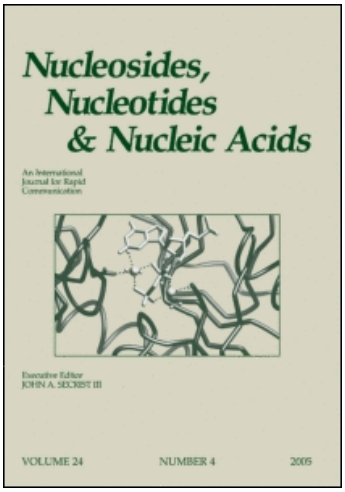

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Thomas E. Rawson[a]; Thomas R. Webb[a]
[a] Department of Medicinal and Biomolecular Chemistry 460 Point San Bruno Blvd., Genentech Inc, South San Francisco, CA

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THE SYNTHESIS OF 5'-HOMO-2'-DEOXYCYTIDINE

Thomas E. Rawson and Thomas R. Webb*

Genentech Inc.
Department of Medicinal and Biomolecular Chemistry
460 Point San Bruno Blvd., South San Francisco, CA 94080

Abstract: Syntheses of some pyrimidine 2'-deoxy-5'-homologs are reported.

The synthesis of 5'-homologs of nucleosides has attracted considerable attention over the years.[1-5] There are several potential applications of this interesting class of compounds; for example, in the case of the 2'-deoxy pyrimidine derivatives, an evaiuation of their anti-cancer or anti-viral activity has not been reported. Another potential application is the incorporation of these modified nucleosides into oligonucleotide analogs. Such derivatives may be resistant to nucleases,[4] but may retain the ability to hybridize to their analogous complementary sequence. We reasoned that an improved synthesis of some new members of this class of compounds would facilitate the synthesis of other 5'-homonucleosides. We therefore undertook the synthesis of the previously unknown title compound via the uridine-5'-homo-2'-deoxynucleoside, by the adaptation of known syntheses of similar nucleosides.

The synthesis of 5'-tosylthymidine (**1**) the starting material for the previous synthesis of 5'-homothymidine,[1] was reported by Michelson and Todd in 1955 via a five step synthesis from thymidine,[6,7] in moderate overall yield. We found however that **1** could be prepared in one step in 72% yield, by

