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C_2-Symmetrical Tetrahydroxyazepanes as Inhibitors of Glycosidases and HIV/FIV Proteases

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Abstract—C_2-Symmetrical tetrahydroxyazepanes were synthesized as inhibitors for glycosidases. Tetrahydroxyazepane **1** is a non-specific inhibitor of various glycosidases, while compounds **2**, **3** and **4** specifically inhibit β-*N*-acetylglucosaminidase, β-glucosidase, and *a*-fucosidase, respectively, with K_i in the micromolar range. Compound **1** is not an inhibitor of HIV/FIV proteases, but its 3,6-difluorobenzyl derivatives are moderate inhibitors of both enzymes. Copyright © 1996 Elsevier Science Ltd

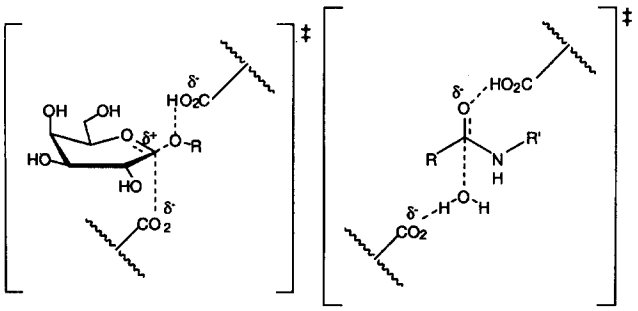


Figure 1. Proposed transition-state structures of glycosidase (left) and HIV protease (right) catalyzed reactions.

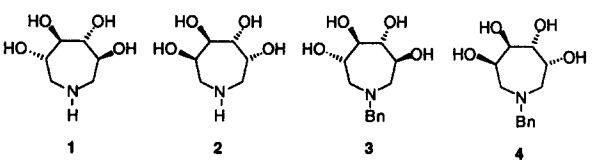
Introduction

Glycosidases and aspartyl proteases share a common mechanism in catalysis (i.e. both utilize two carboxyl groups as general acid and general base in the hydrolytic reactions, Fig. 1).[1,2] All the inhibitors of these two classes of enzymes developed so far are, however, structurally very different. Five- and six-membered iminocyclitols, for example, have been used as transition-state analogue inhibitors of glycosidases[3] and various peptide isosteres, including those with C_2-symmetry, have been developed as inhibitors of the HIV (Human Immunodeficiency Virus) protease.[4] The *N*-containing cyclitols, however, do not inhibit the HIV protease[5] and FIV (Feline Immunodeficiency Virus) protease.[6]

We report here that seven-membered iminocyclitols (tetrahydroxyazepanes) can be used as a common scaffold for the development of glycosidase and HIV/FIV protease inhibitors. These heterocycles are conformationally more flexible than the corresponding six- and five-membered counterparts and may adopt the half-chair or *pseudo*-chair structure to mimic the transition state of the enzymatic glycosidic cleavage.[7] They also may be large enough to act as inhibitors of HIV/FIV proteases through H-bonding interaction with the active-site carboxylate groups. Though several tetrahydroxyazepanes are known, little is known with regard to their biological activities, with the exception that one related compound was reported to have no inhibitory activity against *a*-mannosidase.[8] Since the HIV and FIV proteases are homodimers with C_2-symmetry, and many potent HIV protease inhibitors to date also contain an element of C_2-symmetry,[4] we chose compounds **1**–**4** (Fig. 2) as core structures for the development of inhibitors of glycosidases and the two aspartyl proteases.

Results and Discussion

In our preliminary study,[7] we have reported that compound **1** is a broad spectrum inhibitor of different glycosidases, and compounds **2** and **4** are micromolar inhibitors of β-*N*-acetylglucosaminidase and *a*-fucosidase, respectively. The syntheses of these compounds followed a literature procedure[9] with some modifications. 1,2:5,6-Di-*O*-isopropylidene-D-mannitol (**5**) was used as starting material and was converted to 3,4-di-*O*-allyl-D-mannitol (**7**) in two steps with high yield (Scheme 1). The tetra-ol **7** was then further derivatized via hydroxyl group manipulations followed by an intramolecular S_N2 reaction to give 3,4-di-*O*-allyl-1,2:5,6-dianhydro-L-iditol (bis-epoxide **9**). Compound **7** was also converted via an intramolecular Mitsunobu reaction (Scheme 2) to 1,2:5,6-dianhydro-3,4-di-*O*-allyl-D-mannitol (bis-epoxide **12**). Both epoxides were treated with allylamine in the presence of perchloric acid (Schemes 1 and 2) to give a mixture of C_2-symmetrical tetrahydroxyazepane (**10**) and the partially



1 **2** **3** **4**

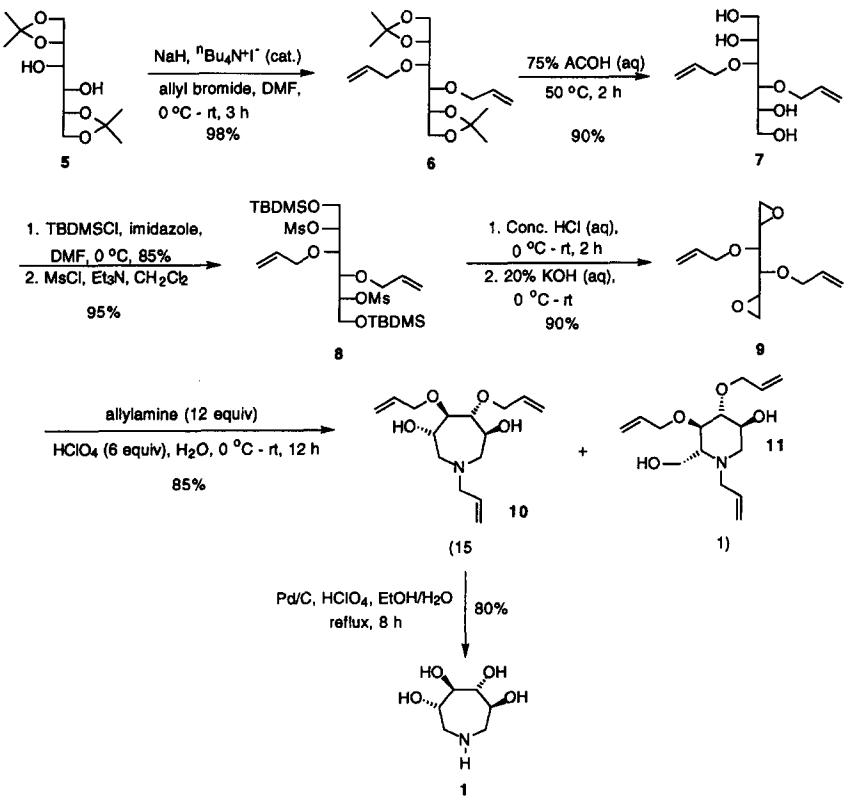
Figure 2. C_2-Symmetrical tetrahydroxyazepanes as glycosidase inhibitors.

protected 1-deoxynojirimycin (**11**) or a mixture of C_2-symmetrical tetrahydroxyazepane (**13**) and the partially protected L-gulo-piperidine (**14**). The ratio of hydroxyazepane to hydroxypiperidine was higher (15:1 to 20:1) than that according to the Depenzay's method (2:1 to 7:1).[9] Utilization of 3,4-di-*O*-allyl derivatives and allylamine as nucleophile may contribute to this improvement. The allyl groups were removed with 10% Pd/C suspended in perchloric acid in methanol at refluxing temperature to give the azepanes **1** and **2** (Schemes 1 and 2).[10] Other conditions were tested to hydrolyse the allyl ethers; however, no satisfactory results were obtained.

N-Benzylated tetrahydroxyazepanes were obtained using the same bis-epoxides as key intermediates (Scheme 3). The conditions used to open bis-epoxides **9** and **12** followed by a spontaneous intramolecular ring closure was the same as that used in the preparation of azepanes **10** and **13**, except that the nucleophile used here was benzylamine. Thus, orthogonally protected tetrahydroxyazepanes **15** and **17** were obtained as major products. Selective removal of ally groups in compounds **15** and **17** under the condition of palladium–Lewis acid promoted hydrolysis[11] afforded the *N*-benzylated tetrahydroxylazepanes **3** and **4**. Due to the difficulty of removing tin-containing contaminants, multiple chromatographic separations are necessary, and the isolated yield was moderate.

Compounds **1–4** were evaluated in vitro for their inhibitory effects against various glycosidases, and the results are listed in Table 1. (3*S*,4*R*,5*R*,6*S*)-3,4,5,6-Tetrahydroxyazepane (**1**) is an inhibitor of all of the glycosidases that were tested; among them **1** inhibits β-galactosidase most efficiently with $K_i = 6.5$ μM. (3*R*,4*R*,5*R*,6*R*)-3,4,5,6-Tetrahydroxyazepane (**2**) is a good inhibitor of β-*N*-acetylglucosaminidase with a K_i of 4.6 μM. It is also interesting to see that hydroxyazepanes **1** and **2** tend to inhibit β-glycosidases better than *a*-glycosidases in general. *N*-Benzylation changed the inhibitory profile of these two hydroxyazepanes. Unlike compound **1**, its *N*-benzylated form or tetrahydroxyazepane **3** does not have potent inhibitory activities to most of the glycosidases tested. However, it still maintained its inhibitory activity against β-glucosidase with a $K_i = 30.5$ μM. Compound **3** also weakly inhibits *a*-mannosidase and α-galactosidase. The *N*-benzylated derivative **2** changed its inhibitory selectivity from β-*N*-acetylglucosaminidase to *a*-fucosidase with $K_i = 23.4$ μM. The different inhibitory selectivity between compounds **1** and **3**, as well as **2** and **4** may indicate that (1) selective recognition of glycosidases may not solely depend upon the stereochemistry of hydroxyl groups of the inhibitor; (2) some glycosidases may have a lipophilic pocket near the active site. Thus, this series of tetrahydroxyazepanes are good molecular probes for the study of glycosidases recognition.

In order to explain the inhibitory activity of these hydroxyazepanes and their structural correlations with five- or six-membered azasugars and their derivatives (Fig. 3), models of these compounds were built. The model of azepane **1** was built based on molecular dynamics simulation using InsightII software.[12] Three

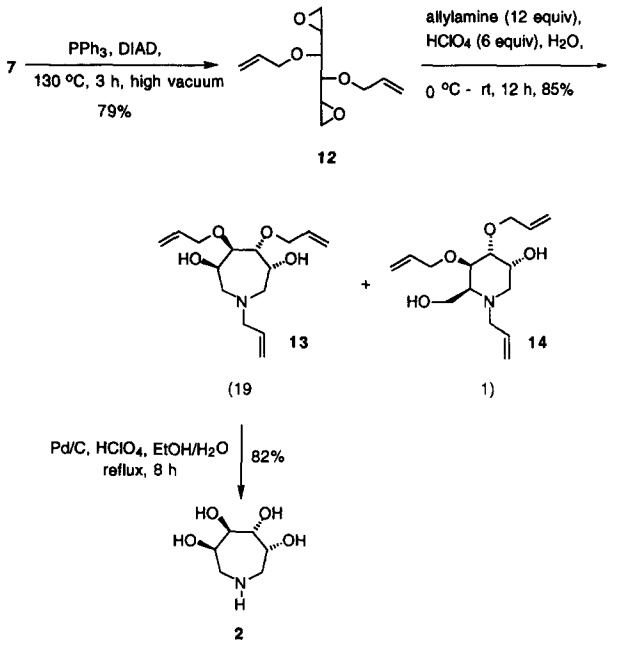


Scheme 1.

