HETEROCYCLES, Vol. 91, No. 4, 2015, pp. 764 - 781. © 2015 The Japan Institute of Heterocyclic Chemistry
Received, 29th January, 2015, Accepted, 24th February, 2015, Published online, 27th February, 2015
DOI: 10.3987/COM-15-13184

SYNTHESIS OF NEW HYDANTOINS BEARING GLUTARIMIDE OR SUCCINIMIDE MOIETY AND THEIR EVALUATION FOR CELL DIFFERENTIATION-INDUCING AND ANTI-ANGIOGENIC ACTIVITIES

Jun-ichi Yamaguchi,[1]* Tomomi Noguchi-Yachide,[2] Yuka Sakaguchi,[1] Chiaki Shibata,[1] Shinnosuke Kanuma,[1] Akiko Yoshizaki,[1] Yuka Takizawa,[1] and Yuichi Hashimoto[2]

1 Department of Applied Chemistry, Faculty of Engineering, Kanagawa Institute of Technology, 1030 Shimo-ogino, Atsugi, Kanagawa 243-0292, Japan; E-mail: yamagu@chem.kanagawa-it.ac.jp. 2 Institute of Molecular and Cellular Biosciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

Abstract — Several derivatives of hydantoin containing glutarimide or succinimide at the 3-position were synthesized. The new hydantoin derivatives had a structure similar to that of thalidomide, and so may possess activity similar to that of thalidomide and/or its analogs, such as effects on cell differentiation and angiogenesis. Some hydantoins showed enhancing effects on all-*trans* retinoic acid (ATRA)-induced cell differentiation of human leukemia cell line HL-60 and anti-angiogenic activity on human umbilical vein endothelial cells (HUVEC). These new hydantoin derivatives were more effective than thalidomide in cell differentiation-inducing activity on HL-60 and anti-angiogenic activity on HUVEC.

INTRODUCTION

Imidazolidine-2,4-dione (**1**), called hydantoin, and its derivatives have been investigated extensively and possess a wide range of biological activities.[1] Phenytoin (5,5-diphenylhydantoin, **2**) and ethotoin (3-ethyl-5-phenylhydantoin, **3**) are first-line medicines for the treatment of certain types of epileptic seizures.[2]

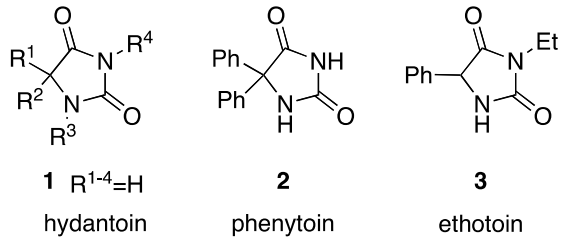
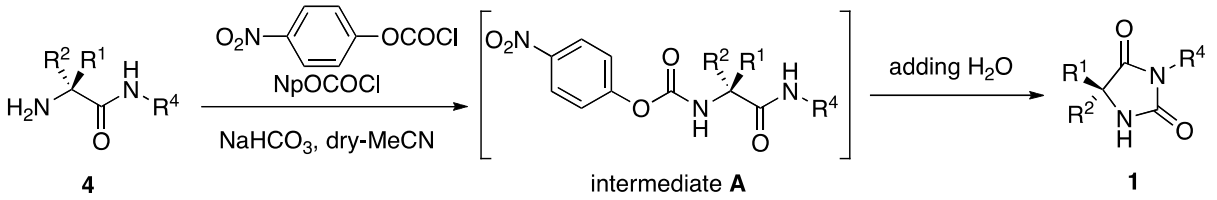


Figure 1. Hydantoin and its derivatives

A previous study reported a method for the one-pot preparation of optically active hydantoin derivatives (**1**) from optically active amino acid amides (**4**) using 4-nitropheny chloroformate (NpOCOCl) (Scheme 1). In the first step, treatment of **4** with NpOCOCl along with an excess of $NaHCO_3$ in dry MeCN resulted in a formation of intermediate **A**. After the addition of H_2O into the reaction mixture, optically active multi-substituted hydantoins (**1**) without racemization were obtained easily (Scheme 1),[3] allowing the synthesis of many types of hydantoin derivatives (**1**). Furthermore, synthesized hydantoin derivatives have been reported to possess the ability to act as a chiral auxiliary as effectively as oxazolidinone (Evans' chiral auxiliary).[4]



Scheme 1. One-pot preparation of multi-substituted hydantoins (**1**) from amino acid amide (**4**)

In contrast, α-(*N*-phthalimide)glutarimide (thalidomide, TDM) has been used as a sedative and/or hypnotic drug, but was banned in the 1960s due to its teratogenic effects. In 1998, TDM was approved as a treatment for Hansen's disease by the United States Food and Drug Administration (FDA), which was followed by investigations into other pharmacological applications of TDM and its derivatives.[5] The pharmacological effects of TDM include anti-tumor-promoting activities such as suppression of cell differentiation[6] and angiogenesis,[7] and many studies have been started to examine these new bioactivities. Since hydantoin (**1**) has a structural resemblance to the phthalimide moiety of TDM, the hydantoin derivatives (**5**) bearing a glutarimide group were expected to have the capacity to exceed the bioactivity of TDM (Figure 2). Compounds containing a glutarimide (piperidine-2,6-dione) moiety and a succinimide (pyrrolidine-2,5-dione) moiety are known to possess bioactivity,[8] so succinimide derivatives (**6**) also were synthesized.

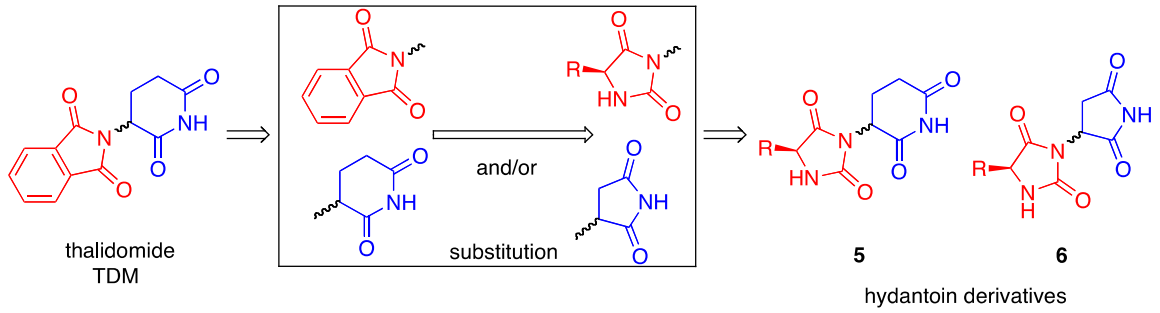
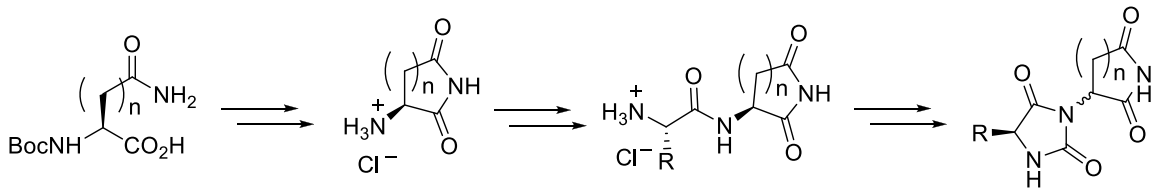


Figure 2. Conception of the present research

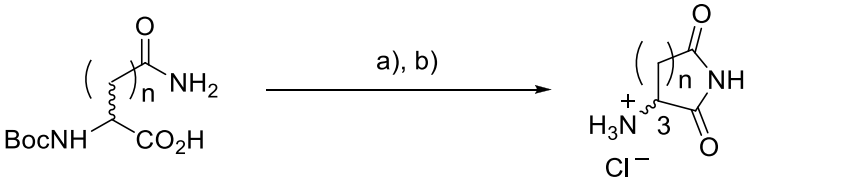
The synthetic plan for the new hydantoin derivatives (**5** or **6**) is shown in Scheme 2. *N*-*tert*-Butoxycarbonyl (Boc)-glutamine (**7**) or *N*-Boc-asparagine (**8**) converted to 2-aminoglutarimide (**9**) or -succinimide (**10**), respectively. Condensation of *N*-Boc amino acid with **9** or **10** and then removal of a Boc group gave the corresponding amino acid amide (**11** or **12**). Finally, the amino acid amide (**11** or **12**) transformed into the desired hydantoin derivative (**5** or **6**).[3]



Scheme 2. Synthetic plan of new hydantoin derivatives (**5** or **6**)

RESULTS AND DISCUSSION

Syntheses of 5 and 6: Fox and co-workers reported that (*S*)-3-aminoglutarimide (**(*S*)-9**) could be prepared without racemization.[9] Therefore, the first attempt involved the preparation of (*S*)-3-amino-glutarimides (**(*S*)-9**) or (*S*)-3-aminosuccinimides (**(*S*)-10**) as hydrogen chloride salts. The treatment of (*S*)-*N*-Boc-glutamine (**(*S*)-7**) or (*S*)-*N*-Boc-asparagine (**(*S*)-8**) with water-soluble carbodiimide (WSC)

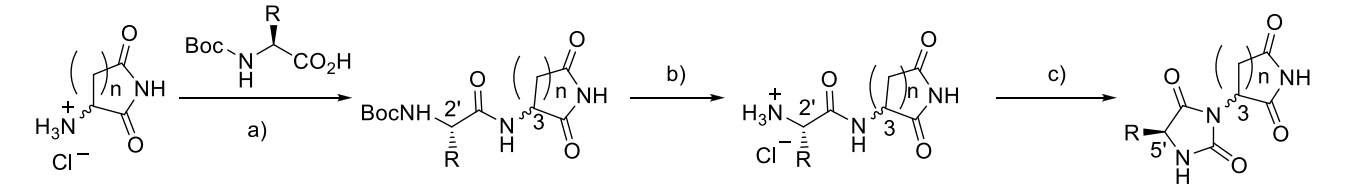


a) WSC HCl, HOSu, DMF, 60 °C, 6 h. b) 4M HCl dioxane.

