Syntheses of Capreomycin Analogs in Relation to Their Antibacterial Activities[1]

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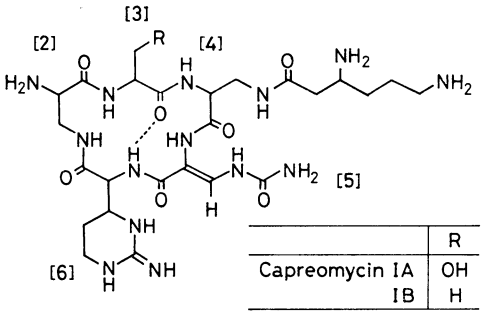
Six analogs of an antituberculous antibiotic capreomycin were synthesized in order to clarify the structure-activity relationship, especially with regard to the significance of the β-amino group in α,β-diaminopropionic acid residue as well as the position of linkage of the branch residue, β-lysine, to the cyclic peptide moiety. All the synthetic products were found to have the same conformations in solution as those of the natural antibiotics in terms of NMR spectra. It was found from their antibacterial activities that an amino group located at β-position of the α,β-diaminopropionic acid residue adjacent to ureidodehydroalanine residue remarkably strengthens the biological activity, and that the position of a branch does not significantly influence the antibacterial potency.

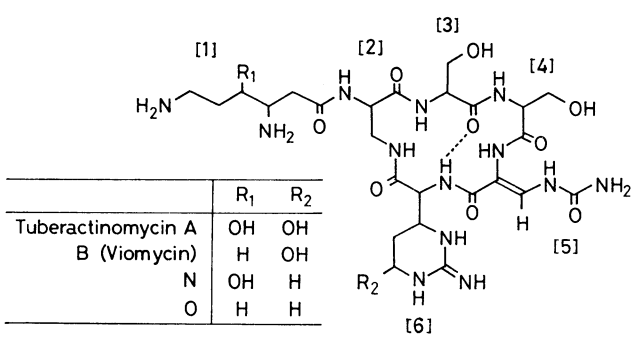
Peptide antibiotics such as, capreomycin,[2] viomycin,[3] and tuberactinomycin,[4] show comparable antituberculous activities. From a structural point of view, these antibiotics constitute one group of branched cyclic pentapeptides with analogous amino acid sequence (Figs. 1 and 2). However, capreomycin, whose chemical structure was established,[5] is structurally unique. The Ser[4] residue[6] in all tuberactinomycins including viomycin (tuberactinomycin B) is replaced by A_2pr[7] residue in capreomycins. This antibiotic contains one more amino group in its molecule than in tuberactinomycin. Capreomycin differs from tuberactinomycin in the position of linkage of the branch part, *i.e.*, β-Lys or γ-Hy-β-Lys,[7] to the cyclic pentapeptide moieties, which is of interest in connection with the structure-activity relationship as well as biosynthesis of capreomycin.

Little is known about structural requirements for the antimicrobial activity of capreomycin so far. Syntheses of several capreomycin analogs have been carried out in the present study in order to clarify how the above-mentioned structural differences between capreomycin and tuberactinomycin are reflected on the antibacterial activity.



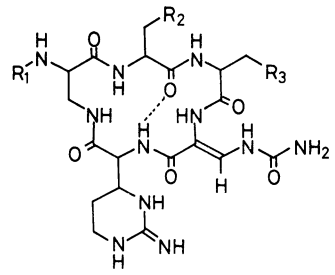
	R
Capreomycin IA	OH
IB	H

Fig. 1. Structures of capreomycins.



	R_1	R_2
Tuberactinomycin A	OH	OH
B (Viomycin)	H	OH
N	OH	H
O	H	H

Fig. 2. Structures of tuberactinomycins.



	R_1	R_2	R_3
Capreomycin IIA (2a)	H	OH	NH_2
IIB (2b)	H	H	NH_2
Pseudocapreomycin IB (4)	β-Lys	H	NH_2
Reversecapreomycin IIB (11a)	H	NH_2	H
[Orn[4]]-Capreomycin IIB (11b)	H	H	$CH_2CH_2NH_2$
Di-β-Lys-capreomycin IIB (13)	β-Lys	H	β-Lys-NH

Fig. 3. Structures of capreomycin analogs synthesized.

Capreomycin analogs and related compounds synthesized are summarized in Fig. 3. Capreomycin IIA and IIB lacking β-Lys residue in capreomycin IA and IB molecules, respectively, were obtained from natural sources as minor components of the antibiotics.[8] Capreomycin IIA and IIB have comparable antibacterial activities to those of IA and IB,[9] whereas a similar cyclic moiety of tuberactinomycin, *i.e.*, tuberactinamine N shows less activity than tuberactinomycin itself.[10] It was suggested that the β-amino group of A_2pr^4 residue[6] in capreomycin II might act to enhance the antibacterial activity. For the sake of confirmation, we compared not only the antimicrobial spectra of tuberactinamine N and capreomycin IIA and IIB synthesized here, under the same conditions, but also those of tuberactinomycin O and so-called pseudocapreomycin IB whose branch part is linked to the second amino acid (A_2pr) residue in a similar way to that of the former. [Orn[4]]-Capreomycin IIB[6] and reversecapreomycin IIB, in which the positions of Ala[3] and A_2pr^4 residue in capreomycin IIB are exchanged, were also synthesized for the purpose of observing the effects of the length of the side chain in the basic amino acid residue and its sequential position.

