Chemical Studies on Tuberactinomycin. XII.[1] Syntheses and Antimicrobial Activities of [Ala^3, Ala^4]-, [Ala^3]-, and [Ala^4]-Tuberactinomycin O[2]

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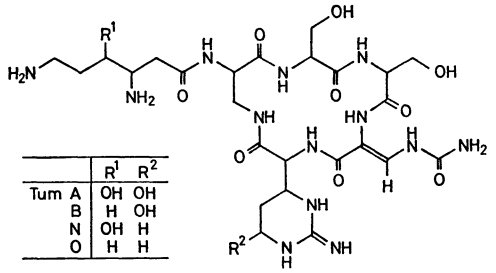
Total syntheses of [Ala^3, Ala^4]-, [Ala^3]-, and [Ala^4]-tuberactinomycin O corresponding to deoxy analogs of natural antibiotic tuberactinomycin O were achieved for the purpose of elucidation of relationship between chemical structure and biological activity. Cyclization of the pentapeptide intermediates at two different positions was investigated. Minimum inhibitory concentrations of the synthetic analogs against *Mycobacterium* or *Corynebacterium* indicated that both hydroxy groups of two serine residues of tuberactinomycins are not required for antibacterial activity, nor participated to drug-resistancy of bacilli. These analogs were found to have the same conformations in solution as those of natural antibiotic from the results of NMR, ORD, and CD measurements.

Chemical structures of tuberactinomycins (Tum),[3] antituberculous peptides isolated from *Streptomyces griseoverticillatus* var. *tuberacticus*,[4] were determined by X-ray analysis as well as chemical degradations in our previous investigations (Fig. 1).[5] Exhaustive study on proton magnetic resonance of tuberactinomycin family added one successful example to conformational analysis of cyclic peptides.[6] Total synthesis of one congener, Tum O, in our recent work contributed to unambiguous establishment of the structures of Tum.[7]



	R^1	R^2
Tum A	OH	OH
B	H	OH
N	OH	H
O	H	H

Fig. 1. Chemical structure of tuberactinomycins.

For the purpose of thorough elucidation of the structure-antibacterial activity relation of this antibiotic, we planned to exchange each amino acid residue in tuberactinomycins with others by full utilization of either semi-synthesis, total synthesis or chemical modification. In the first experiment, the branched part, *i.e.*, γ-Hy-β-Lys[3] in Tum N was replaced by many different acyl groups semi-synthetically.[1,8] From results of this study, it was concluded that the branched part is not necessarily required for the antibacterial activity but has an auxiliary role for enhancement of the activity.[1]

Therefore, in the present investigation, replacement of amino acid residues inside the cyclic moiety of Tum was so designed as to clarify the more significant site than branched part for the biological activity. Since two hydroxy groups of serine residues are common to all the Tum congeners, it seems to be important to see the effect of elimination of these functional groups from the molecule on antibacterial activity. Thus, we carried out the total syntheses of three deoxy analogs, *i.e.*, [Ala^3, Ala^4]-, [Ala^3]-, and [Ala^4]-Tum O.

In our synthetic strategy, prior to completion of the required amino acid sequence, the cyclic peptide moiety was first synthesized, and then β-Lys was introduced to the branched position as in the semi-synthesis of Tum O[8] from tuberactinamine (Tua) N.[3] In this synthetic plan, possible difficulties arising from extremely labile character of β-ureidodehydroalanine (Uda) residue must be overcome by some device. β,β-Diethoxyalanine (Dea) was chosen as a latent form of Uda,[3] so that Uda structure could be recovered at any synthetic step. A durability of Dea[3] residue during the entire course of this peptide synthesis was assured by preliminary experiments using model compounds.

In order to minimize a racemization at the C-terminal residue in the peptide cyclization reaction, Dea (route A) or A_2pr[3] (route B) was chosen as the C-terminal amino acid of the open chain pentapeptide, since Dea is to be converted to achiral Uda residue in the final synthetic step and α-amino group of A_2pr is not participated in the peptide linkage in the cyclic moiety but is able to be protected by Boc[3] group of urethane form.

In the preparations of [Ala^3, Ala^4]-Tum O, we compared both routes each other for relative advantages. However, the results showed no difference between them in respect of yield for the cyclization reaction. Therefore, route A was adopted for the syntheses of [Ala^3]- and [Ala^4]-Tum O.

Synthesis of [Ala^3, Ala^4]-Tum O. The Protected Cyclic Pentapeptide: *Route A*: H–Dea–OEt[9] and H–A_2pr(Z)–OH[10] were prepared according to the literatures. Capreomycidine (Cpd) was obtained from hydrolyzate of the natural Tum N. Whole synthetic scheme *via* route A was shown in Fig. 2. α-Amino group of Cpd was blocked by Nps[3] protecting group, since selective deprotection from Boc group is required prior to the cyclization. Nps–Cpd(NO_2)–OH (**6**) was coupled with a debenzyloxycarbonylated tetrapeptide, which was obtained by hydrogenolysis of **4**, to give a pentapeptide (**7a**). Saponification of **7a** followed by active esterification by means of DCC–HONSu[3] method to afford the pentapeptide active ester (**9a**). After removal of Nps group from **9a** by means of hydrogen chloride in THF, it was then cyclized under high-dilution conditions in pyridine to **10a** in 35% yield. Molecular weight of **10a** was measured to be 743

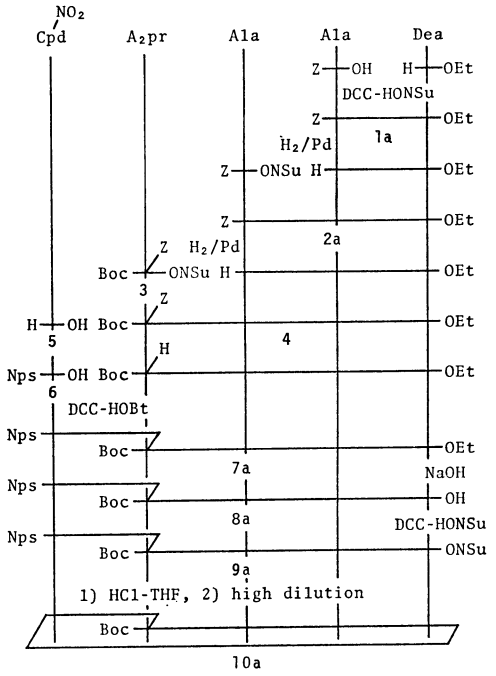


Fig. 2. Synthetic scheme for protected cyclic peptide (**10a**) *via* route A.

(Calcd for $C_{24}H_{46}O_{11}N_{10}\cdot 3/2H_2O$: 714) by means of vapor pressure osmometry, indicating formation of a cyclic monomer.

Route B: Synthetic scheme *via* route B was depicted in Fig. 3. The Nps group of the protected dipeptide (**12**) was selectively cleaved by hydrogen chloride in THF, and then coupled with the protected tripeptide (**14**) by DCC–HOBt[3] method to give the pentapeptide (**15**). This peptide (**15**) was converted into the 1-succinimidyl ester (**17**) after saponification. The active ester (**17**) was treated with hydrogen chloride in THF to remove the Nps group selectively, and then cyclized in pyridine under high-dilution condition in 38% yield. The cyclic peptide (**10a**) thus obtained was identical with the product *via* route A in thin-layer chromatography, high-pressure liquid chromatography, and NMR spectrum.

