Cyclopropane-Based Conformational Restriction of GABA by a Stereochemical Diversity-Oriented Strategy: Identification of an Efficient Lead for Potent Inhibitors of GABA Transports

Kazuaki Nakada, a Mamie Yoshikawa, b Soichiro Ide, b Akihiro Suemasa, a Shuhei Kawamura, a Takaaki Kobayashi, a Eiji Masuda, d Yoshiko Ito, d Wataru Hayakawa, b Takahiro Katayama, b Shizuo Yamada, d Mitsuhiro Arisawa, a Masabumi Minami b and Satoshi Shuto*, a,c

aLaboratory of Organic Chemistry for Drug Development, bLaboratory of Pharmacology, cCenter for Research and Education on Drug Discovery, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Sapporo 060-0812, Japan

dGraduate School of Pharmaceutical Sciences, University of Shizuoka, Yada, Shizuoka 422-8526, Japan

Corresponding Author: Satoshi Shuto

Phone & Fax: +81-11-706-3769

E-mail: shu@pharm.hokudai.ac.jp
GABA

H₂N—CO₂H

$\text{H}_2\text{N}\text{CO}_3\text{H}$

$\text{H}_2\text{N}$

$\text{CO}_3\text{H}$

$\text{HO}_2\text{C}$

$\text{NH}_2$

$\text{+ enantiomers}$

3D-Diverse Conformational Restriction
Abstract

A series of cyclopropane-based conformationally restricted γ-aminobutyric acid (GABA) analogs with stereochemical diversity, i.e., the *trans*- and *cis*-2,3-methano analogs **Ia** and **Ib** and their enantiomers *ent-Ia* and *ent-Ib*, and also the *trans-* and *cis*-3,4-methano analogs **IIa** and **IIb** and their enantiomers *ent-IIa* and *ent-IIb*, were synthesized from the chiral cyclopropane units Type-a and Type-b that we developed. These analogs were systematically evaluated with four GABA transporter (GAT) subtypes. The *trans*-3,4-methano analog **IIa** had inhibitory effects on GAT3 (IC$_{50}$ = 23.9 μM) and betaine-GABA transporter1 (5.48 μM), indicating its potential as an effective lead compound for the development of potent GAT inhibitors due to its hydrophilic and low molecular weight properties and excellent ligand efficiency.

Introduction

Blockade of neurotransmitter uptake by inhibition of their transporters increases the neurotransmitter level in the synaptic cleft to enhance synaptic transmission, which can be an effective strategy for drug therapy of central nervous system (CNS) diseases. Serotonin-selective reuptake inhibitors (SSRI) have been successfully developed as antidepressants, and SSRI are now the most widely used of all antidepressants, due to their effectiveness as well as their clinical safety and tolerability.$^1$

γ-Aminobutyric acid (GABA, **I** in Figure 1) is a major inhibitory neurotransmitter in the CNS, whose activity in the synaptic cleft is terminated upon its reuptake by the transporters of GABAergic neuronal cells and astroglial cells.$^2$ Inhibition of GABA reuptake by its transporters is thought to be an effective drug development strategy targeting the GABAergic neuronal system.$^3$ Four GABA transporter (GAT) subtypes, i.e., GAT1, GAT2, GAT3, and BGT1 (betaine-GABA transporter1) are expressed in brain.$^{2,3}$ Among these subtypes, GAT1 and GAT3 may be druggable targets, since the two subtypes are more
highly expressed than the other two subtypes in brain, and their expression in presynaptic neurons and astrocytes may regulate GABA levels in the synaptic cleft of the GABAergic neuronal system.\textsuperscript{2c,4} Accordingly, GABAergic synaptic transmission is likely to be enhanced effectively by inhibiting the GAT1 and/or GAT3 subtypes. In fact, tiagabine (6, Figure 1), a GAT1-selective inhibitor, is clinically effective for the treatment of epilepsy.\textsuperscript{5}

![Figure 1](image)

**Figure 1.** GABA (1), hydrophilic small molecular leads 2–5 for developing GAT inhibitors, and resulting potent GAT inhibitors 6–9.

Extensive studies have been performed to develop selective inhibitors of GABA transporter subtypes, in which hydrophilic small molecular compounds, such as (R)-nipecotic acid [(R)-2], (S)-nipecotic acid [(S)-2], guvacine (3) and their bioisosteres 4 and 5, have been mainly utilized as leads.\textsuperscript{5,5,7} These studies have produced potent GAT1-selective inhibitors, including the clinical drug tiagabine (6). In these GAT inhibitors reported, (S)-SNAP-5114 (7), a hydrophobic analog of (S)-2, is the most potent GAT3 inhibitor.
known to date, however, inhibitory effect of (S)-SNAP-5114 on GAT3 is not so strong, as it is about 100 times less potent than the effective GAT1 inhibitors, such as tiagabine, on GAT1. In addition, none of inhibitors significantly selective to GAT2 or BGT1 have been developed. Therefore, highly selective inhibitors of GAT3, GAT2, or BGT1 are all useful as pharmacologic tools for investigating GABAergic neurotransmission, and studies using such highly selective inhibitors may disclose druggability of these GABA transporter subtypes. On the basis of these previous results and considerations, we thought new small molecular leads structurally different from 2-5 might be effective to develop inhibitors highly selective to the GABA transporters.

Although transporters, including GABA transporters, can be important targets for drug developments, their structural analysis is often troublesome due to the membranous nature of these proteins compared with that of proteins soluble in blood or cytosol. Therefore, structural data on the target transporters are generally poor, and a method to effectively identify compounds targeting these proteins without their structural data is needed for the drug development. We previously reported a stereochemical diversity-oriented conformational restriction strategy to develop compounds that bind selectively to structure-unknown target proteins. To realize this strategy, we developed versatile chiral cyclopropane units Type-a with different stereochemistries (Figure 2), and a series of the cyclopropane-based conformationally restricted analogs of receptor ligands and enzyme inhibitors with stereochemical diversity were effectively designed and synthesized using these units.

Thus, based on the stereochemical diversity-oriented strategy, we planned to synthesize a series of cyclopropane-based conformationally restricted GABA analogs, i.e., Ia,b and IIa,b and their enantiomers ent-Ia,b and ent-IIa,b (Figure 2), to identify an efficient small molecule lead for developing potent and selective inhibitors of GABA transporters. In this article, we describe the results of these studies in detail.
**Results and Discussion**

**Cyclopropane-Based Conformationally Restricted GABA Analogs.** GABA is conformationally very flexible due to its central “–C(sp³)–C(sp³)–C(sp³)–” backbone, and accordingly, it can assume a variety of conformations, which may make it possible to bind to different proteins, i.e., transporter subtypes as well as receptor subtypes, maybe in its different conformations. Therefore, the conformation binding to one transporter subtype, i.e., the bioactive conformation, might be different from that binding to other transporter subtypes. Proper conformational restriction of GABA may thus allow us to identify transporter inhibitors with subtype-selectivity.

In conformationally restricted GABA analogs selectively bound to the target protein, the ionic functional carboxy and amino groups, which are likely to be essential for binding, would assume a special
arrangement superimposing the bioactive conformation, in which the two functional groups effectively interact with certain amino acid residues in the binding pocket of the protein. The bioactive conformations of GABA for the binding to its each transporter subtype, however, is unknown. Thus, we planned to use a stereochemical diversity-oriented strategy to design conformationally restricted analogs, where the versatile chiral cyclopropane units (Figure 2) can be effectively used as described below.

For the design of conformationally restricted analogs, it is essential that the analogs are as similar as possible to the parent compound in size, shape, and molecular weight (MW). Because of its characteristic rigid and small structural features, a cyclopropane ring is effective for rigidly restricting the conformation of a molecule while leaving intact the chemical and physical properties of the lead compounds.9,10

We designed a series of cyclopropane-based conformationally restricted GABA analogs, which are the trans- and cis-2,3-methano analogs Ia and Ib and their enantiomers ent-Ia and ent-Ib, and also the trans- and cis-3,4-methano analogs IIa and IIb and their enantiomers ent-IIa and ent-IIb.11 In these conformationally restricted analogs having a 2,3- or a 3,4-methanobutyl backbone, the carboxyl and amino moieties are located in a variety of spatial arrangements depending on their regio- and stereochemistries. Accordingly, the spatial location of the carboxy and amino functions of one of the series might produce a bioactive conformation of GABA for binding to a target protein, and therefore, we expected that a new lead useful for the development of the selective inhibitors might be identified.

As a general observation, during the process of lead optimization, MW of the compounds usually increases, where hydrophobic moieties are often added onto the lead structure to result in an increasing logP of compounds, interfering with the desired pharmacokinetic properties of the compounds.12,13 Accordingly, hydrophilic low-MW leads are generally favorable, even if the affinity for the target is not very strong. From this viewpoint, the cyclopropane-based conformationally restricted GABA analogs just have a hydrophilic low-MW structural feature suitable for an efficient lead.
Synthesis. Although much effort has been devoted to developing practical methods for preparing chiral cyclopropanes, stereoselective synthesis of cyclopropane derivatives with the desired stereochemistry is often troublesome.\textsuperscript{14} In fact, although some of the cyclopropane-based conformationally restricted analogs of GABA have been synthesized, these are racemic or obtained by optical resolution.\textsuperscript{11} To solve this problem, we previously developed the chiral cyclopropane units Type-a, comprising four stereoisomeric cyclopropane derivatives \textit{10} and \textit{11}, and their enantiomers \textit{ent-10} and \textit{ent-11} bearing two adjacent carbon substituents in a \textit{trans} or a \textit{cis} orientation, as shown in Figure 2.\textsuperscript{9a,c} These units are generally useful for synthesizing a series of stereoisomeric cyclopropane compounds having an asymmetric \textit{trans}- or \textit{cis}-cyclopropane structure.

\textbf{Scheme 1}

\[ \text{(R)-epichlorohydrin} \xrightarrow{\text{ref. 9a}} \text{PhO}_2\text{S} \xrightarrow{\text{a}} \text{14} \]

\[ \text{PhO}_2\text{S} \xrightarrow{\text{b}} \text{12} + \text{13} \]

Conditions: a) 1) \text{Me(MeO)NH}_2\text{Cl, Et}_2\text{N, AlCl}_3, \text{CH}_2\text{Cl}_2, 2) \text{TrCl, pyridine, CH}_2\text{Cl}_2, 77\%; b) \text{Mg, THF, MeOH, 54\% (12), 36\% (13).} 

\textbf{Scheme 2}
In this study, we developed alternative cyclopropane units Type-b with a Weinreb amide moiety (Figure 2) for systematic synthesis of the target compounds. While the Type-b units, similarly to Type-a units, comprises four stereoisomeric cyclopropane derivatives, the substituents (TrOCH₂- and Me(MeO)NHCH₂-) on cyclopropane are different from those of the Type-a units (TBDPSOCH₂- and CHO-). Therefore the Type-b units effectively compensate for the Type-a units and can be useful for synthesizing a series of chiral cyclopropane compounds with stereochemical diversity, including the target conformationally restricted GABA analogs.

