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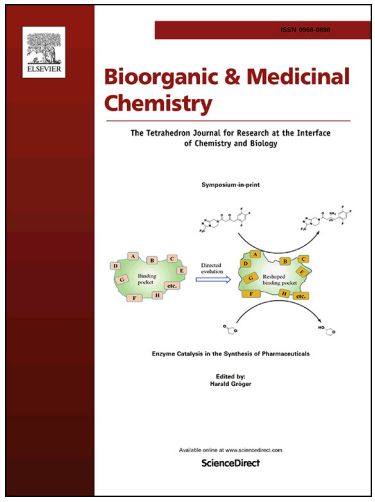

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Tatsuhiko Tsunoda, Mio Takase, Hideyuki Shigemori

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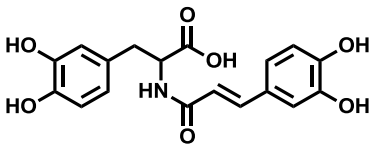
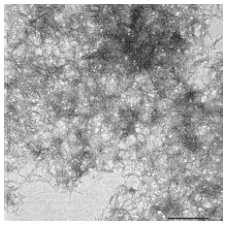


Clovamide



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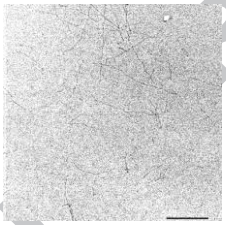

Aβ42+ Clovamide

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Structure–Activity Relationship of Clovamide and Its Related Compounds for the Inhibition of Amyloid β Aggregation

Tatsuhiko Tsunoda,[a] Mio Takase,[a] and Hideyuki Shigemori[b,*]

[a]*Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan*
[b]*Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan*

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ABSTRACT

Alzheimer's disease (AD), a neurodegenerative disorder, is characterized by aggregation of amyloid β-protein (Aβ). Aβ aggregates through β-sheet formation and induces cytotoxicity against neuronal cells. Inhibition of Aβ aggregation by naturally occurring compounds is thus a promising strategy for the treatment of AD. We have already reported that caffeoylquinic acids and phenylethanoid glycosides, which possess two or more catechol moieties, strongly inhibited Aβ aggregation. Clovamide (**1**) containing two catechol moieties, isolated from cacao beans (*Theobroma cacao* L.), is believed to exhibit preventive effects on Aβ aggregation. To investigate the structure-activity relationship of clovamide (**1**) for the inhibition of Aβ aggregation, we synthesized **1** and related compounds **2–11** through reaction between L-DOPA, D-DOPA, L-tyrosine, or L-phenylalanine and caffeic acid, *p*-coumaric acid, or cinnamic acid, and compounds **12** and **13** were derived from **1**. Among tested compounds **1–13**, those containing one or two catechol moieties exhibited potent anti-aggregation activity, whereas the non-catechol-type related compounds showed little or no activity. This suggests that at least one catechol moiety is essential for inhibition of Aβ42 aggregation, and this activity increases depending on the number of catechol moieties. Consequently, clovamide (**1**) and its related compounds may be a promising therapeutic option for inhibiting Aβ-mediated pathology in AD.

1. Introduction

Many age-related degenerative diseases are characterized by the accumulation of amyloid deposits derived from a variety of proteins. Alzheimer's disease (AD) is the most common progressive neurodegenerative disorder.[1–4] The deposits mainly consist of 40- and 42-mer amyloid β-proteins (Aβ40 and Aβ42), which are produced from amyloid β-protein precursor (APP) by two proteases, β- and γ-secretases.[5,6] Aβ42 plays a more important role in the pathogenesis of AD than Aβ40 because of its stronger aggregative ability and neurotoxicity.[4] Accumulated evidence shows that Aβ oligomers (intermediates of Aβ aggregates), but not the monomers nor fibrils, induce cognitive dysfunction and synaptic impairment during AD progression.[7,8] Recently, several small molecules were reported to inhibit Aβ-related pathologies *in vitro* and *in vivo*, especially polyphenols, some of which (resveratrol and epigallocatechin gallate) are currently in preclinical or clinical trials.[9]

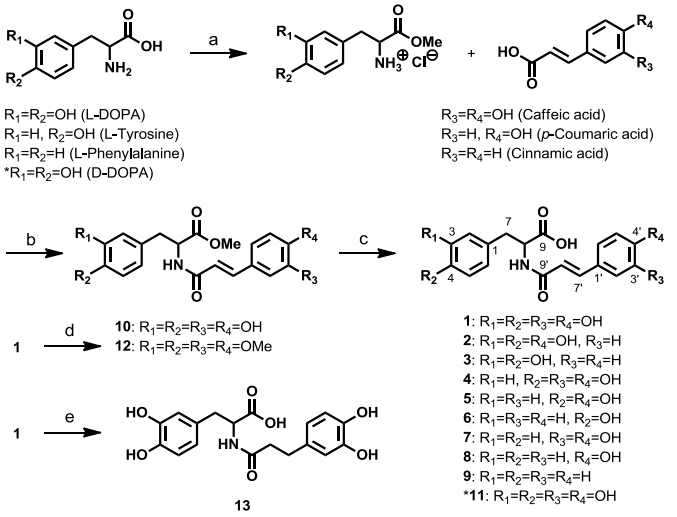
We previously reported that caffeoylquinic acid derivatives, contained in coffee beans and sweet potato, and phenylethanoid glycosides inhibit aggregation of Aβ42. These compounds, which inhibited Aβ42 aggregation, possessed a catechol moiety as a common structure.[10–13] Clovamide (**1**), containing two catechol moieties, was isolated from red clover (*Trifolium pratense*)[14] as a constituent of cacao liquor (*Theobroma cacao*).[15] Various effective properties of **1** were reported: antioxidant activity,[16] anti-inflammatory properties,[17] and neuroprotective effects.[18] However, nothing is known about the inhibitory activity with respect to Aβ42 aggregation. The aim of this study was to evaluate the potential role of **1** in the treatment of AD. For this purpose, we synthesized **1** and related compounds **2–13** and investigated the structure–activity relationship of **1–13** with respect to Aβ42 aggregation using thioflavin T (Th-T) assay and transmission electron microscopy (TEM).

2. Results

2.1. Syntheses of L-clovamide (1) and related compounds 2–13

L-clovamide (**1**) has five key structural groups: two phenolic hydroxyl groups, a carboxyl group, a stereoisomer, and an unsaturated C-C bond.

L-clovamide (**1**) and related compounds **2–11** containing different numbers and structures of catechol moieties were synthesized in three steps *via* a modification of a previously reported method.[16] Firstly, L-DOPA, L-tyrosine, and L-phenylalanine were methylated with $SOCl_2$ in anhydrous MeOH. Each aromatic amino acid hydrochloride was reacted with available caffeic acid, *p*-coumaric acid, and cinnamic acid in CH_2Cl_2/DMF to obtain the corresponding amide. Hydrolysis of methoxy groups with LiOH in THF/H_2O afforded clovamide (**1**) and related compounds **2–10**. Compound **11**, the stereoisomer of **1**, was synthesized using D-DOPA as a starting material instead of L-DOPA. However, all hydroxyl groups of **1** were methylated with MeI and K_2CO_3 in DMF to afford **12** in 85% yield, and the unsaturated C-C bond of **1** was reduced with H_2 and Pd/C,



Scheme 1. Syntheses of compounds **1–13**

(a) $SOCl_2$, MeOH, 0℃ → RT, 43–45 h; (b) **2**, **4**, **5**, **6**, **7**, **8**, **9**: EDC, HOBt, DIEA, CH_2Cl_2/DMF (3:1), RT, 8–17 h / **1**, **3**, **10**: EDC, HOBt, Et_3N, CH_2Cl_2/DMF (3:1), RT, 9–19 h; (c) LiOH, THF/H_2O (2:1), RT, 6 h; (d) MeI, K_2CO_3, DMF, RT, 24 h; (e) H_2, Pd/C, MeOH, RT, 9 h

providing **13** in 96% yield (**Scheme 1**)

