# SYNTHESIS OF 3-(3-AMINO-3-CARBOXYPROPYL)URIDINE (A MODIFIED NUCLEOSIDE IN CERTAIN RNAS) BY FOUR COMPONENT CONDENSATION [1]

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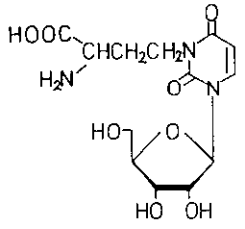
3-(3-Amino-3-carboxypropyl)uridine [a modified nucleoside in certain transfer RNA (viz., Echerichia coli $tRNA_1^{Ile}$)] was prepared by a simultaneous condensation of four components [aldehyde, (2-picolyl 1-oxide)amine, cyclohexenyl-isocyanide and acetic acid] as the key reaction.

Among a large number of naturally occurring nucleosides,[2,3,4] 3-(3-amino-3-carboxypropyl)uridine (1),[5] Polyoxins,[6] Neopolyoxins,[7] Nikkomycins,[8] Sinefungin and A 9141C antibiotic[9] are unique in that



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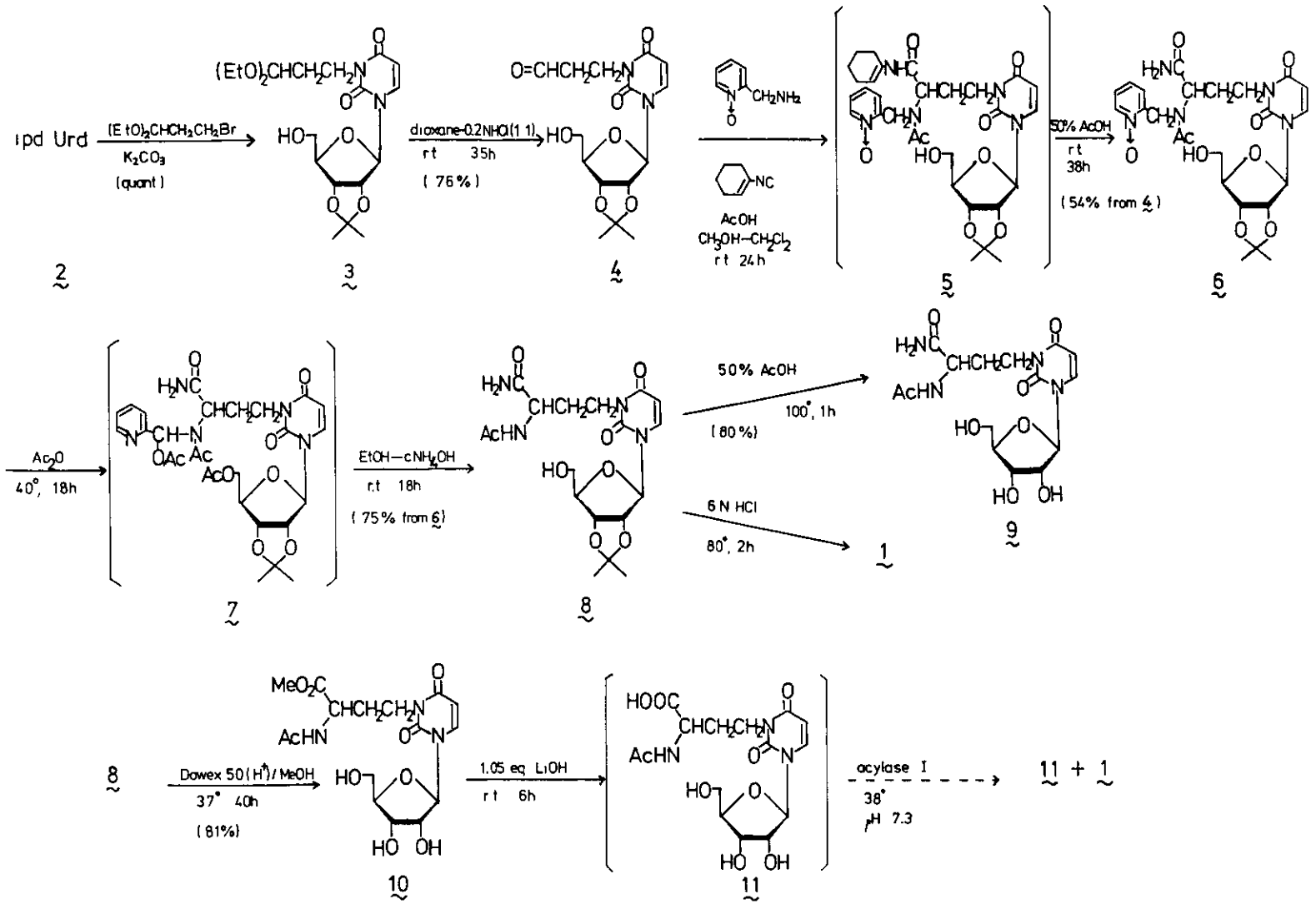
the molecules consist of a hybrid of the nucleoside and the amino acid. With some exceptions (viz, Sinefungin and Nikkomycins), chemical syntheses of most nucleosides of the hybrid type have been achieved by a number of workers. Thus, Ohashi and coworkers have prepared 1 by the alkylation of 2', 3'-O-isopropylideneuridine with ethyl L-α-benzamido-γ-bromobutyrate, followed by deblocking in overall yield of 42%.[10] Seela and Cramer have also prepared 1 and the corresponding 5'-phosphate by a similar procedure.[11]

