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Bioorganic & Medicinal Chemistry 14 (2006) 3405–3420
Bioorganic & Medicinal Chemistry

Synthesis, in vitro pharmacology, and structure–activity relationships of 2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid derivatives as mGluR2 antagonists

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Received 24 November 2005; revised 28 December 2005; accepted 29 December 2005
Available online 20 January 2006

Abstract—Chemical modification of the bicyclo[3.1.0]hexane ring C-3 position led to the discovery of 3-alkoxy-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, 3-benzylthio-, and 3-benzylamino-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid derivatives, metabotropic glutamate receptor 2 (mGluR2) antagonists. In particular, 3-(3,4-dichlorobenzyloxy)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (**15ae**), (1*R*,2*S*,5*R*,6*R*)-2-amino-3-(3,4-dichlorobenzylthio)-6-fluorobicyclo[3.1.0]hexane-2,6-carboxylic acid (**15at**), and (1*R*,2*S*,5*R*,6*R*)-2-amino-3-(*N*-(3,4-dichlorobenzylamino))-6-fluorobicyclo[3.1.0]hexane-2,6-carboxylic (**15ba**) showed high affinity for the mGluR2 receptor (**15ae**: K_i = 2.51 nM, **15at**: K_i = 1.96 nM, and **15ba**: K_i = 3.29 nM) and potent antagonist activity for mGluR2 (**15ae**; IC_{50} = 34.21 nM, **15at**; IC_{50} = 13.34 nM, and **15ba**; IC_{50} = 35.96 nM). No significant agonist activity for mGluR2 was observed with **15ae**, **15at**, or **15ba**. This paper reports on the synthesis, in vitro pharmacological profile, and structure–activity relationships (SARs) of 3-substituted-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid.

1. Introduction

L-Glutamate, a major excitatory neurotransmitter in the mammalian central nervous system, is involved in several physiological and pathological conditions, exerting its effects through either ionotropic glutamate receptors (iGluRs), in which the receptors have an ion channel structure, or metabotropic glutamate receptors (mGluRs), which are members of the GPCR family C and characterized by a large extracellular amino-terminal agonist binding site.[1–4] mGluRs are classified as one of eight subtypes organized into three groups based on sequence homology, signal transduction mechanisms, and pharmacological properties.[3,5–10] Group I mGluRs (mGluR1 and 5) are positively coupled to phospholipase C. Their activation produces phosphoinositide turn over and diacylglycerol within target neurons. Both group II (mGluR2 and 3) and group III mGluRs (mGluR4 and mGluR 6–8) are located presynaptically and negatively coupled to the activity of adenylcyclase.[3,4] mGluRs have been implicated in the pathology of major psychiatric disorders such as depression, anxiety, and schizophrenia[11] due to their critical role as modulators of synaptic transmission, ion channel activity, and synaptic plasticity.[9] Indeed, the efficacy of group II mGluR agonists in animal models and in clinical trials suggests that their inhibition of the glutamatergic system may make agonists of the group II mGluRs effective in the treatment of many diseases and conditions, including schizophrenia,[12–14] anxiety,[15–18] and panic disorders.[19]

In contrast, the efficacy of antagonists for mGluRs has not been clarified in animal models or clinical trials. This may be due to the lack of potent and selective antagonists of mGluRs. Our laboratory initiated efforts to identify potent and selective antagonists for group II mGluRs, reporting that 3-alkoxy-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid derivatives **2** are potent and selective antagonists for group II mGluRs, exhibiting good absorption and blood–brain barrier (BBB) penetration.[20] Compounds **2** were identified through chemical modification of **1**,[12] a typical group

Keywords: mGluR2 antagonist; 2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acids; Synthesis; Structure–activity relationship.
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doi:10.1016/j.bmc.2005.12.061

II mGluR agonist. Compound **1**, a compound incorporating a fluorine atom at the C-6 position of **4** reported by Kew et al.[21] strongly inhibited cAMP formation with the same EC_{50} value as **4**, exhibiting better oral activity than the original compound **4**. Of the compounds which we have reported, 2-amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid **3** (MGS0039) was the most potent group II mGluR antagonist with respect to affinity, selectivity, oral absorption, and BBB penetration, exhibiting dose-dependent antidepressant-like effects in the rat forced swim test and mouse tail suspension test. In a previous paper,[20] we reported that incorporating an hydroxyl or alkoxy group at the C-3 position of group II mGluR agonist **1** produced a group II mGluR antagonist, despite the size of the alkoxy group. Our interest in chemical modification of **2** to identify group II mGluR antagonists focused on the defluorine compounds of **2**. The fluorine atom at α-position of the carboxyl group enhances the acidity of the carboxyl group at the C-6 position on the bicyclo[3.1.0]hexane ring of **2**. We assumed that the defluorination of **2** would dramatically affect the acidity of the compounds and their resulting binding affinity and compound absorption. We were also interested in chemical modifications of **2**, focusing on the incorporation of an *S*-arylthio, *S*-benzylthio (*S*-analogue) and *N*-benzylamino group (*N*-analogue) on the C-3 position of group II mGluR agonists **2**. These transformations may expand the structural diversity of the compounds, since the nitrogen atom is capable of having another substituent, and the sulfur atom has deferent oxidation states (Chart 1).

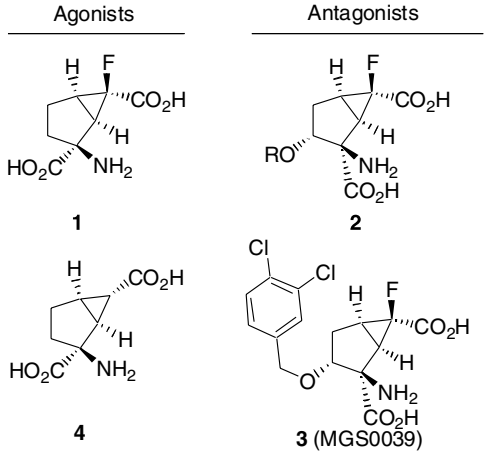
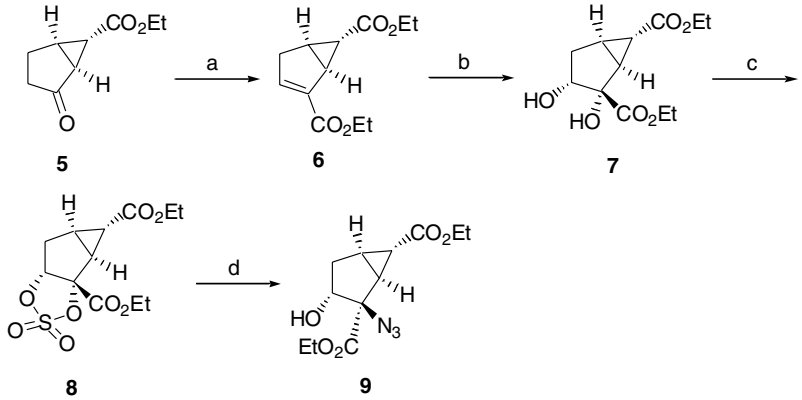


Chart 1. Group II mGluR agonists and antagonists.

Here, we focus on the defluorine compound at the C-6 position of the bicyclo[3.1.0]hexane ring of **2**, *S*-analogues, and *N*-analogues. We discuss the synthesis, in vitro pharmacological profile, structure–activity relationships of 3-alkoxy-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, and pharmacokinetic profiles of **15ae**, **15at**, and **15ba**.

2. Chemistry

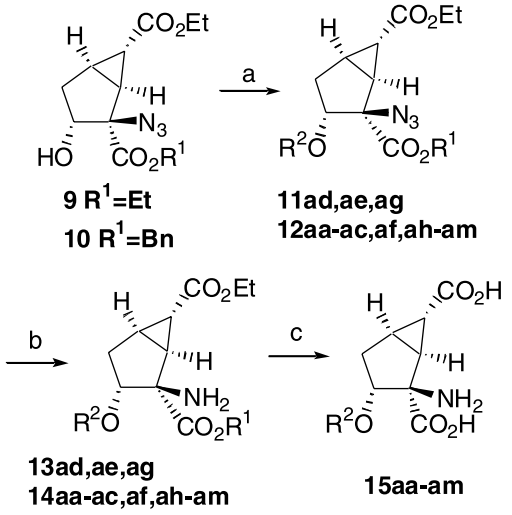
Scheme 1 illustrates the synthesis of the key intermediate **9**. Treating the optically pure compound **5**[22] with lithium bis(trimethylsilyl)amide and *N*-phenylbis(trifluoromethanesulfonimide) produced the corresponding enol triflate. The α,β-unsaturated carbonyl compound **6** was obtained from the enol triflate through a palladium-catalyzed reaction with carbon monoxide in the presence of ethanol. We used the method of transformation from α,β-unsaturated carbonyl compound into β-hydroxy α-amino acid reported by Shao and Goodman,[23] while the key intermediate **9** was synthesized from **6**. Oxidation of the double bond in **6** with OsO_4 and *N*-methylmorphorine *N*-oxide (NMO) resulted in diol **7**. In the reaction, the oxidant approaches the double bond at the opposite side of the fused cyclopropane ring, resulting in **7** as the single product. The reaction of **7** with $SOCl_2$ followed by oxidation with $NaIO_4$ and $RuCl_3$ provided a cyclic sulfate **8**. Regioselective nucleophilic displacement by NaN_3 at the C-2 position of **8** proceeded[24,25] due to the high ability of the cyclic sulfate as a leaving group, followed by hydrolysis, providing the key intermediate **9**.



Scheme 1. Reagents and conditions: (a) i—HMDS, *n*-BuLi, Tf_2NPh, THF, −60 °C; ii—CO, $Pd(OAc)_2$, $(i\text{-Pr})_2NEt$, PPh_3, EtOH, rt; (b) 4% OsO_4, 50% NMO, MeCN, rt; (c) $SOCl_2$, Et_3N, CH_2Cl_2, 4 °C; (d) i—NaN_3, DMF, 50 °C; ii—H_2SO_4, Et_2O, H_2O, rt.

With the key intermediates **9** and **10**[26] in hand, we addressed the synthesis of 3-alkoxy-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid **15aa**–**am**, as shown in Scheme 2. The 3-hydroxy groups in the key intermediates **9** and **10** were etherized with benzyl trichloroacetimidates and a catalytic amount of trifluoromethanesulfonic acid to yield **11ad**, **11ae**, and **11ag** (R^1 = Et), and **12aa**–**ac**, **12af**, and **12ah**–**am** (R^1 = Bn).[27,28] The benzyl trichloroacetimidates were prepared from 1,1,1-trichloroacetonitrile and the corresponding alcohol (R^2OH). For the reaction of **10** with 1-(3,4-dichlorophenyl)ethanol, the mixture of two diastereomers **12al** and **12am** was separated by silica gel chromatography.

Reduction of compounds **11ad**, **11ae**, **11ag**, **12aa**–**ac**, **12af**, and **12ah**–**am** using the Staudinger reaction[29,30] provided compounds **13ad**, **13ae**, **13ag**, **14aa**–**ac**, **14af**, and **14ah**–**am** without reductive cleavage of the benzyl group at the C-3 position. Finally, 2,6-dicarboxylic acid **15aa**–**am** were obtained by hydrolysis of the esters **13ad**, **13ae**, **13ag**, **14aa**–**ac**, **14af**, and **14ah**–**am** with LiOH.



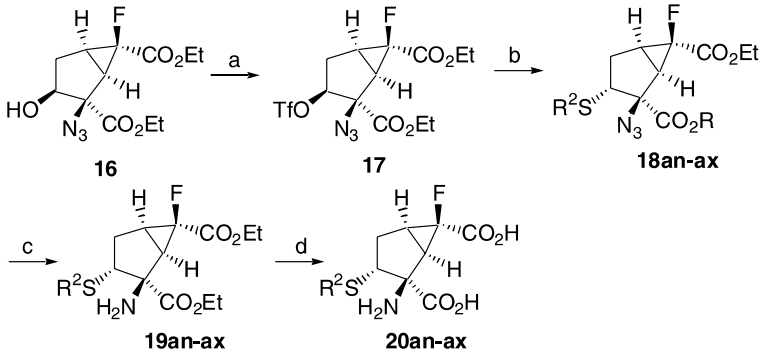
Scheme 2. Reagents and conditions: (a) $R^2OC(=NH)CCl_3$, TfOH, $CHCl_3$, cyclohexane, rt; (b) Me_3P, THF, H_2O, rt; (c) LiOH–H_2O, THF, H_2O, rt.