The synthetic route of the Type-b units is in Scheme 1. Treatment of bicyclic lactone 14, prepared from (R)-epichlorophydrin by our previously reported method,⁹a with the Me(MeO)NH·HCl/Et₃N/AlCl₃ system in CH₂Cl₂ and then with TrCl/pyridine in CH₂Cl₂ gave the Weinreb amide 15. Reductive de-sulfonylation of 15 was performed by treatment with Mg in MeOH/THF to afford trans-unit 12 and cis-unit 13 in 54% and 36% yield, respectively. The enantiomeric units ent-12 and ent-13 were synthesized from the bicyclic lactone ent-14.⁹a

With the Type-b units in hand, the trans-2,3-methano GABA analog Ia was prepared from the unit 12 as shown in Scheme 2. A two-step reduction of the Weinreb amide moiety of 12 with LiAlH₄ and NaBH₄ gave the corresponding alcoholic product, and subsequent treatment with NaN₃, PPh₃, and CBr₄ in DMF gave the azide 16. Catalytic hydrogenation of 16 with H₂ and Pd-C in the presence of Boc₂O in
THF/MeOH produced the Boc-protected amine 17. After acidic removal of the O-Tr protecting group of 17, the resulting alcohol was oxidized by Pinic conditions to give 18, and treatment of 18 with HCl/AcOEt afforded the desired conformationally restricted GABA analog Ia as a hydrochloride.

The cis-2,3-methano analog Ib was prepared from unit 13 as shown in Scheme 3. Unit 13 was converted to the Boc-protected amine 20 via 19 by a procedure similar to that used in the above-mentioned synthesis of Ia. Removal of the O-Tr protecting group and subsequent oxidation with CrO₃ and H₂SO₄ in aqueous acetone gave the lactam 21. Acidic treatments of 21 with TFA followed by HCl furnished the desired conformationally restricted analog Ib as a hydrochloride. The enantiomers ent-Ia and ent-Ib were synthesized from units ent-12 and ent-13, respectively.

