Fused Pyrimidines. 7. Nucleosides of Pyrimidopyrimidinediones and Pteridinediones as Potential Chemotherapeutic Agents

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Several nucleoside derivatives of pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **1** and 2,4(1*H*,3*H*)-pteridinedione **2** were prepared. Treating the appropriate silylated nucleobase with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **3** in the presence of trimethylsilyl triflate gave **4** and **8** which, upon debenzoylation, gave **5** and **9**, respectively. Treatment of **4** with phosphorus pentasulfide afforded the sulfur substituted compound **6**. Again, deprotection gave **7**. The arabinose derivatives were obtained by treating 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranose **10** with the silylated nucleobases to give **11** and **13**. Debenzoylation gave the free arabinonucleosides **12** and **14** respectively. The deoxy derivative **16** was prepared by the reaction of **1** with 1-chloro-3,5-di-*O*-acetyl-2-deoxy-D-ribofuranose **15**. Deacetylation of **16** with methanolic ammonia gave the α-anomer **17**.

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The search for effective antiviral agents is one of the most challenging areas of investigation for medicinal chemists today. The interest in nucleosides as potential antiviral and antitumor agents remains high [1]. Much of this activity has had as the main targets influenza infections (flu), herpes infections and human immuno viruses (HIV) [2]. AZT (3'-Azido-3'-deoxythymidine), 2',3'-dideoxyinosine and 2',3'-dideoxycytidine are some of the clinically approved compounds for the treatment of AIDS and AIDS-related complex. Since then, several approaches have been made in order to find an effective antiviral agent and most of them involve some modification of the sugar moiety. Our continuing interest in fused pyrimidines [3,4,5] containing a second six-membered ring possessing two nitrogen atoms prompted us to explore nucleosides of the pyrimidopyrimidine and pteridine ring systems in which the carbohydrate moiety is attached to the pyrimidine ring. In essence, these can be viewed as analogs of uridine.

Similar nucleosides have been prepared from pyrido[2,3-*d*]pyrimidine-2,4-diones [6], quinazoline-2,4-diones [7,8], as well as 2,4(1*H*,3*H*)-pteridinedione (lumazine) [9]. However, no examples based on the pyrimido[4,5-*d*]pyrimidine-2,4-dione (**1**) structure have been described. We report here the syntheses of several nucleosides containing **1** and pteridine-2,4-dione **2**. Some examples of nucleosides of **2** have been synthesized [9] but not evaluated for biological activity.

Pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **1** (Figure 1) was prepared [10] and silylated by refluxing in 1,1,1,3,3,3-hexamethyldisilazane and chlorotrimethylsilane in the presence of a catalytic amount of ammonium sulfate [11-14]. Condensation of the 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **3** and the silylated nucleobase was carried out according to the Friedel-Crafts catalyzed Hilbert-Johnson reaction as modified by Vorbruggen [12,13]. The reaction was carried out in dry acetonitrile in the presence of trimethylsilyl trifluoromethanesulfonate giving 1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **4** in 52% yield. The reaction was relatively clean with the disubstituted 1,3-bis(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione as the major contaminant, usually in yields less than 5%.

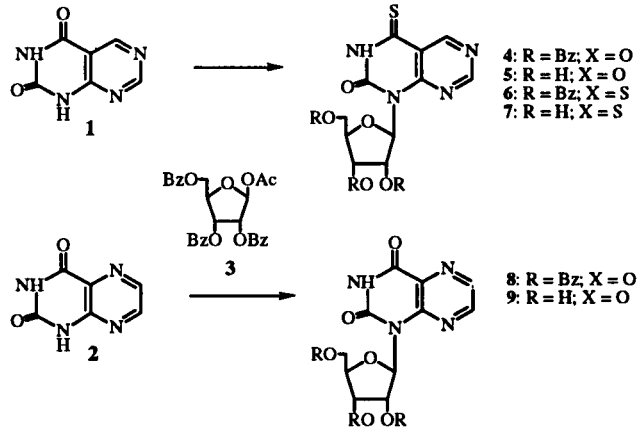

Figure 1

The reaction was also carried out by a one pot reaction [15] in which **1** was suspended in acetonitrile to which was added the sugar **3**, hexamethyldisilazane , chlorotrimethylsilane, and the catalyst trimethylsilyl trifluoromethanesulfonate. The mixture, after a few hours of reflux and the usual workup gave approximately the same yield. Nor did the yield improve when the reaction was carried out with 1-bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranoside using the mercury procedure [9]. Changing the solvent from acetonitrile to 1,2-dichloroethane also did not increase the yield. The protected nucleoside **4** was deblocked by treatment with methanolic ammonia and purified to give 1-β-D-ribofuranosylpyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **5** in 86% yield.

