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Synthesis of both enantiomers of hydroxypipecolic acid derivatives equivalent to 5-azapyranuronic acids and evaluation of their inhibitory activities against glycosidases

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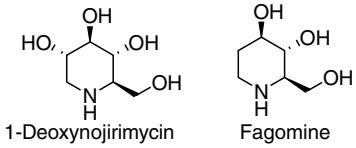
ABSTRACT

We have synthesized 3-hydroxy- and 3,4,5-trihydroxypipecolic acid derivatives corresponding to 5-aza derivatives of uronic acids and evaluated their inhibitory activities against various glycosidases including β-glucuronidase. Compounds **4** and **5** were chosen as common intermediates for the synthesis of 3,4,5-trihydroxypipecolic acids and 3-hydroxypipecolic acids as well as for 3-hydroxybaikiain, a unique natural product isolated from a toxic mushroom. Cross aldol reaction of *N*-Boc-allylglycine derivative with acrolein followed by the ring-closing metathesis gave **4** and **5** as a mixture of diastereomers which could be separated by silica gel column chromatography. By employing lipase-catalyzed kinetic resolution, the synthesis of both L- and D-isomers of 3,4,5-trihydroxy- and 3-hydroxypipecolic acids was achieved. None of the compounds tested showed inhibitory activity against α- and β-glucosidases. On the other hand, L-**23** and L-**29** were found to have potent inhibitory activity against β-glucuronidase. In addition, it is interesting that some uronic-type azasugar derivatives showed moderate inhibitory activities against β-*N*-acetylglucosaminidase.

1. Introduction

Azasugars (iminosugars) are a unique class of compounds that have inhibitory activities against various glycosidases by acting as transition state analogues, and they have potential applications as drugs, including antidiabetics, antiobesities, antivirals and therapeutics, for the treatment of some genetic disorders such as Gaucher disease.[1] we have recently been interested in the development of novel synthetic methods for azasugars and their applications to search for a new glycosidase inhibitor as well as to study the structural–activity relationship (SAR) between azasugars and glycosidases.[2] SAR studies of azasugars are important for designing novel glycosidase inhibitors based on an azasugar scaffold. For instance, we[2a] and others[2b] reported the syntheses of 1-deoxynojirimycin (DNJ), fagomine, isofagomine and their stereoisomers (Chart 1), and through the SAR studies of these analogues, we revealed that an L-isomer of DNJ was capable of acting as a non-competitive inhibitor for α-glucosidase.[2a]

As a part of our continuous synthetic study for azasugars, we became interested in hydoroxylated pipecolic acid derivatives. 3,4,5-Trihydroxypipecolic acids are considered as 5-aza congeners



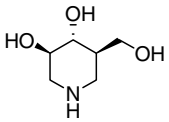


Chart 1.

of uronic acids (Chart 2). It is widely known that D-glucuronic acid, a typical uronic acid, plays a crucial role in drug metabolism known as 'glucuronic acid conjugation'. β-Glucuronidase is an enzyme that hydrolyzes a β-glycosidic bond of a terminal glucuronic acid residue in oligo- and polysaccharides. Inhibitors of β-glucuronidase are clinically important since this class of inhibitors has recently been shown to have a protective effect against antitumor camptotecin derivative CPT-11-induced mucosa damage and diarrhea.[3] The 5-aza analogue of D-glucuronic acid, one of the 3,4,5-tri-

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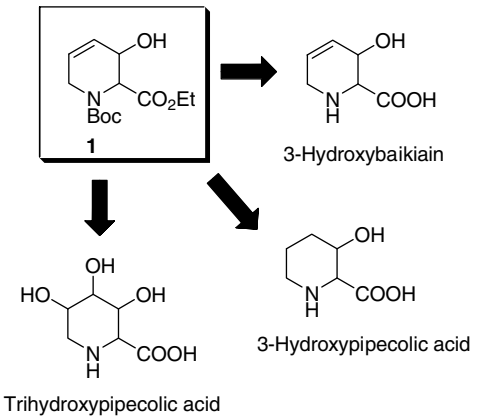


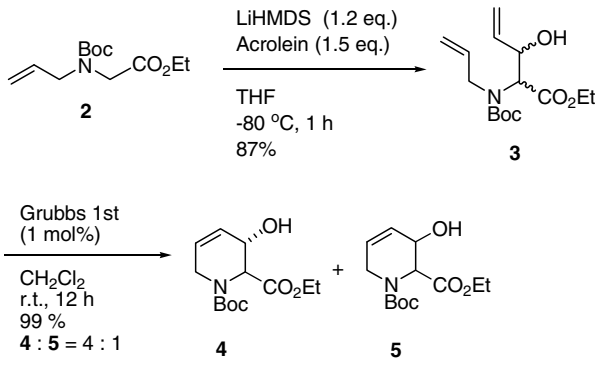
Chart 2.

hydroxypipecolic acid derivatives, is a potential β-glucuronidase inhibitor which may act as a transition-state analogue. However, there have been only a few reports on glycosidase inhibition of uronic acid-type azasugar derivatives.[4]

There is a strong demand for a practical route providing an easy access to 3,4,5-trihydroxypipecolic acid derivatives, and a chiral synthesis constitutes an area of considerable current interest. We envisioned synthesis of 3,4,5-trihydroxypipecolic acids from a precursor **1**, which would be prepared by an enzymatic reaction to obtain both of the enantiomers in one synthetic scheme. Combined with the enzymatic preparation, the conversion of a double bond of **1** to a diol moiety with control of their stereochemistries allows all stereoisomers of trihydroxypipecolic acids to be synthesized. Such a synthetic strategy will be of benefit to SAR study for these analogues. In addition, **1** is also a useful intermediate for synthesizing 3-hydroxypipecolic acids which constitute non-natural variants of a structural motif often encountered in a variety of functional molecules and may be regarded as expanded hydroxylated proline or a conformationally restricted serine derivative.[5] (−)-3-Hydroxybaikiain, the 4,5-dehydro derivative of 2,3-*cis*-3-hydroxy-L-pipecolic acid, has been isolated from a toxic mushroom, *Russula subnigricans* Hongo.[6] However, a simultaneous synthesis of all stereoisomers of 3-hydroxypipecolic acid and the chiral synthesis of their 4,5-dehydro compounds have not been achieved.[7] To the best of our knowledge, their inhibitory activities against glycosidases have never been investigated. Herein, we report a new chiral synthesis of stereoisomers of 3-hydroxy- and 3,4,5-trihydroxypipecolic acids as well as 3-hydoxybaikiain derivatives in conjunction with their glycosidases such as β-glucuronidase inhibitory activities.[8]

2. Chemistry

N-Boc-allylglycine ethyl ester **2**,[9] readily obtainable from ethyl bromoacetate and allylamine in two steps, was treated with lithium hexamethyldisilazide (LiHMDS) to generate a corresponding enolate, a reaction of which with acrolein gave an inseparable mixture of **3** in good yield. Because of the presence of rotarmors caused by the *N*-Boc protecting group, it was difficult to determine the ratio of *syn* and *anti* adducts from its NMR spectrum. A ring-closing metathesis (RCM) reaction of **3** afforded a mixture of **4** and **5**, key intermediates for hydroxypipecolic acids, which were separated by silica gel column chromatography in 80% and 19%, respectively (Scheme 1). Complicated NMR spectra of **4** and **5** caused by a non-bonded interaction of *N*-Boc protecting groups made it difficult to elucidate their relative stereochemistries. Thus, the major isomer **4** was converted to a known compound in three steps and it was determined as a *trans* adduct (data not shown).[10]



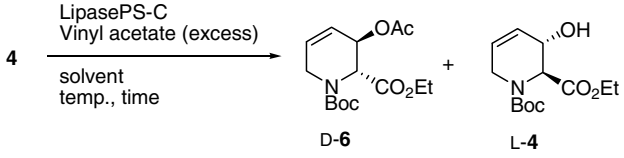
Scheme 1.

Along with our initial plan to obtain both enantiomers of the common intermediate **4**, a kinetic resolution by using a lipase-catalyzed transesterification was tried. Among the lipases tested, lipase PS-C (lipase isolated from *Pseudomonas cepacia* immobilized on ceramic particles, purchased from Amano) gave the best result (Scheme 2). The reaction conditions were further optimized and the results are summarized in Table 1.

When hexane and carbon tetrachloride were used as solvents, the reaction gave D-**6** and L-**4** in good yields, but neither of the ees of an acetate L-**4** was satisfactory (entries 1 and 2).[11] The reaction without a solvent gave a similar result (entry 3). The best result was obtained when diisopropyl ether was used, and the reaction gave 47% of D-**6** with 97%ee and 47% of L-**6** with 99%ee.

Compound L-**4**, a chiral precursor for L-pipecolic acid derivatives, in hand was converted to 2,3-*trans*-3-hydroxybaikiain L-**7** and 2,3-*trans*-3-hydroxypipecolic acid L-**9**. Treatment of L-**4** with 5 N HCl at 120 °C for 3 h gave 2,3-*trans*-3-hydroxybaikiain (3-epi-3-hydroxybaikiain) L-**7** in 99% yield after ion-exchange column chromatography. Similarly, compound L-**8** obtained from hydrogenation of L-**4** was treated with refluxing 5 N HCl to give 2,3-*trans*-3-hydroxypipecolic acid L-**9** in 84% yield (Scheme 3).

Next our target was shifted to 3-hydroxybaikiain, a natural product isolated from a toxic mushroom.[6] To achieve the synthesis of 3-hydroxybaikiain, the synthesis should be starting from the *cis*-isomer **5** by using the same schemes as those described above. Since **5** was the minor product of the sequential cross aldol and RCM reactions, this plan did not seem efficient. Thus, we intended

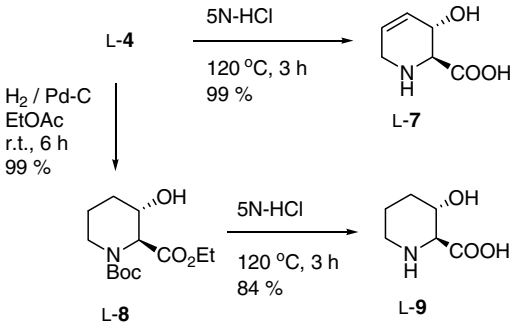


Scheme 2.

Table 1
Summary of lipase PS-C-catalyzed transesterification

Entry	Solvent	Temperature, time	D-**6**		L-**4**	
			Yield[a] (%)	%ee[b]	Yield[a] (%)	%ee[b]
1	Hexane	40 °C, 3 days	44	95	54	87
2	CCl_4	40 °C, 3 days	41	99	53	68
3	None	30 °C, 4 days	45	92	52	70
4	*i*-Pr_2O	40 °C, 3 days	47	97	47	99

[a] Isolated yield.
[b] The ees were determined by HPLC (see Section 5).



Scheme 3.

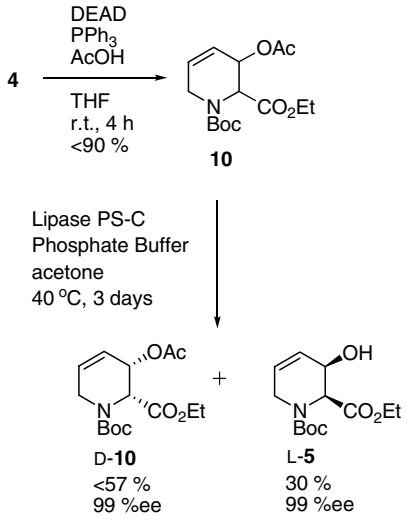
to synthesize a chiral intermediate L-**5**, started from the major isomer **4**, by employing the Mitsunobu reaction and enzymatic hydrolysis.

Compound **4** was treated with triphenylphsphine and acetic acid in the presence of diethyl azodicarboxylate (DEAD) to give inverted acetate **10** which contains a small amount of inseparable impurity. Lipase-mediated hydrolysis of acetate **10** gave a desired chiral synthon L-**5** in 30% yield along with D-**10**. Although D-**10** still contained impurity, both L-**5** and D-**10** showed high enatiomeric purities over 99%ee (Scheme 4).[12]

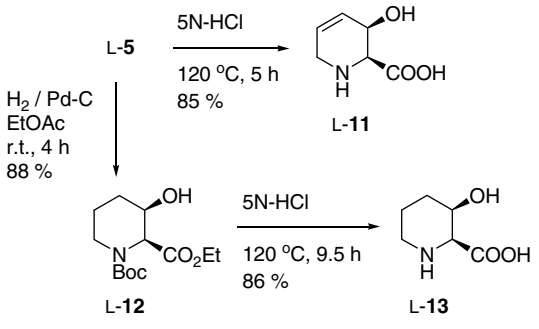
Deprotection of L-**5** by acid treatment gave 3-hydroxybaikiain L-**11** in 85% yield. All of the spectral data of L-**11** were identical to those reported,[6] and the structure of L-**11** was therefore unambiguously determined. Similar to the *trans* isomer, L-**13** was synthesized from L-**5** in two steps as shown in Scheme 5.

