Tetrahedron Letters No. 43, pp 3907 - 3910, 1976. Pergamon Press. Printed in Great Britain.

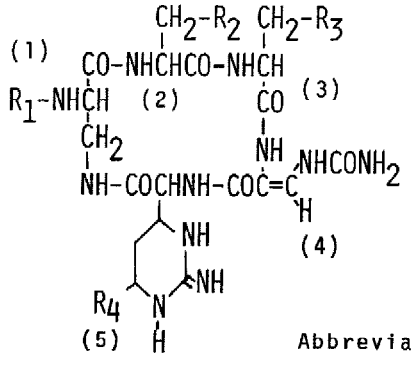
REVISED STRUCTURE AND TOTAL SYNTHESIS OF CAPREOMYCIN

Tetsuo Shiba,* Shinya Nomoto, Tadashi Teshima, and Tateaki Wakamiya
Department of Chemistry, Faculty of Science, Osaka University
Toyonaka, Osaka 560, Japan

(Received in Japan 12 August 1976; received in UK for publication 9 September 1976)

The antituberculous peptide capreomycin (Cpm) which was initially isolated from *Streptomyces capreolus* by Herr *et al.* [1] is composed of four congeners, Cpm IA, IB, IIA, and IIB.[2] Close similarities were shown in chemical and biological characters to the peptide antibiotics tuberactinomycin (Tum) group[3] in which viomycin was included as one congener. In 1971, the chemical structure of Cpm had been proposed by Bycroft *et al.*[4] presumably on the analogy of the incorrect amino acid sequence of viomycin.[5] Since we could not agree with this proposed structure, NMR spectra were investigated in detail in our recent study and the conclusion was obtained that the amino acid sequence in the cyclic peptide moiety of Cpm must be revised as shown in Fig. 1.[6]

The remaining problem to be elucidated for the total structure of Cpm is



	R_1	R_2	R_3	R_4
Capreomycin IA	H	OH	β-Lys-NH	H
IB	H	H	β-Lys-NH	H
Tuberactinomycin A	γ-Hy-β-Lys	OH	OH	OH
B(Viomycin)	β-Lys	OH	OH	OH
N	γ-Hy-β-Lys	OH	OH	H
O	β-Lys	OH	OH	H

Fig.1

Abbreviations; γ-Hy-β-Lys: *threo*-γ-Hydroxy-β-lysine, β-Lys: β-Lysine

All component amino acids are of L-configurations.

only concerned to the location of the β-lysine (β-Lys) residue as a branched part. In contrast to Bycroft's finding that N^{β}-2,4-dinitrophenyl-α,β-diaminopropionic acid (β-DNP-Dpr) was detected in the hydrolyzate of DNP-Cpm IB,[4] we could not repeat this result, but obtained α-DNP-Dpr in the acid hydrolyzates of DNP-Cpm IA as well as IB. Differentiation of α- and β-DNP-Dpr[7] was carried out by paper electrophoresis at pH 1.9.[8] Therefore, we inferred that β-Lys was linked to β-amino group of Dpr^3 in both Cpm IA and IB and not to α-amino group of Dpr^1 (Fig. 1). This assumption was further supported by the exact investigation of NMR spectra.[9] This conclusion seemed to be rather surprising from the view of similarities to Tum.

For the purpose of the confirmation of this newly proposed structure of Cpm, the total syntheses of both Cpm IA and IB were also undertaken in the present study. The synthetic strategy up to the protected cyclic pentapeptides (1) and (2) as shown in Fig. 2 is in accordance with that of Tum O whose total synthesis was recently performed in our laboratory.[10] After the selective deprotection of benzyloxycarbonyl (Z) group on β-amino group of Dpr^3 at 1 and 2, β-Lys residue was introduced to this free amino group to yield the protected hexapeptide (3) and (4) respectively. Although the difficulty was encountered in the reductive removal of the nitro group from the capreomycidine residue in 3 and 4, this was successfully achieved by the cleavage of *t*-butoxycarbonyl (Boc) group preceding the reduction. In the last step of the total synthesis, β,β-diethoxyalanine (Dea) residue was converted to β-ureidodehydroalanine (Uda) in a similar manner to that in the synthesis of Tum O.[10] The final products were identical with natural Cpm IA and IB respectively in all respects, e.g., paper electrophoresis (pH 6.9), thin-layer chromatography,[11] UV and NMR spectra. This result also indicates that the double bond in the Uda residue was selectively introduced to the desired configuration that is the same as the natural one, plausibly by an influence of the conformation of cyclic peptide moiety.

In order to exclude the possibility of the alternative structure where β-Lys is attached to Dpr^1 as in the case of Tum, we further synthesized these peptides through the selective cleavage of Boc group of 1 and 2 in advance to removal of Z group followed by the introduction of β-Lys to Dpr^1 and the conversion of Dea

