Synthesis, SARs, and Pharmacological Characterization of 2-Amino-3 or 6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid Derivatives as Potent, Selective, and Orally Active Group II Metabotropic Glutamate Receptor Agonists

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(+)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (**4**, LY354740), a highly selective and orally active group II metabotropic glutamate receptor (mGluR) agonist, has increased interest in the study of group II mGluRs. Our interest focused on a conformationally constrained form of compound **4**, because it appeared that the rigid form resulted in not only selectivity for group II mGluR but was orally active. Therefore, we introduced a fluorine atom to compound **4**, based on the molecular size (close resemblance to hydrogen atom) and electronegativity (effects on the electron distribution in the molecule) of this atom and carbon−fluorine bond energy. Compound (+)-**7** (MGS0008), the best compound among 3-fluoro derivatives **7**−**10**, retained the agonist activity of compound **4** for mGluR2 and mGluR3 ((+)-**7**: $EC_{50} = 29.4 \pm 3.3$ nM and 45.4 ± 8.4 nM for mGluR2 and mGluR3, respectively; **4**: $EC_{50} = 18.3 \pm 1.6$ nM and 62.8 ± 12 nM for mGluR2 and mGluR3, respectively) and increased the oral activity of compound **4** ((+)-**7**: $ED_{50} = 5.1$ mg/kg and 0.26 mg/kg for phencyclidine (PCP)-induced hyperactivy and PCP-induced head-weaving behavior, respectively; **4**: $ED_{50} = >100$ mg/kg and 3.0 mg/kg for PCP-induced hyperactivity and PCP-induced head-weaving behavior, respectively). In addition, a compound [^{3}H]-(+)-**7** binding study using mGluR2 or 3 expressed in CHO cells was successful ((+)-**7**: $K_i = 47.7 \pm 17$ nM and 65.9 ± 7.1 nM for mGluR2 and mGluR3, respectively; **4**: $K_i = 23.4 \pm 7.1$ nM and 53.5 ± 13 nM for mGluR2 and mGluR3, respectively). On the basis of a successful result of compound **7**, we focused on the introduction of a fluorine atom on the C6 position of compound **4**. (1*R*,2*S*,5*R*,6*R*)-2-Amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid ((−)-**11**) exhibited a high degree of agonist activity for group II mGluRs equal to that of compound **4** or **7** ((−)-**11**: $K_i = 16.6 \pm 5.6$ and 80.9 ± 31 nM for mGluR2 and mGluR3, respectively). Our interest shifted to modification on CH_2 at C4 position of compound **11**, since replacement of the CH_2 group with either an oxygen atom or sulfur atom yielded compound **5** or **6**, resulting in increased agonist activity. We selected a carbonyl group instead of CH_2 at the C4 position of compound **11**. The carbonyl group might slightly change the relative conformation of three functional groups, the amino group and two carboxylic acids, which have important roles in mediating the interaction between group II mGluRs and their ligand, compared with the CH_2 group of **4**, oxygen atom of **5**, and sulfur atom of **6**. (1*R*,2*S*,5*S*,6*S*)-2-Amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic acid monohydrate ((+)-**14**, MGS0028) exhibited a remarkably high degree of agonist activity for mGluR2 ($K_i = 0.570 \pm 0.10$ nM) and mGluR3 ($K_i = 2.07 \pm 0.40$ nM) expressed in CHO cells but not mGluR4, 6, 7, 1a, or 5 expressed in CHO cells ($K_i = >100\,000$ nM). Furthermore, compound (+)-**14** strongly inhibited phencyclidine (PCP)-induced head-weaving behavior ($ED_{50} = 0.090$ μg/kg) and hyperactivity ($ED_{50} = 0.30$ mg/kg) in rats. Thus, (+)-**7** and (+)-**14** are potent, selective, and orally active group II mGluR agonists and might be useful not only for exploring the functions of mGluRs but in the treatment of schizophrenia.

Introduction

L-Glutamate (**1**, Chart 1) is a neurotransmitter at the vast majority of excitatory synapses in the brain. Normal functioning of glutamatergic synapses is required for all major functions of the brain.[1,2] At present, glutamate receptors are broadly classified into two types: the ionotropic glutamate receptors (iGluRs), in which the receptors have an ion channel structure, and the metabotropic glutamate receptors (mGluRs), which are coupled to G-proteins. iGluRs are classified pharmacologically into three subtypes: *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl isoxazole-4-

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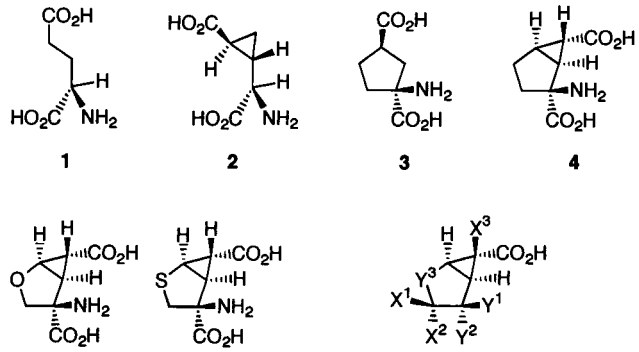
Chart 1



	X^1	X^2	X^3	Y^1	Y^2	Y^3
7	F	H	H	NH_2	CO_2H	CH_2
8	H	F	H	NH_2	CO_2H	CH_2
9	H	F	H	CO_2H	NH_2	CH_2
10	F	F	H	NH_2	CO_2H	CH_2
11	H	H	F	NH_2	CO_2H	CH_2
12	H	H	Me	NH_2	CO_2H	CH_2
13	H	H	F	NH_2	CO_2H	C(α-OH)H
14	H	H	F	NH_2	CO_2H	CO

propionate (AMPA), and kainate. mGluRs are classified into eight subtypes, identified as subtype 1 through subtype 8, which are classified into three groups (I−III) on the basis of their sequence homology, signal transduction mechanisms, and pharmacology.[3−9]

Group I mGluRs (mGluR1 and mGluR5) are positively coupled to phospholipase C, and their activation produces phosphoinositide turnover and diacylglycerol within the target neuron. In contrast, both group II (mGluR2 and mGluR3) and group III mGluRs (mGluR4, and mGluR6−mGluR8) are located in glutamatergic terminals and negatively coupled to the activity of adenyl cyclase.[9,10,18] Accordingly, by inhibiting the glutamatergic system, agonists for group II and/or group III might be useful in the treatment of many diseases and conditions such as schizophrenia,[11] anxiety,[12] and drug addiction,[13] and for protection from excitotoxicity.[14,15]

(1*S*, 3*R*)-1-Aminocyclopentane-1,3-dicarboxylic acid (**3**, ACPD) is a selective agonist for group I and group II mGluRs and activates group I and group II mGluRs at similar concentrations.[10] In vitro electrophysiological data have demonstrated that compound **3** produces membrane depolarization and increases the firing rate of midbrain dopamine neurons.[9] Unilateral microinjection of compound **3** into the dorsal striatum produced circling behavior contralateral to the site of microinjection that appeared to be mediated by dopamine. Similarly, microinjection of compound **3** into the nucleus accumbens induced dopamine-dependent locomotor activity, and haloperidol blocked this compound **3** (ACPD)-induced hyperactivity. In contrast, in an in vivo microdialysis study, application of compound **3** to the nucleus accumbens blocked prefrontal cortex stimulation-induced increases in dopamine release in the nucleus accumbens. Compound **3** also blocked ventral tegmental area stimulation-induced increase in dopamine release in the nucleus accumbens. It has been postulated that agonists of group II mGluRs might indirectly enhance net glutamatergic transmission in brain areas involved in schizophrenia by reducing synaptic excitation of inhibitory GABAergic neurons.[19] (2*S*,3*S*,4*S*)-2-(carboxycyclopropyl)glycine (**2**, L-CCG-1) is a potent agonist for group II mGluRs but possesses agonist effects at group I mGluRs at somewhat higher concentrations.[16,17] Recently, a highly selective group II mGluR agonist, (+)-2-aminocyclo[3.1.0]hexane-2,6-dicarboxylic acid (**4**), has been found to have oral activities in mice in both the elevated plus maze model of anxiety and in the compound **3** (ACPD)-induced limbic seizure model.[20] Furthermore, compound **4** had potent antipsychotic effects in an animal model designed specifically to mimic the glutamatergic dysfunction observed in schizophrenia[11] and drug addiction.[13] More recently, selective group II mGluR agonists, 2-oxa-4-aminobicyclo-[3.1.0]hexane-4,6-dicarboxylic acid (**5**, LY379268) and 2-thia-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid (**6**, LY389795), have been presented.[21]

It was our goal to develop novel, potent, selective, and orally active agonists for group II mGluRs. We were interested in introduction of fluorine atom(s) to compound **4**, based on the molecular size and electronegativity of fluorine and carbon−fluorine bond energy. Fluorine is not a sterically demanding substituent, since sterically, with its small van der Waals radius, it closely resembles hydrogen. In molecules where conformational recognition is important, minimal steric disturbance by a substituent is especially significant. The electronegativity of fluorine can have pronounced effects on the electron distribution in molecules, affecting the basicity or acidity of neighboring groups, dipole moments within molecules, and the overall reactivity and stability of neighboring functional groups. Once introduced, the high carbon−fluorine bond energy renders the substituent relatively resistant to metabolic transformation.[22] Given the nature of fluorine, the C3 or C6 position of compound **4**, an orally active, potent and selective group II mGluR agonist, was selected for introduction to fluorine atom(s), since it was expected that the introduced fluorine atom(s) would directly influence functional groups, carboxylic acid, and amino group at C2 or carboxylic acid at C6. Among compounds **7**−**11** containing fluorine atom(s) at C3 or C6, (+)-(1*S*,2*S*,3*S*,5*R*,6*S*)-2-amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (+)-**7** and (−)-(1*R*,2*S*,5*R*,6*R*)-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (−)-**11** had the best inhibitory effect of *c*AMP formation, with the same EC_{50} value as compound **4**, and compound (+)-**7** showed higher oral activity in laboratory animals than compound **4**.

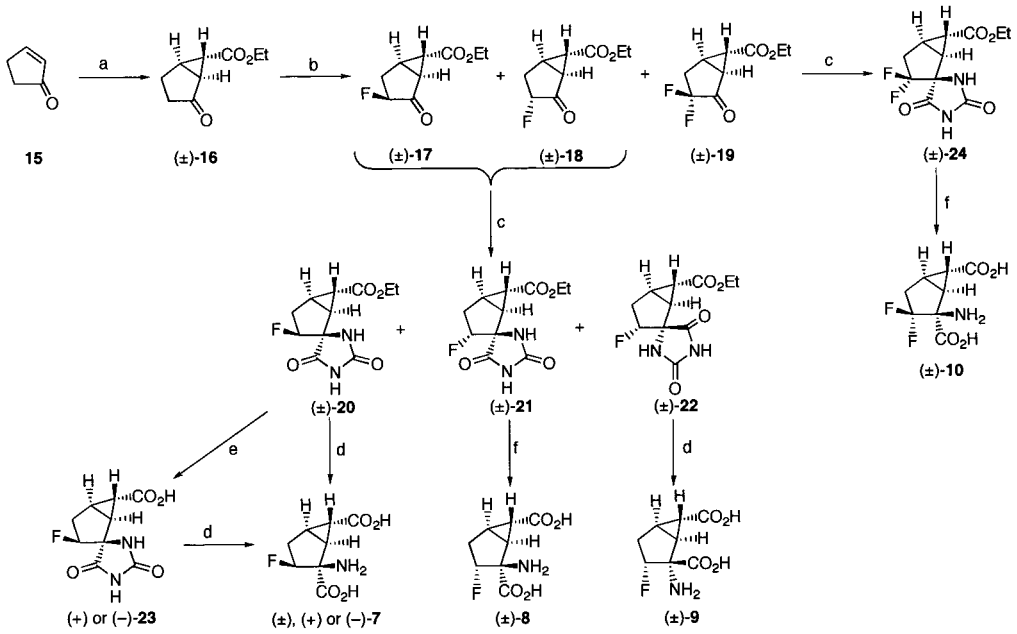
Furthermore, the successful modifications of CH_2 at the C4 position of compound **4** with either an oxygen atom (**5**) or sulfur atom (**6**) aroused our interest in replacement of the CH_2 with a carbonyl group. The chemical modification based on replacement of the C4-CH_2 of compound **11** with a carbonyl group produced (+)-(1*R*,2*S*,5*S*,6*S*)-2-amino-6-fluoro-4-oxobicyclo[3.1.0]-hexane-2,6-dicarboxylic acid monohydrate ((+)-**14**) as the best group II mGluR agonist.

In this paper, we report the synthesis, structure−activity relationships (SARs), and biological activities of compounds **7**−**14**, which have fluorine atom(s) incorporated on the C3 or C6 position of compound **4**.

Chemistry

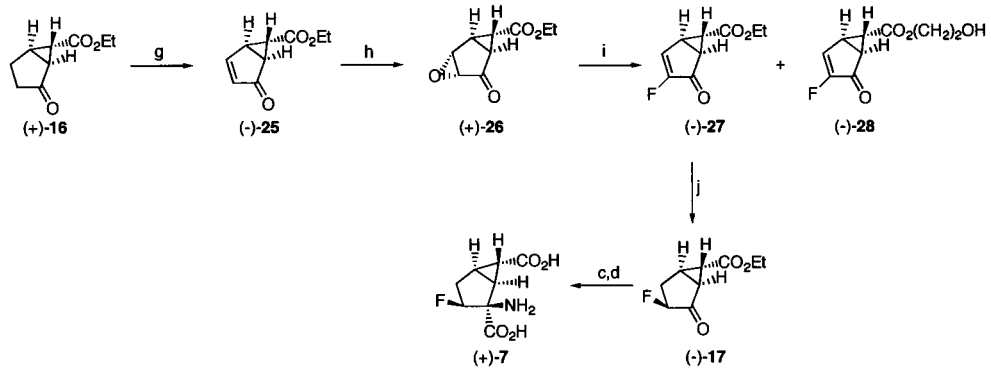
Synthesis of 2-Amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acids. The syntheses of com-

Scheme 1[a]



[a] Reagents and conditions: (a) $EtO_2CCH_2SMe_2$·Br, DBU, $PhCH_3$; (b) (*i*) LHMDS, TMSCl, THF, (*ii*) $(PhSO_2)_2NF$, CH_2Cl_2; (c) $(NH_4)_2CO_3$, KCN, EtOH−H_2O; (d) 60% aq H_2SO_4; (e) (*i*) 2 M aq NaOH, (*ii*) (*R*)-PhCH(NH_2)Me, acetone−H_2O and then 1 M aq HCl; (f) 2.5 or 3.0 M aq NaOH. Method A: a, b. Method B: c, d. Method C: c, f. Method D: e, d.

Scheme 2[a]



[a] Reagents and conditions: (g) (*i*) LHMDS, TMSCl, THF, (*ii*) $Pd(OAc)_2$, MeCN; (h) TBHP, Triton B, PhMe; (i) KF·HF, ethylene glycol; (j) H_2, Pd/C, EtOH; (c) $(NH_4)_2CO_3$, KCN, EtOH−H_2O; (d) 60% aq H_2SO_4. Method E: g−j. Method D: c, d.

pounds (±)-**7**, (+)-**7**, (−)-**7**, (±)-**8**, (±)-**9**, and (±)-**10** are shown in Scheme 1, and the stereospecific syntheses of compounds (+)-**7** and (−)-**7** are depicted in Scheme 2.

Racemic ethyl 2-oxobicyclo[3.1.0]hexane-6-carboxylate (±)-**16** obtained by a known procedure[20] was treated with trimethylsilyl chloride in the absence of lithium bisilanamide, and the resulting silyl enolate was fluorinated with *N*-fluoro-benzenesulfonamide (NFSI)[23,24] to yield a mixture of monofluoro compounds (±)-**17** and (±)-**18**, and difluoro compound (±)-**19** after chromatography (method A). Treatment of lithium enolate[23] of compound (±)-**16** with NFSI resulted in poor yield. The mixture of (±)-**17** and (±)-**18** was reacted under Bucherer−Bergs conditions to yield three hydantoins, (±)-**20**, (±)-**21**, and (±)-**22**, after chromatography and recrystallization. Similarly, hydantoin (±)-**24** was prepared from compound (±)-**19** by treatment with ammonium carbamate and potassium cyanide. Hydantoins (±)-**20**, (±)-**21**, (±)-**22**, and (±)-**24** were hydrolyzed under acidic or basic conditions to yield compounds (±)-**7**, (±)-**8**, (±)-**9**, and (±)-**10**, respectively (method B or C) (Scheme 1).

