CHEMICAL STUDIES ON TUBERACTINOMYCIN. XV[1]

TOTAL SYNTHESIS OF TUBERACTINOMYCIN O[2]

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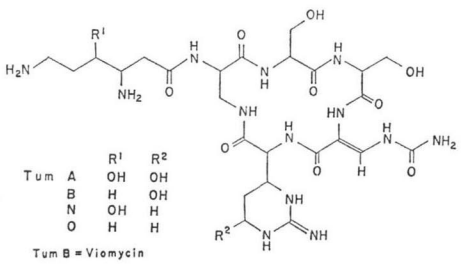
Tuberactinomycin O, one of the four congeners of the antituberculous peptide tuberactinomycin, was totally synthesized. The β-ureidodehydroalanine moiety was constructed from β,β-diethoxyalanine with excess urea in acidic medium after a cyclization reaction of a pentapeptide was finished. Cyclization was carried out by means of the 1-succinimidyl ester method. To the cyclic pentapeptide, β-lysine was introduced as the branched moiety and then deprotected to afford tuberactinomycin O which was completely identified with the natural form of the antibiotic.

Antituberculous peptide, tuberactinomycin (Tum) O, had been isolated as one of the four Tum congeners from *Streptomyces griseoverticillatus* var. *tuberacticus*[3]. X-Ray analysis established the chemical structure of this antibiotic unambiguously as shown in Fig. 1[4]. Furthermore, from NMR study on the tuberactinomycins including Tum O, the conformation with intramolecular hydrogen bond was presented.[5] After these structural studies, a total synthesis of Tum seemed to be worth accomplishing, since synthesis of such an antibiotic involving unusual amino acids, *e.g.*, β-ureidodehydroalanine (Uda), and capreomycidine (Cpd), has not previously been attempted, and its success would afford a valuable tool for investigation of structure-biological activity relationships by syntheses of numerous structural analogs.

In our synthetic principle, tuberactinamine (Tua) N corresponding to the cyclic peptide moiety of Tum N or O, was first synthesized, and then β-lysine residue was introduced to the free α-amino group of α,β-diaminopropionic acid (A_2pr) residue in Tua N in the same manner as in the conversion of Tum N to Tum O *via* Tua N, which was already achieved in our previous work[6]. However the β-ureidodehydroalanine residue was so labile that it would be intolerant of many steps in peptide synthesis. Therefore, this portion was planned to be constructed from the β,β-diethoxyalanine residue at the stage of the cyclic pentapeptide, namely at the last step of synthesis of Tua N in a similar manner as in syntheses of Tum O analogs[7].

In our previous study on Tum analogs[7], the position of the C-terminal residue of the linear pentapeptide was investigated in order to minimize racemization and to improve the yield of the cyclization reaction. It was found that use of β,β-diethoxyalanine (Dea) and A_2pr as C-terminal amino acid made practically no difference. In this study, therefore, the cyclization reaction was carried out between carboxyl group of Dea and amino group of Cpd to conserve the latter amino acid. Synthetic scheme is shown in Figs. 2 and 3.

Fig. 1. Chemical structures of tuberactinomycins.



Dipeptides, **1** and **3**, were the same starting materials used in our previous study[7]. Dipeptide **1** was debenzyloxycarbonylated and coupled with Z-Ser(*t*-Bu)-ONSu* to give tripeptide **2**. Fragment condensation of **3** and debenzyloxycarbonylated product of **2** obtained above was carried out by means of the DCC-HOBt* method to give pentapeptide **4**. The pentapeptide ester **4** was converted to 1-succinimydyl ester **6** by saponification followed by active esterification (DCC-HONSu).* After deprotection of Nps* group from **6** with hydrogen chloride in THF, the free linear peptide was cyclized in pyridine under high dilution condition in approximately 25% yield (at 60°: 24.6%, at room temperature: 25.6%). Compound **7** was ninhydrin-negative on silica gel TLC (Rf 0.65, chloroform - methanol (9: 1)), while deprotected **6** was ninhydrin-positive (Rf 0.01). Molecular weight of **7** measured by vapor pressure osmometry was 811 (Calcd for $C_{35}H_{62}N_{10}O_{13}\cdot H_2O$: 849). Thus, the cyclic peptide so obtained was certified to be a cyclic monomer.

Fig. 2.