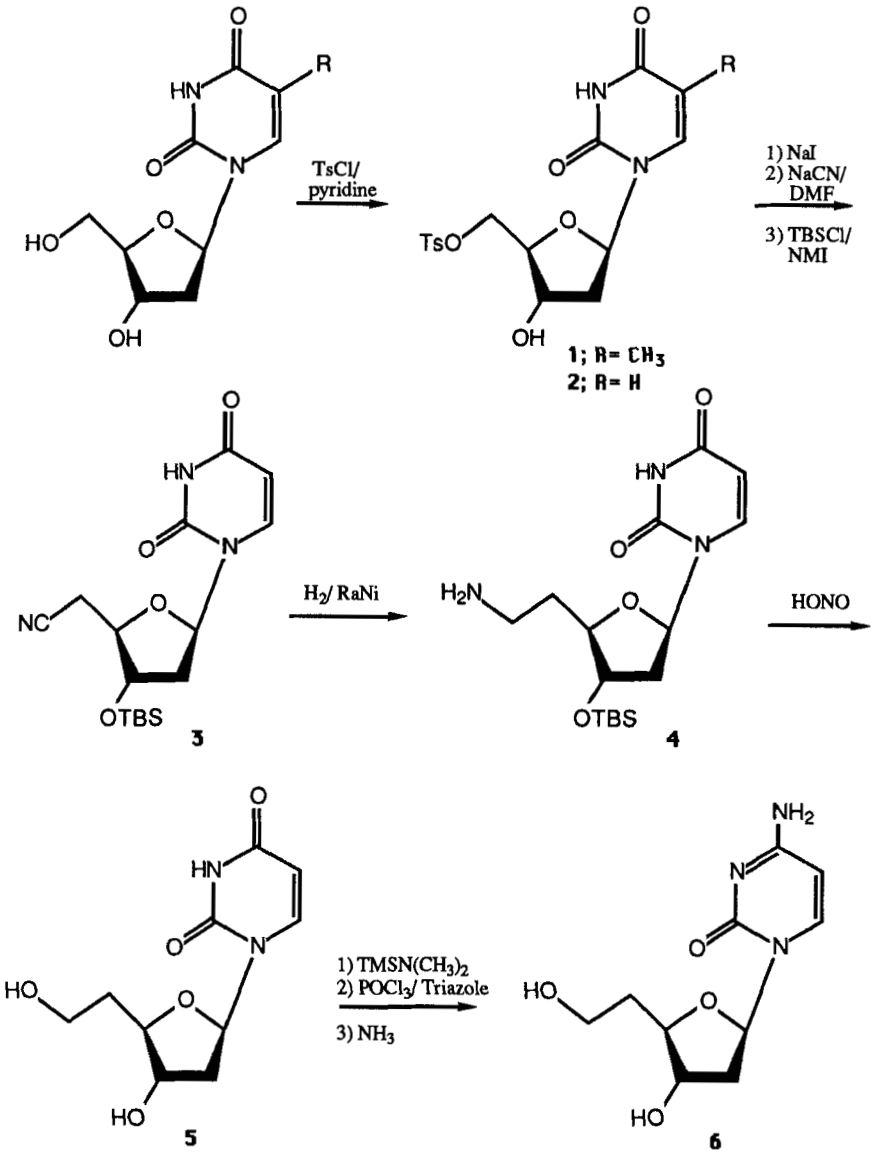
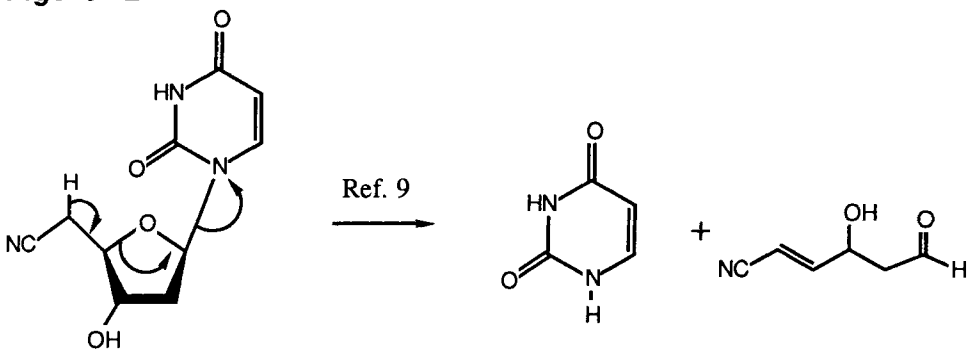


Figure 1; Ts= p-toluenesulfonyl, TBS= t-butyldimethylsilyl, NMI= N-methylimidazole, DMF= dimethylformamide.

treatment of thymidine with tosyl chloride in pyridine. The selective tosylation of primary versus secondary alcohols has many precedents.[8] This procedure was readily applied to the synthesis of **2** and greatly facilitated the synthesis of multigram quantities of **1** or **2**. Todd's procedure for the conversion of **1** to its corresponding iodide was used to prepare the 2',5'-dideoxy-5'-iodouridine.

The direct conversion of this iodide to the nitrile **3** proved to be surprisingly difficult. It has been pointed out that nitriles such as 2',5'-dideoxy-5'-cyanouridine are very sensitive to base promoted depyrimidination (see Figure 2).[9] This observation, together with the expected propensity of the iodide to form O^2:5'-cyclo-3'-2'-deoxyuridine,[4] can explain the difficulties encountered in this transformation. We found that the *in situ* conversion of the 2',5'-dideoxy-5'-cyanouridine to the dichloromethane soluble 3'-t-butyldimethylsilyl ether **3**, facilitated the isolation and purification of this sensitive 5'-deoxy-5'-cyano pyrimidine derivative. The resulting nitrile was then reduced to the amine **4**. The reduction of the 5-methyl derivative of **3** has been reported to require Pd on $BaSO_4$,[1,4] however in our hands this catalyst gave no reduction. Pd on carbon gave reduction, but good results depended on the commercial source (and lot number) of the catalyst. However, consistent reduction to the amine was observed with Raney Nickel. Direct reduction to the imine by diisobutylaluminum hydride (or catalytic hydrogenation), and finally hydrolysis, did not give the desired 6'-aldehyde.

