Advance Publication Cover Page

Four Stereoisomers of 2–Aminomethyl–1–cyclopropanecarboxylic Acid: Synthesis and Biological Evaluation

Masato Oikawa,* Yuka Sugeno, Hideyuki Tukada, Yuichi Takasaki,
Satoshi Takamizawa, and Raku Irie

Advance Publication on the web August 2, 2019
doi:10.1246/bcsj.20190168

Four Stereoisomers of 2–Aminomethyl–1–cyclopropanecarboxylic Acid: Synthesis and Biological Evaluation[#]

Masato Oikawa,* Yuka Sugeno, Hideyuki Tukada, Yuichi Takasaki, Satoshi Takamizawa, Raku Irie

Yokohama City University, Seto 22–2, Kanazawa-ku, Yokohama 236–0027, Japan

E-mail: moikawa@yokohama-cu.ac.jp

Abstract

Here, we report practical method for asymmetric synthesis of cyclopropane-fused GABA analogs. Starting from 2–furaldehyde, the *cis*–isomer (CAMP) was synthesized over 10 steps; (–)– and (+)–CAMP•HCl were synthesized by employing *d*– and *l*–menthol as the chiral auxiliary for total 2.5% and 1.3% yields, respectively. On the other hand, the *trans*–isomer (TAMP) was elaborated via double asymmetric induction, i.e. organocatalytic asymmetric cyclopropanation on chiral substrate. Thus, starting from *l*– and *d*–menthyl acrylate, in combination with quinidine-derived and quinine-derived organocatalysts, (–)– and (+)–TAMP•HCl were synthesized in total 6.6% and 3.7% yields, respectively, over 8 steps each. Configurational analysis of the synthetic intermediates based on ^{13}C NMR is also reported. Preliminary oncological assays showed the weak but specific activities of CAMP and TAMP as the molecular basis of GABA analogs, which are still left unexplored.

Keywords: Amino acids, Organocatalysis, Cytotoxicity

Introduction

Diastereomeric *cis*–2–aminomethyl–1–cyclopropanecarboxylic acid (CAMP) and the *trans*–congener (TAMP), and the enantiomers (Figure 1, **1**–**4**), are conformationally restricted analogs of 4–aminobutyric (γ–aminobutyric) acid (GABA), which is one of the neurotransmitters in the mammalian central nervous system.[1] The neuropharmacology of four analogs **1**–**4** bearing three-membered ring has been well studied to date.[2]

In 2004, Sakai and co–workers have reported isolation of *N*–methylated cyclopropane GABA analog with more substituents, dysibetaine CPa (DBCPa) and CPb (DBCPb) (Figure 1), from Micronesian marine sponge.[3] Since neuroactivities of DBCPa/b could not be investigated due to the limited amount from the marine sponge, we have carried out chemical synthesis and the results have been already reported.[4] We also decided to synthesize CAMP and TAMP as control compounds in the neuropharmacological evaluation of DBCPa/b.[5] Since DBCPa/b were later found, unfortunately, to be neurologically inactive from mice in vivo assays,[4a] we have performed other assays related to oncology on CAMP and TAMP, which, to the best of our knowledge, have not yet been reported. Herein, we report, in full detail, our studies on asymmetric synthesis and the oncological evaluation of four stereoisomers of 2–aminomethyl–1–cyclopropanecarboxylic acid; CAMP and TAMP.[5] Since the method for synthesizing more than subgram quantities of enantiomerically pure specimens of CAMP and TAMP seemed to be not established well, we first studied facile and promising synthesis route for these analogs, as follows.[5-6]

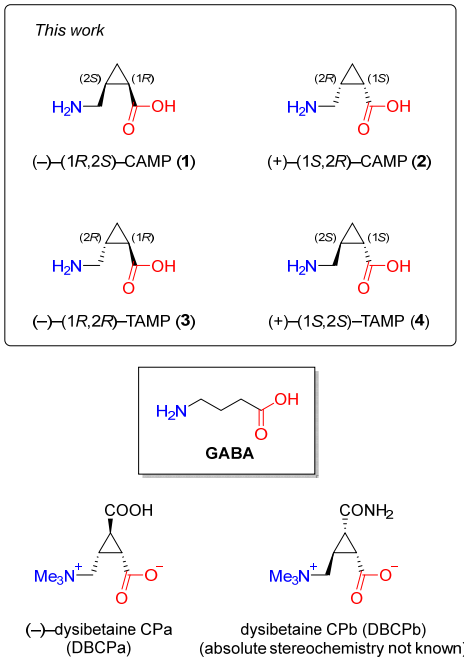
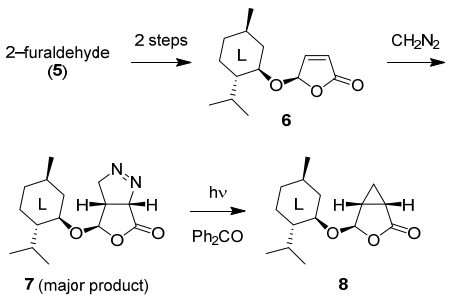


Figure 1. GABA and the analogs conformationally restricted by cyclopropane ring

Results and Discussion

Synthesis of (+)– and (–)–*cis*–2–aminomethyl–1–cyclopropanecarboxylic acid (CAMP)

