Chemoenzymatic Preparation of Novel Cyclic Imine Sugars and Rapid Biological Activity Evaluation Using Electrospray Mass Spectrometry and Kinetic Analysis

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Chemoenzymatic Preparation of Novel Cyclic Imine Sugars and Rapid Biological Activity Evaluation Using Electrospray Mass Spectrometry and Kinetic Analysis

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Abstract: Cyclic imine sugars were prepared by a novel chemoenzymatic strategy in which azido-sugars, constructed by enzymatic aldol reactions, were hydrogenated under acidic conditions. These cyclic imine sugars were found to be potent inhibitors of glycoprocessing enzymes having K_i's in the nanomolar and micromolar range for a variety of glycosidases. In comparison with their fully hydrogenated counterparts the cyclic imine sugars generally showed comparable or better inhibition against the glycosidases tested. Because these cyclic imines are so readily available and since imines are key intermediates in a variety of cycloadditions, condensations, and nucleophilic additions, they are valuable as versatile synthetic intermediates for the preparation of novel iminocyclitols and derivatives. An example of such synthetic utility is demonstrated by the synthesis of amino-iminocyclitol **24** via a three-center, two-component Strecker reaction. A novel method for rapidly screening glycosidase inhibitors using electrospray mass spectrometry is also described and shown to be capable of identifying potent fucosidase inhibitors for detailed kinetic analysis. Also, in the reductive amination of azido-sugars for the preparation of the five-membered ring iminocyclitol **8**, rhodium was found to exhibit superior face selectivity when compared to palladium or platinum catalysts.

The pivotal roles that carbohydrates play in biological processes have become increasingly evident.[1] Efforts to understand and control the bioprocessing of carbohydrates is contributing much to glycobiology and to the development of new therapeutic strategies.[2−8] Among agents capable of controlling glycoprocessing enzymes, iminocyclitols have been shown to be one of the most effective[9−11] as protonated iminocyclitols mimic the oxonium ion transition state of sugar transfer reactions (Figure 1).[11,12] Though several routes are available for the synthesis of these polyhydroxylated heterocycles,[9,10,13,14] the use of aldolases followed by hydrogenation of the azido-sugar products is one of the most versatile and has been used for the preparation of various five-, six-, and seven-membered ring iminocyclitols (Figure 2).[15−18] This paper describes a new strategy that allows facile chemoenzymatic synthesis of cyclic imine sugars which were found to be potent glycosidase inhibitors and useful building blocks for the synthesis of iminocyclitol derivatives and libraries. Also described is a method for rapidly identifying glycoprocessing enzyme inhibitors using electrospray mass spectrometry.

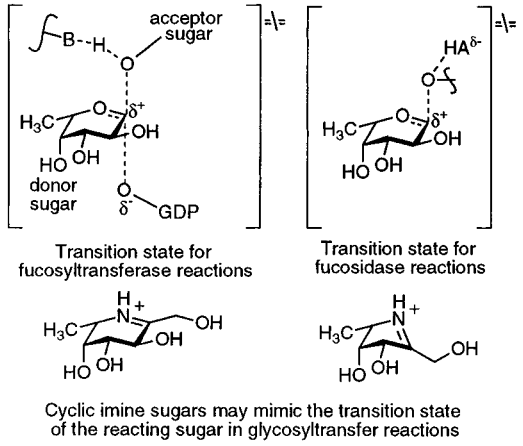

Figure 1. Postulated transition-state structures for glycosyl transferases and glycosidases and comparison with cyclic imine sugars.

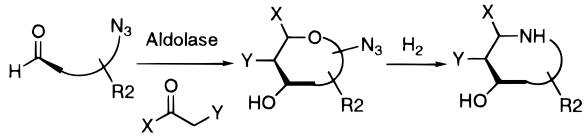

Figure 2. Preparation of azido-sugars and iminocyclitols using aldolases.

[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1997.

(1) Varki, A. *Glycobiology* **1993**, *3*, 97−130.
(2) Sears, P.; Wong, C.-H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 12086−12093.
(3) Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199−210.
(4) Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681−690.
(5) Lee, R. E.; Mikusová, P. J.; Brennan, P. J.; Besra, G. S. *J. Am. Chem. Soc.* **1995**, *117*, 11829−11832.
(6) Nishimura, Y.; Satoh, T.; Adachi, H.; Kondo, S.; Takeuchi, T.; Azetaka, M.; Fukuyasu, H.; Iizuka, Y. *J. Am. Chem. Soc.* **1996**, *118*, 3051−3052.
(7) von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418−423.
(8) Hendrix, M.; Wong, C.-H. *Pure Appl. Chem.* **1996**, *68*, 2081−2087.
(9) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340−347.
(10) Hughes, A. B.; Rudge, A. J. *Nat. Prod. Rep.* **1994**, 135−162.
(11) Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319−384.
(12) Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171−1202.
(13) Fleet, G. W. *J. Chem. Br.* **1989**, *25*, 287−292.
(14) Paulsen, H.; Todt, K. *Adv. Carbohydr. Chem.* **1968**, *23*, 115−232.
(15) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443−473.
(16) Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182−190.
(17) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 412−432, 521−546.
(18) Morís-Varas, F.; Xin-Hua, Q.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 7647−7652.

