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TOTAL SYNTHESIS OF CAPREOMYCIN[1]

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Abstract—Total syntheses have been achieved of capreomycin IA and IB according to our newly proposed structures. The β-lysine residue in the branch was introduced to the cyclic peptide moiety which was prepared by cyclization of the corresponding pentapeptide. Deprotection followed by conversion of β,β-diethoxyalanine residue to β-ureidodehydroalanine residue afforded the desired products, which were identical with natural capreomycins in all respects.

The peptide antibiotic capreomycin, produced by *Streptomyces capreolus*,[2] has been used in clinical therapy as an antituberculous agent. Chemical and biological properties of capreomycin are very similar to those of viomycin and tuberactinomycin whose total structures were established in our previous studies (Fig. 1).[3,4] Recently we revised the structural formula of capreomycin as shown in Fig. 2[5,6] from the one formerly proposed by Bycroft *et al.*[7] It should be noted that the mode of linkage of β-Lys† in the branch of capreomycin is different from that in tuberactinomycin, although the amino acid sequence in the cyclic peptide moiety is quite similar in both compounds.

In this study we confirmed this revised formula by the total syntheses of capreomycin **IA** and **IB**.[1] The synthetic strategy is basically similar to that for tuberactinomycin O[8] or its analogs,[9] although rather more difficult because capreomycin bears two A_2pr residues in its molecule and has a different kind of linkage between the branch and the cyclic moiety.

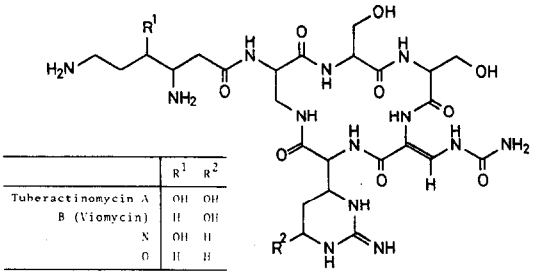
The synthetic scheme for the carboxyl terminal tripeptides **3a** and **3b** is given in Fig. 3. The dipeptide **2** was prepared by the reaction of Boc-L-A_2pr(Z)-ONSu with H-DL-Dea-OEt which was generated from N-benzyloxycarbonyl deriative **1** just before use. The Dea residue at the carbonyl terminal was employed in its DL-form as a synthetic precursor of the unstable Uda, since a chiral center in Dea will be eliminated in the conversion step from Dea to Uda. Although the coupling product of **2** or the following longer peptides could be a mixture of diastereomers concerning the DL form of the Dea residue, no separation of such isomers was actually recognized at any synthetic step. When in a preliminary experiment **2** was treated with TFA to remove the Boc group and then submitted to coupling with Boc-L-Ala-ONSu, the product was found to be a complex mixture, from which the desired compound **3b** was obtained only in a 40% yield after silica gel column chromatography. Such an undesirable result in this step might be attributed to a lability of the acetal group in the Dea residue in acidic condition. In fact, the compound **1** itself, when treated with TFA, gave many spots on thin-layer chromatogram, all of which were ninhydrin-negative materials indicating them to be not debenzyloxycarbonylated products but deacetal compounds. This partial degradation occurred even with hydrogen chloride in THF or AcOEt, but not in ethanolic hydrogen chloride or in 99% HCOOH. Therefore, **2** was treated with ethanolic hydrogen chloride and then allowed to react with the protected active ester of Ser or Ala to afford the tripeptide **3a** or **3b** in a satisfactory yield.

The amino terminal dipeptide **4** is the same intermediate as prepared in the synthesis of tuberactinomycin O.[8] This compound **4** was condensed in turn with the tripeptide ester hydrochlorides **5a** and **5b**, which were prepared from **3a** and **3b** as described above, by means of DCC–HOBt method to give the pentapeptides **6a** and **6b** in a high yield respectively.

The ethyl esters **6a** and **6b** were converted to the corresponding 1-succinimidyl esters **8a** and **8b** through saponification followed by esterification with DCC and HONSu. Nps groups were then very carefully removed with dilute HCl in THF at 0° so that the above-mentioned side reaction arising from degradation of the Dea residue might be suppressed as much as possible. The deprotected products thus obtained were cyclized in a large amount of pyridine at room temperature to give cyclic pentapeptides **9a** and **9b** which were purified by silica gel column chromatography. The yields of the cyclization reactions giving **9a** and **9b** were 24% and 27% respectively.