In order to prepare a more potent analog of this antibiotic than natural capreomycin or tuberactinomycin, and taking into account the fact that the addition of β-lysine branch to the cyclic peptide moiety virtually increases the biological activity in the case of tuberactinomycin, introduction of β-Lys to the free α-amino group of A_2pr^2 residue[6] in capreomycin IB, *viz.*, the preparation of di-β-Lys-capreomycin IIB, was undertaken.

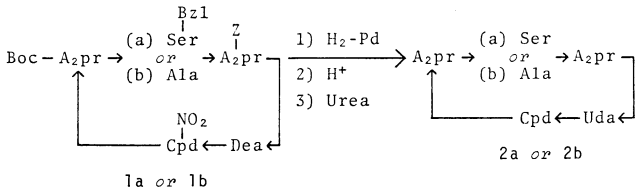


Fig. 4. Synthetic scheme for capreomycin IIA(**2a**) and IIB(**2b**).

Syntheses of Capreomycin IIA and IIB. The protected cyclopentapeptides **1a** and **1b** (Fig. 4), whose syntheses were reported in the total syntheses of capreomycin IA and IB,[5] were deprotected by catalytic reduction followed by acid treatment. The products were immediately treated with excess urea in order to convert the aldehyde group liberated from Dea[7] into Uda[7] residue, affording capreomycin IIA and IIB (**2a** and **2b**).

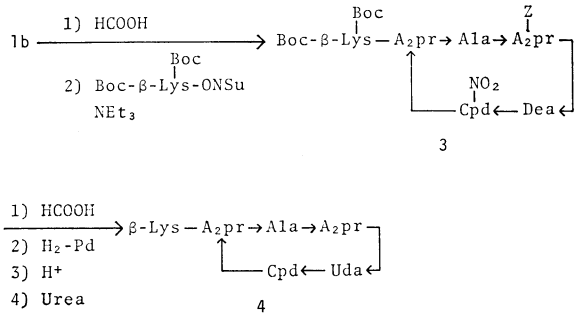


Fig. 5. Synthetic scheme for pseudocapreomycin IB(**4**).

Synthesis of Pseudocapreomycin IB. After cleavage of the Boc[7] group in **1b** (Fig. 5) with 99 % formic acid, Boc–β-Lys(Boc) residue was introduced to the α-amino group of the A_2pr^2 residue by the ONSu[7] active ester method. The product **3** was subsequently converted into pseudocapreomycin IB (**4**) through successive removal of Boc, Z, and nitro groups followed by the transformation of Dea into Uda residue.

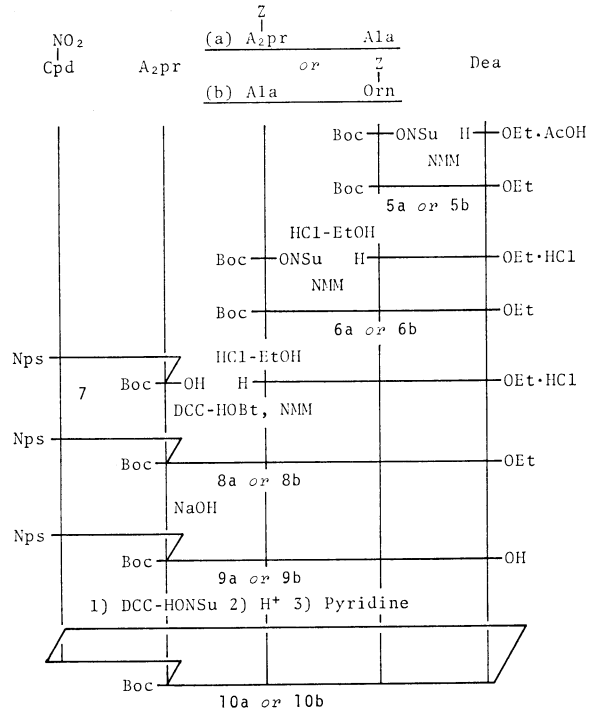


Fig. 6. Synthetic scheme for protected cyclopentapeptides **10a** and **10b**.

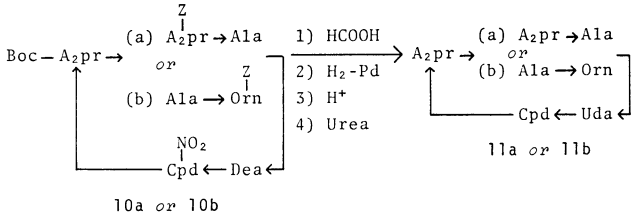


Fig. 7. Synthetic scheme for reversecapreomycin IIB (**11a**) and [Orn[4]]-capreomycin IIB(**11b**).

