This article is dedicated to Professor Satoshi Ōmura in celebration of his 2015 Nobel Prize.

Regular Article

Synthesis and GGCT Inhibitory Activity of *N*-Glutaryl-L-alanine Analogues

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γ -Glutamylcyclotransferase (GGCT) is an important enzyme that cleaves γ -glutamyl-amino acid in the γ -glutamyl cycle to release 5-oxoproline and amino acid. Eighteen *N*-acyl-L-alanine analogues including eleven new compounds have been synthesized and examined for their inhibitory activity against recombinant human GGCT protein. Simple *N*-glutaryl-L-alanine was found to be the most potent inhibitor for GGCT. Other *N*-glutaryl-L-alanine analogues having methyl and dimethyl substituents at the 2-position were moderately effective, while *N*-(3*R*-aminoglutaryl)-L-alanine, the substrate having an (*R*)-amino group at the 3-position or *N*-(*N*-methyl-3-azaglutaryl)-L-alanine, the substrate having an *N*-methyl substituent on the 3-azaglutaryl carbon, in construct, exhibited excellent inhibition properties.

Key words γ -glutamylcyclotransferase (GGCT); *N*-glutaryl-L-alanine; inhibitory activity; synthesis

Human chromosome 7 ORF 24 (C7orf24) was discovered as one of the highly expressed proteins in cancers by proteome analysis of bladder cancer.¹⁾ Initially, it was a hypothetical protein with unknown function, but in 2008, C7orf24 was identified as γ -glutamylcyclotransferase (GGCT).^{2,3)} The enzyme GGCT was first purified from pig liver in 1956,⁴⁾ and later it was highly purified from human brain by Meister and colleagues.⁵⁾ These investigators found that GGCT catalyzes the formation of 5-oxoproline and free amino acids from γ -glutamyl dipeptides.^{6,7)} GGCT plays a role in glutathione homeostasis in the γ -glutamyl cycle^{2,3,8,9)} as shown in Chart 1. In recent years, the study of GGCT has progressed and GGCT has attracted much attention in cancer research.^{10–24)}

GGCT is expressed in various cancer cell lines,^{10–18)} although its actual role in cancer progression is still unknown. Gromov *et al.* reported high-level GGCT expression in malignant tumor tissues as compared to normal tissues.¹²⁾ Silencing of GGCT using small interfering RNA (siRNA) inhibited cancer cell proliferation in an *in vitro* study.^{1,13)} Hama *et al.* reported the antitumor effect of anti-GGCT siRNA on lung cancer xenograft mice.¹⁹⁾ He's group has demonstrated that systemic administration of PEGylated hyaluronic acid-modified liposomal at siRNA could suppress breast tumor growth. Importantly, this treatment induced necrosis of tumor tissue with no obvious toxicity to normal tissues.²⁰⁾ If enzymatic activity of GGCT is critical in the regulation of cancer cell growth,

Chart 1. Major Pathway of γ -Glutamyl Cycle

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GGCT would be an attractive target enzyme in cancer therapy.²¹⁾ Nonetheless, little effort has been dedicated to finding an inhibitor of GGCT enzymatic activity.^{22–24)}

Recently, we initiated an investigation for a GGCT inhibitor to discover small molecules that inhibit GGCT activity. In this paper, we report the synthesis of eighteen *N*-acyl-L-alanine analogues and our evaluation of their inhibitory activity against purified GGCT.

Results and Discussion

Design and Synthesis of GGCT Inhibitors Because the structure of an inhibitor of enzymatic activity often resembles the structure of the natural substrate for the enzyme, we selected compounds that are structurally related to γ -glutamyl-L-alanine (**1**) to be candidates for the GGCT inhibitors. *N*-Glutamyl-L-alanine (**2**) is a deaminated analogue of γ -glutamyl-L-alanine. We designed a series of new inhibitors based on the structure of **2** as shown in Chart 2. They may be classified as; i, *N*-glutaryl-, *N*-succinyl-, and *N*-adipyl-L-alanines, **2**, **3**,

Chart 2. Design of GGCT Inhibitor

and **4**, respectively, having carbon chains of different lengths; ii, two mono methyl esters **5**, **6**, and a dimethyl ester **7**; iii, aromatic ring derivatives **8**, **9**, and **10**; iv, derivatives **11** and **12** with methyl substituent at the 2- and 4-positions, and derivatives **13** and **14** with dimethyl substituent at the 2- and 4-positions, and two diastereomeric amino derivatives **15** and **16** at the 3-position; v, 3-oxa-glutaryl derivative **17**, and 3-aza-glutaryl derivatives **18**, and **19**.

Synthesis of *N*-Acyl-L-alanines **2 to **19**** The synthesis is shown in Charts 3, 4, and 5. The synthesis consists of two steps. Dehydrative coupling of mono ester of alkadiolic acid with L-alanine benzyl ester and successive removal of the benzyl ester group by catalytic hydrogenolysis afforded the desired *N*-acyl-L-alanine products. L-Alanine benzyl ester hydrochloride and the corresponding half ester of alkadiolic acid were treated with 1-ethyl-3-(3-dimethylaminopropyl)-

Chart 3. Synthesis of *N*-Acyl-L-alanine Esters

Chart 4. Synthesis of *N*-(3-Aminoglutaryl)-L-alanine Esters Chart 5. Preparation of **2–6** and **8–19** by Catalytic Hydrogenolysis

carbodiimide (EDCI) in the presence of triethylamine and catalytic *N,N*-dimethyl-4-aminopyridine (DMAP) in methylene chloride at room temperature (r.t.) as shown in Chart 3. The coupling products **2'**, **3'**, **4'**, **6'**, **8'**, **9'**, **10'**, **11'**, **12'**, **17'**, **18'**, and **19'** were obtained in 43 to 91% yields. For the synthesis of

Table 1. GGCT Inhibitory Activity for **2–19**

Entry	Compound	Rate of inhibition ^{a)} (%)
1	2	87
2	3	27
3	4	17
4	5	22
5	6	41
6	7	3
7	8	20
8	9	13
9	10	19
10	11	49
11	12	23
12	13	52
13	14	7
14	15	13
15	16	80
16	17	22
17	18	40
18	19	69

^{a)} Concentration of each inhibitor (1 mM).

13' and **14'**, the amide-bond formation was performed by the reaction of L-alanine benzyl ester hydrochloride with 2,2-dimethylglutaric anhydride. The reaction provided a mixture of **13'**, and **14'**. Separation of the mixture by HPLC gave pure **13'** in 52% yield and **14'** in 11% yield. The synthesis of **5'** and **7** were carried out under the same EDCI-mediated coupling conditions with the corresponding acid in 87 and 43% yields, respectively.

Preparations for amino compounds **15'** and **16'** were achieved in two steps i) the coupling of *N*-Boc protecting half esters with L-alanine benzyl ester hydrochloride by the EDCI-mediated conditions and ii) deprotection of the *tert*-Bu group under the acidic conditions as shown in Chart 4. After the coupling, a trifluoroacetic acid (TFA) salt of **15'** was obtained as a crystalline product by the deprotection of *N*-Boc group and *tert*-Bu ester with TFA in 80% yield. Product **16'** was obtained as an oil in 66% yield by the TFA promoted deprotection of the *N*-Boc group and chromatographic purification.