2.2. Structure–activity relationship of clovamide (1) and related compounds 2–13 on Aβ42 aggregation

To evaluate the activity of clovamide (**1**) and related compounds **2–13**, thioflavin T (Th-T) fluorescence assays were performed for each compound. Compounds **1** (IC_{50} = 5.7 μM), **3** (IC_{50} = 22.7 μM), **4** (IC_{50} = 87.8 μM), **7** (IC_{50} = 72.9 μM), **10** (IC_{50} = 4.9 μM), **11** (IC_{50} = 1.6 μM), and **13** (IC_{50} = 8.7 μM) prevented the aggregation of Aβ42 in a dose-dependent manner (**Figs 1 and S1**), while compounds **2**, **5**, **6**, **8**, **9**, and **12** exhibited little or no activity (**Fig. S1**). The IC_{50} values of these compounds are shown in **Table 1**, in which the ability to prevent Aβ42 aggregation follows the order: **1** ≈ **10** ≈ **11** ≈ **13** > **3** > **4** ≈ **7** > **2**, **5**, **6**, **8**, **9**, and **12**. In addition, compounds possessing two catechol moieties such as **1**, **10**, **11**, and **13** exhibited more potent inhibition of aggregation than those containing only one catechol moiety. These results demonstrate the significance of the catechol moiety in the inhibition of Aβ42 aggregation.

To ensure that the compounds inhibited Aβ42 fibril formation, we observed Aβ42 fibrils directly using TEM (**Figs 2 and S2**). Typical Aβ42 fibril formation was observed in the presence of Aβ42 alone. Compound **2** and non-catechol-type related compounds **5**, **6, 8**, **9**, and **12** exhibited little or no activity. On the other hand, Aβ42 fibrils were reduced in the presence of **1**, **3**, **4**, **7**, **10**, **11**, and **13** in a dose-dependent manner. Remarkably, **1**, **10**, **11**, and **13** containing two catechol moieties significantly reduced Aβ42 fibrils.

3. Discussion

In this study, we investigated the effects of clovamide (**1**) and related compounds **2–13** on fibril formation (**Figs 1 and 2**). The Th-T assay and TEM results showed that at least one catechol moiety must be present for inhibition of Aβ42 aggregation. Moreover, the other chemical moieties of **1**, a carboxyl group, a stereoisomer, and an unsaturated C-C bond, did not contribute to the activity. The activity tends to increase according to the number of catechol moieties (**Table 1**). This tendency is consistent with the results of previous studies showing that polyphenols possessing multiple catechol moieties exhibit higher inhibitory activity against Aβ42 aggregation.[10, 19] The catechol moiety easily undergoes autoxidation to form *o*-quinone.[20] Such covalent modification may destabilize the β-sheet structure in amyloidogenic polypeptides.[21] The reason for **1** and compounds

Table 1. Effects of compounds **1–13** on Aβ42 aggregation

Compounds	IC_{50} (μM)[a]
L-Clovamide (**1**)	5.7
p-Coumaroyl-DOPA (**2**)	>100
Cinnamoyl-DOPA (**3**)	22.7
Caffeoyl-Tyrosine (**4**)	87.8
p-Coumaroyl-Tyrosine (**5**)	>100
Cinnamoyl-Tyrosine (**6**)	>100
Caffeoyl-Phenylalanine (**7**)	72.9
p-Coumaroyl-Phenylalanine (**8**)	>100
Cinnamoyl-Phenylalanine (**9**)	>100
Clovamide-Me (**10**)	4.9
D-Clovamide (**11**)	1.6
Clovamide-5Me (**12**)	>100
Dihydroclovamide (**13**)	8.7

[a]IC_{50} values were calculated from the inhibitory rate (%) of each concentration of derivatives for Aβ42 aggregation estimated using Th-T assay at 24 hours.

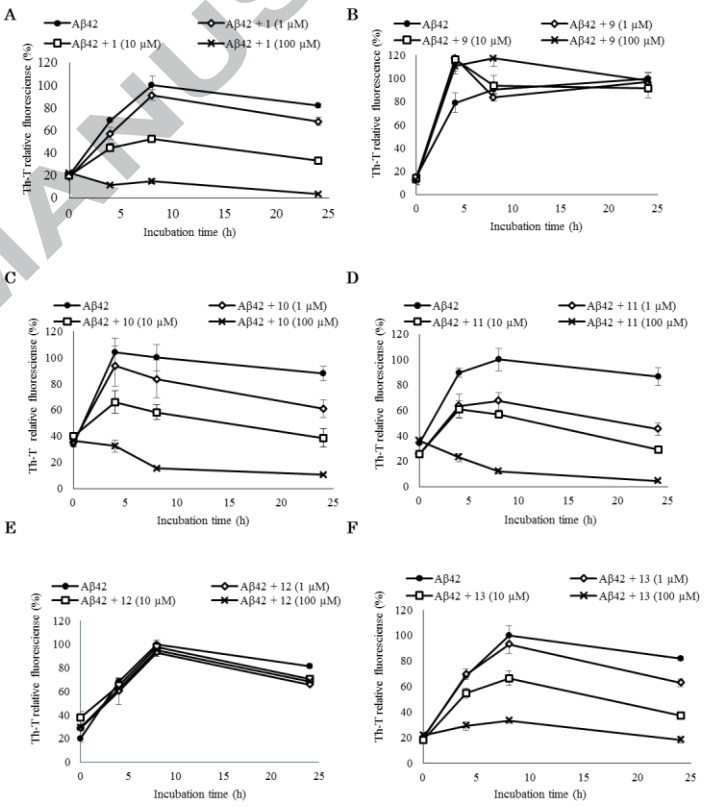


Figure 1. Effect of compounds **1**, **9**, **10**, **11**, **12**, and **13** on aggregation of Aβ42. Fibril formation of Aβ42 (25 μM） was monitored by Th-T fluorescence and presence of 1, 10, and 100 μM of (**A**) Clovamide (**1**), (**B**) Cinnamoyl-Phenylalanine (**9**), (**C**) Clovamide-Me (**10**), (**D**) D-Clovamide (**11**), (**E**) Clovamide-5Me, and (**F**) Dihydroclovamide (**13**). Fluorescence intensity was measured at an excitation wavelength 420 nm and emission wavelength of 485 nm. Values represent the mean ± SD (n = 6).

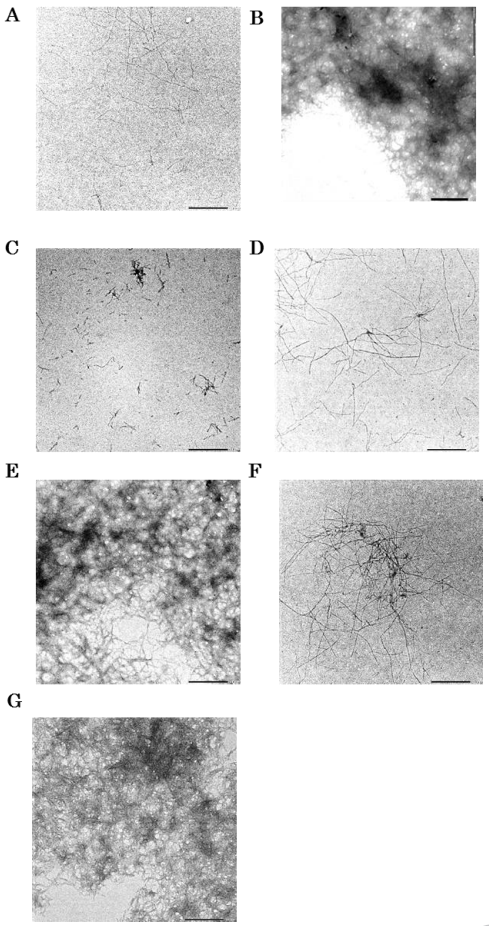


Figure 2. Effects of compounds **1**, **9**, **10**, **11**, **12**, and **13** on Aβ fibrillogenesis by TEM. The fibril formation was observed after 24 h incubation in 50 mM PBS buffer. Scale bars: 500 nm. (**A**) Aβ42 (25 μM) + **1** (100 μM), (**B**) Aβ42 (25 μM) + **9** (100 μM), (**C**) Aβ42 (25 μM) + **10** (100 μM), (**D**) Aβ42 (25 μM) + **11** (100 μM), (**E**) Aβ42 (25 μM) + **12** (100 μM), (**F**) Aβ42 (25 μM) + **13** (100 μM), and (**G**) Aβ42 (25 μM)