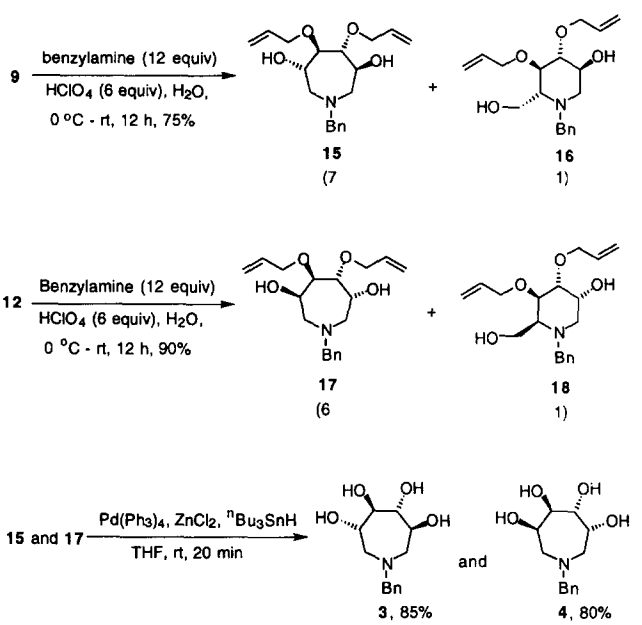
low energy trajectories were obtained (Fig. 4). One of them has one axial hydroxyl group, another has two, and the third has four hydroxyl groups all in the axial position stabilized by an intramolecular hydrogen bonding (Fig. 4). The first two bear a pseudo-chair conformation, while the third one has a boat conformation. Considering the stable conformation of six-membered ring sugar has a chair conformation and the hydroxyl groups are preferred to locate in the equatorial positions, the pseudo-chair conformation with least axial hydroxyl groups was chosen for structural comparisons with five- or six-membered azasugar glycosidase inhibitors. The first compound selected for comparison with azepane **1** was a β-galactosidase inhibitor, (3*S*,4*R*,5*R*,6*R*)-3,4,5-trihydroxy-6-hydroxymethylpiperidine (Fig. 3), with a K_i value of 13 μM.[13] Two ways of molecular fitting were attempted. In the first fitting (Fig. 5a), there is a good fitting between the nitrogen atoms and the 3,4-OHs, as well as the C2–C4 backbones. However, the 6-OH oxygen atom does not fit well to the 1°-OH oxygen in the piperidine. In another fitting (Fig. 5b), the ring nitrogen atoms and the 3,4-OHs, and the 6-OH in **8** and the 5-OH in the piperidine fitted well to each other. Thus, it is possible to overlap most of the pharmacophores in both molecules (three -OHs and one sp^{3+} ring nitrogen atom).

Since compound **1** is also a good inhibitor of β-glucosidase, it is compared with isofagomine (Fig. 3), which is a very good β-glucosidase inhibitor with a K_i value of 0.11 μM.[14] All three hydroxyl groups of isofagomine overlap well with the 3-, 4-, and 5-OHs, in addition the ring nitrogen atoms also are overlapped (Fig. 6). However, the axial 6-OH of **1** is an extra functionality compared to isofagomine, thus it may disturb a tight binding between **1** and the β-glucosidase.

The model of compound **3** was built based on the model of compound **1**, with the ring nitrogen protected with a benzyl group (in the equatorial position) and was energy minimized. It was compared with a benzylamidine derivative (Fig. 3), which is also a β-glucosidase



Scheme 2.



Scheme 3.

Table 1. Inhibition of glycosidases with tetrahydroxyazepanes and analogues

Enzymes	$K_i \pm$S.E.M. (μM)			
	1 (200 μM)	**2** (240 μM)	**3** (200 μM)	**4** (160 μM)
α-Mannosidase from jack beans	25.7 ± 1.3	NI[b]	48%[a]	11%[a]
α-Galactosidase from green coffee beans	67.0 ± 4.5	NI	31%[a]	NI
β-Galactosidase from *Aspergillus niger*	6.5 ± 1.2	NI	3%[a]	NI
α-Glucosidase from yeast	29.4 ± 2.2	21%[a]	NI	6%[a]
β-Glucosidase from sweet almonds	12.8 ± 0.7	NI	30.5 ± 2.5	14%[a]
β-*N*-Acetylglucosaminidase from jack beans	22.7 ± 2.6	4.6 ± 0.4	1%[a]	6%[a]
α-Fucosidase from bovine kidney	44%[a]	16%[a]	5%[a]	23.4 ± 3.8

[a]The assay concentrations for percent inhibition are indicated in parentheses and are performed with weak inhibitors. All others numbers represent K_i values in μM. All tetrahydroxyazepanes are competitive inhibitors.
[b]NI: no inhibition.

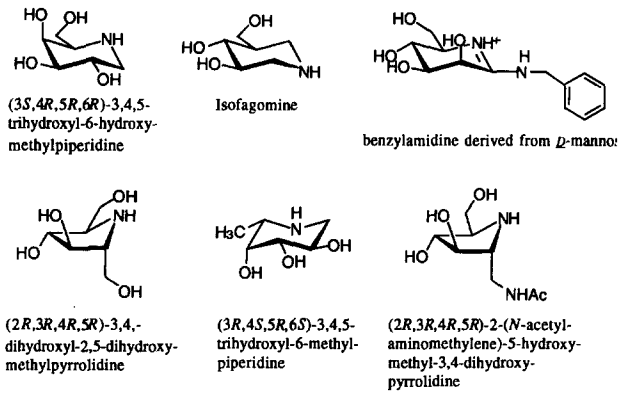


Figure 3. Potent glycosidase inhibitors that are used in structural comparisons with the models or the crystal structure of tetrahydroxylazepanes.

inhibitor with a K_i value of 25 μM.[15] As shown in Figure 7, one of the amidine nitrogen and the ring nitrogen of **3**, as well as two hydroxyl groups in both molecules are well overlapped. The 5-OH group in **3** and the 1°-OH in the amidine are close to each other in this fitting. The benzyl moiety of both molecules also occupied the same space. These structural similarities may explain why these two molecules have a similar inhibitory effect to the β-glucosidase. In addition, both of them inhibit β-mannosidase. The model of **3** also was compared with a more potent C_2-symmetrical β-glucosidase inhibitor, (2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-2,5-dihydroxymethylpyrrolidine (Fig. 3, $K_i = 7.8$ μM)[16] (Fig. 8). Although the comparison of hydroxyl pharmacophores is in a different fashion as in Figure 5, the ring nitrogen atom plus three hydroxyl groups (all equatorial in **3**) in both molecules fitted well to each other. The results may indicate that even if the hydrophilic pharmacophores are arranged in a desired 3-D relationship, an additional lipophilic moiety of the molecule may hamper the inhibition of the β-glucosidase.

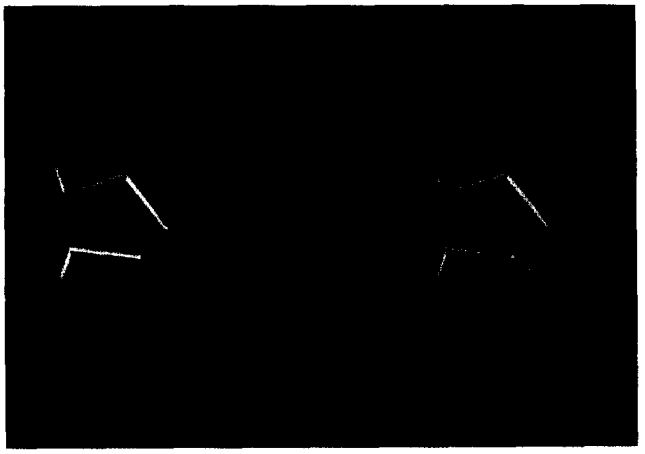


Figure 4. Stereoview of superpositions of three energy minimized trajectories of tetrahydroxyazepane (**1**) obtained from molecular dynamic simulations. Green lines represent the structure with hydroxyl groups all in the axial positions. Yellow lines represent the structure with one hydroxyl group in the axial position. Orange lines represent the structure with two hydroxyl groups in the axial positions.

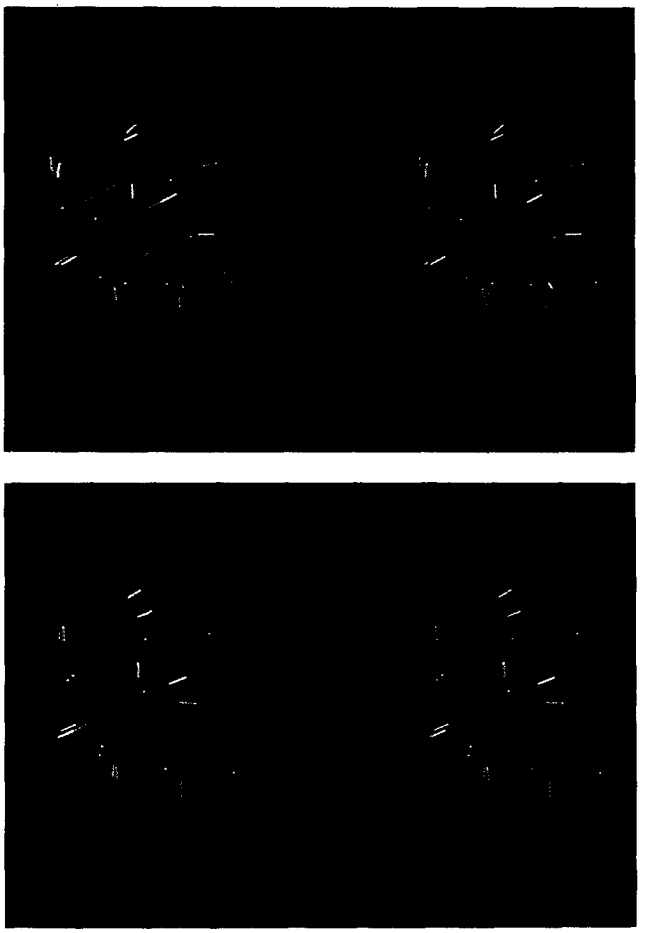


Figure 5. Overlay of compound **1** with (3*S*,4*R*,5*R*,6*R*)-3,4,5-trihydroxy-6-hydroxymethylpiperidine. (a) Seven pairs of atoms were chosen for the fitting: the ring nitrogen atoms; carbon atoms in positions 2, 3, and 4 in both molecules; oxygen atoms of 3,4-OHs; the oxygen atom of the 6-OH in **1** and the oxygen atom of the 1°-OH in the piperidine. (b) Eight pairs of atoms were chosen: the ring nitrogen atoms; the C1s; the C2s; the C6 in **1** with the C5 in the piperidine; the 3-OHs; the 6-OH in **1** with the 5-OH in the piperidine; the oxygen atoms of the 4,5-OHs in **1** and the oxygen atom of the 4-OH in the piperidine.

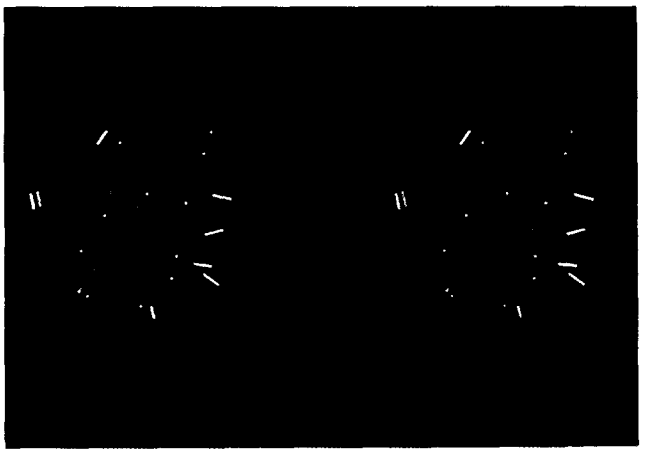


Figure 6. Overlay of compound **1** and isofagomine. Seven pairs of atoms were chosen for the fitting: the ring nitrogen atoms; the 3,4-OHs; the oxygen atom of the 5-OH in **1** with the oxygen atom of the 1°-OH in isofagomine; the C2s; the C3s; and the C4 atoms.

The X-ray crystal structure of azepane **4** was obtained as a *pseudo*-chair conformation but has the 4,5-OHs in axial positions (Fig. 9, Table 2).[17] The crystal structure of **4** was compared with the model of a very potent α-fucosidase inhibitor, (3*R*,4*S*,5*R*,6*S*)-3,4,5-trihydroxy-6-methylpiperidine (Fig. 3, $K_i = 4.8$ nM).[18] Again, the ring nitrogen atom and the three hydroxyl groups of the piperidine fitted well with the ring nitrogen of **4** and the corresponding hydroxyl groups (Fig. 10). Thus, the conformation of **4** corresponding to its crystal structure should represent a putative bioactive conformation. However, the extra hydroxyl groups and lipophilic benzyl groups in **4** may be the cause for its lower inhibitory activity.

