STUDIES ON TOMAYMYCIN. III.

SYNTHESES AND ANTITUMOR ACTIVITY OF TOMAYMYCIN ANALOGS

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(Received for publication June 29, 1983)

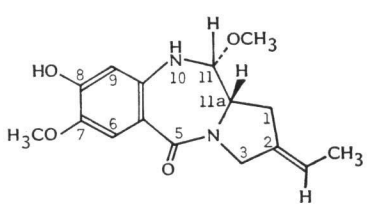
The syntheses and antitumor activity of tomaymycin analogs are described. Structural modification of such parts of tomaymycin as the ethylidenepyrrole ring, aminal bond, and substituents of the benzene ring are discussed.

Tomaymycin[1] is an antitumor antibiotic possessing the pyrrolo[1,4]benzodiazepine skeleton, which is bound to DNA through a covalent aminal linkage between the 2-amino group of guanine and C-11 of tomaymycin[2]. Hydrogen bonding between the 8-hydroxyl group of tomaymycin and a phosphorus group of DNA is also an important binding force[2]. As shown in Table 1, tomaymycin exhibits marked antitumor activity which fascinated us as a synthetic target. In a previous paper, we reported the total syntheses[3] of *E*-(**1E**) and *Z*-tomaymycin (**1Z**) whose structure[4] was determined on the basis of their ^{1}H NMR and ^{13}C NMR spectra. The synthetic *Z*-tomaymycin (**1Z**) exhibited the same antimicrobial activity[3] as that of naturally occurring *E*-tomaymycin (**1E**). We became interested in modification of the active sites of tomaymycin in order to seek more efficient antitumor agents. In this paper, we describe the syntheses of tomaymycin analogs and their antitumor activity.

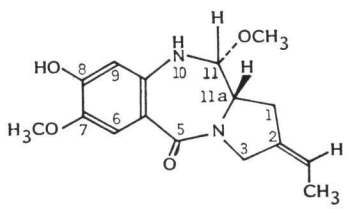
Chemistry

Modification in the Ethylidenepyrrole Ring of Tomaymycin

Modification in the ethylidenepyrrole ring of tomaymycin was accomplished by the general synthetic route shown in Scheme 1. Condensation of 5-methoxy-2-nitro-4-(4-nitrobenzyloxy)benzoyl chloride (**2a**) or 4-benzyloxy-5-methoxy-2-nitrobenzoyl chloride (**2b**) with the modified pyrrole derivatives (**3** or **3'**) according to the SCHOTTEN-BAUMAN method gave the corresponding amides (**4** or **4'**) in good yields. Diphenylmethyl esters (**4'**) were treated with trifluoroacetic acid (TFA) in anisole to give the corresponding acids (**4**) in good yields. Reduction of the modified pyrrole-2-carboxylic acids (**4**) with *N*,*N'*-carbonyldiimidazole and $LiAlH_4$ in tetrahydrofuran (THF) by the method of STAAB *et al.*[5] gave the corresponding aldehydes (**5**) in moderate yields. Catalytic hydrogenation of **5** with 5% Pd-$BaSO_4$ was



1E
E-Tomaymycin



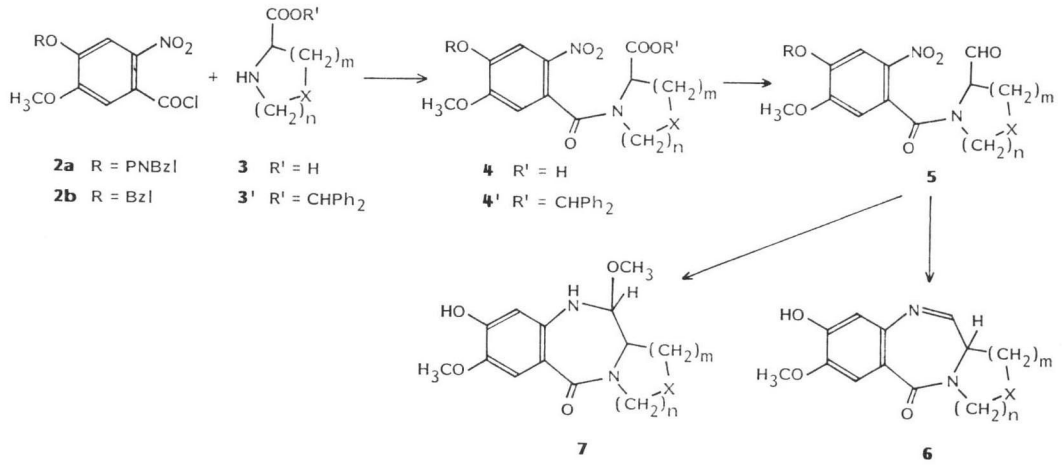
1Z
Z-Tomaymycin

carried out in THF or ethyl acetate (EtOAc) to give the corresponding [1,4]benzodiazepine derivatives (**6**). The similar catalytic hydrogenation of **5** in methanol (MeOH) gave the corresponding methanol adducts of [1,4]benzodiazepine derivatives (**7**).