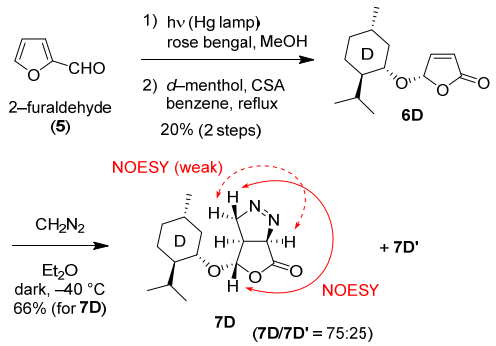
Several methods, reported to date for enantioselective synthesis of CAMP, employ chiral auxiliary (1–phenylethan–1–amine),[7] chiral resolution,[8] polymer-supported reagents,[9] and radical reaction.[10] Meanwhile, our strategies toward asymmetric synthesis of (+)– and (–)–CAMP were based on chiral auxiliary-assisted organic synthesis using menthol.[6a] In 1994, Feringa's group reported diastereoselective synthesis of cyclopropane **8** using *l*–menthol as a chiral auxiliary (Scheme 1).[11]



Scheme 1. Chiral auxiliary-guided cyclopropanation (Feringa, 1994)[11]

Since the procedure described[11] seemed to be not established well, we decided to improve their cyclopropane formation method for synthesis of highly enantiopure *cis*-disubstituted cyclopropane. Eventually, large-scale synthesis of **8** and the enantiomer, followed by 6-step transformation, was successfully developed for the synthesis of both enantiomers of CAMP (**1**, **2**) as follows.[6a]

The starting material, (5*S*)–(*d*–menthyloxy)–2(5*H*)–furan one (**6D**),[12] was readily prepared by rose bengal-sensitized photooxidation of 2–furaldehyde (**5**), followed by CSA-catalyzed condensation with *d*–menthol (Scheme 2).[13] A diastereomeric mixture of 5–(*d*–menthyloxy)–2(5*H*)–furanones (**6D** and the isomer) was formed in a 1:1 ratio. Diastereomerically pure **6D** was obtained by recrystallization from petroleum ether, in 20% yield over 2 steps from 2–furaldehyde (**5**). Treatment of **6D** with diazomethane in Et_2O at –40 °C for two days gave diazene **7D** and the minor isomer **7D'** (for the structure, see Figure 2) in a ratio of 75:25, as judged by ^{1}H NMR analysis of the crude 1,3–dipolar cycloaddition product.



Scheme 2. Preparation of diazene **7D**

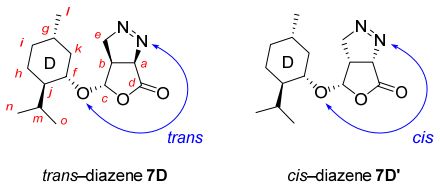


Figure 2. A set of diastereomers obtained in the cycloaddition. The configurations were analyzed by the NMR calculations (see text)

Since diazene products, especially the minor isomer **7D'**, were unstable under both acidic and alkaline conditions, purification by flash column chromatography using neutral silica gel 60N was carefully performed to give pure diazene **7D** in 66% isolated yield. The minor diastereomer **7D'** (for the structure, see Figure 2) was not isolated in pure form, and hence was not used in this synthetic study. The stereochemistry of the major isomer, diazene **7D**, was first speculated to be "*trans*" from NOESY cross peaks (see Scheme 2 and the Supporting Information). The solid poof was obtained by comparison of the ^{13}C NMR chemical shift values with those expected by density functional theory (DFT) calculation.[14] The calculations were performed with Spartan '18 (Wavefunction, Irvine, CA, U.S.A.).[15] The theoretical ^{13}C NMR shifts for *trans*–diazene **7D** and *cis*–diazene **7D'** (Figure 2) were independently obtained by the calculation sequence; 1) conformational search with MMFF94,[16] 2) structural optimizations with HF/3–21G level, 3) energy calculations at ωB97X–D/6–31G* level, 4) structural optimizations with ωB97X–D/6–31G* level, 5) energy calculations at ωB97X–V/6–311+G(2df,2p)[6–311+G*] level with fixing the geometries to generate Boltzmann distribution, 6) empirically corrected calculations of the ^{13}C NMR chemical shifts at ωB97X-D/6-31G* level with ωB97X–D/6–31G* model,[17] and 7) correction of the ^{13}C NMR shift values based on the Boltzmann weighting. Parameters regarding solvents (C_6D_6) were not added in these calculations.

The experimental ^{13}C values for **7D** ($7D_{obs}$), and the calculated ^{13}C values for **7D** ($7D_{calc}$) and **7D'** ($7D'_{calc}$), are summarized in Table 1. It was found that calculated ^{13}C values for **7D** ($7D_{calc}$) agree with the experimental values ($7D_{obs}$); the root mean square deviation (RMS) for $7D_{calc}$ is 0.97 ppm. On the other hand, RMS values for $7D'_{calc}$ is higher (2.60 ppm). Furthermore, analysis using DP4 probability statistics[14a,18] of the calculated ^{13}C shifts with the experimental data showed that the probability ratio for $7D_{calc}$/$7D'_{calc}$ was 100.0%:0.0%. The ^{13}C-based configurational analysis for **7D** was consistent with the NOESY cross peaks observed at H*c*/H*e* (see Scheme 2 and the Supporting Information).

Table 1. Comparison of experimental ^{13}C shifts of *trans*–diazene **7D**, and calculated ^{13}C shifts for *trans*–diazene **7D** and *cis*–diazene **7D'**

Position [a]	Experimental for **7D** [b] ($7D_{obs}$)	Calculated [c]	
		trans–diazene **7D** ($7D_{calc}$)	*cis*–diazene **7D'** ($7D'_{calc}$)
a	93.5	94.3	94.6
b	39.9	40.2	38.1
c	104.3	103.2	96.8
d	167.0	168.0	169.3
e	83.1	82.8	78.7
f	77.7	76.6	74.9
g	31.3	30.1	30.0
h	23.2	24.3	23.3
i	34.3	32.8	32.8
j	48.1	46.6	46.6
k	39.1	38.6	37.6
l	22.3	22.2	22.2
m	25.7	27.0	25.6
n	21.0	20.4	20.4
o	15.9	16.6	15.5
RMS / ppm		0.97	2.60
DP4 / %		100.0	0.0

[a] For numbering, see Figure 2.
[b] Experimental ^{13}C NMR data were collected at 100 MHz in C_6D_6.
[c] Calculated ^{13}C NMR data were obtained employing ωB97X–V/6–311+G(2df,2p)[6–311+G*]//ωB97X–D/6–31G* model. For detail, see text.