The model of tetrahydroxyazepane **2** was directly taken from the crystal structure of **4** except that the benzyl group was removed. The model of **2** was compared with the model of (2*R*,3*R*,4*R*,5*R*)-2-(*N*-acetyl-amino-

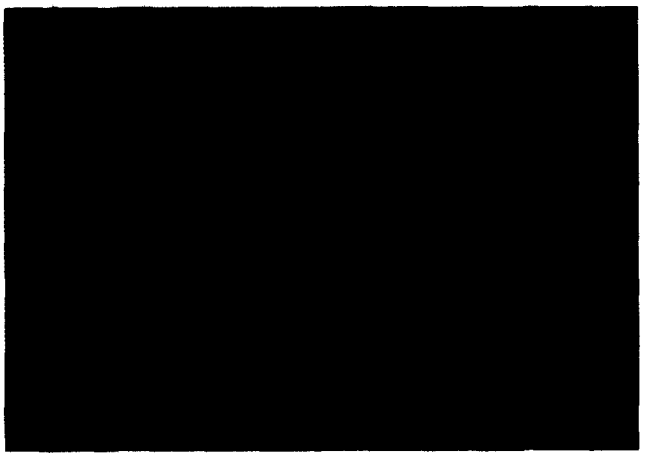


Figure 7. Overlay of compound **3** with a benzylamidine derivative. Seven pairs of atoms were chosen for the fitting, they are: the ring nitrogen atom of **3** with the amidine nitrogen atom (outside the ring); the oxygen atoms of 3,4-OHs in **3** with the 4,5-OH in the amidine, respectively; the oxygen atoms of the 5-OH in the **3** with the oxygen atom of the 1°-OH in the amidine; the C6 atom and the ring nitrogen atom in the amidine; the C3, C4 atoms in **3** and the C4, C5 atoms in the amidine, respectively.

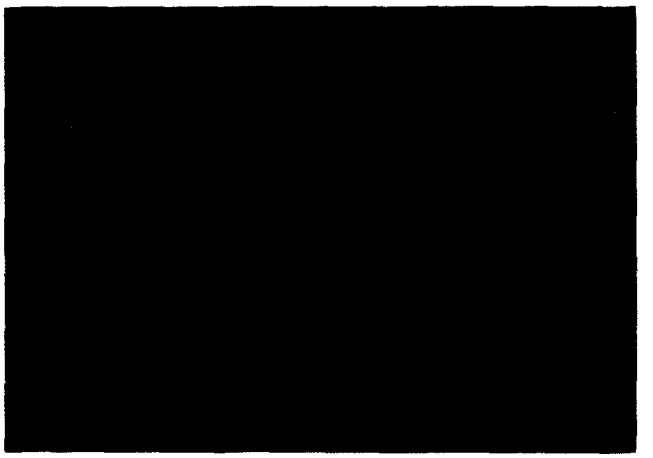


Figure 8. Stereoview of the molecular fitting of compound **3** and the model of (2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-2,5-dihydroxymethylpyrrolidine. Six pairs of atoms were chosen for the fitting: the ring nitrogen atoms; the oxygen atoms of the 4,5-OHs in **3** and the 2,3-OH in the pyrrolidine, respectively; the oxygen atom of 6-OH in **3** and oxygen of the 1°-OH in pyrrolidine; the C2 atoms; the C6 atoms and the C5 atoms.

Table 2. Bond lengths and angles for compound **4**

	Bond lengths [Å]	(standard deviation, $\pm 10^{-3}$ Å)	
O(1)—C(9)	1.432 (4)	O(2)—C(10)	1.438 (4)
O(3)—C(11)	1.423 (5)	O(4)—C(12)	1.441 (4)
N(1)—C(7)	1.474 (5)	N(1)—C(13)	1.479 (5)
N(1)—C(8)	1.484 (5)	C(1)—C(6)	1.377 (7)
C(1)—C(2)	1.386 (8)	C(2)—C(3)	1.385 (9)
C(3)—C(4)	1.358 (10)	C(4)—C(5)	1.388 (7)
C(5)—C(6)	1.379 (6)	C(6)—C(7)	1.511 (6)
C(8)—C(9)	1.534 (6)	C(9)—C(10)	1.516 (6)
C(10)—C(11)	1.536 (5)	C(11)—C(12)	1.507 (6)
C(12)—C(13)	1.531 (6)		
	Bond angle [°]	**(standard deviation, $\pm 10^{-1}$ °)**	
C(7)—N(1)—C(13)	108.0 (3)	C(7)—N(1)—C(8)	112.9 (3)
C(13)—N(1)—C(8)	114.1 (3)	C(6)—C(1)—C(2)	120.8 (5)
C(3)—C(2)—C(1)	119.4 (6)	C(4)—C(3)—C(2)	120.4 (5)
C(3)—C(4)—C(5)	119.9 (5)	C(6)—C(5)—C(4)	120.8 (5)
C(1)—C(6)—C(5)	118.7 (4)	C(1)—C(6)—C(7)	120.9 (4)
C(5)—C(6)—C(7)	120.4 (5)	N(1)—C(7)—C(6)	113.1 (3)
N(1)—C(8)—C(9)	117.7 (3)	O(1)—C(9)—C(10)	109.8 (3)
O(1)—C(9)—C(8)	109.4 (3)	C(10)—C(9)—C(8)	112.6 (3)
O(2)—C(10)—C(9)	108.0 (3)	O(2)—C(10)—C(11)	109.7 (3)
C(9)—C(10)—C(11)	114.5 (3)	O(3)—C(11)—C(12)	108.0 (3)
O(3)—C(11)—C(10)	109.6 (3)	C(12)—C(11)—C(10)	115.9 (3)
O(4)—C(12)—C(11)	109.9 (3)	O(4)—C(12)—C(13)	109.3 (3)
C(11)—C(12)—C(13)	114.6 (3)	N(1)—C(13)—C(12)	114.1 (3)

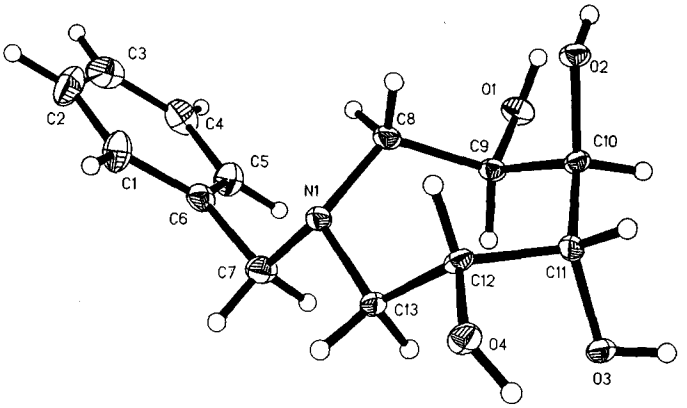


Figure 9. ORTEP representation of the X-ray crystal structure of tetrahtdroxyazepane (**4**).

methylene)-5-hydroxymethyl-3,4-dihydroxypyrrolidine (Fig. 3), a good inhibitor for β-*N*-acetylglucosaminidase ($K_i = 1.9$ μM).[19] As shown in Figure 11, the nitrogen atom and three of the hydroxyl groups of **2** fitted well to the ring nitrogen atom in the pyrrolidine and the hydroxyl groups. The good 3-D pharmacophore correlations between these two molecules ensure their similar bioactivity. The role of the fourth hydroxyl group in **2** is not obvious.

In summary, it seems that the 3-D arrangements of the pharmacophores in tetrahydroxyazepanes are in agreement with those found in the structurally more defined or rigid five- or six-membered ring azasugar based glycosidase inhibitors. The ring nitrogen and three of the four hydroxyl groups may be essential for their biological activity. *N*-Benzylation of tetrahydroxyazepanes changed the inhibitory activity and specificity of these compounds indicating that some of the glycosidases, especially β-glycosidase and α-fucosidase, may have a lipophilic binding pocket close to the active site.

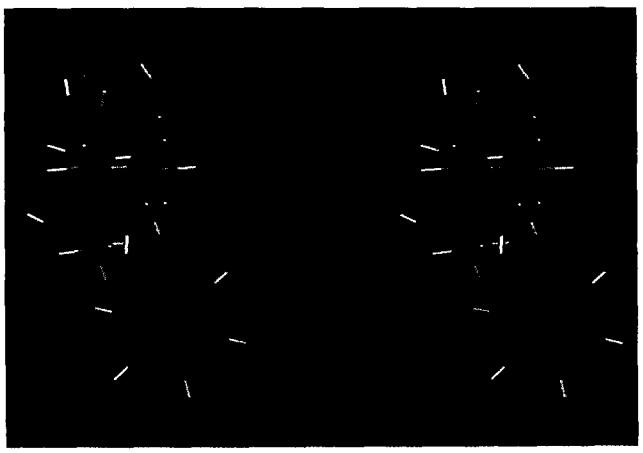


Figure 10. Overlay of the crystal structure of compound **4** and with (3*R*,4*S*,5*R*,6*S*)-3,4,5-trihydroxy-6-methylpiperidine. Eight pairs of atoms were chosen for the fitting: the nitrogen atoms; the 4-OH, 5-OH, and the 6-OH in **4** with the 5-OH, 4-OH, and the 3-OH in the piperidine, respectively; the C4, C5, and the C6 in **4** with the C5, C4 with the C3 atoms in the piperidine, respectively; the C2 atom in **4** and the C7 atom in the piperidine.

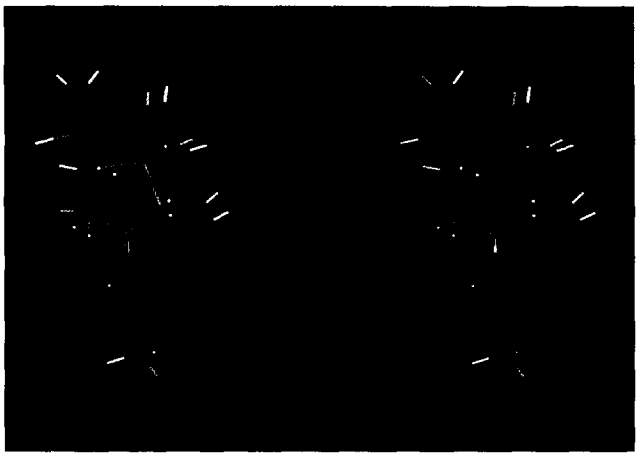


Figure 11. Overlay of compound **2** with (2*R*,3*R*, 4*R*,5*R*)-20(*N*-acetylaminomethylene)-5-hydroxymethyl-3,4-dihydroxypyrrolidine. Four pairs of atoms were chosen for the fitting: the nitrogen atoms; the oxygen atoms of the 3, 4, and the 6-OHs in **2** with the oxygen atoms of 1°-OH, the 4-OH and the 3-OH in the pyrrolidine, respectively.

Another possibility of modeling is to have the NH group of the 7-membered inhibitor and C1 of the 6- or 5-membered heterocycles overlap.

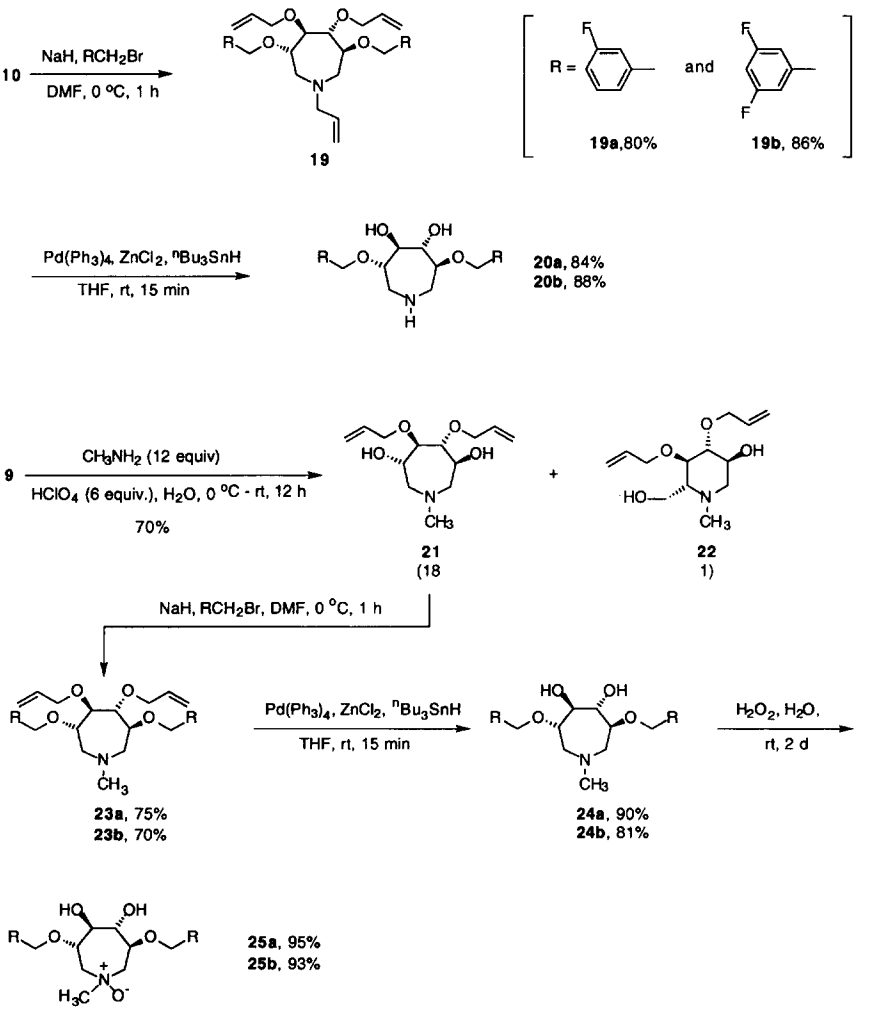
Since two carboxylic groups are functioning as essential groups for processing glycosides and polypeptides in both glycosidases and aspartyl proteases,[1,2] and HIV and FIV proteases are C_2-symmetrical homodimers,[1,3] these C_2-symmetrical iminocyclitols are further exploited as inhibitors of these two proteases. It is known that symmetrical seven-membered ring cyclic urea,[20] cyclic oxamide,[21] and non-symmetric azacyclic urea[22] are very good inhibitors for HIV protease. Compounds **1**–**4** were, however, not inhibitors of these proteases. We then investigated the protected derivatives.

