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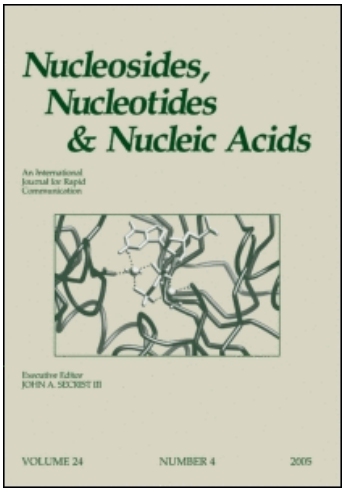

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THE USE OF ACETYL BROMIDE FOR THE MULTIGRAM SYNTHESIS OF THE ANTI-HIV AGENT 2′,3′-DIDEHYDRO-2′,3′-DIDEOXYCYTIDINE (d4C)

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Abstract: Treatment of uridine (**1**) with acetyl bromide produced bromoacetate **2** which was reduced with Zn/Cu to give the 2′,3′-unsaturated uridine (d4U; **3**). Conversion of the uracil moiety of **3** to thioamide **7** with Lawesson's reagent, followed by amination and deprotection with methanolic ammonia, afforded d4C (**9**). This multigram scale process for the synthesis of d4C proceeded in 20% yield from uridine.

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The search for compounds active against human immunodeficiency virus (HIV), the causative agent in acquired immunodeficiency syndrome (AIDS), has identified a class of nucleoside analogues which lack a 3′-OH group. Of these compounds, only one, 3′-azido-3′-deoxythymidine (AZT), is currently approved for AIDS therapy.[1,2] Among the other nucleoside analogues which are presently undergoing clinical evaluation are the 2′,3′-dideoxy compounds, 2′,3′-dideoxyinosine (ddI)[3] and 2′,3′-dideoxycytidine (ddC),[4] and the 2′,3′-unsaturated compound 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T).[5] A closely related unsaturated analogue, 2′,3′-didehydro-2′,3′-dideoxycytidine (d4C, **9**) has also been reported to be active against HIV in vitro.[6] We are interested in the evaluation of d4C for the treatment of HIV infection and therefore required an efficient synthesis which would permit the generation of multigram quantities for advanced biological studies. Previously published syntheses of d4C[6b,7,8] were performed

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on a relatively small scale and are not readily amenable to scaleup. We report an improved synthesis of d4C which allows for the preparation of larger quantities of the target compound.

In previous studies of nucleosides, we have examined various methods for the introduction of a 2′,3′-double bond[9,10] and found that the most efficient process for the conversion of a vicinal diol to the olefin involves reduction of intermediate bromoacetates. The bromoacetates are readily prepared from the ribonucleosides (vide infra). The analogous approach to synthesize d4C would start with cytidine and conversion of the 2′,3′-diol functionality to the olefin would give d4C. Previous studies by Marumoto and Honjo[11] have shown that treatment of either cytidine or $\underline{N}^4$-acetyl cytidine with acetyl halides gave poor yields of the corresponding haloacetates. Consequently, we chose to prepare d4U first from uridine, and then transform the uracil base to cytosine.