**Scheme 3**

```
13 \(\xrightarrow{a}\)\(\xrightarrow{b}\) 19 \(\xrightarrow{c}\)\(\xrightarrow{d}\) 21 \(\xrightarrow{HCl}\) Ib
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Conditions: a) 1) LiAlH₄, THF, 0 °C, 2) NaBH₄, MeOH, THF, 3) NaN₃, PPh₃, CBr₄, DMF, 0 °C–rt, quant.; b) H₂, Pd/C, Boc₂O, THF, MeOH, 84%; c) 1) 80% AcOH aq., 2) CrO₃, H₂SO₄, acetone aq., 0 °C; d) 1) TFA, CH₂Cl₂, 2) 1 N HCl, 3) recrystallization (EtOH), 39%.

**Scheme 4**
Scheme 5

\[
\begin{align*}
\text{OHC} & \xrightarrow{\text{a}} \text{R}_1\text{O} & \text{b} & \begin{cases} \text{R}_1^1 = \text{H}, \text{R}_2^2 = \text{TBDPS} \\
\text{R}_1^1 = \text{Bn}, \text{R}_2^2 = \text{H} \end{cases} \\
\text{OTBDPS} & \text{C} & \text{O}_2\text{H} & \xrightarrow{\text{d}} \text{BnO} & \text{NHBoc} & \text{NH}_2 & \text{llb} \cdot \text{HCl} \\
\text{10} & \text{22} & \text{24} & \text{25} & \text{26} & \text{llb} & \end{align*}
\]

Conditions: a) 1) MeO\text{CH}_{2}\text{PPh}_3\text{Cl}, NaHMDS, THF, 0 °C, 2) HCl aq., THF, 0 °C, 3) NaBH$_4$, THF, MeOH, 66%; b) 1) BnBr, NaH, Bu$_4$NI, DMF, THF, -10 °C, 2) Bu$_4$NF, THF, 84%; c) 1) Dess-Martin periodinane, CH$_2$Cl$_2$, 0 °C, 2) NaClO$_2$, NaH$_2$PO$_4$, 2-methyl-2-butene, t-BuOH aq.; 97%; d) 1) (PhO)$_2$P(O)N$_3$, Et$_3$N, CH$_2$Cl$_2$, 2) t-BuOH, reflux, 75%; e) 1) H$_2$, Pd/C, THF, MeOH, 2) Dess-Martin periodinane, CH$_2$Cl$_2$, 0 °C, 3) NaClO$_2$, NaH$_2$PO$_4$, 2-methyl-2-butene, t-BuOH aq., 91%; f) HCl aq., 76%.

Scheme 5

\[
\begin{align*}
\text{CHO} & \xrightarrow{\text{a}} \text{R}_1\text{O} & \text{b} & \begin{cases} \text{R}_1^1 = \text{H}, \text{R}_2^2 = \text{TBDPS} \\
\text{R}_1^1 = \text{Bn}, \text{R}_2^2 = \text{H} \end{cases} \\
\text{OTBDPS} & \text{C} & \text{O}_2\text{H} & \text{NHBoc} & \text{NH}_2 & \text{llb} \cdot \text{HCl} \\
\text{11} & \text{27} & \text{29} & \text{30} & \text{31} & \text{llb} & \end{align*}
\]

Conditions: a) 1) MeO\text{CH}_{2}\text{PPh}_3\text{Cl}, NaHMDS, THF, 0 °C, 2) HCl aq., THF, 0 °C, 3) NaBH$_4$, THF, MeOH, 72%; b) 1) BnBr, NaH, Bu$_4$NI, DMF, THF, -10 °C, 2) Bu$_4$NF, THF, 95%; c) 1) Dess-Martin periodinane, CH$_2$Cl$_2$, 0 °C, 2) NaClO$_2$, NaH$_2$PO$_4$, 2-methyl-2-butene, t-BuOH aq.; 91%; d) 1) (PhO)$_2$P(O)N$_3$, Et$_3$N, CH$_2$Cl$_2$, 2) t-BuOH, reflux, 59%; e) 1) H$_2$, Pd/C, THF, MeOH, 2) CrO$_3$, H$_2$SO$_4$, acetone aq., 0 °C; 72%; f) HCl aq., 70 °C, 40%.
The trans-3,4-methano analog IIa was prepared from unit 10 as shown in Scheme 4. A Wittig reaction of unit 10 with MeOCH₂Ph₃Cl/NaN(TMS)₂, followed by acidic treatment, gave the corresponding one carbon-elongated aldehyde, which was reduced with NaBH₄ in MeOH to produce the primary alcohol 22. Benzylation of the hydroxyl of 22 and subsequent removal of the O-silyl protecting group gave 23. A two-step oxidation of the primary alcohol moiety of 23 into carboxyl gave 24, of which Crutius rearrangement was then investigated. The carboxylic acid 24 was treated with (PhO)₂PON₃/Et₃N in CH₂Cl₂ to produce the corresponding acid azide, which was heated in t-BuOH under reflux to proceed the rearrangement giving the Boc-protected amine 25. After reductive removal of the O-Bn protecting group, oxidation of the resulting primary alcohol gave 26. Deprotection of the N-Boc group with aqueous HCl afforded the target compound IIa as a hydrochloride.

The cis-3,4-methano analog IIb was prepared from unit 11, according to a procedure similar to that used to synthesize IIa (Scheme 5). The enantiomers ent-IIa and ent-IIb were synthesized from the units ent-10 and ent-11, respectively.

Thus, the target compounds were systematically and effectively synthesized using the Type-a and Type-b cyclopropane units.

**Effects on GABA Transporters.** The inhibitory effects of 100 μM GABA analogs on GABA uptake in GAT1/CHO, GAT2/CHO, GAT3/CHO, and BGT1/CHO cells were examined (Table 1). The analog IIa showed high efficacy for GAT3 and BGT1 and moderate and low efficacy for GAT2 and GAT1, respectively. On the other hand, the other analogs showed only low to moderate efficacies for all of the GAT subtypes. Thus, potency of analog IIa at the four cloned GAT subtypes were compared to some commercial available GAT inhibitors with IC₅₀ values (Table 2). Analog IIa showed high affinity for BGT1, similar to that of NNC 05-2090, a potent BGT1-selective inhibitor. Analog IIa also showed higher affinities at GAT2 and GAT3 compared with those of β-alanine, a GAT2/3-selective inhibitor,
although its affinity at GAT2 and GAT3 was lower than that of SNAP-5114, the most potent GAT2/3 inhibitor known so far.

Table 1. Inhibitory effects (%) of GABA analogs (100 μM) on GABA uptake in GAT subtypes.

<table>
<thead>
<tr>
<th></th>
<th>GAT1</th>
<th>GAT2</th>
<th>GAT3</th>
<th>BGT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>−3.4 ± 4.7</td>
<td>2.9 ± 5.2</td>
<td>−10.1 ± 7.2</td>
<td>57.3 ± 4.0</td>
</tr>
<tr>
<td>ent-Ia</td>
<td>17.1 ± 6.5</td>
<td>19.0 ± 6.6</td>
<td>25.0 ± 2.1</td>
<td>24.9 ± 2.9</td>
</tr>
<tr>
<td>Ib</td>
<td>−1.5 ± 15.5</td>
<td>−0.5 ± 8.8</td>
<td>−4.0 ± 5.0</td>
<td>18.2 ± 4.0</td>
</tr>
<tr>
<td>ent-Ib</td>
<td>−5.9 ± 9.5</td>
<td>46.8 ± 13.5</td>
<td>54.5 ± 3.4</td>
<td>6.6 ± 4.3</td>
</tr>
<tr>
<td>Iia</td>
<td>25.1 ± 8.1</td>
<td>66.8 ± 1.1</td>
<td>92.6 ± 2.2</td>
<td>92.8 ± 0.7</td>
</tr>
<tr>
<td>ent-Iia</td>
<td>53.7 ± 4.8</td>
<td>6.5 ± 18.0</td>
<td>50.5 ± 10.4</td>
<td>46.2 ± 5.0</td>
</tr>
<tr>
<td>Iib</td>
<td>47.4 ± 7.0</td>
<td>4.4 ± 9.1</td>
<td>−1.4 ± 14.1</td>
<td>28.8 ± 18.2</td>
</tr>
<tr>
<td>ent-Iib</td>
<td>12.2 ± 6.7</td>
<td>−9.5 ± 23.2</td>
<td>−17.9 ± 10.7</td>
<td>−0.7 ± 10.2</td>
</tr>
</tbody>
</table>

aData are expressed as means ± SEM.

Table 2. IC$_{50}$ values (μM) of analog IIa and representative GABA ligands on GABA uptake in GAT subtypes.$^a$

<table>
<thead>
<tr>
<th></th>
<th>GAT1</th>
<th>GAT2</th>
<th>GAT3</th>
<th>BGT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIa</td>
<td>&gt; 100</td>
<td>36.9</td>
<td>13.9</td>
<td>5.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(28.2 – 48.4)</td>
<td>(3.62 – 53.4)</td>
<td>(3.77 – 7.97)</td>
</tr>
<tr>
<td>tiagabine</td>
<td>0.049</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(0.016 – 0.155)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SNAP-5114</td>
<td>170</td>
<td>11.9</td>
<td>1.12</td>
<td>71.7</td>
</tr>
<tr>
<td></td>
<td>(43.6 – 661)</td>
<td>(2.00 – 71.2)</td>
<td>(0.64 – 1.94)</td>
<td>(22.6 – 228)</td>
</tr>
<tr>
<td>ß-alanine</td>
<td>209</td>
<td>–</td>
<td>41.2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(124 – 350)</td>
<td>–</td>
<td>(10.0 – 169)</td>
<td>–</td>
</tr>
<tr>
<td>NNC 05-2090</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.10</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(1.96 – 13.3)</td>
</tr>
</tbody>
</table>

aData are expressed as means (95%CI).

**Effects on GABA Receptors.** Binding of GABA analogs to GABA$_B$ receptors was investigated with crude synaptic membranes of rat brain in the presence of isoguvacine (40 μM) to block GABA$_A$ receptors
(Table 3). At a 1 μM concentration, none of the analogs synthesized in this study significantly competed with [3H]GABA for GABA_B receptors at 1 μM. At a 10 μM concentration, \textit{Ia}, \textit{ent-Ia}, and \textit{ent-IIa} had weak inhibitory effects on [3H]GABA binding, although analog \textit{IIa}, identified as a potent GAT inhibitor, was inactive. In the same experiment, (+)-baclofen (10 nM – 10 μM) competed with [3H] GABA for brain GABA_B receptors in a concentration-dependent manner, and the IC\textsubscript{50} value (mean±S.E., n=3) to displace 50% of control specific binding was 0.36±0.16 μM.

We then evaluated the inhibitory effects of 100 μM GABA analogs on [3H]muscimol binding to mouse brain membrane fraction (Figure 3A). Analogs \textit{Ia} and \textit{IIa} completely displaced 5 nM [3H]muscimol binding to the membrane fraction. Scatchard analysis showed that the K\textsubscript{d} value of muscimol was 15.2 nM. Competition binding assay revealed that analog \textit{IIa} showed rather high affinity (Ki values = 0.34 μM (0.25-0.46 μM)) for GABA\textsubscript{A} receptors (Figure 3B).

<table>
<thead>
<tr>
<th></th>
<th>1 μM</th>
<th>10 μM</th>
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<tbody>
<tr>
<td>\textit{Ia}</td>
<td>83.3 ± 0.67</td>
<td>57.0 ± 0.04</td>
</tr>
<tr>
<td>\textit{ent-Ia}</td>
<td>94.7 ± 2.79</td>
<td>64.9 ± 10.3</td>
</tr>
<tr>
<td>\textit{Ib}</td>
<td>127 ± 17.4</td>
<td>125 ± 28.5</td>
</tr>
<tr>
<td>\textit{ent-Ib}</td>
<td>95.9 ± 10.1</td>
<td>99.3 ± 22.5</td>
</tr>
<tr>
<td>\textit{IIa}</td>
<td>112 ± 7.54</td>
<td>106.0 ± 27.5</td>
</tr>
<tr>
<td>\textit{ent-IIa}</td>
<td>98.9 ± 6.70</td>
<td>69.1 ± 8.31</td>
</tr>
<tr>
<td>\textit{IIb}</td>
<td>91.4 ± 11.2</td>
<td>96.2 ± 26.8</td>
</tr>
<tr>
<td>\textit{ent-IIb}</td>
<td>109 ± 7.80</td>
<td>115.4 ± 3.64</td>
</tr>
</tbody>
</table>
