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Cyclopropane-based conformational restriction of GABA by a stereochemical diversity-oriented strategy: Identification of an efficient lead for potent inhibitors of GABA transports

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ABSTRACT

A series of cyclopropane-based conformationally restricted γ-aminobutyric acid (GABA) analogs with stereochemical diversity, that is, 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.

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1. 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, **1** in Fig. 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, that is, 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.[2c,4] Accordingly, GABAergic synaptic transmission is likely to be enhanced effectively by inhibiting the GAT1 and/or GAT3 subtypes. In fact, tiagabine (**6**, Fig. 1), a GAT1-selective inhibitor, is clinically effective for the treatment of epilepsy.[5]

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.[2,5–7] 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.[5–7]

Abbreviations: 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.

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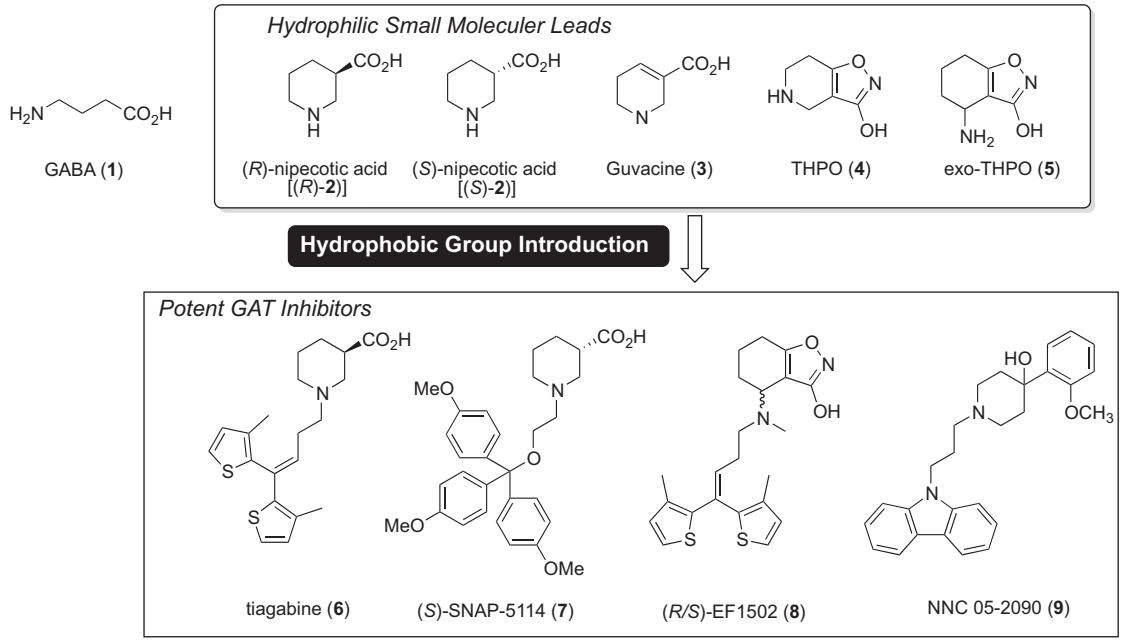

Figure 1. GABA (**1**), hydrophilic small molecular leads **2**–**5** for developing GAT inhibitors, and resulting potent GAT inhibitors **6**–**9**.

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,[8] 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.[9] To realize this strategy, we developed versatile chiral cyclopropane units Type-a with different stereochemistries[9a] (Fig. 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.[9a–c]

Thus, based on the stereochemical diversity-oriented strategy, we planned to synthesize a series of cyclopropane-based conformationally restricted GABA analogs, that is, **Ia**,**b** and **IIa**,**b** and their enantiomers ***ent*-Ia**,**b** and ***ent*-IIa**,**b** (Fig. 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.

2. Results and discussion

2.1. Cyclopropane-based conformationally restricted GABA analogs

GABA is conformationally very flexible due to its central '–C(sp^3)–C(sp^3)–C(sp^3)–' backbone, and accordingly, it can assume a variety of conformations, which may make it possible to bind to different proteins, that is, transporter subtypes as well as receptor subtypes, maybe in its different conformations. Therefore, the conformation binding to one transporter subtype, that is, 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 (Fig. 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.

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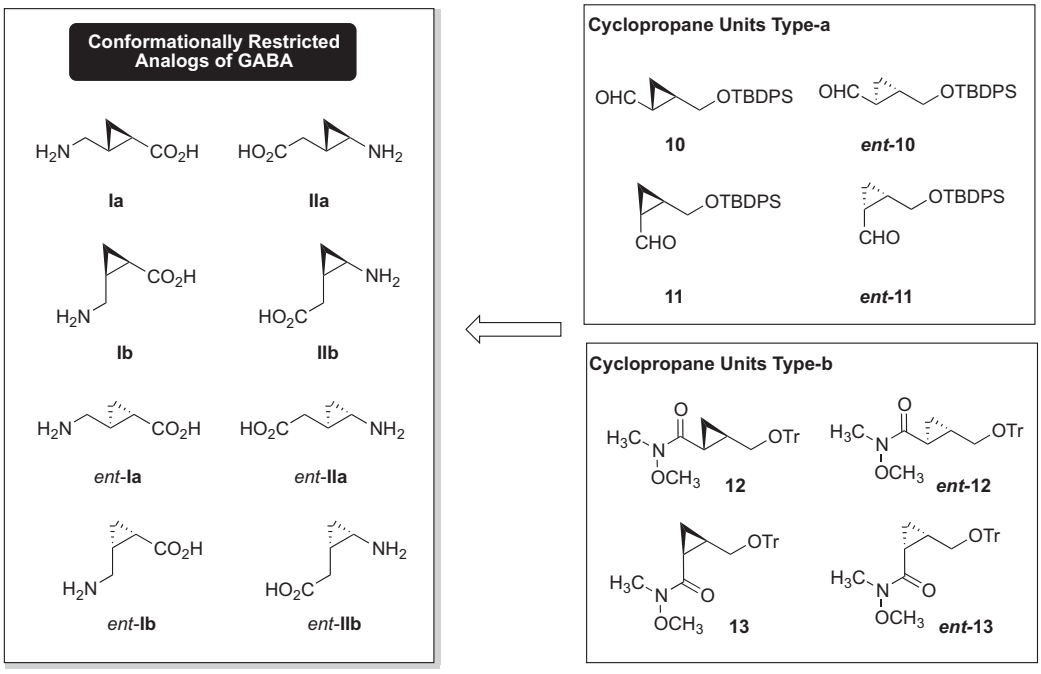
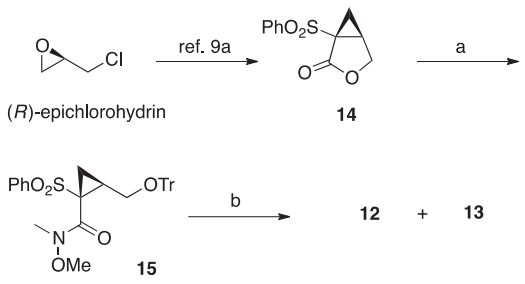


Figure 2. A series of conformationally restricted GABA analogs with stereochemical diversity derived from the chiral cyclopropane units Type-a and Type-b.