A partially protected tetrahydroxyazepane **10** was chosen as starting material. 3-Fluorobenzyl or 3,5-difluorobenzyl groups were introduced to the 3- and 6-OHs by treating **10** with sodium hydride followed by 3-fluorobenzyl bromide or α-bromo-3,5-difluorotoluene to give compounds **19a**, **b** (Scheme 4), respectively. Here fluorinated benzyl groups were used in order to increase the water solubility of the final compounds. Removal of allyl groups with palladium–Lewis acid promoted hydrolysis[11] give two symmetrical compounds **20a**, **b**. Other methods of de-allylation were tried without success. The benzyl derivatives that are attached to the 3- and 6-OHs in **20a**, **b** may serve as binding motif to the P1 and P1′ sites of the HIV/FIV proteases.

In 7-membered ring cyclic urea based HIV protease inhibitors there is a carbonyl group of which the oxygen atom is capable of forming hydrogen bonding with the amide protons of the Ile^{50} and $ILe^{50'}$ residues (flap region) of the HIV protease and the -OH groups are capable of interacting with the Asp^{25} and $Asp^{25'}$ carbonyl groups. The structural water 301 has been replaced with the inhibitor after binding.[20] We also

were interested in introducing a hydroxyl function to the ring to provide a similar interaction. However, various conditions including treatment of dimethyl dioxarane[23] and benzoyl peroxide/Na_2HPO_4 followed by basic hydrolysis[24] did not allow the oxidation of the secondary amine to hydroxyamine in compound **20a** or **b**. Since tertiary amine could be oxidized easily, compounds with a *N*-oxide moiety were designed to bypass this oxidation problem. Bis-epoxide **9** were treated with methylamine in the presence of perchloric acid to provide *N*-methylated tetrahydroxyazepane (**21**) (Scheme 4). The ratio of the 7-membered ring azepane to the 6-membered ring piperidine was about 18:1. 3-Fluorobenzyl or 3,5-difluorobenzyl groups were introduced to the 3,6-OHs of **21** in the same way as that for preparing compounds **19a, b**. Removal of ally groups in **23a, b** provide *N*-methylated tetrahydroxyazepanes (**24a, b**) with benzyl derivatives attached to the 3- and 6-OHs. Oxidation of the tertiary amine in compounds **24a, b** with hydrogen peroxide gave the final *N*-oxides **25a, b** in high yields.[25] It was expected that the oxygen anion in the zwitterion of compounds **25a, b** would act as a good hydrogen bond acceptor.

Compounds **20a, b, 24a, b, 25a, b** were tested for their inhibitory activities against the HIV and FIV protease.[26,27] The results are listed in Table 3. Generally, all compounds that were assayed have an IC_{50} value in the upper micromolar range against both proteases. Tetrahydroxyazepanes **1** and **2** did not show inhibitory activities for the HIV protease. This may indicate that the lipophilic moieties introduced to the 3,6-OHs are crucial for enzyme–inhibitor interactions. The reasons for the weak inhibition of the HIV protease by these compounds may be due to the following: (1) the lipophilic side chains in these compounds might be one bond further from the 7-membered ring scaffold, since all the reported inhibitors have the benzyl groups directly connected to the 7-membered ring scaffolds.[20–22] Thus, the fluorobenzyl binding motifs may not have optimal interactions with the P1 and P1' sites as expected; (2) the lack of another set of lipophilc binding motif at the C2 and C7 positions may significantly decrease the binding affinity, thus the structural simplicity of these hydroxyazepanes do not compromise the structural demand for the HIV protease; (3) although there is a good hydrogen bond acceptor in compounds **25a, b**, the extra *N*-methyl group may interfere with the interaction of the oxygen anion with the amide protons of the Ile^{50} and $ILe^{50'}$; (4) these compounds also may interact with the HIV protease in a different orientation compared to the



Scheme 4.

Table 3. Inhibition of HIV and FIV proteases with tetrahydroxyazepane derivatives

Compound and assay concentration	IC_{50} (μM)	
	HIV protease	FIV protease
20a (80 μM, 40 μM, 20 μM)	570	1400
20b (330 μM, 165 μM, 82 μM)	380	670
24a (250 μM, 125 μM, 62 μM)	675	NI[b]
24b (250 μM, 125 μM, 62 μM)	1425	NI[b]
25a (160 μM, 80 μM, 40 μM)	384	weak inhibition[a]
25b (260 μM, 130 μM, 65 μM)	360	NI[b]

[a]Weak inhibition stands for $IC_{50} > 1.5$ mM.
[b]No inhibition.

cyclic urea based inhibitors. Nonetheless, the inhibition of the HIV protease by these tetrahydroxyazepanes suggested that we may have a correct scaffold. The lipophilic motif should be optimized in order to achieve tight binding.

In the inhibition of FIV protease, we observed a similar trend. Most of the *N*-substituted compounds **24a**, **b**, **25a**, **b** did not show inhibition (Table 3), compounds **20a**, **b** were active, however, their IC_{50} values were inceased compared to their inhibitory activity to the HIV protease. This observation is in agreement with the *a*-keto-amide based inhibitors reported previously.[27] Interestingly, compounds **20a**, **b**, **24a**, **b**, **25a**, **b** are not inhibitors of the glycosidases in this study.

Conclusion

In conclusion, in this study we have explored the novel utility of the C_2-symmetrical tetrahydroxyazepanes as glycosidase inhibitors and as scaffold for the development of HIV protease and FIV protease inhibitors. Work is in progress to prepare new derivatives of 7-membered iminocyclitols to improve inhibition potency.

Experimental

All reactions were performed under an argon atmosphere, unless otherwise mentioned. NMR spectra were recorded on Bruker AMX-400 and AC-250 spectrometers at ambient temperature. ^{1}H chemical shifts were referenced using internal tetramethylsilane or δ_H 4.80 for solutions in D_2O. ^{13}C Chemical shifts were referenced as follows: solutions in $CDCl_3$ δ_c 77.00, CD_3OD δ_c 49.00. Chromatography was carried out either on a column with silica gel 60 or on a preparative TLC plate coated with 500 μM silica gel 60-F_{254}. TLC was recorded using silica gel Kiesegel 60-F_{254} 250 μM, indicated by UV light, ammonium molybdate–ceric sulfate stain or ninhydrin stain. Computer-assisted modeling was carried out on a SiliconGraphic Personal Iris 4D/35 work station using InsightII (version 95.0) software package licensed from Biosym Technologies, San Diego, CA.

3,4-Di-*O*-allyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol (6). To a stirred soln of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**5**, 10.0 g, 38 mmol) and tetrabutylammonium iodide (0.7 g, 1.9 mmol) in 150 mL anhydrous DMF at 0 °C was added sodium hydride (2.3 g, 91 mmol). The resulted gray suspension was allowed to stir at 0 °C for 30 min, followed by 15 min at room temperature. After cooling the suspension to 0 °C, allyl bromide (20.0 mL, 231 mmol) was added slowly via a syringe. The resulting orange-brown suspension was stirred at 0 °C for 30 min, then warmed up to room temperature and stirred for an additional 1.5 h. The reaction mixture was quenched at 0 °C by addition of 150 mL of saturated NaCl (aq) solution and was extracted with ethyl ether (200 mL × 4). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concd to give a yellow–brownish oil. Chromatographic purification gave a yellow oil (12.8 g, yield 98%). R_f 0.32 (EtOAc:hexanes, 2:8, v/v). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.35 (6H, s), 1.41 (6H, s), 3.62 (2H, d, J=5.3 Hz), 3.96 (2H, dd, J=8.2 Hz, J'=6.6 Hz), 4.08 (2H, t, J=7.2 Hz), 4.20 (6H, m), 5.15 (2H, dd, J=11.3 Hz, J'=1.0 Hz), 5.25 (2H, dd, J=17.2 Hz, J'=1.5 Hz), 5.89 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 25.5, 26.7, 66.7, 73.9, 75.8, 79.8, 117.1, 134.7 ppm. FABHRMS calcd for $C_{18}H_{31}O_6$: 343.2121. Found: $[M+H]^+$ 343.2133.

3,4-Di-*O*-allyl-D-mannitol (7). 3,4-Di-*O*-allyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol (**6**, 13.43 g, 39.2 mmol) was dissolved in 250 mL of a 75% acetic acid solution in water mixture (v/v). The resulting soln was heated to 50 °C and stirred for 2 h. All volatiles were removed to give an off-white solid. The crude product was purified by column chromatography using a gradient of methanol from 4 to 12% in dichloromethane (v/v). The purified product was a white solid (9.3 g, yield 90%). R_f 0.20 (methanol:dichloromethane, 1:9, v/v). ^{1}H NMR (400 MHz, CD_3OD): δ 3.63 (2H, dd, J=11.1 Hz, J'=4.8 Hz), 3.73 (4H, m), 3.80 (2H, dd, J=11.1 Hz, J'=2.4 Hz), 4.14 (2H, dd, J=12.4 Hz, J'=5.8 Hz), 4.23 (2H, tdd, J=1.0 Hz, J'=5.6 Hz, J''=12.4 Hz), 5.11 (2H, dd, J=10.4 Hz, J'=1.3 Hz), 5.27 (2H, ddd, J=1.5 Hz, J'=3.1 Hz, J''=17.2 Hz), 5.95 (2H, m) ppm. ^{13}C NMR (CD_3OD, TMS): δ 64.6, 72.3, 74.7, 79.9, 116.8, 136.5 ppm. FABHRMS calcd for $C_{12}H_{22}O_6Na$: 285.1314. Found: $[M+Na]^+$ 285.1320.

3,4-Di-*O*-allyl-1,6-di-*O*-*tert*-butyldimethylsilyl-D-mannitol. 3,4-Di-*O*-allyl-D-mannitol (**7**, 6.77 g, 25.8 mmol) and imidazole (4.40 g, 64.6 mmol) were dissolved in 20 mL anhydrous DMF, and the soln was cooled to 0 °C. *tert*-Butyldimethylsilyl chloride (9.13 g, 59.4 mmol) was added to the above soln in one portion while stirring. The reaction mixture was stirred at 0 °C for 2.5 h. The reaction was quenched by addition of 15 mL of satd $NaHCO_3$ (aq) soln and was then extracted with dichloromethane (60 mL × 4). The organic layers were combined, dried, filtered, and concd to give a light-yellow oil. The product was further purified by column chromatography using a gradient of ethyl acetate from 7 to 10% in hexanes. The purified product was a light-

yellow oil weighing 12.41 g (yield, 98%). R_f 0.56 (EtOAc:hexanes, 3:7, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.10 (12H, s), 0.93 (18H, s), 2.66 (2H, d, J=6.1 Hz), 3.68 (2H, dd, J=4.4 Hz, J'=1.4 Hz), 3.81(6H, m), 4.12 (2H, tdd, J=1.3 Hz, J'=5.9 Hz, J''=12.4 Hz), 4.20 (2H, tdd, J=1.3 Hz, J'=5.8 Hz, J''=12.4 Hz), 5.14 (2H, ddd, J=1.2 Hz, J'=2.9 Hz, J''=10.3 Hz), 5.24 (2H, ddd, J=1.6 Hz, J'=3.3 Hz, J''=17.2 Hz), 5.93 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ −5.4, 18.3, 25.9, 63.9, 70.5, 73.4, 78.1, 117.0, 134.9 ppm. FABHRMS calcd for $C_{24}H_{50}O_6Si_2Na$: 513.3044. Found: $[M+Na]^+$ 513.3020.

