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Synthesis of all stereoisomers of 3-hydroxypipecolic acid and 3-hydroxy-4,5-dehydropipecolic acid and their evaluation as glycosidase inhibitors

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Abstract—A highly practicable synthesis of both enantiomers of 3-hydroxypipecolic acid derivatives **1**, **2**, **3**, **4** is described. Screening of these molecules for glycosidase inhibition has been examined. Compound **3** was shown to be a potent inhibitor of β-*N*-acetylglucosaminidase as well as *Escherichia coli* β-glucuronidase.

Functionalized chiral, non-racemic piperidines are common structural units found in many biologically and medicinally important natural and non-natural products.[1] Among them, pipecolic acid, the next higher homolog of proline, has received considerable attention as a proline analogue. In particular, 3-hydroxypipecolic acids **1** and **2**, six-membered cyclic-amino-hydroxy acids, constitute non-natural variants of a structural motif often encountered in a variety of functional molecules, and they may be regarded as expanded hydroxylated proline or a conformationally restricted serine derivative and may affect physiological and pathological processes.[2] In addition, (−)-3-hydroxybaikiain **4**, the 4,5-dehydro derivative of **2**, has been isolated from a toxic mushroom, *Russula subnigricans* Hongo.[3] The piperidine unit of 3-hydroxypipecolic acid is found in a number of biologically important products. For example, the *cis*-isomer **2** forms a part of the structure of tetrazomine **5**, an antitumor antibiotic,[4] while the *trans*-isomer **1** is a precursor of (−)-swainsonine **6**, which has shown potent and specific α-D-mannosidase inhibitory activity,[5] and one-carbon homologated analogue of **1** is also found in the structure of febrifugine **7**, a potent antimalarial agent[6] (Fig. 1). In addition, pipecolic acids **1**–**4** might be precious scaffolds to be incorporated into conformationally restricted peptidomimetics of biological relevance.[7]

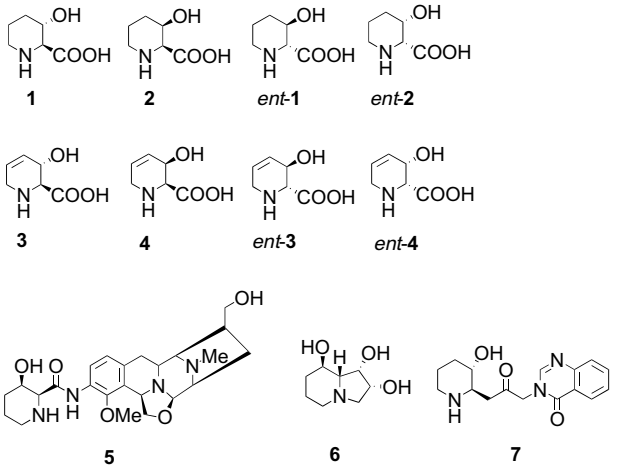

Figure 1. Structures of pipecolic acids and their derivatives.

Over the past several years, we have been interested in the synthesis of polyhydroxylated piperidines (azasugars), which show attractive biological activities such as glycosidase inhibition.[8] In the other hand, glycosidase inhibitory activities using uronic types changed from a hydroxymethyl to a carboxyl has been little reported