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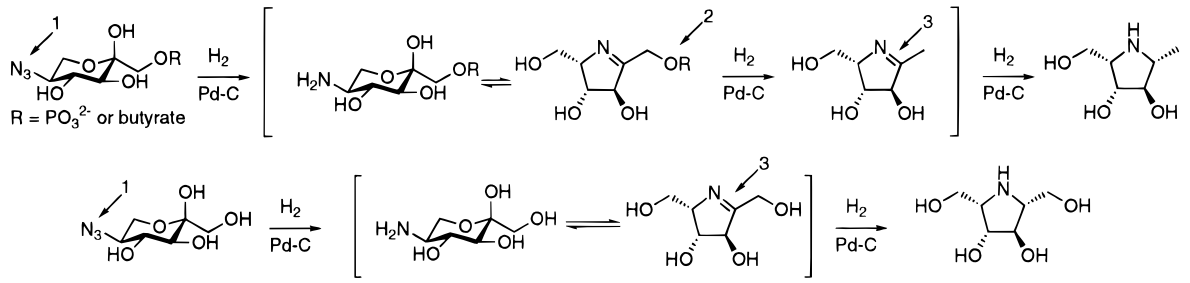
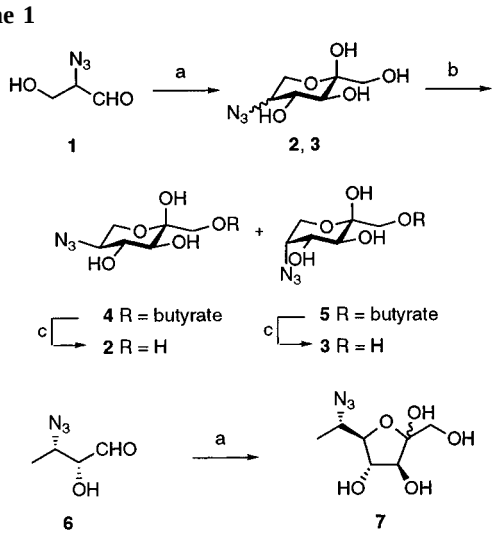


Figure 3. The steps involved in the hydrogenation of an azido-sugar to an iminocyclitol.

Scheme 1



[a] (a) (i) DHAP, rabbit muscle aldolase, pH 6.7, 25 °C, (ii) acid phosphatase, pH 5.0, 37 °C, 78% for two steps: (b) vinyl butyrate, PPL, THF, 85%; (c)[22] IRA 400 (OH), MeOH, 99%.

Azido-sugars obtained from aldolase reactions are usually converted to iminocyclitols by catalytic hydrogenation under hydrogen pressures of 50 psi or more. This one-pot hydrogenation procedure consists of two or three reduction steps: (1) reduction of azide to amine; (2) reductive deoxygenation in the case of phosphates and butyrates and; (3) intramolecular reductive amination (Figure 3).[19,20]

The utility of azido-sugars would be greatly expanded if the hydrogenation process could be stopped after the first reduction step. Trapping this intermediate would give access to cyclic imine sugars whose shape and charge should mimic the transition-state stabilized by glycoprocessing enzymes (Figure 1). Additionally, since imines are versatile intermediates capable of various reactions such as cycloadditions, condensations, and nucleophilic additions, the cyclic imine sugars should be useful for the synthesis of iminocyclitol derivatives and libraries.

We performed our studies on azido-sugars **2**−**5** and **7** which were prepared according to Scheme 1. The known enzymatic aldol condensation of 2-azido-3-hydroxy propanal (**1**) and dihydroxyacetone phosphate (DHAP) gave a diastereomeric mixture of **2** and **3** which were separated by silica gel chromatography after enzymatic butyration of the primary hydroxyl group. Azido-sugar **7** was prepared chemoenzymatically according to published procedures.[21]

Since hydrogenation of azido-sugar **2** to iminocylitol **8** had been performed using hydrogen pressures of 50 psi or more in previous reports,[23−26] we initially anticipated that the reductive amination step might be avoided by using a lower pressure of hydrogen. However, we found that an atmospheric pressure (15 psi) of hydrogen or even formic acid was sufficient to convert the azido-sugar **2** to iminocyclitol **8** within 10 min (Figure 4). In the case of the butyrated compound **4**, hydrogenation gave the deoxygenated iminocyclitol, as in the case of phosphate sugars[25] (Figure 3). Different catalysts were also investigated but each gave either complete hydrogenation or no reaction. Because the rearrangement of the amino-carbonyl sugar to its cyclic imine form is a fast intramolecular process and the subsequent reduction also very facile, it was not possible to prevent over-reduction under these conditions. We also observed that, among various catalysts, rhodium-alumina gave a higher face selectivity in the reductive amination of azido-sugar **2** to iminocyclitol **8** as seen in Figure 4. Unlike the completely face-selective reductive amination frequently observed for the preparation of six-membered ring iminocyclitols, palladium- or platinum-catalyzed reductive amination for the preparation of many five-membered ring iminocyclitols such as **8**[24−26] results in only 85−95% face selectivity at best. Rhodium is a superior catalyst for achieving high face-selectivity in the preparation of **8** and perhaps is useful for the highly face-selective reductive amination of other azido-sugars.

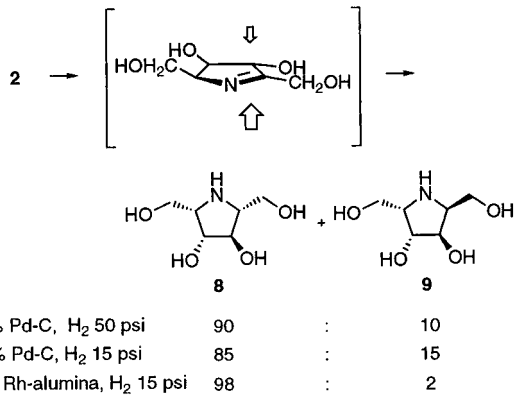


Figure 4. Face selectivity of the reductive amination depending on catalyst and reaction conditions.

To prevent the azido-sugars from full hydrogenation to iminocyclitols, an intermediate had to be trapped in some form. We decided to try trapping the amino-carbonyl sugar intermediate as its hydrochloride salt. Protonation of the amine would greatly disfavor rearrangement to the cyclic imine, a step required for the subsequent reductive amination, and should

(19) Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, J. A. J.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187−6196.

