Nanomolar versus Millimolar Inhibition by Xylobiose-Derived Azasugars: Significant Differences between Two Structurally Distinct Xylanases

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Abstract: The synthesis of xylobiose-derived nitrogen-containing inhibitors of xylanase is described starting with accessible precursors through efficient synthetic schemes. Four disaccharides were identified as powerful competitive inhibitors of the retaining family 10 xylanase, Cex, from *Cellulomonas fimi*, namely imidazole (K_i = 150 nM), lactam oxime (K_i = 370 nM), isofagomine (K_i = 130 nM), and deoxynojirimycin (K_i = 5800 nM) derivatives of xylobiose. By contrast, none of the compounds inhibited the family 11 xylanase, Bcx, from *Bacillus circulans*, to an appreciable extent. Two possible explanations are provided for the discrimination exhibited by the imidazole and the lactam oxime. One explanation relates to the different active site locations of the acid/base residue in the two enzymes: anti to the C1−O5 bond for the family 10 Cex and syn for the family 11 Bcx. The other explanation concerns proposed differences in the transition state conformation for the two enzymes: half-chair for Cex and boat for Bcx. The reasons for the difference in inhibition values between Cex and Bcx for the isofagomine and deoxynojirimycin derivatives are less clear-cut but may be ascribed to destabilizing steric and electrostatic interactions between the inhibitors and an essential tyrosine residue in the active site of Bcx.

Xylanases (EC 3.2.1.8) are glycosidases that catalyze the hydrolytic cleavage of β-1,4-linked polymers of D-xylose. Their potential use in biomass conversion, fuel production, and bread baking has attracted considerable interest.[1,2] Further, they already enjoy widespread use in the paper industry as agents to facilitate lignin removal, thereby reducing the need for bleaching chemicals.[3,4] As a consequence, considerable effort has been expended in the study of xylanases, both in their cloning and expression and in studies of structure, stability, and catalytic mechanism, as reviewed in refs 5−9. Within the sequence-based classification of glycosidases, most xylanases are found in families 10 and 11, with a few being classified into families 5 and 43.[10] Despite the fact that members of families 10 and 11 catalyze the same reaction, cleavage of the glycosidic bond with net retention of anomeric configuration, there are significant differences between the two groups, both in three-dimensional structure and in the substrate specificity. Family 10 xylanases are members of the so-called clan GH-A grouping of glycosidase families, the catalytic domains of which fold as a $(\beta/\alpha)_8$ barrel, with similar locations of the key active site groups.[10,11] Many members of this family are capable of cleaving both xylan and cellulose. Family 11 xylanases, smaller enzymes that only cleave xylan, belong to clan GH−C and fold as a primarily β-sheet jelly roll.

The catalytic mechanism of retaining glycosidases was initially proposed by Koshland, who suggested that the active-site contains two key catalytic groups, one with the role of acid/base and the other functioning as a nucleophile (Figure 1).[12] It has since been shown in the vast majority of cases that these two groups are carboxyl groups and that a covalent intermediate is formed, which then undergoes hydrolysis to afford a hemiacetal with net retention of anomeric stereochemistry.[7,8] The transition states leading to and from the covalent intermediate have substantial oxacarbenium ion character, as indicated by kinetic isotope effects and by the effects of electron-withdrawing substituents on the sugar ring upon reaction rate.[7,8,13] Detailed structural insights into stable species along the reaction coordinate have now been obtained for several glycosidases by solving structures of the free enzyme, the Michaelis complex, the 2-deoxy-2-fluoroglycosyl−enzyme intermediate, and the product complex. Such studies on the retaining clan GH-A cellulase Cel5A from *Bacillus agaradhaerens* revealed substantial distortion of the sugar moiety in the −1 binding subsite in the Michaelis complex into a $^1S_3$ skew-boat and relaxation of this conformation into a $^4C_1$ chair in the covalent intermedi-

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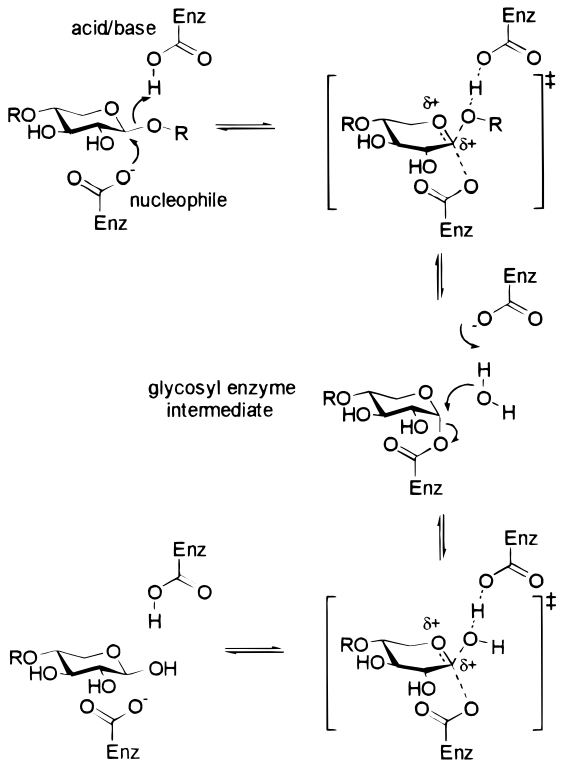
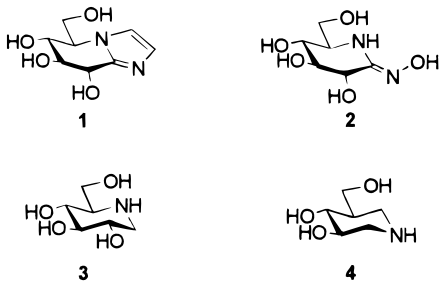


Figure 1. Hydrolytic mechanism of a retaining β-xylanase proceeding through an intermediate in a chair conformation.

ate.[14] This was accompanied by almost no change in the position of the protein atoms. A similar $^4C_1$ chair was observed for the 2-deoxy-2-fluoro-cellobiosyl− and 2-deoxy-2-fluoroxylobiosyl−enzyme intermediates formed on the family 10 xylanase/cellulase Cex from *Cellulomonas fimi*[15,16] as well as the cellobiosyl−enzyme intermediate formed on a double mutant of Cex without need for the C-2 fluorine substituent.[17] Interestingly, the equivalent 2-deoxy-2-fluoroxylobiosyl−enzyme intermediate trapped on family 11 xylanases accommodates the −1 sugar in a distorted $^{2,5}B$ conformation.[18,19] Such a conformation is attained more easily for xylosides compared to glucosides, owing to the lack of a bulky hydroxymethyl substituent that is forced into a pseudoaxial orientation in the latter case. The $^{2,5}B$ conformation places O5, C5, C1, and C2 in a plane, just as is required to accommodate the double bond character that develops between C1 and O5 in the proposed transition state. It has therefore been speculated that family 11 xylanases carry out catalysis via a boat transition state, rather than a half-chair, and that this feature dictates the absolute specificity for xylan hydrolysis exhibited by enzymes of this family.[18,19]

To gain further insight into the details of the hydrolytic mechanism of glycosidases, specific inhibitors, ideally transition state analogues, are necessary which can act as mechanistic and structural probes. A diverse array of extremely potent, basic, nitrogen-containing inhibitors has been developed over the years, and these have been found to be of great utility in the study of the glycosidase mechanism.[20,21] Examples of some of the more successful and general inhibitors include molecules having an sp^2-hybridized nitrogen atom in place of the glycosidic oxygen, such as the imidazole (**1**)[22,23] and the lactam oxime (**2**),[24,25] and



various amidines and amidrazones.[26] Another class of inhibitors includes those having an sp^3-hybridized nitrogen atom in place of the sugar endocyclic oxygen or carbon 1, such as deoxynojirimycin (**3**)[20,27] and isofagomine (**4**).[28,29] Most of these inhibitors have, at some stage, been proposed to act as mimics of the transition states of the enzyme-catalyzed mechanism of glycoside hydrolysis, although only a few studies have attempted to determine the degree to which some of these compounds actually resemble the transition state.[30]

The planar nature of the sugar ring and the build-up of positive charge on C1 and the ring oxygen are key features of the hypothetical transition state that are mimicked to varying degrees by the aforementioned inhibitors. Additionally, it has been suggested that the trajectory of protonation of the glycosidic oxygen in the first step of the catalytic mechanism is an important feature.[31] This trajectory is of course dictated by the positioning of the acid catalyst; thus, a classification of some glycosidases into two classes based on a syn- or anti-protonation trajectory, relative to the C1−O5 bond, has been proposed by Heightman and Vasella (Figure 2).[32] Inhibitors such as the imidazole (**1**) and lactam oxime (**2**) should therefore function as good inhibitors of anti-protonators. In this vein, the X-ray crystal structure of a cellobiose-derived imidazole bound to Cel5A from *B. agaradhaerens*, an anti-protonator, has been determined, and this structure clearly shows the lateral protonation of this inhibitor.[33]

Although there are many nitrogen-containing inhibitors known for monosaccharidases, there are but few of the corre-

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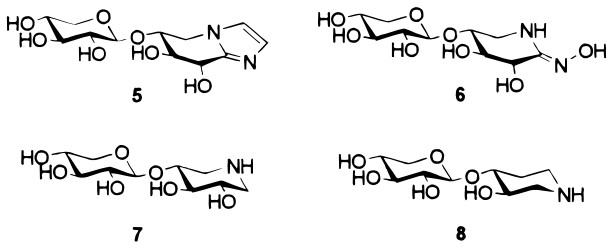
sponding inhibitors known for glycan hydrolases. Glycan hydrolases have a low affinity for monosaccharide substrates and inhibitors and typically require at least disaccharide glycosides as minimal substrates as a consequence of their multiple binding subsites. The development of the corresponding disaccharide inhibitors with residues that occupy the −1 and −2 binding subsites is synthetically challenging and has been a stumbling block for the preparation of effective inhibitors for glycan hydrolases. The syntheses of several cellobiose- and cellotriose-derived cellulase inhibitors have been described in recent years.[26,34−37] Further, several natural product inhibitors which bind in the −1 and −2 subsites have been known for some time; examples include molecules such as the adiposins and amylostatins,[38] which inhibit amylase and are closely related to acarbose, and allosamidin and its derivatives, which are inhibitors of some chitinases.[39] However, aside from recent studies on Cel5A with a cellobiose-derived imidazole,[33] an earlier complex of hevamine, a Chitinase from *Hevea brasiliensis*, with allosamidin,[40] and the structure of bulgecin A with a lytic transglycosylase from *Escherichia coli*,[41] there have been no structural studies of complexes of β-glycanases with tight-binding inhibitors and relatively few kinetic studies with a single enzyme and a series of inhibitors.

The xylanases Cex and Bcx of families 10 and 11, respectively, provide an ideal system for such model studies. Not only are high-resolution crystal structures of both enzymes and their glycosyl−enzyme intermediates available but also the enzymatic reactions likely proceed via different transition states. Further, they differ in their protonation trajectory of the glycosidic oxygen in the catalytic mechanism, the family 10 Cex being an anti-protonator and the family 11 Bcx being a syn-protonator. In addition, the synthetic chemistry necessary for the introduction of the nitrogen atom(s) into potential inhibitors is more accessible for xylosides due to the absence of a hydroxymethyl substituent and thus of a stereogenic center at C5.

To conduct detailed kinetic and structural studies of xylanases, the synthesis of a number of disaccharide inhibitors was undertaken. The compounds of interest were the xylobiose-derived imidazole (**5**), lactam oxime (**6**), deoxynojirimycin (**7**), and isofagomine (**8**) each of which was chosen as being representative of known, potent, nitrogen-containing inhibitors.



Synthesis of the Imidazole (5), the Lactam Oxime (6), and Xylobiodeoxynojirimycin (7). The syntheses of the imidazole

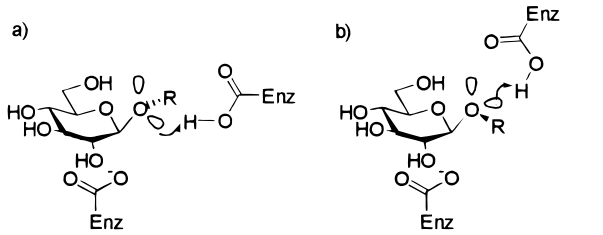
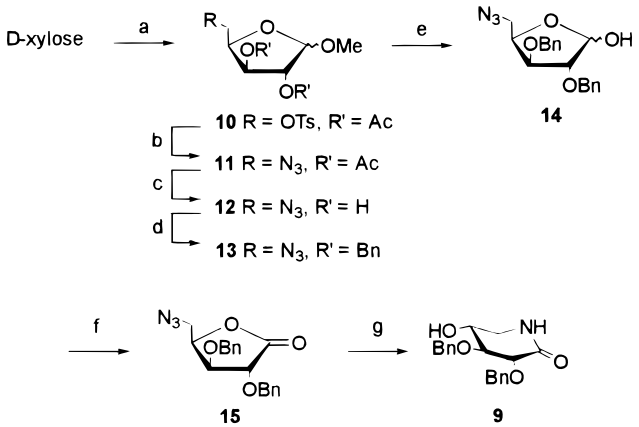


Figure 2. Protonation trajectories of the glucosidic bond in the catalytic hydrolysis mechanism proposed by Heightman and Vasella:[32] (a) anti-protonation and (b) syn-protonation.

Scheme 1[a]



[a] (a) Reference 42. (b) NaN_3, DMPU, 70°, 45% from D-xylose. (c) NaOMe, MeOH, 90%. (d) BnBr, NaH, THF. (e) H_2SO_4, H_2O, AcOH, 43% over two steps. (f) CrO_3, Pyr. (g) Bu_3P, H_2O, THF, 65% over two steps.

(**5**), the lactam oxime (**6**), and the deoxynojirimycin derivative (**7**) required the partially protected lactam (**9**) as a common precursor. D-Xylose was converted to the known tosylate (**10**)[42] and then treated with NaN_3 in DMPU to afford the azide (**11**) (Scheme 1). Deacetylation with NaOMe in MeOH gave the diol (**12**), which was benzylated to furnish the dibenzyl ether (**13**). Hydrolysis of the methyl glycosides with H_2SO_4 in AcOH/H_2O gave the hemiacetal (**14**), which was oxidized with CrO_3/pyridine, affording the lactone (**15**). Staudinger reduction of **15** occurred with spontaneous cyclization to afford the desired lactam (**9**).[43]

To convert the lactam (**9**) to the imidazole (**5**) required activation of the carbonyl group as a thionolactam. Thionation of **9** with Lawesson's reagent was troublesome and furnished the thionolactam (**16**) in poor yield. A higher yield was achieved when **9** was acetylated to give **17** prior to thionation, affording **16** in 82% over two steps (Scheme 2). Conversion of **16** to the imidazole (**18**) following a protocol of Granier et al.,[22] using aminoacetaldehyde dimethyl acetal in the presence of mercuric acetate, followed by treatment with aqueous acid, proceeded smoothly (78% yield) and led also to the D-*lyxo* imidazole **19** (11% yield). D-Xylosylation of **18** with the trichloroacetimidate (**20**)[44] was sluggish, giving the disaccharide (**21**) in a yield of 15% (Scheme 3). In this case the reaction was most likely complicated by the presence of the basic nitrogen of the imidazole moiety. Deprotection of **21** by sequential catalytic hydrogenolysis and hydrolysis of the acetate protecting groups afforded the xylobiose-derived lactam (**5**) in 80% yield.