The D-isomers of 3-hydroxypipecolic acids were the final target of this series and were to be obtained from common intermediates D-**4** and D-**5**. Compound D-**4** was readily obtained from D-**6** by hydrolysis of an acetyl group treated with lithium hydroxide in 97% yield. Deacetylation of D-**10** with LiOH was rather complicated since a competitive β-elimination product **14** and a transesterification product **15** were accompanied. The reaction, however, gave pure D-**5** in 60% yield after purification by silica gel column chromatography. By using the same schemes as those mentioned above, the syntheses of D-enantiomers of 3-hydroxybaikiains (D-**7** and D-**11**) and 3-hydroxypipecolic acids (D-**9** and D-**13**) from D-**4** and D-**5** were achieved (see Section 5) (Schemes 6 and 7).

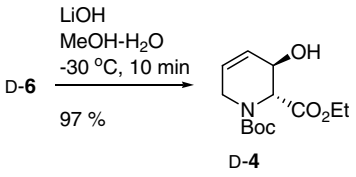
As a final series of hydroxypipecolic acids for SAR study, we aimed to synthesize all stereoisomers of 3,4,5-trihydroxypipecolic acids. First, *cis*-dihydroxylation of L-**4** by OsO_4 was tried. The reaction of L-**4** with catalytic potassium osmate in the presence of *N*-methylmorpholine *N*-oxide (NMO) gave a diastereomeric mixture of triol derivatives, which were separated by silica gel column chromatography to give L-**16** and L-**17** in 32% and 59% yields, respectively. The structures of L-**16** and L-**17** were firmly elucidated by converting to the corresponding nojirimycin derivatives.[13] As a result, the major L-**16** had relative stereochemistry of 3,4-*cis* and L-**17** was a 3,4-*trans* isomer. The resulting triols L-**16** and L-**17** were treated with refluxing 5 N HCl to give trihydroxypipecolic acids L-**18**[14] and L-**19**.



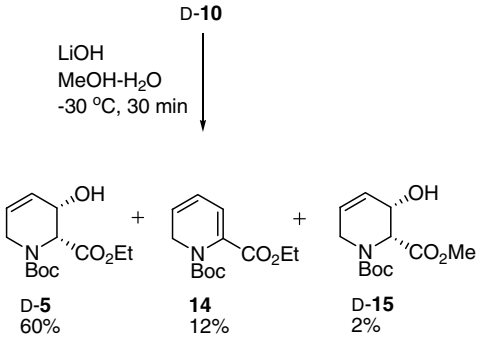
Scheme 4.



Scheme 5.



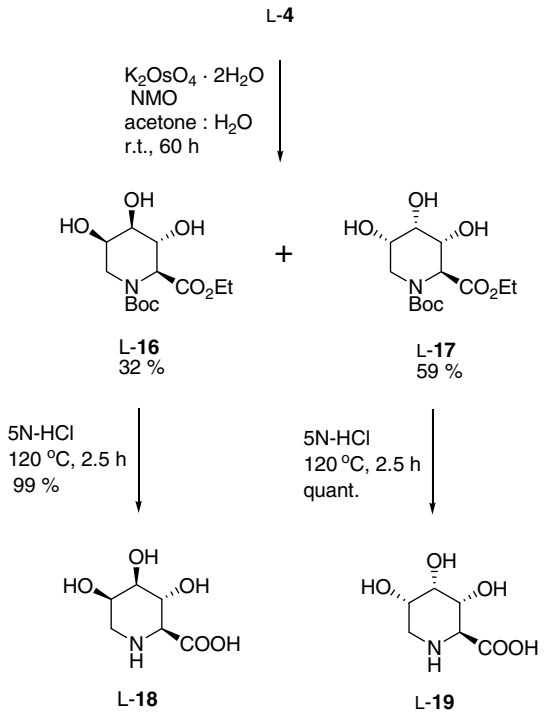
Scheme 6.



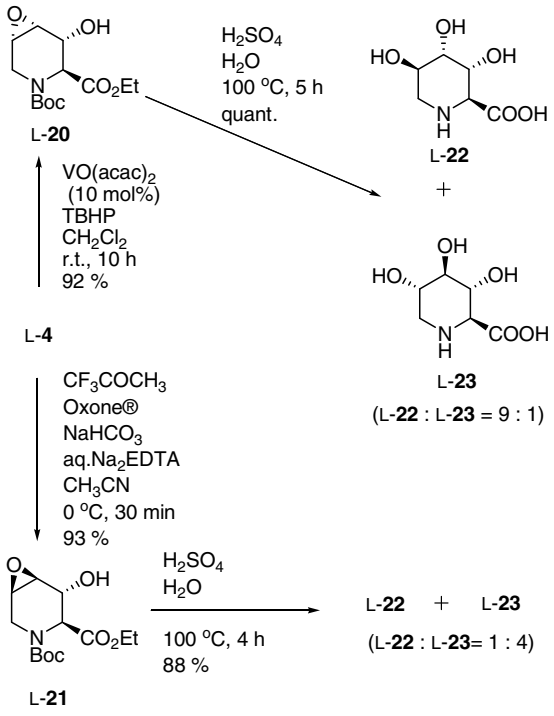
Scheme 7.

In comparison with the structure of azasugar, L-**18** and L-**19** corresponded to 5-azaderivatives of D-allo- and D-mannopyranuronic acids, respectively (Scheme 8).

3,4-*trans*-Diol derivatives were next synthesized via a 3,4-epoxy derivative. To our delight, the direction of epoxide formation could be controlled by choosing reaction conditions: the reaction of L-**4** with vanadyl acetylacetonate and *tert*-butyl hydroperoxide gave 3,4-*syn*-epoxide L-**20** exclusively, and treatment of L-**4** with 3-methyl-3-(trifluoromethyl)dioxirane, generated in situ from trifluoroacetone and Oxone®, gave 3,4-*trans*-epoxide L-**21** dominantly. It is likely that the former epoxidation proceeds through a chelation-controlled mechanism. Deprotection and hydrolytic opening of the oxirane ring could be done in one step by acid treatment. Thus, 3,4-*syn*-epoxide L-**20** was treated with refluxing 5 N H_2SO_4 to give a 9:1 mixture of L-**22** and L-**23**[15] in 92% yield. Fortunately, these products could be separated by repeated recrystallization. The dominant



Scheme 8.



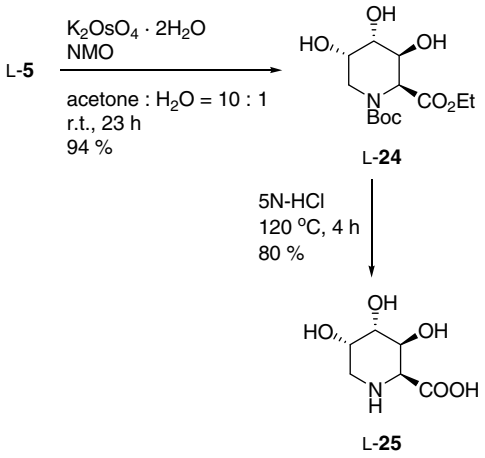
Scheme 9.

formation of L-**22** can simply be explained by the fact that nucleophilic attack of water is likely to occur at the less-hindered C-5 position via *trans*-diaxial-opening of the epoxide ring. A similar reaction of 3,4-*trans*-epoxide L-**21**, on the other hand, gave a 1:4 mixture of L-**22** and L-**23**, in 88% yield, which are equal to 5-aza-D-altro- and 5-aza-D-glucopyranuronic acids, respectively. The former L-**23** was a natural product isolated from the seeds of *Baphia racemosa*.[15] All of the instrumental data for L-**23** were identical to those reported. The result suggests that hydrolytic cleavage of the oxirane ring of L-**21** also proceeded by way of nucleophilic attack at the less-hindered C-5 position. It is notable that either of the 5-azasugar derivatives corresponding to D-altro- and D-glucopyranuronic acids could selectively be synthesized from the same intermediate (Scheme 9).

As described in the synthesis of 3-hydroxypipecolic acids, it was expected that the above-mentioned syntheses could be applied to the *syn*-intermediate L-**5** to prepare the other four stereoisomers of 5-azapyranuronic acids.

An attempt to synthesize 5-aza derivatives of D-gulo- and D-tallopyranuronic acids was made. The reaction of L-**5** with potassium osmate and NMO gave a sole product L-**24**, which was further subjected to acid hydrolysis. The resulting product was revealed by instrumental analysis data to be identical to those of the known D-isomer of trihydroxypipecolic acid[16] and was determined as L-**25**, corresponding to 5-aza-D-gulopyranuronic acid (Scheme 10). Many efforts were made to obtain 5-azapyranuronic acid having D-tallo configurations; however, the formation of any trace of the desired product was not observed. The exclusive formation of L-**24** was ascribed to an 1,3-allylic strain between ethoxycarbonyl and Boc groups which forces the ethoxycarbonyl group to occupy a pseudoaxial position. As a result, the approaching of the reagent from a β-face of L-**5** was impeded. Owing to the 1,3-allylic strain in L-**5** mentioned above, a hydroxyl group at C3 was also forced to occupy a pseudoequatorial position, suppressing the process of *cis*-dihydroxylation through a chelation-controlled mechanism.

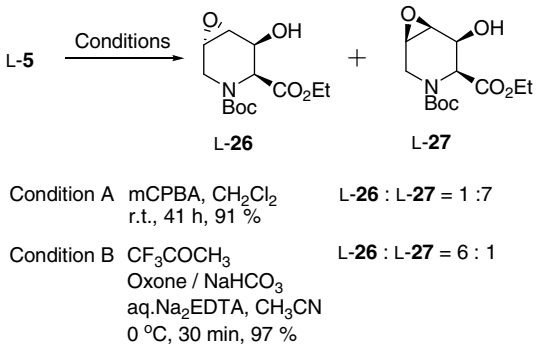
Finally, the synthesis of 5-aza-D-ido- and 5-aza-D-galactopyranuronic acids was tried via epoxy derivatives. First, we at-



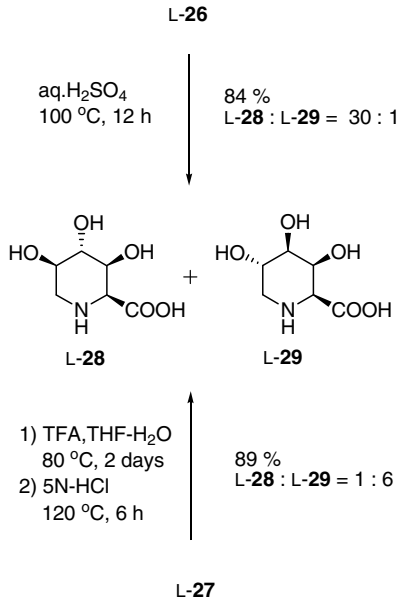
Scheme 10.

tempted to obtain a 3,4-*cis*-epoxide derivative by the reaction using vanadyl acetylacetonate. However, the reaction gave a troublesome complex mixture. For the same reason as that described above, it is thought that the pseudoequatorial hydroxyl group at the 3-position could no longer be an anchor for the formation of a vanadyl complex. In contrast, it has been reported that epoxidation using mCPBA more selectively gave a *cis*-epoxide in a cyclohex-2-enol system when an allylic hydroxyl group occupied a pseudoequatorial rather than a pseudoaxial position.[17] This encouraged us to try the reaction of L-**5** with mCPBA, which gave a 1:7 mixture of L-**26** and L-**27** in 91% yield (condition A, Scheme 11). The major product of L-**5** obtained by trifluoromethyldioxirane-mediated epoxidation was L-**26**, as expected, with a ratio of 6:1 to L-**26**/L-**27** (condition B, Scheme 11).

Deprotection and hydrolytic cleavage of epoxide of L-**26** by the treatment of refluxing 5 N HCl dominantly gave 5-aza-D-idopyranuronic acid L-**28** with a trace amount of L-**29** in 85% yield. The



Scheme 11.



Scheme 12.

result can be interpreted by the fact that the *trans*-diaxial-opening of epoxide favoring nucleophilic attack at the C5 position of L-**26** gave rise to L-**28**. For a similar reason, the acid treatment of L-**27** using the same condition gave a 2:1 mixture of L-**28**/L-**29** (data not shown). Interestingly, when the reaction was performed using trifluoroacetic acid instead of 5 N H_2SO_4, 5-aza-D-galactopyranuronic acid L-**29** was dominantly obtained from L-**5** in 89% yield (L-**28**/L-**29** = 1:6, Scheme 12). The reason for the change in selectivity of the products when a different acid was used is not clear.

We have also synthesized an L-series of 5-azapyranuronic acids D-**18**, D-**19**, D-**22**, D-**23**, D-**25**, D-**28**, and D-**29** from D-**4** and D-**5** (see Section 5).

