# New Electronic Analogs of the Sialyl Cation: N-Functionalized 4-Acetamido-2,4-dihydroxypiperidines. Inhibition of Bacterial Sialidases

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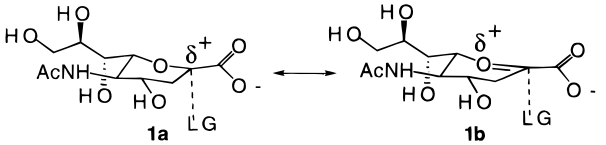
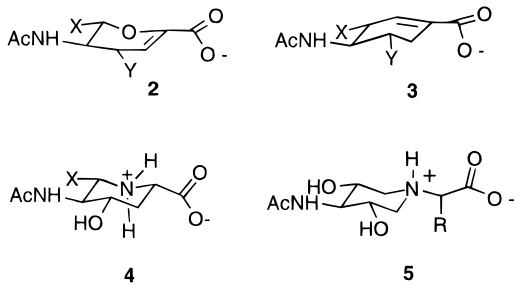
## Introduction

Sialic acids (*N*-acetylneuraminic acid, NeuAc) are nine-carbon ketoses which have the unusual feature of a highly acidic carboxyl group ($pK_a \sim 2.8$) immediately adjacent to the anomeric carbon. Sialic acids are typically found in terminal glycosidic linkages of cell surface glycoproteins and glycolipids and are represented in species as diverse as bacteria, trypanosomes, and higher members of the animal kingdom, including humans.[1,2] A variety of biological phenomena are associated with recognition of sialosides, including viral replication, escape of immune detection, and cell adhesion[3] providing considerable interest in the development of inhibitors of sialyltransferases and sialidases for mechanistic and clinical applications.[4]

Kinetic and kinetic isotope effect analyses of viral and bacterial sialidases have led to the conclusion that as in solution,[5] the enzymatic reaction involves catalysis proceeding through transition states having oxocarbenium ion character.[6] Sialidase transition states resemble the resonance pair **1a**/**b** which has development of partial




positive charge at the C-2 anomeric carbon and an associated flattening of the pyranosyl ring about atoms C6−O6−C2−C3. Outstanding progress has been made for the design and synthesis of sialidase (or neuraminidase) geometric transition state analog inhibitors, particularly for the influenza enzyme.[7] These inhibitors, as exemplified by 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acids **2**, and more recently reported carbocyclic analogs **3**[7g] utilize unsaturation to flatten the pyranosyl ring as a mimic of the transition state **1a**/**b**. Sialidase electronic transition state inhibitors reported thus far mimic oxocarbenium ion character by replacing the pyranosyl ring oxygen with a basic nitrogen atom (piperidines **4**), which on protonation would mimic the positive formal charge placed on oxygen in structure **1b**.[8]




An alternate design strategy would mimic the electronic charge distribution of resonance contributor **1a** with inhibitors **5**, which place the formal charge at a site electronically equivalent to the glycosidic carbon of oxocarbenium ion resonance contributor **1a**. Recent experimental work has shown that placement of the charge mimic in the position analogous to the anomeric carbon of a glycosidase substrate can be an effective strategy for glycosidase inhibition, e.g., isofagomine.[9]

We are evaluating an approach which employs a central "core" structure like **5**, upon which additional functionality could be installed to tailor the inhibitor for its particular target. For example, sialidase inhibitors might incorporate aglycon functionality to complement transition state mimicry, or sialyltransferase inhibitors[10] might include structural features of the leaving group (CMP) or acceptor sugar.[11a,b] At issue in the present work is synthesis of the core structure; previously reported inhibitors of the types **2**−**4** do not readily lend themselves to these design requirements. Given that hydrophobic aryl sialosides are good substrates for sialidases,[12] we wished to test the efficacy of inhibitors which included a

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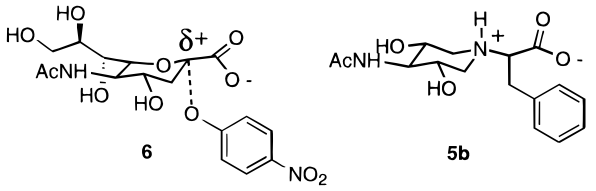
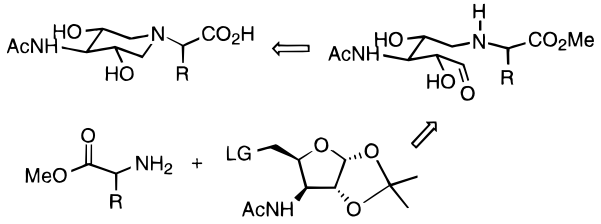
hydrophobic aglycon mimic. Structures **6** and **5b** illustrate the correspondence between the transition state for cleavage of an aryl sialoside and an inhibitor including functionality which mimics the departing aryl aglycon.



Compounds **5** replace the glycerol C7−C9 side chain found in NeuAc with a hydroxyl group. This feature affords the opportunity for preparation of side chain analogs[7g] and allows synthesis of the core inhibitor structure with relative synthetic ease. The sialic acid carboxylate group is an important component of sialidase binding interactions;[13] this functionality is included in inhibitors **5** with an intervening methylene linker.[14] We report here the synthesis of *trans,trans*-4-acetamido-3,5-dihydroxypiperidines **5a** and (±)-**5b** and, to demonstrate their potential for development of inhibitors of sialic acid transferring enzymes, show that they are competitive inhibitors for bacterial sialidases from *Salmonella typhimurium*, *Clostridium perfringens*, and *Vibrio cholerae*.

