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A NOVEL AMINO ACID ANTIBIOTIC TAN-950

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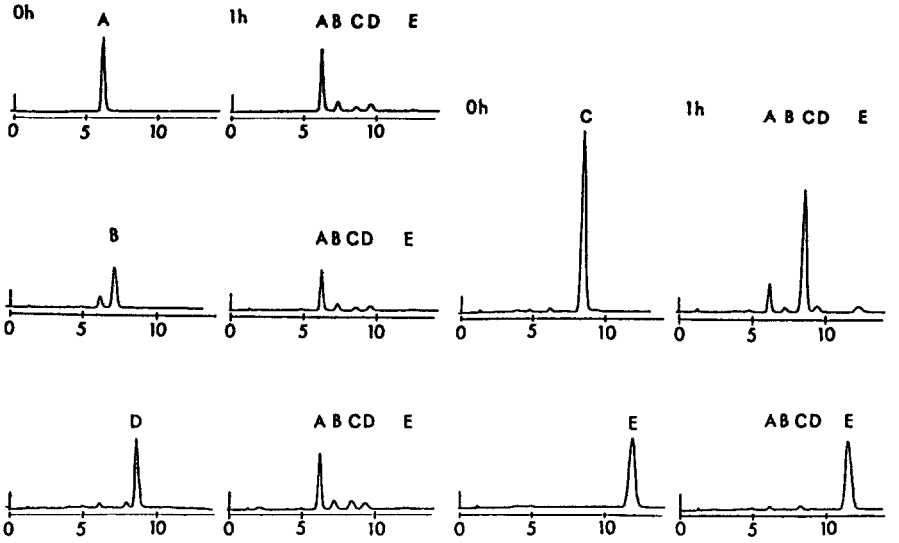
Abstract: A novel antifungal antibiotic, TAN-950 A, was isolated from the culture filtrate of Streptomyces platensis A-136. Its structure was determined to be (S)-2-amino-3-(isoxazolin-5-on-4-yl)-propanoic acid. Minor components, TAN-950 B, C, D and E, were found to be present in an equilibrium mixture containing TAN-950 A. Their structures were determined from spectral analyses, X-ray crystallographic analysis and synthesis from L-glutamic acid.

A new amino acid antibiotic, TAN-950 A (**1**), was isolated from the broth filtrate of Streptomyces platensis, in the course of screening for new antifungal antibiotics.[1,2] TAN-950 A showed protective effects against Candida albicans infection in mice. It was found to have high affinity for excitatory amino acid receptors in rat brain.[3] This paper deals with the structure elucidation of TAN-950 components and the synthesis of **1**.

Compound **1** was isolated by column chromatographies using a cation-exchange resin (Amberlite IR-120^R), adsorptive resins (Diaion HP-20^R and Diaion SP-207^R), anion-exchange Sephadex (QAE-Sephadex A-25^R) and microcrystalline cellulose. Upon QAE-Sephadex A-25^R chromatography, fractions containing mainly TAN-950 B (**2**), C (**3**), D (**4**) and E (**5**) were obtained and desalted with Amberlite IR-120^R to give a mixture of **1-5**. The ratio of the peak areas estimated by HPLC analysis was **1**:**2**:**3**:**4**:**5** = 2:1:2:1:2.[4] We tried to separate **2-4** by preparative HPLC, but could not because isomerization proceeded rapidly. Fig. 1 shows the HPLC patterns of **1-5** just after separation by preparative HPLC and after the samples had been allowed to stand at 60°C for an hour. The isomerization rates of **2** and **4** were faster than those of **3** and **5**. A mixture of **1-5** in 0.5 N sodium hydroxide became converted to **1** almost quantitatively in an hour at 60°C. This indicates that in an alkaline solution, the conversion to **1** is favored in the equilib-

Fig. 1. HPLC patterns of TAN-950 A-E (1-5) after preparative HPLC



rium.

Compound **1** was obtained as the mono sodium salt and the molecular formula was determined to be $C_6H_7N_2O_4Na$ on the basis of elemental analysis, secondary ion mass spectrum (SIMS) and NMR data (Tables 1 and 2). The UV spectrum indicated a maximum at 253 nm, suggesting the presence of an α,β-unsaturated carbonyl group. Coupling constants of ^1H NMR data showed that a methine (δ 3.86) and a methylene (δ 2.80 and 2.70) were connected. The chemical shift of the methine carbon (δ 58.49) and the amphoteric property shown in the isolation procedure suggested that the methine was an α-carbon of an amino acid.

