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COMMUNICATIONS TO THE EDITOR

A Facile Synthesis of D-Galactose-type *Gem*-Diamine 1-*N*-Iminosugar: A New Family of Galactosidase Inhibitor

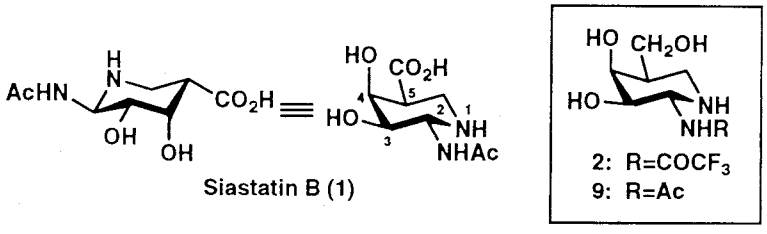
Sir:

Currently there is significant interest in the synthesis and isolation of glycosidase inhibitors due to their practical potential for the prevention and treatment of a variety of diseases, including cancer, diabetes and AIDS.[1~3] Various types of inhibitors have been designed based on the mechanism of the enzyme-catalyzed reaction and the structure of natural inhibitors.[3,4] We proposed a new type of glycosidase inhibitor, *gem*-diamine 1-*N*-iminosugars, modeled on natural siastatin B (**1**) in which an anomeric carbon atom is replaced by a nitrogen atom.[5] The *gem*-diamine 1-*N*-iminosugars, especially 2-trifluoroacetamido-1-*N*-iminosugars, have been proved to be very potent and specific glycosidase inhibitors.[5~8] Uronic acid-type *gem*-diamine 1-*N*-iminosugars also showed potent suppression of experimental and spontaneous pulmonary metastasis of tumor cells in mice.[6,7,9] We here report the extension of our study on *gem*-diamine 1-*N*-iminosugars to the synthesis of D-galactose-type 1-*N*-iminosugar **2** and its inhibitory activity against glycosidases.

Siastatin B (**1**) has the same configuration as D-galactose as 1-*N*-iminosugar (Fig. 1) and is easily obtainable from *Streptomyces* culture.[10] Therefore, we chose **1** as a starting material for the facile synthesis of **2**. The synthesis of **2** was begun with the known derivative **3**[11] obtained from **1**. Upon protection of the carboxyl group, **3** gave the MEM ester **4**,† which was reduced with $NaBH_4$ to the alcohol **5**† in good yield. Treatment of **5** with hydrazine hydrate afforded the amine **6**† in 83% yield. Conventional trifluoroacetylation of **6** furnished the trifluoroacetamide **7**,† which was converted into the triol **8**† by hydrogenolysis in good yield. Removal of the *t*-Boc group with 4 M hydrogen chloride in dioxane afforded the desired D-galactose-type 2-trifluoroacetamido-1-*N*-iminosugar **2**.†

The inhibitory effect of **2** on various glycosidases was next examined (Table 1).†† As expected, **2** showed strong inhibition against galactosidases, particularly β-D-galactosidase (IC_{50} 0.05 μg/ml). This result can be rationalized in that **2** should closely mimick a glycopyranosyl cation **10**, one of the presumed reaction intermediates (the chair-like and the flattened conformational cation **10** and **11**, respectively) in the transition state of the enzymatic glycoside cleavage (Fig. 2).[12] β-D-Glucosidase and α-*N*-acetylgalactosaminidase were also inhibited with an IC_{50} of 0.14 and 0.65 μg/ml, respectively. However, β-*N*-acetylglucosaminidase was not affected at 100 μg/ml. These results indicate that β-glucosidase may roughly recognize the configuration of the 4-OH group of the

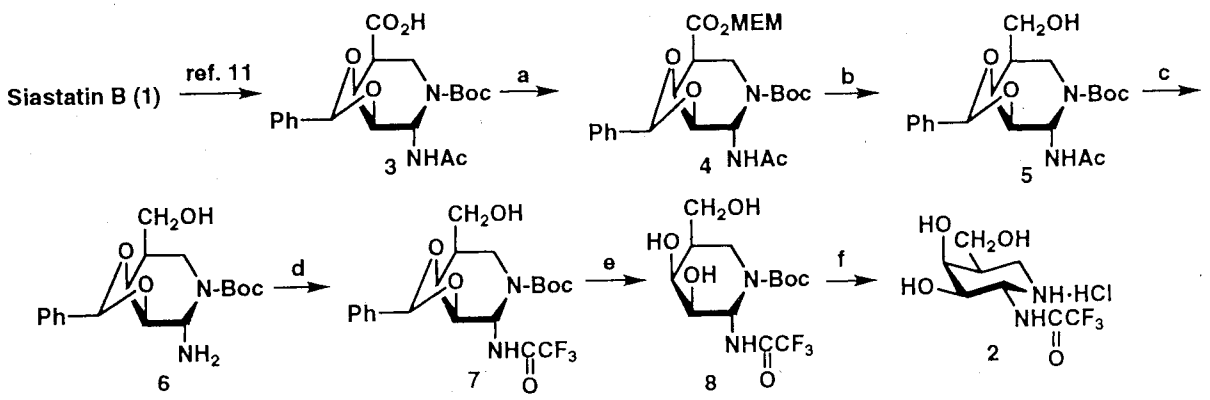
Fig. 1. Structures of siastatin B and D-galactose-type *gem*-diamine 1-*N*-iminosugars.