However, as a part of our continuing synthetic studies of natural products by application of

four component condensation[12] (Ugi reaction) involving amines and isocyanides of 2-picolyl 1-oxide series[13], the synthesis of 3-(3-amino-3-carboxylpropyl)uridine(1) was attempted by the use of this condensation reaction as the key reaction, emphasis being laid upon a comparatively large scale preparation.

Synthetic sequence of our approach to 1 is shown in Scheme 1. 2',3'-O-Isopropyrideneuridine (2) (12 g, 42.3 mmol) was reacted with 3-bromopropanal diethyl acetal (14 g, 66.4 mmol) in DMF (150 ml) in the presence of potassium carbonate (19.5 g) at 70° for 3 days. After usual work-up including silica gel column chromatography (silica:450g, eluting system:$CHCl_3$-$CH_3OH$ 1000:20), 2',3'-O-isopropylidene 3-(3,3-diethoxypropyl)uridine(3) was obtained in a quantitative yield. The structure was confirmed by nmr [nmr ($CDCl_3$)$\delta$ : 4.62 ppm (t, 1H, $(EtO)_2CH$-)] and mass spectroscopy [ms (m/e) 399 ($M^+$-15)]. The peak is characteristic of 2',3'-O-isopropylidenenucleoside. Hydrolysis of 3 (17.5 g, 42.3 mmol) in dioxane-0.2N HCl (1:1; 40 ml) at room temperature for 3.5 hr afforded the corresponding aldehyde (4), yield being 76%. Nmr ($CDCl_3$)$\delta$: 9.75 ppm (s, 1H, CH=O), ms (m/e):325 ($M^+$-15). 2',3'-O-Isopropylidene-3-(2-formylethyl)uridine (4, 11.0 g, 30.7 mmol) was allowed to react with (2-picolyl 1-oxide)amine[13] (3.8 g, 30.7 mmol) (2-picolyl 1-oxide will be referred to as "op", hereafter), cyclohexenyl isocyanide (3.3 g, 30.8 mmol),[14] and acetic acid (1.9 ml) (whose molar ratios being 1:1:1), in MeOH-$CH_2Cl_2$ (1:1; 60 ml) at room temperature for 24 hr to afford a crude product (5). The structure was deduced on the basis of the presence of cyclohexenyl [$\delta$ : 1.6 ppm (4H), 2.1 ppm (4H), 5.8 ppm (1H)], "op" ($\delta$ : 7.35 ppm (3H), 8.25 ppm (1H), 4.9 ppm (2H)], acetyl [$\delta$ : 2.1 ppm (3H)] and isopropylidene group [$\delta$ : 1.4 ppm (3H), 1.6 ppm (3H)] in nmr spectra ($CDCl_3$). Removal of the cyclohexenyl group in 5 could be effected by the treatment with 50% aq. acetic acid (50 ml) at room temperature for 38 hr to afford 6. The structure was confirmed by spectral data: [ms(m/e), 515 ($M^+$-18), 473 ($M^+$-60); nmr ($CDCl_3$) spectra showed the presence of "op" group [$\delta$ : 7.35 ppm (2H), 7.56 ppm (1H), 8.24 ppm (1H), 4.89 ppm (2H)], acetyl ($\delta$ : 2.15 ppm (3H), and isopropylidene ($\delta$ : 1.35 ppm (3H), 1.57 ppm (3H) group]. Anal. Calcd for $C_{24}H_{31}N_5O_9$ 1/2 $CDCl_3$: C, 49.60; H, 5.31; N, 11.80. Found: C, 49.55; H, 5.54; N, 11.65. Treatment of 6 (5 g, 9.4 mmol) with excess acetic anhydride (20 ml) at 40° for 18 hr, followed by separation by the aid of column afforded 7, which in turn was treated with ethanolic ammonia (20 ml) at room temperature for 18 hr. Work-up including silica gel column chromatography (silica:100 g; eluting system being $CHCl_3$-$CH_3OH$ 1000:20) afforded 8 as a homogeneous foam. Ms (m/e)426 ($M^+$), 411 ($M^+$-15); nmr ($CDCl_3$) spectral data ($\delta$ : 7.40 ppm (d, 1H, H6, J = 8Hz), 7.0 ppm 6.8 ppm (bs, 2H, $-CONH_2$), 5.77 ppm (d, 1H, H5, J = 8Hz), 5.60 ppm (d, 1H, H1')] was also consistent with the assigned structure. Anal. Calcd for $C_{18}H_{26}N_4O_8 \cdot 1/2\ CHCl_3$: C, 45.70; H, 5.45; N, 11.53; Cl, 10.97. Found: C, 45.48; H, 5.41; N, 11.14; Cl, 11.12. Yield was 75% on the basis of 6. Hydrolysis of isopropylidene group in 8 by a conventional method (50% aq. AcOH, 100°, 1 hr) afforded, after recrystallization from aq. EtOH an analytical sample of 9, mp 124-130° (dec.), yield being 80%.


ipd Urd
(EtO)2CHCH2CH2Br
K2CO3
(quant)
dioxane-0.2NHCl(1:1)
rt 35h
(76%)
AcOH
CH3OH-CH2Cl2
rt 24h
50% AcOH
rt
38h
(54% from 4)
Ac2O
40°, 18h
EtOH—cNH2OH
rt 18h
(75% from 6)
50% AcOH
100°, 1h
(80%)
6 N HCl
80°, 2h
Dowex 50(H+)/MeOH
37° 40h
(81%)
1.05 eq LiOH
rt 6h
acylase I
38°
pH 7.3

Scheme 1

Anal. Calcd for $C_{15}H_{22}N_4O_8 \cdot 1/5\ H_2O$: C, 46.20; H, 5.75; N, 14.37. Found: C, 46.31; H, 5.91; N, 14.01.