(20) Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280−6289.

(21) Qiao, L.; Murray, B. W.; Shimazaki, M.; Schultz, J.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 7653−7662.

(22) Pathak, V. P. *Syn. Commun.* **1993**, *23*, 83−85.

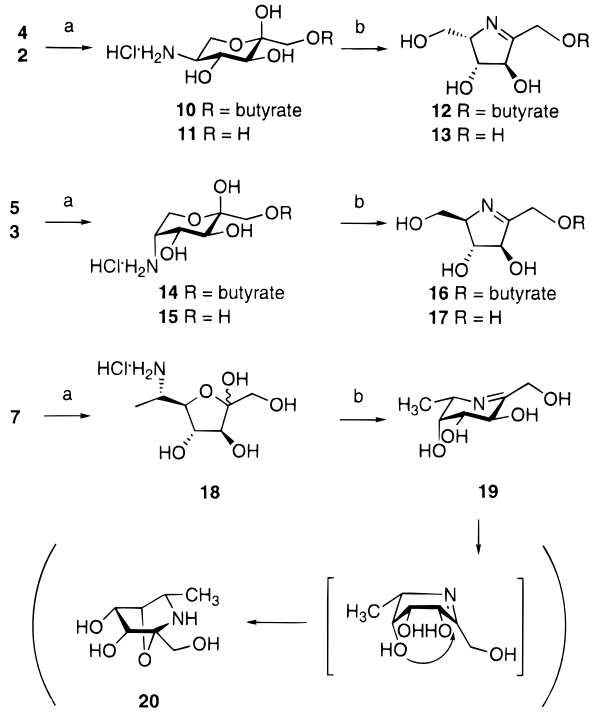
(23) Pederson, R. L.; Kim, M.-J.; Wong, C.-H. *Tetrahedron Lett.* **1988**, *29*, 4645−4648.

(24) Ziegler, T.; Straub, A.; Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 76−717.

(25) Kajimoto, T.; Chen, L.; Liu, K. K.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6678−6680.

(26) Hung, R. R.; Straub, J. A.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 3849−3855.

Scheme 2

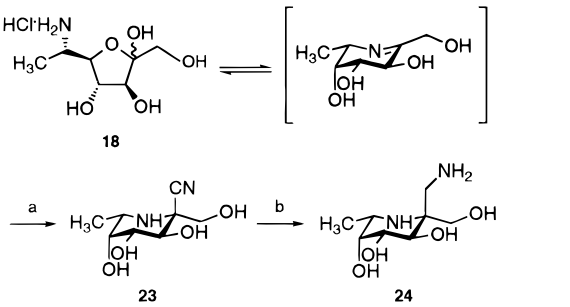


[a] (a) H_2 (1 atm), Pd-C, aq HCl, quant; (b) excess NaOH.

consequently stop the hydrogenation after the first step. Indeed, when **2** was hydrogenated in excess of aqueous hydrochloric acid, the amino-carbonyl sugar was trapped as its hydrochloride salt **11** (Scheme 5). Similarly, **3** and **7** were converted to the hydrochloride salt of their corresponding amino-carbonyl sugars. Even the butyrated compounds **10** and **14** were prepared without debutyration (Scheme 2). It should be noted that the butyrated azido-sugars did not give butyrated iminocyclitols but rather deoxy iminocyclitols when hydrogenated under the usual nonacidic conditions (Figure 3). When the hydrochloride salts were neutralized (pD 6.0) or put into basic solutions (excess NaOD), the compounds immediately reached an equilibrium in which the cyclic imine form was the major species present. The ratio of cyclic imine/amino-carbonyl sugar, however, differed depending on conformational factors. Upon adding base, compounds **14** and **15** formed exclusively the cyclic imines, whereas compounds **10** and **11** existed as a 70:30 mixture of the cyclic imine form and free amine-carbonyl sugar. The difference in the ratio of cyclic imine to amino-carbonyl is explained by the axial amine group in compounds **14** and **15**. This axial amine destabilizes the amino-carbonyl form, **14** and **15**, whereas compounds **10** and **11** contain all equatorial substituents. When compound **18** is neutralized, 75% exists as the cyclic imine and 25% exists in the bicyclic form **20**, which can arise through intramolecular addition of a hydroxyl to the imine (Scheme 2).[14,18] When using cyclic imines in biological studies or as a synthetic building block, it is desirable that the compound be prepared as the relatively stable amino-carbonyl salts until use as cyclic imine sugars are unstable.[14] Aqueous solutions of the amine-carbonyl salts were quite stable and could be stored for weeks at −20 °C without decomposition or decrease in inhibitory activity. Thus, trapping of the amino-carbonyl sugar as the hydrochloride salt not only prevented over-reduction to iminocyclitols but also prevented decomposition.

Imines are very useful intermediates suitable for various synthetic manipulations and have been utilized extensively in the preparation of libraries.[27] Because various azido-sugars[9,14,15,17] are readily prepared by chemoenzymatic methods

Scheme 3



[a] (a) KCN, dioxane, H_2O, 77%; (b) H_2, PtO_2, concentrated HCl, EtOH, 99%.