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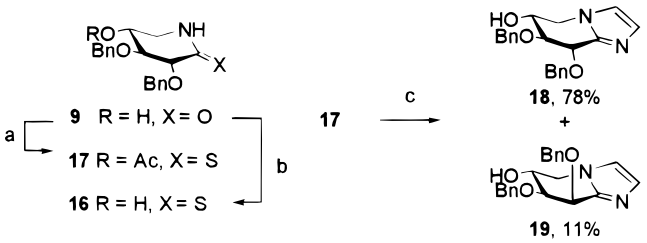
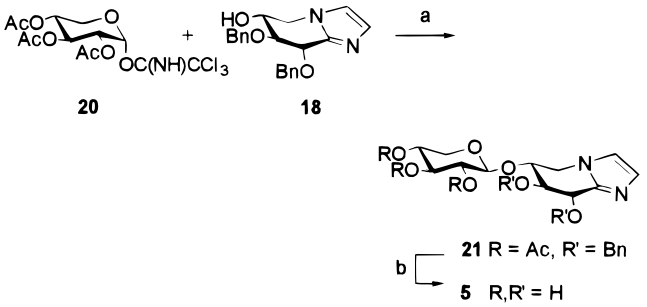
Scheme 2[a]



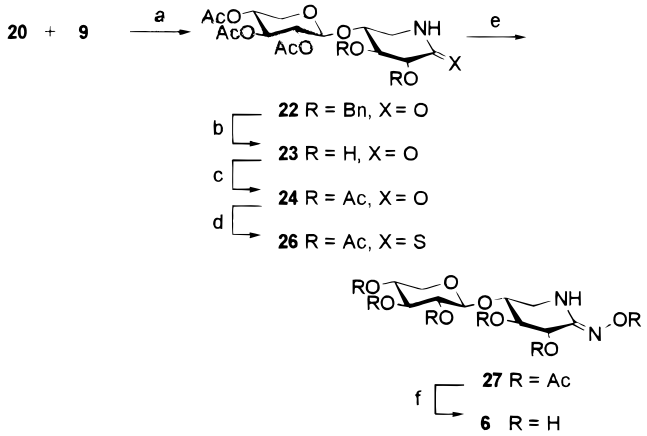
[a] Reagents: (a) (i) Ac_2O, Pyr; (ii) Lawesson's reagent, C_6H_6, 82%. (b) Lawesson's reagent, C_6H_6, 29%. (c) (i) aminoacetaldehyde dimethyl acetal, $Hg(OAc)_2$, THF; (ii) toluene, H_2O, TsOH.

Scheme 3[a]



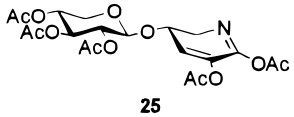
[a] Reagents: (a) $BF_3.Et_2O$, $(CH_2Cl)_2$, 15%. (b) (i) Pd/C, H_2, MeOH; (ii) aqueous NH_3, MeOH, 80%.

Scheme 4[a]



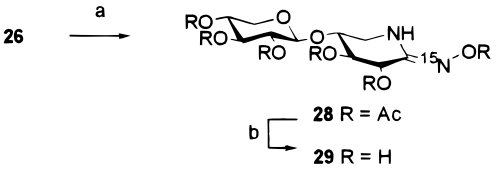
[a] Reagents: (a) $BF_3.Et_2O$, $(CH_2Cl)_2$, 68%. (b) Pd/C, MeOH. (c) Ac_2O, Pyr, 94% over two steps. (d) Lawesson's reagent, C_6H_6, 89%. (e) (i) $NH_2OH.HCl$, $NaHCO_3$, MeOH; (ii) Ac_2O, Pyr, 66%. (f) NaOMe, MeOH, 89%.

To achieve the synthesis of **6**, the D-xylonolactam (**9**) was D-xylosylated with **20** in the same manner as for the imidazole (**18**), providing **22** in 68% yield (Scheme 4). Hydrogenolysis of **22** furnished the intermediate diol (**23**), which was treated briefly with pyridine and acetic anhydride to give **24** (94% yield). In this case, a prolonged treatment of the diol (**23**) with pyridine and acetic anhydride afforded imidate (**25**) as the major



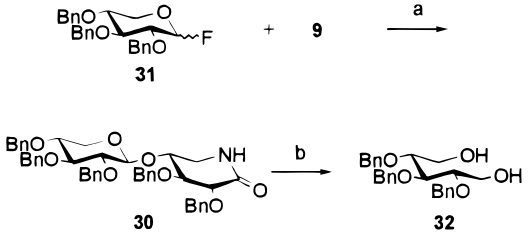
product. The lactam (**24**) was treated with Lawesson's reagent to afford the thionolactam (**26**) as an unstable yellow foam. Treatment of **26** with hydroxylamine in methanol and acetylation of the residue after removal of the solvent afforded the oxime

Scheme 5[a]



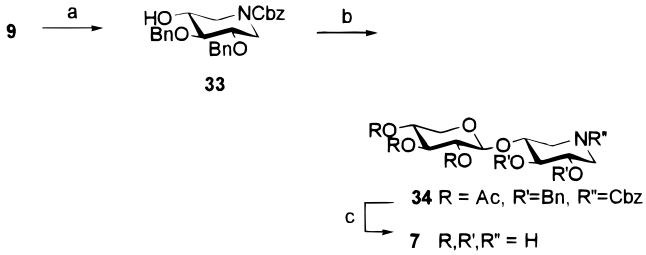
[a] Reagents: (a) (i) $^{15}NH_2OH.HCl$, $NaHCO_3$, MeOH; (ii) Ac_2O, Pyr, 62%. (b) NaOMe, MeOH, 82%.

Scheme 6[a]



[a] Reagents: (a) BF_3·Et_2O, CH_2Cl_2. (b) $LiAlH_4$, Et_2O.

Scheme 7[a]



[a] Reagents: (a) (i) $LiAlH_4$, THF; (ii) H_2O then CbzCl, 83%. (b) **20**, $BF_3.Et_2O$, $(CH_2Cl)_2$, 68%. (c) (i) NaOMe, MeOH; (ii) Pd/C, H_2, EtOH, 78%.

ester (**27**). Compound **27** was cleanly deacetylated with sodium methoxide in methanol to afford the lactam oxime (**6**). In an identical manner, the thionolactam (**26**) was treated with an excess of $^{15}NH_2OH$ to afford, first, the ^{15}N-labeled oxime ester (**28**) and, subsequently, the ^{15}N-labeled lactam oxime (**29**) (Scheme 5). The oxime (**6**) was assigned the (*Z*)-configuration on the basis of a small chemical shift difference (δ 3.25 ppm) between the ^{13}C NMR resonance for C1 in the lactam oxime (**6**) and the oxime ester (**27**).[25] In addition, the ^{15}N NMR spectrum of (**27**) showed a single resonance, δ 252.8, consistent with the assignment of the tautomer with an exocyclic C=N bond to **6**.[25]

The conversion of the lactam (**9**) into the xylobiose-derived deoxynojirimycin (**7**) was next addressed. While reduction of a benzylated disaccharide such as **30** with $LiAlH_4$ seemed appealing, a preliminary, small-scale experiment with **30** (prepared from **9** and **31**[45]) indicated that the reduction caused elimination, affording the xylitol (**32**) (Scheme 6). Instead, reduction of the lactam (**9**) with lithium aluminum hydride followed by quenching of the reaction mixture with water and addition of benzyl chloroformate allowed for the direct isolation of the Cbz derivative (**33**) (Scheme 7). D-Xylosylation of **33** with the trichloroacetimidate (**20**) afforded the disaccharide (**34**). Deprotection of **34** was achieved by sequential treatment with catalytic sodium methoxide in methanol and, subsequently, catalytic hydrogenolysis to afford **7** in 78% yield.

Synthesis of the Isofagomine (8). Synthesis of the isofagomine (**8**) demanded an efficient synthesis of the piperidine (**35**).

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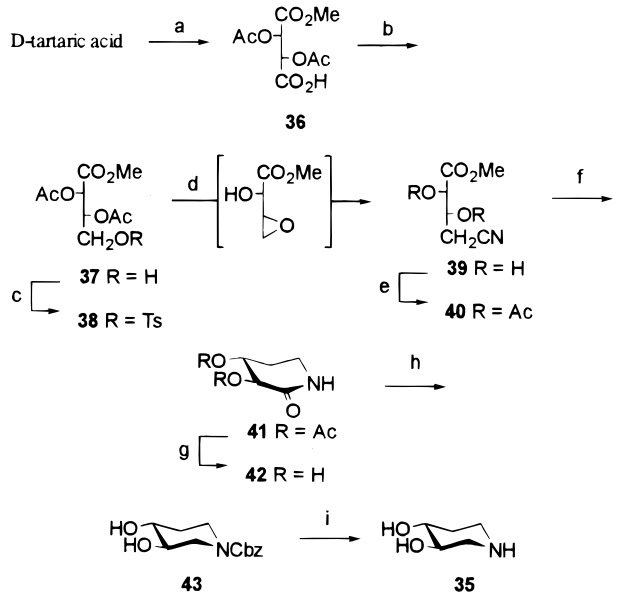
Scheme 8[a]



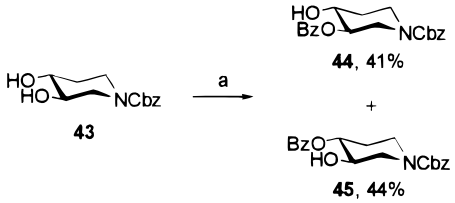
[a] (a) References 46, 47. (b) BH_3·Me_2S, THF, 55%. (c) TsCl, Pyr, 97%. (d) KCN, MeOH. (e) Ac_2O, Pyr, 47% over two steps. (f) PtO_2, H_2, MeOH, 64%. (g) NaOMe, MeOH, 90%. (h) (i) TMSCl, HMDS, MeCN; (ii) BH_3·Me_2S, dioxane; (iii) 1 M HCl, reflux; (iv) $NaHCO_3$, CbzCl, 55%. (i) Pd/C, H_2, EtOH.

The synthesis of **35** has been achieved by Ichikawa et al. from D-lyxose, but their route was complicated by deoxygenation steps and was somewhat lengthy.[29] It was of interest to prepare **35** from a precursor that contained only the two necessary stereogenic centers to simplify the synthesis sufficiently to provide reasonable quantities of **35**. D-Tartaric acid was converted in two steps to the known methyl ester (**36**)[46,47] (Scheme 8). Reduction of **36** with borane methyl sulfide gave the alcohol (**37**)[48] which was cleanly converted to the unstable tosylate (**38**). Treatment of **38** with potassium cyanide in MeOH provided the nitrile (**39**), presumably by way of an intermediate epoxide. However, it proved more convenient to acetylate the crude reaction mixture after treatment of **38** with potassium cyanide and to isolate the diacetate (**40**). Hydrogenation of **40** over PtO_2 proceeded in moderate yield with cyclization to afford the lactam (**41**), which was converted to the diol (**42**) upon treatment with NaOMe in MeOH. Reduction of **42** according to a procedure modified from that of Godskesen et al.[49] directly gave the carbamate (**43**), which was treated with hydrogen over Pd/C completing a synthesis of D-*xylo*-isofagomine (**35**).

To complete the synthesis of **8**, the carbamate (**43**) was treated with 1-benzoyloxybenzotriazole to afford the separable monobenzoates **44** and **45** (Scheme 9). D-Xylosylation of **44** with the trichloroacetimidate (**20**) afforded the disaccharide (**46**), which was transesterified to afford the carbamate (**47**) (Scheme 10). Hydrogenolysis of **47** furnished the xylobiose-derived isofagomine (**8**) in good yield.

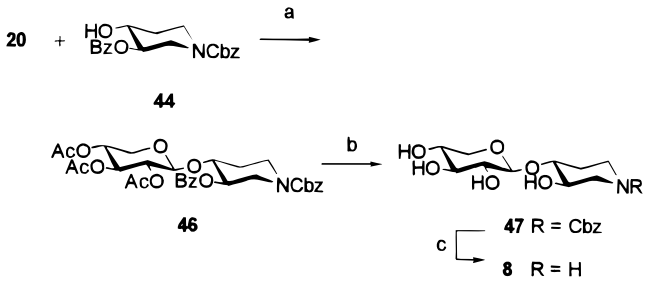
With quantities of **45** in hand we decided to prepare the xylobiose-derived fagomine (**48**). In a fashion analogous to that above, **45** was treated with the trichloroacetimidate (**20**) to provide the disaccharide (**49**) (Scheme 11). This compound

Scheme 9[a]



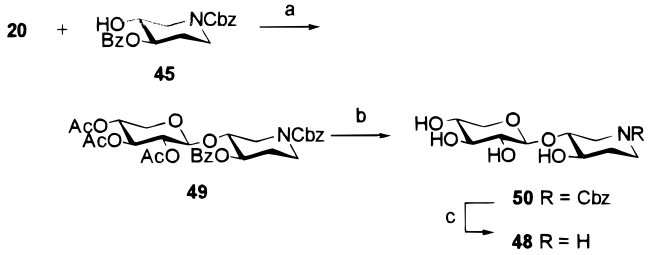
[a] Reagents: (a) 1-benzoyloxybenzotriazole, Et_3N, CH_2Cl_2.

Scheme 10[a]



[a] Reagents: (a) BF_3·Et_2O, $(CH_2Cl)_2$, 44%. (b) NaOMe, MeOH, 81%. (c) Pd/C, EtOH, 83%.

Scheme 11[a]



[a] Reagents: (a) BF_3·Et_2O, $(CH_2Cl)_2$. (b) NaOMe, MeOH, 45% over two steps. (c) Pd/C, EtOH, 79%.

Table 1. Inhibition Constants for Cex and Bcx with Various Nitrogen-Containing Inhibitors

	K_i (μM)	
compound	Cex	Bcx
xylobiose	4800	80000
5	0.15	520
6	0.37	1400
7	5.8	1500
8	0.13	1100
47	190	no inhibition
48	110	3100
50	790	no inhibition

could be purified with some care away from contaminating trichloroacetamide; on a larger scale it proved more convenient to treat the crude reaction mixture with NaOMe in MeOH and isolate the tetrol (**50**) instead. As before, hydrogenolysis of **50** cleanly furnished **48** in good yield.

Xylanase Inhibition. Each of the inhibitors was evaluated for its ability to inhibit the family 10 xylanase Cex, from *Cellulomonas fimi* and the family 11 xylanase Bcx, from *Bacillus circulans* (Table 1). Compounds **47** (K_i = 190 μM) and **50** (K_i = 790 μM) exhibited rather weak inhibition against Cex, consistent with the nonbasic character of these compounds. The xylobiodeoxynojirimycin (**7**) was a good inhibitor of Cex, displaying a K_i of 5.8 μM, whereas the fagomine derivative (**48**), the 2-deoxy version of **7**, was a much poorer inhibitor (K_i = 110 μM). The imidazole (**5**) and isofagomine (**8**) (Figure 3) both showed extremely strong competitive inhibition of Cex

(46) Shriner, R. L.; Furrow, C. L. *Org. Synth. Collect. Vol.* **1963**, *4*, 242−243.
(47) Lucas, H. J.; Baumgarten, W. *J. Am. Chem. Soc.* **1941**, *63*, 1653−1657.
(48) Umemura, E.; Tsuchiya, T.; Umezawa, S. *J. Antibiot.* **1988**, *41*, 530−537.
(49) Godskesen, M.; Lundt, I.; Madsen, R.; Winchester, B. *Bioorg. Med. Chem.* **1996**, *4*, 1857−1865.

