Studies on 2-Aziridinecarboxylic Acid. VIII.[1] Total Synthesis of Actinomycin D and Its Serine Analogue *via* Ring-opening Reaction of 1-Benzyloxycarbonyl-2-aziridinecarboxylic Acid Moiety

Kiichiro NAKAJIMA,* Takumi TANAKA, Masahiro NEYA, and Kenji OKAWA
Department of Chemistry, Faculty of Science, Kwansei Gakuin University, Nishinomiya 662
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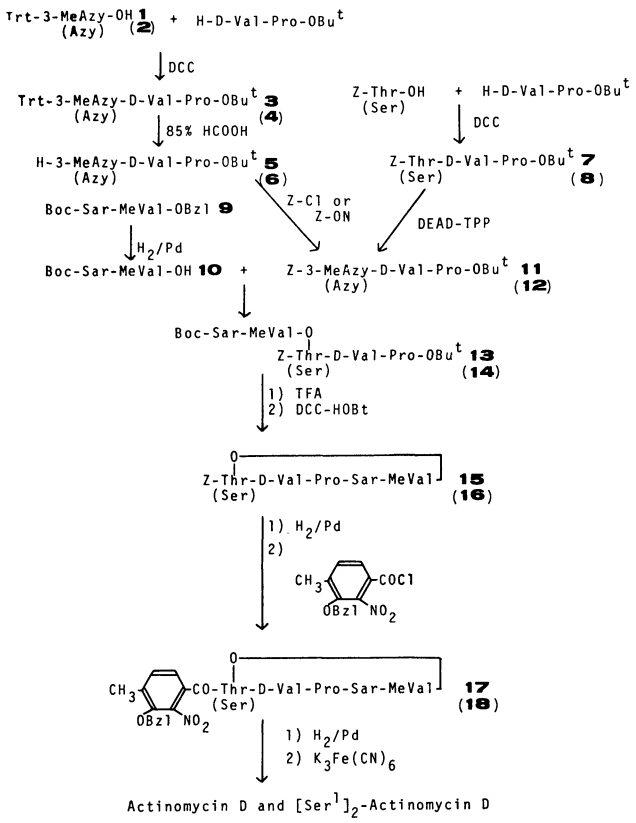
Direct synthesis of peptides containing 1-benzyloxycarbonyl-2-aziridinecarboxylic acid were carried out by the reaction of β-hydroxy-α-amino acid peptides with DEAD–TPP reagents, and key *O*-peptide intermediates of actinomycin D synthesis were prepared *via* a ring-opening reaction of the aziridine with *N*-protected dipeptide. Peptide lactone was prepared by the DCC–HOBt method, and the subsequent debenzyl procedure and oxydation procedure gave the title compounds in good yields.

In a previous paper,[2] we reported the synthesis of *O*-peptides *via* the ring-opening reaction of 1-aminoacyl-2-aziridinecarboxylic acid peptides with some carboxylic acids. We used this reaction for the total synthesis of actinomycin D,[3] but some problems remain with respect to the yield and the generality in the synthesis of the peptides containing 2-aziridinecarboxylic acid (Azy) and *O*-peptides.

This paper reports the direct synthesis of 1-benzyloxycarbonyl-Azy peptides from the corresponding serine and threonine peptides. By an application of the ring-opening reaction to the above Azy peptides, total synthesis of actinomycin D and its analogue were successfully carried out.

1-Benzyloxycarbonyl-Azy peptides are usually prepared from *N*-terminus-free Azy peptides derived from *N*-trityl-β-hydroxy-α-amino acid or peptides. But this rocedure requires many steps to obtain 1-benzyloxycarbonyl- or 1-*t*-butoxycarbonyl-Azy peptides.[4] Recently, A. K. Bose and co-workers reported[5] that the reaction of Z–Ser–$NHC_6H_4CH_3$ with diethyl azodicarboxylate (DEAD)-triphenylphosphin (TPP) reagents gave the β-lactam derivative in 53% yield, while the corresponding threonine derivative gave only the 3-methyl-2-aziridinecarboxylic acid (3-MeAzy) in 53% yield. We applied this DEAD–TPP procedure to synthesize Azy peptides, which are the significant intermediates in *O*-peptide synthesis.