Derivatization and degradation experiments of **1** were also carried out (Fig. 2). Compound **1** gave an N-benzoyl derivative (**6**), which showed the presence of an amino group. In the ^1H-^1H COSY spectrum of **6**, an amide proton (δ 8.71) showed an interaction with the methine (δ 4.52) indicating that the amino group in **1** was attached to the methine. On acid hydrolysis of **1** in 6 N hydrochloric acid, L-glutamic acid (**7**) was obtained, suggesting that five carbons of **1** were bound linearly. The remaining one carbon was also connected according to data from correlation spectroscopy *via* long range couplings (COLOC) in **1** (Fig. 3). The methylene protons (H-3) exhibited cross-peaks with all other carbons. An olefinic proton (δ 7.98) also showed interactions with C-4 and C-6 indicating that C-4 and C-5 formed a trisubstituted double bond.

Upon treatment with diazomethane, **6** afforded compounds **8** and **9**. ^{13}C

Table 1. ^{13}C NMR spectral data of TAN-950 A (1) and related compounds

Position	1[a]	6[b]	12a[b]
C-6	180.58 s	172.66 s	174.91 s
C-1	177.09 s	171.71 s	172.15 s
C-5	155.54 d	151.94 d	146.83 d
C-4	82.85 s	93.36 s	40.89 d
C-2	58.49 d	52.21 d	53.10 d
C-3	26.51 t	23.64 t	28.52 t
Bz(CO)		166.25 s	
Aromatic		133.77 s	147.11 s
"		131.33 d	143.38 s
"		128.20 d	128.54 d
"		127.22 d	123.48 d
PNB(CH_2)			65.05 t

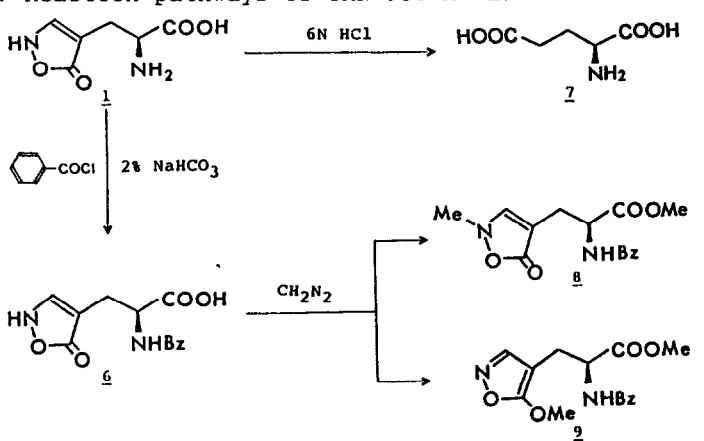
a:In D_2O. b:In DMSO-d_6.

Table 2. ^{1}H NMR spectral data of TAN-950 A (1) and related compounds

Position	1[a]	6[b]	12a[b]
H-5	7.98 (s)	8.32 (s)	7.29 (s)
H-2	3.86 (dd, 4.4,7.3)	4.52 (ddd, 5.2,7.8, 9.3)	4.32 (d, 8.3)
H-3	2.80 (dd, 4.4,15.6)	2.76 (dd, 5.2,14.8)	2.52 (m)
	2.70 (dd, 7.3,15.6)	2.67 (dd, 9.3,14.8)	2.34 (m)
H-4			3.24 (dt, 6.2,9.2)
Aromatic		7.85 (2H,m)	8.25 (d, 8.6)
		7.50 (3H,m)	7.68 (d, 8.6)
NH		8.71 (d, 7.8)	8.38 (br)
COOH		12.66 (br)	
N=OH			10.83 (s)
PNB(CH_2)			5.32 (s)

Coupling constants in Hz are given in parentheses.
a: In D_2O. b: In DMSO-d_6.

Fig. 2. Reaction pathways of TAN-950 A (1)



NMR data indicated that **8** had an N-methyl (δ 39.92) and an O-methyl group (δ 52.51) and **9** had two O-methyl groups (δ 58.17 and 52.65). In the COLOC spectrum of **8** (J = 4Hz, in $CDCl_3$), a cross-peak was found between the N-methyl protons (δ 3.37) and C-5 (δ 151.92), which indicated that the nitrogen atom was bound to C-5. Taking into account the molecular formula of **8**, the carbonyl group on C-6 must be linked to the nitrogen to form an isoxazolin-5-one ring. Isoxazolin-5-ones give 5-methoxyisoxazoles in addition to N-methylisoxazolin-5-ones by diazomethane treatment.[5] These findings indicated the structures of these compounds to be as shown in Fig. 2.