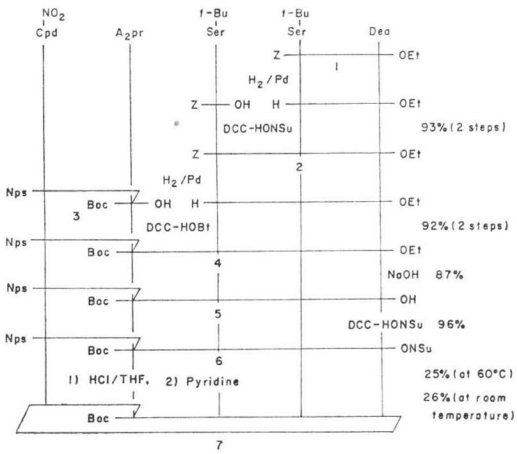
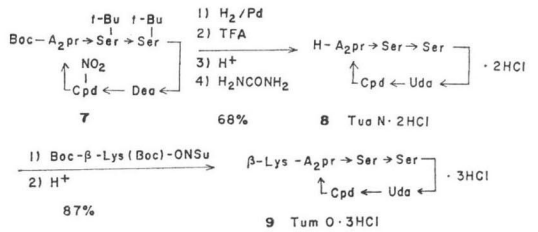


Fig. 3.



Deprotection of **7** was carried out successively by hydrogenolysis for nitro group, and acidolysis by TFA for *t*-Bu and Boc groups.* Deprotected cyclic peptide was then heated under reflux in acetone - 2 M hydrochloric acid (1: 1) for 10 minutes, and immediately treated with excess urea in the same medium at room temperature to give the desired product, Tua N·2HCl (**8**), in satisfactory yield. The key intermediate thus obtained was clearly identified with Tua N derived from natural Tum N, in all respects (mp, [α], UV spectrum, NMR spectrum,** and biological activities) (Tables 1 and 2).

We should emphasize that the signal of the β-proton of Uda was observed at δ 8.02 as one singlet solely in NMR spectrum of synthetic Tua N (in D_2O). This indicated that the configuration of the double bond selectively introduced in Uda was a natural form, *i.e.*, Z configuration fortunately. This result may have arisen from the situation that the ureido group was sterically controlled by a definite conformation of the residual cyclic moiety. Conversion of Tua N to Tum O was carried out, as in our previous work[6], by introduction of Boc-β-Lys(Boc)-ONSu* to α-amino group of A_2pr and deprotection of Boc groups. The product so obtained was identical with natural Tum O in all respects (mp, [α], UV spectrum, NMR spectrum, and biological activities) (Tables 1 and 2, Fig. 4). The total synthesis of tuberactinomycin O was thus accomplished for the first time.

* Abbreviations according to IUPAC-IUB commission, J. Biol. Chem. 247: 977 (1972), are used. Z: benzyloxycarbonyl, Nps: *o*-nitrophenylsulfenyl, Boc: *t*-butoxycarbonyl, *t*-Bu: *t*-butyl, HONSu: *N*-hydroxysuccinimide, HOBt: 1-hydroxybenzotriazole, DCC: dicyclohexylcarbodiimide, Dea: β,β-diethoxyalanine, A_2pr: α,β-diaminopropionic acid, THF: tetrahydrofuran, DMF: *N,N*-dimethylformamide.

** NMR spectrum of Tua N was described in the experimental section.

Table 1. Comparisons of natural and synthetic compounds.

		Tua N		Tum O	
		Synthetic	Natural	Synthetic	Natural
mp (decomp.)		263~264°C	263~264°C	240~242°C	240~242°C
$[\alpha]$ (c 0.5, H_2O)		$[\alpha]_{365}^{18}$ −54.0°	$[\alpha]_{365}^{18}$ −50.8°	$[\alpha]_{D}^{16}$ −16.0°	$[\alpha]_{D}^{16}$ −16.2°
λ_{max} nm (ε)	H_2O	268(26,600)	268(22,000)[6]	268(25,500)	268.5(23,800)[3]
	0.1 M HCl	268(26,700)	268(22,000)[6]	268(26,500)	269 (24,900)[3]
	0.1 M NaOH	285(17,000)	286(14,000)[6]	286(17,400)	288 (13,200)[3]

Table 2. Antimicrobial activities of natural and synthetic compounds.