The second step for all benzyl esters **2'** to **19'** is carried out in ethanol under a hydrogen atmosphere in the presence of Pearlman's catalyst (10 mol%) at r.t. The reaction proceeded quite well to afford the desired products in excellent yield (80–98%) except **18** (49%).

Inhibitory Activity for GGCT The activity of GGCT was evaluated by the method originally reported by Board and Hutchinson.²⁵⁾ with slight modifications. We used purified recombinant GGCT proteins from *E. coli*, which was isolated with high purity. The results are listed in Table 1. We tested three amides of dioic acid **2** to **4** with carbon chains of different length. Glutaryl-L-alanine (**2**) showed 87% inhibition of GGCT activity (entry 1). In comparison with **2**, compounds with one less and one additional carbon, *N*-succinyl-L-alanine (**3**) and *N*-adipinyl-L-alanine (**4**), were poorly effective (entries 2, 3). A methyl ester at the alanine unit (**5**) showed 22% inhibition (entry 4) and a methyl ester at the glutaryl unit (**6**) exhibited 41% of activity (entry 5). While, dimethyl ester **7** displayed scarcely any inhibition (entry 6). These results show that carboxylic acid units in the alanine and glutaryl terminals

are important for the inhibition. Ring-containing substrates **8** to **10** were only minimally effective, showing at best only 20% inhibition (entries 7, 8, 9). Compounds with mono and dimethyl groups substituents at the 2-position, **11** (49%) and **13** (52%), showed 2–7 times greater inhibitory activity than those located at the 4-position, **12** (23%) and **14** (7%) in entries 10–13. Crossley *et al.* reported that (*R*)-3-aminoglutaryl compound **16** was twice as effective an inhibitor as (*S*)-3-amino compound **15** in the inhibition of GGCT activity.²⁴⁾ Our current study using purified GGCT, supported this observation, with 80% inhibition for **16** but being 6 times more effective than with 13% inhibition for **15** (entries 14, 15). These results indicated that the stereochemistry of the substituent at the 3-position is important for the activity. *N*-Methyl-3-aza analogue **19** could assume a similar conformation as **16** at the 3-position and showed 69% of inhibition (entry 18). On the other hand, simple 3-oxa and 3-aza analogues **17** and **18** were less effective than **19** (entries 16, 17).

In conclusion, we have prepared eighteen analogues of *N*-acyl-L-alanines and revealed their inhibitory activity against GCCT. Compounds **2**, **16**, and **19** exhibited good inhibition. In particular, the simplest analogue, *N*-glutaryl-L-alanine, was found to have the most potent inhibitory activity against GCCT. The discovery of these new inhibitors will be important for further investigation of GGCT in biochemistry and cancer science.

Experimental

General Melting points were determined on a Yanagimoto micro-melting point apparatus and were not corrected. All reactions were run under an atmosphere of nitrogen. Solvents and reagents were dried prior to use. Et₂O and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl. CH₂Cl₂ was distilled from P₂O₅ and toluene was distilled from CaH₂. ¹H-NMR spectra were recorded on JEOLJNM-ALM-270 (270 MHz) and Agilent Unity Inova XL-400 (400 MHz). Proton chemical shifts were internally referenced to the residual proton resonance in CDCl₃ (δ 7.26) or CD₃OD (δ 3.31). ¹³C-NMR spectra were recorded on Agilent Unity Inova XL-400 (100 MHz). Carbon chemical shifts were internally referenced to the deuterated solvent signals in CDCl₃ (δ 77.00). MS were recorded on JEOL JMC-GC MATE. Electron impact (EI) spectra were performed at 70 eV for low and high resolution mass spectra. Chiral HPLC analyses were performed on a JASCO PU-2080 using UV-2075 detector. Analytical TLC was performed on Merck silica gel 60F₂₅₄ plates. Flash column chromatography was performed with Merck silica gel 60 (40–63 μ m pore size).

Open reading frame (orf) of GGCT was purchased from Integrated DNA Technologies (IA, U.S.A.). pENTR Directional TOPO cloning vector (K 2400-20), *E. coli* Expression System with Gateway Technology (11803-012) and ProBond Purification system (K-850-01) were purchased from Invitrogen (CA, U.S.A.).

Preparation of *N*-Acyl-L-alanines

Typical Procedure for Amide-Bond Formation of Carboxylic Acid with L-Alanine Benzyl Ester To a mixture of the corresponding carboxylic acid (1.2 mmol) and L-alanine benzyl ester hydrochloride (1.0 mmol) in CH₂Cl₂ (4.4 mL), Et₃N (3.0 mmol), DMAP (0.35 mmol) and

1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.44 mmol) were added successively at r.t. The mixture was stirred for several hours to few days at r.t. until the starting material was consumed on TLC. Water was added and the mixture was extracted with ether. The extract was washed with water, brine and dried over MgSO₄. Solvent was removed and the crude product was purified by column chromatography on silica gel eluted with an appropriate ratio of ethyl acetate and hexane. In the case of **10'** and **11'** EtOAc was used for the extraction instead of ether.

N-Glutaryl-L-alanine Dibenzy Ester (**2'**)

White crystals, yield 83%. mp 50–52°C. *R*_f=0.24 (40% EtOAc in hexane). $[\alpha]_D^{17}$ –1.3 (*c*=1.0, CHCl₃). ¹H-NMR (CDCl₃) δ : 7.40–7.32 (10H, m), 6.03 (1H, d, *J*=7.6 Hz, NH), 5.17 (2H, ABq, *J*=12.7 Hz), 5.12 (2H, s), 4.63 (1H, quint, *J*=7.6 Hz), 2.43 (2H, t, *J*=7.3 Hz), 2.26 (2H, t, *J*=7.0 Hz), 2.03–1.92 (2H, m), 1.39 (3H, d, *J*=7.0 Hz). ¹³C-NMR (CDCl₃) δ : 172.94, 172.87, 171.5, 135.9, 135.3, 128.60, 128.55, 128.4, 128.24, 128.21, 128.1, 67.1, 66.2, 48.0, 35.1, 33.1, 20.7, 18.4. EI-MS *m/z*: 383 (*M*⁺). High resolution (HR)-MS *m/z*: 383.1734 (Calcd for C₂₂H₂₅NO₅: 383.1732). *Anal.* Calcd for C₂₂H₂₅NO₅: C, 68.91; H, 6.57; N, 3.65. Found: C, 68.61; H, 6.48; N, 3.65.

N-Succinyl-L-alanine Dibenzy Ester (**3'**)

White crystals, yield 75%. mp 74–75°C. *R*_f=0.59 (50% EtOAc in hexane). $[\alpha]_D^{21}$ –10.1 (*c*=0.1, CHCl₃). ¹H-NMR (CDCl₃) δ : 7.39–7.29 (10H, m), 6.24 (1H, d, *J*=6.8 Hz), 5.17 (2H, ABq, *J*=12.4 Hz), 5.12 (2H, s), 4.63 (1H, m), 2.79–2.64 (2H, m), 2.53 (2H, t, *J*=6.4 Hz), 1.38 (3H, d, *J*=6.8 Hz). ¹³C-NMR (CDCl₃) δ : 172.8, 172.6, 170.8, 135.7, 135.3, 128.6, 128.5, 128.4, 128.20, 128.16, 128.09, 67.1, 66.5, 48.1, 30.7, 29.4, 18.4. EI-MS *m/z*: 369 (*M*⁺). HR-MS *m/z*: 369.1581 (Calcd for C₂₁H₂₃NO₅: 369.1576). *Anal.* Calcd for C₂₁H₂₃NO₅: C, 68.28; H, 6.28; N, 3.79. Found: C, 68.55; H, 6.25; N, 3.85.

