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SYNTHESIS OF C_2-SYMMETRICAL POLYHYDROXYAZEPANES AS INHIBITORS OF GLYCOSIDASES

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Abstract: Two C_2-symmetrical bis-epoxides were prepared from D-mannitol and were subjected to nucleophilic displacements with allylamine and benzylamine. Initial intermolecular epoxide opening, followed by a preferred intramolecular 7-*endo*-*tetragol* cyclization, afforded protected polyhydroxyazepanes as major products. Compound **15** was found to inhibit seven different glycosidases with K_i in the micromolar range.

Inhibition of glycosidases has many therapeutical potentials, including the treatment of cancer, diabetes, and AIDS.[1] Due to such significance, extensive research has been directed toward the syntheses and biological studies of six-membered and five-membered ring azasugars and their analogues. We report here the investigation of seven-membered ring azasugars (Figure 1) as inhibitors of glycosidases. The flexibility of the seven-membered ring makes these compounds possibly capable of mimicking the putative oxonium ion transition state that is generated during the carbohydrates hydrolysis by glycosidases. Since many glycosidase inhibitors are substituted at the secondary ring-amine moiety,[1d] in this study we also synthesized the *N*-benzylated derivatives of polyhydroxyazepanes for inhibition analysis.

Almost every reported approach that was utilized in the synthesis of polyhydroxyazepanes contains a key step of mono- or bis-epoxide opening by benzylamine.[2] Usually the products are a mixture of six-membered ring and seven membered ring azasugars in a ratio of 1:1 to 1:7, with the exception that a bis-epoxide (1,2:5,6-dianhydro-3,4-isopropylidenehexitol) opening by benzylamine in refluxing gave only seven-membered ring azasugar in 64-88% yield.[2d] However, since the bis-epoxides used were not diastereomerically pure, the products also contained mixtures of diastereoisomers. In this study, Depezay's protocol[2c] is modified to synthesize 1,6-dideoxy-1,6-imino-D-mannitol (D-manno-azepane) and L-ido-azepane and their *N*-benzylated derivatives (Figure 1).

