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A SYNTHESIS OF ψ-CYTIDINE*

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ABSTRACT

A practical, seven-step synthesis of 5-(β-D-ribofuranosyl)cytosine (**1**, ψ-cytidine) was achieved. ψ-Uridine was converted into the 2′,3′-isopropylidene acetal **2** in 85% yield. Tosylation of **2** to the 5′-sulfonate **3**, followed by deacetonation, afforded 5′-*O*-tosyl-ψ-uridine (**6**) in good yield. Treatment of **6** with 1,1′-carbonyldiimidazole afforded the 2′,3′-cyclic carbonate **10**, which was converted into 4,5′-anhydro-2′,3′-*O*-carbonyl-ψ-uridine (**11**) by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *N*,*N*-dimethylformamide. 4,5′-Anhydro-ψ-uridine (**7**) was obtained in quantitative yield from **11** by decarbonylation in aqueous pyridine. Ammonolysis of **7** afforded ψ-cytidine (**1**) in good yield.

INTRODUCTION

In 1973, David and Lubineau[1] synthesized 5-(β-D-ribofuranosyl)cytosine (**1**, ψ-cytidine) in low yield by condensation of 2,5-dilithio-2,4-bis(trimethylsilyl)cytosine with 2,4:3,5-di-*O*-benzylidene-D-ribose. Our attempts to synthesize this *C*-nucleoside[2] by an alternative route through annelation of 2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)-3-methoxyacrylonitrile with urea failed, because the protected *C*-nucleoside intermediate decomposed during deprotection in acidic media. We have since made several attempts to synthesize ψ-cytidine, which was recently reported to have some antileukemic activity[3].

RESULTS AND DISCUSSION

For the synthesis of ψ-cytidine (**1**), ammonolysis of 4,5′-anhydro-ψ-uridine (**7**) appeared to be the method of choice. Unfortunately, the reported procedure[4] for synthesis of 2,5′-anhydrouridine from uridine by using triphenylphosphine and diethyl azodicarboxylate failed for ψ-uridine, which was recovered in almost quantitative yield from the mixture. 2′,3′-*O*-Isopropylidene-5′-*O*-tosyl-ψ-uridine (**3**)

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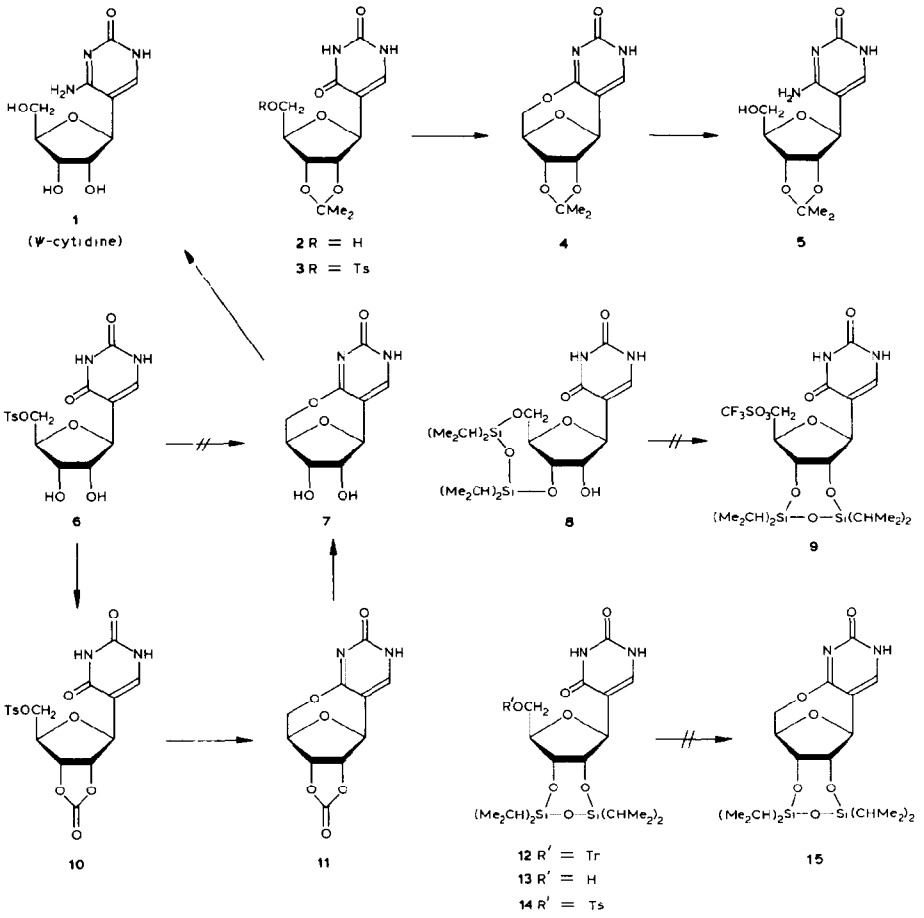