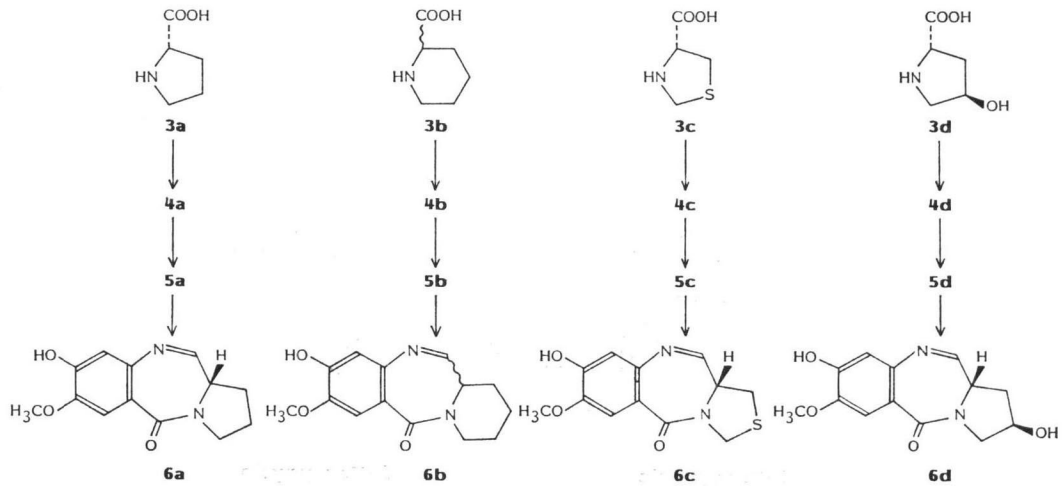
According to the synthetic route shown in Scheme 2, commercially available L-proline (**3a**), D,L-2-piperidinecarboxylic acid (**3b**), L-thioproline (**3c**), and L-hydroxyproline (**3d**)[8] were converted to the corresponding [1,4]benzodiazepine derivatives (**6a**~**d**) respectively.

According to the synthetic route shown in Scheme 3, *N*-*t*-butoxycarbonyl-4-oxo-L-proline diphenylmethyl ester (**8**)[4] was reacted with methoxyamine to give (*E*,*Z*)-*N*-*t*-butoxycarbonyl-4-methoxyimino-L-proline diphenylmethyl ester (**3''e**) in 53% yield. The WITTIG reaction of **8** with cyanomethylenetriphenylphosphorane[8] gave (*E*,*Z*)-*N*-*t*-butoxycarbonyl-4-cyanomethylene-L-proline diphenylmethyl ester (**3''f**) in 89% yield. The *t*-butoxycarbonyl group of **3''e** and **3''f** was removed by treatment with MeOH saturated with hydrogen chloride gas to give **3'e** in 65% yield and **3'f** in 62% yield respectively. **3'e** and **3'f** were converted to the corresponding [1,4]benzodiazepine derivatives (**7e**) and (**6f**) respectively

Scheme 1.



Scheme 2.



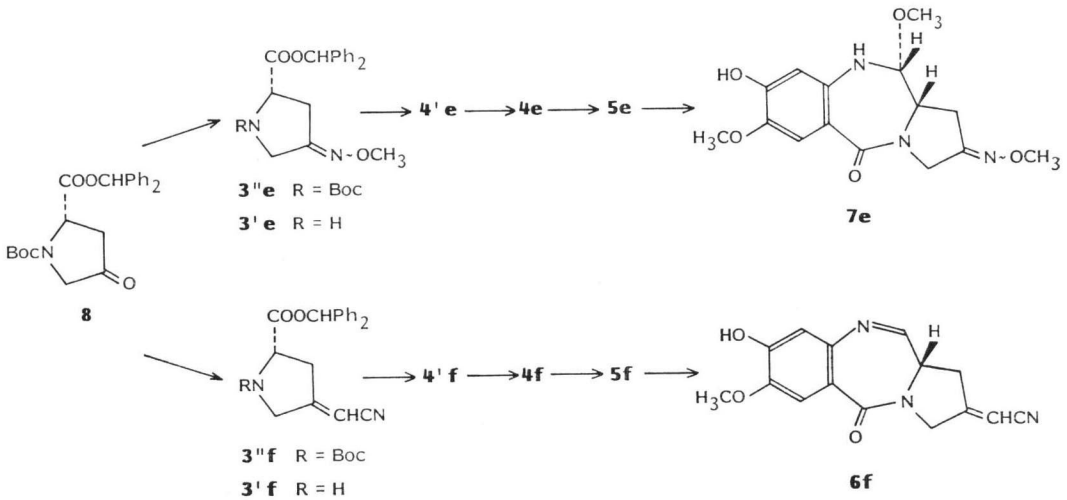
by a similar synthetic procedure to that shown in Scheme 1.

According to the synthetic route shown in Scheme 4, *N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-oxo-L-proline (**4g**) was prepared by oxidation of **4d** with JONES reagent[7] in 53% yield. Esterification of **4d** with palmitic anhydride gave **4h** in 86% yield. **4g** and **4h** were converted to the corresponding [1,4]benzodiazepine derivatives (**6g**) and (**7h**) respectively by a similar synthetic procedure as shown in Scheme 1. Similarly **7d** was prepared from **4d**.

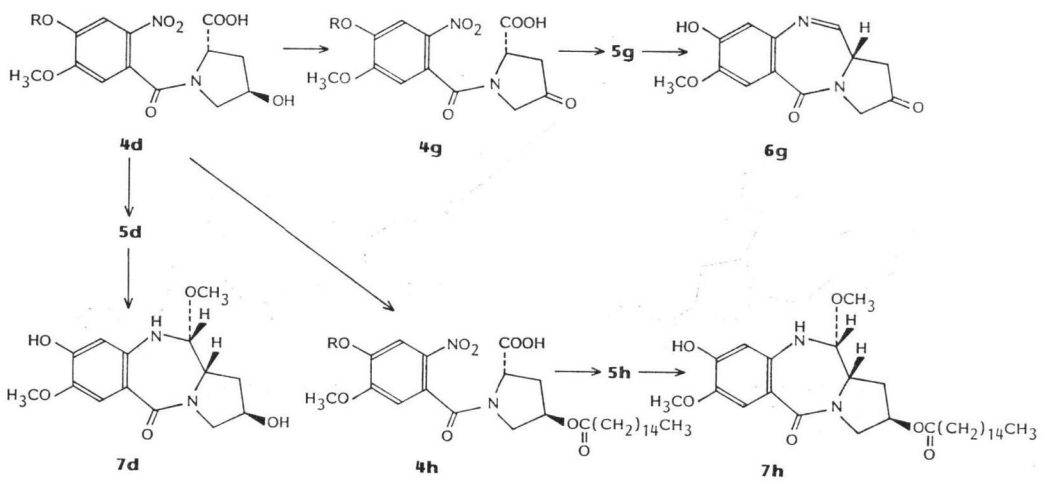
Modification at C-11 (aminal carbon) of Tomaymycin

