Synthesis of L-Epicapreomycidine

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A cyclic guanidino amino acid, L-epicapreomycidine, isolated from hydrolyzates of protease inhibitors such as chymostatin and elastatinal was synthesized. This amino acid is a diastereomer of L-capreomycidine which is a component amino acid of tuberactinomycin N,O and capreomycin. A new synthetic route from β-hydroxy-DL-ornithine *via* an aziridine compound to DL-capreomycidine was applied to the synthesis of L-epicapreomycidine starting from *erythro*-β-hydroxy-L-ornithine. A convenient and one-step technique to obtain L-*erythro* form from a mixture of four diastereomers of β-hydroxyornithine by means of enzymatic reaction was newly exploited. The synthetic L-epicapreomycidine obtained was identical with natural one in all respects, thus confirming the proposed structure.

A cyclic guanidino amino acid, L-epicapreomycidine, was found as a component amino acid of peptides produced by microorganism, *i.e.*, chymostatin and elastatinal which are known as protease inhibitors.[1] This amino acid is a diastereomer of L-capreomycidine which is a constituent of antibiotics capreomycin[2] or tuberactinomycin N and O.[3] DL-Capreomycidine and DL-epicapreomycidine had been synthesized by Bycroft *et al.* by the method based on an oximation of methyl (2-amino-4-pyrimidinyl)acetate followed by hydrogenation.[4]

Owing to the disadvantage in this method by which one of the diastereomers can not be secured purposely and optically active isomers for each diastereomer are not easily obtained unless an efficient technique of an optical resolution for such peculiar guanidino amino acid is developed.

In our previous work, we exploited an entirely novel approach to synthesize DL-[5] and also L-capreomycidine[6] stereoselectively employing N^{δ}-benzyloxycarbonyl-*threo*-β-hydroxyornithine as a key intermediate. In the present study, where *erythro* isomer of optically pure β-hydroxyornithine was taken as an intermediate in a similar synthetic strategy, epicapreomycidine of the natural steric configuration was successfully obtained for the first time.

3-(Benzyloxycarbonylamino)propionaldehyde **3**[7] was condensed with bis(glycinato)copper(II) to give a mixture of *erythro* and *threo* N^{δ}-benzyloxycarbonyl-β-hydroxy-DL-ornithine in a ratio of 1:1. Since use of aqua[*N*-(1-carboxylatoethylidene)glycinato]copper(II) as in the preparation of L-capreomycidine[8] has been found to give a *threo*-rich mixture, the usual copper complex of glycine was used in this case where *erythro* isomer is a desirable isomer. After fractional recrystallization of the condensed product from water was repeated six times, a pure DL-*erythro* isomer **4a** was obtained. Acetyl derivative **5a** derived from **4a** was treated with the acylase to give L-*erythro* isomer. However, since the tedious fractional recrystallization caused a low yield of the desired product, the product of the coupling reaction was directly subjected to the enzymatic resolution without fractionations of the diastereomers. Interestingly, the deacetylation of *erythro* isomer was found to proceed much faster than that of *threo* one. Thus, when the reaction was stopped just before the *threo* isomer became susceptible to the enzyme, N^{δ}-benzyloxycarbonyl-*erythro*-β-hydroxy-L-ornithine was obtained in an optically pure form without any further purification through single reaction procedure. This new procedure is very advantageous in a sense that not only the optical resolution, but also the diastereomeric separation took place simultaneously in a one-step enzymatic reaction.

Erythro isomer of β-hydroxy-L-ornithine derivative **6a** was converted to L-epicapreomycidine according to a series of the reaction which is the same in principle to that for the synthesis of L-capreomycidine.[6] Thus, successive procedures of N^{α}-tosylation, methyl esterification, amidation and *O*-mesylation gave an intermediate **10**. The acid amide form is requisite for the purpose of *O*-mesylation followed by aziridine ring formation to prevent an elimination of β-hydroxyl function.