Figure 2



The conversion of **4** into the 2'-deoxy-5'-homolog **5**, was accomplished by nitrous acid treatment. This compound was then converted into the corresponding cytidine derivative **6** by transient protection of the hydroxyl groups with trimethylsilyl dimethylamine, conversion of this product to the triazolide, and finally treatment of this triazolide with anhydrous ammonia.[10,11]

This synthesis represents a convenient procedure for the preparation of nucleoside analogs **5** and **6**. These compounds (**5** and **6**) showed no protection against the cytopathic effects of human immunodeficiency virus in the ATH8 *in vitro* assay at concentrations as high as 100 mM.[11,12]

Experimental Section

Deoxyuridine was purchased from Sigma Chemical Company and all other reagents were from Aldrich Chemical Company. ^{1}H-NMR spectra were recorded on a 80-MHz IBM NR/80 spectrometer with tetramethylsilane as the internal standard. Mass spectra were obtained by the use of the positive-ion fast atom bombardment (FAB) technique. Thin layer chromatography (tlc) was performed on EM DC-Alufolien kieselgel-60 F_{254} plates, column chromatography was done with EM kieselgel-60 (70-230 mesh). All concentrations were performed under reduced pressure.

5'-O-p-Toluenesulfonylthymidine[7]. A solution of thymidine (4.84 g, 20 mmol) in 100 ml of dry pyridine was treated with a solution of p-toluenesulfonyl chloride (8.0 g, 42 mmol) in 80 ml of dry pyridine, by dropwise addition over a 0.5 h period at room temp. This solution was allowed to set at 5° C for 48 h, then 10 ml of water was added and the solution was stirred at room temp. for 0.5 h. The reaction mixture was then concentrated to ca. 50 ml, 10ml of methanol was then added, followed by enough water to give a turbid solution. Crystallization began and more water was added (200 ml total). After 1 h the mixture was filtered, the residue was dried and then recrystallized from methanol/dichloromethane/toluene. Yield 5.7 g (72%) m.p. = 169-175° C (decomposition, lit.[7] = 172° C).

2'-Deoxy-5'-O-p-toluenesulfonyluridine (**2**). To a stirred solution of 2'-deoxyuridine (19.0 g, 83 mmol) in 150 ml of pyridine at 0°C was added a solution of p-toluenesulfonyl chloride (19.9 g, 104 mmol) in 100 ml of pyridine over 0.5 hours. The reaction mixture was warmed to 20° C and the reaction followed by tlc (silica, 95:5 CH_2Cl_2:CH_3OH), product R_f = 0.22. After four hours, 40 ml of water was added and stirring continued for 15 minutes. The mixture was taken up in ethyl acetate, washed with cold 1N HCl pH 3 to remove pyridine, brine, then dried over Na_2SO_4, filtered and concentrated. Yield 26.57 g crude product. Recrystallization from ethyl acetate/hexane gave 17.93 g (56%) **2** as white crystals, mp 166-167° C (dec). ^{1}H NMR (d_6-DMSO-TMS) d 2.08-2.11 (2H, m, H-2'), 2.40 (3H, s, p-Tol-CH3), 3.83 (1H, m, H-3'), 4.11-4.21 (1 + 2H, m, H-

4' + H-5'), 5.42 (1H, d, 3'-OH), 5.57 (1H, d, J = 7.5 Hz, H-5), 6.09 (1H, t,J= 3 Hz, 1'), 7.44 (1H, d, J=7.5 Hz, H-6), 7.46 (2H, d, J=8 Hz, p-tol-Ar), 7.77 (2H, d, J=8 Hz, p-tol-Ar), 11.34 (1H, s, 3-NH). UV_{max} - 225, 263 nm. Anal. ± 0.2% ($C_{16}H_{18}N_2O_7S$) C, H, N.

