

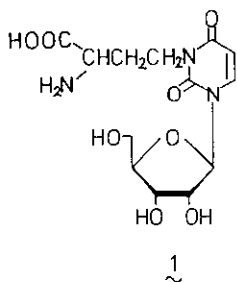
SYNTHESIS OF 3-(3-AMINO-3-CARBOXYPROPYL)URIDINE (A MODIFIED NUCLEOSIDE IN CERTAIN RNAS) BY FOUR COMPONENT CONDENSATION ¹

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3-(3-Amino-3-carboxypropyl)uridine [a modified nucleoside in certain transfer RNA (*viz.*, *Escherichia coli* tRNA^{11e})] 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 antibiotic⁹ are unique in that

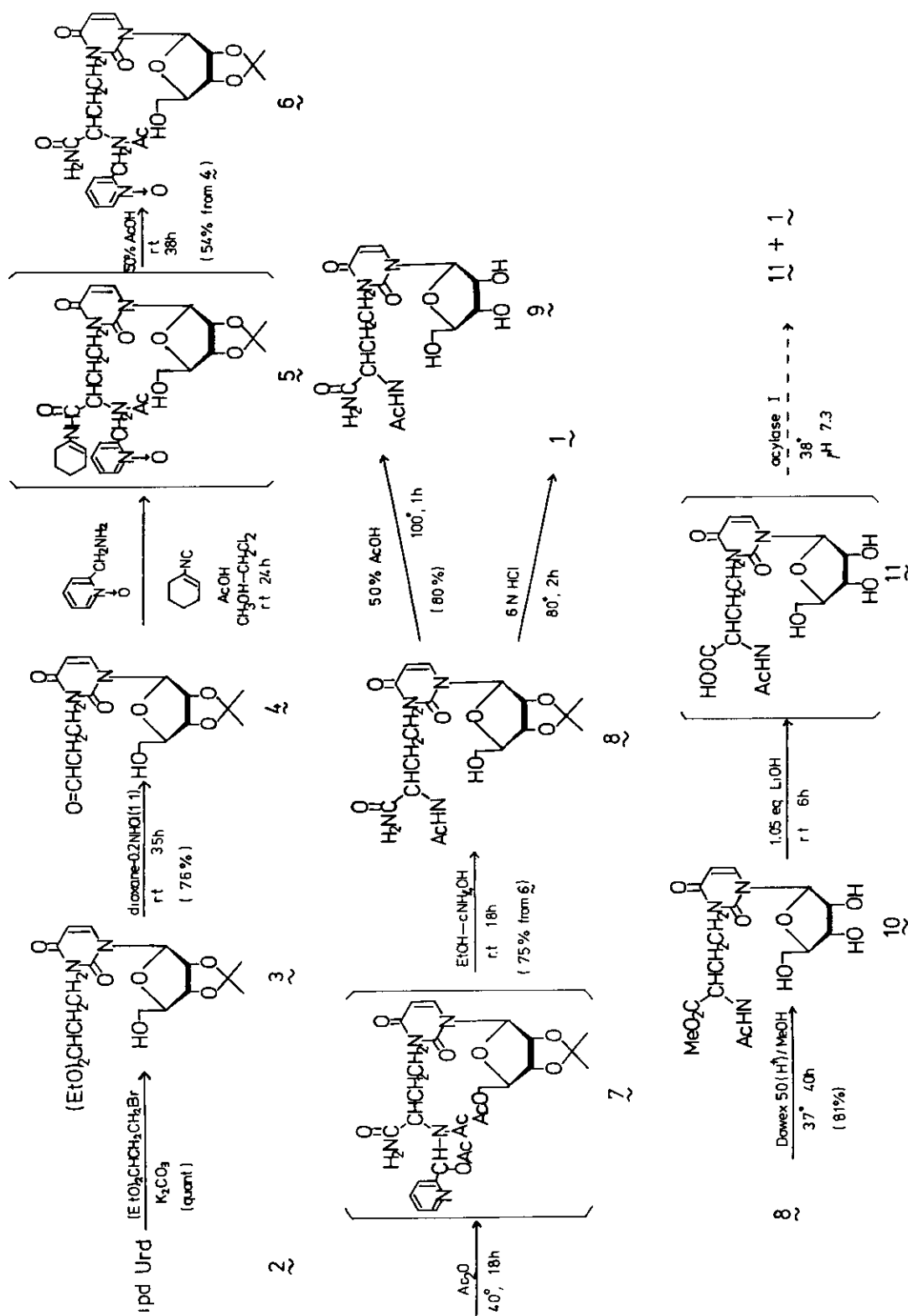


the molecules consist of a hybrid of the nucleoside and the amino acid. With some exceptions (*viz.*, Sinefungin and Nikkomycins), chemical syntheses of most nucleosides of the hybrid type have been achieved by a number of workers. Thus, Ohashi and coworkers have prepared **1** by the alkylation of 2', 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 condensation¹² (Ugi reaction) involving amines and isocyanides of 2-picolyl 1-oxide series¹³, 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-Isopropylideneuridine (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:CHCl₃-CH₃OH 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 (CDCl₃) δ : 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 (CDCl₃) δ : 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)amine¹³ (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-CH₂Cl₂ (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 [δ : 1.6 ppm (4H), 2.1 ppm (4H), 5.8 ppm (1H)], "op" [δ : 7.35 ppm (3H), 8.25 ppm (1H), 4.9 ppm (2H)], acetyl [δ : 2.1 ppm (3H)] and isopropylidene group [δ : 1.4 ppm (3H), 1.6 ppm (3H)] in nmr spectra (CDCl₃). 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 (CDCl₃) spectra showed the presence of "op" group [δ : 7.35 ppm (2H), 7.56 ppm (1H), 8.24 ppm (1H), 4.89 ppm (2H)], acetyl [δ : 2.15 ppm (3H), and isopropylidene [δ : 1.35 ppm (3H), 1.57 ppm (3H) group]. Anal. Calcd for C₂₄H₃₁N₅O₉ 1/2 CDCl₃: 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 CHCl₃-CH₃OH 1000:20) afforded 8 as a homogeneous foam. Ms (m/e) 426 (M⁺), 411 (M⁺-15); nmr (CDCl₃) spectral data (δ : 7.40 ppm (d, 1H, H6, J = 8Hz), 7.0 ppm 6.8 ppm (bs, 2H, -CONH₂), 5.77 ppm (d, 1H, H5, J = 8Hz), 5.60 ppm (d, 1H, H1')] was also consistent with the assigned structure. Anal. Calcd for C₁₈H₂₆N₄O₈·1/2 CHCl₃: 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%.



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 ($DMSO-d_6$) δ : 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 $C_{16}H_{23}N_3O_9 \cdot 1/4 CHCl_3$: 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: λ_{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 I (pig kidney) in order to resolve the racemate.

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