Modification at C-11 of tomaymycin was accomplished by addition to (11a*S*) (*E*)-2-ethylidene-8-hydroxy-7-methoxy-5-oxo-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**9**)[8] of mercaptan, amine, or alcohol as shown in Scheme 5. Addition of ethyl mercaptan to **9** gave (11*R*, 11a*S*)(*E*)-2-ethylidene-11-ethylthio-1,2,3,10,11,11a-hexahydro-8-hydroxy-7-methoxy-5-oxo-5*H*-pyrrolo[2,1-c][1,4]-benzodiazepine (**10a**) in 60% yield. Addition of benzyl mercaptan to **9** gave (11*R*,11a*S*)(*E*)-11-benzyl-

Scheme 3.



Scheme 4.



thio-2-ethylidene-1,2,3,10,11,11a-hexahydro-8-hydroxy-7-methoxy-5-oxo-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**10b**) in 18% yield. Addition of diethylamine to **9** gave (11*R*,11a*S*)(*E*)-11-diethylamino-2-ethylidene-1,2,3,10,11,11a-hexahydro-8-hydroxy-7-methoxy-5-oxo-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**11**) in 43% yield. Addition of MeOH to **9** was reported in the previous paper[8].

Modification of Substituents on the Benzene Ring of Tomaymycin

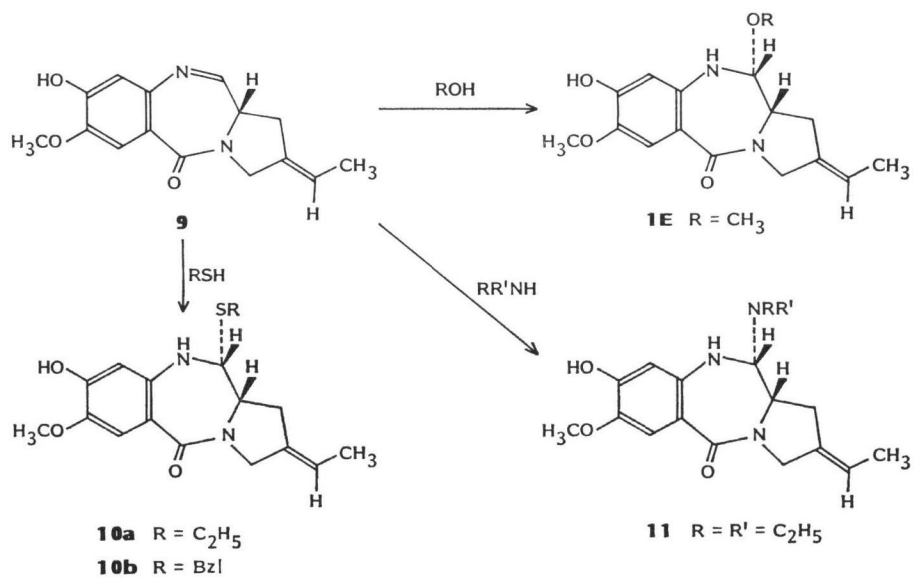
Methylation of tomaymycin (**1E**) with diazomethane gave (11*R*,11a*S*)(*E*)-2-ethylidene-1,2,3,10,11,11a-hexahydro-5-oxo-7,8,11-trimethoxy-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**13**)[8].

According to the synthetic route shown in Scheme 6, (11*R*,11a*S*)-9,11-dimethoxy-1,2,3,10,11,11a-hexahydro-6-hydroxy-5-oxo-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**19**) was prepared. Benzylation of 6-hydroxy-3-methoxy-2-nitrobenzoic acid (**14**)[9] gave 6-benzyloxy-3-methoxy-2-nitrobenzoic acid (**15**) in 91% yield. Treatment of **15** with thionyl chloride gave 6-benzyloxy-3-methoxy-2-nitrobenzoyl chloride (**16**) in 89% yield. Condensation of **16** with L-proline (**3a**) gave *N*-(6-benzyloxy-3-methoxy-2-nitrobenzoyl)-L-proline (**17**) in 96% yield. Reduction of **17** with the STAAB reagent[5] gave the corresponding aldehyde (**18**) in 92% yield. Catalytic hydrogenation of **18** with 10% Pd-carbon in a mixture of EtOAc and MeOH (2: 1) gave **19** in 75% yield.