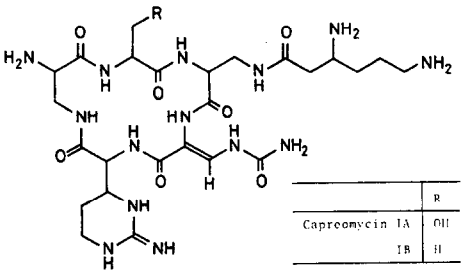
In the next step of the synthesis (Fig. 4), reductive cleavage of the Z group at the β-amino group of the A_2pr residue was accompanied by partial removal of the nitro group at the Cpd residue and presumably also by N,N-acyl migration at the A_2pr residue giving a complex reaction product. This defect was overcome by carrying out the reduction with a simultaneous addition of Boc-β-Lys(Boc)-ONSu[10–12] in DMF. Thus, the desired hexapeptide **10a** or **10b** was successfully obtained in a fairly good yield. In these conditions, even the O-benzyl group (δ 7.33, s, 5H and δ 4.51, s, 2H) of the Ser residue is sufficiently resistant to removal, indicating that the Z group is more susceptible to reduction than the benzyl group. Immediately after cleavage of the Z group a β-Lys residue could be smoothly introduced to the free amino group. It might be expected that the three bulky Boc groups in the molecule of the hexapeptide **10a** would protect the benzyl group as well as the nitro group from

†Abbreviations: Cpd; capreomycidine (the guanidino amino acid in Fig. 2), A_2pr; α,β-diaminopropionic acid, Ser; serine, Ala; alanine, Dea; β,β-diethoxyalanine, β-Lys; β-lysine, Uda; β-ureidodehydroalanine, Z; benzyloxycarbonyl, Boc: *tert*-butoxycarbonyl, Nps; *o*-nitrophenylsulfenyl, DCC; dicyclohexylcarbodiimide, HONSu; 1-hydroxysuccinimide, HOBt; 1-hydroxybenzotriazole, NMM; N-methylmorpholine, TFA; trifluoroacetic acid. All amino acids are of L-configurations unless otherwise stated.



	R^1	R^2
Tuberactinomycin A	OH	OH
B (Viomycin)	H	OH
N	OH	H
O	H	H

Fig. 1. The structures of tuberactinomycins.



	R
Capreomycin IA	OH
IB	H

Fig. 2. The structures of capreomycins.

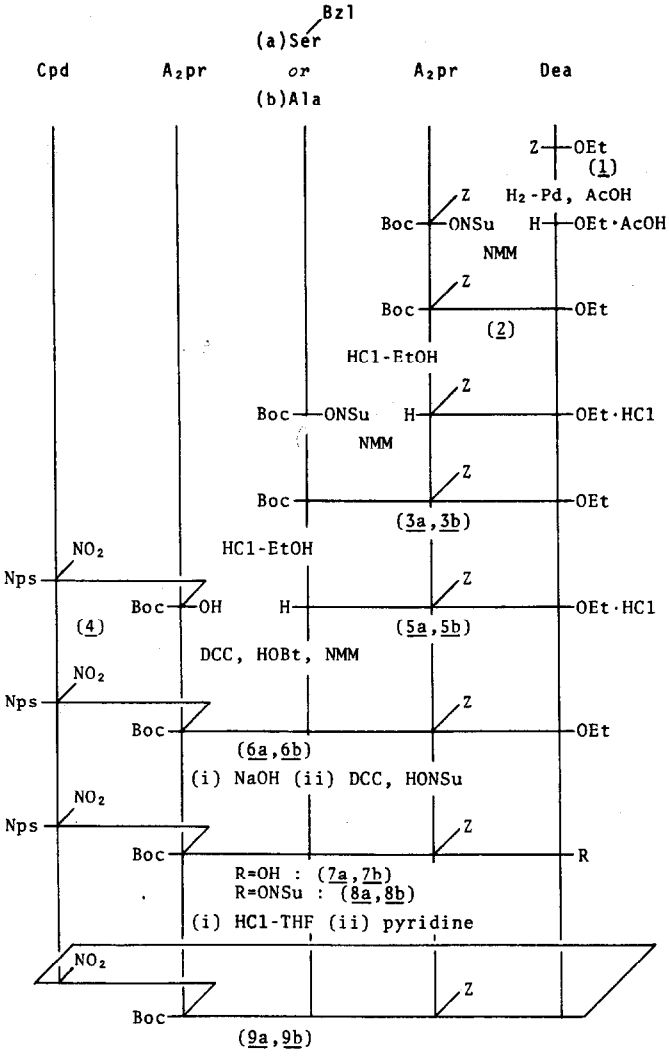


Fig. 3. Synthesis of the cyclic pentapeptides.

hydrogenolyis, and in fact in the final step of the synthesis all attempts to remove the nitro and benzyl groups reductively failed, even using PtO_2 catalyst and longer reaction time. The Boc groups were therefore cleaved with 99% formic acid prior to the reduction. As expected, the nitro group was then very readily removed by hydrogenolysis. The reduced product, without being isolated, was treated with acid in order to hydrolyze the acetal of the Dea residue. The product was then immediately allowed to react with an excess of urea (Fig. 5) to afford the final product **11a** or **11b** in an excellent yield.

The synthetic hexapeptides **11a** and **11b** thus synthesized were identical chromatographically with the natural capreomycins **IA** and **IB** respectively. Identity of the synthetic and natural specimens was also found in physicochemical properties, as seen in Table 1 of the experimental section. Furthermore, the NMR spectra both in D_2O and in H_2O of the synthetic and natural products were practically superimposable (Figs. 6 and 7).