Syntheses of Reversecapreomycin IIB and [Orn[4]]-Capreomycin IIB. The synthetic schemes for these compounds

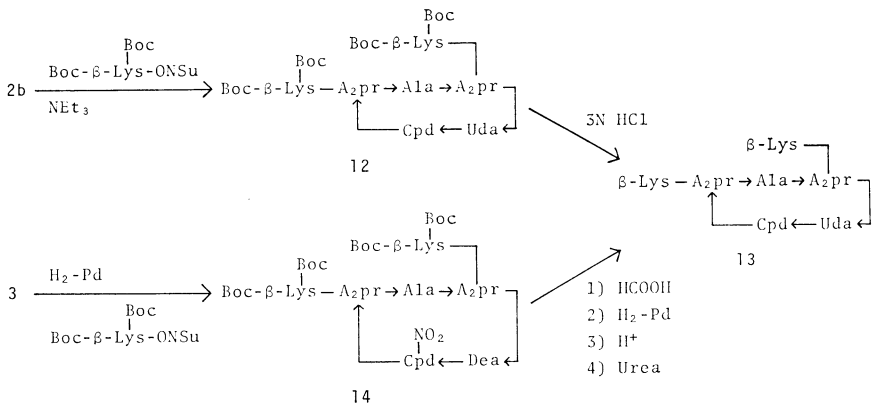


Fig. 8. Synthetic scheme for di-β-Lys-capreomycin IIB (**13**).

are given in Figs. 6 and 7. The C-terminal tripeptides (**6a**) and (**6b**) were prepared by the stepwise elongation method from the C-terminal utilizing ONSu active esters. Cleavage of Boc groups in dipeptides (**5a**) and (**5b**) was carried out in ethanol, otherwise severe decomposition occurred at diethyl acetal group of Dea residue.

The N-terminal dipeptide **7**[12] was condensed with deprotected C-terminal tripeptides (**6a**) and (**6b**) by means of DCC-HOBt[7] to afford linear pentapeptides (**8a**) and (**8b**), which were then cyclized by the ONSu active ester method. Conversion of the protected cyclic pentapeptides (**10a**) and (**10b**) thus obtained into the final products (**11a**) and (**11b**) were carried out as in the preparation of capreomycin IIA (**2a**) or IIB (**2b**).

Synthesis of Di-β-Lys-capreomycin IIB. This analog was prepared in the following two ways. Route A (Fig.

Table 1.[a] Chemical shifts (δ) of capreomycin analogs in D_2O–TFA (4: 1)

Position of amino acid residue		Cpm IA	Cpm IB	Cpm IIA	Cpm IIB
1	α-CH_2	2.63(dd)	2.50(dd)		
		2.85(dd)	2.81(dd)		
	β-CH	3.8 (m)	3.7 (m)		
	γ-CH_2	1.8 (m)	1.8 (m)		
	δ-CH_2	1.8 (m)	1.8 (m)		
	ε-CH_2	3.10(m)	3.08(m)		
2	α-CH	4.3—4.5(m)	4.2—4.5(m)	4.3—4.6(m)	4.3—4.6(m)
	β-CH_2	3.3 (m)	3.3(m)	3.3 (m)	3.3 (m)
		3.8 (m)	3.8(m)	4.1 (m)	4.1 (m)
3	α-CH	4.86(t)	4.67(q)	4.84(t)	4.68(q)
	β-CH_2	3.84(d)		3.95(d)	
	β-CH_3		1.43(d)		1.45(d)
4	α-CH	4.3—4.5(m)	4.2—4.5(m)	4.3—4.5(m)	4.3—4.5(m)
	β-CH_2	3.7—4.3(m)	3.7—4.2(m)	3.7—4.2(m)	3.79(dd)
					3.8—4.2(m)
5	β-CH	8.04(s)	8.03(s)	8.05(s)	8.04(s)
6	α-CH	5.01(d)	4.96(d)	5.01(d)	4.95(d)
	β-CH	4.5 (m)	4.5 (m)	4.5 (m)	4.5 (m)
	γ-CH_2	1.6—2.3(m)	1.6—2.3(m)	1.6—2.3(m)	1.6—2.3(m)
	δ-CH_2	3.3 (m)	3.3 (m)	3.3 (m)	3.3 (m)

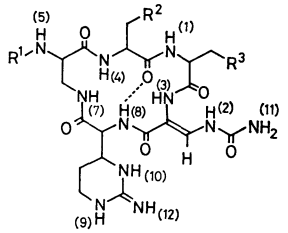
Position of amino acid residue		Pseudo Cpm IB	Reverse Cpm IIB	[Orn[4]]-Cpm IIB	Di-β-Lys-Cpm IIB
1	α-CH_2	2.65(dd)			2.7—3.0(m)
		2.83(dd)			
	β-CH	3.7 (m)			3.8 (m)
	γ-CH_2	1.8 (m)			1.8 (m)
	δ-CH_2	1.8 (m)			1.8 (m)
	ε-CH_2	3.08(m)			3.10(m)
2	α-CH	4.68(dd)	4.3—4.6(m)	4.1—4.4(m)	4.68(dd)
	β-CH_2	3.2 (m)	3.3 (m)	3.4 (m)	3.4 (m)
		4.1 (m)	4.1 (m)	4.1 (m)	4.0 (m)
3	α-CH	4.74(q)	5.18(dd)	4.68(q)	4.69(q)
	β-CH_2		3.60(dd)		
			4.0—4.4(m)		
	β-CH_3	1.45(d)		1.47(d)	1.43(d)
4	α-CH	4.3—4.5(m)	4.33(q)	4.1—4.5(m)	4.3—4.6(m)
	β-CH_2	3.6—4.1(m)		2.0 (m)	3.6—4.1(m)
	β-CH_3		1.46(d)		
	γ-CH_2			1.8 (m)	
	δ-CH_2			3.08(t)	
5	β-CH	8.03(s)	8.05(s)	8.06(s)	8.02(s)
6	α-CH	4.97(d)	5.02(s)	4.97(d)	5.97(d)
	β-CH	4.5 (m)	4.5 (m)	4.4 (m)	4.4 (m)
	γ-CH_2	1.6—2.3(m)	1.6—2.3(m)	1.6—2.3(m)	1.6—2.3(m)
	δ-CH_2	3.3 (m)	3.3 (m)	3.3 (m)	3.3 (m)