Scheme 1. Reagents and conditions: (a) (1) $Me(MeO)NH_2Cl$, Et_3N, $AlCl_3$, CH_2Cl_2, (2) TrCl, pyridine,CH_2Cl_2, 77%; (b) Mg, THF, MeOH, 54% (**12**), 6% (**13**).

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 log*P* 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.

2.2. Synthesis

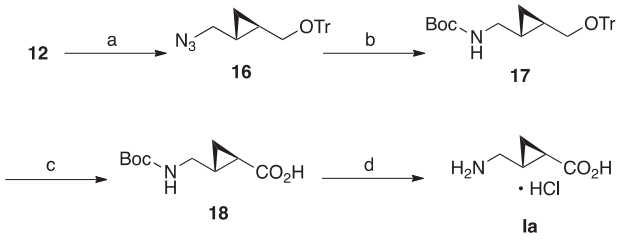
Although much effort has been devoted to developing practical methods for preparing chiral cyclopropanes, stereoselective synthesis of cyclopropane derivatives with the desired stereochemistry is often troublesome.[14] In fact, although some of the cyclopropane-based conformationally restricted analogs of GABA have been synthesized, these are racemic or obtained by optical resolution.[11] To solve this problem, we previously developed the chiral cyclopropane units Type-a, comprising four stereoisomeric cyclopropane derivatives **10** and **11**, and their enantiomers ***ent*-10** and ***ent*-11** bearing two adjacent carbon substituents in a *trans* or a *cis* orientation, as shown in Figure 2.[9a,c] These units are generally useful for synthesizing a series of stereoisomeric cyclopropane compounds having an asymmetric *trans*- or *cis*-cyclopropane structure.

In this study, we developed alternative cyclopropane units Type-b with a Weinreb amide moiety (Fig. 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 ($TrOCH_2$– and $Me(MeO)NHCH_2$–) on cyclopropane are different from those of the Type-a units (TBDPS-OCH_2– 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.

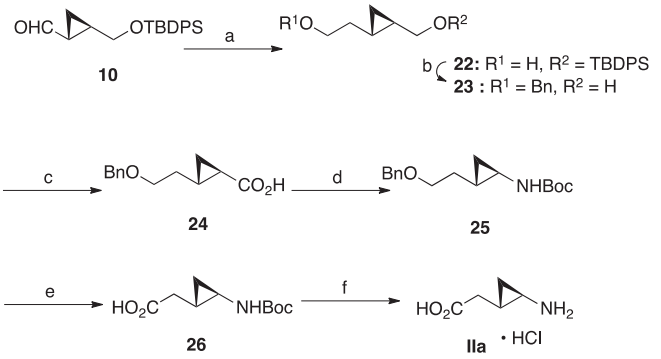
The synthetic route of the Type-b units is in Scheme 1. Treatment of bicyclic lactone **14**, prepared from (*R*)-epichlorophydrin by our previously reported method,[9a] with the $Me(MeO)NH{\cdot}HCl$/Et_3N/$AlCl_3$ system in CH_2Cl_2 and then with TrCl/pyridine in CH_2Cl_2 gave the Weinreb amide **15**. 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 $LiAlH_4$ and $NaBH_4$ gave the corresponding alcoholic product, and subsequent treatment with NaN_3, PPh_3, and CBr_4 in DMF gave the azide **16**. Catalytic hydrogenation of **16** with H_2 and Pd-C in the presence of Boc_2O 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



Scheme 2. Reagents and conditions: (a) (1) $LiAlH_4$, THF, 0 °C, (2) $NaBH_4$, THF, MeOH, (3) NaN_3, PPh_3, CBr_4, DMF, 0 °C to rt, 70%; (b) H_2, Pd/C, Boc_2O, THF, MeOH, 89%; (c) (1) 80% AcOH aq, (2) Dess–Martin periodinane, CH_2Cl_2, (3) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH aq, 84%; (d) HCl, AcOET, 89%.



Scheme 4. Reagents and conditions: (a) (1) $MeOHCH_2PPh_3Cl$, NaHMDS, THF, 0 °C, (2) HCl aq, THF, 0 °C, (3) $NaBH_4$, THF, MeOH, 66%; (b) (1) BnBr, NaH, Bu_4NI, DMF, THF, −10 °C, (2) Bu_4NF, THF, 84%; (c) (1) Dess–Martin periodinane, CH_2Cl_2, 0 °C, (2) $NaClO_2$, NaH_2PO_4, 2-methyl-2-utene, *t*-BuOH aq 97%; (d) (1) $(PhO)_2P(O)N_3$,Et_3N, CH_2Cl_2, (2) *t*-BuOH, reflux, 75%; (e) (1) H_2, Pd/C, THF, MeOH, (2) Dess–Martin periodinane, CH_2Cl_2, 0 °C, (3) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH aq, 91%; (f) HCl aq, 76%.

above-mentioned synthesis of **Ia**. Removal of the *O*-Tr protecting group and subsequent oxidation with CrO_3 and H_2SO_4 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.

The *trans*-3,4-methano analog **IIa** was prepared from unit **10** as shown in Scheme 4. A Wittig reaction of unit **10** with $MeOCH_2Ph_3PCl/NaN(TMS)_2$, followed by acidic treatment, gave the corresponding one carbon-elongated aldehyde, which was reduced with $NaBH_4$ 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 $(PhO)_2PON_3/Et_3N$ in CH_2Cl_2 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.

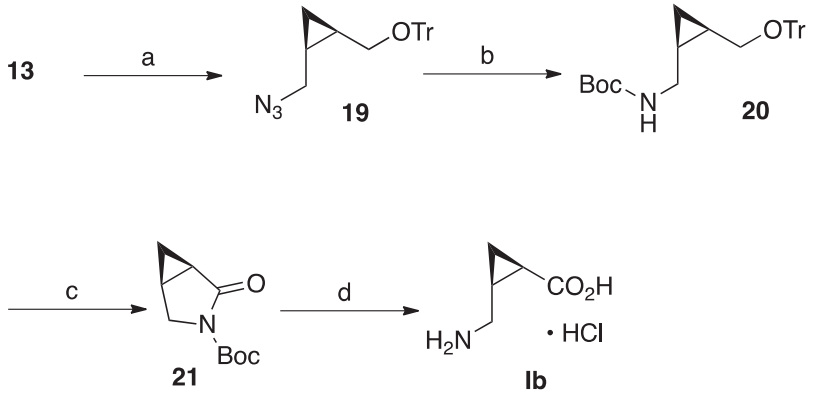
Thus, the target compounds were systematically and effectively synthesized using the Type-a and Type-b cyclopropane units.