2',5'-Dideoxy-5'-iodouridine. To a stirred solution of of NaI (18.8 g, 125 mmol) in 150 ml acetone was added 2'-deoxy-5'-O-p-toluenesulfonyluridine (19.2 g, 50 mmol). This was heated to reflux. After eight hours the reaction was cooled and the precipitated salts removed by filtration. The filtrate was concentrated then partitioned between ethyl acetate and brine, the brine was extracted four times with ethyl acetate, and the combined ethyl acetate washes were dried (Na_2SO_4) and concentrated. This mixture was crystallized from ethanol to give 11.38 g of product, mp 163°-164°C (dec.). ^1H NMR (DMSO-TMS) d 2.10 (1H, m, H-2'), 2.23 (1H, m, H-2'), 3.37 (1H, m, H-5'), 3.48 (1H, m, H-5'), 3.80 (1H, m, H-4'), 4.14 (1H, m, H-3'), 5.50 (1H, s, 3'-OH), 5.65 (1H, d, J=7.5 Hz, H-5), 6.19 (1H, t, J=7 Hz, H-1'), 7.66 (1H, d, J=7.5 Hz, H-6), 11.35 (1H, s, 3-NH). Anal. ($C_9H_{11}N_2O_4I$) C, H, N, I.

2',5'-Dideoxy-3'-0-t-butyldimethysilyl-5'-cyanouridine(**3**). To a stirred solution of 2',5'-dideoxy-5'-iodouridine (16.75 g, 49 mmol) in 100 ml DMF at room temp. was added NaCN (3.64 g, 74 mmol). Stirring was continued at 20°C and reaction progress followed by tlc (silica , 9:1 CH_2Cl_2:CH_3OH), product R_f = 0.31. After 20 hours, reaction was complete. The stirred reaction mixture was treated with 50 ml N-methylimidazole followed by (14.9 g, 99 mmol) t-butyldimethylsilyl chloride. The reaction was followed by tlc (silica, 9:1 CH_2Cl_2:CH_3OH), product R_f = 0.71, and was complete in 1 hour. The reaction mixture was partitioned between water and 1:1 ether:ethyl acetate. The ether:ethyl acetate extract was dried over Na_2SO_4, filtered and concentrated to yield 10.9g (62%) slightly impure **3**. The crude product was used in the next step but could be recrystallized from hexane/ethyl acetate to yield 9.4 g (54%) white powder, mp 139-140°C. ^1H NMR (DMSO-TMS) δ 0.09 (6H, S, 3'-OSi CH_3), 0.85 (9H, S, 3'-OSi-t-butyl), 2.10 (1H, m, H-2'), 2.32 (1H, m, H-2'), 2.93 (2H, m, H-5'), 3.91 (1H, m, H-4'), 4.31 (1H, m, H-4'), 4.31 (1H, m, H-3'), 5.76 (1H, d, J = 7.5 Hz, H-5), 6.15 (1H, t, H-1'), 7.65 (1H, d, J = 7.5 Hz, H-6), 11.38 (1H, s, 3-NH). UV_{max}-261nm. Anal. ($C_{16}H_{25}N_3O_4Si$) C, H, N.