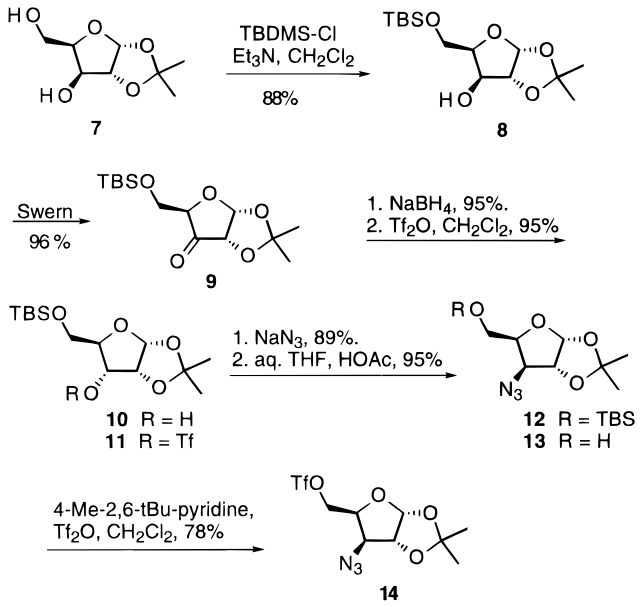
## Results and Discussion

**Synthesis of Inhibitors.** The route to the required *trans,trans*-3,5-dihydroxy-4-acetamidopiperidines **5a** and (±)-**5b** was based on the plan that reductive cyclization of a 5-*N*-alkylxylose derivative (**19**, Scheme 2) would readily afford piperidines with the desired relative stereochemistry; the 5-*N*-alkylxylose compounds would be prepared by *N*-alkylation of primary amines with a xyloside derivatized at C-5 for nucleophilic displacement, as outlined in Scheme 1.



The synthesis started from 1,2-isopropylidene-α-D-xylofuranose[15] (**7**), (Scheme 1), which was selectively protected in 94% yield as the *tert*-butyldimethylsilyl ether

**Scheme 1. Synthesis of Azido Triflate 14**



**8**. The stereochemistry of the secondary alcohol was then inverted by Swern oxidation[16] to the ketone **9** and stereoselective reduction[17] with $NaBH_4$ to ribofuranoside **10**.

We anticipated that the required *xylo*-stereochemistry for azide **12** could be obtained by displacement of a suitable derivative of the C-3 hydroxyl in alcohol **10**. Reaction of ribo triflate **11** (obtained from **10** by reaction with triflic anhydride and pyridine) with sodium azide afforded the protected azide **12** in 85% yield from **10**. Unlike the reported reaction for the corresponding xylo triflate, no elimination products were observed.[18] The *xylo*-configuration of azide **12** was established by X-ray crystallographic analysis of a diacetyl derivative.[19]

Desilylation of **12** afforded alcohol **13**, which was then converted to the triflate **14**. The hindered base 2,6-di-*tert*-butyl-4-methylpyridine had to be used in order to obtain the triflate in high yield.[20] Triflate **14** was sufficiently stable to purify it by column chromatography on silica gel, but decomposed slowly at 4 °C, and so was used immediately after purification. The synthesis of the key intermediate azido triflate **14** from commercially available 1,2-isopropylidene-α-D-xylose was achieved in 44% overall yield for seven steps and can be prepared at the gram scale.

The purified triflate **14** was converted to the secondary amines **15a,b** by displacement with glycine methyl ester and D/L phenylalanine methyl ester, respectively,[21] as shown in Scheme 2. The displacements proceeded smoothly at room temperature, with yields of 80% for

(11) (a) We do not intend to imply that an aglycon or nucleophile is not part of the transition state for the reaction; it is a convenient artifice of inhibitor design to separate these peripheral transition state structural features from the core *N*-acetylneuraminyl unit which serves as a charge mimic. (b) For a pertinent example of glycosyltransferase inhibitor design, see: Hashimoto, H.; Endo, T.; Kajihara, Y. *J. Org. Chem.* **1997**, *62*, 1914−1915.

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(14) Direct attachment of a carboxylate residue to nitrogen is not appropriate as the resulting carbamic acid would not be stable. The methylene bridge between the carboxylate and secondary nitrogen increases the distance between the carboxylate and anomeric carbon mimic by ∼1.1 Å relative to the C−COO− bond length for *N*-acetylneuraminic acid.

(15) A similar transformation of a 1,2-isopropylidene xylofuranoside to the ribofuranoside has been reported: Ritzmann, G.; Klein, R. S.; Hollenberg, D. H.; Fox, J. J. *Carbohydr. Res.* **1975**, *39*, 227−236.

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(19) Obtained by reduction of azide **12** to the amine with Raney nickel, N-acetylation, desilylation, and O-acetylation. X-ray: Abboud, K.; Parr, I. B.; Horenstein, B. A. Manuscript in prepration.