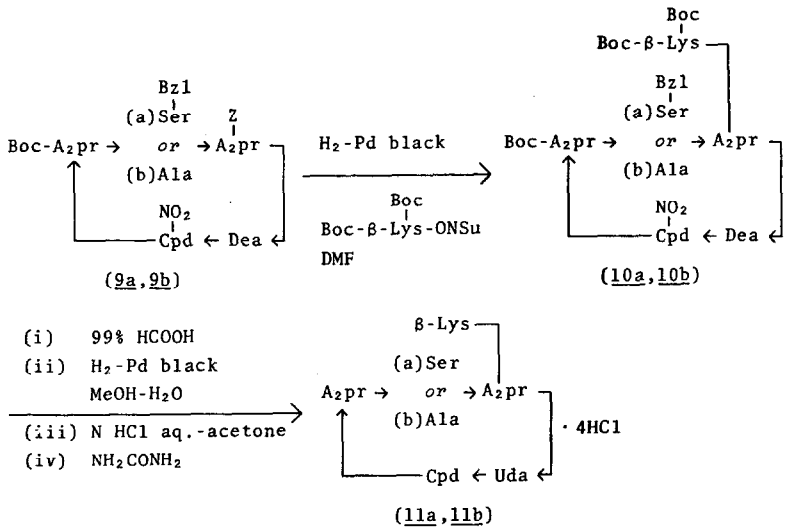


Fig. 4. Introduction of the β-Lys residue to the cyclic peptide and subsequent deprotection.

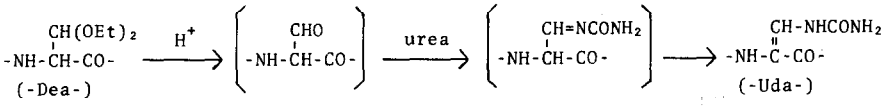


Fig. 5. Conversion of the Dea residue to Uda.

Table 1. Physicochemical properties of the natural and synthetic capreomycins IA and IB

		Capreomycin IA		Capreomycin IB	
		synthetic	natural	synthetic	natural
R_f*		0.25	0.25	0.29	0.29
m.p. (dec.)		240-5°	246-8°	250-3°	253-5°
$[\alpha]_D^{22}$ (c0.5, H_2O)		-22.0°	-21.9	-42.5°	-44.6°
UV	0.1N HCl	269 nm (ε23,400)	269 nm (ε24,000)	268 nm (ε22,000)	268 nm (ε22,700)
	H_2O	268 (23,200)	268 (23,900)	268 (21,900)	268 (22,300)
	0.1N NaOH	288 (15,800)	287 (15,900)	290 (13,100)	290 (14,400)

* Tlc (Silica gel, phenol - water - 28% ammonia, 30:10:1)

Of particular note is the fact that the signal of the olefin proton of the Uda residue in the NMR spectrum of the synthetic material appeared as a sharp singlet having the same chemical shift as the natural product. This fortunate fact may indicate that the double bond of the Uda residue was selectively introduced with the same olefin configuration as the natural type, possibly under the influence of some environmental factors in the molecule of the cyclic peptide.

Finally, the antibacterial spectra of the synthetic products were in good agreement with those of the respective natural products (Table 2). All these results clearly show that the synthetic products are completely identical to the natural capreomycins **IA** and **IB** in all respects. Consequently, the structure of capreomycin was explicitly established for the first time by the present synthetic study.

EXPERIMENTAL

All m.ps are uncorrected. The following instruments were used for measurements: Hitachi 124 Spectrophotometer, Perkin-Elmer 141 Polarimeter, Knauer Osmometer, Varian XL-100-15 NMR Spectrometer. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate and TMS were used as internal references in D_2O and H_2O and in organic solvents respectively.

Since all peptides containing DL-Dea residue are a mixture of diastereomers as described in the text, they show rather wide ranges of m.p. and their specific rotations fluctuate from batch to batch.

Z-DL-Dea-OEt (1)

To a soln of crude H-DL-Dea-OEt[13] (10.0 g, 48.7 mmol) in $CHCl_3$ (100 ml), Z-Cl (9.10 g, 53.6 mmol) and NEt_3 (5.40 g, 53.6 mmol) were added dropwise with vigorous stirring at 0° for 30 min. After being stirred at 0° for 2 hr and then at room temp. for 5 hr, the soln was washed with 10% citric acid aq, 10% $NaHCO_3$ aq and water successively, and dried over Na_2SO_4. The

Table 2. Antibacterial activities of the natural and synthetic capreomycins IA and IB

Minimum inhibitory concentration (γ/ml)

