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CHEMOENZYMATIC SYNTHESIS OF IMINOCYCLITOL DERIVATIVES: A USEFUL LIBRARY STRATEGY FOR THE DEVELOPMENT OF SELECTIVE FUCOSYLTRANSFER ENZYMES INHIBITORS

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Abstract: A chemoenzymatic strategy has been developed for the synthesis of libraries of iminocyclitol derivatives for the discovery of new and selective fucosidase inhibitors.

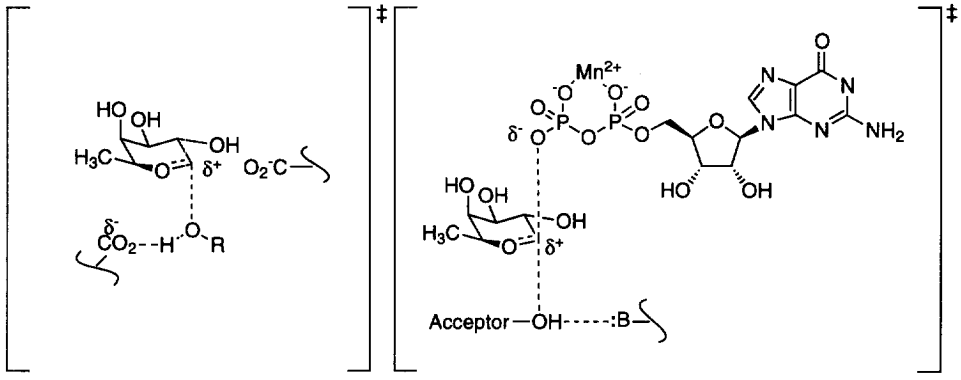

Figure 1. Postulated transition-state structures of fucosidase and fucosyltransfersae reactions

As glycobiology research has been progressed[1] the importance of inhibitors of glycoenzymes has increased.[2] In recent years much attention has been given to the development of selective glycosidase inhibitors, because such compounds may have potential as drugs against infectious diseases and diabetes or may function as immunomodulators.[3] It is known that fucose-containing oligosaccharides play a key role in cancer development and inflammatory diseases.[4] For these reasons we are interested in the synthesis of fucosidase and fucosyltransferase inhibitors not only for mechanistic studies, but also as prospective antiinflammatory and antitumor agents. However, efforts have been focused mainly on compounds that mimic the transition state of the monosaccharide moiety and these molecules often act as broad spectrum inhibitors with low selectivity.

Little attention has been given to inhibitors that mimic both the glycone and the leaving group (aglycone) during glycoside cleavage, although such compounds should be promising drug candidates with greater specificity.[5] We expected that additional interactions with hydrophilic and/or hydrophobic binding groups adjacent to the cleavage site would be essential for selective binding to fucosidases and should therefore improve inhibition potency and increase selectivity.[6] We have recently described the chemoenzymatic synthesis

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of a novel cyclic imine azasugar **4**,[7] which is a nanomolar inhibitor of α-fucosidase from bovine epididymis. Since imines are very useful intermediates suitable for various synthetic transformations such as cycloadditions, condensations and nucleophilic additions, and have been used extensively in the preparation of libraries,[8] compound **4** as well as its reduced form may find use as template for the constraction of libraries which may lead to the discovery of new inhibitors selective for certain fucosidases and fucosyltransferases (Figure 2).[9]