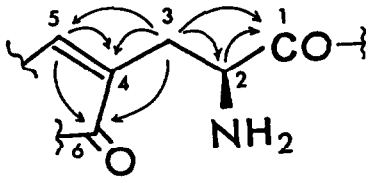
The structure of **1** was finally confirmed by X-ray crystallographic analysis of **6** (Fig. 4) to be (S)-2-amino-3-(isoxazolin-5-on-4-yl)-propanoic acid. An analogous compound has been isolated as a D-glucopyranosyl derivative from Pisum sativum L. seedling.[6]

When the mixture of **1-5** was allowed to react with 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON) in 50% aqueous dioxane, only the N-Boc derivative of **1** (**10**) was obtained (Fig. 5). Upon treatment with p-nitrobenzyl (PNB) bromide, the mixture of **1-5** gave three spots on TLC (silica gel; solvent system of ethyl acetate:methanol = 19:1). The substance giving an Rf value of 0.73 was easily determined to be a tri-PNB derivative of **1** (**11**) considering the reactivity of **1** with PNB bromide.

The substance giving an Rf value of 0.67 was a mono-PNB ester (**12a**, $C_{13}H_{13}N_3O_6$). The ^1H-^1H COSY data indicated the presence of a partial structure, $=\overset{|}{C}H\overset{|}{C}HCH_2\overset{|}{C}H-$. Further structure elucidation of **12a** was based on a COLOC experiment (Fig. 6). An exchangeable proton with deuterium oxide (δ 8.38) showed coupling with C-2, C-4 and C-6. As C-6 also showed correlations with H-2 and H-3, the C-6 carbonyl was found to be amidated to form a five-membered ring. Another exchangeable proton with deuterium oxide (δ 10.83) showed an interaction with C-5 (δ 146.83) indicating the presence of an oxime moiety. These findings indicated the structure of **12a** to be the oxime derivative of pyroglutamic acid as shown in Fig. 6. The substance giving an Rf value of 0.56 was deduced to have the same molecular formula as **12a**, but the ^1H-^1H COSY spectrum showed three series of the $=\overset{|}{C}H\overset{|}{C}HCH_2\overset{|}{C}H-$ group. These data indicated that it contained three compounds (**12b, c** and **d**) having structures similar to **12a**.

The characteristic chemical shifts of aldoxime protons were present and could be used to assign the stereochemistry of the oxime group. An aldehydic proton in the Z oxime has been reported to lie at higher field than that of the E form by about 0.6-0.7 ppm.[7,8] In the ^1H NMR spectrum of the mixture of **1-5**, H-5 protons of **2-5** were observed at δ 7.45, 7.40, 6.95 and 6.90 in the ratio of 2:2:1:1, respectively. As the ratio of **2**, **3**, **4** and **5** was estimated to be 1:2:1:2 from HPLC analysis, **2** and **4** had the Z oxime

Fig. 3. COLOC data of TAN-950 A (1)



→ $^{1}H \longrightarrow {}^{13}C$ Long range coupling
(J = 7 Hz, in D_2O)

Fig. 4. Molecular structure of 6

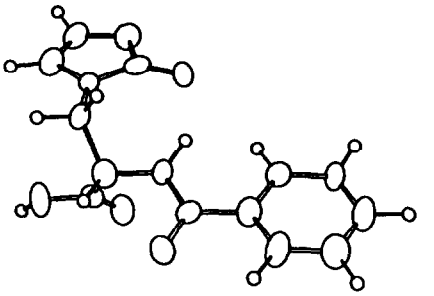


Fig. 5. Reaction pathways of TAN-950 A-E mixture (1-5)

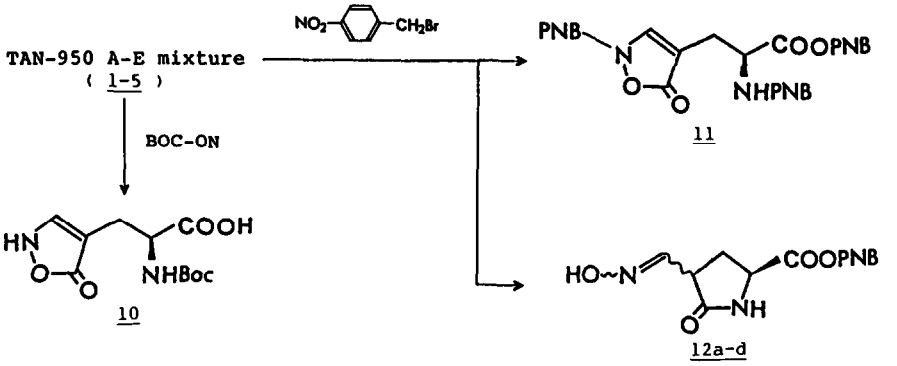
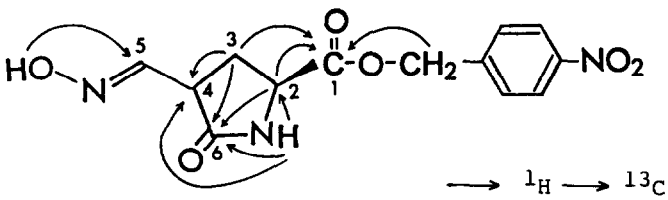
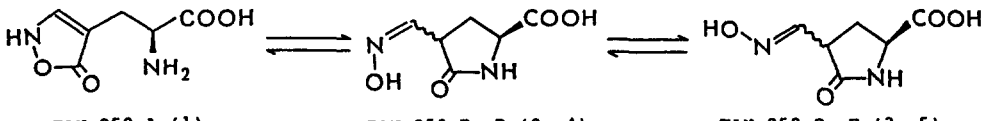