† **4**: $[\alpha]_D^{23}$ +22° (*c* 0.91, MeOH), **5**: $[\alpha]_D^{23}$ +87° (*c* 0.93, MeOH), **6**: $[\alpha]_D^{23}$ +26° (*c* 0.81, MeOH), **7**: $[\alpha]_D^{23}$ +68° (*c* 0.96, MeOH), **8**: $[\alpha]_D^{23}$ +46° (*c* 0.59, MeOH), **2**: $[\alpha]_D^{23}$ +39° (*c* 0.64, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 2.06~2.15 (1H, m, 5-H), 3.18 (1H, br t, *J*=12.2 Hz, H-6ax), 3.22 (1H, dd, *J*=5.4, 12.2 Hz, H-6eq), 3.58 (1H, dd, *J*=7.3, 10.7 Hz, $-CH_2OH$), 3.69 (1H, dd, *J*=6.4, 10.7 Hz, $-CH_2OH$), 3.90 (1H, dd, *J*=2.9, 10.3 Hz, H-3), 4.08~4.12 (1H, m, H-4), 5.07 (1H, d, *J*=10.3 Hz, H-2).

†† All enzymes were purchased from Sigma Chemical Co., St. Louis. All enzyme assays were similarly evaluated as described previously.[8]

Scheme 1. Synthesis of D-galactose-type 2-trifluoroacetamido-1-*N*-iminosugar.



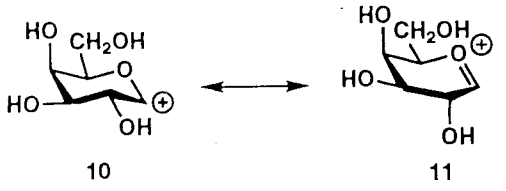
(a) $CH_3OCH_2CH_2OCH_2Cl$, *i*-Pr_2NEt, DMF, rt, 98% (b) $NaBH_4$, CF_3CH_2OH/THF, rt, 94% (c) $H_2NNH_2 \cdot xH_2O$, 70°C, 83% (d) CF_3CO_2Et, *i*-Pr_2NEt, DMF, 60°C, 73% (e) H_2/10% Pd-C, MeOH, rt, 92% (f) 4 M HCl/dioxane, rt, 80%.

Table 1. Inhibitory activity of siastatin B (**1**), **2** and **9** against glycosidases.

Enzyme	IC_{50} (μg/ml)		
	1	**2**	**9**
α-D-Galactosidase[a]	>100	0.1	6
β-D-Galactosidase[a]	>100	0.05	4
α-D-Glucosidase[b]	>100	>100	>100
β-D-Glucosidase[c]	>100	0.14	19
α-D-Mannosidase[d]	>100	>100	>100
β-D-Mannosidase[e]	>100	38	>100
β-D-Glucuronidase[f]	15.5	>100	>100
α-D-*N*-Acetylgalacto-saminidase[g]	>100	0.65	0.08
β-D-*N*-Acetylgluco-saminidase[h]	>100	>100	0.65

[a] *Aspergillus niger*, [b] Baker's yeast, [c] Almonds, [d] Jack beans, [e] Snail, [f] Bovine liver, [g] Chicken liver, [h] Bovine epididymis.

Fig. 2. The presumed reaction intermediates (**10** and **11**) in a transition state of hydrolysis by D-galactosidase.



inhibitors for enzyme-inhibitor interaction. On the other hand, the binding groups equivalent to the 2-NHAc groups in *N*-acetylgalacto- and glucosaminide are likely to play important roles for specificity and potency of the inhibitors for the corresponding enzymes. This result was also supported by the observation of the strong and comparable inhibition for *N*-acetylgalactosaminidase (IC_{50} 0.08 μg/ml) and *N*-acetylglucosaminidase (IC_{50} 0.65 μg/ml) with the previous 2-acetamido-1-*N*-iminosugar **9**[8] of galactose-type. In addition, **2** inhibited β-glycosidases more potently than α-glycosidases. These results suggest that the 2-trifluoroacetamide group of **2** favorably interacts with the amino acid residue of β-glycosidases instead of water molecule which participates in hydrolysis.[13] Further biological evaluations (anti-HIV, antimetastatic, *etc.*) of compound **2** are in progress.

In summary, a *gem*-diamine 1-*N*-iminosugar of D-galactose-type, a new type of glycosidase inhibitor, has been synthesized from siastatin B which has isolated from *Streptomyces* culture. The analogue was proved to be a potent inhibitor of β-D-galactosidase (IC_{50} 0.05 μg/ml), α-D-galactosidase (IC_{50} 0.1 μg/ml), and β-D-glucosidase (IC_{50} 0.14 μg/ml). The chemical modification of natural siastatin B (**1**) presented here should offer a useful

approach to *gem*-diamine 1-*N*-iminosugars which can be regarded as carbohydrate mimics, promising to be potent glycosidase inhibitors. Thus, this 1-*N*-iminosugar is potent inhibitor of *β*-D-galactosidase and further supports the hypothesis of our design an the new type inhibitor.

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