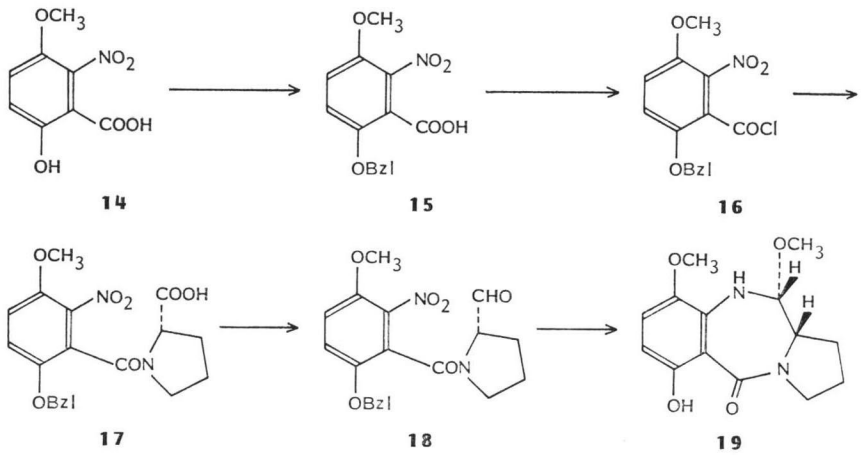
Antitumor Activity of Tomaymycin (1E)

Antitumor activity of tomaymycin is shown in Table 1. Tomaymycin has potent antitumor activity against L 1210 leukemia transplanted intraperitoneally in DBA 2/C57 BL mice. In the case of intraperitoneal administration of tomaymycin (Dose 125 μg/kg/day) in mice for five consecutive days, increased life span (ILS) is 26~50%. Tomaymycin shows complete suppression of growth of transplantable ascites tumors such as Sarcoma 180 and Ehrlich carcinoma. In the case of intraperitoneal administration of tomaymycin (Dose 31.3~125 μg/kg/day) for five consecutive days, inhibition of growth is 76~99%. However, tomaymycin does not inhibit the growth of the solid form of these tumors.

Scheme 5.



Scheme 6.



Antitumor Activity of Tomaymycin Analogs

Antitumor activity of tomaymycin analogs on P388 leukemia was evaluated either by the method of ILS effect or log cell killing. The ILS effect of tomaymycin analogs on P388 leukemia in BDF_1 mice is shown in Table 2. A number of tomaymycin analogs modified in the ethylidenepyrrole ring or the benzene ring of tomaymycin led to decrease of potency in antitumor activity. Three analogs, **6a**, **6d**, and **7d** were active against P388 leukemia. Especially, **6a** has effective antitumor activity in a wide range (12.5 ~ 150 mg/kg/day). Other analogs, **7a**, **6b**, **6c**, **6f**, **6g**, and **19** were inactive against P388 leukemia on an intermittent dosage schedule.

Table 1. Antitumor activity of tomaymycin in mouse.

Tumor[a]	Mouse[b] strain	Dose[c] (μg/kg/day)	Inhibition[d] ratio (%)
Leukemia L1210 (ascites)	DBA 2/C57BL	125	26~50
		62.5	11~25
Sarcoma 180 (solid)	ICR	250	toxic
		125	0
Sarcoma 180 (ascites)	ICR	125	99
		62.5	99
		31.3	76~99
		15.6	11~25
Ehrlich carcinoma (solid)	ICR	125	0
		62.5	0
Ehrlich carcinoma (ascites)	ICR	125	99
		62.5	99
		31.3	51~75
		15.6	0
Lewis lung carcinoma (solid)	C57BL	125	25
		62.5	0

[a] Tumor inoculum: 1×10^6 cells implanted i.p. or s.c. on day 0.
[b] Number of mouse: 5
[c] Agent administered i.p. every day on days 1 to 5.
[d] ILS % against L1210, inhibition expressed $(1-T/C)\times100$ against other tumors.

Table 2. Antitumor activity of tomaymycin analogs on P388 leukemia (i.p.-i.p.) by ILS effect.

Compound	Dose (mg/kg/day)	Schedule (day)	No. of BDF_1 mice	ILS (%)
6a	200	1~5	10	toxic
	150	1~5	10	52
	100	1~5	10	34
	50	1~5	10	30
	25	1~5	10	30
	12.5	1~5	10	19
7d	50	1~4	10	39
6d	50	1~4	10	20
7a	20	1, 2, 4, 6, 7	3	no
6b	50	1, 2, 4, 6, 7	3	no
6c	50	1, 2, 4, 6, 7	3	no
6f	50	1, 2, 4, 6, 7	3	no
6g	50	1, 2, 4, 6, 7	3	no
19	50	1, 2, 4, 6, 7	3	no

Tumor inoculum: 1×10^5 ascites cells implanted i.p. on day 0. Agents were administered i.p. on scheduled days.

ILS %: (MST treated/MST control$-1)\times100$. MST=median survival time.

Log cell killing analysis on P388 leukemia in BDF_1 mice of tomaymycin analogs is shown in Table 3. Multiplication of P388 leukemia cell (1×10^5) transplanted intraperitoneally into BDF_1 mice produces 7×10^7 cells after four days. Tomaymycin (**1E**) and its analogs, **7e** and **13** inhibited completely multiplication of P388 leukemia cells. An analog, **7h** is a weak inhibitor of multiplication of P388 leukemia cells. An analog modified in a substituent on the benzene ring of tomaymycin, **13** has the same potency as that of tomaymycin in an efficient dose. However, analogs modified in the ethylidenepyrrole ring of tomaymycin, **7e** and **7h** have less potency than that of tomaymycin in an efficient dose.

Table 3. Antitumor activity of tomaymycin analogs on P388 leukemia in BDF_1 mice (i.p.-i.p.) by log cell killing method.

