Synthesis of (*S*)-5-(4,5-Dihydroxypentyl)uracil from Pseudouridine and Clarification of Stereochemistry[1]

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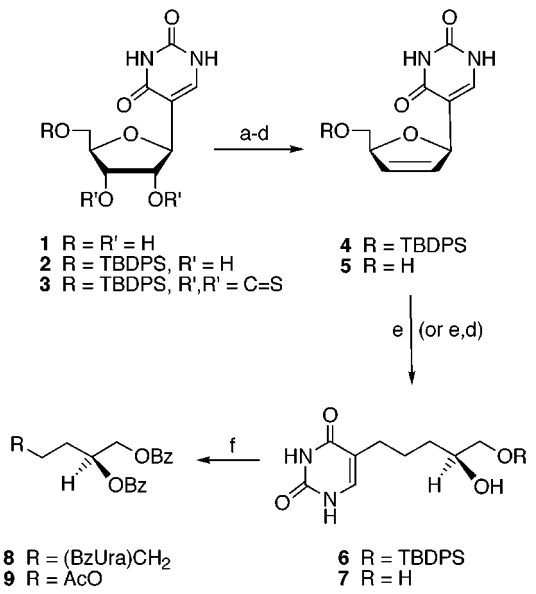
Approximately half of the thymine bases in the large transducing bacteriophage SP-15 of *Bacillus subtilis* were replaced by (*S*)-5-(4,5-dihydroxypentyl)uracil (DHPU, **7**).[2,3] Incorporation of DHPU with its chiral dihydroxypentyl side chain in place of thymine confers novel physical properties on SP-15 DNA including a lower melting temperature (T_m = 61.5 °C), increased buoyant density (CsCl), and alkaline lability.[2] Phosphoglucuronidation of one hydroxyl on the pentyl side chain and glucosidation of the other occurs, and this hypermodification is responsible for the observed increase in buoyant density.[2,3] Presence of the hypermodified DHPU has also been associated with high levels of resistance to endonucleases[4] and DNA T4 ligase,[5] although potential roles of DHPU in the control of bacteriophage transcription and translation are not well understood.[6−8]

DHPU was isolated from SP-15 DNA after formic acid hydrolysis.[2] Neither elucidation of its biosynthetic pathway nor identification of the sequence of steps leading to its incorporation into SP-15 DNA have been established.[6−10] Potential biosynthetic precursors including uracil,[6] ribose,[9] and glutamic acid[10] have been suggested. An obvious candidate, pseudouridine (**1**), was discounted as a precursor since its Cahn−Ingold−Prelog configuration descriptor at C4′ (*R*) was noted to be opposite to that of DHPU at C4′ (*S*).[10] However, C−I−P configuration descriptors are based on rules with C3′ of higher priority than C5′ in pseudouridine (**1**), but C5′ of higher priority than C3′ in DHPU (no hydroxyl group on C3′). Thus, the absolute stereochemistry at C4′ is the *same* in both **1** and DHPU.

Scheme 1[a]



[a] (a) TBDPSCl/pyridine. (b) $(Imidazole)_2CS$/DMF/Δ. (c) $P(OEt)_3$/Δ. (d) NH_4F/MeOH/Δ. (e) H_2/Pd−C. (f) BzCl/pyridine.

(*S*)-DHPU (**7**) was synthesized in seven steps from (*S*)-malic acid.[10] Two syntheses of the racemic product have involved an analogous route[10] or generation of an initial intermediate by photochemical addition of vinylene carbonate to uracil.[11] We now describe a synthesis of DHPU from pseudouridine [5-(β-D-ribofuranosyl)uracil, **1**] that employs hydrogenolysis of the benzylic C1′−O4′ bond with retention of configuration at C4′ (Scheme 1).