2.3. Effects on GABA transporters

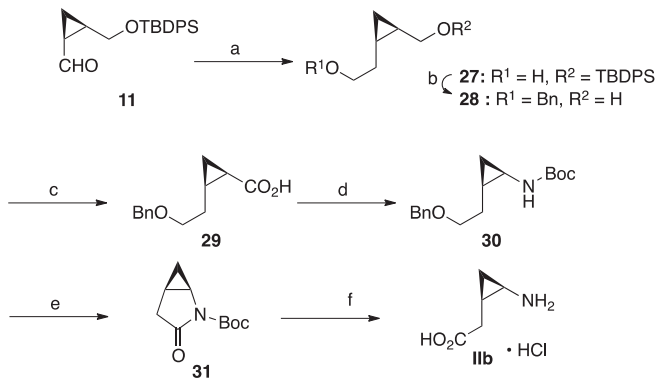
The inhibitory effects of 100 μM GABA analogs on GABA uptake in GAT1/CHO, GAT2/CHO, GAT3/CHO, and BGT1/CHO cells were examined (Table 1). The analog **IIa** showed high efficacy for GAT3 and BGT1 and moderate and low efficacy for GAT2 and GAT1, respectively. On the other hand, the other analogs showed 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 IC_{50} 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.

2.4. Effects on GABA receptors

Binding of GABA analogs to GABAB receptors was investigated with crude synaptic membranes of rat brain in the presence of isoguvacine (40 μM to block GABAA receptors (Table 3).[15] At a 1 μM concentration, none of the analogs synthesized in this study significantly competed with [^{3}H]GABA for GABAB receptors at 1 μM. At a 10 μM concentration, **Ia**, ***ent*-Ia**, and ***ent*-IIa** had weak inhibitory effects on [^{3}H]GABA binding, although analog **IIa**, identified as a potent GAT inhibitor, was inactive. In the same experiment, (±)-baclofen (10 nM–10 μM) competed with [^{3}H] GABA for brain GABAB receptors in a concentration-dependent manner, and the IC50 value (mean ± SE, $n = 3$) to displace 50% of control specific binding was 0.36 ± 0.16 μM.



Scheme 3. Reagents and conditions: (a) (1) $LiAlH_4$, THF, 0 °C, (2) $NaBH_4$, MeOH, THF, (3) NaN_3, PPh_3, CBr_4, DMF, 0 °C to rt, quant.; (b) H_2, Pd/C, Boc_2O, THF, MeOH, 84%; (c) (1) 80% AcOH aq, (2) CrO_3, H_2SO_4, acetone aq, 0 °C; (d) (1) TFA, CH_2Cl_2, (2) 1 N HCl, (3) recrystalization (EtOH), 39%.



Scheme 5. Reagents and conditions: (a) (1) $MeOHCH_2PPh_3Cl$, NaHMDS, THF, 0 °C, (2) HCl aq, THF, 0 °C, (3) $NaBH_4$, THF, MeOH, 72%; (b) (1) BnBr, NaH, Bu_4NI, DMF, THF, −10 °C, (2) Bu_4NF, THF, 95%; (c) (1) Dess–Martin periodinane, CH_2Cl_2, 0 °C, (2) $NaClO_2$, NaH_2PO_4, 2-methyl-2-utene, *t*-BuOH aq; 91% (d) (1) $(PhO)_2P(O)N_3$,Et_3N, CH_2Cl_2, (2) *t*-BuOH, reflux, 59%; (e) (1) H_2, Pd/C, THF, MeOH, (2) CrO_3, H_2SO_4, acetone aq, 0 °C; 72%; (f) HCl aq, 70 °C, 40%.

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

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

[a] Data are expressed as means ± SEM.

We then evaluated the inhibitory effects of 100 μM GABA analogs on [^{3}H]muscimol binding to mouse brain membrane fraction (Fig. 3A). Analogs **Ia** and **IIa** completely displaced 5 nM [^{3}H]muscimol binding to the membrane fraction. Scatchard analysis showed that the K_d value of muscimol was 15.2 nM. Competition binding assay revealed that analog **IIa** showed rather high affinity (K_i values = 0.34 μM (0.25–0.46 μM)) for $GABA_A$ receptors (Fig. 3B).

2.5. 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 (Fig. 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** ($F_{4,47} = 1.96$, $p = 0.12$; Fig. 4B). Furthermore, both analog **IIa** and tiagabine dose-dependently inhibited PTZ-induced tonic convulsions (ED_{50} = 189 ± 104 and 43.7 ± 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).

Table 2
IC_{50} values (μM) of analog **IIa** and representative GABA ligands on GABA uptake in GAT subtypes[a]

	GAT1	GAT2	GAT3	BGT1
IIa	>100	36.9 (28.2–48.4)	13.9 (3.62–53.4)	5.48 (3.77–7.97)
Tiagabine	0.049 (0.016–0.155)	—	—	—
SNAP-5114	170 (43.6–661)	11.9 (2.00–71.2)	1.12 (0.64–1.94)	71.7 (22.6–228)
β-alanine	—	209 (124–350)	41.2 (10.0–169)	—
NNC 05-2090	—	—	—	5.10 (1.96–13.3)

[a] Data are expressed as means (95%CI).

2.6. 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.[11] 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.

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.[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.[13] Although the inhibitory effects of **IIa** on GAT3 and BGT1 are not very strong and it also binds to $GABA_A$ receptor, **IIa** 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 **IIa** together with those of tiagabine and

Table 3
Inhibitory effects (%) of GABA analogs on GABA binding to rat brain $GABA_B$ receptors

	1 μM	10 μM
Ia	83.3 ± 0.67	57.0 ± 0.04
***ent*-Ia**	94.7 ± 2.79	64.9 ± 10.3
Ib	127 ± 17.4	125 ± 28.5
***ent*-Ib**	95.9 ± 10.1	99.3 ± 22.5
IIa	112 ± 7.54	106.0 ± 27.5
***ent*-IIa**	98.9 ± 6.70	69.1 ± 8.31
IIb	91.4 ± 11.2	96.2 ± 26.8
***ent*-IIb**	109 ± 7.80	115.4 ± 3.64