Scheme 3 shows the synthesis of **15an**–**ax**, bicyclo[3.1.0]hexane ring system having aryl-, benzyl-, and alkylthio groups. Compounds **15an**–**ax** were synthesized from **16**.[20]

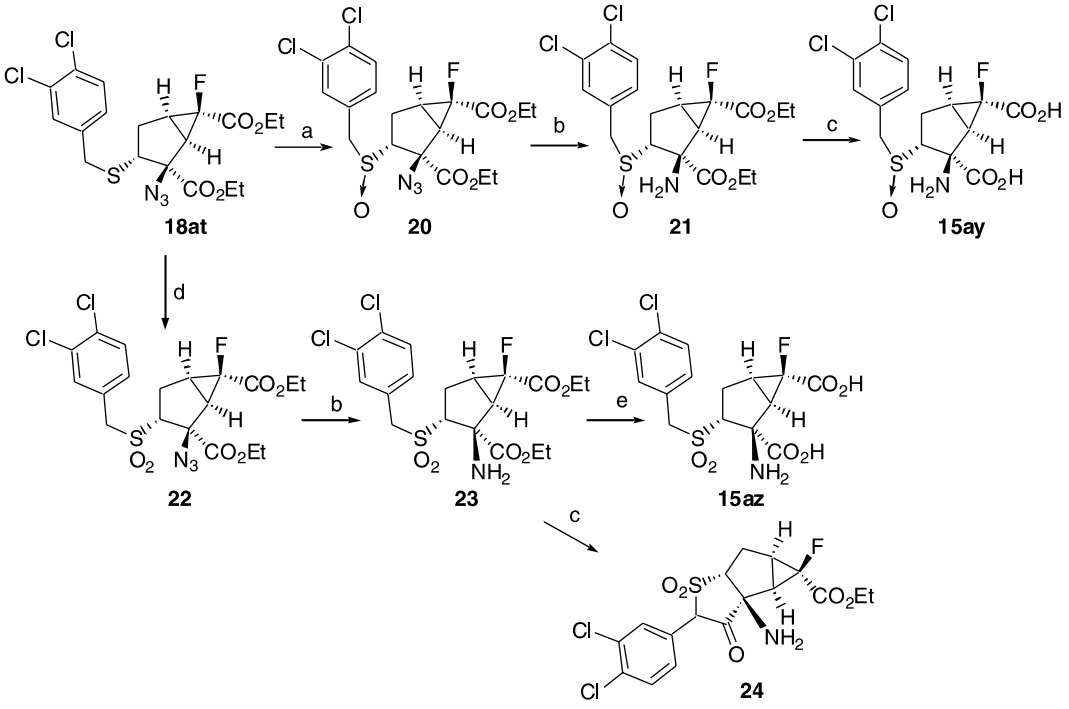
Compound **16** was treated with anhydride and pyridine to yield a key intermediate **17** to introduce a thioether moiety. Nucleophilic substitution reaction of **17** with various sodium benzyl and alkyl thiorates was allowed to proceed to render the corresponding thioethers (**18an**–**ax**). A Staudinger reaction[29,30] was then performed by trimethylphosphine in the presence of a small amount of water in THF to obtain **19an**–**ax**. Finally, hydrolysis of the ester groups of C-3 and C-6 positions in **19an**–**ax** with lithium hydroxide provided **15an**–**ax**.

Scheme 4 illustrates the preparation of **15ay** and **15az**, sulfinyl and sulfonyl derivatives. *m*-CPBA was used for oxidation of the thioether moiety, since the oxidation state could be controlled by the amount of *m*-CPBA. Oxidation of **18at** with 1.2 and 2.5 equiv of *m*-CPBA provided **20** and **22** in 84% and 91% yields, respectively. Compound **20** was transformed into **15ay** through reduction by the Staudinger reaction and hydrolysis by lithium hydroxide. After **23** was obtained by the same reduction from **20**, cyclization reaction of **23** occurred to give **24** instead of **15az** under the hydrolysis conditions using lithium hydroxide. The cyclization reaction may be attributable to the high acidity of the proton at the benzyl position in **23**. Thus, we attempted hydrolysis of **23** under acid conditions to avoid the cyclization reaction. This produced a satisfactory yield of **15az**, after which no **23** was detected.

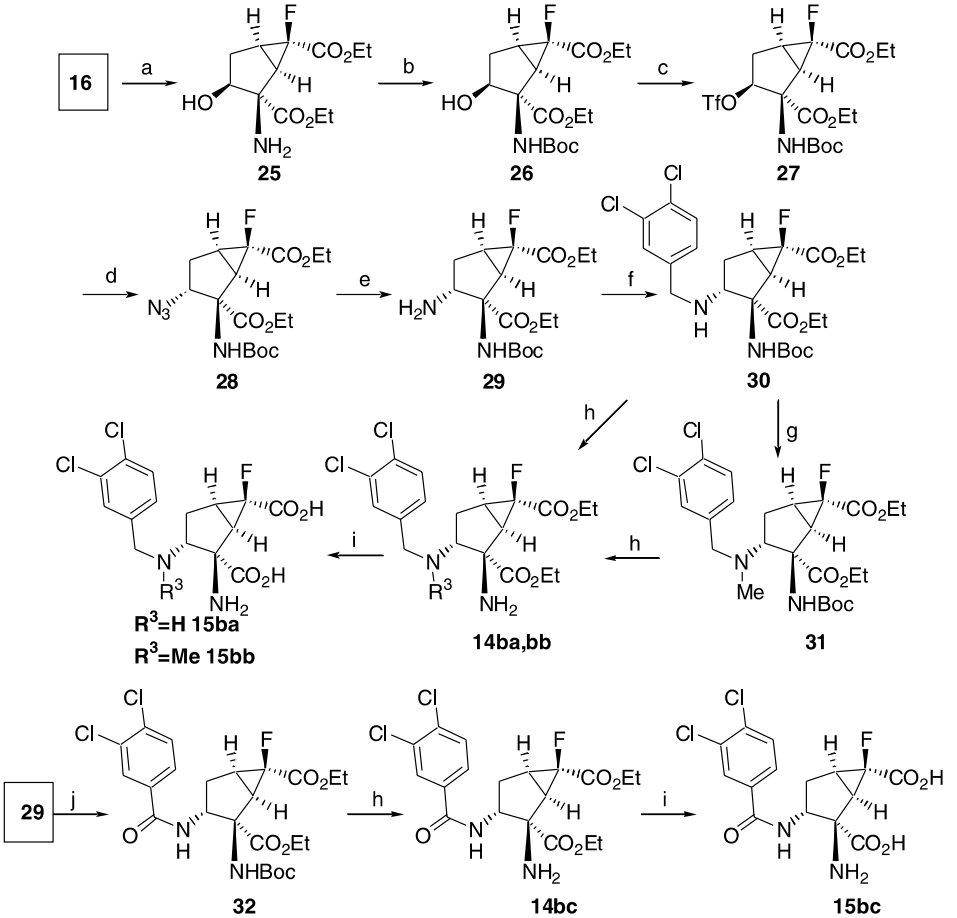
Scheme 5 shows the synthesis of **15ba**, **15bb**, and **15bc** compounds having an amino group and having an amide moiety at the C-3 position of the bicyclo[3.1.0]hexane ring. Reduction of the azide group in **16** and the protection of the generated amino group with a Boc group were performed first, since the synthesis of **15ba** and **15bb** required distinguishing between the two amino groups at the C-3 and C-2 positions of **15ba** and **15bb**. Reduction of azide group of **16** by the Staudinger reaction was allowed to proceed to give **25**. Treatment of Boc_2O and sodium bicarbonate in THF provided **26**, after which compound **26** was treated with



Scheme 3. Reagents and conditions: (a) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (b) R^2SH, EtONa, DMF, rt; (c) Me_3P, THF, H_2O, rt; (d) LiOH–H_2O, THF, H_2O, rt.



Scheme 4. Reagents and conditions: (a) *m*-CPBA (1.2 equiv), CH_2Cl_2, rt; (b) Me_3P, THF, H_2O, rt; (c) LiOH–H_2O, THF, H_2O, rt; (d) *m*-CPBA (2.5 equiv), CH_2Cl_2, rt; (e) 60% H_2SO_4, 130 °C.



Scheme 5. Reagents and conditions: (a) Me_3P, THF, H_2O, rt; (b) Boc_2O, satd $NaHCO_3$, THF, rt; (c) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (d) NaN_3, DMF, rt; (e) H_2, Pd/C, EtOH, rt; (f) 3,4-dichlorobenzyl bromide, pyridine, $CHCl_3$, rt; (g) MeI, K_2CO_3, DMF, rt; (h) 4 M HCl/AcOEt, 0 °C; (i) LiOH–H_2O, THF, H_2O, rt; (j) 3,4-dichlorobenzoyl chloride, rt.

trifluoromethanesulfonyl anhydride and pyridine to obtain **27**. Transformation from **27** into **28** was achieved by nucleophilic substitution with sodium azide, followed by hydrogenation of the introduced azide group with 5% Pd/C as a catalyst. The *N*-mono-substituted compound **30** was produced via benzylation of an amino group at the C-3 position of **29** with 3,4-dichlorobenzyl bromide and pyridine in chloroform. The *N*-di-substituted compound **31** was synthesized from **30** by methyl iodide and K_2CO_3 in DMF. Following removal of the Boc group in **30** and **31**, hydrolysis of **14ba** and **14bb** with lithium hydroxide provided **15ba** and **15bb**.

The treatment of **29**, the intermediate of synthesis of **15ba** and **15bb**, with 3,4-dichlorobenzoyl chloride and pyridine in chloroform, gave **32**. The removal of the Boc group and hydrolysis of **32** in the same manner from **30** to **15ba** provided **15bc**.

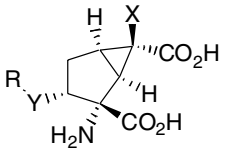
3. Results and discussion

3.1. Pharmacological profile studies

The binding affinity of **15aa**–**bc** for mGluR2 was evaluated by [^{3}H]-(1*S*,2*S*,3*S*,5*R*,6*S*)-2-amino-3-fluorobicyclio[3.1.0]hexane-2,6-dicarboxylic acid ([^{3}H]-MGS0008) binding using CHO cells stably expressing mGluR2.[12] Table 1 gives the K_i values obtained for mGluR2.

The agonist activity of **15ae**, **15aj**, **15an**, **15at**, and **15ay**–**ba** was evaluated by measuring the agonist-dependent inhibition of forskolin-induced cyclic AMP (cAMP) formation in mGluR2 expressing cells. The antagonist activities of the compounds were measured with 30 μM glutamic acid. Table 2 shows the agonist and antagonist activities of the compounds.

Table 1. Binding affinity for mGluR2



15	X	Y	R	Affinity K_i (nM)
aa	H	O	2-Naphtyl-CH_2	4.02 (2.53)[a]
ab	H	O	2-Ph-PhCH_2	9.27 (4.07)[a]
ac	H	O	4-Cl-PhCH_2	8.46 (3.17)[a]
ad	H	O	3-NC-PhCH_2	8.17 (2.94)[a]
ae	H	O	3,4-Cl_2-PhCH_2	2.51 (2.38)[a]
af	H	O	3,4-F_2-PhCH_2	4.84 (2.27)[a]
ag	H	O	3-Cl-4-F-PhCH_2	7.59 (3.57)[a]
ah	H	O	Ph_2CH	6.05
ai	H	O	(4-Cl-Ph)$_2$CH	8.65
aj	H	O	(4-F-Ph)$_2$CH	2.24
ak	H	O	(3,4-Cl_2-Ph)CH	8.60
al	H	O	(*R**)-3,4-Cl_2-Ph(Me)CH	5.02
am	H	O	(*S**)-3,4-Cl_2-Ph(Me)CH	3.86
an	F	S	Thiophen-2-yl-CH_2	6.37
ao	F	S	2-Ph-PhCH_2	3.77
ap	F	S	4-MeO-PhCH_2	3.77
aq	F	S	4-F-PhCH_2	2.13
ar	F	S	4-*t*-Bu-PhCH_2	21.90
as	F	S	3-CF_3-PhCH_2	5.15
at	F	S	3,4-Cl_2-PhCH_2	1.96
au	F	S	3-Cl-2,6-F_2-PhCH_2	4.92
av	F	S	Ph(Me)CH	5.70
aw	F	S	(4-F-Ph)$_2$CH	6.37 (3.75)[a]
ax	F	S	3,4-Cl_2-Ph–	3.69
ay	F	SO	3,4-Cl_2-PhCH_2	11.28
az	F	SO_2	3,4-Cl_2-PhCH_2	14.61
ba	F	NH	3,4-Cl_2-PhCH_2	3.29
bb	F	N-Me	3,4-Cl_2-PhCH_2	6.98
bc	F	N	3,4-Cl_2-PhCO	168.01
3 (Ref. 20)				2.61
LY341495 (Ref. 31)				2.90

[a] The figure within parentheses is the K_i value of the corresponding fluorinated compound **2**.[20]

Table 2. Antagonist and agonist activities

15	Antagonist activity IC_{50} (nM)	Agonist activity EC_{50} (nM)
ae	34.21	>100,000
aj	38.87	>100,000
an	100.69	>100,000
at	13.34	>100,000
ay	55.19	>100,000
az	689.07	>100,000
ba	35.96	>100,000

At first, to examine the effects of defluorination of **2** having the substituent/s on benzyl group at the C-3 position of the bicyclo[3.1.0]hexane ring on affinity for mGluR2, we investigated some corresponding defluorinated compounds to **2**, for which high mGluR2 affinities had been reported in a previous paper.[20] Compounds **15aa**–**af** showed K_i values of 4.02 nM (2.53 nM), 9.27 (4.07), 8.46 (3.17), 8.17 (2.94), 2.51 (2.38), 4.84 (2.27), and 7.59 (3.57), respectively (the figure within parentheses is the K_i value of the corresponding fluorinated compound **2**[20]). Compounds **15aa**–**ad**, **15af**, and **15ag** showed between 1.6- and 2.8-fold lower affinity than the corresponding **2**, but **15ae** showed almost the same affinity for mGluR2. Compound **15ae** was the corresponding defluorinated compound of MGS0039, the most potent compound in the series of fluorinated compounds at the C-6 position. We then examined the introduction of a methyl and substituted phenyl group on the methylene of the benzyl group at the C-3 position of the bicyclo[3.1.0]hexane ring. Compounds **15ai** and **15ak**–**am** also tended to show lower affinities for mGluR2 compared to the corresponding **2**, but **15ai** showed only high affinity.

