Structure and Synthesis of E-64, a New Thiol Protease Inhibitor[†]

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E-64, a new thiol protease inhibitor, was isolated from a mold, as described previously.¹⁾ This was proved to consist of each one mole of L-leucine, agmatine and L-*trans*-epoxysuccinic acid by the analysis of the digestion products of E-64 by pronase. Moreover, the absolute structure of E-64 was assumed to be N-[N-(L-3-*trans*-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatine and the identity was established by the comparison with its optical isomer which was obtained synthetically. The difference of optical activity of *trans*-epoxysuccinic acid gave no effect on papain inhibitory activity.

As described in the preceding paper,¹⁾ E-64, a novel thiol protease inhibitor, was isolated from a culture extract of *Asp. japonicus* TPR-64 and its physicochemical and biochemical properties were clarified. This paper is concerned with the estimation of the structure of E-64 and with the establishment thereof by chemical synthesis of the related compounds including its optical isomer. The structure-activity relationship of E-64 will be described in the subsequent paper

As mentioned previously,¹⁾ E-64 can be obtained as white needles of whose molecular formula was established as C₁₅H₂₇O₅N₅ (M.W. 357). This is almost neutral electrophoretically showing approximately similar behavior to that of L-leucine. Positive color reactions of E-64 indicated the presence of guanidyl, epoxide, 1,2-dicarboxyl groups and a peptide bond with a good correspondence with its IR spectrum.

E-64 was cleaved almost completely into three constituents with pronase E, all of which were isolated as crystals, as mentioned below, whereas hydrolysis with HCl resulted in the disappearance of epoxide. Table I shows the analytical data of these three constituents.

Component A has a molecular formula of

C₆H₁₃O₂N and gives a positive ninhydrin test. This is active optically, $[\alpha]_D^{20} - 8.5$ ($c=2$, H₂O). In addition, from both the elution pattern on an amino acid autoanalyzer and PMR analysis, this was suggested to be L-leucine and the identity was confirmed by direct comparison with an authentic sample.

Component B, strongly basic and positive with ninhydrin and Sakaguchi reactions, was identified as agmatine by spectral comparison in PMR with an authentic sample. In addition, the result of elemental analysis was identical with the calculated value of agmatine·2HCl.

Component C, strongly acidic, white prisms, mp 173~175°C, $[\alpha]_D^{30} + 78$ ($c=1$, H₂O), was clarified to possess epoxide and 1,2-dicarboxyl groups from the positive color reactions and suggested to be *trans*-epoxysuccinic acid judging from its molecular formula. The identity was confirmed by the comparison with DL-*trans*-epoxysuccinic acid synthesized by the method of K. Sakaguchi *et al.*²⁾ on elemental analysis, TLC and spectral analyses (IR, PMR, MS). R. Kuhn *et al.*³⁾ reported that (+) malic acid was prepared from (–)*trans*-epoxysuccinic acid, an optical isomer of component C. Another report⁴⁾ also pointed out that the oxiran ring of (–)*trans*-epoxysuccinic acid was rapidly opened by a simple S_N2 mechanism with inversion of configuration to give *erythro*-β-hydroxy-L-aspartic acid when it was warmed

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TABLE I. PROPERTIES OF COMPONENT A, B, C

	A		B		C	
mp	300°C		178~178.5°C		173~175°C	
Nature	neutral, plate		basic, plate		acidic, prism	
Elemental analysis	Found	Calcd.	Found	Calcd.	Found	Calcd.
N	10.68	10.67	27.43	27.58	—	—
C	54.92	54.94	29.78	29.56	36.52	36.37
H	9.71	9.99	7.68	7.94	3.27	3.06
Cl	—	—	34.40	34.91	—	—
Molecular formula	$C_6H_{18}O_2N$		$C_5H_{14}N_4 \cdot 2HCl$		$C_4H_4O_5$	
Positive color reaction	ninhydrin		ninhydrin Sakaguchi		thiosulfate (epoxide) Feigl (dicarboxylic acid)	
$[\alpha]_D^{20}$	-8.5 ($c=2$, H_2O)		—		+78 ($c=1$, H_2O)	
Contents in E-64	32.8%		35.8%		—	

TABLE II. PROTON MAGNETIC RESONANCE ABSORPTIONS ASSIGNED TO E-64

Solvent	Chemical shift, ppm	Area	Multiplicity	Assignment
D ₂ O + DCl	0.95	6	br. s	a
	1.55	7	br. s	b
	3.20	4	br. s	c
	3.66	1	d	d
	3.78	1	d	d
	4.30	1	t	e
DMSO-d ₆	0.85	6	br. s	a
	1.42	7	br. s	b
	3.05	4	br. s	c
	4.25	1	t	e
	7.45	4	br	f
	8.30	3	br	g

with aqueous ammonia. Component C was, therefore, inferred to be L-*trans*-epoxysuccinic acid with the absolute configuration of (2S, 3S).