O-Mesyl amide **10** was treated with diethylamine to give an aziridine derivative **11**. The aziridine ring in **11** was then opened with ammonia, and benzyloxycarbonyl group was removed by hydrogenolysis to give triamino amide derivative **12**.

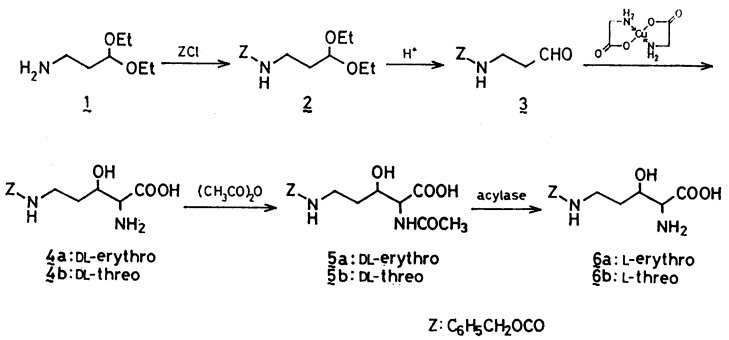

Fig. 1.

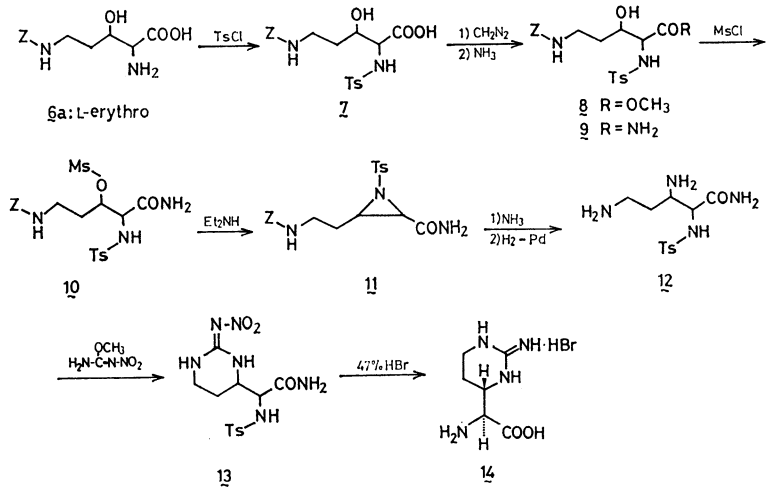

Fig. 2.

Concerning the stereoselectivity, the fact that *erythro* form of β-hydroxyornithine derivative **10** afforded *trans* aziridine **11** which turned to *erythro* form of triaminopentanamide derivative **12** was well understood by double S_N2 reaction mechanism as described in the synthesis of DL-capreomycidine.[5] The *trans* configuration of the aziridine compound **11** was supported by NMR data which showed a coupling constant of $J=$ 6 Hz for two protons at the aziridine ring.

Two free amino groups in **12** were coupled with *O*-methyl-*N*-nitroisourea in the presence of sodium hydroxide to give cyclic nitroguanidino derivative **13**. All the protecting groups of **13** were finally removed by hydrolysis with 47% hydrobromic acid to obtain epicapreomycidine hydrobromide **14**. Synthetic compound is identical with natural epicapreomycidine in all respects (mp, $[M]_D$, NMR, TLC, PC, amino acid analysis) (Table 1).

Table 1. Comparison of synthetic and natural epicapreomycidine hydrobromide

			Epicapreomycidine		Capreomycidine
			Synthetic	Natural	
Mp (dec) (°C)[a]			198—205	199—202	218—230
$[M]_D$ (*c* 1.19, 6 M HCl)			+21.0°	+18.4°	+44°
R_f	TLC[b]	a	0.11	0.11	0.11
		b	0.50	0.50	0.50
	PC[b]	c	0.34	0.34	0.34
		d	0.16	0.16	0.16
Amino acid analysis (min)[c]			215	215	205

a) Melting points were compared for diflavianates. b) Developing solvent: a) 1-butanol–acetic acid–water (4: 1: 2), b) phenol–water–28% ammonia (30: 10: 1), c) *t*-butyl alcohol–acetic acid–water (2: 1: 1), d) 1-butanol–ethyl methyl ketone–28% ammonia–water (5: 3: 3: 1). c) Following conditions were applied; 27 cm column, pH 5.28, 0.35 M sodium citrate buffer.