These findings suggest a fluorine atom must be introduced to increase binding affinity, and introducing a 3,4-dichlorobenzyl group and bis(4-fluorophenyl)methyl group at the C-3 position is particularly effective in increasing binding affinity for mGluR2. Compounds **15ae** and **15aj** with high binding affinities for mGluR2 also showed high antagonist activity (**15ae**: IC_{50} = 34.21 nM, **15aj**: IC_{50} = 38.87 nM), but no significant agonist activity (ED_{50} > 100,000 nM).

We next examined chemical modifications, focusing on incorporating *S*-analogues and *N*-analogues of the group II mGluR antagonist **2**.

We began by investigating chemical modification of the substituent on the benzene ring of benzyl group at the C-3 position of the bicyclo[3.1.0]hexane ring. Introducing single substituents, a phenyl group (**15ao**), methoxy group (**15ap**), fluorine atom (**15aq**), and trifluoromethyl group (**15as**), resulted in good affinity for mGluR2. Compounds **15ao**, **15ap**, and **15as** showed binding affinities higher than those of the corresponding *O*-benzyl compounds **2**. The K_i value of **15ar** having a bulky alkyl group was lower than those of other single substituted compounds. In the *S*-benzyl compounds, **15at** with a 3,4-dichlorobenzyl group also showed marked binding affinity for mGluR2. Introduction of three halogen atoms, two fluorine atoms, and a chlorine atom in **15au** at the benzene ring slightly reduced mGluR2 affinity (K_i = 4.92 nM).

We then examined the introduction of alkyl and phenyl groups at the benzyl position. The K_i value of the 1-bis(4-fluorophenyl)methyl compound **15aw** for mGluR2 was 6.37 nM, lower than that of the corresponding *O*-benzyl compound (K_i = 3.75.[20]) The binding affinity of **15av** was evaluated as a mixture of two unseparated diasteromers by column chromatography (K_i = 5.70 nM).

In general, incorporating *S*-benzyl compounds increased binding affinities for mGluR2 compared to *O*-benzyl compounds. The 3,4-dichlorobenzyl group also showed marked effectiveness in the *S*-benzyl compounds.

Previously, we reported[20] on the introduction of an *O*-benzyl group by benzylation with trichloroacetimidate of the corresponding benzyl alcohol, preventing synthesis of the *O*-aryl compounds. Thus, we were interested in the binding affinity for mGluR2 of *O*-aryl compound. Introduction of the *S*-benzyl group was performed by nucleophilic displacement with the corresponding benzylmercaptan under basic conditions, and the synthesis of *S*-aryl compound succeeded. We reported that *S*-benzyl compounds generally showed good binding affinity compared to *O*-benzyl compounds. For this reason, we examined *S*-aryl compounds, and **15ax** showed high binding affinity for mGluR2. However, the K_i value of **15ax** was slightly lower than those of **15ae** and **15at**.

As the next step in chemical modification, we investigated the transformation of benzyl thioether moiety of **15at**. We synthesized a sulfinyl compound (**15ay**) and sulfonyl compound (**15az**), each having a sulfur atom of two deferent oxidation states. Their K_i values were lower than those of **15at** (**15ay**: K_i = 11.28 nM, **15ba**: K_i = 14.61 nM).

Our final chemical modification focused on incorporating the *N*-alkyl group at the C-3 position of group II mGluR antagonists **2**. Compound **15ba**, an *N*-benzyl compound corresponding to **3**, exhibited high binding affinities (K_i = 3.29 nM) for mGluR2, identical to **3**. Compound **15bb**, *N*,*N*-disubstituted compound, exhibited 2.5-fold lower mGluR2 affinity than **15ba**. Compound **15bc**, a benzoyl compound, exhibited much lower affinity for mGluR2 (K_i = 168.01 nM). These findings suggest that the oxygen atom of ether bond in **3** can be replaced by a sulfur or nitrogen atom to produce nearly the same binding affinity for mGluR2, while increase of the size of around sulfur or nitrogen atom reduced mGluR2 binding affinity.

Finally, we performed evaluations of plasma levels for the three compounds **15ae**, **15at**, and **15ba**. All three showed high affinity for mGluR2, while retaining a 3,4-dichlorobenzyl group.

Table 3 gives plasma levels and pharmacokinetic parameters for **15ae**, **15at**, and **15ba** following oral administration to rats at a dose of 10 mg/kg.

Table 3. Pharmacokinetic parameters of **15ae**, **at**, and **ba** following oral administration to rats

Compound	**15ae**			**15at**[a]	**15ba**[a]	**3** (Ref. 20)	LY341495 (Ref. 31)
Dose (mg/kg)	3	10	30	10	10	10	10
T_{max} (h)	3.3 ± 1.2	4.7 ± 3.1	5.3 ± 2.3	3.33 ± 1.15	3.33 ± 1.15	6	0.5
C_{max} (ng/mL)	81.9 ± 23.1	147.0 ± 39.5	519.3 ± 49.9	116 ± 51.0	96.7 ± 8.62	492	64
$t_{1/2}$ (h)	4.0 ± 0.4	4.0 ± 0.5	3.2 ± 0.4	—	—	2.3	2.64
AUC_{inf} (ng h/mL)	702.5 ± 75.6	1385.1 ± 57.4	5919.1 ± 585.3	—	—	6810	284
AUC_{0-6h} (ng h/mL)	—	—	—	500 ± 213	455 ± 28.9	—	—

Each value represents the mean ± SD of three animals.
[a] $t_{1/2}$ and AUC_{inf} could not be estimated for limited sampling points.

Following oral administration of **15ae**, maximum plasma concentrations (C_{max}) of 81.9, 147.0, and 519.3 ng/mL were reached at 3.3–5.3 h at dose of 3, 10, and 30 mg/kg, respectively. C_{max} values and corresponding exposure, as measured by AUC_{inf}, increased less than dose, proportionally. Plasma levels of **15at** and **15ba** following oral administration were also evaluated at 10 mg/kg in rats. The C_{max} levels of these compounds were 116 and 96.7 ng/mL, somewhat lower than that of **15ae**, and the corresponding AUC_{0-6h} values were 500 and 455 ng h/mL, respectively.

At oral administration, all compounds, which were studied at 10 mg/kg (po) in rat, exhibited the higher plasma concentrations than those for the known group II mGluR antagonist (2*S*)-amino-2-((1*S*,2*S*)-2-carboxycycloprop-1-yl)-3-(9-xanthyl)propionic acid (LY341495).[31,32]

4. Conclusions

In this paper, we have reported the syntheses and SARs. Overall, *S*-analogues showed good mGluR2 binding affinity compared to *O*-benzyl group, while introducing a bulky alkyl group in benzene moiety and increase of the size of around sulfur or nitrogen atom did not improve the binding affinity. The fluorine atom and the 3,4-dichlorobenzyl group (**15ae**, **15at**, and **15ba**) played important roles in increasing binding affinity. Of the compounds discussed, **15ae**, **15aj**, **15an**, **15at**, and **15ay**–**ba** also showed antagonist activity. In particular, **15at** with a 3,4-dichlorobenzyl group showed the highest mGluR2 affinity among known group II mGluR antagonists, and the presence of a 3,4-dichlorobenzyl group and fluorine atom at the 6 position of bicyclo[3.1.0]hexane ring effectively increased affinity for mGluR2 receptor.

Preliminarily examined at a dose of 10 mg/kg (po) in rats, compounds **15ae**, **15at**, and **15ba** exhibited plasma concentrations higher than that of known group II mGluR antagonist (LY341495), but the plasma concentrations of those compounds did not reach the plasma concentration of **3**. Given the high binding affinity for mGluR2, those compounds have the potency of group II mGluR2 antagonists, although they require further improvements in plasma concentrations. We believe studies of SARs and the resulting pharmacokinetic profiles will provide new opportunities to explore and improve upon new group II mGluR2 antagonists.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Varian Gemini 2000 (200 MHz), Varian Unity Inova 300 (300 MHz) or JEOL Lambda500 (500 MHz). Chemical shifts are reported in parts per million relative to TMS or sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TMSP) as an internal standard. Mass spectra (MS) were obtained on a JEOL JMS-SX102 or Micromass Platform LC. Optical rotations were determined with a JASCO DIP-360 polarimeter. Elemental analyses were performed on a Perkin-Elmer 2400.

5.1.1. (1*S*,5*R*,6*S*)-6-Bicyclo[3.1.0]hex-2-ene-2,6-dicarboxylic acid diethyl ester (6). To a mixture of HMDS (9.0 mL, 42.8 mmol) and THF (46 mL) was added BuLi (2.66 M hexane solution, 16.1 mL, 42.8 mmol) under nitrogen atmosphere at −70 to −68 °C. After the mixture was stirred for 0.5 h, a THF (23 mL) solution of **6** (6.00 g, 35.7 mmol) was added to the mixture at −70 to −60 °C and stirred for 1 h. A THF (700 mL) solution of *N*-phenylbis(trifluoromethanesulfonimide) (213 g, 0.597 mol) at −70 to −60 °C was added to the mixture and stirred for 2 h. The mixture was stirred for 2 h, after removing the dry ice–acetone bath. The reaction mixture was diluted with ether. The ethereal solution was washed with saturated $NaHCO_3$ (3×) and brine, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (hexane/AcOEt = 20:1) to get a colorless oil (12.68 g). A mixture of the obtained colorless oil (12.68 g), diisopropylethylamine (7.3 mL, 42.2 mmol), triphenylphosphine (663 mg, 2.53 mmol), ethanol (68 mL), DMF (67.8 mL), and $Pd(OAc)_2$ (285 mg, 1.27 mmol) was stirred for 18 h at room temperature under carbon monoxide atmosphere. The mixture was diluted with 1 N HCl and extracted with ether (3×). The combined ethereal layer was washed with saturated $NaHCO_3$ (4×) and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 20:1) to yield **7** (5.86 g, 73%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 1.26 (3H, t, J = 7.1 Hz), 1.31 (3H, t, J = 6.8 Hz), 2.22–2.30 (1H, m), 2.59–2.91 (4H, m), 4.08–4.29 (4H, m), 6.53 (1H, s); MS (ESI, Pos) *m/z* 247 (M^++Na); $[\alpha]_D^{26}$ +145.5 (*c* 0.95, $CHCl_3$).

5.1.2. (1*S*,5*R*,6*S*)-2,3-Dihydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (7). A mixture of **7** (5.86 g, 26.1 mmol), MeCN (150 mL), H_2O (54 mL), 50% aqueous NMO (10.8 mL, 52.2 mol), and 4% aqueous OsO_4 (8.3 mL, 1.31 mmol) was stirred for 1 h at room temperature. $NaHSO_3$ was added to the reaction mixture with ice-cooling stirred for 30 min. The mixture was filtered using Celite Pad, and the filtrate was extracted with AcOEt (2×). The combined AcOEt layer was washed with saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 2:1) to yield **7** (5.43 g, 81%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.25 (3H, t, J = 7.3 Hz), 1.33 (3H, t, J = 7.0 Hz), 1.73–2.16 (4H, m), 2.27–2.45 (2H, m), 3.83 (1H, s), 4.00–4.15 (1H, m), 4.12 (2H, q, J = 7.0 Hz), 4.21–4.41 (2H, m); MS (ESI, Pos) m/z 281 (M^++Na); $[\alpha]_D^{27}$ −89.7 (c 0.32, $CHCl_3$).