Fig. 6. COLOC data of 12a



→ $^{1}H \longrightarrow {}^{13}C$ Long range coupling
(J = 6.3 Hz, in DMSO-d_6)

Fig. 7. Equilibrium machanism of TAN-950 A-E (1-5)



TAN-950 A (1) TAN-950 B, D (2, 4) TAN-950 C, E (3, 5)

form and **3** and **5** had the E form (Fig. 7). The difference in isomerization rates of **2-5**, which we described previously, can be explained by the stereochemistry of the oxime group. As **2** and **4** have Z oxime, the hydroxyl group is located near the amide group. Thus the hydroxyl group can easily attack the carbonyl of the amide group to afford **1**. The oxime orientation of **12a** was also determined to be E, because H-5 protons were observed at δ 7.29 in **12a** and at δ 7.28, 6.71 and 6.67 in the mixture of **12b-d**.

Taking into account the equilibrium mechanism described above, the synthesis of **1** was performed using a pyroglutamic acid analogue as a key intermediate (Fig. 8). N-Boc-L-Glutamic acid dimethyl ester (**13**) and N-Boc-L-pyroglutamic acid methyl ester (**14**) were prepared from L-glutamic acid by methods like those described in literature.[9,10] When **13** was reacted with lithium diisopropylamide (LDA) or lithium bis-(trimethylsilyl)amide and successively with isopropyl formate, it gave a formyl derivative of pyroglutamic acid (**15**). Compound **14** afforded **15** in a similar manner. Upon treatment with hydroxylamine, **15** gave an oxime derivative (**16**), which was the N-Boc methyl ester form of TAN-950 B-E. When **16** was treated with one equivalent of sodium hydroxide, isomerization proceeded, as expected, to afford **17**. Hydrolysis of **17** afforded N-Boc-TAN-950 A (**10**), which could be obtained from **16** directly. Deprotection of the Boc group of **10** gave **1**. The optical purity of the synthesized compound **1** was determined to be 88% ee from that of glutamic acid obtained by 6 N hydrochloric acid hydrolysis. The ratio of D to L glutamic acid was detected by HPLC using a chiral mobile phase.[11] An enantiomer of TAN-950 A (**18**) was also obtained from D-glutamic acid by almost the same method as described above. Its optical purity was 72% ee.

TAN-950 A is active *in vitro* against yeasts such as *C. albicans*, *C. tropicalis* or *Saccharomyces cerevisiae*.[1] The antifungal activity of TAN-950 B, C, D and E increases in proportion to the equilibrium rate. This suggests that the antifungal activity shown by the minor components is derived mainly from TAN-950 A. The enantiomer of TAN-950 A (**18**) did not show any antifungal activity. TAN-950 A also showed protective effects against *C. albicans* infection in mice.[1]

Furthermore, TAN-950 A had high affinity for excitatory amino acid receptors in rat brain as shown in Table 3.[3] The receptors can be divided into at least three subtypes: kainate (K), quisqualate (Q) and N-methyl-D-aspartate (N) subtypes. TAN-950 A effectively elicited the firing of the hippocampal CA1 neurons *in vitro*. The mixture of TAN-950 A-E showed less affinity for Q and K subtypes and more affinity for the N subtype than TAN-950 A. Studies on the modification and pharmacology of TAN-950 A are now in progress.[12]

Fig. 8. Synthetic route of TAN-950 A (1)