The relative stereochemistries of the fluorine atom and hydantoin of compound (±)-**20**, (±)-**21**, or (±)-**22** were determined by NOE analysis (Figure 1). In compound (±)-**21**, NOEs were observed between the $H_{3\beta}$ and $H_{6\beta}$, $H_{3\beta}$ and amide H, and $H_{6\beta}$ and amide H. On the other hand, no NOEs were observed between $H_{3\alpha}$ and $H_{6\beta}$, or between $H_{3\alpha}$ and amide H of compound (±)-**20**. NOEs were observed between $H_{1\alpha}$ and amide H, and $H_{3\beta}$ and $H_{6\beta}$ of the racemic diastereoisomer (±)-**22** of (±)-**21**.

Optically pure compounds (+)-**7** and (−)-**7** were produced by acidic hydrolysis of carboxylic acids (+)-**23** and (−)-**23** afforded by selective saponification of ester of compound (±)-**20** under basic conditions followed by optical resolution by treatment with (*R*)- or (*S*)-1-phenylethylamine (method D) (Scheme 1).

In addition to the above resolution, optical compound (+)-**7** was stereospecifically afforded from optical compound (+)-**16** via key intermediate (−)-**27** as shown in Scheme 2. Racemic ethyl 2-oxobicyclo[3.1.0]hexane-6-carboxylate (±)-**16** was separated by chiral HPLC[25] to yield the known optical compound (+)-**16** and its enan-

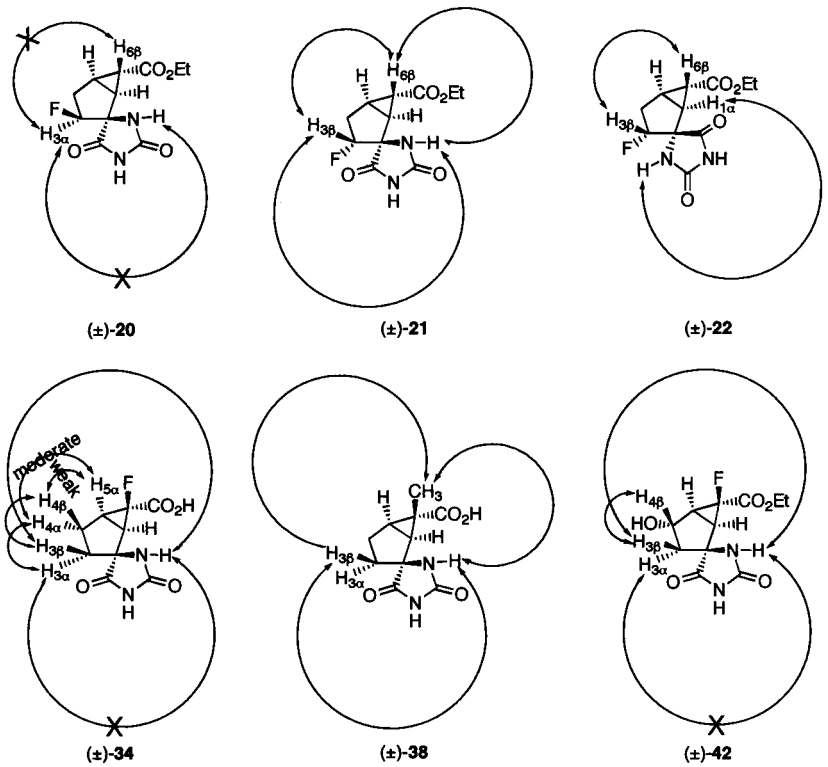
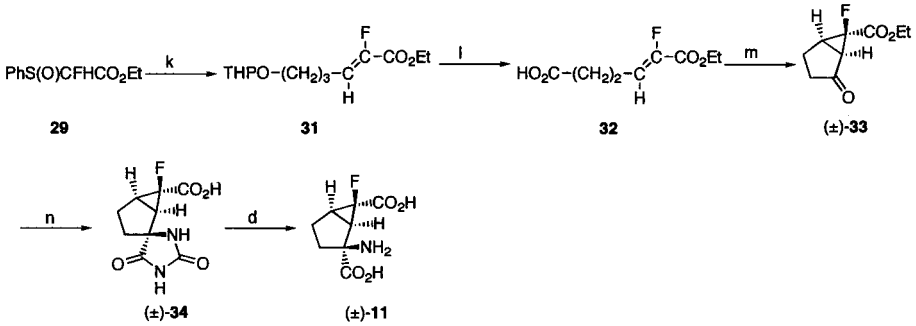


Figure 1. NOEs of compounds (±)-**20**−(±)-**22**, (±)-**34**, (±)-**38**, and (±)-**42**.

Scheme 3[a]



[a] Reagents and conditions: (k) THPO-$(CH_2)_4$-Br (**30**), NaH, DMF; (l) Jone's reagent; (m) (*i*) $(COCl)_2$, hexane, reflux, (*ii*) CH_2N_2, Et_2O, (*iii*) $Cu(TBS)_2$, PhH, reflux; (n) (*i*) 1 N aq NaOH, (*ii*) KCN, $(NH_4)_2CO_3$, EtOH−H_2O; (d) 60% H_2SO_4, 140−150 °C. Method F: k, l. Method G: m. Method H: n, d.

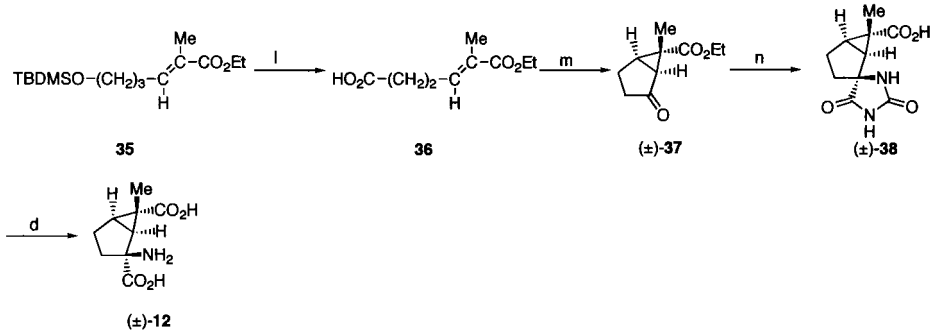
tiomer (−)-**16**.[26] Treatment of optically active compound (+)-12 with trimethylsilyl chloride (TMSCl) under basic conditions using lithium bis(trimethylsilyl)amide (LHMDS) followed by palladium acetate[27] afforded enone compound (−)-**25**, which was stereoselectively epoxidated by *tert*-butyl hydroperoxide (TBHP)[28,29] in the presence of Triton B to yield epoxide (+)-**26**. Fluorination of epoxide (+)-**26** with potassium hydrogen difluoride[28,30] in ethylene glycol yielded key intermediate (−)-**27** and its ester exchanger (−)-**28**. Hydrogenation of vinyl fluoride (−)-**27** with palladium on carbon induced stereoselective progress, yielding (−)-**17** (method E), which was derived to compound (+)-**7** by two steps, formation of hydantoin under Bucherer−Bergs conditions followed by hydrolysis of both the hydantoin and ester under acidic conditions (method D).

Compound [^{3}H]-(+)-**7** was prepared in the same manner as for preparation of (+)-**7** from (−)-**17** after compound (−)-**27** was hydrogenated by tritium gas in the absence of 10% Pd/$CaCO_3$.[31]

Synthesis of 2-Amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acids. The syntheses of compounds (±)-**11**, (+)-**11**, (−)-**11**, (±)-**12**, (±)-**13**, (±)-**14**, (+)-**14**, and (−)-**14** are shown in Schemes 3−7.

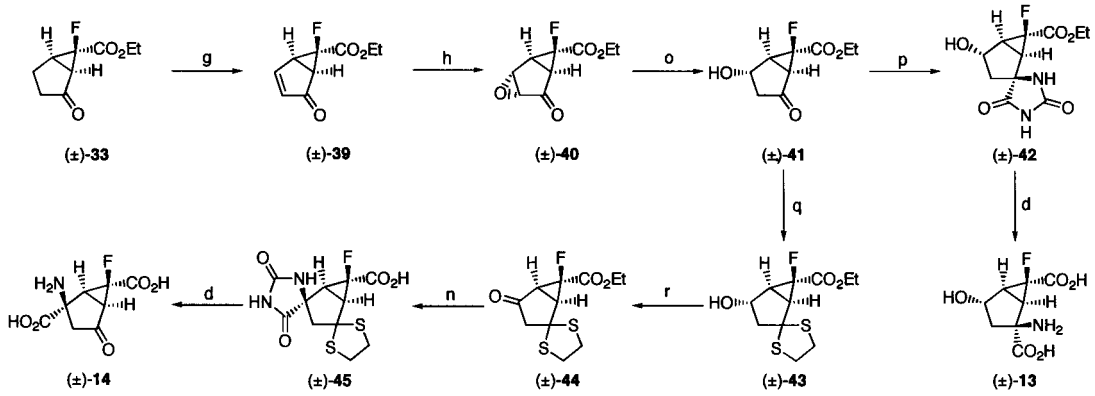
Racemic ethyl 6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate (±)-**33** was obtained by intermolecular reaction using bis(*N*-*tert*-butylsalicylaldimine)copper(II)[32,33] between the double bond and diazomethyl group produced by the treatment of compound **32** with oxalyl chloride followed by diazomethane (method G). Ethyl (*Z*)-2-fluoro-5-carboxy-2-pentenate **32** was stereoselectively prepared by coupling ethyl phenylsulfinylfluoroacetate **29**[34] with 1-bromo-4-tetrahydropyranyloxybutane **30**[35] in the presence of sodium hydride followed by oxidation with Jone's reagent (method F). After hydrolysis of the ethyl ester of compound (±)-**33**, the resulting carboxylic acid was reacted under Bucherer−Bergs conditions to yield hydantoin (±)-**34** as a single product. The direct treatment of compound (±)-**33** under Bucherer−Bergs conditions resulted mainly in amida-

Scheme 4[a]



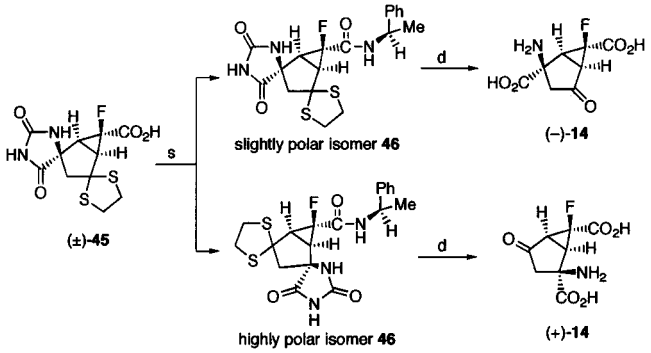
[a] Reagents and conditions: (l) Jone's reagent; (m) (*i*) $(COCl)_2$, hexane, reflux, (*ii*) CH_2N_2, Et_2O, (*iii*) $Cu(TBS)_2$, PhH, reflux; (n) (*i*) 1 N aq NaOH, (*ii*) KCN, $(NH_4)_2CO_3$, EtOH−H_2O; (d) 60% H_2SO_4, 140−150 °C. Method G: m. Method H: n, d. Method I: l.

Scheme 5[a]



[a] Reagents and conditions: (g) (*i*) LHMDS, TMSCl, THF. (*ii*) $Pd(OAc)_2$, MeCN; (h) TBHP, Triton B, PhMe; (o) $(PhSe)_2$, $NaBH_4$, AcOH, EtOH; (p) (*i*) 1 N aq NaOH, (*ii*) KCN, $(NH_4)_2CO_3$, EtOH−H_2O, (*iii*) EtOH, EDC·HCl, DMAP, DMF; (d) 60% H_2SO_4, 140−150 °C; (q) (*i*) TBSCl, imidazole, DMF, (*ii*) $HS(CH_2)_2SH$, $BF_3{\cdot}Et_2O$, $CHCl_3$; (r) DMSO, DCC, Py-TFA; (d) (*i*) 1 N aq NaOH, (*ii*) KCN, $(NH_4)_2CO_3$, EtOH−H_2O. Method J: g, h, o. Method K: p, d. Method L: q, r. Method H: n, d.

Scheme 6[a]



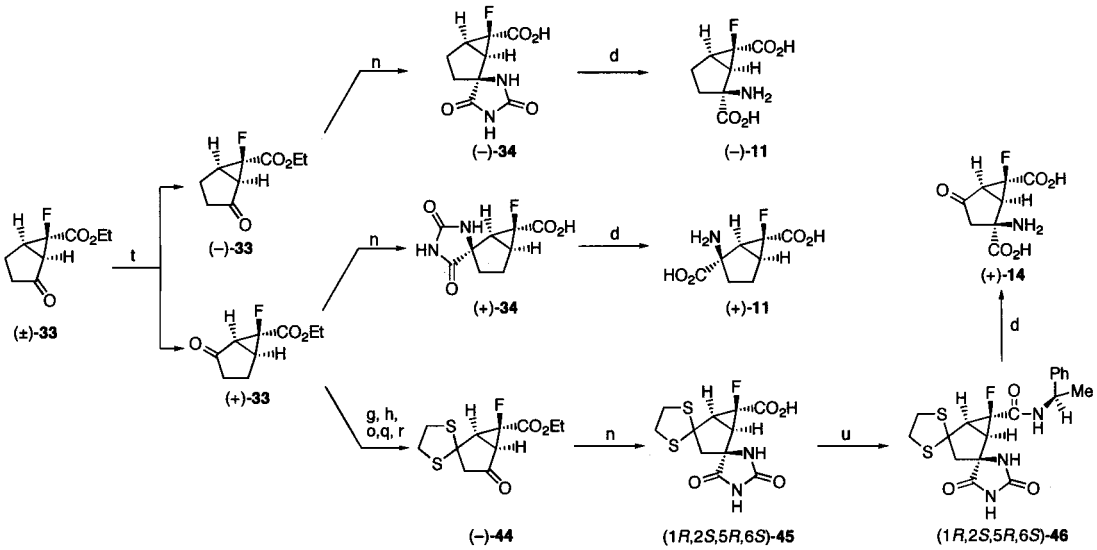
[a] Reagents and conditions: (s) (*i*) (*R*)-(+)-1-phenylethylamine, EDC·HCl, HOBt, DMF, (*ii*) silica gel chromatography; (d) 60% H_2SO_4, 140−150 °C. Method M: s, d.

tion of the ethyl ester of compound (±)-**33**. Compound (±)-**34** was hydrolyzed under acidic conditions to yield compound (±)-**11** (method H) (Scheme 3). The relative stereochemistry of compound (±)-**34** was supported by NOE analysis (Figure 1). Moderate and weak NOEs were observed between $H_{4\alpha}$ and $H_{5\alpha}$ and $H_{4\beta}$ and $H_{5\alpha}$, respectively. Furthermore, the observation of NOEs between $H_{3\beta}$ and H_{amide} and $H_{3\beta}$ and $H_{4\beta}$ but not between $H_{3\alpha}$ and H_{amide} supported the streochemistry of (1*RS*,2*SR*,5*RS*,6*RS*).

6-Methyl compound (±)-**12** was synthesized from ethyl (*E*)-2-methyl-6-(tetrahydropyran-2-yl)oxy-2-hexenate **35**[36] in the same manner as for synthesis of compound (±)-**11** (methods G and H) after oxidizing compound **35** with Jone's reagent (method I) (Scheme 4). The relative stereochemistry of compound (±)-**38** was determined by NOEs which were observed between $H_{3\beta}$ and H_{Me}, H_{amide} and H_{Me}, and $H_{3\beta}$ and H_{amide} (Figure 1).