3, 4-Di-*O*-allyl-1, 6-di-*O*-*tert*-butyldimethylsilyl-2,5-di-*O*-methanesulfonyl-D-mannitol (8). To a soln of 3,4-di-*O*-allyl-1,6-di-*O*-*tert*-butyldimethylsilyl-D-mannitol (9.79 g, 20.0 mmol) in 120 mL of freshly distilled dichloromethane was added triethylamine (11.20 mL, 80.4 mmol). The solution was cooled to 0 °C and methanesulfonyl chloride (4.90 mL, 60.1 mmol) was added slowly via a syringe. The reaction mixture was stirred at 0 °C for 65 min. Then 50 mL of ethyl ether was added to the reaction followed by addition of 200 mL distilled water. The above mixture was extracted with dichloromethane (60 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered and concd to give a yellow oil. The product was purified by chromatography using a gradient of ethyl acetate from 8 to 18% in hexanes. The purified product was a colorless oil (12.52 g, yield 97%). R_f 0.47 (EtOAc:hexanes, 3:7, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.10 (12H, s), 0.91 (18H, s), 3.08 (6H, s), 3.86 (2H, d, J=3.2 Hz), 3.90 (2H, dd, J=11.8 Hz, J'=6.3 Hz), 4.18 (2H, tdd, J=1.2 Hz, J'=5.9 Hz, J''=12.1 Hz), 4.24 (2H, tdd, J=1.2 Hz, J'=5.7 Hz, J''=12.1 Hz), 4.80 (2H, m), 5.19 (2H, dd, J=10.4 Hz, J'=1.3 Hz), 5.29 (2H, ddd, J=1.4 Hz, J'=3.0 Hz, J''=17.2 Hz), 5.92 (2H, m) ppm.^{13}C NMR ($CDCl_3$): δ −5.4, 18.3, 25.8, 38.7, 61.8, 73.5, 77.9, 83.1, 117.7, 134.1 ppm. FABHRMS calcd for $C_{26}H_{54}O_{10}S_2Si_2Cs$: 779.1751. Found: $[M+Cs]^+$ 779.1728.

3,4-Di-*O*-allyl-1,2:5,6-dianhydro-L-iditol (9). To a suspension of 3,4-di-*O*-allyl-1,6-di-*O*-*tert*-butyldimethylsilyl-2,5-di-*O*-methanesulfonyl-D-mannitol (**8**, 10.03 g, 15.5 mmol) in 140 mL of methanol was added (dropwise) concd HCl (aq, 5.14 mL) at 0 °C. The soln was stirred at 0 °C for 60 min and another 2 h at room temperature. The reaction was then recooled to 0 °C and 20% KOH (aq, 25.7 mL) was added. The reaction was stirred at 0 °C for 20 min and 3 h at room temperature to become a white suspension. The reaction was then diluted with 100 mL of distilled water and was extracted with dichloromethane (120 mL × 4). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concd to give a yellow oil. The product was purified by chromatography using a gradient of ethyl acetate from 8 to 18% in hexanes. The purified product was a light-yellowish oil (3.2 g, yield 90%). R_f 0.35 (EtOAc:hexanes, 3:7, v/v). IR (film) υ 2993, 2870, 1646, 1459, 1421, 1255, 1123, 1086, 996, 925, 854, 834, 815 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (2H, dd, J=4.8 Hz, J'=2.2 Hz), 2.82 (2H, dd, J=4.8 Hz, J'=3.8 Hz), 4.09 (2H, tdd, J=1.4 Hz, J'=5.9 Hz, J''=12.9 Hz), 4.28 (2H, tdd, J=1.6 Hz, J'=5.3 Hz, J''=12.9 Hz), 5.18 (2H, ddd, J=1.3 Hz, J'=2.2 Hz, J''=10.4 Hz), 5.28 (2H, ddd, J=1.6 Hz, J'=3.3 Hz, J''=17.2 Hz), 5.90 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 43.3, 52.1, 71.5, 80.5, 117.2, 134.4 ppm. FABHRMS calcd for $C_{12}H_{18}O_4Na$: 249.1103. Found: $[M+Na]^+$ 249.1111. Anal. calcd for $C_{12}H_{18}O_4$: C, 63.70; H, 8.02. Found, C, 63.49; H, 7.92.

General procedure for preparing 2,3,4,5-tetrahydroxyazepane derivatives and piperidine derivatives from bis-epoxides

Using the preparation of (3*S*,4*R*,5*R*,6*S*)-*N*-ally-4,5-di-*O*-allyl-3,4,5,6-tetrahydroxyazepane (**10**) and *N*-allyl-3,4-di-*O*-allyl-1-deoxynojirimycin (**11**) as an example. To a suspension of 3,4-di-*O*-allyl-1,2:5,6-dianhydro-L-iditol (**9**, 0.83 g, 3.67 mmol) in 15 mL of distilled water was added allylamine (3.34 mL, 44.1 mmol). The resulted suspension was cooled to 0 °C and added perchloric acid (1.90 mL, 22.0 mmol) dropwise via a syringe. The reaction mixture was stirred at 0 °C for 2 h and an additional 20 h at room temperature. Five milliliters of satd NH_4Cl (aq) soln was added to the reaction, followed by addition of 15 mL distilled water. The aqueous solution was extracted with dichloromethane (25 mL × 4). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concd to give a yellow oil as product. The products were further separated by column chromatography using a gradient of methanol from 0 to 4% in dichloromethane.

(3*S*,4*R*,5*R*,6*S*)-*N*-Ally-4,5-di-*O*-allyl-3,4,5,6-tetrahydroxyazepane (10). The major product (**10**) that was isolated was an light-yellow oil at room temperature and was a off-white solid upon freezing (0.83 g, yield 80%). R_f 0.37 (methanol:dichloromethane, 5:95, v/v). IR (film) υ 3447, 2909, 2841, 1645, 1464, 1420, 1339, 1246, 1126, 1080, 1052, 995, 922, 832 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.60 (2H, ddd, J=1.0 Hz, J'=8.2 Hz, J''=12.7 Hz), 2.86 (2H, ddd, J=1.1 Hz, J'=2.1 Hz, J''=12.6 Hz), 3.18 (2H, tdd, J=1.2 Hz, J'=6.7 Hz, J''=13.0 Hz), 3.23 (2H, tdd, J=1.3 Hz, J'=6.5 Hz, J''=13.0 Hz), 3.44 (2H, dd, J=4.2 Hz, J'=1.8 Hz), 3.56 (2H, s, br), 3.73 (2H, m), 4.14 (2H, ddd, J=1.3 Hz, J'=5.8 Hz, J''=12.5 Hz), 4.24 (2H, ddd, J=1.4 Hz, J'=5.5 Hz, J''=12.5 Hz), 5.19 (4H, m), 5.29 (2H, ddd, J=1.6 Hz, J'=3.3 Hz, J''=17.2 Hz), 5.82 (1H, m), 5.93 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 57.2, 62.3, 67.8, 72.5, 86.5, 117.2, 118.8, 134.5 ppm. FABHRMS calcd for $C_{15}H_{25}NO_4Na$: 306.1681. Found: $[M+Na]^+$ 306.1687. Anal. calcd for $C_{15}H_{25}NO_4$: C, 63.58; H, 8.89; N, 4.94. Found, C, 63.56; H, 8.77; N, 5.03.

***N*-Allyl-3,4-di-*O*-allyl-1-deoxynojirimycin (11).** The minor product (**11**) was isolated as an off-white solid (0.054 g, yield 5%). R_f 0.05 (methanol:dichloromethane, 5:95, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (1H, dd, J=11.3 Hz, J'=10.1 Hz), 2.29 (1H, ddd,

$J=1.7$ Hz, $J'=3.0$ Hz, $J''=9.1$ Hz), 3.06 (1H, dd, $J=14.3$ Hz, $J'=7.7$ Hz), 3.11 (1H, dd, $J=11.3$ Hz, $J'=4.6$ Hz), 3.17 (1H, t, $J=8.8$ Hz), 3.39–3.45 (2H, m), 3.58 (1H, ddd, $J=4.7$ Hz, $J'=8.8$ Hz, $J''=10.0$ Hz), 3.71–3.77 (1H, m), 3.86 (1H, dd, $J=11.8$ Hz, $J'=3.1$ Hz), 4.11–4.27 (2H, m), 4.31–4.41 (2H, m), 5.16–5.25 (2H, m), 5.26–5.34 (2H, m), 5.84 (1H, m), 5.96 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 55.3, 55.4, 57.4, 64.6, 69.2, 73.7, 73.9, 77.9, 86.4, 117.0, 117.2, 118.8, 133.4, 134.6, 134.9 ppm. FABHRMS calcd for $C_{15}H_{25}NO_4Na$: 306.1681. Found: $[M+Na]^+$ 306.1690.

(3*S*,4*R*,5*R*,6*S*)-3,4,5,6-Tetrahydroxyazepane (1). Compound **10** (33.9 mg, 0.12 mmol) was dissolved in 2 mL of a methanol and water mixture (4:1, v/v). The resulting soln was degassed and 23 mg of 10% Pd/C and 55 μL of 2.3 M of perchloric acid solution (0.13 mmol) were added. The reaction mixture was heated to reflux for 8 h. The Pd/C was filtered and all solvents were evapd. The crude product was then eluted from a Dowex ion-exchange [$(NH_4)^+$ form, 200–400 mesh] column with gradient of ammonium hydroxide from 0 to 1 M. The eluent was lyophilized to give a light yellow-colored solid (15.6 mg, yield 80%). R_f 0.39 (ethyl alcohol:conc NH_4OH, 2:1, v/v). ^{1}H NMR (400 MHz, D_2O): δ 2.80 (2H, dd, $J=14.2$ Hz, $J'=7.6$ Hz), 3.07 (2H, $J=14.2$ Hz, $J'=4.0$ Hz), 3.52 (2H, dd, $J=5.6$ Hz, $J'=2.2$ Hz), 3.72 (2H, m) ppm. ^{13}C NMR (D_2O): δ 53.0, 74.1, 80.0 ppm. FABHRMS calcd for $C_6H_{14}NO_4$: 164.0923. Found: $[M+H]^+$ 164.0927.

1,2:5,6-Dianhydro-3,4-di-*O*-allyl-D-mannitol (12). 3,4-Di-*O*-allyl-D-mannitol (**7**, 2.50 g, 9.5 mmol) and triphenylphosphine (5.75 g, 21.9 mmol) were suspended in 30 mL anhydrous benzene and was heated to reflux. Benzene was distilled until ca 5 mL was left in the reaction container. The reaction mixture was cooled to room temperature and DIAD (5.07 mL, 23.9 mmol) was added. The reaction was stirred at room temperature for 30 min and then was heated to 130 °C under vacuum for 3 h. The crude product was then purified with column chromatography to give a light yellow-colored oil as pure product (1.7 g, yield 79%). R_f 0.36 (EtOAc:hexanes, 3:7, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.78 (2H, dd, $J=5.3$ Hz, $J'=2.7$ Hz), 2.86 (2H, dd, $J=5.3$ Hz, $J'=3.8$ Hz), 3.16 (2H, m), 3.37 (2H, dd, $J=7.3$ Hz, $J'=2.6$ Hz), 4.08 (2H, tdd, $J=1.3$ Hz, $J'=6.1$ Hz, $J''=12.8$ Hz), 4.21 (2H, tdd, $J=1.4$ Hz, $J'=5.5$ Hz, $J''=12.8$ Hz), 5.18 (2H, ddd, $J=1.3$ Hz, $J'=2.8$ Hz, $J''=10.4$ Hz), 5.26 (2H, ddd, $J=1.6$ Hz, $J'=3.2$ Hz, $J''=17.2$ Hz), 5.90 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 46.3, 50.3, 72.4, 78.4, 117.4, 134.7 ppm. FABHRMS calcd for $C_{12}H_{18}O_4Na$: 249.1103. Found: $[M+Na]^+$ 249.1113.

(3*R*,4*R*,5*R*,6*R*)-*N*-Ally-4, 5-di-*O*-allyl-3,4,5,6-tetrahydroxyazepane (13). The procedure for preparing compound **13** was the same as that for compound **10**, except the bis-epoxide used was 1,2:5,6-dianhydro-3,4-di-*O*-allyl-D-mannitol (**12**). Yield 81%. R_f 0.41 (methanol:dichloromethane, 1:9, v/v). IR (film) υ 3419, 2912, 2862, 1645, 1462, 1420, 1339, 1267, 1225, 1080, 996, 921, 867, 831 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.70 (2H, ddd, $J=0.8$ Hz, $J'=6.3$ Hz, $J''=13.2$ Hz), 2.82 (2H, ddd, $J=0.8$ Hz, $J'=3.6$ Hz, $J''=13.2$ Hz), 3.17 (2H, td, $J=1.2$ Hz, $J'=6.5$ Hz), 3.68 (2H, dd, $J=1.3$ Hz, $J'=1.6$ Hz), 4.06 (2H, m), 4.13 (2H, tdd, $J=1.4$ Hz, $J'=5.9$ Hz, $J''=12.7$ Hz), 4.23 (2H, tdd, $J=1.5$ Hz, $J'=5.4$ Hz, $J''=12.7$ Hz), 5.17 (4H, m), 5.29 (2H, ddd, $J=1.6$ Hz, $J'=3.3$ Hz, $J''=17.2$ Hz), 5.84 (1H, m), 5.94 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 57.0, 62.4, 68.9, 72.3, 80.6, 117.0, 118.1, 118.3, 135.0 ppm. FABHRMS calcd for $C_{15}H_{26}NO_4$: 284.1862. Found: $[M+H]^+$ 284.1855.