N-Adipyl-L-alanine Dibenzy Ester (**4'**)

White crystals, yield 46%. mp 35–36°C. *R*_f=0.40 (50% EtOAc in hexane). $[\alpha]_D^{22}$ –12.1 (*c*=0.1, CHCl₃). ¹H-NMR (CDCl₃) δ : 7.39–7.31 (10H, m), 6.08 (1H, d, *J*=6.4 Hz), 5.17 (2H, ABq, *J*=12.4 Hz), 5.11 (2H, s), 4.64 (1H, m), 2.37 (2H, t, *J*=7.2 Hz), 2.22 (2H, t, *J*=6.4 Hz), 1.69–1.66 (4H, m), 1.41 (3H, d, *J*=7.2 Hz). ¹³C-NMR (CDCl₃) δ : 173.2, 173.0, 172.0, 135.9, 135.3, 128.6, 128.5, 128.4, 128.2 (2C), 128.1, 67.1, 66.2, 48.0, 35.9, 33.8, 24.8, 24.3, 18.4. EI-MS *m/z*: 397 (*M*⁺). HR-MS *m/z*: 397.1894 (Calcd for C₂₃H₂₇NO₅: 397.1889). *Anal.* Calcd for C₂₃H₂₇NO₅: C, 69.50; H, 6.85; N, 3.52. Found: C, 69.49; H, 6.94; N, 3.63.

N-(1-Benzoyloxy-1-oxo-pentanoyl)-L-alanine Methyl Ester (**5'**)

Colorless oil, yield 87%. *R*_f=0.26 (50% EtOAc in hexane). $[\alpha]_D^{21}$ –0.1 (*c*=1.0, CHCl₃). ¹H-NMR (CDCl₃) δ : 7.39–7.30 (5H, m), 6.04 (1H, d, *J*=6.4 Hz), 5.12 (2H, s), 4.59 (1H, m), 3.75 (3H, s), 2.45 (2H, t, *J*=7.3 Hz), 2.27 (2H, t, *J*=7.3 Hz), 2.02–1.95 (2H, m), 1.38 (3H, d, *J*=7.3 Hz). ¹³C-NMR (CDCl₃) δ : 173.5, 173.0, 171.6, 135.9, 128.5, 128.22, 128.19, 66.2, 52.4, 47.9, 35.1, 33.1, 20.7, 18.4. EI-MS *m/z*: 307 (*M*⁺). HR-MS *m/z*: 307.1421 (Calcd for C₁₆H₂₁NO₅: 307.1419).

N-(1-Methoxy-1-oxo-pentanoyl)-L-alanine Benzyl Ester (**6'**)²⁶⁾

Colorless oil, yield 55%. *R*_f=0.33 (50% EtOAc in hexane). $[\alpha]_D^{20}$ –1.5 (*c*=0.1, CHCl₃). ¹H-NMR (CDCl₃) δ : 7.39–7.31 (5H, m), 6.09 (1H, d, *J*=7.3 Hz), 5.18 (2H, ABq, *J*=12.4 Hz), 4.65 (1H, m), 3.67 (3H, s), 2.38 (2H, t, *J*=7.3 Hz), 2.28 (2H,

t, $J=7.3$ Hz), 2.00–1.92 (2H, m), 1.41 (3H, d, $J=7.3$ Hz). ^{13}C -NMR (CDCl_3) δ : 173.6, 172.9, 171.6, 135.3, 128.6, 128.4, 128.1, 67.1, 51.6, 48.0, 35.1, 32.7, 20.7, 18.4. EI-MS m/z : 307 (M^+). HR-MS m/z : 307.1421 (Calcd for $\text{C}_{16}\text{H}_{21}\text{NO}_5$: 307.1419).

N-Glutaryl-L-alanine Dimethyl Ester (7)

White crystals, yield 43%. mp 28–30°C. $R_f=0.20$ (50% EtOAc in hexane). $[\alpha]_{\text{D}}^{20} +1.4$ ($c=0.1$, CHCl_3). ^1H -NMR (CDCl_3) δ : 6.16 (1H, d, $J=6.0$ Hz), 4.60 (1H, m), 3.75 (3H, s), 3.68 (3H, s), 2.40 (2H, t, $J=7.2$ Hz), 2.29 (2H, t, $J=7.2$ Hz), 1.97 (2H, m), 1.41 (3H, d, $J=7.2$ Hz). ^{13}C -NMR (CDCl_3) δ : 173.6, 173.5, 171.6, 52.4, 51.6, 47.9, 35.1, 32.9, 20.7, 18.4. EI-MS m/z : 231 (M^+). HR-MS m/z : 231.1101 (Calcd for $\text{C}_{10}\text{H}_{17}\text{NO}_5$: 231.1107). *Anal.* Calcd for $\text{C}_{10}\text{H}_{17}\text{NO}_5$: C, 51.94; H, 7.41; N, 6.06. Found: C, 51.68; H, 7.32; N, 6.00.

N-(3-Isophthalyl)-L-alanine Dibenzyl Ester (8')

White crystals, yield 91%. mp 105–107°C. $R_f=0.75$ (50% EtOAc in hexane). $[\alpha]_{\text{D}}^{22} +20.4$ ($c=0.1$, CHCl_3). ^1H -NMR (CDCl_3) δ : 8.45 (1H, t, $J=2.4$ Hz), 8.21 (1H, dt, $J=7.6$, 0.8 Hz), 8.03 (1H, dt, $J=7.6$, 1.1 Hz), 7.53 (1H, t, $J=7.6$ Hz), 7.48–7.32 (10H, m), 6.82 (1H, d, $J=7.0$ Hz), 5.40 (2H, s), 5.22 (2H, ABq, $J=12.3$ Hz), 4.85 (1H, m), 1.55 (3H, d, $J=7.0$ Hz). ^{13}C -NMR (CDCl_3) δ : 172.8, 165.9, 165.3, 135.5, 135.1, 133.9, 132.3, 131.6, 130.1, 128.42, 128.36, 128.34, 128.12, 128.10, 128.0 (2C), 127.8, 66.9, 66.7, 48.5, 17.6. EI-MS m/z : 417 (M^+). HR-MS m/z : 417.1579 (Calcd for $\text{C}_{25}\text{H}_{23}\text{NO}_5$: 417.1576). *Anal.* Calcd for $\text{C}_{25}\text{H}_{23}\text{NO}_5$: C, 71.93; H, 5.55; N, 3.36. Found: C, 71.70; H, 5.29; N, 3.42.