a) Abbreviation: Cpm, capreomycin.

Table 2.[a] Chemical shifts (δ) of NH protons of capreomycin analogs in H_2O (pH 2.5)

	Cpm IA	Cpm IB	Cpm IIA	Cpm IIB	Pseudo Cpm IB	Reverse Cpm IIB	[Orn^4]-Cpm IIB	Di-β-Lys-Cpm IIB
1	9.33(d)	9.72(d)	9.60(d)	9.50(d)	9.43(d)	9.61(d)	9.39(d)	9.20(d)
2	9.24(d)	9.24(d)	9.33(d)	9.30(d)	9.29(d)	9.25(d)	9.28(d)	9.19(d)
3	8.82(s)	8.76(s)	9.10(s)	9.10(s)	9.05(s)	8.88(s)	8.82(s)	8.77(s)
4	8.64(d)	8.68(d)	8.73(d)	8.73(d)	8.59(d)	8.78(d)	8.78(d)	8.58(d)
5					8.44(d)			8.41(d)
6[b]	8.22(t)	8.15(t)						8.12(t)
7[b]	8.10(t)	8.15(t)	8.19(t)	8.08(t)	7.95(t)	8.27(t)	8.09(t)	8.00(t)
8	7.61(d)	7.62(d)	7.50(d)	7.49(d)	7.50(d)	7.68(d)	7.72(d)	7.61(d)
9	7.46(s)	7.42(s)	7.44(s)	7.44(s)	7.44(s)	7.41(s)	7.39(s)	7.40(s)
10	7.46(s)	7.42(s)	7.31(s)	7.18(s)	7.23(s)	7.41(s)	7.17(s)	7.40(s)
11	6.48(s)	6.49(s)	6.43(s)	6.34(s)	6.44(s)	6.45(s)	6.49(s)	6.46(s)
12	6.29(s)	6.34(s)	6.29(s)	6.27(s)	6.29(s)	6.23(s)	6.31(s)	6.30(s)

a) Abbreviation: Cpm, capreomycin. b) The chemical shifts of protons 6 and 7 are exchangeable.



R^3: β-Lys-NH- in Cpm IA, IB and di-β-Lys-Cpm IIB

8): Boc-β-Lys(Boc) residue was introduced simultaneously to both the α-amino group of A_2pr^2 and β-amino group of A_2pr^4 in capreomycin IIB (**2b**). Subsequently, four Boc groups in the product **12** were removed to afford di-β-Lys-capreomycin IIB (**13**). Route B (Fig. 8): when the protected hexapeptide (**3**, Fig. 5) was hydrogenated in the presence of Boc-β-Lys(Boc)-ONSu, a heptapeptide **14** was directly obtained in a good yield without cleavage of the nitro group as well as *N*,*N*-acyl migration at the A_2pr^4 residue.[5] The hexapeptide **14** was then subjected to deprotection followed by conversion of Dea into Uda residue.

The advantage of route B over route A lies in its applicability to a possible need in which analogs possessing different kinds of amino acid residues at two branch positions are required.

NMR Analysis. The NMR spectral data of capreomycin analogs prepared in this study are summarized in Tables 1 and 2, together with those of natural antibiotics for comparison. Assignment of each signal was performed by means of decoupling technique and a comparison with the spectra of natural antibiotics.[11,12]

The cyclic peptide moieties of tuberactinomycin and capreomycin as well as tuberactinomycin analogs have a stable and rigid conformation with an intramolecular hydrogen bond in commom as shown in Figs. 1 and 2.[11a] This is shown in particular by constant chemical shifts of each α-NH and α-CH signal in all the cyclic moieties. The fact that all capreomycin analogs show similar NMR spectral patterns to those of the natural ones, especially with respect to signals of α-NH and α-CH, indicates the conformational similarity of the analogs to the natural antibiotics.