Moreover, it was suggested from the molecular weight that E-64 consisted of each one mole of these three components. This was also supported by the analysis of the signals on PMR which were reasonably assigned, as depicted in Table II. In this, the two protons on the oxiran ring of component C, afforded singlet signal, whereas the same protons on E-64 gave double doublet. This seems to indicate that the circumstances around these two protons on E-64 are different each other.

that is, one of carboxyl groups may exist in a combined state, but another one in a free.

In addition, E-64 was negative for ninhydrin test and approximately neutral electrophoretically, as mentioned above.

On the basis of these results, the structure of E-64 can be assumed most reasonably to be N-[N-(1-3-*trans*-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatine(compound I). The synthesis of compound I was carried out independently through two synthetic schemes (Figs. 1 and 2). Herein, L-leucine and DL-*trans*-epoxysuccinic acid were employed for convenience as starting materials with an expectation that coexisting

FIG. 1. Synthetic Scheme of E-64 (Scheme 1).

diastereoisomer will be separated favorably in the course of the procedure.

Table III shows the properties of two products synthesized independently *via* the above two routes, Schemes 1 and 2, and the products were named Synthetic 1 and 2, respectively. These two compounds agreed with natural E-64 on TLC and elemental analyses, and PMR and IR spectra coincided completely except delicate differences illustrated in Fig. 3. Furthermore, both compounds gave an excellent agreement with E-64 on ID₆₀ for papain for 0.3×10^{-9} mole.

On the basis of these results, it seems likely that the two synthetic compounds obtained here are identical with natural E-64 in their structural formulae, but not in the absolute configuration of *trans*-epoxysuccinic acid moiety. This seems to contribute to minute

differences in IR and PMR spectra. Comparison of PMR spectra between natural E-64 and synthetic 2 afforded the difference in the chemical shift of the signals due to the two protons on the oxiran ring, which were at 3.66, 3.78 and 3.71, 3.78 ppm, respectively, as illustrated in Fig. 3. Upon this, it seems likely that *trans*-epoxysuccinic acid of synthetic 2 is mostly in D-form in contrast to that of natural E-64. Moreover, synthetic 1 appears to be a mixture of D and L. The same speculation can also apply to the interpretation of slight differences on the IR spectra indicated by the arrows in Fig. 3.

As mentioned above, the synthesis of the intended compound (compound I) could not be attained, but its optical isomer was obtained as a result. The primary purpose, the elucidation of the structure of E-64, which was de-

FIG. 2. Synthetic Scheme of E-64 (Scheme 2).

TABLE III. PHYSICOCHEMICAL PROPERTIES OF SYNTHETIC E-64

	Synthetic 1		Synthetic 2	
Synthetic scheme	(1)		(2)	
mp ^{a)}	166~170°C (decomp.)		173~175°C (decomp.)	
[α] _D ^{b)}	-69.5° (c=0.456 0.1 N HCl)		-80.6° (c=0.456 0.1 N HCl)	
Elemental analysis	Found	Calcd.	Found	Calcd.
N	17.95	17.80	17.82	17.80
C	45.75	45.79	45.73	45.79
H	7.91	7.94	7.90	7.94
Molecular formula	C ₁₅ H ₂₇ N ₅ O ₈ ·2H ₂ O		C ₁₅ H ₂₇ N ₅ O ₈ ·2H ₂ O	
TLC (cellulose)				
BuOH: AcOH: H ₂ O=4: 1: 2	agreement with E-64		agreement with E-64	
n-ProOH: H ₂ O=7: 3	"		"	
Positive color reaction	Sakaguchi, thiosulfate		Sakaguchi, thiosulfate	

^{a)} Natural E-64=180~182°C^{b)} Natural E-64=+24.4

FIG. 3. Comparisons among Natural E-64, Synthetic 1 and 2 on PMR and IR Spectra.

termed to be N-[N-(*L-trans*-carboxyoxiran-2-carbonyl)-*L*-leucyl]-agmatine, has, however, been achieved through the information on the related compound obtained synthetically.