The reaction of **4** with phosphorus pentasulfide in pyridine afforded 1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-oxo(1*H*)-pyrimido[4,5-*d*]pyrimidine-4(3*H*)-thione **6** in 73% yield after chromatographic purification. This procedure was cleaner than the reaction with Lawesson's reagent [16]. Deblocking of **6** with methanolic ammonia gave 1-β-D-ribofuranosyl-2-oxo(1*H*)-pyrimido[4,5*d*]pyrimidine-4(3*H*)-thione **7** in 64% yield after hplc purification.

Even though nucleosides of pteridinedione have been reported we wanted to include them in our study in order to evaluate any differences in biological activity based on the two isomers. Reaction of **2** with **3** under the same conditions used for the formation of **4** afforded 1(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,4(1*H*,3*H*)-pteridinedione **8** in 50% yield. It was observed that 1,3-bis(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,4(1*H*,3*H*)-pteridinedione was formed in 18% yield. Compound **8** was debenzoylated to obtain the free nucleoside **9** in 60% yield. The spectral data were in agreement with the spectra reported in the literature [9].

Most nucleotides and nucleosides which show biological activity are in the β configuration. In recent years there have been a number of reports on α nucleosides [17-20] and some of these exhibit antimetabolic properties. Hence, we prepared some α-arabinonucleosides. 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranose **10** was prepared according to reported procedures [21] and allowed to react with **1** in presence of hexamethyldisilazane, chlorotrimethylsilane and trimethylsilyl trifluoromethanesulfonate. (Figure 2) After the usual work up, chromatographic separation and recrystallization, 1-(2',3',5'-tri-*O*-benzoyl-α-D-arabinofuranosyl)pyrimido-[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **11** was obtained in 48% yield [22]. Debenzoylation of **11** in methanolic ammonia gave **12** in 60% yield. Compound **13** was prepared by reacting in the same manner the silylated nucleobase **2** with **10** in acetonitrile in 68% yield [23]. The 1,3 bis-disubstituted compound was obtained in much less yield (~5%) compared to the ribose derivative. Debenzoylation of **13** gave **14** in 77% yield after purification.

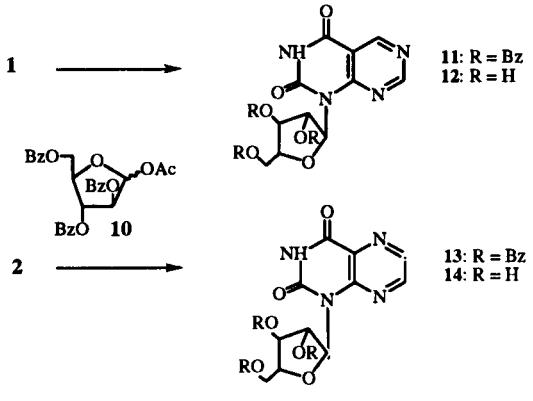

Figure 2

1-Chloro-3,5-di-*O*-acetyl-2-deoxy-D-ribofuranose **15** was prepared according to a known procedure [24]. Initially, the reaction of **15** with the silylated nucleobase **1** did not yield the expected product with trimethylsilyl trifluoromethanesulfonate or tin tetrachloride as catalyst under our experimental conditions (See Figure 3). The reaction was successful using the mercuration procedure previously described [9,25]. The silylated nucleobase **1** was taken up in toluene and refluxed with a mixture of mercury salts. The chlorosugar derivative was then added and after a few hours reflux the product **16** was obtained by the usual workup and chromatographic purification in 14% yield as an anomeric mixture. The ratio was found to be ~9:1 (α:β). Deacetylation was carried out in methanolic ammonia and hplc separation gave 1-(2'-deoxy-α-D-ribofuranosyl)-pyrimido[4,5-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione **17** in 84% yield. The configuration of these compounds were determined by nmr [26]. Also, the arabinose compounds are expected to form in the α-configuration in accord with the suggested mechanism [25,27].