Test organism	Capreomycin IA		Capreomycin IB	
	synthetic	natural	synthetic	natural
Staphylococcus aureus ATCC 6538P	100	50	100	100
Staphylococcus epidermidis sp-al-1	50	50	100	50
Streptococcus pyogenes N.Y.5	100	100	100	100
Sartina lutea ATCC 9341	100	100	100	100
Micrococcus flavus ATCC 10240	100	100	100	100
Bacillus subtilis ATCC 6633	12.5	12.5	12.5	12.5
Escherichia coli B	50	50	100	100
Salmonella typhosa H 901	25	25	50	50
Salmonella paratyphi PA 41-N-22	100	100	100	100
Salmonella enteritidis Gaertner	100	50	100	100
Shigella sonnei E33	50	50	100	50
Mycobacterium ATCC 607	6.3	12.5	6.3	6.3

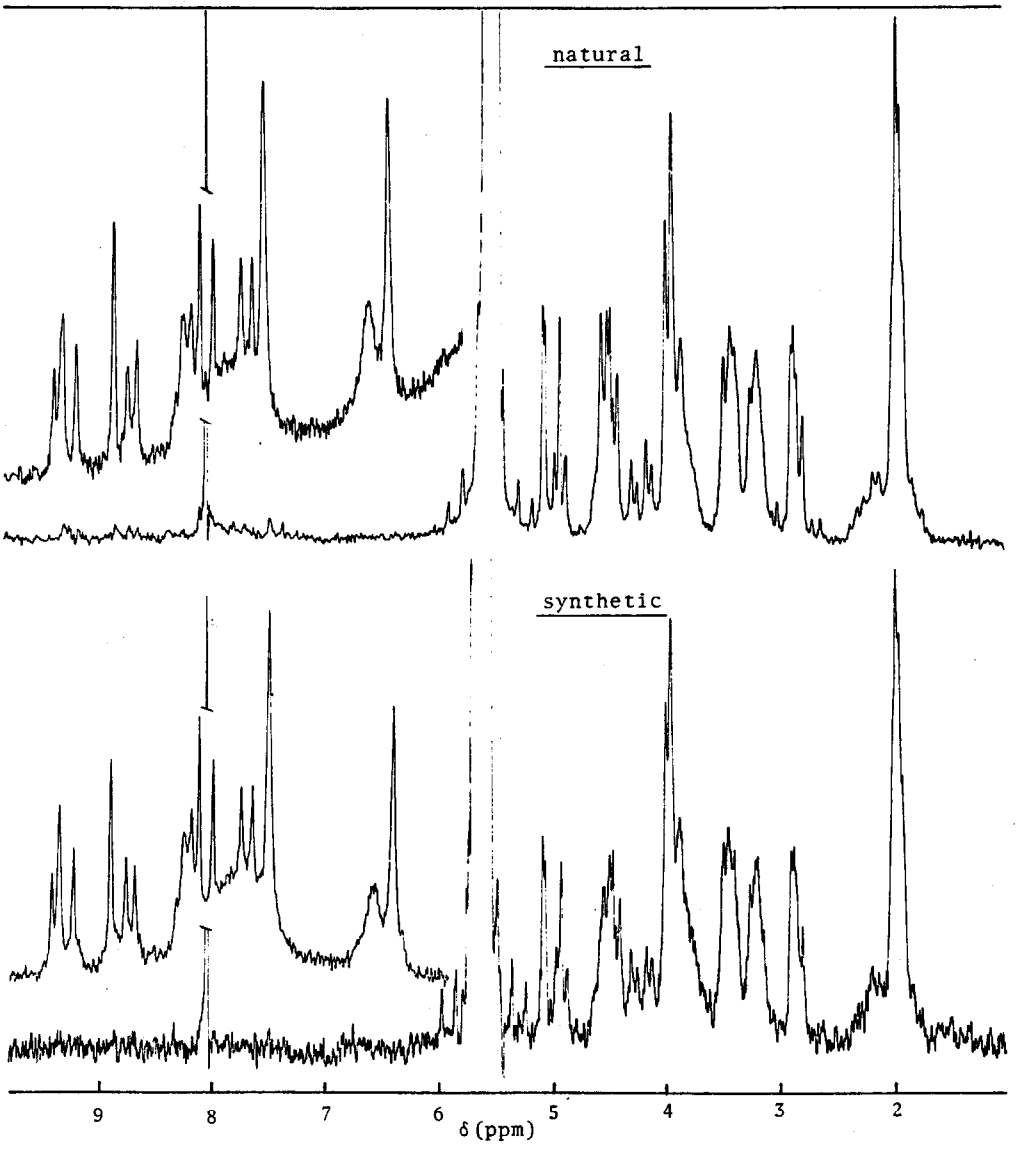


Fig. 6. NMR spectra (100 MHz) of natural and synthetic capreomycin IA in D_2O–TFA (4:1) and in H_2O (the amide proton region, δ6–10).