EXPERIMENTAL

General methods. IR spectra were recorded on a Model DS 70G spectrometer, Japan Spectroscopic Co., Japan and PMR spectra were measured at 60 MHz with a Model R-20 spectrometer, Hitachi-Perkin-Elmer Co. Optical rotations were determined on a Model DIP-4 polarimeter, Japan Spectroscopic Co. Amino-acid analysis was carried out by use of a Hitachi KLA-5 amino acid autoanalyzer.

Assay of inhibitory activity for papain. Inhibitory activity for papain was determined by the method described previously.¹⁾

Hydrolysis of E-64 by pronase E and isolation of the products. A water solution (35 ml) containing both E-64 (300 mg) and pronase E (40 mg) which was purchased from Junsei Pure Chem. Co. and further purified through a Sephadex G-25 column was incubated at 30°C for two days and followed by the gelfiltration on a Sephadex G-25 column in order to eliminate the used enzyme. The low molecular fractions were combined and concentrated to 10 ml. This was submitted to a column of phosphocellulose (H⁺

type, 100 ml) and eluted into three fractions, that is the acidic fraction (non-adsorbed), the neutral (eluted with water) and the basic (eluted with 0.1 N HCl). Evaporation of the neutral fraction gave white powder, which on crystallization from aqueous ethanol was obtained as white plates and designated as Component A (65.5 mg, 68%). From the basic fraction, white plates were obtained in the same manner and named Component B (100 mg, 60%). The acidic fraction was acidified with H₂SO₄ and extracted with ethylether (20 ml × 2). The ethylether layer was concentrated and followed by addition of petroleum ether to give white prisms designated as Component C (62 mg, 56%).

Benzyl phenyl carbonate. Benzyl phenyl carbonate was prepared according to the method of H. Zahn *et al.*⁵⁾

1-Nitroguanyl-3, 5-dimethyl-pyrazole. 1-Nitroguanyl-3, 5-dimethyl-pyrazole was synthesized from guanidine nitrate via nitroguanidine and nitroamino-guanidine.⁶⁻⁸⁾

DL-Ethyl hydrogen trans-epoxysuccinate (II). DL-*trans*-Epoxysuccinic acid was prepared from fumaric acid by the method of Payne *et al.*⁹⁾ A solution of DL-*trans*-epoxysuccinic acid (19.7 g) and conc. sulfuric acid (6 g) in abs. EtOH (200 ml) was refluxed for 6 hr and then was brought to pH 7 with 10% Na₂CO₃ solution. After removal of ethanol by evaporation, the residue was suspended into 2% Na₂CO₃ solution and extracted with AcOEt (100 ml × 2). The organic layer was washed with 2% HCl and sat. NaCl in turn, dried over MgSO₄ and evaporated to an oil. The fractional distillation of the resulting oil gave 16.7 g of diethyl epoxysuccinate as a colorless oil (bp 84~91°C, 3 mmHg). To an ethanolic solution (50 ml) of diethyl epoxysuccinate (13.1 g), was added an ethanolic solution (120 ml) of KOH (3.94 g) under ice cooling and stirred for 2 hr at the same temperature. The deposited solid was filtered off and crystallized from aqueous EtOH to give white prisms of DL-ethyl potassium *trans*-epoxysuccinate (11.75 g, mp 283°C (decomp.)). *Anal.* Calcd. for C₆H₇O₅K: C, 36.36; H, 3.56. Found: C, 36.13; H, 3.75. PMR (D₂O) δ: 1.29 (3H, t, *J* = 6.6 Hz), 3.55 (2H, d, d, *J* = 2.0 Hz), 4.25 (2H, q, *J* = 6.6 Hz). The aqueous solution (50 ml) of DL-ethyl potassium *trans*-epoxysuccinate (10.46 g) was saturated with NaCl, acidified with conc. HCl and extracted with AcOEt (30 ml × 3). The combined organic layer was washed with sat. NaCl and evaporated to a colorless oil of (II) (6.95 g). bp 116~119°C (0.3 mmHg).

