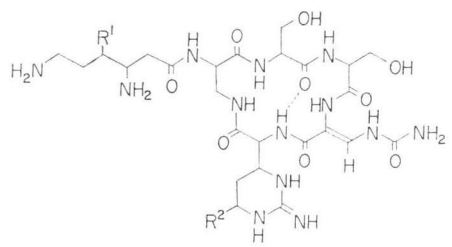
CHEMICAL STUDIES ON TUBERACTINOMYCIN. XVI SYNTHESIS OF TUBERACTINOMYCIN N

Sir:

The antituberculous peptide, tuberactinomycin N, has been isolated as a major component of tuberactinomycin congeners (Fig. 1) from *Streptomyces griseoverticillatus* var. *tuberacticus*[1]. Tuberactinomycin N was selectively cleaved at a branched position by the acid treatment in the presence of excess urea to afford its cyclic peptide moiety, tuberactinamine N. Reintroduction of β-lysine residue to a free amino group of tuberactinamine N gave tuberactinomycin O, thus achieving a conversion of tuberactinomycin N to O[2]. After a determination of the entire structure of tuberactinomycin O by X ray analysis,[1a] its total synthesis was also accomplished recently in our laboratory[3,4].

Fig. 1.



	R^1	R^2
Tuberactinomycin A	OH	OH
B*	H	OH
N	OH	H
O	H	H

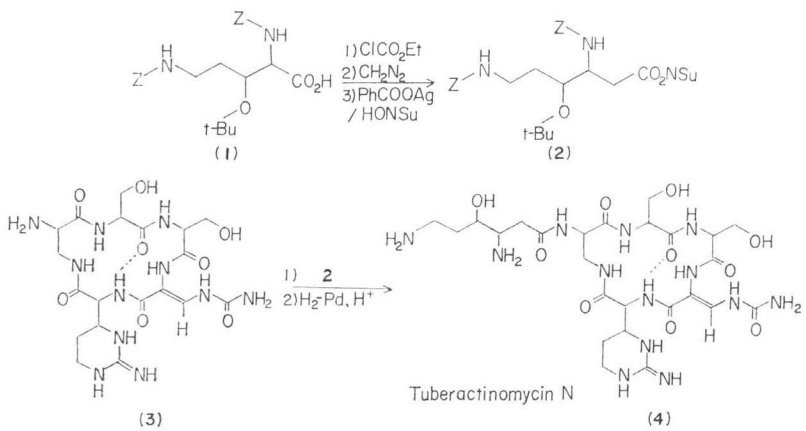
*Tuberactinomycin B: Viomycin

As the next objective of our synthetic study, a total synthesis of tuberactinomycin N was selected. Tuberactinamine N has been already synthesized as a key intermediate in total synthesis of tuberactinomycin O[3]. The stereochemistry of the branched part of tuberactinomycin N, although not unambiguously established, was supposed to be *threo*-γ-hydroxy-L-β-lysine from its behavior in acid hydrolysis[5].

In the present study, authentic *threo*-γ-hydroxy-L-β-lysine in the form of its active ester **2** was introduced at the amino group of tuberactinamine N in a manner similar to that in the synthesis of tuberactinomycin O[2,3,4] as shown in Fig. 2. The protected active ester, N^{β},N^{ε}-dibenzyloxycarbonyl-O-*t*-butyl-L-β-lysine 1-succinimidyl ester (**2**), was derived through the ARNDT-EISTERT reaction[6] from β-hydroxy-L-ornithine derivative **1** as an intermediate in the synthesis of the free amino acid *threo*-γ-hydroxy-L-β-lysine, which will be reported in detail elsewhere, and then coupled with tuberactinamine N followed by deprotections to give tuberactinomycin N.