N-(6-Benzyloxycarbonylpicolinyl)-L-alanine Benzyl Ester (9')

Colorless oil, yield 46%. $R_f=0.40$ (30% EtOAc in hexane). $[\alpha]_{\text{D}}^{22} +14.3$ ($c=0.1$, CHCl_3). ^1H -NMR (CDCl_3) δ : 8.68 (1H, d, $J=7.8$ Hz), 8.34 (1H, d, $J=7.8$ Hz), 8.21 (1H, d, $J=7.8$ Hz), 7.94 (1H, td, $J=8.0$, 1.8 Hz), 7.49 (2H, d, $J=7.3$ Hz), 7.41–7.30 (8H, m), 5.44 (2H, s), 5.22 (2H, ABq, $J=12.3$ Hz), 4.84 (1H, m), 1.57 (3H, d, $J=7.3$ Hz). ^{13}C -NMR (CDCl_3) δ : 172.2, 164.0, 163.0, 149.5, 146.5, 138.4, 135.3, 128.52, 128.45, 128.37, 128.28, 128.21, 128.04, 127.95, 127.3, 125.3, 67.3, 66.9, 48.3, 18.0. CI-MS m/z : 419 ($\text{M}+\text{H}^+$). HR-MS m/z : 419.1611 (Calcd for $\text{C}_{24}\text{H}_{23}\text{N}_2\text{O}_5$: 419.1607).

N-{2-(3-Hydroxyphenyl)acetyl}-L-alanine Benzyl Ester (10')

Colorless oil, yield 95%. $R_f=0.31$ (50% EtOAc in hexane). $[\alpha]_{\text{D}}^{20} -22.6$ ($c=0.1$, CHCl_3). ^1H -NMR (CDCl_3) δ : 7.36–7.28 (5H, m), 7.15 (1H, t, $J=7.8$ Hz), 6.76–6.72 (3H, m), 6.30 (1H, t, $J=6.4$ Hz), 5.11 (2H, ABq, $J=12.3$ Hz), 4.61 (1H, m), 3.51 (2H, s), 1.35 (3H, d, $J=6.9$ Hz). ^{13}C -NMR (CDCl_3) δ : 172.8, 171.5, 157.0, 135.6, 135.2, 130.2, 128.7, 128.5, 128.2, 121.0, 116.5, 114.8, 67.4, 48.5, 43.4, 18.2. EI-MS m/z : 313 (M^+). HR-MS m/z : 313.1316 (Calcd for $\text{C}_{18}\text{H}_{19}\text{NO}_4$: 313.1314).

Preparation of *N*-(4-Methylglutaryl)-L-alanine Dibenzyl Ester (11') and *N*-(2-Methylglutaryl)-L-alanine Dibenzyl Ester (12')

11' (Diastereomeric mixtures). White crystals, yield 42%. mp 68–69°C. $R_f=0.67$ (50% EtOAc in hexane). ^1H -NMR (CDCl_3) δ : 7.39–7.30 (10H, m), 6.09 (1H, d, $J=7.3$ Hz), 5.15 (2H, ABq, $J=12.3$ Hz), 5.11 (2H, s), 4.61 (1H, m), 2.47–2.26 (3H, m), 1.94 (1H, m), 1.73 (1H, m), 1.38 (3H, d, $J=7.3$ Hz), 1.13 (3H, d, $J=6.9$ Hz). ^{13}C -NMR (CDCl_3) δ : 175.4, 173.5, 173.1, 136.1, 135.5, 128.80, 128.77, 128.6, 128.5, 128.4, 128.3, 67.3, 66.5, 48.1, 40.0, 31.9, 29.5, 18.4, 17.8. EI-MS m/z : 397 (M^+). HR-MS m/z : 397.1893 (Calcd for $\text{C}_{23}\text{H}_{27}\text{NO}_5$: 397.1889). *Anal.* Calcd for $\text{C}_{23}\text{H}_{27}\text{NO}_5$: C, 69.50; H, 6.85; N, 3.52. Found:

C, 69.68; H, 6.69; N, 3.53.

12' (Diastereomeric mixtures). White crystals, yield 40%. mp 80–81°C. $R_f=0.72$ (50% EtOAc in hexane). ^1H -NMR (CDCl_3) δ : 7.41–7.30 (10H, m), 6.03 (1H, d, $J=7.8$ Hz), 5.17 (2H, ABq, $J=11.9$ Hz), 5.11 (2H, s), 4.62 (1H, m), 2.44–2.25 (3H, m), 1.96 (1H, m), 1.75 (1H, m), 1.38 (3H, d, $J=7.3$ Hz), 1.13 (3H, d, $J=6.8$ Hz). ^{13}C -NMR (CDCl_3) δ : 175.7, 173.8, 173.6, 136.5, 136.0, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 67.8, 67.0, 48.6, 40.7, 32.5, 29.8, 19.1, 18.2. EI-MS m/z : 397 (M^+). HR-MS m/z : 397.1886 (Calcd for $\text{C}_{23}\text{H}_{27}\text{NO}_5$: 397.1889). *Anal.* Calcd for $\text{C}_{23}\text{H}_{27}\text{NO}_5$: C, 69.50; H, 6.85; N, 3.52. Found: C, 69.46; H, 6.74; N, 3.52.

N-2-{2-(Benzyloxy)-2-oxoethoxy}acetyl-L-alanine Benzyl Ester (17')

Colorless oil, yield 54%. $R_f=0.54$ (50% EtOAc in hexane). $[\alpha]_{\text{D}}^{25} -30.4$ ($c=0.1$, CHCl_3). ^1H -NMR (CDCl_3) δ : 7.54–7.31 (10H, m), 5.19 (2H, s), 5.17 (2H, ABq, $J=11.9$ Hz), 4.68 (1H, m), 4.20 (2H, ABq, $J=16.4$ Hz), 4.10 (2H, ABq, $J=14.7$ Hz), 1.44 (3H, $J=7.3$ Hz). ^{13}C -NMR (CDCl_3) δ : 172.4, 169.6, 168.6, 135.4, 135.1, 128.71, 128.65, 128.63, 128.5, 128.4, 128.1, 71.1, 68.7, 67.1, 67.0, 47.6, 18.1. EI-MS m/z : 385 (M^+). HR-MS m/z : 385.1527 (Calcd for $\text{C}_{21}\text{H}_{23}\text{NO}_6$: 385.1525).