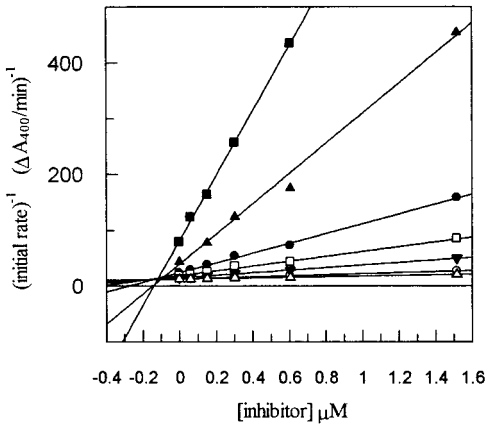


Figure 3. Dixon plot of inhibition of *C. fimi* xylanase by the xylobiose-derived isofagomine **8**. The concentrations of the substrate, 2,4-dinitrophenyl β-cellobioside, used were 0.011 (■), 0.028 (▲), 0.071 (●), 0.142 (□), 0.285 (▼), 0.712 (○), and 1.42 (△) mM.

(K_i = 150 and 130 nM, respectively), with the lactam oxime (**6**) being an only slightly poorer inhibitor (K_i = 370 nM). Interestingly, while substitution of the basic nitrogen of **8** with a benzyloxycarbonyl group, giving **47**, caused a 1000-fold reduction in inhibitory potency, the same substitution on **48** (namely **50**) merely caused a 2-fold reduction in potency. This is quite surprising given the supposed importance of protonation of the nitrogen in inhibitors of the deoxynojirimycin type. However, this different response to *N*-acylation of the isofagomine (**8**) (to give **47**) compared to the fagomine (**48**) (to give **50**) is reminiscent of the consequences of *N*-alkylation noted by Ichikawa.[29]

Interestingly, compounds **5**−**8** and **48** were all rather poor inhibitors of Bcx. The best of these compounds was the imidazole (**5**), which inhibited Bcx with a K_i of 0.4 mM. Compounds **47** and **50** showed no inhibition of Bcx at concentrations up to 5 mM.

Discussion

The principal series of inhibitors tested, compounds **5**−**8**, can be separated into two groups. Compounds **5** and **6** have an sp^2-hybridized anomeric carbon with an exocyclic double bond off C1; thus, planarity is attained on the "amidine" structure of the ring nitrogen, anomeric carbon, and endocyclic nitrogen. Compounds **7** and **8** contain an sp^3-hybridized nitrogen at positions equivalent to the ring oxygen or the anomeric carbon, respectively. All of these compounds proved to be remarkably effective inhibitors of *C. fimi* Cex, with K_i values in the nano- to micromolar range. Unlike the well-studied cellobiohydrolase Cel7A from *Trichoderma reesei*, which binds to its disaccharide product, cellobiose, relatively tightly (K_i = 20 μM),[34] Cex binds to its disaccharide product, xylobiose, quite poorly (K_i = 4.8 mM). This product affinity is similar to that seen for cellotriose with Cel5A from *B. agaradhaerens* (K_i = 4.5 mM). As a consequence, these inhibitors bind to Cex some 6.0−3.7 × 10^4-fold more tightly (as expressed in terms of the K_i ratio) than does the equivalent disaccharide. Thus, they are bound much more tightly, relatively, than does the cellobiose-derived imidazole to Cel5A (K_i ratio = 55), and indeed the same inhibitor binds some 6.5-fold *worse* to the *T. reesei* Cel7A than does cellobiose. The inhibition values measured for Cex therefore represent the highest relative affinities yet seen for any xylanase or cellulase with a small molecule.

By contrast, the same inhibitors bind relatively poorly to Bcx with K_i ratios (relative to xylobiose) in the 26−150-fold range.

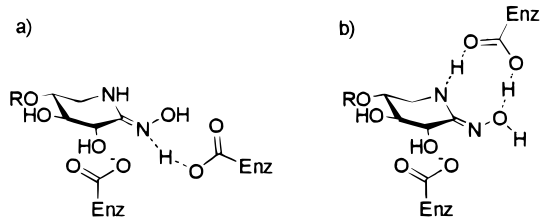


Figure 4. Protonation modes of the lactam oxime suggested by Vonhoff et al.:[34] (a) anti-protonation and (b) syn-protonation.

While these relative affinities are indeed in the range observed for *B. agaradhaerens* Cel5A and are in fact much better than those seen for the *T. reesei* Cel7A, they are still very poor compared to those found for Cex. Initial analysis would suggest two likely causes of this difference in affinities: differences in the protonation trajectory and differences in transition state conformation.

Cex belongs to family 10 of the glycosyl hydrolase classification scheme, thus clan GH-A, and has therefore been classified as an anti-protonator.[32] The imidazole (**5**) and the lactam oxime (**6**) therefore presumably experience favorable interactions of their exocyclic nitrogen atoms with the acid/base residue, and this is reflected in the tight binding of **5** and **6** to Cex (Figure 4a). By contrast, Bcx belongs to family 11 (and clan GH-C) and has been classified as a syn-protonator.[32] In this case, the lone pair on nitrogen in **5** and **6** must be directed away from the acid/base group and no hydrogen bonding is then possible. This might well account for a good portion of the difference in affinities noted here. Some caution in this analysis is necessary, though since, as noted earlier, it may be possible for the oxygen atom of the lactam oxime to engage in productive binding with a syn-carboxyl group (Figure 4b),[34] though the K_i values measured for **5** and **6** give no support to this hypothesis. Further mimicry of the transition state may be achieved by protonation of the "glycosidic" nitrogens of **5** and **6** by the acid/base/residue, thus imitating the partially protonated glycosidic oxygen at the transition state. One important caveat here is that at the transition state the substrate aglycon must be directed in a pseudoaxial orientation. By contrast, however, the "glycosidic" nitrogens of **5** and **6** are in the plane of the sugar ring; thus, some difficulty in matching the transition state may be expected. Nonetheless, in the X-ray structure of the complex of a cellobiose-derived imidazole with Cel5A from *B. agaradhaerens* it was observed that, despite the less than ideal positioning of the "glycosidic" nitrogen atom, the acid/base residue was able to form a short (2.58 Å) hydrogen bond with this atom.[33] Thus, the in-plane orientation of the "glycosidic" nitrogens of **5** and **6** seems unlikely to have a major impact on the formation of a strong hydrogen bond with the acid/base residue of Cex.

Another possible reason for the relatively high affinities of **5** and **6** for Cex but not for Bcx is that the $^4H_3$ conformation they are assumed to adopt is nicely complementary to that of the $^4H_3$ transition state stabilized by Cex (Figure 1), but not the $^{2,5}B$ transition state of Bcx (Figure 5). It is of interest to note here that while single-crystal X-ray structures of D-gluconolactam and the D-*gluco*-lactam oxime **2** show these molecules to be in conformations close to that of a $^4H_3$ chair,[25,50] expected for the transition state of the enzyme-catalyzed reaction, the X-ray structure determined for the complex of the cellobiose-derived imidazole with Cel5A showed the imidazopyridine ring of the inhibitor to be in the ^4E conformation,[33] even though, by analogy with D-gluconolactam and the D-*gluco*-lactam oxime

(50) Ogura, H.; Furuhata, K.; Takayanagi, H.; Tsuzuno, N.; Iitaka, Y. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2687−2688.

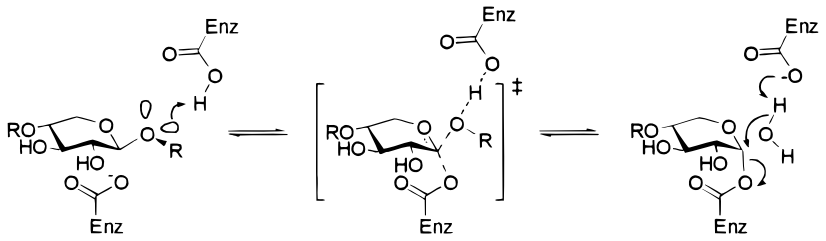


Figure 5. Formation of the xylobiosyl−enzyme intermediate on Bcx with the sugar moiety in the −1 subsite in a $^{2,5}B$ conformation.

2, this molecule is expected to be in the $^4H_3$ conformation in the solid state. On this basis it is quite possible that the imidazole **5** and the lactam oxime **6** may actually bind to Cex in ^4E conformations rather than the $^4H_3$ conformation and thus may in fact not mimic the conformation of the transition state exactly even in the case of Cex.

Also of particular note is the large difference in relative affinities seen for Cex and Bcx with compounds **7** and **8**. These compounds presumably owe their high affinity for Cex to being protonated in the active site of the enzyme and interacting with the charged acid/base or nucleophile. As mentioned earlier, Bcx has the acid/base syn to the C1−O5 bond of the substrate. Such a location could place this residue in close proximity to the nitrogen of **7**; thus, strong interactions might be expected. Likewise, the nucleophile of Bcx should be directed toward C1 of the substrate (in order to form a glycosyl enzyme) and thus should be able to interact with the nitrogen of **8**. A possible reason why this might not be true is that the inhibitors might not readily adopt the conformation preferred by the enzyme. At first glance both **7** and, particularly, **8** seem conformationally mobile; thus, it is difficult to see why these compounds should not be able to adopt a suitable conformation in the active site that provides for a good interaction between the basic amine and either the acid/base or nucleophile residues. However, even in cyclohexane, the difference in free energy[51] of a boat versus a chair conformation is approximately 6.4 kcal mol^{-1}. Thus, such differences in conformational free energies might well explain the difference in affinities of **7** and **8** for the two classes of enzymes. Quite possibly then, xylobiose analogues with the reducing-end sugar ring constrained into a $^{2,5}B$ conformation would serve as much better inhibitors.

Another crucial difference between Cex and Bcx is the presence of an essential tyrosine residue in the active site of Bcx whose oxygen atom lies in close proximity to the sugar endocyclic oxygen. Mutation of this residue, Tyr69 to Phe, provides a mutant enzyme that is a severely disabled catalyst.[52] A role for this residue in catalysis has been suggested wherein a hydrogen bond forms between Tyr69 and the anomeric oxygen of the glycosyl enzyme as the catalytic nucleophile departs from this intermediate.[18] A partial negative charge thus develops on the phenolic oxygen that could serve to stabilize the partial positive charge that develops on the sugar as it approaches the transition state. Possibly, unfavorable interactions of Tyr69 with each of these inhibitors may cause the high K_i values observed with Bcx.

The inhibition by the fagomine derivative (**48**) gives some idea of the importance of interactions with the 2-OH group in **7** as removal of this group causes a 20-fold reduction in affinity. It is therefore interesting to speculate on the potential for reintroducing a hydroxyl group into an isofagomine structure to improve still further the extremely powerful inhibition by **8**.

(51) Stoddart, J. F. *Stereochemistry of carbohydrates*; John Wiley & Sons: New York, 1971; p 53.

(52) Joshi, M. Personal communication.

Summary

We have prepared a series of potent, nanomolar inhibitors of a family 10 xylanase, Cex, which, surprisingly, are relatively ineffective inhibitors of a family 11 xylanase, Bcx. All syntheses were conducted from readily available precursors in a highly stereoselective manner. These new inhibitors all exhibit competitive inhibition consistent with the formation of a noncovalent enzyme−inhibitor complex in the active site. The behavior of two of these inhibitors, namely the imidazole (**5**) and the lactam oxime (**6**), is consistent with the classification of the family 10 enzyme, Cex, as an anti-protonator and with the family 11 enzyme, Bcx, as a syn-protonator. However, the differences in affinities for the two classes of xylanases may also have their origins in the different conformations of the transitions states of the two enzyme-catalyzed reactions, Bcx favoring a boat conformation and Cex preferring a half-chair conformation. The efficient syntheses of these new inhibitors lay open the way for detailed structural evaluation of the mode of interaction of these inhibitors with these two enzymes and kinetic evaluation of the degree to which these compounds resemble the transition state of glycoside hydrolysis.

Experimental Section

General. All melting points are uncorrected. Organic extracts were dried over $MgSO_4$ and concentrated in vacuo. ^1H and ^{13}C NMR spectra were recorded on Bruker instruments and were referenced using the solvent peak. NMR spectra were in $CDCl_3$ unless otherwise noted. ^{15}N NMR spectra were recorded on a Varian instrument and were referenced to $^{15}NH_3$. Flash chromatography was performed on Merck silica gel 60.[53] Thin-layer chromatography was performed on Merck silica gel 60 F_{254} plates. Microanalyses were performed by Mr. Peter Borda at the University of British Columbia.

Methyl 2,3-Di-*O*-acetyl-5-azido-5-deoxy-D-xylofuranosides (11). A solution of **10**[42] (70 g, prepared from 30 g of D-xylose, 130 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (500 g) was treated with NaN_3 (56 g, 860 mmol) at 70 °C for 18 h. The mixture was diluted with H_2O (2 L), stirred for 1 h, and extracted with EtOAc (3 × 200 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ and brine, dried, and evaporated. Flash chromatography (1:3 EtOAc−C_6H_6) of the residue gave **11** as a clear oil (24.8 g, 45% from D-xylose); R_f 0.35 (1:2 EtOAc−C_6H_6). ^1H NMR (400 MHz) of α-**11**: δ 2.07, 2.07 (2 s, 6 H, Ac), 3.26−3.33 (m, 2 H, H5,5), 3.35 (s, 3 H, OMe), 4.38 (ddd, q, $J_{3,4} \approx J_{4,5} \approx J_{4,5}$ 6.0 Hz, H4), 4.98 (dd, 1 H, $J_{1,2}$ 4.7, $J_{2,3}$ 5.5 Hz, H2), 5.10 (d, 1 H, $J_{1,2}$ 4.5 Hz, H1), 5.44 (dd, t, 1 H, $J_{2,3} \approx J_{3,4}$ 6.2 Hz, H3). ^1H NMR (400 MHz) of β-**11**: δ 2.06, 2.07 (2s, 3 H, Ac), 3.26−3.33 (m, 1 H, H5), 3.38 (s, 3 H, OMe), 3.49 (dd, 1 H, $J_{4,5}$ 8.0, $J_{5,5}$ 12.8 Hz, H5), 4.17 (ddd, dt, 1 H, $J_{3,4}$ 5.8, $J_{4,5} \approx J_{4,5}$ 7.8 Hz, H4), 4.83 (br s, 1 H, H2), 5.04 (br s, 1 H, H1), 5.26 (dd, 1 H, $J_{2,3}$ 1.7, $J_{3,4}$ 6.2 Hz, H3). ^{13}C NMR (75.5 MHz): δ 20.43, 20.54 (CH_3CO), 50.12, 51.33 (C5), 55.42, 55.47 (OMe), 74.23, 74.86, 75.05, 76.96, 79.28, 80.68 (C2,3,4), 99.81, 107.06 (C1), 169.34, 169.68, 170.05 (CO). DCI HRMS (NH_3, CH_4): calcd for *m/z* $[M + H]^+$ 274.1039, found 274.1042.