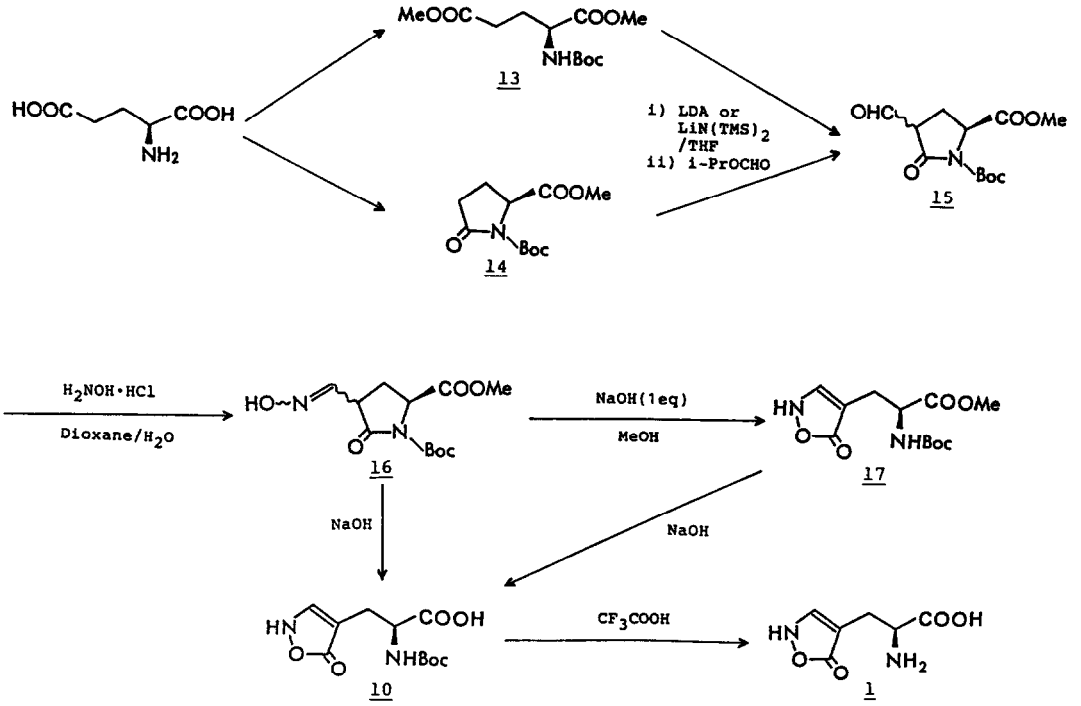


Table 3. Receptor binding and neuronal excitation in rat brain

Compound	Receptor binding K	Q (IC_{50}: μM)	N	Excitation of neurons MEC (μM)	subtype
TAN-950 A	3.6	0.28	19	300	Q/K
TAN-950 A-E	7.9	3.8	6.1	300	Q/K,N

MEC: Minimum effective concentration

Experimental

Melting points were determined using a Yamato melting point apparatus model MP-21. The optical rotations and UV spectra were recorded on a Jasco DIP-181 instrument and a Hitachi 320 spectrophotometer, respectively, and measured at 20-25° C in MeOH unless otherwise stated. The IR spectra were measured with a Hitachi 285 grating spectrophotometer in KBr pellets. The NMR spectra were obtained using a Bruker AC-300. As internal standard, 3-(trimethylsilyl)propionic acid d_4 sodium salt was used in D_2O and tetramethylsilane was used in $CDCl_3$ and DMSO-d_6. The SIMS were measured with a

Hitachi M-80A mass spectrometer with a xenon ion beam source in a glycerol matrix.

Isolation of **1** and the mixture of **1-5**: The isolation procedure was described in another paper.[1] **1**: $[\alpha]_D$ -70° (c 0.52, H_2O). UV(H_2O): 253 nm (ε 8,060). IR: 3430, 1640, 1500 cm^{-1}. SIMS: m/z 195 $(M+H)^+$. Anal. Calcd for $C_6H_7N_2O_4Na \cdot H_2O$: C, 33.97; H, 4.28; N, 13.21; Na, 10.66. Found: C, 33.64; H, 4.31; N, 12.72; Na, 11.0. HPLC: Column, ODS, YMC-Pack AQ-312 (Yamamura Chem. Lab.); Mobile phase, 2.5mM $n\text{-}Bu_4N^+OH^-$ /0.02M phosphate buffer (pH 6.0); Flow rate, 2 ml/min; Detection, UV absorbance at 214 nm; Retention time (min), **1**; 6.2, **2**; 7.3, **3**; 8.5, **4**; 9.5, **5**; 12.3.