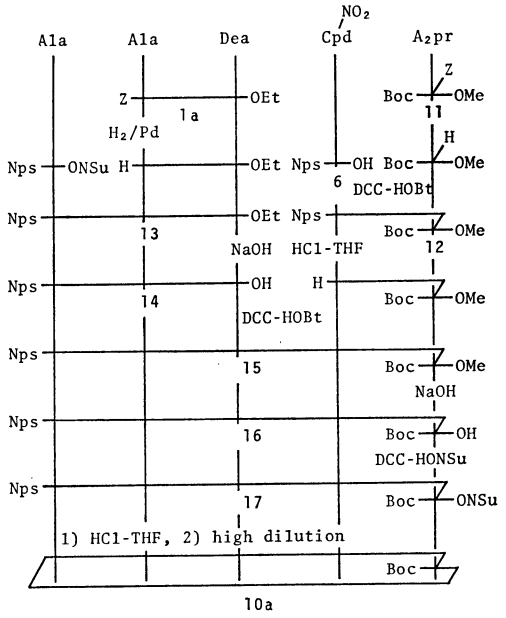


Fig. 3. Synthetic scheme for protected cyclic peptide (**10a**) *via* route B.

A Conversion to [Ala³, Ala⁴]-Tum O: A conversion of Dea residue in **10a** to Uda residue, being a key step throughout this synthesis, was carried out as follows. The deprotected product obtained by hydrogenolysis of **10a** was heated in acetone–1 M hydrochloric acid (1 : 1) under reflux for 8—10 min, and then excess of urea was added. By this procedure, the cyclic Uda peptide (**18a**) was obtained in a good yield, whose structure was confirmed by NMR (olefinic proton: δ 8.0(s) in D_2O) and UV spectra (λ_{max}: 268 nm (neutral or acidic medium), 286 (basic medium)). At the final step of the synthesis, introduction of Boc–β-Lys(Boc)–ONSu to the amino group of **18a** followed by deprotection gave the expected product, [Ala³, Ala⁴]-Tum O (**19a**) in a satisfactory yield (Fig. 4).

Synthesis of [Ala³]- and [Ala⁴]-Tum O. [Ala³]- and [Ala⁴]-Tum O were synthesized according to the route A for [Ala³, Ala⁴]-Tum O. Cyclization reaction

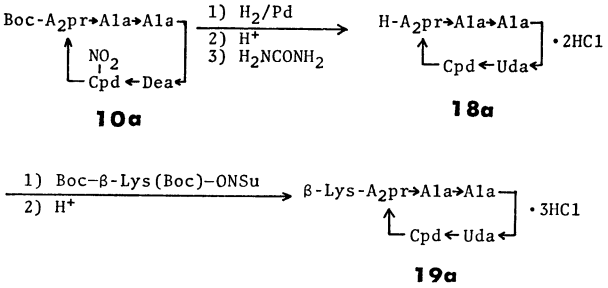


Fig. 4. Conversion of **10a** to **19a**.

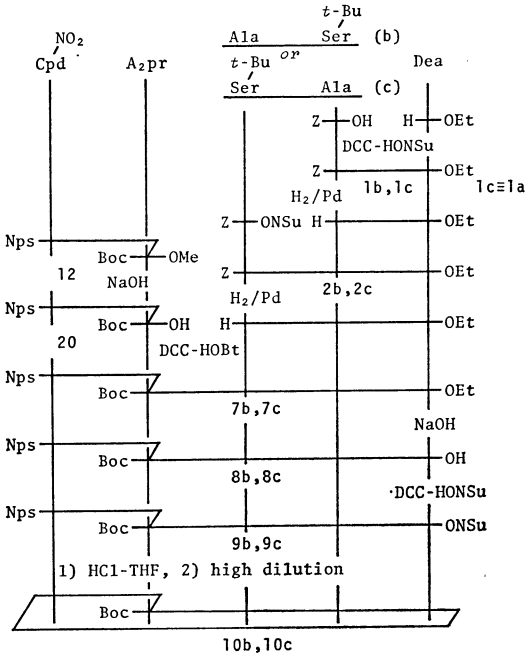


Fig. 5. Synthetic scheme for **19b** and **19c**. b: -Ala³–Ser⁴–, c: -Ser³–Ala⁴–.

Table 1. Chemical shifts of **18a**, **18b**, and **18c** in D_2O

		18a	**18b**	**18c**	Tua N
A_2pr	α-CH	4.4 (1H)	4.3 (1H)	4.4 (1H)	4.40 (1H,q)
	β-CH_2	3.3 (1H)	3.3 (1H)	3.30 (1H,dd)	3.30 (1H)
		4.1 (1H)	4.1 (1H)	4.15 (1H,dd)	4.12 (1H,q)
Ser^3	α-CH			4.82 (1H,t)	4.84 (1H,t)
	β-CH_2			3.92 (2H,d)	3.95 (2H,d)
Ala^3	α-CH	4.60 (1H,q)	4.70 (1H,q) [a]		
	β-CH_3	1.40 (3H,d)	1.50 (3H,d)		
Ser^4	α-CH		4.3 (1H)		4.32 (1H,q)
	β-CH_2		3.92 (1H,dd)		3.90 (1H,dd)
			4.10 (1H,dd)		4.20 (1H,dd)
Ala^4	α-CH	4.23 (1H,q)		4.32 (1H,q)	
	β-CH_3	1.40 (3H,d)		1.45 (3H,d)	
Uda	β-CH	8.00 (1H,s)	8.04 (1H,s)	8.03 (1H,s)	8.04 (1H,s)
Cpd	α-CH	4.95 (1H,d)	4.95 (1H,d)	5.01 (1H,d)	5.01 (1H,d)
	β-CH	4.4 (1H,m)	4.4 (1H,m)	4.4 (1H,m)	4.45 (1H,m)
	γ-CH_2	1.8 (1H,m)	1.8 (1H,m)	1.8 (1H,m)	1.8 (1H,m)
		2.1 (1H,m)	2.1 (1H,m)	2.1 (1H,m)	2.1 (1H,m)
	δ-CH_2	3.3 (2H,m)	3.3 (2H,m)	3.3 (2H,m)	3.32 (2H,m)

Abbreviations; s: singlet, d: doublet, dd: double doublet, t: triplet, q: quartet. a) Chemical shift in D_2O+TFA.

was carried out between the carboxyl group of Dea and the amino group of Cpd. The synthetic scheme for these analogs is shown in Fig. 5.

Tripeptides (**2b**, **c**) were prepared by stepwise elongation method from their C-terminal residue using the *t*-Bu group for protection of hydroxyl group of Ser. Dipeptide (**20**) was coupled with either of deprotected tripeptides obtained from **2b** and **2c** by DCC–HOBt method to give a pentapeptide (**7b**) or (**7c**). These ethyl esters (**7b**, **c**) were converted to their 1-succinimidyl esters (**9b**, **c**) *via* free carboxylic acids respectively. Each active ester was treated with hydrogen chloride in THF to remove Nps group selectively and then cyclized in pyridine under high dilution method to give **10b** in 32% or **10c** in 31% yield. Molecular weights of these peptides (**10b**, **c**) measured by means of vapour pressure osmometry (844 and 811 respectively, calcd for $C_{31}H_{54}N_{10}O_{12}\cdot H_2O$: 777) indicated that a cyclic monomer was formed in both cases. Removal of all protecting groups and conversion of Dea to Uda residue in cyclic peptides (**10b**, **c**) were performed. Resulting Uda peptides (**18b**, **c**) were coupled with Boc–β-Lys(Boc)–ONSu and the products were deblocked to give desired compounds, [Ala^3]- and [Ala^4]-Tum O (**19b**, **c**) (Fig. 6).