(53) Still, W. C.; Kahn, M.; Mitra, A. J. *J. Org. Chem.* **1978**, *43*, 2923−2925.

Methyl 5-Azido-5-deoxy-D-xylofuranosides (12). A solution of **11** (5.00 g, 18.3 mmol) in MeOH (100 mL) was treated with NaOMe (100 mg, 1.8 mmol) at 25 °C for 30 min. Evaporation and flash chromatography (1:1 EtOAc−hexane then EtOAc) of the residue gave **12** (3.12 g, 90%); R_f 0.35 (α-**12**) and 0.40 (β-**12**, EtOAc). ^{1}H NMR (400 MHz) of α-**12**: δ 2.60 (d, 1 H, *J* 5.0 Hz), 2.77 (d, 1 H, *J* 7.2 Hz, 2OH, 3OH), 3.43 (dd, 1 H, $J_{4,5}$ 5.1, $J_{5,5}$ 12.9 Hz, H5), 3.47 (s, 3 H, OMe), 3.51 (dd, 1 H, $J_{4,5}$ 4.4, $J_{5,5}$ 12.9 Hz, H5), 4.07−4.10 (m, 1 H), 4.22−4.30 (m, 2 H, H2,3,4), 4.96 (d, 1 H, $J_{1,2}$ 4.5 Hz, H1). ^{1}H NMR (400 MHz) of β-**12**: δ 2.46 (d, 1 H, $J_{2,OH} \approx$ 3.1 Hz, 2OH), 2.87 (d, 1 H, $J_{3,OH}$ 10.9 Hz, 3OH), 3.40 (s, 3 H, OMe), 3.47−3.53 (m, 2 H, H5,5), 4.04 (dd, 1 H, $J_{3,OH} \approx$ 10.5, $J_{3,4} \approx$ 4.1 Hz, H3), 4.18 (d, 1 H, $J_{2,OH} \approx$ 2.4 Hz, H2), 4.41 (ddd, q, 1 H, $J_{3,4} \approx J_{4,5} \approx J_{4,5}$ 4.8 Hz, H4), 4.85 (s, 1 H, H1). ^{13}C NMR (75.5 MHz): δ 50.53, 51.26 (C5), 55.19, 55.43 (OMe), 75.71, 75.97, 76.53, 77.92, 79.60, 81.05 (C2,3,4), 101.19, 108.46 (C1). DCI MS (NH_3): *m*/*z* 190 (2, $[M + H]^+$), 158 (7), 134 (6), 133 (100), 115 (13).

Methyl 5-Azido-2,3-di-*O*-benzyl-5-deoxy-D-xylofuranosides (13). (a) With BnBr and Ag_2O. A solution of **12** (0.50 g, 2.64 mmol) in DMF was treated with BnBr (1.6 mL, 13.5 mmol) and Ag_2O (1.55 g, 6.7 mmol) at 25 °C for 18 h. Pyridine (1 mL) was added and the mixture was filtered through Celite. The filtrate was diluted with water and extracted with Et_2O. The combined organic phases were washed with 1 M H_2SO_4, saturated aqueous $NaHCO_3$, and brine and dried, and the solvent was evaporated. Flash chromatography of the residue gave **13** (0.68 g, 70%).

(b) With BnBr and NaH. A solution of BnBr (24 mL, 202 mmol) in THF (150 mL) was treated with NaH (60% in oil, 11 g, ca. 275 mmol), cooled to 0 °C, and treated dropwise with a solution of **12** (12.8 g, 67.7 mmol) in THF (50 mL) at such a rate that the temperature of the solution did not exceed 10 °C. When gas evolution ceased, the mixture was heated to 60 °C for 5 min, cooled to 25 °C, treated with MeOH (30 mL) and pyridine (30 mL), and stirred overnight. Water was added and the mixture was extracted with toluene. The combined organic phases were washed with 1 M H_2SO_4, saturated aqueous $NaHCO_3$, and brine and dried, and the solvent was evaporated to give crude **13** as an oil (33.0 g) an analytical sample of which was purified by flash chromatography; R_f 0.31 (β-**13**), 0.38 (α-**13**; 1:3 EtOAc−hexane). ^{1}H NMR (400 MHz) of α-**13**: δ 3.38 (s, 3 H, OMe), 3.38−3.41 (m, 2 H, H5,5), 4.00−4.02 (m, 1 H, H2), 4.28−4.30 (m, 2 H, H3,4), 4.52 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.66 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.56 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.63 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.80 (d, 1 H, $J_{1,2}$ 4.2 Hz, H1), 7.25−7.37 (m, 10 H, Ph). ^{1}H NMR (400 MHz) of β-**13**: δ 3.37 (dd, 1 H, $J_{4,5}$ 4.7, $J_{5,5}$ 13.0 Hz, H5), 3.41 (s, 3 H, OMe), 3.54 (dd, 1 H, $J_{4,5}$ 8.5, $J_{5,5}$ 13.0 Hz, H5), 4.01 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.2 Hz, H2), 4.08 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 6.4 Hz, H3), 4.35 (ddd, 1 H, $J_{3,4}$ 6.4, $J_{4,5}$ 8.4, $J_{4,5}$ 4.5 Hz, H4), 4.42 (d, 1 H, *J* 12.2 Hz, CH_2Ph), 4.58 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.49 (d, 1 H, *J* ≈ 12.8 Hz, CH_2Ph), 4.55 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.90 (d, 1 H, $J_{1,2}$ 1.7 Hz, H1), 7.25−7.37 (m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 50.95, 51.75 (C5), 55.08, 55.24 (OMe), 71.94, 72.10, 72.27, 72.49 (*C*H_2Ph), 75.38, 79.66, 80.94, 81.67, 83.77, 86.56 (C2,3,4), 100.35, 108.01 (C1), 127.54−128.30, 137.24, 137.34, 137.60 (Ph). DCI HRMS (NH_3, CH_4): calcd for *m*/*z* $[M - 1]^+$ 368.1610, found 368.1617.

5-Azido-2,3-di-*O*-benzyl-5-deoxy-D-xylofuranoses (14). A solution of crude **13** (32.0 g) in AcOH (400 mL) was treated with 1 M H_2SO_4 (80 mL) and heated to 50−60 °C for 6 h. Water (1 L) was added and the mixture was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phases were washed with saturated aqueous K_2CO_3, saturated aqueous $NaHCO_3$, and brine and dried, and the solvent was evaporated. Flash chromatography (1:5 EtOAc−hexane) of the residue gave **14** as an oil (10.3 g, 43% from **12**); R_f 0.15 (1:3 EtOAc−hexane). ^{1}H NMR (400 MHz): δ 3.42 (dd, 0.5 H, $J_{4,5}$ 6.1, $J_{5,5}$ 12.6 Hz, H5), 3.50 (dd, 0.5 H, $J_{4,5}$ 6.5, $J_{5,5}$ 12.5 Hz, H5), 3.47 (dd, 0.5 H, $J_{4,5} \approx$ 6.4, $J_{5,5} \approx$ 13.9 Hz, H5), 3.60 (dd, 0.5 H, $J_{4,5}$ 6.7, $J_{5,5}$ 12.5 Hz, H5), 3.94 (dd, 0.5 H, $J_{2,3}$ 2.5, $J_{3,4}$ 4.1 Hz, H3), 4.00 (dd, 0.5 H, $J_{2,3}$ 2.2, $J_{3,4}$ 4.4 Hz, H3), 3.98−3.99 (m, 1 H, H2), 4.30 (ddd, td, 0.5 H, $J_{3,4}$ 4.8, $J_{4,5} \approx J_{4,5}$ 6.2 Hz, H4), 4.33 (ddd, dt, 0.5 H, $J_{3,4}$ 4.4, $J_{4,5} \approx J_{4,5}$ 6.5 Hz, H4), 4.44 (d, 0.5 H, *J* 11.9 Hz, CH_2Ph), 4.51 (d, 0.5 H, *J* ≈ 10.8 Hz, CH_2Ph), 4.48 (d, 0.5 H, *J* 11.3 Hz, CH_2Ph), 4.61 (d, 0.5 H, *J* 12.0 Hz, CH_2Ph), 4.52 (d, 0.5 H, *J* 12.0 Hz, CH_2Ph), 4.58 (d, 0.5 H, *J* 11.8 Hz, CH_2Ph), 4.56 (d, 0.5 H, *J* 11.8 Hz, CH_2Ph), 4.61 (d, 0.5 H, *J* 11.9 Hz, CH_2Ph), 5.28 (s, 0.5 H), 5.45 (d, 0.5 H, $J_{1,2}$ 4.0 Hz, H1), 7.25−7.40 (m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 50.07, 50.72 (C5), 71.80, 72.05, 72.38, 72.94 (*C*H_2Ph), 76.66, 79.81, 80.46, 80.73, 81.11, 84.67 (C2,3,4), 95.94, 101.29 (C1), 127.63−128.52, 136.56, 137.05, 137.16 (Ph). DCI HRMS (NH_3, CH_4): calcd for *m*/*z* $[M + H]^+$ 356.1610, found 356.1611.

5-Azido-2,3-di-*O*-benzyl-5-deoxy-D-xylono-1,4-lactone (15). Pyridine (150 mL) was added to CrO_3 (14.5 g, 145 mmol) and the mixture was stirred for 1 h, then treated with a solution of **14** (10.3 g, 29.0 mmol) in pyridine (150 mL) and stirred at 25 °C overnight. Et_2O (1 L) was added and the mixture was filtered through Celite. The filtrate was washed with 1 M H_2SO_4, saturated aqueous $NaHCO_3$, and brine, dried and the solvent evaporated to give crude **15** as an oil (9.31 g) an analytical sample of which was purified by flash chromatography; R_f 0.23 (1:3 EtOAc−hexane). ^{1}H NMR (400 MHz): δ 3.58 (dd, 1 H, $J_{4,5}$ 4.3, $J_{5,5}$ 13.3 Hz, H5), 3.64 (dd, 1 H, $J_{4,5}$ 4.6, $J_{5,5}$ 13.3 Hz, H5), 4.31 (dd, t, 1 H, $J_{2,3} \approx J_{3,4}$ 6.3 Hz, H3), 4.37 (d, 1 H, $J_{2,3}$ 5.8 Hz, H2), 4.52 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.64 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.62 (ddd, dt, 1 H, $J_{3,4} \approx J_{4,5}$ 6.6, $J_{4,5}$ 4.5 Hz, H4), 4.71 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 5.04 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 7.23−7.40 (m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 49.91 (C5), 72.51, 72.56 (*C*H_2Ph), 76.64, 77.10, 78.49 (C2,3,4), 127.82−128.49, 136.56 (Ph), 172.20 (CO). DCI HRMS (NH_3, CH_4): calcd for *m*/*z* $[M + H]^+$ 353.1375, found 353.1364.

5-Amino-2,3-di-*O*-benzyl-5-deoxy-D-xylono-1,5-lactam (9). A solution of **15** (8.84 g, 25.0 mmol) in 10:1 THF−H_2O (550 mL) was treated with Bu_3P (7.2 mL, 29.2 mmol) and stirred at 25 °C for 24 h. Evaporation of the solvent and flash chromatography (EtOAc) of the residue gave a solid which was crystallized from EtOAc−petroleum ether. Flash chromatography (EtOAc) of the mother liquor gave **9** (5.3 g, 65% from **14**); mp 107−108 °C. R_f 0.19 (EtOAc). ^{1}H NMR (400 MHz): δ 2.80 (br s, 1 H, 4OH), 3.16 (ddd, 1 H, $J_{4,5}$ 2.1, $J_{5,5}$ 12.2, $J_{5,NH}$ 6.8 Hz, H5), 3.42 (ddd, 1 H, $J_{4,5}$ 4.5, $J_{5,5}$ 12.2, $J_{5,NH}$ 3.1 Hz, H5), 3.71 (dd, t, 1 H, $J_{2,3} \approx J_{3,4}$ 6.8 Hz, H3), 3.89 (ddd, td, 1 H, $J_{3,4} \approx J_{4,5}$ 6.8, $J_{4,5}$ 4.8 Hz, H4), 3.94 (d, 1 H, $J_{2,3}$ 6.6 Hz, H2), 4.58 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 5.12 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 4.75 (d, 1 H, *J* 11.3 Hz, CH_2Ph), 4.77 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 6.14 (br s, 1 H, NH), 7.21−7.42 (m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 44.35 (C5), 66.83, 77.86, 80.71 (C2,3,4), 73.63, 74.35 (*C*H_2Ph), 127.94−128.51, 137.33, 137.56 (Ph), 170.89 (CO). DCI MS (NH_3): *m*/*z* 323 (6, $[M + H]^+$), 221 (10), 130 (10), 116 (6), 115 (100), 114 (13), 98 (21), 91 (27). Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.84; H, 6.42; N, 4.21.

5-Amino-2,3-di-*O*-benzyl-5-deoxy-D-xylonothio-1,5-lactam (16). A solution of **9** (100 mg, 0.31 mmol) in C_6H_6 (4 mL) was treated with Lawesson's reagent (86 mg, 0.21 mmol) at 25 °C for 36 h. Evaporation of the solvent and flash chromatography (1:1 EtOAc−hexane) of the residue gave **16** (32 mg, 29%); R_f 0.22 (1:1 EtOAc−hexane). ^{1}H NMR (400 MHz): δ 2.73 (br s, 1 H, 4OH), 3.35 (br d, 1 H, *J* 13.6 Hz, H5), 3.55 (br d, 1 H, *J* 12.8, H5), 3.75 (dd, t, 1 H, $J_{2,3} \approx J_{3,4}$ 4.5 Hz, H3), 3.99 (br d, 1 H, $J_{3,4} \approx$ 4.3 Hz, H4), 4.30 (d, 1 H, $J_{2,3}$ 4.3 Hz, H2), 4.46 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.61 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.83 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 5.14 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 7.20−7.44 (m, 20 H, Ph), 8.37 (br s, 1 H, NH). ^{13}C NMR (75.5 MHz): δ 48.62 (C5), 66.10, 78.00, 80.71 (C2,3,4), 72.61, 74.05 (*C*H_2Ph), 127.85−128.51, 136.86, 137.16 (Ph), 199.51 (CS). DCI MS (NH_3): *m*/*z* 344 (4, $[M + H]^+$), 238 (4), 237 (28), 146 (5), 133 (5), 132 (8), 131 (100), 130 (12), 91 (82).