***N*-Allyl-3,4-di-*O*-allyl-L-gulo-piperidine (14).** Compound **14** was isolated as a light-yellow oil. Yield 4%. R_f 0.45 (methanol:dichloromethane, 1:9, v/v). IR (film) υ 3402, 3073, 2917, 2854, 2360, 1640, 1452, 1420, 1342, 1259, 1134, 1072, 993, 921, 669 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.41 (1H, s, br), 2.69 (1H, dd, $J=12.8$ Hz, $J'=6.2$ Hz), 2.80 (1H, dd, $J=12.8$ Hz, $J'=3.3$ Hz), 2.99 (1H, dd, $J=10.1$ Hz, $J'=5.2$ Hz), 3.41 (2H, dd, $J=6.5$ Hz, $J'=0.9$ Hz), 3.59 (1H, dd, $J=14.1$ Hz, $J'=3.5$ Hz), 3.70 (1H, dd, $J=11.4$ Hz, $J'=5.5$ Hz), 3.85–3.90 (2H, m), 4.02 (1H, m), 4.06–4.22 (4H, m), 5.15–5.23 (4H, m), 5.26–5.33 (2H, m), 5.80–5.97 (3H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 50.7, 57.6, 58.8, 58.9, 67.3, 71.5, 72.1, 76.4, 76.8, 117.4, 117.9, 118.1, 134.5, 134.6, 135.1 ppm. FABHRMS calcd for $C_{15}H_{25}NO_4Na$: 306.1681. Found: $[M+H]^+$ 306.1689.

(3*R*,4*R*,5*R*,6*R*)-3,4,5,6-Tetrahydroxyazepane (2). The procedure for preparing compound **2** is the same as that of compound **1**, except the azepane **13** was used as starting material. Compound **2** was obtained as a off-white solid. Yield 80%. R_f 0.34 (ethyl alcohol:conc NH_4OH, 2:1, v/v). ^{1}H NMR (400 MHz, D_2O): δ 2.79 (2H, dd, $J=14.4$ Hz, $J'=6.4$ Hz), 2.85 (2H, $J=14.4$ Hz, $J'=3.5$ Hz), 3.75 (2H, s), 3.95 (2H, m) ppm. ^{13}C NMR (D_2O): δ 47.5, 69.4, 75.6 ppm. FABHRMS calcd for $C_6H_{14}NO_4$: 164.0923. Found: $[M+H]^+$ 164.0925.

(3*S*,4*R*,5*R*,6*S*)-*N*-Benzyl-4,5-di-*O*-allyl-3,4,5,6-tetrahydroxyazepane (15). The procedure for preparing compound **15** is the same as that for compound **10**, except benzylamine was used here in place of allylamine. Compound **15** was obtained as a yellow oil after purification. Yield 66%. R_f 0.59 (methanol:dichloromethane, 5:95, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.61 (2H, ddd, $J=0.8$ Hz, $J'=8.2$ Hz, $J''=12.6$ Hz), 2.88 (2H, ddd, $J=1.0$ Hz, $J'=1.9$ Hz, $J''=12.6$ Hz), 3.44 (2H, dd, $J=4.2$ Hz, $J'=1.8$ Hz), 3.55 (2H, s, br), 3.69 (1H, d, $J=13.1$ Hz), 3.74 (1H, d, $J=13.1$ Hz), 4.13 (2H, tdd, $J=1.3$ Hz, $J'=5.8$ Hz, $J''=12.5$ Hz), 4.23 (2H, tdd, $J=1.4$ Hz, $J'=5.5$ Hz, $J''=12.6$ Hz), 5.18 (2H, ddd, $J=1.3$ Hz, $J'=2.6$ Hz, $J''=10.4$ Hz), 5.28 (2H, ddd, $J=1.6$ Hz, $J'=3.2$ Hz, $J''=17.2$ Hz), 5.92 (2H, m), 7.25–7.35 (5H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 57.3, 63.5, 67.8, 72.5, 86.4, 117.2, 127.6, 128.5, 129.1, 134.5, 137.6 ppm. FABHRMS calcd for $C_{19}H_{28}NO_4$: 334.2018. Found: $[M+H]^+$ 334.2011.

***N*-Benzyl-3,4-di-*O*-allyl-1-deoxynojirimycin (16).** Compound **16** was obtained as a yellow oil after purification. Yield 9%. R_f 0.34 (methanol:dichloromethane, 5:95, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13 (1H, dd, J=11.3 Hz, J'=9.8 Hz), 2.37 (1H, dd, br, J=7.5 Hz), 2.36 (2H, m), 3.05 (1H, dd, J=11.3 Hz, J'=4.4 Hz), 3.21 (1H, t, J=8.6 Hz), 3.33 (1H, d, J=13.5), 3.50 (1H, d, J=8.7 Hz), 3.54 (1H, m), 3.81 (1H, dd, br, J=11.5 Hz, J'=6.2 Hz), 4.00 (1H, dd, J=11.9 Hz, J'=3.1 Hz), 4.07 (1H, d, J=13.5 Hz), 4.20 (2H, m), 4.35 (2H, m), 5.19 (2H, m), 5.30 (2H, m), 5.96 (2H, m) 7.24–7.35 (5H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 55.1, 57.0, 57.8, 65.5, 69.0, 73.6, 73.8, 78.0, 85.8, 117.1, 117.2, 127.4, 128.5, 128.8, 134.6, 134.9, 137.7 ppm. FABHRMS calcd for $C_{19}H_{28}NO_4$: 334.2018. Found: $[M+H]^+$ 334.2027.

(3*R*,4*R*,5*R*,6*R*)-*N*-Benzyl-4,5-di-*O*-allyl-3,4,5,6-tetrahydroxyazepane (17). The procedure for preparing compound **17** was the same as that for compound **10**, except the bis-epoxide used was 1,2:5,6-dianhydro-3,4-di-*O*-allyl-D-mannitol (**12**) and the amine used was benzylamine. Compound **17** was isolated as a yellow oil. Yield 78%. R_f 0.29 (methanol:dichloromethane, 5:95, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.73 (2H, dd, J=12.9 Hz, J'=6.4 Hz), 2.83 (2H, dd, J=13.2 Hz, J'=3.4 Hz), 3.29 (2H, s), 3.70 (4H, m), 4.05 (2H, m), 4.12 (2H, tdd, J=1.4 Hz, J'=5.8 Hz, J''=12.7 Hz), 4.22 (2H, tdd, J=1.5 Hz, J'=5.4 Hz, J''=12.7 Hz), 5.17 (2H, ddd, J=1.3 Hz, J'=2.9 Hz, J''=10.4 Hz), 5.29 (2H, ddd, J=1.6 Hz, J'=3.3 Hz, J''=17.2 Hz), 5.93 (2H, m), 7.24–7.35 (5H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 57.2, 63.6, 68.8, 72.2, 80.8, 117.0, 127.4, 128.5, 128.9, 134.9, 138.3 ppm. FABHRMS calcd for $C_{19}H_{28}NO_4$: 334.2018. Found: $[M+H]^+$ 334.2028.

***N*-Benzyl-3,4-di-*O*-allyl-L-gulo-piperidine (18).** Compound **18** was isolated as a yellow oil. Yield 12%. R_f 0.50 (methanol:dichloromethane, 5:95, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.46 (1H, s, br), 2.71 (1H, dd, J=13.8 Hz, J'=4.5 Hz), 2.79 (1H, dd, J=13.6 Hz, J'=2.8 Hz), 3.10 (1H, dd, J=11.6 Hz, J'=6.0 Hz), 3.56 (1H, dd, J=8.1 Hz, J'=3.5 Hz), 3.69 (1H, dd, J=11.2 Hz, J'=6.8 Hz), 3.85–4.00 (5H, m), 4.07–4.22 (4H, m), 5.20 (2H, m), 5.30 (2H, m), 5.92 (2H, m), 7.23–7.38 (5H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 49.4, 58.1, 59.2, 59.8, 67.9, 71.4, 72.2, 75.6, 77.6, 117.2, 117.4, 127.2, 128.4, 129.0, 134.6, 134.7, 139.1 ppm. FABHRMS calcd for $C_{19}H_{28}NO_4$: 334.2018. Found: $[M+H]^+$ 334.2026.

(3*S*,4*R*,5*R*,6*S*)-*N*-Benzyl-3,4,5,6-tetrahydroxyazepane (3). To a soln of compound **15** (18.9 mg, 0.06 mmol) in anhydrous THF, anhydrous zinc chloride (39 mg, 0.29 mmol) was added. The resulting white suspension was stirred at room temperature for 10 min and then tetrakis(triphenylphosphin)palladium (0) (16.4 mg, 0.014 mmol) was added. The resulting light-yellow suspension was stirred at room temperature for 10 min and tributyltin hydride (63 mL, 0.23 mmol) was added via a syringe. The reaction mixture was stirred at room temperature for 30 min. All volatiles were removed and 1.0 mL of 1 N HCl (aq) soln added to the residue and was then evaporated. Then 1 mL of satd $NaHCO_3$ (aq) was added to the resulted yellow residue and evapd to dryness. The white solid was extracted with methanol (5 mL × 4). The organic solvent was evapd and the crude product purified by preparative TLC (methanol:ethyl acetate, 1:9, v/v). The isolated product was a light-yellow oil (12.2 mg, yield 85%). R_f 0.30 (methanol:ethyl acetate, 1:9, v/v). ^{1}H NMR (400 MHz, CD_3OD): δ 2.54 (2H, dd, J=12.9 Hz, J'=8.0 Hz), 2.82 (2H, dd, J=13.0 Hz, J'=4.5 Hz), 3.42 (2H, dd, J=5.6 Hz, J'=2.3 Hz), 3.55 (2H, m), 3.62 (1H, d, J=13.2 Hz), 3.70 (1H, d, J=13.2 Hz), 7.21–7.36 (5H, m) ppm. ^{13}C NMR (CD_3OD): δ 60.2, 64.5, 73.3, 77.6, 128.3, 129.4, 130.1, 140.1 ppm. FABHRMS calcd for $C_{13}H_{20}NO_4$: 254.1392. Found: $[M+H]^+$ 254.1397.

(3*R*,4*R*,5*R*,6*R*)-*N*-Benzyl-3,4,5,6-tetrahydroxyazepane (4). The procedure for preparing compound **4** was the same as that for compound **3**, except the starting material used was **17** instead of **15**. Compound **4** was isolated as a light-yellow oil. Yield 80%. R_f 0.31 (methanol:ethyl acetate, 1:9, v/v). ^{1}H NMR (400 MHz, CD_3OD): δ 2.70 (2H, dd, J=13.0 Hz, J'=7.2 Hz), 2.81 (2H, dd, J=13.0 Hz, J'=4.6 Hz), 3.62 (1H, d, J=13.0 Hz), 3.70 (1H, d, J=13.0 Hz), 3.88 (2H, s, br), 3.99 (2H, m), 7.22–7.37 (5H, m) ppm. ^{13}C NMR (CD_3OD): δ 58.1, 64.4, 70.2, 74.5, 128.4, 129.5, 130.2, 140.0 ppm. FABHRMS calcd for $C_{13}H_{20}NO_4$: 254.1392. Found: $[M+H]^+$ 254.1398. An X-ray crystal structure was obtained. Compound **4** was crystallized from water as a colorless, plate-like crystal. The crystal was mounted along with the largest dimension and data were collected with a Rigaku AFC6R diffractometer equipped with a copper rotating anode and a highly oriented graphite monochromator. A constant scan speed of 8°/min in ω was used and the weak reflections [$I<5\sigma(I)$] were rescanned to a maximum of six times and the counts accumulated to assure good counting statistics. The intensities of three monitor reflections measured after every 200 reflections did not change significantly during 13 h of X-ray exposure. Unit cell dimensions and standard deviations were obtained by least squares fit to 25 reflections ($50<2\theta<80°$). The data were corrected for Lorentz and polarization effects and not for absorption because of low value of m. The system absences ($0k0$, $k=2n+1$) indicated a choice between the space groups $P2_1$ and $P2_1/m$. Since the compound is enantiomeric, the former space group was used. The structure was solved by direct methods using SHELX86. All nonhydrogen atoms were refined anisotropically by the full matrix least-squares method. Unit cell dimesions are: a=6.279 (1) Å, b=9.604 (2) Å, c=10.310 (1) Å; α=90°, β=92.86 (1)°, γ=90°. Two molecules were found in the unit cell. They were linked via an intermolecular hydrogen bond, the bond length of H(3A)—O4 is 1.938 Å. Bond lengths and bond angles of the X-ray crystal structure of **4** are listed in Table 2.