Test organisms	MIC (μg/ml)			
	Tua N		Tum O	
	Synthetic	Natural	Synthetic	Natural
Staphylococcus aureus ATCC 6538P	>100	>100	>100	>100
Staphylococcus epidermidis sp-al-1	>100	>100	>100	>100
Streptococcus pyogenes N.Y. 5	50	50	>100	100
Sarcina lutea ATCC 9341	>100	>100	>100	>100
Micrococcus flavus ATCC 10240	>100	>100	>100	>100
Bacillus subtilis ATCC 6633	>100	>100	25	25
Escherichia coli NIHJ-JC 2	>100	>100	>100	>100
Escherichia coli B	>100	>100	50	50
Salmonella typhosa H 901	>100	>100	25	25
Salmonella paratyphi PA 41-N-22	>100	>100	100	>100
Salmonella enteritidis Gaertner	>100	>100	100	100
Shigella sonnei E33	>100	>100	50	50
Serratia marcescens	>100	>100	>100	>100
Pseudomonas aeruginosa IAM 1095	>100	>100	>100	>100
Mycobacterium ATCC 607	12.5	12.5	6.3	6.3

Experimental

All melting points are uncorrected. NMR spectra were obtained with a Varian XL-100-15 spectrometer using sodium dimethylsilapentanesulfonate as an internal standard. The optical rotations were measured 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 developing solvents, 10% ammonium acetate - 10% ammonia - acetone (9: 1: 10) (solvent A) and chloroform - methanol (9: 1) (solvent B). Paper electrophoresis (PEP) was carried out at 750 volt and 1 mA/cm for 1.5 hours on Toyo Roshi No. 51 paper using buffer solution of pyridine - acetic acid - water (30: 4: 966). All amino acids used, except for β,β-diethoxylalanine, were of L-configuration.

Z-Ser(t-Bu)-Ser(t-Bu)-Dea-OEt (**2**)*

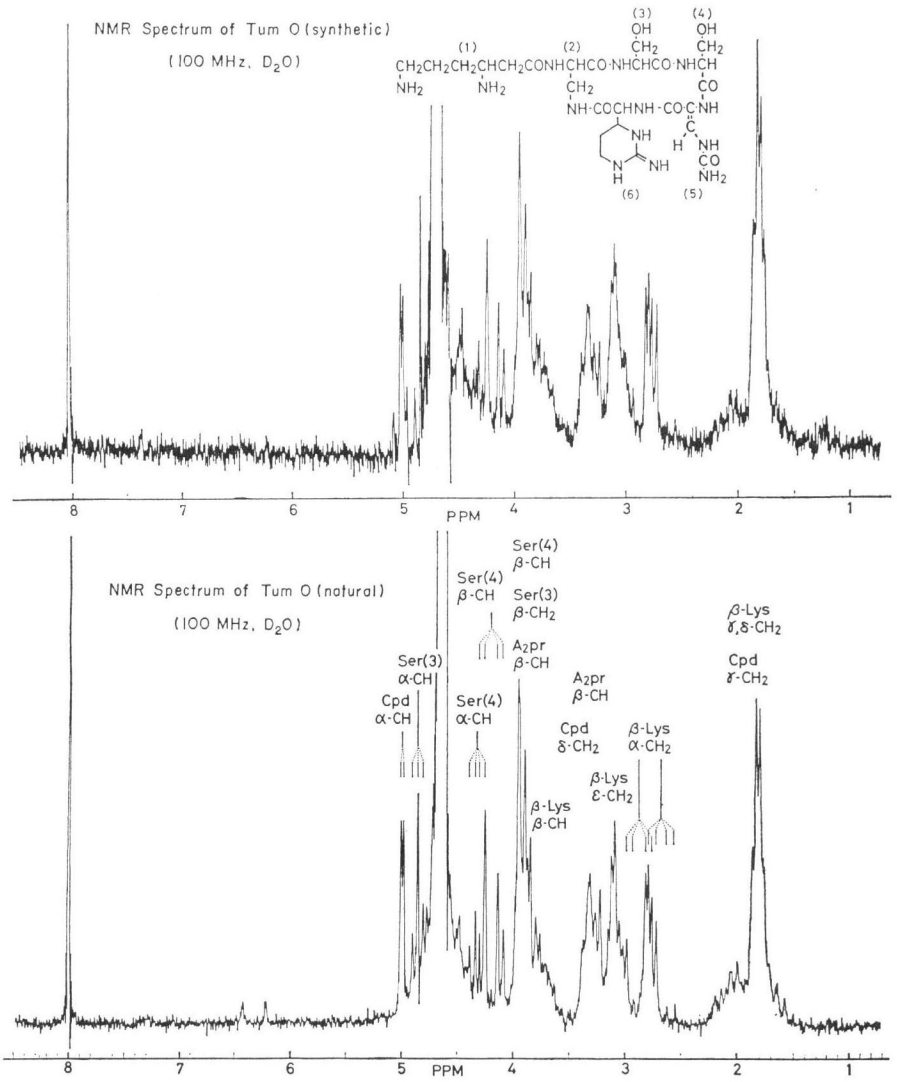
Into a solution of Z-Ser(t-Bu)-Dea-OEt* (**1**) (16.9 g, 35.0 mmol) in DMF (60 ml), hydrogen was bubbled in the presence of palladium black (50 mg). After deprotection had been completed, the product solution was added to a solution of Z-Ser(t-Bu)-ONSu in THF, which was prepared from Z-Ser(t-Bu)-OH (11.4 g, 38.5 mmol), HONSu (4.87 g, 42.3 mmol), and DCC (8.73 g, 42.3 mmol) in THF (100 ml). The reaction mixture was stirred at 0°C for 2 hours, and then at room temperature overnight. An insoluble material was filtered off, and the filtrate was concentrated *in vacuo*. A solution of the residue in