5.1.3. (1*S*,2*S*,3*R*,5*R*,6*S*)-3,3-Dioxo-tetrahydro-2,4-dioxa-3λ^6-thiacyclopropa[*a*]pentalene-1,1b-dicarboxylic acid diethyl ester (8). $SOCl_2$ (2.47 mL) was added to a mixture of **7** (5.86 g, 22.7 mmol), CH_2Cl_2 (82 mL), and Et_3N (6.96 mL, 49.9 mmol) with ice-cooling and the mixture was stirred for 15 min. H_2O was added to the reaction mixture, and CH_2Cl_2 layer was separated. The CH_2Cl_2 layer was washed with H_2O and saturated brine, dried with $MgSO_4$, and evaporated. The residue was dissolved with CCl_4 (42 mL), MeCN (42 mL), and H_2O (50 mL). $NaIO_4$ (6.31 g, 29.5 mol) and $RuCl_3{\cdot}H_2O$ (50 mg) were added to the solution with ice-cooling and stirred for 2 h. The mixture was filtered using Celite Pad, and the filtrate was partitioned into organic and aqueous layers, and the aqueous layer was extracted with ether. The combined organic layer was washed with saturated brine, dried with $MgSO_4$, evaporated, and chromatographed (hexane/AcOEt = 4:1) to yield **8** (6.37 g, 88%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.26 (3H, t, J = 7.3 Hz), 1.37 (3H, t, J = 7.3 Hz), 1.71 (1H, dd, J = 3.5, 3.5 Hz), 2.26–2.38 (1H, m), 2.56–2.68 (3H, m), 4.14 (2H, q, J = 7.3 Hz), 4.29–4.47 (2H, m), 5.35 (1H, dd, J = 6.2, 6.2 Hz); MS (ESI, Pos) m/z 343 (M^++Na); $[\alpha]_D^{24}$ −35.7 (c 0.27, $CHCl_3$).

5.1.4. (1*S*,2*S*,3*R*,5*R*,6*S*)-2-Azido-3-hydroxybicyclo-[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (9). A mixture of **8** (6.37 g, 19.9 mmol), DMF (78 mL), H_2O (8 mL), and NaN_3 (2.33 g, 35 mmol) was stirred for 1 h at 50 °C. The reaction mixture was concentrated under reduced pressure. The residue was dissolved with ether (400 mL) and H_2O (11 mL). 20% H_2SO_4 (31 mL) was added dropwise to the mixture with ice-cooling and vigorously stirred for 20 h at room temperature. The organic layer of the mixture was separated, washed with saturated brine, dried with $MgSO_4$, evaporated, and chromatographed (hexane/AcOEt = 4:1) to yield **9** (88.5 g, 91%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.27 (3H, t, J = 7.3 Hz), 1.38 (3H, t, J = 7.3 Hz), 1.83 (1H, dd, J = 3.1, 3.1 Hz), 1.99–2.46 (4H, m), 3.73–3.90 (1H, m), 4.14 (2H, q, J = 7.0 Hz), 4.36 (2H, q, J = 7.0 Hz); MS (ESI, Pos) m/z; 306 (M^++Na); $[\alpha]_D^{27}$ −49.1 (c 0.22, $CHCl_3$).

5.1.5. General method for the synthesis of 15aa–ac and 15ae–ak (method A)

5.1.5.1. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Azido-3-(3,4-dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (11ae). A THF (153 mL) solution of 3,4-dichlorobenzyl alcohol (200 g, 1.13 mol) was added to a THF (227 mL) suspension of NaH (60%, 4.52 g, 113 mmol) and stirred for 0.5 h at room temperature. Trichloroacetonitrile (113 mL, 1.13 mol) was added to the mixture with ice-cooling and stirred for 1.5 h at room temperature. Pentane (150 mL) and methanol (3.7 mL) were added to the reaction mixture. After stirring for 30 min at room temperature, a formed precipitate was filtered off. The filtrate was concentrated under reduced pressure to yield crude 3,4-dichlorobenzyl-2,2,2-trichloroacetoimidate (358 g) as a brown viscous liquid.

Trifluoromethanesulfonic acid (161 μL) was added to the mixture of 3,4-dichlorobenzyl-2,2,2-trichloroacetoimidate (1.47 g, 4.56 mmol), **9** (860 mg, 3.04 mol), $CHCl_3$ (3.6 mL), and cyclohexane (7.2 mL) under nitrogen atmosphere. After the mixture was stirred for 1 h at room temperature, a formed precipitate was filtered off. Saturated $NaHCO_3$ was added to the filtrate with ice-cooling. The mixture was extracted with $CHCl_3$ (2×). The $CHCl_3$ layer was washed with saturated brine, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (hexane/AcOEt = 15:1) to yield **11ae** (440 mg, 33%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.27 (3H, t, J = 7.0 Hz), 1.32 (3H, t, J = 7.5 Hz), 1.75 (1H, t, J = 3.1 Hz), 2.03–2.38 (4H, m), 3.54 (1H, dd, J = 7.47 Hz), 4.14 (4H, q, J = 7.2 Hz), 4.23–4.46 (3H, m), 4.58 (1H, d, J = 12.3 Hz), 7.09 (1H, dd, J = 8.4, 2.0 Hz), 7.36 (1H, d, J = 2.0 Hz), 7.40 (1H, d, J = 8.4 Hz); MS (ESI, Pos) m/z; 464 (M^++Na); $[\alpha]_D^{24}$ +2.5 (c 0.70, $CHCl_3$).

5.1.5.2. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(3,4-dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (13ae). A mixture of **11ae** (540 mg, 1.22 mmol), Me_3P (1 M THF solution, 1.34 mL), THF (18 mL), and H_2O (1.4 mL) was stirred for 22 h at room temperature. Ether was added to the reaction mixture, and the organic layer of the mixture was separated. The organic layer was washed with saturated $NaHCO_3$ and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 3:1–1:1) to yield **13ae** (160 mg, 39%) as a pale yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.25 (3H, d, J = 7.5 Hz), 1.30 (3H, d, J = 7.47 Hz), 1.72 (1H, dd, J = 3.1, 3.1 Hz), 1.97–2.37 (4H, m), 3.46 (1H, dd, J = 8.6, 7.3 Hz), 4.06–4.54 (4H, m), 4.41 (1H, d, J = 12.3 Hz), 4.51 (1H, d, J = 12.3 Hz), 7.08 (1H, dd, J = 8.4, 1.8 Hz), 7.35 (1H, d, J = 1.8 Hz), 7.39 (1H, d, J = 8.4 Hz); MS (ESI, Pos) m/z 438 (M^++Na); $[\alpha]_D^{23}$ +12.6 (c 0.33, $CHCl_3$).

5.1.5.3. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(3,4-dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ae). A mixture of **13ae** (160 mg, 0.384 mmol), THF (4 mL), H_2O (2 mL), and $LiOH{\cdot}H_2O$ (48 mg, 1.15 mmol) was stirred for five days at room temperature. The reaction mixture was acidified with 1 N HCl

and stirred for 1 h at room temperature. The solution was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O–50% aqueous THF–10% aqueous pyridine) to yield **15ae** (66 mg, 49%) as a white powder. Mp > 250 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.61 (1H, t, J = 3.0 Hz), 1.97–2.41 (4H, m), 3.78 (1H, dd, J = 8.5, 7.9 Hz), 4.50 (1H, d, J = 12.1 Hz), 4.55 (1H, d, J = 12.1 Hz), 7.27–7.31 (1H, m, J = 12.1 Hz), 7.53–7.58 (2H, m); MS (ESI, Nega) m/z 358 (M^-−1); $[\alpha]_D^{28}$ +5.1 (c 2.00, 1 N NaOH); Anal. Calcd for $C_{15}H_{15}Cl_2NO_5$: C, 50.02; H, 4.20; N, 3.89. Found: C, 49.99; H, 4.21; N, 3.80.

5.1.5.4. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(2-naphthylmethyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15aa). Starting from **10** and 2-naphthenylmethyl alcohol, **15aa** was obtained as a white powder. Mp > 213 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.57–1.63 (1H, m), 1.95–2.06 (2H, m), 2.14–2.25 (1H, m), 2.33–2.43 (1H, m), 3.83 (1H, dd, J = 8.9, 8.9 Hz), 4.68–4.80 (2H, m), 7.53–7.64 (3H, m), 7.88–8.01 (4H, m); MS (ESI, Nega) m/z 340 (M^-−1); $[\alpha]_D^{28}$ +24.1 (c 0.26, 1 N NaOH); Anal. Calcd for $C_{19}H_{19}NO_5{\cdot}0.2H_2O$: C, 66.15; H, 5.67; N, 4.06. Found: C, 66.16; H, 5.58; N, 3.84.

5.1.5.5. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(2-phenylbenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ab). Starting from **10** and 2-phenylbenzyl alcohol, **15ab** was obtained as a white powder. Mp > 205 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.53–1.56 (1H, m), 1.90–2.21 (4H, m), 3.56 (1H, dd, J = 7.5, 7.5 Hz), 4.43 (1H, d, J = 10.9 Hz), 4.50 (1H, d, J = 10.9 Hz), 7.39–7.57 (9H, m); MS (ESI, Nega) m/z 366 (M^-−1); $[\alpha]_D^{26}$ −12.9 (c 0.17, 1 N NaOH); Anal. Calcd for $C_{21}H_{21}NO_5{\cdot}0.75H_2O$: C, 66.22; H, 5.95; N, 3.68. Found: C, 66.25; H, 5.78; N, 3.68.

5.1.5.6. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(4-chlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ac). Starting from **10** and 4-chlorobenzyl alcohol, **15ac** was obtained as a white powder. Mp > 205 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.61 (1H, dd, J = 3.3, 3.3 Hz), 1.99–2.05 (2H, m), 2.11–2.20 (1H, m), 2.34–2.40 (1H, m), 3.78 (1H, dd, J = 7.6, 7.6 Hz), 4.78 (2H, s), 7.35 (2H, d, J = 8.5 Hz), 7.43 (2H, d, J = 8.5 Hz); MS (ESI, Nega) m/z 324 (M^-−1); $[\alpha]_D^{27}$ +5.1 (c 0.58, 1 N NaOH); Anal. Calcd for $C_{15}H_{16}ClNO_5{\cdot}0.1H_2O$: C, 55.00; H, 4.99; N, 4.28. Found: C, 54.81; H, 4.91; N, 4.27.

5.1.5.7. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(3,4-difluorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15af). Starting from **10** and 3,4-difluorobenzyl alcohol, **15af** was obtained as a white powder. Mp > 298 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.60–1.64 (1H, m), 1.98–2.08 (2H, m), 2.11–2.20 (1H, m), 2.34–2.41 (1H, m), 3.78 (1H, dd, J = 7.3, 7.3 Hz), 4.51 (2H, s), 7.11–7.32 (3H, m); MS (ESI, Nega) m/z 326 (M^-−H); $[\alpha]_D^{26}$ −3.1 (c 0.37, 1 N NaOH); Anal. Calcd for $C_{15}H_{15}ClNO_5{\cdot}0.3H_2O$: C, 51.60; H, 4.50; N, 4.01. Found: C, 51.56; H, 4.36; N, 3.90.

5.1.5.8. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(3-chloro-4-fluorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ag). Starting from **9** and 3-chloro-4-fluorobenzyl alcohol, **15ag** was obtained as a white powder. Mp > 270 °C (decomp.). ^{1}H NMR (500 MHz, D_2O, TMSP) δ 1.60–1.65 (1H, m), 1.96–2.21 (3H, m), 2.36–2.43 (1H, m), 3.81 (1H, t, J = 8.1 Hz), 4.64 (2H, s), 7.17–7.24 (1H, m), 7.35–7.40 (1H, m), 7.46–7.52 (1H, m); MS (ESI, Nega) m/z 342 (M^-−1); $[\alpha]_D^{23}$ +3.6 (c 0.12, 1 N NaOH); Anal. Calcd for $C_{15}H_{15}F_2NO5{\cdot}0.1H_2O$: C, 54.75; H, 4.66; N, 4.26. Found: C, 54.61; H, 4.61; N, 4.22.

5.1.5.9. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(diphenylmethoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ah). Starting from **10** and diphenylmethyl alcohol, **15ah** was obtained as a white powder. Mp > 228 (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.49 (1H, dd, J = 2.8, 2.8 Hz), 1.93–2.05 (2H, m), 2.14–2.33 (2H, m), 3.75 (1H, dd, J = 7.8, 7.8 Hz), 5.55 (1H, s), 7.30–7.48 (10H, m); MS (ESI, Nega) m/z 366 (M^-−1); $[\alpha]_D^{29}$ −14.3 (c 0.38, 1 N NaOH); Anal. Calcd for $C_{21}H_{21}NO_5{\cdot}0.7H_2O$: C, 66.37; H, 5.94; N, 3.69. Found: C, 66.53; H, 5.84; N, 3.83.