N-Carbobenzoxy-1, 4-diaminobutane carbonate (IV). HCl salt of IV was prepared from III (22.3 g) and benzyl phenyl carbonate (57.1 g) by the method similar to that described by J. S. Rao.¹⁰⁾ A suspension of HCl salt of IV in water (200 ml) was made alkali with NaOH (10 g) and extracted with AcOEt (100 ml × 3).

The organic layer was washed with sat. NaCl, concentrated to about 150 ml and treated with dry ice to deposit a solid. The solid was filtered off after chilling for a few hours and washed with AcOEt and water in turn. Recrystallization from EtOH gave colorless solid of IV (36.76 g), mp 127~133°C.

N-Carbobenzoxy-*N*^o-nitroagmatine (V). A solution of IV (8.0 g), 1-nitroguanyl-3, 5-dimethylpyrazole⁶⁾ (6.2 g) and triethylamine (2.84 g) in EtOH (80 ml) was heated under reflux for 2 hr and then chilled overnight. The deposited solid was filtered off and washed with a small amount of EtOH. Recrystallization from EtOH gave 7.55 g of a colorless solid of V, mp 122~124°C.

N^o-Nitroagmatine hydrobromide (VI). VI was synthesized from V by the method similar to that described by W. D. J. Johnson *et al.*¹³⁾ and obtained as colorless solid, mp 174~176°C (decomp.).

N-(Boc-L-leucyl)-*N*^o-nitroagmatine (VII). A solution of VI (3.9 g) and triethylamine (1.69 g) in water (9 ml) was added to a cooled solution of Boc-L-Leu-OSu¹²⁾ (4.99 g) in DME (dimethoxyethane) (50 ml) to give a suspension which become clear on 5.5 hr stirring. The solution was concentrated to about 15 ml, diluted with 2.5% Na₂CO₃ solution (40 ml) and then the whole was extracted with AcOEt (40 ml × 2). The AcOEt layer was washed with 1 M citric acid and sat. NaCl and evaporated to dryness, followed by chromatography on a silicagel column (200 g). Elution with AcOEt in acetone (2:1) subsequent to AcOEt in isopropylether (2:1) afforded 5.7 g of glass of VII which was determined by IR and PMR analyses.

N-L-Leucyl agmatine·2HCl (VIII). A solution VII (3.15 g) in MeOH (40 ml), acetic acid (8 ml) and water (4 ml) was stirred under H₂ stream in the presence of 10% Pd-C (0.4 g) at room temperature for 4 hr. After removal of the catalyst by filtration, the filtrate was evaporated to an oil. An aqueous solution of water layer gave 2.99 g of a caramel of an acetic acid salt of *N*-Boc-L-leucyl agmatine. The salt was dissolved in 99% formic acid (120 ml) and then the solution was evaporated keeping the temperature below 30°C after stirring at room temperature for 3 hr. An aqueous solution (40 ml) of the residue was washed with AcOEt and applied to a column of Amberlite IRC-50 (H⁺, 48 ml). After washing with water, the column was eluted with 2 N HCl and the eluate was evaporated to give 1.8 g of glass of VIII, which was identified by IR and PMR analyses.

N-[*N*-(D- and L-3-trans-Ethoxycarbonyloxiran-2-carbonyl)-L-leucyl] agmatine·HCl (IX). To a solution of II (1.0 g) and VIII (1.7 g) in DMF (Dimethyl formamide) (11 ml) were added a solution of diethylphosphoryl cyanide¹³⁾ (1.05 g) in DMF (11 ml) and that

of triethylamine (1.38 g) in DMF (2 ml) in turn under cooling. The suspension was stirred under ice cooling for an hour and at room temperature for further 4 hr. After removal of the deposited solid by filtration, ethylether (200 ml) was added to the filtrate and the whole was left overnight in a refrigerator. The resulting oil was separated by decantation and subjected to a silicagel column (70 g) followed by the development with 33% EtOH in CHCl₃. The fractions positive with Sakaguchi reaction were combined and evaporated to give 0.75 g of a colorless solid of IX which was identified by IR and PMR analyses.