Treatment of pseudouridine (**1**) with *tert*-butyldiphenylsilyl chloride in pyridine gave 5-(5-*O*-TBDPS-β-D-ribofuranosyl)uracil (**2**, 76%). Thiocarbonyldiimidazole/DMF/90 °C converted **2** into its cyclic 2′,3′-*O*-thionocarbonate **3** (94%) which was heated with triethyl phosphite[12] (110 °C) to give 5-[5(*S*)-(TBDPS)oxymethyl-2,5-dihydrofuran-2(*R*)-yl]uracil (**4**, 94%). Deprotection of **4** (NH_4F/MeOH)[13] gave 5-[2,5-dihydro-5(*S*)-hydroxymethylfuran-2(*R*)-yl]uracil (**5**, 71%). Concomitant hydrogenolysis of the C1′−O4′ bond and hydrogenation of the furanyl double bond occurred with Pd−C to give 5-[5-(TBDPS)oxy-4(*S*)-hydroxypentyl]uracil (**6**, 54%). Deprotection of **6** (NH_4F/MeOH) gave the desired (*S*)-5-(4,5-dihydroxypentyl)uracil (**7**, 99%). Treatment of **5** with H_2/Pd−C also gave **7**. The mp, UV, NMR, and HRMS data for **7** were consistent with those reported previously for (*S*)-DHPU.[10]

Treatment of **7** with benzoyl chloride/pyridine gave an amorphous tribenzoyl derivative **8** (80%). The bisignate CD spectrum of **8** (Figure 1) has a positive first Cotton

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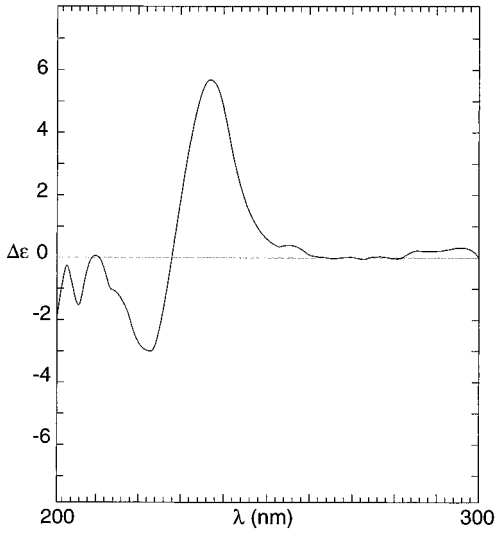

Figure 1. Circular dichroism spectrum of **8** in MeOH solution.

effect (236 nm, +5.65) and a negative second Cotton effect (222 nm, −3.07) indicative of exciton splitting[14] for the vicinal (4′ and 5′) benzoyl chromophores. The signed order of Cotton effects in the CD spectrum of **8** is consistent with positive (+)-chirality between the primary (5′) and secondary (4′) benzoyl chromophores with an (*S*)-configuration at C4′ as found with (*S*)-4-*O*-acetyl-1,2-di-*O*-benzoylbutane-1,2,4-triol (**9**) and analogous derivatives[15] (and assuming weak exciton splitting interactions with the *N*-benzoyluracil chromophore, which is in a 1,4 relationship with the chiral center at C4′).

Synthesis of (*S*)-5-(4,5-dihydroxypentyl)uracil (DHPU, **7**) from pseudouridine (**1**) was achieved in five steps with retention of configuration at C4′. This establishes pseudouridine as a prime precursor candidate for the biosynthesis of DHPU by *B. subtilis* bacteriophage SP-15.

Experimental Section

Uncorrected melting points were determined with a hot-stage apparatus. UV spectra were recorded with solutions in H_2O (pH 7.0) unless otherwise indicated. NMR spectra of solutions in Me_4Si/Me_2SO-d_6 were recorded at 270 or 400 MHz (^1H) or 25 MHz (^{13}C). Mass spectra were determined at 70 eV with chemical ionization (CI, CH_4) unless otherwise indicated. All chemicals and solvents were of reagent quality, and pyridine was dried by reflux over and distillation from CaH_2. Column chromatography was performed with silica gel (100−200 mesh).

5-[5-*O*-(*tert*-Butyldiphenylsilyl)-*β*-D-ribofuranosyl]uracil (2). TBDPSCl (6.15 g, 22 mmol) was added to a suspension of **1** (3.66 g, 15 mmol) in dried pyridine (250 mL), and the mixture was stirred for 48 h at ambient temperature. Volatiles were evaporated, and the residue was chromatographed (MeOH/$CHCl_3$, 1:99). Crystallization of the solid residue ($CHCl_3$) gave **2** (5.49 g, 76%; colorless needles): mp 114−115 °C; ^1H NMR δ 1.05 (s, 9H), 3.73 (q, J = 5.7 Hz, 1H), 3.88 (br d, 2H), 3.97−4.01 (m, 2H), 4.57 (d, J = 4.7 Hz, 1H), 4.85 (br s, 1H), 5.03 (br s, 1H), 7.34 (s, 1H), 7.43−7.73 (m, 10H), 11.09 (br, 2H); ^{13}C NMR δ 18.72, 26.62, 64.41, 70.73, 73.66, 78.69, 82.90, 110.92, 127.77−135.02 (arom), 139.12, 151.17, 163.46; MS m/z 483 (M + 1). Anal. Calcd for $C_{25}H_{30}N_2O_6Si\cdot H_2O$ (500.6): C, 59.98; H, 6.44; N, 5.60. Found: C, 60.22; H, 6.26; N, 5.65.