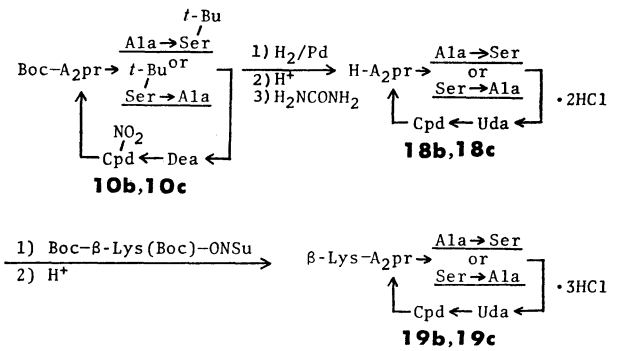


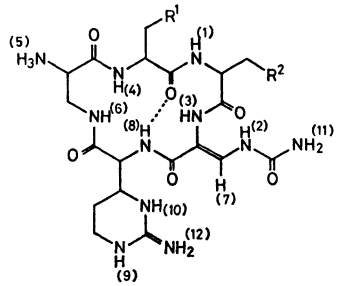
Fig. 6. Synthetic scheme for **19b** and **19c** (continued). b: -Ala^3-Ser^4-, c: -Ser^3-Ala^4-.

Conformational Analyses. Conformational analyses of the compounds synthesized in this investigation were carried out by means of NMR, ORD, and CD studies. Each signal in NMR spectra of [Ala^3, Ala^4]-, [Ala^3]-, and [Ala^4]-Tua N[11] (**18a**—**c**) could be assigned by

Table 2. Chemical shifts of **18a**, **18b**, and **18c** in low-field region in H_2O at pH 2.5 (at 40 °C)

	18a	**18b**	**18c**	Tua N
1	9.17 (d)	9.28 (d)	9.27 (d)	9.33 (d)
2	9.13 (d)	9.25 (d)	9.24 (d)	9.24 (d)
3	8.68 (s)	8.82 (s)	8.81 (s)	8.83 (s)
4	8.63 (d)	8.78 (d)	8.72 (d)	8.67 (d)
5	—	—	—	—
6	7.69 (t)	8.09 (t)	8.22 (t)	8.15 (t)
7	8.00 (d)	8.03 (d)	8.03 (d)	8.00 (d)
8	7.69 (d)	7.74 (d)	7.86 (d)	7.72 (d)
9	7.43 (s)	7.45 (s)	7.41 (s)	7.37 (s)
10	7.28 (s)	7.25 (s)	7.37 (s)	7.31 (s)
11	6.44 (s)	6.46 (s)	6.39 (s)	6.34 (s)
12	6.30 (s)	6.31 (s)	6.29 (s)	6.24 (s)

Abbreviations; s: singlet, d: doublet, t: triplet.



	R^1	R^2
[Ala^3,Ala^4]-Tua N	H	H
[Ala^3]-Tua N	H	OH
[Ala^4]-Tua N	OH	H
Tua N	OH	OH

Fig. 7.

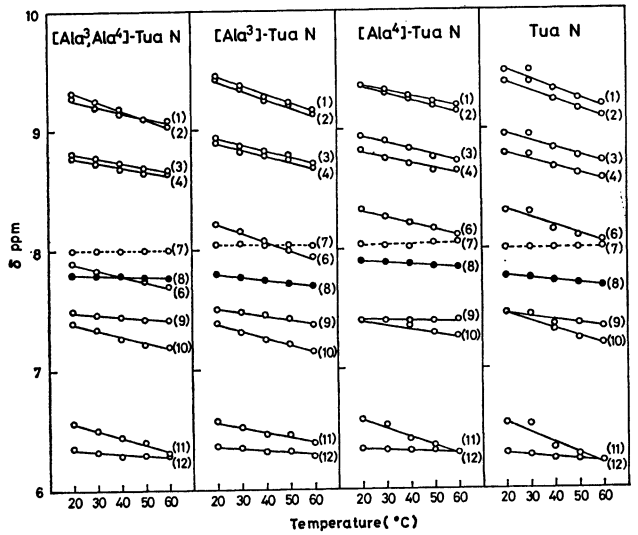


Fig. 8. Temperature dependence of chemical shifts in low-field region of NMR of **18a**, **18b**, and **18c**, as measured in H_2O at pH 2.5. o---o: olefinic proton, •—•: α-amide proton of Cpd, o—o: other NH protons.

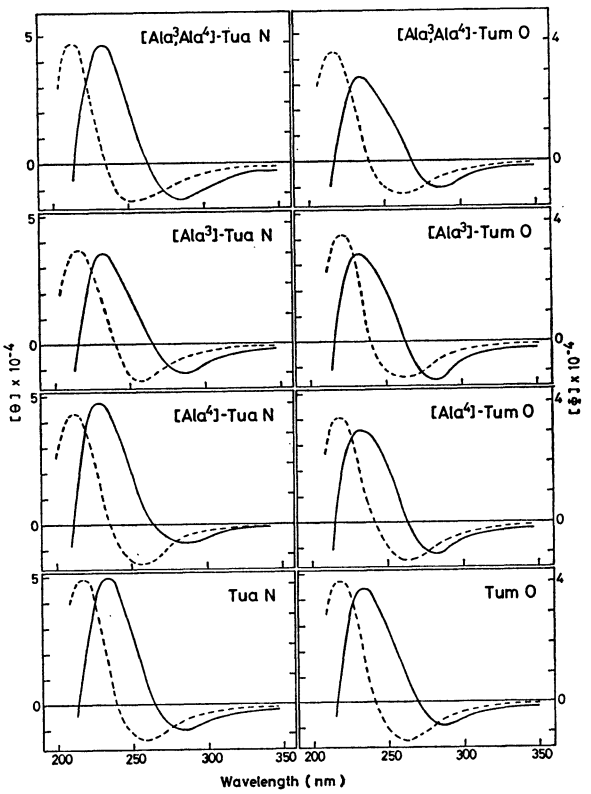


Fig. 9. ORD and CD curves of Tua N and O analogs. ——: ORD, ------: CD.

decoupling method and comparison with natural ones[6] as shown in Table 1, 2, and Fig. 7. These assignments are very consistent with those for natural Tums, and rather abnormal differences in chemical shifts of α-CH or α-NH in Ser³ and Ser⁴ residues of natural sample were reconfirmed by this study.

Temperature dependence of chemical shifts as depicted in the Fig. 8 suggested that amide proton of Cpd may be participated to an intramolecular hydrogen bond as in natural tuberactinomycins.[6] Slower rate in deuterium exchange of the amide proton of Cpd compared to other protons, supported the existence of an intramolecular hydrogen bond mentioned above, too. Furthermore, for comparison between the synthetic and the natural peptides in terms of the conformation of whole molecules, ORD and CD curves were measured. Cyclic peptide moieties, *i.e.* **18a**—**c** which were synthesized in this study, showed principally the same patterns as that of Tua N. Similarly, ORD and CD curves of Tum O analogs resemble those of natural Tums as shown in Fig. 9. Therefore, it could be concluded that all cyclic peptides prepared here have the same conformations as the natural one including the intramolecular hydrogen bond.