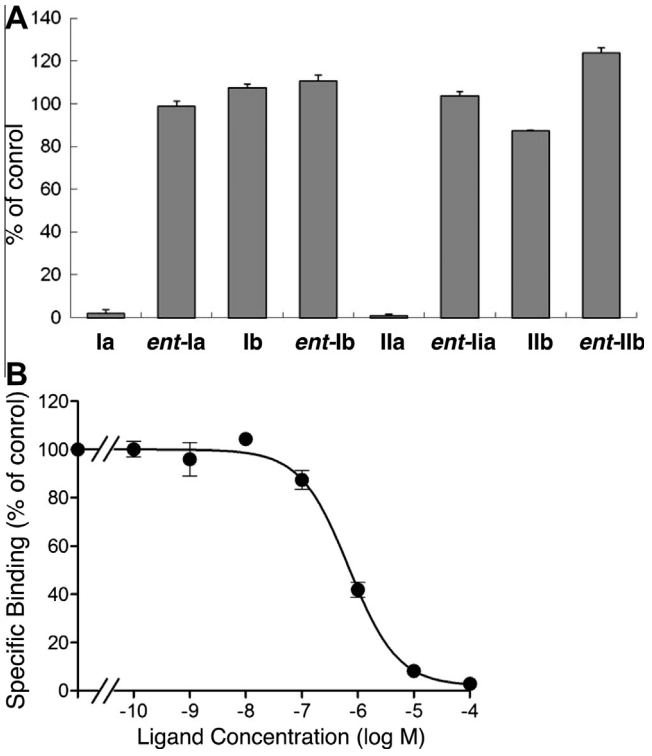


Figure 3. Binding efficacies of GABA analogs on $GABA_A$ receptors. (A) Inhibitory effects of 100 μM GABA analogs on [^{3}H]muscimol (5 nM) binding to mouse brain membrane fraction. Data are expressed as mean ± SEM. (B) Displacement of specific binding of [^{3}H]muscimol to mouse brain membrane fraction by analog **IIa**. Data are expressed as mean ± SEM.

(*S*)-SNAP and their low MW leads, (*R*)- or (*S*)-nipecotic acid [(*R*)-**2** or (*S*)-2]. As a GAT3 inhibitor, **IIa** has an excellent LE of 0.89, which are significantly higher than those of (*R*)-**2** (LE = 0.65) or (*S*)-**2** (LE = 0.40). As a BGT1 inhibitor, **IIa** also has an excellent LE of 0.90. These results showed that **IIa** can be a favorable lead for developing potent GAT3 and/or BGT1 inhibitors.

2.7. Conclusion

We used the chiral cyclopropane units effectively to synthesize a series of conformationally restricted GABA analogs and identified a GAT3/BGT1 inhibitor **IIa**, 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.

3. Experimental

Chemical shifts are reported in ppm downfield from Me_4Si (^{1}H) and MeOH (^{13}C). Thin-layer chromatography was done on Merck coated plate $60F_{254}$. 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.

3.1. (1*R*,2*S*)-2-(Triphenylmethoxy)methyl-1-(*N*-methoxy-*N*-methyl)carbamoyl-1-phenylsulfonylcyclopropane (15)

A mixture of **14**[8a] (7.3 g, 31 mmol) and $AlCl_3$ (8.2 g, 61 mmol) in CH_2Cl_2 (77 mL) was stirred at 0 °C for 30 min. Also, a mixture of Me(OMe)NH (12.0 g, 123 mmol) and Et_3N (17.1 mL, 123 mmol) in CH_2Cl_2 (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 $CHCl_3$ and aqueouse HCl (1 M), and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), 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 CH_2Cl_2 (62 mL) was stirred at room temperature for 12 h. The resulting mixture was partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), 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: ^{1}H NMR (400 MHz, $CDCl_3$, 60 °C) 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, –C*H*aHbOTr), 3.15 (3H, s, NCH_3), 3.32–3.36 (1H, m, –CHa*H*bOTr), 3.60 (3H, s, $NOCH_3$), 7.17–7.84 (20H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 16.7, 26.5, 48.7, 60.3, 61.9, 87.4, 127.1, 127.8, 128.8, 133.6, 143.8; LRMS (FAB) *m*/*z* 564 $(M+Na)^+$; $[\alpha]_D^{19}$ −37.3° (*c* 1.09, $CHCl_3$).

3.2. (1*R*,2*R*)-2-(Triphenylmethoxymethyl)-1-(*N*-methoxy-*N*-methyl)carbamoylcyropropane (12) and (1*S*,2*R*)-2-(Triphenylmethoxymethyl)-1-(*N*-methoxy-*N*-methyl)carbamoylcyropropane (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 1 h. The resulting mixture was poured into cooled aqueous HCl (1 M), which was extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), 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. Compound **12**: ^{1}H NMR (400 MHz, $CDCl_3$) 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, NCH_3 and –CHaHbOTr, *J* = 6.3, 9.5 Hz), 3.72 (3H, s, $NOCH_3$), 7.18–7.43 (15H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $(M+Na)^+$; $[\alpha]_D^{23}$ −32.7° (*c* 0.96, $CHCl_3$); Anal. Calcd for $C_{26}H_{27}NO_3{\cdot}0.2CHCl_3$: C, 73.98; H, 6.45; N, 3.29. Found: C, 73.90; H, 6.51; N, 3.37. Compound **13**: ^{1}H NMR (400 MHz, $CDCl_3$) 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, –C*H*aHbOTr, *J* = 8.6, 10.4 Hz), 3.16 (3H, s, NCH_3), 3.46 (1H, dd, –CHa*H*bOTr, *J* = 5.0, 10.4 Hz), 3.77 (3H, s, $NOCH_3$), 7.17–7.45 (15H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $(M+Na)^+$; $[\alpha]_D^{24}$ +9.2° (*c* 0.84, $CHCl_3$); Anal. Calcd for $C_{26}H_{27}NO_3{\cdot}0.1H_2O$: C, 77.43; H, 6.80; N, 3.47. Found: C, 77.33; H, 6.78; N, 3.47.

