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From Natural Product-Inspired Pyrrolidine Scaffolds to the Development of New Human Golgi α-Mannosidase II Inhibitors

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Natural products are an important source of inspiration in drug discovery, medicinal chemistry, and chemical biology.[1,2] An ongoing challenge, however, is the efficient preparation of natural product-based molecules that incorporate a large number of stereogenic centers. Difficult stereoselective reactions and tedious separation processes are frequently required.[3]

Many polyhydroxylated pyrrolidines and their derivatives, which bear identical functional groups of different spatial configurations, are known to exhibit a variety of different biological properties and inhibitory activities against various glycoprocessing enzymes, which are involved in diseases such as diabetes and cancer, viral infections, and lysosomal storage disorders.[4,5] Because of their broad-spectrum biological activities, this type of molecules can be considered a privileged scaffold.[6] For example, synthetic ADMDP (aminodeoxy-DMDP) with the configuration pattern of (2*R*,3*R*,4*R*,5*R*), inspired from naturally occurring DMDP (2,5-dideoxy-2,5-imino-D-mannitol) or DAB (1,4-dideoxy-1,4-imino-D-arabinitol),[7,8] was successfully synthesized by us and others, and its derivatives have been extensively applied as potent β-hexosaminidase and α-glucosidase inhibitors.[9–11] The C-2 aminomethyl moiety of the pyrrolidine ring can be considered a diversity position to which various functional moieties or substituents can be conjugated, which has the potential to improve inhibitory potency and selectivity.[12]

According to general mechanisms of enzymatic glycoside hydrolysis and transglycosylation,[4] the pyrrolidine skeleton might mimic the sp^2-like hybridization of the glycosyl unit; and the protonated N1-nitrogen can mimic the charge distribution at the anomeric position. However, the corresponding relationships between the stereo-configurations of multiple hydroxy groups of pyrrolidine iminosugars and pyranose-based glycans have yet to be comprehensively established. Obviously, a systematic correlation study is limited by difficulties associated with acquiring and modifying all possible polyhydroxylated pyrrolidines. Indeed, two interesting questions can be posed: 1) Can all sixteen stereoisomeric ADMDP-based scaffolds with four stereogenic centers (Figure 1) be prepared? and 2) How can we apply this unique set of scaffolds as a powerful tool to efficiently answer questions of biological relevance?

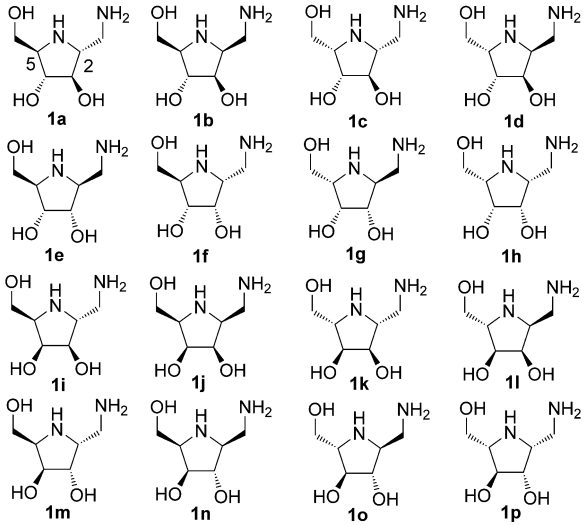

Figure 1. Chemical structures of all sixteen ADMDP-based scaffolds.