3. Biological

Since 3-hydroxypipecolic acid derivatives are designed as aza-sugar analogues of uronic acid derivatives, they are expected to act as transition state analogues and to be inhibitors for β-glucuronidase as mentioned earlier. In addition, there are a few reports on the inhibitory effect of pipecolic acid derivatives on glycosidases. The effect arising by replacement of 5-hydroxymethyl to a carboxyl group on glycosidase inhibitory activities is also of interest to us. Thus, we examined inhibitory activities of the obtained 3-hydroxy- and 3,4,5-trihydroxypipecolic acid derivatives against various glycosidases, including β-glucuronidase. None of the compounds tested showed inhibitory activity against glucosidases, galactosidases, and mannosidases (data not shown). The results clearly showed that these enzymes strictly recognize the 5-hydroxymethyl group. Some of the compounds tested, on one hand, showed inhibitory activities against β-glucuronidase, β-*N*-acetylglucosaminidase, and α-*N*-acetylgalactosaminidase. The results are summarized in Tables 2 and 3. 3-Hydroxypipecolic acids L-**9** and L-**13** showed moderate inhibitory activity against *Escherichia coli* β-glucuronidase, and their enantiomers D-**9** and D-**13** also showed similar results. Only weak inhibition by D-**9** and D-**13** against the same enzyme isolated from bovine liver was observed. Unexpectedly, the 3-hydroxypipecolic acid derivatives showed weak to moderate inhibitory activity against β-*N*-acetylglucosaminidase. It is noteworthy that L-**7** showed the most potent inhibitory activity: its IC_{50} values were 720 and 750 μM against the enzyme isolated from bovine kidney and human placenta, respectively.

Recently, the dynamic modification of cytosolic and nuclear proteins by *O*-glycosylation with β-*N*-acetylglucosamine (*O*-GlcNAc) on serine and threonine residues has been found to be one of the post-translational modifications.[18] It has also been shown that *O*-GlcNAc is stimulated by high glucose flux and implicated to type II diabetes.[19] The cycle of addition/removal of β-*N*-acetylglucosamine is rapid and is catalyzed by different enzymes similar to protein phosphorylation/dephosphorylation catalyzed by kinases and phosphatases.[20] The enzyme for the addition process of *N*-acetylglucosamine is *O*-GlcNAc transferase, and the removal process is catalyzed by *O*-GlcNAcase. The *O*-GlcNAcase inhibitor PUGNAc has been used for investigating the biological function of *O*-GlcNAc.[21] β-*N*-Acetylglucosaminidase, which is also known as β-*N*-acetylhexosaminidase, is functionally different to *O*-GlcNAcase.[22] However, PUGNAc inhibits both *O*-GlcNAcase and β-*N*-acetylglucosaminidase.[23] Moreover, the study to find a selective inhibitor for *O*-GlcNAcase is proceeding.[24] These facts suggest that

Table 2
Inhibition rates of 3-hydroxypipecolic acid derivatives against β-glucuronidase, β-*N*-acetylglucosaminidase, and α-*N*-acetylgalactosaminidase at 1000 μM

Compound	Inhibition rate (IC_{50} value)				
	β-Glucuronidase		β-*N*-Acetylglucosaminidase		α-*N*-Acetylgalactosaminidase
	Bovine liver	*E. coli*	Bovine kidney	Human placenta	Chicken liver
L-**7**	19%	2%	**58**% (**720** μM)	**60**% (**750** μM)	25%
L-**9**	0.89%	31%	38%	36%	5.2%
L-**11**	8.6%	3.3%	7.3%	17%	0.2%
L-**13**	0.18%	24%	20%	30%	2.5%
D-**7**	0.2%	0.9%	15%	14%	3.2%
D-**9**	0.62%	20%	32%	25%	1.3%
D-**11**	10%	0.9%	0.8%	14%	1.3%
D-**13**	15%	23%	47%	46%	3.2%

Table 3
Inhibition rates of 3,4,5-trihydroxypipecolic acid derivatives against β-glucuronidase, β-*N*-acetylglucosaminidase, and α-*N*-acetylgalactosaminidase at 1000 μM

Compound	Configuration	Inhibition rate (IC_{50} value)				
		β-Glucuronidase		β-*N*-Acetylglucosaminidase		α-*N*-Acetylgalactosaminidase
		Bovine liver	*E. coli*	Bovine kidney	Human placenta	Chicken liver
L-**18**	D-Allo	14%	1%	0.5%	28%	3.8%
L-**19**	D-Manno	2.1%	0.6%	14%	26%	4.5%
L-**22**	D-Altro	20%	1.1%	17%	42%	11%
L-**23**	D-Gluco	**95% (70 μM)**	**72% (215 μM)**	13%	43%	0.2%
L-**25**	D-Gulo	8.8%	2.3%	5.5%	7.6%	21%
L-**28**	D-Ido	17.5%	2.6%	4.8%	6.3%	4.2%
L-**29**	D-Galacto	**87% (86 μM)**	21%	1.9%	2.6%	31%
D-**18**	L-Allo	12%	2.6%	18%	39%	4.4%
D-**19**	L-Manno	3.1%	1.3%	10%	26%	8.2%
D-**22**	L-Altro	6.8%	5.2%	27%	42%	4%
D-**23**	L-Gluco	2.7%	5.4%	11%	50%	1.3%
D-**25**	L-Gulo	9.4%	1.2%	1.7%	2.1%	7.2%
D-**28**	L-Ido	15.8%	4.2%	4.4%	5.4%	3.4%
D-**29**	L-Galacto	7.1%	6.3%	0.5%	0.8%	10%

a compound possessing inhibitory activity against β-*N*-acetylglucosaminidase may also have inhibitory activity against *O*-GlcNAcase. Therefore, the finding that L-**7** showed inhibitory activity against β-*N*-acetylglucosaminidase is interesting because L-**7** may be a new lead for designing novel *O*-GlcNAcase inhibitors.

Stereochemistries of L-**7** 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 L-**7** and L-**11**, all stereoisomers of 3-hydroxypipecolic acids L-**9**, L-**13**, D-**9**, and D-**13** showed inhibitory activity against β-*N*-acetylglucosaminidase as well as *E. coli* β-glucuronidase. Although most of the compounds tested showed negligible inhibitory activity against α-*N*-acetylgalactosaminidase obtained from chicken liver, only 4,5-dehydro-3-hydroxypipecolic acid L-**7** showed a weak inhibitory activity against the same enzyme. However, the reason for this is unclear (Table 2).

In the case of 3,4,5-trihydroxypipecolic acids, L-**23** was found to have the most potent inhibitory activity against β-glucuronidase among the compounds tested. As mentioned above, L-**23** is a natural product and was already reported to have an inhibitory activity against human liver β-glucuronidase.[4a] The IC_{50} value of L-**23** for *E. coli* β-glucuronidase is 215 μM, which is comparable to that previously reported (Table 3).[4a] For the same enzyme from bovine liver, L-**23** showed a more potent inhibitory activity (IC_{50} = 70 μM). The structure of L-**23** was designed as a 5-aza analogue of D-glucuronic acid, which should have the function of a transition-state inhibitor

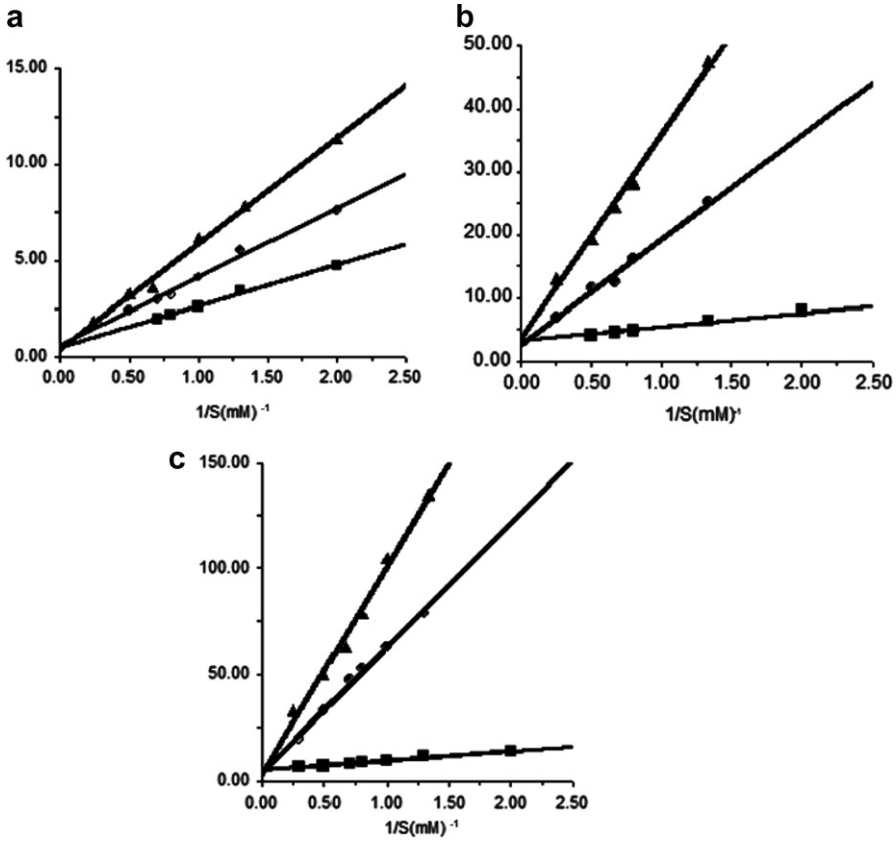


Figure 1. Lineweaver–Burk plots of L-**23** and L-**29** inhibition of bovine liver β-glucuronidase (a and b) and *E. coli* β-glucuronidase (c). Concentrations of L-**23** (a) and L-**29** (b) were 0 (■), 100 μM (•), and 250 μM (▲). The calculated K_i values were 6.5 μM and 15.4 μM, respectively. Concentrations of L-**23** were 0 (■), 250 μM (•), and 500 μM (▲). The calculated K_i values were 400 μM.

for enzymes recognizing D-glucuronic acid. Thus, the finding that L-**23** had inhibitory activity against β-glucuronidase meets our expectation. On the other hand, it is interesting that L-**29**, which has D-galacto configurations as the azasugar, showed an inhibitory activity against bovine liver β-glucuronidase (IC_{50} = 86 μM) comparable to that of L-**23**.

To investigate the mode of inhibition against β-glucuronidase by L-**23** and L-**29**, the reaction was analyzed by Lineweaver–Burk plots. From the results shown in Figure 1, it is clear that both L-**23** and L-**29** inhibit β-glucuronidase in a competitive manner. The K_i values of L-**23** and L-**29** to the enzyme from bovine liver were 6.5 and 15.4 μM, respectively. In addition, the K_i value of L-**23** to the same enzyme from *E. coli* was much weaker (400 μM). The fact that L-**23** competitively inhibits β-glucuronidases from bovine liver as well as from *E. coli* may also be evidence for its action as a transition-state analogue of D-glucuronic acid as mentioned above. Moreover, the result reveals that L-**29**, similar to L-**23**, can bind to an active site of β-glucuronidase and act as a transition-state inhibitor. The results also suggest that β-glucuronidase should not strictly recognize C-4 configurations of these inhibitors.

Inhibitors of β-glucuronidase are expected to become drugs that suppress side-effects of the camptotecin derivative CPT-11,[3] an antitumor drug used for the treatment of small cell lung carcinoma and other solid tumors. One of the serious side-effects of this drug is diarrhea. There are two types of diarrhea induced by CPT-11. Acute diarrhea occurring at the early stage of administration is caused by the anticholinesterase effect of CPT-11.[25] Delayed diarrhea occurring at a later stage is more serious. CPT-11 is changed to an active form SN-38 by the action of carboxyesterase in the liver. SN-38 is further changed to inactive SN-38 glucuronate conjugate, which is excreted into bile.[26] By the action of β-glucuronidase from intestinal bacteria, deconjugation of SN-38 glucuronide gives rise to SN-38, which causes the severe delayed diarrhea in patients treated with CPT-11.[26] In Japan, Kampo medicine containing baicalin, a β-glucuronidase inhibitor, is co-administrated with CPT-11 to suppress delayed diarrhea.[3] Therefore, compounds L-**23** and L-**29**, showing inhibitory activity against β-glucuronidase, are noteworthy. Although they showed slightly weaker inhibitory activity against the *E. coli* β-glucuronidase than the bovine liver one, they have a possibility of becoming lead compounds for designing novel therapeutics for CPT-11-induced diarrhea. From our previous results for azasugars (DNJ series), some of the L-enantiomers of azasugar could act as non-competitive inhibitors for glycosidases.[2] However, none of the L-enantiomers of 3,4,5-trihydroxypipecolic acid derivatives showed inhibitory activity against β-glucuronidase.

Against β-*N*-acetylglucosaminidase, both L- and D-enantiomers of **18**, **19**, **22**, and **23**, which have 2,3-*trans* stereochemistry, showed moderate inhibitory activity. These results are in contrast to those for 3-hydroxypipecolic acid derivatives: both 2,3-*cis* and 2,3-*trans* derivatives have moderate inhibitory activity. L-**29** was the only derivative that showed a moderate inhibitory activity against β-*N*-acetylgalactosaminidase. Indeed, L-**29** has D-galacto configuration, but lacks 2-acetamido and 5-hydroxymethyl groups compared with *N*-acetylgalactosamine. The inadequate structural similarity of L-**29** to parental *N*-acetylgalactosamine affects the inhibitory activity.