had been converted into the 4,5′-anhydro-*C*-nucleoside[5,6] **4**, and treated with ammonia to give a cytosine derivative[5]. Using a modification of the procedure of Michelson and Cohn[5] for acetonation of ψ-uridine, we prepared the 2′,3′-isopropylidene acetal **2** in 85% yield. Compound **2** was then converted in two steps into the known 4,5′-anhydro-2′,3′-*O*-isopropylidene-ψ-uridine (**4**), which, after treatment with ammonia, gave 2′,3′-*O*-isopropylidene-ψ-cytidine (**5**). The isopropylidene group in **5**, which is a part of a stable bicyclo[3.3.0] structure, resisted acid-catalyzed deacetonation, and decomposed under more-vigorous conditions. Attempts to deacetonate **4** prior to treatment with ammonia afforded a mixture of ψ-uridine and **2**, showing that the 4,5′-anhydro linkage is more susceptible to acid hydrolysis than the isopropylidene group.

Deacetonation of **3** in hot, aqueous acetic acid afforded 5′-*O*-tosyl-ψ-uridine (**6**), but the latter did not undergo anhydronucleoside formation by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile or in boiling *N,N*-dimethylformamide. Apparently, a cyclic structure involving O-2′ and O-3′ is

necessary to bring C-5′ close enough to the C-4 carbonyl group for formation of the anhydro linkage. Therefore, we tried to prepare 2′,3′-*O*-(1,1,3,3-tetraisopropyldisiloxanyl)-ψ-uridine 5′-triflate (**9**) from 3′,5′-*O*-tetraisopropyldisiloxanyl-ψ-uridine (**8**), and then convert **9** into the 4,5′-anhydro-*C*-nucleoside **15**. Although the 1,3-dimethyl analog of **8** was converted quantitatively into the corresponding 2′,3′-*O*-disiloxanyl-5′-triflate[7], compound **8** was found to give an intractible mixture when treated with trifluoromethanesulfonic anhydride in pyridine. In order to prepare **9**, 5′-*O*-trityl-ψ-uridine[8] was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane to afford in high yield the crystalline, protected nucleoside **12**, which was detritylated to give **13**. Compound **13** was treated with trifluoromethanesulfonic anhydride in pyridine–dichloromethane, but an intractible mixture resulted. Compound **13** was converted into the 5′-*p*-toluenesulfonate **14** which, however, failed to cyclicize to **15** by treatment with DBU in acetonitrile under reflux for 2 h. Apparently, the flexibility of the seven-membered, cyclic disiloxanyl structure is such that it does not promote the close proximity of C-5′ to C-4 necessary for cyclization.