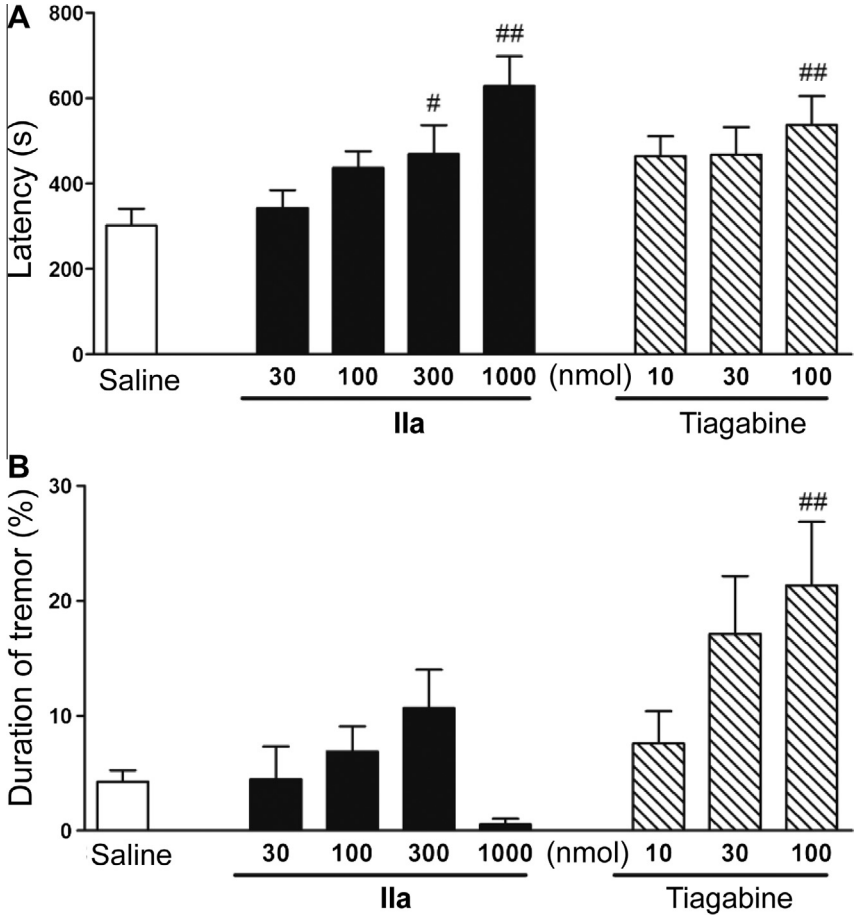


Figure 4. Anticonvulsant effects of analog **IIa** and tiagabine on seizures induced by pentylenetetrazole (PTZ; 100 mg/kg, sc) in mice. (A) The latency of clonic convulsion and (B) the duration of tremor were examined in mice intracerebroventricularly injected with saline (n = 17), analog **IIa** (30 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.

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

Compound	LE (IC_{50}, mM)			
	GAT1	GAT3	BGT1	MW
IIa	—	0.83 (13.9)	0.90 (5.48)	115
(*R*)-nipecotic acid [**(*R*)-2**][a]	0.80 (5.9)	0.65 (51)	—	129
Tiagabin (**6**)	0.40 (0.049)	—	—	375
(*S*)-nipecotic acid [**(*S*)-2**][a]	0.60 (116)	0.40 (2320)	—	129
(*S*)-SNAP-5114 [**7**][a]	0.13 (388)	0.20 (5)	0.23 (71.7)	506

[a] IC_{50} data were taken from Ref. 7.

3.3. (1*R*,2*R*)-1-Azidomethyl-2-(triphenylmethoxy)methylcyclopropane (16)

A mixture of **12** (5.13 g, 12.8 mmol) and $LiAlH_4$ (1.45 g, 38.3 mmol) in THF (60 mL) was stirred at 0 °C for 1 h, to which H_2O (1.4 mL), aqueous NaOH (15%, 4.35 mL), and H_2O (4.35 mL) was succesively added. The resulting mixture was filtrated and evaporated to give an oil (4.8 g). A mixture of the oil and $NaBH_4$ (967 mg, 25.6 mmol) in THF/MeOH (1:1, 64 mL) was stirred at room temperature for 5 h, and then aqueous HCl (1 M) was aded. The resulting mixture was extracted with $CHCl_3$, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), 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, Ph_3P (9.14 g, 34.8 mmol), and CBr_4 (5.78 g, 17.4 mmol) in DMF (67 mL) was stirred at 0 °C for 40 min. To the mixture was added NaN_3 (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 H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), 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: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.48–0.55 (2H, m, H-3), 0.95–1.07 (2H, m, H-1 and H-2), 2.94 (1H, dd, –C*H*a*H*bO, J = 6.3, 9.7 Hz), 3.03 (1H, dd, –CHa*H*bO, J = 6.3, 9.7 Hz), 3.11–3.17 (2H, m, –CH_2N$_3$), 7.20–7.46 (15H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) 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 $(M+Na)^+$; $[\alpha]_D^{23}$ −12.0° (c 1.15, $CHCl_3$); Anal. Calcd for $C_{24}H_{23}N_3O$: C, 78.02; H, 6.27; N, 11.37. Found: C, 78.02; H, 6.33; N, 11.22.

3.4. (1*R*,2*R*)-1-(*N*-*t*-Butoxycarbonyl)aminomethyl-2-(triphenylmethoxy)methylcyclopropane (17)

A mixture of **16** (569 mg, 1.54 mmol), Boc_2O, and Pd-C (10%, 190 mg) in THF/MeOH (1:1, 15 mL) was stired under H_2 (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: ^{1}H NMR (500 MHz, $CDCl_3$) 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(CH_3)$_3$), 2.74–2.78 (1H, m, –C*H*aHbO), 2.85–2.90 (1H, m, –C*H*aHbN), 3.06–3.09 (1H, m, –CHa*Hb*O), 3.15–3.18 (1H, m, –CHa*Hb*N), 4.68 (1H, brs, NH), 7.14–7.45 (15H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) 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)$^+$; $[\alpha]_D^{23}$ −10.8° (*c* 0.90, $CHCl_3$).

3.5. (1*R*,2*R*)-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 H_2O was added. The resulting solution was extracted with AcOEt, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), 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 CH_2Cl_2 (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 Na_2S_2O 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_2SO_4), 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), $NaClO_2$ (1.3 mg, 14 mmol), and $NaH_2PO_4{\cdot}2H_2O$ (1.1 g, 7.0 mmol) in *t*-BuO/H_2O H (4:1, 35 mL) was stirred at room temperature for 13 h. After addition of saturated NaCl, the resulting mixture was extracted with $CHCl_3$, and the organic layer was dried (Na_2SO_4), 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: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86–0.94 (1H, m, H-3), 1.23–1.26 (1H, m, H-3), 1.44 (9H, s, –C(CH_3)$_3$), 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); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 18.7, 23.1, 28.3, 43.0, 79.6, 155.8, 179.4.

3.6. (1*R*,2*R*)-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: ^{1}H NMR (400 MHz, D_2O) 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); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.5, 19.7, 19.8, 42.9, 178.2; LRMS (FAB) *m*/*z* 116 (M+H)$^+$; $[\alpha]_D^{23}$ −65.8° (*c* 0.97, 1 *N* HCl), [lit[10a] $[\alpha]_D^{26}$ −65.5° (*c* 0.95, 1 N HCl)]; mp 132.0–137.0 °C (lit.[10a]: 131–135 °C); Anal. Calcd for $C_5H_{10}ClNO_2$: C, 39.62; H, 6.65; N, 9.24. Found: C, 39.39; H, 6.70; N, 8.96.

3.7. (1*S*,2*R*)-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, $CDCl_3$) δ 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, –C*H*aHbN$_3$, *J* = 8.6, 9.7 Hz), 2.90 (1H, dd, –C*H*aHbO, *J* = 8.0, 13.2 Hz), 3.23 (1H, dd, –CHa*Hb*O, *J* = 6.9, 13.0 Hz), 3.37 (1H, dd, –CHa*Hb*N$_3$, *J* = 6.3, 9.7 Hz), 7.21–7.47 (15H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) δ 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)$^+$; $[\alpha]_D^{24}$ +25.0° (*c* 1.28, $CHCl_3$); Anal. Calcd for $C_{24}H_{23}N_3O$: C, 78.02; H, 6.27; N, 11.37. Found: C, 77.75; H, 6.30; N, 11.07.