Antibacterial Activities. The minimum inhibitory concentrations of capreomycin analogs were determined by a serial agar dilution method (Table 3). Both capreomycin IIA and IIB showed apparently greater activities than the corresponding tuberactinamine N,

Table 3. Minimum inhibitory concentrations (mcg/ml) of capreomycin analogs

Test organism	Cpm IA	Cpm IB	Tum O	Tua N	Cpm IIA	Cpm IIB	Pseudo Cpm IB	Reverse Cpm IIB	[Orn^4]-Cpm IIB	Di-β-Lys-Cpm IIB
Staphylococcus epidermidis sp-al-1	100	>100	>100	>100	>100	>100	50	>100	>100	50
Streptococcus pyogenes 1022	>100	>100	>100	>100	50	50	50	100	100	>100
Streptococcus agalactiae 1020	>100	>100	—	—	50	100	>100	>100	100	>100
Corynebacterium diphtheriae P.W.8	3.1	3.1	3.1	50	6.3	6.3	1.6	100	25	1.6
Bacillus subtilis ATCC 6633	25	25	50	>100	50	25	6.3	100	50	3.1
Escherichia coli B	50	100	100	>100	50	100	50	>100	>100	100
Klebsiella pneumoniae ATCC 10031	25	50	25	>100	25	25	25	>100	100	50
Salmonella typhosa H 901	25	50	100	>100	50	50	25	>100	>100	50
Shigella sonnei E 33	50	100	50	>100	100	100	100	>100	>100	100
Proteus vulgaris OX 19	50	50	50	>100	50	100	50	>100	>100	100
Mycobacterium ATCC 607	6.3	6.3	6.3	50	25	12.5	12.5	100	25	3.1

a) Abbreviations: Cpm, capreomycin; Tum tuberactinomycin; Tua, tuberactinamine.

being comparable to Tum O, whereas activities of both reversecapreomycin IIB and [Orn^4]-capreomycin IIB proved to be weaker than that of capreomycin IIB, being comparable to that of tuberactinamine N. On the other hand, pseudocapreomycin IB and di-β-Lys-capreomycin IIB showed even slightly greater activities than those of capreomycin IB or tuberactinomycin O.

Structure-activity Relationship. Both capreomycin IIA and IIB lacking the β-Lys branch showed stronger activities than tuberactinamine N which is also a cyclic peptide having no branch part. It is suggested that the β-amino group of A_2pr^4 residue promotes biological potency, although no such effect could be recognized for the same β-amino group of A_2pr^4 residue in pseudocapreomycin IB as compared with tuberactinomycin O. A possible explanation is as follows: the antibacterial potency of tuberactinomycin or capreomycin I might reach the upper limit in this sort of peptide antibiotics, since no synthetic analog has been found remarkably more active than the natural antibiotics so far.

Weaker activities of both [Orn^4]- and reversecapreomycin IIB apparently indicate the significance of the location of β-amino group of A_2pr^4 residue for its function as an activity-enhancing group.

A comparable activity of pseudocapreomycin IB to natural capreomycin IA or IB may imply that the effectiveness of β-Lys residue does not differ so much at either position of A_2pr residues for the enhancement of antibacterial activity. However, even if two β-Lys residues were attached to both amino groups, no significant increase of the activity was observed as seen in the case of di-β-Lys-capreomycin IIB.

Experimental

All the melting points are uncorrected. NMR spectra were recorded with a Varian XL-100-15 spectrometer using sodium dimethylsilapentanesulfonate as an internal standard. Specific rotations were measured with a Perkin-Elmer 141 Polarimeter. Molecular weights were determined with a Knauer vapor pressure osmometer using DMF as a solvent.

*Capreomycin IIA (**2a**).* A protected cyclopentapeptide **1a**[5] (170 mg, 0.180 mmol) was treated with 99 % formic acid (5 ml) at room temperature for 1 h. After removal of formic acid by evaporation, the product was hydrogenated with palladium black in methanol. The filtrate from catalyst was concentrated *in vacuo*. The residue was dissolved in 1 M hydrochloric acid–acetone (1: 1) (5 ml) and heated under reflux for 10 min. To the cooled solution was added urea (500 mg, 8.33 mmol) and the mixture was stirred at room temperature overnight. After evaporation of the solvent *in vacuo*, ethanol was added to the residue to form a white precipitate, which was reprecipitated from water–methanol–ethanol, yield 99 mg (84.6 %), mp 250 °C (dec), $[\alpha]_D^{23}$ +9.3° (*c* 2.8, H_2O). Found: C, 34.77; H, 5.60; N, 25.13; Cl, 15.35 %. Calcd for $C_{19}H_{35}N_{12}O_7Cl_3 \cdot 1/2H_2O \cdot 1/2CH_3OH$: C, 34.70; H, 5.67; N, 24.90; Cl, 15.76 %.

*Capreomycin IIB (**2b**).* The compound was prepared from a protected cyclopentapeptide **1b**[5] (150 mg, 0.179 mmol) in a similar manner to that for **2a**, yield 93 mg (81.6 %), mp 252 °C (dec), $[\alpha]_D^{23}$ −24.9° (*c* 0.57, H_2O). Found: C, 31.99; H, 6.15; N, 23.82; Cl, 15.24 %. Calcd for $C_{19}H_{35}N_{12}O_6Cl_3 \cdot 4H_2O$: C, 32.32; H, 6.14; N, 23.81; Cl, 15.07 %.