Scheme 3. Preparation of imide derivatives (**9** or **10**)

HCl and *N*-hydroxysuccinimide (HOSu) in DMF resulted in the formation of 2-(*N*-Boc-amino)glutarimide or –succinimide, respectively. Removal of a Boc group from the imide yielded the (*S*)-cyclic imides (**(*S*)-9** and **(*S*)-10**), respectively. (*R*)-3-Aminoglutarimide hydrochloride (**(*R*)-9**) and (*R*)-3-aminosuccinimide hydrochloride (**(*R*)-10**) also were prepared using the same method. HPLC analysis of **9** and **10** indicated that no racemization of the cyclic imides (**9** and **10)** occurred.

Condensation of (*S*)-**9** with (*S*)-*N*-Boc-amino acid was performed using a WSC HCl - 1-hydroxybenzotriazole (HOBt) method in the presence of *N*-methylmorpholine (NMM) in DMF to give (3*S*,2'*S*)-*N*-Boc-amino-acylaminoglutarimide (**(3*S*,2'*S*)-13**). (3*S*,2'*S*)-*N*-Boc-amino-acylamino-succinimide **(3*S*,2'*S*)-14**) also was prepared using the same reaction conditions used to prepare **(3*S*,2'*S*)-13**. The *N*-Boc group of **(3*S*,2'*S*)-13** and **(3*S*,2'*S*)-14** was removed by treatment with a dioxane solution of HCl to give the amino acid amide (**(3*S*,2'*S*)-11** or **(3*S*,2'*S*)-12**, respectively) as the HCl salt. Several of these HCl salts were hygroscopic, so these compounds were isolated as free amines. Their diastereomers, **(3*R*,2'*S*)-11** or **(3*R*,2'*S*)-12**, were prepared from **(*R*)-9** and **(*R*)-10**, respectively. Comparison of the ^{1}H NMR spectra of compounds **11** and **12** indicated no racemization occurred at the 3-position of glutarimide or succinimide. In the final transformation, the new hydantoin derivatives **5** and **6** were produced by the synthetic method for hydantoin derivatives using 4-nitropheny chloroformate.[3] However, partial racemization of several hydantoin derivatives (**5** and **6**) occurred in the final step; therefore, **5** and **6** were produced as diastereomer mixtures. For example, hydantoin formation of **(3*S*,2'*S*)-11-F** resulted in an 83:17 mixture of hydantoin derivatives **(3*S*,5'*S*)-5** and **(3*R*,5'*S*)-5**. As shown in Table 1, the ratio of diastereomers was determined by HPLC analysis or integration of ^{1}H NMR spectra. We have reported that no racemization occurred at a 5-position of a hydantoin ring in a hydantoin formation used NpOCOCl.[3] Therefore, these compounds (**5** and **6**) can occurred on a racemization at not the 5'-position of hydantoin but the 3-position of glutarimide or succinimide moiety. Figure 3 shows six new hydantoin derivatives: **5-F** and **6-F** synthesized from (*S*)-phenylalanine; **5-V** and **6-V** synthesized from (*S*)-valine; and **5-L** and **6-L** synthesized from (*S*)-leucine. For the examination of bioactivity, the hydantoin derivatives were used as a mixture of diastereomers (ratio *ca*. 1:1, Table 2).



a) WSC HCl, HOBt, NMM, DMF, overnight. b) 4M HCl dioxane. c) i. NpCOCl, $NaHCO_3$, MeCN, rt, 3 h; ii. adding H_2O.

Scheme 4. Synthesis of hydantoin derivatives **5** or **6**

Table 1. Synthesis of **5** or **6** from **7** or **8**

Entry	n	R		Stereochemistry **13** or **14**	Yield/% **13** or **14**	Salt or Free **11** or **12**	Yield/% **5** or **6**	Ratio of **5** or **6** (3*S*,5'*S*):(3*R*,5'*S*)
1	2	-CH_2Ph	F	(3*S*,2'*S*)	96	HCl	70	83:17 [a)]
2	2	-CH_2Ph	F	(3*R*,2'*S*)	91	HCl	86	8:92 [a)]
3	1	-CH_2Ph	F	(3*S*,2'*S*)	84	HCl	85	100:0 [b)]
4	1	-CH_2Ph	F	(3*R*,2'*S*)	83	Free	21	0:100 [b)]
5	2	-*i*-Pr	V	(3*S*,2'*S*)	81	HCl	66	85:15 [a)]
6	2	-*i*-Pr	V	(3*R*,2'*S*)	81	HCl	71	17:83 [a)]
7	1	-*i*-Pr	V	(3*S*,2'*S*)	84	Free	71	77:23 [a)]
8	1	-*i*-Pr	V	(3*R*,2'*S*)	39	Free	67	0:100 [a)]
9	2	-*i*-Bu	L	(3*S*,2'*S*)	96	HCl	73	85:15 [a)]
10	2	-*i*-Bu	L	(3*R*,2'*S*)	71	HCl	78	0:100 [a)]
11	1	-*i*-Bu	L	(3*S*,2'*S*)	93	Free	74	100:0 [b)]
12	1	-*i*-Bu	L	(3*R*,2'*S*)	42	Free	32	0:100 [b)]

a) Determined by HPLC analysis. Conditions: HPLC: column; Inertsil®ODS-3, 4.6 x 300 mm, Speed; 1.0 mL/min, Detection; 220 or 254 nm. Eluent and retention time were described in Table 2. b) Determined by integration of ^{1}H NMR spectra.

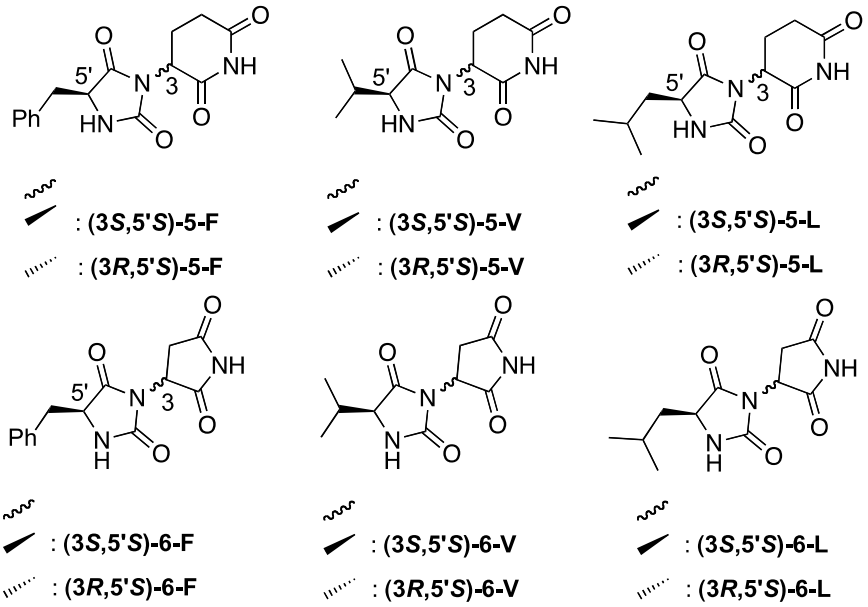


Figure 3. Synthesized hydantoin derivatives **5** or **6**

Effects on ATRA-Induced HL-60 Cell Differentiation: First, effects on HL-60 cell differentiation-inducing activity was investigated. Activity was estimated in terms of the nitroblue

tetrazolium (NBT)-reducing activity of cells as reported previously.[10] All hydantoin derivatives (**5** and **6**) were used as a mixture of diastereomers, and a mixture ratio was determined by HPLC analysis or integration of ^{1}H NMR (Table 2). All the hydantoin derivatives, as well as TDM, showed no differentiation-inducing activity on HL-60 cells. However, they enhanced HL-60 cell differentiation induced by low concentration of ATRA. In our experimental conditions, untreated HL-60 cells contained 2–5% NBT-positive cells, and treatment of the cells with 1.6 nM ATRA resulted in 20% NBT-positive cells. Addition of 100 μM TDM enhanced the cell differentiation-inducing activity elicited by 1.6 nM ATRA, *i.e.*, the population of NBT-positive cells was increased to 27%. As shown in Figure 4, every new hydantoin derivative exhibited enhancing activity on HL-60 cell differentiation-inducing activity at 100 μM in the presence of 1.6 nM ATRA under the experimental conditions used with the population of NBT-positive cells were 33–49%, and which were more active than TDM (27%). The activity of the hydantoin derivatives **5-F** and **6-F**, which contain a benzyl group at the 5-position of the hydantoin ring was greater than that of the other hydantoin derivatives.