Scheme 1.

The synthetic routes used in this study of actinomycin D and its analogue are summarized in Scheme 1. In the usual procedure, the key intermediate (2*S*,3*S*)-Z–3-MeAzy–D-Val–Pro–OBu^t (**11**) and (2*S*)-Z–Azy–D-Val–Pro–OBu^t (**12**) were prepared from the corresponding 1-trityl-3-MeAzy and 1-trityl-Azy-tripeptides (**3**, **4**). **3** and **4** were prepared by the coupling of (2*S*,3*S*)-Trt–3-MeAzy–OH (**1**) or (2*S*)-Trt–Azy–OH (**2**) with D-Val–Pro–OBu^t by the DCC method, followed by detritylation and 1-benzyloxycarbonylation with Z–Cl[1] or Z–ON[1] to obtain **11** and **12**. In the direct synthetic procedure of **11** and **12** by using DEAD–TPP procedure, the reactions of both Z–Thr (**7**) and Z–Ser tripeptide (**8**) with DEAD–TPP reagents gave only the corresponding **11** and **12** without any β-lactam in good yield (*ca.* 80%). The products of each procedure were completely identified. Next, we carried out the ester formation reaction between Boc–Sar–MeVal–OH (**10**) and **11** or **12** in CH_2Cl_2 solution at 50 °C for 7 d without boron trifluoride etherate, which had been necessary for the ring-opening reaction of 1-aminoacyl- or 1-benzoyl-Azy peptides.[2,3] The desired pentapeptide esters (**13**, **14**) were obtained in about 80% yields. After the *t*-butoxycarbonyl and *t*-butyl ester had been removed by treatment with trifluoroacetic acid, cyclization (2 mmol in 1 L THF, at 25 °C for 7 d) was carried out with the aid of DCC-HOBt[1] (10 mmol each) to obtain the cyclic pentapeptide lactones (**15**, **16**) in 66.2% (Thr) and 42.9% (Ser) yields. The benzyloxycarbonyl group of **15** and **16** was removed by catalytic hydrogenolysis. The reaction of the resulting N-terminus free pentapeptide lactones with 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride[6] gave the corresponding **17** and **18** in 90 and 95% yields. Subsequent debenzylation of **17** and **18** by catalytic hydrogenolysis, followed by oxydation with potassium hexacyanoferrate(III) in a mixture of

TABLE 1. PHYSICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF ACTINOMYCIN D AND [SER^1]$_2$-ACTINOMYCIN D

Characteristic	Synthetic actinomycin D	Natural actinomycin D[a)]	[Ser^1]$_2$-actinomycin D	[Ser^1]$_2$-actinomycin D[b)]
Melting point θ_m/°C	243—245	241—243	253—255	269—273
Optical rotation([α]$_D^{23}$/°)	−340.6[c)]	−323[d)]	−452[d)]	−435±15[d)]
UV absorption	24300 (443)	24400 (443)	24200 (450)	24950 (450)
[ε in methanol (λ/nm)]	33600 (240)	34100 (240)	37400 (238)	36600 (239)
IR absorption ($\tilde{\nu}$/cm^{-1}, KBr)	1745(lactone C=O)	1745	1740	
	1620—1670 (amide)	1620—1670	1610—1660	
	1580 (chromophore)	1580	1575	
	1195 (lactone COC)	1195	1195	
Antibacterial activity (MIC, g/ml)[e)]				
S. aureus JC-1 209P	0.78	0.78	3.13	—
B. subtilis ATCC-663	0.39	≦0.2	3.13	20% activity
E. coli NIHJ JC-2	>100	>50	>100	—
K. pmuno NCTC-418	>100	>50	>100	—
P. aeruginosa IAM-1095	100	50	100	—
P. vulgaris IAM-1025	>100	>50	>100	—

a) From the data of J. Meienhofer[9)]. b) From the data of H. Brockmann *et al.*[8)] c) In methanol at *c* 0.23. d) In methanol at *c* 0.25. e) Ref. 7.

methanol and 0.067 M phosphate buffer, pH 7.1, gave actinomycin D and [Ser^1]$_2$-actinomycin D in 74 and 51% yields, respectively.