3.8. (1*S*,2*R*)-1-(*N*-*t*-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, $CDCl_3$) δ –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 –C(CH_3)$_3$), 2.04–2.11 (1H, m, –C*H*aHbO), 2.26–2.31 (1H, m, –C*H*aHbN), 3.67–3.72 (2H, m, –CHa*Hb*O and –CHa*Hb*N), 5.45 (1H, br, NH), 7.20–7.49 (15H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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)$^+$; $[\alpha]_D^{24}$ −0.6° (*c* 0.68, $CHCl_3$).

3.9. (3*R*,4*S*)-*N*-*t*-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_2O was added. The resulting solution was extracted with AcOEt, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_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_2SO_4 (0.8 mL) in H_2O (1.8 mL) was added slowly at 0 °C. To the resulting orange solution was added *i*-PrOH until the color of the solution was disappeared, and the rusultiong mixture was partitioned between Et_2O and H_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), 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: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77–0.79 (1H, m, H-6), 1.17–1.20 (1H, m, H-6), 1.50 (9H, s, –C(CH_3)$_3$), 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); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 12.3, 21.5, 28.0, 48.0, 82.6, 150.4, 173.8.

3.10. (1*S*,2*R*)-1-Aminomethyl-2-carboxycyclopropane hydrochloride (Ib, HCl salt)

A solution of **21** (601 mg, 3.0 mmol) and TFA (1.1 mL) in CH_2Cl_2 (10 mL) was stirred at room temperature for 30 min, and then evaporated. A solution of the residual oil in aqueous HCl (1 M, 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: ^{1}H NMR (500 MHz, D_2O) 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)$^+$; $[\alpha]_D^{24}$ −38.3° (*c* 0.95, 1 N HCl), [lit[10b] $[\alpha]_D^{26}$ −38.5° (*c* 1, 1 N HCl)]; mp 243–244 °C (lit.[10b]: 244–246 °C); Anal. Calcd for $C_5H_{10}ClNO_2$: C, 39.62; H, 6.65; Cl, 23.39; N, 9.24. Found: C, 39.49; H, 6.55; Cl, 23.48; N, 9.30.

3.11. (1*S*,2*R*)-2-(*t*-Butyldiphenylsilyloxy)methyl-1-(2-hydroxyethyl)cyclopropane (22)

To a suspension of $MeOCH_2PPh_3Cl$ chloride (12.3 g, 36 mmol) in THF (81 mL) was added $NaN(Si(CH_3)_3)_2$ (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 °C, and the resulting mixture was stirred at the same temperature for 3 h. After addition of aqueous saturated NH_4Cl, the solvent of the mixture was evaporated, and the residue was partitioned between AcOEt and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), 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 $NaHCO_3$ (100 mL), and the resulting solution was extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to give an oil (4.4 g). A mixture of the oil and $NaBH_4$ (206 mg, 5.5 mmol) in THF/MeOH (4:1, 136 mL) was stirred at room temperature for 5 h, and then aqueous HCl (1 M) was aded. The resulting mixture was extracted with $CHCl_3$ and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), 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: ^{1}H NMR (500 MHz, $CDCl_3$) 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(CH_3)$_3$), 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, –C*Ha*HbOTBDPS, J = 9.2, 10.3 Hz), 3.72–3.80 (2H, m, H-2′), 3.89 (1H, dd, –CHa*Hb*OTBDPS, J = 5.2, 10.3 Hz), 7.36–7.69 (10H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) 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)$^+$; $[\alpha]_D^{22}$ −0.2° (c 1.28, $CHCl_3$); Anal. Calcd for $C_{22}H_{30}O_2Si$: C, 74.53; H, 8.53. Found: C, 74.39; H, 8.73.

3.12. (1*S*,2*R*)-1-(2-Benzyloxy)ethyl-2-hydroxymethylcyclopropane (23)

A mixture of **22** (3.0 g, 8.5 mmol), BnBr (2.01 mL, 17 mmol), Bu_4NI (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 NH_4Cl, the solvent was evaporated, and the residue was partitioned between AcOEt and H_2O. The organic layer was washed with brine, dried (Na_2SO_4) 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 (1.0 M 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: ^{1}H NMR (500 MHz, $CDCl_3$) 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, –C*Ha*HbOH), 3.52–3.57 (3H, m, H-2′ and –CHa*Hb*OH), 4.51 (2H, s, –CH_2Ph), 7.25–7.38 (5H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) 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)$^+$; $[\alpha]_D^{22}$ +1.6° (c 0.93, $CHCl_3$); Anal. Calcd for $C_{13}H_{18}O_2{\cdot}0.15H_2O$: C, 74.71; H, 8.83. Found: C, 74.84; H, 8.97.

3.13. (1*S*,2*R*)-*trans*-1-(2-Benzyloxy)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_2Cl_2 (26 mL) was stirred at 0 °C for 45 min. After addition of a mixture of saturated aqueous Na_2S_2O 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_2SO_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 $NaH_2PO_4{\cdot}2H_2O$ (805 mg, 5.2 mmol) in t-BuOH/H_2O (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_2SO_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: ^{1}H 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_2Ph), 7.25–7.36 (5H, m, aromatic); ^{13}C 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)$^+$; $[\alpha]_D^{23}$ −45.9° (c 1.03, $CHCl_3$); Anal. Calcd for $C_{13}H_{16}O_3{\cdot}0.05CHCl_3$: C, 69.28; H, 7.15. Found: C, 69.10; H, 7.17.

3.14. (1*S*,2*R*)-1-(2-Benzyloxy)ethyl-2-(*t*-buthoxycarbonyl)aminocyclopropane (25)

A mixture of **24** (1.35 g, 6.1 mmol), $(PhO)_2PON_3$ (3.95 mL, 18 mmol), and Et_3N (1.28 mL, 9.2 mmol) in CH_2Cl_2 (61 mL) was stirred at room temperature for 3.5 h. The reaction mixture was partitioned between AcOEt and H_2O, and the organic layer was washed with brine, dried (Na_2SO_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: ^{1}H 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, –CH_2-Ph), 4.57–4.72 (1H, br, –NH), 7.26–7.34 (5H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) 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)$^+$; $[\alpha]_D^{2222}{}_D$ −25.0° (c 0.70, $CHCl_3$); Anal. Calcd for $C_{17}H_{25}NO_3$: C, 70.07; H, 8.65; N, 4.81. Found: C, 69.77; H, 8.45; N, 4.65.

