SEMISYNTHESIS OF DI-β-LYSYLCAPREOMYCIN IIA, A CAPREOMYCIN ANALOG EFFECTIVE AGAINST VIOMYCIN-RESISTANT MYCOBACTERIUM[1]

Sir:

In the course of our studies on the peptide antibiotics tuberactinomycin (Tum) and capreomycin (Cpm), two compounds among more than one hundred synthetic analogs were of particular interests in their activities against viomycin-resistant mutants of *Mycobacterium smegmatis*.[2] These are palmitoyltuberactinamine N (Pal-Tua N) and di-β-lysylcapreomycin IIA (di-β-Lys-Cpm IIA). Needless to say, that finding a useful antibiotic against drug-resistant strains of microorganism is urgently expected in practical chemotherapy.

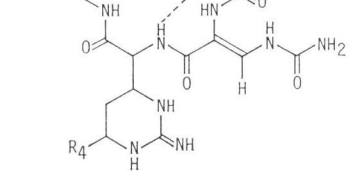
Of the two analogs, Pal-Tua N was easily prepared semisynthetically by the palmitoylation of tuberactinamine N (Tua N), the cyclic peptide moiety which is readily available from natural Tum N.[3] On the other hand, di-β-Lys-Cpm IIA had to be obtained by total synthesis in several steps. Since considerable amounts are required to elucidate details of biological activities and action mechanism, particularly for the latter compound, we had to develop a semisynthetic procedure for di-β-Lys-Cpm IIA starting from commercially available Cpm.

Of the four *pKa'* values of Cpm I in aqueous DMF[4], the lowest one was deduced from a comparison with those of Tums or Tua N to be of α-amino group of α,β-diaminopropionic acid at position 2 (A_2pr^2) (Table 1). The difference of *pKa'* values of Cpm I suggested the possibility of selective acylation of the α-amino group of A_2pr^2 which might be exposed simply by an addition of one equivalent of base to the disulfate of the antibiotics.

Based on the above consideration, the direct introduction of a β-lysine residue to the α-amino group of A_2pr^2 was first attempted by the coupling of di-Boc-L-β-Lys-OSu[3] with Cpm IA in the presence of one equivalent of triethylamine (TEA). The sample of Cpm IA used was prepared by

Abbreviations: HONb = *N*-hydroxy-5-norbornene-2,3-dicarboximide; HOSu = *N*-hydroxysuccinimide; ZONb = *N*-benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide; ZOSu = *N*-benzyloxycarbonyloxysuccinimide; ZCl = benzyloxycarbonyl chloride; BocON = 2-*t*-butoxycarbonyloxyimino-2-phenylacetonitrile; $(Boc)_2O$ = di-*t*-butyl dicarbonate.

Fig. 1. Chemical structures of tuberactinomycins, capreomycins, and their derivatives.



		R_1	R_2	R_3	R_4
Tuberactinomycin	A	γ-Hy-β-lys[a]	OH	OH	OH
	B	β-Lys[b]	OH	OH	OH
	N	γ-Hy-β-lys[a]	OH	OH	H
	O	β-Lys[b]	OH	OH	H
Tuberactinamine	N	H	OH	OH	H
Palmitoyl-tuberactinamine	N	$CH_3(CH_2)_{14}CO$	OH	OH	H
Capreomycin	IA	H	OH	β-Lys-NH[b]	H
	IB	H	H	β-Lys-NH[b]	H
Di-β-lysyl-capreomycin	IIA	β-Lys[b]	OH	β-Lys-NH[b]	H

[a] γ-Hy-β-lys: γ-hydroxy-L-β-lysine; [b] β-Lys: L-β-lysine.

Table 1. *pKa'* values of capreomycin, tuberactinomycins, and tuberactinamine N.

	pKa' values[a]		
	α-NH_2 in A_2pr^2	β- and ε-NH_2 in β-Lys	Guanidinoamino acid
Capreomycin I[b]	6.4	7.9, 9.4	12.8
Tuberactinomycin A	—	7.2, 10.3	>10
B	—	7.3, 10.1	>11
N	—	7.3, 10.1	>11
O	—	7.4, 9.8	>12
Tuberactinamine N	6.0	——	11.1

