α-Fucosidase Inhibitors

# Rapid Diversity-Oriented Synthesis in Microtiter Plates for In Situ Screening: Discovery of Potent and Selective α-Fucosidase Inhibitors**

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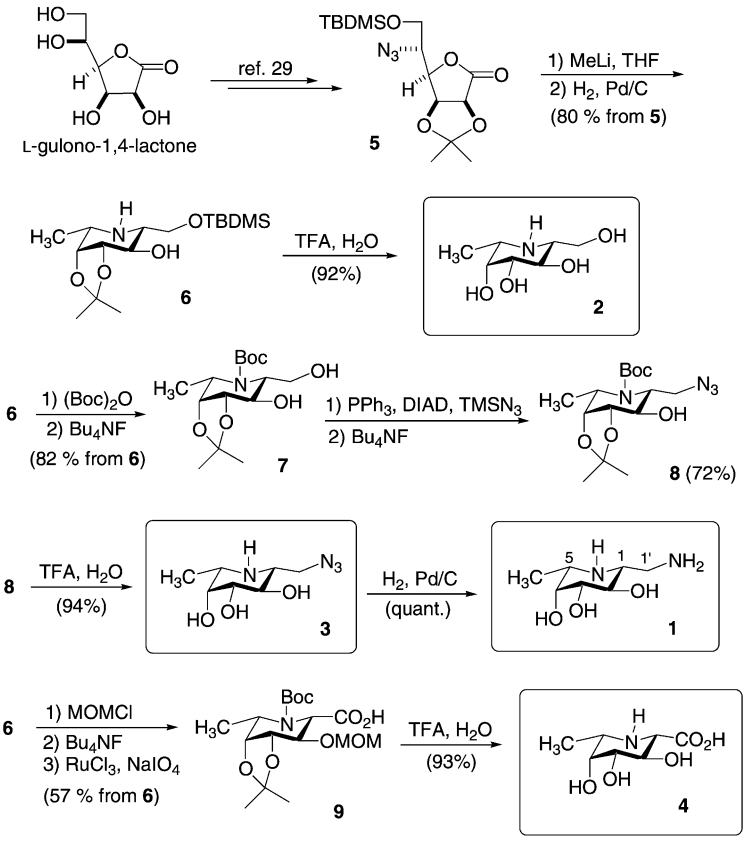
Polyhydroxypiperidines and pyrrolidines (also called iminocyclitols or aza-sugars) have attracted increasing attention because of their inhibitory activity against various glycosidases.[1,2] The glucose-type iminocyclitol deoxynojirimycin (DNJ), for example, has been used for the treatment of non-insulin-dependent diabetes (miglitol, targeting the intestinal disaccharidases).[3] Recent studies further indicate that DNJ and its derivatives are effective against hepatitis B[4,5] and C,[6] as well as glycosphingolipid storage disorders (such as Gaucher disease).[7,8] The efficacy of iminocyclitols is attributed to their mimicry of the transition state of enzymatic glycosidic cleavage.

Of the members of the glycosidase family, α-fucosidase is involved in the hydrolytic degradation of numerous fucose-containing glycoconjugates. The existence of this enzyme is associated with a variety of essential functions. For example, the abnormal accumulation of fuco-glycoconjugates, which results from the absence or deficiency of α-fucosidase, leads to the genetic neurovisceral storge disease fucosidosis.[9] An aberrant distribution of intracellular and extracellular α-fucosidase is also found in cystic fibrosis[10,11] and colon–rectal cancer.[12,13] Though the physiological functions of α-fucosidase are not completely understood, potent fucosidase inhibitors may be used as probes for the study of fucosidases with regard to their functions and for the development of potential therapeutic agents.