3.15. (1*S*,2*R*)-2-(*t*-Buthoxycarbonyl)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 stired under H_2 (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 CH_2Cl_2 (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 Na_2S_2O 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_2SO_4), and evaporated to give an oil (228 mg). A mixture of the oil, 2-methyl-2-butene (831 mL, 7.8 mmol), $NaClO_2$ (353 mg, 3.9 mmol), and $NaH_2PO_4{\cdot}2H_2O$ (307 mg, 2.0 mmol) in t-BuOH/H_2O (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 (Na_2SO_4), and evaporated. The residue was purified by column chromatography (silica gel; hexane/AcOEt, 1:1) to give **26** (192 mg, 91%) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$) 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(CH_3)$_3$), 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); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.3, 15.8, 28.2, 29.2, 37.7, 80.9, 157.8, 174.5; LRMS (FAB) m/z 216 (M+H)$^+$; $[\alpha]_D^{20}$ +44.5° (c 0.90, $CHCl_3$); Anal. Calcd for $C_{10}H_{17}NO_4{\cdot}0.1H_2O$: C, 55.34; H, 7.99; N, 6.45. Found: C, 55.09; H, 7.88; N, 6.20.

3.16. (1*S*,2*R*)-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 stireed at room temperature for 35 min, and then evaported. The residue was triturated with Et_2O to give **IIa** (HCl salt,

230 mg, 1.5 mmol, 76%) as white powder: ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (500 MHz, D_2O) δ 10.1, 13.2, 28.5, 36.2, 177.5; LRMS (FAB) *m*/*z* 116 (M+H)$^+$; $[\alpha]_D^{21}$ −21.3° (*c* 0.99, 1 *N* HCl); mp 104–106 °C; Anal. Calcd for $C_5H_{10}ClNO_2{\cdot}0.1H_2O$: C, 39.15; H, 6.70; N, 9.13. Found: C, 39.08; H, 6.51; N, 9.10.

3.17. (1*R*,2*R*)-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**: ^{1}H NMR (500 MHz, $CDCl_3$) 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(CH_3)$_3$), 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, –C*Ha*HbOTBDPS, *J* = 10.3, 11.5 Hz), 3.73–3.82 (2H, m, H-2′), 3.92 (1H, dd, –CHa*Hb*OTBDPS, *J* = 4.6, 11.5 Hz), 7.38–7.72 (10H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) 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)$^+$; $[\alpha]_D^{21}$ +12.2° (*c* 1.46, $CHCl_3$); Anal. Calcd for $C_{22}H_{30}O_2Si$: C, 74.53; H, 8.53. Found: C, 74.27; H, 8.52.

3.18. (1*R*,2*R*)-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**: ^{1}H NMR (400 MHz, $CDCl_3$) 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, –C*Ha*H-bOH, *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 –CHa*Hb*OH, *J* = 6.3, 10.4 Hz), 4.56 (2H, s, –CH_2Ph), 7.27–7.38 (5H, m, aromoatic); ^{13}C NMR (100 MHz, $CDCl_3$) 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)$^+$; $[\alpha]_D^{22}$ +21.4° (*c* 1.38, $CHCl_3$); Anal. Calcd for $C_{13}H_{18}O_2{\cdot}0.1H_2O$: C, 75.04; H, 8.82. Found: C, 75.11; H, 8.92.

3.19. (1*R*,2*R*)-1-(2-Benzyloxy)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**: ^{1}H NMR (500 MHz, $CDCl_3$) 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, –CH_2Ph), 7.26–7.36 (5H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) 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)$^+$; $[\alpha]_D^{22}$ −50.1° (*c* 1.12, $CHCl_3$); Anal. Calcd for $C_{13}H_{16}O_3{\cdot}0.1H_2O$: C, 70.31; H, 7.35. Found: C, 70.00; H, 7.27.

3.20. (1*R*,2*R*)-1-(2-Benzyloxy)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**: ^{1}H NMR (500 MHz, $CDCl_3$) 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(CH_3)$_3$), 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, –CH_2Ph), 5.35 (1H, brs, –NH), 7.27–7.35 (5H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) 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)$^+$; $[\alpha]_D^{21}$ −49.4° (*c* 1.08, $CHCl_3$); Anal. Calcd for $C_{17}H_{25}NO_3$: C, 70.07; H, 8.65; N, 4.81. Found: C, 70.07; H, 8.49; N, 4.69.

3.21. (4*R*,5*R*)-*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 H_2 (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 CrO_3 oxide (360 mg, 3.6 mmol) and H_2SO_4 (0.33 mL) in H_2O (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 rusultiong mixture was partitioned between Et_2O and H_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), 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, $CDCl_3$) δ 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, $CDCl_3$) δ 5.4, 14.9, 28.0, 35.0, 36.4, 83.0, 150.4, 173.6; LRMS (FAB) *m*/*z* 198 (M+H)$^+$; $[\alpha]_D^{22}$ −36.5° (*c* 0.64, $CHCl_3$).

3.22. (1*R*,2*R*)-*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_2O to give **IIb** (HCl salt, 70 mg, 40%) as a white solid: ^{1}H NMR (500 MHz, D_2O) δ 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_2O) δ 9.8, 11.5, 27.2, 32.6, 177.3; LRMS (FAB) *m*/*z* 116 (M+H)$^+$; $[\alpha]_D^{22}$ −21.5° (*c* 0.83, 1 *N* HCl); mp 165–169 °C; Anal. Calcd for $C_5H_{10}ClNO_2{\cdot}0.25H_2O$: 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.

3.23. (1*S*,2*R*)-2-(Triphenylmethoxy)methyl-1-(*N*-methoxy-*N*-methyl)carbamoyl-1-phenylsulfonylcyclopropane (*ent*-15)

$[\alpha]_D^{22}$ +37.7° (*c* 1.22, $CHCl_3$) Anal. Calcd for $C_{32}H_{31}NO_5S{\cdot}0.45CHCl_3$: 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.

3.24. (1*S*,2*S*)-2-(Triphenylmethoxymethyl)-1-(*N*-methoxy-*N*-methyl)carbamoylcycropropane (*ent*-12)

$[\alpha]_D^{20}$ +33.3° (*c* 0.95, $CHCl_3$).

3.25. (1*R*,2*S*)-2-(Triphenylmethoxymethyl)-1-(*N*-methoxy-*N*-methyl)carbamoylcycropropane (*ent*-13)

$[\alpha]_D^{22}$ −9.3° (*c* 0.99, $CHCl_3$).

3.26. (1*S*,2*S*)-1-Azidomethyl-2-(triphenylmethoxy)methylcyclopropane (*ent*-16)

$[\alpha]_D^{20}$ +12.4° (*c* 0.94, $CHCl_3$); Anal. Calcd for $C_{24}H_{23}N_3O{\cdot}0.1H_2O$: C, 77.64; H, 6.30; N, 11.32. Found: C, 77.60; H, 6.35; N, 11.21.

3.27. (1*S*,2*S*)-1-(*N*-*t*-Butoxycarbonyl)aminomethyl-2-(triphenylmethoxy)methylcyclopropane (*ent*-17)

$[\alpha]_D^{21}$ +11.0° (*c* 1.06, $CHCl_3$).