Experimental

All melting points are uncorrected. NMR spectra were measured with a Varian XL-100-15 spectrometer. The optical rotations were measured with a Perkin-Elmer 141 polarimeter. TLC was carried out by the ascending method on silica gel G using developing solvents, 1-butanol–acetic acid–water (4: 1: 2) (solvent A) and phenol–water–28% ammonia (30: 10: 1) (solvent B). Paper chromatography was carried out on Toyo Roshi No. 51 paper using developing solvents, *t*-butyl alcohol–acetic acid–water (2: 1: 1) (solvent C) and 1-butanol–ethyl methyl ketone–28% ammonia–water (5: 3: 3: 1) (solvent D). Amino acid analysis was carried out with a Hitachi KLA-5 Amino Acid Analyzer (55 cm column, 0.20 M sodium citrate buffer pH 3.25 (92 min) and 4.25, flow rate 60 ml/h). An acylase was gifted from Tanabe Seiyaku Co., Ltd.

3-(Benzyloxycarbonylamino)propionaldehyde Diethyl Acetal (2). To a solution of 3-aminopropionaldehyde diethyl acetal (**1**) (35.3 g, 0.240 mol) and triethylamine (49.9 ml, 0.360 mol) in 500 ml of chloroform, benzyloxycarbonyl chloride (44.7 g, 0.264 mol) was added dropwise for 1.5 h with stirring at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. Excess benzyloxycarbonyl chloride was decomposed by addition of *N*-(2-aminoethyl)piperazine (4.5 ml, 35 mmol). The reaction mixture was washed with 20% aqueous citric acid, 4% $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a colorless oil; yield 64.5 g (95.7%).

For elemental analysis, a small amount of this oil was distilled.

Found: C, 63.56; H, 8.23; N, 4.84%. Calcd for $C_{15}H_{23}NO_4$: C, 64.03; H, 8.24; N, 4.98%.

3-(Benzyloxycarbonylamino)propionaldehyde (3). A mixture of **2** (27.8 g, 99.0 mmol) in 800 ml of acetone and 100 ml of 0.1 M† HCl was heated under reflux for 1 h, and concentrated *in vacuo* to one eighth volume. The mixture was extracted with ethyl acetate and organic layer was washed with brine. Organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. An oily residue was triturated with hexane to give crystals; yield 17.6 g (85.9%), mp 57—58 °C. For elemental analysis, the product was converted to a 2,4-dinitrophenylhydrazone derivative, mp 161 °C.

Found: C, 52.56; H, 4.48; N, 18.07%. Calcd for $C_{17}H_{17}N_5O_6$: C, 52.71; H, 4.42; N, 18.08%.

N^δ-Benzyloxycarbonyl-β-hydroxy-DL-ornithine (4). To a suspension of bis(glycinato)copper(II) (208 g, 981 mmol) and Na_2CO_3 (104 g, 981 mmol) in 1.8 l of H_2O, a solution of **3**

† 1 M = 1 mol dm^{-3}.

(81.2 g, 392 mmol) in 400 ml of dichloromethane was added dropwise at 60 °C for 15 min. The reaction mixture was stirred at 60 °C for 1.5 h and neutralized with HCl on cooling in ice bath. H_2S was bubbled through the solution with stirring for 1.5 h. Copper sulfide was filtered off and washed with 4 l of 1 M HCl. The filtrate and washings were combined and aerated to remove H_2S. The solution was neutralized with ammonia to give crystals; yield 15.7 g (14.2%) (*erythro*: *threo* (1: 1)). From the mother liquor, a further crop was recovered; yield 4.8 g (4.3%) (*erythro*: *threo* (1: 1.3)). The ratio of *erythro* and *threo* isomers were obtained by amino acid analysis (retention time: *threo*, 186 min; *erythro*, 197 min).