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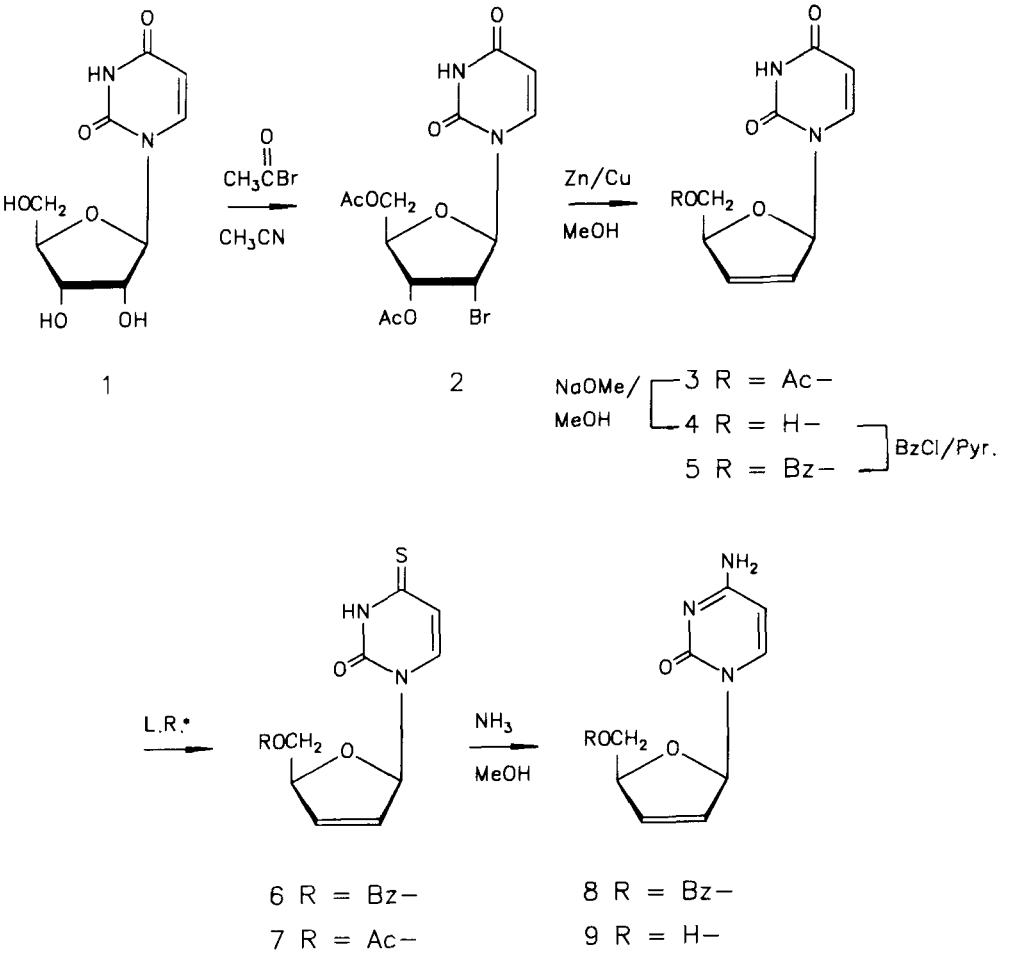
A suspension of uridine (**1**) was heated at reflux in acetonitrile, and acetyl bromide was added dropwise to give a nearly quantitative yield of bromoacetate **2** (Scheme).[11] The use of acetyl bromide is a very attractive alternative to previous studies which employed acetoxyisobutyryl bromide for the generation of 2′,3′-bromoacetates.[12,13] Acetyl bromide is less expensive and easier to handle than acetoxyisobutyryl bromide, and the product **2** is obtained in an excellent yield on at least a 60 g scale. Acetoxyisobutyryl bromide affords the product in lower yield protected as a mixture of 5′-acetoxyisobutyrate and 5′-acetate.

Reduction of bromoacetate **2** with Zn/Cu in methanol afforded 5′-$\underline{O}$-acetyl-d4U (**3**) in 40% yield. Uracil was the major side product (35%) in this reaction, and it could be separated from the product by column chromatography followed by recrystallization. Deprotection of **3** with sodium methoxide in methanol gave d4U (**4**) in 85% yield.