The synthetic actinomycin D was indistinguishable from the natural substance as to physical properties and biological activity against some bacteria, although [Ser^1]$_2$-actinomycin D had only about 10% of the natural activity against gram-positive bacteria. The physical and biological data are summarized in Table 1. The CD curves of the synthetic products are shown in Fig. 1. The CD curve of the synthetic actinomycin D agreed with that of the natural product[7)] in methanol solution, and its serine analogue also showed a very similar pattern. Thus, the serine analogue seemed to have a very similar conformation in a methanol solution, although it had only 10% activity against gram-positive bacteria, which agrees with Brockmann's data.[8)] As the presence of the methyl group of threonine seems to be significant for the appearance of the antibacterial activity, we are now trying to synthesize a variety of actinomycin peptide analogues for a study of the biological activity-structure relationship.

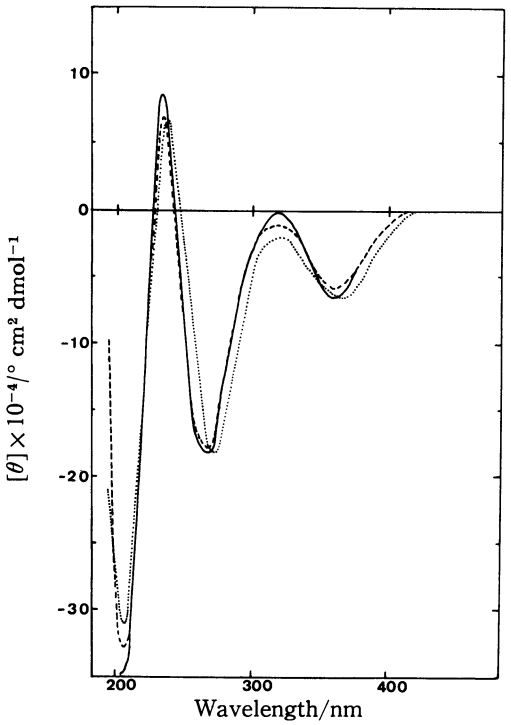


Fig. 1. CD spectra of natural actinomycin D (——), synthetic actinomycin D (– – –), and [Ser^1]$_2$-actinomycin D (·····) in methanol.

This study showed that the DEAD–TPP method for synthesizing Azy peptides is very convenient and shortens the step of the conventional Azy peptide synthetic procedure. Also, the method using the ring-opening reaction of aziridine is very useful for efficiently synthesizing peptide lactones.

Experimental

Melting points are uncorrected. Infrared spectra were obtained on a Hitachi EPI-G3 spectrometer, ultraviolet spectra on a Shimadzu UV-200 spectrophotometer. Optical rotations were determined at the D line on a Perkin-Elmer 141 polarimeter. The NMR spectra were obtained with a Hitachi R-20B High-resolution NMR Spectrometer, the chemical shifts being obtained using TMS as the internal reference. The homogeneity of the products was checked by thin-layer chromatography on silica-gel plates.

(2S)-1-Trityl-2-aziridinecarboxylic Acid (2). To a solution of (2*S*)-1-trityl-2-aziridinecarboxylic acid methyl ester[10)] (3.43 g, 10 mmol) in CH_3CN (40 ml), was added drop by drop a solution of 2 M LiOH (14.3 ml, 28.6 mmol) at 20 °C with stirring. After the solution was stirred for 24 h at 20 °C, the solvent was removed and the residue was dissolved in ethyl acetate. The solution was washed with 10% citric acid and water, dried over Na_2SO_4, and concentrated *in vacuo*. The obtained **2** was unstable and then was used immediately in the next coupling procedure.