Recently we have established a novel combinatorial approach to five-membered iminocyclitols based on reductive amination and the Strecker reaction,[14] and a potent inhibitor has been identified from the library. The process, that is, synthesis and isolation of individual compounds for testing, is however very slow. To further facilitate the discovery of new glycosidase inhibitors, we aimed to develop a new method for the rapid derivatization of an iminocyclitol core designed for a specific glycosidase family (for example, fuconojirimycin derivatives for fucosidases in this study) without protecting group manipulation and under such conditions that the product could be used directly for screening in situ without isolation. This concept was first demonstrated by the development of new HIV protease inhibitors.[15] Herein we report the generation of a library based on fuconojirimycin (FNJ) in a microtiter plate, followed by the direct in situ evaluation of these reaction mixtures as fucosidase inhibitorse without product isolation. This approach has led to the discovery of the most potent inhibitors of α-fucosidases from bovine kidney and *Corynebacterium sp*.

Previous work on the development of glycosidase inhibitors indicated the existence of an additional binding component in the inhibitor.[2,16–25] This study was thus aimed at generating an FNJ derivative **1** (Scheme 1) as a core structure to mimic the transition state of the fucose moiety. A β-aminomethyl group was attached to the C1-position for identification of a new binding component throught the subsequent in situ amide-bond formation and screening. Since 1-deoxy-FNJ has been shown to be a potent inhibitor against α-fucosidases ($K_i = 9.8$ nM for the α-fucosidase from human neutrophil and 6.2 nM from bovine epididymal),[26]




***Scheme 1.*** Synthesis of fuconojirimycin derivatives **1**–**4**. TBDMS = *tert*-butyldimethylsilyl, TFA = trifluoroacetic acid, Boc = *tert*-butoxycarbonyl, DIAD = diisopropyl-azodicarboxylate, TMS = trimethylsilyl, MOM = methoxymethyl.

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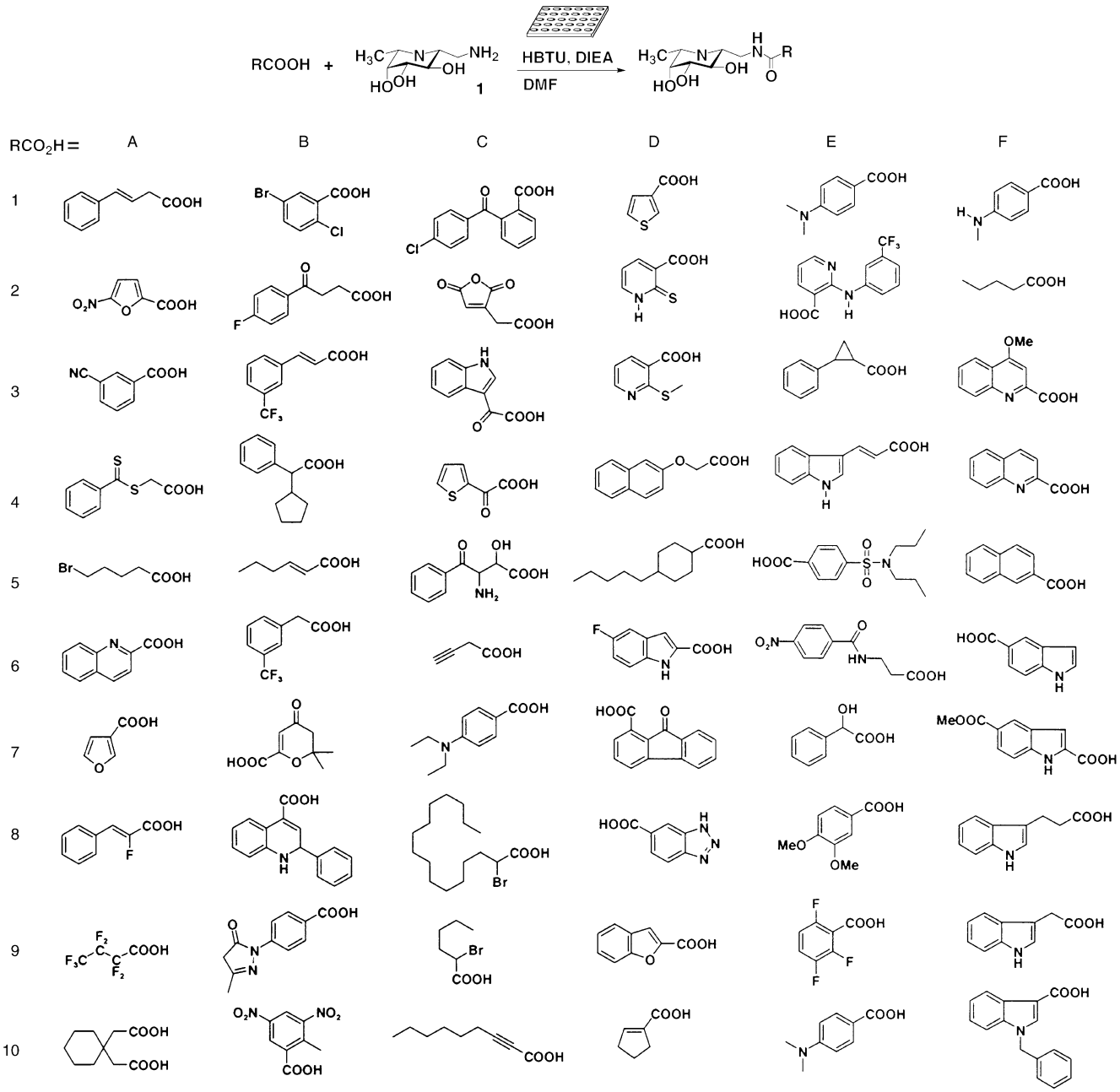
further derivatization of this core may lead to a better and more-selective inhibitor of a specific fucosidase. In addition, it has been suggested that the inhibitory potency of **1**, in comparison with that of FNJ, was not affected by the tether extending from C1 or the anomeric stereochemistry,[27] and the β-linked FNJ amine may also be used for the identification of GDP-β-fucose (the glycosyl donor of α-fucosyltransferases) mimics as fucosyltransferase inhibitors.[28]

