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SYNTHESIS OF 3-METHYLPSEUDOURIDINE AND 2′-DEOXY-3-METHYLPSEUDOURIDINE*

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ABSTRACT

The first chemical synthesis of 3-methyl-ψ-uridine (**5**) and its 2′-deoxy analogue (**9**) has been achieved. ψ-Uridine was trimethylsilylated and the crude product was treated with acetyl chloride, to give the 1-acetyl derivative (**3**). Crude **3** was methylated with dimethoxymethyldimethylamine and then saponified, to give crystalline **5** in 82% overall yield. Treatment of **5** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane afforded the 3′,5′-protected product, which was converted into the 2′-*O*-[(imidazol-1-yl)thiocarbonyl] derivative **7**. Reduction of **7** with tributyltin hydride followed by deblocking of the product gave crystalline 2′-deoxy-3-methyl-ψ-uridine (**9**) in 35% yield from **5**.

INTRODUCTION

Among *N*-methylated nucleosides, 3-methyl-ψ-uridine and its 2′-deoxy analogue are of particular interest. The N-3 position of such nucleosides cannot take part in hydrogen bonding, but these nucleosides may still be able to interact with adenine nucleosides by hydrogen bonding through the N-1 position, provided that the methylated *C*-nucleosides assume the *syn* conformation.

Methods of selective alkylation at the N-1 position and both N-1 and N-3 positions have been developed. 1-Methyl-ψ-uridine, a natural product isolated[1] from the culture broth of *Streptomyces platensis*, has been synthesized[2,3] by selective methylation of ψ-uridine. The 2′-deoxy analogue, which has been synthesized[4,5] as a *C*-nucleoside isostere of thymidine, exhibited activity[4] against mouse mastocytoma P-815 cells and *Streptococcus faecium in vitro*. A simple method of 1,3-

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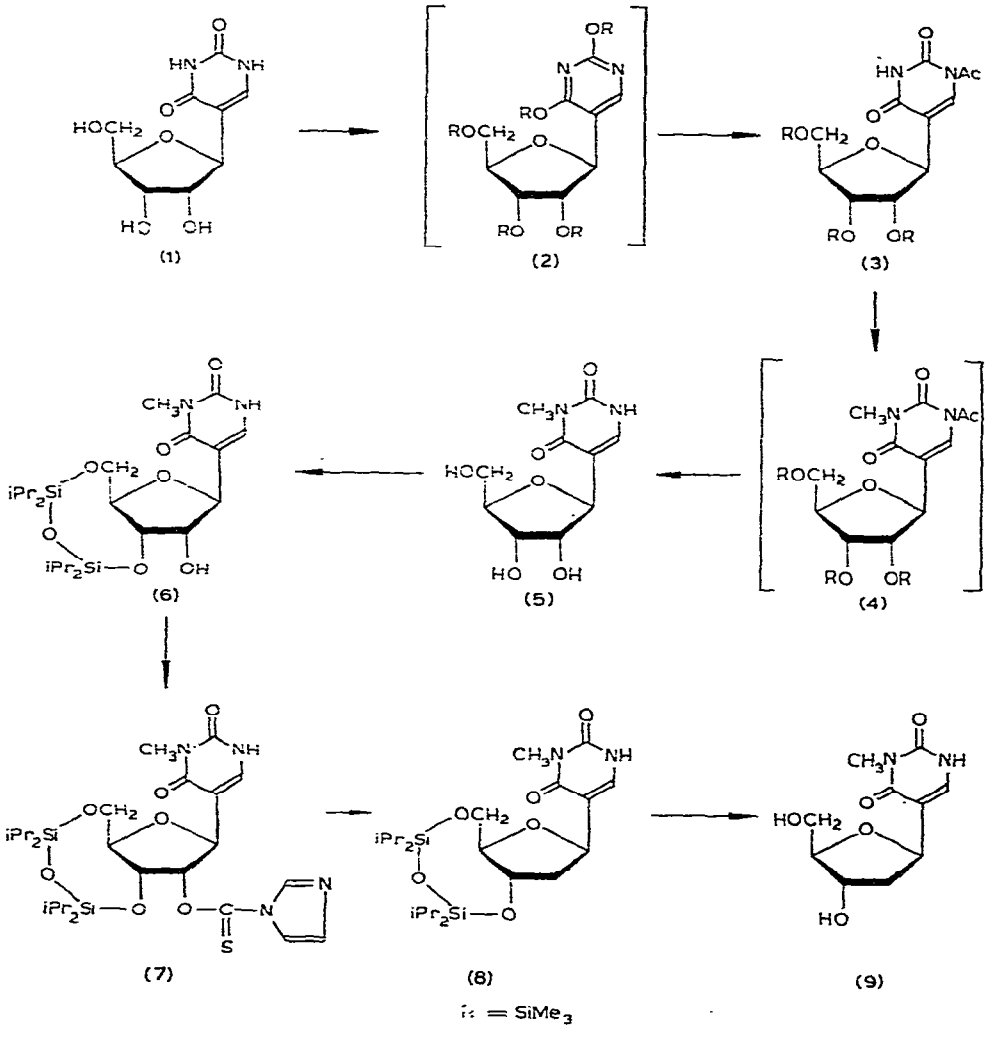
dimethylation of ψ-uridine has been developed[6] in connection with the synthesis of ψ-isocytidine, an antileukemic *C*-nucleoside[7], and its 2′-deoxy analogue[4]. However, no regioselective method for 3-methylation of ψ-uridine has been described. We now report on the synthesis of 3′-methyl-ψ-uridine by regioselective methylation. A synthesis of 2′-deoxy-3-methyl-ψ-uridine is also described.

