Nucleosides XCII. A Facile Synthesis of 5-(β-D-Ribofuranosyl)-isocytosine (ψ-Isocytidine)

*Chung K. Chu, Kyoichi A. Watanabe, and Jack J. Fox**

Laboratory of Organic Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, N.Y., 10021

Received April 29, 1975

Sir:

Several reports have appeared on the synthesis of pseudouridine [5-(β-D-ribofuranosyl)uracil, ψ-uridine] (2) and ψ-cytidine (3). All these methods involve the condensation of a suitably protected sugar with a preformed pyrimidin-5-yllithium derivative. These procedures are complicated and the yields are low. As part of our program of preparation of nucleosides bearing a carbon to carbon linkage between the sugar and aglycon (*C*-nucleosides), we investigated the synthesis of 5-ribofuranosylpyrimidines. Of special interest is the synthesis of the hitherto-unknown ψ-isocytidine (Figure 1) which may be

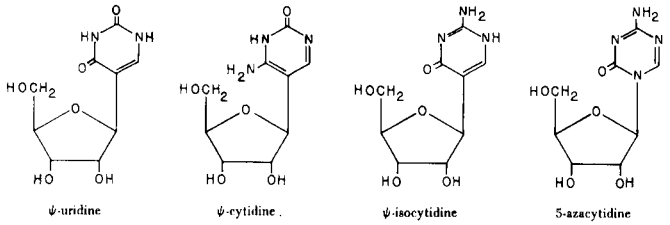

Figure 1

viewed as an analog of cytidine or as a carbon isostere of 5-azacytidine (4a) [a nucleoside antibiotic (4b) which is also an anti-leukemic agent (4c)]. This report describes a facile 4-step synthesis of ψ-isocytidine.

The key intermediate (Figure 2) for the synthesis of ψ-isocytidine **5** is the 2-(D-ribofuranosyl)-2-formylacetate derivative **3** (R = alkyl, R′ = H). Although sodio derivatives of malonic esters (5,6) and other active methylene compounds (7) have been condensed with protected D-ribosyl halides to afford *C*-glycosyl derivatives, ring closure of these (*e.g.* with urea) gives only 6-substituted pyrimidine *C*-5-nucleosides (5). Attempts to prepare **3** by condensation of ethyl formylacetate or its dimethyl acetal with halogenoses were not successful (8). The synthesis of the sodium enolate **3** (R′ = Na) was readily achieved in good yield by formylation of methyl or ethyl 2-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-D-ribofuranosyl)acetate **2** with ethyl formate and sodium hydride. The pmr spectrum of of **3** was generally similar to that for **2** except for the

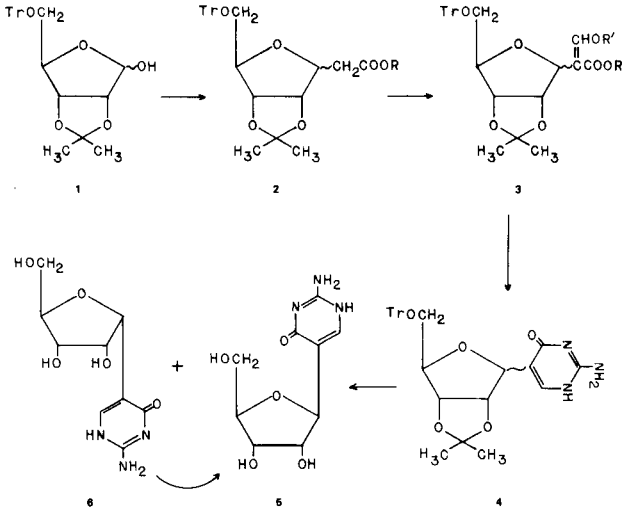

Figure 2

absence of the methylene signal (δ = 2.68). Though the configuration at C-1′ of **3** is not established, its general structure is confirmed by conversion to **5** (*vide infra*). Compound **2** was prepared in high yield by condensation of 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranose **1** (5,9) with (alkoxycarbonylmethylene)triphenylphosphorane (10-13).