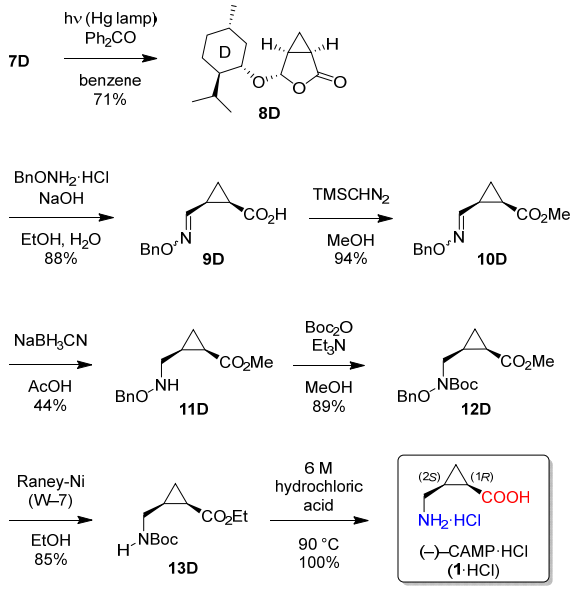
As shown in Table 2, the configurational analysis was also conducted on the experimental ^{13}C values for the diastereomer **7D'** ($7D'_{obs}$). The RMS and DP4 analyses have assigned that the stereochemistry of the minor diazene **7D'** is "*cis*" but not "*trans*".

In total, the major diazene **7D** and the minor diazene **7D'** were concluded to be the *trans* and the *cis* isomers, respectively, from the ^{13}C NMR analyses.[14b,19] The assignments were finally confirmed by leading to (–)–CAMP (see below).

Table 2. Comparison of experimental ^{13}C shifts of *cis*–diazene **7D'**, and calculated ^{13}C shifts for *trans*–diazene **7D** and *cis*–diazene **7D'**

Position [a]	Experimental for **7D'** [b] ($7D'_{obs}$)	Calculated [c]	
		trans–diazene **7D** ($7D_{calc}$)	*cis*–diazene **7D'** ($7D'_{calc}$)
a	93.5	94.3	94.6
b	39.5	40.2	38.1
c	93.7	103.2	96.8
d	168.0	168.0	169.3
e	78.9	82.8	78.7
f	77.0	76.6	74.9
g	31.2	30.1	30.0
h	23.1	24.3	23.3
i	34.3	32.8	32.8
j	47.8	46.6	46.6
k	36.0	38.6	37.6
l	22.3	22.2	22.2
m	25.5	27.0	25.6
n	20.8	20.4	20.4
o	15.6	16.6	15.5
RMS / ppm		2.86	1.34
DP4 / %		0.0	100.0

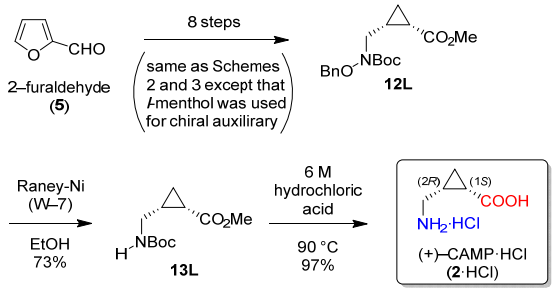
[a] For numbering, see Figure 2.
[b] Experimental ^{13}C NMR data were collected at 100 MHz in C_6D_6.
[c] Calculated ^{13}C NMR data were obtained employing ωB97X–V/6–311+G(2df,2p)[6–311+G*]//ωB97X–D/6–31G* model. For detail, see text.



Scheme 3. Synthesis of (–)–CAMP•HCl (**1**•HCl)

Next, as shown in Scheme 3, 1,3–elimination of nitrogen (N_2) from diazene **7D** gave cyclopropane **8D** in 71% yield upon photoirradiation (high-pressure mercury lamp, benzophenone, benzene). To introduce a nitrogen functionality of CAMP, *d*–menthyl acetal **8D** was treated with benzyloxyamine to give oxime ether **9D** in 88% yield. Here, several hydrogenation methods, using 10% Pd/C, Raney–Ni (W–7), and PtO_2, were attempted to directly reduce oxime ether group to amine. However, most of them were unsuccessful because of the decomposition of the cyclopropane ring under hydrogenation conditions. After screening various conditions for the reduction,[20] the stepwise reduction protocol was finally found to be fruitful. Thus, esterification of **9D** with $TMSCHN_2$ gave methyl ester **10D** (94% yield), whose C=N bond was then reduced by $NaBH_3CN$ in AcOH to afford benzyloxyamine **11D** in 44% yield. Protection of amine **11D** with Boc group (Boc_2O, Et_3N, MeOH) gave **12D** in 89% yield. To cleave the N–O bond, **12D** was treated with freshly prepared Raney–Ni (W–7) in EtOH[21] to give desired **13D** with concomitant transesterification in 85% yield. The transesterification would be induced by residual sodium hydroxide, which had been used for preparation of Raney–Ni (W–7). Global deprotection under acidic conditions (6 M HCl, 90 °C) successfully delivered desired (–)–CAMP•HCl (**1**•HCl) in quantitative yield. Spectroscopic data were in good agreement with those reported.[7-10] In total, starting from 2–furaldehyde (**5**), the synthesis of (–)–CAMP was successfully accomplished in 2.5% yield over 10 steps.