After the successful introduction of the 2′,3′-double bond, transformation of the base moiety from uracil to cytosine (**4** to **9**) was examined. The 5′-OH was protected as the benzoyl ester **5**[14] in 95%

SCHEME



1 2

6 R = Bz–
7 R = Ac–

8 R = Bz–
9 R = H–

*L.R. = Lawesson's Reagent

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yield by treatment of **4** with benzoyl chloride in pyridine. The benzoate was initially chosen as a protecting group over the acetate of **3** because of its greater stability. This stability allowed for the investigation of a variety of methods for the conversion of uracil to cytosine. The goal was to introduce the amine functionality at C-4 by activating that position and then displacing the activating group with ammonia. This conversion was first attempted using the triazole method of Sung.[15] Although this method has previously been successfully employed in a synthesis of d4C on a small scale (0.5g),[6b] we could not achieve comparable yields upon scaleup of the reaction (>10 g of 5). Alternative methods for the introduction of an amine at C-4 were examined. Fox et al. have reported that P_2S_5 can be employed in the conversion of uracil nucleosides to their corresponding thioamides, which can then be ammonolyzed to their cytosine counterparts.[16] Unfortunately, exposure of nucleoside **5** to P_2S_5 in pyridine as described furnished none of the desired thioamide **6** as detected by either TLC or ^1H NMR spectroscopy.

An alternative reagent to P_2S_5 for the generation of thioamides is to employ Lawesson's reagent.[17-19] Treatment of uridine **5** with Lawesson's reagent in refluxing chloroform afforded thiouridine **6** as a bright yellow solid in 60% yield.[20] ^{13}C NMR spectroscopy indicated a characteristic downfield shift of C-4 from 164 to 190 ppm upon introduction of the sulfur atom.

Amination and deprotection of thioamide **6** with methanolic ammonia at 80 °C for 5 h in a steel bomb produced d4C (**9**) in 77% yield. When shorter reaction times were employed (2 h), benzoyl d4C (**8**) was isolated, indicating that the sulfur at C-4 on thiouracil **6** was displaced faster than the cleavage of the benzoate at the 5′ position.

Next, an attempt was made to streamline the synthesis by avoiding the deprotection/protection involved in removal of the acetate at the 5′-position and subsequent benzoylation. The success of the thioamide approach to d4C by using Lawesson's reagent on benzoate **5** prompted us to attempt the same sequence with acetate **3**. Treatment of acetate **3** with 0.6 equiv of Lawesson's reagent in refluxing dichloroethane gave

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thioamide **7** in 80% yield. As before, a downfield shift from 164 to 190 ppm was observed for C-4 upon introduction of the sulfur atom in acetate **7**. Compound **7** was subsequently aminated and deprotected in one reaction to afford a 62% yield of d4C (**9**). The major side reaction appeared to be glycosidic cleavage, as 4-thiouracil could be isolated from the reaction mixture.

Purification of **9** was achieved using flash chromatography on silica gel with 20:80:1 methanol-dichloromethane-ammonium hydroxide as the eluant. As a result of having to employ such a polar eluting phase, the product was contaminated with silica ash. Adsorption of the nucleoside onto a column of Diaion HP-20 resin, followed by desorption of the product with methanol/water, and lyophilization of the product to dryness, afforded ash-free, analytically pure d4C (**9**). The compound was identical by mp and ^{1}NMR spectroscopy with the data that have been reported for **9**.[6b,7,21]

In summary, a four step sequence has been successfully employed starting with uridine and producing analytically pure d4C in 20% overall yield. The results of biological studies conducted with this material will be reported elsewhere.

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EXPERIMENTAL

Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. TLC was performed on silica gel 60 F-254 plates purchased from E. Merck Co., and column chromatography was performed as described[22] on flash silica gel (40-μM particle size, J. T. Baker Co.). The internal diameter of the flash column employed is given in mm. The height of the column bed was kept constant at approximately 120 mm. Elemental analyses were performed by the Analytical Research Department, Bristol-Myers Squibb Co., Wallingford, CT. Mass spectra were recorded on a Finnegan 4500 and IR spectra were recorded on a Perkin-Elmer 1800. ^{1}H and ^{13}C NMR spectra were recorded on a Gemini-300 spectrometer. Chemical shifts are reported in delta units

(δ) downfield from the internal standard, tetramethylsilane. Coupling constants are given in hertz.