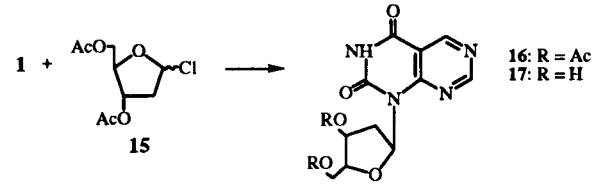

Figure 3

All of the unprotected nucleosides prepared in this study have been subjected to a variety of biological screens. Overall, they provided no viable chemotherapeutic agents. Through the auspices of the Drug Synthesis and Chemistry Branch of the National Institutes of Health compounds **5**, **7**, **9**, **12**, **14**, and **17** were evaluated for *in vitro* anti-HIV activity using a standard protocol [28]. None of the compounds tested exhibited any appreciable activity against HIV.

Antiviral testing with respiratory viruses and herpes viruses of the same set of compounds was conducted through the Virology Branch of the NAID at NIH at contract laboratories and by Dr. John Drach at the University of Michigan Dental School. Again, there was a lack of significant activity against a host of cell cultures, including HSV-1, HSV-2, HCMV, Varicella Zoster virus, and Epstein-Barr virus.

However, compound **14** demonstrated significant activity against HBV in culture [29]. While other nucleosides have been shown to possess activity against Hepatitis B virus, this compound exhibits behavior unlike that of other nucleosides. It is active against DNA outside of the cells but not against DNA inside the cells. This observation is being explored further. The isomer, **12**, failed to exhibit similar activity.

EXPERIMENTAL

Melting points are uncorrected and were determined in open capillary tubes using a Thomas-Hoover apparatus. Purity of products was determined by hplc and nmr. Infrared spectra were determined in KBr wafers on a Perkin Elmer 1600 Series FTIR. Mass spectra were measured on a Hewlett Packard 5995A GC/MS instrument, using a direct insertion probe. The ^{1}H nmr and ^{13}C nmr spectra were recorded on a General Electric QE 300 instrument at 300 MHz and 75 MHz, respectively, with tetramethylsilane as the internal standard unless otherwise specified. All values are reported in ppm relative to tetramethylsilane. Relative integrals of peak areas are in agreement with the assigned structures. Ultraviolet spectra were recorded on a Cary 1 UV-Visible spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Column chromatographic purifications were done with silica gel (Mallinckrodt, 60-200 mesh for gravity and Fisher Scientific, 230-425 mesh for flash chromatography). All the solvents used were dried according to standard procedures [30]. The hplc analysis was carried out using a Partisil M9 10/50 ODS-2 (reverse phase) column. The integrated hplc unit consists of a LKB 2151 variable wavelength detector, LDC/Milton Roy Constametric III pump and a HP 3396A integrator.

Pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**1**).

This compound was prepared by an established procedure [10] in 65 % yield, mp >300°; ir: ν 3200-3900 (NH), 1734, 1680 (C=O) cm^{-1}; uv (*p*H 1): λ max 205 nm (ε 15,600), 268 (4700); (*p*H 7): λ max 206 nm (ε 18,100), 240 (ε 5800), 280 (ε 3800); (*p*H 11): λ max 208 nm (ε 21,000), 270 (ε 13,800), 316 (ε 3100); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 9.00(s, 2H, H-5,H-7), 11.5 (br s, 2H, NH); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 108.9, 150.8, 156.8, 158.2, 161.7, 162.2; ms: m/z 164 (100), 137 (24), 121 (13), 93 (29).

2,4(1*H*,3*H*)-Pteridinedione (**2**).

This compound was prepared according to known procedures [31,32] in 60% yield, mp >300°; ir ν 3460-2800 (NH), 1720, 1670 (C=O) cm^{-1}; uv (*p*H 1: λ max 204 nm (ε 14,100), 229 (ε 15,800), 324 (ε 8500); (*p*H 7): λ max 204 nm (ε 13,200), 229 (ε 15,600), 325 (ε 9600); (*p*H 11): λ max 206 nm (ε 17,000), 230 (ε 21,600), 273(ε 5100), 325 (ε 12,700); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.51 (s, 1H, H-6), 8.63(s, 1H, H-7), 11.76 (br s, 2H, NH); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 128.5, 141.0, 149.0, 150.6, 150.8, 161.5; ms: m/z 164 (100), 121 (47), 93 (42).

1-(2',3',5'-Tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimido-[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**4**).