4. Conclusion

Our plan was initially made for the synthesis of all stereoisomers of 3-hydroxy- and 3,4,5-trihydroxypipecolic acid derivatives to supply samples for SAR study of glycosidase inhibitors. To achieve this, we have developed a simple, facile, and inexpensive method for accessing hydroxypipecolic acid derivatives. The method we developed consists of (1) cross aldol-type condensation between acrolein and allylglycine derivatives, (2) RCM reaction, (3) lipase-catalyzed kinetic resolution, and (4) additional *cis*- or *trans*-dihydroxylation of alkene. The use of lipase reaction makes it convenient to prepare both L- and D-enantiomers of pipecolic acid derivatives. As a result, we have synthesized all stereoisomers of 3-hydroxypipecolic acids, including 4,5-dehydro-3-hydroxypipecolic acids. Also, the synthesis of L- and D-enantiomers of 3,4,5-trihydroxypipecolic acids having allo, manno, altro, gluco, gulo, ido, and galacto configurations as azasugars. Among the stereoisomers of 3,4,5-trihydroxypipecolic acids, only the derivative possessing a tallo configuration could not be obtained. The compounds obtained were evaluated for their inhibitory activities against various glycosidases, including β-glucuronidase. None of the compounds tested showed inhibitory activity against α- and β-glucosidases. L-**23**, an azasugar analogue of D-glucuronic acid, was found to have potent inhibitory activity against β-glucuronidase as expected. L-**29**, 5-aza-galactopyranuronic acid, also showed inhibitory activity against β-glucuronidase, comparable to that of L-**23**. In addition, it is interesting that some uronic-type azasugar derivatives showed moderate inhibitory activities against β-*N*-acetylglucosaminidase. These analogues are expected to be new lead compounds for designing inhibitors for β-glucuronidase as well as β-*N*-acetylglucosaminidase.

5. Experimental

Melting points are uncorrected. NMR spectra were recorded at 400 MHz (^{1}H), 100 MHz (^{13}C) using $CDCl_3$, CD_3OD, and D_2O. As an internal standard, tetramethylsilane was used for $CDCl_3$ and CD_3OD and 1,4-dioxane was used for D_2O. Mass spectra were obtained by EI or FAB mode. Silica gel for chromatography was Fuji Silysia PSQ 100B. When the reagents sensitive to moisture were used, the reaction was performed under argon atmosphere.

5.1. *tert*-Butyl 1-(ethoxycarbonyl)-2-hydroxybut-3-enylallylcarbamate (3)

To a solution of 1 M LiHMDS (25 mL, 25 mmol) in THF (200 mL) was slowly added a solution of **2**[9] (5.0 g, 21 mmol) in THF (100 mL) at −80 °C. After being stirred for 30 min, acrolein (2.1 mL, 31 mmol) was added and the mixture was stirred at the same temperature for 1 h. The reaction mixture was quenched by the addition of satd NH_4Cl and partitioned between AcOEt and H_2O. The organic layer was washed with brine and dried over Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 9:1) to give **3** (6.4 g, 87%) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.33 (3H, m), 1.46 (9H, d, J = 10.7 Hz), 3.54–3.70 (1H, m), 3.76–4.03 (1H, m), 4.10–4.28 (3H, m), 4.76–4.82 (1H, m), 5.12–5.27 (3H, m), 5.33–5.42 (1H, m), 5.77–5.89 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 13.8, 27.9, 27.9, 51.7, 51.8, 60.9, 61.2, 63.2, 63.8, 71.4, 80.2, 80.6, 80.7, 116.6, 116.8, 117.2, 117.8, 133.6, 133.9, 135.7, 136.5, 137.2, 153.7, 154.8, 171.3, 171.9; IR (neat): 3474.1, 2980.5, 1739.9, 1699.0 cm^{-1}; EI-MS (m/z): 300 (M^++1); Anal. Calcd for $C_{15}H_{25}NO_5$: C, 60.18; H, 8.42; N, 4.68. Found: C, 59.94; H, 8.36; N, 4.71.

5.2. *tert*-Butyl 2-ethyl 2,3-*trans*-2,3-dihydro-3-hydroxypyridine-1,2(6*H*)-dicarboxylate (4) and *tert*-butyl 2-ethyl 2,3-*cis*-2,3-dihydro-3-hydroxypyridine-1,2(6*H*)-dicarboxylate (5)

To a solution of **3** (6.0 g, 20 mmol) in dry CH_2Cl_2 (500 mL) was added Grubbs' catalyst 1st (166 mg, 0.2 mmol). After the mixture was stirred at room temperature for 12 h, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 4:1) to give more polar **4** (4.4 g, 80%) and less polar **5** (1.0 g, 19%) as a syrup.

4: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (3H, t, J = 7.3 Hz), 1.49 (9H, d, J = 11.6 Hz), 1.87–1.96 (1H, m), 3.78–3.92 (1H, m), 4.09–4.26 (3H, m), 4.60 (0.5H, s), 4.67 (0.5H, s), 4.93 (0.5H, s), 5.06 (0.5H, s), 5.85–5.99 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 13.8, 27.9, 28.0, 41.5, 42.3, 58.9, 60.3, 60.9, 63.6, 63.8, 80.3, 80.4, 123.8, 123.9, 128.0, 128.2, 155.7, 156.0, 169.1; IR (neat): 3444.6, 2979.2, 1738.9, 1732.4, 1694.6, 1682.7 cm^{-1}; EI-MS (m/z): 271 (M^+); HRMS Calcd for $C_{13}H_{21}NO_5$: 271.1420. Found: 271.1419.

5: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (3H, t, J = 7.3 Hz), 1.49 (9H, d, J = 1.9 Hz), 3.54–3.66 (1H, m), 3.97–4.27 (4H, m), 4.44–4.46 (1H, m), 5.04 (0.5H, d, J = 5.3 Hz), 5.21 (0.5H, d, J = 5.3 Hz), 5.61–5.69 (1H, m), 5.88 (1H, d, J = 10.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.6, 13.7, 27.8, 41.4, 42.1, 54.1, 55.6, 61.0, 65.6, 80.2, 80.3, 123.2, 123.4, 129.3, 129.7, 154.1, 154.6, 171.2, 171.3; IR (neat): 3475.0, 2979.2, 1739.7, 1704.7 cm^{-1}; EI-MS (m/z): 271 (M^+); HRMS Calcd for $C_{13}H_{21}NO_5$: 271.1420. Found: 271.1432.

5.3. (2*R*,3*R*)-1-*tert*-Butyl 2-ethyl 3-acetoxy-2,3-dihydropyridine-1,2(6*H*)-dicarboxylate (D-6) and (2*S*,3*S*)-1-*tert*-butyl 2-ethyl 3-hydroxy-2,3-dihydropyridine-1,2(6*H*)-dicarboxylate (L-4)

To a solution of **4** (2.1 g, 7.6 mmol) in diisopropyl ether (33 mL) were added lipase PS-C Amano II (3.8 g, purchased from Wako Co. Ltd) and vinyl acetate (65 mL, 708 mmol). The mixture was kept at 40 °C for 3 days. Insoluble materials were removed by suction and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 4:1) to give less polar D-**6** (1.1g, 47%, and 97%ee) and more polar L-**4** (978 mg, 47%, 99%ee).

D-**6**: syrup; $[\alpha]_D^{21}$ −163.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.24–1.28* (3H, m), 1.49 (9H, d, J = 16.9 Hz), 2.07 (3H, s), 3.80 (0.5H, d, J = 17.9, 1.9 Hz), 3.90 (0.5H, d, J = 19.3, 1.9 Hz), 4.15–4.26 (3H, m), 5.04 (0.5H, d, J = 1.9 Hz), 5.15 (0.5H, d, J = 1.9H), 5.61 (0.5H, d, J = 5.8 Hz), 5.68 (0.5H, d, J = 5.3 Hz), 5.93–6.07 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 14.0, 20.9, 20.9, 28.2, 28.2, 41.3, 42.2, 55.9, 57.0, 61.5, 66.0, 66.2, 80.5, 80.6, 120.2, 120.5, 130.1, 130.9, 131.4, 154.8, 155.7, 168.3, 168.4, 170.2; IR (neat): 2979.5, 1732.1, 1694.9, 1668.0 cm^{-1}; EI-MS (m/z): 313 (M^+); HRMS Calcd for $C_{15}H_{23}NO_6$: 313.1525. Found: 313.1523.

L-**4**: white crystal; mp 61–63 °C; $[\alpha]_D^{21}$ +52.5° (*c* 0.99, $CHCl_3$).

5.4. Determination of optical purity of L-4 and D-6

To a solution of L-**4** (60 mg, 0.22 mmol) in CH_2Cl_2 (5 mL) was added TFA (0.8 mL, 10.5 mmol). After the mixture was stirred at room temperature for 1 h, the solvents were removed under reduced pressure. The residue was dissolved in 1,4-dioxane/H_2O = 1:1 (5 mL). To this solution were added *p*-toluenesulfonyl chloride (70 mg, 0.22 mmol) and $NaHCO_3$ (278 mg, 3.3 mmol). The mixture was stirred at room temperature for 3 h diluted with AcOEt, and washed with H_2O. The water layer was extracted by AcOEt three times and the combined organic layer was washed with brine and then dried (Na_2SO_4). After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 4:1) to give (2*S*,3*S*)-ethyl 3-hydroxy-1-tosyl-1,2,3,6-tetrahydropyridine-2-carboxylate (59 mg, 82%). $[\alpha]_D^{25}$ +64.8° (*c* 1.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (3H, t, J = 7.2 Hz), 2.26 (1H, br s), 2.42 (3H, s), 3.86–3.92 (2H, m), 3.97–4.05 (2H, m), 4.09–4.15 (1H, m), 4.52 (1H, br s), 4.89 (1H, d, J = 1.9 Hz), 5.87–5.91 (1H, m), 5.94–5.98 (1H, m), 7.30 (2H, d, J = 8.2 Hz), 7.73 (2H, d, J = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 21.5, 42.5, 60.8, 61.4, 64.7, 124.8, 127.4, 128.0, 129.5, 135.8, 143.7, 168.0; IR (neat): 3501.0, 2982.9, 1746.8, 1732.2, 1335.4, 1162.5 cm^{-1}; EI-MS (m/z): 325 (M^+); HRMS Calcd for $C_{15}H_{19}NO_5S$: 325.098. Found: 325.0989. By HPLC analysis of the compound thus obtained using chiral column, enatiomeric excess of L-**4** was determined as 99%ee: column: CHIRALPAK IA, hexane/THF = 85:15, flow rate 1.0 mL/min, retention time 26.2 (major) and 31.8 (minor) min at 33 °C, detection: 254 nm.

By the same method, enatiomeric excess of D-**6**, after deacetylation (vide infra), was determined as 97%ee.

(2*R*,3*R*)-Ethyl 3-hydroxy-1-tosyl-1,2,3,6-tetrahydropyridine-2-carboxylate: $[\alpha]_D^{28}$ −62.7° (*c* 1.19, $CHCl_3$).

5.5. (2*S*,3*S*)-3-Hydroxybaikiain (L-7)

A mixture of L-**4** (300 mg, 1.11 mmol) and 5 N HCl (32 mL) was kept at 120 °C for 3 h. The solvent was removed under reduced pressure and the residue was dissolved in H_2O (50 mL). The mixture was applied to a top of ion-exchange column (DOWEX 50W X8) and eluted with H_2O and then with 0.5 N NH_3. The eluate of 0.5 N NH_3 was concentrated to dryness under reduced pressure to give L-**7** (157 mg, 99%) as a crystal: mp 232–237 °C (decomp.); $[\alpha]_D^{26}$ +60.6° (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ *3.60 (1H,* dd, J = 17.4, 2.4 Hz), 3.68 (1H, d, J = 5.8 Hz), 3.74 (1H, dd, J = 17.4, 2.4 Hz), 4.48–4.51 (1H, m), 5.77–5.81 (1H, m), 5.85–5.89 (1H, m); ^{13}C NMR (100 MHz, D_2O) δ 40.7, 60.3, 63.1, 123.2, 127.1, 171.8; IR (KBr): 3403, 1647, 1409, 1091, 1059 cm^{-1}; EI-MS (m/z): 143 (M^+); Anal. Calcd for $C_6H_9NO_3{\cdot}0.15H_2O$: C, 49.41; H, 6.43; N, 9.60. Found: C, 49.44; H, 6.36; N, 9.64.

5.6. (2*S*,3*S*)-1-*tert*-Butyl 2-ethyl 3-hydroxypiperidine-1,2-dicarboxylate (L-8)

A mixture of L-**4** (518 mg, 1.9 mmol) and Pd–C (5%) (129 mg) in AcOEt (52 mL) was stirred at room temperature for 15 h under H_2 atmosphere. Insoluble materials were removed by Celite filtration and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 4:1) to give L-**8** (514 mg, 99%) as a crystal: mp 86–88 °C; $[\alpha]_D^{21}$ −31.5° (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (3H, t, J = 7.0 Hz), 1.46–1.53 (11H, m), 1.79–1.82 (2H, m), 2.26 (0.5H, br s), 2.38 (0.5H, br s), 2.91 (0.5H, br s), 3.05 (0.5H, br s), 3.91 (0.5H, br s), 4.01 (0.5H, br s), 4.21 (2H, q, J = 7.0 Hz), 4.42 (1H, s), 4.73 (0.5H, br s), 4.79 (0.5H, br s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 18.0, 27.3, 28.1, 40.6, 41.7, 60.2, 60.9, 65.0, 80.0, 156.0, 156.4, 169.7; IR (neat): 3445.1, 2978.5, 1739.2, 1682.9 cm^{-1}; EI-MS (m/z): 273 (M^+); Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.14; H, 8.57; N, 5.08.