2′-Bromo-2′-deoxy-3′,5′-di-O-acetyl-uridine (2)

To a refluxing suspension of 60.00 g (0.246 mol) of uridine (**1**, Sigma) in 1.5 L of CH_3CN under an atmosphere of nitrogen was added dropwise 140 mL (1.91 mol) of acetyl bromide (Aldrich) over a period of 30 min. TLC (10% MeOH/CH_2Cl_2) analysis conducted immediately after addition of the acetyl bromide indicated that the reaction was complete. The reaction was cooled, and the solvents were removed in vacuo to give a brownish oil. The residue was dissolved in 1 L of CH_2Cl_2 and washed with 500 mL of water. The organic layer was dried over $MgSO_4$ and concentrated to give 95.5 g (100%) of 2′-bromo-2′-deoxy-3′,5′-O-diacetyl-uridine (**2**): mp 55-60 °C (lit.[11] mp 67-76 °C). ^1H NMR ($CDCl_3$, 300 MHz) 7.41 (1H, d, J = 8.3, H6), 6.17 (1H, d, J = 5.6, H1′), 5.79 (1H, d, J = 8.3, H5), 5.11 (1H, t, J = 5.3, H3′), 4.55 (1H, t, J = 5.7, H2′), 4.37 (3H, m, H4′, H5′), 2.14 (3H, s, CH_3), 2.10 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) 170.1 (C=O,acetate), 169.7 (C=O, acetate), 163.0 (C4), 150.1 (C2), 139.0 (C6), 103.3 (C5), 90.9 (C1′), 79.9 (C4′), 70.6 (C3′), 63.1 (C5′), 48.4 (C2′), 20.7 (CH_3), 20.6 (CH_3). IR (cm^{-1}) 3430, 1750, 1700, 1235. MS m/z (relative intensity; methane DCI) 393,391 (M^+, 80), 311 (50), 281,279 (100). Anal. Calcd for $C_{13}H_{15}N_2O_7Br$: C, 39.91; H, 3.86; N, 7.16. Found: C, 39.84; H, 4.03; N, 6.79.

5′-O-Acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine (3)

To a rapidly mechanically stirred solution of 1.5 L of HOAc was added 450 g of Zn/Cu couple (Fairfield Chem. Co.). The heterogeneous black mixture was heated to reflux for 1 h, the heat was removed and the solid was collected and washed with methanol three times. The black Zn/Cu couple was added to a stirred solution of 78.5 g (0.200 mol) of crude bromoacetate **2** in 1 L of methanol. After 20 min, the solid was filtered and washed with methanol. The filtrate was

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concentrated and the resulting oil was purified on a 100 mm flash chromatography column, eluting with 5% MeOH/CH_2Cl_2. The fractions containing the desired product (R_f 0.6 in 10% MeOH/CH_2Cl_2) were pooled, concentrated, and recrystallized from 10% iPrOH/toluene to afford 19.8 g of 5′-O-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine (**3**; 40%) as a tan solid: mp 124-127 °C (lit.[10] mp 127-129 °C). ^1H NMR ($CDCl_3$, 300 MHz) 9.9 (1H, br s, NH), 7.45 (1H, d, J = 7.9, H6), 6.95 (1H, m, H1′), 6.25 (1H, dt, J = 6.0, 1.7, H3′), 5.88 (1H, dt, J = 6.0, 2.1, H2′), 5.70 (1H, d, J = 8.1, H5), 5.02 (1H, m, H4′), 4.26 (2H, ABq, H5′), 2.04 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) 171.1 (C=O), 164.4 (C4), 151.6 (C2), 140.6 (C6), 134.1 (C3′), 127.6 (C2′), 103.1 (C5), 90.4 (C1′), 84.8 (C4′), 65.0 (C5′), 20.9 (CH_3).