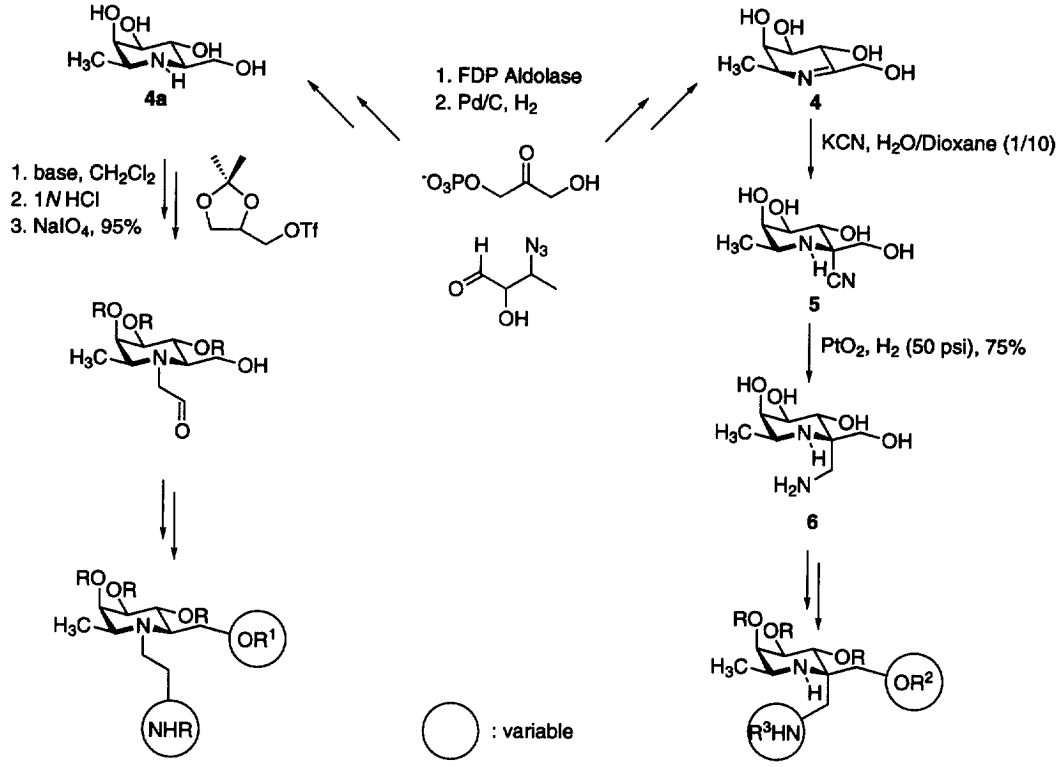
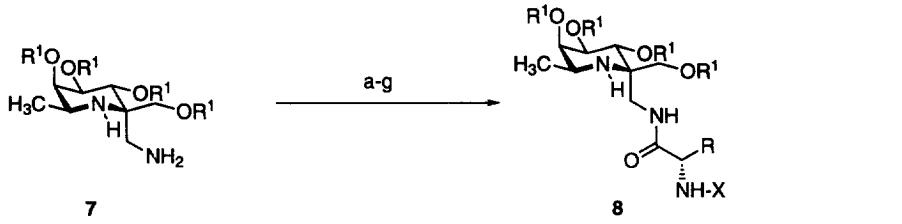


Figure 2. A library approach to the synthesis of iminocyclitol derivatives based on a transition-state mimic of enzymatic fucosyltransfer reactions

This paper describes the synthesis of linkage-specific inhibitors towards α-L-fucosidases based pm the strategy illustrated in Figure 2. The readily available cyclic imine **4** was used as a novel starting material for the preparation of a library of *C*-linked iminocyclitols from which fucosidase inhibitors were found and proven to be more potent, selective and stable than the known inhibitors of the enzymes examined to date.

We incorporated different amino acids and derivatives into the amino-iminocyclitol **6**, which was prepared from **5**,[10] by simple amide bond formation, using the chemoselectively protected derivative **7**.[11]

Starting from the amino-iminocyclitol **6** selective protection of the primary amine was accomplished in 93% yield under standard conditions using Cbz-Cl. Without purification, treatment of the crude product with 1.2 equiv TsOH in CH_2Cl_2 using excess 2,2-dimethoxy-propane leads to the expected bisacetonide, which was directly converted to **7** by hydrogenolysis in 90% yield in two steps.