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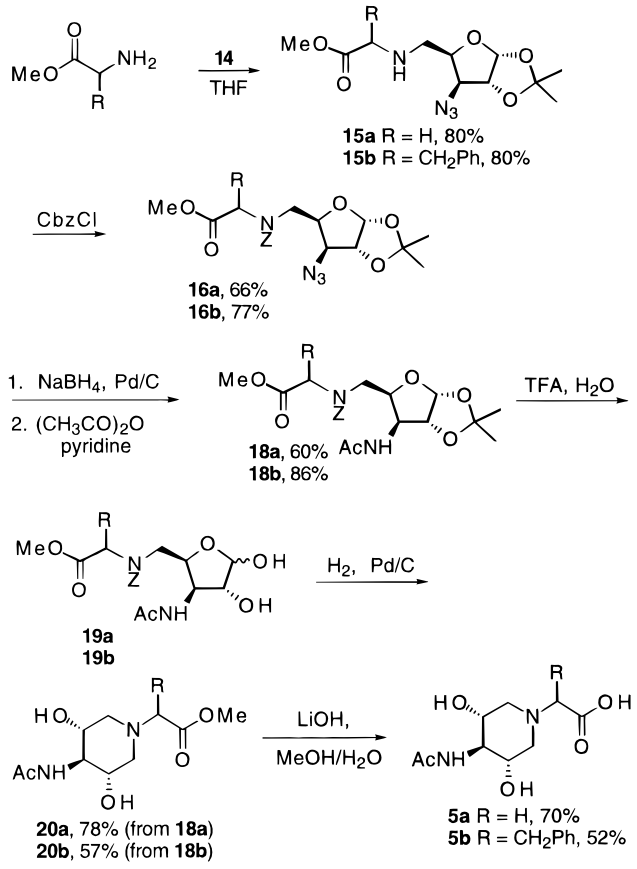
(21) Yenakis, D.; Moll, N.; Gross, B. *Synthesis* **1983**, 541−542.

**Scheme 2. Synthesis of Inhibitors 5a and (±)-5b from Triflate 14**




**15a,b**. Amines **15a,b** were then protected as the CBZ carbamates **16a,b** in 66−89% yields.[22]

The azides **16a,b** were then reduced[23] to the corresponding amines **17a,b** which were acetylated to give the 3-acetamidofuranosides **18a,b** in 60% and 86% overall yields from **16a,b**. The 1,2-isopropylidene ketal of compounds **18a,b** was hydrolyzed to provide the hemiacetals **19a,b** as a mixture of α- and *β*-anomers. From earlier work we found that it was helpful to carry out the hydrolytic removal of the isopropylidene group with the secondary amine blocked as the benzyl carbamate.[24] The hemiacetals **19a,b** were then reductively cyclized under neutral conditions to give the piperidines **20a,b** in 78% and 57% yields from **18a,b**. To the best of our knowledge, this is the first report of the synthesis of piperidines with this substitution pattern. Compounds **20a,b** were converted to the sialidase inhibitors **5a** and (±)-**5b** by alkaline hydrolysis in 70% and 52% respective yields after chromatography. Presently, preparation of each antipode of **5b**, and side chain analogs, is underway.[25a,b]

**Inhibition Studies.** Inhibition of the sialidases from *C. perfringens*, *S. typhimurium*, and *V. cholerae* by dihydroxypiperidines **5a** and (±)-**5b** was determined using the substrate 2-*O*-(*p*-nitrophenyl)-α-D-*N*-acetylneuraminic acid (Table 1).[12a] Figure 1 presents a double-reciprocal plot for the inhibition of the *S. typhimurium* sialidase by (±)-**5b**, from which it is concluded that the inhibition is competitive; the remaining inhibitor/sialidase inhibition kinetics were also well fit to competitive inhibition patterns. The *C. perfringens* sialidase is inhibited to the same extent by **5a** or (±)-**5b**, whereas the *S. typhimurium* enzyme binds the more hydrophobic (±)-**5b** almost 5 times more tightly than **5a**. Compound **5a** did not show inhibition at concentrations below 2 mM, and (±)-**5b** was a modest inhibitor of the *V. cholerae* sialidase, with $K_i = 1.5$ mM.

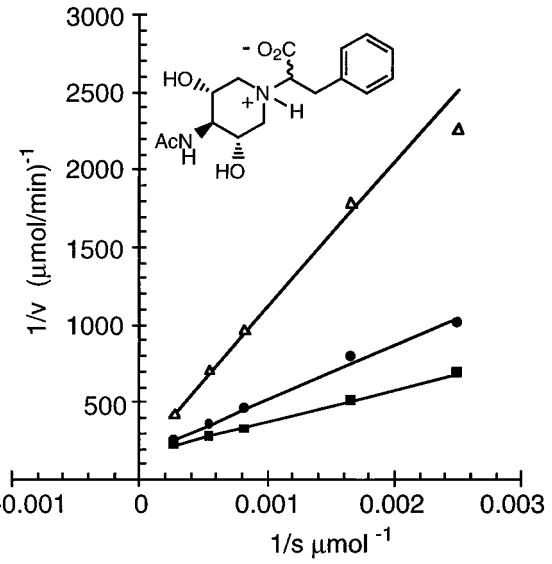



**Figure 1.** Inhibition of *S. typhimurium* sialidase by (±)-**5b**. Double reciprocal plot with 2-*O*-(*p*-nitrophenyl)-α-D-*N*-acetylneuraminic acid at varying concentrations (100−2000 $\mu$M) with fixed concentrations of inhibitor (±)-**5b**: (■) 0, (●); 0.4, and (△) 2 mM. Reactions were initiated with 3.6 $\mu$g of sialidase.