N-Benzoyl TAN-950 A (**6**): To a solution of **1** (517 mg, 2.44 mmol) in 2% $NaHCO_3$ (30 ml) was added benzoyl chloride (350 μl, 2.99 mmol), and the mixture was stirred for 1 hour at room temperature. After adding additional benzoyl chloride (300 μl, 2.56 mmol) to the mixture, the solution was stirred at room temperature for 3 hours. The reaction mixture was washed with EtOAc and adjusted to pH 3 and washed with Et_2O. The aqueous layer was treated in the usual manner (extracted with EtOAc, washed with a saturated saline solution, dried over anhydrous Na_2SO_4 and concentrated). The resulting residue was precipitated with Et_2O to give a white powder of **6** (460 mg, y.68%). The powder (100 mg) was crystallized from MeOH - Et_2O to afford crystals of **6** (70 mg). mp 147-148.5° C (dec). $[\alpha]_D$ -28° (c 0.50). UV: 226 nm (ε 14,200), 257 (11,300). IR: 1745, 1650, 1580, 1540 cm^{-1}. FDMS: m/z 277 $(M+H)^+$. Anal. Calcd for $C_{13}H_{12}N_2O_5$: C, 56.52; H, 4.38; N, 10.14. Found: C, 56.50; H, 4.47; N, 10.12.

X-ray crystallographic analysis of **6**: A colorless columnar crystal of dimensions 0.1x0.2x0.7 mm was chosen for X-ray experiment. Data were collected on a Rigaku AFC-5 diffractometer equipped with a graphite monochromated MoKα radiation (λ =0.7107 Å). The crystal is orthorhombic, $P2_12_12_1$, with cell dimensions a=17.991(3), b=10.216(1), c=6.746(2) Å and V=1239.9(4) $Å^3$. In all, 1308 reflections were measured, of which 769 with $F>2.0\sigma(F)$ were judged to be observed. The structure was solved by direct methods using program MULTAN78[13] from which the locations of all non-hydrogen atoms were obtained. The structure was refined using the full-matrix least-squares method (X-ray76[14]) and the positions of all hydrogen atoms were determined from a difference Fourier map. Non-hydrogen atoms were treated anisotropically, whereas hydrogen atoms were refined with isotropic thermal parameters. The final R was 0.059.

Prepation of L-glutamic acid (**7**) from **1**: A solution of **1** (200 mg) in 6N HCl (10 ml) was refluxed for 7 hours. The reaction mixture was evaporated to

dryness and the residue was chromatographed on a column of Dowex 1X2 (50-100 mesh, AcO^-type, 20 ml) eluting with 0.2N-0.5N AcOH. The eluate was concentrated and chromatographed on activated carbon (5 ml) eluting with H_2O and 20% MeOH. The eluate was concentrated and the residue was crystallized from H_2O-EtOH to give crystals of **7** (36 mg, y.26%). $[\alpha]_D$ +12° (c 0.52, H_2O) [cf. L-Glu: $[\alpha]_D$ +12.0° (H_2O)]. Anal. Calcd for $C_5H_9NO_4$: C, 40.82; H, 6.17; N 9.52. Found: C, 40.52; H, 6.13; N, 9.32. The physicochemical data were identical with those of L-glutamic acid.

N'-Methyl N-benzoyl TAN-950 A methyl ester (**8**) and O-methyl N-benzoyl TAN-950 A methyl ester (**9**): To a solution of **6** (270 mg) in MeOH (10 ml) was added an ether solution of diazomethane. The mixture was allowed to stand at room temperature for 30 minutes. The reaction mixture was concentrated and applied to a column of silica gel (15 g). The fractions eluted with hexane-EtOAc (1:1) and (1:4) were separately concentrated to give a white powder of **9** (138 mg, y.46%) and colorless oil of **8** (142 mg, y.48%), respectively. **9**: $[\alpha]_D$ -39° (c 0.49). UV: 227 nm (ε 17,500). IR: 1760, 1640, 1515 cm^{-1}. SIMS: m/z 305 $(M+H)^+$. Anal. Calcd for $C_{15}H_{16}N_2O_5$: C, 59.21; H, 5.30; N, 9.21. Found: C, 59.16; H, 5.32; N, 9.16. **8**: $[\alpha]_D$ -27° (c 0.54). UV: 226 nm (ε 12,200) and 270 (11,200). IR: 1740, 1665, 1605, 1535 cm^{-1}. SIMS: m/z 305 $(M+H)^+$. Anal. Calcd for $C_{15}H_{16}N_2O_5$: C, 59.21; H, 5.30; N, 9.21. Found: C, 59.59; H, 5.22; N, 8.77.