ethyl acetate was washed successively with 10% citric acid, saturated sodium hydrogencarbonate, and water. The organic layer was dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. Residual oil was purified with silica gel column chromatography (silica gel column 27 mm × 500 mm). From the eluate (700 ~ 1,280 ml) with benzene - ethyl acetate (9: 1), a colorless oil was obtained. Yield 20.4 g (93.1%), $[\alpha]_D^{22}$ +15.1° (*c* 1.0, DMF).***

Found: C, 59.59; H, 8.45; N, 6.41%.
Calcd for $C_{31}H_{51}N_8O_{10}$: C, 59.50; H, 8.22; N, 6.72%.

In order to obtain a crystalline derivative of the oily product, it was converted to its hydrazide. Thus, to a solution of **2** (100 mg, 0.160 mmol) in DMF (1 ml), hydrazine hydrate (160 mg, 3.20 mmol) was added. The mixture was allowed to stand overnight at room temperature and concentrated *in vacuo*. The residue was recrystallized from ethanol-water, yield 63 mg (64%), mp 158 ~ 160°C, $[\alpha]_D^{23}$ +14.2° (*c* 1.0, DMF)***.

Fig. 4. NMR spectra of synthetic and natural Tum O.



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

Found: C, 56.57; H, 8.08; N, 11.33%.
Calcd for $C_{29}H_{49}N_5O_9$: C, 56.94; H, 8.07; N, 11.45%.

Boc-A_2pr(Nps-Cpd(NO_2))-Ser(*t*-Bu)-Ser(*t*-Bu)-Dea-OEt (**4**).

H-Ser(*t*-Bu)-Ser(*t*-Bu)-Dea-OEt was obtained by hydrogenolysis of **2** (561 mg, 0.898 mmol) in a similar manner as in the preparation of **2**. To a solution of the above product in DMF (3 ml), Boc-A_2pr(Nps-Cpd(NO_2))-OH (**3**)[7] (500 mg, 0.898 mmol), HOBt (133 mg, 0.988 mmol) and then DCC (204 mg, 0.988 mmol) were added with stirring at 0°C for 2 hours and at room temperature overnight. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in ethyl acetate. An insoluble material was filtered off and the filtrate was washed with 10% citric acid, and then with saturated sodium hydrogen carbonate and water. The organic layer was dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. Oily yellow product was triturated with hexane, to give solid (850 mg, 91.9%) which was reprecipitated from ethyl acetate-hexane, yield 730 mg (78.9%), mp 125~127°C, $[\alpha]_D^{23}$ +54.5° (*c* 1.0, DMF).***

Found: C, 49.91; H, 7.00; N, 14.65; S, 3.18%.
Calcd for $C_{48}H_{71}N_{11}O_{16}S$: C, 50.13; H, 6.95; N, 14.96; S, 3.11%.

Boc-A_2pr(Nps-Cpd(NO_2))-Ser(*t*-Bu)-Ser(*t*-Bu)-Dea-OH (**5**).