**Table 1**

| sialidase | $K_i$, $\mu$M **5a** | $K_i$, $\mu$M (±)-**5b** | $K_m$, $\mu$M[a] |
|---|---|---|---|
| *S. typhimurium* | 2500 ± 400 | 560 ± 30 | 1200 ± 100 |
| *C. perfringens* | 400 ± 100 | 500 ± 70 | 280 ± 30 |
| *V. cholerae* | >2000[b] | 1500 ± 200 | 2700 ± 740 |

Compounds **5a** and (±)-**5b** represent the core structure of a sialic acid oxocarbenium ion analog, lacking the C6−C9 glycerol side chain found in the natural sialidase substrates and *p*-nitrophenyl glycoside substrate employed in this work. Despite this, the data presented in Table 1 show that significant inhibition may be obtained, particularly for (±)-**5b**. For both the *S. typhimurium* and *V. cholerae* sialidases, (±)-**5b** binds approximately 2 times tighter than the *p*-nitrophenyl glycoside substrate (with the assumption that $K_m \approx K_d$, based on the observation of intrinsic kinetic isotope effects[6]), while for the *C. perfringens* sialidase **5a,b** are bound with slightly lower affinity than substrate. The $K_i$'s for **5a** and (±)-**5b** are modest, being in the $10^{-4} - 10^{-3}$ M range. The following observations lead us to suggest that inhibitors **5**, which lack a glycerol side chain, are important leads for further

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(23) Kokotos, G.; Constantinou-Kokotou, V. *J. Chem. Res., Miniprint* **1992**, 311.

(24) Acid hydrolysis of the ketal in analogous N-unprotected compounds afforded products which appeared to have aromatized. We have not fully characterized these reaction products; their aromatic nature was evident from $^1$H NMR spectra which showed a multiplet at $\delta$ 7.5−8.0 consisting of four hydrogens. For an example of aromatization of a poly(hydroxypiperidine), see: Nishimura, Y. In *Studies in Natural Products Chemistry*; Rahman, A. U., Ed.; Elsevier: Amsterdam, 1992; pp 495−578.

(25) (a) Racemic compounds were synthesized since we did not wish to make initial assumptions regarding which stereoisomer might ultimately afford the better inhibitor. (b) Synthesis of side chain analogs raises the synthetic complexity by requiring discrimination between the two hydroxyl groups of compounds **5** or precursors.

development of more potent sialidase inhibitors containing a side chain. Vasella and colleagues[8d] have measured $K_i$'s for a series of piperidine-based inhibitors (structures **4**) for the *V. cholerae* sialidase, in which the side chain substituent X was varied from the full glycerol functionality to hydroxymethyl, methyl, and hydrogen. The respective $K_i$'s were 0.029, 6.1, 9.6, and 10 mM, demonstrating that $10^2-10^3$ binding affinity is realized for a full side chain versus truncants. Similarly, a recent study of NeuAc-2-en analogs **2** in which the side chain X was varied in length showed a systematic variation in $K_i$ with chain length against influenza sialidase, consistent with an earlier report.[7e,26] Also, side chain recognition is important for the *S. typhimurium* and influenza sialidases, since the X-ray crystal structures show specific interactions with the glycerol side chain.[13]

The three sialidases tested show different sensitivity to the presence of a hydrophobic aglycon mimic in the inhibitor. The inhibition data suggest that the active site of the *C. perfringens* enzyme can accommodate a relatively large hydrophobic group (i.e., the phenyl ring of (±)-**5b**) but that no net energetic advantage over binding **5a** exists. Inhibitors **5a**/**5b** bind weakly to the *V. cholerae* sialidase, though the measurable $K_i$ for (±)-**5b** suggests that it may benefit from a modest hydrophobic binding effect relative to **5a**. This is in contrast to the results for the *S. typhimurium* enzyme, which binds (±)-**5b** almost 5 times more tightly than **5a** which demonstrates that favorable hydrophobic interactions may indeed be utilized as a component of inhibitor design for the *S. typhimurium* sialidase.[27] It is also noteworthy that (±)-**5b** binding ($560 \pm 30\ \mu$M) to the *S. typhimurium* sialidase is comparable to that of the geometric transition state analog 2,3-dehydro-*N*-acetyl neuraminic acid (380 $\mu$M),[28] making (±)-**5b** one of the best inhibitors for the *S. typhimurium* sialidase. Each antipode of inhibitor (±)-**5b** is likely to have a different $K_i$; the observed data reflect the net inhibition. As mentioned, we are now preparing each enantiomer of **5b** separately to address this issue.

In conclusion, we have described a synthetic route to novel N-functionalized 4-acetamido-2,4-dihydroxypiperidines which allows for the flexible N-substitution based on the starting amine employed. This is a new structural class of sialidase inhibitor which combines transition state analogy with the ability to include aglycon mimicry. These compounds competitively inhibit bacterial sialidases with $K_i$'s ranging from $\sim 10^{-4}-10^{-3}$ M, despite the lack of the glycerol side chain analogous to the C7−C9 tail of *N*-acetylneuraminic acid. The results indicate that (1) location of the oxocarbenium ion charge mimic in a position analogous to the glycosyl carbon and (2) inclusion of hydrophobic aglycon functionality are viable strategies for the development of sialidase inhibitors. The inhibitors reported represent the first generation of their type and have served to demonstrate the potential of the approach; the next generation of inhibitors will be those that refine binding interactions for sialidases.

## Experimental Section

**General.** Starting materials and reagents were purchased from Sigma, Aldrich, or Fisher Scientific and were used without further purification. Sialidases from *S. typhimurium*, *C. perfringens*, and *V. cholerae* were purchased from Sigma. Solvents were obtained from Fisher Scientific as ACS reagent grade. THF was dried immediately before use by distillation from Na/benzophenone under nitrogen. Moisture sensitive reactions were performed under an atmosphere of nitrogen using standard techniques. Analytical TLC was performed on silica gel 60F-245 plates. Column chromatography was performed with Davisil grade 633, type 60 A, silica gel (200−425 mesh). EI, CI, and FAB mass spectra were recorded at the University of Florida. Chemical ionization methods (CI) used either ammonia or isobutane, and FAB MS used nitrobenzyl alcohol as matrix. NMR and other spectral data for all compounds are presented in the Supporting Information.