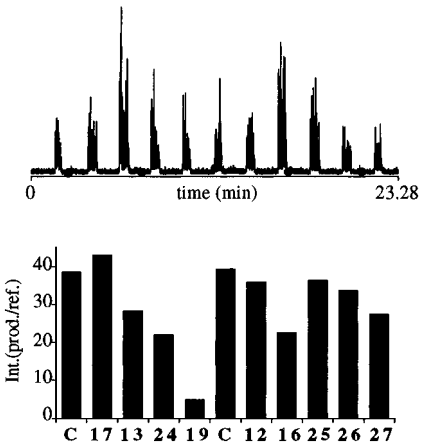


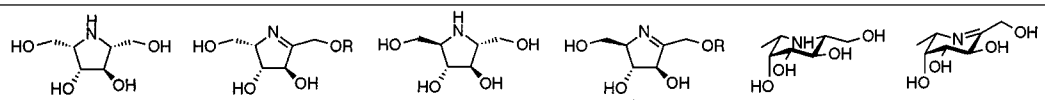
Figure 5. Screening of fucosidase inhibitors using electrospray mass spectrometry. (Top) Total ion current spectrum. (Bottom) A bargraph of product ion to reference ion intensity ratio generated from the mass spectrum for each of the potential inhibitors and two control samples that contained no inhibitor: **C**, control; **25**, deoxygalactonojirimycin; **26**, deoxynojirimycin; and **27**, deoxymannojirimycin.

and the reduction procedure is so facile, the cyclic imine sugars can be accessed as general chiral synthons for the synthesis of other interesting nitrogen heterocycles. As an example of such applications, we prepared a novel amino-iminocyclitol **24** by a three-center, two-component Strecker reaction followed by hydrogenation. This iminocylitol derivative possesses a highly nucleophilic primary amine, a primary hydroxyl group, and a secondary ring nitrogen which is known for its unusually low pK_a and nucleophilicity.[28] These functional groups make **24** a useful building block for the preparation of azafucoside libraries and tricomponent glycosyltransferase inhibitors that mimics the whole nucleotide-donor sugar-acceptor sugar transition state (Figure 2).[29] Other useful transformations of the cyclic-imine sugars are under investigation and will be reported in due course.

With a facile method available for creating libraries from imino-sugars, an efficient assaying method to determine the ability of these compounds to inhibit glycoprocessing enzyme inhibitors is required. For such purposes, we recently developed a method for screening galactosyltransferase inhibitors using quantitative electrospray mass spectrometry and showed that the method was capable of rapidly screening inhibitors and

(27) Gordon, E. M.; Gallop, M. A.; Patel, D. V. *Acc. Chem. Res.* **1996**, *29*, 144−154.
(28) Hanozet, G.; Pircher, H.-P.; Vanni, P.; Oesch, B.; Semenza, G. *J. Biol. Chem.* **1981**, *256*, 3703−3711.
(29) Palcic, M. M.; Heerze, L. D.; Srivastava, O. P.; Hindsgaul, O. *J. Biol. Chem.* **1989**, *264*, 17174−17181.

Table 1. Comparison of the Inhibition (K_i) of Various Glycosidases by Various Iminocyclitols and Cyclic Imines (Values in Micromolar)



8 | **13** R = H, **12** R = butyrate | **21** | **17** R = H, **16** R = butyrate | **22** | **19**

Enzyme	α-glucosidase (Brewer's yeast)	β-glucosidase (almond)	α-galactosidase (green coffee bean)	β-galactosidase (*E. coli*)	α-mannosidase (Jack bean)	α-fucosidase (bovine epididymis)
8[32]	**2.8**	**19**	50	no inhibition	3100	not determined
13	280±25	**16±1**	39±3	no inhibition	127±4	**5.5±0.1**
12	220±44	no inhibition	no inhibition	317±37	no inhibition	95±11
21[32]	**3.3**	**7.8**	no inhibition	no inhibition	no inhibition	no inhibition
17	**2.6±0.2**	**13±0.7**	no inhibition	276±51	**17±1**	381±71
16	167±29	no inhibition	no inhibition	253±44	no inhibition	336±54
22[21]	not determined	not determined	not determined	not determined	not determined	**0.0056**
19	no inhibition	no inhibition	no inhibition	no inhibition	no inhibition	**0.0069±0.0020**

measuring inhibition potency.[30] We found that this strategy is also applicable to the rapid screening of glycosidase inhibitors. In a representative case, using *p*-nitrophenyl-α-L-fucoside as the substrate, the rate of fucosidase catalyzed hydrolysis was measured by monitoring the formation of *p*-nitrophenol in the presence of inhibitors (1 μM), and as a result, **19** was identified to be the best inhibitor among the compounds tested (Figure 5). By using automated LC-MS which requires a reaction solution of 10 μL or less, we were able to screen a sample every 2 min. In addition to being sensitive, simple, fast, suitable for complete automation, and nonradioactive, this screening strategy is straightforward and readily applicable to various enzymes-inhibitor systems.[30] This novel screening method should greatly speed up the discovery of new inhibitors.

Cyclic aldimine sugars or their hydrates,[11,31,32] nojirimycin[33] and FR-900483[34,35] for example, are known to be potent inhibitors of glycosidases and as immunomodulators.[34,35] In many cases these cyclic aldimines show superior inhibition compared to the fully hydrogenated iminocyclitols.[11] Yet, the biological activity of cyclic ketimine sugars, which possess an extra hydroxymethylene group or "aglycon" have not been studied previously. To further examine the inhibition activities of the cyclic ketimines against various glycosidases, conventional inhibition analysis was performed (Table 1), and it was found that the cyclic ketimine sugars are comparable to or better than that of the fully hydrogenated iminocyclitols,[21,36] except for the inhibition of α-glucosidase by compound **13**. Most notable was the potent inhibition of α-mannosidase by **17**, as the fully hydrogenated version **21** showed no inhibitory activity against these enzymes. The presence of a butyrate moiety greatly influenced the inhibition properties, perhaps due to masking of a hydroxyl group and additional bulk. The six-membered ring ketimine **19**, identified as the most potent fucosidase inhibitor using electrospray mass spectrometry, also exhibited the most potent inhibitory activity against α-L-fucosidase from bovine epididymis with a K_i of 6.9 nM. Because the rearrangement of the amino-carbonyl sugar to the cyclic imine form is fast under nonacidic conditions, direct use of aqueous solutions of the hydrochloric salts of the amino-carbonyl sugars gave the same inhibition as using pre-formed cyclic imines. The acidic solutions were more convenient to use since they were more stable compared to the basic or neutral solutions of the inhibitors.