Finally, 5′-*O*-tosyl-ψ-uridine 2′,3′-carbonate (**10**) which, like compound **3**, contains a more conformationally rigid bicyclo[3.3.0] system, was prepared by treatment of **6** with 1,1′-carbonyldiimidazole. As expected, **10** was converted very readily into the 4,5′-anhydro-*C*-nucleoside **11**, which was obtained crystalline in 95% yield. Treatment of **11** with methanolic ammonia gave a mixture of ψ-cytidine (**1**) and urea. Isolation of pure **1** was rather difficult. The 2′,3′-*O*-carbonyl group of **11** was therefore removed by boiling in aqueous pyridine[9] to provide 4,5′-anhydro-ψ-uridine (**7**) in quantitative yield. Ammonolysis of **7** afforded the desired, crystalline ψ-cytidine (**1**) in 83% yield. The identity of **1** was confirmed by its ^{1}H-n.m.r. spectrum in deuterium oxide; the data were indistinguishable from those reported for ψ-cytidine by David and Lubineau[1].

EXPERIMENTAL

General methods. — Melting points were determined on a Thomas–Hoover apparatus (capillary method) and are uncorrected. The elemental analyses were performed by M-H-W Laboratories, Phoenix, Arizona. ^{1}H-N.m.r. spectra for solutions in dimethyl sulfoxide-d_6 were recorded on a JEOL PFT-100 spectrometer with tetramethylsilane as the internal standard. Chemical shifts are reported on the δ scale. Values given for coupling constants are first order. Woelm silica gel (70–230 mesh) was used for column chromatography.

2′,3′-O-Isopropylidene-ψ-uridine (**2**). — ψ-Uridine (10.0 g, 41 mmol) was dissolved in warm *N,N*-dimethylformamide (200 mL), and the solution was cooled to room temperature. To the solution was added a mixture of acetone (400 mL), conc. hydrochloric acid (2 mL), and 2,2-dimethoxypropane (60 mL), and the mixture was stirred for 3 days at room temperature. The mixture was evaporated *in vacuo*, and the residue crystallized from 2-propanol to give **2** (6 g). The mother liq-

uor was concentrated, and chromatographed on a column of silica gel with 9:1 (v/v) chloroform–ethanol as the eluent to give 3.9 g of **2**, to provide a total yield of 85%, m.p. 229–230°, undepressed on admixture with an authentic sample[5,6].

2′,3′-O-Isopropylidene-5′-O-tosyl-ψ-uridine (**3**). — A mixture of **2** (9.9 g, 35 mmol) and *p*-toluenesulfonyl chloride (7.74 g, 40.6 mmol) in pyridine (100 mL) was stirred overnight at room temperature, and then diluted with ice–water (50 mL). The mixture was evaporated *in vacuo*, and the residue partitioned between chloroform (500 mL) and water (200 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated to give 14.5 g (95%) of crude **3**. A portion of the crude sample (500 mg) was purified by column chromatography (chloroform–ethanol, 9:1, v/v) to give pure **3**, m.p. 180–182° [lit.[5] m.p. 172–174° (dec.)]; ^{1}H-n.m.r.: δ 1.23, 1.43 (2 s, 6 H, CMe_2), 2.41 (s, 3 H, Ts-*Me*), 4.00–4.16 (m, 3 H, H-4′,5′,5″), 4.52–4.75 (m, 3 H, H-1′,2′,3′), 7.41 (s, 1 H, H-6), 7.45 (d, 2 H, aryl), 7.79 (d, 2 H, aryl), 10.97 (s, 1 H, NH exchangeable), and 11.17 (s, 1 H, NH exchangeable).

4,5′-Anhydro-2′,3′-O-isopropylidene-ψ-uridine (**4**). — To a solution of **3** (217 mg, 0.5 mmol) in acetonitrile (10 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (84 mg, 0.55 mmol), and the solution was heated under reflux for 24 h, during which time colorless crystals precipitated. The mixture was filtered while hot, and the filtrate evaporated *in vacuo*. The residue crystallized from acetone–ether to give 115 mg (86%) of **4**; ^{1}H-n.m.r.: δ 1.27, 1.42 (2 s, 6 H, CMe_2), 4.40 (m, 2 H, H-5′,5″), 4.55 (m, 2 H, H-3′,4′), 4.86 (s, 1 H, H-1′), 4.93 (d, 1 H, $J_{2',3'}$ 6.0 Hz, H-2), and 8.04 (s, 1 H, H-6).