Antimicrobial Activities. Minimum inhibitory concentrations of [Ala³, Ala⁴]-, [Ala³]-, and [Ala⁴]-Tum O (**19a**—**c**) were listed in Table 3. Spectral patterns in their antibacterial activities of all these analogs were very similar to those of Tum N. It should be noted that [Ala⁴]-Tum O (**19c**) manifested even stronger activities than those of natural Tum N against some of test organisms, *i.e.*, *Bacillus subtilis*, *Salmonella paratyphi*, and *Salmonella enteritidis*, whereas [Ala³, Ala⁴]-Tum O (**19a**) was slightly weaker than natural antibiotics. Although

Table 3. Minimum inhibitory concentrations[a] of **19a**, **19b**, and **19c**

Test Organisms[b]	**19a**	**19b**	**19c**	Tum O	Tum N
Corynebacterium diphtheriae P.W. 8	12.5	6.3	6.3	6.3	6.3
Bacillus subtilis ATCC 6633	50	25	12.5	25	25
Escherichia coli NIHJ	100	50	12.5	12.5	12.5
Escherichia coli B	100	100	50	50	50
Salmonella typhosa H 901	100	100	25	25	25
Salmonella paratyphi PA 41-N-22	>100	>100	25	>100	>100
Salmonella enteritidis Gaertner	>100	>100	25	100	100
Shigella sonnei E33	>100	>100	100	50	50
Klebsiella pneumoniae ATCC 10031	100	100	25	25	25
Proteus vulgaris OX 19	>100	100	50	50	50
Mycobacterium ATCC 607	12.5	6.3	6.3	6.3	6.3

a) μg/ml. b) **19a**, **19b**, and **19c** were inactive to the following organisms: *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* sp-al-1, *Streptococcus pyogenes* N.Y. 5, *Sarcina lutea* ATCC 9341, *Micrococcus flavus* ATCC 10240, *Shigella flexineri* type 3a, *Serratia marcescens*, *Pseudomonas aeruginosa* IAM 1095.

Table 4. Minimum inhibitory concentrations[a] of **19a**, **19b**, and **19c** against human tubercule bacilli

	19a	**19b**	**19c**	Tum N
Human tubercule bacillus	12.5	<12.5	<12.5	<12.5
Human tubercule bacillus (100 μg-resistant to Tum B)	>100	>100	100	100
BCG (100 μg-resistant to Tum N)	>100	>100	>100	>100

a) μg/ml.

these synthetic analogs were also quite active against sensitive species of human tubercule bacillus, they showed no significant activity to Tum-resistant species of human tubercule bacillus and BCG. (Table 4).

Relationship between Structure and Activities. From all results of comparisons in biological activities of the synthetic peptides, it could be emphasized conclusively that both hydroxyl groups at Ser[3] and Ser[4] residues are not necessarily required for antibacterial activity of Tums, although the presence of these hydroxyl groups seemed to strengthen the activities, especially the hydroxyl group of Ser[3] playing more important role than that of Ser[4]. However, it is apparent that these hydroxyl groups may not be concerned with the drug resistancy of human tubercule bacilli.

Experimental

All melting points are uncorrected. NMR spectra were obtained with a Varian XL-100-15 spectrometer using sodium dimethylsilapentanesulfonate as an internal standard. ORD and CD spectra were obtained with a JASCO Model ORD/UV-5 in water. The specific rotations were obtained with a Perkin-Elmer 141 Polarimeter. Molecular weights were obtained with a Knauer vapor pressure osmometer using methanol as a solvent. UV spectra were recorded on a Hitachi 124 Spectrophotometer. TLC was carried out by the ascending method on silica gel G using a developing solvent chloroform–methanol (7: 1).

*Z–Ala–Dea–OEt (**1a**).* To a solution of Z–Ala–OH (14.6 g, 65.2 mmol) and H–Dea–OEt[9] (13.4 g, 65.2 mmol) in ethyl acetate (40 ml), a solution of HONSu[3] (9.01 g, 78.2 mmol) in dioxane (30 ml) and then DCC (14.8 g, 71.7 mmol) were added under stirring in an ice bath. Stirring was continued at 0 °C for 2 h, thereafter at room temperature overnight. After the addition of acetic acid (500 mg, 8.30 mmol), *N,N'*-dicyclohexylurea was filtered off and filtrate was concentrated *in vacuo*. A solution of the residue in ethyl acetate was washed with saturated aqueous sodium hydrogencarbonate and water. Organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo* to yield an oily product, yield 24.9 g (92.9%).

For characterization a portion of **1a** was converted into the crystalline hydrazide. Thus, to a solution of **1a** (1.40 g, 3.41 mmol) in DMF (20 ml), hydrazine hydrate (3.42 g, 68.3 mmol) was added. The mixture was allowed to stand for two days at room temperature and then concentrated *in vacuo*. The residue was triturated with water, and a crystalline product was filtered off, yield 0.920 g (68.1%). It was recrystallized from ethanol–ether–hexane, mp 208—209 °C (dec), $[\alpha]_D^{29}$ −1.2 °C (*c* 1.0, DMF).[12] Found: C, 54.21; H, 7.17; N, 14.19%. Calcd for $C_{18}H_{28}N_4O_6$: C, 54.53; H, 7.12; N, 14.13%.

*Z–Ala–Ala–Dea–OEt (**2a**).* Into a solution of **1a** (11.5 g, 28.0 mmol) and acetic acid (3.36 g, 56.0 mmol) in ethanol (200 ml), hydrogen was bubbled in the presence of palladium black. After debenzyloxycarbonylation had been completed, catalyst was filtered off and the filtrate was concentrated *in vacuo*. To a solution of the residue in ethyl acetate, triethylamine (3.30 g, 30.0 mmol) and Z–Ala–ONSu (8.96, 28.0 mmol) were added with stirring at 0 °C. The mixture was stirred at 0 °C for 1 h, thereafter at room temperature overnight. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in chloroform. The organic solution was washed with saturated aqueous sodium hydrogencarbonate and water, and then dried over anhydrous sodium sulfate. A crystalline residue obtained after concentration *in vacuo* was recrystallized from chloroform–ethyl acetate, yield 11.7 g (86.8%), mp 186—188 °C. $[\alpha]_D^{28}$ −5.6 ° (*c* 0.9, DMF). Found: C, 57.32; H, 7.37; N, 8.94%. Calcd for $C_{23}H_{35}N_3O_8$: C, 57.37; H, 7.33; N, 8.73%.

*Boc–A$_2$pr(Z)–ONSu (**3**).* To a suspension of H–A$_2$pr(Z)–OH[10] (2.38 g, 10.0 mmol) in water (20 ml), triethylamine (2.1 ml, 15 mmol) and *t*-butyl 4,6-dimethylpyrimidyl-2-thiolcarbonate (2.64 g, 11.0 mmol)[13] in dioxane (6 ml) were added. The reaction mixture was stirred for 20 h. After addition of saturated aqueous sodium hydrogencarbonate, the solution was washed with ethyl acetate. The aqueous layer was acidified with citric acid and the product was transferred to ethyl acetate. The organic layer was washed with water, and then dried over anhydrous sodium sulfate, and then concentrated *in vacuo* to give an oily residue. To its solution in THF (30 ml), HONSu (1.15 g, 10.0 mmol) and DCC (2.06 g, 10.0 mmol) were added with stirring at 0 °C. The mixture was stirred at 0 °C for 2 h, and then at room temperature overnight. The *N,N'*-dicyclohexylurea formed was filtered off and the filtrate was concentrated *in vacuo*. Oily residue was triturated with hexane, and the crystalline product thus obtained (3.71 g, 85.2%) was recrye stallized from dioxane–hexane, yield 2.77 g (63.6%), mp 90—92 °C, $[\alpha]_D^{29}$ −30.7 ° (*c* 1.9, DMF). Found: C, 54.78; H, 5.80; N, 9.68%. Calcd for $C_{20}H_{25}N_3O_8$: C, 55.17; H, 5.79; N, 9.65%.