2′,3′-Didehydro-2′,3′-dideoxyuridine (4)

To a suspension of 2.00 g (7.94 mmol) of 5′-O-acetyl-d4U (**3**) in 100 mL of methanol was added 0.500 g (9.25 mmol) of sodium methoxide. The reaction was stirred for 2 h at 22 °C and neutralized with Dowex 50 x 8 [H^+] (200 mesh) ion exhange resin which had been washed with methanol. The resin was filtered and washed with methanol. The filtrate was concentrated in vacuo, and the residue was purified on a 40 mm flash column, eluting with methanol/methylene chloride/ammonium hydroxide 10/90/1. The product was obtained as a white solid (1.43 g, 85%): mp 155 °C(lit. mp[14] 154-155 °C). ^1H NMR (d_6-DMSO, 300 MHz) 7.70 (1H, d, J = 7.9, H6), 6.77 (1H, t, J = 1.6, H1′), 6.37 (1H, dt, J = 6.0, 1.6, H3′), 5.87 (1H, dt, J = 6.0, 1.6, H2′), 5.54 (1H, dd, J = 8.0, 2.1, H5), 4.94 (1H, t, J = 5.1, OH), 4.37 (1H, m, H4′), 3.54 (2H, m, H5′). ^{13}C NMR (d_6-DMSO, 75Hz) 163 (C4), 151 (C2), 141 (C6), 135 (C3′), 126 (C2′), 101 (C5), 89 (C1′), 87 (C4′), 62 (C5′).

5′-O-Benzoyl-2′,3′-didehydro-2′,3′-dideoxyuridine (5)

D4U (**4**) (10.0 g, 47.6 mmol) was dissolved in 250 mL of pyridine and treated with 7.9 mL of benzoyl chloride (68 mmol) at 50-55 °C. TLC analysis showed completion of the reaction after 5 h. The

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pyridine was removed in vacuo by azeotropic distillation with toluene. The residue was suspended in H_2O and extracted with EtOAc. The organic layer was dried over $MgSO_4$ to afford a 95% yield of benzoate **5** which was used without further purification in the subsequent reaction. ^1H NMR (d_6-DMSO, 300 MHz) 7.5-8.0 (5H, m, aromatic), 7.35 (1H, d, J = 7.5, H-6), 6.81 (1H, s, H-1'), 6.50 (1H, d, J = 6.0, H3'), 6.05 (1H, d, J = 6.0, H2'), 5.15 (1H, d, J = 7.5, H5), 5.1 (1H, m, H4'), 4.5 (2H, m, H5'). ^{13}C NMR ($CDCl_3$, 75 MHz) 166.1 (OC=O), 163.5 (C4), 150.7 (C2), 140.0 (C6), 133.6 (C3'), 130.1, 129.6, 128.6, 128.4 (aromatic), 127.1 (C2'), 102.6 (C5), 89.8 (C1'), 84.9 (C4'), 64.6 (C5').

5'-O-Benzoyl-2',3'-didehydro-2',3'-dideoxy-4-thiouridine (6)