(+)–CAMP was also synthesized employing *l*–menthol as the chiral auxiliary (Scheme 4). It should be noted that, in the synthesis of (+)–CAMP, reduction of the N–O bond of **12L** (Raney–Ni (W–7), EtOH) did not accompany transesterification, using strictly neutralized Raney–Ni prepared carefully. The synthesis of more than 30 mg of (+)–CAMP•HCl (**2**•HCl) was thus accomplished in 1.3% yield over 10 steps. The spectroscopic data were identical with those reported.[7-10]



Scheme 4. Synthesis of (+)–CAMP•HCl (**2**•HCl)

Synthesis of (+)– and (–)–*trans*–2–aminomethyl–1–cyclopropanecarboxylic acid (TAMP)

Three works are known for the synthesis of enantiomerically pure TAMP.[8,22] Our strategies toward synthesis of (+)– and (–)–TAMP are based on a combination of chiral auxiliary-guided synthesis and organocatalyst-mediated asymmetric cyclopropanation.[6b] In 2003[23] and 2004,[24] Gaunt's group reported enantioselective organocatalytic cyclopropanation via ammonium ylides derived from **15** and **16** (Figure 3). We decided to employ their method, also using menthol as a chiral auxiliary, for construction of highly enantiopure *trans*-disubstituted cyclopropane.[6b]

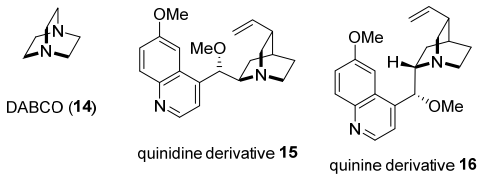
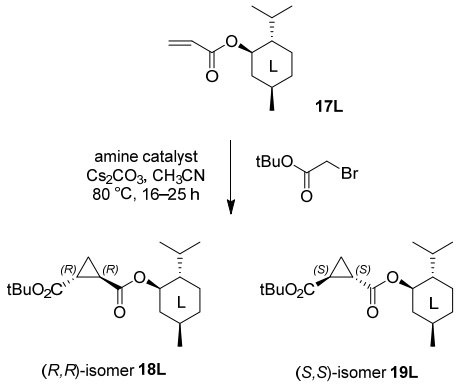


Figure 3. Amine catalysts for formation of ammonium ylide in the cyclopropanation

First, effects of four amine catalysts including $BnEt_3NCl$, 1,4–diazabicyclo[2.2.2]octane (DABCO, **14**), **15**,[23] and **16**[23] (Figure 3), on the diastereoselectivity in the cyclopropanation of *l*–menthyl acrylate (**17L**) with *tert*–butyl bromoacetate, were studied. *l*–Menthyl acrylate (**17L**) was prepared by esterification of *l*–menthol with acryloyl chloride in 94% yield.[25] As shown in Table 3, cyclopropanation using $BnEt_3NCl$ as a catalyst did not proceed after 31 h (entry 1).

Table 3. Asymmetric cyclopropanation on chiral acrylate ester mediated by four amine catalysts



Entry	Amine catalyst	Yield [a]	**18L**/**19L** [b] (*R*,*R*)/(*S*,*S*)
1	$BnEt_3NCl$ (1 equiv)	0%	–
2	DABCO **14** (1 equiv)	35%	56:44
3	quinidine derivative **15** (0.2 equiv)	42% (89%)	75:25
4 [c]	quinidine derivative **15** (0.2 equiv)	69% (99%)	75:25
5	quinine derivative **16** (0.2 equiv)	34% (71%)	7:93
6 [c]	quinine derivative **16** (0.2 equiv)	55% (71%)	7:93

[a] In parentheses are the yield based on recovered starting material **17L**.

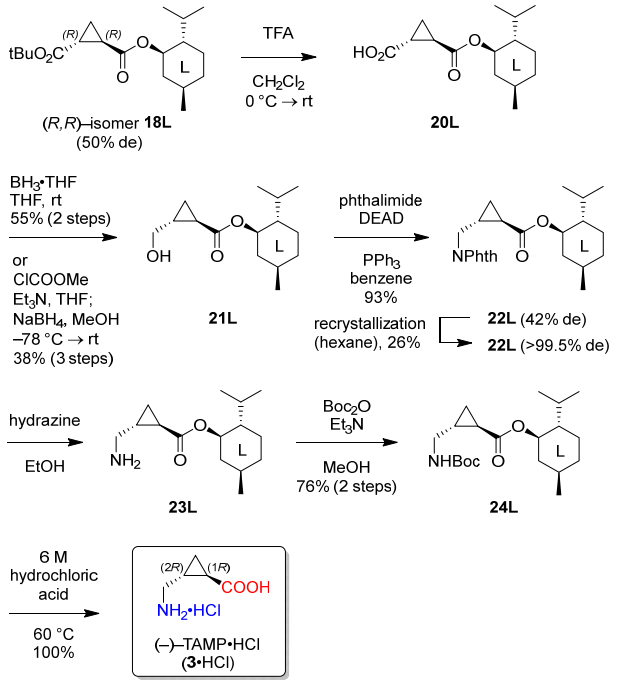
[b] Diastereoselectivity determined by chiral HPLC analysis (CHIRALPAK ID–3, 0.46 × 25 cm, H_2O/CH_3CN = 40:60).

[c] Reaction conducted by slow addition procedure.