2′,3′-O-Isopropylidene-ψ-cytidine (**5**). — A solution of **4** (2.30 g, 8.65 mmol) in methanolic ammonia (saturated at 0°) was heated overnight at 120° in a sealed, steel vessel. After cooling, crystalline **5** (2.42 g, 99%) was collected by filtration, m.p. 259–260° (dec); ^{1}H-n.m.r.: δ 1.28, 1.51 (2 s, 6 H, CMe_2), 3.59 (m, 2 H, H-5′,5″), 4.00 (m, 1 H, H-4′), 4.45 (d, 1 H, $J_{1',2'}$ 6.1 Hz, H-1′), 4.58 (t, 1 H, $J_{1',2'}$ $J_{2',3'}$ 6.1 Hz, H-2′), 4.73 (dd, 1 H, $J_{3',4'}$ 2.7 Hz, H-3′), 5.34 (t, 1 H, OH exchangeable), and 7.46 (s, 1 H, H-6).

Anal. Calc. for $C_{12}H_{17}N_3O_5$: C, 50.88; H, 6.05; N, 14.83. Found: C, 50.69; H, 6.09; N, 14.65.

5′-O-Tosyl-ψ-uridine (**6**). — A solution of crude **3** (14.0 g) in 80% aqueous acetic acid (250 mL) was boiled under reflux for 10 min and then evaporated *in vacuo*. Traces of acetic acid were removed by several evaporations of ethanol from the residue, which was triturated with ethanol to give crude **6** (13.5 g) as an amorphous solid. A small sample was purified by column chromatography with 9:1 (v/v) chloroform–ethanol as the eluent to provide analytically pure **6** as a foam; ^{1}H-n.m.r.: δ 2.42 (s, 3 H, Ts-*Me*), 3.79 (m, 3 H, H-4′,5′,5″), 4.13 (m, 2 H, H-2′,3′), 4.44 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 5.02 (m, 2 H, OH exchangeable), 7.25 (s, 1 H, H-6), 7.47 (d, 2 H, Ts), 7.79 (d, 2 H, Ts), and 11.04 (s, 2 H, NH exchangeable).

Anal. Calc. for $C_{16}H_{18}N_2O_8S$: C, 48.24; H, 4.55; N, 7.03. Found: C, 48.23; H, 4.80; N, 6.87.

2′,3′-O-(1,1,3,3-Tetraisopropyldisiloxanyl)-5′-O-trityl-ψ-uridine (**12**). — A

mixture of ψ-uridine (4.9 g, 20 mmol) and chlorotriphenylmethane (6.67 g, 24 mmol) in dry pyridine (100 mL) was stirred at room temperature. Additional amounts of chlorotriphenylmethane were added after 24 and 48 h (2 g each), and stirring was continued for 3 more days. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane[10] (7.2 g, 22 mmol) was added to the mixture, and stirring was continued for 24 h. The mixture was evaporated *in vacuo*, and traces of pyridine were removed by several evaporations of toluene from the residue, which was then dissolved in chloroform (500 mL). The chloroform solution was dried (Na_2SO_4), evaporated, and the residue crystallized from methanol to give 13.9 g (95%) of **12**, m.p. 135–140°; ^{1}H-n.m.r.: δ 1.05 (m, 28 H, CH*Me*$_2$), 3.20 (m, 2 H, H-5′,5″), 3.92 (m, 1 H, H-4′), 4.55 (m, 3 H, H-1′,2′,3′), 7.28–7.37 (m, 16 H, aromatic and H-6), 10.92 (s, 1 H, NH exchangeable), and 11.15 (s, 1 H, NH exchangeable).

Anal. Calc. for $C_{40}H_{52}N_2O_7Si_2$: C, 65.90; H, 7.19; N, 3.84. Found: C, 65.68; H, 7.13; N, 3.85.