*Boc–A$_2$pr(Z)–Ala–Ala–Dea–OEt (**4**).* Hydrogenolysis of **2a** (7.00 g, 14.5 mmol) was carried out with addition of acetic acid (1.75 g, 29.1 mmol) in ethanol–dioxane (250 ml) as in the preparation of **2a** from **1a**. To a solution of the residual oil of CH_3COOH·H–Ala–Dea–OEt and **3** (6.33 g, 14.5 mmol) in ethyl acetate (200 ml) *N*-methylmorpholine (2.94 g, 29.1 mmol) was added with stirring at 0 °C. The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The residue obtained after concentration *in vacuo* was dissolved in chloroform, and washed with 10% aqueous citric acid, saturated aqueous sodium hydrogencarbonate, and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crystalline residue was recrystallized from chloroform–ethyl acetate–ether, yield 16.9 g (87.2%), mp 187—191 °C, $[\alpha]_D^{29}$ −9.2 ° (*c* 2.1, DMF). Found: C, 55.60; H, 7.41; N, 10.55%. Calcd for $C_{31}H_{49}N_5O_{11}$: C, 55.76; H, 7.40; N, 10.49%.

*H–Cpd(NO$_2$)–OH (**5**).* To a mixture of fuming nitric acid (*d*=1.52) (1.5 ml) and 50% fuming sulfuric acid (1.2 ml), H–Cpd–OH·HCl[14] (740 mg, 3.55 mmol) was added portionwise over 30 min with stirring at −20 °C, and then concentated

sulfuric acid (1 ml) was added. The mixture was stirred under the same conditions for 45 min, thereafter poured onto ice. On neutralization of the solution with sodium hydrogencarbonate, a crystalline product was separated out, yield 540 mg (70.0%). It was recrystallized from water, yield 535 mg (69.3%), mp 250 °C (dec), $[\alpha]_D^{29}$ +28.0° (*c* 1.0, 6 M HCl). Found: C, 32.82; H, 5.25; N, 32.22%. Calcd for $C_6H_{11}N_5O_4$: C, 33.18; H, 5.11; N, 32.25%.

Nps-Cpd(NO₂)-OH (6). To a solution of **5** (2.00 g, 9.21 mmol) in 1 M aqueous sodium hydroxide (10.1 ml) and dioxane (40 ml), Nps-Cl (1.92 g, 1.01 mmol) and 1 M aqueous sodium hydroxide (10.1 ml) were added over 15 min simultaneously. The solution was diluted with water, and acidified with citric acid. A yellow crystalline product separated out was filtered off. A small amount of the product was recovered from the filtrate by extraction with ethyl acetate. Both crops were combined and recrystallized from acetone–hexane, yield 2.55 g (74.7%), mp 180—181 °C (dec), $[\alpha]_D^{29}$ +31.3° (*c* 2.0, DMF). Found: C, 38.91; H, 3.94; N, 22.56; S, 8.56%. Calcd for $C_{12}H_{14}N_6O_6S$: C, 38.92; H, 3.81; N, 22.69; S, 8.66%.

Boc-A₂pr(Nps-Cpd(NO₂))-Ala-Ala-Dea-OEt (7a). To a solution of **4** (700 mg, 1.05 mmol) in DMF (10 ml), hydrogen was bubbled in the presence of palladium black. After completion of hydrogenolysis, the catalyst was filtered off. To the filtrate, **6** (383 mg, 1.05 mmol), HOBt (180 mg, 1.33 mmol) and then DCC (236 mg, 1.14 mmol) were added with stirring at 0 °C. The stirring was continued at 0 °C for 2 h and then at room temperature overnight. After addition of acetic acid (20 mg, 0.33 mmol), *N,N'*-dicyclohexylurea was filtered off. The filtrate was concentrated *in vacuo*, and a solution of the residue in ethyl acetate was washed with 10% aqueous citric acid, saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The yellow solid obtained was reprecipitated from dioxane–ether, yield 760 mg (90.0%), mp 152—165 °C (dec), $[\alpha]_D^{27}$ +40.5° (*c* 2.2, DMF). Found: C, 47.80; H, 6.40; N, 16.80; S, 3.45%. Calcd for $C_{35}H_{55}N_{11}O_{14}S \cdot 1/2C_4H_8O_2$: C, 47.78; H, 6.39; N, 16.57; S, 3.45%.

Boc-A₂pr(Nps-Cpd(NO₂))-Ala-Ala-Dea-OH (8a). To a suspension of **7a** (1.73 g, 1.95 mmol) in ethanol (2 ml), 2 M aqueous sodium hydroxide (1.47 ml, 2.94 mmol) was added with stirring at room temperature and the stirring was continued for 1 h. After the solution was diluted with water (15 ml), it was washed with ethyl acetate, acidified with citric acid, and then extracted with ethyl acetate. The organic layer was washed with water, and dried over anhydrous sodium sulfate. The residue obtained after concentration *in vacuo* was reprecipitated with dioxane–ether, yield 1.46 g (87.0%), mp 154—166° (dec), $[\alpha]_D^{27}$ +39.4° (*c* 1.9, DMF). Found: C, 45.24; H, 5.86; N, 17.66; S, 3.88%. Calcd for $C_{33}H_{51}N_{11}O_{14}S \cdot H_2O$: C, 45.25; H, 6.10; N, 17.59; S, 3.66%.

Boc-A₂pr(Nps-Cpd(NO₂))-Ala-Ala-Dea-ONSu (9a). To a solution of **8a** (960 mg, 1.12 mmol) and HONSu (150 mg, 1.34 mmol) in dioxane (20 ml), DCC (280 mg, 1.34 mmol) was added with stirring at 0 °C. The mixture was stirred at 0 °C for 2 h and then at room temperature for 6 h. After addition of acetic acid (20 mg, 0.33 mmol), *N,N'*-dicyclohexylurea was filtered off. The filtrate was concentrated *in vacuo*, and the residue was reprecipitated from dioxane–ether, yield 920 mg (86.0%), mp 159—160 °C (dec), $[\alpha]_D^{27}$ +39.5° (*c* 2.0, DMF). Found: C, 45.63; H, 5.69; N, 17.39; S, 3.26%. Calcd for $C_{37}H_{54}N_{12}O_{16}S \cdot H_2O$: C, 45.67; H, 5.80; N, 17.27; S, 3.30%.

Cyclo[Boc-A₂pr-Ala-Ala-Dea-Cpd(NO₂)] (10a) via *Route A.* To a solution of **9a** (880 mg, 0.921 mmol) in THF (10 ml), 0.28 M hydrogen chloride in THF (9.86 ml, 2.76 mmol) was added dropwise over 15 min, and the stirring was continued for 30 min. Anhydrous ether was added to the mixture to obtain a precipitate of the free amino active ester, yield 730 mg (94.8%). It was immediately cyclized without purification. Thus, a solution of the product in DMF (100 ml) was added slowly into pyridine (800 ml) by use of high-dilution apparatus over 72 h at 55 °C with stirring. After stirring was continued for additional 24 h, the reaction mixture was concentrated *in vacuo*, and the residue was purified by silica gel chromatography. From the eluate with chloroform–methanol, a white solid was obtained [R_f 0.70 on TLC, ninhydrin negative], yield 212 mg (33.5%). For elemental analysis, the product thus obtained was reprecipitated from ethyl acetate, mp >250 °C, $[\alpha]_D^{19}$ −26.0° (*c* 0.94, DMF). Found: C, 45.81; H, 6.75; N, 19.52%, mol wt, 743. Calcd for $C_{27}H_{46}N_{10}O_{11} \cdot 3/2H_2O$: C, 45.43; H, 6.92; N, 19.63%, mol wt, 714.

Boc-A₂pr(Z)-OMe (11). To a solution of Boc-A₂pr-(Z)-OH, which was prepared from H-A₂pr(Z)-OH (13.77 g, 57.9 mmol) according to the method described for **3**, in ethyl acetate (100 ml), an ethereal solution of diazomethane was added, until yellow color remained. After excess diazomethane was decomposed with acetic acid, the mixture was concentrated *in vacuo*. The trituration of the oily residue with hexane in an ice-salt bath gave crystalline product, yield 18.47 g (90.5%). For elemental analysis, it was recrystallized from hexane. mp 50—52 °C $[\alpha]_D^{14}$ −8.8° (*c* 1.0, DMF). Found: C, 58.02; H, 6.92; N, 7.90%. Calcd for $C_{17}H_{24}N_2O_6$: C, 57.94; H, 6.87; N, 7.95%.