A mixture of dry **1** (0.66 g, 4.0 mmoles), ammonium sulfate (10 mg), 1,1,1,3,3,3-hexamethyldisilazane (5 ml) and chlorotrimethylsilane (3 ml) was heated under reflux for 4 hours. Excess 1,1,1,3,3,3-hexamethyldisilazane and chlorotrimethylsiane were removed by distillation. Xylene (25 ml) was added to the residue and the solvent again removed by distillation. The residue was dissolved in acetonitrile (30 ml) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**3**) (2.22 g, 4.4 mmoles) was added. The mixture was cooled to 0° and trimethylsilyl trifluoromethane-sulfonate (1.1 ml, 5.7 mmoles) was added dropwise with stirring. The solution was gradually warmed to room temperature and stirred for an additional 3 hours. Methanol (2 ml) was added and after stirring for 30 minutes, the solvents were evaporated. The residue was dissolved in ethyl acetate (100 ml) and the organic layer washed successively with saturated sodium bicarbonate (2 x 25 ml), water (2 x 20 ml), saturated aqueous sodium chloride (30 ml) and dried over anhydrous sodium sulfate. The solvent was evaporated and the crude product chromatographed (hexane:ethyl acetate, 6:4) and the product recrystallized from ether to yield the title compound, 1.26 g (52%), mp 110-112°; ir: ν 1725 (C=O), 3070-3430 (NH) cm^{-1}; uv (*p*H 1): λ max 205 nm (ε 31,000), 227 (ε 19,000), 275 (ε 7,000); (*p*H 7): λ max 205 nm (ε 31,000), 228 (ε 18,700), 274 (ε 8,000); (*p*H 11): λ max 206 nm (ε 80,000), 233 (ε 25,000), 274 (ε 8,000); ^{1}H nmr (deuteriochloroform): δ 4.65-4.90 (m, 3H, H-5',H-4'), 6.18-6.32 (m, 2H, H-2',H-3'), 7.2-8.2 (m, 16H, aromatic CH, H-1'), 9.03 (br s, 1H, NH), 9.10 (s, 1H, H-5), 9.34 (s, 1H, H-7); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 63.6, 71.1, 73.8, 79.2, 87.5, 109.7, 128.3, 128.4, 128.8, 129.6, 129.8, 129.9, 133.1, 133.4, 148.9, 156.6, 158.6, 159.3, 161.8, 165.3, 165.6, 166.2.

Anal. Calcd. for $C_{32}H_{24}N_4O_9$: C, 63.16; H, 3.98; N, 9.21. Found: C, 63.19; H, 3.98; N, 9.19.

1-β-D-Ribofuranosylpyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**5**).

A solution of **4** (1.0 g, 1.65 mmoles) in methanolic ammonia (25 ml, saturated at 0°) was stirred at room temperature for 24 hours in a pressure bottle. After evaporation, the residue was triturated with acetone (3 x 15 ml) and recrystallized from methanol to give 0.42 g, (86%) of **5**, mp 210-212°; ir δ 3030-3430 (NH, OH), 1680 (C=O) cm^{-1}; uv (*p*H 1): λ max 214 nm (ε 10,750), 275 (ε 5,700); (*p*H 7): λ max 209 nm (ε 18,500), 238 (ε 9,200), 280 (ε 4870); (*p*H 11): λ max 213 nm (ε 22,200), 237 (ε 15,850), 273 (ε 6200); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.35-3.75 (m, 3H, H-4',H-5'), 4.23 (m, 1H, H-3'), 4.6 (m, 2H, H-2',OH), 4.9- 5.1 (m, 2H, OH), 6.53 (s, 1H, H-1'), 9.14 (s, 1H, H-5), 9.17 (s, 1H, H-7) and 11.8 (br s, 1H, NH).

Anal. Calcd. for $C_{11}H_{12}N_4O_6 \cdot 0.33CH_3OH$: C, 44.35; H, 4.39; N, 18.26. Found: C, 44.29; H, 4.44; N, 18.33.

1-(2',3',5'-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-oxo(1*H*)-pyrimido-[4,5-*d*]pyrimidine-4(3*H*)-thione (**6**).