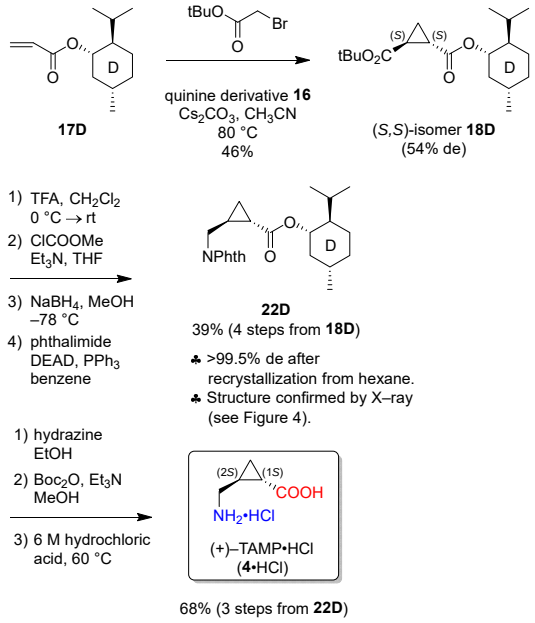
In the presence of 1.0 equiv of DABCO (**14**, entry 2),[23] *tert*–butyl bromoacetate reacted with *l*–menthyl acrylate (**17L**) to form *trans*–cyclopropanes **18L** and **19L** in 35% yield. The diastereomeric ratio was determined to be **18L**/**19L** = 56:44 by chiral HPLC analysis (CHIRALPAK ID–3, 0.46 × 25 cm, H_2O/CH_3CN = 40:60). The structures were determined later by X–ray crystallographic analysis of the derivative **22D** (see below), and also by leading to (+)– and (–)–TAMP finally. Changing the cyclopropanation catalyst to quinidine derivative **15** (0.2 equiv) gave *trans*–cyclopropane in 42% yield (89% based on recovered starting material **17L**) with the diastereomer ratio of **18L**/**19L** = 75:25 (entry 3). Furthermore, slow mixing procedure[24] greatly improved the yield to 69% without loss of the diastereoselectivity (entry 4). We also found that the use of quinine-derived catalyst **16** (0.2 equiv) in the reaction afforded *trans*–cyclopropane in 34% yield (63% based on recovered starting material **17L**) with higher diastereomer ratio of 7:93 (entry 5). Finally, the cyclopropanation yield with catalyst **16** was improved to 55% by the slow mixing procedure (entry 6), as also observed in the cases where quinidine derivative **15** was employed (entries 3 and 4). From these observations and the empirical rule in cyclopropanation described by Gaunt,[24] the reaction of *l*–menthyl acrylate (**17L**) using quinidine derivative **15** (entries 3 and 4) is mismatched asymmetric cyclopropanation, whereas the reaction of **17L** employing quinine derivative **16** (entries 5 and 6) is likely stereochemically matched (double asymmetric induction effect). Since sterically demanding acrylate has been reported to be poorly reactive in the ammonium ylide-mediated cyclopropanation,[24] the low isolated yield observed in Table 3 was likely attributed to the menthyl group. To our disappointment, however, all attempts to reduce the undesirable steric repulsion between the acrylate and the ammonium ylide were unfruitful; for example, the use of sterically less demanding benzyl bromoacetate in combination with the catalyst **15** resulted in much lower yield (18%) than that shown in Table 3 (entry 3), since hydrolytic decomposition of the benzyl ester also took place under such harsh alkaline conditions (Cs_2CO_3, MeCN, 80 °C) (data not shown). Our efforts still continue to improve the isolation yield in the asymmetric cyclopropanation.

Here, we next show the synthesis of (–)–TAMP from *trans*–cyclopropane **18L**, which was obtained as a mixture with **19L** (**18L**/**19L** = 75:25, Table 3, entries 3, 4). We anticipated practical separation of the diastereomers would be possible later in the synthesis, and, hence, decided to start the synthesis toward (–)–TAMP using the cyclopropane **18L** of 50% de, as shown in Scheme 5. Thus, the *tert*–butyl ester **18L** (50% de) was removed by TFA to give carboxylic acid **20L**, which, in turn, was reduced to alcohol **21L** (BH_3•THF, or ClCOOMe followed by $NaBH_4$[26]). The alcohol **21L** was still an inseparable mixture with the (*S*,*S*)–diastereomer. To introduce nitrogen functionality of TAMP, alcohol **21L** was then transformed into phthalimide **22L** (42% de) under Mitsunobu conditions (phthalimide, DEAD, PPh_3, benzene) in 93% yield.[27] Here, the phthalimide **22L** was found to be a crystalline solid and was successfully purified by recrystallization from hot hexane to be >99.5% de.

With diastereomerically pure, fully protected (–)–TAMP **22L** in hand, protecting group manipulation was then conducted. The *N*–phthaloyl group was first removed by hydrazine monohydrate in EtOH to give pure *N*–Boc amine **24L** in 76% yield after reprotection (Boc_2O, Et_3N), ready for the final deprotection. Finally, acidic hydrolysis by 6 M HCl at 60 °C successfully delivered the desired (–)–TAMP•HCl (**3**•HCl) quantitatively. Spectroscopic data including the $[\alpha]_D$ value were in good agreement with those reported.[8,22b] In total, the synthesis of (–)–TAMP•HCl (**3**•HCl) was accomplished in 6.6% yield over 8 steps from *l*–menthol.



Scheme 5. Synthesis of (–)–TAMP•HCl (**3**•HCl)



Scheme 6. Synthesis of (+)–TAMP•HCl (**4**•HCl)

The enantiomeric (+)–TAMP was also synthesized in this study by employing, A) cyclopropane **19L** of 86% de obtained in Table 3 (entries 5, 6), or B) a combination of *d*–menthol chiral auxiliary and quinine–derived organocatalyst **16** for construction of the (*S*,*S*)–*trans*–cyclopropane core. The latter procedure B is shown in Scheme 6. Cyclopropanation of *d*–menthyl acrylate (**17D**), prepared from *d*–menthol and acryloyl chloride in 86% yield, by using quinine-derived catalyst **16** proceeded in 46% yield to give (*S*,*S*)–isomer **18D** as a major diastereomer. The diastereomeric ratio of **18D** was determined to be 77:23, which, without separation, was subjected to the same reaction sequence as for the synthesis of (–)–TAMP (Scheme 5) to give rise to diastereomerically pure phthalimide **22D** (>99.5% de) after recrystallization. Here, structural analysis of **22D** was conducted by X–ray crystallography (Figure 4) to confirm the structure. Gaunt's empirical rule for the stereoselectivity in the two chiral alkaloid-catalyzed cyclopropanations was thus found to be applicable to also the cases where chiral substrates are employed (Table 3, entries 3–6). The synthesis of (+)–TAMP•HCl (**4**•HCl) was finally accomplished in 3.7% yield over 8 steps starting from *d*–menthol. The procedure established in this study has successfully allowed to synthesize 253 mg of (+)–TAMP•HCl (**4**•HCl).