The key intermediate (±)-**41** for both (±)-**13** and (±)-**14** was prepared by four steps, treatment with trimethylsilyl chloride (TMSCl) under basic conditions using LHMDS followed by palladium acetate,[37] stereoselective epoxidation by TBHP[28,29] in the presence of Triton B, and regiospecific reduction of α,*β*-epoxy carbonyl compound (±)-**40** using benzeneselenol generated in situ by reduction of diphenyl diselenide $(PhSe)_2$ with sodium borohydride in the presence of acetic acid[37] (method J). Compound (±)-**13** was prepared by acidic hydrolysis of compound (±)-**42**, which was derived by hydrolysis of the ester, treatment under Bucherer−Bergs conditions, and then esterification of carboxlic acid for purification by silica gel chromatography (method K) (Scheme 5). The relative stereochemistry of compound (±)-**13** was determined by NOE analysis of precursor (±)-**42**. NOEs between $H_{3\beta}$ and $H_{4\beta}$ and $H_{3\beta}$ and H_{amide} were observed, but not between $H_{3\alpha}$ and H_{amide} (Figure 1). For ketone (±)-**44**, compound (±)-**43**, which was produced from compound (±)-**41** by protection of hydroxyl group with *tert*-butyldimethylsilyl (TBS) group followed by thioketalyzation (TBS−O bond was cleaved by workup), was oxidized by dimethylsufoxide (DMSO) and dicyclohexylcarbodiimide (DCC) in the presence of pyridine and

Scheme 7[a]



[a] Reagents and conditions: (t) chiral HPLC; (n) (*i*) 1 N aq NaOH, (*ii*) KCN, $(NH_4)_2CO_3$, EtOH−H_2O; (d) 60% H_2SO_4, 140−150 °C; (g) (i) LHMDS, TMSCl, THF, (*ii*) $Pd(OAc)_2$, MeCN; (h) TBHP, Triton B, PhMe; (o) $(PhSe)_2$, $NaBH_4$, AcOH, EtOH; (q) (*i*) TBSCl, imidazole, DMF, (*ii*) $HS(CH_2)_2SH$, $BF_3{\cdot}Et_2O$, $CHCl_3$; (r) DMSO, DCC, Py-TFA; (u) (*i*) (*R*)-(+)-1-phenylethylamine, EDC·HCl, HOBt, DMF, (*ii*) silica gel chromatography. Method N: t. Method H: n, d. Method J: g, h, o. Method L: q, r. Method O: u, d.

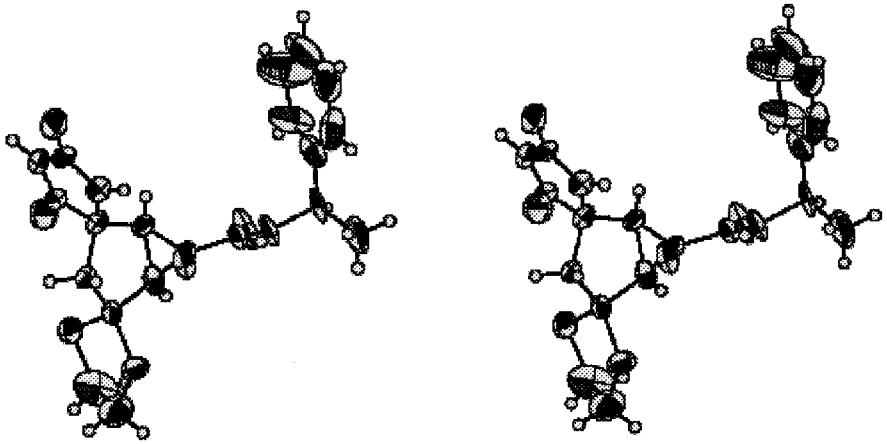


Figure 2. Stereoview of slightly polar isomer **46**·MeOH from the X-ray crystallograph. Solvent molecule (MeOH) is omitted for clarity.

trifluoroacetic acid[38,39] (method L). Ketone (±)-**44** was derived to compound (±)-**14** using method H (Scheme 5).

Optical isomer (+)-**14** or (−)-**14** was prepared by hydrolysis of highly polar isomer **46** or slightly polar isomer **46** produced by coupling (±)-**45** with (*R*)-(+)-1-phenylethylamine using 3-ethyl-1-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole hydrate (HOBt·H_2O) followed by separation of the two diastereomers using silica gel chromatography (method M) (Scheme 6). The X-ray finding for the slightly polar isomer **46** suggested that the stereochemistry of slightly polar isomer **46** was (1*S*,2*R*,5*R*,6*R*) (Figure 2).

Furthermore, optical isomer (+)-**11**, (−)-**11**, or (+)-**14** was prepared from optical isomer (−)-**33** or (+)-**33**, which was resolved optically by chiral HPLC (method N).[40] Treatment of (−)-**33** and (+)-**33** under conditions of method H yielded optical isomers (−)-**11** and (+)-**11**, respectively. On the other hand, compound (+)-**14** was obtained from optical isomer (+)-**33** by methods J, L, H (n), and O. The purification of compound (1*R*,2*S*,5*R*,6*S*)-**45** was unsuccessful, and this compound was derived to amide (1*R*,2*S*,5*R*,6*S*)-**46** (= slightly polar isomer **46**) followed by purification (Scheme 7).

Results and Discussion

Agonist and Antagonist Activities of Compounds 7−14. Agonist activities were evaluated by measuring agonist-dependent inhibition of forskolin-induced cyclic AMP (cAMP) formation in mGluR2-, mGluR3-, mGluR4-, mGluR6-, and mGluR7-expressing cells,[41] by measuring D-myo Ins (1,4,5) P_3 formation in mGluR1a-expressing cells[42,43] and by determining intracellular concentration of Ca^{2+} in mGluR5-expressing cells.[44,45] Antagonist activities were measured with 30 μM glutamic acid present.

The agonist and antagonist activities of derivatives **7−14** and standard agonist **4** or standard antagonist **48** for mGluRs2 and mGluRs3 expressed in CHO cells are shown in Table 1.

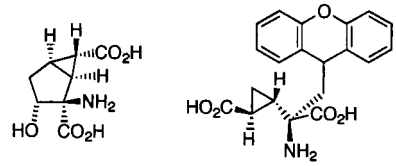
Racemic (±)-**7**, a compound with fluorine atom(s) incorporated on **4**, demonstrated high activity as a

Table 1. Agonist and Antagonist Activities of Compounds **7**−**14** and Standards **4** and **48**

	$EC_{50} \pm$ SEM (nM)[a]			
	agonist activity[b]		antagonist activity[c]	
compd	mGluR2	mGluR3	mGluR2	mGluR3
(±)-**7**	64.7 ± 9.3	NT[d]	NT[d]	NT[d]
(±)-**7**	29.4 ± 3.3	45.4 ± 8.4	>100 000	>100 000
(−)-**7**	2640 ± 290	NT[d]	NT[d]	NT[d]
(±)-**8**	>100 000	NT[d]	17 100	NT[d]
(±)-**9**	4490 ± 400	NT[d]	>100 000	NT[d]
(±)-**10**	>100 000	NT[d]	36 200	NT[d]
(±)-**11**	34.2 ± 6.3	NT[d]	NT[d]	NT[d]
(±)-**11**	1120 ± 200	NT[d]	NT[d]	NT[d]
(−)-**11**	16.6 ± 5.6	80.9 ± 31	>100 000	>100 000
(±)-**12**	>100 000	NT[d]	>100 000	NT[d]
(±)-**13**	21.0 ± 3.3	NT[d]	>100 000	NT[d]
(±)-**14**	1.26 ± 0.2	NT[d]	NT[d]	NT[d]
(+)-**14**	0.570 ± 0.10	2.07 ± 0.40	>100 000	>100 000
(−)-**14**	94.7 ± 10	NT[d]	NT[d]	NT[d]
4	18.3 ± 1.6	62.8 ± 12	NT[d]	NT[d]
48	NT[d]	NT[d]	50.3 ± 22	100 ± 16

[a] EC_{50} values represent the means of 3−7 separate experiments obtained from 5−7 concentrations of each compound, run in duplicate. [b] Compounds (+)-**7**, (−)-**11**, and (+)-**14** exhibited no agonist activities for mGluR1a, mGluR4−mGluR7 expressed in CHO cells (ED_{50} = >100 000 nM). [c] Compounds (+)-**7**, (−)-**11**, and (+)-**14** exhibited no antagonist activities for mGluR1a, mGluR4, mGluR6, and mGluR7 expressed in CHO cells (ED_{50} = >100 000 nM). [d] Not tested.

Chart 2



47 **48**

mGluR2 agonist for suppression of forskolin-stmulated cAMP accumulation in CHO cells, with an EC_{50} value of 64.7 ± 9.3 nM. This agonist activity was determined to be highly stereoselective. Enantiomer (+)-**7** (EC_{50} = 29.4 ± 3.3 nM) exhibited approximately 90-fold higher agonist activity than the corresponding inactive enantiomer (−)-**7** (EC_{50} = 2640 ± 290 nM) in mGluR2 expressed in CHO cells. Furthermore, enantiomer (+)-**7** exhibited a degree of high agonist activity for mGluR3-expressing CHO cells (EC_{50} = 45.4 ± 8.4 nM) but not for mGluRs4, 6, 7, 1a, and 5 expressed in CHO cells (EC_{50} = >100 000 nM), and it exhibited no clear antagonist activity for mGluRs1a and 2−7 (EC_{50} = >100 000 nM). These findings suggested that enantiomer (+)-**7** was a potent and selective agonist for group II mGluRs. In contrast, it is quite interesting that the C3-diastereomer (±)-**8** of compound (±)-**7** exhibited no agonist or antagonist activities for mGluR2 expressed in CHO cells (EC_{50} = >100 000 nM). The conspicuous difference in activity between (±)-**7** and (±)-**8** might not be due to steric hindrance by the incorporated fluorine atom alone, since derivative **47** (Ro 65-3479) (Chart 2), a compound with a hydroxyl group incorporated on the C3 position of **4**, exhibited high affinities for both mGluRs2 and mGluR3.[51] These results suggest that the conspicuous decrease in agonist activity of (±)-**8** for mGluR2 might be due to the electrophilicity of the incorporated fluorine atom, and that the stereochemistry of the fluorine atom might influence the electron density of amino and/or carboxylic groups at the C2 position. The C2 diastereomer (±)-**9** of (±)-**8** exhibited low agonist activity for mGluR2 (EC_{50} = 4490 ± 400 nM), but higher than that of (±)-**8**. Concerning the structure−activity relationships of compounds **4**, **5**, and **6**, the importance of C2 stereochemisty for the activity for group II mGluRs has already been reported.[20,21] Notably, however, the C3 stereochemistry of the fluorine atom more strongly agonist influences activity than C2 stereochemisty ((±)-**7** vs (±)-**8**, and (±)-**8** vs (±)-**9**). Similarly, the difluoro compound (±)-**10** exhibited no agonist or antagonist activity for mGluR2 expressed in CHO cells (EC_{50} = >100 000 nM). This lack of activity might be due to α-fluorine atom of (±)-**10**, since compound (±)-**8** with a fluorine atom incorporated on the α-side of C3 exhibited neither agonist nor antagonist activities for mGluR2 expressed in CHO cells.

Racemic (±)-**11**, a compound with a fluorine atom incorporated on the C6 of (±)-**4**, demonstrated high activity as a mGluR2 agonist for suppression of forskolin-stmulated cAMP accumulation in CHO cells with an EC_{50} value of 34.2 ± 6.3 nM. This agonist activity was determined to be highly stereoselective. Enantiomer (−)-**11** (EC_{50} = 16.6 ± 5.6 nM) exhibited approximately 67-fold higher agonist activity than the corresponding inactive enantiomer (+)-**11** (EC_{50} = 1120 ± 200 nM) for mGluR2 expressed in CHO cells. Furthermore, enantiomer (−)-**11** exhibited a high degree of agonist activity for mGluR3-expressing CHO cells (EC_{50} = 80.9 ± 31 nM) but not for mGluR4, mGluR6, mGluR7, mGluR1a, or mGluR5 expressed in CHO cells (EC_{50} = >100 000 nM), and it exhibited no clear antagonist activity for mGluR1a and mGluRs2−7 (EC_{50} = >100 000 nM). In contrast, compound (±)-**12**, with a methyl group incorporated on the C6 of (±)-**4**, exhibited neither agonist nor antagonist activities for mGluR2 expressed in CHO cells (EC_{50} = >100 000 nM). The dramatic difference in activity between compounds (±)-**11** and (±)-**12** might depend on differences in steric hindrance and/or electron density between the fluorine atom and methyl group.

As a next step, the C4 methylene of compound (±)-**11** was modified. The incorporation of a hydroxyl group on α-site of the C4 methylene did not significantly decrease forskolin-stimulated cAMP formation via mGluR2 expressed in CHO cells (EC_{50} value of 21.0 ± 3.3 nM (compound (±)-**13**)). Notably, however, the replacement of C4 methylene with a carbonyl group greatly enhanced agonist suppression of forskolin-stimulated cAMP accumulation via mGluR2 expressed in CHO cells ((±)-**14**: EC_{50} = 1.26 ± 0.2 nM). Compound (+)-**14** demonstrated great and stereospecific activity as an agonist for mGluR2 and mGluR3 (EC_{50} = 0.570 ± 0.10 nM and 2.07 ± 0.40 nM for mGluR2 and mGluR3, respectively), but had no agonist effect on mGluR1a or mGluR4-mGluR7 (EC_{50} = >100 000 nM) and no antagonist effect on mGluR1a or mGluR2-mGluR7 (EC_{50} = >100 000 nM). This agonist activity was approximately 165-fold that of the corresponding inactive enantiomer (−)-**14** (EC_{50} = 94.7 ± 10 nM) for mGluR2 expressed in CHO cells. Compared with compounds (−)-**8**, the greatly enhanced agonist activity of (+)-**14** might depend on the slight variation caused by the conversion of C4 methylene group into carbonyl group: the relative conformation of three functional groups (one amino group and two carboxylic acids) and/or the electron

Table 2. Competition by Compounds (+)-**7**, (−)-**8**, and (+)-**11** and Standards **4** and **48** for [^{3}H]-(+)-**7** Binding to mGluR2 or mGluR3 Expressed in CHO Cells

	$K_i \pm$ SEM (nM)[a]	
compd	mGluR2	mGluR3
(+)-**7**	47.7 ± 17	65.9 ± 7.1
(−)-**11**	22.5 ± 7.3	41.7 ± 7.1
(+)-**14**	3.30 ± 0.31	3.62 ± 1.6
4	23.4 ± 7.1	53.5 ± 13
48	3.72 ± 0.43	4.11 ± 1.6

[a] K_i values represent the means of 3 or 4 separate experiments obtained from 5−7 concentrations of each compound, run in duplicate.

density of molecular. The degree of structural similarity among compounds **5**, **6**, and (+)-**14** might be higher than that between compounds (−)-**11** and (+)-**14**, since compounds **5**, **6**, and (+)-**14** might have more planarity in the five-member ring than compounds **4** and (−)-**11**. At any rate, compound (+)-**14** might be the best agonist for group II mGluRs ever presented.

[^{3}H]-(+)-7 Binding to mGluR2 Expressed in CHO Cells. The binding of [^{3}H]-(+)-**7** (34 Ci/mmol) was performed according to the method previously described with minor modifications using CHO cells stably expressing mGluR2 or mGluR3.[46] Binding data of group II mGluR antagonist (2*S*)-2-amino-2-((1*S*,2*S*)-2-carboxycycloprop-1-yl)-3-(9-xanthyl)propanoic acid **48** (LY-341495)[52,53] and group II mGluR agonists **4**,[20] (+)-**7**, (−)-**11**, and (+)-**14** are shown in Table 2.

Compound **4** and **48** inhibited [^{3}H]-**7** binding with K_i values of 23.4 ± 7.1 and 3.72 ± 0.43 nM for mGluR2 expressed in CHO cells, and 53.5 ± 13.5 and 4.11 ± 1.6 nM for mGluR3 expressed in CHO cells, respectively. The binding of [^{3}H]-(+)-**7** was inhibited by compounds (+)-**7** and (−)-**11** with K_i values of 47.7 ± 17 and 22.5 ± 7.3 nM for mGluR2, and 65.9 ± 7.1 and 41.7 ± 7.1 nM for mGluR3, respectively.

