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Polyoxin Analogs. I. Synthesis of Aminoacyl Derivatives of 5′-Amino-5′-deoxyuridine

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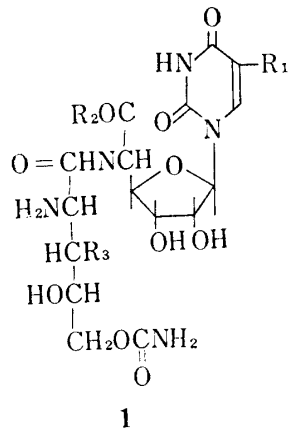
5′-δ-Carbamoyloxy-L-α-aminovaleramido-5′-deoxyuridine, a polyoxin analog, was prepared by the reaction of 5′-amino-5′-deoxy-2′, 3′-O-isopropylideneuridine and N-carbobenzoxy-L-α-aminovalero-δ-lactone, followed by carbamoylation and the removal of the protective groups. Several other ω-substituted aminoacyl derivatives were also prepared utilizing the activated ester method and the mixed anhydride method. All these compounds showed no polyoxin-activity.

Recently, we have reported the isolation[2] and the structure[3] of a new class of peptide uracil nucleoside antibiotics, polyoxins A—M. Biologically active polyoxins have been shown to have the general structure **1**, and we sugested[3a] it to be an analog of uridine diphosphate N-acetylglucosamine because polyoxins inhibited the uptake of glucosamine into cell wall of *Cochliobolus miyabeanus*.[4] Indeed, it has recently been shown[5] that polyoxin D is a competitive inhibitor of UDP-N-acetylglucosamine in a chitin synthetase system of *Neurospora crassa*.



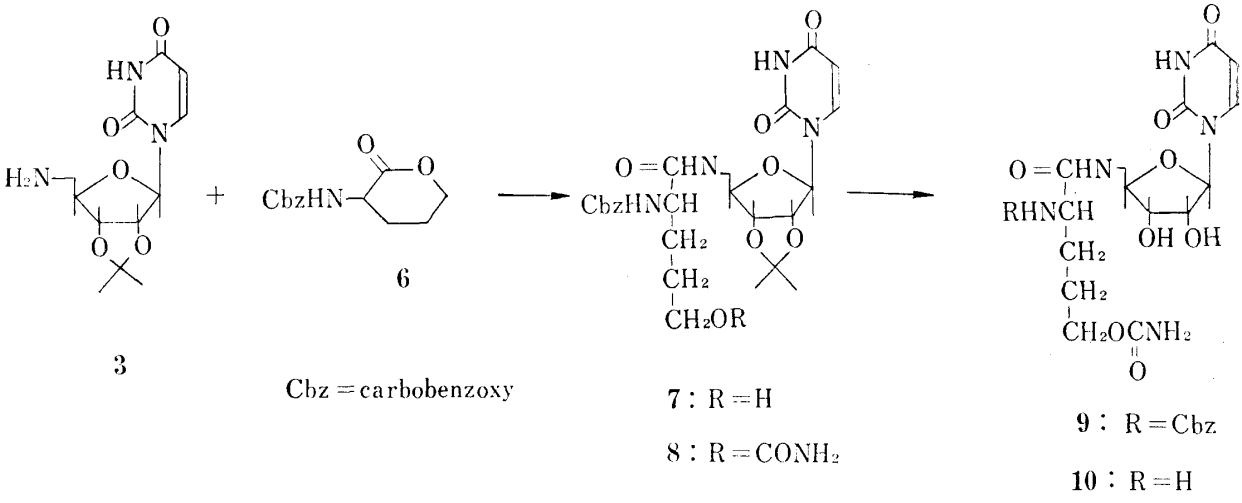
1

	Polyoxins		R_1	R_2	R_3
a	A		CH_2OH		OH
b	B		CH_2OH	HO	OH
d	D		COOH	HO	OH
e	E		COOH	HO	H
f	F		COOH		OH
g	G		CH_2OH	HO	H
h	H		CH_3		OH
j	J		CH_3	HO	OH
k	K		H		OH
l	L		H	HO	OH
m	M		H	HO	H

Chart 1

1) Location: *Yamato-machi, Kitaadachi-gun, Saitama.*
2) *a*) K. Isono, J. Nagatsu, Y. Kawashima, and S. Suzuki, *Agr. Biol. Chem.* (Tokyo), **29**, 848 (1965); *b*) K. Isono, J. Nagatsu, K. Kobinata, K. Sasaki, and S. Suzuki, *ibid.*, **31**, 190 (1967); *c*) K. Isono, K. Kobinata, and S. Suzuki, *ibid.*, **32**, 792 (1968).
3) *a*) K. Isono, K. Asahi, and S. Suzuki, *J. Am. Chem. Soc.*, **91**, 7490 (1969); *b*) K. Isono, S. Suzuki, M. Tanaka, T. Nanbata, and K. Shibuya, *Tetrahedron Letters*, **1970**, 425.
4) S. Sasaki, N. Ohta, I. Yamaguchi, S. Kuroda, and T. Misato, *Nippon Nogeikagaku Kaishi*, **42**, 633 (1968).
5) A. Endo and T. Misato, *Biochem. Biophys. Res. Commun.*, **37**, 718 (1969).