5.1.5.10. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(bis(4-chlorophenyl)methoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ai). Starting from **10** and bis(4-chlorophenyl)methyl alcohol, **15ai** was obtained as a white powder. Mp > 222 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.45–1.53 (1H, m), 1.91–2.05 (2H, m), 2.15–2.26 (2H, m), 3.73 (1H, dd, J = 8.1, 8.1 Hz), 5.53 (1H, s), 7.31–7.45 (8H, m); MS (ESI, Nega) m/z 434 (M^-−1); $[\alpha]_D^{28}$ −14.3 (c 0.38, 1 N NaOH); Anal. Calcd for $C_{21}H_{19}Cl_2NO_5{\cdot}0.8H_2O$: C, 55.96; H, 4.61; N, 3.11. Found: C, 56.20; H, 4.89; N, 2.95.

5.1.5.11. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(bis(4-fluorophenyl)methoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15aj). Starting from **10** and bis(4-fluorophenyl)methyl alcohol, **15aj** was obtained as a white powder. Mp > 295 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.49 (1H, dd, J = 3.8, 3.8 Hz), 1.88–2.04 (2H, m), 2.17–2.24 (2H, m), 3.73 (1H, dd, J = 8.4, 8.4 Hz), 5.54 (1H, s), 7.09–7.17 (2H, m), 7.35–7.42 (2H, m); MS (ESI, Nega) m/z 402 (M^-−1); $[\alpha]_D^{25}$ −12.8 (c 0.18, 1 N NaOH); Anal. Calcd for $C_{21}H_{19}F_2NO_5{\cdot}1.5H_2O$: C, 58.60; H, 5.15; N, 3.25. Found: C, 58.78; H, 4.85; N, 3.28.

5.1.5.12. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(bis(3,4-dichlorophenyl)methoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ak). Starting from **10** and bis(4-fluorophenyl)methyl alcohol, **15ak** was obtained as a white powder. Mp > 295 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.56–1.69 (1H, m), 2.02–2.48 (4H, m), 3.49–3.72 (1H, m), 5.39–5.58 (1H, m), 7.20–7.69 (6H, m); MS (ESI, Nega) m/z 502 (M^-−1); $[\alpha]_D^{24}$ −3.43 (c 0.34, 1 N NaOH).

5.1.6. Method B

5.1.6.1. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Azido-3-(2-cyanobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (11ad). A THF (1.6 mL) solution of 3-cyanobenzyl

alcohol (1.0 g, 7.51 mmol) was added to a THF (1.1 mL) suspension of NaH (60%, 30 mg, 0.75 mmol) and stirred for 0.5 h at room temperature. Trichloroacetonitrile (0.75 mL, 7.51 mol) was added to the mixture with ice-cooling and stirred for 2 h at room temperature. Pentane (1.3 mL) and methanol (27 μL) were added to the reaction mixture. After stirring for 15 min at room temperature, a formed precipitate was filtered off. The filtrate was concentrated under reduced pressure to yield crude 3-cyanobenzyl-2,2,2-trichloroacetoimidate (2.02 g) as a brown viscous liquid.

Trifluoromethanesulfonic acid (50 μL) was added to the mixture of 3-cyanobenzyl-2,2,2-trichloroacetoimidate (471 mg, 1.70 mmol), **9** (320 mg, 1.13 mmol), $CHCl_3$ (1.4 mL), and cyclohexane (2.8 mL) under nitrogen atmosphere, and stirred for 1 h. Then trifluoromethanesulfonic acid (50 μL) was added to the reaction mixture. After the mixture was stirred for 2 h at room temperature, a formed precipitate was filtered off. Saturated $NaHCO_3$ was added to the filtrate with ice-cooling. The mixture was extracted with $CHCl_3$ (2×). The $CHCl_3$ layer was washed with saturated brine, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (hexane/AcOEt = 10:1) to yield **11ad** (210 mg, 46%) as a pale yellow oil. ^1H NMR (200 MHz, $CDCl_3$, TMS) δ 1.27 (3H, t, J = 7.5 Hz), 1.31 (3H, t, J = 7.0 Hz), 1.76 (1H, t, J = 3.1 Hz), 3.55 (1H, dd, J = 8.6, 7.3 Hz), 4.14 (2H, q, J = 7.5 Hz), 4.23–4.41 (2H, m), 4.46 (1H, d, J = 12.3 Hz), 4.61 (2H, s), 4.66 (1H, d, J = 12.3 Hz), 7.40–7.71 (4H, m); MS (ESI, Pos) m/z 421 (M^++Na).

5.1.6.2. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(2-cyanobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (13ad). A mixture of **11ad** (210 mg, 0.525 mmol), Me_3P (1 M THF solution, 0.58 mL), THF (7 mL), and H_2O (0.6 mL) was stirred for 20 h at room temperature. Ether was added to the reaction mixture, and the organic layer of the mixture was separated. The organic layer was washed with saturated $NaHCO_3$ and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 1:1–1:2) to yield **13ad** (59 mg, 30%) as a colorless oil. ^1H NMR (200 MHz, $CDCl_3$, TMS) δ 1.25 (3H, t, J = 7.0 Hz), 1.30 (3H, t, J = 7.0 Hz), 1.73 (1H, dd, J = 3.1, 3.1 Hz), 1.98–2.37 (4H, m), 3.48 (1H, dd, J = 8.8, 7.5 Hz), 4.11 (2H, q, J = 7.0 Hz), 4.27 (2H, m), 4.48 (1H, d, J = 12.3 Hz), 4.59 (1H, d, J = 12.3 Hz), 7.37–7.59 (4H, m); MS (ESI, Pos) m/z 395 (M^++Na); $[\alpha]_D^{24}$ +2.5 (c 0.71, $CHCl_3$); Anal. Calcd for $C_{16}H_{16}N_2O_5 \cdot 0.3H_2O$: C, 59.73; H, 5.20; N, 8.71. Found: C, 59.75; H, 5.15; N, 8.59.

5.1.6.3. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(2-cyanobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ad). A mixture of **13ad** (96 mg, 0.258 mmol), THF (4 mL), H_2O (2 mL), and $LiOH \cdot H_2O$ (27 mg, 0.643 mmol) was stirred for five days at room temperature. HCl (1 N) was added to the reaction mixture and stirred for 1 h at room temperature. The solution was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O–50% aqueous THF–10% aqueous pyridine) and reverse-phase silica gel (Wakogel®50C18, H_2O–30% MeCN) to yield **15ad** (46 mg, 5%) as a white powder. Mp > 230 °C (decomp.). ^1H NMR (300 MHz, D_2O, TMSP) δ 1.57–1.66 (1H, m), 1.94–2.45 (4H, m), 3.75–3.88 (1H, m), 4.60 (2H, s), 7.64 (4H, m); MS (ESI, Nega) m/z 315 (M^{--1}); $[\alpha]_D^{28}$ −1.1 (c 0.20, 1 N NaOH).

5.1.7. Method C

5.1.7.1. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Azido-3-((*R)-1-(3,4-dichlorophenyl)ethoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (15al) and (1*S*,2*R*,3*R*,5*R*,6*S*)-2-azido-3-((*S**)-1-(3,4-dichlorophenyl)ethoxy)bicyclo[3.1.0]-hexane-2,6-dicarboxylic acid diethyl ester (15am).** A THF (0.3 mL) solution of 1-(3,4-dichlorophenyl)ethanol (500 mg, 2.62 mol) was added to a THF (0.3 mL) suspension of NaH (60%, 10 mg, 0.25 mmol) and stirred for 0.5 h at room temperature. Trichloroacetonitrile (0.26 mL, 2.62 mol) was added to the mixture with ice-cooling and stirred for 2 h at room temperature. Pentane (0.46 mL) and methanol (9.3 μL) were added to the reaction mixture. After stirring for 10 min at room temperature, a formed precipitate was filtered off. The filtrate was concentrated under reduced pressure to yield crude 1-(3,4-dichlorophenyl)ethyl-2,2,2-trichloroacetoimidate (860 mg) as a brown viscous liquid.

Trifluoromethanesulfonic acid (14 μL) was added to the mixture of 1-(3,4-dichlorophenyl)ethyl-2,2,2-trichloroacetoimidate (548 mg, 1.64 mmol), **10** (375 mg, 1.09 mol), CH_2Cl_2 (1.4 mL), and cyclohexane (2.8 mL) under nitrogen atmosphere. After the mixture was stirred for 1 h at room temperature, a formed precipitate was filtered off. Saturated $NaHCO_3$ was added to the filtrate with ice-cooling. The mixture was extracted with $CHCl_3$ (2×). The $CHCl_3$ layer was washed with saturated brine, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified with silica gel column chromatography to yield **15al** (196 mg, 38%) and **15am** (238 mg, 46%).

Compound **15al**: R_f = 0.72 (hexane/AcOEt = 3:1, TLC; $60F_{254}$). ^1H NMR (300 MHz, D_2O, TMSP) δ 1.14–1.29 (6H, m), 1.63 (1H, dd, J = 2.6, 2.6 Hz), 1.90–2.29 (4H, m), 3.33 (1H, dd, J = 8.1, 8.1 Hz), 4.09 (2H, q, J = 7.2 Hz), 4.58 (1H, q, J = 6.2 Hz), 5.25 (1H, d, J = 11.9 Hz), 5.42 (1H, d, J = 11.9 Hz), 6.98-7.06 (1H, m), 7.24–7.49 (7H, m); MS (ESI, Pos) m/z 540 (M^++Na); $[\alpha]_D^{26}$ +78.5 (c 3.2, $CHCl_3$).

Compound **15am**: R_f = 0.65 (hexane/AcOEt = 3:1, TLC; $60F_{254}$), ^1H NMR (300 MHz, D_2O, TMSP) δ 1.18–1.29 (6H, m), 1.65 (1H, dd, J = 3.3, 3.3 Hz), 2.01–2.35 (4H, m), 3.44 (1H, dd, J = 7.9, 7.9 Hz), 4.10 (2H, q, J = 7.3 Hz), 4.32 (1H, q, J = 6.6 Hz), 5.16 (1H, d, J = 12.3 Hz), 5.33 (1H, d, J = 12.3 Hz), 7.03–7.11 (1H, m), 7.30–7.44 (7H, m); MS (ESI, Pos) m/z 540 (M^++Na); $[\alpha]_D^{27}$ −15.5 (c 3.2, $CHCl_3$).

5.1.7.2. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-((*R)-1-(3,4-dichlorophenyl)ethoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (14al).** A mixture of **12al** (189 mg, 0.360 mmol), Me_3P (1 M THF solution, 0.4 mL), THF (5.4 mL), and H_2O (0.5 mL) was stirred for 13 h at room temperature. Ether was added to the reaction mixture,

and the organic layer of the mixture was separated. The organic layer was washed with saturated $NaHCO_3$ and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 2:1–1:1) to yield **14al** (150 mg, 85%) as a colorless oil. ^1H NMR (200 MHz, $CDCl_3$, TMS) δ 1.21 (3H, t, J = 7.2 Hz), 1.23 (3H, d, J = 7.2 Hz), 1.59–1.62 (1H, m), 1.82–2.25 (4H, m), 3.22 (1H, dd, J = 8.6, 7.3 Hz), 3.72–3.78 (1H, m), 4.07 (2H, q, J = 7.2 Hz), 5.15 (1H, d, J = 12.3 Hz), 5.32 (1H, d, J = 12.3 Hz), 7.06 (1H, d, J = 8.4 Hz), 7.30–7.41 (7H, m); (ESI, Pos) m/z 622 (M^++Na); $[\alpha]_D^{28}$ +50.8 (c 1.19, $CHCl_3$).

5.1.7.3. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-((*S)-1-(3,4-dichlorophenyl)ethoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (14am).** Starting from **12am**, **14am** was obtained as an oil. Yield 89%. ^1H NMR (200 MHz, $CDCl_3$, TMS) δ 1.21 (3H, t, J = 7.2 Hz), 1.23 (3H, d, J = 7.2 Hz), 1.59–1.62 (1H, m), 1.82–2.25 (4H, m), 3.22 (1H, dd, J = 8.6, 7.3 Hz), 3.72–3.78 (1H, m), 4.07 (2H, q, J = 7.2 Hz), 5.15 (1H, d, J = 12.3 Hz), 5.32 (1H, d, J = 12.3 Hz), 7.06 (1H, d, J = 8.4 Hz), 7.30–7.41 (7H, m); (ESI, Pos) m/z 622 (M^++Na); $[\alpha]_D^{29}$ −39.1 (c 1.7, $CHCl_3$)

5.1.7.4. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-((*R)-1-(3,4-dichlorophenyl)ethoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15al).** A mixture of **14al** (146 mg, 0.30 mmol), THF (4.5 mL), H_2O (0.5 mL), and $LiOH{\cdot}H_2O$ (37 mg, 0.88 mmol) was stirred for four days at room temperature. HCl (1 N) was added to the reaction mixture and stirred for 10 min at room temperature. The solution was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O–50% aqueous THF–10% aqueous pyridine) to yield **15al** (87 mg, 77%) as a white powder. Mp > 277 °C (decomp.). ^1H NMR (300 MHz, D_2O, TMSP) δ 1.36 (3H, d, J = 6.1 Hz), 1.47–1.51 (1H, m), 1.87–2.11 (4H, m), 3.67 (1H, dd, J = 8.1 Hz), 4.54 (1H, q, J = 6.1 Hz), 7.26 (1H, d, J = 8.5 Hz), 7.52 (1H, s), 7.55 (1H, d, J = 8.5 Hz); MS (ESI, Nega) m/z 372 (M^-−1); $[\alpha]_D^{28}$ +88.4 (c 0.40, 1 N NaOH); Anal. Calcd for $C_{16}H_{17}Cl_2NO_5{\cdot}0.2H_2O$: C, 50.86; H, 4.64; N, 3.71. Found: C, 50.81; H, 4.58; N, 3.66.