Boc-A₂pr(Nps-Cpd(NO₂))-OMe (12). Compound **11** (4.75 g, 13.5 mmol) was hydrogenolyzed over palladium black in DMF (30 ml). To the filtrate from the catalyst, **6** (5.00 g, 13.5 mmol), HOBt (2.03 g, 15.0 mmol), and then DCC (3.10 g, 15.0 mmol) were added with stirring at 0 °C. The stirring was continued at 0 °C for 2 h and then at room temperature overnight. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in ethyl acetate. Insoluble material was filtered off and the filtrate was washed with 10% aqueous citric acid, saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The yellow solid was recrystallized from acetone–hexane, yield, 6.33 g (82.2%), mp 155—160 °C, $[\alpha]_D^{28}$ +89.8° (*c* 1.0, DMF). Found: C, 42.71; H, 5.37; N, 18.73; S, 5.47%. Calcd for $C_{21}H_{30}N_8O_9S \cdot H_2O$: C, 42.85; H, 5.48; N, 19.04; S, 5.45%.

Nps-Ala-Ala-Dea-OEt (13). As in the preparation of **2a**, **13** was synthesized from Z-Ala-Dea-OEt (1.00 g, 2.44 mmol) and Nps-Ala-ONSu (828 mg, 2.44 mmol). The yellow oily product was triturated with hexane to yield crystalline product (962 mg, 78.7%), which was recrystallized from ethyl acetate–hexane, yield 768 mg (62.7%). mp 128—131 °C, $[\alpha]_D^{29}$ −29.3° (*c* 1.0, DMF). Found: C, 50.33; H, 6.49; N, 11.08; S, 6.34%. Calcd for $C_{21}H_{32}N_4O_8S$: C, 50.39; H, 6.44; N, 11.19; S, 6.41%.

Nps-Ala-Ala-Dea-OH (14). To a suspension of **13** (750 mg, 1.50 mmol) in ethanol (5 ml), 1 M aqueous sodium hydroxide was added for saponification. The subsequent procedure was practically the same as that for **8a**. Trituration of the residual product with hexane gave a yellow solid, yield 699 mg, (98.7%). For elemental analysis, the solid was reprecipitated from ethyl acetate–hexane, mp 148—149 °C (dec), $[\alpha]_D^{28}$ −32.0° (*c* 1.0, DMF). Found: C, 47.93; H, 5.99; N, 11.51; S, 6.60%. Calcd for $C_{19}H_{28}N_4O_8S$: C, 48.29; H, 5.97; N, 11.86; S, 6.79%.

Boc-A₂pr(Nps-Ala-Ala-Dea-Cpd(NO₂))-OMe (15). To a suspension of **12** (500 mg, 0.876 mmol) in methanol (10

ml), 0.2 M hydrogen chloride in THF (4.5 ml, 0.90 mmol) was added with stirring at room temperature. After 45 min, additional 0.2 M hydrogen chloride in THF (1.0 ml, 0.20 mmol) was added, and the solution was stirred for additional 15 min. Addition of ether and hexane to the reaction mixture gave a white precipitate, yield 339 mg (85.3%). To a solution of this solid, **14** (353 mg, 0.747 mmol) and HOBt (111 mg, 0.822 mmol) in chloroform (25 ml), triethylamine (76 mg, 0.747 mmol) and DCC (170 mg, 0.822 mmol) were added with stirring at 0 °C. Afterwards, the reaction mixture was treated as in the preparation of **7a**. The solid obtained (565 mg, 74.0%) was reprecipitated from dioxane–ether, yield 382 mg (50.0%). mp 203—204 °C (dec), $[\alpha]_D^{28}$ −33.5° (*c* 1.0, DMF). Found: C, 47.02; H, 6.23; N, 16.77; S, 3.92%. Calcd for $C_{34}H_{53}N_{11}O_{14}S \cdot 1/2C_4H_8O_2$: C, 47.20; H, 6.27; N, 16.82; S, 3.50%.

*Boc–A_2pr(Nps–Ala–Ala–Dea–Cpd(NO_2))–OH (**16**).* The methyl ester **15** (900 mg, 1.03 mmol) was saponified with 1 M aqueous sodium hydroxide (1.13 ml, 1.13 mmol) in ethanol (9 ml) at room temperature for 2 h according to the usual procedure like in the preparation of **8a**. Yield 810 mg (93.9 %). For elemental analysis, it was recrystallized from dioxane–ether, mp 173—174 °C (dec), $[\alpha]_D^{28}$ −32.4° (*c* 1.0, DMF). Found: C, 46.37; H, 6.34; N, 16.72; S, 3.77%. Calcd for $C_{33}H_{51}N_{11}O_{14}S \cdot 1/2C_4H_8O_2$: C, 46.60; H, 6.15; N, 17.08; S, 3.56%.

*Boc–A_2pr(Nps–Ala–Ala–Dea–Cpd(NO_2))–ONSu (**17**).* The acid **16** (1.00 g, 1.17 mmol) was converted to **17** with HONSu (150 mg, 1.28 mmol) and DCC (260 mg, 1.28 mmol) in THF (50 ml) as in the synthesis of **9a**, A yellow solid was obtained, yield 960 mg (86.3%). For elemental analysis, this solid was reprecipitated from dioxane–hexane, mp 162—163 °C (dec), $[\alpha]_D^{23}$ −31.7° (*c* 1.0, DMF). Found: C, 46.90; H, 6.04; N, 16.81; S, 3.44%. Calcd for $C_{37}H_{54}N_{12}O_{16}S \cdot 1/2C_4H_8O_2$: C, 46.89; H, 5.85; N, 16.82; S, 3.21%.

*Cyclo[Boc–A_2pr–Ala–Ala–Dea–Cpd(NO_2)] (**10a**)* via *Route B.* To a solution of **17** (960 mg, 1.01 mmol) in THF (100 ml), 0.194 M hydrogen chloride in THF (11.4 ml, 2.21 mmol) was added with stirring at room temperature. After 1 h, the solution was concentrated *in vacuo* and trituration of the residue with anhydrous ether gave a solid, yield 810 mg (95.7%). The product thus obtained, was immediately cyclized without purification. The cyclization procedure was the same as that for **10a** *via* route A except for the use of the reaction conditions at 60 °C for 55 h. Purification by silica gel chromatography gave a white solid [R_f 0.70 on TLC ninhydrin negative], yield 251 mg (36.1%). For elemental analysis, the product thus obtained was reprecipitated from ethyl acetate, mp >250 °C, $[\alpha]_D^{29}$ −31.2° (*c* 0.94, DMF). Found: C, 45.49; H, 6.70; N, 19.67%. Calcd for $C_{27}H_{46}N_{10}O_{11} \cdot 3/2H_2O$: C, 45.43; H, 6.92; N, 19.63%.