[(2S)-*1-Trityl-2-aziridinylcarbonyl*]-D-*valylproline* t-*Butyl Ester*(**4**). Z-D-Valylproline *t*-butyl ester (4.05 g, 10 mmol) was hydrogenated over palladium in MeOH (50 ml) for 2 h to give N$^\alpha$-free dipeptide. To a solution of 2 (3.17 g, 10 mmol) and N$^\alpha$-free

dipeptide in CH_2Cl_2 (60 ml) was added a solution of DCC (2.06 g, 10 mmol) in CH_2Cl_2 (30 ml) at −10 °C with stirring. The reaction mixture was stirred for 3 h at −10 °C, and then overnight in a refrigerator. After *N,N'*-dicyclohexylurea (DCurea) was removed by filtration, the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and the solution wss washed with 10% citric acid, 1 M $NaHCO_3$, and water, dried over Na_2SO_4, and concentrated *in vacuo*. 5.34 g (93.8%) of **4** was obtained as an amorphous powder, $[\alpha]_D^{23}$ −90.8° (*c* 1.0, MeOH).

Found: C, 73.53; H, 7.77; N, 7.38%. Calcd for $C_{35}H_{43}O_4N_3$: C, 73.79; H, 7.61; N, 7.38%.

N-*(Benzyloxycarbonyl)threonyl*-D-*valylproline* t-*Butyl Ester (7)*. *N*-(Benzyoxycarbonyl)-D-valylproline *t*-butyl ester (27 g, 66.7 mmol) was hydrogenated over palladium (10 g) in MeOH (200 ml) for 3 h to give N^{α}-free dipeptide. To a solution of *N*-(benzyloxycarbonyl)threonine (16.9 g, 66.7 mmol) and N^{α}-free dipeptide in CH_2Cl_2 (200 ml) was added a solution of DCC (13.8 g, 66.7 mmol) in CH_2Cl_2 (50 ml) at −10 °C. The reaction mixture was stirred at −10 °C for 3 h and was then kept overnight in a refirgerator. After removal of DCurea by filtration, the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and the solution was washed with 10% citric acid, 1 M $NaHCO_3$, water, then dried over Na_2SO_4 and concentrated *in vacuo*. The residue was crystallized from ethyl acetate–hexane; 24 g (71%) of **7** was obtained: mp 91—92.5 °C, $[\alpha]_D^{23}$ −29.5° (*c* 1.1, MeOH).

Found: C, 61.85; H, 7.92; N, 8.21%. Calcd for $C_{26}H_{39}O_7N_3$: C, 61.76; H, 7.77; N, 8.31%.

N-*(Benzyloxycarbonyl)seryl*-D-*valylproline* t-*Butyl Ester (8)*. To a solution of *N*-(benzyloxycarbonyl)serine (957 mg, 4 mmol) and N^{α}-free dipeptide (from 1.62 g, 4 mmol of *N*-(benzyloxycarbonyl)-D-valylproline *t*-butyl ester) in CH_2Cl_2 (20 ml), was added a solution of DCC (825 mg, 4 mmol) in CH_2Cl_2 (20 ml) at −10 °C with stirring. After the reaction mixture was worked up as described in **7**, the residue was crystallized from ethyl acetate–hexane; 1.6 g (81.3%) of **8** was obtained: mp 141—142 °C, $[\alpha]_D^{23}$ −25.1° (*c* 1.0, MeOH).

Found: C, 60.90; H, 7.62; N, 8.45%. Calcd for $C_{25}H_{37}O_7N_3$: C, 61.08; H, 7.59; N, 8.55%.