Figure 3. Binding efficacies of GABA analogs on GABA_A receptors. (A) Inhibitory effects of 100 μM GABA analogs on [³H]muscimol (5 nM) binding to mouse brain membrane fraction. Data are expressed as mean ± SEM. (B) Displacement of specific binding of [³H]muscimol to mouse brain membrane fraction by analog IIa. Data are expressed as mean ± SEM.
Figure 4. Anticonvulsant effects of analog \textbf{IIa} and tiagabine on seizures induced by pentylenetetrazole (PTZ; 100 mg/kg, s.c.) in mice. (A) The latency of clonic convulsion and (B) the duration of tremor were examined in mice intracerebroventricularly injected with saline (n = 17), analog \textbf{IIa} (30 nmol [n = 6], 100 nmol [n = 12], 300 nmol [n = 13], 1000 nmol [n = 4]), or tiagabine (10 nmol [n = 12], 30 nmol [n = 12], 100 nmol [n = 15]). # p < 0.05, ## p < 0.001, significantly different from saline-injected mice. Data are expressed as mean ± SEM.
Anticonvulsive effect of analog IIa. The anticonvulsive effect of the GAT inhibitor IIa identified in this study was examined using pentylenetetrazol (PTZ)-induced seizures in mice. Intracerebroventricular injection of analog IIa and tiagabine dose-dependently prolonged the latency of clonic convulsions induced by PTZ (Figure 4A). One-way factorial analysis of variance (ANOVA) revealed that the latency of PTZ-induced clonic convulsions was significantly prolonged by the pretreatment with analog IIa ($F_{4,47} = 3.82, p < 0.01$) and tiagabine ($F_{3,52} = 3.77, p < 0.05$). On the other hand, one-way factorial ANOVA revealed that the duration of the tremor was significantly increased by pretreatment with tiagabine ($F_{3,52} = 4.48, p < 0.01$), but not significantly changed by pretreatment with analog IIa ($F_{4,47} = 1.96, p = 0.12$; Figure 4B). Furthermore, both analog IIa and tiagabine dose-dependently inhibited PTZ-induced tonic convulsions ($ED_{50} = 189 \pm 104$ and $43.7 \pm 17.3$ nmol, respectively). Tonic convulsions were significantly inhibited by the pretreatment with 1000 nmol of analog IIa (the number of mice showed tonic convulsions = 0/4) and 100 nmol of tiagabine (4/15) compared with the saline-pretreated group (10/17; $p < 0.05$, chi-square test).

Discussion. Although the synthesis of some of the cyclopropane-based conformationally restricted analogs of GABA have been reported, these were limited to the 1,3-methono analogs and were not prepared stereoselectively and/or prepared as a racemate. We successfully systematically synthesized not only the 2,3-methano analogs, but also the 3,4-methano analogs stereoselectively as optically pure forms from the chiral cyclopropane units, which clearly shows that the units are very useful for preparing various conformationally restricted chiral cyclopropane analogs with stereochemical diversity.

Among the newly synthesized 2,3-methano GABA analogs we identified a GAT inhibitor IIa. The anticonvulsive effect of IIa was investigated to confirm whether or not the compound was effective in vivo through the GABAergic system. Thus, IIa effectively prolonged the latency of clonic convulsions in the mouse model. Although the effect ($ED_{50} = 189$ nM) was somewhat weaker than that of the potent
GAT1 inhibitor tiagabine (6, ED$_{50}$ = 43.7 nM), tremor, which is a typical side effect of tiagabine, was not observed at all by the treatment with IIa. These results suggest an advantage of GAT3 as a drug target molecule for the GABAergic system due to its intensive expression, particularly in astrocytes to regulate GABA levels in the synaptic cleft. However, these are only preliminary results, and further pharmacologic studies are required, because, for considering the in vivo effect of IIa, its binding to BGT1 and GABA$_A$ receptor must be taken into account.

**Table 4.** LE values of IIa and known inhibitors to GAT1 and GAT2.

<table>
<thead>
<tr>
<th>compound</th>
<th>LE (IC$_{50}$, mM)</th>
<th>GAT1</th>
<th>GAT3</th>
<th>BGT1</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IIa</strong></td>
<td>-</td>
<td>0.83 (13.9)</td>
<td>0.90 (5.48)</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>(R)-nipecotic acid [(R)-2]$^a$</td>
<td>0.80 (5.9)</td>
<td>0.65 (51)</td>
<td>-</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>tiagabine (6)</td>
<td>0.40 (0.049)</td>
<td>-</td>
<td>-</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>(S)-nipecotic acid [(S)-2]$^a$</td>
<td>0.60 (116)</td>
<td>0.40 (2320)</td>
<td>-</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>(S)-SNAP-5114 [7]$^a$</td>
<td>0.13 (388)</td>
<td>0.20 (5)</td>
<td>0.23 (71.7)</td>
<td>506</td>
<td></td>
</tr>
</tbody>
</table>

$^a$IC$_{50}$ data were taken from ref. 7.

It is important to note that only IIa has potent inhibitory effects on GAT3 and BGT1 among the eight stereo- and regioisomeric conformationally restricted analogs, which suggests that our stereochemical diversity-oriented conformational restriction strategy worked effectively in this study without the
structural data of the target protein. These results show that subtle changes in stereochemical restriction can affect the pharmacological profiles of compounds and that a systematic study by utilizing the stereochemical diversity-oriented conformational restriction strategy allows for exhaustive investigation of the bioactive conformation of compounds in order to develop the desired ligands that bind to the target protein.

The pharmacological potency within a chemical series is often well correlated with MW, and accordingly, lead compounds should be estimated by considering the potency with respect to MW.\textsuperscript{12} Recently, ligand efficacy (LE), which is a way of normalizing potency and MW of a compound to provide a useful comparison between compounds with range of MW and activities, has become a useful parameter for lead assessment.\textsuperscript{13} Although the inhibitory effects of II\textsubscript{a} on GAT3 and BGT1 are not very strong and it also binds to GABA\textsubscript{A} receptor, II\textsubscript{a} has vital importance as a lead for developing useful GAT3 or BGT1 inhibitors, due to its hydrophilic and low-MW properties, namely from the viewpoint of LE. Table 4 summarizes the LE and MW of II\textsubscript{a} together with those of tiagabine and (S)-SNAP and their low MW leads, (\textit{R})- or (\textit{S})-nipecotic acid [(\textit{R})-2 or (\textit{S})-2]. As a GAT3 inhibitor, II\textsubscript{a} has an excellent LE of 0.89, which are significantly higher than those of (\textit{R})-2 (LE = 0.65) or (\textit{S})-2 (LE = 0.40). As a BGT1 inhibitor, II\textsubscript{a} also has an excellent LE of 0.90. These results showed that II\textsubscript{a} can be a favorable lead for developing potent GAT3 and/or BGT1 inhibitors.

\textbf{Conclusion.} We used the chiral cyclopropane units effectively to synthesize a series of conformationally restricted GABA analogs and identified a GAT3/BGT1 inhibitor II\textsubscript{a}, which can be an effective lead compound for the optimization due to its favorable LE and hydrophilic properties. Thus, the stereochemical diversity-oriented conformational restriction strategy was effective in this study.
Experimental

Chemical shifts are reported in ppm downfield from Me₄Si (¹H) and MeOH (¹³C). Thin-layer chromatography was done on Merck coated plate 60F₂₅₄. Silica gel chromatography was done on Merck silica gel 60. Reactions were carried out under an argon atmosphere. Purity of the final compounds was determined by combustion analysis.

(1R,2S)-2-(Triphenylmethoxy)methyl-1-(N-methoxy-N-methyl)carbamoyl-1-phenylsulfonylcyclopropane (15). A mixture of 14a (7.3 g, 31 mmol) and AlCl₃ (8.2 g, 61 mmol) in CH₂Cl₂ (77 mL) was stirred at 0 °C for 30 min. Also, a mixture of Me(OMe)NH (12.0 g, 123 mmol) and Et₃N (17.1 mL, 123 mmol) in CH₂Cl₂ (77 mL) was stirred at room temperature for 1 h and filtered with a glass filter. To the filtrate was added the above reaction mixture, and the resulting mixture was stirred at room temperature for 5 h. The mixture was partitioned between CHCl₃ and aqueous HCl (1M), and the organic layer was washed with saturated aqueous NaHCO₃ and brine, dried (Na₂SO₄), and evaporated to give an oil (11.0 g). A mixture of the oil, TrCl (10.3 g, 37 mmol) and pyridine (9.9 mL, 123 mmol) in CH₂Cl₂ (62 mL) was stirred at room temperature for 12 h. The resulting mixture was partitioned between CHCl₃ and H₂O, and the organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 2:1 then 1:1) to give 15 (12.8 g, 24 mmol, 77%) as a white amorphous solid: ¹H-NMR (400 MHz, CDCl₃, 60 ºC) δ 1.47−1.52 (1H, m, H-3), 1.87−1.92 (1H, m, H-3), 2.17−2.21 (1H, m, H-2), 2.97−3.01 (1H, m, −CHaHbOTr), 3.15 (3H, s, NCH₃), 3.32−3.36 (1H, m, −CHaHbOTr), 3.60 (3H, s, NOCH₃), 7.17−7.84 (20H, m, aromatic); ¹³C-NMR (100MHz, CDCl₃) δ 14.1, 16.6, 25.5, 48.7, 60.3, 61.9, 87.4, 127.1, 127.8, 128.8, 133.6, 143.8; LRMS (FAB) m/z 564 (M+Na)⁺; [α]D¹⁹ −37.3° (c 1.09, CHCl₃).

(1R,2R)-2-(Triphenylmethoxymethyl)-1-(N-methoxy-N-methyl)carbamoylcyclopropane (12) and (1S,2R)-2-(Triphenylmethoxymethyl)-1-(N-methoxy-N-methyl)carbamoylcyclopropane (13). A