[*Ala*^{3}, *Ala*^{4}]-*Tua N·2HCl (**18a**).* To a solution of **10a**, obtained either by route A or B (300 mg, 0.427 mmol) and acetic acid (0.2 ml) in a mixture of ethanol (50 ml) and water (15 ml), hydrogen was bubbled in the presence of palladium black for removal of the nitro group. The filtrate from catalyst was concentrated *in vacuo*, a solution of the residue in acetone–1 M hydrochloric acid (1: 1) (10 ml) was heated under reflux for 8 min. After cooling, the solution was allowed to stand with addition of urea (600 mg, 10.0 mmol) at room temperature overnight. The mixture was concentrated *in vacuo*, trituration of the residue with ethanol–ether gave a precipitate (207 mg, 81.3%). This precipitate was recrystallized from water–methanol, yield 126 mg (49.5%). mp >250 °C $[\alpha]_D^{28}$ −23.8° (*c* 1.0, H_2O). λ_{max}; 268 nm (H_2O, ε 25 700), 267 nm (0.1 M HCl, ε 25 400), 301 nm (1 M NaOH, ε 18 400). Found: C, 38.03; H, 5.97; N, 25.86; Cl, 11.68%. Calcd for $C_{19}H_{33}N_{11}O_6Cl_2 \cdot H_2O$: C, 38.00; H, 5.87; N, 25.66; Cl, 11.81%.

[*Ala*^{3}, *Ala*^{4}]-*Tum O·3HCl (**19a**).* To a suspension of **18a** (50 mg, 0.0858 mmol) in DMF (3 ml), Boc–β-Lys(Boc)–ONSu[8,15] (46 mg, 0.103 mmol) and triethylamine (10.4 mg, 0.103 mmol) were added with stirring at room temperature, and stirring was continued overnight. The solution was concentrated *in vacuo*. Trituration of the residue with THF gave a gelatinous solid, which was collected by centrifugation. The precipitate thus obtained was dissolved in water and washed with ethyl acetate. The aqueous layer was concentrated *in vacuo*. For deprotection, the residue was dissolved in 6 M hydrochloric acid (1 ml) and allowed to stand for 30 min. Addition of ethanol and ether to the mixture gave a white precipitate (58 mg, 91%), which was recrystallized from water–methanol, yield 40 mg (62.4%), mp 250 °C (dec), $[\alpha]_D^{22}$ −25.4° (*c* 0.52, H_2O), λ_{max}; 268 nm (H_2O, ε 23 400), 267 nm (0.1 M HCl, ε 22 500), 295 nm (1 M NaOH, ε 16 700). Found: C, 39.26; H, 6.29; N, 23.62; Cl, 13.72%. Calcd for $C_{25}H_{46}N_{13}O_7Cl_3 \cdot H_2O$: C, 39.24; H, 6.32; N, 23.80; Cl, 13.90%.

*Z–Ser(t-Bu)–Dea–OEt (**1b**).* Coupling of Z–Ser(*t*-Bu)–OH (13.2 g, 44.7 mmol) and H–Dea–OEt (9.17 g, 44.7 mmol) in a manner similar to that for **1a** gave an oily product **1b**, yield 20.8 g (96.5%).

For characterization a portion of **1b** was converted into the crystalline hydrazide as in the case of **1a**, mp 136—144 °C, $[\alpha]_D^{14}$ +10.3° (*c* 1.0, DMF).[12] Found: C, 56.31; H, 7.93; N, 12.00%. Calcd for $C_{22}H_{36}N_4O_7$: C, 56.39; H, 7.74; N, 11.96%.

*Z–Ala–Ser(t-Bu)–Dea–OEt (**2b**).* Hydrogenolysis of **1b** (10.0 g, 20.7 mmol) in DMF (40 ml), and coupling of its product with Z–Ala–OH (4.26 g, 20.7 mmol) by the succinimidyl ester method was performed in a similar manner to that for preparation of **2a**. Acetic acid (242 mg, 4.20 mmol) and *N*-(2-aminoethyl)-piperazine (1.00 g, 7.75 mmol) were used to destroy unreacted DCC and the succinimidyl ester respectively. Yield 8.90 g (77.4%). For elemental analysis, the product thus obtained was recrystallized from ethyl acetate–hexane, mp 104—109 °C, $[\alpha]_D^{19}$ +8.22° (*c* 2.13, DMF). Found: C, 58.16; H, 7.81; N, 7.55%. Calcd for $C_{27}H_{43}N_3O_9$: C, 58.57; H, 7.83; N, 7.59%.

*Z–Ser(t-Bu)–Ala–Dea–OEt (**2c**).* Z–Ser(*t*-Bu)–OH (10.0 g, 33.9 mmol) was coupled with $CH_3COOH \cdot$H–Ala–Dea–OEt prepared from **1c** (13.9 g, 33.9 mmol) by the succinimidyl ester method using *N*-methylmorpholine (6.85 g, 67.7 mmol). Yield 13.5 g (72.0%). For elemental analysis, the product thus obtained was recrystallized from ethyl acetate–hexane, mp 131—145 °C, $[\alpha]_D^{19}$ +5.55° (*c* 2.11, DMF). Found: C, 58.78; H, 7.95; N, 7.80%. Calcd for $C_{27}H_{43}N_3O_9$: C, 58.57; H, 7.83; N, 7.59%.

*Boc–A_2pr(Nps–Cpd(NO_2))–OH (**20**).* Saponification of **12** (3.00 g, 5.26 mmol) in a similar manner to that for **8a** gave a yellow solid, yield 2.49 g (85.0%). For elemental analysis, it was converted to a dicyclohexylammonium salt, and recrystallized from methanol–ether, mp 213—214 °C (dec), $[\alpha]_D^{23}$ +63.8° (*c* 1.0, DMF). Found: C, 51.57; H, 6.96; N, 16.79; S, 4.40%. Calcd for $C_{32}H_{51}N_9O_9S \cdot 1/2CH_3OH$: C, 51.77; H, 7.09; N, 16.72; S, 4.25%.

*Boc–A_2pr(Nps–Cpd(NO_2))–Ala–Ser(t-Bu)–Dea–OEt (**7b**).* Coupling of **20** (3.00 g, 5.39 mmol) and the debenzyloxycarbonylation product of **2b** (2.98 g, 5.39 mmol) in a similar manner as in the preparation of **7a** gave a yellow solid, yield 4.67 g (90.5%), mp 154—164°, $[\alpha]_D^{19}$ +48.6° (*c* 1.9, DMF). Found: C, 48.46; H, 6.58; N, 15.95; S, 3.28%. Calcd for $C_{39}H_{63}N_{11}O_{15}S$: C, 48.89; H, 6.63; N, 16.08; S, 3.35%.

*Boc–A_2pr(Nps–Cpd(NO_2))–Ser(t-Bu)–Ala–Dea–OEt (**7c**).*

Similarly, **20** (3.00 g, 5.39 mmol) was coupled with the deprotected product of **2c** (2.98 g, 5.39 mmol) to give a yellow solid, yield 4.63 g (89.7%), mp 143—153°, $[\alpha]_D^{19}$ +51.6° (*c* 1.9, DMF). Found: C, 48.14; H, 6.54; N, 15.81; S, 3.43%. Calcd for $C_{39}H_{63}N_{11}O_{15}S \cdot H_2O$: C, 47.99; H, 6.71; N, 15.79; S, 3.29%.

*Boc-A_2pr(Nps-Cpd(NO_2))-Ala-Ser(t-Bu)-Dea-OH (**8b**).* Saponification of **7b** (3.17 g, 3.31 mmol) gave a yellow solid in a similar manner as in the preparation of **8a**, yield 2.72 g (88.3%), mp 143 °C (dec), $[\alpha]_D^{19}$ +53.6° (*c* 1.6, DMF). Found: C, 46.74; H, 6.37; N, 16.17; S, 3.22%. Calcd for $C_{37}H_{59}N_{11}O_{15}S \cdot H_2O$: C, 46.88; H, 6.49; N, 16.25; S, 3.38%.

*Boc-A_2pr(Nps-Cpd(NO_2))-Ser(t-Bu)-Ala-Dea-OH (**8c**).* Similarly, saponification of **7c** (4.62 g, 4.82 mmol) gave a yellow solid, yield 3.98 g (88.8%), mp 140—141 °C (dec), $[\alpha]_D^{19}$ +49.2° (*c* 2.0, DMF). Found: C, 46.70; H, 6.40; N, 16.18; S, 3.33%. Calcd for $C_{37}H_{59}N_{11}O_{15}S \cdot H_2O$: C, 46.88; H, 6.49; N, 16.25; S, 3.38%.