Solvolysis of 8 (250 mg, 0.59 mmol) with absolute methanol (20 ml) in the presence of Dowex 50W ($H^+$ form) at 37° for 40 hr gave rise to 10. Nmr (DMSO-$d_6$) $\delta$ : 3.61 ppm (S, 3H, $OCH_3$). Ms (m/e) 401 ($M^+$), 342 ($M^+$-$CO_2CH_3$). The assigned structure was also consistent with combustion values. Anal. Calcd for $C_{16}H_{23}N_3O_9$ 1/4 $CHCl_3$: C, 45.26; H, 5.40; N, 9.75. Found: C, 44.88; H, 5.51; N, 9.43. The nucleoside derivative 8 (170 mg, 0.4 mmol) was treated with 6N HCl (1 ml) at 80° for 2 hr. The ninhydrin-test positive fraction was isolated by cellulose column chromatography (column size: 2.2 cm x 47 cm; eluting system: $H_2O$-0.2M TEAB). UV: $\lambda$max (264 nm) of the product did not shift in $H_2O$, acidic and alkaline media. The compound (1) obtained had a mobility (Pep. 0.05M TEAB, pH 7.5 100V/cm, 1.5 hr) of 7 cm, compared to a mobility of 4.5 cm for 8. PPC (BuOH-AcOH-$H_2O$ 4:1:2) showed that the sample was homogeneous (Rf 0.23). TLC (avicel, solvent system: BuOH-AcOH-$NH_4OH$ 4:1:2), Rf 0.23. In the same conditions, uridine had Rf-values of 0.42. The nucleoside 11 obtained by alkaline hydrolysis of 10 is being subjected to hydrolysis with acylase 1 (pig kidney) in order to resolute the racemate.

Acknowledgement. The authors wish to thank Mr. K. Kobayashi and Miss Y. Kakizaki for technical assistance. This work was supported in part by a Grant-in Aid for special project research from the Minitry of Education, Sciences and Culture, Japan which is gratefully acknowledged.

References

1. a) Studies on the chemical synthesis of potential antimetabolites. XXVI: For XXV, see, K. Tsuchida, K. Ikeda, and Y. Mizuno, Chem. Pharm. Bull., in press. b) A part of this work has been presented in the 8th Symposium on Nucleic Acids Chemistry held in Sapporo, August, 1980.
2. R. H. Hall, "The Modified Nucleosides in Nucleic Acids", Columbia University press, New York, N.Y., 1971.
3. S. Nakamura and H, Kondo, Heterocycles, 8, 583 (1977).
4. R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley-Interscience; New York, N.Y. 1970.
5. a) B. G. Barrell, and F. Sanger, FEBS Lett., 3, 275 (1969). b) S. Friedman, H. Li Janet, K. Nakanishi, and G. Van Lear, Biochemistry, 13, 2932 (1974).
6. K. Isono, K. Asai, and S. Suzuki, J. Am. Chem. Soc., 91, 7490 (1969).
7. M. Uramoto, K. Kobinata, K. Isono, E. E. Jenkins, J. A. McCloskey, and T. Higashijima, Nucleic Acids Res., 8, s569 (1980).
8. H. Hagenmaier, A. Keakeisenm H. Zähner, and W. A. König, Liebig's Ann. Chem., 1979, 1494.

9. a) R L. Hamill and M. M. Hoehn, J. Antibiotics, 26, 463 (1973); b) R. S. Gordee, T. F. Butler, J. Antibiotics, 26, 466 (1973); c) R. L. Hamill, C. B. Carrell, S. M. Nash, and R. Nagarajan, 17th Annu. ICAAC meeting. Abstracts of Paper No. 48 (1977).

10. Z. Ohashi, M. Maeda, J. A. McCloskey, and S. Nishimura, Biochemistry, 13, 2620 (1974).

11. F. Seela, and F. Cramer, Chem. Ber., 109, 82 (1976).

12. P. Hoffman, G. Gokel, and D. Merquarding, in "Isonitrile Chemistry", I. Ugi, Ed., Academic Press. New York, 1971, pp 19-35.

13. Y. Mizuno, W. Limn, K. Tsuchida, and K. Ikeda, J. Org. Chem., 37, 39 (1972).

14. I. Ugi and F. K. Rosendahl, Liebig's Ann. Chem., 666, 65 (1963).

Received, 1st September, 1980