To a suspension of **5** (1.22 g, 2 mmoles) in dry pyridine (50 ml) was added phosphorus pentasulfide (1.12 g, 2.5 mmoles) and the reaction mixture was heated under reflux for 12 hours with the exclusion of moisture. The solvent was evaporated and ice-cold water (50 ml) added cautiously. After stirring for 30 minutes, chloroform (200 ml) was added, the organic layer separated, washed successively with water (2 x 200 ml), saturated sodium chloride solution (100 ml), dried (sodium sulfate), solvent evaporated and the residue chromatographed (hexane:ethyl acetate, 6:4) to yield 0.93 g (73%) of the title compound. This was recrystallized from ether, mp 124-126°; ir: ν 3400 (-NH), 1724 (C=O), 1100 (C=S) cm^{-1}; uv (*p*H 1): λ max 202 nm (ε 17,000), 229 (30,000), 341 (6500); (*p*H 7): λ max 202 nm (ε 21,000), 228 (ε 26,000), 361 (ε 4000); (*p*H 11): λ max 205 nm (ε 1,000,000), 233 (ε 26,000); ^{1}H nmr (deuteriochloroform): δ 4.65-4.90 (m, 3H, H-4',H-5'), 6.20 (t, J = 7.8 Hz, 1H, H-3'), 6.33 (m, 1H, H-2'), 7.2-8.1 (m, 16H, aromatic CH, H-1'), 8.70 (br s, 1H, NH), 9.07 (s, 1H, H-5), and 9.56 (s, 1H, H-7); ^{13}C nmr (deuteriochloroform): δ 63.5, 71.1, 73.6, 79.3, 87.5, 114.5, 128.3, 128.4, 128.8, 129.6, 129.8, 133.1, 133.5, 133.6, 146.4, 153.2, 161.0, 161.3, 165.3, 165.6, 166.2, 187.0.

Anal. Calcd. for $C_{32}H_{24}N_4O_8S \cdot 1H_2O$: C, 59.81; H, 4.05; N, 8.72. Found: C, 60.05; H, 4.03; N, 8.38.

1-β-D-Ribofuranosyl-2-oxo(1*H*)-pyrimido[4,5-*d*]pyrimidine-4(3*H*)-thione (**7**).

In a manner similar to that described for **5**, debenzoylation of **6** (1.25 g, 2.0 mmoles) with methanolic ammonia (100 ml) gave 0.40-g (64%) of **7**, after the hplc purification, mp 187-190°; ir: ν 1115 (C=S), 2900-3400 (OH, NH) cm^{-1}; uv (*p*H 1): λ max 211 nm (ε 22,000), 239 (ε 11,800), 296 (ε 5200); (*p*H 7): λ max 209 nm (ε 21,500), 239 (ε 13,900), 283 (ε 6580); (*p*H 14): λ max 214 nm (ε 24,000), 240 (ε 11,000), 269 (ε 6160); ^1H nmr (dimethyl sulfoxide-d_6): δ 3.73-3.35 (m, 3H, H-4',H-5'), 4.2 (m, 1H, H-3'), 4.6 (m, 2H, H-2',OH), 4.86 (m, 1H, OH), 5.03 (m, 1H, OH), 6.53 (s, 1H, H-1'), 8.47 (br s, 1H, NH), 9.04 (s, 1H, H-5), 9.30 (s, 1H, H-7).

Anal. Calcd. for $C_{11}H_{12}N_4O_5S \cdot 1NH_3 \cdot 0.25H_2O$: C, 39.58; H, 4.64; N, 20.98. Found: C, 39.08; H, 4.55; N, 20.94.

1-(2',3',5'-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2,4(1*H*,3*H*)-pteridinedione (**8**).

This compound was prepared [9] from **2** (0.33 g, 2.0 mmoles) in a manner similar to that described for **4**, to yield 0.61 g (50%) of the title compound, mp 145-147°; ir: ν 3060-3430 (NH), 1720 (C=O) cm^{-1}; uv (*p*H 1): λ max 203 nm (ε 32,000), 229 (ε 62,000), 314 (ε 8700); (*p*H 7): λ max 203 nm (ε 30,000), 229 (ε 60,000), 314 (ε 8500); (*p*H 11): λ max 207 nm (ε 56,000), 228 (ε 55,000), 319 (ε 8500); ^1H nmr (dimethyl sulfoxide-d_6): δ 4.5-4.9 (m, 3H, H-4',H-5'), 6.3 (m, 2H, H-2',H-3'), 7.3-8.1 (m, 16H, aromatic CH,H-1'), 8.67 (d, J = 2.2 Hz, 1H, H-6), 8.75 (d, J = 2.2 Hz, 1H, H-7) 12.1 (br s, 1H, NH); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 63.3, 70.1, 73.6, 77.8, 86.1, 128.4, 128.5, 128.7, 129.0, 129.1, 129.2, 133.3, 133.5, 133.7, 140.6, 146.5, 147.8, 149.2, 159.5, 164.4, 164.6, 165.3.