10, **11**, and **13**, which contain two catechol moieties, exhibiting the highest activity could be attributed to the number of autoxidized catechol moieties. Gazit proposed that blocking of β-sheet formation by polyphenols could originate from π-π stacking interactions between Aβ42 and polyphenols.[22] The π-orbital of the aromatic ring of the catechol moiety in **1** would also contribute to the π-π stacking effects, inhibiting Aβ42 aggregation.

According to the result for **3**, the catechol moiety derived from L-DOPA exhibits a greater contribution than those in **4** and **7**, which are derived from caffeic acid, with respect to inhibitory activity against Aβ42 aggregation (**Table 1**). This result might be related to the flexibility of the catechol unit (hybrid orbits of the catechol moiety bonding carbon atom). Based on the structure–activity relationship in the inhibitory activity against Aβ42 agglutination of curcumin and curcumin related compounds, it is known that the length of the linker region and the flexibility of the substructure are related to the intensity of the inhibitory activity against Aβ42 aggregation.[23] It is relevant to consider the flexibility of the catechol moiety, which is important in the expression of activity. The orbital of the 7'-position carbon atom in the catechol moiety derived from caffeic acid is sp^2-hybridized, while that of the 7-position carbon atom in the catechol moiety derived from L-DOPA is sp^3-hybridized. The catechol moiety bonded to the 7-position carbon atom can rotate in a three-dimensional manner. Since the catechol moiety directly binds to Aβ42, it can inhibit Aβ42 aggregation. Taken together, it is possible that a more flexible catechol moiety was involved in inhibiting Aβ42 aggregation. It is known from the structure–activity relationships of curcumin and curcumin related compounds that the presence of aromatic rings and the number of phenolic hydroxyl groups on aromatic rings affect inhibition of Aβ42 aggregation.[23, 24] Moreover, rosmarinic acid, the amide isostere of clovamide (**1**), and its derivatives also indicated the importance of phenolic hydroxyl groups in inhibiting Aβ42 aggregation.[25] Taxifolin, a flavonoid compound containing a catechol moiety, was reported to inhibit Aβ aggregation, while dihydrokaempferol, a compound related to taxifolin having a phenolic hydroxyl group only at the 4'-position, exhibited relatively low inhibitory activity against Aβ42 aggregation.[21] From these reports, the weak activity of **2**, despite its catechol moiety, is presumed to originate from the phenolic hydroxyl group at the 4'-position. Further structural analysis of clovamide and related compounds to elucidate the mechanism of inhibitory activity against Aβ42 aggregation could help to develop more potent inhibitors for AD therapy.

4. Materials and methods

4.1. General Procedure

NMR spectra were recorded on an AVANCE 500 spectrometer (Bruker, USA), operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR in CD_3OD and DMSO-d_6. The resonances at δ_H 3.35 and δ_H 2.49 of the residual CD_2HOD and CD_3SOCD_2H and those at δ_C 49.0 and δ_C 39.5 of CD_3OD and DMSO-d_6 were used as internal references for the ^{1}H and ^{13}C NMR spectra, respectively. ESI-MS was performed on a Synapt G2 mass spectrometer (Waters, USA). Optical rotations were recorded on a Jasco DIP-1000 digital polarimeter, while UV spectra were recorded on a Hitachi double beam spectrophotometer U-2000A. Syntheses were conducted under a nitrogen atmosphere.

4.2. Syntheses of clovamide (1) and related compounds 2–13

L-DOPA methyl ester hydrochloride (a)

$SOCl_2$ (947 μL, 12.7 mmol) was added to anhydrous MeOH (18 mL) in an ice bath. After 30 min, L-3,4-dihydroxyphenylalanine (1.00 g, 5.07 mmol) was added, and the mixture was stirred at room temperature for 56 h. After removal of the solvent, the residue was purified by silica gel chromatography ($CHCl_3$:MeOH = 14:1) to afford L-DOPA methyl ester hydrochloride (**a**, 1.03 g, 93%) as a white powder.

L-Tyrosine methyl ester hydrochloride (b)

$SOCl_2$ (906 μL, 12.5 mmol) was added to anhydrous MeOH (18 mL) in an ice-water bath. After 30 min, L-tyrosine (0.906 g, 5.00 mmol) was added, and the mixture was stirred at room temperature for 36 h. After removal of the solvent, the residue was purified by silica gel chromatography ($CHCl_3$:MeOH = 14:1 → 12:1 → 5:1 → 0:1) to afford L-tyrosine methyl ester hydrochloride (**b**, 0.876 g, 90%) as a white powder.

L-Phenylalanine methyl ester hydrochloride (c)

$SOCl_2$ (723 μL, 10.0 mmol) was added to anhydrous MeOH (12 mL) in an ice-water bath. After 30 min, L-phenylalanine

(0.661 g, 4.0 mmol) was added, and the mixture was stirred at room temperature for 45 h. The residue was evaporated to afford L-phenylalanine methyl ester hydrochloride (**c**, 0.867 g, quant.) as a white powder.

Synthesis of L -clovamide (1)

Caffeic acid (140.7 mg, 0.78 mmol) was dissolved in CH_2Cl_2/DMF (1:3, 3.0 mL), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) (247 µL, 147 mmol) and 1-hydroxybenzotriazole (HOBt) (75.4 mg, 0.56 mmol) were added to the solution. After the mixture was cooled in an ice-water bath for 15 min, Et_3N (156 µL, 1.12 mmol) and compound **a** (138.3 mg, 0.56 mmol) were added to the mixture. After stirring at room temperature for 19 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo.* The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 9:1) to afford clovamide methyl ester (clovamide-Me, **10**, 84.7 mg, 40%) as a yellow powder: ^{1}H NMR (CD_3OD) δ 7.36 (1H, d, *J* = 15.7 Hz, H-7'), 6.99 (1H, d, *J* = 1.9 Hz, H-2), 6.89 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 6.75 (1H, d, *J* = 8.2 Hz, H-5), 6.64 (1H, d, *J* = 1.9 Hz, H-2'), 6.67 (1H, d, *J* = 8.1 Hz, H-5'), 6.53 (1H, dd, *J* = 8.1, 1.9 Hz, H-6'), 6.41 (1H, d, *J* = 15.7 Hz, H-8'), 4.69 (1H, m, H-8), 3.68 (3H, s, MeO-9'), 3.02 (1H, dd, *J* = 13.9, 5.9 Hz, H-7a), 2.93 (1H, dd, *J* = 13.9, 6.6 Hz, H-7b); ^{13}C NMR (CD_3OD) δ 174.6 (C, C-9), 169.8 (C, C-9'), 149.5 (C, C-4'), 147.3 (C, C-3'), 146.8 (C, C-3), 145.9 (C, C-4), 143.8 (CH, C-7'), 130.2 (C, C-1), 128.9 (C, C-1'), 123.1 (CH, C-6'), 122.4 (CH, C-6), 118.4 (CH, C-8'), 118.0 (CH, C-2), 117.2 (CH, C-5), 117.1 (CH, C-5'), 115.9 (CH, C-2'), 56.5 (CH, C-8), 53.4 (CH_3, CO-9) 39.1 (C, C-7); UV λ_{max} (MeOH) nm (ε): 208 (12400), 220 (12100), 291 (9700), 324 (10700); HR-ESI-MS (negative ion) *m/z:* 372.1100 $[M-H]^-$ (calcd for $C_{19}H_{18}NO_7$, 372.1083); $[\alpha]_D^{20}$ +20° (*c*= 1.0, MeOH).