N-*(t-Butoxycarbonyl)sarcosyl*-N-*methylvaline Benzyl Ester (9)*. To a solution of *t*-butoxycarbonylsarcosine (6.62 g, 35 mmol) and *N*-methylvaline benzyl ester (7.73 g, 35 mmol) in THF (100 ml) was added a solution of DCC (7.22 g, 35 mmol) in THF (80 ml) at −10 °C with stirring. After being stirred at 5 °C overnight, the DCurea was filtered off. The filtrate was concentrated *in vacuo* and the residue was dissolved in ethyl acetate, the solution was washed with 10% citric acid, 1 M $NaHCO_3$, water, then dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified with silica-gel column chromatography (ethyl acetate–hexane, 1 : 5 v/v). 13.15 g (95.7%) of **9** was obtained as a syrup: $[\alpha]_D^{23}$ −81.6° (*c* 1.0, MeOH).

Found: C, 64.33; H, 8.19; N, 7.28%. Calcd for $C_{21}H_{32}O_5N_2$: C, 64.26; H, 8.22; N, 7.14%.

N-*(t-Butoxycarbonyl)sarcosyl*-N-*methylvaline (10)*. H_2 gas was bubbled through a solution of **9** (9 g, 23 mmol) in MeOH (80 ml) containing palladium (5 g) for 2 h with stirring. After the catalyst was filtered out, the filtrate was concentrated *in vacuo*. 6.95 g (100%) of **10** was obtained as a syrup: $[\alpha]_D^{23}$ −80.4° (*c* 1.0, MeOH).

Found: C, 55.49; H, 8.72; N, 9.35%. Calcd for $C_{14}H_{26}O_5N_2$: C, 55.61; H, 8.67; N, 9.27%.

[*(2S, 3S)-1-Benzyloxycarbonyl-3-methyl-2-aziridinylcarbonyl*]-D-*valylproline* t-*Butyl Ester (11)*. Via *Usual Procedure*: To a solution of **5**[3] (from 7.07 g, 12.1 mmol of **3**[3]) in $CHCl_3$ (40 ml) was added Et_3N (1.68 ml, 12.1 mmol) and Z–ON[1] (3.39 g, 12.1 mmol) at 25 °C with stirring. After the solution was stirred for 3 d, the solvent was removed *in vacuo*. The residue was worked up as described in **4**, and the crude product was purified by silica-gel column chromatography ($CHCl_3$) to give 4.91 g (83.1%) of **11** as a syrup: $[\alpha]_D^{23}$ −76.0° (*c* 0.9, MeOH). NMR ($CDCl_3$) δ: 1.28 (3H d, J=5.8 Hz, =$CHCH_3$), 2.83 (1H m, Azy; β-proton), 3.18 (1H d, J=6.6 Hz, Azy; α-proton). Z–ON can be replaced with Z–Cl.[1]

Found: C, 64.05; H, 7.65; N, 8.62%. Calcd for $C_{26}H_{37}O_6N_3$: C, 64.02; H, 7.83; N, 8.42%.

Via *DEAD–TPP Procedure*: To a solution of DEAD (174 mg, 1.0 mmol) and TPP (262 mg, 1.0 mmol) in an ice-cold dry THF (10 ml) was added **7** (200 mg, 0.4 mmol) at 0 °C with stirring. After being stirred for 1 h, the reaction was stopped by the addition of water (5 ml). The product was extracted with ethyl acetate and the solution was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in ether and the crystal which appeared, *N,N'*-bis(ethoxycarbonyl)hydrazine, were filtered off, then the filtrate was concentrated *in vacuo*. The residue was subjected to silica-gel column chromatography (AcOEt–hexane, 1 : 2 v/v). 169 mg (87.6%) of **11** was obtained as a syrup: $[\alpha]_D^{23}$ −74.9° (*c* 1.1, MeOH). The NMR spectrum of this product was identical with that from **11**.