RESULTS AND DISCUSSION

Our strategy in preparing 3-methyl-ψ-uridine (5) was to block the N-1 position of ψ-uridine (**1**) with a readily removable protecting-group, methylate at N-3, and then remove the N-1 substituent. The acetyl group was chosen as the N-1 protecting-group, and it was introduced by following the method of 1-methyl-ψ-



uridine preparation[2], which consists of pertrimethylsilylation of **1** and treatment of the product (**2**) with methyl iodide. The methylation at N-1 would proceed by an electrophilic substitution closely related to the Hilbert–Johnson mechanism[8]. When acetyl chloride instead of methyl iodide was used as the electrophilic reagent, the 1-acetyl-ψ-uridine derivative (**3**) was obtained, exclusively, as a yellowish solid which showed a single acetyl signal at δ 2.75 in the ^{1}H-n.m.r. spectrum. Without purification, **3** was treated with dimethoxymethyldimethylamine in boiling benzene, to give the 1-acetyl-3-methyl-ψ-uridine derivative (**4**). The ^{1}H-n.m.r. spectrum of **4** exhibited an acetyl and a methyl signal at δ 2.75 and 2.93, respectively. Deprotection of **4** with methanolic ammonia afforded 3-methyl-ψ-uridine (**5**) as colourless crystals in 82% overall yield from **4**. The u.v.-spectral characteristics of **5** were similar to those of 3-methyluracil[9]. Although **5** has been reported as one of the many products of methylation of ψ-uridine with dimethyl sulfate[10] or diazomethane[11], it has never been characterized, except by u.v. spectroscopy.

3-Methyl-ψ-uridine (**5**) was treated with 1,1,3,3-tetraisopropyldisiloxane[12], and the amorphous product, 3-methyl-3′,5′-*O*-tetraisopropyldisiloxanyl-ψ-uridine (**6**, 54%), was converted into the amorphous 2′-*O*-[(imidazol-1-yl)thiocarbonyl] derivative **7** in 91% yield. Compound **7** was trimethylsilylated* and then reduced with tributyltin hydride in the presence of 2,2′-azobis(2-methylpropionitrile)[13,14]. The protected 2′-deoxy-3-methyl-ψ-uridine (**8**) was obtained in 71% yield as a foam. Deprotection of **8** with tetrabutylammonium fluoride afforded crystalline 2′-deoxy-3-methyl-ψ-uridine (**9**) in 65% yield. The u.v.-spectral behaviour of **9** was very similar to that of the *ribo* analogue **5**. The ^{1}H-n.m.r. spectrum of **9** was almost identical to that of 2′-deoxy-1-methyl-ψ-uridine[4,5], except that the N-methyl and H-6 signals of the former (δ 3.13 and 7.43) appeared upfield relative to those of the latter (δ 3.25 and 7.63). This difference in chemical shifts is consistent with the structural assignment of the aglycon, since the signals of the corresponding protons of 1-methylthymine (δ 3.20 and 7.50) appear upfield relative to those for 3-methylthymine (δ 3.33 and 7.74, respectively). The β configuration of **9** is established by the identical chemical shifts of the lactol ring protons of **9** and those of the known β-*C*-nucleoside, 2′-deoxy-1-methyl-ψ-uridine.

EXPERIMENTAL

General. — Melting points were determined on a Thomas–Hoover apparatus (capillary method) and are uncorrected. The elementary analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee. N.m.r. spectra were determined 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 (20–230 mesh) was used for column chromatography.