To a suspension of 15.0 g (47.8 mmol) of benzoate **5** in 250 mL of $CHCl_3$ was added 0.5 equiv (23.9 mmol, 9.67g) of Lawesson's reagent (Aldrich). (The $CHCl_3$ employed in this reaction was first passed through a column of activated alumina to removed the ethanol present as a stabilizer.) The reaction mixture was refluxed, and the yellow suspension became homogeneous within 10 min. After heating at reflux for 6 h, the reaction mixture was concentrated overnight by evaporation in a fume hood under a stream of N_2. The residue was purified by flash chromatography, eluting the product with 90/10 CH_2Cl_2/EtOAc. Thiouridine **6** was isolated as a bright yellow solid (9.46 g, 60%): mp 128-129 °C (lit.[20] mp 75-77 °C). ^1H NMR (d_6-DMSO, 300 MHz) 7.90 (2H, m, aromatic), 7.67 (1H, m, aromatic), 7.53 (2H, m, aromatic), 7.27 (1H, d, J = 7.5, H6), 6.77 (s, 1H, H1'), 6.55 (1H, m, H3'), 6.05 (1H, d, J = 7.5, H5), 5.85 (1H, m, H2'), 5.15 (1H, m, H4'), 4.46 (2H, m, H5'). ^{13}C NMR (d_6-DMSO, 75 MHz) 190.5 (C4), 165.5 (C=O), 148.0 (C2), 133.7, 129.4, 129.3, 128.9 (aromatic), 135.7 (C6), 134.1 (C3'), 126.5 (C2'), 112.7 (C5), 90.2 (C1'), 84.6 (C4'), 65.3 (C5'); IR (KBr) cm^{-1} 3350-3150, 3150-2800, 1725, 1615, 1470; MS m/z (relative intensity) 331 (M+H, 2.5), 283 (3.25), 129 (100), 81 (42), Anal. Calcd for $C_{16}H_{14}N_2O_4S_1$: C, 58.17; H, 4.27; N, 8.48; S, 9.70. Found: C, 57.92; H, 4.28; N, 8.38; S, 9.82.

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5′-O-Acetyl-2′,3′-didehydro-2′,3′-dideoxy-4-thiouridine (7).

To a stirred solution of 14.0 g (55.5 mmol) of 5′-O-acetyl-d4U (**3**) in 600 mL of dichloroethane under a nitrogen atmosphere was added 13.6 g (33.6 mmol) of Lawesson's reagent (Aldrich). The mixture was heated to reflux for 1 h. TLC analysis at that time showed that no starting material remained. The reaction was cooled and the solvents were removed by rotary evaporation in a well-ventilated hood. The crude solid was purified on a 50 mm flash column, eluting with 15% ethyl acetate/CH_2Cl_2. The fractions containing the desired product were combined and concentrated by rotary evaporation, then high vacuum (0.05 mm) for 18 h to give 11.8 g of a bright yellow solid of 5′-O-acetyl-4-thio-d4U (**7**, 80%). ^{1}H NMR ($CDCl_3$, 300 MHz) 9.6 (1H, br s, NH), 7.31 (1H, d, J = 7.6, H6), 6.91 (1H, m, H1′), 6.36 (1H, d, J = 7.6, H5), 6.27 (1H, dt, J = 6.0, 1.6, H3′), 5.91 (1H, d, J = 6.0, H2′), 5.06 (1H, m, H4′), 4.30 (2H, ABq, H5′), 2.03 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) 190.8 (C4), 171.1 (C=O), 148.8 (C2), 135.1 (C6), 134.3 (C3′), 127.4 (C2′), 113.9 (C5), 90.9 (C1′), 85.1 (C4′), 64.8 (C5′), 21.0 (CH_3). Anal. Calcd for $C_{11}H_{12}N_2O_4S \cdot 0.41\ H_2O$: C, 47.93; H, 4.69; N, 10.16; S, 11.63. Found: C, 48.06; H, 4.39; N, 9.79; S, 11.72.

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2′,3′-Didehydro-2′,3′-dideoxycytidine (d4C; 9)

(A) From benzoate **6**: In a 200 mL stainless steel bomb was suspended 9.46 g (28.7 mmol) of 5′-O-benzoyl-2′,3′-didehydro-2′,3′-dideoxy-4-thiouridine (**6**) in 100 mL of methanol. The bomb was externally cooled to 5°C, and ammonia gas was bubbled through the mixture for 20 min. The bomb was sealed and heated in an oil bath at 100 °C. After 5 h, the reaction was cooled, and the solvents were removed under a stream of nitrogen in a fume hood. The oily residue was purified by flash chromatography eluting with 90/10 CH_2Cl_2/MeOH and then with 75/25 CH_2Cl_2/MeOH. The product **9** was isolated as a tan solid in 77% yield (4.61 g). ^{1}H NMR (d_6-DMSO, 300 MHz) 7.65 (d, 1H, J = 7.4, H6), 7.12 (broad s, 2H, NH_2), 6.86 (s, 1H, H1′), 6.31 (d, 1H, J = 6.0, H-3′), 5.85 (d, 1H, J = 6.0, H2′), 5.66 (d, 1H, J = 7.4, H5),