This crude ester (60.0 mg, 0.16 mmol) was dissolved in THF/H_2O (2:1, 5.0 mL) before adding LiOH・H_2O (16.9 mg, 0.40 mmol) and stirring at 45°C for 9 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated. The residue was purified by silica gel column chromatography using hexane:EtOAc = 1:4 to remove some non-polar compounds, then $CHCl_3$:MeOH = 2:1 → 1:1 was used to obtain clovamide (**1**, 26.9 mg, 47%) as a pale yellow powder: ^{1}H NMR (CD_3OD) δ: 7.31 (1H, d, *J* = 15.7 Hz, H-7'), 6.98 (1H, d, *J* = 1.9 Hz, H-2), 6.88 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 6.74 (1H, d, *J* = 8.2 Hz, H-5), 6.66 (1H, d, *J* = 2.0 Hz, H-2'), 6.62 (1H, d, *J* = 8.0 Hz, H-5'), 6.53 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.38 (1H, d, *J* = 15.7 Hz, H-8'), 4.58 (1H, m, H-8), 3.08 (1H, dd, *J* = 13.8, 4.9 Hz, H-7a), 2.93 (1H, dd, *J* = 13.8, 6.6 Hz, H-7b). ^{13}C NMR (CD_3OD) δ: 177.5 (C, C-9), 169.5 (C, C-9'), 149.5 (C, C-4'), 147.4 (C, C-3'), 146.8 (C, C-3), 145.8 (C, C-4), 143.2 (CH, C-7'), 131.2 (C, C-1), 129.1 (C, C-1'), 123.0 (CH, C-6'), 122.6 (CH, C-6), 119.1 (CH, C-8'), 118.3 (CH, C-2), 117.2 (CH, C-5'), 117.0 (CH, C-5), 115.8 (CH, C-2'), 57.4 (CH, C-8), 39.1 (C, C-7). UV λ_{max} (MeOH) nm (ε): 208 (22100), 290 (14700), 322 (15000). HR-ESI-MS (negative ion) *m/z:* 358.0907 $[M-H]^-$ (calcd for $C_{18}H_{16}NO_7$, 358.0927). $[\alpha]_D^{30}$ +23° (*c* = 1.0, MeOH).

Synthesis of *p*-Coumaroyl-DOPA (2)

EDC (152 µL, 0.86 mmol), *p*-coumaric acid (141.0 mg, 0.86 mmol), and HOBt (116.1 mg, 0.86 mmol) were dissolved in CH_2Cl_2/DMF (1:3, 3.0 mL). The solution was cooled in an ice-water bath. After 15 min, Et_3N (149 µL, 0.86 mmol) and compound **a** (129.6 mg, 0.61 mmol) were added to the solution. After stirring at room temperature for 8 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo.* The residue was purified by silica gel column chromatography to afford *p*-coumaroyl-DOPA methyl ester (17.1 mg, 7%) as a yellow powder.

This crude ester (11.2 mg, 0.03 mmol) was dissolved in THF/H_2O (2:1, 0.80 mL) before adding LiOH・H_2O (4.0 mg, 0.09 mmol) and stirring at 45°C for 6 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated. The residue was purified using Sep-Pak C18 (12 cc, MeOH:H_2O = 40:60 → 100:0) to afford *p*-coumaroyl-DOPA (**2**, 5.0 mg, 47%) as a dark brown powder: ^{1}H NMR (CD_3OD) δ: 7.40 (1H, d, *J* = 15.7 Hz, H-7'), 7.39 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.77 (2H, d, *J* = 8.5 Hz, H-3', 5'), 6.67 (1H, d, *J* = 2.0 Hz, H-2), 6.65 (1H, d, *J* = 8.1 Hz, H-5), 6.55 (1H, dd, *J* = 8.1, 2.0 Hz, H-6), 6.45 (1H, d, *J* = 15.7 Hz, H-8'), 4.65 (1H, m, H-8), 3.08 (1H, dd, *J* = 13.9, 5.2 Hz, H-7a), 2.93 (1H, dd, *J* = 13.9, 7.8 Hz, H-7b). ^{13}C NMR (CD_3OD) δ 177.5 (CH, C-3', 5'), 176.9 (C, C-9), 169.5 (C, C-9'), 161.3 (C, C-4'), 146.9 (C, C-3), 145.9 (C, C-4), 142.9 (CH, C-7'), 131.4 (CH, C-2', 6'), 131.0 (C, C-1), 128.6 (C, C-1'), 122.6 (CH, C-6), 119.2 (CH, C-8'), 118.3 (CH, C-2), 117.0 (CH, C-5), 57.0 (CH, C-8), 39.1 (C, C-7). UV λ_{max} (MeOH) nm (ε): 204 (19800), 291 (10800), 309 (10300), 399 (500). HR-ESI-MS (negative ion) *m/z:* 342.0998 $[M-H]^-$ (calcd for $C_{18}H_{16}NO_6$, 342.0978). $[\alpha]_D^{29}$ -10° (*c* = 1.0, MeOH).

Synthesis of Cinnamoyl-DOPA (3)

Cinnamic acid (88.1 mg, 0.59 mmol) was dissolved in CH_2Cl_2/DMF (1:3, 3.0 mL). EDC (105 µL, 0.89 mmol) and HOBt (80.3 mg, 0.59 mmol) were added to the solution. After the mixture was cooled in an ice-water bath for 15 min, Et_3N (166 µL, 1.18 mmol) and compound **a** (147.2 mg, 0.59 mmol) were added to the mixture. After stirring at room temperature for 9 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo.* The residue was purified by silica gel column chromatography to afford cinnamoyl-DOPA methyl ester (83.7 mg, 41%) as a yellow powder.

This crude ester (64.3 mg, 0.19 mmol) was dissolved in THF/H_2O (2:1, 2.0 mL) before adding LiOH・H_2O (23.7 mg, 0.56 mmol) and stirring at 45°C for 6 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated. The residue was purified by silica gel column chromatography using hexane:EtOAc = 7:3 → 6:4 → 5:5 to remove some non-polar compounds, then $CHCl_3$:MeOH = 8:2 was used to afford cinnamoyl-DOPA (**3**, 20.0 mg, 33%) as a dark brown powder: ^{1}H NMR (CD_3OD) δ: 7.52 (2H, d, *J* = 8.5 Hz, H-2', 6'), 7.49 (1H, d, *J* = 15.7 Hz, H-7'), 7.35 (3H, m, H-3', 4', 5'), 6.69 (1H, d, *J* = 2.0 Hz, H-2), 6.67 (1H, d, *J* = 8.0 Hz, H-5), 6.65 (1H, d, *J* = 15.7 Hz, H-8'), 6.56 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 4.72 (1H, m, H-8), 3.10 (1H, dd, *J* = 14.1, 5.2 Hz, H-7a), 2.93 (1H, dd, *J* = 14.1, 8.4 Hz, H-7b). ^{13}C NMR (CD_3OD) δ: 175.7 (C, C-9), 169.2 (C, C-9'), 147.0 (C, C-3), 146.0 (C, C-4), 143.0 (CH, C-7'), 137.0 (C, C-1'), 131.7 (CH, C-4'), 130.7 (C, C-3', 5'), 130.6 (C, C-1), 129.7 (CH, C-2', 6'), 122.5 (CH, C-6), 122.2 (CH, C-8'), 118.1 (CH, C-5), 117.1 (CH, C-2), 56.4 (CH, C-8), 38.8 (C, C-7). UV λ_{max} (MeOH) nm (ε): 207 (11500), 220 (11000), 272 (9600). HR-ESI-MS (negative ion) *m/z:* 326.1026 $[M-H]^-$ (calcd for $C_{18}H_{16}NO_5$, 326.1028). $[\alpha]_D^{29}$ -28° (*c*= 1.0, MeOH).