In summary, we have described a new chemoenzymatic strategy for the preparation of cyclic imine sugars that are potent inhibitors of various glycosidases and useful synthetic intermediates for the synthesis of iminocyclitol derivatives and libraries. We have also described a novel method for the rapid screening of glycosidase inhibitors using electrospray mass spectroscopy. The method proved to be useful for rapidly generating lead inhibitors for detailed kinetic analysis. As inhibitors of glycoprocessing enzymes are useful as pharmaceuticals,[4−7,37] the ability to generate libraries of iminosugars and to rapidly screen such compounds for biological activity is of medicinal importance. We are currently using these methods to synthesize and identifiy potent glycosyltransferase inhibitors.

Experimental Section

General Methods. The reagents used were purchased from Aldrich, Sigma, or TCI America and were of the highest purity available. The solvents were reagent grade and used as supplied except for THF which was distilled before use. Solvent evaporation was performed under reduced pressure below 30 °C using a Büchi rotary evaporator, followed by evacuation (<0.1 mmHg) to constant sample weight. High resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE instrument with fast atom ion bombardment (FAB). ^{1}H NMR spectra were obtained at 250, 400, or 500 MHz and ^{13}C NMR at 62.5, 100, or 125 MHz on a Bruker AMC-250, AMX-400, or AMX-500 instrument. Silica gel 60 (230−240 mesh) from Mallinckrodt was used in chromatography.

Hydrogenation of Azido-Sugar 2 to Iminocyclitol 8 Using Rhodium. A mixture of azido-sugar **2** (7 mg, 0.03 mmol) and 5% rhodium-

(30) Wu, J.; Takayama, S.; Wong, C.-H.; Siuzdak, G. *Chem. Biol*. in press.
(31) Wong, C.-H.; Provencher, L.; Porco, J. A.; Jung, S.-H.; Wang, Y.-F.; Chen, L.; Wang, R.; Steensma, D. H. *J. Org. Chem*. **1995**, *60*, 1492−1501.
(32) Witte, J. F.; McClard, R. W. *Tetrahedron Lett.* **1991**, *32*, 3927−3930.
(33) Inouye, S.; Tsuruoka, T.; Ito, T.; Niida, T. *Tetrahedron* **1967**, *23*, 2125−2144.
(34) Shibata, T.; Nakayama, O.; Tsurumi, Y.; Okuhara, M.; Terano, H.; Kohsaka, M. *J. Antibiot.* **1988**, *41*, 296−301.
(35) Kayakiri, H.; Takasse, S.; Setoi, H.; Uchida, I.; Terano, H.; Hashimoto, M. *Tetrahedron Lett*. **1988**, *29*, 1725−1728.
(36) Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett*. **1985**, *26*, 3127−3130.
(37) Jacob, G. S. *Curr. Biol*. **1995**, *5*, 605−611.
(38) Grover, A. K.; Cushley, R. J. *Biochim. Biophys. Acta* **1977**, *482*, 109−124.

alumina (4 mg) in H_2O (0.5 mL) was hydrogenated under an atmospheric pressure of hydrogen using a balloon with vigorous stirring for 3 h. The mixture was filtered through Celite, and the H_2O was lyophilized to give the title compound in quantitative yield. The face selectivity was determined by the integration of the signal for 2 protons of **9** at 4.24 ppm and the signal for 1 proton of **8** at 4.12 ppm.[20]

Enzymatic Acylation of Azido-Sugar Mixture 2, 3. To the azido-sugar mixture (107 mg, 0.52 mmol) in THF (3 mL) was added vinyl butyrate (1 mL) and porcine pancreatic lipase (PPL) (200 mg), and the mixture was stirred at room temperature for 5 days. Filteration through Celite followed by removal of the volatiles gave an oil which was chromatographed (EtOAc/hexanes/MeOH = 500/200/1) to yield diastereomerically pure **4** (100 mg, 69%) and diastereomerically pure **5** (23 mg, 16%) as pale yellow oils.

4: ^{1}H-NMR (400 MHz, CD_3OD): δ 0.95 (t, 3H, J = 7.4 Hz, butyrate), 1.64 (sex, 2H, J = 7.4 Hz, butyrate), 2.33 (t, 2H, J = 7.4 Hz, butyrate), 3.34−3.44 (m, 1H, H-5), 3.41 (d, 1H, J = 9.2 Hz, H-3), 3.55−3.65 (m, 2H, H-$6_{ax,\ eq}$), 3.71 (t, 1H, J = 9.4 Hz, H-4), 4.08 (d, 1H, J = 11.3 Hz, H-1_a), 4.11 (d, 1H, J = 11.3 Hz, H-1_b). ^{13}C-NMR (62.5 MHz, CD_3OD): δ 13.9, 19.4, 36.8, 61.4, 63.4, 66.0, 72.9, 74.6, 97.9, 174.812. HRMS (FAB$^+$) for $C_{10}H_{17}N_3O_6 + Na^+$: calcd 298.1015, found 298.1025.