**1,2-*O*-Isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-xylofuranose (8).** 1,2-*O*-Isopropylidene-D-xylofuranose (7.608 g, 40 mmol) was dissolved in $CH_2Cl_2$ (120 mL) and cooled in an ice bath. Triethylamine (7.8 mL, 56 mmol) and TBDMSCl (7.3 g, 48 mmol) were added. The reaction was stirred at 0 °C for 30 min, then warmed to rt, and stirred for 18 h at which time additional triethylamine (0.7 mL, 5 mmol) and TBDMSCl (0.7 g, 4.6 mmol) were added to the reaction mixture with stirring continued for 5 h. The reaction mixture was concentrated *in vacuo*, and the residue was suspended in $CH_2Cl_2$ (40 mL) and filtered. $CHCl_3$ (120 mL) was added, and the solution was washed with 1 M HCl (4 × 30 mL) and saturated aqueous NaCl (1 × 30 mL) and dried ($Na_2SO_4$). Chromatography on silica gel (1% methanol in $CHCl_3$) afforded compound **8** (10.77 g, 88%) as an oil. $^1$H NMR analysis of **8** showed a small amount of disilylated product which was removed in the next step of the synthesis: $[\alpha]^{20}_D$ −9.3 ($c$ = 10 g/100 mL, $CHCl_3$); FAB HRMS exact mass calcd for $MH^+$ $C_{14}H_{29}SiO_5$ 305.1784, found 305.1784.

**1,2-*O*-Isopropylidene-3-oxo-5-*O*-(*tert*-butyldimethylsilyl)-D-xylofuranose (9).** Oxalyl chloride (3.4 mL, 39 mmol) was dissolved in dry $CH_2Cl_2$ (105 mL) and cooled to −55 °C. DMSO (5.5 mL, 77.9 mmol) was added such that the temperature remained below −50 °C, and the mixture was stirred for 2 min. A solution of **8** (10.77 g, 35.4 mmol) in dry $CH_2Cl_2$ (35 mL) was added slowly over 5 min, and the mixture was stirred at −50 °C for 30 min. Triethylamine (16.3 mL, 117 mmol) in dry $CH_2Cl_2$ (30 mL) was added and the mixture stirred at −55 °C for a further 40 min. The reaction mixture was warmed to rt, stirred for 2.5 h, then poured into 160 mL of water, and extracted with $CHCl_3$ (130 mL). The organic layer was then washed with saturated aqueous NaCl (1 × 80 mL) and dried over $Na_2SO_4$. The crude product was crystallized from hexane to give **9** as a waxy solid (10.3 g, 96%): mp 32 °C; $[\alpha]^{20}_D$ +114 ($c$ = 10 g/100 mL, $CHCl_3$); FAB HRMS exact mass calcd for $MH^+$ $C_{14}H_{27}SiO_5$ 303.1627, found 303.1599.

**1,2-*O*-Isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranose (10).** Ketone **9** (6.89 g, 22.8 mmol) was dissolved in ethanol (150 mL) and water (50 mL) and cooled in an ice bath. $NaBH_4$ (5.61 g, 148.3 mmol) was then added, and stirring was continued for 3.5 h. The reaction mixture was poured into water (400 mL) and extracted with ethyl acetate (8 × 150 mL). The combined organic layers were then dried ($Na_2SO_4$) and concentrated *in vacuo* to afford **10** (6.60 g, 95%): $[\alpha]^{20}_D$ +25.7 ($c$ = 4.1 g/100 mL, $CHCl_3$).

**1,2-*O*-Isopropylidene-3-[(trifluoromethanesulfonyl)oxy]-5-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranose (11).** Triflic anhydride (1.8 mL, 10.6 mmol) was dissolved in 15 mL of dry $(CH_2Cl)_2$ and added to a solution of pyridine (1.2 mL, 15.6 mmol) in 10 mL of $(CH_2Cl)_2$ at −10 °C. After 3 min a precipitate formed. A solution of **10** (3.2 g, 10 mmol) in 16 mL of $(CH_2Cl)_2$ was added at −10 °C, and the mixture was stirred for 2.25 h at this temperature. Aqueous 5% $NaHCO_3$ (40 mL) was added; the reaction mixture was warmed to rt and stirred for 30 min before being cast into $CHCl_3$ (60 mL). The organic phase was washed with 5% $NaHCO_3$ (40 mL) and saturated aqueous NaCl

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(30 mL) and then dried ($Na_2SO_4$). After being concentrated *in vacuo*, the residue was dissolved in toluene (50 mL) and re-evaporated; this procedure was repeated three times providing the triflate **11** (4.33 g, 95%) as a red oil.