mixture of 15 (12.8 g, 24 mmol) and Mg (11.5 g, 0.47 mol) in MeOH/THF (1:2, 120 mL) was stirred at room temperature for 10 min and then at 0 °C for 1h. The resulting mixture was poured into cooled aqueous HCl (1 M), which was extracted with AcOEt. The organic layer was washed with saturated aqueous NaHCO3 and brine, dried (Na2SO4), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 19:1 then 12:1) to give 12 (5.1 g, 12.8 mmol, 54%) and 13 (3.4 g, 8.5 mmol, 36%) as oils.

12: 1H-NMR (400 MHz, CDCl3) δ 0.76–0.81 (1H, m, H-3), 1.20–1.25 (1H, m, H-3), 1.69–1.77 (1H, m, H-2), 2.05–2.15 (1H, m, H-1), 2.89–2.94 (1H, dd, –CHaHbOTr, J = 6.9, 9.5 Hz), 3.16–3.21 (4H, s and dd, NCH3 and –CHaHbOTr, J = 6.3, 9.5 Hz), 3.72 (3H, s, NOCH3), 7.18–7.43 (15H, m, aromatic); 13C-NMR (100 MHz, CDCl3) δ 12.3, 15.6, 21.8, 32.5, 61.6, 65.6, 86.3, 126.9, 127.7, 128.6, 144.1; LRMS (FAB) m/z 424 (M+Na)+; [α]23D –32.7º (c 0.96, CHCl3); Anal. Calcd for C26H27NO3•0.2CHCl3: C, 73.98; H, 6.45; N, 3.29. Found: C, 73.90; H, 6.51; N, 3.37.

13: 1H-NMR (400 MHz, CDCl3) δ 0.86–0.90 (1H, m, H-3), 0.99–1.01 (1H, m, H-3), 1.42–1.50 (1H, m, H-1), 2.22–2.30 (1H, m, H-2), 3.13 (1H, dd, –CHaHbOTr, J = 8.6, 10.4 Hz), 3.16 (3H, s, NCH3), 3.46 (1H, dd, –CHaHbOTr, J = 5.0, 10.4 Hz), 3.77 (3H, s, NOCH3), 7.17–7.45 (15H, m, aromatic); 13C-NMR (100 MHz, CDCl3) δ 9.3, 15.3, 21.2, 32.5, 61.6, 62.0, 86.5, 126.8, 127.6, 128.7, 144.3; LRMS (FAB) m/z 424 (M+Na)+; [α]24D +9.2º (c 0.84, CHCl3); Anal. Calcd for C26H27NO3•0.1H2O: C, 77.43; H, 6.80; N, 3.47. Found: C, 77.33; H, 6.78; N, 3.47.

(1R,2R)-1-Azidomethyl-2-(triphenylmethoxy)methylcyclopropane (16). A mixture of 12 (5.13 g, 12.8 mmol) and LiAlH4 (1.45 g, 38.3 mmol) in THF (60 mL) was stirred at 0 °C for 1 h, to which H2O (1.4 mL), aqueous NaOH (15%, 4.35 mL), and H2O (4.35 mL) was successively added. The resulting mixture was filtrated and evaporated to give an oil (4.8 g). A mixture of the oil and NaBH4 (967 mg, 25.6 mmol) in THF/MeOH (1:1, 64 mL) was stirred at room temperature for 5 h, and then aqueous HCl (1M) was added. The resulting mixture was extracted with CHCl3, and the organic layer was washed with saturated aqueous NaHCO3 and brine, dried (Na2SO4), and evaporated. The residue was purified by
column chromatography (silica gel; hexane/AcOEt, 5:1–1:1) to give an oil (3.0 g). A mixture of the oil, Ph3P (9.14 g, 34.8 mmol), and CBr4 (5.78 g, 17.4 mmol) in DMF (67 mL) was stirred at 0 °C for 40 min. To the mixture was added NaN3 (5.66 g, 8.71 mmol), and the resulting mixture was stirred at room temperature for 18 h and then evaporated. The residue was partitioned between AcOEt and H2O, and the organic layer was washed with brine, dried (Na2SO4), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 10:1–1:1) to give 16 (3.3 g, 8.93 mmol, 3 steps 70%) as an oil: 1H-NMR (400 MHz, CDCl3) δ 0.48–0.55 (2H, m, H-3), 0.95–1.07 (2H, m, H-1 and H-2), 2.94 (1H, dd, −CHaHbO, J = 6.3, 9.7 Hz), 3.03 (1H, dd, −CHaHbO, J = 6.3, 9.7 Hz), 3.11–3.17 (2H, m, −CH2N3), 7.20–7.46 (15H, m, aromatic); 13C-NMR (125 MHz, CDCl3) δ 8.6, 15.7, 17.3, 54.9, 66.3, 86.3, 126.9, 127.7, 128.6, 144.2; LRMS (FAB) m/z 392 (M+Na)+; [α]23D −12.0º (c 1.15, CHCl3); Anal. Calcd for C24H23N3O: C, 78.02; H, 6.27; N, 11.37. Found: C, 78.02; H, 6.33; N, 11.22.

(1R,2R)-1-(N-t-Butoxycarbonyl)aminomethyl-2-(triphenylmethoxy)methylcyclopropane (17). A mixture of 16 (569 mg, 1.54 mmol), Boc2O, and Pd-C (10%, 190 mg) in THF/MeOH (1:1, 15 mL) was stirred under H2 (1 atm) for 14 h. The resulting mixture was filtered with Celite and evaporated, and the residue was purified by column chromatography (silica gel; hexane/AcOEt, 10:1) to give 17 (605 mg, 1.4 mmol, 89%) as a white amorphous soli: 1H-NMR (500 MHz, CDCl3) δ 0.36–0.44 (2H, m, H-3), 0.76–0.87 (1H, m, H-1), 0.90–0.96 (1H, m, H-2), 1.45 (9H, s, −C(CH3)3), 2.74–2.78 (1H, m, −CHaHbO), 2.85–2.90 (1H, m, −CHaHbN), 3.06–3.09 (1H, m, −CHaHbO), 3.15–3.18 (1H, m, −CHaHbN), 4.68 (1H, brs, NH), 7.14–7.45 (15H, m, aromatic); 13C-NMR (125 MHz, CDCl3) δ 8.6, 17.0, 17.3, 28.4, 44.5, 66.8, 79.1, 86.2, 126.9, 127.7, 128.6, 144.3, 155.8; LRMS (FAB) m/z 466 (M+Na)+; [α]23D −10.8º (c 0.90, CHCl3).

(1R,2R)-1-(N-t-Butoxycarbonyl)aminomethyl-2-carboxycyclopropane (18). A solution of 17 (1.8 g, 4.1 mmol) in aqueous AcOH (80%, 41 mL) was stirred at room temperature for 16 h, and then H2O was added. The resulting solution was extracted with AcOEt, and the organic layer was washed with saturated
aqueous NaHCO₃ and brine, dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 3:1–1:5) to give an oil (709 mg). A mixture of the oil and Dess-Martin periodinane (1.7 g, 4.0 mmol) in CH₂Cl₂ (35 mL) was stirred at 0 °C for 1 h and then at room temperature for 1 h. After addition of a mixture of saturated aqueous Na₂S₂O₃ and saturated aqueous NaHCO₃ (1:1), the resulting mixture was extracted with CHCl₃, and the organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 3:1–1:1) to give an oil (700 mg). A mixture of the oil, 2-methyl-2-butene (3.0 mL), NaClO₂ (1.3 mg, 14 mmol), and NaH₂PO₄·2H₂O (1.1 g, 7.0 mmol) in t-BuO/H₂O H (4:1, 35 mL) was stirred at room temperature for 13 h. After addition of saturated NaCl, the resulting mixture was extracted with CHCl₃, and the organic layer was dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 2:1–1:2) to give 18 (724 mg, 3.4 mmol, 84%) as an oil: ¹H-NMR (500 MHz, CDCl₃) δ 0.86–0.94 (1H, m, H-3), 1.23–1.26 (1H, m, H-3), 1.44 (9H, s, C(CH₃)₃), 1.51–1.54 (1H, m, H-2), 1.62–1.67 (1H, m, H-1), 3.02–3.04 (1H, m, H-1´), 3.18–3.21 (1H, m, H-1´), 4.70 and 5.70 (1H, br, −NH); ¹³C-NMR (125 MHz, CDCl₃) δ 14.2, 18.7, 23.1, 28.3, 43.0, 79.6, 155.8, 179.4.

(1R,2R)-1-Aminomethyl-2-carboxycyclopropane hydrochloride (Ia, HCl salt). A solution of 18 (338 mg, 1.6 mmol) in AcOEt including HCl (4 M) was stirred at room temperature for 10 min, and then evaporated to give Ia (HCl salt, 216 mg, 89%) as white powder: ¹H-NMR (400 MHz, D₂O) δ 1.03–1.07 (1H, m, H-3), 1.27–1.31 (1H, m, H-3), 1.68–1.73 (2H, m, H-1 and H-2), 2.90–2.93 (1H, dd, H-1´, J = 7.7, 13.1 Hz), 3.00–3.04 (1H, dd, H-1´, J = 6.8, 13.1 Hz); ¹³C-NMR (125 MHz, CDCl₃) δ 14.5, 19.7, 19.8, 42.9, 178.2; LRMS (FAB) m/z 116 (M+H)+; [α]²⁰D −65.8° (c 0.97, 1 N HCl), [lit¹⁰a]²⁰D −65.5° (c 0.95, 1 N HCl); m.p. 132.0–137.0 °C (lit.¹⁰a: 131–135 °C); Anal. Calcd for C₅H₁₀ClNO₂: C, 39.62; H, 6.65; N, 9.24. Found: C, 39.39; H, 6.70; N, 8.96.
(1S,2R)-1-Azidomethyl-2-(triphenylmethoxy)methylcyclopropane (19). Compound 19 (3.1 g, 8.4 mmol, quant., oil) was prepared from 13 (190 mg, 0.50 mmol) as described for the preparation of 16: $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 0.15–0.18 (1H, m, H-3), 0.83–0.87 (1H, m, H-3), 1.18–1.25 (1H, m, H-2), 1.28–1.34 (1H, m, H-1), 2.75 (1H, dd, $-\text{CH}_a\text{H}_b\text{N}_3$, $J$ = 8.6, 9.7 Hz), 2.90 (1H, dd, $-\text{CH}_a\text{H}_b\text{O}$, $J$ = 8.0, 13.2 Hz), 3.23 (1H, dd, $-\text{CH}_a\text{H}_b\text{O}$, $J$ = 6.9, 13.0 Hz), 3.37 (1H, dd, $-\text{CH}_a\text{H}_b\text{N}_3$, $J$ = 6.3, 9.7 Hz), 7.21–7.47 (15H, m, aromatic); $^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta$ 8.8, 14.4, 15.3, 51.1, 63.4, 86.4, 126.9, 127.8, 128.6, 144.1; LRMS (FAB) $m/z$ 392.2 (M+Na)$^+$; $[\alpha]^{24}_D$ +25.0º ($c$ 1.28, CHCl$_3$); Anal. Calcd for C$_{24}$H$_{23}$N$_3$O: C, 78.02; H, 6.27; N, 11.37. Found: C, 77.75; H, 6.30; N, 11.07.

(1S,2R)-1-(N-tert-Butoxycarbonyl)aminomethyl-2-(triphenylmethoxy)methylcyclopropane (20). Compound 20 (515 mg, 1.16 mmol, 84%, a white amorphous solid) was prepared from 19 (512 mg, 1.39 mmol) as described for the preparation of 17: $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ –0.11– –0.77 (1H, m, H-3), 0.63–0.68 (1H, m, H-3), 1.15–1.19 (1H, m, H-2), 1.29–1.36 (10H, m, H-1 and $-\text{C(CH}_3)_3$), 2.04–2.11 (1H, m, $-\text{CH}_a\text{H}_b\text{O}$), 2.26–2.31 (1H, m, $-\text{CH}_a\text{H}_b\text{N}$), 3.67–3.72 (2H, m, $-\text{CH}_a\text{H}_b\text{O}$ and $-\text{CH}_a\text{H}_b\text{N}$), 5.45 (1H, br, NH), 7.20–7.49 (15H, m, aromatic); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 7.6, 14.6, 15.4, 28.4, 40.2, 63.9, 78.5, 86.9, 127.0, 127.9, 128.3, 143.9, 155.9; LRMS (FAB) $m/z$ 466 (M+Na)$^+$; $[\alpha]^{24}_D$ –0.6º ($c$ 0.68, CHCl$_3$).

(3R,4S)-N-tert-Butoxycarbonyl-3,4-methano-2-pyrrolidone (21). A solution of 20 (2.3 g, 5.2 mmol) in aqueous AcOH (80%, 52 mL) was stirred at room temperature for 11 h, and then H$_2$O was added. The resulting solution was extracted with AcOEt, and the organic layer was washed with saturated aqueous NaHCO$_3$ and brine, dried (Na$_2$SO$_4$), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 3:1–1:5) to give an oil (906 mg). To a solution of the oil in acetone (44 mL), a mixture of CrO$_3$ (879 mg, 8.79 mmol), and H$_2$SO$_4$ (0.8 mL) in H$_2$O (1.8 mL) was added slowly at 0 ºC. To the resulting orange solution was added i-PrOH until the color of the solution was disappeared, and the resulting mixture was partitioned between Et$_2$O and H$_2$O. The organic layer was washed with