Table 2. Mixture ratio of diastereomer and data

	Equipment	Ratio (3*S*,5'*S*):(3*R*,5'*S*)	Retention Time (min) (3*S*,5'*S*)/(3*R*,5'*S*)	Eluent MeCN:0.1%TFAaq.
5-F	HPLC	46:54	73.1/78.2	10:90
5-V	HPLC	55:45	48.2/56.3	5:95
5-L	HPLC	47:53	55.3/59.3	10:90
6-F	^{1}H NMR	57:43		
6-V	HPLC	61:39	27.2/30.4	5:95
6-L	^{1}H NMR	50:50		

Conditions: HPLC: column; Inertsil®ODS-3, 4.6 x 300 mm, Speed; 1.0 mL/min, Detection; 220 or 254 nm. ^{1}H NMR: solvent: DMSO-d_6.

Anti-angiogenic Activity: Next, the anti-angiogenic activity of the hydantoin derivatives (**5** and **6**) was examined using an assay to determine tube formation-inhibitory activity toward HUVEC cells as described previously.[7] A 100 μM solution of **5** or **6** was treated with the HUVEC cells. Results are shown in Figure 5. Nearly all of the hydantoin derivatives exhibited weaker activity compared to TDM, except for **5-V** and **6-V**. Among all of the new hydantoin derivatives, **6-V** possessed the most potent tube formation inhibitory activity on HUVEC.

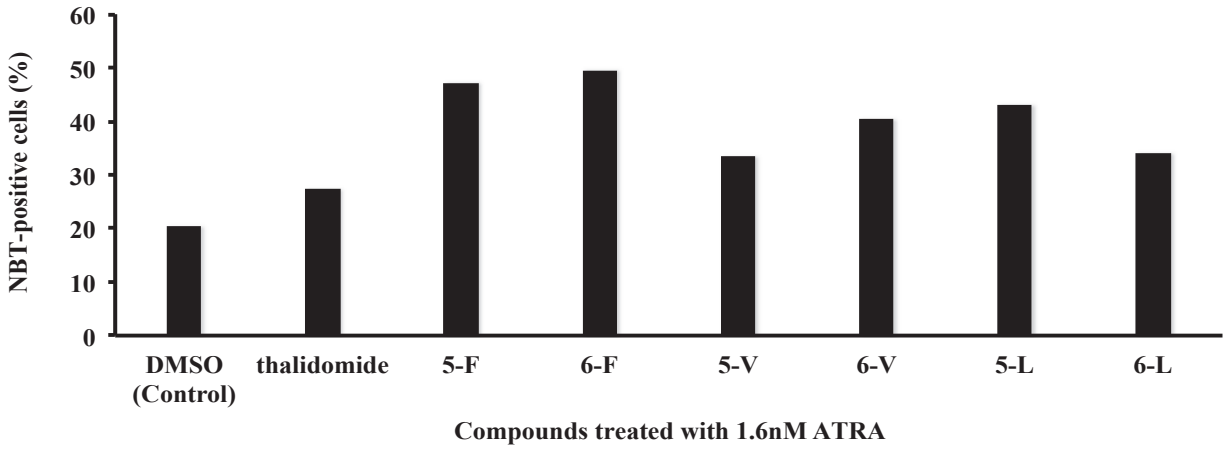


Figure 4. Effects of **5** or **6** (100 μM) on ATRA-induced HL-60 Cell differentiation

Vertical scale: The percentage of NBT-positive cells treated with 100 μM of indicated compounds in the presence of 1.6 nM ATRA. The percentage of NBT-positive cells in the presence of 1.6 nM ATRA alone was 27%.

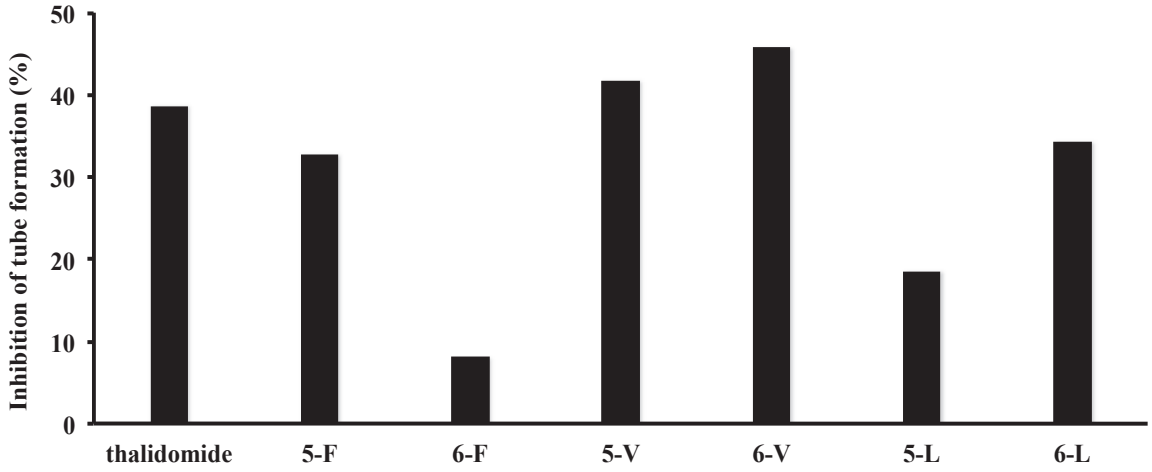


Figure 5. HUVEC tube formation-inhibiting activity of **5** or **6** (100 μM)

Vertical scale: The inhibitory degree (%) of tube formation measured by means of the number of pixels using MetaMorph software (Universal Imaging, Downingtown, PA) after photographing cultured HUVECs.

In conclusion, the synthesis of new hydantoin derivatives containing a glutarimide or succinimide moiety revealed that these products possessed moderate bioactivity. Further investigations based on these hydantoins are in progress.

EXPERIMENTAL

All melting points were determined using Yanaco melting point apparatus (MP-500D) and are uncorrected. Optically rotation was recorded on JASCO P-2200 Polarimeter. IR spectra were recorded

on a JASCO FT/IR-4100 spectrometer in the range from 4000 to 400 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on JEOL ECX400 spectrometer. Chemical Shifts are reported in ppm (parts per million) either tetramethylsilane (TMS) or residual solvent signal as an internal reference. Coupling constants (*J*) are given in Hz. High-resolution MS spectra were measured on LCT Premier XE (Micromass®). HPLC was measured on JASCO system (PU-2089, UV-970 or UV-2075, AS-2055) equipped with reversed-phased column (Inertsil®ODS-3, 4.6 x 300 mm).

General procedure for preparation of 3-(*tert*-butoxycarbonylamino)piperidine-2,6-dione or -pyrrolidine-2,5-dione: To a DMF solution of the starting material was added HOSu and WSC hydrochloride. The reaction mixture was heated at 80 °C for 6 h. After removal of DMF under reduced pressure, the organic material was extracted with EtOAc. The extract was washed with 10% citric acid, 5% aq. $NaHCO_3$, and brine. After dried over Na_2SO_4, the extract was condensed under reduced pressure. The residue was purified with reprecipitation from MeOH-Et_2O-hexane.

(*S*)-3-(*tert*-Butoxycarbonylamino)piperidine-2,6-dione: mp 155-156 °C (mp 152-153 °C)[8]; $[\alpha]^{25}_D$ 55.1 (*c* 1.0, MeOH) ($[\alpha]^{20}_D$ -52.2 (*c* 0.63, MeOH))[8]; IR (KBr): 1668, 1609 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.47 (9H, s), 1.87 (1H, ddd, *J*= 18.8, 12.8, and 5.0 Hz), 2.50-2.55 (1H, m), 2.68 (1H, ddd, *J*= 18.8, 13.8 and 5.4 Hz), 2.81 (1H, brd, *J*= 18.8 Hz), 4.20-4.40 (1H, m), 5.2 (1H, brs), 7.84 (1H, brs); ^{13}C NMR (CD_3OD, 100 MHz) δ 26.03, 28.68, 32.21, 52.29, 80.66, 158.03, 174.01, 174.96; HRMS Found: 251.0978; Calcd: 251.1008 ($C_{10}H_{16}N_2O_4Na^+$).

(*R*)-3-(*tert*-Butoxycarbonylamino)piperidine-2,6-dione: mp 153-155 °C; $[\alpha]^{25}_D$ +46.2 (*c* 0.75, MeOH); IR (KBr): 1731, 1695 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.82 (9H, s), 2.36 (1H, qd, *J*= 12.8 and 4.6 Hz), 2.48 (1H, dtd, *J*= 12.4, 5.5 and 2.8 Hz), 3.01 (1H, ddd, *J*= 17.8, 4.6, and 2.8 Hz), 3.10 (1H, ddd, *J*= 17.8, 12.4, and 5.5 Hz), 4.67 (1H, dd, *J*= 12.4 and 5.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 26.03, 28.68, 32.21, 52.29, 80.66, 158.04, 174.01, 174.95; HRMS Found: 251.0973; Calcd: 251.1008 ($C_{10}H_{16}N_2O_4Na^+$).

(*S*)-2-(*tert*-Butoxycarbonylamino)pyrrolidine-2,5-dione: mp 172-173 °C; $[\alpha]^{25}_D$ -37.9 (*c* 1.0, MeOH); IR (KBr): 1713 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.45 (9H, s), 2.87 (1H, dd, *J*= 17.8 and 6.4 Hz), 3.10 (1H, dd, *J*= 17.8 and 9.2 Hz), 4.20-4.40 (1H, m), 5.22 (1H, brd, *J*= 5.5 Hz), 8.05 (1H, brs); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.22, 37.05, 51.26, 81.22, 155.18, 174.00, 175.82; HRMS Found: 237.0818; Calcd: 237.0851 ($C_9H_{14}N_2O_4Na^+$).