2′,3′-O-(1,1,3,3-Tetraisopropyldisiloxanyl)-ψ-uridine (**13**). — Compound **12** (7.0 g, 9.6 mmol) was dissolved in chloroform (20 mL) and 10% trifluoroacetic acid in chloroform (50 mL) was added. After 15 min at room temperature, the mixture was made neutral with methanolic ammonia, and then evaporated *in vacuo*. The residue was suspended in chloroform (300 mL), and the solution was washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel with 19:1 (v/v) chloroform–ethanol as the eluent to give 4.7 g (80%) of **13** as a colorless foam; ^{1}H-n.m.r.: δ 1.02 (m, 28 H, CH*Me*$_2$), 3.45–3.78 (m, 3 H, H-4′,5′,5″), 4.19–4.42 (m, 2 H, H-2′,3′), 4.61 (d, 1 H, $J_{1',2'}$ 3.0 Hz, H-1′), 4.93 (t, 1 H, 5′-OH exchangeable), 7.59 (s, 1 H, H-6), 10.86 (s, 1 H, NH exchangeable), and 11.11 (s, 1 H, NH exchangeable).

Anal. Calc. for $C_{21}H_{38}N_2O_7Si_2$: C, 51.82; H, 7.87; N, 5.75. Found: C, 51.72; H, 7.87; N, 5.61.

2′,3′-O-(1,1,3,3-Tetraisopropyldisiloxanyl)-5′-O-tosyl-ψ-uridine (**14**). — A mixture of **13** (5.0 g, 10.3 mmol) and *p*-toluenesulfonyl chloride (3.0 g, 26 mmol) in pyridine (100 mL) was stirred overnight at room temperature. The mixture was evaporated *in vacuo* and the residue dissolved in chloroform (300 mL). The solution was washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel [19:1 (v/v) chloroform–ethanol] to provide 6.0 g (91%) of **14** as a colorless foam; ^{1}H-n.m.r.: δ 1.02 (m, 28 H, CH*Me*$_2$), 2.41 (s, 3 H, Me), 4.02 (m, 1 H, H-4′), 4.17 (m, 3 H, H-3′,5′,5″), 4.39 (m, 1 H, H-2′), 4.57 (s, 1 H, H-1′), 7.28 (s, 1 H, H-6), 7.46 (d, 2 H, aromatic), 7.79 (d, 2 H, aromatic), 10.96 (s, 1 H, NH exchangeable), and 11.15 (s, 1 H, NH exchangeable).

Anal. Calc. for $C_{28}H_{44}N_2O_9Si_2$: C, 52.47: H, 6.92: N, 4.37. Found: C, 52.60: H, 7.03; N, 4.45.

2′,3′-O-Carbonyl-5′-O-tosyl-ψ-uridine (**10**). — A solution of **6** (13.0 g) and 1,1′-carbonyldiimidazole (6.0 g, 1.1 equiv.) in *N,N*-dimethylformamide (50 mL) was stirred overnight at room temperature. The mixture was partitioned between ethyl acetate (500 mL) and water (100 mL). The organic layer was separated,

washed with water, dried (Na_2SO_4), and evaporated. The residue was triturated with ethanol (50 mL) to give crystalline **10** (10.25 g, 74%), m.p. 235–236°; ^{1}H-n.m.r.: δ 2.41 (s, 3 H, Ts-*Me*), 4.12–4.37 (m, 3 H, H-4′,5′,5″), 4.84 (d, 1 H, $J_{1',2'}$ 2.7 Hz, H-1′), 5.13 (dd, 1 H, $J_{2',3'}$ 7.8, $J_{3',4'}$ 3.0 Hz, H-3′), 5.28 (dd, 1 H, H-2′), 7.43 (d, 2 H, Ts), 7.52 (s, 1 H, H-6), 7.75 (d, 2 H, Ts), 11.10 (s, 1 H, NH exchangeable), and 11.28 (s, 1 H, NH exchangeable).