As part of a program of the preparation and biological evaluation of analogs of polyoxins toward the elucidation of the structure–activity relationship, we report herein the synthesis of ω-substituted aminoacyl derivatives of 5′-amino-5′-deoxyuridine, simple and easily accessible 5′-decarboxylated analogs of polyoxins. It seemed to be feasible that 5′-carboxyl was not essential for biological activity because polyoxins A, F, H and K whose 5′-carboxyl were masked with the additional amino acid, 3-ethylidene-L-azetidine-2-carboxylic acid through amide linkage, were also as active as polyoxins B, D, E, G, J, L and M which had the free 5′-carboxyl group. It was also expected that the removal of an ionic carboxyl would give a favorable effect to overcome the permeability barrier of cell membrane.[6]



3

6

Cbz = carbobenzoxy

7 : R = H

8 : R = $CONH_2$

9 : R = Cbz

10 : R = H

Chart 2

5′-Azido-5′-deoxy-2′,3′-O-isopropylideneuridine (**2**) prepared by the method of Horwitz, *et al.*[7] from uridine, was hydrogenated over palladium in ethanol to afford 5′-amino-5′-deoxy-2′,3′-O-isopropylideneuridine (**3**). Since **3** was obtained as amorphous powder and was difficult to purify, the hydrogenation product was directly used for the coupling with amino acids without further purification. Characterization of **3** was achieved by the preparation of crystalline 5′-acetamido-5′-deoxyuridine (**4**).

δ-Hydroxy-L-α-aminovaleric acid[8] was utilized as a primary candidate for amino acid to be coupled with **3**, because it constituted a 4-deoxy analog of a deoxypolyoxamic acid[3a] (2-amino-2,3-dideoxy-L-xylonic acid) moiety of polyoxins. In view of the difficulty for activation of the carboxyl group with the intact δ-hydroxy group, a δ-lactone was directly used for the coupling with aminonucleoside (**3**). Thus N-carbobenzoxy-δ-hydroxy-L-α-aminovaleric acid (**5**) prepared from δ-hydroxy-L-α-aminovaleric acid was treated with dicyclohexylcarbodiimide to afford the crystalline N-carbobenzoxy-δ-lactone (**6**), which was brought to react with an equimolar of **3** in anhydrous dioxane in the presence of catalytic amount of triethylamine. The reaction was conducted 4 hr at refluxing temperature and amide **7** was isolated as colorless foam after purification on silica gel chromatography with benzene–acetone. The yield varied from 45 to 53%.[9] Carbamoylation of the newly generated ω-hydroxy group of **7** was effected by the reaction of equimolar phosgene in the presence of equimolar pyridine[10] in

6) According to a private communication from Dr. E. Cabib of the National Institutes of Health, U.S.A., polyoxins A and M are powerful inhibitors of yeast chitin synthetase, although they showed no effect on growth of yeast. It was assumed to be ascribable to the permeability barrier of yeast cell membrane.

7) J.P. Horwitz, A.J. Tomson, J.A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3045 (1962).

8) M. Goodman and A.M. Felix, *Biochemistry*, **3**, 1529 (1964).

9) The main by-product was proved to be 5′-deoxy-5′,6-epimino-5,6-dihydro-2′,3′-O-isopropylideneuridine formed by the intramolecular nucleophilic addition of 5′-amino group across the 5,6-double bond of uracil. The structure and the reaction of this compound will be reported in a separate paper.

10) M.P. Mertes and E.A. Coats, *J. Med. Chem.*, **12**, 154 (1969).

anhydrous dioxane at room temperature followed by treatment with concentrated ammonia. O-Carbamoyl nucleoside **8** was obtained as colorless foam after purification on silica gel chromatography. The yield of this step was 60—70%. O-Carbamoyl group was indicated by a positive *p*-(N,N-dimethylamino)benzaldehyde test.[11] Acid hydrolysis gave crystalline 5′-δ-carbamoyloxy-N-carbobenzoxy-L-α-aminovaleramido-5′-deoxyuridine (**9**), mp 178—182°, from aqueous ethanol. The infrared (IR) spectrum indicated the following assignable bands: 3410, 3300, 3200 (OH, NH), 1728 (COOH), 1705 sh, 1685 (urethanes and uracil carbonyls), 1643 (amide I), 1667, 1639 cm^{-1} (amide II). The removal of the carbobenzoxy group was finally achieved by the catalytic hydrogenolysis over palladium in 80% methanol with the addition of a few drops of acetic acid.[12] Aminoacylnucleoside, **10**, was obtained as amorphous white powder of the acetate salt, $[\alpha]_D^{25}$ +14.0° (c=0.935, H_2O). It showed positive ninhydrin, *p*-(N,N-dimethylamino)benzaldehyde,[11] and periodate-benzidine tests.[13] The best overall yield from **3** was 26%. The IR spectrum showed a broad band at 1695 cm^{-1} (uracil carbonyl, carbamoyl, and amide I) and a band at 1555 cm^{-1} (amide II).