saturated aqueous NaHCO₃ and brine, dried (Na₂SO₄), and evaporated. The residue was purified by
column chromatography (silica gel; hexane/AcOEt, 2:1–1:5) to give 21 (615 mg, 3.1 mmol, 60%) as a
white amorphous solid: ¹H-NMR (500 MHz, CDCl₃) δ 0.77–0.79 (1H, m, H-6), 1.17–1.20 (1H, m, H-6),
1.50 (9H, s, –C(CH₃)₃), 1.86–1.91 (1H, m, H-3), 2.00–2.04 (1H, m, H-4), 3.71 (1H, d, H-5, J = 10.9 Hz),
3.79 (1H, m, H-5); ¹³C-NMR (100 MHz, CDCl₃) δ 11.6, 12.3, 21.5, 28.0, 48.0, 82.6, 150.4, 173.8.

(1S,2R)-1-Aminomethyl-2-carboxycyclopropane hydrochloride (Ib, HCl salt). A solution of 21
(601 mg, 3.0 mmol) and TFA (1.1 mL) in CH₂Cl₂ (10 mL) was stirred at room temperature for 30 min,
and then evaporated. A solution of the residual oil in aqueous HCl (1M, 30 mL) was stirred at 70 °C for
12 h, and then evaporated to give while powder. Recrystalization of the powder from EtOH gave
colorless crystals of Ib (HCl salt, 182 mg, 39%) as HCl salt: ¹H-NMR (500 MHz, D₂O) δ 1.01–1.06 (1H, m,
H-3), 1.28–1.32 (1H, m, H-3), 1.57–1.67 (1H, m, H-1), 1.87–1.93 (1H, m, H-2), 3.18–3.29 (2H, m, H-1’);
LRMS (FAB) m/z 116 (M+H)⁺; [α]D²⁴ −38.3° (c 0.95, 1 N HCl) , [lit¹⁰b [α]D²⁶ −38.5° (c 1, 1 N HCl)]; m.p. 243–244 ºC (lit.¹⁰b : 244–246 ºC); Anal. Calcd for C₅H₁₀ClNO₂: C, 39.62; H, 6.65; Cl, 23.39;

(1S,2R)-2-(t-Butyldiphenylsilyloxy)methyl-1-(2-hydroxyethyl)cyclopropane (22). To a suspension
of MeOCH₂PPh₃Cl chloride (12.3 g, 36 mmol) in THF (81 mL) was added NaN(Si(CH₃)₃)₂ (1.9 M in
THF, 17.1 mL, 33 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. To the
resulting solution was added a solution of 10 (5.51 g, 16.3 mmol) in THF (80 mL) at 0 ºC, and the
resulting mixture was stirred at the same temperature for 3 h. After addition of aqueous saturated NH₄Cl,
the solvent of the mixture was evaporated, and the residue was partitioned between AcOEt and H₂O. The
organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was purified by
column chromatography (silica gel; hexane/AcOEt, 49:1) to give an oil (5.0 g). To a solution of the oil in
THF (56 mL) was added a mixture of aqueous HCl (12 M, 17 mL) and THF (80 mL) at 0 C, and the
resulting mixture was stirred at the same temperature for 35 smin. To the mixture was added aqueous
saturated NaHCO₃ (100 mL), and the resulting solution was extracted with AcOEt. The organic layer was washed with brine, dried (Na₂SO₄), and evaporated to give an oil (4.4 g). A mixture of the oil and NaBH₄ (206 mg, 5.5 mmol) in THF/MeOH (4:1, 136 mL) was stirred at room temperature for 5 h, and then aqueous HCl (1M) was added. The resulting mixture was extracted with CHCl₃ and the organic layer was washed with saturated aqueous NaHCO₃ and brine, dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 8:1–3:1) to give **22** (3.8 g, 66%) as an oil: ¹H-NMR (500 MHz, CDCl₃) δ 0.27–0.33 (2H, m, H-3), 0.53–0.59 (1H, m, H-1), 0.93–1.01 (1H, m, H-2), 1.06 (9H, s, −C(CH₃)₃), 1.18–1.25 (1H, m, H-1´), 1.77–1.82 (1H, m, H-1´), 2.53 (1H, br s, −OH), 3.08 (1H, dd, −CH₃HbOTBDPS, J = 9.2, 10.3 Hz), 3.72–3.80 (2H, m, H-2´), 3.89 (1H, dd, −CH₃HbOTBDPS, J = 5.2, 10.3 Hz), 7.36–7.69 (10H, m, aromatic); ¹³C-NMR (125 MHz, CDCl₃) δ 8.3, 14.4, 19.1, 20.6, 26.8, 35.9, 63.3, 68.3, 127.6, 129.6, 133.4, 135.6; LRMS (FAB) m/z 377.2 (M+Na)⁺; [α]₂²²D −0.2º (c 1.28, CHCl₃); Anal. Calcd for C₂₂H₃₀O₂Si: C, 74.53; H, 8.53. Found: C, 74.39; H, 8.73.

**1S,2R**-1-(2-Benzyloxy)ethyl-2-hydroxymethylcyclopropane (23). A mixture of **22** (3.0 g, 8.5 mmol), BnBr (2.01 mL, 17 mmol), Bu₄NI (625 mg, 1.7 mmol), and NaH (60%, 1.69 g, 42 mmol) in DMF/THF (1/1, 170 mL) was stirred at -10 °C for 25 h. After addition of aqueous saturated NH₄Cl, the solvent was evaporated, and the residue was partitioned between AcOEt and H₂O. The organic layer was washed with brine, dried (Na₂SO₄) and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 30:1) to give an oil (3.17 g). A mixture of the oil and TBAF (1.0M in THF, 10.7 mL, 10.7 mmol) was stirred at room temperature for 3 h and then evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 4:1–1.1) to give **23** (1.46 g, 7.09 mmol, 84%) as an oil: ¹H-NMR (500 MHz, CDCl₃) δ 0.34–0.43 (2H, m, H-3), 0.67–0.73 (1H, m, H-1), 0.86–0.92 (1H, m, H-2), 1.42–1.49 (1H, m, H-1´), 1.62–1.69 (1H, m, H-1´), 1.71–1.77 (1H, br s, −OH), 3.28–3.31 (1H, m, −CH₃HbO), 3.52–3.57 (3H, m, H-2´ and −CH₃HbOH), 4.51 (2H, s, −CH₂Ph), 7.25–7.38 (5H, m, aromatic); ¹³C-NMR (125 MHz, CDCl₃) δ 9.6, 14.3, 21.0, 33.4, 67.0, 70.2, 72.9, 127.6, 127.7,
128.3, 138.3; LRMS (EI) m/z 205 (M−H)+; [α]$_{D}^{22}$ +1.6º (c 0.93, CHCl$_3$); Anal. Calcd for C$_{13}$H$_{18}$O$_2$•0.15H$_2$O: C, 74.71; H, 8.83. Found: C, 74.84; H, 8.97.

**(1S,2R)-trans-1-(2-Benzylxylo)ethyl-2-carboxycyclopropane (24).** A mixture of 23 (528 mg, 2.6 mmol) and Dess-Martin periodinane (1.67 g, 3.9 mmol) in CH$_2$Cl$_2$ (26 mL) was stirred at 0 °C for 45 min. After addition of a mixture of saturated aqueous Na$_2$S$_2$O and saturated aqueous NaHCO$_3$ (1:1), the resulting mixture was extracted with CHCl$_3$, and the organic layer was washed with brine, dried (Na$_2$SO$_4$), and evaporated to give an oil (583 mg). A mixture of the oil, 2-methyl-2-butene (2.17 mL, 21 mmol), NaClO$_2$ (926 mg, 10 mmol), and NaH$_2$PO$_4$•2H$_2$O (805 mg, 5.2 mmol) in t-BuOH/H$_2$O (24:1, 25 mL) was stirred at room temperature for 2 h. The resulting mixture was partitioned between AcOEt and aqueous HCl (1 M), and the organic layer was washed with brine, dried (Na$_2$SO$_4$), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 4:1) to give 24 (547 mg, 97%) as an oil: ¹H-NMR (400 MHz, CDCl$_3$) δ 0.81–0.85 (1H, m, H-3), 1.23–1.28 (1H, m, H-1), 1.40–1.44 (1H, m, H-3), 1.52–1.58 (1H, m, H-2), 1.59–1.66 (2H, m, H-1’), 3.54 (2H, t, H-2’, J = 6.8 Hz), 4.51 (2H, s, −CH$_2$Ph), 7.25–7.36 (5H, m, aromatic); ¹³C-NMR (100 MHz, CDCl$_3$) δ 16.0, 19.8, 21.0, 33.2, 69.4, 73.0, 127.6, 127.6, 128.4, 138.3, 180.7; LRMS (EI) m/z 220 (M)+; [α]$_{D}^{23}$ −45.9º (c 1.03, CHCl$_3$); Anal. Calcd for C$_{13}$H$_{16}$O$_3$•0.05CHCl$_3$: C, 69.28; H, 7.15. Found: C, 69.10; H, 7.17.

**(1S,2R)-1-(2-Benzylxylo)ethyl-2-(t-butoxycarbonyl)aminocyclopropane (25).** A mixture of 24 (1.35 g, 6.1 mmol), (PhO)$_2$PON$_3$ (3.95 mL, 18 mmol), and Et$_3$N (1.28 mL, 9.2 mmol) in CH$_2$Cl$_2$ (61 mL) was stirred at room temperature for 3.5 h. The reaction mixture was partitioned between AcOEt and H$_2$O, and the organic layer was washed with brine, dried (Na$_2$SO$_4$), and evaporated. A solution of the residual oil in t-BuOH (61 mL) was heated under reflux for 19 h, and then evaporated. The residue was purified by column chromatography (silica gel; AcOEt/hexane, 7:1) to give 25 (1.33 g, 75%) as an oil: ¹H-NMR (400 MHz, CDCl$_3$) δ 0.53–0.56 (1H, m, H-3), 0.63–0.67 (1H, m, H-3), 0.91–0.97 (1H, m, H-1), 1.44–1.50 (10H, m, H-1’ and −C(CH$_3$)$_3$), 1.64–1.69 (1H, m, H-1’), 2.25–2.30 (1H, m, H-2), 3.57 (2H, dd, H-2’, J =
6.3, 6.8 Hz), 4.52 (2H, s, −CH₂Ph), 4.57–4.72 (1H, br, −NH), 7.26–7.34 (5H, m, aromatic); ¹³C-NMR (100 MHz, CDCl₃) δ 13.5, 17.7, 28.4, 29.1, 32.5, 69.6, 72.9, 79.3, 127.5, 127.6, 128.3, 138.6, 156.4; LRMS (FAB) m/z 314 (M+Na)⁺; [α]²²D −25.0° (c 0.70, CHCl₃); Anal. Calcd for C₁₇H₂₅NO₃: C, 70.07; H, 8.65; N, 4.81. Found: C, 69.77; H, 8.45; N, 4.65.

(1S,2R)-2-(t-Butoxycarbonyl)amino-1-(carboxymethyl)cyclopropane (26). A mixture of 25 (1.33 mg, 4.6 mmol) and Pd-C (10%, 664 mg) in THF/MeOH (1:4, 46 mL) was stirred under H₂ (1 atm) at room temperature for 3 h. The resulting mixture was filtered with Celite and evaporated, and the residue was purified by column chromatography (silica gel; hexane/AcOEt, 2:1) to give an oil (949 mg). A mixture of the oil and Dess-Martin periodinane (584 mg, 1.4 mmol) in CH₂Cl₂ (10 mL) was stirred at 0 °C for 1 h and then at room temperature for 1 h. After addition of a mixture of saturated aqueous Na₂S₂O₃ and saturated aqueous NaHCO₃ (1:1), the resulting mixture was extracted with CHCl₃, and the organic layer was washed with brine, dried (Na₂SO₄), and evaporated to give an oil (228 mg). A mixture of the oil, 2-methyl-2-butene (831 mL, 7.8 mmol), NaClO₂ (353 mg, 3.9 mmol), and NaH₂PO₄·2H₂O (307 mg, 2.0 mmol) in t-BuOH/H₂O (4:1, 10 mL) was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt and aqueous HCl (1 M), and the organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 1:1) to give 26 (192 mg, 91%) as an oil: ¹H-NMR (400 MHz, CDCl₃) δ 0.64–0.69 (1H, m, H-3), 0.85–0.91 (1H, m, H-3), 1.14–1.19 (1H, m, H-1), 1.45 (9H, s, −C(CH₃)₃), 1.96–2.05 (1H, m, H-1’), 2.33–2.35 (1H, m, H-2), 2.80–2.85 (1H, m, H-1’), 5.08 (1H, brs, −NH); ¹³C-NMR (100 MHz, CDCl₃) δ 13.3, 15.8, 28.2, 29.2, 37.7, 80.9, 157.8, 174.5; LRMS (FAB) m/z 216 (M+H)⁺; [α]²⁰D +44.5° (c 0.90, CHCl₃); Anal. Calcd for C₁₀H₁₇NO₄·0.1H₂O: C, 55.34; H, 7.99; N, 6.45. Found: C, 55.09; H, 7.88; N, 6.20.