Compound	Dose (mg/kg/day)	Schedule (day)	No. of P388 leukemia cells in mice on day 4
1E	0.1	1~3	toxic
	0.01	1~3	2.0×10^5
	0.001	1~3	8.6×10^8
7e	10	1~3	5.0×10^5
	1	1~3	1.5×10^8
	0.1	1~3	6.5×10^7
7h	10	1~3	1.0×10^8
	1	1~3	4.0×10^7
	0.1	1~3	6.0×10^7
13	1	1~3	toxic
	0.1	1~3	1.5×10^5
	0.01	1~3	1.8×10^5
	0.001	1~3	4.2×10^7
Adriamycin	0.01	1~3	4.5×10^5
Control	none	—	7.0×10^7

Tumor inoculum: 1×10^5 ascites cells implanted i.p. on day 0. Agents were administered i.p. every day on days 1 to 3.

Antiphage Effect of Tomaymycin Analogs

Tomaymycin analogs modified at the methoxy group at C-11 (aminal) or at a substituent on the benzene ring of tomaymycin were evaluated for antiphage effect on SP10 phage. Demethanolated tomaymycin (**9**) and 8-*O*-methyltomaymycin (**13**) have the same antiphage activity as that of tomaymycin. Ethylthio analog (**10a**) and benzylthio analog (**10b**) have one half the potency of tomaymycin. Dimethylamino analog (**11**) is 125-fold less active than tomaymycin.

Table 4. Antiphage activity of tomaymycin analogs on SP10 phage.

	1E	**9**	**10a**	**10b**	**11**	**13**	Anthramycin
ID_{50} (μg/ml)	0.2	0.2	0.4	0.4	25	0.2	2.5

Experimental

Melting points were taken with an Arther H. Thomas melting point apparatus and are uncorrected. IR spectra were measured on a Hitachi 260-10 spectrophotometer. NMR spectra were measured at 100 MHz on a Jeol-MH 100 NMR spectrometer and at 60 MHz on a JNM-PMK 60 NMR spectrometer using Me_4Si as an internal standard. Mass spectra were measured on a Hitachi M-80 and Jeol JMS-D 300 mass spectrometer.

(*E*,*Z*)-*N*-*t*-Butoxycarbonyl-4-methoxyimino-L-proline Diphenylmethyl Ester (**3″e**)

A solution of **8** (15.0 g), methoxyamine hydrochloride (7.76 g), and triethylamine (50 ml) in ethanol (100 ml) was stirred for 6 hours at 50°C and evaporated *in vacuo*. To the residue were added EtOAc and H_2O. The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was chromatographed on silica gel. After elution with *n*-hexane, subsequently with benzene, and then with diisopropyl ether (IPE), the IPE fraction was evaporated *in vacuo*. The residue was recrystallized from IPE to give **3″e** (8.50 g, 53%); mp 112~113°C; IR (Nujol) 1745, 1695 cm^{-1}; NMR (DMSO-d_6) δ 1.1~1.6 (9H, m), 2.7~3.1 (2H, m), 3.83 (3H, s), 4.20 (2H, s), 4.5~4.8 (1H, m), 6.87 (1H, s), 7.30 (10H, s).

(*E*,*Z*)-*N*-*t*-Butoxycarbonyl-4-cyanomethylene-L-proline Diphenylmethyl Ester (**3″f**)

A solution of **8** (11.9 g) and cyanomethylenetriphenylphosphorane[6] (9.64 g) in THF (100 ml) was refluxed for 5 hours and evaporated *in vacuo*. The residue was dissolved in benzene and chromatographed on silica gel. The eluent with chloroform was evaporated *in vacuo* to give **3″f** (11.2 g, 89%)

as syrup: IR (film) 2230, 1740, 1700 cm^{-1}; NMR ($CDCl_3$) δ 1.2~1.6 (9H, m), 2.7~3.3 (2H, m), 4.2~4.5 (2H, m), 4.5~4.9 (1H, m), 5.1~5.4 (1H, m), 6.9 (1H, s), 7.33 (10H, s).

(*E,Z*)-4-Cyanomethylene-L-proline Diphenylmethyl Ester (**3'f**)

To a solution of **3''f** (9.17 g) in MeOH (50 ml) was added MeOH (20 ml) saturated with hydrogen chloride at 3°C. The solution was stirred at room temperature for 4 hours, cooled with an ice-water bath, adjusted to pH 8.0 with triethylamine, and evaporated *in vacuo*. To the residue was added EtOAc. Insoluble material was filtered off. The filtrate was washed with H_2O, dried over $MgSO_4$, treated with charcoal, filtered, and evaporated *in vacuo* to give **3'f** (4.32 g, 62%) as brown oil: IR (Nujol) 3340, 2200, 1720 cm^{-1}; NMR (DMSO-d_6, 100 MHz) δ 2.7~3.0 (2H, m), 3.4~3.8 (2H, m), 3.9~4.2 (1H, m), 5.5~5.7 (1H, m), 6.8 (1H, s), 7.2~7.5 (10H, m).

(*E,Z*)-4-Methoxyimino-L-proline Diphenylmethyl Ester (**3'e**)

t-Butoxycarbonyl group of **3''e** was removed as described above to give **3'e** as brown oil in 65% yield: IR (film) 3330, 1735 cm^{-1}; NMR ($CDCl_3$) δ 2.7~3.0 (3H, m), 3.6~3.8 (2H, m), 3.85 (3H, s), 3.9~4.3 (1H, m), 6.90 (1H, s), 7.33 (10H, s).

General Procedure for Condensation of **3** with **2a** or **2b**.

A solution of 5-methoxy-2-nitro-4-(4-nitrobenzyloxy)benzoyl chloride (**2a**) or 4-benzyloxy-5-methoxy-2-nitrobenzoyl chloride (**2b**) (15 mmol) in dry THF (20 ml) was added to an ice-cooled solution of **3** (15 mmol) and triethylamine (15 mmol) in H_2O (10 ml) with stirring. The mixture was stirred at room temperature for 20 minutes and evaporated *in vacuo*. The concentrated aqueous solution was adjusted to pH 2.0 with 15% hydrochloric acid. The resulting precipitate was filtered, washed with brine, dried over P_2O_5, and recrystallized from ethanol to give **4**.