3.28. (1*S*,2*S*)-1-Aminomethyl-2-carboxycyclopropane hydrochloride (*ent*-IIa, HCl salt)

$[\alpha]_D^{22}$ +68.6° (*c* 0.97, 1 *N* HCl aq); mp 141.0–145.0 °C; Anal. Calcd for $C_5H_{10}ClNO_2$: C, 39.62; H, 6.65; Cl, 23.39; N, 9.24. Found: C, 39.34; H, 6.44; Cl, 23.14; N, 9.18.

3.29. (1*R*,2*S*)-1-Azidomethyl-2-(triphenylmethoxy)methylcyclopropane (*ent*-19)

$[\alpha]_D^{22}$ −24.3° (*c* 1.86, $CHCl_3$); Anal. Calcd for $C_{24}H_{23}N_3O$: C, 78.02; H, 6.27; N, 11.37. Found: C, 77.74; H, 6.24; N, 11.24.

3.30. (1*R*,2*S*)-1-(*N*-*t*-Butoxycarbonyl)aminomethyl-2-(triphenylmethoxy)methylcyclopropane (*ent*-20)

$[\alpha]_D^{22}$ +1.2° (*c* 1.01, $CHCl_3$).

3.31. (1*R*,2*S*)-*cis*-1-Aminomethyl-2-carboxycyclopropane (*ent*-IIb, HCl salt)

Mp 241.2–243.0 °C (lit.[10b]: 241–242 °C); Anal. Calcd for $C_5H_{10}ClNO_2$: 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.

3.32. (1*R*,2*S*)-2-(*t*-Butyldiphenylsilyloxy)methyl-1-(2-hydroxyethyl)cyclopropane (*ent*-22)

$[\alpha]_D^{23}$ +0.2° (*c* 1.25, $CHCl_3$); Anal. Calcd for $C_{22}H_{30}O_2Si$: C, 74.53; H, 8.53. Found: C, 74.16; H, 8.57.

3.33. (1*R*,2*S*)-*trans*-1-(2-Benzyloxy)ethyl-2-hydroxymethylcyclopropane (*ent*-23)

$[\alpha]_D^{24}$ −1.5° (*c* 1.29, $CHCl_3$).

3.34. (1*R*,2*S*)-1-(2-Benzyloxy)ethyl-2-carboxycyclopropane (*ent*-24)

$[\alpha]_D^{24}$ +43.9° (*c* 1.30, $CHCl_3$).

3.35. (1*R*,2*S*)-1-(2-Benzyloxy)ethyl-2-(*t*-buthoxycarbonyl)aminocyclopropane (*ent*-25)

$[\alpha]_D^{22}$ +25.0° (*c* 1.30, $CHCl_3$).

3.36. (1*R*,2*S*)-*trans*-2-(*t*-Buthoxycarbonyl)amino-1-(carboxymethyl)cyclopropane (*ent*-26)

$[\alpha]_D^{22}$ −43.3° (*c* 1.20, $CHCl_3$); Anal. Calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.50; H, 7.77; N, 6.37.

3.37. (1*R*,2*S*)-*trans*-2-amino-1-(carboxymethyl)cyclopropane hydrochloride (*ent*-IIa, HCl salt)

$[\alpha]_D^{22}$ +20.6° (*c* 1.10, 1 N HCl); mp 103–105 °C; Anal. Calcd for $C_5H_{10}ClNO_2{\cdot}0.2H_2O$: 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.

3.38. (1*S*,2*S*)-2-(*t*-Butyldiphenylsilyloxy)methyl-1-(2-hydroxyethyl)cyclopropane (*ent*-27)

$[\alpha]_D^{24}$ −11.5° (*c* 1.57, $CHCl_3$); Anal. Calcd for $C_{22}H_{30}O_2Si$: C, 74.53; H, 8.53. Found: C, 74.41; H, 8.62.

3.39. (1*S*,2*S*)-*cis*-1-(2-Benzyloxy)ethyl-2-hydroxymethylcyclopropane (*ent*-28)

$[\alpha]_D^{24}$ −20.6° (*c* 1.27, $CHCl_3$).

3.40. (1*S*,2*S*)-*cis*-1-(2-Benzyloxy)ethyl-2-carboxycyclopropane (*ent*-29)

$[\alpha]_D^{24}$ +49.8° (*c* 1.45, $CHCl_3$); Anal. Calcd for $C_{13}H_{16}O_3{\cdot}0.15H_2O$: C, 70.03; H, 7.37. Found: C, 70.03; H, 7.33.

3.41. (1*S*,2*S*)-*cis*-1-(2-Benzyloxy)ethyl-2-(*t*-buthoxycarbonyl)aminocyclopropane (*ent*-30)

$[\alpha]_D^{22}$ +49.1° (*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.

3.42. (4*S*,5*S*)-*N*-*t*-Butoxycarbonyl-4,5-methano-2-pyrrolidone (*ent*-31)

$[\alpha]_D^{24}$ +36.4° (*c* 0.69, $CHCl_3$).

3.43. (1*S*,2*S*)-*cis*-2-Amino-1-(carboxymethyl)cyclopropane hydrochloride (*ent*-IIb, HCl salt)

^{1}H NMR (400 MHz, D_2O) δ 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_2O) δ 9.8, 11.5, 27.2, 32.6, 177.2; LRMS (FAB) *m*/*z* 116 $(M+H)^+$; $[\alpha]_D^{22}$ +22.8° (*c* 0.82, 1 N HCl); mp 175–179 °C; Anal. Calcd for $C_5H_{10}ClNO_2$: 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.

3.44. 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.

3.45. 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).

3.46. Stable expression of human GABA transporter subtypes in Chinese hamster ovary (CHO) cells

In order to produce cell lines stablely 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% CO_2. After 7–14 days, single colonies were isolated and further cultivated in selection medium. The cells stablely expressing GABA transporters were selected using [^{3}H] GABA uptake assay.

3.47. [^{3}H]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 $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 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 [^{3}H]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 [^{3}H]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 [^{3}H]GABA uptake were evaluated using the following equation; inhibitory effect = {1 − [(total GABA uptake in the presence of 100 μM analog) − (non-specific 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_{50} 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.

3.48. [^{3}H]muscimol binding assay on $BABA_A$ recepotor

To prepare mouse brain membrane fruction, 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 [^{3}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 [^{3}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_d 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 fom the binding assays are expressed as the mean (95% CI) of three independent experiments, each of which was performed in duplicate.

3.49. [^{3}H]GABA binding assay on $BABA_B$ recepotor

The assay was performed by the procedure reported previously.[15]

3.50. 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 occurence 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 frozon 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.

3.51. 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. ED_{50} values of anticonvulsant effects were calculated using a log-probit method. The data are expressed as means ± SEMs.

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