5.7. (2*S*,3*S*)-3-Hydroxypipecolic acid (L-9)

By the same procedure for the synthesis of L-**7**, L-**9** (111 mg, 84%) was obtained from L-**8** (249 mg, 0.91 mmol). Mp 244–251 °C (decomp.); $[\alpha]_D^{21}$ +12.8° (*c* 0.85, H_2O) [lit.[6]86%ee: $[\alpha]_D$ +11.6° (*c* 0.8, H_2O)]; ^{1}H NMR (400 MHz, D_2O) δ *1.47–1.60* (2H, m), 1.74–1.89 (2H, m), 2.90–2.97 (1H, m), 3.16–3.22 (1H, m), 3.45 (1H, d, J = 6.8 Hz), 3.97–4.01 (1H, m); ^{13}C NMR (100 MHz, D_2O) δ 18.3, 28.1, 42.5, 61.7, 65.8, 171.6; IR (KBr): 3282.8, 2983.9, 2959.4, 1624.1, 1599.1, 1401.3 cm^{-1}; FAB-MS (m/z): 146 (M^++1); Anal. Calcd for $C_8H_{11}NO_3{\cdot}1.1H_2O$: C, 43.68; H, 8.06; N, 8.49. Found: C, 43.66; H, 7.88; N, 8.47.

5.8. *tert*-Butyl 2-ethyl 3-acetoxy-2,3-dihydropyridine-1,2(6*H*)-dicarboxylate (10)

To a solution of **4** (4.5 g, 17 mmol) in THF (88 mL) were added PPh_3 (8.7 g, 33 mmol), AcOH (2.4 mL, 41 mmol), and DEAD (15 mL, 33 mmol) at 0 °C. After the mixture was stirred at room temperature for 4 h, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 9:1) to give **10** (<4.6 g, <90%) containing small

amounts of inseparable impurity: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (3H, t, J = 7,3 Hz), 1.47 (9H, s), 2.10 (3H, s), 3.91–4.18 (4H, m), 5.22 (0.5H, d, J = 5.3 Hz), 5.38 (0.5H, d, J = 5.3 Hz), 5.49 (1H, s), 5.64 (1H, d, J = 10.6 Hz), 5.86–5.93 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 20.7, 28.0, 41.7, 42.3, 51.5, 53.0, 60.7, 66.0, 80.6, 80.8, 121.9, 122.2, 122.3, 126.3, 126.5, 154.2, 154.7, 169.0, 169.5, 169.6, 169.9; IR (neat): 3452.2, 2981.8, 1747.5, 1713.5, 1370.8, 1232.1, 1163.4, 1030.4, 755.9 cm^{-1}; EI-MS (m/z): 313 (M^+); HRMS Calcd for $C_{15}H_{23}NO_6$: 313.1525. Found: 313.1529.

5.9. (2*R*,3*S*)-1-*tert*-Butyl 2-ethylcetoxy-2,3-dihydropyridine-1,2(6*H*)-dicarboxylate (D-10) and (2*S*,3*R*)-1-*tert*-butyl 2-ethyl 2,3-dihydro-3-hydroxypyridine-1,2(6*H*)-dicarboxylate (L-5)

To a solution of **10** (206 mg, 0.66 mmol) were added Lipase PS-C (340 g) and 0.1 mol/L phosphate buffer solution (pH 7) (7.0 mL). The mixture was kept at 40 °C for 3 days. Insoluble materials were removed by Celite filtration and the filtrate was extracted with AcOEt three times. The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (n-hexane/ethyl acetate = 4:1) to give less polar D-**10** (<118 mg, <57%, and 99%ee, containing small amounts of inpurity) and L-**5** (55 mg, 30%, and 99%ee). The optical purities of the products were determined after converting an *N*-tosyl derivative as described above.

L-**5**: $[\alpha]_D^{21}$ −30.9° (c 1.20, $CHCl_3$).

N-Tosyl derivative [(2*S*,3*R*)-Ethyl 3-hydroxy-1-tosyl-1,2,3,6-tetrahydropyridine-2-carboxylate]: $[\alpha]_D^{28}$ −3.4° (c 1.53, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (3H, t, J = 7.3 Hz), 2.43 (3H, s), 3.96 (1H, dd, J = 17.4, 2.4 Hz), 3.85–3.93 (1H, m), 3.97–4.08 (2H, m), 4.48 (1H, s), 4.96 (1H, d, J = 5.8 Hz), 5.67 (1H, dd, J = 10.1, 2.4 Hz), 5.80 (1H, d, J = 10.1 Hz), 7.30 (2H, d, J = 7.7 Hz), 7.69 (2H, d, J = 7.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 1.0, 13.7, 21.5, 29.7, 42.4, 56.0, 61.6, 66.4, 123.3, 127.3, 129.6, 129.8, 136.1, 143.7, 170.2; EI-MS (m/z): 326 (M^++1); HRMS Calcd for $C_{15}H_{19}NO_5S$: 325.0984. Found: 325.0981; HPLC: CHIRALPAK IA, hexane/THF = 85:15, flow rate 1.0 mL/min, retention time 20.5 (minor), 32.5 (major) min, 34 °C, detection: 254 nm.

5.10. (2*S*,3*R*)-3-Hydroxybaikiain (L-11)

By the same procedure as for the synthesis of L-**7**, L-**11** (61 mg, 85%) was obtained from L-**5** (136 mg, 0.50 mmol) as a crystal. mp 285–287 °C; $[\alpha]_D^{26}$ −335.3° (c 1.0, H_2O) [lit.[6] $[\alpha]_D^{20}$ −332.7° (c 0.3, H_2O)]; ^{1}H NMR (400 MHz, D_2O) δ *3.54–3.60* (1H, m), 3.64 (1H, dd, J = 4.3, 1.4 Hz), 3.70 (1H, d, J = 2.9 Hz), 4.48 (1H, t, J = 3.9 Hz), 5.82–5.86 (1H, m), 5.99–6.04 (1H, m); ^{13}C NMR (100 MHz, D_2O) δ 43.1, 61.5, 61.6, 124.4, 127.6, 172.3; IR (KBr): 3325.1, 3003.9, 2391.4, 1647.0, 1397.3, 1091.8 cm^{-1}; EI-MS (m/z): 143 (M^+); Anal. Calcd for $C_6H_9NO_3{\cdot}0.1H_2O$: C, 49.72; H, 6.40; N, 9.66. Found: C, 49.46; H, 6.40; N, 9.48.

5.11. (2*S*,3*R*)-1-*tert*-Butyl 2-ethyl3-hydroxypiperidine-1,2-dicarboxylate (L-12)

By the same procedure as for the synthesis of L-**8**, L-**12** (191 mg, 88%) was obtained from L-**5** (216 mg, 0.80 mmol). $[\alpha]_D^{21}$ −60.2° (c 1.22, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, t, J = 7.2 Hz), 1.47 (11H, s), 1.71 (1H, br s), 2.01 (1H, d, J = 11.6 Hz), 2.58–2.74 (1H, m), 3.72 (2H, s), 3.88–4.02 (1H, m), 4.26 (2H, d, J = 7.2 Hz), 4.92–5.06 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 23.5, 23.7, 28.3, 30.3, 40.2, 41.4, 57.2, 58.3, 61.5, 68.9, 69.1, 80.5, 154.8, 154.9, 172.1; IR (neat): 3454.2, 2979.4, 1732.8, 1694.9, 1393.4, 1368.0, 1152.8 cm^{-1}; EI-MS (m/z): 273 (M^+); HRMS Calcd for $C_{13}H_{23}NO_5$: 273.1576. Found: 273.1562.

5.12. (2*S*,3*R*)-3-Hydroxypipecolic acid (L-13)

By the same procedure for the synthesis of L-**7**, L-**13** (82 mg, 86%) was obtained from L-**12** (168 mg, 0.66 mmol) as a crystal. mp 270–274 °C (decomp.); $[\alpha]_D^{27}$ −60.0° (c 0.57, H_2O) [lit.[27] 82%ee: $[\alpha]_D$ −52.8° (c 0.6, H_2O)]; ^{1}H NMR (400 MHz, D_2O) δ *1.55–1.66* (2H, m), 1.77–1.89 (2H, m), 2.81–2.89 (1H, m), 3.28 (1H, dd, J = 12.7, 2.4 Hz), 3.52 (1H, d, J = 1.4 Hz), 4.35 (1H, s); ^{13}C NMR (100 MHz, D_2O) δ 16.7, 29.5, 44.4, 63.1, 64.9, 173.1; IR (KBr): 3400.8, 3168.6, 3053.8, 1623.0, 1408.2, 1393.2 cm^{-1}; FAB-MS (m/z): 146 (M^++1); HRMS Calcd for $C_6H_{12}NO_3$: 146.0817. Found: 146.0820; Anal. Calcd for $C_6H_{11}NO_3$: C, 49.65; H, 7.64; N, 9.65. Found: C, 49.30; H, 7.80; N, 9.59.

5.13. (2*R*,3*R*)-1-*tert*-Butyl 2-ethyl 3-hydroxy-2,3-dihydropyridine-1,2(6*H*)-dicarboxylate (D-4)

To a solution of D-**6** (50 mg, 0.16 mmol) in MeOH (1.2 mL) was added a solution of LiOH (19 mg, 0.80 mmol) in H_2O (0.4 mL) at −30 °C. After being stirred at the same temperature for 10 min, the mixture was neutralized by AcOH and then allowed to warm to room temperature. The whole was extracted with $CHCl_3$ four times and the combined organic layer was washed with brine and then dried (Na_2SO_4). After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (n-hexane/ethyl acetate = 1:1) to give D-**4** (42 mg, 97%). $[\alpha]_D^{21}$ −54.1° (c 0.98, $CHCl_3$).

5.14. (2*R*,3*S*)-1-*tert*-Butyl 2-ethyl2,3-dihydro-3-hydroxypyridine-1,2(6*H*)-dicarboxylate (D-5), *tert*-butyl ethyl pyridine-1,2(6*H*)-dicarboxylate (14) and (2*R*,3*S*)-1-*tert*-butyl 2-methyl 2,3-dihydro-3-hydroxypyridine-1,2(6*H*)-dicarboxylate (D-15)

To a solution of D-**10** (500 mg, 1.6 mmol) in MeOH (12 mL) was added a solution of LiOH (196 mg, 8.0 mmol) in H_2O (4 mL) at −30 °C. After being stirred at the same temperature for 30 min, the mixture was neutralized by AcOH and then allowed to warm to room temperature. The whole mixture was extracted with $CHCl_3$ four times and the combined organic layer was washed with brine and then dried (Na_2SO_4). After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (n-hexane/ethyl acetate = 9:1) to give D-**4** (261 mg, 60%), **14** (48 mg, 12%), and D-**15** (8 mg, 2%), respectively.

D-**4**: $[\alpha]_D^{21}$ +29.6° (c 0.94, $CHCl_3$).

14: $[\alpha]_D^{27}$ +0.69° (c 0.95, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (3H, t, J = 7.3 Hz), 1.45 (9H, s), 4.25 (2H, q, J = 7.3 Hz), 4.29–4.30 (2H, m), 5.90–5.94 (1H, m), 6.05–6.10 (1H, m), 6.44 (1H, d, J = 4.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 27.9, 42.7, 60.9, 81.6, 120.0, 122.5, 127.2, 130.5, 152.6, 164.1; IR (neat): 2980.7, 1714.9, 1355.0, 1275.0, 1166.9, 1075.4, 764.3 cm^{-1}; EI-MS (m/z): 253 (M^+); HRMS Calcd for $C_{13}H_{19}NO_4$: 253.1314. Found: 253.1302.

D-**15**: $[\alpha]_D^{27}$ −37.6° (c 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (9H, s), 3.49–3.67 (1H, m), 3.75 (3H, s), 3.94–4.10 (2H, m), 4.45 (1H, br s), 5.06 (0.5H, d, J = 5.3 Hz), 5.25 (0.5H, d, J = 5.3 Hz), 5.61–5.69 (1H, m), 5.87 (1H, d, J = 10.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2, 41.6, 42.3, 52.4, 54.3, 55.9, 66.0, 66.1, 80.8, 123.6, 123.9, 129.8, 130.2, 154.3, 154.9, 172.3; IR (neat): 3480.5, 2977.3, 1743.9, 1695.5, 1404.1, 1164.1, 1005.9, 893.8 cm^{-1}; EI-MS (m/z): 257 (M^+); HRMS Calcd for $C_{12}H_{19}NO_5$: 257.1263. Found: 257.1254.

5.15. (2*S*,3*R*,4*R*,5*R*)-1-*tert*-Butyl 2-ethyl 3,4,5-trihydroxypiperidine-1,2-dicarboxylate (L-16) and (2*S*,3*R*,4*S*,5*S*)-1-*tert*-butyl 2-ethyl 3,4,5-trihydroxypiperidine-1,2-dicarboxylate (L-17)

To a solution of L-**4** (504 mg, 1.9 mmol) in acetone (14 mL) were added NMO (1.5 mL) and a solution of $K_2OsO_4{\cdot}2H_2O$ (6.9 mg,

0.019 mmol) in H_2O (1.4 mL) at 0 °C. The mixture was stirred at room temperature for 60 h. The reaction was quenched by the addition of solid $Na_2S_2O_3$ (871 mg). The mixture was stirred for 30 min and dried over Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 19:1) to give more polar L-**16** (179 mg, 32%) and less polar L-**17** (336 mg, 59%).