(*R*)-3-(*tert*-Butoxycarbonylamino)pyrrolidine-2,5-dione: mp 150 °C (decomp.); $[\alpha]^{25}_D$ +35.4 (*c* 1.0, MeOH); IR (KBr): 1709 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.43 (9H, s), 2.93 (1H, dd, *J*= 12.8 and 6.0 Hz), 3.00 (1H, dd, *J*= 12.8 and 9.2 Hz), 4.34 (1H, dd, *J*= 9.2 and 6.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz)

δ 28.71, 37.54, 52.17, 81.06, 157.63, 178.15, 179.90; HRMS Found: 237.0855; Calcd: 237.0851 ($C_9H_{14}N_2O_4Na^+$).

General procedure for removal of Boc group: The 4*M*-HCl-dioxane (3.5 mL) of (*S*)-3-Boc-amino-piperidine-2,6-dione (152 mg) was stirred for 1 h at room temperature. After removal of dioxane under reduced pressure, the residue was purified with reprecipitation from MeOH-Et_2O-hexane. (*S*)-3-Amino-piperidine-2,6-dione hydrochloride (**(*S*)-9**) (113 mg, quant.) was given.

HPLC Conditions of racemization check of **9** and **10**: Column: Chiralpak IA (4.6 mm x 250 mm, Daicel); Eluent: **9**: hexane: *i*-PrOH: MeOH: Et_2NH=60: 10: 30: 0.1, 1 mL/min. **10**: hexane: EtOH: MeOH= 40: 20: 20, 1 mL/min.; Detection: 220 nm; Retention time: **(*S*)-9**: 17.2 min, **(*R*)-9**: 21.4 min, **(*S*)-10**: 10.3 min, **(*R*)-10**: 15.1 min.

(*S*)-3-Aminopiperidine-2,6-dione hydrochloride, (*S*)-9: mp 218-220 °C; $[\alpha]^{25}_D$ -49.3 (*c* 1.0, MeOH), ($[\alpha]^{24}_D$ -53.5 (*c* 1.0, MeOH))[11]; IR (KBr): 1703, 1673 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 2.05-2.15 (1H, m), 2.45-2.50 (1H, m), 2.90-3.00 (2H, m), 4.10-4.20 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 23.81, 31.30, 50.94, 170.68, 173.46; HRMS Found:129.0661; Calcd: 129.0664 ($C_5H_9N_2O_2^+$).

(*R*)-3-Aminopiperidine-2,6-dione hydrochloride, (*R*)-9: mp 218-221 °C; $[\alpha]^{25}_D$ +40.9 (*c* 1.0, MeOH); IR (KBr) 1702 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.90-2.05 (1H, m), 2.20-2.30 (1H, m), 2.60-2.70 (2H, m), 4.17 (1H, dd, *J*= 13.3 and 5.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 23.81, 31.30, 50.93, 170.69, 173.46; HRMS Found:129.0659; Calcd: 129.0664 ($C_5H_9N_2O_2^+$).

(*S*)-2-Aminopyrrolidine-2,5-dione hydrochloride, (*S*)-10: mp 206-208 °C; $[\alpha]^{25}_D$ -74.9 (*c* 1.0, MeOH); IR (KBr): 1728 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 2.69 (1H, dd, *J*= 17.9 and 5.9 Hz), 3.12 (1H, dd, *J*= 17.9 and 9.2 Hz), 4.39 (1H, dd, *J*= 9.2 and 5.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 35.51, 50.22, 175.39, 175.50; HRMS Found: 115.0509; Calcd: 115.0508 ($C_4H_7N_2O_2^+$).

(*R*)-2-Aminopyrrolidine-2,5-dione hydrochloride, (*R*)-10: mp 214-216 °C (decomp.); $[\alpha]^{25}_D$ +99.8 (*c* 1.0, MeOH); IR (KBr): 1727 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 2.69 (1H, dd, *J*= 17.8 and 6.4 Hz), 3.13 (1H, dd, *J*= 17.8 and 9.6 Hz), 4.39 (1H, dd, *J*= 9.6 and 6.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 35.5, 50.22, 175.42, 175.60; HRMS Found: 115.0510; Calcd: 115.0508 ($C_4H_7N_2O_2^+$).

General procedure for condensation of the staring material with *N*-Boc-amino acid: To a DMF (5 mL) solution of (*S*)-3-aminopiperidine-2,6-dione hydrochloride (**(*S*)-9**) (101 mg), *N*-Boc-phenylalanine (177 mg), HOBt hydrate (109 mg), DIEA (0.116 mL) was added WSC hydrochloride (133 mg) at room temperature. The reaction mixture was stirred for over night, and DMF was removed under reduced pressure. The organic materials were extracted with EtOAc, and the extract was washed with 10% citric

acid, 5% aq. $NaHCO_3$, and brine. After dried over Na_2SO_4, the extract was condensed under reduced pressure. The residue was purified with preparative TLC ($CHCl_3$:MeOH=9:1), and the desired compound (223 mg, 98%) was given.

(3*S*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylphenylalanylamino)piperidine-2,6-dione, (3*S*,2'*S*)-13-F: mp 179 °C (decomp.); $[\alpha]^{25}_D$ -35.7 (*c* 0.75, MeOH); IR (KBr): 1713, 1681, 1656 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.41 (9H, s), 1.77 (1H, qd, *J*= 12.8 and 5.0 Hz), 2.45 (1H, m), 2.65-2.85 (2H, m), 3.10 (2H, d, *J*= 6.0 Hz), 4.35-4.50 (2H, m), 4.90-5.05 (1H, m), 6.80-6.85 (1H, brs), 7.15-7.30 (5H, m), 8.16 (1H, brs); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.62, 28.23, 31.10, 38.15, 50.84, 55.56, 80.50, 127.09, 128.71, 129.27, 136.11, 155.30, 170.73, 171.09, 171.85; HRMS Found: 398.1683; Calcd: 398.1692 ($C_{19}H_{25}N_3O_5Na^+$).

(3*R*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylphenylalanylamino)piperidine-2,6-dione, (3*R*,2'*S*)-13-F: mp 197 °C (decomp.); $[\alpha]^{25}_D$ +29.1 (*c* 0.75, MeOH); IR (KBr): 1785, 1722, 1682, 1654 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.36 (9H, s), 1.88 (1H, ddd, *J*= 17.4, 12.8, and 4.6 Hz), 2.03 (1H, dddd, *J*= 17.8, 12.3, 12.3, and 2.8 Hz), 2.59 (1H, ddd, *J*= 17.8, 4.6, and 2.8 Hz), 2.72 (1H, ddd, *J*= 17.8, 12.3, and 5.5 Hz), 2.85 (dd (1H, dd, *J*= 14.2 and 8.7 Hz), 3.12 (1H, dd, *J*= 14.2 and 6.0 Hz), 4.30-4.40 (1H, m), 4.59 (1H, dd, *J*= 12.3 and 5.5 Hz), 6.20-6.25 (1H, brs), 6.69 (1H, brd, *J*= 8.2 Hz), 7.15-7.30 (5H, m); ^{13}CNMR(CD_3OD, 100 MHz) δ 25.54, 28.64, 39.53, 51.07, 57.43, 80.67, 127.67, 129.39, 130.45, 138.60, 157.50, 173.10, 174.29, 174.74; HRMS Found: 398.1683; Calcd: 398.1692 ($C_{19}H_{25}N_3O_5Na^+$).

(3*S*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylvalinyl)piperidine-2,6-dione, (3*S*,2'*S*)-13-V: mp 161 °C (decomp.); $[\alpha]^{25}_D$ -53.4 (*c* 0.75, MeOH); IR (KBr): 1718, 1683, 1654 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.93 (3H, d, *J*= 6.9 Hz), 0.98 (3H, d, *J*= 6.4 Hz), 1.44 (9H, s), 1.84 (1H, qd, *J*= 12.8 and 5.5 Hz), 2.05-2.25 (1H, m), 2.45-2.60 (1H, m), 2.65-2.85 (2H, m), 3.90-4.05 (1H, m), 4.54 (1H, dt, *J*= 12.8 and 6.4 Hz), 5.04 (1H, d, *J*= 8.7 Hz), 6.78 (1H, brs), 8.20 (1H, brs); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.74, 19.27, 24.79, 28.29, 30.77, 31.16, 50.77, 59.98, 80.23, 155.77, 170.99, 171.12, 172.19; HRMS Found: 350.1703; Calcd: 350.1692 ($C_{15}H_{25}N_3O_5Na^+$).

(3*R*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylvalinyl)piperidine-2,6-dione, (3*R*,2'*S*)-13-V: mp 96 °C; $[\alpha]^{25}_D$ +5.6 (*c* 0.75, MeOH); IR (KBr) 1709 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.93 (3H, d, *J*= 6.8 Hz), 0.98 (3H, d, *J*= 6.8 Hz), 1.88 (1H, qd, *J*= 12.4 and 5.5 Hz), 2.17 (1H, dsept, *J*= 6.8 and 6.8 Hz), 2.45-2.60 (1H, m), 2.65-2.85 (2H, m), 4.05-4.15 (1H, m), 4.55-4.70 (1H, m), 5.14 (1H, d, *J*= 8.2 Hz), 7.05 (1H, s), 8.60 (1H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ 18.25, 19.75, 25.27, 28.70, 32.011, 32.15, 61.52, 80.60, 157.88, 172.98, 174.50, 174.83. HRMS Found: 350.1703; Calcd: 350.1692 ($C_{15}H_{25}N_3O_5Na^+$).