5: ^{1}H-NMR (400 MHz, CD_3OD): δ 0.88 (t, 3H, J = 7.4 Hz, butyrate), 1.58 (sex, 2H, J = 7.4 Hz, butyrate), 2.68 (t, 2H, J = 7.4 Hz, butyrate), 3.56 (dd, 1H, J = 1.5 and 12.5 Hz, H-6_{ax}), 3.66 (d, 1H, J = 9.7 Hz, H-3), 3.74−3.75 (m, 1H, H-5), 3.94 (dd, 1H, J = 3.9 and 9.8 Hz, H-4), 3.98 (d, 1H, J = 12.5 Hz, H-6_{eq}), 4.00 (d, 1H, J = 11.2 Hz, H-1_a), 4.07 (d, 1H, J = 11.2 Hz, H-1_b). ^{13}C-NMR (62.5 MHz, CD_3OD): δ 13.9, 19.4, 36.9, 62.1, 64.2, 65.9, 69.8, 71.5, 98.3, 174.9. HRMS (FAB$^+$) for $C_{10}H_{17}N_3O_6 + Na^+$: calcd 298.1015, found 298.1020.

5-Amino-5-deoxy-L-*xylo*-2-hexulopyranoside Hydrochloride (11). A mixture of azido-sugar **2** (10 mg, 0.05 mmol) and 10% palladium−carbon (3 mg) in 0.5 N HCl_{aq} (200 uL) was hydrogenated under an atmospheric pressure of hydrogen, using a balloon, with vigorous stirring for 10 min. The mixture was filtered through Celite, and the H_2O was lyophilized to give the title compound in quantitative yield as a colorless solid. ^{1}H-NMR (400 MHz, D_2O): δ 3.27 (dt, 1H, J = 8.4 and 10.2 Hz, H-5), 3.51 (d, 1H, J = 11.8 Hz, H-1_a), 3.55 (d, 1H, J = 9.3 Hz, H-3), 3.71 (d, 1H, J = 11.8 Hz, H-1_b), 3.86 (dd, 1H, J = 9.4 and 10.3 Hz, H-4), 3.90 (d, 2H, J = 8.4 Hz, H-$6_{ax,\ eq}$). ^{13}C-NMR (100 MHz, D_2O): δ 51.8, 58.8, 63.6, 70.3, 70.9, 98.5. HRMS (FAB$^+$) for $C_6H_{13}N_1O_5 + H^+$: calcd 180.0872, found 180.0879.

2,*N*-Dehydro-2,5-dideoxy-2,5-imino-L-*gulo*-hexitol (13). Addition of 7 μL of 10 N NaOD to the NMR tube of the D_2O solution of **11** resulted in immediate formation of the cyclic imine **13**. ^{1}H-NMR (500 MHz, D_2O): δ 3.81 (dd, 1H, J = 4.0 and 9.2 Hz, H-6_a), 3.86 (dd, 1H, J = 4.0 and 9.2 Hz, H-6_b), 4.01−4.08 (m, 1H, H-5), 4.24 (t, 1H, J = 3.6 Hz, H-4), 4.42 (s, 2H, H-$1_{a,b}$), 4.61 (d, 1H, J = 3.6 Hz, H-3). ^{13}C-NMR (125 MHz, D_2O): δ 60.4, 60.6, 72.5, 78.8, 83.0, 182.2.

5-Amino-5-deoxy-D-*arabino*-2-hexulopyranoside Hydrochloride (15). A mixture of azido-sugar **3** (9 mg, 0.044 mmol) and 10% palladium−carbon (3 mg) in 1 N HCl_{aq} (100 μL) was hydrogenated under an atmospheric pressure of hydrogen, using a balloon, with vigorous stirring for 10 min. The mixture was filtered through Celite, and the H_2O was lyophilized to give the title compound in quantitative yield as a colorless solid. ^{1}H-NMR (400 MHz, D_2O): δ 3.51 (d, 1H, J = 9.6 Hz, H-1_a), 3.62 (d, 1H, J = 8.4 Hz, H-3), 3.63−3.66 (m, 1H, H-5), 3.69 (d, 1H, J = 9.6 Hz, H-1_b), 3.75 (d, 1H, J = 11.2 Hz, H-6_{ax}), 4.12 (dd, 1H, J = 4.0 and 8.4 Hz, H-4), 4.17 (d, 1H, J = 11.2 Hz, H-6_{eq}). ^{13}C-NMR (100 MHz, D_2O): δ 52.4, 58.5, 63.4, 66.4, 67.2, 98.0. HRMS (FAB$^+$) for $C_6H_{13}N_1O_5 + H^+$: calcd 180.0872, found 180.0877.

2,*N*-Dehydro-2,5-dideoxy-2,5-imino-D-*gluco*-hexitol 17. Addition of 7 μL of 10 N NaOD to the NMR tube of the D_2O solution of **15** resulted in immediate formation of the cyclic imine **17**. ^{1}H-NMR (500 MHz, D_2O): δ 3.70−3.75 (m, 2H, H-$6_{a,b}$), 3.79−3.82 (m, 1H, H-5), 4.00 (t, 1H, J = 4.5 Hz, H-4), 4.40 (s, 2H, H-$1_{a,b}$), 4.56 (d, 1H, J = 4.5 Hz, H-3). ^{13}C-NMR (125 MHz, D_2O): δ 60.3, 62.2, 75.9, 80.4, 83.4, 182.0.

1-Butyroyl 5-Amino-5-deoxy-L-*xylo*-2-hexulopyranoside Hydrochloride (10). A mixture of azido-sugar **4** (7.5 mg, 0.027 mmol) and 10% palladium−carbon (3 mg) in 0.5 N HCl_{aq} (140 μL) was hydrogenated under an atmospheric pressure of hydrogen, using a balloon, with vigorous stirring for 5 min. The mixture was filtered through Celite, and the H_2O was lyophilized to give the title compound in quantitative yield as a colorless solid. ^{1}H-NMR (500 MHz, D_2O): δ 0.86 (t, 3H, J = 7.5 Hz, butyrate), 1.57 (sex, 2H, J = 7.5 Hz, butyrate), 2.36 (t, 2H, J = 7.5 Hz, butyrate), 3.25 (dt, 1H, J = 6.5 and 10.5 Hz, H-5), 3.50 (d, 1H, J = 9.5 Hz, H-3), 3.85 (dd, 1H, J = 9.5 and 10.5 Hz, H-4), 3.86 (dd, 1H, J = 6.5 and 11.2 Hz, H-6_{ax}), 3.89 (t, 1H, J = 11.2 Hz, H-6_{eq}), 4.12 (d, 1H, J = 11.5 Hz, H-1_a), 4.20 (d, 1H, J = 11.5 Hz, H-1_b). ^{13}C-NMR (125 MHz, D_2O): δ 13.6, 18.7, 36.3, 52.0, 59.2, 65.5, 70.3, 71.9, 97.5, 176.5. HRMS (FAB$^+$) for $C_{10}H_{19}N_1O_6 + H^+$: calcd 250.1290, found 250.1286.