Synthesis of Caffeoyl-Tyrosine (4)

Caffeic acid (144.0 mg, 0.80 mmol) was dissolved in CH_2Cl_2/DMF (1:3, 3.0 mL). EDC (142 μL, 0.80 mmol), HOBt (108.0 mg, 0.80 mmol), DIEA (230 μL, 1.33 mmol), and compound **b** (130.0 mg, 0.67 mmol) were added to the solution. After stirring at room temperature for 10 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo.* The residue was purified by silica gel column chromatography and with Sep-Pak C18 to afford caffeoyl-tyrosine methyl ester (26.4 mg, 10%) as a yellow powder.

This crude ester (9.9 mg, 0.03 mmol) was dissolved in THF/H_2O (2:1, 0.80 mL) before adding LiOH・H_2O (5.8 mg, 0.14 mmol) and stirring at 45°C for 9 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated. The residue was purified using Sep-Pak C18 (0.7 cc, MeOH:H_2O = 3:7 → 5:5 → 1:0) to afford caffeoyl-tyrosine (**4**, 4.5 mg, 47%) as a brown powder: ^{1}H NMR (CD_3OD) δ: 7.34 (1H, d, *J* = 15.7 Hz, H-7'), 7.05 (2H, d, *J* = 8.7 Hz, H-2, 6), 6.99 (1H, d, *J* = 2.0 Hz, H-2'), 6.89 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 6.77 (2H, d, *J* = 8.7 Hz, H-3, 5), 6.75 (1H, d, *J* = 8.2 Hz, H-5'), 6.40 (1H, d, *J* = 15.7 Hz, H-8'), 4.69 (1H, m, H-8), 3.13(1H, dd, *J* = 14.1, 5.3 Hz, H-7a), 2.93 (1H, dd, *J* = 14.1, 8.4 Hz, H-7b). ^{13}C NMR (CD_3OD) δ: 175.1 (C, C-9), 169.8 (C, C-9'), 158.1 (C, C-4), 149.6 (C, C-4'), 147.5 (CH, C-3'), 143.6 (CH, C-7'), 132.1 (C, C-2, 6), 130.0 (C, C-1), 129.1 (C, C-1'), 123.0 (CH, C-6'), 118.8 (CH, C-8'), 117.2 (CH, C-5'), 117.0 (CH, C-3, 5), 115.9 (CH, C-2'), 56.5 (CH, C-8), 39.7 (C, C-7). UV λ_{max} (MeOH) nm (ε): 203 (18600), 287 (6200), 294 (6200), 321 (6700); HR-ESI-MS (negative ion) *m/z:* 342.0980 $[M-H]^-$ (calcd for $C_{18}H_{16}NO_6$, 342.0978). $[\alpha]_D^{29}$ -17° (*c* = 1.0, MeOH).

Synthesis of *p*-Coumaroyl-Tyrosine (**5**)

EDC (170 μL, 0.96 mmol) and HOBt (129.6 mg, 0.96 mmol) were added to a solution of *p*-coumaric acid (157.4 mg, 0.96 mmol) in CH_2Cl_2/DMF (1:3, 3.0 mL). After cooling the solution in an ice-water bath for 15 min, DIEA (166 μL, 0.96 mmol) and compound **b** (156.0 mg, 0.80 mmol) were added. After stirring at room temperature for 10 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo.* The residue was purified by silica gel column chromatography and with Sep-Pak C18 to afford caffeoyl-tyrosine methyl ester (54.0 mg, 20%) as a yellow powder.

This crude ester (21.1 mg, 0.06 mmol) was dissolved in THF/H_2O (2:1, 0.80 mL) before adding LiOH・H_2O (7.8 mg, 0.19 mmol) and stirring at 45°C for 6 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated to afford *p*-coumaroyl-tyrosine (**5**, 20.2 mg, quant.) as a white powder: ^{1}H NMR (CD_3OD) δ: 7.41 (1H, d, *J* = 15.7 Hz, H-7'), 7.37 (2H, d, *J* = 8.6 Hz, H-2', 6'), 7.03 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.77 (1H, d, *J* = 8.6 Hz, H-3, 5), 6.70 (1H, d, *J* = 8.6 Hz, H-3', 5'), 6.45 (1H, d, *J* = 15.7 Hz, H-8'), 4.70 (1H, m, H-8), 3.13 (1H, dd, *J* = 14.1, 5.3 Hz, H-7a), 2.93 (1H, dd, *J* = 14.1, 8.5 Hz, H-7b). ^{13}C NMR (CD_3OD) δ 175.7 (C, C-9), 169.8 (C, C-9'), 161.4 (C, C-4), 158.1 (C, C-4'), 143.2 (CH, C-7'), 132.1 (CH, C-2, 6), 131.5 (CH, C-2', 6'), 129.9 (C, C-1), 128.5 (C, C-1'), 118.7 (CH, C-8'), 117.5 (CH, C-3', 5'), 117.0 (CH, C-3, 5), 56.3 (CH, C-8), 38.6 (C, C-7). UV λ_{max} (MeOH) nm (ε): 223 (10200), 293 (7900), 309 (7900), 444 (400). HR-ESI-MS (negative ion) *m/z:* 326.1026 $[M-H]^-$ (calcd for $C_{18}H_{16}NO_5$, 326.1028). $[\alpha]_D^{30}$ -40° (*c* = 1.0, MeOH).

Synthesis of Cinnamoyl-Tyrosine (**6**)

Cinnamic acid (99.8 mg, 0.67 mmol) was dissolved in CH_2Cl_2/DMF (1:3, 3.0 mL). EDC (119 μL, 0.67 mmol) and HOBt (91.0 mg, 0.67 mmol) were added to the solution. After the solution was cooled in an ice-water bath for 15 min, DIEA (194 μL, 1.12 mmol) and compound **b** (130.0 mg, 0.56 mmol) were added. After stirring at room temperature for 3 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo.* The residue was purified with Sep-Pak C18 to afford caffeoyl-tyrosine methyl ester (35.5 mg, 20%) as a white powder.

This crude ester (24.1 mg, 0.07 mmol) was dissolved in THF/H_2O (2:1, 0.80 mL) before adding LiOH・H_2O (15.5 mg, 0.37 mmol) and stirring at 45°C for 4.5 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated to afford cinnamoyl-tyrosine (**6**, 22.2 mg, 96%) as a white powder: ^{1}H NMR (CD_3OD) δ: 7.54 (2H, d, *J* = 8.0 Hz, H-2', 6'), 7.49 (1H, d, *J* = 15.8 Hz, H-7'), 7.36 (3H, m, H-3', 4', 5'), 7.06 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.77 (2H, d, *J* = 8.6 Hz, H-3, 5), 6.65 (1H, d, *J* = 15.8 Hz, H-8'), 4.69 (1H, m, H-8), 3.15 (1H, dd, *J* = 14.1, 5.2 Hz, H-7a), 2.93 (1H, dd, *J* = 14.1, 8.7 Hz, H-7b). ^{13}C NMR (CD_3OD) δ: 175.6 (C, C-9), 169.2 (C, C-9'), 158.1 (C, C-4), 143.1 (CH, C-7'), 137.0 (C, C-1'), 132.1 (CH, C-2, 6), 131.7 (C, C-1), 130.7 (CH, C-3', 5'), 129.8 (CH, C-4'), 129.7 (CH, C-2', 6'), 122.2 (CH, C-8'), 117.0 (CH, C-3, 5), 56.4 (CH, C-8), 38.6 (C, C-7). UV λ_{max} (MeOH) nm (ε): 203 (16000), 216 (11200), 276 (9300), 392 (300). HR-ESI-MS (negative ion) *m/z:* 310.1044 $[M-H]^-$ (calcd for $C_{18}H_{16}NO_4$, 310.1079). $[\alpha]_D^{29}$ -24° (*c* = 1.0, MeOH).