(3*S*,4*R*,5*R*,6*S*)-*N*-Allyl-4,5-di-*O*-allyl-3,6-di-*O*-(3′-fluorobenzyl)-3,4,5,6-tetrahydroxyazepane (19a). Compound **10** (98.5 mg, 0.35 mmol) and tetrabutylammonium

iodide (6.4 mg, 0.017 mmol) were dissolved in 8 mL anhydrous DMF and the resulting soln was cooled to 0 °C. Sodium hydride powder (21 mg, 0.88 mmol) was added to the above soln to form a suspension which was stirred at 0 °C for 20 min, 30 min at room temperature and was recooled to 0 °C. 3-Fluorobenzyl bromide (94 μL, 0.76 mmol) was added to the above suspension, the reaction mixture was then allowed to stir at 0 °C for 40 min. The reaction was quenched with 20 mL of water followed by extraction with dichloromethane (20 mL × 4). The combined organic phases was dried (over anhydrous $MgSO_4$), filtered, and concd. The crude product was purified with column chromatography with a gradient of 5 to 13% of ethyl acetate in hexanes (v/v). The product was obtained as a yellow oil (139 mg, yield 80%). R_f 0.38 (ethyl acetate:hexanes, 3:7, v/v). IR (film) υ 3076, 2852, 1617, 1591, 1488, 1450, 1346, 1255, 1137, 1088, 994, 923, 781, 747, 684 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.67 (2H, dd, $J = 13.3$ Hz, $J' = 7.2$ Hz), 2.76 (2H, dd, $J = 13.3$ Hz, $J' = 3.0$ Hz), 3.14 (2H, d, br, $J = 6.4$ Hz), 3.59 (2H, dd, $J = 5.83$ Hz, $J' = 1.8$ Hz), 3.70 (2H, m), 4.15 (2H, tdd, $J = 1.4$ Hz, $J' = 5.6$ Hz, $J'' = 12.5$ Hz), 4.26 (2H, tdd, $J = 1.5$ Hz, $J' = 5.4$ Hz, $J'' = 12.5$ Hz), 4.61 (2H, d, $J = 12.1$ Hz), 4.68 (2H, d, $J = 12.1$ Hz), 5.14–5.19 (4H, m), 5.28 (2H, ddd, $J = 1.6$ Hz, $J' = 3.4$ Hz, $J'' = 17.2$ Hz), 5.86 (1H, m), 5.94 (2H, m), 6.96 (2H, m), 7.10 (4H, m), 7.25–7.30 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 55.1, 62.1, 71.6, 72.9, 79.9, 83.2, 114.2, 114.3, 114.4, 114.5, 116.6, 117.9, 123.0, 129.7, 129.8, 135.2, 135.6, 141.4, 141.5 ppm. FABHRMS calcd for $C_{29}H_{35}F_2NO_4Cs$: 632.1588. Found: $[M+Cs]^+$ 632.1568.

(3*S*,4*R*,5*R*,6*S*)-*N*-Allyl-4,5-di-*O*-allyl-3,6-di-*O*-(3′,5′-difluorobenzyl)-3,4,5,6-tetrahydroxyazepane (19b). The procedure for preparing **19b** was the same as that for **19a**, except 3,5-difluorotoluene was used in place of 3-fluorobenzyl bromide. Yield 86%. R_f 0.51 (ethyl acetate:hexanes, 3:7, v/v). ^1H NMR (400 MHz, $CDCl_3$): δ 2.68 (2H, dd, $J = 13.4$ Hz, $J' = 7.1$ Hz), 2.76 (2H, dd, $J = 13.4$ Hz, $J' = 3.0$ Hz), 3.15 (2H, dd, $J = 6.5$ Hz, $J' = 1.1$ Hz), 3.59 (2H, dd, $J = 5.9$ Hz, $J' = 1.8$ Hz), 3.69 (2H, m), 4.14 (2H, tdd, $J = 1.4$ Hz, $J' = 5.7$ Hz, $J'' = 12.5$ Hz), 4.27 (2H, tdd, $J = 1.5$ Hz, $J' = 5.4$ Hz, $J'' = 12.5$ Hz), 4.59 (2H, d, $J = 12.6$ Hz), 4.65 (2H, d, $J = 12.6$ Hz), 5.15–5.20 (4H, m), 5.28 (2H, ddd, $J = 1.7$ Hz, $J' = 3.4$ Hz, $J'' = 17.2$ Hz), 5.85 (1H, m), 5.93 (2H, m), 6.70 (2H, m), 6.89 (4H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 54.9, 62.1, 71.1, 72.9, 80.2, 83.0, 102.4, 102.6, 102.9, 109.8, 109.9, 110.0, 110.1, 116.7, 118.1, 135.0, 135.6, 142.9, 143.0 ppm. FABHRMS calcd for $C_{29}H_{33}F_4NO_4Cs$: 668.1400. Found: $[M+Cs]^+$ 668.1420.

(3*S*,4*R*,5*R*,6*S*)-3,6-Di-*O*-(3′-fluorobenzyl)-3,4,5,6-tetrahydroxyazepane (20a). To a soln of compound **19a** (11.6 mg, 0.02 mmol) in 5 mL of anhydrous THF was added anhydrous zinc chloride (9.5 mg, 0.07 mmol). After 10 min of stirring at room temperature, tetrakis-(triphenylphosphine)palladium (0) (7 mg, 0.006 mmol) was added. The reaction mixture was stirred for 15 min and tributyltin hydride (26 μL, 0.09 mmol) was added. The reaction was stirred for 15 min and organic solvent was removed, followed by addition of 1 mL of water and 1 mL of 1 N HCl (aq). All volatiles were removed and 2 mL of satd $NaHCO_3$ (aq) was added and then evapd to give a white solid that was extracted with methanol, filtered, and evapd. The crude product was purified with preparative TLC (500 μm, developed in methanol:dichloromethane, 1:9, v/v). The isolated product was a yellow oil weighed 7.4 mg. Yield 84%. R_f 0.29 (methanol:dichloromethane, 1:9, v/v). ^1H NMR (400 MHz, $CDCl_3$): δ 2.91 (2H, dd, $J = 14.1$ Hz, $J' = 6.5$ Hz), 3.08 (2H, dd, $J = 14.1$ Hz, $J' = 4.1$ Hz), 3.27 (2H, s, broad), 3.47 (2H, m), 3.79 (2H, dd, $J = 4.5$ Hz, $J' = 2.1$ Hz), 4.65 (4H, s), 6.98 (2H, m), 7.09 (4H, m), 7.31 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 50.8, 71.4, 75.3, 81.6, 114.4, 114.6, 114.7, 114.8, 123.1, 130.0, 140.6, 140.7, 161.7, 164.1 ppm. FABHRMS calcd for $C_{20}H_{24}F_2NO_4$: 380.1673. Found: $[M+H]^+$ 380.1685.

(3*S*,4*R*,5*R*,6*S*)-3,6-Di-*O*-(3′,5′-difluorobenzyl)-3,4,5,6-tetrahydroxyazepane (20b). Compound **20b** was prepared analogously to compound **20a**, using the starting material **19b**. Compound **20b** was isolated as a yellow oil. Yield 88%. R_f 0.50 (methanol:dichloromethane, 1:9, v/v). ^1H NMR (400 MHz, $CDCl_3$): δ 2.92 (2H, dd, $J = 14.1$ Hz, $J' = 6.5$ Hz), 3.09 (2H, dd, $J = 14.1$ Hz, $J' = 4.2$ Hz), 3.23 (2H, s br), 3.46 (2H, m), 3.79 (2H, dd, $J = 4.6$ Hz, $J' = 2.1$ Hz), 4.65 (4H, s), 6.73 (2H, m), 6.88 (4H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 51.2, 70.9, 75.1, 82.0, 102.8, 103.0, 103.3, 109.9, 110.0, 110.1, 110.2, 142.1, 142.2, 161.9, 164.2 ppm. FABHRMS calcd for $C_{20}H_{22}F_4NO_4$: 416.1485. Found: $[M+H]^+$ 416.1502.

(3*S*,4*R*,5*R*,6*S*)-4,5-Di-*O*-allyl-*N*-methyl-3,4,5,6-tetrahydroxyazepane (21). Compound **21** was prepared analogously to compound **10**, using methylamine in place of allylamine. Compound **21** was isolated as a light-yellow oil. Yield 66.5%. R_f 0.44 (methanol:dichloromethane, 2:8, v/v). IR (film) υ 3427, 3079, 2943, 2902, 2857, 2810, 1646, 1464, 1424, 1333, 1245, 1164, 1127, 1080, 1034, 996, 924, 834 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.45 (3H, s), 2.51 (2H, dd, $J = 12.3$ Hz, $J' = 8.5$ Hz), 2.82 (2H, d, broad, $J = 12.6$ Hz), 3.43 (2H, dd, $J = 4.2$ Hz, $J' = 1.7$ Hz), 3.74 (2H, m), 4.14 (2H, tdd, $J = 1.3$ Hz, $J' = 5.7$ Hz, $J'' = 12.5$ Hz), 4.24 (2H, tdd, $J = 1.4$ Hz, $J' = 5.5$ Hz, $J'' = 12.5$ Hz), 5.19 (2H, m), 5.29 (2H, ddd, $J = 1.6$ Hz, $J' = 3.2$ Hz, $J'' = 17.2$ Hz), 5.93 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 47.8, 59.8, 67.6, 72.5, 86.3, 117.3, 134.5 ppm. FABHRMS calcd for $C_{13}H_{24}NO_4$: 258.1705. Found: $[M+H]^+$ 258.1695.

3,4-Di-*O*-allyl-*N*-methyl-1-deoxynojirimycin (22). Compound **22** was separated from **21** as a minor product. Yield 3.5%. R_f 0.44 (methanol:dichloromethane, 2:8, v/v). ^1H NMR (400 MHz, $CDCl_3$): δ 1.98 (1H, d, br, $J = 9.4$ Hz), 2.21 (1H, t, $J = 10.6$ Hz), 2.35 (3H, s), 2.92 (1H, d, br, $J = 13.2$ Hz), 3.05 (1H, dd, $J = 10.9$ Hz, $J' = 3.9$ Hz), 3.17 (1H, t, $J = 9.1$ Hz), 3.44 (1H, t, $J = 9.3$ Hz), 3.48 (1H, s, br), 3.63 (1H, m), 3.76 (1H, dd, $J = 11.8$ Hz, $J' = 1.0$ Hz), 3.83 (1H, dd, $J = 11.8$ Hz, $J' = 2.7$ Hz), 4.19 (2H, m), 4.37 (2H, m), 5.19 (2H, m), 5.31 (2H, m), 5.95 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ

41.7, 57.5, 59.5, 67.8, 69.0, 72.3, 73.9, 77.6, 86.7, 117.1, 117.4, 134.6, 134.9 ppm. FABHRMS calcd for $C_{13}H_{24}NO_4$: 258.1705. Found: $[M+H]^+$ 258.1708.

(3*S*,4*R*,5*R*,6*S*)-4,5-Di-*O*-allyl-3,6-di-*O*-(3′-fluorobenzyl)-*N*-methyl-3,4,5,6-tetrahydroxyazepane (23a). Compound **23a** was prepared analogously to **19a**, using the starting material **21**. Compound **23a** was isolated as a colorless oil. Yield 75%. R_f 0.41 (methanol:dichloromethane, 5:95. v/v). IR (film) υ 2856, 1617, 1591, 1488, 1449, 1348, 1254, 1137, 1085, 923, 862, 780, 747, 683 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.39 (3H, s), 2.64 (2H, dd, $J=13.2$ Hz, $J'=7.0$ Hz), 2.70 (2H, dd, $J=13.2$ Hz, $J'=3.0$ Hz), 3.61 (2H, dd, $J=8.3$ Hz, $J'=2.1$ Hz), 3.75 (2H, m), 4.13 (2H, tdd, $J=1.4$ Hz, $J'=5.6$ Hz, $J''=12.6$ Hz), 4.24 (2H, tdd, $J=1.5$ Hz, $J'=5.4$ Hz, $J''=12.6$ Hz), 4.62 (2H, d, $J=12.2$ Hz), 4.70 (2H, d, $J=12.2$ Hz), 5.16 (2H, ddd, $J=1.3$ Hz, $J'=3.0$ Hz, $J''=10.4$ Hz), 5.28 (2H, ddd, $J=1.6$ Hz, $J'=3.4$ Hz, $J''=17.2$ Hz), 6.96 (2H, m), 7.11 (4H, m), 7.23–7.25 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 47.7, 57.3, 71.6, 79.0, 82.2, 114.2, 114.4, 114.5, 114.6, 116.8, 129.7, 129.8, 134.9 ppm. FABHRMS calcd for $C_{27}H_{34}F_2NO_4$: 474.2456. Found: $[M+H]^+$ 474.2469.