N-{*N*-Benzyloxycarbonyl-*N*-(benzyloxycarbonylmethyl)-glycinyll}-L-alanine Benzyl Ester (18')

Colorless oil, yield 43%. $R_f=0.53$ (50% EtOAc in hexane). $[\alpha]_{\text{D}}^{25} -17.4$ ($c=0.1$, CHCl_3). A 4:3 mixture of rotamers. ^1H -NMR (CDCl_3) δ : 7.99 (4/7H, d, $J=7.3$ Hz, NH), 7.61 (3/7H, d, $J=7.3$ Hz, NH), 7.32–7.15 (15H, m), 5.15–5.01 (2H, m), 5.12 (2H, ABq, $J=11.9$ Hz), 5.04 (2H, s), 5.09–5.02 (2H, m), 4.53 (3/7H, quint, $J=7.3$ Hz), 4.47 (4/7H, quint, $J=7.3$ Hz), 4.16 (3/7H, $J=16.9$ Hz), 4.07 (4/7H, $J=17.9$ Hz), 4.04 (3/7H, $J=17.9$ Hz), 4.01 (4/7H, $J=17.4$ Hz), 3.95 (4/7H, $J=17.9$ Hz), 3.86 (3/7H, $J=17.9$ Hz), 3.83 (1H, $J=17.9$ Hz), 1.34 (9/7H, d, $J=7.3$ Hz), 1.27 (12/7H, d, $J=7.3$ Hz). ^{13}C -NMR (CDCl_3) δ : 172.5 (3/7C), 172.3 (4/7C), 170.9 (4/7C), 170.4 (3/7C), 168.7 (4/7C), 168.5 (3/7C), 155.9 (1C), 135.82 (4/7C), 135.75 (3/7C), 135.6 (4/7C), 135.5 (3/7C), 134.91 (3/7C), 134.88 (4/7C), 128.71, 128.68, 128.64, 128.57, 128.51, 128.4, 128.3, 128.11, 128.05, 127.98, 68.3 (3/7C), 68.2 (4/7C), 67.8 (4/7C), 67.5 (3/7C), 67.0 (3/7C), 66.96 (4/7C), 53.5 (4/7C), 53.1 (3/7C), 51.7 (4/7C), 50.7 (3/7C), 48.3 (3/7C), 48.2 (4/7C), 17.6 (3/7C), 17.5 (4/7C). FAB-MS m/z : 519 ($\text{M}+\text{H}^+$). HR-MS m/z : 519.2135 (Calcd for $\text{C}_{29}\text{H}_{31}\text{N}_2\text{O}_7$: 519.2131).

N-{*N*-(Benzyloxycarbonylmethyl)-*N*-methylglycinyll}-L-alanine Benzyl Ester (19')

White crystals, yield 52%. mp 76–78°C. $R_f=0.33$ (50% EtOAc in hexane). $[\alpha]_{\text{D}}^{25} -38.5$ ($c=0.1$, CHCl_3). ^1H -NMR (CDCl_3) δ : 7.76 (1H, d, $J=7.8$ Hz), 7.42–7.30 (10H, m), 5.16 (2H, ABq, $J=12.3$ Hz), 5.15 (2H, s), 4.66 (1H, m), 3.38 (2H, ABq, $J=17.4$ Hz), 3.22 (2H, ABq, $J=16.5$ Hz), 2.43 (3H, s), 1.40 (3H, d, $J=6.9$ Hz). ^{13}C -NMR (CDCl_3) δ : 172.6, 170.5, 170.3, 135.48, 135.46, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 67.0, 66.6, 60.4, 58.5, 47.6, 43.0, 18.1. EI-MS m/z : 398 (M^+). HR-MS m/z : 398.1846 (Calcd for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_5$: 398.1842). *Anal.* Calcd for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_5$: C, 66.32; H, 6.58; N, 7.03. Found: C, 66.18; H, 6.39; N, 7.10.

Preparation of *N*-(4,4-Dimethylglutaryl)-L-alanine Benzyl Ester (13') and *N*-(2,2-Dimethylglutaryl)-L-alanine Benzyl Ester (14') A mixture of L-alanine benzyl ester hydrochloride (474 mg, 2.2 mmol), 3,3-dimethylglutaric anhydride (375 mg, 2.64 mmol), DMAP (54 mg, 0.44 mmol) and

triethylamine (0.9 mL, 2.64 mmol) in CH_2Cl_2 (7.5 mL) was stirred at r.t. overnight. The mixture was quenched with water, acidified with 1 M HCl and extracted with EtOAc several times. The combined extract was washed with brine and dried over MgSO_4 . The crude product was purified by reverse phase HPLC (C-18) eluted with 60% methanol in water to give **13'** (365 mg) in 52% yield and **14'** (76 mg) in 11% yield.

13': White crystals, mp 64–66°C. $R_f=0.26$ (50% EtOAc in hexane). $[\alpha]_D^{24} -7.7$ ($c=0.5$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 7.32–7.25 (5H, m), 6.35 (1H, d, $J=7.3$ Hz), 5.11 (2H, ABq, $J=11.9$ Hz), 4.58 (1H, m), 2.20–2.16 (2H, m), 1.85–1.80 (2H, m), 1.34 (3H, d, $J=7.3$ Hz), 1.14 (6H, s). $^{13}\text{C-NMR}$ (CDCl_3) δ : 181.9, 174.0, 173.3, 135.2, 128.6, 128.4, 128.1, 67.2, 48.1, 41.6, 35.7, 32.1, 25.0, 18.1. FAB-MS m/z : 322 ($\text{M}+\text{H}^+$). HR-MS m/z : 322.1650 (Calcd for $\text{C}_{17}\text{H}_{24}\text{NO}_5$: 322.1654). *Anal.* Calcd for $\text{C}_{17}\text{H}_{24}\text{NO}_5$: C, 63.54; H, 7.21; N, 4.36. Found: C, 63.52; H, 7.44; N, 4.57.

14': Colorless oil. $R_f=0.17$ (50% EtOAc in hexane). $[\alpha]_D^{20} -31.6$ ($c=0.1$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 7.37–7.32 (5H, m), 6.20 (1H, d, $J=6.9$ Hz), 5.17 (2H, ABq, $J=12.3$ Hz), 4.59 (1H, m), 2.35–2.31 (2H, m), 1.89–1.85 (2H, m), 1.41 (3H, d, $J=6.9$ Hz), 1.20 (6H, s). $^{13}\text{C-NMR}$ (CDCl_3) δ : 179.0, 177.3, 173.8, 135.9, 129.3, 129.2, 128.9, 67.9, 48.9, 42.2, 36.0, 30.4, 25.9, 18.9; FAB-MS m/z : 322 ($\text{M}+\text{H}^+$); HR-MS m/z : 322.1658 (Calcd for $\text{C}_{17}\text{H}_{24}\text{NO}_5$: 322.1654).

TFA Salt of (S)-N-(3-Aminoglutaryl)-L-alanine Benzyl Ester (15') After the typical procedure of amino-bond formation for (S)-tert-butyl 3-(N-tert-butoxycarbonyl)-aminoglutaric acid with L-alanine benzyl ester, the crude product was dissolved in CH_2Cl_2 (7 mL) and reacted with TFA (3 mL) for 10 h at r.t. After the condensation solid residue was crystallized by EtOAc. White crystals, yield 95%. mp 144–146°C. $R_f=0.05$ (20% MeOH in CHCl_3). $[\alpha]_D^{22} -51.2$ ($c=0.1$, CH_3OH). $^1\text{H-NMR}$ (CD_3OD) δ : 7.37–7.32 (5H, m), 5.17 (1H, ABq, $J=11.9$ Hz), 4.47 (1H, q, $J=7.3$ Hz), 3.85 (1H, m), 2.78–2.60 (4H, m), 1.39 (3H, d, $J=7.3$ Hz). $^{13}\text{C-NMR}$ (CD_3OD) δ : 174.0, 173.3, 171.4, 163.0 (q, $J_{\text{C-F}}=34$ Hz), 137.2, 129.6, 129.4, 129.2, 118.2 (q, $J_{\text{C-F}}=292$ Hz), 68.0, 48.7, 46.8, 37.2, 36.6, 17.1. FAB-MS m/z : 309 ($\text{M}+\text{H}^+$). HR-MS m/z : 309.1452 (Calcd for $\text{C}_{15}\text{H}_{21}\text{N}_2\text{O}_5$: 309.1450). *Anal.* Calcd for $\text{C}_{15}\text{H}_{21}\text{F}_3\text{N}_2\text{O}_7$: C, 48.34; H, 5.01; N, 6.63. Found: C, 48.32; H, 4.96; N, 6.57.