4-*O*-Acetyl-5-amino-2,3-di-*O*-benzyl-5-deoxy-D-xylonothio-1,5-lactam (17). A solution of **9** (1.00 g, 3.05 mmol) in EtOAc (30 mL) was treated with pyridine (2.5 mL) and Ac_2O (1.5 mL) at 25 °C for 24 h and evaporated. The residue was dissolved in C_6H_6 (15 mL) and treated with Lawesson's reagent (860 mg, 2.13 mmol) at 25 °C for 24 h. Evaporation of the solvent and flash chromatography (1:2 EtOAc−hexane) of the residue gave **17** (970 mg, 82%); R_f 0.41 (1:1 EtOAc−hexane). ^{1}H NMR (400 MHz): δ 1.97 (s, 3 H, Ac), 3.39 (ddd, dt, 1 H, $J_{5,5}$ 13.7, $J_{NH,5} \approx J_{4,5}$ 4.7 Hz, H5), 3.62 (ddd, dt, 1 H, $J_{5,5}$ 13.7, $J_{NH,5} \approx J_{4,5}$ 4.2 Hz, H5), 3.80 (dd, 1 H, $J_{2,3}$ 4.9, $J_{3,4}$ 3.4 Hz, H3), 4.23 (d, 1 H, $J_{2,3}$ 5.1 Hz, H2), 4.58 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.64 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.70 (d, 1 H, *J* 11.3 Hz, CH_2Ph), 5.09 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 5.06 (ddd, q, $J_{4,5} \approx J_{4,5} \approx J_{3,4}$ 4.2 Hz, H4), 7.23−7.43 (m, 10 H, Ph), 8.49 (br s, 1 H, NH). ^{13}C NMR (75.5 MHz): δ 20.89 (Me),

44.47 (C5), 72.81, 73.71 (*C*H_2Ph), 70.45, 79.44, 82.78 (C2,3,4), 127.88−127.92, 137.28, 137.37 (Ph), 170.07 (CO), 201.01 (CS). DCI HRMS (NH_3, CH_4): calcd for *m*/*z* $[M + H]^+$ 386.1426, found 386.1431.

(6*R*,7*S*,8*S*)-7,8-Dibenzyloxy-6-hydroxy-5,6,7,8-tetrahydroimidazo-[1,2-*a*]pyridine (18) and (6*R*,7*S*,8*R*)-7,8-Dibenzyloxy-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (19). A solution of **14** (800 mg, 2.08 mmol) in THF (8 mL) was treated with aminoacetaldehyde dimethyl acetal (1.12 mL, 10.4 mmol) and $Hg(OAc)_2$ (856 mg, 2.69 mmol) at 5 °C for 45 min and evaporated. The residue was dissolved in 11:1 toluene−H_2O (48 mL) and treated with TsOH·H_2O (148 mg, 0.78 mmol) at 65 °C for 18 h. EtOAc was added, the mixture was washed with saturated aqueous $NaHCO_3$ and brine and dried, and the solvent was evaporated. The residue was purified by flash chromatography (3:1 EtOAc−hexane) to give **18** (570 mg, 78%) and **19** (80 mg, 11%); data of **18**: R_f 0.27 (EtOAc), mp 100−101 °C. ^{1}H NMR (400 MHz): δ 4.05−4.11 (m, 2 H), 4.17−4.23 (m, 2 H, H5,5, H6,7), 4.53 (d, 1 H, *J* 11.9 Hz, C*H*$_2$Ph), 4.65 (d, 1 H, *J* 11.9 Hz, C*H*$_2$Ph), 4.76 (d, 1 H, *J* 11.7 Hz, C*H*$_2$Ph), 4.93 (d, 1 H, *J* 11.7 Hz, C*H*$_2$Ph), 4.78 (d, 1 H, $J_{7,8}$ 2.9 Hz, H8), 6.88 (d, 1 H, $J_{2,3}$ 1.0 Hz), 7.14 (d, 1 H, $J_{2,3}$ 1.0 Hz, H2,3), 7.18−7.33 (m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 48.75 (C5), 66.59, 70.87, 75.71 (C6,7,8), 71.63, 72.48 (*C*H_2Ph), 119.48 (C3), 127.75, 127.88, 128.01, 128.08, 128.42, 128.49, 137.06, 137.10 (Ph), 129.13 (C2), 141.33 (C8a). DCI MS (NH_3): *m*/*z* 352 (4), 351 (18, $[M + H]^+$), 320 (6), 259 (8), 214 (25), 197 (13), 138 (66), 137 (46), 121 (42), 92 (8), 91 (100). Anal. Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 71.70; H, 6.34; N, 7.98. Data of **19**: R_f 0.13 (EtOAc), mp 142−144 °C. ^{1}H NMR (400 MHz): δ 3.01 (br s, 1 H, OH), 3.62 (dd, 1 H, $J_{6,7}$ 9.7, $J_{7,8}$ 3.5 Hz, H7), 3.69 (dd, 1 H, $J_{5,5}$ 12.1, $J_{4,5}$ 9.3 Hz, H5), 4.35 (dd, 1 H, $J_{5,5}$ 12.1, $J_{4,5}$ 6.6 Hz, H5), 4.43 (d, 1 H, *J* 11.7 Hz, C*H*$_2$Ph), 4.63 (d, 1 H, *J* 11.7 Hz, C*H*$_2$Ph), 4.66 (ddd, td, 1 H, $J_{5,6} \approx J_{6,7}$ 9.4, $J_{5,6}$ 4.8 Hz, H6), 4.69 (d, 1 H, *J* 11.9 Hz, C*H*$_2$Ph), 4.77 (d, 1 H, *J* 12.0 Hz, C*H*$_2$Ph), 4.82 (d, 1 H, $J_{7,8}$ 3.5 Hz, H8), 6.84 (d, 1 H, $J_{2,3}$ 1.1 Hz), 7.09 (d, 1 H, $J_{2,3}$ 1.2 Hz, H2,3), 7.24−7.34, 7.40−7.44 (2m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 48.37 (C5), 64.12, 67.37, 79.33 (C6,7,8), 70.72, 71.26 (*C*H_2Ph), 119.17 (C3), 127.64, 127.97, 128.01, 128.27, 128.33, 128.46, 137.18, 137.80 (Ph), 129.54 (C2), 143.08 (C8a). DCI MS (NH_3): *m*/*z* 351 (3, $[M + H]^+$), 320 (5), 259 (6), 214 (29), 138 (50), 137 (36), 121 (33), 92 (8), 91 (100).

(6*R*,7*S*,8*S*)-6-(Tri-*O*-acetyl-*β*-D-xylopyranosyloxy)-7,8-dibenzyloxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (21). BF_3·Et_2O (34 μL, 0.27 mmol) was added to a mixture of **18** (233 mg, 0.665 mmol), 2,3,4-tri-*O*-acetyl-D-xylopyranosyl trichloroacetimidate **20**[44] (443 mg, 1.00 mmol), and molecular sieves (4 Å) in 1,2-dichloroethane (10 mL) and the mixture stirred at −15 °C for 1 h. The mixture was filtered and the filtrate was washed with saturated aqueous $NaHCO_3$ and brine and dried. The solvent was evaporated and the residue was purified by flash chromatography (3:1 EtOAc−hexane) and crystallized from EtOAc/Et_2O/hexane to give **21** (60 mg, 15%); R_f 0.20 (3:1 EtOAc−hexane). ^{1}H NMR (400 MHz, C_6D_6): δ 1.59 (s, 3 H, Ac), 1.65 (s, 3 H, Ac), 1.66 (s, 3 H, Ac), 3.06 (dd, 1 H, $J_{5',5'}$ 12.0, $J_{4',5'}$ 7.7 Hz, H5′), 3.36 (dd, 1 H, $J_{5,5}$ 12.5, $J_{4,5}$ 7.2 Hz, H5), 3.56 (dd, 1 H, $J_{5,5}$ 12.5, $J_{4,5}$ 4.6 Hz, H5), 3.79 (ddd, td, 1 H, $J_{5,6} \approx J_{6,7}$ 7.1, $J_{5,6}$ 4.6 Hz, H6), 4.01 (dd, 1 H, $J_{6,7}$ 7.0, $J_{7,8}$ 4.2 Hz, H7), 4.02 (dd, 1 H, $J_{5',5'}$ 12.0, $J_{4',5'}$ 4.3 Hz, H5′), 4.39 (d, 1 H, $J_{1',2'}$ 6.0 Hz, H1′), 4.52 (d, 1 H, *J* 11.6 Hz, C*H*$_2$Ph), 4.57 (d, 1 H, *J* 11.6 Hz, C*H*$_2$Ph), 4.77 (d, 1 H, $J_{7,8}$ 4.0 Hz, H8), 4.94 (ddd, td, 1 H, $J_{4',5'} \approx J_{3',4'}$ 7.7, $J_{4',5'}$ 4.8 Hz, H4′), 5.02 (d, 1 H, *J* 11.9 Hz, C*H*$_2$Ph), 5.25 (d, 1 H, *J* 11.9 Hz, C*H*$_2$Ph), 5.09 (dd, $J_{1',2'}$ 6.0, $J_{2',3'}$ 7.9 Hz, H2), 5.31 (t, 1 H, $J_{3',4'} \approx J_{2',3'}$ 7.8 Hz, H3′), 6.30 (d, 1 H, $J_{2,3}$ 0.9 Hz, H2 or H3), 7.06−7.27 (m, 9 H, 8 aromatic H, H2 or H3), 7.50 (br d, $J_{vic} \approx$ 7.2 Hz, 2 aromatic H). ^{13}C NMR (75.5 MHz, C_6D_6): δ 20.59, 20.74 (Me), 44.97 (C5), 61.60 (C5′), 68.28, 70.14, 70.38, 72.96, 74.05, 80.29 (C6,7,8,2′,3′,4′), 72.14, 73.41 (*C*H_2Ph), 98.86 (C1′), 118.33 (C3), 127.56, 127.87, 128.04, 128.25, 128.36, 137.55, 137.99 (Ph), 129.33 (C2), 143.55 (C8a), 169.27, 169.76, 169.92 (CO). DCI MS (NH_3): *m*/*z* 610 (9), 609 (25, $[M + H]^+$), 517 (21), 444 (21), 443 (79), 395 (49), 198 (27), 197 (73), 122 (27), 121 (100), 91 (82). Anal. Calcd for $C_{32}H_{36}N_2O_{10}$: C, 63.15; H, 5.96; N, 4.60. Found: C, 62.98; H, 6.07; N, 4.57.

(6*R*,7*S*,8*S*)-7,8-Dihydroxy-6-(*β*-D-xylopyranosyloxy)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (5). A solution of **21** (38 mg, 62 μmol) in MeOH (1.5 mL) and AcOH (1 mL) was treated with Pd black (50 mg) and H_2 (ca. 1 bar) at 25 °C overnight. More AcOH (1 mL) and Pd black (50 mg) were added, and the mixture was again treated with H_2 (ca. 1 bar) at 25 °C overnight and then filtered and evaporated. The residue [R_f 0.18 (18:2:1 EtOAc−MeOH−H_2O)] was dissolved in MeOH (2 mL) and treated with aqueous NH_3 (25%) at 25 °C for 3 h. The solvent was evaporated and the residue purified by flash chromatography (4:2:1 EtOAc−MeOH−H_2O) to give **5** (15 mg, 80%); R_f 0.17 (4:2:1 EtOAc−MeOH−H_2O). ^{1}H NMR (400 MHz, D_2O): δ 3.28 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 9.3 Hz, H2′), 3.31 (dd, 1 H, $J_{5',5'}$ 11.6, $J_{4',5'}$ 10.5 Hz, H5′), 3.44 (dd, t, 1 H, $J_{2',3'} \approx J_{3',4'}$ 9.2 Hz, H3′), 3.62 (ddd, 1 H, $J_{4',5'}$ 10.4, $J_{3',4'}$ 9.0, $J_{4',5'}$ 5.4 Hz, H4′), 3.97 (dd, 1 H, $J_{5',5'}$ 11.6, $J_{4',5'}$ 10.4 Hz, H5′), 4.08 (dd, 1 H, $J_{4,5}$ 6.1, $J_{5,5}$ 12.7 Hz, H5), 4.08 (dd, 1 H, $J_{6,7}$ 7.4, $J_{7,8} \approx$ 6.0 Hz, H7), 4.33 (ddd, 1 H, $J_{5,6} \approx$ 6.6, $J_{5,6}$ 4.8, $J_{6,7}$ 7.7 Hz, H6), 4.37 (dd, 1 H, $J_{4,5}$ 4.7, $J_{5,5}$ 12.6 Hz, H5), 4.53 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H1′), 4.67 (d, 1 H, $J_{7,8}$ 5.8 Hz, H8), 7.06, 7.07 (2s, H2,3). LSIMS HRMS (thioglycerol): calcd for $[M + H]^+$ 303.1192, found 303.1194.

4-*O*-(Tri-*O*-acetyl-*β*-D-xylopyranosyl)-5-amino-2,3-di-*O*-benzyl-5-deoxy-D-xylono-1,5-lactam (22). BF_3·Et_2O (270 μL, 2.2 mmol) was added to a solution of **9** (706 mg, 2.16 mmol) and 2,3,4-tri-*O*-acetyl-D-xylopyranosyl trichloroacetimidate **20**[44] (1.36 g, 3.24 mmol) in 1,2-dichloroethane (10 mL) at 0 °C and the solution was allowed to warm to room temperature and left to stand for 6 h. The solution was washed with saturated aqueous $NaHCO_3$ and brine and then dried and the solvent evaporated. The residue was purified by flash chromatography (9:1 EtOAc−hexane then EtOAc) to afford **22** as a white crystalline solid (861 mg, 68%); mp 150−158 °C, R_f 0.16 (3:1 EtOAc−hexane). ^{1}H NMR (400 MHz): δ 2.00, 2.01, 2.03 (3s, 9 H, Ac), 3.22 (ddd, 1 H, $J_{5,5}$ 12.9, $J_{4,5}$ 6.8, $J_{5,NH}$ 3.3 Hz, H5), 3.29 (dd, 1 H, $J_{4',5'}$ 8.1, $J_{5',5'}$ 12.0 Hz, H5′), 3.37 (ddd, dt, 1 H, $J_{5,5}$ 13.0, $J_{4,5} \approx J_{5,NH}$ 4.0 Hz, H5), 3.83 (dd, t, 1 H, $J_{2,3} \approx J_{3,4}$ 6.2 Hz, H3), 3.94 (d, 1 H, $J_{2,3}$ 6.8 Hz, H2), 4.03 (ddd, td, 1 H, $J_{3,4} \approx J_{4,5}$ 6.5, $J_{4,5} \approx$ 4.4 Hz, H4), 4.10 (dd, 1 H, $J_{4',5'}$ 4.8, $J_{5',5'}$ 12.0 Hz, H5′), 4.62 (d, 1 H, $J_{1',2'}$ 6.2 Hz, H1′), 4.67 (d, 1 H, *J* 11.3 Hz, C*H*$_2$Ph), 4.71 (d, 1 H, *J* 11.4 Hz, C*H*$_2$Ph), 4.69 (d, 1 H, *J* 11.7 Hz, C*H*$_2$Ph), 5.00 (d, 1 H, *J*, 11.5 Hz, C*H*$_2$Ph), 4.84 (dd, 1 H, $J_{2',3'}$ 8.1, $J_{1',2'}$ 6.3 Hz, H2′), 4.89 (ddd, td, 1 H, $J_{3',4'} \approx J_{4',5'}$ 8.0, $J_{4',5'}$ 4.8 Hz, H4′), 5.10 (dd, t, 1 H, $J_{2',3'} \approx J_{3',4'}$ 8.0 Hz, H3′), 6.43 (br s, 1 H, NH), 7.23−7.33 (m, 8 aromatic H), 7.35−7.38 (m, 2 aromatic H). ^{13}C NMR (75.5 MHz): δ 20.63, 20.67, 20.71 (Me), 41.15 (C5), 61.70 (C5′), 68.37, 70.45, 70.68, 75.24, 78.52, 81.55 (C2,3,4,2′,3′,4′), 73.64, 73.95 (*C*H_2Ph), 98.58 (C1), 127.69−128.31, 137.68, 137.76 (Ph), 169.32, 169.77, 169.94 (OCO), 171.45 (NCO). DCI MS (NH_3): *m*/*z* 586 (2, $[M + H]^+$), 259 (6), 204 (10), 202 (9), 201 (10), 157 (16), 140 (13), 139 (14), 98 (41), 97 (32), 92 (9), 91 (100). Anal. Calcd for $C_{30}H_{35}NO_{11}$: C, 61.53; H, 6.02; N, 2.39. Found: C, 61.37; H, 5.93; N, 2.28.