Crude enolate **3** was treated with guanidine and sodium methoxide in methanol to afford only one nucleosidic product as detected by tlc (14). The protected nucleoside **4** was readily isolated in ~ 30% yield in crystalline form after column chromatography using silica gel G; m.p. 251-253°; uv λ max (ethanol): 291 nm, λ max (*p*H 12) 277 nm, λ max (*p*H 1) 259 nm. (The uv spectral behavior resembles that for isocytosine) (15). Pmr data (DMSO-d_6): isopropylidene CH_3 δ = 1.20 (s, 3H); 1.31 (s, 3H); H5′, 5″ 2.54 (m, 2H); H4′, 4.14 (m, 1H); H3′, 4.61 (d, 1H, $J_{3',2'} \cong 6$ Hz); H2′, 4.76 (q, 1H, $J_{2',3'} \cong 6$ Hz, $J_{2',1'} \cong 3.5$ Hz); H1′, 4.96 (d, 1H, $J_{1',2'} \cong 3.5$ Hz); NH_2, 6.49 (broad s, 2H), trityl, 7.36 (m, 15H); H6, 7.55 (s, 1H); NH, 10.96 (broad s, 1H). The configuration of **4** at C-1′ is not established. The H1′-H2′

coupling constant (3.5 Hz in DMSO-d_6) does not permit definitive assignment. On the basis of Imbach's rule (16), the difference in chemical shifts of the two methyl signals of the isopropylidene group (11 Hz) *suggests* that compound **4** is the α-isomer. Treatment of **4** with ~ 10% methanolic hydrogen chloride afforded ~ 90% yield of mixed (αβ) isomeric free nucleosides from which the *beta* isomer, ψ-isocytidine **5**, crystallized from the reaction mixture as its hydrochloride salt in ~ 20% yeild, m.p. 215-216° dec.; $[\alpha]_D^{25}$ + 120° (c 0.1 water); uv: λ max (*p*H 3.6) 268 nm (ε, 9,000), shoulder 290 (8,000), λ min (*p*H 3.6) 247 (6,200), λ max (0.1*N* HCl) 262 (14,000), λ min (0.1*N* HCl) 241 (8,800), λ max (0.1*N* NaOH) 277 (13,600), λ min (0.1 NaOH) 253 (5,700). From the mother liquor, crystalline hydrochloride of the α-isomer **6** was obtained (70%), m.p. 182-183° dec.; $[\alpha]_D^{25}$ -164° (c 0.1 water); uv: λ max (*p*H 3.7) 265 nm (ε, 10,900) shoulder 290 (6,700), λ min (*p*H 3.7) 248 (8,300); λ max (0.1*N* HCl) 262 (16,000), λ min (0.1*N* HCl) 242 (10,800), λ max (0.1*N* NaOH) 278 (15,600), λ min (0.1*N* NaOH) 253 (6,100). The configuration of C-1′ for ψ-isocytidine **5** and the α-isomer **6** was established by comparison to their pmr spectra (*in* deuterium oxide) with ψ-uridine and its α-isomer. The spectrum of **5** was almost identical with that of ψ-uridine while that for **6** was almost identical to the α-isomer of ψ-uridine (17). As reported for α and β isomers of pseudouridine (18), the signal for H-1′ of ψ-isocytidine **5** appears upfield (δ = 4.72) relative to that for **6** (δ = 5.04), while the signal of H-6 of **5** (δ = 7.75) occurs downfield relative to that of **6** (δ = 7.65). Most noteworthy is the fact that the Δδ values for H-1′ (0.32) and for H-6 (0.10) for the ψ-isocytidine isomers **5** and **6** are identical with the corresponding Δδ values for H-1′ and H-6 of α and β isomers of pseudouridine (17). Compounds **4**, **5**, and **6** gave proper elemental analyses.

Upon treatment with dilute deuterium chloride in deuterium oxide at room temperature, pure α-isomer **6** underwent a slow epimerization at C-1′ to ψ-isocytidine **5** as indicated by pmr. The H-6 signal of **6** decreased slowly with concomitant increase of a new signal corresponding to the H-6 signal of **5**. The rest of the spectrum consists of signals for both **5** and **6**. No evidence for the formation of pyranosyl isomers was found. Thus the yield of the desired ψ-isocytidine is readily raised to ~ 80% from **4** by acid-catalyzed isomerization of **6** → **5** regardless of the configuration at C-1′ of **3** or **4**. Compound **3** should be a versatile intermediate for the syntheses of pseudouridine type nucleosides of potential biochemical and chemotherapeutic value. Chemical efforts toward these goals are underway.