As shown in Scheme 1, FNJ analogues **1**–**4** were synthesized from L-gulono-1,4-lactone by a modified procedure developed by Fleet et al.,[27,29,30] and azido-lactone **5** was prepared in four steps in an overall 40% yield.[29] The nucleophilic addition of methyllithium and subsequent reductive amination afforded **6** with an exclusive new *S* stereogenic center in 80% yield (two steps from **5**). β-Homofuconojirimycin **2** was obtained in 92% yield by acidic deprotection. To selectively convert the C1′-hydroxy group into another functionality, compound **6** was transformed to diol **7**. Mitsunobu reaction of **7** to transform the primary alcohol to an azide and removal of the trimethylsilyl group afforded the desired product **8** (72% in two steps). Deprotection followed by reduction gave 1-aminomethyl-FNJ (**1**). In addition, compound **6** was converted into the carboxyl derivative **4** through protection and oxidation with $RuCl_3/NaIO_4$ (54% from **6**).

The α-fucosidase from bovine kidney[31] was chosen for the inhibition studies of iminocyclitols **1**–**4**. Compounds **1**–**3** showed strong inhibitory effects with $IC_{50}$ values of 25, 30, and




***Scheme 2.*** The reaction of 1-aminomethyl-FNJ (**1**) with a library of 60 carboxylic acids in a microtiter plate for direct screening of fucosidase inhibition in situ.

24 nM, respectively, in contrast to the three- to fourfold lower activity of the carboxylic analogue **4** (92 nM). Compound **1** was then used for the subsequent diversity-oriented reaction with various acids in the presence of (1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 1 equiv) and diisopropyl ethylamine (DIEA, 2 equiv) in DMF, followed by aqueous dilution and screening without purification. Each assay was performed in a well of a microtiter plate containing 10 nM of the reaction mixture, and the remaining enzyme activity was measured spectroscopically by detection of the released *p*-nitrophenol at 400 nm in the presence of *p*-nitrophenyl-α-L-fucopyranoside (the substrate).