N$^{\delta}$-Benzyloxycarbonyl-erythro-*β-hydroxy*-DL-*ornithine (**4a**).* Crude product (*erythro*: *threo* (1: 1.3)) (13.9 g) of **4** was recrystallized six times from H_2O (250—300 ml per g of the crude crystals) to give a pure *erythro* isomer **4a**: yield 1.41 g (10.1%), mp 248—248.5 °C (dec).

Found: C, 55.29; H, 6.41; N, 9.86%. Calcd for $C_{13}H_{18}N_2O_5$: C, 55.31; H, 6.43; N, 9.92%.

N$^{\alpha}$-Acetyl-N$^{\delta}$-benzyloxycarbonyl-erythro-*β*-*hydroxy*-DL-*ornithine (**5a**).* To a solution of **4a** (539 mg, 1.90 mmol) in 1 M NaOH (1.90 ml, 1.90 mmol), acetic anhydride (0.32 ml, 3.40 mmol) and 1 M NaOH (3.80 ml, 3.80 mmol) were added dropwise at the same time with stirring at 0 °C. Keeping the pH of the solution at 9 by addition of 1 M NaOH, the mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The reaction mixture was acidified with HCl and extracted with ethyl acetate. The organic layer was washed with H_2O and dried over anhydrous $MgSO_4$. The oily residue after concentration *in vacuo* was triturated with ethyl acetate and ether to give crystals: yield, 413 mg (67.0%). They were recrystallized from ethyl acetate; yield, 312 mg (50.6%), mp 123—125 °C.

Found: C, 55.46; H, 6.21; N, 8.58%. Calcd for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.22; N, 8.64%.

N$^{\delta}$-Benzyloxycarbonyl-erythro-*β-hydroxy*-L-*ornithine (**6a**).* To **5a** (283 mg, 0.873 mmol) was added 0.1 M LiOH until a clear solution was obtained, and the solution was adjusted to pH 7.1 with 0.1 M LiOH. To the solution, 1.5 ml of 0.01 M cobalt chloride and the acylase (35 mg, 20 units/mg) were added, and the solution was incubated at 37 °C for 20 h. When crystals of free *erythro*-L-form were separated out, pH of the mixture decreased to 6.4. After the solution was adjusted again to pH 7.1, the incubation was continued for further 60 h. After cooling in ice bath, crystals deposited were collected by filtration; yield 95 mg (77%). They were recrystallized from H_2O; yield, 75 mg (61%), mp 237 °C (dec), $[\alpha]_D^{20}$ +6.2° (*c* 1.0, 2 M HCl).

Found: C, 55.40; H, 6.40; N, 9.88%. Calcd for $C_{13}H_{18}N_2O_5$: C, 55.31; H, 6.43; N, 9.92%.

Convenient Preparation of N$^{\delta}$-*Benzyloxycarbonyl*-erythro-*β-hydroxy*-L-*ornithine (**6a**) from a Mixture of Four Diastereomers.* N$^{\delta}$-Benzyloxycarbonyl-*β*-hydroxy-DL-ornithine (**4**) (*erythro*: *threo* (1: 1)) (21.0 g, 74.5 mmol) was acetylated in the manner similar to that in the preparation of **5a** to give a mixture of *erythro* and *threo* isomers of *N$^{\alpha}$*-acetyl-*N$^{\delta}$*-benzyloxycarbonyl-*β*-hydroxy-DL-ornithine (**5**) as a colorless oil. The enzymatic reaction of this mixture with the acylase was carried out in the manner similar to that in the reaction of **5a**. After the reaction mixture was cooled in an ice bath, crystals separated out were collected by filtration. They were found to be pure *erythro* isomers from the result of amino acid analysis; yield 2.08 g (39.6%).