Human Golgi α-mannosidase II (hGMII) is a key enzyme in *N*-glycan processing, and its inhibition is a known anticancer strategy.[13] Several naturally occurring or synthetic inhibitors toward various GMIIs have been reported (Figure 2).[14–17] For example, swainsonine is a potent GMII inhibitor and also reduces certain tumors and hematological dysfunctions, but its use is associated with side effects such as co-inhibition of lysosomal α-mannosidases that limit its clinical study.[13,17] DMJ (1,5-dideoxy-1,5-imino-D-mannitol) is a common mannosidase inhibitor but is not a potent dGMII inhibitor ($K_i = 400\ \mu M$).[14] Interestingly, recent research shows several β-glucosidase inhibitors that can also inhibit dGMII by binding similar locations with mannose-

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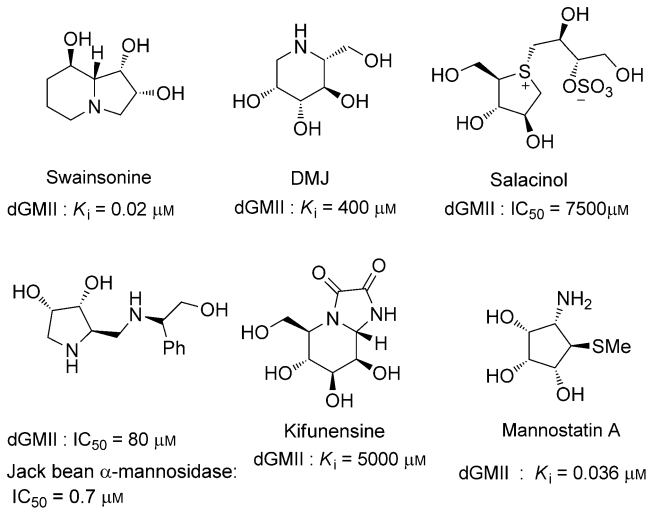
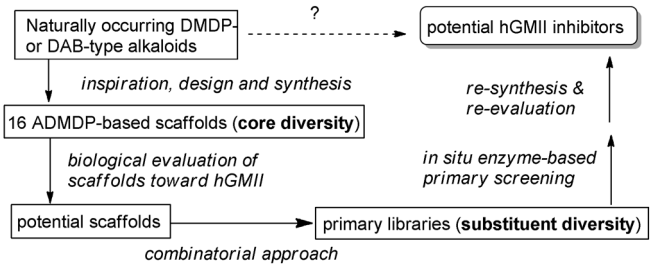


Figure 2. Examples of reported inhibitors against Golgi α-mannosidase II (dGMII = Drosophila GMII).

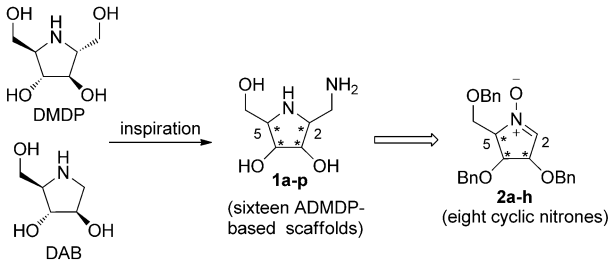
containing substrates.[14] Additionally, other molecules possessing distinct features including scaffolds and configurations of hydroxy groups exhibit potent inhibitory activities against mannosidases from jack bean or almonds.[18–21] Nevertheless, systematic exploration of a whole series of novel polyhydroxylated pyrrolidine-based scaffolds with regard to the development of new and potent hGMII inhibitors has yet to be reported.

Herein, we describe the preparation of all sixteen ADMDP isomers as primary scaffolds, followed by efficiently developing potent and selective hGMII inhibitors. The general strategy is illustrated in Scheme 1.

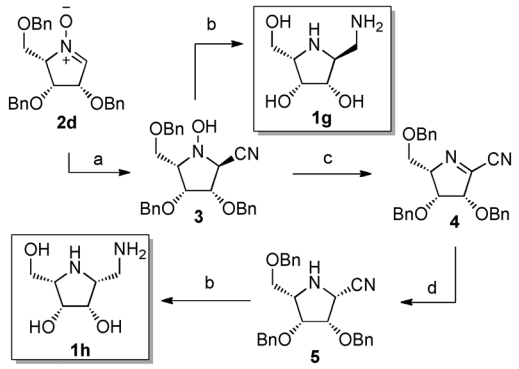


Scheme 1. Strategy for the development of hGMII inhibitors inspired from naturally occurring pyrrolidine-based alkaloids.