N-Boc TAN-950 A (**10**): To a solution of **1** (6.36 g, 30 mmol) in 50% aqueous dioxane (150 ml) were added Et_3N (6.3 ml, 45 mmol) and BOC-ON (purity 97%, 11.4 g, 45 mmol), and the mixture was stirred for 3 hours at room temperature. The reaction mixture was concentrated, diluted with H_2O (300 ml) and washed with EtOAc (3 X 150 ml). After adding NaCl, the aqueous layer was adjusted to pH 2.7 and treated in the usual manner. The resulting residue was precipitated with hexane-Et_2O to give a white powder of **10** (6.32 g, y.77%). $[\alpha]_D$ -29° (c 0.54). UV: 260 nm (ε 9,200). IR: 1675, 1580, 1510 cm^{-1}. ^1H NMR (DMSO-d_6) δ ppm 12.50 (1H,br), 8.23 (1H,s) 7.06 (1H,d,J=8.2Hz), 4.02 (1H,ddd,J=5.1, 8.2, 9.4Hz), 2.57 (1H,dd,J=5.1, 14.6Hz), 2.43 (1H,dd, J=9.4, 14.6Hz), 1.36 (9H,s). SIMS: m/z 273 $(M+H)^+$. Anal. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.51; H, 6.05; N, 10.23.

The mixture of **1-5** (20 mg) gave **10** (4.7 mg) by almost the same method as described above.

TAN-950 A tri-PNB derivative (**11**) and TAN-950 B-E mono-PNB esters (**12a-d**): To a solution of the mixture of **1-5** (4.8 g) in DMF (50 ml) was added PNB bromide (5.0 g), and the mixture was stirred for 1.5 hours at room tempera-

ture. After adding additional PNB bromide (5.0 g) to the mixture, the solution was stirred for 6 hours at room temperature. The reaction mixture was concentrated and the residue was treated in the usual manner. The resulting residue was precipitated with hexane to give a crude powder (10.2 g). The crude powder was applied to a column of silica gel (250 g). The fractions eluted with hexane-EtOAc (1:4) and EtOAc were separately concentrated to give a crude powder of **11** (690 mg) and a crude powder of **12a-d** (2.4 g), respectively. These were separately purified by chromatography on silica gel eluting with $CHCl_3$-EtOAc to afford a powder of **11** (390 mg), a powder of **12a** (150 mg) and a mixture of **12b-d** (880 mg), respectively. **11**: IR: 1740, 1605, 1515 cm^{-1}. ^1H NMR (DMSO-d_6) δ ppm 8.50 (1H,s), 8.22, 8.18, 8.15 (each 2H,d,J=8.6Hz), 7.63, 7.60, 7.56 (each 2H,d, J=8.6Hz), 5.24 (2H,ABq,J= 14.0Hz), 4.99 (2H,s), 3.93 (1H,br.d,J=15.0Hz), 3.78 (1H,br.d,J=15.0Hz), 3.51 (1H,m), 2.94 (1H,br.), 2.54 (2H,m). SIMS: m/z 578 $(M+H)^+$. Anal. Calcd for $C_{27}H_{33}N_5O_{10}$: C, 56.15; H, 4.01; N, 12.13. Found: C, 56.18; H, 3.91; N, 12.03. **12a**: $[\alpha]_D$ +45° (c 0.52); IR: 1755, 1705, 1610, 1520 cm^{-1}. SIMS: m/z 308 $(M+H)^+$. Anal. Calcd for $C_{13}H_{13}N_3O_6$: C, 50.82; H, 4.26; N, 13.68. Found: C, 50.82; H, 3.98; N, 13.62. **12b-d**: SIMS: m/z 308 $(M+H)^+$. Anal. Calcd for $C_{13}H_{13}N_3O_6$: C, 50.82; H, 4.26; N, 13.68. Found: C, 50.67; H, 4.15; N, 13.63.

Synthesis of TAN-950 A (1)

γ-Formyl-N-Boc-L-pyroglutamic acid methyl ester (**15**): Method I: To a solution of **13** (40.7 g, 148 mmol) in anhydrous tetrahydrofuran (THF, 800 ml) was added 1.65 M LDA / THF-hexane (1:4) solution (197 ml, 326 mmol) at -78° C under an argon atmosphere. The resulting mixture was stirred at -40° C for 50 minutes. The mixture was cooled to -78 C, and isopropyl formate (22.2 ml, 222 mmol) was added to the solution. The solution was gradually warmed to -20° C over 3 hours. Isopropanol (56 ml), Et_2O (50 ml) and hexane (350 ml) were added to the reaction mixture. The solution was poured into a mixture of 1N HCl (760 ml) and H_2O (300 ml). The organic layer was extracted with 3% Na_2CO_3 (200 ml). The aqueous layer was washed with EtOAc (800 ml), adjusted to pH 2.8 and treated in the usual manner to give a paste of **15** (12.3 g, y.31%). The paste was crystallized from Et_2O-hexane. mp 106-108.5° C. IR: 1780, 1760, 1700, 1670 cm^{-1}. SIMS: m/z 377 $(M+H+DAE)^+$: measured in a mixed solvent of MeOH and diethanolamine (DAE). Anal. Calcd for $C_{12}H_{17}NO_6$: C, 53.13; H, 6.32; N, 5.16. Found: C, 53.18; H, 6.26; N, 5.06.