*Trimethylsilylation prior to reduction is necessary to avoid α,β-isomerization (K. Pankiewicz, A. Matsuda, and K. A. Watanabe, unpublished results).

3-Methyl-ψ-uridine (**5**). — A mixture of ψ-uridine (**1**; 6.1 g, 25 mmol) and ammonium sulfate (~5 mg) in hexamethyldisilazane (75 ml) was stirred and heated to reflux. When the reaction mixture became clear (~1 h), it was allowed to cool to room temperature. The excess of hexamethyldisilazane was removed by evaporation *in vacuo*, and a solution of the oily residue in acetonitrile (100 ml) was treated with acetyl chloride (6 ml, 83 mmol). The mixture was stirred for 2.5 h at room temperature and then concentrated to dryness *in vacuo*, and traces of acetyl chloride were removed by several co-evaporations with benzene. The crude product, probably 1-acetyl-2,2′,3′,5′-tetra-*O*-trimethylsilyl-ψ-uridine (**3**), was obtained as a yellowish powder; ^{1}H-n.m.r. data ($CDCl_3$) δ 2.74 (3 H, s, NAc).

A solution of crude **3** in benzene (100 ml) containing dimethoxymethyldimethylamine (10 ml, 75 mmol) was heated at reflux for 1 h, and then concentrated *in vacuo*, to give the 1-acetyl-3-methyl derivative **4** as a tan solid; ^{1}H-n.m.r. data ($CDCl_3$): δ 2.75 (3 H, s, NAc) and 2.93 (3 H, s, NMe). Compound **4** was treated with methanolic ammonia (100 ml, saturated at 0°) overnight at room temperature. Concentration of the mixture to half its volume *in vacuo* caused precipitation of crystalline 3-methyl-ψ-uridine (**5**, 3.51 g) which was collected by filtration. The filtrate was mixed with silica gel (50 g), and the mixture was dried *in vacuo* and placed on the top of a column (20 × 4.5 cm) of silica gel. The column was washed with 8% ethanol in chloroform (v/v, 500 ml). An additional crop of **5** (1.78 g) was eluted from the column with 16% ethanol in chloroform. The total yield of **5** was 5.29 g (82%); m.p. 148–149°; $\lambda_{max}^{H_2O}$ 262 nm (ε 8500), $\lambda_{min}^{H_2O}$ 234 nm (ε 2800); ^{1}H-n.m.r. data (dimethyl sulfoxide-d_6): δ 2.52 (s, 3 H, NMe), 3.5–4.5 (m, 5 H, H-2′,3′,4′,5′,5′), 4.52 (d, 1 H, H-1′, $J_{1',2'}$ 3.4 Hz), 4.7–4.9 (m, 3 H, OH, exchangeable), 7.59 (s, 1 H, H-6), and 11.19 (broad s, 1 H, NH, exchangeable); pK_a 9.05.

Anal. Calc. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.55; H, 5.74; N, 10.84.

3-Methyl-3′,5′-O-tetraisopropyldisiloxanyl-ψ-uridine (**6**). — A mixture of **5** (2.6 g, 10 mmol) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[12,13] (3.48 g, 11 mmol) in pyridine (30 ml) was stirred at room temperature for 6 h and then evaporated *in vacuo*. The residue was partitioned between chloroform (100 ml) and water (50 ml). The organic layer was separated, washed with water (2 × 50 ml), dried (Na_2SO_4), and concentrated to dryness. The residue was chromatographed over a column (30 × 3 cm) of silica gel with benzene–ethyl acetate (5:3), to give **6** (2.7 g, 54%) as a foam; ^{1}H-n.m.r. data (dimethyl sulfoxide-d_6): δ 1.02 (m, 28 H, 4 iPr), 3.12 (s, 3 H, NMe), 3.6–4.0 (m, 5 H, H-2′,3′,4′,5′,5′), 4.56 (s, 1 H, H-1′), 5.11 (d, 1 H, OH, exchangeable), 7.31 (s, 1 H, H-6), and 11.32 (s, 1 H, NH, exchangeable).

Anal. Calc. for $C_{22}H_{40}N_2O_7Si_2$: C, 52.77; H, 8.05; N, 5.59. Found: C, 52.55; H, 8.15; N, 5.41.