*Cyclo[Boc–β-Lys(Boc)–A_2pr–Ala–A_2pr(Z)–Dea–Cpd(NO_2)] (**3**).* Compound **1b**[5] (300 mg, 0.359 mmol) was treated with 99 % formic acid at room temperature for 2 h. Formic acid was removed *in vacuo* and the residue was dried over sodium hydroxide under reduced pressure. The white powder thus obtained was then coupled with Boc–β-Lys(Boc)–ONSu[10] (191 mg, 0.431 mmol) in DMF (2 ml) in the presence of triethylamine (54 mg, 0.538 mmol) at room temperature for 40 h. To the reaction mixture was added chloroform to form a gelatinous precipitate (335 mg, 87.7 %). The product was reprecipitated from DMF–ether for elemental analysis, mp 240 °C (dec), $[\alpha]_D^{23}$ −43.7° (*c* 0.90, DMF). Found: C, 50.36; H, 6.81; N, 17.13 %. Calcd for $C_{46}H_{73}N_{13}O_{16} \cdot 3/2H_2O$: C, 50.63; H, 7.02; N, 16.69 %.

*Pseudocapreomycin IB (**4**).* Deprotection of **3** (110 mg, 0.132 mmol) and conversion of Dea into Uda residue were carried out as in the preparation of **2a**, yield 62 mg (74 %), mp 245 °C (dec), $[\alpha]_D^{23}$ −30.7° (*c* 0.61, H_2O). Found: C, 37.44; H, 6.09; N, 24.27 %. Calcd for $C_{25}H_{48}N_{14}O_7Cl_4$: C, 37.60; H, 6.06; N, 24.56 %.

*Boc–Orn(Z)–Dea–OEt (**5b**).* Z–Dea–OEt[5b] (12.4 g, 36.6 mmol) was hydrogenated with palladium black catalyst in ethanol (100 ml) in the presence of acetic acid (4.40 g, 73.2 mmol) to give an oil of H–Dea–OEt·AcOH. This was then dissolved in ethyl acetate (30 ml) and added dropwise to an ice-cooled solution of Boc–Orn(Z)–ONSu (17.0 g, 36.6 mmol) in ethyl acetate (30 ml) with stirring. Subsequently, *N*-methylmorpholine (4.44 g, 43.9 mmol) was added dropwise and the mixture was stirred at 0 °C for 2 h and at room temperature for 16 h. The solution was washed with 10 % aqueous citric acid, saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to afford a white solid (17.1 g, 84.7 %). It was recrystallized from ethyl acetate–hexane, mp 62—63 °C, $[\alpha]_D^{23}$ −5.9° (*c* 2.4, DMF). Found: C, 57.91; H, 7.96; N, 7.27 %. Calcd for $C_{27}H_{43}N_3O_9 \cdot 1/2H_2O$: C, 57.67; H, 7.89; N, 7.47 %.

*Boc–A_2pr(Z)–Ala–Dea–OEt (**6a**),* A protected tripeptide **6a** was obtained from Boc–A_2pr(Z)–ONSu[12] (10.0 g, 22.9 mmol) and Z–Ala–Dea–OEt (**5a**)[12] (9.40 g, 22.9 mmol) in a similar way to that in the preparation of **5b**, yield 11.7 g (85.3 %), mp 94—96 °C, $[\alpha]_D^{23}$ −10.7° (*c* 2.4, DMF). Found: C, 56.69; H, 7.48; N, 9.45%. Calcd for $C_{28}H_{44}N_4O_{10}$: C, 56.36; H, 7.43; N, 9.39 %.

*Boc–Ala–Orn(Z)–Dea–OEt (**6b**).* Compound **5b** (6.00 g, 10.8 mmol) was treated with 28 ml of 5.8 M hydrogen chloride in ethanol at room temperature for 90 min. Benzene (300 ml) was added to the solution, which was then lyophilized. After the same procedure had been repeated twice, the resulting white powder was dried over sodium hydroxide under reduced pressure. To a solution of the deprotected dipeptide thus obtained and Boc–Ala–ONSu (3.10 g, 10.8 mmol) in ethyl acetate (70 ml) was added *N*-methylmorpholine (1.32 g, 13.0 mmol) dropwise at 0 °C with stirring. After being stirred at 0 °C for 2 h and then at room temperature for 16 h, the solution was washed with 10 % aqueous citric acid, saturated aqueous sodium hydrogencarbonate and brine successively, and dried over anhydrous magnesium sulfate. When ethyl acetate was removed *in vacuo*, a white solid (6.06 g, 89.5 %) was obtained. For elemental analysis, the product was recrystallized from ethylacetate–hexane, mp 70—75 °C, $[\alpha]_D^{23}$ −4.3° (*c* 2.3, DMF). Found: C, 57.49; H, 7.68; N, 8.96%. Calcd for $C_{30}H_{48}N_4O_{10}$: C, 57.68; H, 7.74; N, 8.97 %.