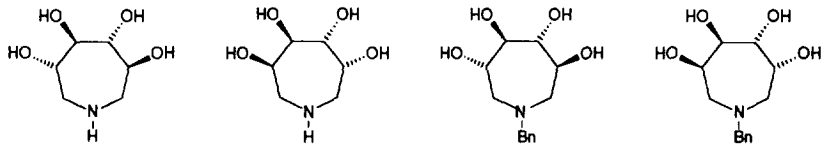
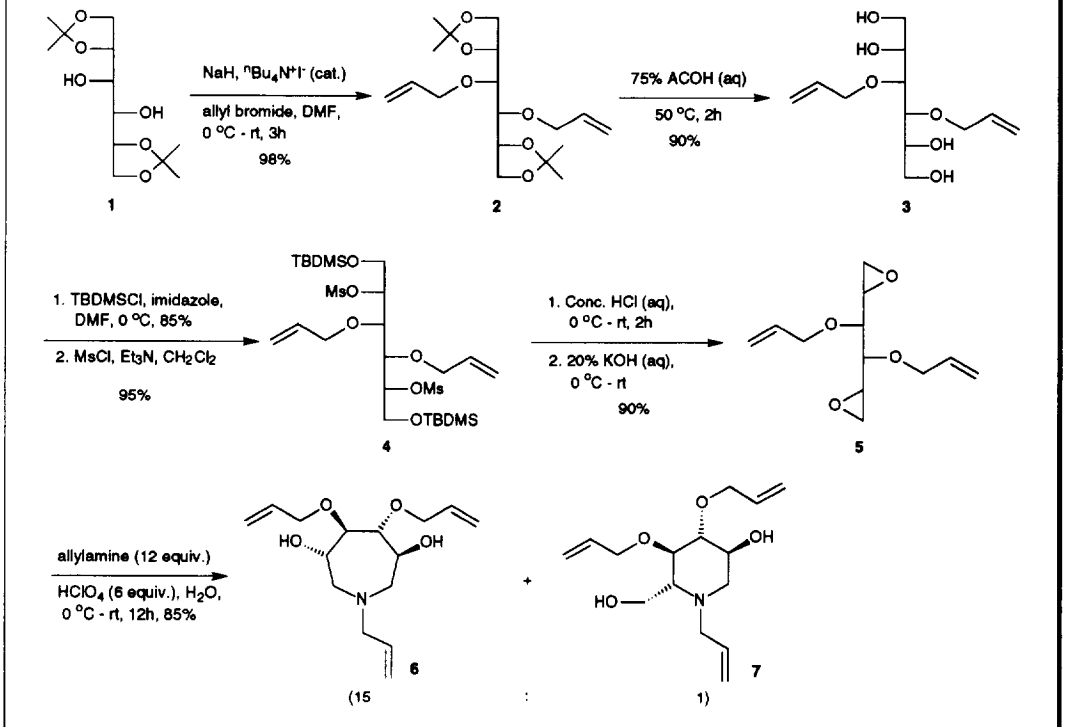
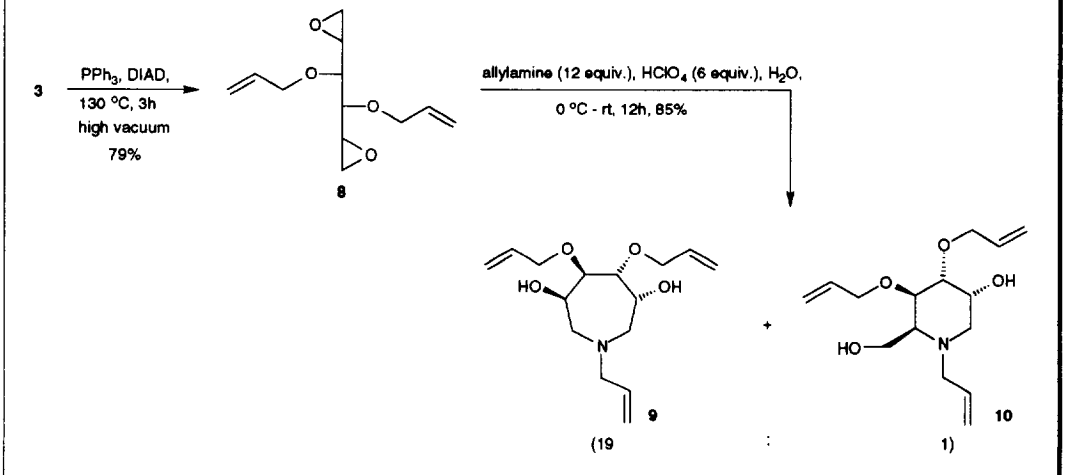


Figure 1. C_2-symmetrical seven-membered ring azasugars and their *N*-benzylated analogues.

Scheme 1



Scheme 2



Commercially available 1,2:5,6-*di*-*O*-isopropylidene-*D*-mannitol (**1**) was used as starting material. C_2-symmetrical bisepoxides were prepared from **1** on multigram scales. The -OH groups of **1** were protected with allyl groups throughout the synthesis. The reason we chose the allyl group instead of the more labile benzyl group is because the *N*-substituted seven-membered ring azasugar has a *N*-benzyl moiety, and conditions used in the removal of *O*-benzyl at the final stage of synthesis will remove the *N*-benzyl as well. 1,2:5,6-*di*-*O*-Isopropylidene-3,4-*di*-*O*-allyl-*D*-mannitol (**2**, Scheme 1) was prepared by treating diol **1** with sodium hydride followed by allyl bromide in the presence of tetrabutylammonium iodide. Acidic hydrolysis of **2** gave a common starting material , 3,4-*di*-*O*-allyl-*D*-mannitol (**3**), for the synthesis of bis-epoxides.

In one pathway (Scheme 1), tetrol **3** was selectively silylated at the primary 1,6-hydroxyl functions and consecutively mesylated at the secondary 2,5-hydroxyl functions to give a fully protected *D*-mannitol **4**. Subsequent removal of silyl groups in **4** followed by a base promoted intramolecular S_N2 displacement caused inversion of configuration at the 2- and 5-positions gave 1,2:5,6-dianhydro-3,4-*di*-*O*-allyl-*L*-iditol (bis-epoxide **5**).[3] On the other hand (Scheme 2), using Mitsunobu conditions,[4] the primary alcohol functions in tetrol **3** were activated followed by intramolecular nucleophilic attack by the 2,5-hydroxyl groups to afford the configuration retained 1,2:5,6-dianhydro-3,4-*di*-*O*-allyl-*D*-mannitol (bis-epoxide **8**).