(3*S*, 4*R*, 5*R*, 6*S*)-4, 5-Di-*O*-allyl-3, 6-Di-*O*-(3′,5′-difluorobenzyl)-*N*-methyl-3,4,5,6-tetrahydroxy-azepane (23b). Compound **23b** was prepared analogously to **19b**, using the starting material **21**. Compound **23b** was isolated as a colorless oil. Yield 75%. R_f 0.33 (methanol:dichloromethane, 1:9, v/v). IR (film) υ 2846, 1625, 1597, 1459, 1321, 1117, 1082, 986, 923, 874, 848, 770, 669 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.40 (3H, s), 2.68 (4H, m), 3.62 (2H, m), 3.76 (2H, m), 4.11 (2H, tdd, $J=1.4$ Hz, $J'=5.7$ Hz, $J''=12.6$ Hz), 4.25 (2H, tdd, $J=1.5$ Hz, $J'=5.3$ Hz, $J''=12.6$ Hz), 4.61 (2H, d, $J=12.6$ Hz), 4.66 (2H, d, $J=12.6$ Hz), 5.18 (2H, ddd, $J=1.3$ Hz, $J'=2.9$ Hz, $J''=10.4$ Hz), 5.28 (2H, ddd, $J=1.7$ Hz, $J'=3.3$ Hz, $J''=17.2$ Hz), 5.92 (2H, m), 6.70 (2H, m), 6.90 (4H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 47.8, 57.3, 71.0, 72.4, 79.8, 82.4, 102.4, 102.7, 102.9, 109.8, 109.9, 110.0, 110.1, 116.8, 134.8 ppm. FABHRMS calcd for $C_{27}H_{32}F_4NO_4$: 510.2267. Found: $[M+H]^+$ 510.2253.

(3*S*,4*R*,5*R*,6*S*)-3,6-Di-*O*-(3′-fluorobenzyl)-*N*-methyl-3,4,5,6-tetrahydroxyazepane (24a). Compound **24a** was prepared in a similar fashion to **20a**, except the starting material used was **23a**. Compound **24a** was isolated as a yellow oil. Yield 90%. R_f 0.45 (methanol:dichloromethane, 1:9, v/v). ^1H NMR (400 MHz, $CDCl_3$): δ 2.39 (3H, s), 2.59 (2H, ddd, $J=0.6$ Hz, $J'=6.9$ Hz, $J''=13.4$ Hz), 2.84 (2H, dd, $J=13.4$ Hz, $J'=4.6$ Hz), 3.35 (2H, s), 3.56 (2H, m), 3.77 (2H, m), 4.66 (4H, s), 6.98 (2H, m), 7.10 (4H, m), 7.30 (2H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 48.3, 59.7, 71.5, 75.0, 80.2, 114.4, 114.5, 114.6, 114.7, 123.1, 129.9, 130.0 ppm. FABHRMS calcd for $C_{21}H_{26}F_2NO_4$: 394.1830. Found: $[M+H]^+$ 394.1842.

(3*S*,4*R*,5*R*,6*S*)-3,6-Di-*O*-(3′,5′-difluorobenzyl)-*N*-methyl-3,4,5,6-tetrahydroxyazepane (24b). Compound **24b** was prepared in a similar fashion to **20b**, except the starting material used was **23b**. Compound **24b** was isolated as a light-yellow oil. Yield 81%. R_f 0.37 (methanol:chloroform, 0.5:9.5, v/v). IR (film) υ 3456, 3412, 2888, 2810, 1627, 1596, 1460, 1364, 1320, 1117, 984, 963, 850, 668 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.41 (3H, s), 2.60 (2H, dd, $J=13.3$ Hz, $J'=6.9$ Hz), 2.85 (2H, dd, $J=13.3$ Hz, $J'=4.5$ Hz), 3.32 (2H, s, br), 3.55 (2H, m), 3.78 (2H, dd, $J=5.1$ Hz, $J'=2.2$ Hz), 4.63 (2H, d, $J=12.6$ Hz), 4.65 (2H, d, $J=12.6$ Hz), 6.72 (2H, m), 6.88 (4H, m) ppm. ^{13}C NMR ($CDCl_3$): δ 48.3, 59.8, 71.0, 74.9, 80.6, 102.7, 103.0, 103.2, 109.9, 110.0, 110.1, 110.2, 142.2, 161.7, 164.2 ppm. FABHRMS calcd for $C_{21}H_{23}F_4NO_4Cs$: 562.0618. Found: $[M+Cs]^+$ 562.0602.

(3*S*,4*R*,5*R*,6*S*)-3,6-Di-*O*-(3′-fluorobenzyl)-*N*-methyl-3,4,5,6-tetrahydroxyazepane *N*-oxide (25a). Compound **24a** (13.3 mg, 0.03 mmol) was suspended in 2 mL of distilled water and 20 mg of 50% hydrogen peroxide was added. The reaction mixture was allowed to stir for 2 days at room temperature. The water was evapd to give a white powder. The crude product was purified by prep. TLC (500 *m*m, developed with 15% methanol in dichloromethane, v/v). The product was obtained as a white powder (13.2 mg, yield 95%). R_f 0.16 (methanol: dichloromethane, 1:9, v/v). ^1H NMR (400 MHz, CD_3OD): δ 3.22 (3H, s), 3.51–3.76 (7H, m), 3.96 (1H, m), 4.72 (4H, m), 7.00 (2H, m), 7.21 (4H, m), 7.34 (2H, m) ppm. ^{13}C NMR (CD_3OD): δ 60.1, 68.9, 70.2, 72.0, 72.5, 75.6, 76.0, 77.1, 79.2, 115.5, 115.7, 115.9, 131.2 ppm. FABHRMS calcd for $C_{21}H_{25}F_2NO_5Cs$: 542.0755. Found: $[M+Cs]^+$ 542.0767.

(3*S*,4*R*,5*R*,6*S*)-3,6-Di-*O*-(3′,5′-difluorobenzyl)-*N*-methyl-3,4,5,6-tetrahydroxyazepane *N*-oxide (25b). Compound **25b** was prepared in a similar way to **25a**, except the starting material used was **24b**. Compound **25b** was isolated as a white powder. Yield 93%. R_f 0.38 (methanol:dichloromethane, 2:8, v/v). ^1H NMR (400 MHz, CD_3OD): δ 3.28 (3H, s), 3.49–3.75 (7H, m), 3.98 (1H, t, $J=7.7$ Hz), 4.73 (4H, m), 6.84 (2H, m), 7.05 (4H, m) ppm. ^{13}C NMR (CD_3OD): δ 60.4, 68.9, 70.0, 71.4, 71.7, 75.7, 76.3, 77.4, 78.8, 103.3, 103.4, 103.5, 103.7, 103.8, 103.9, 111.2, 111.3, 111.5, 111.6 ppm. FABHRMS calcd for $C_{21}H_{23}F_4NO_5Cs$: 578.0567. Found: $[M+Cs]^+$ 578.0548.

Computational methods for minimization and molecular dynamics calculations

Molecules were built under the Builder–Fragment Get protocol. Atom types and hybridization were modified. The potential of the newly generated molecule was then fixed and was energy minimized under the Builder–Optimize protocol. A typical energy minimization was carried out using Consistent Valence Force Field[28] with overlap = 0.01, dielectric constant = 1.0, 1000 or 2000 iterations, and maximum derivative less than 0.001 kcal/atom. Various control variables were set as *ff* convergence = 5.0, *sd* convergence = 10.0, *cg* convergence = 1.0. The minimizations were carried out in the following cycle:[12] (1) using the steepest descent method for iterations steps until the maximum deriva-

tive is less than *ff* convergence kcal/Å with no cross terms and no morse function; (2) using the steepest descent method for iterations steps until the maximum derivative is less than *sd* convergence kcal/Å; (3) using the conjugate gradient method for iterations steps until the maximum derivative is less than *cg* convergence kcal/Å; and (4) the using va09a method (quasi-Newton–Raphson method) for iterations steps until the maximum derivative is less than derivative kcal/Å without cross terms and no morse function/no charges.

The model of compound **1** was first built and minimized using the above procedure. The dynamic simulations of compound **1** were performed at a time-step of 1 fs using the subset defined in step one of minimizations. After an initial heating period of 100 iterations, the temperature was increased to 500 K and dynamics were performed for a 100 ps period. A frame was saved every 100 fs. The low-energy trajectories were obtained and the energy was minimized using the above Builder–Optimize protocol, three representative trajectories are superimposed in Figure 4.

Inhibition analysis of tetrahydroxyazepanes against various glycosidases

α-Mannosidase (EC 3.2.1.24) from jack beans, α-galactosidase (EC 3.2.1.22) from green coffee beans, β-galactosidase (EC 3.2.1.23) from *Aspergillus niger*, α-glucosidase (EC 3.2.1.20) type VII from yeast, β-glucosidase (EC 3.2.1.21) from sweet almonds, β-*N*-acetylglucosaminidase (EC 3.2.1.30) from jack beans, and α-fucosidase (EC 3.2.1.111) from bovine kidney were purchased from Sigma Chemical Company. The assay soln concentration of compounds **1**, **2**, **3**, and **4** were 200, 240, 200, and 160 μM, respectively. The amount of the enzyme used was 0.05 units. All inhibition analyses were performed at 37 °C in 0.1 M HEPES buffer, pH 6.8, in the presence of 0.2–2 μM of *p*-nitrophenyl-glycoside, unless otherwise mentioned. In the case of α-fucosidase, the assay was carried out at 37 °C in 50 mM sodium acetate buffer, pH 6.0. The optical absorbance at 400 nm was measured to determine the amount of liberated *p*-nitrophenoxide over a 3 min period. Inhibition constants were derived by nonlinear regression analysis with FORTRAN Compo program for competitive inhibition.[29]

Inhibition analysis of tetrahydroxyazepanes against HIV and FIV proteases

For determination of IC_{50} values for HIV protease, a backbone engineered HIV-1 protease was prepared by total synthesis.[30] Final concentration of the protease (450 nM) was added to a solution (152 μL final volume) containing tetrahydroxyazepane (inhibitor), 28 μM fluorogenic peptide substrate (Abz-Thr-Ile-Phe-(*p*-NO_2)-Gln-Arg-NH_2),[26,27] and 1.8% dimethyl sulfoxide in assay buffer: 100 mM MES buffer containing 0.5 mg/mL BSA (bovine serum album) at pH 5.5. A K_m of 37 ± 8 μM was reported for the synthetic HIV protease against the above fluorogenic substrate.[26] The essay concentrations of each inhibitor are listed in Table 3. The soln was mixed and incubated over 5 min at 37 °C during which time the rate of substrate cleavage was monitored by continuously recording the change in fluorescence of the assay solution. An excitation filter of 325 nm and an emission filter of 420 nm were used. For each inhibitor concentration, three measurements were carried out. These data were converted into μM substrate cleaved per minute, using a predetermined standard calibration curve of change in fluorescence against concentration of substrate cleaved.

The inhibitory effects of compounds **20a**, **20b**, **24a**, **b**, **25a**, **b** on the enzymatic activity of recombinantly derived FIV protease[27] were studied using a fluorogenic assay based on the enzymatic cleavage of a novel peptide based substrate [Arg-Ala-Leu-Thr-Lys(aminobenzyl)-Val-Gln-Phe-Val-Gln-Ser-Lys-Gly-Arg]. Inhibition studies were carried out at a single substrate concentration (20 μM) in a 50 mM sodium citrate/100 mM sodium phosphate buffer (pH 5.3) containing 2% DMSO and 1 M sodium chloride. The same wavelengths were used for detections. Under these conditions the fluorogenic substrate displayed a K_m of 14 μM and a k_{cat} of 0.9 s^{-1}. IC_{50} values were determined by least squares analysis of Dixon Plots.[31]

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