1-Butyroyl 2,*N*-Dehydro-2,5-dideoxy-2,5-imino-L-*gulo*-hexitol (12). Addition of 7 μL of 10 N NaOD to the NMR tube of the D_2O solution of **10** resulted in immediate formation of the cyclic imine **12**. ^{1}H-NMR (500 MHz, D_2O): δ 0.85 (t, 3H, J = 7.5 Hz, butyrate), 1.50 (sex, 2H, J = 7.5 Hz, butyrate), 2.11 (t, 2H, J = 7.5 Hz, butyrate), 3.81 (dd, 1H, J = 5.0 and 11.5 Hz, H-6_a), 3.85 (dd, 1H, J = 5.0 and 11.5 Hz, H-6_b), 4.03−4.08 (m, 1H, H-5), 4.23 (dd, 1H, J = 4.0 and 6.0 Hz, H-4), 4.41 (s, 2H, H-$1_{a,b}$), 4.59 (d, 1H, J = 4.0 Hz, H-3). ^{13}C-NMR (125 MHz, D_2O): δ 14.0, 20.1, 40.4, 60.4, 60.7, 72.6, 78.9, 83.1, 182.3, 185.0.

1-Butyroyl 5-Amino-5-deoxy-D-*arabino*-2-hexulopyranoside Hydrochloride (14). A mixture of azido-sugar **5** (8.4 mg, 0.030 mmol) and 10% palladium−carbon (3 mg) in 0.33 N HCl_{aq} (186 μL) was hydrogenated under an atmospheric pressure of hydrogen, using a balloon, with vigorous stirring for 5 min. The mixture was filtered through Celite, and the H_2O was lyophilized to give the title compound in quantitative yield as a colorless solid. ^{1}H-NMR (500 MHz, D_2O): δ 0.86 (t, 3H, J = 7.5 Hz, butyrate), 1.56 (sex, 2H, J = 7.5 Hz, butyrate), 2.38 (t, 2H, J = 7.5 Hz, butyrate), 3.60 (d, 1H, J = 10.0 Hz, H-3), 3.65−3.68 (m, 1H, H-5), 3.76 (dd, 1H, J = 1.5 and 14.0 Hz, H-6_{ax}), 4.14 (dd, 1H, J = 5.0 and 10.0 Hz, H-4), 4.19 (dd, 1H, J = 2.0 and 14.0 Hz, H-6_{eq}), 4.19 (s, 2H, 1-$H_{a,b}$). ^{13}C-NMR (125 MHz, D_2O): δ 13.6, 18.6, 36.3, 53.0, 59.5, 66.4, 66.8, 68.9, 97.6, 177.1. HRMS (FAB$^+$) for $C_{10}H_{17}N_1O_5 + H^+$: calcd 250.1291, found 250.1295.

1-Butyroyl 2,*N*-Dehydro-2,5-dideoxy-2,5-imino-D-*gluco*-hexitol (16). Addition of 7 μL of 10 N NaOD to the NMR tube of the D_2O solution of **14** resulted in immediate formation of the cyclic imine **16**. ^{1}H-NMR (500 MHz, D_2O): δ 0.86 (t, 3H, J = 7.5 Hz, butyrate), 1.52 (sex, 2H, J = 7.5 Hz, butyrate), 2.12 (t, 2H, J = 7.5 Hz, butyrate), 3.69−3.75 (m, 2H, H-$6_{a,b}$), 3.77−3.81 (m, 1H, H-5), 3.98 (t, 1H, J = 4.5 Hz, H-4), 4.40 (s, 2H, H-$1_{a,b}$), 4.55 (d, 1H, J = 4.5 Hz, H-3). ^{13}C-NMR (125 MHz, D_2O): δ 14.1, 20.2, 40.4, 60.4, 62.2, 76.1, 80.5, 83.6, 182.5, 185.0.

6-Amino-6,7-dideoxy-L-*galacto*-2-septulofuranoside Hydrochloride (18). The title compound was obtained in a manner analogous to the procedure for compound **11**. ^{1}H-NMR (500 MHz, D_2O): δ 1.16 (d, 3H, J = 6.8 Hz, H-7), 3.31 (dq, 1H, J = 8.8 and 6.9 Hz, H-6), 3.37 (d, 1H, J_{AB} = 12.2 Hz, H-1), 3.40 (d, 1H, J_{AB} = 12.2 Hz, H-1), 3.54 (dd, 1H, J = 8.9 and 6.8 Hz, H-5), 3.91 (d, 1H, J = 7.9 Hz, H-3), 3.99 (dd, 1H, J = 7.8 and 6.8 Hz, H-4). ^{13}C-NMR (125 MHz, D_2O): δ 14.64, 52.65, 63.00, 76.58, 77.40, 81.46, 102.92. HRMS (FAB$^+$) for $C_7H_{15}N_1O_5 + H^+$: calcd 194.1028, found 194.1022.