(3*S*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylleucinyl)piperidine-2,6-dione, (3*S*,2'*S*)-13-L: mp 205 °C (decomp.); $[\alpha]^{25}_D$ -53.5 (*c* 0.75, MeOH); IR (KBr) 1718, 1681, 1655 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.86 (3H, d, *J*= 6.9 Hz), 0.88 (3H, d, *J*= 6.9 Hz), 1.45 (2H, t, *J*= 6.9 Hz), 1.85-1.95 (2H, m), 2.45-2.55 (1H, m),

2.72 (1H, dt, *J*= 17.0 and 8.7 Hz), 3.97 (1H, q, *J*= 7.8 Hz), 4.54 (1H, q, *J*= 8.7 Hz), 6.83 (1H, d, *J*= 8.7 Hz), 8.03 (1H, d, *J*= 8.7 Hz), 10.75 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 21.49, 23.07, 24.19, 28.20, 40.87, 48.93, 52.72, 77.93, 155.27, 0172.08, 172.62, 172.93; HRMS Found: 364.1849; Calcd: 364.1848 ($C_{16}H_{27}N_3O_5Na^+$).

(3*R*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylleucinyl)piperidine-2,6-dione, (3*R*,2'*S*)-13-L: mp 109 °C (decomp.); $[\alpha]^{25}_D$ -3.1 (*c* 0.50, MeOH); IR (KBr) 1707 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.86 (3H, d, *J*= 6.9 Hz), 0.88 (3H, d, *J*= 7.3 Hz), 1.20-1.50 (2H, m), 1.39 (9H, s), 1.55-1.70 (1H, m), 1.85-1.95 (2H, m), 2.45-2.55 (1H, m), 2.60-2.80 (2H, m), 3.95-4.05 (1H, m), 4.45-4.55 (1H, m), 6.87 (1H, d, *J*= 8.7 Hz), 8.18 (1H, d, *J*= 8.2 Hz), 10.82 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 21.46, 22.91, 24.18, 28.13, 30.67, 40.82, 49.06, 52.58, 77.90, 155.18, 171.83, 172.37, 172.78; HRMS Found: 364.1841; Calcd: 364.1848 ($C_{16}H_{27}N_3O_5Na^+$).

(3*S*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylphenylalanylamino)pyrrolidine-2,5-dione, (3*S*,2'*S*)-14-F: mp 151-153 °C (decomp); $[\alpha]^{25}_D$ -22.1 (*c* 0.75, MeOH); IR (KBr) 1825, 1784, 1736, 1709, 1658 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.35 (9H, s), 2.57 (1H, dd, *J*= 17.9 and 6.0 Hz), 2.82 (1H, dd, *J*= 13.7 and 8.7 Hz), 2.92 (1H, dd, *J*= 17.9 and 9.2 Hz), 3.10 (1H, dd, *J*= 13.7 and 5.5 Hz), 4.31 (1H, dd, *J*= 8.7 and 5.5 Hz), 4.47 (1H, dd, *J*= 9.2 and 6.7 Hz), 6.20-6.25 (1H, brs), 6.68 (1H, brd, *J*= 8.2 Hz), 7.15-7.30 (5H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 27.35, 35.77, 37.95, 50.17, 55.80, 79.40, 126.44, 128.14, 129.15, 137.17, 156.25, 173.27, 176.75, 177.52; HRMS Found: 384.1351; Calcd: 384.1535 ($C_{18}H_{23}N_3O_5Na^+$).

(3*R*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylphenylalanylamino)pyrrolidine-2,5-dione, (3*R*,2'*S*)-14-F: mp 147-149 °C; $[\alpha]^{25}_D$ +12.5 (*c* 0.75, MeOH); IR (KBr) 1781, 1721, 1648, 1657 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.35 (9H, s), 2.52 (1H, dd, *J*= 17.9 and 5.5 Hz), 2.81 (1H, dd, *J*=14.2 and 9.1 Hz), 2.94 (1H, dd, *J*= 17.9 and 9.2 Hz), 3.10 (1H, dd, *J*= 14.2 and 5.5 Hz), 4.20-4.35 (1H, m), 4.52 (1H, dd, *J*= 9.2 and 5.5 Hz), 6.25-6.30 (1H, brs), 6.70 (1H, brd, *J*= 7.8 Hz), 7.15-7.30 (5H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 28.61, 37.25, 39.15, 51.38, 57.10, 80.62, 127.66, 129.38, 130.45, 138.54, 157.56, 174.67, 178.03, 178.76, 384.1533; Calcd: 384.1535 ($C_{18}H_{23}N_3O_5Na^+$).

(3*S*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylvalinylamino)pyrrolidine-2,5-dione, (3*S*,2'*S*)-14-V: mp 98-100 °C; $[\alpha]^{25}_D$ -17.0 (*c* 0.25, MeOH); IR (KBr) 1741, 1695 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.93 (3H, d, *J*= 6.9 Hz), 0.96 (3H, d, *J*= 6.9 Hz), 1.45 (9H, s), 2.04 (1H, septd, *J*= 6.9 and 6.4 Hz), 2.65 (1H, dd, *J*= 17.9 and 6.0 Hz), 2.99 (1H, dd, *J*= 17.9 and 9.6 Hz), 3.86 (1H, d, *J*= 6.4 Hz), 4.54 (1H, dd, *J*= 9.6 and 6.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 18.31, 19.66, 32.05, 37.11, 51.40, 61.44, 80.69, 157.94, 174.81, 177.98, 178.70; HRMS Found: 336.1526; Calcd: 336.1535 ($C_{14}H_{23}N_3O_5Na^+$).

(3*R*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylvalinylamino)pyrrolidine-2,5-dione, (3*R*,2'*S*)-14-V: mp 164-165 °C; $[\alpha]^{25}_D$ +28.0 (*c* 1.0, MeOH); IR (KBr) 1728, 1658 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.95 (3H, d,

J= 6.9 Hz), 0.98 (3H, d, *J*= 6.9 Hz), 1.45 (9H, s), 2.05-2.20 (1H, m), 2.85-2.95 (1H, m), 3.10 (1H, dd, *J*= 18.3 and 9.6 Hz), 3.90 (1H, dd, *J*= 8.7 and 6.9 Hz), 4.45-4.55 (1H, m), 5.01 (1H, d, *J*= 8.2 Hz), 6.91 (1H, brs), 8.28 (1H, brs); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.50, 19.64, 28.68, 32.02, 37.10, 51.69, 61.15, 80.57, 157.95, 174.86, 178.13, 178.67; HRMS Found: 336.1534; Calcd: 336.1535 ($C_{14}H_{23}N_3O_5Na^+$).

(3*S*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylleucinylamino)pyrrolidine-2,5-dione, (3*S*,2'*S*)-14-L: mp 97-99 °C; $[\alpha]^{25}_D$ -10.6 (*c* 1.0, MeOH); IR (KBr): 1722, 1665 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.92 (3H, d, *J*= 7.3 Hz), 0.95 (3H, d, *J*= 6.9 Hz), 1.44 (9H, s), 1.50-1.55 (2H, m), 1.65-1.75 (1H, m), 2.64 (2H, dd, *J*= 17.4 and 5.5 Hz), 2.98 (3H, dd, *J*= 17.4 and 8.7 Hz), 4.00-4.10 (1H, m), 4.53 (1H, dd, *J*= 8.7 and 5.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.91, 23.40, 25.90, 28.70, 37.17, 42.20, 51.37, 54.45, 80.67, 157.84, 176.13, 178.00, 178.85; HRMS Found: 350.1690; Calcd: 350.1692 ($C_{15}H_{25}N_3O_5Na^+$).

(3*R*,2'*S*)-3-(*N*-*tert*-Butoxycarbonylleucinylamino)pyrrolidine-2,5-dione, (3*R*,2'*S*)-14-L: mp 163-165 °C; $[\alpha]^{25}_D$ -7.9 (*c* 0.50, MeOH); IR (KBr) 1721, 1663 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.93 (3H, d, *J*= 6.4 Hz), 0.95 (3H, d, *J*= 6.4 Hz), 1.43 (9H, s), 1.45-1.55 (2H, m), 1.65-1.75 (1H, m), 2.65 (1H, dd, *J*= 17.8 and 6.0 Hz), 2.97 (1H, dd, *J*= 17.8 and 9.8 Hz), 4.07 (1H, dd, *J*= 10.1 and 5.5 Hz), 4.46 (1H, dd, *J*= 9.1 and 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.94, 23.42, 25.82, 28.69, 37.18, 42.04, 51.59, 54.09, 80.58, 157.88, 176.11, 178.12, 178.83; HRMS Found: 350.1691; Calcd: 350.1692 ($C_{15}H_{25}N_3O_5Na^+$).

General procedure for removal of Boc group: The 4*M*-HCl-dioxane (28 mL) solution of (3*R,*2'*S*)-3-(*N*-*tert*-butoxycarbonylphenylalanylamino)piperidine-2,6-dione (**(3*R*,2'*S*)-13-F**) (2.07 g) was stirred for 1 h at room temperature. After removal of dioxane under reduced pressure, the residue was purified with reprecipitation from MeOH-Et_2O-hexane. (3*R,*2'*S*)-3-(Phenylalanylamino)piperidine-2,6-dione Hydrochloride (**(3*R*,2'*S*)-11-F**) (1.73 g, quant.) was given. If a corresponding ammonium chloride salt was hygroscopic, it was washed with 5% $NaHCO_3$ and then the organic material was extracted with EtOAc. After drying with Na_2SO_4 and condensed under reduced pressure, the desired material was given as free amine.