Preliminary studies (20) of ψ-isocytidine **5** against leukemia 5178Y cells *in vitro* show a 50% inhibition of growth at 0.8 μg./ml. whereas with 5-azacytidine the ID_{50} is 1.0 μg./ml. This inhibition by ψ-isocytidine (like that by 5-azacytidine) is blocked by uridine and cytidine but not by 2′-deoxycytidine. The α-isomer **6** is considerably less effective as a growth inhibitor of these cells.

REFERENCES

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 08748), and by the Montgomery Fund (C. K. C.).

(2) R. Shapiro and R. W. Chambers, *J. Am. Chem. Soc.*, **83**, 3920 (1961); D. M. Brown, M. G. Burdon and R. P. Slatcher, *J. Chem. Soc.*, 1051 (1968); W. A. Asbun and S. B. Binkley, *J. Org. Chem.*, **31**, 2215 (1966); *ibid.*, **33**, 140 (1968); U. Lerch, M. G. Burdon and J. G. Moffatt, *ibid.*, **36**, 1507 (1971).

(3) A. M. Michelson and W. E. Cohn, *Biochemistry*, **1**, 490 (1962); S. David and A. Lubineau, *Carbohyd. Res.*, **29**, 15 (1973).

(4a) A. Piskala and F. Sorm, *Collect. Czech. Chem. Commun.*, **29**, 2060 (1964); (b) M. E. Bergy and R. R. Herr, *Antimicrobial Agents Chemotherapy*, 625 (1966); (c) O. Hrodek and J. Vesley, *Neoplasia*, **18**, 493 (1971).

(5) H. Ohrui and J. J. Fox, *Tetrahedron Letters*, 1951 (1973).

(6) S. Hanessian and A. G. Pernet, *Can. J. Chem.*, **52**, 1266 (1974).

(7) T. Ogawa, A. G. Pernet and S. Hanessian, *Tetrahedron letters*, 3534 (1973); S. Hanessian and A. G. Pernet, *Can. J. Chem.*, **52**, 1280 (1974).

(8) C. K. Chu, K. A. Watanabe and J. J. Fox, unpublished experiments.

(9) R. S. Klein, H. Ohrui and J. J. Fox, *J. Carbohyd. Nucleosides, Nucleotides*, **1**, 265 (1974).

(10) Hanessian *et al.* (11) reported reaction of ethoxycarbonylmethylene)triphenylphosphorane with 2,3-*O*-isopropylidene-D-ribofuranose. Buchanan *et al.* (12) also reported the reaction using tri-*O*-benzyl-D-ribofuranose. More recently, Ohuri *et al.* (13) performed the Wittig reaction of 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranose and obtained compound **2**.

(11) S. Hanessian, T. Ogawa and Y. Guindon, *Carbohyd. Res.*, **38**, C12 (1974).

(12) J. G. Buchanan, A. R. Edgar, M. J. Power and P. D. Theaker, *ibid.*, **38**, C22 (1974).

(13) H. Ohrui, G. H. Jones, J. G. Moffat, M. L. Maddox, A. T. Christensen and S. K. Bryam, *J. Am. Chem. Soc.*, in press. The authors are indebted to Dr. Moffat of Institute of Molecular Biology, Syntex Research, Palo Alto, California for procedural data on the synthesis of **2** (R = CH_3) prior to publication.

(14) Tlc was performed on microscope slides coated with Merck silica gel GF_{254} and benzene-methanol (4:1) as solvent. Substances were detected by uv-absorption and spraying and charring with 20% ethanolic sulfuric acid.

(15) M. M. Stimson, *J. Am. Chem. Soc.*, 71, 1470 (1949).

(16) J. L. Imbach and B. L. Kam, *J. Carbohyd. Nucleosides, Nucleotides*, **1**, 271 (1974).

(17) The pmr spectra of α and β ψ-uridine were reported by R. Deslauriers and I. C. P. Smith, *Can. J. Biochem.*, **50**, 766 (1972).

(18) F. E. Hruska, A. A. Grey and I. C. P. Smith, *J. Am. Chem. Soc.*, **92**, 4088 (1970).

(19) The authors are indebted to Dr. J. H. Burchenal, K. Ciovacco and K. Calaher of this Institute for these preliminary data.