Synthesis of Caffeoyl-Phenylalanine (**7**)

Caffeic acid (156.8 mg, 0.87 mmol) was dissolved in CH_2Cl_2/DMF (1:3, 3.0 mL). EDC (154 μL, 0.87 mmol) and HOBt (117.6 mg, 0.87 mmol) were added to the solution. After the solution was cooled in an ice-water bath for 15 min, DIEA (251 μL, 1.45 mmol) and compound **c** (130.0 mg, 0.73 mmol) were added. After stirring at room temperature for 17 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo.* The residue was purified by silica gel column chromatography and with Sep-Pak C18 to afford caffeoyl-tyrosine methyl ester (12.8 mg, 5%) as a yellow powder.

This crude ester (12.8 mg, 0.04 mmol) was dissolved in THF/H_2O (2:1, 0.80 mL) before adding LiOH・H_2O (5.8 mg, 0.19 mmol) and stirring at 45°C for 3 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated. The residue was purified using Sep-Pak C18 (6 cc, MeOH:H_2O = 5:5 → 1:0) to afford caffeoyl-phenylalanine (**7**, 4.6 mg, 37%) as a brown powder: ^{1}H NMR (CD_3OD) δ: 7.34 (1H, d, *J* = 15.7 Hz, H-7'), 7.23 (5H, m, H-2, 3, 4, 5, 6), 6.98 (1H, d, *J* = 2.1 Hz, H-2'), 6.88 (1H, dd, *J* = 8.7, 2.1 Hz, H-6'), 6.74 (1H, d, *J* = 8.7 Hz, H-5'), 6.39 (1H, d, *J* = 15.7 Hz, H-8'), 4.77 (1H, m, H-8), 3.24 (1H, dd, *J* = 14.0, 5.2 Hz, H-7a), 3.02 (1H, dd, *J* = 14.0, 8.8 Hz, H-7b). ^{13}C NMR (CD_3OD) δ: 175.7 (C, C-9), 169.8 (C, C-9'), 143.6 (CH, C-7'), 132.3 (C, C-1), 131.1 (CH, C-3, 5), 130.2 (CH, C-2, 6), 129.0 (C, C-1'), 128.6 (C, C-4, 4'), 123.0 (CH, C-6'), 118.7 (CH, C-8'), 117.2 (CH, C-5'), 115.9 (CH, C-2'), 56.2 (CH, C-8), 39.4 (C, C-7). UV λ_{max} (MeOH) nm (ε): 204 (18800), 296 (9800), 323 (10900), 428 (700). HR-ESI-MS (negative ion) *m/z:* 326.1047 $[M-H]^-$ (calcd for $C_{18}H_{16}NO_5$, 326.1028). $[\alpha]_D^{29}$ -20° (*c* = 1.0, MeOH).

Synthesis of *p*-Coumaroyl-Phenylalanine (**8**)

EDC (128 μL, 0.72 mmol) and HOBt (97.7 mg, 0.72 mmol) were added to a solution of *p*-coumaric acid (118.7 mg, 0.72 mmol) in CH_2Cl_2/DMF (1:3, 3.0 mL). The solution was cooled in an ice-water bath. After 15 min, DIEA (209 μL, 1.21 mmol) and compound **c** (130.0 mg, 0.60 mmol) were added to the solution. After stirring at room temperature for 8 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL × 1) and brine (150 mL × 1), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo*. The residue was purified with Sep-Pak C18 to afford caffeoyl-tyrosine methyl ester (31.4 mg, 16%) as a white powder.

This crude ester (27.1 mg, 0.08 mmol) was dissolved in THF/H_2O (2:1, 0.80 mL) before adding LiOH・H_2O (17.5 mg, 0.42 mmol) and stirring at 45°C for 3 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated. The residue was purified using Sep-Pak C18 (35 cc, MeOH:H_2O = 40:60 → 100:0) to afford *p*-coumaroyl-phenylalanine (**8**, 27.2 mg, quant.) as a white powder: ^{1}H NMR (CD_3OD) δ: 7.41 (1H, d, *J* = 15.7 Hz, H-7'), 7.38 (2H, d, *J* = 8.6 Hz, H-2', 6'), 7.23 (6H, m, H-1, 2, 3, 4, 5, 6), 6.77 (2H, d, *J* = 8.6 Hz, H-3', 5'), 6.44 (1H, d, *J* = 15.7 Hz, H-8'), 4.77 (1H, m, H-8), 3.24 (1H, dd, *J* = 14.0, 5.1 Hz, H-7a), 2.93 (1H, dd, *J* = 14.0, 8.8 Hz, H-7b). ^{13}C NMR (CD_3OD) δ: 175.6 (C, C-9), 169.8 (C, C-9'), 161.4 (C, C-4), 143.3 (CH, C-7'), 139.2 (C, C-1), 131.5 (CH, C-2, 6), 131.1 (CH, C-3, 5), 130.2 (C, C-2', 6'), 128.6 (C, C-1'), 128.4 (C, C-4), 118.7 (CH, C-8'), 117.5 (CH, C-3' 5'), 56.1 (CH, C-8), 39.4 (C, C-7). UV λ_{max} (MeOH) nm (ε): 203 (15800), 299 (8700), 310 (8600), 404 (500). HR-ESI-MS (negative ion) *m/z:* 310.1044 [M-H]$^-$ (calcd for $C_{18}H_{16}NO_4$, 342.1079). $[\alpha]_D^{30}$ -37° (*c* = 1.0, MeOH).

Synthesis of Cinnamoyl-Phenylalanine (9)

Cinnamic acid (111.3 mg, 0.75 mmol) was dissolved in 3 mL CH_2Cl_2/DMF (1:3, 3.0 mL), to which was added EDC (217 μL, 0.75 mmol) and HOBt (101.5 mg, 0.75 mmol). The solution was cooled in an ice-water bath. After 15 min, DIEA (217 μL, 1.25 mmol) and compound **c** (135.0 mg, 0.63 mmol) were added. After stirring at room temperature for 17 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo*. The residue was purified by silica gel column chromatography to afford caffeoyl-tyrosine methyl ester (86.2 mg, 45%) as a white powder.

This crude ester (50.5 mg, 0.16 mmol) was dissolved in THF/H_2O (2:1, 0.8 mL) before adding LiOH・H_2O (20.6 mg, 0.49 mmol) and stirring at 45°C for 6 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated to afford cinnamoyl-phenylalanine (**9**, 25.3 mg, 53%) as a white powder: ^{1}H NMR (CD_3OD) δ: 7.53 (2H, d, *J* = 8.6 Hz, H-2', 6'), 7.48 (1H, d, *J* = 15.8 Hz, H-7'), 7.36 (3H, m, H-3', 4', 5'), 7.24 (5H, m, H-2, 3, 4, 5, 6), 6.63 (1H, d, *J* = 15.8 Hz, H-8'), 4.79 (1H, m, H-8), 3.24 (1H, dd, *J* = 14.0, 5.2 Hz, H-7a), 3.02 (1H, dd, *J* = 14.0, 8.8 Hz, H-7b). ^{13}C NMR (CD_3OD) δ: 175.4 (C, C-9), 169.2 (C, C-9'), 143.1 (CH, C-7'), 139.2 (C, C-1), 137.0 (C, C-1'), 131.1 (CH, C-3, 5), 130.7 (CH, C-3', 5'), 130.2 (CH, C-2, 6), 128.6 (C, C-4), 122.1 (CH, C-8'), 56.1 (CH, C-8), 39.3 (C, C-7). UV λ_{max} (MeOH) nm (ε): 216 (23400), 263 (21000). HR-ESI-MS (negative ion) *m/z:* 294.1154 [M-H]$^-$ (calcd for $C_{18}H_{16}NO_3$, 294.1130). $[\alpha]_D^{30}$ -23° (*c* = 1.0, MeOH).