Of the 60 compounds generated from the amide-forming reaction (Scheme 2), several potent inhibitors were found. For the purpose of easy understanding, the potency was expressed as % inhibition at one concentration of the reaction product. As shown in the inhibition profile of these reaction products (Figure 1), the

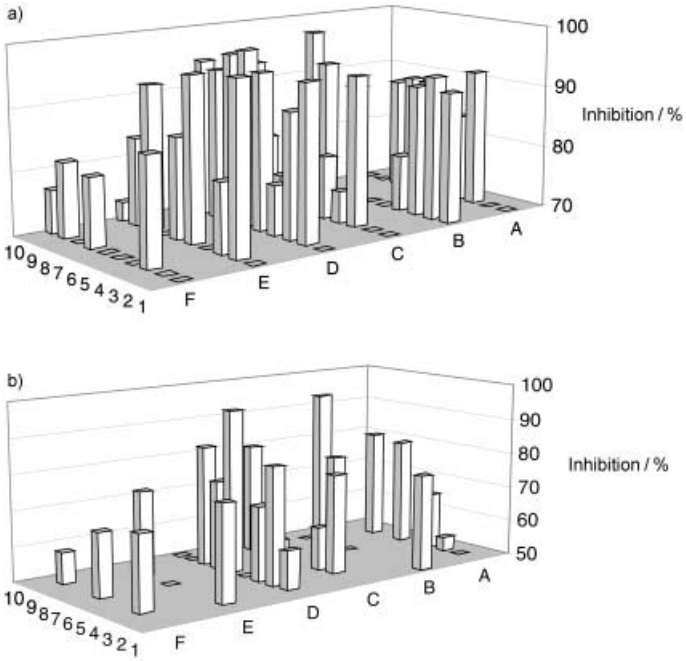
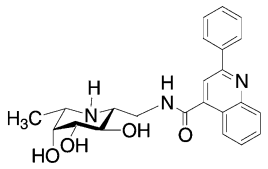


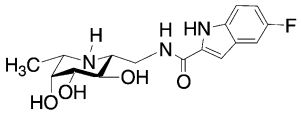

***Figure 1.*** Inhibition profile of the reactions of **1** with 60 different acids at concentartions of a) 50 nM and b) 10 nM.

enzyme indeed prefers a hydrophobic aglycon to a hydrophilic one (for example, the product with B1 versus the one with C5). About two thirds of the reaction products were more potent than or comparable to **1**. The α-fucosidase inhibition was thus greatly enhanced by introduction of an appropriate hydrophobic moiety. Furthermore, the inhibitory results suggest that the enzyme is more interactive with an aromatic than an alkyl aglycon (for example, the reaction with B5 or A10) or partially alkyl derivative (for example, the reaction with B4 or C5). The enzyme seems to prefer a hetroaromatic component (especially a nitrogen-containing hetrocycle such as a quinoline type (for example, A6 and B8) and an indole type (D6)), as well as a fused aromatic ring (for example, the reaction with D4 or D7).

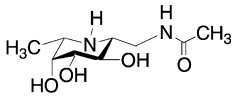
As a consequence of the strongest inhibitory potency exhibited by the aforementioned screening, the acids B8 and D6 were treated with 1-aminomethyl-FNJ **1**, and the products were chromatographed to afford **10** and **11**, respectively. The



**10**



**11**



**12**

inhibitions of **10** and **11** were studied and the $K_i$ values were determined to be 0.50 and 0.60 nM, respectively; thus both are competitive against the nitrophenyl substrate. Neither acid B8 nor acid D6 alone showed any significant inhibition. The coupling reagents (HBTU and *N*-hydroxybenzotriazole (HOBT)) used for the formation of an amide bond and the reaction side product showed little inhibition.[32] Both **10** and **11** were also examined as inhibitors of glycosidases, including α- and β-glucosidases, α- and β-galactosidases, as well as α- and β-mannosidases (Table 1), and no significant inhibition were observed, thus indicating their high selectivity for α-fucosidase.