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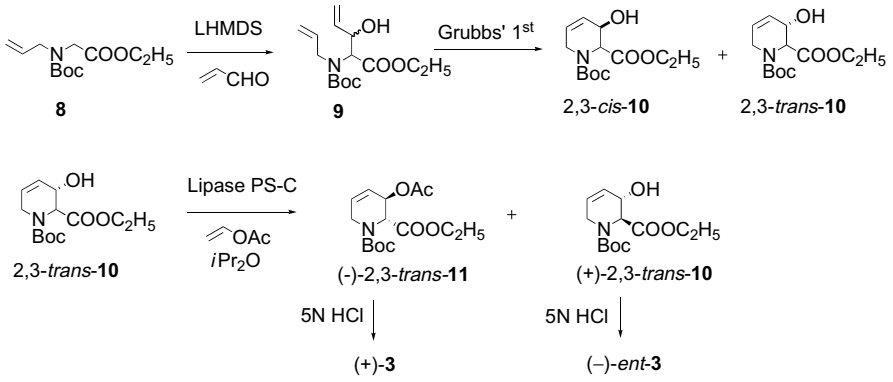
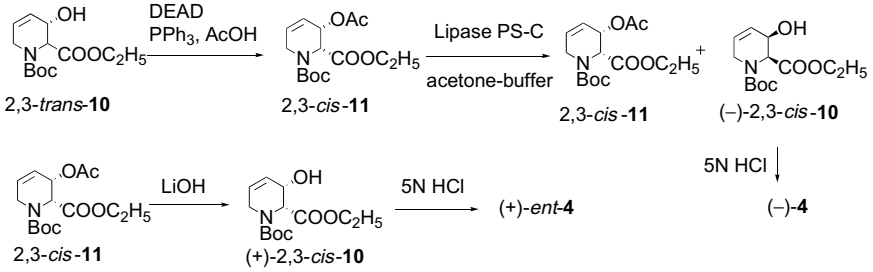
compared with azasugars.[9] A practical route providing an easy access to the title compounds is therefore highly desirable, and a chiral synthesis constitutes an area of considerable current interest. However, a simultaneous synthesis of all stereoisomers (**1**, *ent*-**1**, **2**, and *ent*-**2**) of 3-hydroxypipecolic acid and the chiral synthesis of their 4,5-dehydro compounds (**3**, *ent*-**3**, **4**, and *ent*-**4**) have not been achieved up to now. Accordingly, we became interested in developing a simple and feasible route to 3-hydroxypipecolic acid and 4,5-dehydro-3-hydroxypipecolic acid. In this letter, we report a new chiral synthesis of all stereoisomers of **1** and **3** in conjunction with their inhibitory activities of glycosidases such as β-glucuronidase, β-*N*-acetylglucosaminidase, and α-*N*-acetylgalactosaminidase.

Our simple synthetic approach to **3** began with aldol condensation between *tert*-butyl (ethoxycarbonyl)methylallylcarbamate **8**[11] and acrolein. Treatment of **8** with LHMDS followed by the addition of acrolein at −80 °C in THF gave a diastereomeric isomer of allyl alcohol **9** in 87% yield.[12] Grubbs' catalyst[13] could be used directly on **9** to afford the ring-closing metathesis products **10** in high yields, which were separated to 2,3-*cis*-**10** and 2,3-*trans*-**10** in a ratio of **1** to **4** in 99% yield.[14] With a desired 2,3-*trans*-piperidenol as a major product in hand, the lipase-catalyzed transesterification of 2,3-*trans*-**10** with vinyl acetate was carried out. Of the various lipases tested, resolution of 2,3-*trans*-**10** was best achieved with lipase PS (*Pseudomonas cepacia*), immobilized on ceramic particles in diisopropyl ether at 40 °C, which gave the acetate (−)-2,3-*trans*-**11** in 47% yield, along with the unreacted alcohol (+)-2,3-*trans*-**10** in 47% yield. The enatiomeric purity of (+)-2,3-*trans*-**10** was 99% ee, as determined by chiral HPLC analysis after replacing the *N*-protecting group with tosyl. The ee of the acetate (−)-2,3-*trans*-**11** was determined to be 97% after deacetylation of (−)-2,3-*trans*-**11** with LiOH in CH_3OH–H_2O followed by a procedure similar to that described above. Global deprotection of (+)-2,3-*trans*-**10** and (−)-2,3-*trans*-**11** with 5 N HCl at 120 °C provided the desired (+)-**3** ($[\alpha]_D$ +60.6° (*c* 1.0, H_2O)) and (−)-*ent*-**3** (−58.7° *c* 1.0, H_2O) in 99% and 94% yields, respectively (Scheme 1).