Table 5. Physical data and yields of **4**.

Condensation	Yield (%)	mp (°C)	IR ν_{max}^{Nujol} (cm^{-1})
3a+**2a**→**4a**	69	116	1720
3b+**2b**→**4b**	66	130	1710
3c+**2a**→**4c**	79	115	1730
3'e+**2a**→**4'e**	98	145	1740
3'f+**2b**→**4'f**	94	107	1720

(*E,Z*)-*N*-[5-Methoxy-2-nitro-4-(4-nitrobenzyloxy)benzoyl]-4-methoxyimino-L-proline (**4e**)

To an ice-cooled mixture of **4'e** (5.45 g) and anisole (3 ml) was added trifluoroacetic acid (9 ml). The solution was stirred at room temperature for 30 minutes. To the solution was added IPE and the resulting precipitate was separated by filtration to give **4e** (4.09 g, 100%): IR (Nujol) 1735, 1650 cm^{-1}; NMR (DMSO-d_6, 100 MHz) δ 2.7~3.3 (2H, m), 3.72, 3.84 (3H, two s), 4.0 (3H, s), 4.1~4.6 (2H, m), 4.6~5.0 (1H, m), 5.43 (2H, s), 7.04 (1H, s), 7.72 (2H, d, J=9 Hz), 7.84 (1H, s), 8.26 (2H, d, J=9 Hz).

(*E,Z*)-*N*-(4-Benzyloxy-5-methoxy-2-nitrobenzoyl)-4-cyanomethylene-L-proline (**4f**)

Diphenylmethyl group of **4'f** was removed as described above to give **4f** quantitatively: IR (Nujol) 2240, 1745, 1650 cm^{-1}; NMR (DMSO-d_6) δ 2.9~3.3 (2H, m), 3.5~4.0 (2H, m), 3.97 (3H, s), 4.1~4.5 (1H, m), 5.27 (2H, s), 5.6~6.0 (1H, m), 7.03 (1H, s), 7.6 (5H, s), 7.87 (1H, s).

N-(4-Benzyloxy-5-methoxy-2-nitrobenzoyl)-4-oxo-L-proline (**4g**)

To a suspension of *N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-hydroxy-L-proline (**4d**) (3.3 g) in acetone (100 ml) was added JONES reagent[7] (9 ml) under bubbling N_2 gas. The mixture was stirred at room temperature for 10 minutes and evaporated *in vacuo*. To the concentrated solution were added EtOAc and H_2O. The organic layer was separated, washed with brine, dried over $MgSO_4$, treated with charcoal, filtered, and evaporated *in vacuo* to give **4g** (1.75 g, 53%) as powder: IR (Nujol) 1760, 1640 cm^{-1}: NMR (DMSO-d_6, 100 MHz) δ 2.2~3.4 (2H, m), 3.4~4.0 (2H, m), 3.94 (3H, s), 4.1~4.6 (1H, m), 5.24 (2H, s), 7.0 (1H, s), 7.42 (5H, s), 7.8 (1H, s).

N-(4-Benzyloxy-5-methoxy-2-nitrobenzoyl)-4-palmytoyloxy-L-proline (**4h**)

A solution of **4d** (4.16 g) and palmitic anhydride (5.94 g) in pyridine (300 ml) was stirred at 80°C for 15 hours and evaporated *in vacuo*. To the residue were added EtOAc and H_2O, the organic layer was separated, dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate with chloroform was evaporated *in vacuo*. The residue was triturated with a mixture of *n*-hexane and IPE (1: 1) to give **4h** (5.60 g, 86%): NMR ($CDCl_3$) δ 0.9 (3H, t, J=3 Hz), 1.3

(26H, br. s), 2.1~2.6 (4H, m), 3.2~3.6 (2H, m), 3.98 (3H, s), 4.5~4.9 (1H, m), 5.2 (2H, s), 7.27 (1H, s), 7.4 (5H, s), 7.77 (1H, s).

General Procedure for Reduction of **4** with STAAB Reagent

A mixture of **4** (10 mmol) and *N,N'*-carbonyldiimidazole (20 mmol) in dry THF (50 ml) was stirred at 40°C for one hour. To the resulting solution was added $LiAlH_4$ (10 mmol) by portions at −10°C with stirring under a nitrogen atmosphere. The mixture was stirred at −10°C for 10 minutes and then H_2O (10 ml) was added. The resulting precipitate was filtered off and the filtrate was evaporated *in vacuo*. The residue was dissolved in EtOAc. The solution was washed with brine, dried over $MgSO_4$, treated with charcoal, and evaporated *in vacuo*. The residue was dissolved in EtOAc, chromatographed on silica gel, and eluted with a mixture of IPE - EtOAc (1 : 4) to give **5**.

Table 6. Physical data and yields of **5**.

Compound	Yield (%)	CHO	
		IR ν_{max}^{Nujol} (cm^{-1})	NMR (ppm)
5a	53	1720	9.59 (d, J=3 Hz)
5b	92	1730	9.57, 9.68 (two br.s)
5c	34	1740	9.67 (br.s)
5e	23	1690	10.16 (br.s)
5f	91	1730	9.67 (br.s)
5g	76	1730	9.65 (br.s)
5h	66	1710	9.83 (d, J=2 Hz)

(11a*S*)-8-Hydroxy-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**6a**)

A solution of **5a** (1.5 g) in a mixture of EtOAc (150 ml) and MeOH (10 ml) was shaken at room temperature for 8 hours in the presence of 5% Pd-$BaSO_4$ (1.8 g) under one atmosphere of hydrogen. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. The residue was triturated with IPE to give **6a** (0.7 g, 73%): mp 143~145°C (dec.); IR (Nujol) 3400, 1600, 1510 cm^{-1}; NMR (DMSO-d_6, 100 MHz) δ 1.5~2.2 (4H, m), 3.1~3.6 (2H, m), 3.8 (3H, s), 3.9~4.2 (1H, m), 6.98 (1H, s), 7.18 (1H, s), 7.3 (1H, s), 9.9~10.6 (1H, br. s); Mass m/z 246 (M^+).

