + 0.4% acetic acid) to yield **1** (72 mg, 48%) as a solid: $^1$H NMR (400 MHz, $D_2O$) $\delta$ 4.91 (1 H, d, $J_{3,2}$ = 4.0 Hz, H3), 4.67 (1 H, d, $J_{1',2'}$ = 8.4 Hz, H1′), 4.09 (1 H, d, $J_{1,2}$ = 3.6 Hz, H1), 3.98 (1 H, dd, $J_{2',3'}$ = 10.7, $J_{2',1'}$ = 8.4 Hz, H2′), 3.89 (2 H, m, H2, H4′), 3.79 (1 H, dd, $J_{6a',6b'}$ = 11.8, $J_{6a',5'}$ = 8.2 Hz, H6a′), 3.74 (1 H, dd, $J_{3',4'}$ = 3.3, $J_{3',2'}$ = 10.7 Hz, H3′), 3.69 (1 H, dd, $J_{6b',5'}$ = 4.0, $J_{6b',6a'}$ = 11.8 Hz, H6b′), 3.64 (1 H, dd, $J_{5',6a'}$ = 8.2, $J_{5',6b'}$ = 4.0 Hz, H5′), 3.46 (3 H, s, OMe), 2.01 (3 H, s, Ac) (assignments based on COSY experiment); $^{13}$C NMR (100 MHz, $D_2O$) $\delta$ 185.41, 179.60, 139.39, 132.73, 100.59, 100.21, 75.62, 71.29, 70.18, 68.08, 66.62, 61.21, 56.67, 52.68, 22.65; HRMS (LSIMS-, thioglycerol) $m/z$ 408.1142, calcd for $C_{15}H_{22}NO_{12}$ $[M - 1]^-$ 408.1141; IR (KBr) 3412, 2940, 1740, 1704, 1408, 1068 $cm^{-1}$.

**Phenyl 2,3,4-Tri-*O*-acetyl-β-D-xylopyranoside (16).**[24] A solution of HBr (19 mL, 5.7 M in acetic acid) was added to a solution of 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose **15** (11.26 g, 35.4 mmol) in dry $CH_2Cl_2$ (30 mL) at 0 °C. The reaction mixture was then allowed to warm to ambient temperature, stirred for 3 h, poured into an ice/$H_2O$ mixture, and diluted with $CH_2Cl_2$. The aqueous phase was extracted once with $CH_2Cl_2$, and the combined organic phases were washed with saturated $NaHCO_3$ (3×) and $H_2O$ (1×), dried over $MgSO_4$, and concentrated, yielding 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl bromide as a white solid (11.39 g, 95%). The bromide was then dissolved in $CH_2Cl_2$ (85 mL) to which was added phenol (6.32 g, 67.2 mmol, 2 equiv), tetrabutylammonium hydrogen sulfate (11.4 g, 33.6 mmol, 1 equiv), and 1 M NaOH (85 mL). The mixture was rapidly stirred for 4 h, diluted with EtOAc, washed with 1 M NaOH (4×), $H_2O$ (1×), and brine (2×), dried over $MgSO_4$, and concentrated. The residue was recrystallized from ethanol to yield **16** (5.18 g, 42% overall) as plate-like crystals: $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.27 (2 H, dd, $J$ = 7.9, 8.8 Hz), 7.03 (1 H, tt, $J$ = 7.9, 0.9 Hz), 6.97 (2 H, dd, $J$ = 8.8, 0.9 Hz), 5.26−5.13 (3 H, m), 4.99 (1 H, ddd, $J$ = 7.9, 7.9, 5.1 Hz), 4.20 (1 H, dd, $J$ = 12.0, 5.1 Hz), 3.50 (1 H, dd, $J$ = 12.0, 7.9 Hz), 2.05 (9 H, s); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 179.86, 179.73, 179.27, 156.58, 129.53, 123.04, 117.83, 98.56, 70.75, 70.19, 68.49, 61.84, 20.65. Anal. Calcd for $C_{18}H_{20}O_8$: C, 57.95; H, 5.72. Found: C, 57.79; H, 5.74.