Preparation of (R)-N-(3-Aminoglutaryl)-L-alanine Dibenzyl Ester (16') After the typical procedure of amino-bond formation for (R)-benzyl 3-(N-tert-butoxycarbonyl)aminoglutaric acid with L-alanine benzyl ester, the crude product was dissolved in CH_2Cl_2 (7 mL) and reacted with TFA (3 mL) for 10 h at r.t. Solvent was removed and the crude product was purified by column chromatography on silica gel eluted with 20% MeOH in CHCl_3 . Colorless oil, yield 71%. $R_f=0.69$ (17% MeOH in CHCl_3). $[\alpha]_D^{15} -27.3$ ($c=0.8$, CH_3OH). $^1\text{H-NMR}$ (CDCl_3) δ : 7.41–7.33 (10H, m), 5.23 (2H, ABq, $J=11.9$ Hz), 5.14 (2H, ABq, $J=12.3$ Hz), 4.54 (1H, m), 3.92 (1H, tt, $J=11.9$, 5.5 Hz), 2.99–2.92 (2H, m), 2.76 (1H, dd, $J=17.9$, 2.3 Hz), 2.41 (1H, dd, $J=13.3$, 3.2 Hz), 1.49 (3H, d, $J=7.3$ Hz). $^{13}\text{C-NMR}$ (CD_3OD) δ : 174.0, 171.44, 171.42, 137.2, 137.0, 129.61, 129.58, 129.45, 129.42, 129.36, 129.2, 68.1, 68.0, 49.6, 46.7, 37.1, 36.9, 17.1. FAB-MS m/z : 399 ($\text{M}+\text{H}^+$). HR-MS m/z : 399.1915 (Calcd for $\text{C}_{22}\text{H}_{27}\text{N}_2\text{O}_5$: 399.1920).

Typical Procedure for Deprotection of Benzyl Ester to Carboxylic Acid A mixture of benzyl ester (0.5 mmol) in ethanol (2.8 mL) and Pearlman's catalyst (14 mg, 20% on

carbon) was stirred under a hydrogen atmosphere. After the reaction was completed by being monitored on TLC, catalyst was filtered off and solvent was removed to give the desired carboxylic acid product.

N-Glutaryl-L-alanine (**2**)²⁷⁾

White crystals, yield 86%. mp 104–105°C. $R_f=0.20$ (17% MeOH in CHCl_3). $[\alpha]_D^{18} -6.3$ ($c=1.0$, CH_3OH). $^1\text{H-NMR}$ (CD_3OD) δ : 4.32 (1H, q, $J=7.8$ Hz), 2.33–2.21 (4H, m), 1.90–1.79 (2H, m), 1.33 (3H, d, $J=8.1$ Hz). $^{13}\text{C-NMR}$ (CD_3OD) δ : 176.0, 175.2, 174.4, 34.9, 33.1, 21.3, 16.7. EI-MS m/z : 203 (M^+). HR-MS m/z : 203.0800 (Calcd for $\text{C}_8\text{H}_{13}\text{NO}_5$: 203.0793). *Anal.* Calcd for $\text{C}_8\text{H}_{13}\text{NO}_5$: C, 47.29; H, 6.45; N, 6.89. Found: C, 47.22; H, 6.48; N, 6.80.

N-Succinyl-L-alanine (**3**)²⁸⁾

White crystals, yield 97%. mp 140–141°C. $R_f=0.14$ (17% MeOH in CHCl_3). $[\alpha]_D^{22} -24.1$ ($c=0.1$, CH_3OH). $^1\text{H-NMR}$ (CD_3OD) δ : 4.28 (1H, q, $J=7.6$ Hz), 2.56–2.38 (4H, m), 1.33 (3H, d, $J=7.2$ Hz); $^{13}\text{C-NMR}$ (CD_3OD) δ : 176.3 (2C), 174.3, 48.4, 31.3, 30.2, 17.7. Chemical ionization (CI)-MS m/z : 190 ($\text{M}+\text{H}^+$). HR-MS m/z : 190.0721 (Calcd for $\text{C}_7\text{H}_{12}\text{NO}_5$: 190.0715). *Anal.* Calcd for $\text{C}_7\text{H}_{11}\text{NO}_5$: C, 44.45; H, 5.86; N, 7.40. Found: C, 44.14; H, 5.78; N, 7.26.

N-Adipinyl-L-alanine (**4**)

White crystals, yield 86%. mp 110–112°C. $R_f=0.05$ (17% MeOH in CHCl_3). $[\alpha]_D^{22} -32.0$ ($c=0.1$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 4.31 (1H, q, $J=7.2$ Hz), 2.27–2.24 (2H, m), 2.22–2.17 (2H, m), 1.59 (4H, t, $J=3.2$ Hz), 1.32 (3H, d, $J=7.2$ Hz). $^{13}\text{C-NMR}$ (CD_3OD) δ : 177.3, 176.2, 175.6, 49.3, 36.3, 34.6, 26.3, 25.5, 17.7. CI-MS m/z : 218 ($\text{M}+\text{H}^+$). HR-MS m/z : 218.1024 (Calcd for $\text{C}_9\text{H}_{16}\text{NO}_5$: 218.1028). *Anal.* Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5$: C, 49.76; H, 6.96; N, 6.45. Found: C, 49.53; H, 6.91; N, 6.33.

N-Glutaryl-L-alanine Methyl Ester (**5**)²⁷⁾

Colorless oil, yield 88%. $R_f=0.24$ (17% MeOH in CHCl_3). $[\alpha]_D^{22} -1.99$ ($c=1.0$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 6.22 (1H, d, $J=7.3$ Hz), 4.61 (1H, quint, $J=7.8$ Hz), 3.76 (3H, s), 2.45 (2H, t, $J=7.6$ Hz), 2.33 (2H, t, $J=7.0$ Hz), 1.98 (2H, m), 1.41 (3H, d, $J=7.8$ Hz). $^{13}\text{C-NMR}$ (CDCl_3) δ : 177.5, 173.9, 172.3, 52.5, 48.0, 34.9, 32.8, 20.5, 18.2. EI-MS m/z : 217 (M^+). HR-MS m/z : 217.0958 (Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5$: 217.0950). *Anal.* Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5$: C, 49.76; H, 6.96; N, 6.45. Found: C, 49.76; H, 6.71; N, 6.41.