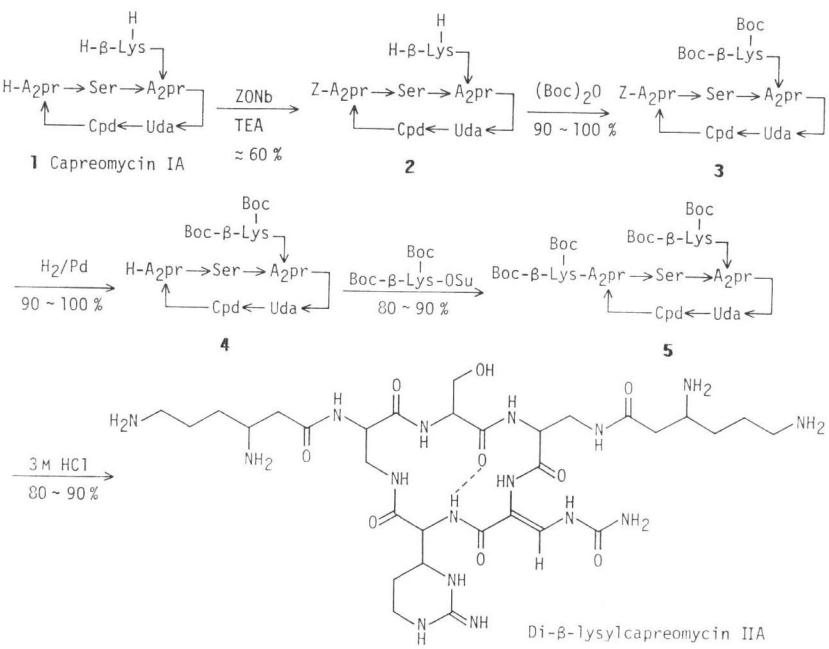
a) The values were of the titration in aqueous solution except those of capreomycin I which was titrated in 66% aqueous DMF.

b) The values were cited from the reference 5.

column chromatographic separation of commercial Cpm, namely a mixture of Cpm IA and IB.[5] In this straightforward coupling procedure, however, hydrolysis of active ester in aqueous DMF solution preceded the desired coupling reaction. Although the use of a large excess of the reagent might make the coupling possible, selective acylation can not be expected. For this reason, we decided to protect both amino groups of β-lysine in the original peptide.

First, the α-amino group of A_2pr^2 was protected with the benzyloxycarbonyl (Z) group. Acylation with ZONb proceeded smoothly, probably because of the higher reactivity of this compound compared with di-Boc-β-Lys active esters. Additional reasons for the usefulness of ZONb are in its stability in aqueous solution which is higher than that of ZOSu and also the lower acidity of HONb liberated in the coupling reaction, relative to that of HOSu or HCl formed from ZOSu or ZCl. Thus, the use of ZONb and TEA for selective acylation gave the mono-acyl derivative satisfactorily albeit in a moderate yield. In order to ascertain the position of the newly introduced

Scheme 1.



acyl group, mono-Z-Cpm IA (**2**) was 2,4-dinitrophenylated in the usual manner. When the Dnp derivative thus obtained was hydrolyzed, only di-Dnp-β-lysine was detected and no other Dnp amino acids appeared. This result clearly indicated that the α-amino group of A_2pr^2 was benzyloxycarbonylated as we expected. The yield of mono-acylation was not high because of the incompleteness of the reaction and formations of a small amount of di- or triacylated by-products which, however, were easily removed by reprecipitation.

The mono-acyl compound **2** was next *t*-butoxycarbonylated with BocON or $(Boc)_2O$. Purification of the product, **3**, was effectively achieved by gel chromatography on a Sephadex LH-20 column with 50% methanol as eluent. Removal of Z group from fully protected derivative **3** was achieved by hydrogenation in the presence of Pd catalyst which yielded the desired key intermediate **4**. Coupling of di-Boc-β-Lys-OSu with **4** proceeded successfully to yield the protected di-β-Lys-Cpm IIA. The final deprotection was carried out with 3 M HCl. The desired product, di-β-Lys-Cpm IIA, was precipitated by an addition of ethanol. The product was purified by chromatography in order to obtain a sample for elemental analysis. Found: C 32.26, H 6.94, N 19.32, Cl 15.38%. Calcd. for $C_{31}H_{56}N_{16}O_9 \cdot 5HCl \cdot 10H_2O$: C 32.12, H 7.04, N 19.33, Cl 15.29%.

Di-β-Lys-Cpm IIA thus prepared was subjected to biological tests. Minimum inhibitory concentrations (MIC) against general microorganisms were very similar to those of Cpms or Tums. The preliminary test against drug-resistant *Mycobacterium* strains showed significant effectiveness compared with the activity of natural Cpms or Tums. Details of the biological tests will be reported elsewhere. Our semisynthetic study leading to di-β-Lys-Cpm IIA opened the way for the preparation of new Cpm derivative against drug-resistant mutants.

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