(11a*S*)-5,11a-Dihydro-8-hydroxy-7-methoxy-5-oxo-1*H*,3*H*-thiazolo-[4,3-c][1,4]benzodiazepine (**6c**)

A solution of **5c** in a mixture of EtOAc and MeOH (3: 1) was hydrogenated as described above to give **6c** in 23% yield: mp 187~188°C (dec.); IR (Nujol) 3350, 1610 cm^{-1}; NMR (DMSO-d_6, 100 MHz) δ 3.0~3.5 (2H, m), 3.76 (3H, s), 3.9~4.8 (3H, m), 7.0 (1H, s), 7.16 (1H, s), 7.62 (1H, br.s).

(12a*R*, *S*)-1, 2, 3, 4, 6, 12a-Hexahydro-9-hydroxy-8-methoxy-6-oxo-pyrido[2,1-c] [1,4]benzodiazepine (**6b**)

A solution of **5b** in a mixture of EtOAc and MeOH (40: 1) was hydrogenated as described above to give **6b** in 89% yield: mp 112~113°C (dec.); IR (Nujol) 1600 cm^{-1}; NMR (DMSO-d_6) δ 1.0~2.0 (6H, m), 3.16 (2H, s), 3.87 (3H, s), 4.0~5.0 (1H, m), 6.97 (1H, s), 7.47 (1H, s), 7.54 (1H, d, J=3Hz), 10.0~10.3 (1H, br.s).

(11a*S*)(*E,Z*)-2-Cyanomethylene-8-hydroxy-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**6f**)

A solution of **5f** in a mixture of EtOAc and MeOH (15: 1) was hydrogenated as described above to give **6f** in 33% yield: IR (Nujol) 2240, 1620, 1600 cm^{-1}; NMR (DMSO-d_6) δ 3.2~4.0 (4H, m), 3.8 (3H, s), 4.1~4.5 (1H, m), 5.0~5.3 (1H, m), 7.0 (1H, s), 7.2 (1H, d, J=4 Hz), 7.76 (1H, s).

(11a*S*)-2,5-Dioxo-8-hydroxy-7-methoxy-2,3,5,11a-tetrahydro-1*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**6g**)

A solution of **5g** in a mixture of EtOAc and MeOH (30: 1) was hydrogenated as described above to give **6g** in 32% yield: IR (Nujol) 1740, 1600 cm^{-1}; NMR (DMSO-d_6, 100 MHz) δ 3.0~4.0 (4H, m), 3.76 (3H, s), 4.0~4.5 (1H, m), 6.66 (1H, s), 6.80 (1H, d, J=4 Hz), 7.60 (1H, s).

(11*R*,11a*S*)(*E,Z*)-7, 11-Dimethoxy-1, 2, 3, 10, 11, 11a-hexahydro-8-hydroxy-2-methoxyimino-5-oxo-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**7e**)

A solution of **5e** in MeOH was hydrogenated as described above to give **7e** in 46% yield: IR (Nujol) 1620, 1520 cm^{-1}; NMR (DMSO-d_6) δ 2.5~3.1 (2H, m), 3.1~4.1 (2H, m), 3.80 (3H, s), 3.83 (3H, s), 4.1~4.7 (1H, m), 6.58 (1H, s), 7.02 (1H, s), 7.24 (1H, d, J=3 Hz), 10.23 (1H, s).

(2*R*,11*R*,11a*S*)-7,11-Dimethoxy-1, 2, 3, 10, 11, 11a-hexahydro-8-hydroxy-5-oxo-2-palmytoyloxy-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**7h**)

A solution of **5h** in MeOH was hydrogenated as described above to give **7h** in 18% yield after column chromatography on silica gel eluted with EtOAc: mp 68~70°C; IR (Nujol) 1710, 1600 cm^{-1}; NMR (DMSO-d_6, 100 MHz) δ 0.84 (3H, t, J=3 Hz), 1.24 (26H, br.s), 2.0~2.4 (4H, m), 3.0~3.8 (2H, m), 3.64 (3H, s), 3.9 (3H, s), 4.9~5.3 (2H, m), 6.52 (1H, s), 6.84 (1H, s), 7.52 (1H, br.s).

(11*R*,11a*S*)(*E*)-2-Ethylidene-11-ethylthio-1, 2, 3, 10, 11, 11a-hexahydro-8-hydroxy-7-methoxy-5-oxo-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**10a**)

To a solution of **9** (0.27 g) in methylene chloride (CH_2Cl_2) (5 ml) was added ethyl mercaptan (1.5 ml). The solution was kept at room temperature for 6 days and evaporated *in vacuo*. To the residue were added water and CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give **10a** (0.2 g, 60%) as yellow crystals from methanol: mp 70~74°C (dec.); IR (Nujol) 3560, 3350, 1620, 1590 cm^{-1}.