Synthesis of D-clovamide (11)

$SOCl_2$ (230 μL, 3.17 mmol) was added to anhydrous MeOH (4.5 mL) in an ice bath. After 30 min, D-3,4-dihydroxy-phenylalanine (250.0 mg, 1.27 mmol) was added, and the mixture was stirred at room temperature for 42 h. After removal of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 14:1 → 0:1) to afford D-DOPA methyl ester hydrochloride (316.9 mg, quant.) as a white powder.

Caffeic acid (143.3 mg, 0.80 mmol) was dissolved in CH_2Cl_2/DMF (1:3, 3.0 mL), to which was added EDC (252 μL, 1.42 mmol) and HOBt (76.8 mg, 0.57 mmol). After the mixture was cooled in an ice-water bath for 15 min, Et_3N (158 μL, 1.14 mmol) and D-DOPA methyl ester hydrochloride (120.0 mg, 0.57 mmol) were added. After stirring at room temperature for 12 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc = 1:2 → MeOH) to afford D-clovamide methyl ester (32.9 mg, 16%) as a yellow powder.

This crude ester (17.2 mg, 0.05 mmol) was dissolved in THF/H_2O (2:1, 2.0 mL) before adding LiOH・H_2O (5.8 mg, 0.14 mmol) and stirring at 45°C for 6 h. The mixture was acidified with Dowex® 50W×8 resin, filtered, and evaporated. The residue was purified by silica gel column chromatography using hexane:EtOAc = 3:7 to remove some non-polar compounds, before using $CHCl_3$:MeOH = 3:7 → 0:1 and Sep-Pak C18 (12 cc, MeOH:H_2O = 1:9 → 1:0) to obtain D-clovamide (**11**, 3.6 mg, 20%) as a brown powder: ^{1}H NMR (CD_3OD) δ: 7.33 (1H, d, *J* = 15.7 Hz, H-7'), 6.98 (1H, d, *J* = 1.9 Hz, H-2), 6.88 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 6.74 (1H, d, *J* = 8.2 Hz, H-5), 6.67 (1H, d, *J* = 1.9 Hz, H-2'), 6.64 (1H, d, *J* = 8.1 Hz, H-5'), 6.54 (1H, dd, *J* = 8.1, 1.9 Hz, H-6'), 6.39 (1H, d, *J* = 15.7 Hz, H-8'), 4.62 (1H, m, H-8), 3.08 (1H, dd, *J* = 13.9, 4.8 Hz, H-7a), 2.93 (1H, dd, *J* = 13.9, 7.5 Hz, H-7b). ^{13}C NMR (CD_3OD) δ: 177.5 (C, C-9), 169.4 (C, C-9'), 149.5 (C, C-4'), 147.4 (C, C-3'), 146.8 (C, C-3), 145.8 (C, C-4), 143.1 (CH, C-7'), 131.3 (C, C-1), 129.2 (C, C-1'), 123.0 (CH, C-6'), 122.7 (CH, C-6), 119.3 (CH, C-8'), 118.3 (CH, C-2), 117.2 (CH, C-5'), 117.0 (CH, C-5), 115.8 (CH, C-2'), 57.6 (CH, C-8), 39.2 (C, C-7). UV λ_{max} (MeOH) nm (ε): 207 (22000), 290 (12300). HR-ESI-MS (negative ion) *m/z:* 358.0907 [M-H]$^-$ (calcd for $C_{18}H_{16}NO_7$, 358.0927). $[\alpha]_D^{30}$ -23° (*c* = 1.0, MeOH).

Synthesis of clovamide-5Me (12)

L-clovamide (**1**, 55.6 mg, 0.15 mmol) was dissolved in DMF (3.0 mL). K_2CO_3 (213.9 mg, 1.55 mmol) and MeI (96.4 μL, 1.55 mmol) were added to the mixture. After stirring at room temperature for 24 h, the mixture was poured into H_2O (50 mL), extracted with EtOAc (50 mL × 3), washed with 5% $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and the solvent removed *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 9:1) to afford clovamide-5Me (**12**, 32.9 mg, 16%) as a white powder: ^{1}H NMR (DMSO-d_6) δ: 8.38 (1H, d, *J* = 7.8 Hz, NH), 7.35 (1H, d, *J* = 15.8 Hz, H-7'), 7.15 (1H, d, *J* = 1.9 Hz, H-2), 7.11 (1H, dd, *J* = 8.4, 1.9 Hz, H-6), 6.98 (1H, d, *J* = 8.4 Hz, H-5), 6.85 (1H, d, *J* = 2.2 Hz, H-2'), 6.84 (1H, d, *J* = 8.1 Hz, H-5'), 6.75 (1H, dd, *J* = 8.1, 2.2 Hz, H-6'), 6.60 (1H, d, *J* = 15.8 Hz, H-8'), 4.60 (1H, m, H-8), 3.79 (3H, s, MeO-3), 3.78 (3H, s, MeO-4), 3.72 (3H, s, MeO-3'), 3.70 (3H, s, MeO-4'), 3.64 (3H, s, MeO-9'), 2.93 (1H, dd, *J* = 13.9, 5.5 Hz, H-7b), 2.88 (1H, dd, *J* = 13.9, 4.7 Hz, H-7b). ^{13}C NMR (DMSO-d_6) δ: 172.2 (C, C-9), 165.2 (C, C-9'), 150.2 (C, C-4), 148.9 (C, C-3), 148.4 (C, C-3'), 147.5 (C, C-4'), 139.5 (CH, C-7'), 129.5 (C, C-1), 127.5 (C, C-1'), 121.5 (CH, C-6'), 121.5 (CH, C-6), 119.1 (CH, C-8'), 109.9 (CH, C-2), 111.7 (CH, C-5'), 111.6 (CH, C-5), 112.9 (CH, C-2'), 53.9 (CH, C-8), 36.4 (C, C-7). UV λ_{max} (MeOH) nm (ε): 233 (6200), 287 (5300), 329 (5500), 439 (500). HR-ESI-MS (negative ion) *m/z:* 428.1693

[M-H]$^-$ (calcd for $C_{23}H_{26}NO_7$, 428.1709). $[\alpha]_D^{26}$ +73° (*c* = 1.0, MeOH).