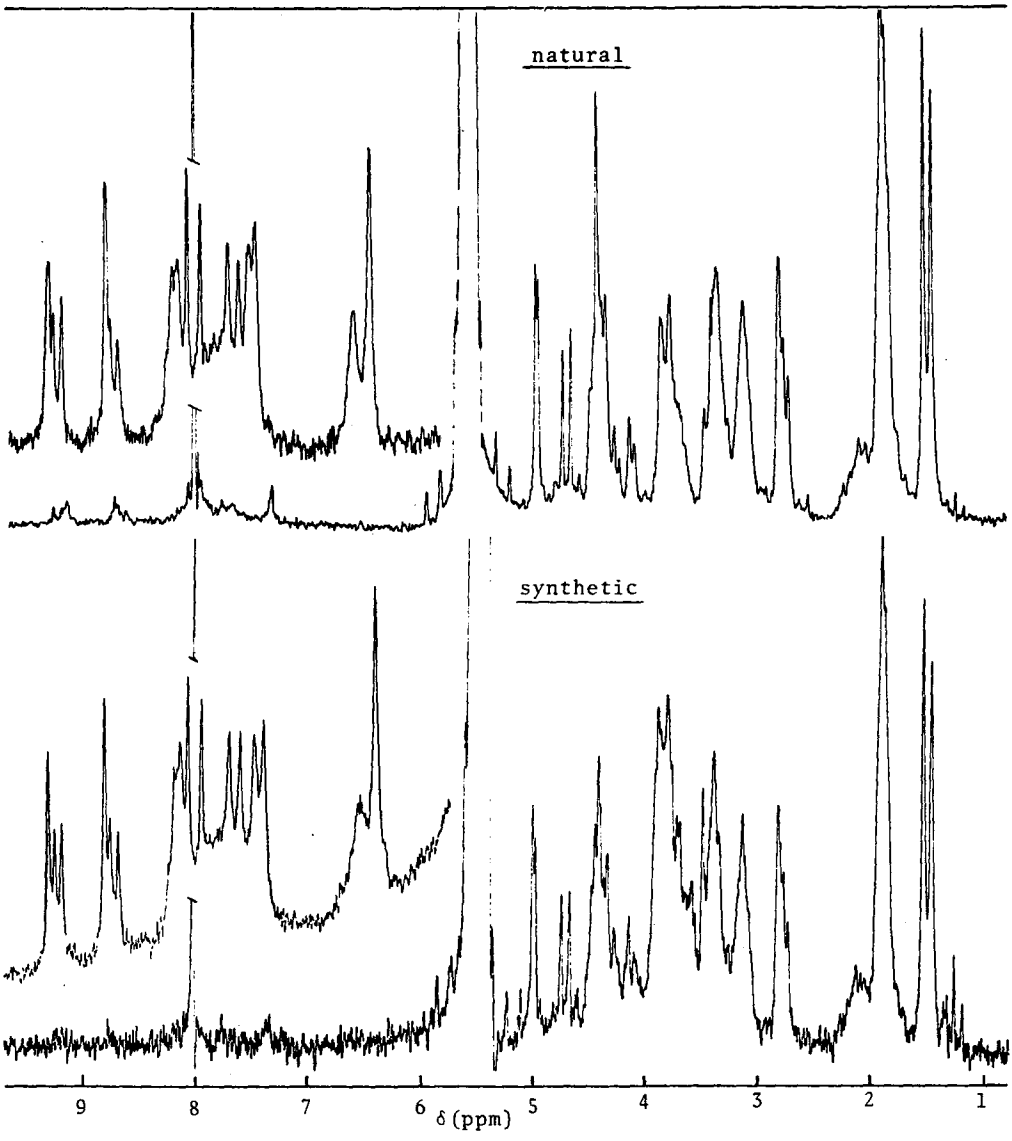


Fig. 7. NMR spectra (100 MHz) of natural and synthetic capreomycin **IB** in D_2O–TFA (4:1) and in H_2O (the amide proton region, δ6–10).

filtered soln was evaporated to leave a brown oil, which was purified by column chromatography on silica gel (benzene–AcOEt) to afford a colorless oil (9.00 g, 54.5%). NMR (δ in CCl_4): 1.14 (t, 6H, $CH(OCH_2\underline{CH_3})_2$); 1.24 (t, 3H, $COOCH_2\underline{CH_3}$); 3.5 (m, 4H, $CH(O\underline{CH_2}CH_3)_2$); 4.14 (q, 2H, $COO\underline{CH_2}CH_3$); 4.45 (dd, 1H, NHC<u>H</u>–CO); 4.65 (d, 1H, $C\underline{H}(OCH_2CH_3)_2$); 5.05 (s, 2H, $C_6H_5\underline{CH_2}$); 5.52 (d, 1H, NH); 7.25 (s, 5H, C_6H_5).

This product was converted to a crystalline hydrazide for elemental analysis as follows; a soln of **1** (1.00 g, 2.95 mmol) and hydrazine hydrate (3.00 g, 59.0 mmol) in DMF (10 ml) was allowed to stand at room temp. for 3 days. After evaporation of excess hydrazine *in vacuo*, the soln was poured into water (200 ml) to form a white ppt, which was recrystallized from AcOEt to give needles (0.640 g, 66.7%), m.p. 139–140°. (Found: C, 55.28; H, 7.07; N, 12.97. $C_{15}H_{23}N_3O_5$ requires: C, 55.37; H, 7.13; N, 12.92%).