[*(2S)-1-Benzyloxycarbonyl-2-aziridinylcarbonyl*]-D-*valylproline* t-*Butyl Ester (12)*. Via *Usual Procedure*: To a solution of **4** (2.34 g, 4.12 mmol) in abs. MeOH (30 ml) was added ice-cold 85% formic acid (15 ml). After this mixture was stirred for 3 h, the solvent was removed *in vacuo*. The residue was partitioned between ether and water. 1 M $NaHCO_3$ was added to the aqueous solution, producing an alkaline solution, and the isolated oil was extracted with ethyl acetate. After this solution was dried over Na_2SO_4, the solvent was removed *in vacuo* to give N^{α}-free tripeptide (**6**), which was used in the following reaction without further purification. To a solution of **6** in $CHCl_3$ (20 ml) was added Et_3N (0.55 ml, 3.91 mmol) and Z–Cl[1] (0.63 ml, 3.91 mmol) at −5 °C with stirring. After being stirred for 1 h, the reaction mixture was worked up as described in **11**; 1.62 g (83.3%) of **12** was obtained as syrup: $[\alpha]_D^{23}$ −26.0° (*c* 0.9, MeOH), NMR δ: 2.42 (1H q, J_{gem}=0.8 Hz, J_{trans}=3.0 Hz, Azy; β-proton), 2.53 (1H q, J_{gem}=0.8 Hz, J_{cis}=6.6 Hz, Azy; β-proton), 3.14 (1H q, J_{trans}=3.0 Hz, J_{cis}=6.6 Hz, Azy; α-proton).

Found: C, 63.15; H, 7.90; N, 8.59%. Calcd for $C_{25}H_{35}O_6N_3$: C, 63.41; H, 7.45; N, 8.87%.

Via *DEAD–TPP Procedure*: To a solution of DEAD (177 mg, 1.02 mmol) and TPP (267 mg, 1.02 mmol) in ice-cold dry THF (10 ml) was added **8** (200 mg, 0.407 mmol) at 0 °C with stirring. After the mixture was stirred for 1 h, the reaction was stopped by the addition of water (5 ml). The solution was worked up as described in **11**. The crude product was subjected to silica-gel column chromatography (AcOEt–hexane, 1 : 2 v/v). 161 mg (83.5%) of **12** was obtained as a syrup: $[\alpha]_D^{23}$ −25.4° (*c* 0.9, MeOH). The NMR spectrum of this product was identical with that of **12** given earlier.

[O-(t-*Butoxycarbonylsarcosyl*-N-*methylvalyl*)-N-*benzyloxycarbonylthreonly*]-D-*valylproline* t-*Butyl Ester (13)*. A solution of **11** (3.67 g, 7.53 mmol) and **9** (7.27 g, 24 mmol) in CH_2Cl_2 (60 ml) was refluxed for 7 d, and the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed with 1 M $NaHCO_3$, and water, dried over Na_2SO_4, and then concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$). 4.75 g (80%) of **13** was obtained as an amorphous powder: $[\alpha]_D^{23}$ −30.1° (*c* 1.0, MeOH).

Found: C, 60.92; H, 8.36; N, 8.81%. Calcd for $C_{40}H_{63}O_{11}N_5$: C, 60.82; H, 8.04; N, 8.87%.

[O-(t-*Butoxycarbonylsarcosyl*-N-*methylvalyl*)-N-*benzyloxycarbonylseryl*]-D-*valylproline* t-*Butyl Ester* (***14***). A solution of **12** (1.09 g, 2.3 mmol) and **9** (2.09 g, 6.9 mmol) in CH_2Cl_2 (20 ml) was refluxed for 7 d and the reaction mixture was worked up as described for **13**. 1.44 g (80.9%) of **14** was obtained as an amorphous powder: $[\alpha]_D^{23}$ −47.4° (*c* 1.0, MeOH).

Found: C, 60.16; H, 8.10; N, 9.13%. Calcd for $C_{39}H_{61}O_{11}N_5$: C, 60.37; H, 7.92; N, 9.03%.