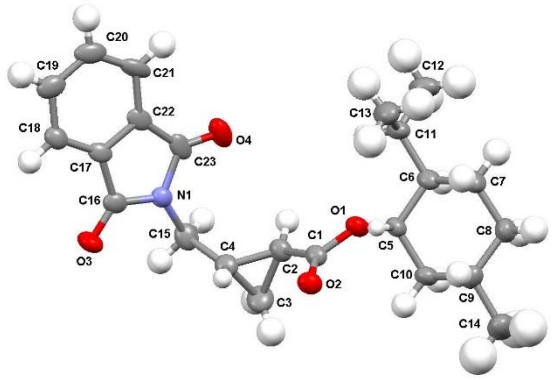


Figure 4. Molecular structure of **22D** determined by single crystal X–ray diffraction measurement at 183 K. ORTEP representation is drawn at 50% probability level for the ellipsoid.

Oncological activity of four isomers for 2–aminomethyl–1–cyclopropanecarboxylic acid

GABA is one of the neurotransmitters, and neuroactivity of the cyclopropane analogs **1**–**4** has been well investigated; (–)–CAMP (**1**) and (+)–CAMP (**2**) were characterized as an antagonist and a full agonist for GABAc receptor, respectively, whereas (–)–TAMP (**3**) is the partial agonist.[2b] In this study, with all four stereoisomers in hand, we evaluated cytotoxicity and other in vitro activities as a preliminary study toward oncological application of GABA analogs. While GABAergic system gene expression has been previously studied in patients with neuroblastoma,[28] no structure–activity relationships (SAR) study has been reported on GABA analogs yet. Results for our preliminary evaluations are as follows.

Cytotoxicity was first evaluated on eight cell lines (HEK293T, MRC5, MRC5 high-density, MDA–MB231, LNCaP, A549, PC3, and Jurkat cells) by CellTiter–Glo® Luminescent Cell Viability Assay (Promega). Selected results are shown in Figure S1 (see the Supporting Information). It was found some compounds are cytotoxic at 0.3 μM with low potency. For example, (+)–CAMP (**2**) inhibited 16% of proliferation of the human embryonic kidney (HEK293T) cells, while other isomers were totally inactive. Both (–)–TAMP (**3**) and (+)–TAMP (**4**) inhibited MRC5 (high density) (the normal human lung fibroblast cell line) proliferation with potency of 7% and 4%, respectively, while (–)–CAMP (**1**) and (+)–CAMP (**2**) made no effect. To A549 cells (the human epithelial of lung carcinoma), only (+)–TAMP (**4**, 0.3 μM) showed 13% of inhibition on the proliferation. Only a weak cytotoxicity was thus observed with the GABA analogs, however, the effects were apparently specific to the cell lines.

Cytotoxicity was also assayed on 53 human cancer cell lines panel. Selected results are shown in Figure S2. It was found (+)–CAMP (**2**) and (–)–TAMP (**3**) showed

antiproliferation activity on Caov–4 (17% inhibition at 0.3 μM) and DMS–53 (12% inhibition at 3 μM), respectively, while other analogs were inactive. It is again interesting that the effects of the GABA analog diastereomers are specific to the cancer cells.

Activating transcription factor (ATF6) is a type II transmembrane protein containing a cytosolic cAMP-responsive element-binding protein and ATF basic leucine zipper domain, and observed as one of the unfolded protein response (UPR) components in carcinoma of liver and uterus.[29] It has been suggested ATF6α, one of the two homologues of ATF6 in the mammalian genome, promotes hepatocarcinogenesis by regulation of target genes. UPR components are one of the targets for cancer treatment, and some drugs including retaspimycin hydrochloride (IPI–504) inactivates the transcription factors such as ATF6.[30] Here, we have evaluated inhibition of ATF6 signaling by the four GABA analogs **1**–**4** by a reporter gene assay in HEK293 cells, and found that 10 μM of (+)–CAMP (**2**) weakly inhibits 17% of the signal (Figure S3). Other analogs made no effect.

p53 is the tumor suppressor protein.[31] Upon carcinogenesis, p53 leads the cell to apoptotic cell death. By a reporter gene assay in HEK293 cells, here, (+)–CAMP (**2**) was found to weakly inhibit 23% and 28% of the p53 signaling at 0.3 and 10 μM, respectively (Figure S3). Other analogs were totally inactive.[32]

From these cytotoxic assays and reporter gene assays, specific actions of GABA analog stereoisomers (**1**–**4**) in oncological aspects were shown as a preliminary SAR data. Among them, (+)–CAMP (**2**) was found to show weak, but definitive and diverse activities.

Conclusion

In this work, we have established a new synthetic entry to the four stereoisomers of 2–aminomethyl–1–cyclopropanecar boxylic acid. The *cis* analogs (CAMP) were synthesized over 10 steps starting from 2–furaldehyde (**5**), through diastereoselective cyclopropane formation via diazene intermediate **7D**/**7L** employing *d*– or *l*–menthol as a chiral auxiliary. On the other hand, the *trans* analogs (TAMP) were synthesized by enantioselective organocatalytic cyclopropanation on *d*– or *l*–menthyl acrylate as a key step, where we observed double asymmetric induction effect. The enantioselective synthesis of TAMP was thus successfully achieved over 8 steps starting from *d*– or *l*–menthol.