Based on our synthetic design (Scheme 2 and Figure S1 in the Supporting Information), all sixteen ADMDP-typed isomers **1a–p** can be prepared from the corresponding eight five-membered chiral tri-*O*-benxyl cyclic nitrones **2a–h** as key intermediates (see the Supporting Information).[9] Each cyclic nitrone can act as a precursor to two desired products bearing the 2,3-*cis* and 2,3-*trans* configuration, dependent on the chemical transformations used.[9] For example (Scheme 3), the highly diastereoselective nucleophilic addition of TMSCN to cyclic nitrone **2d** in methanol at 50 °C



Scheme 2. General strategy for the preparation of scaffolds **1a–p** from eight cyclic nitrones **2a–h**.



Scheme 3. Typical synthetic method for the preparation of new polyhydroxylated pyrrolidines **1g** and **1h** from cyclic nitrone **2d**. Reagents and conditions: a) trimethylsilyl cyanide, MeOH, 50 °C, 10 h; b) 1. Raney nickel, $H_{2(g)}$, Boc_2O, MeOH, RT, 2 h; 2. $Pd(OH)_2$, $H_{2(g)}$, MeOH, RT, 10 h; 3. TFA, DCM, 0 °C, 10 min; c) MsCl, Et_3N, THF, 0 °C, 1 min; d) $NaBH_4$, THF/MeOH (4:1), 0 °C, 18 h.

gave the 2,3-*trans* isomer **3** as a major product (87%, d.r. ≥ 20:1), which could be converted directly to **1g** in good yield (86%). On the other hand, the C-2 nitrile group in **3** was inverted through an elimination–reduction sequence to afford **5** (83% over two steps). Subsequently, global hydrogenolysis of **5** gave **1h** in 92% yield. Following the similar protocol with a different cyclic nitrone, all desired scaffolds **1a–p** were obtained in a yield ranging 30–35% from the corresponding cyclic nitrones **2a–h**. Notably, **1g**, **1j**, **1m**, **1n**, and **1p** are novel compounds.

With all sixteen scaffolds in hand, their biological evaluation could commence. Recombinant hGMII was prepared (see the Supporting Information) and used for inhibition studies. At a concentration of 1 mM, only **1i** showed inhibitory activity against hGMII with a potency of more than 80% (Figure 3). This finding suggests that the (2*R*,3*R*,4*S*,5*R*) configuration of pyrrolidine **1i** is critical to inhibitory potency against hGMII. By contrast, its C2 epimer **1j** was dramatically less active (approximately 20% inhibition), thus indicating that the orientation of the aminomethyl moiety significantly influences the inhibitory potency. Notably, though the four scaffolds **1i**, **1j**, **1k**, and **1l** share the same 3,4-diol configuration (3*R*,4*S*), the inhibition activity of **1i** was more potent than that of the other compounds. These observations emphasize that all four stereogenic centers play an important role in the hGMII inhibitory activity.

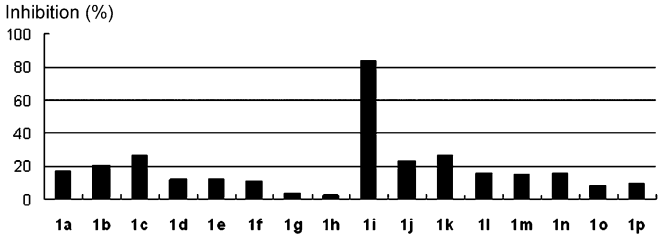


Figure 3. The inhibition activity of sixteen scaffolds **1a–p** at 1 mM against hGMII.