(3*S*,2'*S*)-3-(Phenylalanylamino)piperidine-2,6-dione hydrochloride, (3*S*,2'*S*)-11-F: mp 169 °C (decomp.); $[\alpha]^{25}_D$ -19.4 (*c* 0.10, MeOH); IR (KBr) 1699 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.05-2.20 (2H, m), 2.68 (1H, dt, *J*= 14.2 and 3.2 Hz), 2.70-2.85 (1H, m), 3.10 (1H, dd, *J*= 14.2 and 8.2 Hz), 3.33 (1H, dd, *J*= 14.2 and 5.5 Hz), 4.16 (1H, dd, *J*= 8.2 and 5.5 Hz), 4.62 (1H, dd, *J*= 10.5 and 7.3 Hz), 7.30-7.40 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.38, 32.01, 38.56, 51.37, 55.71, 128.86, 130.11, 130.65, 135.43, 169.80, 172.85, 174.62; HRMS Found: 276.1344; Calcd: 276.1348 ($C_{14}H_{18}N_3O_3^+$).

(3*R*,2'*S*)-3-(Phenylalanylamino)piperidine-2,6-dione hydrochloride, (3*R*,2'*S*)-11-F: mp 154 °C (decomp.); $[\alpha]^{25}_D$ +81.5 (*c* 1.52, MeOH); IR (KBr) 1699 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.80 (1H,

qd, *J*= 12.8 and 4.6 Hz), 1.85-1.95 (1H, m), 2.59 (1H, ddd, *J*= 17.8, 4.6, and 2.3 Hz), 2.72 (1H, ddd, *J*= 17.8, 12.8, and 5.5 Hz), 3.12 (1H, dd, *J*= 13.7 and 7.4 Hz), 3.18 (1H, dd, *J*= 13.7 and 7.3 Hz), 4.11 (1H, dd, *J*= 7.4 and 7.3 Hz), 4.66 (1H, dd, *J*= 12.8 and 5.5 Hz), 7.25-7.40 (5H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 25.40, 31.85, 38.60, 50.90, 55.86, 128.83, 130.05, 130.59, 135.56, 169.67, 173.20, 174.46; HRMS Found: 276.1351; Calcd: 276.1348 ($C_{14}H_{18}N_3O_3^+$).

(3*S*,2'*S*)-3-(Valinylamino)piperidine-2,6-dione hydrochloride, (3*S*,2'*S*)-11-V: mp 172 °C (decomp.); $[\alpha]^{25}_D$ -14.4 (*c* 0.25, MeOH); IR (KBr) 1673, 1706, 1729 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.11 (3H, d, *J*= 6.4 Hz), 1.13 (3H, d, *J*= 6.4 Hz), 2.08 (1H, qd, *J*= 12.8 and 4.6 Hz), 2.15 (1H, dddd, *J*= 12.8, 5.5, 5.0, and 2.3 Hz), 2.24 (1H, septd *J*= 6.4 and 5.5 Hz), 2.69 (1H, ddd, *J*= 17.4, 4.6, and 2.3 Hz), 2.89 (1H, ddd, *J*= 17.4, 12.8, and 5.0 Hz), 3.72 (1H, d, *J*= 5.5 Hz), 4.72 (1H, dd, *J*= 12.8 and 5.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 18.01, 18.79, 25.44, 31.55, 32.05, 51.17, 59.85, 169.60, 172.74, 174.63; HRMS Found: 228.1345; Calcd : 228.1348($C_{10}H_{18}N_3O_3^+$).

(3*R*,2'*S*)-3-(Valinylamino)piperidine-2,6-dione hydrochloride, (3*R*,2'*S*)-11-V: mp 163 °C (decomp.); $[\alpha]^{25}_D$ +73.4 (*c* 0.25, MeOH); IR (KBr) 1695 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.97 (3H, d, *J*= 6.0 Hz), 0.99 (3H, d, *J*= 6.0 Hz), 2.00-2.20 (3H, m), 2.59 (1H, dt, *J*= 17.4 and 3.2 Hz), 2.65-2.75 (1H, m), 3.63 (1H, d, *J*= 6.0 Hz), 4.59 (1H, dd, *J*= 10.5 and 7.3 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 18.01, 18.83, 25.53, 31.48, 32.02, 51.33, 59.83, 169.66, 173.10, 174.57; HRMS Found: 228.1346; Calcd: 228.1348 ($C_{10}H_{18}N_3O_3^+$).

(3*S*,2'*S*)-3-(Leucinylamino)piperidine-2,6-dione hydrochloride, (3*S*,2'*S*)-11-L: mp 161 °C (decomp.); $[\alpha]^{25}_D$ -15.7 (*c* 0.25, MeOH); IR (KBr) 1704 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.02 (3H, d, *J*= 8.2 Hz), 1.03 (3H, d, *J*= 8.2 Hz), 1.65-1.85 (3H, m), 2.00-2.20 (2H, m), 2.65-2.85 (2H, m), 3.88 (1H, dd, *J*= 8.2 and 5.5 Hz), 4.68 (1H, dd, *J*= 12.8 and 5.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 22.10, 23.07, 25.35, 25.45, 32.04, 41.82, 51.23, 53.07, 170.84, 172.85, 174.57; HRMS Found: 242.1497; Calcd: 242.1505 ($C_{11}H_{20}N_3O_3^+$).

(3*R*,2'*S*)-3-(Leucinylamino)piperidine-2,6-dione hydrochloride, (3*R*,2'*S*)-11-L: mp 148 °C (decomp.); $[\alpha]^{25}_D$ +56.8 (*c* 0.50, MeOH); IR (KBr) 1703 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.00 (3H, d, *J*= 6.0 Hz), 1.02 (3H, d, *J*= 6.0 Hz), 1.70-1.80 (3H, m), 2.00-2.20 (2H, m), 2.68 (1H, ddd, *J*= 18.3, 5.0, and 3.2 Hz), 2.79 (1H, ddd, *J*= 18.3, 11.9, and 6.4 Hz), 3.91 (1H, t, *J*= 7.8 Hz), 4.69 (1H, dd, *J*= 11.9 and 6.4 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 22.14, 23.05, 25.48, 32.04, 51.26, 53.10, 68.14, 170.87, 173.26, 174.52; HRMS Found: 242.1504; Calcd: 242.1505 ($C_{11}H_{20}N_3O_3^+$).

(3*S*,2'*S*)-3-(Phenylalanylamino)pyrrolidine-2,5-dione hydrochloride, (3*S*,2'*S*)-12-F: mp 200-201 °C (decomp.); $[\alpha]^{25}_D$ +36.5 (*c* 0.50, MeOH); IR (KBr) 1747, 1690 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.57 (1H, dd, *J*= 17.9 and 5.9 Hz), 2.92 (1H, dd, *J*= 17.9 and 9.2 Hz), 3.10 (1H, dd, *J*= 14.2 and 7.8 Hz), 3.20

(1H, dd, *J*= 14.2 and 6.9 Hz), 4.08 (1H, dd, *J*= 7.8 and 6.9 Hz), 4.46 (1H, dd, *J*= 9.2 and 5.9 Hz), 7.25-7.40 (5H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 36.71, 38.47, 51.68, 55.37, 128.96, 130.16, 130.55, 135.30, 169.71, 177.53, 178.59; HRMS Found: 262.1192; Calcd: 262.1192 ($C_{13}H_{16}N_3O_3^+$).

(3*R*,2'*S*)-3-(Phenylalanylamino)pyrrolidine-2,5-dione, (3*R*,2'*S*)-12-F: mp 232-235 °C (decomp.); $[\alpha]^{25}_D$ +75.8 (*c* 1.0, MeOH); IR (KBr) 1669 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.54 (1H, dd, *J*= 16.5 and 6.4 Hz), 2.63 (1H, dd, *J*= 16.5 and 4.1 Hz), 3.00 (1H, dd, *J*= 13.7 and 5.0 Hz), 3.19 (1H, ddd, *J*= 5.0, 4.1, and 1.4 Hz), 3.24 (1H, dd, *J*= 13.7 and 4.1 Hz), 4.28 (1H, ddd, *J*= 6.4, 4.1, and 1.4 Hz), 7.15-7.30 (5H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 37.72, 40.46, 51.93, 57.64, 128.33, 129.50, 131.36, 136.51, 169.87, 169.95, 174.20; HRMS Found: 284.1008; Calcd: 284.1011 ($C_{13}H_{15}N_3O_3Na^+$).

(3*S*,2'*S*)-3-(Valinylamino)pyrrolidine-2,5-dione, (3*S*,2'*S*)-12-V: mp 229 °C (decomp.); $[\alpha]^{25}_D$ +0.6 (*c* 1.0, MeOH); IR (KBr) 1670, 1643 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.96 (3H, d, *J*= 6.9 Hz), 1.04 (3H, d, *J*= 7.3 Hz), 2.20-2.30 (1H, m), 2.56 (1H, dd, *J*= 15.6 and 9.2 Hz), 2.93 (1H, dd, *J*= 15.6 and 3.7 Hz), 3.85 (1H, dd, *J*= 3.7 and 1.4 Hz), 4.40 (1H, ddd, *J*= 9.2, 3.7, and 1.4 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 17.36, 19.07, 33.35, 40.64, 52.99, 61.29, 169.38, 169.74, 174.31; HRMS Found: 214.1200; Calcd: 214.1192 ($C_9H_{16}N_3O_3^+$).