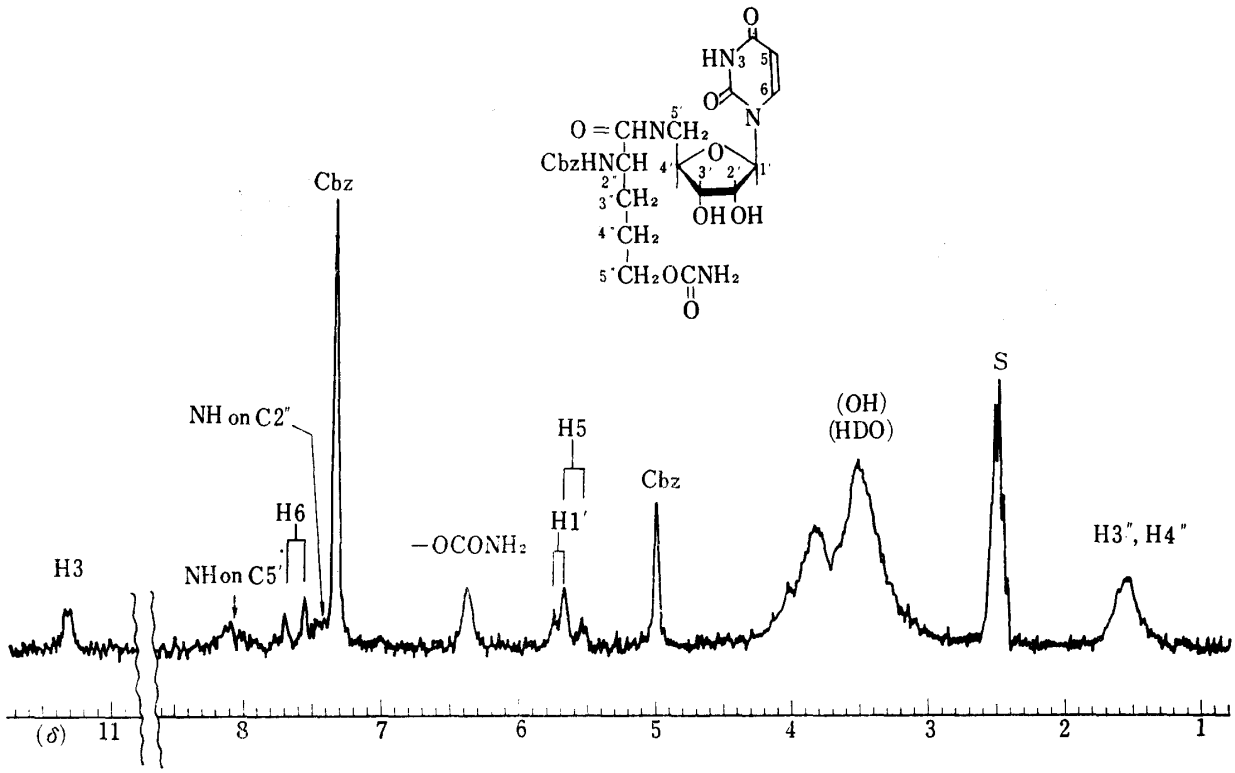


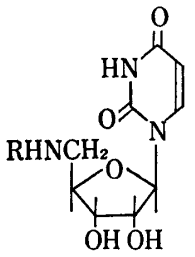
Fig. 1. H^1-NMR Spectrum of 5′-N-Carbobenzoxy-δ-carbamoyloxy-L-α-aminovaleramido-5′-deoxyuridine (**9**) in DMSO-d_6; Internal Standard, TMS.

The nuclear magnetic resonance (NMR) spectrum of **9** in DMSO-d_6 is reasonable for the structure, which with assignment is shown in Fig. 1. A long-range coupling between C-5 proton and N-3 proton (J=2.5 Hz) of uracil derivatives was well documented.[14] The NMR of **10** in D_2O indicated in addition an acetate-methyl signal at δ 1.92 and no signal attributable to a carbobenzoxy group.

11) R.M. Fink, R.E. Cline, C. McGaughey, and K. Fink, *Anal. Chem.*, **28**, 4 (1956).
12) If acetic acid was eliminated, the hydrogenation product inclined to decompose into an unidentified ninhydrin-negative substance during hydrogenation and the subsequent treatment.
13) J.A. Cionelli and F. Smith, *Anal. Chem.*, **26**, 1132 (1954).
14) *a*) S. Gronowitz, B. Norrman, B. Gestblom, B. Mathiasson, and R.A. Hoffman, *Arkiv Kemi*, **22**, 65 (1964); *b*) A.F. Cooke and J.G. Moffatt, *J. Am. Chem. Soc.*, **89**, 2697 (1967); *c*) R.J. Cushley, I. Wempen, and J.J. Fox, *ibid.*, **90**, 709 (1968); *d*) A.J.H. Nollet, G.J. Koomen, W.F.A. Grose, and U.K. Pandit, *Tetrahedron Letters*, **1967**, 4607.

An aliquot of **7** was subjected to hydrolysis to yield **11**, followed by hydrogenation to afford aminoacyl nucleoside **17**.

Considering the structures of aminoacyl pyrimidine nucleoside antibiotics, blasticidin S[15] and gougerotin[16] as well as polyoxins, it seemed also to be interesting to substitute the amino acid with various ω-substituted amino acids. Thus L-glutaminyl (**18**), L-lysyl (**19**), L-arginyl (**20**), L-citrullyl (**21**) as well as L-alanyl (**22**) derivatives of 5′-amino-5′-deoxyuridine were prepared utilizing either the standard *p*-nitrophenylester method[17] or the mixed anhydride method.[18] The latter method was adopted for the preparation of aminoacyl derivatives of 1-(3-amino-3-deoxy-β-D-glucopyranosyl)uracil.[19] Carbobenzoxy group was used to protect the amino group and nitro-L-arginine[20] was employed for protection of a guanido group. Properties of all the aminoacylnucleosides and the corresponding N-carbobenzoxy derivatives were described in the experimental part. Because of the difficulties encountered in purification of free aminoacylnucleosides, characterization was more intensively made with the carbobenzoxy derivatives (**11**—**16**). After hydrogenation over palladium in 80% methanol added with few drops of acetic acid,[12] aminoacyl nucleosides acetate (**17**—**22**) were obtained as amorphous white powder from water–ethanol–ether.



11: R=COCH(NHCbz)$CH_2CH_2CH_2OH$
12: R=COCH(NHCbz)$CH_2CH_2CONHH_2$
13: R=COCH(NHCbz)$CH_2CH_2CH_2CH_2NHCbz$
14: R=COCH(NHCbz)$CH_2CH_2CH_2NHC(=NNO_2)NH_2$
15: R=COCH(NHCbz)$CH_2CH_2CH_2NHCONH_2$
16: R=COCH(NHCbz)CH_3
17: R=δ-hydroxy-L-norvalyl(AcOH)
18: R=L-glutaminyl(AcOH)
19: R=L-lysyl(AcOH)
20: R=L-arginyl(AcOH)
21: R=L-citrullyl(AcOH)
22: R=L-alanyl(AcOH)

Chart 3

Antimicrobial activity of the aminoacyl nucleosides **10** and **17**—**22** was tested by the conventional pulp-agar plate diffusion method. No inhibitory effect was observed, however, when 10 mg/ml solutions were tested for the following microorganisms; *Pellicularia sasakii, Piricularia oryzae, Alternaria kikuchiana, Screrotinia cinerea, Glomerella cingulata, Diaporsche citri, Penicillium chrysogenum, Candida albicans, Mycobacterium tuberculosis* 607, *Mycobacterium phlei, Bacillus subtilis, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Xanthomonas oryzae.*

Comparing the structure **10** with that of polyoxin M[3b] (**1m**) which constitutes the smallest molecule among the active polyoxins, it appears that the lack of 5′-carboxyl and/or 4″-hydroxyl in **10** caused the loss of activity. The carboxyl may be expected to be the important group, because it would place a negative charge in a position analogous to that of the phosphate groups in UDP-N-acetylglucosamine.