**1,2-*O*-Isopropylidene-3-deoxy-3-azido-5-*O*-(*tert*-butyldimethylsilyl)-D-xylofuranose (12).** Triflate **11** (4.33 g, 10 mmol) was dissolved in a suspension of $NaN_3$ (3.75 g, 57.7 mmol, in 50 mL of ethanol) at rt. The reaction mixture was heated to 65−70 °C for 14 h, after which time additional $NaN_3$ (3.75 g, 57.7 mmol) was added followed by reflux for 24 h. The reaction mixture was then cooled to rt and stirred for 4 days. The reaction mixture was concentrated and then partitioned between water (30 mL) and $CHCl_3$ (100 mL). The organic fraction was then washed with water (2 × 30 mL) and saturated aqueous NaCl (1 × 30 mL) and dried ($Na_2SO_4$). Column chromatography on silica gel (4:6 hexane/$CHCl_3$) afforded azide **12** (2.912 g, 89%) as an oil: FAB HRMS exact mass calcd for $MH^+$ $C_{14}H_{28}SiN_3O_4$ 330.1849, found 330.1824.

**1,2-*O*-Isopropylidene-3-deoxy-3-azido-D-xylofuranose (13).** The azide **12** (1.84 g, 5.6 mmol) was dissolved in 20 mL of 50% aqueous THF, and $CH_3COOH$ (15 mL) was added. The reaction mixture was stirred (rt, 18 h); then the reaction was carefully quenched by the addition of water (60 mL) and sufficient $Na_2CO_3$ to raise the pH above 9. The mixture was extracted with $CHCl_3$ (3 × 30 mL), and the combined organic phases were dried ($Na_2SO_4$). Column chromatography on silica gel (1:3 ethyl acetate/hexane) afforded alcohol **13** (1.14 g, 95%) as an oil: $[\alpha]^{20}_D$ −44 ($c$ = 3.87 g/100 mL, $CHCl_3$); FAB HRMS exact mass calcd for $MH^+$ $C_8H_{14}N_3O_4$ 216.0984, found 216.1016.

**1,2-*O*-Isopropylidene-3-deoxy-3-azido-5-[(trifluoromethanesulfonyl)oxy]-D-xylofuranose (14).** 4-Methyl-2,6-di-*tert*-butylpyridine (1.28 g, 6.3 mmol) was dissolved in 10 mL of dry $(CH_2Cl)_2$ under $N_2$ and then cooled to −78 °C. Triflic anhydride (1.05 mL, 6.3 mmol) was added to the solution which was then stirred for 5 min. A solution of **13** (1.12 g, 5.2 mmol) in 12 mL of dry $(CH_2Cl)_2$ was added to the triflate solution which was then stirred at −10 °C for 30 min. The reaction mixture was warmed to rt, 10 mL of hexane was added, and the solution was loaded onto a silica gel column. Elution with 3:7 hexane/$CHCl_3$ afforded triflate **14** (1.41 g, 78%): FAB HRMS exact mass calcd for $MH^+$ $C_9H_{13}SN_3F_3O_6$ 348.0477, found 348.0477.

**1,2-*O*-Isopropylidene-3,5-dideoxy-3-azido-5-*N*-(1′-carbomethoxyethyl)-D-xylofuranose (15a).** Triflate **14** (1.41 g, 4.05 mmol) was dissolved in 10 mL of dry THF, glycine methyl ester (1.36 g, 15.3 mmol) in 12 mL of THF was added, and the reaction mixture was stirred at rt for 14.5 h. The reaction mixture was concentrated *in vacuo*, and the crude product was then purified by column chromatography on silica gel (1:1 hexane/ethyl acetate) to provide **15a** (0.93 g, 80%) as a yellow oil: $[\alpha]^{20}_D$ −33 ($c$ = 5 g/100 mL, $CHCl_3$); FAB HRMS exact mass calcd for $MH^+$ $C_{11}H_{19}N_4O_5$ 287.1355, found 287.1354.

**1,2-*O*-Isopropylidene-3,5-dideoxy-3-azido-5-*N*-(1′-carbomethoxy-2′-benzylethyl)-D-xylofuranose (15b). 15b** was prepared as for **15a**, using **14** (3.94 mmol) in 7.9 mL of THF and DL-phenylalanine methyl ester (1.41 mL, 7.88 mmol) in THF (20 mL). Workup as for **16a** and column chromatography (1:3 ethyl acetate/hexane) gave **15b** (1.19 g, 80%) as an oil: FAB HRMS exact mass calcd for $MH^+$ $C_{18}H_{25}N_4O_5$ 377.1825, found 377.1825.

**1,2-*O*-**Isopropylidene-3,5-dideoxy-3-azido-5-***N***-(1′-carbomethoxyethyl)-5-*N*-[(benzyloxy)carbonyl]-D-xylofuranose (16a).** The amine **15a** (0.92 g, 3.2 mmol) was dissolved in 18 mL of dioxane and cooled in an ice bath. $Na_2CO_3$ (2.19 g, 17.7 mmol) in 54 mL of water was added to this solution followed by dropwise addition of benzyl chloroformate (4.57 mL, 32 mmol) to the rapidly stirred mixture. The reaction mixture was then stirred at rt for 17 h. The mixture was poured into 10% $Na_2CO_3$ (100 mL) and extracted with ethyl acetate (4 × 50 mL). The combined organic fractions were dried ($Na_2SO_4$) and concentrated *in vacuo*. The crude product was then purified twice by column chromatography on silica gel (8:2 hexane/ethyl acetate, then 4% methanol in toluene) to afford **16a** (0.893 g, 66%) as an oil: $[\alpha]^{20}_D$ −37 ($c$ = 5 g/100 mL, $CHCl_3$); FAB HRMS exact mass calcd for $MH^+$ $C_{19}H_{25}N_4O_7$ 421.1723, found 421.1705.