Next, an inversion of hydroxyl of 2,3-*trans*-**10** using the Mitsunobu reaction was performed to give the acetate 2,3-*cis*-**11** in <90% yield, which contained a small amount of impurities. Resolution of the contaminated acetate 2,3-*cis*-**11** with enzymatic hydrolysis using the same lipase in 0.1 M phosphate buffer afforded the acetate 2,3-*cis*-**11** and the alcohol (−)-2,3-*cis*-**10** (30% yield and 99% ee). Unfortunately, the small amount of impurities in the acetate 2,3-*cis*-**11** remained at this stage. Subsequently, hydrolysis of the acetate with LiOH in CH_3OH–H_2O gave pure (+)-2,3-*cis*-**10** (31% yield from 2,3-*trans*-**10** and 99% ee). Finally, global deprotection of (−)-2,3-*cis*-**10** and (+)-2,3-*cis*-**10** with 5 N HCl afforded the desired (−)-**4** {$[\alpha]_D$ −335.3° (*c* 1.0, H_2O)}, lit.[3] {$[\alpha]_D$ −332.7° (*c* 0.3, H_2O)}, and (+)-*ent*-**4** {$[\alpha]_D$ +343.3° (*c* 0.74, H_2O)}, in 85% and 88% yields, respectively. The optical rotation and spectral characteristics of (−)-**4** were in good agreement with those reported in the literature[3] (Scheme 2).



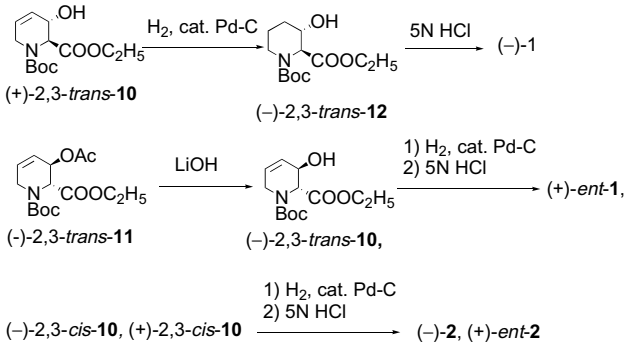
Scheme 1. Synthesis of **3**.



Scheme 2. Synthesis of **4**.

With the homochiral four piperidenols **10** in hand, our objective was directed to their conversion to four 3-hydroxypipecolic acids **1** and **2**. Hydrogenation of (+)-2,3-*trans*-**10** with Pd–carbon gave the piperidenol (−)-2,3-*trans*-**12** in 99% yield, which was deprotected with 5 N HCl to provide (+)-**1** {($[\alpha]_D$ +16.2° (*c* 1.0, 10% H_2O)) in 84% yield. Deacetylation of (−)-2,3-*trans*-**11** with LiOH afforded (−)-2,3-*trans*-**10** (97%), which was transformed into (+)-*ent*-**1** in a two-step sequence (hydrogenation and deprotection) in 86% yield. In a similar manner, (−)-**2** {($[\alpha]_D$ −59.6° (*c* 0.57, 10% H_2O)), and (+)-*ent*-**2** were obtained from (−)-2,3-*cis*-**10** and (+)-2,3-*cis*-**10** in a two-step sequence in 76%, and 76% yields, respectively. The spectral data for all 3-hydroxypipecolic acids were in excellent agreement with data reported in the literature[10] (Scheme 3).

As mentioned earlier, there are few reports on the inhibitory effect of pipecolic acid derivatives on glycosidases. Thus, we examined inhibitory activities of the obtained 3-hydroxypipecolic acid derivatives against various glycosidases, including β-glucuronidase. Since the 3-hydroxypipecolic acid derivatives are designed as an azasugar analogue of uronic acid derivatives, they are thought to act as a transition state analogue and to be a potential inhibitor of β-glucuronidase. In addition, β-glucuronidase inhibitors are important since this class of inhibitors recently exhibited attractive effects such as a protective effect against antitumor camptothecin derivative (CPT-11)-induced mucosa damage and diarrhea in treatment of advanced non-small-cell lung cancer.[15] A



Scheme 3. Synthesis of **1** and **2**.