**Phenyl (5*S*)-2,3,4-Tri-*O*-acetyl-5-bromo-β-D-xylopyranoside (17).** A mixture of **16** (4.82 g, 13.7 mmol) and NBS (7.30 g, 41.0 mmol, 3 equiv) in dry $CCl_4$ (250 mL) was irradiated with one 250 W heat lamp and one 200 W light bulb (reflux). After 2.5 h an additional 2.4 g of NBS was added, and the reaction mixture irradiated for a further 2.5 h, cooled, filtered, and concentrated. The residue was dissolved in $CH_2Cl_2$, washed with saturated $NaHCO_3$ (1×) and $H_2O$ (1×), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (toluene:EtOAc 14:1), giving **17** (1.47 g, 25%) as a pale yellow solid: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.30 (2 H, dd, J 8.5, J 7.6 Hz, $Ph_{meta}$), 7.06 (1 H, tt, J 7.6, J 1.2 Hz, $Ph_{para}$), 6.99 (2 H, dd, J 8.5, J 1.2 Hz, $Ph_{ortho}$), 6.61 (1 H, d, $J_{5,4}$ 4.0 Hz, H5), 5.71 (1 H, dd, $J_{1,2}$ 8.2, $J_{1,3}$ 0.6 Hz, H1), 5.64 (1 H, dd, $J_{3,4} = J_{3,2}$ 9.7 Hz, H3), 5.33 (1 H, dd, $J_{2,3}$ 9.7, $J_{2,1}$ 8.2 Hz, H2), 4.91 (1 H, dd, $J_{4,3}$ 9.7, $J_{4,5}$ 4.0 Hz, H4), 2.10 (3 H, s, Ac), 2.05 (6 H, s, 2 Ac).

**Phenyl (5*S*)-2,3,4-Tri-*O*-acetyl-5-azido-β-D-xylopyranoside (18).** Sodium azide (265 mg, 4.08 mmol, 2 equiv) was added to a solution of **17** (880 mg, 2.04 mmol) in dry DMF (6.5 mL) at 0 °C and stirred for 20 min, before the reaction was allowed to warm to ambient temperature and stirred for a further 1.5 h. The reaction was diluted with EtOAc and washed with $H_2O$ (1×). The aqueous phase was extracted once with EtOAc, and the combined organic phases were washed with $H_2O$ (1×) and brine (3×), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (PE:EtOAc 3:1 to 2.75:1), giving **18** (359 mg, 45%) as a pale yellow syrup which crystallized over time: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.31 (2 H, dd, J 7.6, J 8.5 Hz, $Ph_{meta}$), 7.08 (1 H, tt, J 7.3, J 0.9 Hz, $Ph_{para}$), 7.02 (2 H, dd, J 8.5, J 0.9 Hz, $Ph_{ortho}$), 5.33 (1 H, dd, $J_{2,3}$ 8.8, $J_{2,1}$ 6.7 Hz, H2), 5.27 (1 H, d, $J_{1,2}$ 6.7 Hz, H1), 5.26 (1 H, dd, $J_{3,4} = J_{3,2}$ 8.8 Hz, H3), 5.12 (1 H, dd, $J_{4,3}$ 8.8, $J_{4,5}$ 7.6 Hz, H4), 4.87 (1 H, d, $J_{5,4}$ 7.6 Hz, H5), 2.07, 2.06, 2.04 (9 H, 3 s, 3 Ac); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 180.00, 179.21, 156.47, 129.70, 123.64, 118.14, 97.94, 85.46, 71.52, 70.91, 70.82, 20.57; IR (thin film) 2120 ($N_3$), 1857 (C═O) $cm^{-1}$.