4-*O*-(Tri-*O*-acetyl-*β*-D-xylopyranosyl)-5-amino-2,3-di-*O*-acetyl-5-deoxy-D-xylono-1,5-lactam (24). A suspension of **22** (690 mg, 1.18 mmol) and Pd on carbon (10%, 200 mg) in MeOH (50 mL) was treated with H_2 (ca. 1 bar) at 25 °C overnight. The mixture was filtered through Celite, the solvent was evaporated, and the residue was dissolved in pyridine (6 mL) and treated with Ac_2O (2 mL) at room temperature for 1 h. Water (1 mL) was added and the mixture was stirred for 5 min. Workup (CH_2Cl_2; water, 1 M HCl, saturated aqueous $NaHCO_3$) followed by evaporation and flash chromatography (9:1 EtOAc−hexane then EtOAc) gave **24** as a clear oil (545 mg, 94%); R_f 0.17 (EtOAc). ^{1}H NMR (400 MHz): δ 2.00, 2.02, 2.03, 2.06, 2.10 (5s, 15 H, Ac), 3.28 (ddd, 1 H, $J_{4,5}$ 7.6, $J_{5,5}$ 12.5, $J_{5,NH}$ 2.3 Hz, H5), 3.42 (dd, 1 H, $J_{5',5'}$ 12.2, $J_{4',5'}$ 7.2 Hz, H5′), 3.46 (ddd, dt, 1 H, $J_{4,5} \approx J_{5,NH}$ 4.0, $J_{5,5}$ 12.7 Hz, H5), 4.05 (ddd, td, 1 H, $J_{3,4} \approx J_{4,5} \approx$ 7.5, $J_{4,5}$ 4.5 Hz, H4), 4.12 (dd, 1 H, $J_{4',5'}$ 4.5, $J_{5',5'}$ 12.1 Hz, H5′), 4.64 (d, 1 H, $J_{2,3}$ 5.5 Hz, H2), 4.76 (dd, 1 H, $J_{2,3}$ 5.5, $J_{3,4}$ 7.3 Hz, H3), 4.85 (ddd, td, 1 H, $J_{3',4'} \approx J_{4',5'}$ 7.2, 4.5 Hz, H4′), 5.09 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H1′), 5.06 (dd, t, 1 H, $J_{2',3'} \approx J_{3',4'}$ 7.3 Hz, H3′), 5.34 (dd, t, 1 H, $J_{1',2'} \approx J_{2',3'}$ 8.0 Hz, H2′), 6.65 (br s, 1 H, NH). ^{13}C NMR (75.5 MHz): δ 20.44, 20.48, 20.61, 20.69 (5 C, Me), 41.30 (C5), 61.11 (C5′), 67.94, 69.73, 69.82, 70.58, 71.98, 72.38 (C2,3,4,2′,3′,4′), 97.97 (C1′), 167.48 (C1), 169.17, 169.42, 169.71, 169.77, 169.97 (5 C, CO). DCI HRMS (NH_3, CH_4): calcd for $[M + H]^+$ 490.1561, found 490.1560.

4-*O*-(Tri-*O*-acetyl-*β*-D-xylopyranosyl)-5-amino-2,3-di-*O*-acetyl-5-deoxy-D-xylonothio-1,5-lactam (26). A suspension of Lawesson's reagent (407 mg, 1.01 mmol) and **24** (492 mg, 1.01 mmol) in dry benzene (10 mL) was heated under reflux under nitrogen for 2 h. The

mixture was cooled and applied directly to a column of silica gel. Flash chromatography (70−90% EtOAc−petrol) afforded **26** as an unstable yellow foam (376 mg, 89%). ^{1}H NMR (400 MHz): δ 2.01, 2.03, 2.04, 2.06, 2.15 (5s, 15 H, Ac), 3.34−3.44 (m, 1 H, H5), 3.43 (dd, 1 H, $J_{4',5'}$ 7.1, $J_{5',5'}$ 12.1 Hz, H5′), 3.59 (dt, 1 H, $J_{4,5} \approx J_{5,NH}$ 3.6, $J_{5,5}$ 13.8 Hz, H5), 4.08−4.18 (m, 1 H, H4), 4.13 (dd, 1 H, $J_{4',5'}$ 4.4 Hz, H5′), 4.67 (d, 1 H, $J_{2,3}$ 5.5 Hz, H2), 4.77 (dd, 1 H, $J_{3,4}$ 7.3 Hz, H3), 4.87 (dt, 1 H, $J_{3',4'} \approx J_{4',5'}$ 4.5, $J_{4',5'}$ 7.1 Hz, H4′), 5.08 (t, 1 H, $J_{2',3'}$ 7.3 Hz, H3′), 5.22 (dd, 1 H, $J_{1',2'}$ 7.7 Hz, H2), 5.43 (d, 1 H, H1′), 8.45 (br s, 1 H, NH). DCI HRMS (NH_3, CH_4): calcd for $[M + H]^+$ 506.1332, found 506.1320.

4-*O*-(Tri-*O*-acetyl-*β*-D-xylopyranosyl)-2,3-tri-*O*-acetyl-D-xylono-(*Z*)-hydroximo-1,5-lactam (27). A mixture of the thionolactam **26** (80 mg, 158 μmol), $NaHCO_3$ (200 mg, 2.38 mmol), and NH_2OH·HCl (164 mg, 2.38 mmol) in MeOH (3 mL) was stirred at room temperature for 1 h and then was heated under reflux for 1 h. The solvent was evaporated and the residue was treated with Ac_2O (1 mL) and pyridine (3 mL) at room temperature for 1 h. Water (1 mL) was added and the mixture was stirred for 10 min. The mixture was diluted with CH_2Cl_2, and the organic phase was washed with water and saturated $NaHCO_3$ and dried. The solvent was evaporated and the residue was purified by flash chromatography (4:1 EtOAc−petrol then EtOAc) to give the oxime acetate **27** as a clear oil (57 mg, 66%). Crystallization of a portion gave analytically pure needles, mp 147−148 °C (EtOH). ^{1}H NMR (400 MHz): δ 2.00, 2.01, 2.02, 2.05, 2.08, 2.13 (5s, 15 H, 5 Ac), 3.30 (ddd, 1 H, $J_{4,5}$ 7.8, $J_{5,5}$ 12.4, $J_{5,NH}$ 1.5, H5), 3.39 (dd, 1 H, $J_{4',5'}$ 7.8, $J_{5',5'}$ 12.0 Hz, H5′), 3.46 (dt, 1 H, $J_{4,5} \approx J_{5,NH}$ 4.8 Hz, H5), 3.97 (dt, 1 H, $J_{3,4} \approx J_{4,5}$ 4.8 Hz, H4), 4.09 (dd, 1 H, $J_{4',5'}$ 4.7 Hz, H5′), 4.62 (d, 1 H, $J_{1',2'}$ 6.2 Hz, H1′), 4.80 (dd, 1 H, $J_{2',3'}$ 7.8 Hz, H2′), 4.87 (dt, 1 H, $J_{3',4'} \approx J_{4',5'}$ 7.8 Hz, H4′), 5.08 (t, 1 H, H3′), 5.30 (t, 1 H, $J_{2,3} \approx J_{3,4}$ 4.8 Hz, H3), 5.38 (d, 1 H, H2), 5.50 (br s, 1 H, NH). ^{13}C NMR (75.5 MHz): δ 19.68, 20.62, 20.66, 20.69, 20.76 (6 C, Me), 41.38 (C5), 61.57 (C5′), 67.59, 68.17, 70.18, 70.41, 71.62, 74.53 (C2,3,4,2′,3′,4′), 99.07 (C1′), 151.67 (C1), 168.62, 168.93, 169.08, 169.24, 169.73, 169.93 (6 C, CO). Anal. Calcd for $C_{22}H_{30}N_2O_{14}$: C, 48.35; H, 5.53; N, 5.13. Found: C, 48.37; H, 5.56; N, 5.01. DCI HRMS (NH_3, CH_4): calcd for $[M + H]^+$ 547.1776, found 547.1770.

4-*O*-(Tri-*O*-acetyl-*β*-D-xylopyranosyl)-2,3-tri-*O*-acetyl-D-xylono-(*Z*)-(^{15}N)hydroximo-1,5-lactam (28). The thionolactam (85 mg) (**26**) was treated with $^{15}NH_2OH$·HCl as for **27** above to afford the labeled oxime acetate (**28**) as needles (57 mg, 62%); mp 146−147 °C (EtOH). The ^{13}C NMR (75.5 MHz) spectrum was identical to that recorded for the material above. Anal. Calcd for $C_{22}H_{30}N_2O_{14}$: C, 48.27; H, 5.52; N, 5.12. Found: C, 48.46; H, 5.47; N, 4.97. DCI HRMS (NH_3, CH_4): calcd for $[M + H]^+$ 548.1746, found 548.1750.

D-Xylobiono-(*Z*)-hydroximo-1,5-lactam (6). A solution of the oxime acetate (**27**) (25 mg) in MeOH (2 mL) was treated with NaOMe (1 mL of 1 mg/mL NaOMe in MeOH) and the solution was allowed to stand at room temperature for 2 h. The solution was treated with cation-exchange resin (Amberlite IR-120, H^+ form) until it became neutral and the solvent was evaporated. The residue was purified by flash chromatography (7:2:1 then 4:2:1 EtOAc−MeOH−H_2O) to give the lactam oxime (**6**) as a pale yellow oil (12 mg, 89%). ^{1}H NMR (400 MHz, D_2O): δ 3.18 (dd, 1 H, $J_{4,5}$ 7.6, $J_{5,5}$12.5 Hz, H5), 3.22−3.55 (m, 2 H, H2′,5′), 3.46 (t, $J_{2',3'} \approx J_{3',4'}$ 9.3 Hz, H3′), 3.51 (dd, 1 H, $J_{4,5}$ 4.9 Hz, H5), 3.60 (ddd, 1 H, $J_{3',4'}$ 9.3, $J_{4',5'}$ 5.5 Hz, 10.3, H4′), 3.79 (t, 1 H, $J_{2,3} \approx J_{3,4}$ 6.9 Hz, H3), 3.95 (dd, 1 H, $J_{5',5'}$ 11.6 Hz, H5′), 3.92−4.01 (m, 1 H, H4), 4.17 (br d, 1 H, H2), 4.47 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H1′). ^{13}C NMR (75.5 MHz, D_2O): δ 41.91 (C5), 65.49, 69.10, 69.46, 72.99, 74.17, 75.81, 76.96 (C2,3,4,2′,3′,4′,5′), 101.98 (C1′), 154.79 (C1). LSIMS HRMS (glycerol): calcd for $[M + H]^+$ 295.1141, found 295.1140.

D-Xylobiono-(*Z*)-(^{15}N)hydroximo-1,5-lactam (29). A solution of the labeled oxime acetate (**28**) (45 mg) in dry MeOH (5 mL) was made basic with NaOMe and the solution was left to stand at room temperature for 2 h. The solvent was evaporated and the residue purified by flash chromatography (7:2:1 then 4:2:1 EtOAc−MeOH−H_2O) to give the lactam oxime (**29**) as a pale yellow oil (20 mg, 82%). The ^{1}H NMR (400 MHz) spectrum was identical to that recorded for the material above. ^{13}C NMR (75.5 MHz, D_2O): δ 41.90 (C5), 65.46 (C5′), 69.02 ($J_{2,N}$ 6.5 Hz, C2), 68.42, 72.96, 74.08, 75.78, 76.86 (C3,4,2′,3′,4′), 101.95 (C1′), 154.92 ($J_{1,N}$ 5.6 Hz, C1). ^{15}N NMR (50.7 MHz, D_2O) δ 252.8. LSIMS HRMS (thioglycerol): calcd for $[M + H]^+$ 296.1112, found 296.1101.

2,3-Di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-xylitol (33). A mixture of the lactam (**9**) (264 mg, 807 μmol) and $LiAlH_4$ (240 mg, 6.3 mmol) in dry THF (20 mL) was heated under reflux for 2 h. Water (10 mL) was cautiously added and the mixture was stirred for 20 min. Benzyl chloroformate (160 μL, 1.13 mmol) was added and the mixture was stirred overnight. The mixture was diluted with aqueous HCl (1 M) and extracted with $CHCl_3$. The organic phase was washed with saturated aqueous $NaHCO_3$ and dried. The solvent was evaporated and the residue was purified by flash chromatography (30−40% EtOAc−petrol) to give the carbamate (**33**) as a clear oil (302 mg, 83%). Crystallization of a portion gave an analytically pure microcrystalline powder; mp 81−82° (Et_2O/petrol). ^{1}H NMR (400 MHz): δ 3.15−3.93 (m, 7 H, C1,1,2,3,4,5,5), 4.45−4.83 (m, 6 H, PhCH_2OCH), 5.16, s, PhCH_2OCO), 7.18−7.45 (m, 15 H, Ph). ^{13}C NMR (75.5 MHz): δ 42.78, 43.46, 46.74 (2 C, C1,5), 67.26 (CH_2OCO), 67.69, 71.48, 73.12 (C2,3,4), 75.04, 75.24, 77.84, 78.17 (2 C, CH_2-OCH), 127.61−128.45, 136.53−137.87 (Ph), 155.99 (CO). Anal. Calcd for $C_{27}H_{29}NO_5$: C, 72.46; H, 6.53; N, 3.13. Found: C, 72.23; H, 6.66; N, 3.24. DCI HRMS (NH_3, CH_4): calcd for $[M + H]^+$ 448.2124, found 448.2120.