Boc-L-A$_2$pr(Z)-DL-Dea-OEt (**2**)

Hydrogenolysis of **1** (8.15 g, 24.0 mmol) with Pd black catalyst in EtOH (30 ml) in the presence of AcOH (2.17 g, 36.0 mmol) gave an oil of H-DL-Dea-OEt·AcOH after evaporation of the solvent. This was then dissolved in AcOEt (20 ml) and added dropwise to an ice-cooled soln of Boc-L-A_2pr(Z)-ONSu[9] (9.50 g, 21.8 mmol) in AcOEt (50 ml) with stirring. After dropwise addition of N-methylmorpholine (2.42 g, 24.0 mmol), the mixture was stirred at 0° for 2 hr and at room temp. for 16 hr and then worked up as described in the preparation of **1** to give a white powder (9.49 g, 82.7%). Recrystallization from AcOEt and hexane gave needles, m.p. 38–45°, $[\alpha]_D^{15}$ – 16.6° (c 1.5, DMF). (Found: C, 57.04; H, 7.50; N, 8.05. $C_{25}H_{39}N_3O_9$ requires: C, 57.13; H, 7.48; N, 8.00%).

Boc-L-Ser(Bzl)-L-A$_2$pr(Z)-DL-Dea-OEt (**3a**)

The protected dipeptide **2** (3.15 g, 6.00 mmol) was treated with 20 ml of 4.5N HCl in EtOH at room temp. for 1.5 hr. Benzene (*ca.* 300 ml) was added to the soln, which was then lyophilized. After the same procedure had been repeated twice, the product was dried over NaOH under reduced pressure to give a white powder. To a soln of the product thus obtained and Boc-L-Ser(Bzl)-ONSu (2.35 g, 6.00 mmol) in AcOEt (20 ml), N-methylmorpholine (0.911 g, 9.00 mmol) in AcOEt was added dropwise at 0° with stirring. After being stirred at 0° for 2 hr and at room temp. for 16 hr, the soln was worked up as described above to give a white powder (3.38 g, 80.1%). Recrystallization

from AcOEt and hexane gave needles, m.p. 92–108°, $[\alpha]_D^{22}$–10.6° (*c*, 2.1, DMF). (Found: C, 59.79; H, 7.17; N, 7.85. $C_{35}H_{50}N_4O_{11}$ requires: C, 59.81; H, 7.17; N, 7.97%).

Boc–L–Ala–L–A₂pr(Z)–DL–Dea–OEt (**3b**)

This compound was obtained from **2** (5.26 g, 10.0 mmol) and Boc–L–Ala–ONSu (2.86 g, 10.0 mmol) according to the preparation of **3a**, yield 5.04 g (84.4%). It was recrystallized from AcOEt–hexane to give needles, m.p. 83–87°, $[\alpha]_D^{20}$–18.3° (*c*, 2.0, DMF). (Found: C, 56.13; H, 7.14; N, 9.28. $C_{28}H_{44}N_4O_{10}$ requires: C, 56.36; H, 7.43; N, 9.39%).

Boc – L – A₂pr(Nps – L – Cpd(NO₂)) – L – Ser(Bzl) – L – A₂pr(Z) – DL – Dea – OEt (**6a**)

The protected tripeptide **3a** (3.77 g, 5.39 mmol) was treated with HCl to remove the Boc group as in the preparation of **3**. The product **5a** was dissolved in DMF (20 ml) together with **4**[9] (3.00 g, 5.39 mmol), HOBt (1.02 g, 7.55 mmol), N-methylmorpholine (0.818 g, 8.09 g) and DCC (1.33 g, 6.47 mmol) at 0°. The soln was stirred at 0° for 2 hr and at room temp. for 16 hr, then filtered and evaporated *in vacuo*. The residual oil was worked up in the usual way to afford a yellow powder (5.47 g, 88.9%), m.p. 160–170° (dec) from THF–Et_2O, $[\alpha]_D^{22}$ + 37.4° (*c*, 1.8, DMF). (Found: C, 52.04; H, 5.98; N, 14.57; S, 2.71. $C_{50}H_{68}N_{12}O_{17}S\cdot H_2O$ requires: C, 51.80; H, 6.09; N, 14.50; S, 2.77%).†

Boc – L – A₂pr(Nps – L – Cpd(NO₂)) – L – Ala – L – A₂pr(Z) – DL – Dea – OEt (**6b**)

This compound was obtained as a yellow powder from **4**[9] (2.90 g, 4.86 mmol) and **5b** (2.70 g, 4.86 mmol) according to the preparation of **6a**: yield 4.54 g (90.3%), m.p. 134–163° (dec), $[\alpha]_D^{20}$ + 37.8° (*c*, 2.1, DMF). (Found: C, 49.37; H, 6.11; N, 15.69; S, 2.97. $C_{43}H_{62}N_{12}O_{16}S\cdot H_2O$ requires: C, 49.04; H, 6.13; N, 15.96; S, 3.04%).