15) *a*) J.J. Fox and K.A. Watanabe, *Tetrahedron Letters*, **1966**, 897; *b*) H. Yonehara and N. Otake, *ibid.*, **1966**, 3785.
16) J.J. Fox, Y. Kuwada, and K.A. Watanabe, *Tetrahedron Letters*, **1968**, 6029.
17) *a*) M. Bodanszky, *Nature*, **175**, 685 (1955); *b*) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
18) *a*) R.A. Boissonas, *Helv. Chim. Acta*, **34**, 874 (1951); *b*) J.R. Vaughan, Jr., *J. Am. Chem. Soc.*, **74**, 6137 (1952); *c*) J.R. Vaughan Jr. and R.L. Osato, *ibid.*, **74**, 676 (1952); *d*) T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).
19) H.A. Friedman, K.A. Watanabe, and J.J. Fox, *J. Org. Chem.*, **32**, 3775 (1967).
20) K. Hofmann, W.D. Peckham, and A. Rheiner, *J. Am. Chem. Soc.*, **78**, 238 (1956).

Experimental

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were run on a Perkin-Elmer 521 grating infrared spectrophotometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. NMR spectra were run on a JNM-C60 NMR spectrometer. Chemical shifts were measured to an internal standard TMS or DSS, and are recorded as δ values. Coupling constants were expressed in Hz. Thin-layer chromatography were developed on either Silica Gel G (E. Merck AG) or Avicel SF microcrystalline cellulose for free aminoacyl nucleosides. Nucleosides were detected under ultraviolet ray (254 mμ). 2′,3′-Diols were detected with a periodate-benzidine spray reagent[13] and amino groups were detected with a ninhydrin test. Lemieux's reagent[21] was also used to detect nucleosides. Mallinckrodt's Silicic Acid AR was employed for column chromatography. All samples for elemental analysis of aminoacyl nucleosides (acetate) were dried over P_2O_5 at 56° and those of their derivatives were dried at 100° just before analysis.

5′-Acetamido-5′-deoxyuridine (**4**)——A solution of 400 mg of 5′-azido-5′-deoxy-2′,3′-O-isopropylideneuridine[7] (**2**) in 10 ml of EtOH was hydrogenated over 40 mg of palladium black under 3.5 kg/cm^2 pressure at room temperature for 3 hr. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to dryness, yielding white foam of **3** which was dried *in vacuo* over P_2O_5. The residue was dissolved in 4 ml of pyridine and added with 1 ml of acetic anhydride and the solution was allowed to stand overnight at room temperature. The reaction solution was concentrated to dryness and the residue was dissolved in 5 ml of 50% AcOH and refluxed for 30 min. The hydrolyzate was passed through a column of Dowex 50W-X8 (H, 3 ml) and the eluate was concentrated to dryness. The residue was recrystallized from aqueous EtOH, affording 214 mg of **4**, mp 235—236° (decomp., $[\alpha]_D^{28}$ +22.6° (c=1.123, H_2O). Calcd. for $C_{11}H_{15}O_6N_3$: C, 46.31; H, 5.30; N, 14.73. Found: C, 46.31; H, 5.28; N, 14.75. NMR (DMSO-d_6): 1.82 (3H, s, C$\underline{H}_3$), 5.64 (1H, q, H-5, $J_{5,6}$=8.0, $J_{3,5}$=2.2), 5.71 (1H, d, H-1′, $J_{1',2'}$=5.0), 7.67 (1H, d, H-6, $J_{5,6}$=8.0), 8.02 (1H, diffusedt, N$\underline{H}$ on C-5′, J=5.5), 11.50 (1H, broad s, H-3).

N-Carbobenzoxy-δ-hydroxy-L-α-aminovaleric Acid (**5**)——To an ice-cooled solution of 799 mg of δ-hydroxy-L-α-aminovaleric acid[8] in 6 ml of 1N NaOH, 3.42 g of carbobenzoxy chloride (30% toluene solution) and 7 ml of 1N NaOH were added dropwise under stirring over 50 min. After stirring additional 2 hr, the reaction mixture was washed with ether and passed through a column of 16 ml of Dowex 50W-X8 (H-form). The effluent and washings were combined and concentrated to small volume. Crystals appeared were collected and recrystallized from aqueous ethanol, yielding 1.15 g of **5**, mp 119—122°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1500. *Anal.* Calcd. for $C_{13}H_{17}O_5N$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.49; H, 6.51; N, 5.25.

N-Carbobenzoxy-L-α-aminovalero-δ-lactone (**6**)——To an ice cooled solution of 1.14 g of **5** in 200 ml of EtOAc was added 883 mg of DCC, and the resulting solution was left to stand overnight at room temperature. Urea was filtered off and the filtrate was concentrated *in vacuo* to dryness. The syrup obtained was purified on silica gel chromatography with benzene–ethyl acetate (8: 2). From the main fractions, 838 mg of crystals of **6** were obtained, mp 97—101°. Mass Spectrum *m/e*: 249.1037 (M$^+$). Calcd. for $C_{13}H_{15}O_4N$: 249.1001. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (lactone), 1720, 1510.