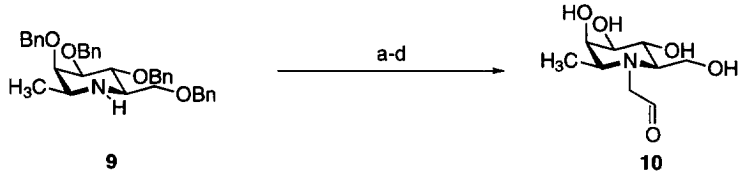


8a X = H; R = H; R^1 = H
8b X = H; R = CH_2OH; R^1 = H
8c X = Cbz; R = H; R^1 = H
8d X = Cbz; R = CH_2OBn; R^1 = isopropylidene

Scheme 1. (a) 3.2 equiv $NaHCO_3$, 1.1 equiv Cbz-Cl, 25 °C; 24 h, dioxane/water 1/1, 93%; (b) 2,2-dimethoxypropane, 1.2 equiv *p*-TsOH H_2O, 25 °C; 24 h, 93%; (c) 1 atm. H_2, MeOH, $Pd(OH)_2$/C-10%, 25 °C; 24 h, 96%; (d) HOBt/EDC, 0 °C to rt, 24 h, 1.2 equiv Cbz-Gly-OH, 90%; (e) THF/3 M HCl 1/1, 60 °C, 1 h, quant; (f) HOAc/water, 1/1, 1 atm. H_2, rt, $Pd(OH)_2$/C-20%, Degussa-Type, 24 h, quant; (g) 1.2 equiv Cbz-Ser(OBn)-OSu, CH_2Cl_2, 2 equiv NEt_3, 0 °C to rt; 24 h, 78%.

Amine **7** could then be used for amide bond formation. It should be noted that the ring nitrogen which is known for its unusually low pK_a and nucleophilicity was not acylated even in the presence of 4 equiv activated amino acid.[12] A similar observation was reported for the reaction with related structures.[13] For the synthesis of **8a** and **8c**, amine **7** was treated with a moderate excess (1.2 equiv) of Cbz-glycine using HOBt/EDC for activation.[11] Upon acid induced cleavage of the acetonides using 3 M HCl, **8c** was isolated in 90% yield after P-2 biogel filtration. Carbamate cleavage by hydrogenolysis using $Pd(OH)_2$/C-20% Degussa-Type in HOAc/H_2O followed by P-2 biogel filtration gave **8a** in quantitative yield. For the synthesis of **8b**, we used the commercially available serine derivative Cbz-Ser-(OBn)-OSu. Treatment of **7** with 1.2 equiv of the activated serine derivative in CH_2Cl_2 using NEt_3 as base led to **8d** in 78% yield after purification on silica gel. Acid induced cleavage of the acetal followed by hydrogenation using $Pd(OH)_2$/C 10%-Degussa-type and P-2 biogel filtration afforded pure **8b** in quantitative yield.

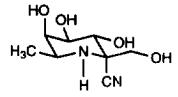
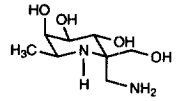
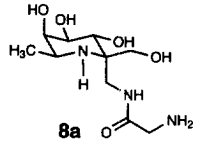
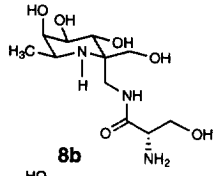
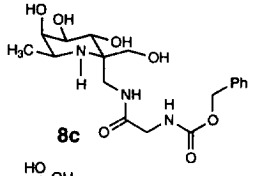
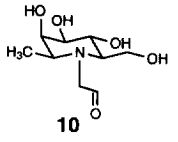
Although several β-1-*C*-alkyl derivatives of **4a** are known as β-alkyl deoxymannojirimycins, and are known to be fucosidase inhibitors,[14] none of the compounds is more active than **4a**.[14a,b] In order to create more potent inhibitors starting from **4a** we hypothesized that derivatization of the ring nitrogen should result in additional binding, improving the inhibition properties as shown in the case of nojirimycin.[3a,5]



Scheme 2. (a) 10 equiv isopropylidineglycerol, 10 equiv $EtN(iPr)_2$, 10 equiv Tf_2O, CH_2Cl_2, –20 °C to rt, 1 h; (b) THF/3 M HCl, 2/1, 60 °C, 1 h; (c) THF/H_2O, 1/1, 1.2 equiv $NaIO_4$, 0 °C, 30 min; (d) HOAc/H_2O, 1/1, 1 atm H_2, 24 h $Pd(OH)_2$/C-20% Degussa-Type, rt, then P-2 biogel, 0.2 mM NH_4HCO_3, 52% over four steps.