Both C_2-symmetrical bis-epoxides **5** and **8** were treated with excess allylamine (12 equiv.) and perchloric acid (6 equivalents). The in situ generated allylammonium perchloride should serve as Lewis acid to enhance the epoxide opening. The first epoxide should be opened by allyamine regiospecifically, however, the second intramolecular epoxide opening faced the competition of 6-*exo-tetragol* ring closure vs. 7-*endo-tetragol* ring closure (Figure 2).

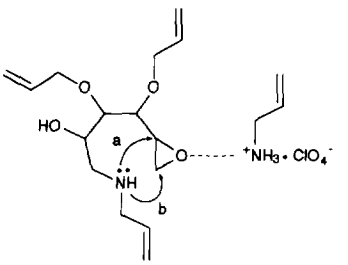
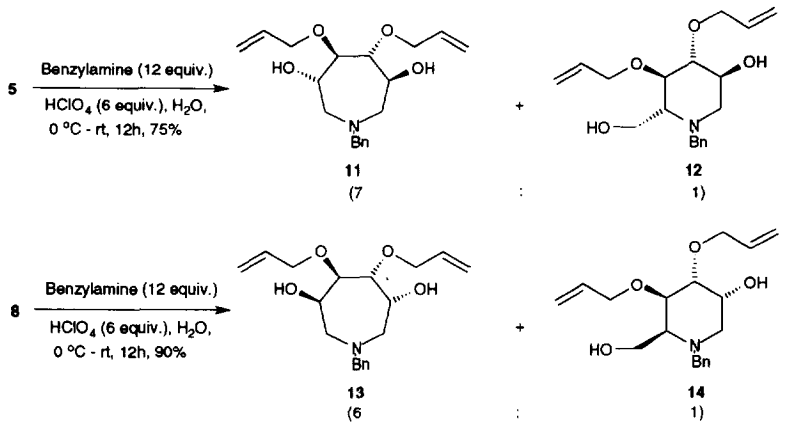


Figure 2. (a) 6-*exo-tet*-Nucloephilic attack of epoxide will give polyhydroxypiperidine as product; (b) 7-*endo-tet*-nulceophilic attack of epoxide will furnish 7-membered ring azasugar.

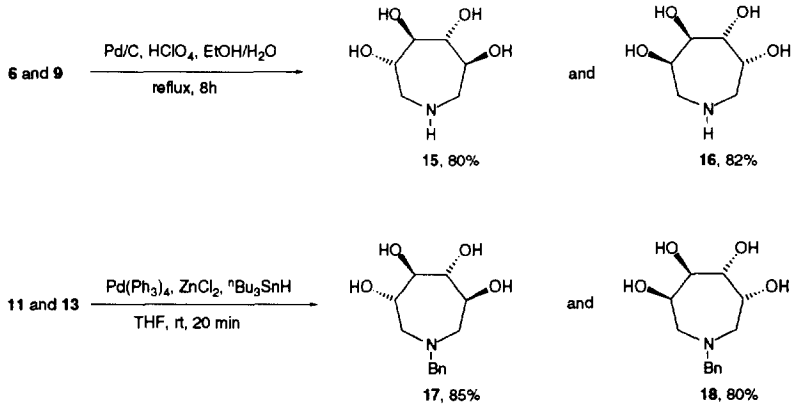
The reactions shown in Schemes 1 and 2 prefer to undergo 7-*endo-tet* cyclization to give seven-membered ring azasugars **6** and **7** as major products. Surprisingly, the ratio of seven-membered ring product vs. six-membered ring product ranged from 15:1 to 19:1. These ratios are significantly improvements over Depezay's best case of 7:1.[2c] Thus, using 3,4-*di*-*O*-allyl protecting groups instead of benzyl groups certainly has a positive effect on selectivity. The nucleophile (allylamine) used in this reaction also is an important determinant of the regioselectivity. In the case of synthesis of *N*-benzylated derivatives of polyhydroxyazepanes, benzylamine was used in the opening of bis-epoxides **6** and **7** (Scheme 3). The ratio of isolated azepanes **11** and **13** vs. piperidines **12** and **14** was significantly decreased, which was around 7:1. Nevertheless, in all cases, seven-membered ring azepanes were obtained as major products, consistent with the Baldwin rule.[5]