Boc – L – A₂pr(Nps – L – Cpd(NO₂)) – L – Ser(Bzl) – L – A₂pr(Z) – DL–Dea – OH (**7a**)

To a suspension of **6a** (4.20 g, 3.68 mmol) in EtOH (5 ml), 2N NaOH (2.80 ml) was added. After being stirred at room temp. for 1 hr, the solution was diluted with water (50 ml) and 10% citric acid aq. (10 ml) was added. The soln was extracted three times with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated *in vacuo* to give a yellow powder (3.39 g, 82.7%), m.p. 132° (dec) from THF–Et_2O, $[\alpha]_D^{22}$ + 33.5° (*c*, 0.9, DMF). (Found: C, 51.43; H, 5.89; N, 14.88; S, 2.65. $C_{48}H_{64}N_{12}O_{17}S\cdot H_2O$ requires: C, 50.97; H, 5.88; N, 14.86; S, 2.83%).

Boc – L – A₂pr(Nps – L – Cpd(NO₂)) – L – Ala – L – A₂pr(Z) – DL – Dea – OH (**7b**)

This compound was obtained from **6b** (2.97 g, 2.87 mmol) according to the preparation of **7a**, yield 2.62 g (90.7%), m.p. 140–148° (dec), $[\alpha]_D^{22}$ + 35.3° (*c*, 1.7, DMF). (Found: C, 48.14; H, 5.79; N, 16.37; S, 2.90. $C_{41}H_{58}N_{12}O_{16}S\cdot H_2O$ requires: C, 48.04; H, 5.90; N, 16.40; S, 3.13%).

Boc – L – A₂pr(Nps – L–Cpd(NO₂)) – L–Ser(Bzl) – L – A₂pr(Z) – DL – Dea – ONSu (**8a**)

To a soln of **7a** (3.48 g, 3.13 mmol) in THF (10 ml), HONSu (0.432 g, 3.75 mmol) and DCC (0.744 g, 3.75 mmol) were added with stirring at room temp. and stirring was continued for 4 hr. The mixture was filtered, and the filtrate was evaporated *in vacuo*. A residual yellow powder was washed well with Et_2O and collected by filtration, yield 3.64 g (96.3%), m.p. 121° (dec) from THF–Et_2O, $[\alpha]_D^{22}$ + 29.9° (*c*, 1.8, DMF). IR (Nujol): 1820, 1785 and 1742 cm^{-1} (COONSu). (Found: C, 51.01; H, 5.52; N, 14.92; S, 244. $C_{52}H_{67}N_{13}O_{19}S\cdot H_2O$ requires: C, 50.85; H, 5.66; N, 14.83; S, 2.61%).

Boc – L – A₂pr(Nps – L – Cpd(NO₂)) – L – Ala – L – A₂pr(Z) – DL – Dea – ONSu (**8b**)

This compound was obtained from **7b** (2.62 g, 2.60 mmol) according to the method for preparation of **8a**: yield 2.69 g (93.7%), m.p. 137° (dec), $[\alpha]_D^{22}$ + 32.7° (*c*, 2.0, DMF). IR (Nujol): 1815, 1785 and 1740 cm^{-1} (COONSu). (Found: C, 48.97; H, 5.80; N, 16.06; S, 2.62. $C_{45}H_{61}N_{13}O_{18}S\cdot 1/2H_2O$ requires: C, 48.55; H, 5.61; N, 16.36; S, 2.88%).

Cyclo[Boc – L – A₂pr – L – Ser(Bzl) – L – A₂pr(Z) – DL – Dea – L – Cpd(NO₂)] (**9a**)

To a soln of **8a** (3.17 g, 2.17 mmol) in THF (3 ml), 3.3 ml of 2.0 N HCl in THF was added dropwise at 0° with stirring to remove the Nps group of **8a** selectively. Stirring was continued at 0° for 30 min and then Et_2O (150 ml) was added. A ppt which formed was collected by filtration, washed with Et_2O and dried over NaOH under reduced pressure. The product was slowly poured into pyridine (3 l) at room temp. with vigorous stirring over a period of 48 hr. After subsequent stirring for 16 hr, the soln was evaporated *in vacuo*. The residual oil was dissolved in $CHCl_3$ and the desired cyclopentapeptide was isolated by purification on silica gel column chromatography using a mixture of $CHCl_3$ and MeOH (19:1) as an eluting solvent; yield 0.486 g (23.8%), m.p. >250° from DMF–Et_2O, $[\alpha]_D^{7}$–39.1° (*c*, 1.0, DMF). (Found: C, 52.68; H, 6.24; N, 16.11; molecular weight, 880,‡ $C_{42}H_{59}N_{11}O_{14}\cdot H_2O$ requires: C, 52.55; H, 6.40; N, 16.05%; mol. wt, 960).