N-[*N*-(D- and L-3-trans-Carboxyoxiran-2-carbonyl)-L-leucyl] agmatine (I+I'). To a methanolic solution (5 ml) of IX (0.64 g), was added 2 N KOH solution (1.5 ml) under ice cooling and left at room temperature for 3 hr. After neutralization with 10% HCl, the solution was evaporated to dryness. An aqueous solution of the residue was applied to an active charcoal column. After washing with water, the elution was carried out with aqueous acetone (1:1). The eluate was evaporated to give a solid. Recrystallization from hot water-EtOH afforded 0.15g of colorless solid, which was found to be a mixture of I and I', mp 185~190°C (decomp.). *Anal.* Calcd. for C₁₈H₂₇N₅O₅·2H₂O: C, 45.79; H, 7.94; N, 17.80. Found: C, 45.75, H, 7.91; N, 17.95. PMR δ: 0.8~1.0 (6H, br. s), 1.4~1.8 (7H, br. s), 3.0~3.4 (4H, br. s), 3.66, 3.78, and 3.71, 3.78 (2H, two pair of d. d, *J*=1.8 Hz), 4.32 (1H, t, *J*=7.2 Hz). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3285 (NH), 1642 (C=O), 1560, 1545, 1465, 1388, 895. $[\alpha]_D^{16}$ -69.5 (*c*=0.463, 0.1 N HCl).

N-(Boc-L-leucyl) *N'*-carbobenzoxy-L, 4-diaminobutane (X). To a suspension of IV (8.53 g) and triethylamine (3.04 g) in water (12 ml) and DMF (80 ml), a solution of Boc-L-Leu-O-Su (9.86 g) in DMF (70 ml) was added and stirred at room temperature for 1.5 hr. After concentration to 30 ml, the residue was shaken with 2% Na₂CO₃ solution (100 ml) and then with AcOEt (150 ml × 2). The organic layer was washed with 1 M citric acid and sat. NaCl in turn and evaporated to an oil which solidified later. The solid was submitted to a silicagel column (500 g) and eluted with AcOEt: CHCl₃: isopropylether=20/10/3. The purified fractions were combined and evaporated to a solid, from which on recrystallization from isopropylether-AcOEt was obtained 12.03 g of X as colorless crystals, mp 88~89.5°C. *Anal.* Calcd. for C₂₈H₃₇N₅O₅: C, 63.42; H, 8.56; N, 9.65. Found: C, 63.72; H, 8.80; N, 9.45. $[\alpha]_D^{16}$ -24.3 (*c*=0.503, dioxane),

N-Carbobenzoxy-*N'*-L-leucyl-L, 4-diaminobutane (XI). A solution of X (7.0 g) in 99% formic acid (183 ml) was left at room temperature for 3 hr. After removal of formic acid by evaporation *in vacuo* keeping the temperature below 30°C, the resulting oil was taken in ethylether (100 ml) and allowed to stand overnight in a

refrigerator to give colorless solid which on recrystallization from AcOEt was obtained as colorless needles of the formic acid salt of XI (5.5 g), mp 101~102°C, $[\alpha]_D^{18} + 9.1$ ($c=0.506$, MeOH). *Anal.* Calcd. for $C_{18}H_{29}N_3O_8 \cdot HCOOH$: C, 59.82; H, 8.19; N, 11.02. Found: C, 60.04; H, 8.30; N, 11.09. An aqueous solution of the salt was treated with NaOH and then extracted with $CHCl_3$ (50 ml $\times$ 3). After washing with water, the organic layer was evaporated to give a colorless solid of XI (4.8 g).

N-Carbobenzoxy-*N'*-[*N*-(*D*- and *L*-3-*trans*-ethoxycarbonyloxiran-2-carbonyl)-*L*-leucyl]-*L*, 4-diaminobutane (XII). To a solution of XI (2.835 g) and II (1.36 g) in DMF (40 ml), were added a solution of diethylphosphoryl cyanide (1.52 g) in DMF (10 ml) and triethylamine (1.1 g) at 0°C in turn. After stirring at room temperature for 4 hr, the solution was diluted with AcOEt (150 ml) and benzene (100 ml). The organic layer was washed with 5% HCl, water, sat. $NaHCO_3$ and sat. NaCl in turn was evaporated to dryness. The resulting solid was reprecipitated from AcOEt-isopropylether to give a colorless powder of XII (3.11 g), mp 104~107°C, $[\alpha]_D^{14} - 23.1^\circ$ ($c=0.506$, dioxane), *Anal.* Calcd. for $C_{24}H_{36}N_3O_7$: C, 60.36; H, 7.39; N, 8.80. Found: C, 60.07; H, 7.54; N, 8.75.