5′-N-Carbobenzoxy-δ-hydroxy-L-α-aminovaleramido-5′-deoxy-2′,3′-O-isopropylideneuridine (**7**)——Compound **3** prepared by hydrogenation of 309 mg of **2** as described before was dissolved in 6 ml of anhydrous dioxane and to this a solution of **6** (250 mg) in 6 ml of dioxane and 3 drops of Et_3N were added and the resulting solution was refluxed for 4 hr. The reaction solution was concentrated to dryness and the residue was purified on silica gel chromatography (benzene–acetone, 1:1). From the fraction having UV absorption but no ninhydrin reaction, 281 mg of **7** was obtained as colorless foam. It gave only one spot on silica gel TLC (benzene–acetone, 1:1) with an *Rf* value of 0.41. NMR ($CDCl_3$–D_2O): 1.33 (3H, s, C$\underline{H}_3$), 1.53 (3H, s, C$\underline{H}_3$), 3.64 (4H, m, 5′,5″-C$\underline{H}_2$), 4.27 (2H, m), 4.81 (1H, m) (2′,3′,4′-C$\underline{H}$), 5.07 (2H, s, Cbz), 5.11 (1H, m, H-2″), 5.38 (1H, d, H-1′, $J_{1',2'}$=2.0), 5.72 (1H, d, 5-H, $J_{5,6}$=8.0), 7.22 (1H, d, H-6), 7.31 (5H, s, Cbz).

5′-N-Carbobenzoxy-δ-carbamoyloxy-L-α-aminovaleramido-5′-deoxyuridine (**9**)——To an ice-cooled solution of 210 mg of **7** in 20 ml of anhydrous dioxane added with 35 mg of anhydrous pyridine, 1 ml of 4.8% $COCl_2$ solution in toluene was added dropwise under stirring. After stirring for 30 min in an ice-bath and additional 2 hr at room temperature, the reaction mixture was concentrated *in vacuo* to yield syrup. It was dissolved in 2 ml of dioxane and the solution was ice-cooled and added with 4 ml of ice-cooled conc. NH_4OH under stirring. It was then brought to room temperature and after 30 min, the reaction mixture was concentrated to dryness. The residue was extracted with acetone and NH_4Cl was filtered off. The filtrate was concentrated to dryness, yielding 226 mg of pale-yellow foam. It was purified on silica gel chromatography (benzene–acetone, 1:1). From the main fraction, 160 mg of colorless foam of **8** was obtained. It gave only one spot on silica gel TLC (benzene–acetone, 1:1) with an *Rf* value of 0.50 and was positive to *p*-(N,N-dimethylamino)benzaldehyde test.[10] Compound **8** (130 mg) was refluxed in 3 ml of 50% acetic acid for 30 min. The hydrolyzate was concentrated to dryness and the residue was

21) R.U. Lemieux and H.F. Bauer, *Anal. Chem.*, **26**, 920 (1954).

recrystallized from aqueous ethanol, affording 80 mg of crystals of **9**, mp 178—182°. It showed only one spot on cellulose TLC (butanol–acetic acid–water, 4:1:2) with an *Rf* value of 0.66 positive to *p*-(N,N-dimethylamino)benzaldehyde and periodate–benzidine tests. *Anal.* Calcd. for $C_{23}H_{29}O_{10}N_5$: C, 51.58; H, 5.46; N, 13.08. Found: C, 51.23; H, 5.48; N, 13.07. NMR (DMSO-d_6) (Fig. 1): 1.56 (4H, m, 3″,4″-C$\underline{H}_2$), 5.00 (2H, s, Cbz), 5.61 (1H, diffused d, H-5), 5.72 (1H, d, H-1′, J=4.8), 6.40 (2H, diffused s, -OCON$\underline{H}_2$), 7.32 (5H, s, Cbz), ~7.4 (1H, m, N$\underline{H}$ on C-2″), 7.63 (1H, d, H-6, $J_{5,6}$=8.3), ~8.1 (1H, m, N$\underline{H}$ on C-5′), 11.32 (1H, diffused d, H-3, $J_{3,5}$=2.5). 2′,3′,4′-C$\underline{H}$, 5′-C$\underline{H}_2$, 2″-C$\underline{H}$, 5″-C$\underline{H}_2$, and 2′,3′-O$\underline{H}$ were involved in 3.0—4.3 HDO region and not resolved. IR ν_{max}^{KBr} cm^{-1}: 3410, 3300, 3200, 3043, 3030, 2950, 1728, 1705, 1685, 1667, 1643, 1639, 1468, 1420, 1393, 1370, 1350, 1327, 1284, 1270, 1247, 1095, 1605, 1045, 1025, 920, 853, 820, 780, 756, 727, 700, 570, 545, and 440.

5′-δ-Carbamoyloxy-L-α-aminovaleramido-5′-deoxyuridine (10)——Compound **9** (50 mg) was dissolved in 20 ml of 80% MeOH containing 4 drops of acetic acid along with 10 ml g of palladium black. It was hydrogenated under 3.5 kg/cm^2 pressure for 3 hr at room temperature. The catalyst was removed by filtration and the filtrate was concentrated to small volume and treated with EtOH and ether. Precipitates were collected by filtration and washed with EtOH–ether, affording 35 mg of white amorphous powder of **10** acetate, $[\alpha]_D^{25}$ +14.0° (c=0.935, H_2O). Cellulose TLC (BuOH–AcOH–H_2O, 4:1:2) indicated only one UV absorbing ninhydrin ad periodate positive spot with an *Rf* value 0.18.[22] *Anal.* Calcd. for $C_{15}H_{23}O_8N_5 \cdot C_2H_4O_2 \cdot 1/2C_2H_6O$: C, 44.62; H, 6.24; N, 14.46. Found: C, 45.51; H, 5.99; N, 14.18. IR ν_{max}^{KBr} cm^{-1}: 3350, 1695, 1555, 1480, 1412, 1340, 1263, 1218, 1080, 820, 768, 565.