Previous reports indicated that the inhibition activity was diminished to a great extent when the *exo*-cyclic amino group was converted into an amide by acylation.[20,33] To understand

***Table 1:*** Inhibition activity against various glycosidases.

| Enzyme | Inhibitor | % Inhibition at 1 mM |
|---|---|---|
| α-fucosidase[a] | **10** | 100[h] ($K_i$ = 0.50 nM) |
| | **11** | 100[h] ($K_i$ = 0.60 nM) |
| | **1** | $K_i$ = 25 nM |
| α-glucosidase[b] | **10** | 20 |
| | **11** | n.i. |
| | **1** | 6 |
| β-glucosidase[c] | **10** | 20 |
| | **11** | n.i. |
| | **1** | 3 |
| α-galactosidase[d] | **10** | 18 |
| | **11** | 36 |
| | **1** | 46 |
| β-galactosidase[e] | **10** | n.i. |
| | **11** | 9 |
| | **1** | n.i. |
| α-mannosidase[f] | **10** | 41 |
| | **11** | 19 |
| | **1** | n.i. |
| β-mannosidase[g] | **10** | 2 |
| | **11** | 2 |
| | **1** | n.i. |

[a] Bovine kidney (Sigma Co. or Glyko Inc.). [b] *Saccharomyces sp.* (Toyobo Co.). [c] *Caldocellum saccharolyticum*, a recombinant protein (Sigma Co.). [d] *Aspergillus niger* (Sigma Co.). [e] *Escherichia coli* (Toyobo Co.). [f] Jack beans (Sigma Co.). [g] Snail acetone powder (Sigma Co.). [h] 100% inhibition was observed when the inhibitor concentration is equal to or higher than 12 nM. n.i. = no inhibition

if the amide played a role in the inhibition, amide **12** was prepared (in quantitative yield by reaction of compound **1** with acetyl chloride) and found to have a fourfold lower activity ($K_i = 100$ nM). This result illustrates that the enzyme inhibition can be significantly enhanced by introduction of a hydrophobic amide group, even though the existence of an alkyl-amide bond has a negative effect.

The α1,2-fucosidase from *Corynebacterium sp.* was then investigated with the same library. The results revealed a preferred aglycon of fused (for example, D4, F4, F5, and F9) or polarized aromatic compounds (for example, B6, D7, and E6), with the exception of D5. The most potent inhibitions were found in the reaction with D7 ($IC_{50} = 5.6$ nM), F4 ($IC_{50} = 4.1$ nM), and F9 ($IC_{50} = 6.3$ nM). Compounds **10** and **11**, the best inhibitors previously described, are less active than the positive control **1** in this study. To obtain more sensitive detection in the enzyme assay, 4-methylumbelliferyl-α-L-fucopyranoside was also used to confirm the previous results. The compound was found to be a better substrate for the bacterial α1,2-fucosidase ($K_m = 12$ μM) than *p*-nitrophenyl-α-L-fucopyranoside ($K_m = 35$ μM), but inactive toward the bovine kidney α-fucosidase.[34] Similar $IC_{50}$ values were obtained for the α1,2-fucosidase from *Corynebacterium sp.*

In conclusion, the method described here represents the first rapid search for an optimal group attached to the core of a common transition-state mimic to bind the hydrophobic site of a glycosyltransfer enzyme using a simple amide-bond forming reaction followed by screening in situ without product isolation. The two compounds (**10** and **11**) are the most potent and selective inhibitors reported so far ($K_i = 0.50$ and 0.60 nM, respectively). The same library was screened for inhibitors of the *Corynebacterium* fucosidase and a different group of inhibitors was identified. This approach is thus proven to be very effective and simple for the rapid identification of selective potent inhibitors of enzymes within the same family.

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