Compound (+)-**14** inhibited potently the binding of [^{3}H]-**7** with K_i values of 3.30 ± 0.31 and 3.62 ± 1.6 nM for mGluR2 and mGluR3 expressed in CHO cells, respectively. The binding affinity of compound (+)-**14** might be highest among group II mGluR agonists ever presented. Furthermore, [^{3}H]-(+)-**7** may be an excellent ligand for examination of the physiological roles played by mGluRs.

Behavioral Pharmacological Studies. The effects of group II mGluR agonists **4**, (+)-**7**, and (+)-**14** on PCP-nduced hyperactivity and head-weaving behavior in rats are shown in Table 3. It was recently found that PCP-induced head-weaving behavior in rats was inhibited by intraperitoneal administration of **4**.[11] Oral administration of **4** (ED_{50} = 3.0 mg/kg) inhibited PCP-induced head-weaving behavior in rats as well. Compound (+)-**7** (ED_{50} = 0.26 mg/kg) more strongly inhibited PCP-induced head-weaving behavior in rats than **4**. Furthermore, PCP-induced hyperactivity in rats was antagonized by oral administration of (+)-**7** (ED_{50} = 5.1 mg/kg), but not by oral administration of **4** (ED_{50} > 100 mg/kg). These results suggested that PCP-induced head-weaving behavior is a sensitive method for screening of group II mGluR agonists and that the introduction of a fluorine atom clearly increased the oral activity. The enhanced oral activity of (+)-**7** might be due to a fluorine substituent effect, high electronegativity, large carbon−fluorine bond energy, and/or increase in lipophilicity.

PCP-induced head-weaving behavior in rats was very strongly inhibited by oral administration of **(+)-14** (ED_{50} = 0.090 μg/kg). The oral activity of this compound was much stronger than those of compounds **4** and **7**. Furthermore, PCP-induced hyperactivity in rats was antagonized by oral administration of compound (+)-**14** (ED_{50} = 0.30 mg/kg) more strongly than by compounds **4** and **7**. Its oral efficacy suggested that compound (+)-**14** might be the best agonist for oral activity ever presented and useful in the treatment of the schizophrenia.

Conclusions

Compounds (+)-**7** (MGS0008), a compound with a fluorine atom incorporated on the C3 position of **4**, is an orally active and highly selective group II mGluR agonist. Compound (+)-**7** successfully overcame the low oral activity of **4** due to incorporation of a fluorine atom. Notably, however, the stereochemistry of the incorporated fluorine atom dramatically affected the in vitro activity for mGluR2 expressed in CHO cells ((±)-**7** vs (±)-**8**). Compound (+)-**14** (MGS0028), which was produced by both incorporation of a fluorine atom on the C6 position and replacement of the C4-methylene with a carbonyl group from compound **4**, is a potent and selective group II mGluR agonist and has great oral activity. PCP-induced hyperactivity and head-weaving behavior in rats were potently antagonized by the oral administration of compound (+)-**14**.

These findings suggest that potent, selective, and orally active mGluR agonists (+)-**7** and (+)-**14** might be useful not only for exploring the functions of group II mGluRs but in the treatment of schizophrenia. Furthermore, tritium labeled [^{3}H]-(+)-**7** might be useful for examining the physiological significance of the mGluRs.

Experimental Section

Chemistry. Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (NMR) spectra were obtained using a Varian Gemini 2000 (200 MHz) or Varian Unity Inova 300 (300 MHz) spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Shimadzu Profile (EI and CI), JEOL JMS-SX102 (FAB) or Micromass Platform LC (IonSpray and ES). Optical rotations were determined with a JAS.CO DIP-360 polarimeter and are reported at the sodium D-line (589 nm). Elemental analyses were performed by a Perkin-Elmer 2400 (carbon, hydrogen, and nitrogen) or Yokogawa IC7000P (halogen and sulfur). Chiral HPLC (resolution and analysis) was performed with a Gilson Model 305 and/or 306 Piston Pump, 806 Manometric Module, 115 Variable Wavelength Detector and Erma ERC-3322 Degasser. Analytical thin-layer chromatography was conducted on precoated silica gel 60 F_{254} plates (Merck). Silica gel (C-200, 100−200 mesh (Wako Pure Chemical)) was used for column chromatography, using the solvent systems (volume ratios) indicated below.

Ethyl (1*SR*,3*SR*,5*RS*,6*SR*)-3-Fluoro-2-oxobicyclo[3.1.0]-hexane-6-carboxylate ((±)-17), Ethyl (1*SR*,3*RS*,5*RS*,6*SR*)-3-Fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((±)-18), and Ethyl (1*SR*,5*RS*,6*SR*)-3,3-Difluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((±)-19). A solution of ethyl (1*SR*,5*RS*,6*SR*)-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((±)-**16**) (6.60 g, 39.3 mmol) in tetrahydrofuran (THF) (150 mL)

Table 3. Effects of Standard **4** and Compounds (+)-**7** and (+)-**14** on PCP-Induced Hyperactivity and Head-Weaving Behavior in Rats

	PCP-induced hyperactivity					PCP-induced head-weaving behavior				
compd	mg/kg	*N*	total counts	% inhibition	ED_{50} (mg/kg)	μg/kg	*N*	number of head-weavings	% inhibition	ED_{50} (μg/kg)
4 (LY354740)					>100					3000
	vehicle	6	64700 ± 13000			vehicle	6	38.5 ± 2.69		
	10	6	40600 ± 11600	37		1000	6	33.0 ± 3.98	14	
	30	6	43800 ± 13300	32		3000	6	19.4 ± 4.63	50	
	100	6	36000 ± 18400	44		10000	6	6.50 ± 1.58	83	
(+)-**7**					5.1					260
	vehicle	6	54700 ± 10200			vehicle	6	39.3 ± 5.71		
	3	6	40600 ± 12100	26		100	6	25.5 ± 8.27	35	
	10	6	11300 ± 3770	79		300	6	21.0 ± 7.19	47	
	30	6	1850 ± 621	97		1000	6	7.50 ± 1.70	81	
(+)-**14**					0.30					0.090
	vehicle	6	59800 ± 9670			vehicle	6	34.1 ± 2.80		
	0.1	6	38500 ± 10400	36		0.03	6	27.1 ± 4.75	21	
	0.3	6	27700 ± 8040	54		0.1	6	16.2 ± 5.15	52	
	1	6	26100 ± 5920	56		0.3	6	4.88 ± 2.10	86	
	3	6	7760 ± 3330	87						

was added dropwise to a solution of lithium bis(trimethylsilyl)-amide (LHMDS) prepared by treatment of 1,1,1,3,3,3-hexamethyl disilazane (HMDS) (7.70 g, 47.6 mmol) with 1.54 M solution of *n*-butyllithium in hexane (30.9 mL, 47.6 mmol) in 150 mL of THF, at −75 °C under a nitrogen atmosphere. After the mixture was stirred for 1 h at this temperature, chlorotrimethylsilane (TMSCl) (7.50 mL, 59.4 mmol) was added, and the reaction mixture was stirred for 1 h at room temperature. After concentration of the reaction mixture under reduced pressure, anhydrous hexane was added to the residue, the resulting inorganic salts were filtered off, and the filtrate was concentrated under reduced pressure.

To a solution of the above residue in CH_2Cl_2 (66 mL) was added *N*-fluorobenzenesulfonimide (NFSI) (15.00 g, 47.6 mmol), and the mixture was stirred at room temperature for 16.5 h. After the reaction mixture was washed twice with water, the organic phase was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and chromatographed on silica gel (hexane/CH_2Cl_2/AcOEt 60:4:1) to yield a mixture of (±)-**17** and (±)-**18** (4.32 g) ((±)-**17**/(±)-**18** 1:3) as colorless oil and (±)-**19** (2.04 g, 25% yield) as a colorless oil.

Mixture of (±)-**17** and (±)-**18**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (3 H × 3/4, t, J = 7.2 Hz), 1.29 (3 H × 1/4, t, J = 7.2 Hz), 2.11−2.79 (5 H, m), 4.18 (2 H, q, J = 7.2 Hz), 4.51 (1 H × 1/4, dd, J = 51 Hz, 8.1 Hz), 4.58 (1 H × 3/4, dt, J = 51 Hz, 8.1 Hz); MS (FAB) (Pos) m/z 187 ($M^+ + 1$).

(±)-**19**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (3 H, t, J = 7.1 Hz), 2.42−2.80 (5 H, m), 4.20 (2 H, q, J = 7.1 Hz); MS (ion spray) (Nega) m/z 203 ($M^+ - 1$).

Ethyl (1*SR*,5*RS*,6*SR*)-3,3-Difluoro-2-oxobicyclo[3.1.0]-hexane-6-carboxylate ((±)-19). A solution of a mixture of (±)-**17** and (±)-**18** (1.30 g, 6.98 mmol) in THF (6.5 mL) was added dropwise to a solution of LHMDS prepared by treatment of HMDS (1.40 g, 8.3 mmol) with 1.54 M solution of *n*-butyllithium in hexane (5.0 mL, 7.7 mmol) in THF (26 mL), at −75 °C under a nitrogen atmosphere. After the mixture was stirred for 1 h at this temperature, TMSCl (1.30 mL, 10.3 mmol) was added, and the reaction mixture was stirred for 1 h at room temperature. After concentration of the reaction mixture under reduced pressure, anhydrous hexane was added to the residue, the resulting inorganic salts were filtered off, and the filtrate was concentrated under reduced pressure.

The residue was dissolved in CH_2Cl_2 (13 mL); to this was added NFSI (3.30 g, 10.5 mmol), and the mixture was stirred at room temperature for 5 h. After the reaction solution was washed twice with water, the organic phase was dried (Na_2SO_4), filtered off the desiccant, concentrated under reduced pressure, and then chromatographed on silica gel (hexane/AcOEt 15:1) to yield (±)-**19** (0.39 g, 27% yield) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (3 H, t, J = 7.1 Hz), 2.42−2.80 (5 H, m), 4.20 (2 H, q, J = 7.1 Hz); MS (ion spray) (Nega) m/z 203 ($M^+ - 1$).

Ethyl (1*SR*,2*SR*,3*SR*,5*RS*,6*SR*)-2-Spiro-5′-hydantoin-3-fluorobicyclo[3.1.0]hexane-6-carboxylate ((±)-20), Ethyl (1*SR*,2*SR*,3*RS*,5*RS*,6*SR*)-2-Spiro-5′-hydantoin-3-fluorobicyclo[3.1.0]hexane-6-carboxylate ((±)-21), and Ethyl (1*SR*,2*RS*,3*RS*,5*RS*,6*SR*)-2-Spiro-5′-hydantoin-3-fluorobicyclo[3.1.0]hexane-6-carboxylate ((±)-22). A mixture of the mixture of (±)-**17** and (±)-**18** (4.84 g, 26.0 mmol), ammonium carbonate (6.25 g, 65.0 mmol), and potassium cyanide (1.86 g, 28.6 mmol) in a mixed solvent of water (26 mL) and EtOH (38 mL) was stirred at 35 °C for 37 h. After the reaction mixture was cooled to room temperature, water (31 mL) was added, stirring was continued for 2.5 h with ice-cooling, and the resulting crystal was collected by filtration to yield 2.10 g of the first crystal as a mixture of diastereomers (±)-**20**, (±)-**21**, and (±)-**22** (5:6:1).[54] With ice-cooling, concentrated HCl was added to the filtrate to adjust its pH to 1.0, and the resulting crystal was collected by filtration to yield 2.00 g of the second crystal as a mixture of diastereomers (±)-**20**, (±)-**21**, and (±)-**22** (4:6:11).[54] The first crystal was chromatographed on silica gel ($CHCl_3$/MeOH 100:1) to yield nonpolar diastereomer (±)-**20** (0.61 g) and a mixture of two polar diastereomers (±)-**21** and (±)-**22** (0.55 g) (including about 25% diastereomer (±)-**22**,[57] with the same R_f value for diastereomers (±)-**21** and (±)-**22**). The nonpolar diastereomer (±)-**20** (0.61 g) was purified by recrystallization from a mixture of water and EtOH (1:1) to yield (±)-**20** (0.52 g, 8% isolated yield) as a colorless crystal: mp 265−269 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.19 (3 H, t, J = 7.0 Hz), 1.95−2.46 (5 H, m), 4.06 (2 H, q, J = 7.0 Hz), 4.81 (1 H, dd, J = 52 Hz, 5.1 Hz), 8.44 (1 H, s), 10.91 (1 H, s); MS (EI) m/z 256 (M^+); Anal. ($C_{11}H_{13}FN_2O_4$) C, H, F, N.

Also, the mixture of higher-polar diastereomers (±)-**21** and (±)-**22** (0.55 g) was recrystallized from a mixture of water and EtOH (1:1) to yield (±)-**21** (0.37 g, 6% isolated yield) as a colorless crystal: mp 195−199 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.18 (3 H, t, J = 7.1 Hz), 1.85−2.43 (5 H, m), 4.05 (2 H, q, J = 7.1 Hz), 4.70 (1 H, dt, J = 52 Hz, 8.0 Hz), 8.21 (1 H, s), 10.83 (1 H, s); MS (EI) m/z 256 (M^+); Anal. ($C_{11}H_{13}FN_2O_4$) C, H, F, N.

The second crystal was washed with AcOEt to remove inorganic material, and the filtrate was concentrated under reduced pressure. The residue was recrystallized twice from a mixture of water and EtOH (1:1). The combined filtrate was concentrated under reduced pressure, and the residue was chromatographed on silica gel ($CHCl_3$/MeOH 100:1), completely removing the aforesaid nonpolar diastereomer (±)-**20**. The resulting crystal (0.25 g) of polar diastereomer (±)-**22** (including about 10% polar diastereomer (±)-**21**) was recrystallized from a mixture of water and EtOH (1:1) to yield (±)-**22** (0.18 g, 3% isolated yield) as a colorless crystal: mp 165−170 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (3 H, t, J = 7.1 Hz), 1.81−2.17 (4 H, m), 2.36 (1 H, dd, J = 13 Hz, 7.2 Hz), 3.95−4.11 (2 H, m), 4.90 (1 H, ddd, J = 51 Hz, 8.9 Hz, 7.2

Hz), 8.54 (1 H, s), 10.87 (1 H, s); MS (EI) *m/z* 256 (M^+); Anal. ($C_{11}H_{13}FN_2O_4$) C, H, F, N.

Ethyl (1*SR*,2*SR*,5*RS*,6*SR*)-2-Spiro-5′-hydantoin-3,3-difluorobicyclo[3.1.0]hexane-6-carboxylate ((±)-24). A mixture of (±)-**19** (1.65 g, 8.1 mmol), ammonium carbonate (1.78 g, 18.5 mmol), and potassium cyanide (0.60 g, 9.2 mmol) in a mixture (12 mL) of water and EtOH (1:2.5) was stirred at 35 °C for 9 days. To the mixture was added water (1 mL), and the resulting mixture was stirred for 2 h with ice-cooling. The resulting precipitate was collected by filtration to yield (±)-**24** (1.05 g, 47% yield) as a colorless crystal: mp 209−213 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.19 (3 H, t, J = 7.0 Hz), 1.85−1.89 (1 H, m), 2.00−2.08 (1 H, m), 2.15−2.27 (1 H, m), 2.33−2.50 (1 H, m), 2.55−2.86 (1 H, m), 4.07 (2H, q, J = 7.0 Hz), 8.49 (1 H, s); MS (EI) *m/z* 274 (M^+); Anal. ($C_{11}H_{12}F_2N_2O_4$) C, H, F, N.

(1*SR*,2*SR*,3*RS*,5*RS*,6*SR*)-2-Amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((±)-8). Compound (±)-**21** (300 mg, 1.2 mmol) was dissolved in 3 M aqueous NaOH solution (2.50 mL), and the mixture was heated at reflux for 16 h. The reaction mixture was cooled to room temperature and filtered through a glass filter. After the filtrate was brought to pH 3 with concentrated HCl, it was chromatographed on AG1-X8 cation-exchange resin (Bio-Rad) (0.1 M AcOH to 3 M AcOH) to yield (±)-**8** (51 mg, 21% yield) as a colorless crystal: mp 230 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.23−2.24 (1 H, m), 2.56−2.96 (4 H, m), 5.15 (1 H, dt, J = 52 Hz, 7.5 Hz); MS (CI) *m/z* 204 (M^+ + 1); Anal. ($C_8H_{10}FNO_4$) C, H, F, N.