(3*R*,2'*S*)-3-(Valinylamino)pyrrolidine-2,5-dione, (3*R*,2'*S*)-12-V: mp 235 °C (decomp.); $[\alpha]^{25}_D$ -5.9 (*c* 1.0, MeOH); IR (KBr) 1678, 1634 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.96 (3H, d, *J*= 6.8 Hz), 1.38 (3H, d, *J*= 6.8 Hz), 2.28 (1H, septd, *J*= 6.8 and 3.7 Hz), 2.73 (1H, dd, *J*= 16.5 and 6.4 Hz), 2.85 (1H, dd, *J*= 16.5 and 4.6 Hz), 3.81 (1H, dd, *J*= 3.7 and 0.9 Hz), 4.29 (1H, ddd, *J*= 6.4, and 4.6, and 0.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 17.09, 18.97, 33.92, 38.26, 52.32, 61.72, 170.24, 170.34, 174.45; HRMS Found: 261.013; Calcd: 236.1011 ($C_9H_{15}N_3O_3Na^+$).

(3*S*,2'*S*)-3-(Leucinylamino)pyrrolidine-2,5-dione, (3*S*,2'*S*)-12-L: mp 257-262 °C (decomp.); $[\alpha]^{25}_D$ -16.6 (*c* 0.25, MeOH); IR (KBr) 1673 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.95 (3H, d, *J*= 6.4 Hz), 0.98 (3H, d, *J*= 6.4 Hz), 1.65-1.90 (3H, m), 2.65 (1H, dd, *J*= 16.0 and 8.2 Hz), 2.84 (1H, dd, *J*= 16.0 and 4.1 Hz), 3.96 (1H, ddd, *J*= 8.2, 5.0, and 1.4 Hz), 4.32 (1H, ddd, *J*= 8.2, 4.1, and 0.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 21.95, 23.57, 25.25, 39.93, 44.82, 53.17, 54.49, 169.66, 170.97, 174.08; HRMS Found: 228.1349; Calcd: 228.1348 ($C_{10}H_{18}N_3O_3^+$).

(3*R*,2'*S*)-3-(Leucinylamino)pyrrolidine-2,5-dione, (3*R*,2'*S*)-12-L: mp 249 °C (decomp.); $[\alpha]^{25}_D$ +3.2 (*c* 0.50, MeOH); IR (KBr) 1677, 1658 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.96 (3H, d, *J*= 6.4 Hz), 0.97 (3H, d, *J*= 6.4 Hz), 1.70 (2H, t, *J*= 5.5 Hz), 1.84 (1H, septt, *J*= 6.4 and 5.5 Hz), 2.75 (1H, dd, *J*= 16.5 and 6.4 Hz), 2.85 (1H, dd, *J*= 16.5 and 4.6 Hz), 3.98 (1H, td, *J*= 5.5 and 0.9 Hz), 4.30 (1H, ddd, *J*= 6.4, 4.6, and 0.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 22.29, 23.41, 25.32, 37.99, 43.46, 52.46, 54.95, 170.09, 171.46, 174.41; HRMS Found: 250.1168; Calcd: 250.1168 ($C_{10}H_{17}N_3O_3Na^+$).

General procedure for transformation of 15 or 16 into hydantoin 5 or 6: To a dry-MeCN (15 mL) suspension of (3*S*,2*S'*)-3-(phenylalanylamino)piperidine-2,6-dione hydrochloride (**(3*S*,2*S'*)-11-F**) (102 mg) and $NaHCO_3$ (214 mg) was added 4-nitrophenyl chloroformate (106 mg) at room temperature. After being stirred for 3 h, water (9 mL) was added to the reaction suspension. The reaction suspension changed into a yellow solution, and then the reaction mixture was stirred for 3 h. After removal of MeCN under the reduced pressure, the organic materials were extracted with EtOAc. The extract was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$:MeOH=9:1) to give (3*S*,5'*S*)-3-(5-benzyl-2,4-dioxoimidazolidin-3-yl)piperidine-2,6-dione (**(3*S*,5'*S*)-5-F**) (67.6 mg). When a large scale reaction was performed, a residue was purified by reprecipitation from MeOH-Et_2O-hexane or Silica-gel column chromatography.

(3*S*,5'*S*)-3-(5-Benzyl-2,4-dioxoimidazolidin-3-yl)piperidine-2,6-dione, (3*S*,5'*S*)-5-F: mp 143-145 °C (decomp.); $[\alpha]^{25}_D$ -17.2 (*c* 0.50, MeOH); IR (KBr) 1715 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.45-1.50 (1H, m), 2.24 (1H, qd, *J*= 13.3 and 4.6 Hz), 2.55 (1H, dq, *J*= 17.4 and 2.7 Hz), 2.68 (1H, ddd, *J*= 17.4, 13.3, and 5.5 Hz), 3.05 (1H, dd, *J*= 13.8 and 4.8 Hz), 3.12 (1H, dd, *J*= 13.8 and 4.6 Hz), 4.46 (1H, dd, *J*= 5.9 and 4.6 Hz), 4.75 (1H, dd, *J*= 12.8 and 5.5 Hz), 7.15-7.30 (5H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 21.81, 31.24, 38.05, 49.89, 58.32, 127.65, 129.04, 129.27, 134.95, 155.31, 167.25, 170.36, 172.07; HRMS Found: 324.0953; Calcd: 324.0960 ($C_{14}H_{13}N_3O_4Na^+$).

(3*R*,5'*S*)-3-(5-Benzyl-2,4-dioxoimidazolidin-3-yl)piperidine-2,6-dione, (3*R*,5'*S*)-5-F: mp 195-197 °C (decomp.); $[\alpha]^{25}_D$ -75.3 (*c* 0.25, acetone); IR (KBr) 1713 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.45-1.55 (1H, m), 2.36 (1H, qd, *J*= 12.8 and 4.6 Hz), 2.59 (1H, ddd, *J*= 17.4, 5.0, and 4.6 Hz), 2.69 (1H, ddd, *J*= 17.4, 5.0, and 4.6 Hz), 3.04 (1H, dd, *J*= 14.2 and 6.0 Hz), 3.12 (1H, dd, *J*= 14.2 and 4.6 Hz), 4.40 (1H, dd, *J*= 6.0 and 4.6 Hz), 3.65 (1H, dd, *J*= 12.8 and 5.5 Hz), 7.15-7.30 (5H, m) ; ^{13}C NMR (CD_3OD, 100 MHz) δ 22.74, 31.85, 38.09, 50.64, 59.03, 128.18, 129.42, 130.95, 136.16, 157.79, 170.85, 174.71, 174.71; HRMS Found: 324.0966; Calcd: 324.0960 ($C_{15}H_{15}N_3O_4Na^+$).

(3*S*,5'*S*)-3-(*5*-Isopropyl-2,4-dioxoimidazolidin-3-yl)piperidine-2,6-dione, (3*S*,5'*S*)-5-V: mp 117-120 °C; $[\alpha]^{25}_D$ +36.1 (*c* 1.0, MeOH); IR (KBr) 1712, 1767 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 0.95 (3H, d, *J*= 6.8 Hz), 1.05 (3H, d, *J*= 7.3 Hz), 1.95-2.05 (1H, m), 2.10-2.25 (1H, m), 2.55-2.85 (3H, m), 4.04 (1H, d, *J*= 3.7 Hz), 4.85-4.90 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 16.30, 19.16, 23.19, 31.61, 31.99, 50.69, 63.47, 158.46, 170.84, 174.53, 175.16; HRMS Found: 276.0960; Calcd: 276.0960 ($C_{11}H_{15}N_3O_4Na^+$).

(3*R*,5'*S*)-3-(5-Isopropyl-2,4-dioxoimidazolidin-3-yl)piperidine-2,6-dione, (3*R*,5'*S*)-5-V: mp 171-172 °C; $[\alpha]^{25}_D$ -28.0 (*c* 1.0, MeOH); IR (KBr) 1712, 1770 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 0.94 (3H, d,

J= 6.9 Hz), 1.05 (3H, d, *J*= 6.9 Hz), 1.95-2.05 (1H, m), 2.10-2.25 (1H, m), 2.55-2.85 (3H, m), 4.04 (1H, d, *J*= 3.6 Hz), 4.85-4.90 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 16.30, 19.16, 23.19, 31.61, 31.99, 50.69, 63.47, 158.46, 170.84, 174.55, 175.16; HRMS Found: 276.0962; Calcd: 276.0960 ($C_{11}H_{15}N_3O_4Na^+$).

(3*S*,5'*S*)-3-(5-Isobutyl-2,4-dioxoimidazolidin-3-yl)piperidine-2,6-dione, (3*S*,5'*S*)-5-L: mp 171-174 °C; $[\alpha]^{25}_D$ -8.9 (*c* 1.0, MeOH); IR (KBr) 1694, 1738 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.97 (3H, d, *J*= 6.4 Hz), 0.98 (3H, d, *J*= 8.2 Hz), 1.50-1.70 (2H, m), 1.80-1.90 (1H, m), 2.00-2.10 (1H, m), 2.60-2.85 (3H, m), 4.16 (1H, dd, *J*= 8.7 and 4.1 Hz), 4.80-4.90 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 21.87, 23.10, 23.66, 25.78, 32.03, 42.12, 50.77, 56.82, 158.07, 171.06, 174.53, 176.24; HRMS Found: 290.1108; Calcd: 290.1117 ($C_{12}H_{17}N_3O_4Na^+$).