N-(*Benzyloxycarbonyl*) *threonyl*-D-*valylprolysarcosyl*-N-*methylvaline Lactone* (***15***). A solution of **13** (1.51 g, 1.91 mmol) in TFA (20 ml) was stirred at 0 °C for 4 h; it was then concen trated *in vacuo*. The residue was dissolved in ethyl acetate and concentrated *in vacuo* (this procedure was repeated twotimes), and the residue was stored over KOH for 12 h in desiccator. The residue was dissolved in dry THF (100 ml), and Et_3N (0.27 ml, 1.91 mmol) was added at −5 °C; the mixture was diluted with THF (900 ml) and HOBt[1] (1.23 g, 9.53 mmol) was added. To a solution of the above mixture was added drop by drop a solution of DCC (1.97 g, 9.53 mmol) in THF (50 ml) at 0 °C for a period of 4 h with stirring. After the mixture was stirred for 5 d at 25 °C, the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and the DCurea which appeared was filtered off. The filtrate was washed with 10% citric acid and 1 M $NaHCO_3$, and water, and then concentrat ed *in vacuo*. The residue was subjected to silica-gel column chromatography ($CHCl_3$–MeOH, 50 : 1 v/v). 0.78 g (66.2%) of **15** was obtained as an amorphous powder: $[\alpha]_D^{23}$ −25.4° (*c* 1.0, MeOH).

Found: C, 60.29; H, 7.68; N, 11.21%. Calcd for $C_{31}H_{45}O_8N_5$: C, 60.47; H, 7.37; N, 11.37%.

N-(*Benzyloxycarbonyl*) *seryl*-D-*valylprolylsarcosyl*-N-*methylvaline Lactone* (***16***). A solution of **14** (1.76 g, 2.27 mmol) in TFA (30 ml) was stirred at 0 °C for 4 h, and then was concentrated *in vacuo*. The residue was dissolved in ethyl acetate and concentrated *in vacuo* (this procedure was repeated two times), and the residue was stored over KOH for 12 h in desiccator. The residue was dissolved in dry THF (100 ml), and Et_3N (0.32 ml, 2.27 mmol) was added at −5 °C, this solution was diluted with THF (1.1 L), and HOBt (1.53 g, 11.35 mmol) was added. To a solution of this mixture, was added drop by drop a solution of DCC (2.34 g, 11.35 mmol) in THF (60 ml) at 0 °C for a period of 4 h with stirring. The reaction mixture was stirred at 25 °C for 5 d, and was worked up as described in **15**. The product was crystallized from ethyl acetate–hexane; 0.58 g (42.9%) of **16** was obtained: mp 228—231 °C, $[\alpha]_D^{23}$ −33.3° (*c* 1.0, MeOH).

Found: C, 60.01; H, 7.34; N, 11.92%. Calcd for $C_{30}H_{43}O_8N_5$: C, 59.89; H, 7.20; N, 11.64%.

(*3-Benzyloxy-4-methyl-2-nitrobenzoyl*) *threonyl*-D-*valylprolylsarcosyl*-N-*methylvaline Lactone* (***17***). A solution of **15** (342 mg, 0.56 mmol) in MeOH (15 ml) was hydrogenated over palladium black. After 2 h, the mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (10 ml) and then cooled to 0 °C. To the $CHCl_3$ solution was added Et_3N (0.093 ml, 0.67 mmol) and 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride[6] (204 mg, 0.67 mmol) with stirring in the dark at 0 °C. After the reaction mixture was stirred for 1 h, the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed with 10% citric acid, 1 M $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified with silica-gel column chromatography ($CHCl_3$) to give 375 mg (90.0%) of **17** as an amorphous powder: $[\alpha]_D^{23}$ −12.2° (*c* 0.5, MeOH).

Found: C, 60.70; H, 6.85; N, 11.32%. Calcd for $C_{38}H_{50}O_{10}N_6$: C, 60.79; H, 6.71; N, 11.19%.

(*3-Benzyloxy-4-methyl-2-nitrobenzoyl*) *seryl*-D-*valylprolylsarcosyl*-N-*methylvaline Lactone* (***18***). A solution of **16** (425 mg, 0.71 mmol) in MeOH (20 ml) was hydrogenated over palladium black. After 2 h, the mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in THF (10 ml) and then cooled to 0 °C. To the solution was added Et_3N (0.12 ml, 0.85 mmol) and 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride[6] (259 mg, 0.85 mmol) with stirring in the dark at 0 °C. After being stirred for 1 h, the reaction mixture was worked up as described in **17**. 492 mg (95.0%) of **18** was obtained as an amorphous powder: $[\alpha]_D^{23}$ +19.9° (*c* 0.5, MeOH).