(1*SR*,2*SR*,5*RS*,6*SR*)-2-Amino-3,3-difluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((±)-10). Compound (±)-**24** (97 mg, 0.35 mmol) was dissolved in aqueous 2.5 M NaOH solution (1.0 mL), and the mixture was heated at reflux for 24 h. The reaction mixture was cooled to room temperature and filtered through a glass filter. After the filtrate was brought to pH 3 with concentrated HCl, it was chromatographed on AG1-X8 cation-exchange resin (Bio-Rad) (0.5 M AcOH to 2 M AcOH) to yield (±)-**10** (44 mg, 56% yield) as a colorless crystal: mp 250 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.46 (1 H, brs), 2.63−2.90 (3H, m), 3.01−3.12 (1H,m); MS (CI) *m/z* 222 (M^+ + 1); Anal. ($C_8H_9F_2NO_4 \cdot 1/2H_2O$) C, H, F, N.

(1*SR*,2*SR*,3*SR*,5*RS*,6*SR*)-2-Amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((±)-7). A solution of (±)-**20** (100 mg, 0.39 mmol) in aqueous 60% H_2SO_4 (1.50 mL) was stirred at 140 °C for 12 h. The reaction solution was then cooled to room temperature and brought to pH 8 with 5 M aqueous NaOH. The resulting mixture was chromatographed on AG1-X8 cation-exchange resin (Bio-Rad) (0.1 M AcOH to 2 M AcOH) to yield (±)-**7** (20 mg, 25% yield) as a colorless crystal: mp 160 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.49 (1H, brs), 2.59−3.06 (4 H, m), 5.40 (1 H, dd, J = 52 Hz, 5.3 Hz); MS (CI) *m/z* 204 (M^+ + 1); Anal. ($C_8H_{10}FNO_4$) C, H, F, N.

(1*SR*,2*RS*,3*RS*,5*RS*,6*SR*)-2-Amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((±)-9). A solution of (±)-**22** (150 mg, 0.59 mmol) in aqueous 60% H_2SO_4 (2.3 mL) was stirred at 140 °C for 13 h. The reaction solution was then cooled to room temperature and brought to pH 8 with an aqueous 5 M NaOH. The resulting mixture was chromatographed on AG1-X8 cation-exchange resin (Bio-Rad) (0.1 M AcOH to 2 M AcOH) to yield (±)-**9** (59 mg, 49% yield) as a colorless crystal: mp 220 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.33 (1 H, brs), 2.54−2.89 (4 H, m), 5.42−5.59 (1 H, m); MS (CI) *m/z* 204 (M^+ + 1); Anal. ($C_8H_{10}FNO_4$) C, H, F, N.

(1*SR*,2*SR*,3*SR*,5*RS*,6*SR*)-2-Spiro-5′-hydantoin-3-fluorobicyclo[3.1.0]hexane-6-carboxylic Acid ((±)-23). A mixture of (±)-**20** (2.20 g, 8.59 mmol) in an aqueous 2 M NaOH was stirred at room temperature. After 2 h, its pH was adjusted to 1.0 by adding concentrated HCl. The resulting crystal was isolated by filtration to yield (±)-**23** (1.81 g, 93% yield) as a colorless crystal: mp 319 °C (decomposed); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.85−2.44 (5 H, m), 4.80 (1 H, dd, J = 52 Hz, 5.3 Hz), 8.44 (1 H, m), 10.88 (1 H, s), 12.30 (1 H, brs); MS (FAB) (Nega) *m/z* 227 (M^+ − 1); Anal. ($C_9H_9FN_2O_4$) C, H, F, N.

(1*S*,2*S*,3*S*,5*R*,6*S*)-2-Spiro-5′-hydantoin-3-fluorobicyclo-[3.1.0]hexane-6-carboxylic Acid ((+)-23) and (1*R*,2*R*,3*R*,5*S*,6*R*)-2-Spiro-5′-hydantoin-3-fluorobicyclo[3.1.0]hexane-6-carboxylic Acid ((−)-23). Compound (±)-**23** (1.80 g, 7.89 mmol) was stirred at 55 °C in a mixture (26 mL) of acetone and water (8:5), and after adding (*R*)-(+)-1-phenylethylamine (0.96 g, 7.89 mmol), it was stirred for 15 h at room temperature. The resulting crystals were filtered to yield (*R*)-(+)-1-phenylethylamine salt of (+)-**23** (1.30 g, 47% yield) as a colorless crystal. To a suspension of 1.20 g of the salt in water (15 mL), 1 M HCl was added to adjust to pH 1.0, and the mixture was stirred at room temperature for 14 h. The resulting crystal was isolated by filtration to yield (+)-**23** (0.65 g, 82% yield) as a colorless crystal. The filtrate was further chromatographed on AG50W−X8 anion-exchange resin (Bio-Rad) (1 M acetic acid) to yield (+)-**23** (0.06 g, 8% yield) as a colorless crystal: mp 330 °C (decomposed); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.85−2.44 (5 H, m), 4.80 (1 H, dd, J = 52 Hz, 5.3 Hz), 8.44 (1 H, m), 10.88 (1 H, s), 12.30 (1 H, brs); MS (FAB) (Nega) *m/z* 227 (M^+); $[\alpha]_D^{22}$ = + 36.8° (c = 0.20, MeOH); Anal. ($C_9H_9FN_2O_4$) C, H, F, N.

The filtrate of resolution ((*R*)-(+)-1-phenylethylamine salt) was concentrated under reduced pressure. A mixture of the resulting crystal (1.3 g) in water (17 mL) was adjusted to pH 1.0 by addition of 1 N HCl and was stirred at room temperature. After 4 h, the resulting crystal was collected by filtration, yielding 0.81 g of crystals. The filtrate was chromatographed on AG50W-X8 anion-exchange resin (Bio-Rad), (1 M AcOH), yielding 0.08 g of crystals. The combined crystals (0.89 g, 3.88 mmol) were dissolved in a mixture of acetone and water (8:5) (13 mL) at 55 °C. (*S*)-(−)-1-Phenylethylamine (0.47 g, 3.88 mmol) was added to the solution, and the mixture was stirred at room temperature for 15 h. The precipitated crystals were filtered to yield (*S*)-(−)-1-phenylethylamine salt of (−)-**23** as a colorless crystal (1.10 g, 85% yield).

This salt was transformed to a free form by treatment with 1 M HCl in the same fashion as for preparation of (+)-**23** to yield (−)-**23** (0.58 g, 86% yield) as a colorless crystal. The filtrate was chromatographed on AG50W-X8 anion-exchange resin (Bio-Rad), (1 M AcOH) to yield (−)-**23** (0.07 g, 10% yield) as a colorless crystal: mp 328 °C (decomposed); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.85−2.44 (5 H, m), 4.80 (1 H, dd, J = 52 Hz, 5.3 Hz), 8.44 (1 H, m), 10.88 (1 H, s), 12.30 (1 H, brs); MS (FAB) (Nega) *m/z* 227 (M^+); $[\alpha]_D^{22}$ = − 37.5° (c = 0.20, MeOH); Anal. ($C_9H_9FN_2O_4$) C, H, F, N.

(1*S*,2*S*,3*S*,5*R*,6*S*)-2-Amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((+)-7). Compound (+)-**23** (0.60 g, 2.63 mmol) was dissolved in 10 mL of aqueous 60% H_2SO_4, and it was stirred at 140 °C for 2 days. The reaction solution was cooled to room temperature and then brought to pH 8 by addition of aqueous 5 M NaOH. The mixture was chromatographed on AG1-X8 cation-exchange resin (Bio-Rad) (0.1 M AcOH to 2 M AcOH) to yield (+)-**7** (0.34 g, 64% yield)[55] as a colorless crystal: mp 200 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.49 (1H, brs), 2.59−3.06 (4 H, m), 5.40 (1 H, dd, J = 52 Hz, 5.3 Hz); MS (CI) *m/z* 204 (M^+ + 1); $[\alpha]_D^{22}$ = +58.6° (c = 0.20, 1 M HCl); Anal. ($C_8H_{10}FNO_4$) C, H, F, N.

(1*R*,2*R*,3*R*,5*S*,6*R*)-2-Amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((−)-7). Compound (−)-**23** (0.58 g, 2.54 mmol) was reacted as for preparation of (+)-**7** to yield (−)-**7** (0.37 g, 73% yield)[55] as a colorless crystal: mp 200 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.49 (1H, brs), 2.59−3.06 (4 H, m), 5.40 (1 H, dd, J = 52 Hz, 5.3 Hz); MS (CI) *m/z* 204 (M^+ + 1); $[\alpha]_D^{22}$ = −59.4° (c = 0.20, 1 M HCl); Anal. ($C_8H_{10}FNO_4$) C, H, F, N.

Ethyl (1*S*,5*R*,6*S*)-2-Oxobicyclo[3.1.0]hex-3-ene-6-carboxylate ((−)-25). A solution of (+)-**16**[25] (8.58 g, 51.0 mmol) in THF (126 mL) was added dropwise to a solution of LHMDS prepared by treatment of HMDS (9.88 g, 61.2 mmol) with 1.61 M solution of *n*-butyllithium in hexane (38.0 mL, 61.2 mmol) in THF (126 mL), at −75 °C under a nitrogen atmosphere. After the mixture was stirred for 1 h at this temperature,

TMSCl (9.70 mL, 76.8 mmol) was added, and the reaction mixture was stirred for 1 h at room temperature. After concentration of the reaction solution under reduced pressure, anhydrous hexane was added to the residue, the resulting inorganic salts were filtered off, and the filtrate was concentrated under reduced pressure.

A mixture of the above residue and $Pd(OAc)_2$ (12.6 g, 56.1 mmol) in MeCN (126 mL) was stirred at room temperature for 16 h. To the mixture was added Et_2O, and the resulting mixture was filtered through Celite. The filtrate was concentrated under reduced pressure, chromatographed on silica gel (hexane/AcOEt 8:1), and then recrystallized from a mixture of hexane and iso-PrOH (9:1) to yield (−)-**25** (7.26 g, 86% yield) as colorless crystal: mp 97−99 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26 (3 H, t, J = 7.0 Hz), 2.27 (1 H, t, J = 2.6 Hz), 2.63 (1 H, m), 2.96 (1 H, dt, J = 4.8 Hz, 2.6 Hz), 4.15 (2 H, q, J = 7.0 Hz), 5.74 (1 H, d, J = 5.7 Hz), 7.61 (1 H, dd, J = 5.7 Hz, 2.6 Hz); MS (CI) m/z 167 ($M^+ + 1$); $[\alpha]_D^{25} = -278°$ (c = 0.42, CH_2Cl_2).

Ethyl (1*S*,3*R*,4*R*,5*R*,6*S*)-3,4-Epoxy-2-oxobicyclo[3.1.0]-hex-3-ene-6-carboxylate ((+)-26). To a solution of (−)-**25** (7.00 g, 42.1 mmol) in toluene (47 mL) was added aqueous 70% *tert*-butyl hydroperoxide (9.3 mL, 72.2 mmol) and 10% benzyltrimethylammonium hydroxide in MeOH (3.5 mL, 1.9 mmol), and then the mixture was stirred at room temperature for 30 min. The reaction mixture was partitioned between AcOEt and water. The separated water phase was extracted with AcOEt. The combined organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was recrystallized from AcOEt to yield (+)-**26** (5.50 g, 73% yield) as a colorless crystal: mp 131−136 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (3 H, t, J = 7.0 Hz), 2.09 (1 H, t, J = 3.1 Hz), 2.21 (1 H, ddt, J = 5.3 Hz, 2.4 Hz, 1.1 Hz), 2.96 (1 H, m), 3.25 (1 H, dt, J = 2.4 Hz, 1.1 Hz), 4.00 (1 H, t, J = 2.4 Hz), 4.17 (2 H, q, J = 7.0 Hz); MS (CI) m/z 183 ($M^+ + 1$); $[\alpha]_D^{25} = +12.2°$ (c = 0.41, CH_2Cl_2).

Ethyl (1*S*,5*R*,6*S*)-3-Fluoro-2-oxobicyclo[3.1.0]hex-3-ene-6-carboxylate ((−)-27) and 2-Hydroxyethyl (1*S*,5*R*,6*S*)-3-Fluoro-2-oxobicyclo[3.1.0]hex-3-ene-6-carboxylate ((−)-28). A suspension of (+)-**26** (5.45 g, 30.0 mmol) and HF·KF (23.3 g, 300 mmol) in ethylene glycol (82 mL) was stirred at 130 °C for 2 h under a nitrogen atmosphere. The mixture was partitioned between ice−water and $CHCl_3$. The separated organic phase was dried ($MgSO_4$), concentrated under reduced pressure, and chromatographed on silica gel (hexane/AcOEt 6:1−1:1−3:2) to yield (−)-**27** (1.01 g, 18% yield) as a colorless oil and (−)-**28** (1.67 g, 28% yield) as a colorless oil.

(−)-**27**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (3 H, t, J = 7.0 Hz), 2.48 (1 H, dt, J = 3.1 Hz, 0.7 Hz), 2.58 (1 H, m), 2.81 (1 H, m), 4.17 (2 H, q, J = 7.0 Hz), 6.91 (1 H, m); MS (CI) m/z 185 ($M^+ + 1$); $[\alpha]_D^{25} = -96.8°$ (c = 0.43, CH_2Cl_2).

(−)-**28**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.72−1.92 (1 H, br s), 2.54 (1 H, t, J = 3.0 Hz), 2.61 (1 H, m), 2.84 (1 H, m), 3.80−3.92 (2 H, m), 4.23−4.30 (2 H, m), 6.92 (1 H, m); MS (CI) m/z 201 ($M^+ + 1$); $[\alpha]_D^{25} = -181°$ (c = 0.41, CH_2Cl_2).

Ethyl (1*S*,3*S*,5*R*,6*S*)-3-Fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((−)-17). A suspension of (−)-**27** (0.80 g, 4.3 mmol) and 5% Pd on carbon (80 mg) in EtOH (8 mL) was stirred under a hydrogen atmosphere. Filtration through Celite, concentration under reduced pressure, and then chromatography on silica gel (hexane/AcOEt 6:1) yielded (−)-**17** (0.61 g, 75% yield) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29 (3 H, t, J = 7.2 Hz), 2.10−2.70 (5 H, m), 4.18 (2 H, q, J = 7.2 Hz), 4.51 (1 H, dd, J = 51.0 Hz, 8.1 Hz); MS (IC) m/z 187 ($M^+ + 1$); $[\alpha]_D^{22} = -11.7°$ (c = 0.45, CH_2Cl_2).

Ethyl (1*S*,2*S*,3*S*,5*R*,6*S*)-2-Spiro-5′-hydantoin-3-fluorobicyclo[3.1.0]hexane-6-carboxylate ((+)-20). A mixture of ((−)-**17**) (280 mg, 1.50 mmol), ammonium carbonate (360 mg, 3.75 mmol), and potassium cyanide (110 mg, 1.69 mmol) in a mixture (3.7 mL) of water and EtOH (2:3) was stirred at 35 °C for 1.5 days. The mixture was concentrated under reduced pressure and partitioned between AcOEt and saturated brine. The separated organic phase was dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed on silica gel ($CHCl_3$/MeOH 80:1−60:1) to yield (+)-**20** (232 mg, 61% yield) as a colorless crystal: mp 279−282 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.19 (3 H, t, J = 7.0 Hz), 1.95−2.46 (5 H, m), 4.06 (2 H, q, J = 7.0 Hz), 4.81 (1 H, dd, J = 52 Hz, 5.1 Hz), 8.44 (1 H, s), 10.91 (1 H, s); MS (EI) m/z 256 (M^+); $[\alpha]_D^{25} = +30.1°$ (c = 0.12, MeOH); Anal. ($C_{11}H_{13}FN_2O_4$) C, H, F, N.