*Boc-A_2pr-(Nps-Cpd(NO_2))-Ala-Ser(t-Bu)-Dea-ONSu (**9b**).* The free acid **8b** (2.98 g, 3.20 mmol) was converted to 1-succinimidyl ester as in the preparation of **9a**, yield 3.13 g (95.1%), mp 141—150 °C (dec), $[\alpha]_D^{19}$ +42.6° (*c* 1.1, DMF). Found: C, 46.84; H, 6.01; N, 15.90; S, 2.92%. Calcd for $C_{41}H_{62}N_{12}O_{17}S \cdot H_2O$: C, 47.12; H, 6.17; N, 16.08; S, 3.07%.

*Boc-A_2pr-(Nps-Cpd(NO_2))-Ser(t-Bu)-Ala-Dea-ONSu (**9c**).* Compound **8c** (4.30 g, 4.62 mmol) was changed to 1-succinimidyl ester as in the preparation of **9a**; yield 4.55 g (95.8 %), mp 145—155 °C (dec), $[\alpha]_D^{19}$ +49.5° (*c* 1.8, DMF). Found: C, 47.21; H, 6.10; N, 16.18; S, 3.02%. Calcd for $C_{41}H_{62}N_{12}O_{17}S \cdot H_2O$: C, 47.12; H, 6.17; N, 16.08; S, 3.07%.

*Cyclo[Boc-A_2pr-Ala-Ser(t-Bu)-Dea-Cpd(NO_2)] (**10b**).* Cleavage of the Nps group of **9b** (1.50 g, 1.46 mmol) and the subsequent cyclization were carried out like in the preparation of **10a**, yield 335 mg (30.2%), mp >250 °C, $[\alpha]_D^{17}$ −18.2° (*c* 1.2, DMF). Found: C, 48.21; H, 7.11; N, 18.01% mol wt, 844. Calcd for $C_{31}H_{54}N_{10}O_{12} \cdot H_2O$: C, 47.93; H, 7.27; N, 18.03%, mol wt, 777.

*Cyclo[Boc-A_2pr-Ser(t-Bu)-Ala-Dea-Cpd(NO_2)] (**10c**).* The cyclic peptide, **10c** was synthesized from **9c** (4.40 g, 4.28 mmol) as in the preparation of **10b**, yield 985 mg (30.3%), mp >250 °C, $[\alpha]_D^{17}$ −29.4° (*c* 1.1, DMF). Found: C, 48.13; H, 7.15; N, 18.17%, mol wt, 811. Calcd for $C_{31}H_{54}N_{10}O_{13} \cdot H_2O$: C, 47.93; H, 7.27; N, 18.03%, M. W., 777.

*[Ala³]—Tua N·2HCl (**18b**).* Deprotection of **10b** (370 mg, 0.488 mmol) and conversion of Dea to Uda residue were carried out as in the preparation of **18a**, yield 238 mg (81.5%), mp >250 °C, $[\alpha]_D^{14}$ −16.6° (*c* 0.56, H_2O). λ_{max}; 268 nm (H_2O, ε 20 600), 268 nm (0.1 M HCl, ε 22 100), 290 nm (1 M NaOH, ε 14 800). Found: C, 37.95; H, 5.64; N, 25.43; Cl, 11.62%. Calcd for $C_{19}H_{33}N_{11}O_7Cl_2 \cdot 1/2H_2O$: C, 37.57; H, 5.64; N, 25.37; Cl, 11.67%.

*[Ala⁴]—Tua N·2HCl (**18c**).* This crystalline compound was prepared from **10c** (300 mg, 0.395 mmol) was preapred in a manner similar to that for **18a**, yield 198 mg (83.5%), mp >250 °C, $[\alpha]_D^{14}$ +35.0° (*c* 0.59, H_2O). λ_{max}; 268 nm (H_2O, ε 23 300), 268 nm (0.1 M HCl, ε 24 700), 290 nm (1 M NaOH, ε 17 600). Found: C, 38.07; H, 5.62; N, 25.37; Cl, 11.78%. Calcd for $C_{19}H_{33}N_{11}O_7Cl_2$: C, 38.13; H, 5.56; N, 25.75; Cl, 11.85%.

*[Ala³]—Tum O·3HCl (**19b**).* Boc-β-Lys(Boc)-ONSu (267 mg, 0.602 mmol) was coupled with the free amino group of **18b** (240 mg, 0.401 mmol) followed by deprotection as in the preparation of **19a** to give colorless crystals, yield 250 mg, mp >250 °C, $[\alpha]_D^{14}$ −36.1° (*c* 0.52, H_2O). λ_{max}; 268 nm (H_2O, ε 23 800), 268 nm (0.1 M HCl, ε 24 100), 290 nm (1 M NaOH, ε 16 400). Found: C, 39.12; H, 6.36; N, 22.92; Cl, 13.47%. Calcd for $C_{25}H_{46}N_{13}O_8Cl_3 \cdot 1/2C_2H_5OH \cdot 1/2H_2O$: C, 39.27; H, 6.34; N, 22.90; Cl, 13.38%.

*[Ala⁴]—Tum O·3HCl (**19c**).* Similarly, coupling of Boc-β-Lys(Boc)-ONSu (130 mg, 0.293 mmol) with **18c** (117 mg, 0.196 mmol) and then deprotection gave colorless crystals, yield 124 mg (83.2%), mp 255 °C (dec), $[\alpha]_D^{14}$ −16.4° (*c* 0.53, H_2O). λ_{max}; 268 nm (H_2O, ε 20 300), 268 nm (0.1 M HCl, ε 21 600), 290 nm (1 M NaOH, ε 15 200). Found: C, 39.73; H, 6.39; N, 22.77; Cl, 13.31%. Calcd for $C_{25}H_{46}N_{13}O_8Cl_3 \cdot C_2H_5OH$: C, 40.08; H, 6.48; N, 22.50; Cl, 13.15%.

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2) This study was presented at the 13th Symposium on Peptide Chemistry, Tokyo, November, 1975, p. 106 and the 14th Symposium on Peptide Chemistry, Hiroshima, November, 1976, p. 127.

3) Abbreviations according to IUPAC-IUB commission, *J. Biol. Chem.*, **247**, 977 (1972), are used. Tum: tuberactinomycin, Tua: tuberactinamine, γ-Hy-β-Lys: γ-hydroxy-β-lysine, Dea: β,β-diethoxyalanine, Uda: β-ureidodehydroalanine, A_2pr: α,β-diaminopropionic acid, Cpd: capreomycidine, Boc: *t*-butoxycarbonyl, Z: benzyloxycarbonyl, Nps: *o*-nitrophenylsulfenyl, DCC: dicyclohexylcarbodiimide, HONSu: *N*-hydroxysuccinimide, HOBt: 1-hydroxybenzotriazole, THF: tetrahydrofuran, DMF: *N,N*-dimethylformamide.

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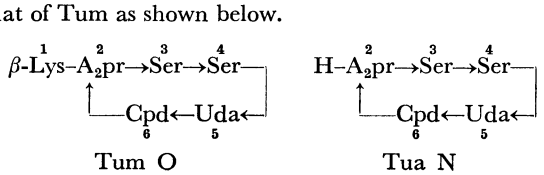

Tum O Tua N

12) DL-Dea was used in this study, while all the other amino acids were of the L-form. Therefore, specific optical rotations measured for Dea peptides may fluctuate to some extent depending on ratios of the diastereomers.

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