N$^{\delta}$-Benzyloxycarbonyl-N$^{\alpha}$-p-*tolylsulfonyl*-erythro-*β*-*hydroxy*-L-*ornithine (**7**).* To a suspension of **6a** (737 mg, 2.61 mmol) in 6.5 ml of THF–H_2O (1: 2) were added triethylamine (0.82 g, 8.1 mmol) and *p*-toluenesulfonyl chloride (763 mg, 4.00 mmol) portionwise during a period of 30 min with stirring at room temperature. After stirring for 2 h, the reaction mixture was concentrated *in vacuo* and an aqueous solution of the residue was extracted with ether. The ether layer was reextracted with aqueous $NaHCO_3$. Both aqueous layers were combined and acidified with HCl to give colorless needles; yield 939 mg (82.4%). Recrystallization from ethanol–ether–hexane, gave a pure sample for elemental analysis; mp 164.5—166.5 °C, $[\alpha]_D^{19}$ +19.4° (*c* 1.13, EtOH).

Found: C, 54.92; H, 5.54; N, 6.39; S, 7.25%. Calcd for $C_{20}H_{24}N_2O_7S$: C, 55.03; H, 5.54; N, 6.42; S, 7.32%.

N$^{\delta}$-Benzyloxycarbonyl-N$^{\alpha}$-p-*tolylsulfonyl*-erythro-*β*-*hydroxy*-L-*ornithine Methyl Ester (**8**).* To a solution of **7** (2.41 g, 5.52 mmol) in 14 ml of THF–dioxane (1: 1), was added excess diazomethane in ether and allowed to react for 1 h. Excess diazomethane was decomposed with acetic acid and the solution was concentrated *in vacuo* to give needles; yield 2.49 g (100%). For elemental analysis, they were recrystallized from ethyl acetate–hexane; mp 108—112 °C, $[\alpha]_D^{19}$ −0.1° (*c* 1.03, MeOH), $[\alpha]_{365}^{19}$ +27.0° (*c* 1.03, MeOH).

Found: C, 55.89; H, 5.95; N, 5.91; S, 6.77%. Calcd for $C_{21}H_{26}N_2O_7S$: C, 55.99; H, 5.82; N, 6.22; S, 7.10%.

N$^{\delta}$-Benzyloxycarbonyl-N$^{\alpha}$-p-*tolylsulfonyl*-erythro-*β*-*hydroxy*-L-*ornithinamide (**9**).* A solution of **8** (2.49 g, 5.53 mmol) in 120 ml of methanol, was saturated with ammonia in a pressure bottle and kept at room temperature for 4 d. Concentration *in vacuo* gave crystals; yield 1.58 g (65.5%). For elemetal analysis they were recrystallized from methanol–ether–hexane; mp 206—208 °C, $[\alpha]_D^{19}$ +15.9° (*c* 1.10, DMF).

Found: C, 55.31; H, 5.74; N, 9.58; S, 7.48%. Calcd for $C_{20}H_{25}N_3O_6S$: C, 55.16; H, 5.79; N, 9.65; S, 7.36%.

N$^{\delta}$-Benzyloxycarbonyl-N$^{\alpha}$-p-*tolylsulfonyl*-O-*methylsulfonyl*-erythro-*β-hydroxy*-L-*ornithinamide (**10**).* To a solution of **9** (661 mg, 1.52 mmol) in 7 ml of pyridine, methanesulfonyl chloride (0.939 ml, 12.1 mmol) was added portionwise with stirring at −25 °C during a period of 2.5 h. After an additional stirring for 2 h, the reaction mixture was concentrated *in vacuo* and extracted with ethyl acetate after addition of 30 ml of H_2O. The organic layer was washed with 5% aqueous citric acid, $NaHCO_3$ and brine successively, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was triturated with hexane to give colorless needles; yield 691 mg (91.5%). Recrystallization from acetone–hexane gave a pure sample for elemental analysis; mp 159—162 °C, $[\alpha]_D^{19}$ +34.3° (*c* 1.00, acetone).