Cyclo[Boc – L – A₂pr – L – Ala – L – A₂pr(Z) – DL – Dea – L – Cpd(NO₂)] (**9b**)

The cyclic peptide (**9b**) was obtained from **8a** (1.50 g, 1.36 mmol) according to the method for preparation of **9a**; yield 0.293 g (26.6%). Recrystallization from hot MeOH gave needles, m.p. > 250°, $[\alpha]_D^{22}$–55.1° (*c*, 0.8, DMF). (Found: C, 49.34; H, 6.41; N, 17.85; mol. wt, 920.‡ $C_{35}H_{53}N_{11}O_{13}\cdot H_2O$ requires: C, 49.23; H, 6.49; N, 18.04%; mol. wt, 854).

Cyclo[Boc – L – A₂pr – L – Ser(Bzl) – L – A₂pr(Boc – L – β – Lys(Boc)) – DL – Dea – L – Cpd(NO₂)] (**10a**)

The cyclic peptide **9a** (150 mg, 0.159 mmol) was hydrogenolyzed in DMF (10 ml) with Pd black catalyst at room temp. in the presence of Boc – L – β – Lys(Boc) – ONSu[10–12] (92 mg, 0.240 mmol) for 14 hr. After the catalyst had been filtered off, $CHCl_3$ (10 ml) and Et_2O (5 ml) were added to the filtrate. A ppt formed and was collected by filtration; yield 150 mg (84.6%), m.p. 242–250° (dec) from DMF–Et_2O, $[\alpha]_D^{7}$–47.0° (*c*, 0.4, DMF). (Found: C, 51.88; H, 7.16; N, 15.79. $C_{50}H_{81}N_{13}O_{17}\cdot H_2O$ requires: C, 52.03; H, 7.25; N, 15.78%).

Cyclo[Boc – L – A₂pr – L – Ala – L – A₂pr(Boc – L – β – Lys(Boc)) – DL – Dea – L – Cpd(NO₂)] (**10b**)

Similarly, the branched cyclic peptide (**10b**) was obtained from **9b** (100 mg, 0.120 mmol), yield 110 mg (89.4%), m.p. 245–247° (dec), $[\alpha]_D^{7}$ – 51.1° (*c*, 1.2, DMF). (Found: C, 48.75; H, 7.29; N, 17.09. $C_{43}H_{75}N_{13}O_{16}\cdot 3/2H_2O$ requires: C, 48.85; H, 7.44; N, 17.22%).

Cyclo[H – L – A₂pr – L – Ser – L – A₂pr(H – L – β – Lys(H)) – Uda – L – Cpd]·4HCl (**11a**) (capreomycin IA)

The protected peptide **10a** (120 mg, 0.106 mmol) was treated with 99% HCOOH (3 ml) at room temp. for 1 hr and the soln was evaporated *in vacuo*. The residual oil was dissolved in a mixture of MeOH and water (9:1) and hydrogenated with Pd black catalyst for 20 hr. The catalyst was filtered off, the filtrate evaporated *in vacuo*, and the residue dissolved in a mixture of N HCl and acetone (1:1). The soln was refluxed for 10 min, then cooled to room temp. Urea (500 mg) was then added, the mixture stirred for 16 hr, then evaporated *in vacuo*. To the residual oil, EtOH (50 ml) was added and the ppt collected by filtration. The white powder thus obtained was reprecipitated from water, MeOH and EtOH; yield 69 mg (80.0%). It was again reprecipi-

†The peptides containing the $Cpd(NO_2)$ residue were usually found to contain water of crystallization, possibly due to the polarity of the nitroguanidino group. This was also the case with Boc – L – A₂pr(Nps – L – $Cpd(NO_2)$) – OMe[9], which failed to crystallize in the absence of water but formed beautiful needles of monohydrate when crystallized from aq. acetone and Et_2O.

‡Determined by vapor pressure osmometry using DMF as a solvent.

tated from the same solvents for analysis. The physicochemical and biological data are given in Tables 1 and 2 and Fig. 6. (Found: C, 36.00; H, 6.07; N, 23.54; Cl, 17.41. $C_{25}H_{48}N_{14}O_8Cl_4 \cdot H_2O$ requires: C, 36.06; H, 6.05; N, 23.55; Cl, 17.03%).

Cyclo[H – L – A_2pr – L – Ala – L – A_2pr(H – L – β – Lys(H)) – Uda – L – Cpd]·4HCl (**11b**) (capreomycin **IB**)

Synthetic capreomycin **IB** (**11b**) was obtained from **10b** (105 mg, 0.102 mmol) according to the method for preparation of **11a**; yield 69 mg (85.0%). The physicochemical and biological data are given in Tables 1 and 2 and Fig. 7. (Found: C, 36.90; H, 6.20; N, 24.05; Cl, 17.74. $C_{25}H_{48}N_{14}O_7Cl_4 \cdot H_2O$ requires: C, 36.77; H, 6.17; N, 24.02; Cl, 17.37%).

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