Method II: To a solution of **14** (112 mg, 0.459 mmol) in anhydrous THF (3 ml) was added a 1M lithium bis(trimethylsilyl)amide hexane solution (0.55 ml, 0.55 mmol) at -78 C under an argon atomosphere. The resulting mixture was

stirred for 20 minutes at -40° C. The mixture was cooled to -78° C, and isopropyl formate (69 μl, 0.688 mmol) was added to the solution. The mixture was gradually warmed to -40° C over 1 hour and then stirred for 3 hours. The reaction mixture was treated in the manner described above to give a white powder of **15** (57 mg, y.46%).

N-Boc-TAN-950 A methyl ester (**17**): To a solution of **15** (378 mg, 1.24 mmol) in H_2O-dioxane (1:9) solution (10 ml) was added hydroxylamine hydrochloride (107 mg, 1.50 mmol), and the mixture was stirred for 1 hour at room temperature. The reaction mixture was treated in the usual manner to give **16** (360 mg, y.91%). SIMS: m/z 392 $(M+H+DAE)^+$ (measured in a mixed solvent of MeOH and DAE). Anal. Calcd for $C_{12}H_{18}N_2O_6$: C, 50.35; H, 6.34; N, 9.79. Found: C, 50.68; H, 6.46; N, 9.24.

To a solution of **16** (76 mg, 0.26 mmol) in MeOH (2.5 ml) was added 2N NaOH (0.10 ml, 0.20 mmol), and the mixture was stirred for 2 hours at room temperature. The reaction mixture was diluted with Et_2O (10 ml) and poured into H_2O (10 ml). The aqueous layer was washed with Et_2O (10 ml), adjusted to pH 3 and treated in the usual manner to afford a white powder of **17** (57 mg, y.75%). $[\alpha]_D$ -19 (c 0.50); UV: 260 nm (ε 7,400). IR: 1710, 1600, 1520 cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm 7.94 (1H,br.s), 5.62 (1H,m), 4.43 (1H,m), 3.76 (3H,s), 2.80 (1H,dd,J=5.3,14.5Hz), 2.71 (1H,dd,J=6.2,14.5Hz), 1.43 (9H,s). Anal. Calcd for $C_{12}H_{18}N_2O_6$: C, 50.35; H, 6.34; N, 9.79. Found: C, 50.11; H, 6.42; N, 8.96.

N-Boc-TAN-950 A (**10**): To a solution of **17** (57 mg, 0.21 mmol) in MeOH (1.5 ml) was added 2N NaOH (0.3 ml, 0.60 mmol), and the mixture was stirred for 2 hours at room temperature. The reaction mixture was diluted with H_2O (10 ml) and washed with Et_2O (2 X 10 ml). The aqueous layer was adjusted to pH 2.5 and treated in the usual manner to give a white powder of **10** (46 mg, y.87%). $[\alpha]_D$ -18° (c 0.57). Anal. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.45; H, 5.89; N, 10.23. The Rf value of TLC, UV and ^1H NMR spectra were identical with those of the authentic sample synthesized from **1**.

TAN-950 A (**1**): A solution of **10** (12 mg) in CF_3COOH (1 ml) was allowed to stand at room temperature for 30 minutes. The reaction mixture was concentrated and the residue was precipitated with Et_2O to give a powder of **1** as the trifluoroacetic acid salt (9.5 mg, y.75%). The retention time of HPLC was identical with that of the natural compound (**1**).

Synthesis of (R)-TAN-950 A (**18**): Compound **18** was synthesized from D-glutamic acid by a method almost the same as that used to synthesize TAN-950 A. $[\alpha]_D$ +55 (c 0.50, H_2O). Anal. Calcd for $C_6H_7N_2O_4Na \cdot 1.3H_2O$: C, 33.13; H, 4.45; N, 12.88; Na, 10.57. Found: C, 33.24; H, 4.55; N, 12.88; Na, 8.7. The retention time of HPLC, UV and ^1H NMR spectra were identical with those

of the natural compound (1).

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