To a suspension of **4** (1.50 g, 1.46 mmol) in ethanol (3 ml), 2 M sodium hydroxide (1.09 ml, 2.19 mmol) was added with stirring at room temperature and stirring was continued for 1.5 hours. After the solution was diluted with water (100 ml), it was washed with ethyl acetate. The organic layer was extracted with water in order to recover sodium salt of **5**, until aqueous layer became colorless. Both aqueous layers were combined, acidified with citric acid, and extracted with ethyl acetate. Organic layer was washed with water, and dried over anhydrous magnesium sulfate. The residue obtained after concentration *in vacuo* was triturated with hexane to give yellow solid, yield 1.27 g (87.2%). For elemental analysis, the product thus obtained was reprecipitated from ethyl acetate-hexane, mp 130~132 °C (decomp.), $[\alpha]_D^{23}$ +51.5° (*c* 1.0, DMF).***

Found: C, 47.77; H, 6.70; N, 14.87; S, 2.96%.
Calcd for $C_{41}H_{67}N_{11}O_{16}S \cdot 3/2H_2O$: C, 47.85; H, 6.86, N, 14.97; S, 3.12%.

Boc-A_2pr(Nps-Cpd(NO_2))-Ser(*t*-Bu)-Ser(*t*-Bu)-Dea-ONSu (**6**).

To a solution of **5** (300 mg, 0.299 mmol) and HONSu (38 mg, 0.329 mmol) in THF (4 ml), DCC (68 mg, 0.329 mmol) was added with stirring at 0°C. The mixture was stirred at 0°C for 2 hours and then at room temperature overnight. N,N'-Dicyclohexylurea was filtered off and filtrate was concentrated *in vacuo*. A solution of the residue thus obtained in ethyl acetate was washed successively with 10% citric acid, saturated sodium hydrogencarbonate, and water. Organic layer was dried over anhydrous magnesium sulfate. Concentration of this solution *in vacuo* gave yellow solid, yield 314 mg (95.5%). This product was reprecipitated from ethyl acetate-ether, yield 234 mg (71.2%), mp 122~124°C, $[\alpha]_D^{23}$ +45.1° (*c* 1.0, DMF).***

Found: C, 48.12; H, 6.37; N, 14.83; S, 2.80%.
Calcd for $C_{45}H_{70}N_{12}O_{18}S \cdot H_2O$: C, 48.38; H, 6.50; N, 15.05; S, 2.87%.

Cyclo[Boc-A_2pr-Ser(*t*-Bu)-Ser(*t*-Bu)-Dea-Cpd(NO_2)] (**7**).

To a solution of **6** (1.46 g, 1.33 mmol) in THF (15 ml), 2.0 M hydrogen chloride in THF (2.62 ml, 5.24 mmol) was added in portions for 1 hour with stirring at room temperature. After stirring had been continued for an additional 1 hour, the reaction mixture was concentrated *in vacuo*. Addition of ether to this residue gave a pale yellow precipitate, yield 1.06 g (89.3%). The product thus obtained was immediately cyclized without purification. A solution of the product in DMF (275 ml) was added slowly into pyridine (2 liters), with stirring, by use of a high dilution apparatus[8] at 60°C for 96 hours. After continued stirring for 20 hours, the reaction mixture was concentrated *in vacuo*, and the residue was purified by silica gel chromatography (silica gel column, 14 mm × 350 mm). From the eluate (200~290 ml) with chloroform - methanol (39: 1), white solid was obtained, yield 272 mg (24.6%). For elemental analysis, it was reprecipitated from methanol - ether, mp >250°C, TLC Rf 0.65 (solvent B), $[\alpha]_D^{15}$ −31.5° (*c* 1.0, DMF).***

Found: C, 49.84; H, 7.47; N, 16.42%. MW, 811.
Calcd for $C_{35}H_{62}N_{10}O_{13}\cdot H_2O$: C, 49.52; H, 7.60; N, 16.50%. MW, 849.

In another experiment, cyclization reaction was carried out under the same conditions mentioned above except at room temperature. In this case, 275 mg (25.6%) of the same cyclization product **7** was obtained starting from the active ester **6** (1.42 g, 1.29 mmol).

Tua N·2HCl (**8**).