**Phenyl (5*S*)-2,3,4-Tri-*O*-acetyl-5-nitro-β-D-xylopyranoside (19).** A solution of **18** (428 mg, 1.09 mmol) in EtOAc (14 mL) and ethanol (35 mL) was stirred with $PtO_2$ (Adam's catalyst, 175 mg) under 1 atm of $H_2$ for 50 min. The catalyst was removed by filtration through a bed of Celite, and the solvent was removed in vacuo, leaving a white solid that was dissolved in HPLC-grade acetone (30 mL) and added dropwise to a freshly prepared solution of dimethyldioxirane[26] in acetone (250 mL, ∼0.06 M). The reaction mixture was stirred at room temperature for 2.5 h and then concentrated under vacuum. The residue was purified by column chromatography (PE:EtOAc 3:1), giving **19** (182 mg, 42%) as a colorless solid. The product was then recrystallized from EtOAc/hexanes to give CR-439 as fine, colorless needles. Crystals were grown from ethanol for X-ray analysis (Note: **19** decomposes on a TLC plate to produce a spot with a larger $R_f$ value; thus, samples should be eluted immediately after spotting to avoid this decomposition): $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.30 (2 H, dd, J 8.5, J 7.6 Hz, $Ph_{meta}$), 7.08 (1 H, tt, J 7.6, J 0.9 Hz, $Ph_{para}$), 7.03 (2 H, dd, J 8.5, J 0.9 Hz, $Ph_{ortho}$), 6.04 (1 H, dd, $J_{4,3}$ 9.4, $J_{4,5}$ 7.3 Hz, H4), 5.56 (1 H, d, $J_{1,2}$ 2.1 Hz, H1), 5.51 (1 H, d, $J_{5,4}$ 7.3 Hz, H5), 5.28 (1 H, dd, $J_{3,4}$ 9.4, $J_{3,2}$ 5,5 Hz, H3), 5.24 (1 H, dd, $J_{2,3}$ 5.5, $J_{2,1}$ 2.1 Hz, H2), 2.11, 2.09, 2.08 (9 H, 3 s, 3 Ac) (assignments based on NOE difference and COSY experiments); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 179.83, 179.37, 178.62, 155.57, 129.65, 123.87, 117.49, 100.59, 99.13, 73.42, 70.30, 67.23, 20.64, 20.61, 20.44. Anal. Calcd for $C_{18}H_{19}NO_{10}$: C, 51.39; H, 4.82; N, 3.53. Found: C, 51.51; H, 4.91; N, 3.57.

**Phenyl (5*S*)-5-Nitro-β-D-xylopyranoside (2).** To a solution of **19** (234 mg, 0.59 mmol) in dry MeOH (10 mL) at 0 °C

was added acetyl chloride (1.0 mL). The reaction mixture was then stirred at 4 °C for 23 h and concentrated in vacuo and the residue purified by column chromatography (PE:EtOAc 1:2 to 1:3) to yield **2** (124 mg, 78%) as a white foam: $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ 7.33 (2 H, dd, $J$ = 7.3, 8.8 Hz, $Ph_{meta}$), 7.12 (2 H, dd, $J$ = 8.8, 1.2 Hz, $Ph_{ortho}$), 7.08 (1 H, tt, $J$ = 7.3, 1.2 Hz, $Ph_{para}$), 5.58 (1 H, d, $J_{5,4}$ = 8.8 Hz, H5), 5.30 (1 H, d, $J_{1,2}$ = 6.7 Hz, H1), 3.93 (1 H, dd, $J_{4,3} = J_{4,5}$ = 8.8 Hz, H4), 3.68 (1 H, dd, $J_{2,3}$ = 8.8, $J_{2,1}$ = 6.7 Hz, H2), 3.62 (1 H, dd, $J_{3,2} = J_{3,4}$ = 8.8 Hz, H3) (assignments based on NOE difference and COSY experiments); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 158.39, 130.53, 124.10, 118.88, 105.65, 101.71, 75.23, 74.62, 73.27; ESI-MS $m/z$ 294.2 $[M + Na]^+$. Anal. Calcd for $C_{11}H_{13}NO_7$: C, 48.71; H, 4.83; N, 5.17. Found: C, 48.48; H, 4.90; N, 5.05.

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**Supporting Information Available:** $^1$H NMR spectra of compounds **1**, **6**−**10**, **13**−**14**, **17**, and **18** and X-ray crystal structure data for compound **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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