5.1.7.5. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-((*S)-1-(3,4-dichlorophenyl)ethoxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15am).** Starting from **14am**, **15am** was obtained as an oil. Yield 13%. Mp > 239 °C (decomp.). ^1H NMR (300 MHz, D_2O, TMSP) δ 1.36 (3H, d, J = 6.5 Hz), 1.46–1.50 (1H, m), 1.95–2.04 (2H, m), 3.49 (1H, dd, J = 7.9, 7.9 Hz), 4.51 (2H, q, J = 6.5 Hz), 7.30 (1H, d, J = 8.4 Hz), 7.55 (1H, s), 7.57 (1H, d, J = 8.4 Hz); MS (ESI, Nega) m/z 372 (M^-−1); $[\alpha]_D^{28}$ −64.0 (c 0.24, 1 N NaOH); HR-MS Calcd for $C_{16}H_{16}Cl_2NO_5$: 372.0406. Found: 372.0424.

5.1.8. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Azido-6-fluoro-3-trifluoromethanesulfonyloxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (17). Tf_2O (78 μL, 0.47 mmol) was added to a mixture of **16** (120 mg, 0.40 mmol), CH_2Cl_2 (4 mL), and pyridine (48 μL, 0.59 mmol) with ice-cooling under nitrogen atmosphere and stirred for 1 h. After Et_2O (30 mL) was added to the reaction mixture, a formed precipitate was filtered off. The filtrate was concentrated under reduced pressure and chromatographed (hexane/AcOEt = 5:1) to yield **17** (166 mg, 96%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 1.35 (3H, t, J = 7.0 Hz), 1.38 (3H, t, J = 7.0 Hz), 2.35–2.50 (2H, m), 2.62–2.86 (2H, m), 4.31 (2H, q, J = 7.0 Hz), 4.27–4.55 (2H, m), 4.94–5.10 (1H, m). MS (FAB, Pos) m/z 434 (M^++1); $[\alpha]_D^{26}$ −41.4 (c 1.1, $CHCl_3$).

5.1.9. Method D

5.1.9.1. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Azido-3-(3,4-dichlorobenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (18at). Sodium (29 mg, 1.28 mmol) was dissolved to EtOH (1.28 mL). 3,4-dichlorobenzyl mercaptan (226 mg, 1.28 mmol) was added to the solution, stirred for 5 min, and evaporated under reduced pressure. A DMF solution (0.46 mL) of **17** (231 mg, 0.533 mmol) was added to the DMF solution (4.6 mL) of the residue and stirred for 30 min at room temperature. Et_2O was added to the reaction mixture, and the ethereal layer was washed with H_2O and saturated brine, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (hexane/AcOEt = 8:1) to yield **18at** (147 mg, 58%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 1.34 (3H, t, J = 7.0 Hz), 1.38 (3H, t, J = 7.0 Hz), 2.20–2.49 (4H, m), 2.99–3.13 (1H, m), 3.68 (1H, d, J = 13.6 Hz), 3.84 (1H, d, J = 13.6 Hz), 4.22–4.51 (4H, m), 7.16 (1H, dd, J = 8.1, 2.0 Hz), 7.34–7.46 (m, 2H); MS (ESI, Pos) m/z 498 (M^++Na); $[\alpha]_D^{24}$ +129.9 (c 0.48, $CHCl_3$).

5.1.9.2. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (19at). A mixture of **18at** (72 mg, 0.151 mmol), Me_3P (1 M THF solution, 166 μL), THF (2.2 mL), and H_2O (220 μL) was stirred for 4 h at room temperature. Ether was added to the reaction mixture, and the organic layer was separated. The organic layer was washed with saturated $NaHCO_3$ and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 3:1–2:1) to yield **29at** (43 mg, 63%) as a pale yellow oil. ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 1.31 (3H, t, J = 7.2 Hz), 1.35 (3H, t, J = 7.2 Hz), 2.08–2.15 (1H, m), 2.24–2.40 (3H, m), 2.86–2.93 (1H, m), 3.73 (1H, d, J = 13.4 Hz), 3.88 (1H, d, J = 13.4 Hz), 4.21–4.37 (4H, m), 7.15 (1H, dd, J = 8.2, 2.2 Hz), 7.36 (1H, d, J = 8.2 Hz), 7.42 (1H, d, J = 2.2 Hz); MS (ESI, Pos) m/z 472 (M^++Na); $[\alpha]_D^{23}$ +94.4 (c 0.25, $CHCl_3$).

5.1.9.3. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15at). A mixture of **19at** (41 mg, 91 μmol), THF (0.8 mL), H_2O (0.4 mL), and $LiOH{\cdot}H_2O$ (10 mg, 0.24 mmol) was stirred for three days at room temperature. $LiOH{\cdot}H_2O$ (2 mg, 0.05 mmol) was added to the reaction mixture and stirred for 2.5 days. The reaction mixture was acidified with 1 N HCl and stirred for 1 h at room temperature. The solution was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O–50% aqueous THF–10% aqueous pyridine) to yield **15at** (25 mg, 69%) as a white powder. Mp > 220 °C (decomp.). ^1H NMR (300 MHz, D_2O, TMSP) δ 2.17–2.48

(4H, m), 3.04–3.13 (1H, m), 3.80 (1H, d, J = 14.9 Hz), 3.85 (1H, d, J = 14.9 Hz), 7.31 (1H, d, J = 8.1 Hz), 7.53 (1H, d, J = 8.1 Hz), 7.59 (1H, s); MS (ESI, Nega) m/z 392 (M^--1); $[\alpha]_D^{30}$ +47.5 (c 0.41, 1 N NaOH); Anal. Calcd for $C_{15}H_{14}Cl_2NO_4S$: C, 45.70; H, 3.58; N, 3.55. Found: C, 45.49; H, 3.60; N, 3.47.

5.1.9.4. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(thiophen-2-ylmethylsulfanyl)-6- fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15an). Starting from **17** and 2-thienylmethanethiol, **15an** was obtained as a white powder. Mp > 230 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMS) δ 2.16–2.32 (2H, m), 2.41–2.50 (2H, m), 3.06–3.18 (1H, m), 4.07 (2H, s), 6.97–7.10 (2H, m), 7.35–7.43 (1H, m); MS (ESI, Nega) m/z 330 (M^--1); $[\alpha]_D^{25}$ +38.7 (c 0.35, 1 N NaOH); Anal. Calcd for $C_{13}H_{14}FNO_4S_2$: C, 47.12; H, 4.26; N, 4.24. Found: C, 47.00; H, 4.30; N, 4.19.

5.1.9.5. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(2-phenylbenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ao). Starting from **17** and 2-phenylbenzyl mercaptan, **15ao** was obtained as a white powder. Mp > 230 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMS) δ 2.08–2.35 (4H, m), 2.87–3.00 (1H, m), 3.78–4.01 (2H, m), 7.26–7.62 (9H, m); MS (ESI, Nega) m/z 440 (M^--1); $[\alpha]_D^{24}$ +49.3 (c 0.21, 1 N NaOH); Anal. Calcd for $C_{21}H_{20}FNO_4S\cdot0.5H_2O$: C, 61.45; H, 5.16; N, 3.41. Found: C, 61.46; H, 5.08; N, 3.44.

5.1.9.6. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(4-methoxybenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ap). Starting from **17** and 4-methoxylbenzyl mercaptan, **15ap** was obtained as a white powder. Mp > 240 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMS) δ 2.16–2.42 (4H, m), 3.03–3.13 (1H, m), 3.77–3.87 (5H, m), 7.00 (2H, d, J = 8.7 Hz), 7.35 (2H, d, J = 8.7 Hz); MS (ESI, Nega) m/z 440 (M^--1); $[\alpha]_D^{26}$ +31.0 (c 0.48, 1 N NaOH); Anal. Calcd for $C_{16}H_{18}NO_5S_2$: C, 54.07; H, 5.11; N, 3.94. Found: C, 53.83; H, 5.12; N, 3.92.

5.1.9.7. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(4-fluorobenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15aq). Starting from **17** and 4-fluorobenzyl mercaptan, **15aq** was obtained as a white powder. Mp > 243 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP) δ 2.13–2.54 (4H, m), 2.97–3.19 (1H, m), 3.84 (2H, s), 7.03–7.29 (2H, m), 7.33–7.55 (2H, m); MS (ESI, Nega) m/z 342 (M^--1); $[\alpha]_D^{25}$ +25.4 (c 0.20, 1 N NaOH); Anal. Calcd for $C_{15}H_{13}Cl_2FNO_5S_2\cdot0.1H_2O$: C, 52.20; H, 4.44; N, 4.06. Found: C, 52.00; H, 4.48; N, 4.06.

5.1.9.8. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(4-*tert*-butylbenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ar). Starting from **16** and 4-*tert*-butylbenzyl mercaptan, **15ar** was obtained as a white powder. Mp > 271 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 1.31 (9H, s), 2.20–2.47 (4H, m), 2.06–3.20 (1H, m), 3.83 (2H, s), 7.32–7.40 (2H, m), 7.46–7.56 (2H, m); MS (ESI, Nega) m/z 380 (M^--1); $[\alpha]_D^{26}$ −1.1 (c 0.63, 1 N NaOH); Anal. Calcd for $C_{19}H_{24}Cl_2FNO_4S\cdot0.2H_2O$: C, 59.26; H, 6.39; N, 3.64. Found: C, 59.40; H, 6.34; N, 3.61.

5.1.9.9. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(3-trifluoromethylbenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15as). Starting from **17** and 3-trifluoromethylbenzyl mercaptan, **15as** was obtained as a white powder. Mp > 271 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 2.16–2.47 (4H, m), 3.04–3.15 (1H, m), 3.89 (1H, d, J = 13.2 Hz), 3.94 (1H, d, J = 13.2 Hz), 7.50–7.78 (4H, m); MS (ESI, Nega) m/z 392 (M^--1); $[\alpha]_D^{24}$ +38.9 (c 0.36, 1 N NaOH); Anal. Calcd for $C_{16}H_{15}F_4NO_4S$: C, 48.85; H, 3.84; N, 3.56. Found: C, 48.75; H, 3.87; N, 3.42.

5.1.9.10. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(2,6-difluoro-3-chlorobenzylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15au). Starting from **17** and 2,6-difluoro-4-chlorobenzyl mercaptan, **15au** was obtained as a white powder. Mp > 234 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 2.09–2.62 (4H, m), 3.11–3.40 (1H, m), 3.79–4.11 (2H, m), 6.87–7.23 (1H, m), 7.32–7.61 (1H, m); MS (ESI, Nega) m/z 394 (M^--1); $[\alpha]_D^{24}$ +66.9 (c 0.23, 1 N NaOH); Anal. Calcd for $C_{15}H_{13}ClF_3NO_4S$: C, 45.52; H, 3.31; N, 3.54. Found: C, 45.47; H, 3.40; N, 3.27.

5.1.9.11. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(2-phenylethylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15av). Starting from **17** and 1-phenylethanethiol, **15av** was a mixture of diasteromers obtained as a white powder. Mp > 307 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 1.48–1.62 (3H, m), 1.82–1.94 (1H, m), 2.05–2.32 (2H, m), 2.43–2.71 (1H, m), 2.83–3.03 (1H, m), 4.04–4.24 (1H, m), 7.27–7.52 (5H, m); MS (ESI, Nega) m/z 338 (M^--1); Anal. Calcd for $C_{16}H_{18}FNO_4S\cdot0.1H_2O$: C, 56.33; H, 5.38; N, 4.11. Found: C, 56.23; H, 5.44; N, 4.21.

5.1.9.12. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(1-bis(4-fluorophenyl)methylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15aw). Starting from **17** and bis(4-fluorophenyl)methanethiol, **15aw** was obtained as a white powder. Mp > 234 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 2.06–2.60 (4H, m), 2.91–3.08 (1H, m), 5.43 (1H, s), 6.99–7.25 (2H, m), 7.39–7.65 (4H, m); MS (ESI, Nega) m/z 436 (M^--1); $[\alpha]_D^{24}$ +15.4 (c 0.36, 1 N NaOH); Anal. Calcd for $C_{21}H_{18}F_3NO_4S\cdot0.5H_2O$: C, 56.50; H, 4.29; N, 3.14. Found: C, 56.54; H, 4.38; N, 3.11.