Synthesis of Dihydroclovamide (13)

L-clovamide (**1**, 55.6 mg, 0.15 mmol) was dissolved in anhydrous MeOH (1.5 mL). Pd/C (4.1 mg) was added to the mixture. After stirring at room temperature for 9 h under a H_2 atmosphere, Pd/C was removed with Celite and the filtrate was evaporated to afford dihydroclovamide (**13**, 40.0 mg, 96%) as a brown powder: ^{1}H NMR (DMSO-d_6) δ: 7.74 (1H, d, *J* = 7.5 Hz, NH), 6.74 (1H, d, *J* = 7.9 Hz, H-5), 6.60 (1H, m, H-2) 6.59 (1H, *J* = 7.2 Hz, H-5'), 6.57 (1H, m, H-2'), 6.41 (1H, m, H-6'), 6.39 (1H, m, H-6), 4.24 (1H, m, H-8), 2.93 (1H, dd, *J* = 13.7, 8.3 Hz, H-7b), 2.87 (1H, dd, *J* = 13.7, 4.7 Hz, H-7a), 2.53 (1H, t, *J* = 8.5 Hz, H-7'), 2.26 (1H, t, *J* = 8.5 Hz, H-8'). ^{13}C NMR (DMSO-d_6) δ: 174.2 (C, C-9), 171.2 (C, C-9'), 145.1 (C, C-4), 144.8 (C, C-3), 143.6 (C, C-3'), 143.4 (C, C-4'), 132.2 (C, C-1'), 129.2 (C, C-1), 120.0 (CH, C-6'), 118.7 (CH, C-6), 115.3 (CH, C-2), 115.8 (CH, C-5'), 115.6 (CH, C-5), 116.8 (CH, C-2'), 54.7 (CH, C-8), 36.8 (CH, C-8'), 37.6 (C, C-7), 30.7 (CH, C-7'). UV λ_{max} (MeOH) nm (ε): 209 (14100), 283 (4100). HR-ESI-MS (negative ion) *m/z:* 360.1088 [M-H]$^-$ (calcd for $C_{18}H_{18}NO_7$, 360.1083). $[\alpha]_D^{20}$ +20° (*c* = 1.0, MeOH).

4.3. Th-T assay

The aggregative ability of Aβ42 was evaluated by the Th-T method developed by Naiki *et al.*[29] The procedure is described elsewhere.[30] Briefly, Aβ42 was dissolved in 0.1% NH_4OH at 250 μM. The resultant solution was diluted 10 times with 50 mM sodium phosphate buffer (PBS) containing 100 mM NaCl (pH 7.4), and 25 μM Aβ42 was incubated with or without test compounds at 37°C. At each time point, 2.5 μL of Aβ42 solution was added to 250 μL of 1 mM Th-T in 50 mM Gly-NaOH (pH 8.5). The fluorescence intensity was measured at 420 nm excitation and 485 nm emission on a Powerscan HT multi-detection microplate reader (Dainippon Sumitomo Pharma).

4.4. Transmission electron microscopy

The procedure was as previously reported[30] with slight modifications. Five microliters of Aβ42 sample after the Th-T assay was spotted onto a glow-discharged, carbon-coated Formvar grid and incubated for 2 min before washing twice with 5 μL of distilled water. The resultant grid was negatively stained twice for 1 min with 5 μL of 0.4% silicotungstic acid. After air-drying for 5 min, samples were subjected to electron microscopy (JEOL JEM-1400).

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at ***.

References and notes

1. Hardy, J.; Allsop, D. *Trends Pharmacol. Sci.* **1991**, *12*, 383.
2. Hardy, J., Selkoe, D. J. *Science* **2002**, *297*, 353.
3. Hamley, I. W. *Chem. Rev.* **2012** *112*, 5147.
4. Haass, C.; Selkoe, D. J. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 101.
5. Masters, C. L.; Simms, G.; Weinman, N. A.; Multhaup, G.; McDonald, B. L.; Beyreuther, K. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 4245.
6. Glenner, G. G.; Wong, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 885.
7. Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, M. S.; Rowan, M. J.; Selkoe, D. J. *Nature* **2002**, *416*, 535.
8. Roychaudhuri, R.; Yang, M.; Hoshi, M. M.; Teplow, D. B. *J. Biol. Chem.* **2009**, *284*, 4749.
9. Gravitz, L. *Nature* **2011**, *475*, S9.
10. Miyamae, Y.; Kurisu, M.; Murakami, K; Han, J.;Isoda, H.; Irie, K.; Shigemori H. *Bioorg. Med. Chem.* **2012**, *20*, 5844.
11. Kurisu, M.; Miyamae, Y.; Murakami, K.; Han, J.; Isoda, H.; Irie, K.; Shigemori, H. *Biosci. Biotechnol. Biochem.* **2013**, *77*, 1329.
12. Kidachi, E.; Kurisu, M.; Miyamae, Y.; Hanaki, M.; Murakami, K.; Irie, K.; Shigemori, H. *Heterocycles* **2016**, *92*, 1976.
13. Aihara, Y.; Kawaguchi, A.; Hanaki, M.; Murakami, K.; Irie, K.; Shigemori, H. *Heterocycles* **2017**, *94*, 1280.
14. Yoshihara, T.; Yoshikawa, H.; Sakamura, S.; Sakuma, T. *Agri. Biol. Chem.* **1974**, *38*, 1107
15. Sanbogi, C.; Osakabe, N.; Natsume, M.; Takizawa, T.; Gomi, S.; Osawa, T. *J. Agric. Food Chem.* **1998**, *46*, 454
16. Arlorio, M.; Locatelli, M.; Travaglia, F.; Coisson, J. D.; Grosso, E. D.; Minassi, A.; Appendino, G.; Martelli, A. *Food Chem.* **2008**, *106*, 967.
17. Zeng, W. H.; Locatelli, M.; Bardelli, C.; Amoruso, A.; Coisson, J. D.; Travaglia, F.; Arlorio, M. *J. Agric. Food Chem.* **2011**, *59*, 5324
18. Fallarini, S.; Miglio, G.; Paoletti, T.; Minassi, A.; Amoruso, A.; Bardelli, C.; Brunelleschi, S.; Lombardi, G. *Br. J. Pharmacol.* **2009**, *157*, 1072.
19. Miyamae, Y.; Han, J.; Sasaki, K.; Terakawa, M.; Isoda, H.; Shigemori, H. *Cytotechnology* **2011**, *63*, 191.
20. Ishii, T.; Mori, T.; Tanaka, T.; Mizuno, D.; Yamaji, R.; Kumazawa, S.; Nakayama, T.; Akagawa, M. *Free Radic. Biol. Med.* **2008**, 45, 1384
21. Sato, M.; Murakami, K.; Uno, M.; Nakagawa, Y.; Katayama, S.; Akagi, K.; Masuda, Y.; Takegoshi, K.; Irie, K. *J. Biol. Chem.*, **2013**, *288*, 23212.
22. Gazit, E. *FASEB J.* **2002**, *16*, 77.
23. Reinke, A. A.; Gestwicki, J. E. *Chem. Biol. Drug. Des.* **2007**, *70*, 206.
24. Endo, H.; Nikaido, Y.; Nakadate, M.; Ise, S.; Konno, H. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5621.
25. Taguchi, R.; Hatayama, K.; Takahashi, T.; Hayashi, T.; Sato, Y.; Sato, D.; Ohta, K.; Nakano, H; Seki, C.; Endo, Y.; Tokuraku, K.; Uwai, K. *Eur. J. Med. Chem.* **2017**, *138*, 1066.
26. Lee, S.; Lee, C.-H.; Kim, E.; Jung, S.-H; Lee, H. K. *Koeran Chem. Soc.* **2007**, *28*, 1781.
27. Murakami, K.; Irie, K.; Ohigashi, H.; Hara, H.; Nagao, M.; Shimizu, T.; Shirasawa, T. *J. Am. Chem. Soc.* **2005**, *127*, 15168.
28. Murakami, K.; Murata, N.; Noda, Y.; Tahara, S.; Kaneko, T.; Kinoshita, N.; Hatsuta, H.; Murayama, S.; Barnham, K. J.; Irie, K.; Shirasawa, T.; Shimizu, T. *J. Biol. Chem.* **2011**, *286*, 44557.
29. Naiki, H.; Gejyo, F. *Methods Enzymol.* **1999**, *309*, 305.
30. Murakami, K.; Irie, K.; Morimoto, A.; Ohigashi, H.; Shindo, M.; Nagao, M.; Shimizu, T.; Shirasawa, T. *J. Biol. Chem.* **2003**, *278*, 46179.