4.94 (t, 1H, J = 5.5, OH), 4.73 (m, 1H, H4′), 3.53 (m, 2H, H5′).[20] ^{13}C NMR (d_6-DMSO, 75 MHz) 165.6 (C4), 155.3 (C2), 141.8 (C6), 134.2 (C3′), 126.8 (C2′), 94.3 (C5), 89.9 (C1′), 87.2 (C4′), 62.7 (C5′); IR (KBr) cm^{-1} 3334, 3192, 1654, 1621, 1490; MS m/z (relative intensity) 210 (M+H, 1.5), 112 (100), 81 (35).

(B) From acetate **7**: In a 200 mL stainless steel bomb was suspended 10.6 g (39.6 mmol) of 5′-O-acetyl-2′,3′-didehydro-2′,3′-dideoxy-4-thiouridine (**7**) in 80 mL of methanol. The bomb was externally cooled to 5 °C, and ammonia gas was bubbled through the mixture for 30 min. The light brown, homogeneous solution was sealed and placed in an oil bath at 100 °C for 2 h. The reaction mixture was cooled, and the solvents were removed overnight under a stream of nitrogen. The crude product was purified on a 100 mm flash chromatography column, eluting with CH_2Cl_2/MeOH/NH_4OH 80/20/1 to afford 6.10 g (74%) of d4C (**9**) as a light yellow foam. Spectroscopic data on this material was consistent with d4C (**9**);[6b,7,21] however, elemental analysis indicated that it was contaminated with silica ash.

DEASHING OF D4C (**9**): 11.82 g of the nucleoside was dissolved in 40 mL of deionized water, the insolubles were filtered, and the pH adjusted to 8 with ammonium hydroxide. The solution was adsorbed onto a column of Diaion HP-20 resin[23] (4.5 x 60 cm, activated by water wash) and eluted with deionized water. After the product began to elute the elution was completed with 3 L of a linear gradient which began with 100 % water and ended with water/methanol 90/10. The appropriate fractions were combined and concentrated to ca. 50 mL on a rotary evaporator at 35 °C. The concentrate was lyophilized to give 9.95 g (84% for deashing step; 62% overall from thioamide **7**) of analytically pure, ash-free d4C (**9**). Anal. Calcd for $C_9H_{11}N_3O_3$: C, 51.67; H, 5.30; N, 20.09. Found: C, 51.58; H, 5.32; N, 20.00.

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20. During the preparation of this manuscript, a report appeared also outlining the preparation of benzoyl thiouridine **6** using Lawesson's reagent. Palomino, E.; Meltsner, B. R.; Kessel, D.; Horwitz, J. P. J. Med. Chem. **1990**, 33, 258. All spectral data given in this manuscript are in agreement with that of Palomino et. al. except for the melting point, which was found to be 52 °C higher than the reported value. Elemental analyses for C,H,N,S were found to be within experimental error in both cases.

21. The proton NMR assignments for the sugar portion of d4C were made by analogy to d4T,[9] in which long-range and one-bond carbon-proton correlation NMR experiments were employed to assign protons. In one report[8] the assignments for H-2′ and H-3′ have been reversed. The ribose assignment given in this report is in agreement with data published by Robins et al. for unsaturated nucleosides.[12]

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23. Diaion HP-20 is a nonionic polymeric resin manufactured by Mitsubishi Chemical Co., Ltd. A similar purification of 2′,3′-dideoxyuridine has been recently reported: Irie, Y., et al. Japan Patent 1 102 095, April 19, **1989**.

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