5′-N-Carbobenzoxy-δ-hydroxy-L-α-aminovaleramido-5′-deoxyuridine (11)——Compound **7** (170 mg) was dissolved in 5 ml of 50% acetic acid and refluxed for 30 min. The hydrolyzate was concentrated to dryness and the residue was purified by silica gel chromatoraphy (benzene–EtOH, 3:1). UV-absorbing periodate–benzidine positive fractions were combined and concentrated to small volume and treated with EtOH~ether affording 63 mg of white powder, mp 170—174°. *Anal.* Calcd. for $C_{22}H_{28}O_9N_4$: C, 53.65; H, 5.73; N, 11.38. Found: C, 52.79; H, 5.94; N, 11.12. NMR (DMSO-d_6): 1.50 (4H, m, 3″,4″-C$\underline{H}_2$), 4.40 (1H, diffused t, O$\underline{H}$ on C-5″), 5.01 (2H, s, Cbz), 5.15, 5.38 (each 1H, diffused d, O$\underline{H}$ on C-2′,3′), 5.62 (1H, d, H-5), 5.73 (1H, d, H-1′, $J_{1'2'}$=5.2), 7.33 (5H, s, Cbz), ~7.35 (1H, m, N$\underline{H}$ on C-2″), 7.63 (1H, d, H-6), ~8.05 (1H, m, N$\underline{H}$ on C-5′), 11.33 (1H, broad s, H-3).

5′-δ-Hydroxy-L-α-aminovaleramido-5′-deoxyuridine (17)——Compound **11** (64 mg) was hydrogenated over 40 mg of palladium black in 20 ml of 80% MeOH containing 3 drops of acetic acid under 3.5 kg/cm^2 pressure for 3 hr at room temperature. The catalyst was removed by filtration and the solution was concentrated to small volume, to which EtOH and ether was added. The precipitates were collected by filtration and washed with EtOH–ether, yielding 35 mg of colorless powder of **17** acetate, $[\alpha]_D^{26}$ +28.6° (c=0.97, H_2O). Cellulose TLC (BuOH–AcOH–H_2O, 4:1:2) indicated only one spot with an *Rf* value of 0.20. *Anal.* Calcd. for $C_{14}H_{22}O_7N_4 \cdot C_2H_4O_2$: C, 45.93; H, 6.26; N, 13.39. Found: C, 44.95; H, 6.44; N, 13.68. IR ν_{max}^{KBr} cm^{-1}: ~3350, 3080, 2925, 2860, ~1690, 1545, 1487, 1450, 1380, 1348, 1290, 1217, 1130, 1055, 925, 865, 820, 765, 610, 490, 430.

5′-N-Carbobenzoxy-L-glutaminylamido-5′-deoxyuridine (12)——Compound **3** prepared by hydrogenation of 465 mg of **2** as described before was dissolved in 10 ml of DMF followed by addition of 597 mg of *p*-nitrophenyl N-carbobenzoxy-L-glutaminate[17b] and 10 drops of Et_3N. After standing overnight at 37°, the reaction solution was concentrated to small volume and ether was added. Precipitated syrup was washed several times with ether and subjected to silica gel chromatography with benzene–acetone (1:1→1:4). From main fraction, 510 mg of colorless foam of carbobenzoxy isopropylidene derivative was obtained, mp 105—110°. *Anal.* Calcd. for $C_{25}H_{33}O_9N_5$: C, 54:84; H, 6.08; N, 12.79. Found: C, 55.21; H, 5.77; N, 12.79.

This compound (430 mg) was dissolved in 7 ml of 50% AcOH and the solution was refluxed for 30 min, then concentrated to dryness. The residue was recrystallized from aqueous EtOH affording 330 mg of crystalline powder, mp 191—192°, $[\alpha]_D^{25}$+3.9° (c=1.270, DMF). *Anal.* Calcd. for $C_{22}H_{27}O_9N_5$: C, 52.27; H, 5.38; N, 13.86. Found: C, 51.76; H, 5.63; N, 13.78. NMR (DMSO-d_6): 1.5—2.4 (4H, m, 3″,4″-C$\underline{H}_2$), ~3.4 (2H, m, 5′-C$\underline{H}_2$), ~3.9 (4H, m, H-2′,3′,4′,2″), 5.01 (2H, s, Cbz), 5.18, 5.40 (each 1 H, diffused d, 2′,3′-O$\underline{H}$), 5.63 (1H, d, 5-H), 5.74 (1H, d, H-1′, J=5.0), 6.75 (2H, broad s, -CON$\underline{H}_2$), ~7.3 (1H, m, N$\underline{H}$ on C-2′), 7.32 (5H, s, Cbz), 7.63 (1H, d, H-6, $J_{5,6}$=8.5), ~8.05 (1H, m, N$\underline{H}$ on C-5′).

5′-L-Glutaminylamido-5′-deoxyuridine (18)——Compound **12** (80 mg) was hydrogenated over 30 mg of palladium black in 100 ml of 80% MeOH containing 4 drops of acetic acid under 3.5 kg/cm^2 pressure for 5 hr at room temperature. The catalyst was filtered off and the filtrate was concentrated to small volume and treated with EtOH and ether. Precipitates were collected by filtration and washed with EtOH–ether affording 50 mg of hydroscopic white powder of **18** acetate. Cellulose TLC (BuOH–AcOH–H_2O, 4:1:2) indicated only one UV-absorbing ninhydrin and periodate positive spot with an *Rf* value of 0.15. $[\alpha]_D^{25}$+26.2° (c=1.058, H_2O). *Anal.* Calcd. for $C_{14}H_{21}O_7N_5 \cdot C_2H_4O_2$: C, 44.54; H, 5.84; N, 16.24. Found: C, 44.88; H, 6.31; N, 16.43. NMR (D_2O): 1.92 (3H, s, $C\underline{H}_3COO^-$), 2.0—2.5 (4H, m, 3″,4″-C$\underline{H}_2$), 5.82 (1H, d, H-1′,

22) In the same run, *Rf* values of polyoxins L and M were 0.11 and 0.14, respectively.