Next, scaffold **1i** was directly coupled with a randomly selected 48-membered acid library **6** by prior activation of the carboxylic acids in the presence of HBTU (1.5 equiv), HOBt (1.5 equiv), and DIEA (3 equiv) in DMSO. After 24 hours, the reactions were analyzed by LC-MS. It was found that amine **1i** had been completely consumed and a significant amount of desired products had been formed (estimate conversion).[10,12] The 48-membered primary library **7** was directly evaluated at 25 μM in an hGMII enzyme-based assay without further purification (Scheme 4). Excitingly, five compounds were found to display more than 60% inhibition in this primary assay. The screening results indicated that the structure of the substitution moiety strongly affects the inhibition potency (Figure 4). For example, when alkyl moieties were used, no significant inhibitory activity was observed. By contrast, compounds containing a fluorine-substituted benzyl moiety such as **7-Ec**, **7-Cd**, and **7-Dd** showed high hGMII inhibitory activities. Notably, neither the acid library **6** nor the coupling reagents showed the any significant inhibition. For comparison purposes, library **8**, which was prepared from scaffold **1a** (the C4-epimer of **1i**), was also synthesized following the same procedure. As expected, no potent inhibitor was found, again emphasizing the importance of the 2*R*,3*R*,4*S*,5*R* stereo-configuration in the scaffolds.

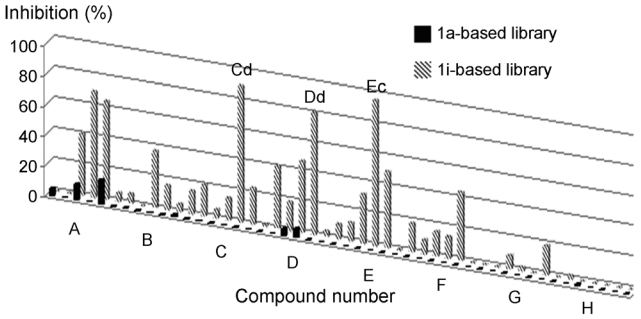
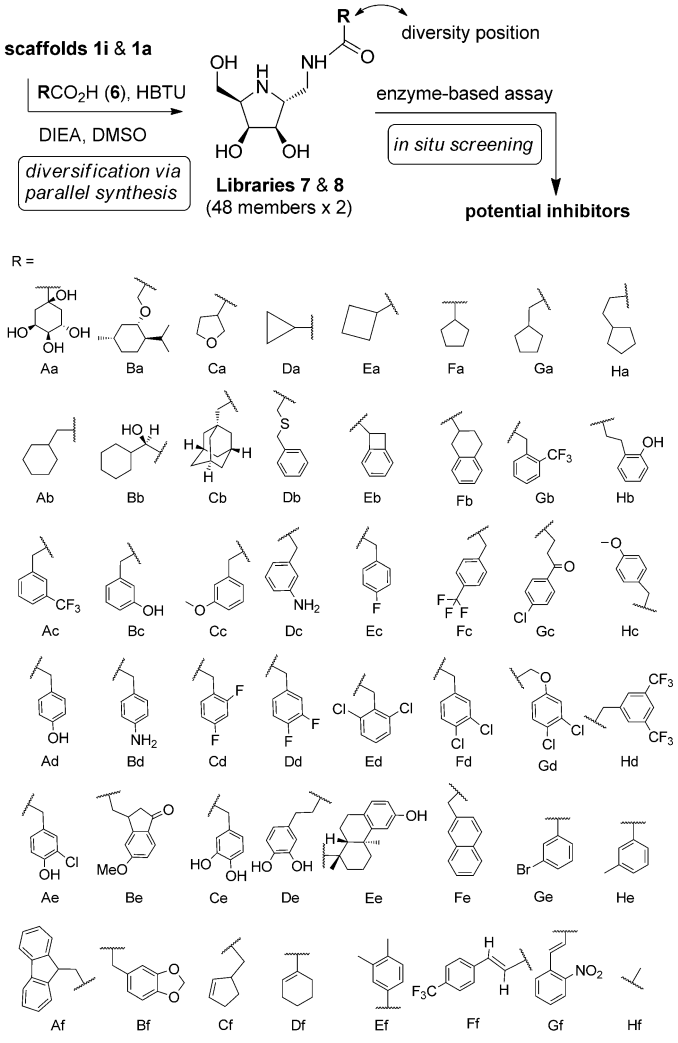


Figure 4. The inhibition activity of library **7** and library **8** at 25 μM against hGMII and chemical structures of selected potent inhibitors **9** and **10**.