During the synthetic studies, configurational analyses based on experimental and theoretical ^{13}C NMR chemical shifts were successfully demonstrated on the intermediary diazenes **7D** and **7D'**. From RMS an DP4 analyses, **7D** and **7D'** were reasonably assigned as *trans*– and *cis*–isomers, respectively, which were later confirmed by leading to (–)–CAMP (**1**). It was thus shown here that the ^{13}C analysis can be applied to bicyclic diazenes, which cannot be readily assigned by NMR analysis based on $J_{H,H}$ coupling constants.

Cytotoxic activities were evaluated on diverse cell lines, and some compounds were found to be active with low potency but with apparent specificity; (+)–CAMP (**2**) and (+)–TAMP (**4**) inhibited proliferation of HEK293T and A549 cell lines, respectively, and both (–)–TAMP (**3**) and (+)–TAMP (**4**) inhibited MRC5 (high density) cells. (+)–CAMP (**2**) and (–)–TAMP (**3**) were found to be effective on the inhibition of the cancer cell proliferation of Caov–4 and DMS–53, respectively, while other analogs made no effect. Because of the low potencies of antiproliferation on cancer cell lines panel (see the Supporting Information for details), we have not yet determined the cytotoxicity mechanism. To address the issues, our work is in progress to improve the activity by synthetically modifying the structures of (+)–CAMP (**2**) and (–)–TAMP (**3**).

Inhibition of ATF6 was evaluated by the reporter gene assay and only (+)–CAMP (**2**) was found to be active among four analogs **1**–**4**. Since some anticancer drugs inactivate ATF6, the observed inhibition by structurally simple GABA analog, (+)–CAMP (**2**), would be of interest. It should be noted here, however, that (+)–CAMP (**2**) was shown to inhibit also the tumor suppressor protein p53.

This paper describes synthesis and oncological activity of conformationally restricted GABA analogs. (+)–CAMP (**2**) was found to have weak but broad activities. The SAR data shown here would provide a molecular basis for the oncological activities of GABA and analogs, which are left unexplored.

Experimental

Procedures for all chemical syntheses are described in the Supporting Information.

Single-crystal X–ray diffraction experiment of (phthalimidomethyl)cyclopropane 22D

Single-crystal X–ray analysis of **22D** was performed on a Bruker SMART APEX CCD area (graphite–monochromated Mo–K*a* radiation (λ = 0.71073 Å)) with a nitrogen flow temperature controller. Data collection was performed at 183 K. Empirical absorption corrections were applied using the SADABS program. The structures were solved by direct methods (SHELXS–97) and refined by full-matrix least squares calculations on F^2 (SHELXL–97) using the SHELX–TL program package. Non-hydrogen atoms were refined anisotropically; hydrogen atoms were fixed at calculated positions and refined using a riding model. Crystallographic data of the structure is summarized in Tables S2–S6. CCDC–1936107 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Cytotoxicity assay

Antiproliferative activities of the GABA analogs **1**–**4** were determined upon treatment for 20 h (HEK293T, Caov–4, DMS–53) or 72 h (MRC5–high density, A549) using the CellTiter–Glo® luminescent Cell Viability Assay (Promega, Madison, WI), according to the manufacture's protocol.

Reporter gene assay (for inhibitory activity)

HEK293 cells seeded in 48-well plates were transfected with pGL4.28 (Promega). Twenty-four hours after transfection, the cells were exposed to the GABA analogs **1**–**4** and thapsigargin (for ATF6,[33] 5 h) or doxorubicin (for p53,[34] 20 h). Luciferase activities were measured using Steady–Glo® Luciferase Assay System (Promega) and a luminescencer.

Acknowledgement

Daicel Co. is gratefully acknowledged for HPLC analysis shown in Table 3. This work was supported by the grant for 2010-2012 Strategic Research Promotion (Nos. T2202, T2309, T2401) of Yokohama City University, Japan.

Supporting Information

Synthetic procedures and characterization data for new intermediates, procedures for single-crystal X–ray diffraction experiment of **22D**, Results for oncological assays, HPLC profiles for diastereomer separation, and ^{13}C NMR calculations are included. This material is available on https://doi.org/10.1246/##.

References

Dedicated to Professor Fuyuhiko Matsuda (Hokkaido University) on the occasion of his 65th birthday.