*Boc–A_2pr(Nps–Cpd(NO_2))–A_2pr(Z)–Ala–Dea–OEt (**8a**).* Removal of Boc group in **6a** (2.36 g, 3.95 mmol) was carried out as in the preparation of **6b**. The deprotected tripeptide obtained above was dissolved in DMF (15 ml) together with dipeptide **7**[12] (2.00 g, 3.59 mmol), HOBt (680 mg, 5.03 mmol),

N-methylmorpholine (436 mg, 4.31 mmol), and DCC (815 mg, 3.95 mmol) at 0 °C with stirring. Stirring was continued at 0 °C for 2 h and at room temperature for 16 h. The solution was filtered and concentrated *in vacuo*. The resulting oily residue was dissolved in ethyl acetate, washed with 10 % aqueous citric acid, saturated aqueous sodium hydrogencarbonate and brine successively and dried over anhydrous magnesium sulfate. The solution was concentrated *in vacuo* to give a yellow solid (3.30 g, 88.7 %). For elemental analysis, it was reprecipitated from THF–ether, mp 136—140 °C (dec), $[\alpha]_D^{23}$ +32.9° (*c* 2.0, DMF). Found: C, 49.19; H, 6.08; N, 15.78; S, 2.96 %. Calcd for $C_{43}H_{62}N_{12}O_{16}S \cdot H_2O$: C, 49.04; H, 6.13; N, 15.96; S, 3.04 %.

*Boc–A_2pr(Nps–Cpd(NO_2))–Ala–Orn(Z)–Dea–OEt (**8b**).* The compound was obtained from **6b** (3.37, 5.39 mmol) and **7**[12] (3.00 g, 5.39 mmol) as a yellow solid according to the method used for **8a**, yield 4.89 g (85.3 %), mp 155—160 °C (dec), $[\alpha]_D^{23}$ +38.0° (*c* 1.8, DMF). Found: C, 50.70; H, 6.33; N, 15.40; S, 3.03 %. Calcd for $C_{45}H_{66}N_{12}O_{16}S$: C, 50.84; H, 6.26; N, 15.81; S, 3.02 %.

*Boc–A_2pr(Nps–Cpd(NO_2))–A_2pr(Z)–Ala–Dea–OH (**9a**).* To a suspension of **8a** (3.00 g, 2.90 mmol) in ethanol (5 ml) was added 2.2 ml of 2 M aqueous sodium hydroxide. After being stirred at room temperature for 30 min, the solution was diluted with water (50 ml), and acidified with 10 % aqueous citric acid. The precipitated oil was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. When the solvent was removed *in vacuo*, a yellow solid (2.45 g, 83.9 %) was obtained. It was reprecipitated from THF–ether for elemental analysis, mp 134—137 °C (dec), $[\alpha]_D^{23}$ +42.5° (*c* 1.7, DMF). Found: C, 48.64; H, 6.05; N, 15.98; S, 3.07 %. Calcd for $C_{41}H_{58}N_{12}O_{16}S \cdot 1/2H_2O \cdot 1/2C_4H_8O$: C, 49.09; H, 6.04; N, 15.98; S, 3.05 %.

*Boc–A_2pr(Nps–Cpd(NO_2))–Ala–Orn(Z)–Dea–OH (**9b**).* This peptide was obtained as a yellow solid from **8b** (4.44 g, 4.18 mmol) according to the method used in the preparation of **9a**: yield 3.91 g (90.5 %), mp 125—130 °C (dec), $[\alpha]_D^{23}$ +34.0° (*c* 1.9, DMF). Found: C, 49.94; H, 6.27; N, 15.18; S, 2.79 %. Calcd for $C_{43}H_{62}N_{12}O_{16}S \cdot 1/2H_2O \cdot 1/2C_4H_8O$: C, 50.04; H, 6.25; N, 15.56; S, 2.97 %.

*Cyclo[Boc–A_2pr–A_2pr(Z)–Ala–Dea–Cpd(NO_2)] (**10a**).* To a solution of **9a** (2.20 g, 2.19 mmol) and HONSu (276 mg, 2.40 mmol) in THF (10 ml), was added DCC (496 mg, 2.40 mmol) with stirring room temperature. After being stirred for 5 h, the solution was filtered and concentrated *in vacuo*. The residual yellow oil was triturated with hexane to give a yellow powder of the ONSu ester of **9a**, yield 2.51 g. Only an oil was obtained in spite of efforts to crystallize the product.

To a solution of the active ester obtained above in THF (3 ml) was added 1.85 ml of 2.7 M hydrogen chloride in THF dropwise with stirring at 0 °C, stirring being continued for 30 min. Ether was added to the solution to obtain a pale yellow powder (1.94 g).

The deprotected peptide active ester thus prepared was then dissolved in DMF (200 ml) and added slowly to pyridine (2 l) with vigorous stirring for 40 h, stirring being continued for 20 h. The solution was then concentrated *in vacuo*. The desired cyclic pentapeptide **10a** was isolated by silica gel column chromatography using a mixture of chloroform and methanol (19: 1) as an eluting solvent. The product was recrystallized from hot methanol to give fine needles (441 mg, 26.5 % based on **9a**), mp >250 °C, $[\alpha]_D^{23}$ −38.1° (*c* 1.2, DMF). Found: C, 49.42; H, 6.32; N, 18.21 %; molecular weight, 781. Calcd for $C_{35}H_{53}N_{11}O_{13} \cdot H_2O$: C, 49.23; H, 6.49; N, 18.04 %; molecular weight, 854.