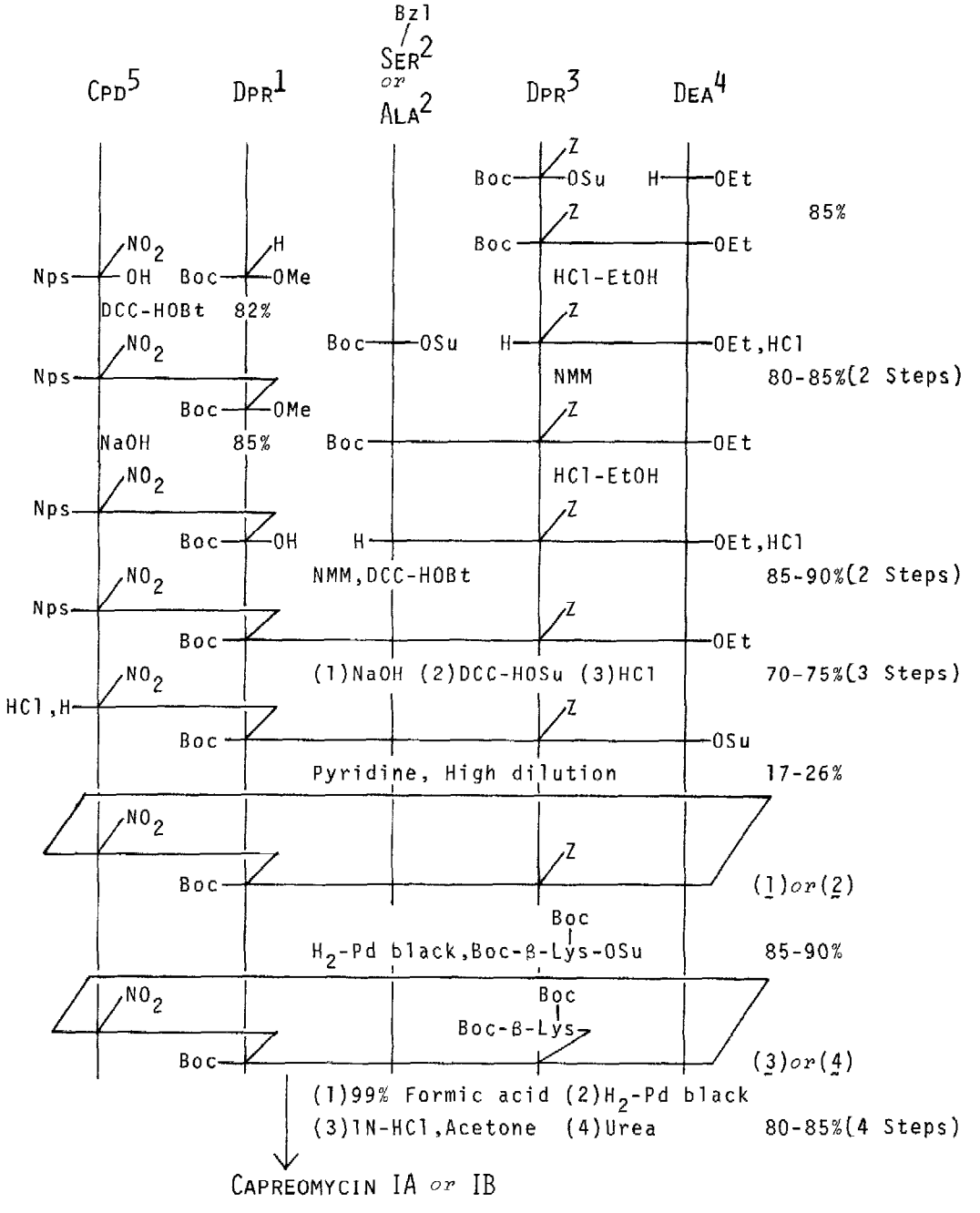


Fig.2

Abbreviations;Cpd:Capreomycidine,Dpr:α,β-Diaminopropionic acid,Dea:β,β-Diethoxy alanine,β-Lys:β-Lysine,Z:Benzyloxycarbonyl,Boc:*tert*-Butoxycarbonyl,Nps:*o*-Nitrophenylsulfenyl,DCC:N,N'-Dicyclohexylcarbodiimide,HOBt:1-Hydroxybenztriazole,HOSu:N-Hydroxysuccinimide,NMM:N-Methylmorpholine

(1),(3):Ser^2 analogs,(2),(4):Ala^2 analogs

to Uda. As expected, the latter products were found to be clearly different from the natural Cpm IA and IB respectively chromatographically.

From the results of this study, the correct structure of capreomycin was first established explicitly. Biosynthetic explanation of Cpm in connection with Tum seemed to be very interesting.

REFERENCES AND FOOTNOTES

1) E. B. Herr, M. E. Haney, G. E. Pittenger and C. E. Higgens, *Proc. Indiana Acad. Sci.*, 69, 134 (1960).

2) Cpm II lacks β-lysine residue of Cpm I. A differs B in only one amino acid residue in cyclic moiety where serine in A is replaced with alanine in B.[4)]

3) H. Yoshioka, T. Aoki, H. Goko, K. Nakatsu, T. Noda, H. Sakakibara, T. Take, A. Nagata, J. Abe, T. Wakamiya, T. Shiba and T. Kaneko, *Tetrahedron Lett.*, 1971, 2043.

4) B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson and T. Webb, *Nature, Lond.*, 231, 301 (1971).

5) T. Noda, T. Take, A. Nagata, T. Wakamiya and T. Shiba, *J. Antibiotics*, 25, 427 (1972).

6) T. Shiba, S. Nomoto and T. Wakamiya, *Experientia*, in print.

7) α-DNP-Dpr and β-DNP-Dpr were prepared from β-Z-Dpr and α-Z-Dpr respectively through 2,4-dinitrophenylations followed by cleavages of Z groups with HBr in acetic acid.

8) T. P. Hettinger, Z. Kurylo-Borowska and L. C. Craig, *Biochemistry*, 7, 4153 (1968).

9) The details will be reported soon elsewhere.

10) T. Teshima, S. Nomoto, T. Wakamiya and T. Shiba, *Tetrahedron Lett.*, 1976, 2343.

11) The developing solvent: phenol-water-28% ammonia water (30 : 10 : 1).