N-[*N*-(*D*- and *L*-3-*trans*-Ethoxycarbonyloxiran-2-carbonyl)-*L*-leucyl]-*L*, 4-diaminobutane acetate (XIII). A solution of XII (1.54 g) in EtOH (24 ml), acetic acid (6 ml) and water (3 ml) was stirred at room temperature under hydrogen atmosphere in the presence of 10% Pd-C (0.15 g) for 3 hr. After removal of catalyst by filtration, the filtrate was evaporated. The aqueous solution of the residue was washed with AcOEt and then evaporated to give a colorless glass of XIII (1.3 g) which was identified by PMR analysis.

N-[*N*-(*D*- and *L*-3-*trans*-Ethoxycarbonyloxiran-2-carbonyl)-*L*-leucyl]-*N*^o-nitroagmatine (XIV). A solution of XIII (3.2 g), triethylamine (0.96 g) and 3,5-dimethyl-1-nitroguanylpzrazole (1.74 g) in EtOH (100 ml) was heated under reflux for 3 hr. After removal of the solvent by distillation, the residue was washed with ethylether and extracted with hot AcOEt. The AcOEt layer was washed with 1 M citric acid and water and then evaporated to give a solid, which was submitted to a silicagel column (30 g). Elution with AcOEt in acetone (2:1) subsequent to AcOEt gave 1.21 g of XIV, which on recrystallization from hot AcOEt-isopropylether was obtained as colorless amorphous solid. mp 135~140°C, $[\alpha]_D^{14} - 21.7$ ($c=0.502$, EtOH), *Anal.* Calcd. for $C_{17}H_{26}N_6O_7$: C, 47.43; H, 7.03; N, 19.52. Found: C, 47.67; H, 7.15; N, 19.26.

N-[*N*-(*D*- and *L*-3-*trans*-Carboxyoxiran-2-carbonyl)-*L*-leucyl]-*N*^o-nitroagmatine (XV). To a methanolic

solution (4 ml) of XIV (0.594 g), was added 2 N KOH solution (0.76 ml) under ice cooling and left at room temperature for 4 hr. The solution was diluted with water (20 ml) and neutralized with Dowex 50W (H^+ , 4 ml). After removal of the resin by filtration, the filtrate was evaporated to dryness. The resulting solid was crystallized from water to give 0.352 g of colorless powder of XV, mp 170~173°C (decomp.), $[\alpha]_D^{12} - 22.2^\circ$ ($c=0.482$, MeOH). *Anal.* Calcd. for $C_{15}H_{26}N_6O_7$: C, 44.77; H, 6.51; N, 20.89. Found: C, 44.53; H, 6.50; N, 20.59.

N-[*N*-(*D*-3-*trans*-Carboxyoxiran-2-carbonyl)-*L*-leucyl]-agmatine (I'). A solution of XV (0.453 g) in MeOH (20 ml), acetic acid (2 ml) and water was treated with 10% Pd-C (0.07 g) at room temperature under stream of H_2 for 5 hr. After removal of the catalyst, the solution was evaporated. The aqueous solution of the residue was applied to a column of charcoal (15 ml). Elution with 50% aqueous acetone subsequent to washing with water gave 0.156 g of a dihydrate of I', which on crystallization from EtOH-water was obtained as colorless crystals, mp 173~175°C (decomp.). *Anal.* Calcd. for $C_{15}H_{27}N_6O_8 \cdot 2H_2O$: C, 45.79; H, 7.94; N, 17.80. Found: C, 45.73; H, 7.90; N, 17.82. PMR ($D_2O + DCl$) δ : 0.8~1.1 (6H, br. s), 1.4~1.8 (7H, br. s), 3.1~3.4 (4H, br. s), 3.71, 3.78 (2H, d, $J=1.8$ Hz), 4.33 (1H, t, $J=6.6$ Hz). IR ν_{max}^{KBr} cm^{-1} : 3236 (NH), 1638 (C=O), 1560, 1454, 1390, 895. $[\alpha]_D^{18} - 80.6^\circ$ ($c=0.454$, 0.1 N HCl), TLC (cellulose): $R_f=0.58$ (BuOH: AcOH: water=4/1/2), 0.67 (*n*-ProOH: water=7/3).

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