4-*O*-(Tri-*O*-acetyl-*β*-D-xylopyranosyl)-2,3-di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-xylitol (34). BF_3·Et_2O (100 μL, 0.79 mmol) was added to a solution of the trichloroacetimidate **20**[44] (1.02 g, 2.42 mmol) and the alcohol (**33**) (773 mg, 1.73 mmol) in dry 1,2-dichloroethane (25 mL) at 0 °C. After 10 min saturated aqueous $NaHCO_3$ (5 mL) was added and the mixture was stirred for 5 min. The organic phase was separated and dried and the solvent was evaporated. The residue was purified by flash chromatography (30−40% EtOAc−petrol) to give the disaccharide (**34**) as a foam (825 mg, 68%). ^{1}H NMR (400 MHz): δ 1.90−2.20 (9 H, m, Ac), 2.59−2.80, 2.74 (m, 1 H, dd, 1 H, *J* 10.1, 13.2 Hz, H1,5), 3.15−5.20 (m, 16 H, H1,2,3,4,5,1′,2′,3′,4′,5′,CH_2Ph), 4.07 (dd, 1 H, $J_{4',5'}$ 5.0, $J_{5',5'}$ 11.9 Hz, H5′), 7.22−7.43 (15 H, Ph). ^{13}C NMR (75.5 MHz): δ 20.55 (3C, CH_3CO), 45.14, 45.82 (C1,5), 67.38 (NCOCH_2), 68.49, 70.86, 71.46, 76.31, 76.83, 83.47 (C2,3,4,2′,3′,4′), 99.39 (C1′), 127.45−128.41, 136.11−138.27 (Ph), 154.78 (NCO), 169.18, 169.65, 169.79 (3C, CO).

Xylobiodeoxynojirimycin: 1,5-Dideoxy-1,5-imino-4-*O*-(*β*-D-xylopyranosyl)-D-xylitol (7). A solution of the disaccharide (**34**) (98 mg, 139 μmol) in dry MeOH (5 mL) was treated with a small piece of sodium metal and the solution left at room temperature for 30 min. The solvent was evaporated and the residue was dissolved in EtOH (10 mL) and aqueous HCl (1 mL of 0.5 M) and treated with Pd/C (10%, 20 mg) under hydrogen overnight. The mixture was filtered and the solvent was evaporated. The residue was purified by ion-exchange chromatography (Dowex 1X-8, OH^- form, eluted with water; BioRad AG 50W-X2, H^+ form, eluted with water then 2 M aqueous NH_3) to afford the amine (**7**) as a white solid (28 mg, 78%). Crystallization afforded an analytically pure powder; mp >200° (dec; H_2O/acetone). ^{1}H NMR (400 MHz, D_2O): δ 2.32−2.45 (2 H, H1,5), 2.98−3.12, 3.14−3.65 (2m, 9 H, H1,2,3,4,5,2′,3′,4′,5′), 3.94 (dd, 1 H, $J_{4',5'}$ 5.4, $J_{5',5'}$ 11.6 Hz, H5′), 4.43 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H1′). ^{13}C NMR (75.5 MHz, D_2O): δ 47.04, 49.29 (C1,5), 65.41 (C5′), 69.44, 71.28, 73.02, 75.88, 76.54, 78.64 (C2,3,4,2′,3′,4′), 101.70 (C1′), Anal. Calcd for $C_{10}H_{19}NO_7$·1/2H_2O: C, 43.79; H, 7.35; N, 5.11. Found: C, 43.96; H, 7.46; N, 4.94. LSIMS HRMS (thioglycerol): calcd for $[M + H]^+$ 266.1240, found 266.1247.

Methyl 2,3-Di-*O*-acetyl-D-threonate (37). A solution of methyl hydrogen di-*O*-acetyl-D-tartrate (**36**)[46,47] (18 g, 73 mmol) in dry THF (150 mL) was treated cautiously with BH_3·Me_2S (14 mL of 10 M, 140 mmol) and the solution was left at room temperature for 3 days. MeOH (10 mL) was added dropwise over 1 h and the solvent was evaporated. The residue was diluted with water and extracted with CH_2Cl_2. The organic extract was dried and the solvent was evaporated. The residue crystallized from Et_2O/petrol to afford the alcohol (**37**) as colorless needles (9.4 g, 55%); mp 75−76 °C (Et_2O/petrol). ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.05, 2.16 (2s, 6 H, Ac), 2.48 (t, 1 H, $J_{4,OH}$ 6.6 Hz, OH), 3.65−3.75 (m, 2 H, H4,4), 3.72 (s, OMe), 5.28−5.36 (m, 2 H, H2,3). ^{13}C NMR (50.1 MHz, $CDCl_3$) δ 20.41, 20.64 (2 C, CH_3CO), 52.68

(CH_3O), 60.39 (C4), 70.42, 72.26 (C2,3), 168.00 (C1), 170.21, 170.27 (CH_3CO). Anal. Calcd for $C_9H_{14}O_7$: C, 46.16; H, 6.03. Found: C, 46.12; H, 6.12.

Methyl 2,3-Di-*O*-acetyl-4-*O*-(*p*-tolylsulfonyl)-D-threonate (38). A solution of the alcohol (**38**) (659 mg, 2.82 mmol) and TsCl (803 mg, 4.22 mmol) in dry pyridine (8 mL) was allowed to stand overnight under N_2. Water (1 mL) was added and the mixture was stirred for 10 min. The mixture was diluted with EtOAc, washed successively with water, 1 M HCl, saturated $NaHCO_3$, and brine, and dried. The solvent was evaporated and the residue was purified by flash chromatography (30−60% EtOAc−petrol) to afford the tosylate (**38**) as a clear oil (1.06 g, 97%) which crystallized. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.97, 2.06 (2s, 6 H, Ac), 2.41 (s, ArCH_3), 3.66 (s, OMe), 4.05−4.15 (m, 2 H, H4,4), 5.16 (d, 1 H, $J_{2,3}$ 3.1 Hz, H2), 5.43 (ddd, 1 H, $J_{3,4}$ 6.4, 6.4 Hz, H3), 7.28−7.35, 7.69−7.76 (2m, 4 H, Ar). ^{13}C NMR (50.1 MHz, $CDCl_3$) δ 20.15, 20.30 (2 C, CH_3CO), 21.52 (ArCH_3), 52.67 (OCH_3), 65.44 (C4), 68.38, 69.68 (C2,3), 127.86, 129.91, 132.21, 145.26 (Ar), 166.95 (C1), 169.20, 169.49 (2 C, CO).

Methyl 4-Cyano-4-deoxy-D-threonate (39). A solution of the tosylate (**38**) (721 mg, 1.86 mmol) and potassium cyanide (603 mg, 9.29 mmol) in dry MeOH (20 mL) was heated at reflux under nitrogen for 2 h. The solvent was evaporated and the residue was applied directly to a column of silica gel and the product was eluted (70−100% EtOAc−petrol) to give the nitrile (**39**) as a white solid (115 mg, 39%). A small portion was recrystallized to afford flakes; mp 91−92 °C (EtOH/Pr^i_2O/petrol). ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.65−2.79 (m, 2 H, H4,4), 3.77 (s, 3 H, Me), 4.18 (d, 1 H, $J_{2,3}$ 2.6 Hz, H2), 4.23 (ddd, 1 H, $J_{3,4}$ 6.1, 7.3 Hz, H3). ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 22.60 (C4), 52.69 (CH_3), 69.80, 73.85 (C2,3), 119.24 (CN), 173.81 (CO). Anal. Calcd for $C_6H_9NO_4$: C, 45.28; H, 5.70; N, 8.80. Found: C, 45.41; H, 5.75; N, 8.66.

Methyl 2,3-Di-*O*-acetyl-4-cyano-4-deoxy-D-threonate (40). A solution of the tosylate (**38**) (5.84 g, 15.1 mmol) and potassium cyanide (4.89 g, 75.3 mmol) in dry MeOH (100 mL) was heated under reflux for 1 h. The solvent was evaporated and the residue was treated with Ac_2O/pyridine (1:3, 40 mL) at room temperature for 1 h. The dark mixture was quenched with water (5 mL) and stirred at room temperature for 5 min. The reaction mixture was diluted with EtOAc, washed sequentially with water, 1 M HCl, saturated $NaHCO_3$, and brine, and then dried, and the solvent was evaporated. The residue was purified by flash chromatography (30−50% EtOAc−petrol) to afford the diacetate (**40**) as a light yellow crystalline solid (1.70 g, 47%). A small portion was recrystallized to afford flakes; mp 75−76 °C (EtOH). ^{1}H NMR (200 MHz): δ 2.06, 2.17 (2s, 6 H, Ac), 2.66−2.87 (m, 2 H, H4,4), 3.72 (s, 3 H, OMe), 5.20 (d, 1 H, $J_{2,3}$ 3.3 Hz, H2), 5.48 (dt, 1 H, $J_{3,4} \approx J_{3,4}$ 6.6 Hz, H3). ^{13}C NMR (50.1 MHz): δ 19.69, 20.32, 20.39 (3C, C4,CH_3CO), 52.92 (OMe), 66.72, 71.29 (C2,3), 115.33 (CN), 166.68 (CO_2CH_3), 169.32, 169.66 ($COCH_3$). Anal. Calcd for $C_{10}H_{13}NO_6$: C, 49.38; H, 5.39; N, 5.76. Found: C, 49.47; H, 5.40; N, 5.70.

5-Amino-2,3-di-*O*-acetyl-4,5-dideoxy-D-*threo*-pentono-1,5-lactam (41). A suspension of the nitrile (**40**) (473 mg, 1.95 mmol) and PtO_2 (55 mg) in MeOH (20 mL) was treated with H_2 overnight. The mixture was filtered, the solvent evaporated, and the residue purified by flash chromatography (90−100% EtOAc−petrol then 5% MeOH-EtOAc) to give the lactam (**41**) as a clear oil (268 mg, 64%). ^{1}H NMR (400 MHz): δ 1.81−2.23 (2 H, H4,4), 1.99, 2.06 (2s, 6 H, Ac), 3.23−3.35 (2 H, m, H5,5), 5.09−5.19 (2 H, m, H2,3), 7.32 (br s, 1 H, NH). ^{13}C NMR (75.5 MHz): δ 20.56, 20.81 (2 C, CH_3CO), 26.77 (C4), 37.58 (C5), 69.53, 71.25 (C2,3), 167.88 (C1), 169.91, 169.95 (2 C, CH_3CO).

5-Amino-4,5-dideoxy-D-*threo*-pentono-1,5-lactam (42). A solution of the lactam (**41**) (3.24 g, 15.1 mmol) in dry MeOH (50 mL) was treated with a small piece of sodium metal at room temperature for 2 h. The solution was neutralized with cation-exchange resin (IR-120, H^+ form) and the solvent was evaporated. The residue was purified by flash chromatography (90% EtOAc−MeOH then 17:2:1 then 7:2:1 EtOAc−MeOH−H_2O) to give the diol (**42**) as a light orange crystalline solid (1.77 g, 90%). A small portion was recrystallized to afford flakes; mp 111−112 °C (EtOH). ^{1}H NMR (400 MHz): δ 1.82−1.95, 2.06−2.14 (2m, 2 H, H4,4), 3.25−3.32 (m, 2 H, H5,5), 3.86−3.95 (m, 2 H, H2,3), 4.75 (br m, 1 H, NH). ^{13}C NMR (75.5 MHz): δ 28.19 (C4), 30.04 (C5), 69.73, 73.16 (C2,3), 173.95 (C1). Anal. Calcd for $C_5H_9NO_3$: C, 45.80; H, 6.92; N, 10.68. Found: C, 46.03; H, 7.05; N, 10.55. DCI HRMS (NH_3, CH_4): calcd for *m*/*z* $[M + H]^+$ 132.0661, found 132.0660.

***N*-Benzyloxycarbonyl-1,4,5-trideoxy-1,5-imino-D-*threo*-pentitol (43).** A mixture of the lactam (**42**) (592 mg, 4.52 mmol), hexamethyldisilazane (2.09 mL, 9.94 mmol), and TMSCl (0.1 mL) in acetonitrile (5 mL) was heated under reflux for 2 h. The solvent was evaporated and the residue was dissolved in dry dioxane (10 mL) and treated with BH_3·Me_2S (2.26 mL, 22.6 mmol) at reflux for 2 h. The solution was cooled and excess aqueous HCl (1 M) was added cautiously and the resultant solution was heated under reflux for 1 h. The bulk of the solvent was evaporated and the residue was treated carefully with saturated aqueous $NaHCO_3$ (30 mL) and then benzyl chloroformate (0.90 mL, 6.3 mmol) and the mixture was stirred vigorously overnight. The mixture was extracted with CH_2Cl_2, and the combined extracts were dried and the solvent was evaporated. The residue was purified by flash chromatography (90−100% EtOAc−petrol then 10% MeOH−EtOAc) to give the diol (**43**) as a white solid (628 mg, 55%); mp 81−82° (EtOH/Pr^i_2O). ^{1}H NMR (400 MHz): δ 1.34−1.51, 1.82−1.95 (2m, 2 H, H5,5), 2.66, 2.70−2.92 (dd, 2 H, *J* 9.9, 13.1 Hz, m, H2,6), 3.30−3.55 (m, 2 H, H2,6), 3.90−4.21 (m, 2 H, H3,4), 5.07 (s, CH_2Ph), 7.25−7.55 (m, 5 H, Ph). ^{13}C NMR (75.5 MHz): δ 31.35 (C5), 42.04, 47.72 (C2,6), 67.36 (CH_2Ph), 71.35, 72.87, 73.36 (C3,4), 127.70, 128.00, 128.39, 136.16 (Ph), 155.33 (CO). Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.37; H, 6.91; N, 5.60. DCI HRMS (NH_3, CH_4): calcd for *m*/*z* $[M + H]^+$ 252.1236, found 252.1231.

D-*xylo*-Isofagomine: 1,4,5-trideoxy-1,5-imino-D-*threo*-pentitol (35). A suspension of the carbamate (**43**) (84 mg, 335 μmol) and Pd/C (10%, 10 mg) in EtOH (5 mL) and aqueous HCl (2 mL of 0.5 M) was treated with H_2 and stirred overnight. The mixture was filtered and the solvent evaporated. The residue was purified by ion-exchange chromatography (Dowex 1X-8, OH^- form, eluted with water; BioRad AG 50W−X2, H^+ form, eluted with water then 2 M aqueous NH_3) to afford the amine (**35**) as an extremely hygroscopic, white solid (32 mg, 82%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.37−1.48 (m, 1 H, H4), 1.92 (dddd, 1 H, $J_{3,4} \sim J_{4,5}$ 3.0, $J_{4,5}$ 4.1, $J_{4,4}$ 13.2 Hz, H4), 2.37 (dd, 1 H, $J_{1,1}$ 12.4, $J_{1,2}$ 9.1 Hz, H1), 2.54 (ddd, 1 H, $J_{4,5}$ 11.3, $J_{5,5}$ 12.9 Hz, H5), 2.94 (dddd, 1 H, $J_{3,5}$ 1.1, $J_{4,5}$ 4.1 Hz, H5), 3.06 (ddd, 1 H, $J_{1,2}$ 1.3, 4.3 Hz, H1), 3.29−3.45 (m, 2 H, H2,3). ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 33.92 (C4), 44.71, 51.34 (C1,5), 73.42, 73.89 (C2,3). LSIMS HRMS (glycerol, MeOH): calcd for *m*/*z* $[M + H]^+$ 118.0868, found 118.0867.