primary screening of the 3-hydroxypipecolic acid derivatives against various glycosidases revealed a rather low level of activity against bovine liver β-galactosidase (EC 3.2.1.23) and *Aspergillus niger* amyloglycosidase (data not shown). On the other hand, some of the compounds tested showed moderate inhibition against β-glucuronidase, β-*N*-acetylglucosaminidase, and α-*N*-acetylgalactosaminidase.[16] The results are summarized in Table 1. 3-Hydroxypipecolic acids **1** and **2** showed moderate inhibitory activity against *Escherichia coli* β-glucuronidase, and their enantiomers *ent*-**1** and -**2** also showed similar results. However, these compounds showed only weak inhibitory activity against the same enzyme obtained from bovine liver (runs 1–4). Unexpectedly, all the compounds showed weak to moderate inhibitory activity against β-*N*-acetylglucosaminidase. Among them, compound **3** showed the most potent inhibitory activity and its IC_{50} values were 0.72 and 0.75 mM against β-*N*-acetylglucosaminidase isolated from bovine kidney and human placenta, respectively (run 5). Recently, reversible O-glycosylation with β-*N*-acetylglucosamine (*O*-GlcNAc) to serine and threonine residues of cytosolic and nuclear proteins has been found to be one of the post-translational modifications such as protein phosphorylation.[17] It has also been shown that *O*-GlcNAc is stimulated by high glucose flux and is implicated in type II diabetes.[18] The *O*-GlcNAcase inhibitor PUGNAc is used as a tool for investigating the biological function of *O*-GlcNAc.[18] Therefore, the result is quite interesting because **3** may be a new lead for designing novel *N*-acetylglucosaminidase inhibitors. The stereochemistries of **3** at 2- and 3-positions accord well with *N*-acetylglucosamine and this may lead to a potent inhibitory activity. In contrast to 4,5-dehydro-3-hydroxypipecolic acids **3** and **4**, all stereoisomers of 3-hydroxypipecolic acids **1**, **2**, *ent*-**1**, and *ent*-**2** showed inhibitory activity against β-*N*-acetylglucosaminidase as well as *E. coli* β-glucuronidase. These results suggest that the recognition of **1** and **2** by these enzymes is different from that of **3** and **4** since their inhibition potency does not depend on their stereochemistry. Although most of the compounds tested show negligible inhibitory activity against α-*N*-acetylgalactosaminidase obtained from chicken liver, only 4,5-dehydro-3-hydroxypipecolic acid **3** showed a weak inhibitory activity against the same enzyme (run 5). However, the reason for this is unclear.

Table 1. Inhibition rates of 3-hydroxypipecolic acid derivatives against β-glucuronidase, β-*N*-acetylglucosaminidase, and α-*N*-acetylgalactosaminidase at 1 mM

Run	Comp	Inhibition rate (IC_{50} value)				
		β-Glucuronidase		β-*N*-Acetylglucosaminidase		α-*N*-Acetylgalactosaminidase
		Bovine liver (%)	*E. coli* (%)	Bovine kidney (%)	Human placenta (%)	Chicken liver (%)
1	**1**	0.89	31	38	36	5.2
2	*ent*-**1**	0.62	20	32	25	1.3
3	**2**	0.18	24	20	30	2.5
4	*ent*-**2**	15	23	47	46	3.2
5	**3**	19	2	58 (0.72 mM)	60 (0.75 mM)	25
6	*ent*-**3**	0.2	0.9	15	14	3.2
7	**4**	8.6	3.3	7.3	17	0.2
8	*ent*-**4**	10	0.9	0.8	14	1.3

In summary, we have achieved a highly feasible synthesis of 3-hydroxypipecolic acid derivatives **1**, **2**, **3**, **4** and their enantiomers. Among the compounds obtained, 4,5-dehydro-3-hydroxypipecolic acid **3** showed inhibitory activity against β-*N*-acetylglucosaminidase. The result revealed that **3** may be a new lead compound for designing novel inhibitors of β-*N*-acetylglucosaminidase, which would be a useful biological tool to investigate the function of *O*-GlcNAc. It is also emphasized that intermediate **10** will serve as a useful synthetic precursor for polyhydroxylated pipecolic acids. Study along this line is ongoing, and the results, including results for the synthesis of a 5-aza analogue of glucuronic acid, will be reported elsewhere.

Acknowledgments

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16. The enzymes β-glucuronidase (from bovine liver; from *E. coli*), β-*N*-acetylglucosaminidase (from bovine kidney; from human placenta), α-*N*-acetylgalactosaminidase (from chicken liver), and *p*-nitrophenyl glycosides were purchased from Sigma Chemical Co. The activities of these glycosidases were determined using an appropriate *p*-nitrophenyl glycoside as a substrate at optimum pH of each enzyme. The reaction mixture (1 mL) contained 10 mM of the substrate and an appropriate amount of the enzyme. The reaction was stopped by adding 2 mL of 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.
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