3-Methyl-3′,5′-O-tetraisopropyldisiloxanyl-2′-O-[(imidazol-1-yl)thiocarbonyl]-ψ-uridine (**7**). — A mixture of **6** (2.7 g, 5.4 mmol) and bis(imidazol-1-yl)thione (2.4 g, 13.5 mmol) in *N*,*N*-dimethylformamide (9 ml) was stirred for 12 h at room

temperature and then partitioned between ethyl acetate (200 ml) and water (50 ml). The organic layer was separated, washed with water (2 × 50 ml), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column (30 × 3 cm) with ethyl acetate–carbon tetrachloride (3:2), to give **7** (3.0 g, 91%) as a foam; ^{1}H-n.m.r. data (dimethyl sulfoxide-d_6): δ 1.03 (m, 28 H, iPr), 3.13 (s, 3 H, NMe), 4.00 (m, 3 H, H-4′,5′,5′), 4.70 (m, 1 H, H-3′), 4.95 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 6.14 (dd, 1 H, $J_{1',2'}$ 1.5, $J_{2',3'}$ 5.5 Hz, H-2′), 7.11 (dd, 1 H, imidazole), 7.49 (s, 1 H, H-6), 7.87 (t, 1 H, imidazole), 8.55 (dd, 1 H, imidazole), and 11.36 (s, 1 H, NH, exchangeable).

Anal. Calc. for $C_{26}H_{42}N_4O_7SSi_2$: C, 51.12; H, 6.93; N, 9.17; S, 5.25. Found: C, 50.81; H, 6.84; N, 8.97; S, 4.90.

2′-Deoxy-3-methyl-3′,5′-O-tetraisopropyldisiloxanyl-ψ-uridine (**8**). — A suspension of **7** (2.0 g, 3.3 mmol) and ammonium sulfate (10 mg) in hexamethyldisilazane (40 ml) was heated under reflux until a clear solution was obtained. The solvent was removed *in vacuo* and the residue dissolved in toluene (30 ml). To the solution was added a mixture of 2,2′-azobis(2-methylpropionitrile) (400 mg) and tri-butyltin hydride (2.3 g) in toluene (30 ml), and the mixture was heated at reflux for 2 h. After removal of the solvent *in vacuo*, the residue was chromatographed on a column (30 × 3 cm) of silica gel with benzene–ethyl acetate (5:2), to give **8** (1.1 g, 70%) as a foam; ^{1}H-n.m.r. data (dimethyl sulfoxide-d_6): δ 1.02 (m, 28 H, iPr), 2.1 (m, 2 H, H-2′a,2′b), 3.12 (s, 3 H, NMe), 3.65 (m, 1 H, H-4′), 3.87 (m, 2 H, H-5′,5′), 4.38 (m, 1 H, H-3′), 4.81 (t, 1 H, $J_{1',2'a} = J_{1',2'b}$ = 6.5 Hz, H-1′), 7.25 (d, 1 H, H-6), and 11.15 (d, 1 H, NH, exchangeable).

Anal. Calc. for $C_{22}H_{40}N_2O_6SSi_2$: C, 54.51; H, 8.32; N, 5.78. Found: C, 54.60; H, 8.62; N, 5.70.

2′-Deoxy-3-methyl-ψ-uridine (**9**). — To a solution of **8** (1.0 g, 2.0 mmol) in tetrahydrofuran (25 ml) was added, dropwise, M tetra-butylammonium fluoride in tetrahydrofuran, and the reaction was monitored by t.l.c. (acetone–chloroform, 3:1). When the reaction was complete, the mixture was concentrated to dryness and the residue chromatographed over a column (30 × 3 cm) of silica gel with acetone. Compound **9** (0.32 g, 65%) crystallized from ethanol–ether; m.p. 175°; ^{1}H-n.m.r. data (dimethyl sulfoxide-d_6): δ 1.75 (m, 1 H, $J_{1',2'a}$ 9.8, $J_{2'a,3'}$ 5.6, $J_{2'a,2'b}$ 12.5 Hz, H-2′a), 2.08 (m, 1 H, $J_{1',2'b}$ 5.8, $J_{2'b,3'}$ 2.1, $J_{2'a,2'b}$ 12.5 Hz, H-2′b), 3.13 (s, 3 H, NMe), 3.41 (m, 2 H, H-5′,5′), 3.70 (m, 1 H, H-4′), 4.13 (m, 1 H, H-3′), 4.84 (dd, 1 H, $J_{1',2'a}$ 9.8, $J_{1',2'b}$ 5.8 Hz, H-1′), and 7.43 (s, 1 H, H-6).

Anal. Calc. for $C_{10}H_{14}N_2O_5$: C, 49.56; H, 5.82; N, 11.61. Found: C, 49.49; H, 5.73; N, 11.53.

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