L-**16**: $[\alpha]_D^{27}$ +6.4° (*c* 0.93, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 1.22 (3H, t, *J* = 6.8 Hz), 1.41 (9H, d, *J* = 21.3 Hz), 3.06–3.21 (1H, m), 3.78–3.87 (3H, m), 4.11 (2H, q, *J* = 6.8 Hz), 4.38 (1H, d, *J* = 12.6 Hz), 4.55 (1H, d, *J* = 24.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4, 14.4, 28.5, 28.6, 41.9, 43.0, 58.4, 59.6, 62.1, 65.0, 65.0, 71.2, 71.6, 71.8, 79.4, 81.6, 157.7, 157.8, 170.8, 170.8; IR (neat): 3418.2, 2979.0, 2938.1, 2530.3, 1731.9, 1673.9, 1416.5, 1164.2, 1026.8 cm^{-1}; EI-MS (*m*/*z*): 305 (M^+); HRMS Calcd for $C_{13}H_{23}NO_7$: 305.1475. Found: 305.1480.

L-**17**: $[\alpha]_D^{27}$ −5.9° (*c* 3.22, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 1.22 (3H, t, *J* = 6.8 Hz), 1.42 (9H, s), 3.12 (1H, br s), 3.37 (1H, t, *J* = 2.9 Hz), 3.83 (1H, br s), 4.12 (1H, s), 4.17 (2H, q, *J* = 6.8 Hz), 4.27 (1H, br s), 4.94 (1H, br s); ^{13}C NMR (100 MHz, CD_3OD) δ 14.5, 28.5, 49.9, 55.9, 62.6, 68.1, 70.2, 71.7, 81.9, 158.1, 170.4; IR (neat): 3390.9, 2979.2, 2934.9, 1739.0, 1698.9, 1417.5, 1368.1, 1250.6, 1091.4 cm^{-1}; EI-MS (*m*/*z*): 305 (M^+); HRMS Calcd for $C_{13}H_{23}NO_7$: 305.1475. Found: 305.1480.

5.16. (2*S*,3*R*,4*R*,5*R*)-3,4,5-Trihydroxypipecolic acid (1-deoxy-5-aza-D-allopyran-uronic acid, L-18)

A mixture of L-**16** (90 mg, 0.29 mmol) and 5 N HCl (8 mL) was kept at 120 °C for 2.5 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (MeOH/10% NH_3 aq = 30:1) to give L-**18** (51 mg, 99%) as a crystal. Mp 231–234 (decomp.); $[\alpha]_D^{27}$ −29.7° (*c* 1.0, H_2O) [lit.[14] $[\alpha]_D^{20}$ −28.3° (*c* 0.64, H_2O)]; ^{1}H NMR (400 MHz, D_2O) δ 3.05 (1H, dd, *J* = 13.5, 2.4 Hz), 3.26 (1H, dd, *J* = 13.5, 4.8 Hz), 3.35 (1H, d, *J* = 9.2 Hz), 3.60 (1H, dd, *J* = 9.2, 2.9 Hz), 3.93 (1H, t, *J* = 9.2 Hz), 4.05–4.07 (1H, m); ^{13}C NMR (100 MHz, D_2O) δ 46.6, 61.7, 66.2, 68.7, 72.6, 172.7; IR (KBr): 3448.7, 3258.2, 1603.8, 1423.2, 1254.8, 1140.5, 1096.4 cm^{-1}; FAB-MS (*m*/*z*): 178 (M^++1); Anal. Calcd for $C_6H_{11}NO_5 \cdot 1.2H_2O$: C, 36.25; H, 6.79; N, 7.05. Found: C, 36.34; H, 6.88; N, 7.01.

5.17. (2*S*,3*R*,4*S*,5*S*)-3,4,5-Trihydroxypipecolic acid (1-deoxy-5-aza-D-mannopyran-uronic acid, L-19)

By the same procedure as for the synthesis of L-**18**, L-**19** (32 mg, quant.) was obtained from L-**17** (55 mg, 0.18 mmol) as a crystal. Mp 229–238 °C (decomp.); $[\alpha]_D^{26}$ +19.2° (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 3.01 (1H, dd, *J* = 12.6, 9.2 Hz), 3.17 (1H, dd, *J* = 12.6, 4.3 Hz), 3.60 (1H, d, *J* = 9.2 Hz), 3.86–3.92 (3H, m); ^{13}C NMR (100 MHz, D_2O) δ 43.1, 57.9, 65.9, 69.1, 69.3, 172.8; IR (KBr): 3402.2, 3222.4, 2473.2, 1612.5, 1400.3, 1372.5, 1154.1, 942.3 cm^{-1}; FAB-MS (*m*/*z*): 178 (M^++1); Anal. Calcd for $C_6H_{11}NO_5 \cdot 0.2H_2O$: C, 39.87; H, 6.36; N, 7.75. Found: C, 39.69; H, 6.46; N, 7.62.

5.18. (1*R*,2*R*,3*S*,6*S*)-4-*tert*-Butyl 3-ethyl 2-hydroxy-7-oxa-4-azabicyclo[4.1.0]heptane-3,4-dicarboxylate (L-20)

To a solution of L-**4** (136 mg, 0.50 mmol) in CH_2Cl_2 (15 mL) were added vanadyl acetylacetonate (14 mg, 0.05 mmol) and *t*-BuOOH (0.35 mL) at 0 °C. The mixture was stirred at room temperature for 10 h. To the mixture, satd $Na_2S_2O_3$ was added and the whole was extracted with CH_2Cl_2 three times. The combined organic layer was washed with satd $NaHCO_3$ and brine and then dried (Na_2SO_4). After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 4:1) to give L-**20** (133 mg, 92%) as a syrup: $[\alpha]_D^{26}$ −10.3° (*c* 1.17, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (3H, t, *J* = 6.8), 1.47 (9H, d, *J* = 8.2 Hz), 2.70 (1H, d, *J* = 10.6 Hz), 3.28–3.48 (2H, m), 3.52 (1H, t, *J* = 4.3 Hz), 4.17 (2H, q, *J* = 6.8 Hz), 4.34 (1H, d, *J* = 15.5 Hz), 4.52 (1H, br s), 4.71 (0.5H, s), 4.87 (0.5H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 27.8, 38.7, 39.7, 51.1, 51.7, 57.7, 59.0, 61.2, 62.1, 80.8, 155.2, 155.6, 168.8; IR (neat): 3472.6, 2979.5, 2934.4, 1738.7, 1699.1, 1367.0, 1250.5, 1058.3 cm^{-1}; EI-MS (*m*/*z*): 287 (M^+); HRMS Calcd for $C_{13}H_{21}NO_6$: 287.1369. Found: 287.1378.

5.19. (1*S*,2*R*,3*S*,6*R*)-4-*tert*-Butyl 3-ethyl 2-hydroxy-7-oxa-4-azabicyclo[4.1.0]heptane-3,4-dicarboxylate (L-21)

To a solution of L-**4** (500 mg, 1.8 mmol) in CH_3CN (14 mL) were added 4×10^{-4} M Na_2EDTA (9.2 mL) and CF_3COCH_3 (1.8 mL) at 0 °C. A mixture of $NaHCO_3$ (1.2 g) and Oxone® (5.8 g) was slowly added to the mixture of L-**4** at 0 °C over 1 h. The mixture was stirred at the same temperature for 30 min. H_2O was added to the mixture and the whole mixture was extracted with CH_2Cl_2 three times. The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 3:2) to give L-**21** (493 mg, 93%) as a syrup: $[\alpha]_D^{27}$ +19.7° (*c* 0.99, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.24–1.30* (3H, m), 1.46 (9H, d, *J* = 15.5 Hz), 3.03 (0.5H, d, *J* = 9.2 Hz), 3.24–3.31 (2.5H, m), 3.78 (1H, dd, *J* = 18.8, 15.5 Hz), 3.93 (1H, dt, *J* = 15.5, 2.9 Hz), 4.16–4.22 (2H, m), 4.58 (0.5H, d, *J* = 2.9 Hz), 4.68 (0.5H, d, *J* = 2.9 Hz), 4.81 (0.5H, d, *J* = 9.2 Hz), 4.89 (0.5H, t, *J* = 3.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 14.1, 28.2, 28.3, 39.5, 40.1, 49.9, 50.1, 51.4, 51.7, 56.8, 58.2, 61.2, 61.2, 66.1, 66.6, 81.1, 81.2, 155.9, 156.3, 168.4, 168.5; IR (neat): 3430.2, 2980.2, 2935.9, 1668.4, 1367.7, 1318.1, 1253.2, 1167.9, 1028.1 cm^{-1}; FAB-MS (*m*/*z*): 288 (M^++1); HRMS Calcd for $C_{13}H_{22}NO_6$: 288.1447. Found: 288.1455.

5.20. (2*S*,3*R*,4*S*,5*R*)-3,4,5-Trihydroxypipecolic acid (1-deoxy-5-aza-D-altropyran-uronic acid, L-22) and (2*S*,3*R*,4*R*,5*S*)-3,4,5-trihydroxypipecolic acid (1-deoxy-5-aza-D-glucopyranuronic acid, L-23)

From L-**20**: a mixture of L-**20** (340 mg, 1.2 mmol), H_2O (4.8 mL) and H_2SO_4 (0.52 mL) was kept at 120 °C for 5 h. The mixture was allowed to cool to room temperature and was applied to a top of ion-exchange column (DOWEX 50W X8). The eluate of 0.5 N NH_3 was concentrated to dryness under reduced pressure to give a 9:1 mixture of L-**22**/L-**23** (211 mg, quant.). Crystallization and recrystallization of the 9:1 mixture from MeOH/acetone/H_2O gave analytically pure L-**22** (77 mg).

From L-**21**: by the same procedure as described above, a 1:4 mixture of L-**22**/L-**23** (58 mg, 88%) was obtained from L-**21** (104 mg, 0.36 mmol). Repeated recrystallizations from MeOH/acetone/H_2O gave analytically pure L-**23** (17 mg).

L-**22**: mp 232–236 °C; $[\alpha]_D^{26}$ −10.3° (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 3.06 (1H, dd, *J* = 13.0, 7.7 Hz), 3.26 (1H, dd, *J* = 13.0, 3.9 Hz), 3.60 (1H, dd, *J* = 6.8, 2.9 Hz), 3.80 (1H, d, *J* = 6.8 Hz), 3.93–3.98 (1H, m),4.25 (1H, d, *J* = 6.8, 2.9 Hz); ^{13}C NMR (100 MHz, D_2O) δ 44.8, 60.3, 65.7, 68.2, 70.6, 171.6; IR (KBr): 3490.1, 3067.3, 1590.6, 1424.4, 1399.8, 1077.4, 710.8 cm^{-1}; FAB-MS (*m*/*z*): 178 (M^++1); Anal. Calcd for $C_6H_{11}NO_5 \cdot H_2O$: C, 36.92; H, 6.71; N, 7.18. Found: C, 36.88; H, 6.70; N, 7.20.

L-**23**: mp 266–272 °C; $[\alpha]_D^{26}$ +19.5° (*c* 0.79, H_2O) [lit.[15] $[\alpha]_D$ +18° (*c* 0.01, H_2O)]; ^{1}H NMR (400 MHz, D_2O) δ 2.81 (1H, dd, *J* = 12.6, 11.6 Hz), 3.34–3.42 (3H, m), 3.58 (1H, dd, *J* = 10.1, 8.7 Hz), 3.64–3.71 (1H, m); ^{13}C NMR (100 MHz, D_2O) δ 45.7, 61.6, 67.5, 70.7, 76.3, 172.3; IR (KBr): 3421.6, 1652.0, 1595.1, 1403.6, 1101.7,

1047.2, 542.5 cm^{-1}; FAB-MS (*m*/*z*): 178 (M^++1); Anal. Calcd for $C_6H_{11}NO_5 \cdot 0.3H_2O$: C, 39.47; H, 6.40; N, 7.67. Found: C, 39.25; H, 6.25; N, 7.73.

5.21. (2*S*,3*S*,4*S*,5*S*)-1-*tert*-Butyl 2-ethyl 3,4,5-trihydroxypiperidine-1,2-dicarboxylate (L-24)

By the same procedure as for the synthesis of L-**16** and L-**17**, L-**24** (214 mg, 0.70 mmol) was obtained as a sole product from L-**5** (202 mg, 0.75 mmol) after silica gel column chromatography (*n*-hexane/ethyl acetate = 1:4). Mp 58–60 °C; $[\alpha]_D^{26}$ −16.2° (*c* 0.90, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 1.26 (3H, t, *J* = 7.2 Hz), 1.45 (9H, s), 3.38–3.51 (1H, m), 3.82 (1H, d, *J* = 9.2 Hz), 3.91–4.04 (3H, m), 4.16 (2H, q, *J* = 7.2 Hz), 4.88 (1H, d, *J* = 17.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 14.6, 28.5, 47.0, 47.8, 59.3, 60.5, 61.9, 67.6, 68.9, 69.2, 71.6, 82.0, 156.9, 157.5, 172.2; IR (KBr): 3424.0, 1732.6, 1704.6, 1394.6, 1368.2, 1254.9, 1169.7, 1086.6 cm^{-1}; EI-MS (*m*/*z*): 305 (M^+); HRMS Calcd for $C_{13}H_{23}NO_7$: 305.1475. Found: 287.1461.