N-(5-Methoxy-5-oxopentanyl)-L-alanine (**6**)²⁶⁾

Colorless oil, yield 98%. $R_f=0.28$ (17% MeOH in CHCl_3). $[\alpha]_D^{22} -6.9$ ($c=0.1$, MeOH). $^1\text{H-NMR}$ (CDCl_3) δ : 6.16 (1H, d, $J=7.3$ Hz), 4.61 (1H, quint, $J=7.3$ Hz), 3.77 (3H, s), 2.45 (2H, t, $J=7.3$ Hz), 2.34 (2H, t, $J=7.0$ Hz), 2.00 (2H, m), 1.41 (3H, d, $J=7.3$ Hz). $^{13}\text{C-NMR}$ (CDCl_3) δ : 175.8, 173.9, 173.0, 51.7, 48.1, 35.0, 20.7, 17.9. EI-MS m/z : 217 (M^+). HR-MS m/z : 217.0947 (Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5$: 217.0950). *Anal.* Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5$: C, 49.76; H, 6.96; N, 6.45. Found: C, 49.49; H, 6.72; N, 6.21.

N-Isophthaloyl-L-alanine (**8**)

White crystals, yield 95%. mp 239–241°C. $R_f=0.10$ (50% EtOAc in hexane). $[\alpha]_D^{20} -0.4$ ($c=0.1$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 8.56 (1H, t, $J=1.8$ Hz), 8.22 (1H, dt, $J=7.9$, 1.8 Hz), 8.11 (1H, dt, $J=7.9$, 1.8 Hz), 7.62 (1H, t, $J=7.9$ Hz), 4.66 (1H, q, $J=7.1$ Hz), 1.57 (3H, d, $J=7.4$ Hz). $^{13}\text{C-NMR}$ (CD_3OD) δ : 176.1, 169.2, 168.9, 135.8, 133.7, 132.8, 132.4, 129.8, 129.7, 50.2, 17.4. EI-MS m/z : 237 (M^+). HR-MS m/z : 237.0640 (Calcd for $\text{C}_{11}\text{H}_{11}\text{NO}_5$: 237.0637). *Anal.* Calcd for $\text{C}_{11}\text{H}_{11}\text{NO}_5$: C, 55.70; H, 4.67; N, 5.90. Found: C, 55.48; H, 4.42; N, 5.77.

***N*-2-(6-Hydroxycarbonylpicolinoyl)-L-alanine (9)²⁹**

White crystals, yield 96%. mp 232–234°C (lit. 232°C). $R_f=0.05$ (17% MeOH in CHCl_3). $[\alpha]_D^{22} +11.6$ ($c=0.1$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 8.23 (2H, d, $J=7.8\text{ Hz}$), 8.08 (1H, t, $J=7.6\text{ Hz}$), 4.54 (1H, q, $J=7.3\text{ Hz}$), 1.50 (3H, d, $J=7.3\text{ Hz}$). $^{13}\text{C-NMR}$ (CD_3OD) δ : 175.8, 167.6, 165.5, 150.9, 148.1, 140.5, 128.6, 126.7, 49.7, 17.4. CI-MS m/z : 239 ($\text{M}+\text{H}^+$). HR-MS m/z : 239.0674 (Calcd for $\text{C}_{10}\text{H}_{11}\text{N}_2\text{O}_5$: 239.0668). Anal. Calcd for $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_5$: C, 50.42; H, 4.23; N, 11.76. Found: C, 50.04; H, 4.03; N, 11.53.

***N*-(3-Hydroxyphenyl)acetyl-L-alanine (10)**

Colorless oil, yield 96%. $R_f=0.07$ (50% EtOAc in hexane). $[\alpha]_D^{25} -73.4$ ($c=0.05$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 7.11 (1H, t, $J=7.8\text{ Hz}$), 6.76 (2H, d, $J=7.8\text{ Hz}$), 6.66 (1H, d, $J=8.7\text{ Hz}$), 4.38 (1H, q, $J=7.3\text{ Hz}$), 3.48 (2H, s), 1.39 (3H, d, $J=7.3\text{ Hz}$). $^{13}\text{C-NMR}$ (CD_3OD) δ : 176.0, 173.8, 158.6, 138.0, 130.5, 121.3, 117.0, 114.8, 49.5, 43.4, 17.7. EI-MS m/z : 223 (M^+). HR-MS m/z : 223.0847 (Calcd for $\text{C}_{11}\text{H}_{13}\text{NO}_4$: 223.0845).

***N*-4-Methylglutaryl-L-alanine (11)**

Colorless oil, yield 90%. $R_f=0.08$ (17% MeOH in CHCl_3). $^1\text{H-NMR}$ (CD_3OD) δ : 4.33 (1H, q, $J=7.3\text{ Hz}$), 2.47–2.27 (3H, m), 1.86 (1H, m), 1.68 (1H, m), 1.38 (3H, d, $J=7.3\text{ Hz}$), 1.11 (3H, d, $J=6.9\text{ Hz}$). $^{13}\text{C-NMR}$ (CD_3OD) δ : 178.7, 177.2, 176.2, 49.0, 40.9, 32.6, 30.5, 18.2, 17.5. EI-MS m/z : 217 (M^+). HR-MS m/z : 217.0953 (Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5$: 217.0950).

***N*-2-Methylglutaryl-L-alanine (12)**

Colorless oil, yield 93%. $R_f=0.06$ (17% MeOH in CHCl_3). $^1\text{H-NMR}$ (CD_3OD) δ : 4.36 (1H, q, $J=7.3\text{ Hz}$), 2.41 (1H, m), 2.32–2.23 (2H, m), 1.86 (1H, m), 1.68 (1H, m), 1.38 (3H, d, $J=7.3\text{ Hz}$), 1.13 (3H, d, $J=6.9\text{ Hz}$). $^{13}\text{C-NMR}$ (CD_3OD) δ : 178.5, 177.0, 176.2, 49.2, 41.0, 32.7, 30.2, 18.1, 17.7. EI-MS m/z : 217 (M^+). HR-MS m/z : 217.0953 (Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5$: 217.0950).

***N*-4,4-Dimethylglutaryl-L-alanine (13)**

Colorless oil, yield 92%. $R_f=0.04$ (17% MeOH in CHCl_3). $[\alpha]_D^{20} -37.2$ ($c=0.1$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 4.34 (1H, q, $J=7.3\text{ Hz}$), 2.25–2.21 (2H, m), 1.85–1.81 (2H, m), 1.37 (3H, d, $J=7.3\text{ Hz}$), 1.18 (6H, s). $^{13}\text{C-NMR}$ (CD_3OD) δ : 181.2, 176.3, 175.6, 42.6, 37.1, 32.6, 25.5, 25.4, 17.6. EI-MS m/z : 231 (M^+). HR-MS m/z : 231.1102 (Calcd for $\text{C}_{10}\text{H}_{17}\text{NO}_5$: 231.1107).

***N*-2,2-Dimethylglutaryl-L-alanine (14)**

Colorless oil, yield 80%. $R_f=0.07$ (17% MeOH in CHCl_3). $[\alpha]_D^{20} -40.5$ ($c=0.1$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 4.36 (1H, q, $J=7.3\text{ Hz}$), 2.34–2.20 (2H, m), 1.90–1.78 (2H, m), 1.39 (3H, d, $J=7.3\text{ Hz}$), 1.19 (3H, s), 1.18 (3H, s). $^{13}\text{C-NMR}$ (CD_3OD) δ : 179.6, 177.4, 176.4, 49.4, 42.5, 36.9, 30.7, 25.5, 25.4, 17.3. EI-MS m/z : 231 (M^+). HR-MS m/z : 231.1105 (Calcd for $\text{C}_{10}\text{H}_{17}\text{NO}_5$: 231.1102).