(1S,2R)-2-amino-1-(carboxymethyl)cyclopropane hydrochloride (IIa, HCl salt). A mixture of 26 (431 mg, 2.0 mmol) and aqueous HCl (4 M, 40 mL) was stirred at room temperature for 35 min, and then
evaporated. The residue was triturated with Et₂O to give IIa (HCl salt, 230 mg, 1.5 mmol, 76%) as white powder: ¹H-NMR (500 MHz, D₂O) δ 0.81–0.85 (1H, m, H-3), 1.03–1.07 (1H, m, H-3), 1.43–1.50 (1H, m, H-1), 2.35–2.47 (2H, m, H-1´), 2.53–2.56 (1H, m, H-1); ¹³C-NMR (500 MHz, D₂O) δ 10.1, 13.2, 28.5, 36.2, 177.5; LRMS (FAB) m/z 116 (M+H)+; [α]²¹D −21.3° (c 0.99, 1 N HCl); m.p. 104–106 ºC; Anal. Calcd for C₅H₁₀ClNO₂•0.1H₂O: C, 39.15; H, 6.70; N, 9.13. Found: C, 39.08; H, 6.51; N, 9.10.

(1R,2R)-2-((t-Butyldiphenylsilyloxy)methyl-1-(2-hydroxyethyl)cyclopropane (27). Compound 27 (oil, 229 mg, 0.65 mmol, 72%) was prepared from 11 (304 mg, 0.90 mmol) as described for the preparation of 22: ¹H-NMR (500 MHz, CDCl₃) δ −0.18−0.14 (1H, m, H-3), 0.58–0.63 (1H, m, H-3), 0.87–0.93 (1H, m, H-1), 1.06 (9H, s, −C(CH₃)₃), 1.11–1.18 (1H, m, H-2), 1.48–1.56 (1H, m, H-1´), 1.78–1.84 (1H, m, H-1´), 2.89 (1H, br s, −OH), 3.38 (1H, dd, −CH₃HbOTBDPS, J = 10.3, 11.5 Hz), 3.73–3.82 (2H, m, H-2´), 3.92 (1H, dd, −CH₃HbOTBDPS, J = 4.6, 11.5 Hz), 7.38–7.72 (10H, m, aromatic); ¹³C-NMR (100 MHz, CDCl₃) δ 7.4, 13.6, 17.6, 19.1, 26.7, 31.2, 63.3, 64.1, 127.7, 129.7, 133.4, 135.6; LRMS (FAB) m/z 355 (M+H)+; [α]²¹D +12.2° (c 1.46, CHCl₃); Anal. Calcd for C₂₂H₃₀O₂Si: C, 74.53; H, 8.53. Found: C, 74.27; H, 8.52.

(1R,2R)-1-(2-Benzyloxy)ethyl-2-hydroxymethylcyclopropane (28). Compound 28 (oil, 935 mg, 4.5 mmol, 95%) was prepared from 27 (1.7 g, 4.8 mmol) as described for the preparation of 23: ¹H-NMR (400 MHz, CDCl₃) δ −0.14–0.10 (1H, m, H-3), 0.61–0.66 (1H, m, H-3), 0.74–0.82 (1H, m, H-1), 1.18–1.26 (1H, m, H-2), 1.43–1.54 (1H, m, H-1´), 1.85–1.92 (1H, m, H-1´), 3.19 (1H, dd, −CH₃HbOH, J = 10.4, 10.9 Hz), 3.49–3.54 (1H, m, H-2´), 3.59–3.64 (1H, m, H-2´), 3.78–3.90 (2H, br s and dd, −OH and −CH₃HbOH, J = 6.3, 10.4 Hz), 4.56 (2H, s, −CH₂Ph), 7.27–7.38 (5H, m, aromatic); ¹³C-NMR (100 MHz, CDCl₃) δ 7.0, 14.0, 18.6, 28.2, 62.1, 70.7, 73.4, 127.9, 128.0, 128.4, 137.4; LRMS (EI) m/z 206 (M)+; [α]²²D +21.4° (c 1.38, CHCl₃); Anal. Calcd for C₁₃H₁₈O₂•0.1H₂O: C, 75.04; H, 8.82. Found: C, 75.11; H, 8.92.
(1R,2R)-1-(2-Benzylxoy)ethyl-2-carboxycyclopropane (29). Compound 29 (oil, 875 mg, 91%) was prepared from 28 (902 mg, 4.4 mmol) as described for the preparation of 24: ¹H-NMR (500 MHz, CDCl₃) δ 0.97–1.00 (1H, m, H-3), 1.10–1.13 (1H, m, H-3), 1.44–1.53 (1H, m, H-1), 1.69–1.74 (1H, m, H-2), 1.86–1.94 (2H, m, H-1’), 3.51 (2H, t, H-2’, J = 6.3 Hz), 4.51 (2H, s, –CH₂Ph), 7.26–7.36 (5H, m, aromatic); ¹³C-NMR (125 MHz, CDCl₃) δ 14.2, 17.8, 20.1, 27.2, 69.9, 72.8, 127.4, 127.5, 128.3, 138.4, 179.6; LRMS (EI) m/z 220 (M)+; [α]D²² −50.1º (c 1.12, CHCl₃); Anal. Calcd for C₁₃H₁₆O₃·0.1H₂O: C, 70.31; H, 7.35. Found: C, 70.00; H, 7.27.

(1R,2R)-1-(2-Benzylxoy)ethyl-2-(t-buthoxycarbonyl)aminocyclopropane (30). Compound 30 (oil, 663 mg, 2.3 mmol, 59%) was prepared from 29 (845 mg, 3.8 mmol) as described for the preparation of 25: ¹H-NMR (500 MHz, CDCl₃) δ 0.20–0.24 (1H, m, H-3), 0.90–0.96 (2H, m, H-1 and H-3), 1.42 (9H, s, –C(CH₃)₃), 1.60–1.65 (1H, m, H-1’), 1.80–1.85 (1H, m, H-1’), 2.52–2.56 (1H, m, H-2), 3.52–3.66 (2H, m, H-2’), 4.54 (2H, s, –CH₂Ph), 5.35 (1H, brs, –NH), 7.27–7.35 (5H, m, aromatic); ¹³C-NMR (125 MHz, CDCl₃) δ 12.5, 15.0, 26.7, 28.3, 28.6, 70.6, 73.2, 78.9, 127.5, 128.4, 129.8, 138.3, 157.1; LRMS (FAB) m/z 292 (M+H)+; [α]D²¹ −49.4º (c 1.08, CHCl₃); Anal. Calcd for C₁₇H₂₅NO₃: C, 70.07; H, 8.65; N, 4.81. Found: C, 70.07; H, 8.49; N, 4.69.

(4R,5R)-N-t-Butoxycarbonyl-4,5-methano-2-pyrrolidone (31). A mixture of 30 (554 mg, 1.9 mmol) and Pd-C (10%, 280 mg) in THF/MeOH (1:4, 15 mL) was stired under H₂ (1 atm) at room temperature for 6 h. The resulting mixture was filtered with Celite and evaporated, and the residue was purified by column chromatography (silica gel; hexane/AcOEt, 3:2) to give an white amorphous solid (363 mg). To a solution of the solid in acetone (18 mL), a mixture of CrO₃ oxide (360 mg, 3.6 mmol) and H₂SO₄ (0.33 mL) in H₂O (1.2 mL) was added slowly at 0 °C. To the resulting orange solution was added i-PrOH until the color of the solution disappeared, and the resulting mixture was partitioned between Et₂O and H₂O. The organic layer was washed with saturated aqueous NaHCO₃ and brine, dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 4:1–3:1) to
give \(31\) (268 mg, 72%) as a white amorphous solid: \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.44–0.47 (1H, m, H-6), 0.98–1.03 (1H, m, H-6), 1.45–1.50 (1H, m, H-4), 1.55 (9H, s, –C(CH\(_3\))\(_3\)), 2.52 (1H, d, H-3, \(J = 18.6\) Hz), 2.89 (1H, dd, H-3, \(J = 7.7, 18.6\) Hz), 3.56–3.59 (1H, m, H-5); \(^{13}\)C-NMR (125 MHz, CDCl\(_3\)) \(\delta\) 5.4, 14.9, 28.0, 35.0, 36.4, 83.0, 150.4, 173.6; LRMS (FAB) \(m/z\) 198 (M+H); \([\alpha]^{22}_{D} +36.5^\circ\) (c 0.64, CHCl\(_3\)).

**(1R,2R)-cis-2-Amino-1-(carboxymethyl)cyclopropane hydrochloride (IIb, HCl salt).** A solution of \(31\) (229 mg, 1.2 mmol) in aqueous HCl (2 M, 24 mL) was stirred at 70 °C for 10 h, and the evaporated. The residue was triturated with Et\(_2\)O to give IIb (HCl salt, 70 mg, 40%) as a white solid: \(^1\)H-NMR (500 MHz, D\(_2\)O) \(\delta\) 0.61–0.65 (1H, m, H-3), 1.09–1.13 (1H, m, H-3), 1.34–1.42 (1H, m, H-1), 2.49 (1H, dd, H-1’, \(J = 8.0, 17.2\) Hz), 2.60 (1H, dd, H-1’, \(J = 7.4, 17.2\) Hz), 2.72–2.75 (1H, m, H-2); \(^{13}\)C-NMR (125 MHz, D\(_2\)O) \(\delta\) 9.8, 11.5, 27.2, 32.6, 177.3; LRMS (FAB) \(m/z\) 116 (M+H); \([\alpha]^{22}_{D} +21.5^\circ\) (c 0.83, 1 N HCl); m.p. 165–169 °C; Anal. Caled for C\(_5\)H\(_{10}\)ClNO\(_2\)•0.25H\(_2\)O: C, 38.47; H, 6.78; Cl, 22.71; N, 8.97. Found: C, 38.22; H, 6.37; Cl, 23.43; N, 8.87.

**(1S,2R)-2-(Triphenylmethoxy)methyl-1-((N-methoxy-N-methyl)carbamoyl-1-phenylsulfonylcyclopropane (ent-15).** \([\alpha]^{22}_{D} +37.7^\circ\) (c 1.22, CHCl\(_3\)) Anal. Caled for C\(_{32}\)H\(_{31}\)NO\(_5\)S•0.45CHCl\(_3\): C, 65.46; H, 5.32; N, 2.35; S, 5.39. Found: C, 65.43; H, 5.31; N, 2.43; S, 5.11.

**(1S,2S)-2-(Triphenylmethoxymethyl)-1-(N-methoxy-N-methyl)carbamoylcycropropane (ent-12):** \([\alpha]^{20}_{D} +33.3^\circ\) (c 0.95, CHCl\(_3\)).

**(1R,2S)-2-(Triphenylmethoxymethyl)-1-(N-methoxy-N-methyl)carbamoylcycropropane (ent-13):** \([\alpha]^{22}_{D} +9.3^\circ\) (c 0.99, CHCl\(_3\)).

**(1S,2S)-1-Azidomethyl-2-(triphenylmethoxy)methylcyclopropane (ent-16):** \([\alpha]^{20}_{D} +12.4^\circ\) (c 0.94, CHCl\(_3\)); Anal. Caled for C\(_{24}\)H\(_{23}\)N\(_3\)O•0.1H\(_2\)O: C, 77.64; H, 6.30; N, 11.32. Found: C, 77.60; H, 6.35; N, 11.21.
(1S,2S)-1-(N-t-Butoxycarbonyl)aminomethyl-2-(triphenylmethoxy)methylcyclopropane (ent-17):
\[ [\alpha]_{D}^{21} +11.0^\circ \text{ (c 1.06, CHCl}_3) \].

(1S,2S)-1-Aminomethyl-2-carboxycyclopropane hydrochloride (ent-1a, HCl salt): \[ [\alpha]_{D}^{22} +68.6^\circ \text{ (c 0.97, 1 N HCl aq.)}; \text{m.p.} 141.0–145.0 ^\circ \text{C}; \text{Anal. Calcd for C}_{5}\text{H}_{10}\text{ClNO}_2: C, 39.62; H, 6.65; Cl, 23.39; N, 9.24. \text{Found: C, 39.34; H, 6.44; Cl, 23.14; N, 9.18.}

(1R,2S)-1-Azidomethyl-2-(triphenylmethoxy)methylcyclopropane (ent-19): \[ [\alpha]_{D}^{22} -24.3^\circ \text{ (c 1.86, CHCl}_3) \]; \text{Anal. Calcd for C}_{24}\text{H}_{23}\text{N}_3\text{O: C, 78.02; H, 6.27; N, 11.37. Found: C, 77.74; H, 6.24; N, 11.24.}

(1R,2S)-1-(N-t-Butoxycarbonyl)aminomethyl-2-(triphenylmethoxy)methylcyclopropane (ent-20): \[ [\alpha]_{D}^{22} +1.2^\circ \text{ (c 1.01, CHCl}_3) \].

(1R,2S)-cis-1-Aminomethyl-2-carboxycyclopropane (ent-1b, HCl salt): \text{m.p. 241.2–243.0 ^\circ \text{C (lit.}^{10b} \text{: 241–242 ^\circ \text{C)}; \text{Anal. Calcd for C}_{5}\text{H}_{10}\text{ClNO}_2: C, 39.62; H, 6.65; Cl, 23.39; N, 9.24. Found: C, 39.65; H, 6.56; Cl, 23.29; N, 9.28.}

(1R,2S)-2-(t-Butyldiphenylsilyloxy)methyl-1-(2-hydroxyethyl)cyclopropane (ent-22): \[ [\alpha]_{D}^{23} +0.2^\circ \text{ (c 1.25, CHCl}_3) \]; \text{Anal. Calcd for C}_{22}\text{H}_{30}\text{O}_2\text{Si: C, 74.53; H, 8.53. Found: C, 74.16; H, 8.57.}

(1R,2S)-trans-1-(2-Benzylxy)ethyl-2-hydroxymethylcyclopropane (ent-23): \[ [\alpha]_{D}^{24} -1.5^\circ \text{ (c 1.29, CHCl}_3) \].

(1R,2S)-1-(2-Benzylxy)ethyl-2-carboxycyclopropane (ent-24): \[ [\alpha]_{D}^{24} +43.9^\circ \text{ (c 1.30, CHCl}_3) \].

(1R,2S)-1-(2-Benzylxy)ethyl-2-(t-buthoxycarbonyl)aminocyclopropane (ent-25): \[ [\alpha]_{D}^{22} +25.0^\circ \text{ (c 1.30, CHCl}_3) \].

(1R,2S)-trans-2-(t-Buthoxycarbonyl)amino-1-(carboxymethyl)cyclopropane (ent-26): \[ [\alpha]_{D}^{22} -43.3^\circ \text{ (c 1.20, CHCl}_3) \]; \text{Anal. Calcd for C}_{10}\text{H}_{17}\text{NO}_4: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.50; H, 7.77; N, 6.37.}
(1R,2S)-trans-2-amino-1-(carboxymethyl)cyclopropane hydrochloride (ent-IIa, HCl salt): $[\alpha]^22_D +20.6^\circ$ (c 1.10, 1 $N$ HCl); m.p. 103–105 °C; Anal. Calcd for C$_3$H$_{10}$ClNO$_2$$ \cdot$0.2H$_2$O: C, 38.70; H, 6.75; Cl, 22.84; N, 9.03. Found: C, 38.75; H, 6.61; Cl, 22.94; N, 9.13.