2'-Deoxy-3'-0-t-butyldimethylsilyl-6'-amino-5'-homouridine (**4**). To a 1 liter stainless steel bomb was added 2'-deoxy-3'-t-butyldimethylsilyl-5'-cyanouridine (**3**) (6.62 g,18.6 mmol), 530 ml methanol saturated with NH_3 and 20 ml of Raney nickel catalyst (50%

slurry in water). The bomb was sealed, stirred, purged and pressurized to 300 psi with H_2. The reaction was periodically sampled and followed by tlc (silica, 50 hexane:45 ether:5 methanol) product R_f = 0.01, UV, ninhydrin. After 30 hours the catalyst was filtered off and the filtrate evaporated. The greenish residue was taken up in ethyl acetate and carefully extracted (to pH = 3) with cold dil. HCl. The ethyl acetate phase was evaporated to recover 2.22 g (34%) of the starting cyano compound. The pH of the aqueous phase was adjusted to 10 and the solution extracted with ethyl acetate. The ethyl acetate was washed with brine, dried over Na_2SO_4, filtered and evaporated to yield 2.2 g (33%) crude product contaminated with nickel. The reduction was run again on recovered starting cyano compound to yield an additional 0.43 g crude amine. The crude amine products were combined and passed through a short silica column (93 CH_2Cl_2, 5 MeOH, 2 TEA; product R_f = 0.2) to remove nickel. Yield = 1.15 g (17%), ^{1}H NMR ($CDCl_3$ - TMS), δ 0.04 (6H, S, 3'-$OSiCH_3$), 0.83 (9H, S, 3'-O-Si-t-butyl), 1.99 (2H, m, H-5'), 2.18 (2H, m, H-2'), 3.07 (2H, m-H-6'), 3.85 (1H, m, H-4'), 4.13 (1H, m, H-3'), 5.76 (1H, d, J = 8Hz, H-5), 6.12 (1H, t, H-1'), 7.56 (1H, d, J = 8Hz, H-6). FAB mass spec., m/z (relative intensity 112 (100, BH^+), 356 (15, MH^+).

2'-Deoxyhomouridine(**5**). A stirred solution of (2.21 g, 32 mmol) $NaNO_2$ in 30 ml water was cooled to 0°C and acidified to pH = 3 with a few drops of 6N HCl. A solution of 2'-deoxy-3'-t-butyldimethylsilyl-6'-amino-6'-deoxy-homouridine(**4**) (1.15 g, 32 mmol) in 10 ml THF was added dropwise over five minutes followed by 20 ml ether. The reaction was followed by tlc (silica, 93 CH_2Cl_2:5 MeOH:2 TEA). After 3 hours, the ether layer was separated. The aqueous phase was extracted again with ether and the combined ether extracts washed with water, brine, dried over Na_2SO_4, filtered and concentrated to a foam. After standing overnight, tlc indicated hydrolysis of the TBS group. The product was purified by flash chromatography (silica, 9 CH_2Cl_2:1 MeOH) product R_f =0.25, to give 0.350 g (45%) 2'-deoxyhomouridine. ^{1}H NMR (CD_3 OD-TMS) δ 1.88 (2H, m, H-5'), 2.25 (2H, m, H-2'), 3.70 (2H, m, H-6'), 3.92 (1H, m, H-3'), 4.18 (1H, m, H-4'), 5.73 (1H, d, J= 7Hz, H-5), 6.18 (1H, t, H-1'), 7.64 (1H, d, J=7Hz, H-6) Exchangeable protons: (DMSO-D_20) δ 4.52 (1H, t, 6'-OH), 5.22 (1H, d, 3'-OH), 11.26 (1H, S, 3-NH). UV_{max} = 263 nm.

2'-Deoxyhomouridine-3',6'-diacetate. Because 2'-deoxy-5'-homouridine was too hygroscopic to provide good elemental analysis, the diacetate was prepared from 15 mg 2'-deoxy-5'-homouridine, 1.0 ml pyridine and 10 drops acetic anhydride. After stirring for 2 hours at 20°C, the reaction mixture was taken up in ether and washed with

dil. HCl to pH = 3. The ether was evaporated and the product purified by prep tlc. Elemental analysis by high res. mass spec.: For MH^+ = $C_{14}H_{19}N_2O_7$; calculated 327.1192; found 327.1195.
^1H NMR ($CDCl_3$) δ 2.06 (6H, m, acetates + H-2' + H-5'), 4.20 (4H, m, H-3' + H-6'), 5.78 (1H, d, J=7Hz, H-5), 6.19 (1H, t, H-1'), 7.40 (1H,d, J=7Hz, H-6), 9.10 (1H, bs, exchangeable, 3-NH).