Found: C, 49.22; H, 5.28; N, 8.19; S, 12.37%. Calcd for $C_{21}H_{27}N_3O_8S_2$: C, 49.11; H, 5.30; N, 8.18; S, 12.49%.

(2S,3R)-5-Benzyloxycarbonylamino-trans-*2,3*-[N-*(p-tolylsulfonyl)epimino]pentanamide (**11**).* A solution of **10** and diethylamine (0.54 ml, 5.2 mmol) in 7 ml of THF was kept at 38—42 °C for 3 h, and then concentrated *in vacuo*. An aqueous solution of the residue was extracted with chloroform. After the extract was dried over anhydrous $MgSO_4$, the solvent was evaporated *in vacuo* to an oil, which was triturated with benzene to give needles; yield 267 mg (56.4%). They were recrystallized from chloroform–hexane; yield 246 mg (52.0%), mp 136—137 °C, $[\alpha]_D^{20}$ +21.8° (*c* 1.06, DMF).

Found: C, 57.18; H, 5.53; N, 9.92; S, 7.53%. Calcd for $C_{20}H_{23}N_3O_3S$: C, 57.54; H, 5.55; N, 10.07; S, 7.68%.

(2S,3R)-erythro-*3,5-Diamino-2-(p-tolylsulfonylamino)pentanamide (**12**).* A solution of **11** (283 mg, 0.678 mmol) in 100 ml of methanol was saturated with ammonia in a pressure bottle, and allowed to stand at 30 °C for 4 d. The residue obtained after concentration *in vacuo* was dissolved in methanol and hydrogenolyzed in the presence of Pd-black. After removal of catalyst, the reaction mixture was concentrated *in vacuo* to give crystals which were recrystallized from metha-

nol–ether; yield 136 mg (52.1%), mp 200—209 °C (dec), $[\alpha]_D^{15}$ +76.5° (*c* 1.06, MeOH).

Found: C, 37.87; H, 6.13; N, 14.58; S, 8.37; Cl, 18.24%. Calcd for $C_{12}H_{22}N_4O_3SCl_2 \cdot 1/2H_2O$: C, 37.70; H, 6.06; N, 14.65; S, 8.39; Cl, 18.55%.

L-*Epicapreomycidine Hydrobromide (14)*. To a solution of **12** (74 mg, 0.20 mmol) in 2 ml of methanol and a trace of H_2O, were added 1 M NaOH (0.40 ml, 0.40 mmol) and *O*-methyl-*N*-nitroisourea (47 mg, 0.40 mmol) portionwise with stirring at 0 °C during a period of 2 h. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. More amount of *O*-methyl-*N*-nitroisourea (20 mg, 0.168 mmol) was added to the reaction mixture. After 3 h, the reaction mixture was acidified with 1 M HCl. The residue obtained after concentration *in vacuo* was triturated with a small amount of H_2O and ethyl acetate to give a nitroguanidino derivative **13** as crystals; yield 40 mg (55%). The product was heated with 8 ml of 47% HBr and 3 ml of anisole under reflux for 5 h. An aqueous layer was separated from anisole layer and concentrated *in vacuo*. A solution of the residue in methanol was neutralized with pyridine. Addition of ethanol to the solution gave colorless prisms of monohydrobromide **14**; yield 23 mg (45%). They were recrystallized from H_2O–ethanol; yield 14 mg (28%). $[\alpha]_D^{20}$ +8.31° (*c* 1:19, 6 M HCl). For elemental analysis, the hydrobromide was converted to diflavianate of yellow prisms; mp 198—205 °C (dec).

Found: C, 38.86; H, 3.19; N, 14.00; S, 7.55%. Calcd for $C_{26}H_{24}N_8O_{18}S_2 \cdot 1/2H_2O$: C, 38.57; H, 3.11; N, 13.84; S, 7.92%.

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