Starting from **4a**, alkylation was accomplished by using the triflate of 2,3-isopropylidene-glycerol prepared in situ.[15] Without purification, acid induced cleavage of the acetal followed by $NaIO_4$ mediated diol oxidation, hydrogenation and P-2 biogel filtration gave **10** as acetal in 52% overall yield. Inhibition data for the newly synthesized compounds are shown in Table 1.[7,16]

Table 1. Comparison of Inhibition (K_i in [ ] and/or IC_{50} in nM) of various α-fucosidases by iminocyclitol derivatives. Inhibition analyses were performed as described recently.[3c] All compounds show satisfactory analytical and spectroscopic data.[17]

	α-Fucosidase *Bovine Kidney*	α-Fucosidase *Bovine Epididymis*	α-1,2-Fucosidase *Arthrobacter oxidans* F_1
 **5**	75	110	122
 **6**	206	160	80
 **8a**	50	170	116
 **8b**	40 **[30]**	20 **[19]**	56 **[47]**
 **8c**	200	40	190
 **10**	11 **[11]**	11 **[8]**	4 **[1.5]**

Compound **10** is the most potent inhibitor of the α-1,2-fucosidase from *Arthrobacter oxidans F1* reported to date (K_i = 1.5 nM).[16] Also very good inhibition was found against the other enzymes. Compound **8c** is four times more selective for the fucosidase from bovine epididymis in comparison with the other glycosidases; however, **8a** is the most selective inhibitor of the fucosidase from bovine kidney. Compound **8b**

shows a similar inhibition potency against all enzymes tested. It should be noted that addition of an amino acid via amide bond formation at the primary amino group of **6** improves inhibition and increases selectivity.

In summary, we have demonstrated a new and effective chemoenzymatic strategy for the synthesis of various iminocyclitol derivatives from which very potent and selective inhibitors of fucosidases have been found. These inhibitors are more stable than **4** and some are more potent and selective than **4**. The strategy should find use in the preparation of libraries for discovery of other new fucosidase and fucosyltransferase inhibitors and may be extended to the development of inhibitors of other glycosyltransfer enzymes.

Acknowledgment: This research was supported by the NIH and NATO-Postgraduiertenstipendium.

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16. Data for selected compounds:

Compound **8b**, colorless hygroscopic solid: ^{1}H NMR (400 MHz, D_2O): δ 1.17 (d, J = 6.6 Hz, 3 H), 3.41 (d , J = 15.6 Hz, 1 H), 3.60 (d, J = 12.3 Hz, 1 H), 3.70 (d, J = 12.3 Hz, 1 H), 3.75 (dd, J = 10.3, 2.7 Hz, 1 H), 3.77–3.85 (m, 5 H), 3.89 (d, J = 10.3 Hz, 1H), 4.00 (t, J = 4.64 Hz, 1 H); ^{13}C NMR (400 MHz, D_2O): δ 16.29, 38.49, 53.75, 56.82, 57.17, 62.31, 62.87, 67.32, 68.53, 71.85, 171.91; HRMS for $C_{11}H_{23}O_6N_3Cs$, $(M + Cs)^+$: calcd 496.4465, found 496.4463. **10**, colorless hygroscopic solid: ^{1}H NMR (400 MHz, D_2O/DMSO-d_6): δ 1.17 (d, J = 6.6 Hz, 3 H), 2.94 (m, 1 H), 3.30 (dd, J = 13.4, 6.6 Hz, 1 H), 3.44 (m, 1 H), 3.50 (dd, J = 9.6, 2.8 Hz, 1 H), 3.61-3.66 (m, 2 H), 3.75 (d, J = 2.8 Hz, 1 H), 3.78–3.81 (m, 1 H); ^{13}C NMR (400 MHz, D_2O/DMSO-d_6): δ 16.23, 44.04, 56.98, 60.47, 62.44, 67.77, 72.12, 75.43, 108.06; HRMS for $C_9H_{17}O_5NNa$, $(M + Na)^+$: calcd 220.1185, found 220.1182.

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