5.22. (2*S*,3*S*,4*S*,5*S*)-3,4,5-Trihydroxypipecolic acid (1-deoxy-5-aza-D-gulopyranuronic acid, L-25)

A mixture of L-**24** (147 mg, 0.48 mmol) and 5 N HCl (13 mL) was kept at 120 °C for 4 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (MeOH/10% NH_3 aq = 10:1) to give L-**25** (68 mg, 80%) as a crystal: mp 252–260 °C (decomp.); $[\alpha]_D^{19}$ −46.4° (*c* 0.83, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.92 (1H, t, *J* = 12.1 Hz), 3.09 (1H, dd, *J* = 12.1, 4.3 Hz), 3.75 (1H, d, *J* = 2.4 Hz), 3.91 (1H, t, *J* = 3.9 Hz), 4.07 (1H, ddd, *J* = 12.1, 4.3, 2.4 Hz), 4.22 (1H, dd, *J* = 4.3, 2.4 Hz); ^{13}C NMR (100 MHz, D_2O) δ 42.2, 58.0, 62.8, 69.4, 69.6, 172.6 [lit.[23] ^{13}C NMR (125 MHz, D_2O) δ 44.2, 59.9, 64.9, 71.4, 71.5, 174.8]; IR (KBr): 3258.0, 3097.3, 1644.8, 1401.2, 1096.7, 1006.2, 648.9 cm^{-1}; FAB-MS (*m*/*z*): 178 (M^++1); Anal. Calcd for $C_6H_{11}NO_5$: C, 40.68; H, 6.26; N, 7.91. Found: C, 40.79; H, 6.16; N, 7.88.

5.23. (1*S*,4*S*,5*S*,6*R*)-3-*tert*-Butyl 4-ethyl 5-hydroxy-7-oxa-3-azabicyclo[4.1.0]heptane-3,4-dicarboxylate (L-26) and (1*R*,4*S*,5*S*,6*S*)-3-*tert*-butyl 4-ethyl 5-hydroxy-7-oxa-3-azabicyclo[4.1.0]heptane-3,4-dicarboxylate (L-27)

Condition A. To a solution of L-**5** (272 mg, 1.0 mmol) in CH_2Cl_2 (3 mL) was added a solution of mCPBA (398 mg, 1.5 mmol) in CH_2Cl_2 (3 mL) at room temperature. After the mixture was stirred at room temperature for 41 h, the reaction was quenched the by addition of satd $NaHCO_3$. The separated organic layer was washed with satd $NaHCO_3$, 10% $Na_2S_2O_3$, and brine and then dried (Na_2SO_4). After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 7:3) to give more polar L-**26** (34 mg, 12%) and less polar L-**27** (227 mg, 79%).

Condition B. By the same procedure as for the synthesis of L-**21**, L-**26** (581 mg, 83%) and L-**27** (101 mg, 14%) were obtained from L-**5** (664 mg, 2.5 mmol) after silica gel column chromatography (*n*-hexane/ethyl acetate = 3:2).

L-**26**: mp 82–84 °C; $[\alpha]_D^{26}$ −74.2° (*c* 1.09, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (3H, t, *J* = 7.2 Hz), 1.46 (9H, s), 3.23–3.37 (3H, m), 3.56 (0.5H, d, *J* = 8.7 Hz), 3.72 (0.5H, d, *J* = 8.7 Hz), 4.05 (1H, br s), 4.17–4.32 (3H, m), 4.75 (0.5H, s), 4.93 (0.5H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 27.9, 39.1, 40.3, 50.0, 50.2, 53.4, 54.0, 54.4, 55.8, 61.3, 64.5, 64.8, 81.0, 154.5, 154.8, 170.3; IR (KBr): 3404.3, 2977.6, 1722.6, 1702.8, 1395.9, 1367.0, 1210.9, 1148.9 cm^{-1}; EI-MS (*m*/*z*): 287 (M^+); HRMS Calcd for $C_{13}H_{21}NO_6$: 287.1369. Found: 287.1378.

L-**27**: mp 83–84 °C; $[\alpha]_D^{26}$ −50.5° (*c* 0.80, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (3H, dt, *J* = 7.3, 2.4 Hz), 1.47 (9H, d, *J* = 15.0), 3.42 (1H, dt, *J* = 21.3, 3.9 Hz), 3.52 (1H, d, *J* = 3.9 Hz), 3.62 (1H, dd, *J* = 15.5, 10.1 Hz), 3.78 (1H, dd, *J* = 16.9, 15.0, 3.9 Hz), 4.18–4.32 (3H, m), 4.72 (0.5H, d, *J* = 5.8 Hz), 4.92 (0.5H, d, *J* = 5.8 Hz), 4.95 (1H, dd, *J* = 11.1, 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 13.9, 28.1, 28.2, 38.8, 39.4, 51.3, 52.1, 52.1, 52.7, 55.2, 55.4, 61.6, 61.6, 69.6, 69.7, 81.0, 81.1, 154.3, 155.0, 172.2, 172.4; IR (KBr): 3455.6, 2977.4, 1720.9, 1698.8, 1401.0, 1369.5, 1174.9, 1115.8 cm^{-1}; EI-MS (*m*/*z*): 287 (M^+); HRMS Calcd for $C_{13}H_{21}NO_6$: 287.1369. Found: 287.1357; Anal. Calcd for $C_{13}H_{21}NO_6$: C, 54.35; H, 7.37; N, 4.88. Found: C, 54.69; H, 7.49; N, 4.71.

5.24. (2*S*,3*S*,4*S*,5*R*)-3,4,5-Trihydroxypipecolic acid(1-deoxy-5-aza-D-idopyranuronic acid, L-28) and (2*S*,3*S*,4*R*,5*S*)-3,4,5-trihydroxypipecolic acid(1-deoxy-5-aza-D-galactopyranuronic acid, L-29)

From L-26: a mixture of L-**26** (144 mg, 0.50 mmol), H_2O (2.0 mL) and H_2SO_4 (0.20 mL) was kept at 100 °C for 12 h. The solvent was removed under reduced pressure and the residue was purified twice by silica gel column chromatography (MeOH/10% NH_3 aq = 50:1), then ion-exchange column (DOWEX 50W X8, eluate: aq 0.5 N NH_3) to give L-**28** (58 mg, 66%) and a 20:1 mixture ofL-**28** and L-**29** (19 mg, 21%).

From L-**27**: a mixture of L-**27** (144 mg, 0.50 mmol), THF (0.9 mL), H_2O (0.6 mL), and TFA (384 μL, 5.0 mmol) was kept at 80 °C for 2 days. The solvent was removed under reduced pressure and the residue was dissolved in 5 N-HCl (5 mL). The mixture was kept at 120 °C for 6 h. The solvents were removed under reduced pressure and the residue was purified by silica gel column chromatography (MeOH/10% NH_3 aq = 50:1) to give L-**29** (39 mg, 44%) and a 1:3 mixture of L-**28** and L-**29** (40 mg, 45%).

L-**28**: $[\alpha]_D^{18}$ −37.6° (*c* 1.16, H_2O); ^1H NMR (400 MHz, D_2O) δ 3.23 (2H, m), 3.80 (1H, d, *J* = 2.4 Hz), 3.86 (1H, d, *J* = 2.4 Hz), 3.90 (1H, t, *J* = 3.4 Hz), 4.14 (1H, s); ^{13}C NMR (100 MHz, D_2O) δ 45.6, 58.9, 66.6, 68.2, 69.3, 172.8; IR (KBr): 3387.7, 1630.0, 1400.7, 1119.3, 1060.3, 680.8, 619.7, 473.2 cm^{-1}; FAB-MS (*m*/*z*): 178 (M^++1); HRMS Calcd for $C_6H_{12}NO_5$: 178.0715. Found: 178.0717; Anal. Calcd for $C_6H_{11}NO_5 \cdot H_2O$: C, 36.92; H, 6.71; N, 7.18. Found: C, 36.95; H, 6.62; N, 7.31.

L-**29**: mp 238–247 °C (decomp.); $[\alpha]_D^{18}$ +19.5° (*c* 0.75, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.67 (1H, t, *J* = 12.1 Hz), 3.31 (1H, dd, *J* = 12.1, 5.3 Hz), 3.52 (1H, dd, *J* = 9.7, 2.9 Hz), 3.66 (1H, d, *J* = 1.4 Hz), 3.89 (1H, ddd, *J* = 13.0, 9.6, 5.3 Hz), 4.29 (1H, dd, *J* = 2.9, 1.4 Hz); ^{13}C NMR (100 MHz, D_2O) δ 46.1, 62.8, 65.3, 69.4, 74.0, 171.9; IR (KBr): 3375.1, 3209.3, 1623.0, 1423.8, 1088.6, 688.9 cm^{-1}; FAB-MS (*m*/*z*): 178 (M^++1); Anal. Calcd for $C_6H_{11}NO_5 \cdot H_2O$: C, 36.92; H, 6.71; N, 7.18. Found: C, 36.80; H, 6.67; N, 7.08.

The D-enantiomers of 3-hydroxy- and 3,4,5-trihydroxypipecolic acids were synthesized from D-**4** and D-**5** by the same methods as for the L-enatiomers (Schemes 13 and 14).

5.25. (2*R*,3*R*)-3-Hydroxybaikiain (D-7)

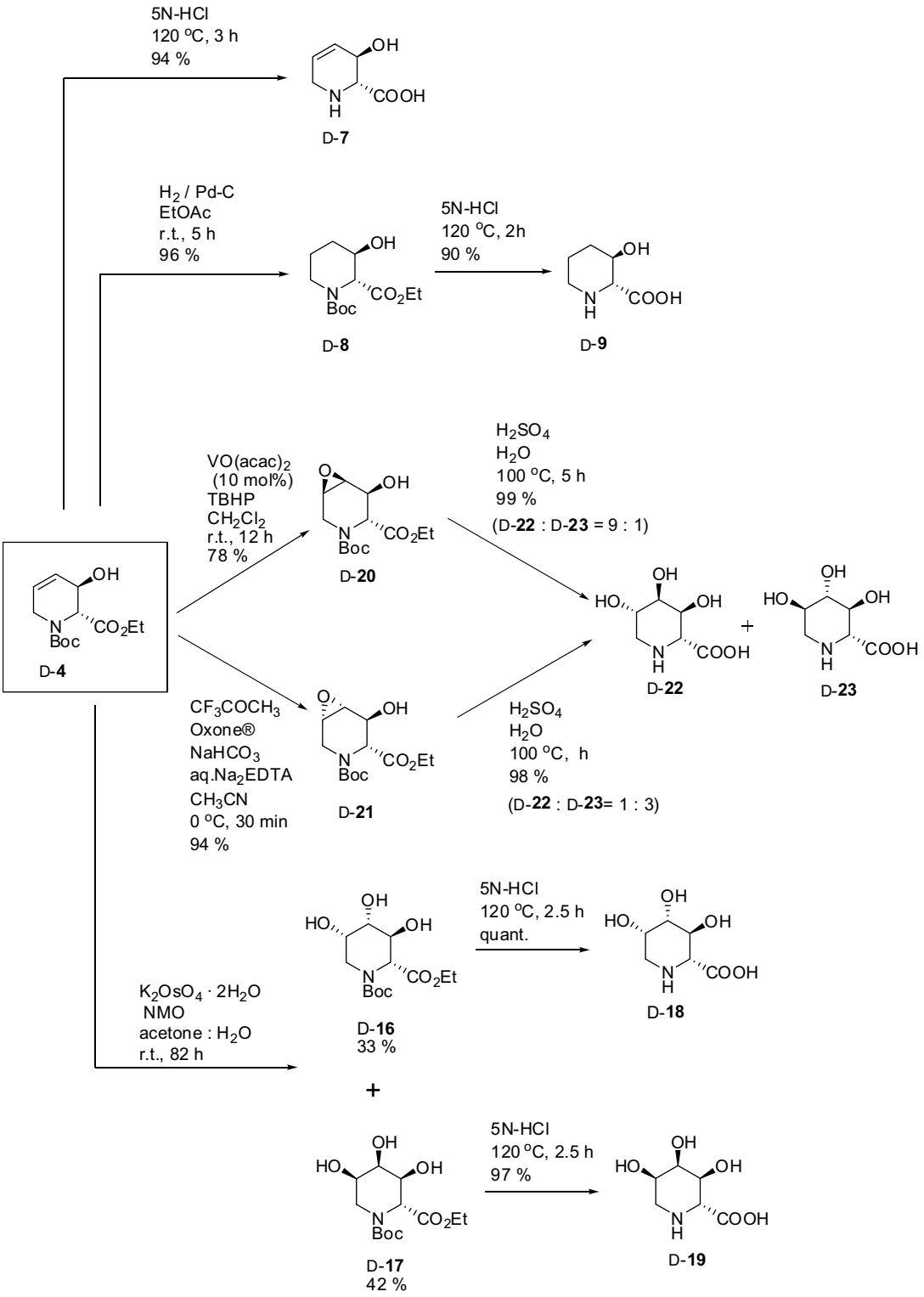
$[\alpha]_D^{25}$ −58.7° (*c* 1.0, H_2O); Anal. Calcd for $C_6H_9NO_3 \cdot H_2O$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.64; H, 7.06; N, 8.67.