(1*S*,2*S*,3*S*,5*R*,6*S*)-2-Amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((+)-7). A mixture of (+)-**20** (120 mg, 0.47 mmol) in aqueous 60% H_2SO_4 (1.8 mL) was stirred at 140 °C for 2 days. The reaction solution was cooled to room temperature and then brought to pH 8 by addition of aqueous 5 M NaOH. The mixture was chromatographed on AG1-X8 cation-exchange resin (Bio-Rad) (0.1 M AcOH to 2 M AcOH) to yield (+)-**7** (75 mg, 79% yield)[55] as a colorless crystal: mp 265−269 °C; mp 200 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.49 (1H, brs), 2.59−3.06 (4 H, m), 5.40 (1 H, dd, J = 52 Hz, 5.3 Hz); MS (CI) m/z 204 ($M^+ + 1$); $[\alpha]_D^{22} = +58.6°$ (c = 0.20, 1 M HCl); Anal. ($C_8H_{10}FNO_4$) C, H, F, N.

Ethyl (*Z*)-2-Fluoro-6-(tetrahydropyran-2-yl)oxy-2-hexenate (31). A solution of ethyl phenylsulfinylfluoroacetate **29**[34] (130 g, 056 mol) and 1-bromo-4-tetrahydropyranyloxybutane **30**[35] (147 g, 0.62 mol) in *N*,*N*-dimethylformamide (DMF) (140 mL) was added dropwise to a suspension of 60% NaH/oil (23.7 g, 0.59 mol) in DMF (700 mL) over 45 min with ice-cooling, and the resulting mixture was stirred at room temperature for 2 h and at 110 °C for 2 h. The reaction mixture was cooled to room temperature, poured into saturated aqueous NH_4Cl, and extracted with a mixture of AcOEt and hexane (2:1). The extract was washed with water and saturated brine, dried (Na_2SO_4), concentrated under reduced pressure, and then chromatographed (hexane/AcOEt 25:1−25:2) to yield **31** (77.9 g, 53% yield) as a light yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.33 (3 H, t, J = 7.1 Hz), 1.46−1.90 (8 H, m), 2.30−2.41 (2 H, m), 3.33−3.57 (2 H, m), 3.72−3.90 (2 H, m), 4.28 (2 H, q, J = 7.1 Hz), 4.57−4.60 (1 H, m), 6.17 (1 H, dt, J = 33.3, 7.8 Hz); MS (CI) (Pos) m/z 261 ($M^+ + 1$).

Ethyl (*Z*)-2-Fluoro-5-carboxy-2-pentenate (32). To a cooled solution of **31** (7.30 g, 28.0 mmol) in acetone (73 mL) in an ice-bath, 8 N Jones' reagent (30 mL) was added dropwise over 20 min. After the mixture was stirred for 2.5 h at room temperature, an excess of Jones' reagent was quenched by the addition of 2-propanol (280 mL) with ice-cooling. The reaction mixture was partitioned between AcOEt and water. The separated water phase was extracted with AcOEt twice. The combined organic phase was washed with water and saturated brine, dried (Na_2SO_4), concentrated under reduced pressure, and then chromatographed (hexane/AcOEt 2:1−1:1) to yield **32** (4.69 g, 88% yield) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (3 H, t, J = 7.1 Hz), 2.46−2.60 (8 H, m), 4.29 (2 H, q, J = 7.1 Hz), 6.03−6.27 (1 H, m); MS (CI) (Pos) m/z 191 ($M^+ + 1$).

Ethyl (1*RS*,5*RS*,6*RS*)-6-Fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((±)-33). A solution of **32** (4.69 g, 24.7 mmol) and $(COCl)_2$ (6.5 mL, 74.5 mmol) in hexane (50 mL) was heated at reflux for 3 h and then concentrated under reduced pressure. A solution of the residue in Et_2O (50 mL) was added to a solution of CH_2N_2 in Et_2O, which was prepared by treatment of a solution of *N*-methylnitrosourea (12.70 g, 124 mmol) in Et_2O with a solution of KOH (40.8 g, 618 mmol) in water (150 mL). The resulting mixture was stirred for 10 min with ice cooling, stirred at room temperature for 1 h, and then concentrated under reduced pressure. The residue was dissolved in benzene (55 mL), and the solution was added dropwise to a solution of bis(*N*-*tert*-butylsalicylaldimine)-copper(II)[32,33] ($Cu(TBS)_2$) (0.41 g, 0.99 mmol) in benzene (600 mL) over 30 min under heating reflux. The mixture was cooled to room temperature, concentrated under reduced pressure, and then chromatographed (hexane/acetone 9:1) to yield (±)-**33** (0.39 g, 27.3% yield) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.33 (3 H, t, J = 7.1 Hz), 2.05−2.55 (4 H, m), 2.59 (1 H, d, J = 6.6 Hz), 2.70−2.77 (1 H, m), 4.30 (2 H, q, J = 7.1 Hz); MS (ion spray) (Pos) m/z 187 ($M^+ + 1$).

(1*RS*,2*SR*,5*RS*,6*RS*)-2-Spiro-5′-hydantoin-6-fluorobicyclo[3.1.0]hexane-6-carboxylic Acid ((±)-34). A mixture of (±)-**33** (256 mg, 1.38 mmol) and 1 N NaOH (1.40 mL, 1.40 mmol) in EtOH (2.60 mL) was stirred for 10 min with ice cooling. To the mixture, 1 N HCl was added dropwise to pH 1, and the resulting mixture was partitioned between AcOEt and saturated brine. The water phase was extracted with AcOEt twice, and the combined organic phase was dried (Na_2SO_4) and then concentrated under reduced pressure. A mixture of the residue, $(NH_4)_2CO_3$ (796 mg, 8.28 mmol), and KCN (277 mg, 4.25 mmol) in a mixture of EtOH and water (1:1) (2.0 mL) was stirred at 55 °C for 8.5 h. After cooling in an ice bath, the reaction mixture was acidified by treatment with concentrated HCl. The resulting mixture was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O) to yield (±)-**34** (310 mg, 99% yield) as a colorless crystal: mp 256 °C (decomposed); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.49−1.70 (1 H, m), 1.93−2.40 (5 H, m), 8.08 (1 H, s), 10.71 (1 H, s); MS (CI) (Pos) *m*/*z* 229 ($M^+ + 1$); Anal. ($C_9H_9FN_2O_4$) C, H, F, N.

(1*RS*,2*SR*,5*RS*,6*RS*)-2-Amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((±)-11). A solution of (±)-**34** (200 mg, 0.88 mmol) in aqueous 60% H_2SO_4 (3.0 mL) was stirred at 140 °C for 6 days. The reaction solution was then cooled to room temperature and brought to pH 8 with 5 M aqueous NaOH. The resulting mixture was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O-50% aqueous THF-10% aqueous pyridine) to yield (±)-**11** (61 mg, 34% yield) as a colorless crystal: mp 225 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.15−2.28 (1 H, m), 2.57 (1 H, dd, J = 13.5, 8.6 Hz), 2.67−2.94 (4 H, m); MS (ion spray) (Nega) *m*/*z* 202 ($M^+ - 1$); Anal. ($C_8H_{10}FNO_4 \cdot 1/2H_2O$) C, H, F, N.

Ethyl (*E*)-2-Methyl-5-carboxy-2-pentenate (36). In a manner similar to the preparation of **32** from **31**, **36** (3.47 g, 76% yield) was obtained from ethyl (*E*)-2-methyl-6-(tetrahydropyran-2-yl)oxy-2-hexenate **35**[36] (10.0 g, 24.4 mmol) and 8 N Jones' reagent (50 mL) in acetone (100 mL), as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (3 H, t, J = 7.0 Hz), 1.87 (3 H, d, J = 1.5 Hz), 2.52 (4 H, d, J = 3.3 Hz), 4.23 (2 H, q, J = 7.0 Hz), 6.70 (1 H, m); MS (CI) (Pos) *m*/*z* 187 ($M^+ + 1$).

Ethyl (1*RS*,5*RS*,6*SR*)-6-Methyl-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((±)-37). In a manner similar to the preparation of (±)-**33** from **32**, (±)-**37** (2.82 g, 84% yield) was obtained from **36** (3.40 g, 18.3 mmol) by treatment with $(COCl)_2$ (4.8 mL, 55.0 mmol) and then a solution of CH_2N_2 in Et_2O followed by $Cu(TBS)_2$ (301 mg, 0.73 mmol), as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25 (3 H, t, J = 7.0 Hz), 1.34 (3 H, s), 1.96−2.55 (6 H, m), 4.13 (2 H, q, J = 7.0 Hz); MS (EI) *m*/*z* 182 (M^+).

(1*RS*,2*SR*,5*RS*,6*SR*)-2-Spiro-5′-hydantoin-6-methylbicyclo[3.1.0]hexane-6-carboxylic Acid ((±)-38). After treatment of (±)-**37** (200 mg, 1.06 mmol) with 1 N NaOH (1.10 mL, 1.10 mmol), 1 N HCl, and then $(NH_4)_2CO_3$ (611 mg, 6.36 mmol) and KCN (207 mg, 3.18 mmol) followed by concentrated HCl in a manner similar to the preparation of (±)-**34** from (±)-**33**, the resulting mixture was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O) to yield (±)-**38** (111 mg, 46% yield) as a colorless crystal: mp 280 °C (decomposed); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.34 (3 H, s), 1.42−1.57 (1 H, m), 1.75−2.16 (5 H, m), 7.96 (1 H, s), 10.66 (1 H, s); MS (ES) (Nega) *m*/*z* 223 ($M^+ - 1$); Anal. ($C_{10}H_{12}N_2O_4$) C, H, N.

(1*RS*,2*SR*,5*RS*,6*SR*)-2-Amino-6-methylbicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((±)-12). A solution of (±)-**38** (120 mg, 0.54 mmol) in 2 N NaOH (1.4 mL) was stirred at reflux for 4 days. The reaction solution was cooled to room temperature and chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O-H_2O/THF (1:1)-10% aqueous pyridine) to yield (±)-**12** (84 mg, 79% yield) as a colorless crystal: mp 245 °C (decomposed); ^{1}H NMR (300 MHz, D_2O) δ 1.36 (3H, s), 1.64−1.75 (1 H, m), 1.92−1.99 (1 H, m), 2.14−2.33 (4 H, m); MS (ES) (Nega) *m*/*z* 198 ($M^+ - 1$); Anal. ($C_9H_{13}NO_4 \cdot 1/3H_2O$) C, H, N.

Ethyl (1*RS*,5*RS*,6*RS*)-6-Fluoro-2-oxobicyclo[3.1.0]hex-3-ene-6-carboxylate ((±)-39). A solution of (±)-**33** (19.5 g, 105 mmol) in THF (230 mL) was added dropwise to a solution of LHMDS prepared by treatment of HMDS (20.3 g, 125 mmol) with 1.61 M solution of *n*-butyllithium in hexane (78 mL, 125 mmol) in THF (230 mL), at −75 °C under a nitrogen atmosphere. After the mixture was stirred for 1 h at this temperature, TMSCl (19.8 mL, 157 mmol) was added, and the reaction mixture was stirred for 1.5 h at room temperature. After concentration of the reaction solution under reduced pressure, anhydrous hexane was added to the residue, the resulting inorganic salts were filtered off, and the filtrate was concentrated under reduced pressure.

A mixture of the above residue and $Pd(OAc)_2$ (25.9 g, 115 mmol) in MeCN (240 mL) was stirred at room temperature for 16 h. To the mixture was added Et_2O, and resulting mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and then chromatographed (hexane/AcOEt 9:1−5:1) to yield (±)-**39** (17.1 g, 89% yield) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (3 H, t, J = 7.3 Hz), 2.78 (1 H, dt, J = 0.6, 5.8 Hz), 3.22 (1 H, dd, J = 2.9, 5.8 Hz), 4.31 (2 H, q, J = 7.3 Hz), 6.07 (1 H, dd, J = 0.6, 5.6 Hz), 7.42 (1 H, ddd, J = 0.6, 2.9, 5.6 Hz); MS (CI) (Pos) *m*/*z* 185 ($M^+ + 1$).

Ethyl (1*RS*,3*RS*,4*RS*,5*SR*,6*RS*)-3,4-Epoxy-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((±)-40). To a solution of (±)-**39** (13.8 g, 74.9 mmol) in toluene (70 mL) was added aqueous 70% *tert*-butyl hydroperoxide (32.9 mL, 256 mmol) and a solution of 40% aqueous benzyltrimethylammonium hydroxide (3.2 mL, 7.56 mmol) in EtOH (10 mL), and the mixture was then stirred at room temperature for 4 h. The reaction mixture was poured into 20% aqueous $Na_2S_2O_3$ cooled in an ice bath and extracted with AcOEt twice. The combined organic phase was washed with saturated brine, dried (Na_2SO_4), concentrated under reduced pressure, and then chromatographed (hexane/AcOEt 8:1−6:1) to yield (+)-**40** (14.7 g, 98% yield) as a colorless crystal: mp 47−48 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (3 H, t, J = 7.3 Hz), 2.50 (1 H, ddt, J = 0.8, 2.4, 6.0 Hz), 3.19 (1 H, dt, J = 0.8, 6.0 Hz), 3.53 (1 H, dt, J = 0.8, 2.4 Hz), 4.02 (1 H, tt, J = 0.8, 2.4 Hz), 4.32 (2 H, q, J = 7.3 Hz); MS (EI) (Pos) *m*/*z* 200 (M^+); Anal. ($C_9H_9FO_4$) C, H, F.

Ethyl (1*RS*,4*SR*,5*SR*,6*RS*)-6-Fluoro-4-hydroxy-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((±)-41). Under a nitrogen atmosphere, to a solution of (±)-**40** (5.63 g, 28.1 mmol) in EtOH (80 mL) was added a solution prepared by the treatment of diphenyl diselenide $(PhSe)_2$ (13.7 g 43.9 mmol) with sodium borohydride (3.19 g, 84.3 mmol) in EtOH (110 mL) followed by acetic acid (0.70 mL, 12.1 mmol),[32] and the resulting mixture was stirred at 35 °C for 2.5 h. The reaction mixture was diluted with AcOEt (380 mL) into which oxygen was passed at room temperature for 5 min. The mixture was washed with half-saturated brine, and the separated water phase was extracted with AcOEt. The combined organic phase was dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed (hexane/AcOEt 4:1−3:1−1:1) to yield (±)-**41** (4.30 g, 76% yield) as a light yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (3 H, t, J = 7.1 Hz), 2.05 (1 H, d, J = 5.1 Hz), 2.30 (1 H, dd, J = 3.5, 19.2 Hz), 2.63 (1 H, dt, J = 5.9, 19.2 Hz), 2.72 (1 H, d, J = 5.9 Hz), 2.85 (1 H, dd, J = 2.1, 5.9 Hz), 4.31 (2 H, q, J = 7.1 Hz), 4.76 (1 H, t, J = 5.1 Hz); MS (EI) (Pos) *m*/*z* 202 (M^+).

Ethyl (1*RS*,2*SR*,4*SR*,5*SR*,6*SR*)-2-Spiro-5′-hydantoin-6-fluoro-4-hydroxybicyclo[3.1.0]hexane-6-carboxylate ((±)-42). After treatment of (±)-**41** (720 mg, 3.56 mmol) with 1 N NaOH (3.70 mL, 3.70 mmol) in EtOH (3.70 mL), to this mixture was added $(NH_4)_2CO_3$ (860 mg, 8.95 mmol) and KCN (260 mg, 3.99 mmol), and the resulting mixture was then stirred at 35 °C for 3 days. The cooled mixture in an ice bath was adjusted to pH 1 by treatment with concentrated HCl and chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O) to yield crude (±)-**21** (440 mg) as a light yellow solid. The solid was treated with EtOH (90 mg, 1.95 mmol), 4-(dimethylamino)pyridine (20 mg, 0.16 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (380 mg, 1.98 mmol) in DMF (3.9 mL) at room temper-

ature 16 h, poured into 1 N HCl, and extracted with $CHCl_3$. The extract was dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed ($CHCl_3$/MeOH 50:1−40:1) followed by recrystallization from EtOH to yield (±)-**42** (153 mg, 16% yield) as a colorless crystal: mp 196 °C (decomposed); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (3 H, t, J = 7.2 Hz), 1.90−2.08 (2 H, m), 2.26 (1 H, dd, J = 1.8, 7.2 Hz), 2.45 (1 H, dd, J = 1.8, 7.2 Hz), 4.17 (2 H, q, J = 7.2 Hz), 4.33 (1 H, dd, J = 5.6, 8.8 Hz), 4.75 (1 H, d, J = 8.8 Hz), 8.13 (1 H, s), 11.00 (1 H, s); MS (ES) (Nega) m/z 271 ($M^+ - 1$); Anal. ($C_{11}H_{13}FN_2O_5 \cdot H_2O$) C, H, F, N.