*Cyclo[Boc–A_2pr–Ala–Orn(Z)–Dea–Cpd(NO_2)] (**10b**).* The cyclopentapeptide was obtained from **9b** (3.70 g, 3.57 mmol) according to the method used for **10a** as fine needles (561 mg, 20.1 %), mp 250 °C, $[\alpha]_D^{23}$ −44.7° (*c* 1.6, DMF). Found: C, 51.04; H, 6.64; N, 17.66 %; molecular weight, 743. Calcd for $C_{37}H_{57}N_{11}O_{13} \cdot 1/2H_2O$: C, 50.91; H, 6.70; N, 17.65 %; molecular weight, 873.

*Reversecapreomycin IIB (**11a**).* This analog was synthesized from **10a** (150 mg, 0.179 mmol) as in the preparation of capreomycin IIA, yield 101 mg (88.6 %), mp >250 °C, $[\alpha]_D^{23}$ +29.2° (*c* 0.26, H_2O). Found: C, 31.15; H, 5.99; N, 22.80; Cl, 14.55 %. Calcd for $C_{19}H_{35}N_{12}O_6Cl_3 \cdot 5.5H_2O$: C, 31.13; H, 6.33; N, 22.93; Cl, 14.51 %.

*[Orn[4]]-Capreomycin IIB (**11b**).* This analog was obtained from **10b** (150 mg, 0.174 mmol) in a similar way to that for the preparation of capreomycin IIA, yield 107 mg (93.0 %), mp 245—248 °C (dec), $[\alpha]_D^{23}$ −51.5° (*c* 1.5, H_2O). Found: C, 33.02; H, 6.24; N, 22.07; Cl, 14.14 %. Calcd for $C_{21}H_{39}N_{12}O_6Cl_3 \cdot 5.5H_2O$: C, 33.14; H, 6.62; N, 22.09; Cl, 13.98 %.

*Di-β-Lys-capreomycin IIB (**13**). Route A*: To a suspension of capreomycin IIB·3HCl (**2b**) (45 mg, 0.071 mmol) in DMF (2 ml) were added Boc-β-Lys(Boc)-ONSu[10] (79 mg, 0.18 mmol) and triethylamine (18 mg, 0.18 mmol) with stirring at room temperature. After being stirred for 48 h, the solution was concentrated *in vacuo*. To the residual oil was added 3 M hydrochloric acid (5 ml). The mixture was stirred at room temperature for 1 h and then neutralized with 4 M aqueous sodium hydroxide. The solution was applied to a column (2.5×80 cm) of Sephadex G10. The desired product **13** was eluted with water, yield 51 mg (75 %). For elemental analysis the product was reprecipitated from water–methanol–ethanol, mp 245 °C (dec), $[\alpha]_D^{23}$ −50.5° (*c* 1.3, H_2O). Found: C, 35.01; H, 6.89; N, 20.23; Cl, 16.44 %. Calcd for $C_{31}H_{61}N_{16}O_8Cl_5 \cdot 6H_2O \cdot CH_3OH$: C, 34.83; H, 7.03; N, 20.31; Cl, 16.07 %.

Route B: The hexapeptide **3** (150 mg, 0.141 mmol) was hydrogenated with palladium black catalyst in DMF (15 ml) in the presence of Boc-β-Lys(Boc)-ONSu[10] (94 mg, 0.21 mmol) for 40 h. To the filtrate from catalyst was added ethyl acetate (50 ml) to form a gelatinous precipitate of **14**, which was collected by centrifugation, yield 147 mg (83.1 %). It was reprecipitated from DMF–chloroform–ether for elemental analysis, mp >250 °C, $[\alpha]_D^{23}$ −43.1° (*c* 0.42, DMF). Found: C, 50.07; H, 7.52; N, 16.61 %. Calcd for $C_{54}H_{95}N_{15}O_{19} \cdot 2H_2O$: C, 50.10; H, 7.71; N, 16.23 %.

Deprotection and conversion of Dea to Uda residue in compound **14** (66 mg, 0.052 mmol) were carried out by a similar procedure to that in the preparation of **2a**, yield 39 mg (78 %). The product thus obtained was identical with the product in route A in thin-layer chromatography (silica gel; phenol–water–concd aqueons ammonia 30: 10: 1; R_f 0.22).

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6) Numbering of positions of the amino acid residues in the cyclic peptide moiety is given in Fig. 1. It is in line with numbering for tuberactinomycin (Fig. 2), *cf.* footnote 11) in Ref.[12].

7) Abbreviations according to IUPAC-IUB recommendation, *J. Biol. Chem.*, **247**, 977 (1972), are used. A_2pr: α,β-diaminopropionic acid, Cpd: capreomycidine, Uda: β-ureidodehydroalanine, β-Lys: β-lysine, Dea: β,β-diethoxyalanine, Boc: *t*-butoxycarbonyl, Z: benzyloxycarbonyl, Nps: *o*-nitrophenylsulfenyl, DCC: dicyclohexylcarbodiimide, HONSu: *N*-hydroxysuccinimide, HOBt: 1-hydroxybenzotriazole. All amino acids used in this study except DL-Dea are of L-configurations.

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