(11*R*,11a*S*)(*E*)-11-Benzylthio-2-ethylidene-1,2,3,10,11,11a-hexahydro-8-hydroxy-7-methoxy-5-oxo-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**10b**)

To a solution of **9** (0.54 g) in CH_2Cl_2 (5 ml) was added benzyl mercaptan (0.26 g). The solution was kept at room temperature for 4 days and evaporated *in vacuo*. The residue was submitted to thin-layer chromatography on silica gel to give **10b** (0.14 g, 18%) as yellow crystals from benzene: mp 143~145°C (dec.); IR (Nujol) 3550, 3340, 1640, 1600, 1570 cm^{-1}; NMR (DMSO-d_6) δ 1.55 (3H, d), 2.60 (2H, m), 3.79 (3H, s), 3.5~4.0 (4H, m), 4.1~4.4 (2H, m), 5.4 (1H, m), 6.35 (1H, s), 7.20 (1H, s), 7.2~7.4 (5H, s).

(11*R*,11a*S*)(*E*)-11-Diethylamino-2-ethylidene-1,2,3,10,11,11a-hexahydro-8-hydroxy-7-methoxy-5-oxo-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine (**11**)

To a solution of **9** (0.54 g) in CH_2Cl_2 (5 ml) was added 40% aqueous diethylamine (1.2 g). The mixture was stirred at room temperature for 5 hours and kept for 4 days. The organic layer was separated, washed with water, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give **11** (0.3 g, 43%) as brown powder: mp 65~68°C (dec.); IR (Nujol) 3650, 3500, 1610 cm^{-1}.

6-Benzyloxy-3-methoxy-2-nitrobenzoic Acid (**15**)

To a mixture of 6-hydroxy-3-methoxy-2-nitrobenzoic acid (**14**) (2.88 g), NaOH (1.08 g) and H_2O (10.8 ml) was added a solution of benzyl bromide (2.78 g) in acetone (5.4 ml). The mixture was stirred at 65°C for 3.5 hours and then poured into H_2O. The aqueous layer was separated, washed with ether, adjusted to pH 3 with 15% hydrochloric acid, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo* to give **15** (3.70 g, 90.5%): mp 171~173°C; IR (Nujol) 1715, 1690, 1370, 1270 cm^{-1}; NMR (DMSO-d_6) δ 3.85 (3H, s), 5.19 (2H, s), 7.25~7.55 (7H, m).

6-Benzyloxy-3-methoxy-2-nitrobenzoyl Chloride (**16**)

To a solution of thionyl chloride (1.45 g) in dry benzene (35 ml) was added **15** (1.89 g) and the mixture was refluxed for one hour. The solvent was then removed *in vacuo* and the residue was triturated with *n*-hexane and filtrated to give **16** (1.78 g, 89%): mp 142~144°C; IR (Nujol) 1775, 1275 cm^{-1}; NMR (DMSO-d_6) δ 3.87 (3H,s), 5.21 (2H, s), 7.44 (7H, m).

N-(6-Benzyloxy-3-methoxy-2-nitrobenzoyl)-L-proline (**17**)

A solution of L-proline (**3a**) (0.76 g) in H_2O (15 ml) and THF (7 ml) was adjusted to pH 9 with triethylamine. To the solution was added dropwise a solution of **16** (1.77 g) in THF (20 ml) at 13°C keeping pH between 8.5~9.0 by adding triethylamine. The reaction mixture was stirred for 40 minutes at the same temperature and THF was removed *in vacuo*. The remained aqueous solution was adjusted to pH 1.0 with conc.HCl to give yellow precipitates of **17** (2.11 g, 96%): IR (Nujol) 1730, 1635, 1450 cm^{-1}; NMR (DMSO-d_6) δ 1.6~2.3 (4H, m), 3.1~3.5 (2H, m), 3.84 (3H, s), 4.1~4.4 (1H, m), 5.18 (2H, s), 7.36 (7H, m).

(2*S*)-*N*-(6-Benzyloxy-3-methoxy-2-nitrobenzoyl)pyrrolidine-2-carbaldehyde (**18**)

To a solution of **17** (700 mg) in dry THF (20 ml) was added *N*,*N*′-carbonyldiimidazole (567 mg)

and the solution was stirred at 40°C for 2 hours. The solution was cooled down to −20°C and $LiAlH_4$ (99 mg) was added, and stirred at the same temperature for 30 minutes. H_2O (5 ml) was added and the mixture was filtered. The filtrate was adjusted to pH 8.5. The solution was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and evaporated to give **18** (620 mg, 92%): IR (Nujol) 1730, 1630 cm^{-1}; NMR (DMSO-d_6) δ 1.7~2.2 (4H, m), 3.2~3.6 (2H, m), 3.89 (3H, s), 4.1~4.5 (1H, m), 5.23 (2H, s), 7.2~7.6 (7H, m), 9.2~9.5 (1H, m).

(11*R*, 11a*S*)-9, 11-Dimethoxy-1, 2, 3, 10, 11, 11a-hexahydro-6-hydroxy-5-oxo-5*H*-pyrrolo[2,1-c][1,4]-benzodiazepine (**19**)

A mixture of **18** (330 mg) and 10% Pd-C (440 mg) in EtOAc (30 ml) and MeOH (15 ml) was stirred in a hydrogen gas stream for one hour. The catalyst was filtered off and the filtrate was evaporated to dryness to give **19** (180 mg, 75%): IR (Nujol) 3380, 1630, 1600 cm^{-1}; NMR ($CDCl_3$) δ 1.67~2.4 (4H, m), 3.3~4.0 (8H, m), 4.1~4.6 (2H, m), 6.1~7.0 (2H, m).

Acknowledgment

We are grateful to Dr. M. KOHSAKA and Dr. K. ENNYU for providing the biological data and to Dr. Y. MORIMOTO and his coworkers for spectral measurements.

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