Scheme 4. Parallel synthesis of the primary libraries **7** and **8**, followed by in situ enzyme-based inhibition evaluation against hGMII.

Two potent inhibitors **9** (=**7-Ec**) and **10** (=**7-Cd**) were resynthesized by coupling of acids **6-Ec** and **6-Cd**, respectively, with scaffold **1i**. Both **9** and **10** were confirmed to be potent competitive inhibitors with an inhibition constant (K_i) of 24 nM and 31 nM, respectively (Figure 5 and Figure S4, Supporting Information). Notably, the fluorine atom at the *para* position of the benzene moiety should play an important role in the inhibitory potency of **9** and **10**.

We then created the three-dimensional (3D) structure of human GMII (hGMII) by using homology modeling of the crystallized structure of Drosophila GMII (PDB code 3DDF).[22] The hydroxy groups at C3 and C4 of the five-membered ring coordinate the zinc ion together with Asp289, Asp177, His175, and His569, and also form hydrogen bonds with Tyr354, Asp426, and Asp570 (Figure 6). The hydroxy group at C6 forms hydrogen bonds with Asp570 and Tyr808. The protonated nitrogen on the pyrrolidine ring has strong electrostatic interactions with Asp289. Notably, the moiety attached at the C2 position shows significant interactions. For example, the aromatic moiety could dock in the pocket formed by Tyr352, Arg313, Tyr354, and Try496 through aromatic interactions and cation–π interactions. Our model shows that bulky substituted groups on the aro-

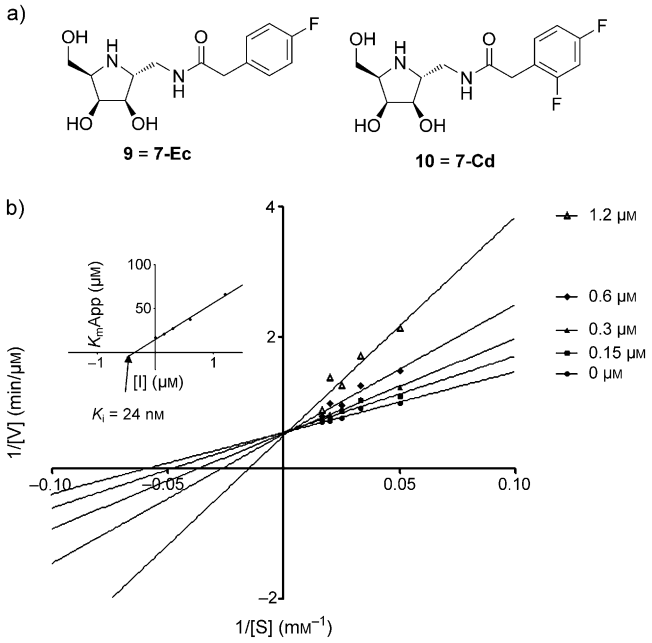


Figure 5. a) Chemical structures of potent hGMII inhibitors **9** and **10** and b) Lineweaver–Burk plot of inhibitor **9**.

matic ring could decrease the overall binding affinity due to steric restrictions in the described pocket. By contrast, the fluorine atom on the aromatic ring would increase the interaction by forming weak hydrogen bonds with Tyr496 and multipolar interaction with the backbone carboxylate of Tyr352. It can be seen that all interactions of the moiety depend on the configuration at the C2 position. Overall, the modeling plausibly and completely explains the observations made in our inhibition study.

The inhibitory activity of compounds **9** and **10** towards a panel of glycosidases,[9,10] including α-glucosidases, β-glucosidase, α-galactosidase, α-mannosidase, and α-rhamnosidase (Table 1), was also examined. Interestingly, although both **9** and **10** were potent inhibitors against hGMII, they displayed only moderate to weak inhibitory activities against other glycosidases including α-mannosidase from Jack bean. This result suggests that both **9** and **10** could be highly selective and potent inhibitors against hGMII.