The ^{1}H NMR (400 MHz, D_2O) spectrum of **35·HCl** (prepared by addition of HCl in MeOH to **35**) was identical to that reported.[29]

2-*O*-Benzoyl-*N*-benzyloxycarbonyl-1,4,5-trideoxy-1,5-imino-D-*threo*-pentitol (45) and 3-*O*-Benzoyl-*N*-benzyloxycarbonyl-1,4,5-trideoxy-1,5-imino-D-*threo*-pentitol (44). A solution of the diol (**43**) (290 mg, 1.16 mmol), 1-benzoyloxybenzotriazole (290 mg, 1.21 mmol), and Et_3N (177 μmol) in dry CH_2Cl_2 (25 mL) was allowed to stand overnight at room temperature under N_2. The solution was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, and dried. The solvent was evaporated and the residue was purified by flash chromatography (20−30% EtOAc−toluene) to give, first, the 3-*O*-benzoate (**44**) (181 mg, 44%). ^{1}H NMR (400 MHz): δ 1.65−1.77, 2.12−2.21 (2m, 2 H, H4,4), 3.10−4.15 (m, 4 H, H1,1,2,5,5), 4.98−5.05 (m, 1 H, H3), 5.13 (s, 2 H, CH_2Ph), 7.27−7.58, 8.00−8.06 (m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 28.05 (C4), 41.24, 47.57 (C1,5), 67.42 (CH_2Ph), 68.19, 68.59, 74.32, 75.01 (2 C, C2,3), 127.90−136.36 (Ph), 155.43 (NCO), 166.35 (*C*OPh). DCI HRMS (CH_4): calcd for *m*/*z* $[M + H]^+$ 356.1498, found 356.1499.

Next to elute was the 2-*O*-benzoate (**45**) (168 mg, 41%). ^{1}H NMR (400 MHz): δ 1.55−1.80, 1.92−2.21 (2m, 2 H, H4,4), 2.50−2.82 (m, 1 H, OH), 3.50−4.20 (m, 4 H, H1,1,2,5,5), 4.85−5.20 (m, 3 H, H3, CH_2Ph), 7.20−7.60, 7.95−8.05 (2m, 5 H, Ph). ^{13}C NMR (75.5 MHz): δ 30.01, 30.72 (1 C, C4), 40.21, 40.53, 43.99, 44.27 (2 C, C1,5), 67.27 (CH_2Ph), 68.03, 68.92, 72.15, 72.75 (2 C, C2,3), 127.75−136.38 (Ph), 155.38 (NCO), 165.96 (*C*OPh). DCI HRMS (CH_4): calcd for *m*/*z* $[M + H]^+$ 356.1498, found 356.1496.

3-*O*-(Tri-*O*-acetyl-β-D-xylopyranosyl)-2-*O*-benzoyl-*N*-benzyloxycarbonyl-1,5-imino-1,4,5-trideoxy-D-*threo*-pentitol (46). BF_3·Et_2O (90

μL, 0.70 mmol) was added to a suspension of powdered 4 Å molecular sieves (2 g), the alcohol (**44**) (437 mg, 1.24 mmol), and the trichloroacetimidate **20**[44] (782 mg, 1.86 mmol) in 1,2-dichloroethane (15 mL) at 0 °C under N_2, and the solution was allowed to stand for 2 h. Et_3N (1 mL) was added, the mixture was filtered through Celite, and the solvent was evaporated. The oil was dissolved in EtOAc, washed with saturated aqueous $NaHCO_3$ and then brine, and dried, and the solvent was evaporated. The residue was purified by flash chromatography (20−25% EtOAc−toluene) to give the disaccharide (**46**) as a clear oil (332 mg, 44%). ^{1}H NMR (400 MHz): δ 2.00, 2.01, 2.03 (3s, 9 H, Ac), 1.60−1.71, 2.00, 2.10 (2m, 2 H, H4,4), 3.25−4.05 (m, 5 H, H1,1,3,5,5), 3.34 (dd, 1 H, $J_{4',5'}$ 8.9, $J_{5',5'}$ 11.7 Hz, H5′), 4.08 (dd, 1 H, $J_{4',5'}$ 5.0 Hz, H5′), 4.62 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H1′), 4.79−5.17 (m, 5 H, H2′,3′,4′,PhCH_2), 7.08−7.58, 7.89−7.96 (2m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 20.66, 20.70 (CH_3CO), 26.35 (C4), 39.15, 43.53 (C1,5), 62.02 (C5′), 67.14 (CH_2Ph), 68.54, 69.12, 70.78, 71.12, 73.12 (C2,3,2′,3′,4′), 99.58 (C1′), 127.62−136.22 (Ph), 155.65 (NCO), 165.17, PhCO), 169.21, 169.74, 170.01 (MeCO). DCI HRMS (CH_4, NH_3): calcd for *m*/*z* [M + H]$^+$ 614.2238, found 614.2243.

***N*-Benzyloxycarbonyl-1,5-imino-1,4,5-trideoxy-3-*O*-(β-D-xylopyranosyl)-D-*threo*-pentitol (47).** A solution of the disaccharide (**46**) (291 mg) in dry MeOH (15 mL) was treated with a small piece of sodium metal, and the solution was allowed to stand overnight. The solution was neutralized with cation-exchange resin (IR-120, H^+ form) and the solvent was evaporated. The residue was purified by flash chromatography (EtOAc then 27:2:1 then 17:2:1 EtOAc−MeOH−H_2O) to give the carbamate (**47**) as a clear oil (148 mg, 81%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.49−1.60, 1.95−2.06 (2m, 2 H, H4,4), 3.10−3.90 (m, 11 H H1,1,2,3,5,5,2′,3′,4′,5′,5′), 4.31 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H1′), 5.09 (s, 2 H, CH_2Ph), 7.26−7.35 (m, 5 H, Ph). ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 28.30, 28.62 (C4), 41.84, 47.96 (C1,5), 66.95 (CH_2Ph), 68.32 (C5′), 69.61, 71.07, 74.62, 77.72, 79.37 (C2,3,2′,3′,4′), 101.41 (C1′), 128.81, 129.05, 129.49, 138.08 (Ph), 157.21 (CO). LSIMS HRMS (thioglycerol): calcd for *m*/*z* [M + H]$^+$ 384.1658, found 384.1660.

Xylobioisofagomine: 1,5-Imino-1,4,5-trideoxy-3-*O*-(β-D-xylopyranosyl)-D-*threo*-pentitol (8). A suspension of the carbamate (**47**) (104 mg), Pd/C (10%, 10 mg), and aqueous HCl (2 mL of 0.5 M) in EtOH (10 mL) was treated with H_2 overnight. The mixture was filtered and the solvent evaporated. The residue was applied to a column of anion-exchange resin (BioRad AG-1 × 8, 200−400 mesh, OH^- form) and eluted with water. The eluant was evaporated, the residue was applied to a column of cation-exchange resin (BioRad AG-50W ×2, 200−400 mesh, H^+ form), and the column was washed with water and then eluted with aqueous ammonia (2 M). The solvent was evaporated to afford the amine (**8**) as a white crystalline solid (56 mg, 83%). This material was recrystallized to afford a white powder; mp 215 °C (dec; H_2O/acetone). ^{1}H NMR (400 MHz): δ 1.34−1.47, 2.03−2.12 (2m, 2 H, H4,4), 2.37 (dd, 1 H, $J_{1,1}$ 12.5, $J_{1,2}$ 9.8 Hz, H1), 2.42−2.53 (m, 1 H, H5), 2.90−3.00, 3.05−3.15 (2m, 2 H, H1,5), 3.24 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.3 Hz, H2′), 3.27 (dd, 1 H, $J_{4',5'}$ 4.8, $J_{5',5'}$ 11.5 Hz, H5′), 3.41 (t, 1 H, $J_{3',4'}$ 9.3 Hz, H3′), 3.51 (ddd, 1 H, $J_{4',5'}$ 5.4 Hz, H4′), 3.55−3.67 (m, 2 H, H2,3), 3.96 (dd, 1 H, H5′), 4.47 (d, 1 H, H1′). ^{13}C NMR (75.5 MHz): δ 29.65 (C4), 42.94, 49.38 (C1,5), 65.35 (C5′), 69.46, 70.16, 73.03, 75.95, 80.28 (C2,3,2′,3′,4′), 100.79 (C1′). Anal. Calcd for $C_{10}H_{19}NO_6$: C, 48.19; H, 7.68; N, 5.62. Found: C, 48.14; H, 7.77; N, 5.60. LSIMS HRMS (thioglycerol): calcd for *m*/*z* [M + H]$^+$ 250.1291, found 250.1279.

4-*O*-(Tri-*O*-acetyl-β-D-xylopyranosyl)-3-*O*-benzoyl-*N*-benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-*threo*-pentitol (49). BF_3·Et_2O (70 μL, 0.55 mmol) was added to a suspension of powdered 4 Å molecular sieves (2 g), the alcohol (**45**) (382 mg, 1.08 mmol), and the trichloroacetimidate (**20**)[44] (683 mg, 1.62 mmol) in 1,2-dichloroethane (15 mL) at 0 °C under N_2, and the solution was allowed to stand for 2 h. Et_3N (1 mL) was added, the mixture was filtered through Celite, and the solvent was evaporated. The oil was dissolved in EtOAc, washed with saturated aqueous $NaHCO_3$ and then brine, and dried, and the solvent was evaporated. The residue was purified by flash chromatography (20−25% EtOAc−toluene) to give the disaccharide (**49**) contaminated with trichloroacetamide, as a clear oil (474 mg). This material could be purified by careful chromatography for characterization; however, the bulk of the material was used directly in the next step. ^{1}H NMR (400 MHz): δ 1.60−1.75, 2.05−2.21 (2m, 2 H, H2,2), 1.97, 2.00 (2s, 9 H, Ac), 2.90−4.15 (m, 7 H, H1,1,4,5,5,5′,5′), 4.60−5.25 (m, 5 H, H3,1′,2′,3′,4′), 5.11 (s, 2 H, CH_2Ph), 7.26−7.58, 7.98−8.04 (2m, 10 H, Ph). ^{13}C NMR (75.5 MHz): δ 20.54, 20.63 (3C, CH_3), 27.01, 27.46, 28.01 (1 C, C2), 40.25, 41.18, 43.44, 44.00, 47.53 (2 C, C1,5), 61.57 (C5′), 67.29, (CH_2Ph), 68.33, 68.49, 70.26, 70.82, 72.40, 73.07, 74.35, 74.87 (C3,4,2′,3′,4′), 98.02, 98.91 (1 C, C1′), 127.80−136.34, (Ph), 155.14, (NCO), 165.20, (PhCO), 169.27, 169.69, 169.87 (3C, CH_3CO). DCI HRMS (CH_4, NH_3): calcd for *m*/*z* [M + H]$^+$ 614.2238, found 614.2244.

***N*-Benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-4-*O*-(β-D-xylopyranosyl)-D-*threo*-pentitol (50).** The material above was treated as for **47** to afford the carbamate (**50**) as a clear oil (185 mg, 45% over two steps). ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.32−1.45 (m, 1 H, H2), 1.95−2.00 (m, 1 H, H2), 3.02−4.01 (m, 10 H, H1,1,3,4,5,5,2′,3′,4′,5′), 3.81 (dd, 1 H, $J_{4',5'}$ 5.3, $J_{5',5'}$ 11.4 Hz, H5′), 4.25−4.31 (br m, 1 H, H1′), 5.02−5.13 (m, 2 H, CH_2Ph), 7.24−7.35 (m, H, Ph). ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 31.37 (C2), 42.04, 45.83 (C1,5), 67.01 (CH_2Ph), 68.43 (C5′), 70.19, 71.00, 74.52, 77.62, 78.64, 79.20 (5 C, C3,4,2′,3′,4′), 103.62, 104.32 (1 C, C1′), 128.87, 129.13, 129.56, 138.03 (Ph), 157.02, 157.46 (1 C, NCO). LSIMS HRMS (thioglycerol): calcd for *m*/*z* [M + H]$^+$ 384.1658, found 384.1648.

2-Deoxyxylobiodeoxynojirimycin: 1,5-Imino-1,2,5-trideoxy-4-*O*-(β-D-xylopyranosyl)-D-*threo*-pentitol (48). The carbamate (**50**) (91 mg) was treated as for **47** above to afford the amine (**48**) as a white crystalline solid (47 mg, 79%). This material was recrystallized to afford a white powder; mp >220° (dec; water/acetone). ^{1}H NMR (400 MHz): δ 1.34−1.48, 1.92−2.02 (2m, 2 H, H2,2), 2.42 (dd, 1 H, $J_{4,5}$ 9.4, $J_{5,5}$ 12.6 Hz, H5), 2.46−2.57, 2.88−2.95 (2m, 2 H, H1,1), 3.17 (dd, 1 H, $J_{4,5}$ 3.9 Hz, H5), 3.23 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.3 Hz, H2′), 3.28 (dd, 1 H, $J_{4',5'}$ 8.4, $J_{5',5'}$ 11.5 Hz, H5′), 3.52 (ddd, 1 H, $J_{4',5'}$ 5.5 Hz, H4′), 3.55−3.66 (m, 2 H, H3,4), 3.93 (dd, 1 H, H5′), 4.44 (d, 1 H, H1′). ^{13}C NMR (75.5 MHz): δ 31.96 (C2), 42.62, 46.73 (C1,5), 65.39 (C5′), 69.43, 70.55, 73.03, 75.87, 79.51 (C3,4,2′,3′,4′), 101.68 (C1′). Anal. Calcd for $C_{10}H_{19}NO_6$: C, 48.19; H, 7.68; N, 5.62. Found: C, 48.11; H, 7.80; N, 5.70. LSIMS HRMS (thioglycerol): calcd for *m*/*z* [M + H]$^+$ 250.1291, found 250.1287.

Kinetic Analysis. (a) Inhibition of *C. fimi* xylanase, Cex. Cex was purified as described previously.[54] Inhibition constants were determined at 37 °C using a 0.05 M NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) and 2,4-dinitrophenyl β-cellobioside as a substrate. Measurements were started by addition of Cex. Measurements of the increase of absorption at 400 nm per min in a continuous assay yielded reaction rates. This increase was linear during all measurements (1−3 min). Michaelis parameters (V_{max} and K_m) were extracted from these data by best fit to the Michaelis−Menten equation using the program Grafit.[55] Estimates of K_i values were obtained by measuring rates in a series of cells at a fixed substrate concentration in the presence of a range of inhibitor concentrations (6−10 concentrations) which encompassed the K_i value ultimately determined, generally from 0.33K_i to 3K_i. The observed rates were plotted in the form of a Dixon plot, and the K_i value was determined by an intersection of this line with a horizontal line drawn through $1/V_{max}$. Full K_i determinations were performed for those inhibitors whose estimated K_i was <10 μM by measurement of rates at a series of seven substrate concentrations (generally from 0.3K_i to 3K_i) in the presence of a range of inhibitor concentrations (typically five concentrations) which bracket the K_i value ultimately determined. Full K_i determinations were usually within 25% of the estimated K_i values. K_i values were calculated from these data by three-dimensional nonlinear regression analysis using the program Grafit. The full K_i determination for xylobiose was performed using 4-nitrophenyl β-D-xylopyranoside as substrate, monitored at 400 nm.

(b) Inhibition of *Bacillus circulans* xylanase, Bcx. Bcx was purified as described previously.[56] Inhibition constants were determined at 40 °C using a 0.02 M 2-morpholinoethanesulfonic acid buffer (pH 6.0)

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containing 0.05 M NaCl, using 2,5-dinitrophenyl β-xylobioside as a substrate and following changes in absorbance at 440 nm. Kinetic studies and data analysis were performed as described above. Full K_i determinations were performed for inhibitors whose estimated K_i was <1.4 mM.

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