(1S,2S)-2-(t-Butyldiphenylsilyloxy)methyl-1-(2-hydroxyethyl)cyclopropane (ent-27): $[\alpha]^24_D -11.5^\circ$ (c 1.57, CHCl$_3$); Anal. Calcd for C$_{22}$H$_{30}$O$_2$Si: C, 74.53; H, 8.53. Found: C, 74.41; H, 8.62.

(1S,2S)-cis-1-(2-Benzylxy)ethyl-2-hydroxymethylcyclopropane (ent-28): $[\alpha]^24_D -20.6^\circ$ (c 1.27, CHCl$_3$).

(1S,2S)-cis-1-(2-Benzylxy)ethyl-2-carboxycyclopropane (ent-29): $[\alpha]^24_D +49.8^\circ$ (c 1.45, CHCl$_3$); Anal. Calcd for C$_{13}$H$_{16}$O$_3$$ \cdot$0.15H$_2$O: C, 70.03; H, 7.37. Found: C, 70.03; H, 7.33.

(1S,2S)-cis-1-(2-Benzylxy)ethyl-2-(t-butoxycarbonyl)aminocyclopropane (ent-30): $[\alpha]^22_D +49.1^\circ$ (c 1.60, CHCl$_3$); Anal. Calcd for C$_{17}$H$_{25}$NO$_3$: C, 70.07; H, 8.65; N, 4.81. Found: C, 69.83; H, 8.46; N, 4.82.

(4S,5S)-N-t-Butoxycarbonyl-4,5-methano-2-pyrrolidone (ent-31): $[\alpha]^24_D +36.4^\circ$ (c 0.69, CHCl$_3$).

(1S,2S)-cis-2-Amino-1-(carboxymethyl)cyclopropane hydrochloride (ent-IIb, HCl salt). $^1$H-NMR (400 MHz, D$_2$O) $\delta$ 0.60–0.64 (1H, m, H-3), 1.08–1.14 (1H, m, H-3), 1.32–1.42 (1H, m, H-1), 2.50 (1H, dd, H-1’, $J = 8.2$, 17.2 Hz), 2.61 (1H, dd, H-1’, $J = 7.7$, 17.2 Hz), 2.69–2.74 (1H, m, H-2); $^{13}$C-NMR (125 MHz, D$_2$O) 9.8, 11.5, 27.2, 32.6, 177.2; LRMS (FAB) $m/z$ 116 (M+H)$^+$; $[\alpha]^22_D +22.8^\circ$ (c 0.82, 1 $N$ HCl); m.p. 175–179 °C; Anal. Calcd for C$_3$H$_{10}$ClNO$_2$: C, 39.62; H, 6.65; Cl, 23.39; N, 9.24. Found: C, 39.32; H, 6.49; Cl, 23.01; N, 9.24.

Animals. Male ICR mice (5 weeks old; Japan SLC, Hamamatsu, Japan) and male SD rats (6 weeks old; Japan SLC) were used. The animals were kept at a constant ambient temperature (22 ± 1 °C) under a 12 h light/dark cycle with free access to food and water. All experiments were approved by the Institutional Animal Care and Use Committee of Hokkaido University.
Drugs. GABA and Pentylenetetrazol (PTZ) were purchased from Sigma Chemical Co. (St. Louis, MO). Tiagabine hydrochloride and SNAP-5114 were purchased from Toronto Research Chemical Inc. (Brisbane, Canada) and Tocris bioscience (Bristol, UK), respectively. β-Alanine was from Wako (Osaka, Japan). NNC 05-2090 was from Santa Cruz (Santa Cruz, CA).

Stable expression of human GABA transporter subtypes in Chinese hamster ovary (CHO) cells. In order to produce cell lines stably expressing GABA transporters, pCMV6-Neo containing each of four GABA transporter subtype cDNAs (ORIGENE, Rockville, MD) was transfected to CHO cells using a transfection reagent Effectene (QIAGEN Co., Hilden, Germany). The cells were cultivated in F-12 medium containing 10 % fetal bovine serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 0.8 mg/mL geneticine at 37°C in a humidified atmosphere with 5% CO2. After 7-14 days, single colonies were isolated and further cultivated in selection medium. The cells stably expressing GABA transporters were selected using [3H]GABA uptake assay.

[3H]GABA uptake assay. Cells grown in 24-well tissue culture plates were preincubated with 500 μL Krebs buffer (25 mM HEPES, 4.7 mM KCl, 2.2 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 10 mM Glucose, 120 mM NaCl, pH7.4) for 5 min at 37°C and then incubated in Krebs buffer containing 500 nM GABA (490–495 nM unlabeled GABA and 5-10 nM [3H]GABA (89.5 Ci/mmol; Perkin Elmer Inc., Boston, MA)) and GABA analogs (1 nM – 1 mM) for 20 min at 37°C. Incubation was stopped by removing Krebs buffer and washing the cells three times with ice–cold phosphate buffered saline, and the cells were solubilized in 250 μL of 0.3 % Triton-X 100. Aliquots were used for measurement of [3H]GABA uptaken by the cells using a liquid scintillation counter (Packard Tri-Carb 1600TR; Packard, Boston, MA, USA) and for determination of the protein concentration. Non-specific uptake was defined in the experiments using incubation buffer containing 120 mM choline chloride instead of NaCl. Inhibitory effects of 100 μM GABA analogs on [3H]GABA uptake were evaluated using the following equation; inhibitory effect = 1 – [(total GABA uptake in the presence of 100 μM analog) – (non-specific uptake)]/[(total GABA uptake in the absence of 100 μM analog) – (non-specific uptake)].
uptake in the presence of 100 μM analog)] / [(total GABA uptake in the absence of analog) – (non-specific uptake in the absence of analog)] × 100 (%). IC<sub>50</sub> values were determined by non-linear regression using a one-competition model (GraphPad Prism 4, GraphPad, San Diego, CA). The results are expressed as means ± SEM or means (95% confidence interval (CI) ) of more than three separate experiments, each of which was carried out in duplicate or triplicate.

**[^H]muscimol binding assay on BABA<sub>A</sub> receptor.** To prepare mouse brain membrane fraction, mice were decapitated, and the brains were removed. The brains were stored at −80°C until use. The brains were homogenized in ice–cold 0.32 M sucrose, and centrifuged at 30,000 × g for 30 min at 4°C. The pellet was washed by 1 mL ice–cold distilled–deionized water and dispersed in 2 mL ice–cold water, and homogenized at 600 rpm for two 10 s bursts at 10 s intervals. The suspension was centrifuged at 30,000 × g for 30 min at 4°C. The pellet was resuspended in ice–cold Tris–HCl buffer (50 mM, pH 7.4), and centrifuged at 30,000 × g for 30 min at 4°C. This step was repeated twice. After the final centrifugation, the pellet was washed and suspended in 1 mL ice–cold Tris–HCl buffer, and stored at −80°C. After thawing, 2 mL Tris–HCl buffer was added to the tissue, and the mixture was centrifuged at 30,000 × g for 30 min at 4 °C three times. The membranes were then suspended in adequate volume of Tris–HCl (100 μg protein/mL, at final concentration), and immediately used or kept frozen at −80°C. For the saturation binding assay, the membrane preparations were incubated for 45 min at 4°C with various concentrations of[^H]muscimol. Nonspecific binding was determined in the presence of 100 μM unlabeled GABA. For the screening or competitive binding assay, the membrane preparations were incubated for 45 min at 4°C with[^H]muscimol (5 or 15.2 nM, respectively) in the presence of a fixed (100 μM) or various concentrations of analogs, respectively. After the incubation, the membrane preparations were rapidly filtrated and washed with ice–cold Tris–HCl buffer twice, and the radioactivity on each filter was measured by a liquid scintillation counter. The K<sub>d</sub> value of muscimol was obtained by Scatchard analysis of the data from the saturation binding assay. Non-linear regression analysis of the
data the competitive binding assay using a one-competition model (GraphPad Prism 4) was conducted to estimate the $K_i$ values. The results from the binding assays are expressed as the mean (95% CI) of three independent experiments, each of which was performed in duplicate.

$[^3]H$GABA binding assay on BABA_B receptor. The assay was performed by the procedure reported previously.¹⁵

Analysis of anticonvulsive effect. Mice were intracerebroventricularly injected with tested drugs in a volume of 5 μl using a Hamiton Gastight syringe (Hamilton, Reno, NV, USA) with a two-step needle (Natsume, Tokyo, Japan). After 15 min, mice were subcutaneously injected with 100 mg/kg PTZ, then the latency of tremor, duration of clonic convulsion and occurrence of tonic convulsion was measured or observed for 15 min. After observation, mice were decapitated under ether anesthesia and the brain was rapidly removed and frozen in powdered dry ice and stocked −80 °C. Coronal sections (50 μm) were prepared on a cryostat (Leica CM3050S; Leica Instruments GmbH, Nussloch, Germany), thaw-mounted onto slides, stained with thionin, and examined by microscopy (×40). Data from mice with the correct placement of injection were used for statistical analyses.

Statistical analyses. Latency of clonic convulsion and duration of tremor were analyzed using an one-way factorial ANOVA followed by Dunnett's multiple comparison post hoc test. Inhibitory effects on tonic convulsion were analyzed using a chi-square test. The data from a EPM test were analyzed by an one-way factorial ANOVA followed by Dunnett's multiple comparison post hoc test. Differences with $p < 0.05$ were considered significant. ED₅₀ values of anticonvulsant effects were calculated using a log-probit method. The data are expressed as means ± SEMs.

Author Information

Corresponding Author: Satoshi Shuto

Phone & Fax: +81-11-706-3769.
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Abbreviations Used

ANOVA, analysis of variance; BGT, betaine-GABA transporter; CHO, Chinese hamster ovary; CNS, central nervous systems; EPM, elevated plus maze; GABA, γ-aminobutyric acid; GAT, GABA transporter; LE, ligand efficiency; PTZ, pentylenetetrazole; SNAP, 1-(2-[tris(4-methoxyphenyl)methoxy]ethyl)-3-piperidinecarboxylic acid; SSRI, serotonin-selective reuptake inhibitors
References and Notes