5.1.9.13. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorophenylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ax). Starting from **17** and 3,4-dichlorobenzyl mercaptan, **15ax** was obtained as a white powder. Mp > 234 °C (decomp.). ^{1}H NMR (500 MHz, D_2O, TMSP): δ 1.60 (1H, dd, J = 3.1, 3.1 Hz), 1.93–1.96 (1H, m), 2.03–2.05 (1H, m), 2.15–2.19 (2H, m), 2.69 (1H, dd, J = 8.5, 9.7 Hz), 3.77 (1H, d, J = 14.0 Hz), 3.81 (1H, d, J = 14.0 Hz), 7.31 (1H, dd, J = 8.5, 1.8 Hz), 7.53 (1H, d, J = 8.5 Hz), 7.58 (1H, d, J = 1.8 Hz); MS (ESI, Nega) m/z 374 (M^--1); $[\alpha]_D^{28}$ +22.0 (c 0.033, 1 N NaOH); Anal.

Calcd for $C_{14}H_{12}Cl_2FNO_4S$: C, 44.22; H, 3.18; N, 3.68. Found: C, 44.45; H, 3.34; N, 3.59.

5.1.10. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Azido-3-(3,4-dichlorophenylsulfinyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (20). After *m*-CPBA (32 mg, 0.12 mmol) was added to a mixture of **18at** (73 mg, 0.153 mmol) and CH_2Cl_2 (1.46 mL) in dry ice bath, the mixture was stirred for 1 h. The mixture was stirred for 3.5 h with ice-cooling and stirred at room temperature for 11 h. After *m*-CPBA (15 mg, 0.06 mmol) was added to the reaction mixture in dry ice bath, the mixture was stirred for 1 h. Then the mixture was stirred for 4 h with ice-cooling. Saturated $NaHCO_3$ was added to the reaction mixture, and the organic layer was separated. The organic layer was washed with saturated brine, dried over with Na_2SO_4, evaporated, and chromatographed ($CHCl_3$/EtOH = 4:1–2:1) to yield **20** (63 mg, 84%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.36 (3H, t, J = 7.0 Hz), 1.38 (3H, t, J = 7.0 Hz), 2.33 (1H, dd, J = 14.1, 8.4 Hz), 2.43–2.61 (2H, m), 2.80–2.97 (1H, m), 3.11–3.24 (1H, m), 3.79 (1H, d, J = 13.2 Hz), 4.09 (1H, d, J = 13.2 Hz), 4.25–4.43 (4H, m), 7.17 (1H, dd, J = 8.4, 2.2 Hz), 7.40–7.50 (2H, m); MS (ESI, Pos) *m*/*z* 514 (M^++Na); $[\alpha]_D^{28}$ +36.0 (*c* 0.49, $CHCl_3$).

5.1.11. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorophenylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (21). A mixture of **20** (61 mg, 0.124 mmol), Me_3P (1 M THF solution, 136 μL), THF (1.7 mL), and H_2O (170 μL) was stirred for 2 h at room temperature. Ether was added to the reaction mixture, and the organic layer was separated. The organic layer was washed with saturated $NaHCO_3$ and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed ($CHCl_3$/EtOH = 50:1) to get residue. The residue was purified with column chromatography (hexane/AcOEt = 2:5) to yield **21** (41 mg, 71%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.34 (3H, t, J = 7.0 Hz), 1.35 (3H, t, J = 7.0 Hz), 2.30–2.43 (3H, m), 2.78–3.12 (2H, m), 3.80 (1H, d, J = 13.2 Hz), 4.19–4.36 (5H, m), 7.17 (1H, dd, J = 8.4, 2.2 Hz), 7.44 (1H, d, J = 8.4 Hz), 7.44 (1H, d, J = 2.2 Hz); MS (ESI, Pos) *m*/*z* 488 (M^++Na); $[\alpha]_D^{29}$ +59.1 (*c* 0.32, $CHCl_3$).

5.1.12. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorophenylsulfanyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15ay). In a manner similar to the preparation of **15at, 15ay** (17 mg, 52%) was obtained from **21** (38 mg, 81.5 μmol) as a white powder. Mp > 160 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 2.16–2.29 (2H, m), 2.44–2.49 (2H, m), 2.77–2.88 (2H, m), 3.44–3.53 (1H, m), 4.05 (1H, d, J = 13.1 Hz), 4.26 (1 H. d, J = 13.1 Hz), 7.29 (1H, d, J = 8.5 Hz), 7.56 (1H, s), 7.60 (1H, d, J = 8.5 Hz); MS (ESI, Nega) *m*/*z* 408 (M^-−1). $[\alpha]_D^{25}$ +79.7 (*c* 0.30, 1 N NaOH); HR MS calcd for $C_{15}H_{13}Cl_2FO_5S$: 407.9876. Found: 407.9893.

5.1.13. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Azido-3-(3,4-dichlorophenylsulfonyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (22). After *m*-CPBA (274 mg, 1.03 mmol) was added to a mixture of **18at** (197 mg, 0.414 mmol), and CH_2Cl_2 (3.94 mL) with ice-cooling, the mixture was stirred for 3 h. Saturated $NaHCO_3$ was added to the mixture, and the organic layer was separated. The organic layer was washed with saturated $NaHCO_3$ and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 5:1) to yield **22** (193 mg, 91%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.36 (3H, t, J = 7.0 Hz), 1.39 (3H, t, J = 7.0 Hz), 2.33–2.58 (3H, m), 2.86–3.05 (1H, m), 3.53 (1H, dd, J = 11.2, 8.1 Hz), 4.24–4.46 (6H, m), 7.28 (1H, dd, J = 8.4, 2.2 Hz), 7.44–7.56 (2H, m); MS (ESI, Pos) *m*/*z* 530 (M^++Na); $[\alpha]_D^{29}$ +7.9 (*c* 0.70, $CHCl_3$).

5.1.14. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorophenylsulfonyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (23). In a manner similar to the preparation of **21**, **23** (346 mg, 97%) was obtained from **22** (377 mg, 0.742 mmol) as a colorless oil. Mp > 160 °C (decomp.). ^{1}H NMR (200 MHz, D_2O, TMSP): δ 1.34 (3H, t, J = 7.0 Hz), 1.36 (3H, t, J = 7.0 Hz), 2.28–2.42 (3H, m), 2.83–3.01 (1H, m), 3.41–3.53 (1H, m), 4.23–4.37 (6H, m), 7.28 (1H, dd, J = 8.4, 1.8 Hz), 7.46 (1H, d, J = 8.4 Hz), 7.55 (1H, d, J = 1.8 Hz); MS (ESI, Pos) *m*/*z* 482 (M^++1); $[\alpha]_D^{29}$ +24.0 (*c* 0.86, $CHCl_3$).

5.1.15. (1*R*,2*S*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorophenylsulfonyl)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15az). A mixture of **23** (108 mg, 0.224 mmol) and 60% H_2SO_4 (1.08 mL) was heated at 130 °C for three days using a sealed tube. After cooling, the mixture was neutralized with 5 N NaOH and stirred for 1 h at room temperature. The solution was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O–50% aqueous THF–10% aqueous pyridine) to yield **15az** (76 mg, 80%) as a white powder. Mp > 230 °C (decomp.). ^{1}H NMR (300 MHz, D_2O): δ 2.33–2.45 (3H, m), 2.82–2.94 (1H, m), 3.98 (1H, dd, J = 10.1, 9.5 Hz), 4.55 (1H, d, J = 15.3 Hz), 4.60 (1H, d, J = 15.3 Hz), 7.37 (1H, d, J = 8.6 Hz), 7.63 (1H, d, J = 8.3 Hz), 7.64 (1H, s); MS (ESI, Nega) *m*/*z* 424 (M^-−1); $[\alpha]_D^{25}$ −5.1 (*c* 0.72, 1 N NaOH); Anal. Calcd for $C_{15}H_{14}Cl_2FNO_6S$: C, 42.27; H, 3.31; N, 3.29. Found: C, 42.60; H, 3.44; N, 3.22.

5.1.16. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-Amino-6-fluoro-3-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (25). A mixture of **16** (245 mg, 0.813 mmol), 1M Me_3P/THF (0.89 mL), THF (7.0 mL), and H_2O (0.7 mL) was stirred for 12 h at room temperature. Et_2O and saturated $NaHCO_3$ were added to the mixture, and the resultant mixture was stirred for 1 h. The aqueous layer was separated and extracted with $CHCl_3$. The combined organic layer was washed with saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 50:1) to yield **25** (163 mg, 73%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ 1.32 (6H, t, J = 7.3 Hz), 2.07–2.23 (2H, m), 2.41 (1H, dd, J = 8.1, 3.3 Hz), 2.71–2.91 (1H, m), 4.10–4.41 (5H, m); MS (ESI, Nega) *m*/*z* 276 (M^++1); $[\alpha]_D^{26}$ +2.8 (*c* 1.5, $CHCl_3$).

5.1.17. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-(*tert*-Butoxycarbonylamino)-6-fluoro-3-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (26). A mixture of **25** (160 mg, 0.581 mmol), Boc_2O (152 mg, 0.697 mmol), saturated $NaHCO_3$ (0.8 mL), and THF (0.8 mL) was stirred for 4 h at room temperature. The mixture was extracted with AcOEt, and the organic layer was washed with saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 2:1) to yield **26** (214 mg, 98%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.29 (3H, t, J = 7.0 Hz), 1.30 (3H, t, J = 7.0 Hz), 1.44 (9H, s), 2.20–2.48 (3H, m), 2.77–2.98 (2H, m), 4.07–4.48 (5H, m), 5.57 (1H, s); MS (ESI, Nega) m/z 398 (M^++Na); $[\alpha]_D^{22}$ −14.0 (c 0.90, $CHCl_3$).

5.1.18. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-(*tert*-Butoxycarbonylamino)-6-fluoro-3-trifluoromethanesulfonyloxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (27). In a manner similar to the preparation of **17** from **16**, **27** (84 mg, 71%) was obtained from **26** (150 mg, 0.296 mmol) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ 1.25–1.41 (6H, m), 1.44 (9H, s), 2.13–2.26 (1H, m), 2.40–2.57(2H, m), 2.97–3.20 (1H, m), 4.14–4.47 (4H, m), 5.32 (1H, s), 5.99 (1H, d, J = 8.4 Hz); MS (ESI, Nega) m/z 506 (M^-−1); $[\alpha]_D^{28}$ +79.8 (c 0.50, $CHCl_3$).

5.1.19. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-(*tert*-Butoxycarbonylamino)-3-azido-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (28). A mixture of **27** (150 mg, 0.296 mmol), NaN_3 (29 mg, 0.443 mmol), and DMF (1,5 mL) was stirred for 4 h at room temperature and stirred for 26 h at 30 °C. Et_2O was added to the mixture, and the ethereal layer was washed with H_2O and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 5:1–3:1) to yield **28** (84 mg, 71%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ 1.29 (3H, t, J = 7.0 Hz), 1.33 (3H, t, J = 7.0 Hz), 1.45 (9H, s), 2.21–2.56 (3H, m), 2.92 (1H, dd, J = 7.7, 2.4 Hz), 3.78–3.88 (1H, m), 4.17–4.41 (4H, m), 5.01 (1H, s); MS (ESI, Pos) m/z 423 (M^++1); $[\alpha]_D^{26}$ +0.79 (c 1.4, $CHCl_3$).

5.1.20. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-(*tert*-Butoxycarbonylamino)-3-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (29). A mixture of **28** (78 mg, 0.195 mmol), 5% Pd/C (8 mg), and EtOH (0.8 mL) was stirred for 12 h under hydrogen atmosphere at room temperature. The mixture was filtered using Celite pad. The filtrate was concentrated under reduced pressure. The residue was purified with silica gel column chromatography (hexane/AcOEt = 50:1–20:1) to give **29** (44 mg, 60%) as a colorless solid. ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ 1.30 (3H, t, J = 7.0 Hz), 1.32 (3H, t, J = 7.0 Hz), 1.44 (9H, s), 2.06–2.27 (2H, m), 2.40–2.55 (1H, m), 2.61–2.72 (1H, m), 3.28–3.47 (1H, m), 4.17–4.41 (4H, m), 5.05 (1H, s); MS (ESI, Pos) m/z 397 (M^++Na); $[\alpha]_D^{27}$ −14.2 (c 1.4, $CHCl_3$).