**1,2-*O*-Isopropylidene-3,5-dideoxy-3-azido-5-*N*-(1′-carbomethoxy-2′-benzylethyl)-5-*N*-[(benzyloxy)carbonyl]-D-xylofuranose (16b). 16b** was prepared as for **16a**, using the amine **15b** (1.161 g, 3.095 mmol) in 100 mL of dioxane and 50 mL of water, with $Na_2CO_3$ (0.826 g, 7.72 mmol) and CbzCl (0.97 mL, 6.79 mmol). Workup and purification as for **16a** gave the product **16b** (1.218 g, 77%) as an oil: FAB HRMS exact mass calcd for $MH^+$ $C_{26}H_{31}N_4O_7$ 511.2193, found 511.2205.

**1,2-*O*-Isopropylidene-3,5-dideoxy-3-amino-5-*N*-(1′-carbomethoxyethyl)-5-*N*-[(benzyloxy)carbonyl]-D-xylofuranose (17a).** Pd/C (10%, 1 mg per 6−10 mg of azide **16**) was suspended in water, and to it a solution of azide **16a** (0.707 g, 1.68 mmol) in 16 mL of methanol was added. $NaBH_4$ (0.191 g, 5.05 mmol) was added in three portions over 15 min at rt. After stirring for a further 30 min, the reaction mixture was filtered through Celite. The Celite was washed with methanol (1 reaction volume) and water (10 reaction volumes). The pH of the combined filtrates was adjusted to pH 7 with 1 M HCl and the methanol removed under reduced pressure. The aqueous layer was then adjusted to pH 1, washed with ethyl acetate (2 × 25 mL), basified to pH 10 with 10% $Na_2CO_3$, extracted with $CHCl_3$ (6 × 30 mL), basified to pH 12 with NaOH, and extracted with ethyl acetate (4 × 20 mL). The organic extracts were combined, dried ($Na_2CO_3$), and then concentrated *in vacuo* to yield the crude amine **17a** as an oil which was used immediately without further purification.

**1,2-*O*-Isopropylidene-3,5-dideoxy-3-azido-5-*N*-(1′-carbomethoxy-2′-benzylethyl)-5-*N*-[(benzyloxy)carbonyl]-D-xylofuranose (17b).** Amine **17b** was prepared from azide **16b** (0.844 g, 1.65 mmol) according to the method above for **17a** using methanol (10 mL), water (40 mL), Pd/C (130 mg), and $NaBH_4$ (188 mg, 4.96 mmol). Workup as for **17a** afforded **17b** as an oil which was used immediately without further purification.

**1,2-*O*-Isopropylidene-3,5-dideoxy-3-acetamido-5-*N*-(1′-carbomethoxyethyl)-5-*N*-[(benzyloxy)carbonyl]-D-xylofuranose (18a).** The crude amine **17a** (663 mg, 1.68 mmol) was dissolved in 20 mL of 1:1 (v/v) dry pyridine/acetic anhydride and stirred for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (50 mL). This was washed with 10% $Na_2CO_3$ (3 × 20 mL), 1 M HCl (3 × 20 mL), and saturated NaCl (1 × 10 mL), and then dried ($Na_2SO_4$) to yield the *N*-acetate **18a** (443 mg, 60% from **16a**) as an oil: FAB HRMS exact mass calcd for $MH^+$ $C_{21}H_{29}N_2O_8$ 437.1924, found 437.1924.

**1,2-*O*-Isopropylidene-3,5-dideoxy-3-acetamido-5-*N*-(1′-carbomethoxy-2′-benzylethyl)-5-*N*-[(benzyloxy)carbonyl]-D-xylofuranose (18b).** Amine **17b** was dissolved in dry pyridine (40 mL) and dry acetic anhydride (50 mL) and stirred for 17 h at rt. Workup and isolation as for **18a** and column chromatography (7:3 ethyl acetate/hexane) afforded *N*-acetate **18b** (747 mg, 86% from **16b**) as a white solid, with partial separation of the diastereoisomers: diastereoisomer A 314 mg, diastereoisomer A+B 267 mg, diastereoisomer B 166 mg. Isomer A: mp 54−56 °C; FAB HRMS exact mass calcd for $MNa^+$ $C_{28}H_{34}NaN_2O_8$ 549.2213, found 549.2227; $MH^+$ $C_{28}H_{35}N_2O_8$ 527.2393, found 527.2369. Isomer B: mp 60−61 °C; $MH^+$ $C_{28}H_{35}N_2O_8$ 527.2393, found 527.2379; $MNa^+$ $C_{28}H_{34}NaN_2O_8$ 549.2213, found 549.2249.

**1,2-Dihydroxy-3,5-dideoxy-3-acetamido-5-*N*-(1′-carbomethoxyethyl)-5-*N*-[(benzyloxy)carbonyl]-D-xylofuranose (19a).** The acetate **18a** (200 mg, 0.45 mmol) was dissolved in TFA (2.27 mL) at 0 °C, and water (0.5 mL) was added dropwise. The reaction mixture was stirred (0 °C, 18 h), then warmed to rt, and cautiously neutralized (9 g of $Na_2CO_3$ in 80 mL of water). This solution was then extracted with ethyl acetate (8 × 25 mL). The combined organic fractions were dried ($Na_2SO_4$) and concentrated *in vacuo* to afford crude **19a** as an oil: 181 mg, quantitative. Hemiacetal **19a** was then used immediately in the next step.