Into a solution of **7** (100 mg, 0.120 mmol) and acetic acid (0.5 ml) in ethanol - water (20 ml-3 ml), hydrogen was bubbled in the presence of palladium-black (30 mg). After hydrogenolysis of the nitro group had been completed, catalyst was filtered off. The filtrate was concentrated *in vacuo* to an oily residue. It was dissolved in TFA (2 ml), and allowed to stand for 30 minutes. Reaction mixture was concentrated *in vacuo*. A solution of the residue in acetone - 2 M hydrochloric acid (1: 1) (10 ml) was heated under reflux for 10 minutes. After cooling, urea (250 mg, 4.17 mmol) was added to the solution and the mixture was allowed to stand overnight at room temperature. It was concentrated *in vacuo*, and ethanol was added to the residue to give a white precipitate. It was crystallized from water - ethanol, yield 50 mg (68%). Recrystallization was repeated from water - ethanol, yield 42 mg (57%), δ 4.4 (1 H, q, A_2pr α-CH), 3.29 (1 H, A_2pr β-CH_2), 4.10 (1 H, A_2pr β-CH_2), 4.80 (1 H, t, Ser^3 α-CH), 3.92 (2 H, d, Ser^3 β-CH_2), 4.30 (1 H, q, Ser^4 α-CH), 3.87 (1 H, dd, Ser^4 β-CH_2), 4.2 (1 H, dd, Ser^4 β-CH_2), 8.02 (1 H, s, Uda β-CH), 4.99 (1 H, d, Cpd α-CH), 4.4 (1 H, m, Cpd β-CH), 1.8 (1 H, m, Cpd γ-CH), 2.1 (1 H, m, Cpd γ-CH), 3.30 (2H, m, Cpd δ-CH_2), TLC: Rf 0.72 (solvent A), PEP: migration distance to cathode 9.7 cm, [Tua N from natural Tum N: δ 4.40 (1 H, q, A_2pr α-CH), 3.30 (1H, A_2pr β-CH_2), 4.12 (1 H, A_2pr β-CH_2), 4.84 (1 H, t, Ser^3 α-CH), 3.95 (2 H, α, Ser^3 β-CH_2), 4.32 (1 H, q, Ser^4 α-CH), 3.90 (1H, dd, Ser^4 β-CH_2), 4.20 (1 H, dd, Ser^4 β-CH_2), 8.04 (1 H, s, Uda β-CH), 5.01 (1 H, d, Cpd α-CH), 4.45 (1 H, m, Cpd β-CH), 1.8 (1 H, m, Cpd γ-CH), 2.1 (1 H, m, Cpd γ-CH), 3.32 (2H, m, Cpd δ-CH_2) TLC: Rf 0.72 (solvent A), PEP: migration distance to cathode 9.7 cm], mp 263~264°C (decomp.), $[\alpha]_{365}^{18}$ −54.0° (*c* 0.50, H_2O).

Found: C, 36.76; H, 5.52; N, 24.79; Cl, 11.25%.
Calcd for $C_{19}H_{33}N_{11}O_8Cl_2\cdot 1/2H_2O$: C, 36.60; H, 5.50; N, 24.71; Cl, 11.37%.

Tum O·3HCl (**9**).

To a suspension of **8** (40 mg, 0.065 mmol) in DMF (2 ml), Boc-β-Lys(Boc)-ONSu (35 mg, 0.078 mmol) and triethylamine (8 mg, 0.078 mmol) were added and stirred at room temperature. The solution was then concentrated *in vacuo*. Trituration of the residue with THF gave a gelatinous solid, which was collected by centrifugation. This precipitate was dissolved in 4 M hydrochloric acid (0.8 ml) and kept at room temperature for 40 minutes. Additions of ethanol and ether gave a white precipitate, yield 44 mg (87%). For elemental analysis, it was recrystallized from water-ethanol, TLC: Rf 0.34 (solvent A), PEP: migration distance to cathode 11 cm, [natural Tum O: TLC: Rf 0.34 (solvent A), PEP: migration distance to cathode 11 cm], mp 240~242°C (decomp.), $[\alpha]_D^{16}$ −16.0° (*c* 0.53, H_2O).

Found: C, 38.18; H, 6.05; N, 22.84; Cl, 13.55%.
Calcd for $C_{25}H_{46}N_{13}O_9Cl_3\cdot 1/2H_2O$: C, 38.10; H, 6.01; N, 23.11; Cl, 13.50%.

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