Anal. Calc. for $C_{17}H_{16}N_2O_9S$: C, 48.11; H, 3.80; N, 6.60. Found: C, 48.32; H, 4.00; N, 6.79.

4,5′-Anhydro-2′,3′-O-carbonyl-ψ-uridine (**11**). — To a solution of **10** (5.0 g, 11.8 mmol) in *N,N*-dimethylformamide (100 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (1.98 g, 13 mmol). The mixture was heated for 1 h at 100°, and then evaporated. The residue was triturated with ethanol (10 mL), and crystalline **11** (2.8 g, 95%) collected by filtration, m.p >310°; ^{1}H-n.m.r.: δ 4.51 (d, 1 H, $J_{4',5'}$ 2.4 Hz, H-4′), 4.68 (m, 2 H, H-5′,5″), 5.17 (s, 1 H, H-1′), 5.25 (d, 1 H, $J_{2',3'}$ 6.7 Hz, H-3′), 5.55 (d, 1 H, H-2′), 8.12 (s, 1 H, H-6), and 11.90 (s, 1 H, NH exchangeable).

Anal. Calc. for $C_{10}H_8N_2O_6$: C, 47.63; H, 3.19; N, 11.11. Found: C, 47.59; H, 3.39; N, 10.98.

4,5′-Anhydro-ψ-uridine (**7**). — A solution of **11** (1.0 g, 4 mmol) in pyridine (25 mL) and water (25 mL) was boiled under reflux for 3 h, and then evaporated *in vacuo*. Traces of pyridine and water were removed by several evaporations of toluene and ethanol from the residue, which was analytically pure, crystalline **7**, m.p. 232–234°; ^{1}H-n.m.r.: δ 3.85–3.96 (m, 2 H, H-5′,5″), 4.21–4.51 (m, 3 H, H-2′,3′,4′), 4.65 (s, 1 H, H-1′), and 8.01 (s, 1 H, H-6).

Anal. Calc. for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.45; N, 12.38. Found: C, 47.59; H, 4.67; N, 12.10.

ψ-Cytidine (**1**). — Compound **7** (226 mg, 1 mmol) was dissolved in methanolic ammonia (presaturated at 0°, 5 mL), and the solution heated for 2 h at 120° in a sealed, steel vessel. After cooling the solution, crystals of ψ-cytidine (**1**) were collected by filtration and washed with methanol to yield 202 mg (83%), m.p. 191–192°; lit.[1] m.p. 161°. The difference in melting points may be due to dimorphism of ψ-cytidine. ^{1}H-n.m.r. data: δ 3.36–3.55 (m, 3 H, H-4′,5′,5″), 3.79–3.93 (m, 2 H, H-2′,3′), 4.27 (d, 1 H, $J_{1',2'}$ 6.7 Hz, H-1′), 5.27 (m, 3 H, OH exchangeable), 6.95 (m, 2 H, NH exchangeable), and 7.35 (s, 1 H, H-6). The ^{1}H-n.m.r. spectrum of this sample in D_2O solution was identical with that reported by David and Lubineau[1]; $[\alpha]_D^{25}$ −64.1° (*c* 0.7, water) [lit.[1] $[\alpha]_D^{25}$ −58.5° (*c* 1, water)]; $\lambda_{max}^{H_2O}$ 271 nm (ε 6000), $\lambda_{min}^{H_2O}$ 250 nm (ε 3700), $\lambda_{max}^{pH\ 1}$ 282 nm (ε 9500), $\lambda_{min}^{pH\ 1}$ 243 nm (ε 800), $\lambda_{max}^{pH\ 13}$ 284 nm (ε 8000), and $\lambda_{min}^{pH\ 13}$ 254 nm (ε 2000).

Anal. Calc. for $C_6H_{13}N_3O_5 \cdot H_2O$: C, 41.37; H, 5.78; N, 16.08. Found: C, 41.38; H, 5.83; N, 16.04.

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