**1,2-Dihydroxy-3,5-dideoxy-3-acetamido-5-*N*-(1′-carbomethoxy-2′-benzylethyl)-5-*N*-[(benzyloxy)carbonyl]-D-xylofuranose (19b).** The acetate **18b** (267 mg, 0.51 mmol) was dissolved in TFA (4.5 mL) at 0 °C, and water (0.5 mL) was added dropwise. The reaction mixture was then stirred between 0 and 4 °C for 21 h before cautiously being added to a solution of $Na_2CO_3$ (6.88 g, 64.9 mmol) in water (60 mL). The solution was then extracted with ethyl acetate (4 × 40 mL). The combined organic fractions were dried ($Na_2SO_4$) and concentrated *in vacuo*. Column chromatography on silica gel (step gradient, 6−10% methanol in $CHCl_3$) afforded **19b** (173 mg, 70%) as an oil which was used immediately for synthesis of **20b**.

***trans,trans*-*N*-(1′-Carbomethoxyethyl)-3,5-dihydroxy-4-acetamidopiperidine (20a).** The hemiacetal **19a** (181 mg, 0.46 mmol) was dissolved in 60 mL of methanol and 10% Pd/C (200 mg) added. This solution was then hydrogenated using a Parr hydrogenation apparatus (rt, 48 psi, 17 h). The solution was filtered through Celite and concentrated *in vacuo*. Recrystallization once from ethyl acetate/hexane and once again from ethanol/diethyl ether afforded **20a** (87 mg, 78%) as a white solid: mp 133−136 °C; FAB HRMS exact mass calcd for $MH^+$ $C_{10}H_{19}N_2O_5$ 247.1294, found 247.1273.

**(±)-*trans,trans*-*N*-(1′-Carbomethoxy-2′-benzylethyl)-3,5-dihydroxy-4-acetamidopiperidine ((±)-20b).** The hemiacetal **19b** (173 mg, 0.35 mmol) was dissolved in methanol (17 mL) and Pd/C (150 mg) added. This solution was then hydrogenated using a Parr hydrogenation apparatus at ambient temperature and 46 psi $H_2$ for 25 h. The solution was filtered through Celite, washed well with methanol, and concentrated *in vacuo*. Column chromatography on silica gel (8% methanol in $CHCl_3$) afforded **20b** (95 mg, 81%) as a white solid: mp 156−159 °C; FAB HRMS exact mass calcd for $MH^+$ $C_{17}H_{25}N_2O_5$ 337.1763, found 337.1768.

***trans,trans*-5-*N*-(1′-Carboxyethyl)-3,5-dihydroxy-4-acetamidopiperidine (5a).** Ester **20a** (25 mg, 0.1 mmol) was dissolved in 1 mL of 80% methanol/water, and $LiOH·H_2O$ (17 mg, 0.4 mmol) was added. The reaction mixture was stirred at rt for 35 min; then 1 M HCl was added dropwise to lower the pH to 1. Concentration *in vacuo* gave the crude product **5a**. This was purified by column chromatography on silica gel (7:2 2-propanol/water) to give the product **5a** (19 mg, 70%) as a white solid: mp 230−234 °C; FAB HRMS exact mass calcd for $MH^+$ $C_9H_{17}N_2O_5$ 233.1137, found 233.1138.

***trans,trans*-5-*N*-(1′-Carboxybenzylethyl)-3,5-dihydroxy-4-acetamidopiperidine ((±)-5b).** Methyl ester (±)-**20b** (18 mg, 0.05 mmol) was dissolved in water (0.15 mL), and methanol (0.60 mL) and $LiOH·H_2O$ (2.5 mg, 0.06 mmol) were added. After stirring at rt for 48 h, the reaction mixture was concentrated to near dryness *in vacuo*. The residue was dissolved in 10 mL of water (pH adjusted to 6) and then purified on Amberlite 120 ($H^+$ form) by initial elution with water followed by a step gradient of 2−5% $NH_4OH$. The product-bearing fractions were combined and concentrated under reduced pressure to give (±)-**5b** (9.5 mg, 52%) as a white solid: mp 195−197 °C; FAB HRMS exact mass calcd for $MNa^+$ $C_{16}H_{22}N_2O_5Na$ 345.1426, found 345.1486; $MH^+$ $C_{16}H_{23}N_2O_5$ 323.1607, found 323.1607.

**Inhibition Studies.** Inhibition constants for **5a** and (±)-**5b** were determined for sialidases from *S. typhimurium*, *C. perfringens*, and *V. cholerae* using 2-*O*-(*p*-nitrophenyl)-α-D-*N*-acetylneuraminic acid as substrate at 30 ± 0.2 °C. The reaction buffers employed for the three enzymes were, respectively, 50 mM Na-acetate, 100 mM NaCl, pH 5.5; 25 mM Na-acetate, pH 5.5; and 100 mM Na-acetate, 150 mM NaCl, 4 mM $CaCl_2$, pH 5.5. Reaction mixtures (700 μL) consisted of the appropriate buffer system containing substrate over a range bracketing $K_m$ and different concentrations of inhibitor **5a** or (±)-**5b**. Each reaction was initiated by addition of 2−5 mU of sialidase followed by removal of time point aliquots at 5, 10, and 15 min. Each aliquot was added to 800 μL of 100 mM $Na_2CO_3$, pH 10, and the absorbance at 400 nm due to *p*-nitrophenolate ($\epsilon$ = 19 200) determined to calculate the initial velocity. Data for initial velocity were obtained in duplicate measurements of each substrate/inhibitor concentration. $K_i$'s were estimated by nonlinear least-squares fit of the initial velocity data to the equation for competitive inhibition.

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**Supporting Information Available:** Spectral data for all compounds (24 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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