N^{α},N^{δ}-Di-benzyloxycarbonyl-O-*t*-butyl-*threo*-β-hydroxy-L-ornithine (248 mg, 0.526 mmol) was allowed to react with ethyl chloroformate (0.050 ml, 0.526 mmol) and N-methylmorpholine (0.059 ml, 0.526 mmol) in ethyl acetate (2 ml) at $-20°C$ with stirring for 1 hour, and then treated with ethereal solution of excess diazomethane to give a diazoketone, IR: 2140 cm^{-1}. To a solution of this diazoketone in tetrahydrofuran (4 ml), were added silver benzoate (40 mg, 0.175 mmol) in 0.4 ml of triethylamine and then N-hydroxysuccinimide (242 mg, 2.10 mmol) in tetrahydrofuran (2 ml). After 4 hours at room temperature, the residue obtained by concentration *in vacuo* was dissolved in ethyl acetate. The solution was filtered and washed with aqueous citric acid solution, saturated sodium hydrogencarbonate, and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in*

Fig. 2.



(1) (2) (3) (4)

vacuo to give a pale yellow oil, IR: 1790, 1815 cm^{-1}. The 1-succinimidyl active ester **2** thus obtained was allowed to react with tuberactinamine N dihydrochloride (**3**) (215 mg, 0.351 mmol) in the presence of triethylamine (0.060 ml, 0.814 mmol) in DMF (4 ml). After the reaction was carried out overnight, a gelatinous residue obtained by concentration *in vacuo* was triturated with tetrahydrofuran, and collected by centrifugation. It was dissolved in 50 ml of 0.5 M hydrochloric acid - acetic acid (1:1). Hydrogenolysis was carried out using palladium-black as a catalyst. The crude product was purified by ion-exchange chromatography (Amberlite IRC CG-50, NH_4^+ form, 0.9×28 cm) with 0.4 M ammonium acetate buffer (pH 9.0) as developing solvent. Fractions containing tuberactinomycin N were neutralized with acetic acid, and diluted with two volumes of water. The solution was passed through an Amberlite IRC CG-50 column (pyridinium form, 0.9×22 cm), and an adsorbed product eluted with 1 M acetic acid. The eluate was neutralized with pyridine and concentrated *in vacuo*. The residue was dissolved in 1 ml of 6 M hydrochloric acid and addition of ethanol and ether gave a white precipitate, yield 159 mg (57.0%, based on tuberactinamine N dihydrochloride). This was reprecipitated from water-ethanol; yield 84.1 mg (30.1%); mp 245~249°C (dec). $[\alpha]_D^{21}$ −18.7° (*c* 1.0, H_2O).

Found: C, 37.02; H, 5.83; N, 22.08; Cl, 12.80%.
Calcd. for $C_{25}H_{46}N_{13}O_{10}Cl_3 \cdot H_2O$: C, 36.93; H, 5.95; N, 22.39; Cl, 13.08%.

Table 1. Comparisons of natural and synthetic tuberactinomycin N.

	Natural	Synthetic
mp (dec) (°C)	242~244	245~249
$[\alpha]_D^{21}$ (*c* 1.0, H_2O)	−19.1°	−18.7°
UV; λ_{max} nm(ε)		
H_2O	268 (2.78×10^4)	268 (2.64×10^4)
0.1 M HCl	268 (2.78×10^4)	268 (2.66×10^4)
0.1 M NaOH	288 (1.75×10^4)	287 (1.72×10^4)
TLC (Rf)*	0.46	0.46
HPLC**	3.4 min	3.4 min

* A developing solvent: 10% ammonium acetate - 10% ammonia - acetone (9:1:10)

** Column: Nucleosil $7C_{18}$, 0.4×13 cm, solvent: acetonitrile - 0.4 M ammonium acetate (1:3) containing 2 mmol/liter sodium lauryl sulfate, pH 5.6, detector: UV (268 nm).

The purified product was identical with natural tuberactinomycin N (**4**) in all respects (mp, $[\alpha]_D$, UV spectrum, NMR spectrum, TLC, and HPLC) (Table 1). This synthesis confirmed the proposed structure of tuberactinomycin N.

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