$J_{1',2'}$=4.3), 5.91 (1H, d, H-5), 7.67 (1H, d, H-6, $J_{5,6}$=8.4). IR ν_{max}^{KBr} cm^{-1}: ~3350, ~1670, 1555, 1415, 1265, 1220, 1110, 1070, 820, 768, 565.

5′-N$^{\alpha}$,N$^{\varepsilon}$-Dicarbobenzoxy-L-lysylamido-5′-deoxyuridine (13)——Compound 3 prepared from 309 mg of azide 2 as foregoing was dissolved in 5 ml of DMF followed by addition of 536 mg of *p*-nitrophenyl N$^{\alpha}$,N$^{\varepsilon}$-dicarbobenzoxy-L-lysinate[23] and 7 drops of Et_3N. After standing 2 days at 37°, the reaction solution was concentrated to small volume and ether was added. Precipitated syrup was washed with ether and subjected to silica gel chromatography (benzene–acetone, 1:1) to afford 405 mg of purified white powder of 5′-N$^{\alpha}$,N$^{\varepsilon}$-dicarbobenzoxy-L-lysylamido-5′-deoxy-2′,3′-O-isopropylideneuridine.

This compound (354 mg) was refluxed for 30 min in 10 ml of 50% AcOH and the hydrolyzate was concentrated to dryness and the residue was recrystallized from aqueous EtOH to afford 250 mg of crystals of 13, mp 151—153°, $[\alpha]_D^{23}$+3.6° (c=1.043, DMF). *Anal.* Calcd. for $C_{31}H_{37}O_{10}N_5$: C, 58.21; H, 5.83; N, 10.95. Found: C, 58.11; H, 5.62; N, 10.91. NMR (DMSO-d_6): 1.1—1.7 (6H, m, 3″,4″,5″-C$\underline{H}_2$), 5.03 (4H, s, Cbz), 5.22, 5.44 (each 1H, d, 2′,3′-O$\underline{H}$), 5.66 (1H, d, H-5), 5.76 (1H, d, H-1′, $J_{1',2'}$=4.6), 7.1—7.5 (2H, m, N$\underline{H}$ on C-2″,6″), 7.35 (10H, s, Cbz), 7.67 (1H, d, H-6, $J_{5,6}$=8.6), 8.08 (1H, m, N$\underline{H}$ on C-5′).

5′-L-Lysylamido-5′-deoxyuridine (19)——Compound 13 (100 mg) was hydrogenated over 30 mg of palladium black in 50 ml of 80% MeOH containing 4 drops of AcOH under 3.5 kg/cm^2 pressure for 3 hr at room temperature. After the conventional treatment as described before, 60 mg of white powder of 19 acetate was obtained, $[\alpha]_D^{23}$+17.7° (c=1.165, H_2O). Cellulose TLC (BuOH–AcOH–H_2O, 4:1:2) indicated only one UV-absorbing ninhydrin and periodate-positive spot with an *Rf* value of 0.13. *Anal.* Calcd. for $C_{15}H_{25}O_6N_5 \cdot 2C_2H_4O_2$: C, 46.43; H, 6.78; N, 14.25. Found: C, 46.51; H, 6.87; N, 14.65, IR ν_{max}^{KBr} cm^{-1}: ~3300, ~1690, 1560, 1410, 1340, 1275, 1220, 1135, 1050, 928, 820, 770, 660, 435.

5′-N$^{\alpha}$-Carbobenzoxy-L-nitroarginylamido-5′-deoxyuridine (14)——N-Carbobenzoxy-L-nitroarginine[20] (936 mg) was dissolved in 27 ml of dry dioxane along with 491 mg of *n*-Bu_3N and the solution was ice-cooled, then added with 288 mg of ethyl chloroformate. After 45 min, compound 3 (prepared from 819 mg of 2 as foregoing) dissolved in 16 ml of dioxane was added. After standing 2 hr at room temperature, the reaction mixture was concentrated to dryness and the resulting syrup was subjected to silica gel chromatography (benzene–acetone, 1:1). UV-absorbing and ninhydrin negative fractions were combined and the solvent was evaporated, affording 986 mg of colorless foam. This compound (600 mg) was refluxed in 20 ml of 50% AcOH for 1 hr. The hydrolyzate was concentrated to dryness and the residue was purified on silica gel chromatography (benzene–MeOH, 4:1). UV-absorbing periodate-positive fractions were combined and concentrated to dryness affording 377 mg of white powder of 14. Cyrstallization was not successful, mp 135—140°. *Anal.* Calcd. for $C_{23}H_{30}O_{10}N_8$: C, 47.75; H, 5.23; N, 19.37. Found: C, 47.42; H, 5.26; N, 19.06. NMR (DMSO-d_6): ~1.55 (4H, m, 3″,4″-C$\underline{H}_2$), 5.01 (2H, s, Cbz), 5.62 (1H, d, H-5), 5.73 (1H, d, H-1′, $J_{1',2'}$=4.8), 7.33 (5H, s, Cbz), ~7.4 (1H, m, N$\underline{H}$ on C-2″), 7.92 (1H, m, N$\underline{H}$ on C-5″), 8.13 (1H, m, N$\underline{H}$ on C-5′).