(1*RS*,2*SR*,4*SR*,5*SR*,6*SR*)-2-Amino-6-fluoro-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((±)-13). In a manner similar to the preparation of (±)-**11** from (±)-**34**, (±)-**13** (17 mg, 15% yield) was obtained from (±)-**42** (140 mg, 0.51 mmol) by treatment with 60% H_2SO_4 (4 mL), as a colorless crystal: mp 220 °C (decomposed): ^{1}H NMR (300 MHz, pyridine-d_6-D_2O 1:1) δ 2.56−2.75 (3 H, m), 2.92 (1 H, dd, J = 1.2, 6.9 Hz), 4.56 (2 H, d, J = 5.4 Hz); MS (ES) (Nega) m/z 218 ($M^+ - 1$); Anal. ($C_8H_{10}FNO_5 \cdot H_2O$) C, H, F, N.

Ethyl (1*RS*,4*RS*,5*RS*,6*SR*)-2,2-Ethylendithio-6-fluoro-4-hydroxybicyclo[3.1.0]hexane-6-carboxylate ((±)-43). To a solution of (±)-**41** (2.28 g, 13.9 mmol) and *tert*-butyldimethylsilyl chloride (TBSCl) (2.50 g, 16.6 mmol) in DMF (14 mL) was added imidazole (1.04 g, 15.3 mmol), and the resulting mixture was stirred for 16 h with ice cooling. The mixture was poured into water and extracted with a mixture of AcOEt and hexane (1:1). The extract was washed with water and saturated brine, dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed (hexane/AcOEt 15:1) to yield ethyl (1*RS*,4*SR*,5*SR*,6*RS*)-4-*tert*-butyldimethylsilyloxy-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate (3.81 g, 86% yield) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.11 (3 H, s), 0.13 (3 H, s), 0.90 (9 H, s), 1.33 (3 H, t, J = 7.1 Hz), 2.21 (1 H, dd, J = 4.0, 19.1 Hz), 2.57 (1 H, dt, J = 5.6, 19.1 Hz), 2.60−2.72 (4 H, m), 4.31 (2 H, q, J = 7.1 Hz), 4.66 (1 H, d, J = 5.6 Hz); MS (CI) (Pos) m/z 317 ($M^+ + 1$).

To a solution of (1*RS*,4*SR*,5*SR*,6*RS*)-4-*tert*-butyldimethylsilyloxy-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate (3.70 g, 11.7 mmol) and 1,2-ethylenedithiol (1.20 mL, 14.0 mmol) in $CHCl_3$ (37 mL), $BF_3 \cdot OEt_2$ (0.44 mL, 3.5 mmol) was added dropwise at room temperature, and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was washed with saturated $NaHCO_3$ and saturated brine, dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed (hexane/AcOEt 4:1−2:1) to yield (±)-**43** (3.21 g, 99% yield) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.32 (3 H, t, J = 7.1 Hz), 2.07 (1 H, d, J = 7.1 Hz), 2.38−2.69 (4 H, m), 3.33−3.45 (4 H, m), 4.27 (2 H, q, J = 7.1 Hz), 4.50 (1 H, dd, J = 5.5, 7.1 Hz); MS (EI) (Pos) m/z 278 (M^+).

Ethyl (1*RS*,5*RS*,6*SR*)-4,4-Ethylenedithio-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((±)-44). To solution of (±)-**43** (3.10 g, 11.1 mmol) and dicyclohexylcarbodiimide (DCC) (9.00, 43.4 mmol) in dimethylsufoxide (DMSO) (116 mL) was added pyridine (1.2 mL) and trifluoroacetic acid (0.6 mL), and the resulting mixture was stirred at room temperature for 16 h. The precipitate in the mixture was filtered, and the filtrate was partitioned between AcOEt and water. The separated organic phase was washed with water and saturated brine, dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed (hexane/AcOEt 7:1−5:1) to yield (±)-**44** (2.60 g, 85% yield) as a colorless crystal: mp 72−74 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (3 H, t, J = 7.1 Hz), 2.79 (1 H, d, J = 6.3 Hz), 2.86−3.08 (2 H, m), 3.18 (1 H, dd, J = 1.9, 6.3 Hz), 3.38−3.53 (4 H, m), 4.31 (2 H, q, J = 7.1 Hz); MS (EI) (Pos) m/z 276 (M^+); Anal. ($C_{11}H_{13}FO_3S_2$) C, H, F, S.

(1*RS*,2*SR*,5*RS*,6*SR*)-2-Spiro-5′-hydantoin-6-fluoro-4,4-ethylenedithiobicyclo[3.1.0]hexane-6-carboxylic Acid ((±)-45). In a manner similar to the preparation of (±)-**42** from (±)-**41**, (±)-**44** (1.30 g, 4.70 mmol) was treated with 1 N NaOH (5.00 mL, 5.00 mmol) in EtOH (5.0 mL), and then $(NH_4)_2CO_3$ (1.13 mg, 11.8 mmol) and KCN (0.34 mg, 5.22 mmol). The reaction mixture was adjusted to pH 1 by the addition of concentrated HCl, and the resulting precipitate was collected by filtration to yield (±)-**45** (1.18 g, 79% yield) as a colorless crystal: mp 287 °C (decomposed); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.37−2.50 (2 H, m), 2.68 (1 H, dd, J = 1.9, 6.9 Hz), 2.76 (1 H, dd, J = 4.2, 15.4 Hz), 3.28−3.50 (4 H, m), 8.10 (1 H, s), 10.78 (1 H, s); MS (ES) (Nega) m/z 317 ($M^+ - 1$); Anal. ($C_{11}H_{11}FN_2O_4S_2$) C, H, F, N, S.

(1*RS*,2*SR*,5*SR*,6*SR*)-2-Amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((±)-14). In a manner similar to the preparation of (±)-**11** from (±)-**34**, (±)-**14** (41 mg, 12% yield) was obtained from (±)-**45** (500 mg, 24.4 mmol) in 60% H_2SO_4 (12 mL) as a light yellow crystal: mp 170 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-d) δ 3.16 (1 H, dd, J = 4.6, 19.5 Hz), 3.45 (1 H, dd, J = 4.6, 19.5 Hz), 3.46 (1 H, d, J = 6.6 Hz), 3.67 (1 H, d, J = 6.6 Hz); MS (ES) (Nega) m/z 216 ($M^+ - 1$); Anal. ($C_8H_8FNO_5$?H_2O) C, H, F, N.

***N*-(*R*)-1-Phenylethyl-(1*R*,2*S*,5*R*,6*S*)-2-spiro-5′-hydantoin-4,4-ethylenedithio-6-fluorobicyclo[3.1.0]hexane-6-carboxylamide (sligtly polar isomer 46) and *N*-(*R*)-1-Phenylethyl-(1*S*,2*R*,5*S*,6*R*)-2-spiro-5′-hydantoin-4,4-ethylenedithio-6-fluorobicyclo[3.1.0]hexane-6-carboxylamide (highly polar isomer 46).** To a solution of (±)-**45** (5.70 g, 17.9 mmol), (*R*)-(+)-1-phenylethylamine (2.60 g, 21.5 mmol), and 1-hydroxybenzotriazole monohydrate (3.40 g, 22.2 mmol) was added EDC·HCl (4.10 g, 21.4 mmol) in DMF (240 mL) with ice cooling. After being stirred for 2 h, the mixture was stirred at room temperature for 16 h and partitioned between AcOEt and 1 N HCl. The separated water phase was extracted with AcOEt three times, and the combined organic phase was washed with saturated brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was chromatographed ($CHCl_3$/MeOH 50:1) to yield slightly polar isomer **46** (3.50 g, 46% yield) and highly polar isomer **46** (3.50 g, 46% yield) as colorless crystals.

Slightly polar isomer **46**: R_f 0.74 ($CHCl_3$/MeOH 9:1); mp 288−289 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.45 (3 H, d, J = 7.0 Hz), 2.34 (1 H, dd, J = 2.6, 6.8 Hz), 2.46 (1 H, m), 2.57 (1 H, dd, J = 2.3, 6.8 Hz), 2.78 (1 H, dd, J = 4.0, 15.2 Hz), 3.34 (4 H, s), 5.04 (1 H, m), 7.23−7.41 (5 H, m), 8.10 (1 H, s), 8.81 (1 H, d, J = 8.4 Hz), 10.77 (1 H, s); MS (ES) (Nega) m/z 420 ($M^+ - 1$); $[\alpha]_D^{26}$ = +62.6° (c = 0.21, MeOH); Anal. ($C_{19}H_{20}FN_3O_3S_2$) C, H, F, N, S.

Highly polar isomer **46**: R_f 0.69 ($CHCl_3$/MeOH 9:1); mp 315−316 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.44 (3 H, d, J = 7.0 Hz), 2.36−2.52 (3 H, m), 2.78 (1 H, dd, J = 4.0, 15.2 Hz), 3.29 (4 H, s), 5.00 (1 H, m), 7.20−7.42 (5 H, m), 8.07 (1 H, s), 8.79 (1 H, d, J = 8.4 Hz), 10.80 (1 H, s); MS (ES) (Nega) m/z 420 ($M^+ - 1$); $[\alpha]_D^{26}$ = +52.6° (c = 0.24, MeOH); Anal. ($C_{19}H_{20}FN_3O_3S_2$) C, H, F, N, S.

(1*S*,2*R*,5*R*,6*R*)-2-Amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((−)-14). In a manner similar to the preparation of (±)-**11** from (±)-**34**, (−)-**14** (40 mg, 28% yield)[56] was obtained from slightly polar isomer **46** (281 mg, 0.67 mmol) in 60% H_2SO_4 (5.2 mL) as a light yellow crystal: mp 175 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-d) δ 3.16 (1 H, dd, J = 4.6, 19.5 Hz), 3.45 (1 H, dd, J = 4.6, 19.5 Hz), 3.46 (1 H, d, J = 6.6 Hz), 3.67 (1 H, d, J = 6.6 Hz); MS (ES) (Nega) m/z 216 ($M^+ - 1$); $[\alpha]_D^{24}$ = −75.1° (c = 0.16, 1 M HCl); Anal. ($C_8H_8FNO_5 \cdot H_2O$) C, H, F, N.

(1*R*,2*S*,5*S*,6*S*)-2-Amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((+)-14). In a manner similar to the preparation of (±)-**11** from (±)-**34**, (+)-**14** (1.22 g, 45% yield)[56] was obtained from highly polar isomer **46** (5.30 g, 12.6 mmol) in 60% H_2SO_4 (96 mL) as a light yellow crystal: mp 175 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-d) δ 3.16 (1 H, dd, J = 4.6, 19.5 Hz), 3.45 (1 H, dd, J = 4.6, 19.5 Hz), 3.46 (1 H, d, J = 6.6 Hz), 3.67 (1 H, d, J = 6.6 Hz); MS (ES) (Nega) m/z 216 ($M^+ - 1$); $[\alpha]_D^{24}$ = +73.2° (c = 0.13, 1 M HCl); Anal. ($C_8H_8FNO_5 \cdot H_2O$) C, H, F.

(1*R*,2*S*,5*R*,6*R*)-2-Spiro-5′-hydantoin-6-fluorobicyclo-[3.1.0]hexane-6-carboxylate ((−)-34). In a manner similar to the preparation of (±)-**34** from (±)-**33**, (−)-**34** (427 mg, 90% yield) was obtained from (−)-**33**[40] (389 g, 2.1 mmol) as a light yellow crystal: mp 311−314 °C (decomposed); ^{1}H NMR (200

MHz, DMSO-d_6) δ 1.49−1.70 (1 H, m), 1.93−2.40 (5 H, m), 8.08 (1 H, s), 10.71 (1 H, s); MS (CI) (Pos) *m*/*z* 229 ($M^+ + 1$); $[\alpha]_D^{26} = -77.3°$ (*c* = 0.41, 1 M NaOH); Anal. ($C_9H_9FN_2O_4$) C, H, F, N.

(1*S*,2*R*,5*S*,6*S*)-2-Spiro-5′-hydantoin-6-fluorobicyclo[3.1.0]-hexane-6-carboxylate ((+)-34). In a manner similar to the preparation of (±)-**34** from (±)-**33**, (+)-**34** (444 mg, 88% yield) was obtained from (+)-**33**[40] (414 g, 2.2 mmol) as a light yellow crystal: mp 311−313 °C (decomposed); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.49−1.70 (1 H, m), 1.93−2.40 (5 H, m), 8.08 (1 H, s), 10.71 (1 H, s); MS (CI) (Pos) *m*/*z* 229 ($M^+ + 1$); $[\alpha]_D^{26} = +77.9°$ (*c* = 0.43, 1 M NaOH); Anal. ($C_9H_9FN_2O_4$) C, H, F, N.

(1*R*,2*S*,5*R*,6*R*)-2-Amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((−)-11). In a manner similar to the preparation of (±)-**11** from (±)-**34**, (−)-**11** (239 mg, 73% yield)[56] was obtained from (−)-**34** (350 mg, 1.5 mmol) as a light yellow crystal: mp 233 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.15−2.28 (1 H, m), 2.57 (1 H, dd, *J* = 8.6, 13.5 Hz), 2.67−2.94 (4 H, m); MS (IonSpray) (Nega) *m*/*z* 202 ($M^+ - 1$); $[\alpha]_D^{26} = -58.8°$ (*c* = 0.14, H_2O); Anal. ($C_8H_{10}FNO_4 \cdot 1/2H_2O$) C, H, F, N.

(1*S*,2*R*,5*S*,6*S*)-2-Amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((+)-11). In a manner similar to the preparation of (±)-**11** from (±)-**34**, (+)-**11** (233 mg, 72% yield)[56] was obtained from (+)-**34** (350 mg, 1.5 mmol) as a light yellow crystal: mp 228 °C (decomposed); ^{1}H NMR (300 MHz, trifluoroacetic acid-*d*) δ 2.15−2.28 (1 H, m), 2.57 (1 H, dd, *J* = 8.6, 13.5 Hz), 2.67−2.94 (4 H, m); MS (IonSpray) (Nega) *m*/*z* 202 ($M^+ - 1$); $[\alpha]_D^{26} = +57.5°$ (*c* = 0.16, H_2O); Anal. ($C_8H_{10}FNO_4 \cdot 1/2H_2O$) C, H, F, N.

Ethyl (1*R*,5*R*,6*R*)-6-Fluoro-2-oxobicyclo[3.1.0]hex-3-ene-6-carboxylate ((+)-39). In a manner similar to the preparation of (±)-**39** from (±)-**33**, (+)-**39** (76.10 g, 89% yield) was obtained from (+)-**33** (86.40 g, 0.46 mol) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (3 H, t, *J* = 7.3 Hz), 2.78 (1 H, dt, *J* = 0.6, 5.8 Hz), 3.22 (1 H, dd, *J* = 2.9, 5.8 Hz), 4.31 (2 H, q, *J* = 7.3 Hz), 6.07 (1 H, dd, *J* = 0.6, 5.6 Hz), 7.42 (1 H, ddd, *J* = 0.6, 2.9, 5.6 Hz); MS (CI) (Pos) *m*/*z* 185 ($M^+ + 1$); $[\alpha]_D^{26} = +430°$ (*c* = 0.34, $CHCl_3$).