(3*R*,5'*S*)-3-(5-Isobutyl-2,4-dioxoimidazolidin-3-yl)piperidine-2,6-dione, (3*R*,5'*S*)-5-L: mp 206-207 °C; $[\alpha]^{25}_D$ -2.6 (*c* 1.0, MeOH); IR (KBr) 1720, 1780 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.97 (3H, d, *J*= 6.8 Hz), 0.98 (3H, d, *J*= 6.9 Hz), 1.55-1.75 (2H, m), 1.85-1.95 (1H, m), 2.00-2.10 (1H, m), 2.55-2.85 (3H, m), 4.16 (1H, dd, *J*= 9.2 and 4.6 Hz), 4.80-4.90 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 21.98, 23.10, 23.62, 26.14, 32.02, 42.06, 50.70, 57.00, 158.02, 171.13, 174.54, 176.25; HRMS Found: 290.1115; Calcd: 290.1117 ($C_{12}H_{17}N_3O_4Na^+$).

(3*S*,5'*S*)-3-(5-Benzyl-2,4-dioxoimidazolidin-3-yl)pyrrolidine-2,5-dione, (3*S*,5'*S*)-6-F: mp 174-175 °C (decomp.); $[\alpha]^{25}_D$ -123.3 (*c* 0.50, MeOH); IR (KBr) 1715 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.05-2.20 (1H, m), 2.75 (1H, dd, *J*= 16.9 and 9.6 Hz), 3.06 (1H, dd, *J*= 14.2 and 5.5 Hz), 3.11 (1H, dd, *J*= 14.2 and 5.5 Hz), 4.46 (1H, dd, *J*= 5.5 and 4.6 Hz), 4.93 (1H, dd, *J*= 9.6 and 5.5 Hz), 7.15-7.35 (5H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 35.29, 35.30, 38.18, 59.66, 128.27, 129.45, 130.92, 136.01, 157.32, 174.45, 176.74, 177.18; HRMS Found: 310.0818; Calcd: 310.0804 ($C_{14}H_{13}N_3O_4Na^+$).

(3*R*,5'*S*)-3-(5-Benzyl-2,4-dioxoimidazolidin-3-yl)pyrrolidine-2,5-dione, (3*R*,5'*S*)-6-F: mp 97-98 °C; $[\alpha]^{25}_D$ -49.3 (*c* 0.50, MeOH); IR (KBr) 1776, 1719 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.25-2.40 (1H, m), 2.70-2.80 (1H, m), 3.50 (1H, dd, *J*= 14.2 and 6.0 Hz), 3.13 (1H, dd, *J*= 14.2 and 4.6 Hz), 4.42 (1H, dd, *J*= 6.0 and 4.6 Hz), 4.85-4.90 (1H, m), 7.15-7.30 (5H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 34.97,37.98, 49.53, 59.09, 128.29, 129.50, 130.89, 136.10, 157.28, 174.52, 176.63, 177.18; HRMS Found: 310.0808; Calcd: 310.0804 ($C_{14}H_{13}N_3O_4Na^+$).

(3*S*,5'*S*)-3-(5-Isopropyl-2,4-dioxoimidazolidin-3-yl)pyrrolidine-2,5-dione, (3*S*,5'*S*)-6-V: mp 167-169 °C (decomp.); $[\alpha]^{25}_D$ -36.5 (*c* 1.0, MeOH); IR (KBr) 1701, 1742 cm^{-1}; ^{1}H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 0.94 (3H, d, *J*= 6.8 Hz), 1.06 (3H, d, *J*= 6.8 Hz), 2.10-2.15 (1H, m), 2.93 (1H, dd, *J*= 17.9 and 6.4 Hz), 3.06 (1H, dd, *J*= 17.9 and 9.6 Hz), 4.00 (1H, d, *J*= 3.6 Hz), 5.01 (1H, ddd, *J*= 9.6, 6.4, and 2.3Hz); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 15.90, 18.70, 30.48, 34.51, 48.29, 156.50, 173.06, 174.32, 174.89; HRMS Found: 262.0806; Calcd: 262.0804 ($C_{10}H_{13}N_3O_4Na^+$).

(3*R*,5'*S*)-3-(5-Isopropyl-2,4-dioxoimidazolidin-3-yl)pyrrolidine-2,5-dione, (3*R*,5'*S*)-6-V: mp 178-180 °C (decomp.); $[\alpha]^{25}_{D}$ +24.1 (*c* 1.0, MeOH); IR (KBr) 1707 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.93 (3H, d, *J*= 6.8 Hz), 1.04 (3H, d, *J*= 6.8 Hz), 2.18 (1H, septd, *J*= 6.8 and 3.2 Hz), 2.84 (1H, dd, *J*= 17.8 and 6.0 Hz), 3.04 (1H, dd, *J*= 17.8 and 9.6 Hz), 4.04 (1H, d, *J*= 3.2 Hz), 5.10 (1H, dd, *J*= 9.6 and 6.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 16.39, 18.92, 31.61, 35.28, 49.61, 63.83, 158.06, 174.86, 176.77, 177.40; HRMS Found: 262.0816; Calcd: 262.0804 ($C_{10}H_{13}N_3O_4Na^+$).

(3*S*,5'*S*)-3-(5-Isobutyl-2,4-dioxoimidazolidin-3-yl)pyrrolidine-2,5-dione, (3*S*,5'*S*)-6-L: mp 167-168 °C (decomp.); $[\alpha]^{25}_{D}$ -78.7 (*c* 0.50, MeOH); IR (KBr) 1705 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.88 (3H, d, *J*= 6.4 Hz), 0.90 (3H, d, *J*= 6.4 Hz), 1.35-1.45 (1H, m), 1.45-1.55 (1H, m), 1.70-1.85 (1H, m), 2.73 (1H, dd, *J*= 17.4 and 5.5 Hz), 2.93 (1H, dd, *J*= 17.4 and 9.6 Hz), 4.10-4.20 (1H, m), 4.99 (1H, dd, *J*= 9.6 and 5.5 Hz), 8.53 (1H, s), 11.48 (1H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ 21.87, 23.10, 23.66, 25.78, 32.03, 42.12, 50.77, 56.82, 158.07, 171.06, 174.53, 176.24; HRMS Found: 276.0965; Calcd: 276.0960 ($C_{11}H_{15}N_3O_4Na^+$).

(3*R*,5'*S*)-3-(5-Isopropyl-2,4-dioxoimidazolidin-3-yl)pyrrolidine-2,5-dione, (3*S*,5'*S*)-6-L: mp 143-145 °C (decomp.); $[\alpha]^{25}_{D}$ +29.3 (*c* 0.50, MeOH); IR (KBr) 1715 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.97 (3H, d, *J*= 6.4 Hz), 0.98 (3H, d, *J*= 6.4 Hz), 1.57 (1H, ddd, *J*= 14.2, 8.7, and 5.5 Hz), 1.68 (1H, ddd, *J*= 14.2, 9.2, and 4.6 Hz), 1.85-1.90 (1H, m), 2.86 (1H, dd, *J*= 17.8 and 5.9 Hz), 3.04 (1H, dd, *J*= 17.8 and 9.6 Hz), 4.17 (1H, dd, *J*= 8.7 and 4.6 Hz), 5.09 (1H, dd, *J*= 9.6 and 5.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 21.93, 23.58, 25.82, 35.33, 41.93, 57.20, 157.65, 175.87, 177.06, 177.38; HRMS Found: 276.0950; Calcd: 276.0960 ($C_{11}H_{15}N_3O_4Na^+$).

REFERENCES

1. Review; M. Meusel and M. Gutschow, *Org. Prep. Proced. Int.*, 2004, **36**, 391.
2. M. A. Rogawski and W. Löscher, *Nat. Rev. Neurosci.*, 2004, **5**, 553.
3. J. Yamaguchi, M. Harada, T. Kondo, T. Noda, and T. Suyama, *Chem. Lett.*, 2003, **32**, 372.
4. J. Yamaguchi, M. Harada, T. Narushima, A. Saitoh, K. Nozaki, and T. Suyama, *Tetrahedron Lett.*, 2005, **46**, 6411.
5. Y. Hashimoto, *Bioorg. Med. Chem.*, 2002, **10**, 461.
6. H. Fujimoto, T. Noguchi, H. Kobayashi, H. Miyachi, and Y. Hashimoto, *Chem. Pharm. Bull.*, 2006, **54**, 855.
7. T. Noguchi, H. Fujimoto, H. Sano, A. Miyajima, H. Miyachi, and Y. Hashimoto, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5509.
8. Q. Li, H. L. Fang, X. Wang, G. Hu, Q. Wang, and W. Xu, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 850.

9. D. J. Fox, J. Reckless, S. G. Warren, and D. J. Grainger, *J. Med. Chem.*, 2002, **45**, 360.
10. H. Kagechika, E. Kawachi, Y. Hashimoto, T. Himi, and K. Shudo, *J. Med. Chem.*, 1988, **31**, 2182; T. Noguchi, C. Shinji, H. Kobayashi, M. Makishima, H. Miyachi, and Y. Hashimoto, *Biol. Pharm. Bull.*, 2005, **28**, 563.
11. E. Sondheimer and R. W. Holley, *J. Am. Chem. Soc.*, 1957, **79**, 3767.