1. a) G. A. R. Johnston, *Curr. Top. Med. Chem.* **2002**, *2*, 903. b) M. Ordóñez, C. Cativiela, *Tetrahedron: Asymmetry* **2007**, *18*, 3. c) R. B. Silverman, *Angew. Chem. Int. Ed.* **2008**, *47*, 3500. d) I. A. Levandovskiy, D. I. Sharapa, T. V. Shamota, V. N. Rodionov, T. E. Shubina, *Future Med. Chem.* **2011**, *3*, 223.
2. a) R. D. Allan, D. R. Curtis, P. M. Headley, G. A. R. Johnston, D. Lodge, B. Twitchin, *J. Neurochem.* **1980**, *34*, 652. b) R. K. Duke, M. Chebib, V. J. Balcar, R. D. Allan, K. N. Mewett, G. A. R. Johnston, *J. Neurochem.* **2000**, *75*, 2602.
3. R. Sakai, K. Suzuki, K. Shimamoto, H. Kamiya, *J. Org. Chem.* **2004**, *69*, 1180.
4. a) M. Oikawa, S. Sasaki, M. Sakai, R. Sakai, *Tetrahedron Lett.* **2011**, *52*, 4402. b) M. Oikawa, S. Sasaki, M. Sakai, Y. Ishikawa, R. Sakai, *Eur. J. Org. Chem.* **2012**, *2012*, 5789. c) M. Sakai, Y. Ishikawa, S. Takamizawa, M. Oikawa, *Tetrahedron Lett.* **2013**, *54*, 5911. d) M. Sakai, K. Tanaka, S. Takamizawa, M. Oikawa, *Tetrahedron* **2014**, *70*, 4587. e) K. Tanaka, M. Sakai, S. Takamizawa, M. Oikawa, *Chem. Lett.* **2015**, *44*, 253.
5. For preliminary studies on this work, see references 6a and 6b.
6. a) M. Oikawa, Y. Sugeno, Y. Ishikawa, H. Tukada, *Synlett* **2013**, *24*, 886. b) Y. Sugeno, Y. Ishikawa, M. Oikawa, *Synlett* **2014**, *25*, 987.
7. R. Galeazzi, G. Mobbili, M. Orena, *Tetrahedron: Asymmetry* **1997**, *8*, 133.
8. R. K. Duke, R. D. Allan, M. Chebib, J. R. Greenwood, G. A. R. Johnston, *Tetrahedron: Asymmetry* **1998**, *9*, 2533.
9. I. R. Baxendale, M. Ernst, W.-R. Krahnert, S. V. Ley, *Synlett* **2002**, *2002*, 1641.
10. V. Rodríguez-Soria, L. Quintero, F. Sartillo-Piscil, *Tetrahedron* **2008**, *64*, 2750.
11. M. T. Rispens, E. Keller, B. de Lange, R. W. J. Zijlstra, B. L. Feringa, *Tetrahedron: Asymmetry* **1994**, *5*, 607.
12. In this paper, compound number with **L** denotes that bears *l*-menthyl ester, and **D** denotes *d*-menthyl ester.
13. O. M. Moradei, L. A. Paquette, *Org. Synth.* **2003**, *80*, 66.
14. a) N. Grimblat, A. M. Sarotti, *Chem. Eur. J.* **2016**, *22*, 12246. b) K. Tanaka, H. Manabe, R. Irie, M. Oikawa, *Bull. Chem. Soc. Jpn.* **2019**, *92*, in press (DOI: 10.1246/bcsj.20190096).
15. *Tutorial and User's Guide, Spartan '18 for Windows, Macintosh and Linux*, Wavefunction Inc., **2018**.
16. T. A. Halgren, *J. Comp. Chem.* **1996**, *17*, 490.
17. W. Hehre, P. Klunzinger, B. Deppmeier, A. Driessen, N. Uchida, M. Hashimoto, E. Fukushi, Y. Takata, *J. Nat. Prod.* **2019**, *published online* (DOI: 10.1021/acs.jnatprod.9b00603).
18. S. G. Smith, J. M. Goodman, *J. Am. Chem. Soc.* **2010**, *132*, 12946.
19. K. Morokuma, R. Irie, M. Oikawa, *Tetrahedron Lett.* **2019**, *60*, 2067.
20. A. Y. Sukhorukov, S. L. Ioffe, *Chem. Rev.* **2011**, *111*, 5004.
21. a) M. Ueda, H. Miyabe, H. Sugino, O. Miyata, T. Naito, *Angew. Chem. Int. Ed.* **2005**, *44*, 6190. b) X. Lu, L. Deng, *Angew. Chem. Int. Ed.* **2008**, *47*, 7710.
22. a) D. J. Aitken, S. D. Bull, I. R. Davies, L. Drouin, J. Ollivier, J. Peed, *Synlett* **2010**, *2010*, 2729. b) K. Nakada, M. Yoshikawa, S. Ide, A. Suemasa, S. Kawamura, T. Kobayashi, E. Masuda, Y. Ito, W. Hayakawa, T. Katayama, S. Yamada, M. Arisawa, M. Minami, S. Shuto, *Bioorg. Med. Chem.* **2013**, *21*, 4938.
23. C. D. Papageorgiou, S. V. Ley, M. J. Gaunt, *Angew. Chem. Int. Ed.* **2003**, *42*, 828.
24. C. D. Papageorgiou, M. A. Cubillo de Dios, S. V. Ley, M. J. Gaunt, *Angew. Chem. Int. Ed.* **2004**, *43*, 4641.
25. a) M. N. Masuno, D. M. Young, A. C. Hoepker, C. K. Skepper, T. F. Molinski, *J. Org. Chem.* **2005**, *70*, 4162. b) K. He, Z. Zhou, G. Zhao, C. Tang, *Heteroat. Chem.* **2006**, *17*, 317.
26. X. Sui, R. Zhu, G. Li, X. Ma, Z. Gu, *J. Am. Chem. Soc.* **2013**, *135*, 9318.
27. O. Mitsunobu, *Synthesis* **1981**, *1981*, 1.
28. S. S. Roberts, M. Mori, P. Pattee, J. Lapidus, R. Mathews, J. P. O'Malley, Y. C. Hsieh, M. A. Turner, Z. Wang, Q. Tian, M. J. Rodland, C. P. Reynolds, R. C. Seeger, S. R. Nagalla, *J. Clin. Oncol.* **2004**, *22*, 4127.
29. a) C. Hetz, *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 89. b) M. Wang, R. J. Kaufman, *Nat. Rev. Cancer* **2014**, *14*, 581.
30. J. Patterson, V. J. Palombella, C. Fritz, E. Normant, *Cancer Chemother. Pharmacol.* **2008**, *61*, 923.
31. L. Xu, J. Massague, *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 209.
32. GABA analogs **1**–**4** were found to be totally inactive in other 15 inhibition assays (SMAD signaling, Wnt signaling, and so on).
33. Y. Wang, J. Shen, N. Arenzana, W. Tirasophon, R. J. Kaufman, R. Prywes, *J. Biol. Chem.* **2000**, *275*, 27013.
34. Y. Wang, K.-M. Debatin, H. Hug, *BMC Mol. Biol.* **2001**, *2*, 8.