Ethyl (1*R*,3*R*,4*R*,5*S*,6*R*)-3,4-Epoxy-6-fluoro-2-oxobicyclo-[3.1.0]hexane-6-carboxylate ((+)-40). In a manner similar to the preparation of (±)-**40** from (±)-**39**, (+)-**40** (72.21 g, 88% yield) was obtained from (+)-**39** (75.60 g, 0.41 mol) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (3 H, t, *J* = 7.3 Hz), 2.50 (1 H, ddt, *J* = 0.8, 2.4, 6.0 Hz), 3.19 (1 H, dt, *J* = 0.8, 6.0 Hz), 3.53 (1 H, dt, *J* = 0.8, 2.4 Hz), 4.02 (1 H, tt, *J* = 0.8, 2.4 Hz), 4.32 (2 H, q, *J* = 7.3 Hz); MS (EI) (Pos) *m*/*z* 200 (M^+); $[\alpha]_D^{26} = +84.2°$ (*c* = 0.28, $CHCl_3$).

Ethyl (1*R*,4*S*,5*S*,6*R*)-6-Fluoro-4-hydroxy-2-oxobicyclo-[3.1.0]hexane-6-carboxylate ((+)-41). In a manner similar to the preparation of (±)-**41** from (±)-**40**, (+)-**41** (17.09 g, 71% yield) was obtained from (+)-**40** (24.00 g, 0.12 mol) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (3 H, t, *J* = 7.1 Hz), 2.05 (1 H, d, *J* = 5.1 Hz), 2.30 (1 H, dd, *J* = 3.5, 19.2 Hz), 2.63 (1 H, dt, *J* = 5.9, 19.2 Hz), 2.72 (1 H, d, *J* = 5.9 Hz), 2.85 (1 H, dd, *J* = 2.1, 5.9 Hz), 4.31 (2 H, q, *J* = 7.1 Hz), 4.76 (1 H, t, *J* = 5.1 Hz); MS (EI) (Pos) *m*/*z* 202 (M^+); $[\alpha]_D^{26} = +79.4°$ (*c* = 0.26, $CHCl_3$).

Ethyl (1*R*,4*R*,5*R*,6*S*)-2,2-Ethylendithio-6-fluoro-4-hydroxybicyclo[3.1.0]hexane-6-carboxylate ((+)-43). In a manner similar to the preparation of ethyl (1*RS*,4*SR*,5*SR*,6*RS*)-4-*tert*-butyldimethylsilyloxy-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate from (±)-**41**, ethyl (+)-(1*R*,4*S*,5*S*,6*R*)-4-*tert*-butyldimethylsilyloxy-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate (73.10 g, 100% yield) was obtained from (+)-**41** (46.70 g, 0.23 mol) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.11 (3 H, s), 0.13 (3 H, s), 0.90 (9 H, s), 1.33 (3 H, t, *J* = 7.1 Hz), 2.21 (1 H, dd, *J* = 4.0, 19.1 Hz), 2.57 (1 H, dt, *J* = 5.6, 19.1 Hz), 2.60−2.72 (4 H, m), 4.31 (2 H, q, *J* = 7.1 Hz), 4.66 (1 H, d, *J* = 5.6 Hz); MS (CI) (Pos) *m*/*z* 317 ($M^+ + 1$); $[\alpha]_D^{26} = +48.2°$ (*c* = 0.28, $CHCl_3$).

The carbonyl group of ethyl (+)-(1*R*,4*S*,5*S*,6*R*)-4-*tert*-butyldimethylsilyloxy-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate (73.00 g, 0.23 mol) was protected with 1,2-ethylenedithiol in the same manner as for preparation of (±)-**43** from ethyl (±)-(1*RS*,4*SR*,5*SR*,6*RS*)-4-*tert*-butyldimethylsilyloxy-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate, to yield **(+)-43** (60.48 g, 94% yield) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.32 (3 H, t, *J* = 7.1 Hz), 2.07 (1 H, d, *J* = 7.1 Hz), 2.38−2.69 (4 H, m), 3.33−3.45 (4 H, m), 4.27 (2 H, q, *J* = 7.1 Hz), 4.50 (1 H, dd, *J* = 5.5, 7.1 Hz); MS (EI) (Pos) *m*/*z* 278 (M^+); $[\alpha]_D^{26} = +44.9°$ (*c* = 0.25, $CHCl_3$).

Ethyl (1*R*,5*R*,6*S*)-4,4-Ethylenedithio-6-fluoro-2-oxobicyclo[3.1.0]hexane-6-carboxylate ((−)-44). In a manner similar to the preparation of (±)-**44** from (±)-**43**, (−)-**44** (45.58 g, 76% yield) was obtained from (+)-**43** (60.00 g, 0.116 mol) as a colorless crystal: mp 87−89 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 (3 H, t, *J* = 7.1 Hz), 2.79 (1 H, d, *J* = 6.3 Hz), 2.86−3.08 (2 H, m), 3.18 (1 H, dd, *J* = 1.9, 6.3 Hz), 3.38−3.53 (4 H, m), 4.31 (2 H, q, *J* = 7.1 Hz); MS (EI) (Pos) *m*/*z* 276 (M^+); $[\alpha]_D^{26} = -42.8°$ (*c* = 0.14, $CHCl_3$); Anal. ($C_{11}H_{13}FO_3S_2$) C, H, F, S.

***N*-(*R*)-1-Phenylethyl-(1*R*,2*S*,5*R*,6*S*)-2-spiro-5′-hydantoin-4,4-ethylenedithio-6-fluorobicyclo[3.1.0]hexane-6-carboxylamide ((1*R*,2*S*,5*R*,6*S*)-46).** After treatment of (−)-**44** (47.39 g, 0.171 mol) with 1 N NaOH (180 mL, 0.180 mol) in EtOH (180 mL), to the mixture were added $(NH_4)_2CO_3$ (41.2 g, 0.429 mol) and KCN (12.3 g, 0.189 mmol), and the resulting mixture was then stirred at 35 °C for 5 days. The cooled mixture in an ice bath was adjusted to pH 1 by the treatment of concentrated HCl and chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O-50% aqueous H_2O) to yielded crude (1*R*,2*S*,5*R*,6*S*)-**45** (51.2 g) as a light yellow solid. The solid was treated with (*R*)-(+)-1-phenylethylamine (23.4 g, 0.193 mol), 1-hydroxybenzotriazole monohydrate (30.8 g, 0.201 mol), and EDC·HCl (34.8 g, 0.182 mol) in DMF (1.08 L) at room temperature for 13 h and the mixture was partitioned between AcOEt and 1 N HCl. The separated water phase was extracted with AcOEt three times, and the combined organic phase was washed with saturated brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was chromatographed ($CHCl_3$/MeOH 50:1−20:1) to yield (1*R*,2*S*,5*R*,6*S*)-**46** (42.6 g, 59% yield): mp 287−289 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.45 (3 H, d, *J* = 7.0 Hz), 2.34 (1 H, dd, *J* = 2.6, 6.8 Hz), 2.46 (1 H, m), 2.57 (1 H, dd, *J* = 2.3, 6.8 Hz), 2.78 (1 H, dd, *J* = 4.0, 15.2 Hz), 3.34 (4 H, s), 5.04 (1 H, m), 7.23−7.41 (5 H, m), 8.10 (1 H, s), 8.81 (1 H, d, *J* = 8.4 Hz), 10.77 (1 H, s); MS (ES) (Nega) *m*/*z* 420 ($M^+ - 1$); $[\alpha]_D^{24} = +63.2°$ (*c* = 0.23, MeOH); Anal. ($C_{19}H_{20}FN_3O_3S_2$) C, H, F, N,S.

(1*R*,2*S*,5*S*,6*S*)-2-Amino-6-fluoro-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid ((+)-14). In a manner similar to the preparation of (±)-**11** from (±)-**34**, (+)-**14** (10.04 g, 43% yield)[56] was obtained from (1*R*,2*S*,5*R*,6*S*)-**46** (45.0 g, 0.107 mol) followed by recrystallization from water as a colorless crystal: mp 175 °C (decomposed); ^{1}H NMR (trifluoroacetic acid-*d*) δ 3.16 (1 H, dd, *J* = 4.6, 19.5 Hz), 3.45 (1 H, dd, *J* = 4.6, 19.5 Hz), 3.46 (1 H, d, *J* = 6.6 Hz), 3.67 (1 H, d, *J* = 6.6 Hz); MS (ES) (Nega) *m*/*z* 216 ($M^+ - 1$); $[\alpha]_D^{23} = +78.7°$ (*c* = 0.65, 1 M HCl); Anal. ($C_8H_8FNO_5 \cdot H_2O$) C, H, F, N.

Pharmacology. Cell Culture. CHO cell lines stably expressing mGluR1a, mGluR2, mGluR3, mGluR4, mGluR6, and mGluR7 were 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 streptomicin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Measurements of Cyclic AMP Formation.[41] Agonist activities for mGluR2, mGluR3, mGluR4, mGluR6, and mGluR7 were evaluated by measuring agonist-dependent inhibition of forskolin-induced cyclic AMP (cAMP) formation in mGluR2-, mGluR3-, mGluR4-, mGluR6-, and mGluR7-expressing cells. Briefly, CHO cells expressing either mGluR2, mGluR3, mGluR4, mGluR6, or mGluR7 were seeded in 96-well plates at a density of 1.26×10^4 cells per well and grown for 2 days. The medium was changed to fresh medium without 2 mM glutamine for 4 to 5 h. The cells were preincubated 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 concentrations of test agents. After incubation for 15 min (mGluR2, mGluR3, and mGluR4) or 30 min (mGluR6 and mGluR7) at 37 °C, the reaction was terminated with ice-cold 100% ethanol and allowed to settle on ice for 40 min. The supernatants were evaporated, and cAMP levels were determined by a cAMP enzymeimmunoassay (EIA) system (RPN 225, Amersham). Antagonist activities were measured with 30 μM glutamic acid present.

Measurements of D-myo Ins (1,4,5) P_3 Formation.[42,43] Agonist activities for mGluR1a were evaluated by measuring D-myo Ins (1,4,5) P_3 formation in mGluR1a-expressing cells. Briefly, CHO cells expressing mGluR1a were seeded in 6-well plates at a density of 3.72×10^5 cells per well and grown for 2 days. The medium was changed to fresh medium without 2 mM glutamine for 4 to 5 h. The cells were preincubated with incubation buffer (20 mM HEPES, 150 mM NaCl, 1.5 mM KCl, 1.8 mM $CaCl_2$, 0.8mM $MgSO_4$, 25 mM glucose, and 10 mM LiCl) (pH 7.4) for 20 min at 37 °C. The reaction was started by replacing the medium with fresh incubation buffer containing various concentrations of test agents. After incubation for 15 s at room temperature, the reaction was terminated with ice-cold 20% perchloric acid and incubated on ice for 20 min. The cells were scraped, and cell suspensions were centrifuged at 15 000 rpm for 10 min. The supernatants were neutralized to pH 7.5 by titrating with ice-cold 1.5 M KOH containing 60 mM HEPES and recentrifuged to remove $KClO_4$ precipitate. The resulting supernatants were evaporated, and the content of Ins (1,4,5) P_3 was quantitatively determined using a D-myo-Inositol 1,4,5-triphosphate (IP_3) [^{3}H] assay system (TRK 1000, Amersham). Antagonist activities were measured with 30 μM glutamic acid present.

Measurements of Intracellular Concentration of Ca^{2+}.[44,45] Intracellular concentrations of Ca^{2+} were determined with the Ca^{2+}-sensitive dye fura 2-AM. mGluR5-expressing CHO cells were plated on dishes (Sumilon, MS-11900) and cultured for 2 days, and the medium was changed to fresh medium without 2 mM glutamine for 4 to 5 h. The cells were loaded with 4 μM fura 2-AM in D-MEM cell culture medium without L-glutamine for 30 min at 37 °C. After loading fura 2-AM, the cells were washed once with 20 mM HEPES buffer (pH 7.4) containing 130 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 0.1% BSA and then scraped from the well. The cells were washed with the buffer twice and suspended in the buffer to obtain a concentration of 8×10^5 cells/mL. The intensity of cell-associated fura 2-AM fluoresence was imaged in a CAF-100 (Nihonbunko). At the end of each incubation, 0.02% Triton X-100 and 0.01 M EGTA were added to measure maximal and minimal fluoresence values, respectively. The intracellular Ca^{2+} concentration was calculated from the ratio of 340/380 nm intensities. MGS compounds were added to the cells with/without 10 μM glutamate.

[^{3}H]-(+)-7 Binding.[46] The binding of [^{3}H]-(+)-**7** (34 Ci/mmol) was performed according to the method previously described with minor modifications. CHO cells stably expressing mGluR2 or 3 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 48000*g* 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 (pH 7.4) by resuspension and recentrifugation. The pellet obtained was then suspended in 50 mM Tris-HCl buffer (pH 7.4), containing 2 or 4 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]-**7**. 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) containing 4 mM $MgCl_2$, using a multicell harvester M-48R (Brandel Biomedical Research and Development Laboratories, Inc., Gaithersburg, MD). Aquasol-2 scintillator (Du Pont/New England Neclear) (10 mL) was added, and filter-bound radioactivity was counted in a liquid scintillation spectrometer (LS6000TA, Beckman Instruments Inc., Fullerton, CA). Nonspecific binding was determined in the presence of 10 μM of compound **4**.

For determination of the equilibrium dissociation constant (K_d), the crude membrane preparation was incubated with 0.5−100 nM of [^{3}H]-**7**. Saturation binding data were analyzed by Scatchard plot analysis, and the K_d and the maximal number of binding sites (B_{max}) were calculated.

In the competitive binding assay, the reaction was carried out using 3.0 nM of [^{3}H]-**7**. The concentration of the test compound that caused 50% inhibition of specific binding of [^{3}H]-**7** (IC_{50} value) was determined from each concentration−response curve. K_i values for each test compound were calculated using the K_d value obtained from Scatchard analysis.

Phencyclidine (PCP)-Induced Hyperactivity in Rats.[47,48] Animals were housed individually in transparent acrylic cages (47 × 28.5 × 29.5 cm) and acclimatized for 60 min with a SCANET apparatus (Neuroscience Inc. Japan) placed in a sound-proof box. Test compounds were administered orally 180 min before the i.p. administration of PCP (5 mg/kg). Immediately after PCP administration, locomotor activity was recorded every 10 min for 120 min. Values are expressed as means ± S.E.M. Data were analyzed by ANOVA, and the significance of differences between groups was determined using Dunnett's test. The total count for the vehicle-treated control group was defined as 100%, the percent inhibition in each group was calculated, and ED_{50} values were determined.

PCP-Induced Head-Weaving Behavior in Rats.[11,49,50] Animals were placed individually in a clear acrylic cage (40.5 × 24.5 × 19.5 cm) and allowed a minimum of 60 min to acclimatize to the new environment. Test compounds were administered orally 60 min before the i.p. administration of PCP (7.5 mg/kg). Immediately after PCP administration, the number of head-weavings (slow, side-to-side or lateral head movement) was counted every 10 min for 30 min. Values are expressed as means ± S. E. M. Data were analyzed by ANOVA, and the significance of differences between groups was determined using Dunnett's test. The total count for the vehicle-treated control group was defined as 100%, the percent inhibition in each group was calculated, and ED_{50} values were determined.

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Supporting Information Available: Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(54) The ratio was determined by comparing the integration volumes of H_{amide} (see Figure 1) of (±)-**20**, (±)-**21**, or (±)-**22**.

(55) The optical purities of (+)-**7** and (−)-**7** were evaluated on CROWNPAK CR(+) (Daisel, two columns (0.4 × 15 cm) connected in series, using eluent of pH 1 aqueous $HClO_4$, UV detection at 208 nm, flow rate of 0.3 mL/min, and column temperature of 2 °C. Compounds (+)-**7** (t_R = 19.4 min) and (−)-**7** (t_R = 23.0 min) each exhibited a single peak on the HPLC chart.

(56) The optical purities of (+)-**14**, (−)-**14**, (+)-**11**, and (−)-**11** were evaluated on CROWNPAK CR(+) (Daisel, two columns (0.4 × 15 cm) connected in series, using eluent of 0.1 M aqueous $HClO_4$, UV detection at 208 nm, flow rate of 0.25 mL/min (for (+)-**14** and (−)-**14**) or 0.30 mL/min (for (+)-**11** and (−)-**11**), and column temperature of 6 °C. (+)-**14** (t_R = 18.6 min), (−)-**14** (t_R = 27.8 min), (+)-**11** (t_R = 36.9 min), and (−)-**11** (t_R = 25.9 min) each exhibited a single peak on the HPLC chart.

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