Scheme 3



Although many conditions were tested, removal of allyl protections in azepanes **6** and **9** to release the 4,5-hydroxyl functions and the secondary amine in the ring was difficult, and resulted in either partially deprotected compounds or decomposed azepanes. However, treatment of **6** and **9** with Pd/C (5% mol) and 1.05 equivalents of perchloric acid in refluxing $EtOH/H_2O$ (4:1, v/v) for 8 hours gave the final polyhydroxylazepanes **15** and **16** in 80% yield.[6] Partially deprotected azepanes were isolated as byproducts (10-15%). Removal of the *O*-allyl protections in azepanes **11** and **13** was effective under the mild conditions using $Pd(Ph_3)_4/ZnCl_2/^nBu_3SnH$ in THF at room temperature.[7] The moderate isolated yield (80%) for **17** and **18** was due to the presence of tributyltin hydride which caused difficulty in product purification.

Scheme 4



The obtained polyhydroxyazepanes (**15**, **16**, and **18**) were evaluated as inhibitors of different glycosidases, the results are listed in Table 1. L-Ido-azepane (**15**) inhibits all the glycosidases that were tested. Moreover, compound **15** (K_i = 6.5 μM) is a better inhibitor of β-galactosidase than deoxygalactojirimycin (K_i > 1 mM),[8] **15** (K_i = 26 μM) also is a better inhibitor of α-mannosidase than 1-deoxymannojirimycin (K_i = 150 μM).[9] D-Manno-azepane (**16**, K_i = 4.6 μM) is better than 1-deoxy-*N*-acetylglucojirimycin (K_i = 9.8 μM)[10] as inhibitor of β-*N*-acetylglucosaminidase. Compound **16** is a weak inhibitor of α-glucosidase and α-fucosidase. *N*-Benzylation of **16** converted its inhibitory specificity from β-*N*-acetylglucosaminidase to α-fucosidase, however, compound **18** (K_i = 23 μM) is weaker than 1-deoxyfucojirimycin (K_i = 4.6 nM)[11] as inhibitor of α-fucosidase. Compound **18** also has weak inhibitory effect toward several other glycosidases (Table 1).

Table 1. Inhibition of glycosidases with polyhydroxyazepanes and analogues.

Enzymes	$K_i \pm$ S.E.M. (μM)		
	15	**16**	**18**
α-Mannosidase from jack beans	25.7 ± 1.3	NI	11%
α-Galactosidase from green coffee beans	67.0 ± 4.5	NI	NI
β-Galactosidase from *Aspergillus niger*	6.5 ± 1.2	NI	NI
α-Glucosidase from yeast	29.4 ± 2.2	21%	6%
β-Glucosidase from sweet almonds	12.8 ± 0.7	NI	14%
β-*N*-Acetylglucosaminidase from jack beans	22.7 ± 2.6	4.6 ± 0.4	6%
α-Fucosidase from bovine kidney[a]	44%	16%	23.4 ± 3.8

The stock solution concentration of compounds **15**, **16**, and **18** are 200 mM, 240 mM, and 160 mM, respectively. NI stands for no inhibition. All inhibition analyses were performed at 37 °C in 0.1 M HEPES buffer, pH = 6.8, in the presence of 0.2-2 mM of *p*-nitrophenyl-glycoside, unless otherwise mentioned.
[a] Assayed at 37 °C in 50 mM sodium acetate buffer, pH = 6.0. All azepanes are competitive inhibitors.

In summary, this study indicated an efficient synthetic pathway to construct either polyhydroxyazepanes and their *N*-benzylated analogues. Preliminary biological studies indicate these seven-membered ring azasugars are inhibitors of glycosidases with the K_i values in the low micromolar range. Our current efforts involve the development of new synthetic methods of preparing various polyhydroxyazepanes as well as utilizing them as potential HIV protease inhibitors. The results are to be reported in due course.

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