5-[5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-*O*-thiocarbonyl-*β*-D-ribofuranosyl]uracil (3). A solution of **2** (5.49 g, 11 mmol) and thiocarbonyldiimidazole (3.0 g, 17 mmol) in DMF (150 mL) was stirred for 6 h at 90 °C under N_2. Volatiles were evaporated, and the residue was chromatographed (MeOH/$CHCl_3$, 1:99). Crystallization of the solid residue ($CHCl_3$) gave **3** (5.4 g, 94%; fine colorless needles): mp 183−185 °C; ^1H NMR δ 1.05 (s, 9H), 3.82 (m, 2H), 4.27 (q, J = 5.0 Hz, 1H), 5.00 (d, J = 3.0 Hz, 1H), 5.62 (dd, J = 4.4, 7.4 Hz, 1H), 5.72 (dd, J = 3.0, 7.4 Hz, 1H), 7.47−7.71 (m, 11H), 11.34 (br s, 2H); ^{13}C NMR δ 18.72, 26.50, 63.24, 80.56, 84.19, 86.53, 88.81, 108.41, 127.83−134.97 (arom), 141.40, 151.00, 163.11, 190.60. Anal. Calcd for $C_{26}H_{28}N_2O_6SSi$ (524.7): C, 59.52; H, 5.38; N, 5.34. Found: C, 59.51; H, 5.42; N, 5.33.

5-[5(*S*)-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-2,5-dihydrofuran-2(*R*)-yl]uracil (4). A solution of **3** (2.0 g, 3.8 mmol) in triethyl phosphite (80 mL) was stirred for 17 h at 110 °C. Volatiles were evaporated, and the residue was chromatographed (MeOH/$CHCl_3$, 1:99) to give **4** (1.6 g, 94%; colorless solid foam): ^1H NMR δ 0.99 (s, 9H), 3.65 (dd, J = 5.4, 10.1 Hz, 1H), 3.74 (dd, J = 4.0 Hz, 1H), 4.87 (m, 1H), 5.57 (br s, 1H), 5.92 (dd J = 5.9 Hz, 1H), 6.04 (dd, 1H), 7.12 (s, 1H), 7.38−7.65 (m, 10H), 10.85 (br, 1H), 11.18 (br, 1H); ^{13}C NMR δ 18.78, 26.62, 66.58, 80.38, 86.35, 112.91, 127.77−135.02 (arom and olefin), 138.18, 151.11, 163.28; MS m/z 449 (M + 1). Anal. Calcd for $C_{25}H_{28}N_2O_4Si\cdot 0.5H_2O$ (457.6): C, 65.62; H, 6.39; N, 6.12. Found: C, 65.36; H, 6.41; N, 5.92.

5-[2,5-Dihydro-5(*S*)-(hydroxymethyl)furan-2(*R*)-yl]uracil (5). A solution of **4** (90 mg, 0.2 mmol) and NH_4F (300 mg) in MeOH (5 mL) was refluxed for 90 min. Volatiles were evaporated, and the residue was chromatographed (MeOH/$CHCl_3$, 1:9). Crystallization of the solid residue (MeOH) gave **5** (30 mg, 71%; fine off-white needles): mp 215 °C; UV_{max} 263 nm (ϵ 8800); ^1H NMR δ 3.47 ("s", 2H), 4.75 (m, 2H), 5.52 (d, J = 3.4 Hz, 1H), 5.92 ("t", 2H), 7.35 (s, 1H), 10.85 (br, 1H), 11.12 (br, 1H); ^{13}C NMR δ 63.89, 80.15, 86.94, 112.97, 128.18, 129.53, 138.94, 151.05, 163.40; MS m/z 211 (M + 1). Anal. Calcd for $C_9H_{10}N_2O_4$ (210.2): C, 51.43; H, 4.80; N, 13.33. Found: C, 51.28; H, 4.63; N, 13.49.