Found: C, 59.96; H, 6.54; N, 11.13%. Calcd for $C_{37}H_{48}O_{10}N_6$: C, 60.31; H, 6.57; N, 11.41%.

Actinomycin D. A solution of **17** (138 mg, 0.183 mmol) in MeOH (12 ml) was hydrogenated over palladium black in the dark. After 2 h, the mixture was filtered and the filtrate was added to an equal volume of stirred 0.067 M phosphate buffer pH 7.1 containing potassium hexacyanoferrate(III) (175 mg, 0.531 mmol). The mixture was stirred at 25 °C for 20 min. It was then partitioned between ethyl acetate and water, the aqueous layer being separated and extracted three times with ethyl acetate. The combined ethyl acetate layer was washed with 1 M $NaHCO_3$, 1 M HCl, and saturated NaCl, dried over Na_2SO_4, and concentrated *in vacuo* to afford a red crystalline residue, which was recrystallized from ethyl acetate–hexane to give actinomycin D, 84 mg (74%). Physical and spectral data are given in Table 1.

Found: C, 58.52; H, 6.94; N, 13.23%. Calcd for $C_{62}H_{86}O_{16}N_{12}\cdot H_2O$: C, 58.48; H, 6.97; N, 13.20%.

[*Ser*[1]]$_2$-*Actinomycin D.* A solution of **18** (184 mg, 0.25 mmol) in MeOH (15 ml) was hydrogenated over palladium black in the dark. After 2 h, the mixture was filtered and the filtrate was added to an equal volume of stirred 0.067 M phosphate buffer pH 7.1 containing potassium hexacyanoferrate(III) (238 mg, 0.724 mmol). The mixture was stirred at 25 °C for 20 min and worked up as described in actinomycin D. [Ser[1]]$_2$-actinomycin D was obtained 78 mg (51.0%) as a red crystalline substance from ethyl acetate–hexane. Physical and spectral data are given in Table 1.

Found: C, 61.09; H, 7.12; N, 14.27%. Calcd for $C_{60}H_{82}O_{16}N_{12}\cdot H_2O$: C, 61.00; H, 7.17; N, 14.23%.

Microbiological Assay. The results of the assay and the microorganisms employed are given in Table 1. The minimum amount of the compound necessary for the complete inhibition of growth was determined by the Stamp method using Mueller Hinton Ager.

References

1) Part VII. K. Nakajima, H. Oda, and K. Okawa, *Bull. Chem. Soc. Jpn.*, **55**, 3232 (1982). Abbreviations of the IUPAC-IUB commission, *J. Biol. Chem.*, **247**, 977 (1972), are used. Trt: trityl, Z: benzyloxycarbonyl, Bzl: benzyl, Z–Cl: benzyloxycarbonyl chloride, Z–ON: 2-benzyloxycarbonyloxyimino-2-phenylacetonitrile, HOBt: *N*-hydroxy benzotriazole. "Azyline" is used as the name of 2-aziridinecarboxylic acid, "Azy" being its abbreviation. 3-MeAzy: 3-methyl-2-aziridinecarboxylic acid.

2) T. Tanaka, K. Nakajima, T. Maeda, A. Nakamura, N. Hayashi, and K. Okawa, *Bull. Chem. Soc. Jpn.*, **52**, 3579 (1979).

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6) This compound was prepared by the reaction of the corresponding carboxylic acid and thionyl chloride. Recrystallized from benzene–heptane, mp 101—102 °C.

7) Natural actinomycin D for bioassay and CD curve measurement was purchased from Banyu Pharmaceutical Co., Ltd.

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10) This compound was prepared by the same prodceure as described by K. Okawa and K. Nakajima, *Biopolymers*, **20**, 1811 (1981), $[\alpha]_D^{23}$ −95.4° (*c* 1.1, MeOH).