5-L-Arginylamido-5′-deoxyuridine (20)——Compound 14 (220 mg) was hydrogenated over 70 mg palladium black in 20 ml of 80% MeOH containing 5 drops of AcOH under 3.5 kg/cm^2 pressure for 3.5 hr at room temperature. After the conventional procedure as foregoing, 129 mg of amorphosu powder of 20 acetate was obtained, $[\alpha]_D^{26}$+14.0° (c=1.00, H_2O). Cellulose TLC (BuOH–AcOH–H_2O, 4:1:2) indicated only one UV-absorbing ninhydrin and periodate-positive spot with an *Rf* value of 0.13. IR ν_{max}^{KBr} cm^{-1}: ~3350, ~1670, 1550, 1410, 1280, 1220, 1130, 820, 768, 655, 430. Satisfactory elemental analysis was not obtained.

5′-N-Carbobenzoxy-L-citrullylamido-5′-deoxyuridine (15)——N$^{\alpha}$-Carbobenzoxy-L-citrulline[24] (309 mg) was dissolved in 5 ml of anhydrous dioxane along with 185 mg of *n*-Bu_3N and the solution was ice-cooled, then, added with 109 mg of ethyl chloroformate. After 30 min, compound 3 (prepared from 309 mg of 2 as foregoing) dissolved in 3 ml of dioxane was added. After standing overnight at room temperature, the reaction mixture was concentrated to dryness and the resulting syrup was subjected to silica gel chromatography (benzene–acetone, 1:4). From UV-absorbing and ninhydrin negative fractions, 404 mg of the product was obtained, which was hydrolyzed by refluxing 30 min in 10 ml of 50% AcOH. After the conventional procedure, 278 mg of white amorphous powder of 15 was obtained, which resisted for crystallization, mp 163—171°. *Anal.* Calcd. for $C_{23}H_{30}O_9N_6$: C, 51.68; H, 5.66; N, 15.72. Found: C, 51.16; H, 5.78; N, 15.59. NMR (DMSO-d_6): ~1.45 (4H, m, 3″,4″-C$\underline{H}_2$), ~2.40 (2H, m, 5″-C$\underline{H}_2$), 5.01 (2H, s, Cbz), 5.40 (2H, broad s, -NHCON$\underline{H}_2$), 5.65 (1H, d, H-5), 5.75 (1H, d, H-1′, $J_{1',2'}$=4.7), 5.96 (1H, m, N$\underline{H}$ on C-5″), 7.36 (5H, s, Cbz), ~7.9 (1H, m, N$\underline{H}$ on C-2″), 7.67 (1H, d, H-6, $J_{5,6}$=8.4), 8.10 (1H, m, N$\underline{H}$ on C-5′), 11.39 (1H, broad s, H-3).

5′-L-Citrullylamido-5′-deoxyuridine (21)——Compound 15 (96 mg) was hydrogenated over 40 mg of palladium black in 20 ml of 80% MeOH containing 4 drops of AcOH under 3.5 kg/cm^2 pressure for 3 hr at room temperature. Conventional treatment gave 70 mg of white amorphous powder of 21 acetate, $[\alpha]_D^{21}$+4.5° (c=1.00, H_2O). Cellulose TLC (BuOH–AcOH–H_2O, 4:1:2) indicated only one UV-absorbing ninhydrin and periodate-positive spot with an *Rf* value of 0.20. IR ν_{max}^{KBr} cm^{-1}: ~3350, ~1680, 1560, 1483, 1445, 1380, 1340, 1286, 1217, 1135, 1065, 820, 767, 560, 430. Satisfactory elemental analysis was not obtained.

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5′-N-Carbobenzoxy-L-alanylamido-5′-deoxyuridine (16)——Carbobenzoxy-L-alanine (446 mg) was dissolved in 9 ml of $CHCl_3$ along with 371 mg of n-Bu_3N and the solution was ice-cooled, then, added with 217 mg of ethyl chloroformate. After 1 hr, compound 3 (prepared from 618 mg of 2 as foregoing) dissolved in 16 ml of dioxane was added, and allowed to react for 2 hr at room temperature. An amorphous coupling product (715 mg) was obtained after purification on silica gel chromatography (benzene–acetone, 3:1). This compound (322 mg) was refluxed 30 min in 10 ml of 50% AcOH. From the hydrolyzate, 230 mg of crystalline **16** was obtained, which was recrystallized from aqueous EtOH, mp 187—190°. *Anal.* Calcd. for $C_{20}H_{24}O_8N_4$: C, 53.57; H, 5.39; N, 12.50. Found: C, 53.65; H, 5.30; N, 12.80. NMR (DMSO-d_6): 1.15 (3H, d, 3″-CH_3), 5.01 (2H, s, Cbz), 5.63 (1H, d, H-5), 5.74 (1H, d, H-1′, $J_{1',2'}=5.4$), 7.34 (5H, s, Cbz), ~7.4 (1H, m, NH on C-2″), 7.65 (1H, d, H-6, $J_{5,6}=8.0$), 8.04 (1H, diffused t, NH on C-5′), 11.39 (broad s, H-3).

5′-L-Alanylamido-5′-deoxyuridine (22)——Compound **16** (120 mg) was hydrogenated over 50 mg of palladium black in 20 ml of 80% MeOH containing 4 drops of AcOH under 3.5 kg/cm² pressure for 3 hr at room temperature. Conventional treatment afforded 46 mg of white amorphous powder of **22** acetate, $[\alpha]_D^{26}+8.5°$ ($c=1.00$, H_2O). Cellulose TLC (BuOH–AcOH–H_2O, 4:1:2) indicated only one UV-absorbing ninhydrin and periodate–benzidine positive spot with an Rf value of 0.27. *Anal.* Calcd. for $C_{12}H_{18}O_6N_4 \cdot C_2H_4O_2$: C, 44.92; H, 5.92; N, 14.97. Found: C, 45.33; H, 6.30; N, 14.71. IR ν_{max}^{KBr} cm^{-1}: ~3300, ~1690, 1560, 1485, 1450, 1410, 1380, 1347, 1285, 1215, 1127, 1025, 930, 820, 767, 720, 656, 430.

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