5-[5-[(*tert*-Butyldiphenylsilyl)oxy]-4(*S*)-hydroxypentyl]uracil (6). A solution of **4** (950 mg, 2.1 mmol) was added to a suspension of 10% Pd−C (300 mg) in MeOH (10 mL), the mixture was stirred under H_2 (1 atm) for 24 h at ambient temperature, and the catalyst was removed by filtration. Volatiles were evaporated, and the residual syrup was chromatographed (MeOH/$CHCl_3$, 1:49). Crystallization of the solid residue ($CHCl_3$) gave **6** (510 mg, 54%; fine colorless needles): mp 168 °C; ^1H NMR δ 0.99 (s, 9H), 1.23−1.59 (m, 4H), 2.18 ("t", 2H), 3.42−3.46 (m, 2H), 3.56 (m, 1H), 4.56 (s, 1H), 7.18 (s, 1H), 7.40−7.65 (m, 10H), 10.64 (br, 1H), 11.00 (br, 1H); ^{13}C NMR δ 18.78, 24.16, 26.03, 32.82, 26.62, 67.86, 70.32, 111.97, 127.77−135.02 (arom), 137.66, 151.41, 164.51; MS m/z 453 (M + 1). Anal. Calcd for $C_{25}H_{32}N_2O_4Si\cdot 0.33H_2O$ (458.6): C, 65.47; H, 7.18; N, 6.11. Found: C, 65.46; H, 7.15; N, 6.03.

(*S*)-5-(4,5-Dihydroxypentyl)uracil (7). A solution of **6** (300 mg, 0.65 mmol) and NH_4F (300 mg) in MeOH (10 mL) was refluxed for 90 min. Workup (as described for **5**) gave **7** (138 mg, 99%; fine colorless needles): mp 225−226 °C (lit.[10b] mp 225−226 °C); UV_{max} 265 nm (ϵ 6600); ^1H NMR δ 1.16−1.24 (m, 1H), 1.34−1.44 (m, 2H), 1.50−1.57 (m, 1H), 2.11−2.19 (m, 2H), 3.20−3.29 (m, 2H), 3.37 ("s", 1H), 4.38 (br s, 2H), 7.18 (s, 1H), 10.66 (br s, 1H), 10.94 (br s, 1H); ^{13}C NMR δ 24.34, 25.98, 32.82, 65.82, 70.91, 111.97, 137.66, 151.29, 164.45; HRMS (EI) m/z 214.0953 (5%, M^+ = 214.0952). Anal. Calcd for $C_9H_{14}N_2O_4$ (214.2): C, 50.46; H, 6.59; N, 13.08. Found: C, 50.04; H, 6.60; N, 12.80.

Catalytic reduction of **5** (as described for **4** → **6**) also gave **7**.

(*S*)-5-(4,5-Di-*O*-benzoyl-4,5-dihydroxypentyl)-*N*-benzoyluracil (8). Benzoyl chloride (158 mg, 1.12 mmol) was added to a solution of **7** (40 mg, 0.187 mmol) in dried pyridine (2 mL), and the mixture was stirred for 1.5 h at 60 °C. Volatiles were evaporated, and the residue was chromatographed (MeOH/$CHCl_3$, 1:99) to give **8** (79.2 mg, 80%; colorless solid foam): UV-(MeOH)$_{max}$ 230, 252 nm (ϵ 28 000, 20 000); CD (MeOH) 236 nm

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($\Delta\epsilon = +5.65$), 222 nm ($\Delta\epsilon = -3.07$); ^{1}H NMR δ 1.59 (m, 2H), 1.83 (m, 2H), 2.33 (m, 2H), 4.45 (dd, $J = 6.7$, 11.8 Hz, 1H), 4.62 (dd, $J = 2.7$, 11.8 Hz, 1H), 5.44 (m, 1H), 7.47−7.96 (m, 16H), 11.48 (br s, 1H); ^{13}C NMR δ 23.96, 26.31, 30.43, 65.44, 71.72, 114.15, 128.36−136.77 (arom), 151.33, 162.88, 166.09, 166.22, 168.80; MS (EI) m/z 526 (M^+).

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