5.26. (2*R*,3*R*)-1-*tert*-Butyl 2-ethyl 3-hydroxypiperidine-1,2-dicarboxylate (D-8)

$[\alpha]_D^{21}$ +33.0° (*c* 1.15, $CHCl_3$).

5.27. (2*R*,3*R*)-3-Hydroxypipecolic acid (D-9)

$[\alpha]_D^{28}$ −12.9° (*c* 1.0, H_2O); Anal. Calcd for $C_6H_{11}NO_3 \cdot 0.8H_2O$: C, 44.66; H, 8.00; N, 8.68. Found: C, 44.42; H, 8.03; N, 8.66.



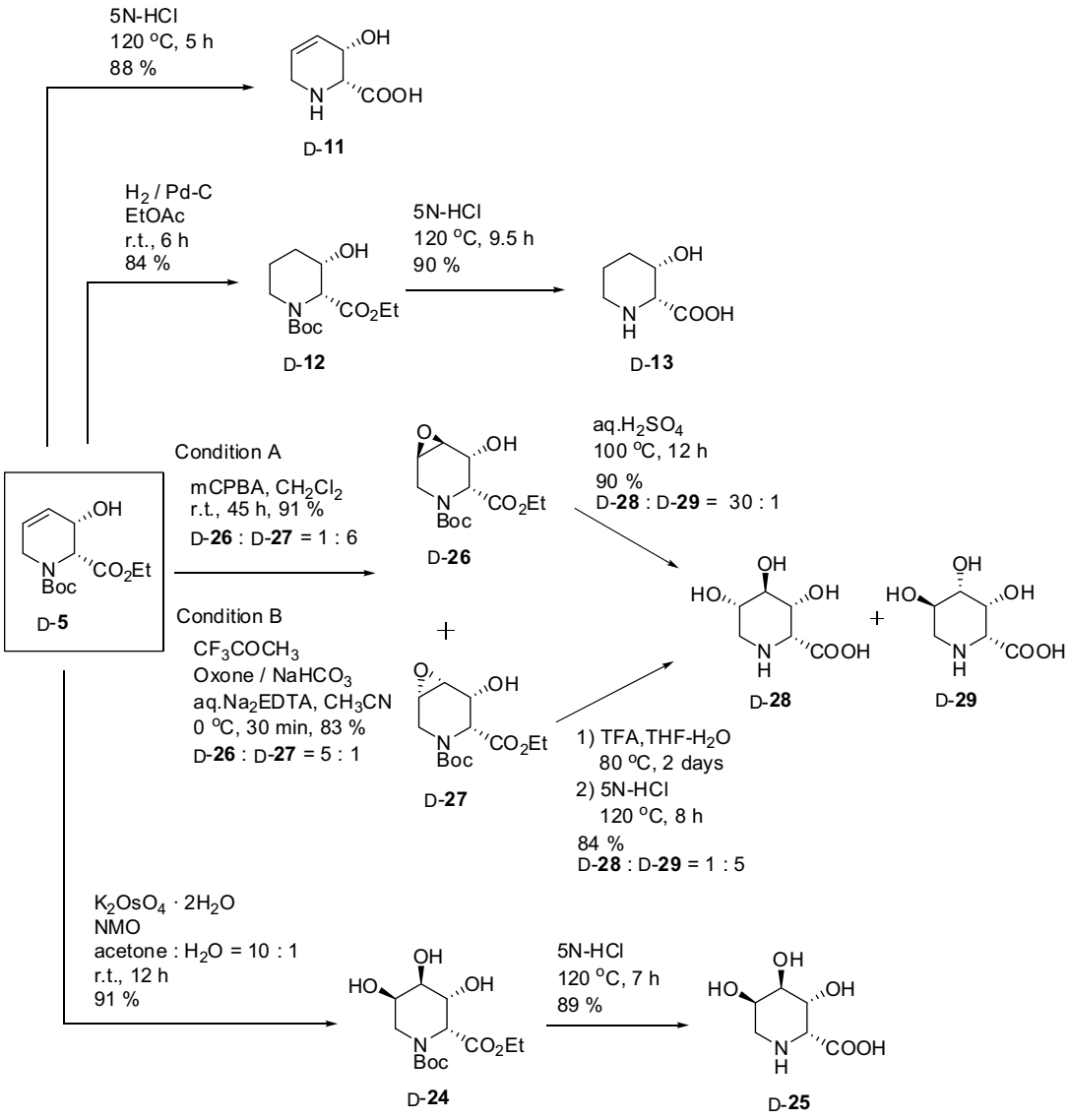
Scheme 13.

5.28. (2*R*,3*S*)-3-Hydroxybaikiain (D-11)

$[\alpha]_D^{27}$ +343.3° (*c* 0.74, H_2O); Anal. Calcd for $C_6H_9NO_3 \cdot 0.1H_2O$: C, 49.72; H, 6.40; N, 9.66. Found: C, 49.77; H, 6.46; N, 9.66.

5.29. (2*R*,3*S*)-1-*tert*-Butyl 2-ethyl 3-hydroxypiperidine-1,2-dicarboxylate (D-12)

$[\alpha]_D^{21}$ +63.2° (*c* 0.98, $CHCl_3$).



Scheme 14.

5.30. (2*R*,3*S*)-3-Hydroxypipecolic acid (D-13)

$[\alpha]_D^{27}$ +51.0 ° (*c* 0.75, H_2O); Anal. Calcd for $C_6H_{11}NO_3$: C, 49.65; H, 7.64; N, 9.65. Found: C, 49.70; H, 7.80; N, 9.67.

5.31. (2*R*,3*S*,4*S*,5*S*)-1-*tert*-Butyl 2-ethyl 3,4,5-trihydroxypiperidine-1,2-dicarboxylate (D-16) and (2*R*,3*S*,4*R*,5*R*)-1-*tert*-butyl 2-ethyl 3,4,5-trihydroxypiperidine-1,2-dicarboxylate (D-17)

D-**16**: $[\alpha]_D^{27}$ −6.8° (*c* 1.42, $CHCl_3$).
D-**17**: $[\alpha]_D^{27}$ +6.0° (*c* 1.80), $CHCl_3$).

5.32. (2*R*,3*S*,4*S*,5*S*)-3,4,5-Trihydroxypipecolic acid (1-deoxy-5-aza-L-allopyran-uronic acid, D-18)

$[\alpha]_D^{27}$ +29.4° (*c* 1.1, H_2O); Anal. Calcd for $C_6H_{11}NO_5{\cdot}0.2H_2O$: C, 39.87; H, 6.36; N, 7.75. Found: C, 40.00; H, 6.20; N, 7.68.

5.33. (2*R*,3*S*,4*R*,5*R*)-3,4,5-Trihydroxypipecolic acid (1-deoxy-5-aza-L-mannopyran-uronic acid, D-19)

$[\alpha]_D^{26}$ −30.0° (*c* 1.1, H_2O); Anal. Calcd for $C_6H_{11}NO_5{\cdot}0.2H_2O$: C, 39.87; H, 6.36; N, 7.75. Found: C, 39.69; H, 6.46; N, 7.62.

5.34. (1*R*,4*R*,5*S*,6*S*)-3-*tert*-Butyl 4-ethyl 5-hydroxy-7-oxa-3-azabicyclo[4.1.0]-heptane-3,4-dicarboxylate (D-20)

$[\alpha]_D^{26}$ +10.6° (*c* 1.0, $CHCl_3$).

5.35. (1*R*,2*S*,3*R*,6*S*)-4-*tert*-Butyl 3-ethyl 2-hydroxy-7-oxa-4-azabicyclo[4.1.0]-heptane-3,4-dicarboxylate (D-21)

$[\alpha]_D^{27}$ −19.4° (*c* 1.17, $CHCl_3$).

5.36. (2*R*,3*S*,4*R*,5*S*)-3,4,5-Trihydroxypipecolic acid(1-deoxy-5-aza-L-altropyran-uronic acid, D-22) and (2*R*,3*S*,4*S*,5*R*)-3,4,5-trihydroxy pipecolic acid(1-deoxy-5-aza-L-glucopyranuronic acid, D-23)

D-**22**: $[\alpha]_D^{26}$ +11.0° (*c* 0.90, H_2O); Anal. Calcd for $C_6H_{11}NO_5{\cdot}0.5H_2O$: C, 38.71; H, 6.50; N, 7.52. Found: C, 39.01; H, 6.70; N, 7.22.
D-**23**: $[\alpha]_D^{26}$ −18.1° (*c* 0.78, H_2O); Anal. Calcd for $C_6H_{11}NO_5{\cdot}1.5H_2O$: C, 35.29; H, 6.91; N, 6.86. Found: C, 35.25; H, 6.79; N, 6.95.

5.37. (2*R*,3*R*,4*R*,5*R*)-1-*tert*-Butyl 2-ethyl 3,4,5-trihydroxypiperidine-1,2-dicarboxylate (D-24)

$[\alpha]_D^{26}$ +16.8° (*c* 1.06, $CHCl_3$).

5.38. (2*R*,3*R*,4*R*,5*R*)-3,4,5-Trihydroxypipecolic acid (1-deoxy-5-aza-L-gulopyranuronic acid, D-25)

$[\alpha]_D^{19}$ +46.4° (*c* 1.23, H_2O) [lit.[16] $[\alpha]_D^{25}$ −5.9° ± 2.2 (*c* 1.25, variable, H_2O)]; Anal. Calcd for $C_6H_{11}NO_5$: C, 40.68; H, 6.26; N, 7.91. Found: C, 40.70; H, 6.18; N, 7.92.

5.39. (1*R*,4*R*,5*R*,6*S*)-3-*tert*-butyl 4-ethyl 5-hydroxy-7-oxa-3-azabicyclo[4.1.0]-heptane-3,4-dicarboxylate (D-26) and (1*S*,4*R*,5*R*,6*R*)-3-*tert*-butyl 4-ethyl 5-hydroxy-7-oxa-3-azabicyclo[4.1.0]heptane-3,4-dicarboxylate (D-27)

D-**26**. $[\alpha]_D^{25}$ +73.4° (*c* 1.23, $CHCl_3$).
D-**27**. $[\alpha]_D^{20}$ +50.7° (*c* 0.90, $CHCl_3$).

5.40. (2*R*,3*R*,4*R*,5*S*)-3,4,5-Trihydroxypipecolic acid(1-deoxy-5-aza-D-idopyranuronic acid, D-28) and (2*R*,3*R*,4*S*,5*R*)-3,4,5-trihydroxypipecolic acid(1-deoxy-5-aza-L-galactopyranuronic acid, D-29)

D-**28**: $[\alpha]_D^{18}$ +35.7° (*c* 0.87, H_2O) [lit.[28] $[\alpha]_D^{25}$ +39.7° (*c* 1.1, H_2O)]
D-**29**: $[\alpha]_D^{19}$ −20.0° (*c* 0.77, H_2O); Anal. Calcd for $C_6H_{11}NO_5{\cdot}H_2O$: C, 36.92; H, 6.71; N, 7.18. Found: C, 37.09; H, 6.65; N, 7.16.

5.41. Evaluation of inhibitory activities against glycosidases

The enzymes β-glucuronidase (from bovine liver, assayed at pH 5.0; from *E. coli*, assayed at pH 6.8), β-*N*-acetylglucosaminidase (from bovine kidney, pH 4.5; from human placenta, assayed at pH 4.5), α-*N*-acetylgalactosaminidase (from chicken liver, pH 4.0), *p*-nitrophenyl glycosides were purchased from Sigma Chemical Co. Glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at optimum pH of each enzyme. The reaction mixture (1 mL) contained 2 mM of the substrate and the appropriate amount of 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. Enzyme inhibition modes and K_i values were determined from Lineweaver–Burk plots.

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10. Compound **4** was converted to a known 2,3-*trans*-2-hydroxy-3-hydroxymethyl-piperidine in three steps: (1) H_2, Pd–C, EtOAc; 99%. (2) $LiAlH_4$, THF; 79%. (3) 5 N HCl, MeOH; 88%.
11. The ee of D-**6** was determined by HPLC using CHIRALPAK IA, Daicel Chemical Ind., Ltd, Japan after deacetylation followed by replacing a Boc to a tosyl group. Similarly, the ee of L-**6** was determined by HPLC after replacing a Boc to a tosyl group (see Section 5).
12. The ees of L-**5** and D-**4** (D-**10**) were determined by the same method as described in Ref. 11.
13. Both L-**16** and L-**17** were converted to 1-deoxymammonojirimycin and 1-deoxyallonojirimycin in two steps (1) $LiAlH_4$, THF; (2) 5 N HCl, MeOH and compared their instrumental data with those reported (see Ref. 2).
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