5.1.21. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-(*tert*-Butoxycarbonylamino)-3-(*N*-3,4-chlorobenzylamino)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (30). After 3,4-dichlorobenzyl bromide (36 mg, 0.150 mmol) was added to a mixture of **29** (51 mg, 0.136 mmol), pyridine (12 μL, 0.150 mmol), and $CHCl_3$ (0.25 mL) with ice-cooling, the mixture was stirred for 19 h at room temperature. H_2O was added to the mixture, and the organic layer was separated. The aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with saturated brine, dried with Na_2SO_4, and evaporated to yield **30** (31 mg, 43%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ 1.23–1.34 (6H, m), 1.44 (9H, s), 2.03–2.26 (2H, m), 2.43 (1H, dd, J = 13.0, 7.3 Hz), 2.83–2.93 (1H, m), 3.02–3.15 (1H, m), 3.71 (1H, d, J = 13.2 Hz), 3.80 (1H, d, J = 13.2 Hz), 4.12–4.39 (4H, m), 4.82 (1H, s), 7.11 (1H, dd, J = 8.1, 2.0 Hz), 7.33–7.45 (2H, m); MS (ESI, Nega) m/z 531 (M^-−1); $[\alpha]_D^{27}$ −15.1 (c 0.52, $CHCl_3$).

5.1.22. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-(*tert*-Butoxycarbonylamino)-3-(*N*-3,4-chlorobenzyl-*N*-methylamino)-6-fluorobicyclo-[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (31). A mixture of **30** (136 mg, 0.255 mmol), MeI (64 mL, 1.02 mmol), K_2CO_3 (71 mg, 0.510 mmol), and DMF was stirred for three days at room temperature. Saturated $Na_2S_2O_3$ was added to the mixture and extracted with AcOEt. The AcOEt layer was washed with saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt = 5:1) to yield **31** (126 mg, 90%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.28 (3H, t, J = 7.0 Hz), 1.29 (3H, t, J = 7.0 Hz), 1.43 (9H, s), 2.11 (3H, s), 2.16–2.58 (3H, m), 2.80–3.07 (2H, m), 3.29 (1H, d, J = 13.6 Hz,), 3.78 (1H, d, J = 13.6 Hz), 4.05–4.43 (4H, m), 4.86 (1H, s), 7.08 (1H, dd, J = 8.4, 1.8 Hz), 7.31–7.41 (2H, m); MS (ESI, Pos) m/z 547 (M^++1); $[\alpha]_D^{25}$ −51.9 (c 0.50, $CHCl_3$).

5.1.23.

5.1.23.1. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-Amino-3-(*N*-3,4-chlorobenzylamino)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (14ba). A mixture of **30** (28 mg, 52.5 μmol) and 4 M HCl/AcOEt was stirred for 6 h with ice-cooling. Saturated $NaHCO_3$ was added to the mixture, and the organic layer was separated. The aqueous layer was extracted with AcOEt. The combined AcOEt layer was washed with saturated brine, dried with Na_2SO_4, and evaporated to yield **14ba** (21 mg, 91%) as a pale yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.31 (3H, t, J = 7.0 Hz), 1.34 (3H, t, J = 6.2 Hz), 2.03–2.28 (3H, m), 2.35–2.51 (1H, m), 2.94–3.08 (1H, m), 3.77 (2H, s), 4.16–4.40 (4H, m), 7.12 (1H, d, J = 7.9 Hz), 7.35 (1H, d, J = 7.9 Hz), 7.40 (1H, s); MS (ESI, Pos) m/z 433 (M^++1); $[\alpha]_D^{24}$ −8.4 (c 0.56, $CHCl_3$).

5.1.23.2. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-Amino-3-(*N*-3,4-chlorobenzyl-*N*-methylamino)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (14bb). Starting from **31**, **14bb** was obtained as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ 1.33 (3H, t, J = 7.0 Hz), 1.35 (3H, t, J = 7.0 Hz), 2.06 (3H, s), 2.03–2.21 (1H, m), 2.23–2.60 (3H, m), 2.68–2.84 (1H, m), 3.22 (1H, d, J = 14.1 Hz), 3.97 (1H, d, J = 14.1 Hz), 4.18–4.32 (4H, m), 7.07 (1H, dd, J = 8.1, 2.0 Hz), 7.30–7.39 (2H, m); MS (ESI, Pos) m/z 447 (M^++1); $[\alpha]_D^{23}$ −24.9 (c 0.84, $CHCl_3$).

5.1.24.

5.1.24.1. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-amino-3-(*N*-3,4-chlorobenzylamino)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic (15ba). In a manner similar to the preparation of **15ay, 15ba** (17 mg, 71%) was obtained from **14ba** (28 mg, 64.6 μmol) as a white powder. Mp > 190 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 2.31–2.77 (4H, m), 3.59–3.74 (1H, m), 4.06 (1H, d, J = 13.5 Hz), 4.15 (1H, m, J = 13.5 Hz), 7.35 (1H, d, J = 7.77 Hz), 7.58–7.64 (2H, m); MS (ESI, Nega) m/z 375 (M^-−1); $[\alpha]_D^{27}$ −14.6 (c 0.29, 1 N NaOH); Anal. Calcd for $C_{15}H_{13}Cl_2FN_2O_4{\cdot}H_2O$: C, 45.59; H, 4.08; N, 7.09. Found: C, 45.67; H, 4.29; N, 6.99.

5.1.24.2. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-Amino-3-(*N*-3,4-chlorobenzyl-*N*-methylamino)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15bb). Starting from **14bb**, **15bb** was obtained as a white powder. Mp > 164 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 2.31–2.41 (1H, m), 2.45–2.53 (1H, m), 2.64 (3H, s), 2.73–2.82 (2H, m), 3.72–3.82 (1H, m), 4.01 (1H, d, J = 13.4 Hz), 4.27 (1H, d, J = 13.4 Hz), 7.35–7.41 (1H, m), 7.61–7.69 (2H, m); MS (ESI, Nega) m/z 389 (M^-−1); $[\alpha]_D^{24}$ −35.2 (c 0.51, 1 N NaOH). HR MS calcd for $C_{16}H_{16}Cl_2FN_2O_4$: 389.0483. Found: 389.0471.

5.1.25.

5.1.25.1. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-(*tert*-Butoxycarbonylamino)-3-(*N*-3,4-chlorobenzoylamino)-6-fluorobicyclo[3.1.0]-hexane-2,6-dicarboxylic acid diethyl ester (32). A mixture of **29** (17 mg, 45.4 μmol), 3,4-dichlorobenzoyl chloride (14 mg, 68.1 μmol), pyridine (7.3 μL, 90.8 μmol), and $CHCl_3$ (0.17 mL) was stirred for 3 h at room temperature. The mixture was concentrated under reduced pressure and chromatographed ($CHCl_3$/AcOEt = 100:1) to yield **3** (21 mg, 84%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ 1.19 (3H, t, J = 7.0 Hz), 1.31 (3H, t, J = 7.3 Hz), 1.41 (9H, s), 2.21–2.64 (3H, m), 2.82–2.91 (1H, m), 4.07–4.37 (4H, m), 4.58-4.75 (1H, m), 6.20 (1H, s), 6.39–6.50 (1H, m), 7.46–7.57 (2H, m), 7.80–7.85 (1H, m); MS (ESI, Nega) m/z 545 (M^-−1). $[\alpha]_D^{23}$ +12.1 (c 0.95, $CHCl_3$).

5.1.25.2. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-Amino-3-(3,4-chlorobenzoylamino)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethyl ester (14bc). Starting from **32**, **14bc** was obtained as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.30 (3H, t, J = 6.8 Hz), 1.33 (3H, t, J = 7.0 Hz), 2.09–2.43 (3H, m), 2.53–2.38 (1H, m), 4.19–4.38 (4H, m), 4.52–4.71 (1H, m), 7.48–7.55 (2H, m), 7.81–7.91 (1H, m).

5.1.25.3. (1*R*,2*R*,3*S*,5*R*,6*R*)-2-Amino-3-(3,4-chlorobenzoylamino)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (15bc). Starting from **14bc**, **15bc** was obtained as a white powder. Mp > 210 °C (decomp.). ^{1}H NMR (300 MHz, D_2O, TMSP): δ 2.33–2.42 (2H, m), 2.57–2.67 (2H, m), 4.46–4.55 (1H, m), 7.58–7.68 (2H, m), 7.87–7.90 (1H, m); MS (ESI, Nega) m/z 389 (M^-−1); $[\alpha]_D^{28}$ +6.0 (c 0.34, 1 N NaOH); Anal. Calcd for $C_{15}H_{13}Cl_2FN_2O_5{\cdot}0.3H_2O$: C, 45.43; H, 3.46, N; 7.06. Found: C, 45.58; H, 3.73; N, 6.91.

5.2.

5.2.1. Pharmacology. *Cell culture:* CHO cell line stably expressing rat mGluR2 was cultured in DMEM supplemented with 10% dialyzed fetal bovine serum, 2 mM glutamine, 1% proline, 1 mM sodium pyruvate, 1 mM succinic acid, 50 U/mL penicillin, and 50 μg/mL streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

5.2.1.1. [^{3}H]-(1*S*,2*S*,3*S*,5*R*,6*S*)-2-Amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid ([^{3}H]MGS0008) binding. The binding of [^{3}H]MGS0008 (34 Ci/mmol) was performed according to the method previously described.[11] CHO cells stably expressing mGluR2 were collected by centrifugation at 1000 rpm for 5 min. The cells were homogenized with 50 mM Tris–HCl buffer (pH 7.4) and centrifuged at 48,000g for 20 min at 4 °C. The pellet was suspended in 50 mM Tris–HCl buffer (pH 7.4), and incubated at 37 °C for 15 min, after which the pellet was washed twice with 50 mM Tris–HCl buffer (pH 7.4) by resuspension and recentrifugation. The pellet obtained was then suspended in 50 mM Tris–HCl buffer (pH 7.4) containing 2 mM $MgCl_2$ and served as a crude membrane preparation.

For a typical binding experiment, the reaction was initiated by incubating 0.5 mL of the crude membrane preparation with [^{3}H]MGS0008. The reaction mixture was incubated for 1 h at 25 °C. The reaction was terminated by rapid filtration through Whatman GF/C glass fiber filters presoaked with 0.3% polyethyleneimine, after which the filters were washed three times with 3 mL of ice-cold 50 mM HEPES buffer (pH 7.4), using a multicell harvester M-48 (Brandel Biomedical Research and Development Laboratories, Inc, Gaithersburg, MD, USA). Aquazol-2 scintillator (Perkin-Elmer, Wellesley, MA, USA) (10 mL) was added, and filter-bound radioactivity was counted in a liquid scintillation spectrometer (LS6000TA, Beckman Instruments Inc, Fullerton, CA, USA). Nonspecific binding was determined in the presence of 10 μM LY354740.

In the competition binding assay, the reaction was carried out using 3.0 nM [^{3}H]MGS0008. The concentration of the test compound that caused 50% inhibition of specific binding of [^{3}H]MGS0008 (IC_{50} value) was determined from each concentration–response curve.

5.2.1.2. Measurements of cAMP formation. Agonist activities for mGluR2 were evaluated by measuring an agonist-dependent inhibition of forskolin-induced cyclic AMP formation in mGluR2 expressing CHO cells. Briefly, CHO cells[11] expressing mGluR2 were seeded in a 96-well plate at a density of 1.26×10^4 cells per well and grown for two days. The medium was changed to fresh medium without 2 mM glutamine, and the cells were incubated for 4–5 h. The cells were pre-incubated with PBS containing 1 mM 3-isobutyl-1-methylxanthine (IBMX) (PBS-IBMX) for 20 min at 37 °C. The reaction was started by replacing the medium with fresh PBS-IBMX containing 10 μM forskolin and various concen-

trations of test agents. After incubation for 15 min at 37 °C, the reaction was terminated by adding ice-cold 100% ethanol and allowed to settle on ice for 40 min. The supernatants were evaporated and cAMP levels were determined by cAMP enzyme immunoassay (EIA) system (Amersham). Antagonist activities of compounds were measured under 30 μM glutamic acid condition, and the test compound was added to the cells 20 min before addition of glutamic acid.

5.3.

5.3.1. Pharmacokinetics evaluation. Each compounds was administered to male Wistar or Sprague–Dawley rats (Charles River, Japan) orally. Blood was collected from the tail vein, and plasma was separated by centrifugation (11,200*g*, 4 °C, 3 min) and stored at −80 °C until bioanalysis. Deproteinized supernatant with methanol of plasma samples was analyzed by liquid chromatography with mass detection on an Agilent ZORBAX SB-C18 column (5 μm, 50 × 2.1 mm). The analyte was eluted with linear gradient mobile phase by increasing acetonitrile concentration in 0.1% acetic acid from 5% to 95% over 4 min at a flow rate of 250 μL/min. Tandem mass spectrometric detection was carried out using TurboIonSpray (AB/MDS Sciex API3000) in positive ion mode. The lower limits of quantitation (LLOQ) were 1 ng/mL.

Acknowledgments

The authors thank Professor Sigetada Nakanishi at Department of Biological Sciences, Kyoto University Faculty of Medicine, for provision of CHO cell line stably expressing rat mGluR2.

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