3',6'-O-Bis(trimethylsilyl)-4-triazolyl-5'-homouridine. A solution of **5** (250 mg, 1.03 mmol) and N,N-dimethylaminotrimethylsilane (1.0 g, 8.5 mmol) in 4 ml dry acetonitrile was stirred at 20°C for 1 hour. Stirring was stopped and the reaction mixture concentrated under vacuum to a foam. Yield = 390 mg (98%). 'H NMR ($CDCl_3$):δ 0.07 (18H,s, $SiCH_3$), 1.70 (1H, m, H5'), 1.84 (1H, m, H-5'), 1.95 (1H, M, H-2'), 2.27 (1H, m, H-2'), 3.67 (2H, m, H-6'), 3.87 (1H, m, H-3'), 4.07 (1H, m, H-4'), 5.70 (1H, d, H-5), 6.10 (1H, t, H-1'), 7.34 (1H, d, H-6).

To a slurry of triazole (1.35 g, 20 mmol) in 6 ml acetonitrile was added phosphorus oxychloride (0.7 g, 4.0 mmol) followed by triethylamine (2.35 g, 23 mmol) and this mixture stirred for five minutes. A solution of of 3',6'-bis(trimethylsilyl)homouridine (0.399 g, 1 mmol) in 3 ml acetonitrile was added and this mixture stirred at 20° C for 1.5 hours. The reaction mixture was then poured into 5% $NaHC0_3$ and extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated to yield 0.30 g (66%) slightly impure product. ^1H NMR ($CDCl_3$): δ 0.08(18H,s,$SiCH_3$), 1.90(2H,m,H-5'), 2.10(2H,m,H-2'), 3.77(2H,m,H-6'), 4.10(2H,m,H-3' + H-4'), 6.15(1H,s,H-1"), 7.08(1H,d,H-5), 8.08(1H,s,triazole), 8.31(1H,d,H6), 9.28(1H,s,triazole).

5'-Homo-2'-deoxycytidine (1-(2'-5'-dideoxy-β-D-allofuranosyl)cytosine, **6**). A solution of 300 mg crude 3',6'-bis-trimethylsilyl-4-triazoyl-5'-homouridine, 5 ml anhydrous NH_3 and 5 ml THF was stirred at 20°C for 2.5 hours in a sealed glass pressure tube. The NH_3 was carefully vented and the remaining solution concentrated to an oil. Purification by flash chromatography (silica: 82 CH_2Cl_2, 16 MeOH, 2 triethylamine) R_f=0.22, gave 52 mg (31%) homocytidine as a hygroscopic foam. ^1H NMR (CD_3OD-TMS): δ 1.90(2H,m,H5'), 2.14(1H,m,H2'), 2.32(1H,m,H2'), 3.72(2H,m,H6'), 3.96(1H,m,H3'), 4.16(1H,m,H4'), 5.97(1H,d,J=7,5 Hz,H5), 6.21(1H,t,J=6 Hz,H1'), 7.69(1H,d,J=7.5 Hz,H6). UV_{max}-274. FAB mass spec = 242(MH^+).

Since the parent compound was too hygroscopic for good elemental analysis it was converted to the picrate in the standard manner. Analysis for $C_{10}H_{15}N_3O_4 \cdot C_6H_3N_3O_7$ calc: C 40.86, H 3.86, N

17.87, found: C 40.53, H 3.73, N 17.87. ^{1}H NMR of picrate (DMS0-d6, TMS): d 1.79(1H,m,H-5'), 2,24(1H,m,H-2'), 3.58(2H,m,H-6'), 3.93(1H,m,H-3'), 4.14(1H,m,H-4'), 6.03(1H,t,H-1'), 6.10(1H,d,H-5), 7.85(1H,d,H-6), 8.23(1H,s,3'-OH,exchangeable), 8.71(2H,s,picrate), 9.42(1H,s,6'-OH,exchangeable).

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