***N*-(S)-(3-Amino)glutaryl-L-alanine (15)²⁴**

White crystals, yield 84%. mp 228–229°C (lit. 217–220°C). $R_f=0.05$ (50% MeOH in CHCl_3). $[\alpha]_D^{17} -22.6$ ($c=0.5$, H_2O). $^1\text{H-NMR}$ (D_2O) δ : 4.25 (1H, q, $J=7.3\text{ Hz}$), 3.93 (1H, m), 2.80–2.63 (4H, m), 1.36 (3H, d, $J=7.3\text{ Hz}$). $^{13}\text{C-NMR}$ (CD_3OD) δ : 176.0, 174.0, 171.5, 48.8, 47.4, 37.4, 37.3, 17.5. FAB-MS m/z : 219 ($\text{M}+\text{H}^+$). HR-MS m/z : 219.0978 (Calcd for $\text{C}_8\text{H}_{15}\text{N}_2\text{O}_5$: 219.0981).

***N*-(R)-(3-Amino)glutaryl-L-alanine (16)²⁴**

White crystals, yield 82%. mp 248–250°C (lit. 232–234°C). $R_f=0.1$ (50% MeOH in CHCl_3). $[\alpha]_D^{17} -24.1$ ($c=0.3$, H_2O). $^1\text{H-NMR}$ (D_2O) δ : 4.25 (1H, q, $J=7.2\text{ Hz}$), 3.96–3.90 (1H, m), 2.73–2.63 (4H, m), 1.37 (3H, d, $J=7.2\text{ Hz}$). $^{13}\text{C-NMR}$ (CD_3OD) δ : 179.2, 175.9, 171.5, 51.0, 46.4, 37.9, 37.3, 17.1. FAB-MS m/z :

219 ($\text{M}+\text{H}^+$). HR-MS m/z : 219.0977 (Calcd for $\text{C}_8\text{H}_{15}\text{N}_2\text{O}_5$: 219.0981).

***N*-(Hydroxycarboxymethoxy)acetyl-L-alanine (17)**

Colorless oil, yield 89%. $R_f=0.08$ (17% MeOH in CHCl_3). $[\alpha]_D^{24} -11.1$ ($c=0.5$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 4.46 (1H, q, $J=7.3\text{ Hz}$), 4.21 (2H, d, $J=2.3\text{ Hz}$), 4.10 (2H, s), 1.44 (3H, d, $J=6.9\text{ Hz}$). $^{13}\text{C-NMR}$ (CD_3OD) δ : 175.9, 174.1, 172.1, 71.5, 69.5, 17.9, 14.6. FAB-MS m/z : 206 ($\text{M}+\text{H}^+$). HR-MS m/z : 206.0670 (Calcd for $\text{C}_7\text{H}_{12}\text{NO}_6$: 206.0665).

***N*-(Hydroxycarboxymethylamino)acetyl-L-alanine (18)**

White crystals, yield 49%. mp 168–169°C. $R_f=0.09$ (MeOH). $[\alpha]_D^{24} -69.5$ ($c=0.5$, H_2O). $^1\text{H-NMR}$ (D_2O) δ : 4.43 (1H, q, $J=7.3\text{ Hz}$), 4.04 (2H, s), 3.80 (2H, s), 1.48 (3H, d, $J=7.3\text{ Hz}$). $^{13}\text{C-NMR}$ (D_2O) δ : 177.8, 171.3, 166.5, 49.9, 49.4, 48.4, 17.1. FAB-MS m/z : 205 ($\text{M}+\text{H}^+$). HR-MS m/z : 205.0826 (Calcd for $\text{C}_7\text{H}_{13}\text{N}_2\text{O}_5$: 205.0824). Anal. Calcd for $\text{C}_7\text{H}_{12}\text{N}_2\text{O}_5$: C, 41.18; H, 5.92; N, 13.72. Found: C, 40.93; H, 5.80; N, 13.47.

***N*-{(Hydroxycarboxymethyl)(methyl)amino}acetyl-L-alanine (19)**

Colorless oil, yield 82%. $R_f=0.10$ (MeOH). $[\alpha]_D^{24} -20.7$ ($c=0.84$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 4.39 (1H, q, $J=7.3\text{ Hz}$), 3.87 (2H, s), 3.65 (2H, s), 2.84 (3H, s), 1.39 (3H, d, $J=7.3\text{ Hz}$). $^{13}\text{C-NMR}$ (CD_3OD) δ : 175.8, 170.5, 167.1, 79.5, 59.5, 58.4, 43.1, 17.7. FAB-MS m/z : 219 ($\text{M}+\text{H}^+$). HR-MS m/z : 219.0977 (Calcd for $\text{C}_8\text{H}_{15}\text{N}_2\text{O}_5$: 219.0981).

Evaluation of Inhibition Rate of *N*-Acyl-L-alanines 2–19 for GGCT

Expression of Recombinant GGCT Human GGCT orf was amplified by PCR from the pIDTSMART-GGCT plasmid and cloned into the pENTR Directional TOPO cloning vector. After creating an entry clone of GGCT, GGCT cloned into expression plasmid of GGCT, pDEST-17 by Gateway technology LR recombination according to the manufacturer's instructions. GGCT orf containing pDEST-17 was transfected into *Escherichia coli* BL21 (DE3) and then expressed as the recombinant protein of GGCT. The GGCT protein was purified by ProBond Purification system and its purity was determined by Coomassie Brilliant Blue (CBB) staining of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and Western blotting.

GGCT Enzymatic Activity The assay for GGCT activity was performed by the method reported by Board and Hutchinson.²⁵ A solution of γ -glutamyl-L-cystein was prepared by mixing of 1M Tris-HCl pH 8.0 (5 μL), 2mM γ -glutamyl-L-cystein solution (5 μL) and water (25 μL). To this solution were added a 10mM solution of each inhibitor in water (5 μL) and GGCT recombinant protein (0.0314 mg/mL) in 10 μL of 100mM Tris-HCl solution (pH 8.0). The whole mixture (total 50 μL) was incubated at 37°C in a heat block for 15 min. After the addition of sulfosalicylic acid dihydrate (25 μL of 200mM solution), the remaining GGCT in the mixture was inactivated by the incubation for 15 min on ice. Naphthalene-2,3-dicarbaldehyde (NDA) solution was prepared freshly by mixing 1mL of DMSO solution of naphthalene-2,3-dicarbaldehyde (10mmol), 7mL of 50mM Tris-HCl solution (pH 10.0) and 2mL of 0.5M NaOH solution. The incubated sample solution (20 μL) was added to 180 μL of NDA solution to form a sample for measurement of fluorescence. The sample was subjected to a fluorescence plate reader (λ_{ex} : 427nm, λ_{em} : 528nm). The activity was cal-

culated by the following equation; [(fluorescence of blank—fluorescence without inhibitor)—(fluorescence of blank—fluorescence in the presence of inhibitor)/(fluorescence of blank—fluorescence without inhibitor)] $\times$ 100. Fluorescence of blank represents the initial fluorescence of added substrate.

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Conflict of Interest The authors declare no conflict of interest.

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