Table 1. Inhibititory activity of **9** and **10** against various glycosidases.

Enzyme	IC_{50} [μM]	
	9	**10**
acid α-glucosidase	n.i.	n.i.[g]
α-glucosidase[a]	9	88
β-glucosylceramidase	54	11
β-glucosidase[b]	93	>200
α-galactosidase[c]	20	17
amyloglucosidase[d]	n.i.	n.i.
α-rhamnosidase[e]	>200	>200
α-mannosidase[f]	29	>200
hGMII	0.3	0.5
	(K_i=24 nM)	(K_i=31 nM)

[a] From *Bacillus stearothermophilus*. [b] From almonds. [c] From green coffee beans. [d] From *Aspergillus niger*. [e] Naringinase from *Penicillium decumbens*. [f] From Jack bean. [g] n.i., no inhibition at 250 μM.

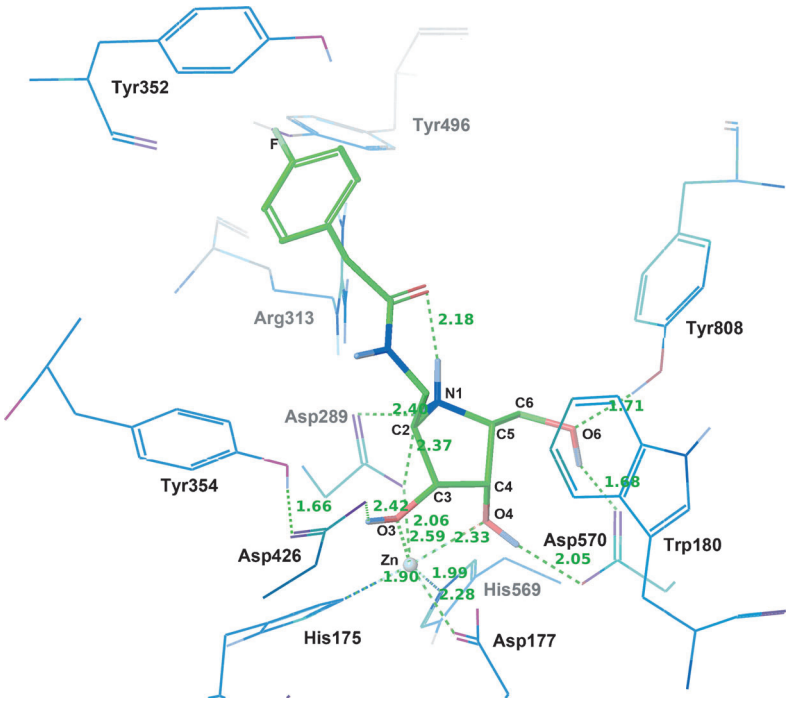


Figure 6. A computational modelling of the complex between hGMII (blue) and inhibitor **9** (green).

In summary, the systematic preparation of all possible sixteen enantiopure ADMDP-based scaffolds has been efficiently achieved from eight five-membered cyclic nitrones through general and flexible transformations. Each scaffold possesses four stereocenters and the *exo*-aminomethyl group, which allows rapid substituent diversity to be accomplished by conjugation with structurally diverse acids. To exemplify the biological utility of these scaffolds, two novel hGMII inhibitors were developed through our concise method. Because these molecules are easily prepared and modified, they are suitable as initial molecules for further investigation of the inhibition selectivity towards other mannosidases including lysosomal and endoplasmic reticulum (ER)

mannosidases in the future. It is hoped that this versatile set of scaffolds as well as the practical development strategy used will become a powerful chemical tool for the exploration of other interesting or disease-associated glycosidases or glycotransferase inhibitors.

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Keywords: alkaloids · combinatorial chemistry · natural products · pyrrolidine-based scaffold · synthetic inhibitors

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