2,*N*-Dehydro-2,6-imino-2,6,7-trideoxy-L-*glycero*-D-*manno*-heptitol (19). Addition of 10 N NaOD to the NMR tube of the D_2O solution of **18** resulted in immediate formation of the cyclic imine **19**. ^{1}H-NMR (500 MHz, D_2O): δ 0.92 (d, 3H, J = 6.6 Hz, H-7), 3.06 (q, 1H, J = 6.7 Hz, H-6), 3.35 (d, 1H, J_{AB} = 11.5 Hz, H-1), 3.43 (d, 1H, J_{AB} = 11.4 Hz, H-1), 3.49 (d, 1H, J = 10.0 Hz, H-3), 3.59 (m, 1H, H-5), 3.63 (dd, 1H, J = 10.0 and 3.2 Hz, H-4). ^{13}C-NMR (125 MHz, D_2O): δ 17.04, 47.08, 65.93, 69.82, 72.31, 73.08, 171.15.

2-Cyano-2,6-imino-2,6,7-trideoxy-L-*glycero*-D-*manno*-heptitol (23). To the amine hydrochloride **18** (243 mg, 1.06 mmol) was added dioxane (8 mL) under Ar, followed by a solution of KCN (104 mg, 1.59 mmol) in water (1.6 mL). The resulting mixture was then stirred at 20 °C for 1 week. The volatiles were evaporated, and the residue chromatographed using a mixture of CH_3CN and H_2O (10:1, R_f = 0.5) to yield the desired compound as a pale yellow solid (166 mg, 77%): ^{1}H-NMR (400 MHz, D_2O): δ 0.95 (d, 3H, J = 6.6 Hz, H-7), 2.93 (dq, 1H, J =

6.5 and 1.3 Hz, H-6), 3.39 (d, 1H, J = 10.0 Hz, H-3), 3.50 (d, 1H, J = 11.4 Hz, H-1), 3.53 (dd, 1H, J = 10.0 and 3.2 Hz, H-4), 4.59 (dd, 1H, J = 3.2 and 1.3 Hz, H-5), 3.81 (d, 1H, J = 11.5 Hz, H-1). ^{13}C-NMR (100 MHz, D_2O): δ 15.95, 50.27, 64.03, 64.34, 67.95, 71.15, 72.56, 119.11. HRMS (FAB$^+$) for $C_8H_{14}N_2O_4 + Na^+$: calcd 225.0851, found 225.0858.

2-Aminomethyl-2,6-imino-2,6,7-trideoxy-L-*glycero*-D-*manno*-heptitol (24). Cyanoazasugar **23** (30 mg, 0.15 mmol) was dissolved in EtOH (10 mL). Concentrated HCl (0.1 mL) and platinum oxide (20 mg) were then added, and the mixture was shaken under H_2 gas (50 psi) for 3 h at room temperature. The catalyst was removed by filtration over Celite, and the cake was washed with a small amount of EtOH followed by evaporation of the filtrate to give the desired compound (41 mg, 99%): ^{1}H-NMR (400 MHz, D_2O): δ 1.20 (d, 3H, J = 6.6 Hz, H-7), 3.31 (d, 1H, J = 13.9 Hz, CH_2N), 3.45 (dq, 1H, J = 6.6 and 1.2 Hz, H-6), 3.60 (d, 1H, J = 13.7 Hz, CH_2N), 3.76−3.77 (m, 2H, H-3 and H-4), 3.81 (d, 1H, J = 12.5 Hz, H-1), 3.85 (bs, 1H, H-5), 3.98 (d, 1H, J = 12.5 Hz, H-1). ^{13}C-NMR (100 MHz, D_2O): δ 13.66, 37.04, 51.07, 61.14, 61.33, 68.21, 69.23, 69.44. HRMS (FAB$^+$) for $C_8H_{18}N_2O_4 + H^+$: calcd 207.1345, found 207.1340.

Screening of Fucosidase Inhibitors Using Electrospray Mass Spectrometry. Each reaction mixture contained *p*-nitrophenyl-α-L-fucopyranoside (600 μM), α-fucosidase from bovine epididymis (2.4 mU/100 μL), and inhibitor (1 μM). The individual reactions were carried out on a 100 μL scale in a 0.5 mL eppendorf tube at 25 °C. After 20 min, a 10 μL aliquot was taken out and mixed with MeOH (200 μL), and an internal standard (300 μM aqueous 2,4-dinitrophenol, 5 μL) was added. The quenched reaction mixtures were directly injected into a PE SCIEX API100 electrospray mass spectrometer via the HP 1090 HPLC autosampler (at a flow rate of 0.2 mL/min, injection/0.4 min) with an orifice potential of 50 V in the negative ionization mode after which the relative peak intensities between 4-nitrophenol and 2,4-dinitrophenol were measured.

General Procedure for Inhibition Assay. Inhibition analyses were performed at 25 °C in 0.1 M HEPES buffer, pH 6.8, except for α-fucosidase (assayed at 25 °C in 50 mM sodium acetate buffer, pH 6.0). The amount of enzyme added in each assay was 0.05 unit/mL. For each inhibitor, four inhibitor concentrations ranging from 0 to 3 times K_i were used. For each of the inhibitor concentrations, four or five substrate concentrations spanning from 0.4 to 4 K_m were used to obtain a set of data. *p*-(Nitrophenyl) glycosides were used as substrates, and the release of *p*-nitrophenol was monitored at 400 nm for 5 min. The earliest portion of this data that showed a steady state[28,38] was used to obtain velocity data. The mode of inhibition was evaluated by double reciprocal analysis. Inhibition constants were derived from a best fit of the initial velocity data to the kinetic equation for competitive inhibition by a nonlinear, least square method using the program Grafit.

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Supporting Information Available: ^{1}H- and ^{13}C-NMR spectra for all new compounds (28 pages). See any current masthead page for ordering and Internet access instructions.

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