1-β-D-Ribofuranosyl-2,4(1*H*,3*H*)-pteridinedione (**9**) [9].

In a manner similar to that described for **5**, debenzoylation of **8**, (0.61 g, 1.0 mmole) gave 0.18 g (60%) of **9**, mp 180-82° (lit [9] 180-182°); ir: ν 3000-3400 (NH, OH), 1680 (C=O) cm^{-1}; uv (*p*H 1): λ max 205 nm (ε 8050), 232 (ε 10,380), 316 (ε 5270); (*p*H 7): λ max 205 nm (ε 8100), 231 (ε 10,200), 317 (ε 5020); (*p*H 11): λ max 210 nm (ε 14,300), 239 (ε 11,300), 323 (ε 5420); ^1H nmr (dimethyl sulfoxide-d_6): δ 3.4-3.5 (m, 2H, H-5'), 3.6-3.7 (m, 1H, H-4'), 4.2-4.3 (m, 1H, H-3'), 4.8 (m, 2H, H-2',OH), 5.0-5.2 (m, 2H, H-5',OH), 6.5 (s, 1H, H-1'), 8.25 (br s, 1H, NH), 8.64 (d, J = 2.2 Hz, H-6), 8.75 (d, J = 2.2 Hz, 1H, H-7); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 62.1, 69.7, 71.0, 84.4, 88.8, 128.7, 140.4, 146.6, 148.4, 149.1, 159.5.

1-(2',3',5'-Tri-*O*-benzoyl-α-D-arabinofuranosyl)pyrimido-[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**11**).

A mixture of **1** (0.66 g, 4.0 mmoles), ammonium sulfate (10 mg), hexamethyldisilazane (5 ml), and chlorotrimethylsiane (3 ml) was heated under reflux for 4 hours. Excess hexamethyldisilazane and chlorotrimethylsilane were removed by distillation and the residue was dissolved in xylene (25 ml), which was subsequently removed by distillation. A solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-arabinofuranose **10** [15] (2.24 g, 4.4 mmoles) in dry acetonitrile (30 ml) was added to the residue. The mixture was cooled to 0° with stirring and trimethylsilane triflate (1.1 ml, 5.7 mmoles) was added. The solution was gradually warmed to room temperature and stirred for an additional 4 hours. Methanol (2 ml) was added and after stirring for 30 minutes, the solvents were evaporated. The residue was dissolved in ethyl acetate (150 ml), the organic layer washed successively with saturated aqueous sodium bicarbonate (2 x 30 ml), water (2 x 20 ml), saturated sodium chloride solution (30 ml), then dried (sodium sulfate). The solvent was evaporated, crude product chromatographed (hexane:ethyl acetate, 6:4) and recrystallized from chloroform/hexane to yield 1.17 g (48%) of the title compound, mp 88-90°; ir: ν 2850-3400 (NH), 1727 (C=O) cm^{-1}; uv (*p*H 1): λ max 202 nm (ε 35,700), 229 (ε 39,400), 273 (ε 6200); (*p*H 7): λ max 202 nm (ε 42,000), 229 (ε 46,300), 273 (ε 6320); (*p*H 11): λ max 208 nm (ε 1,140,200), 227 (ε 50,000), 272 (ε 7,000); ^1H nmr (deuteriochloroform): δ 4.64-4.80 (m, 3H, H-4',H-5'), 5.22-5.28 (m, 1H, H-3'), 6.2 (dd, J = 5.4 Hz, 1H, H-2'), 6.5 (t, J = 4.8 Hz, 1H, H-1'), 7.2-8.2 (m, 16H, aromatic CH, NH), 9.1 (s, 1H, H-5), 9.3 (s, 1H, H-7); ^{13}C nmr (deuteriochloroform): δ 64.7, 76.9, 80.7, 82.3, 87.9, 109.4, 128.7, 128.9, 128.9, 130.0, 130.3, 130.4, 133.4, 133.9, 134.1, 149.8, 157.2, 159.0, 159.7, 162.1, 166.2, 166.4, 166.6.

1-β-D-Arabinofuranosylpyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**12**).

In a manner similar to that described for **5**, debenzoylation of **11** (1.22 g, 2.0 mmoles) with methanolic ammonia (100 ml) gave the crude mixture which was triturated with methanol and recrystallized from water/acetone to give 0.35 g (60%) of the title compound, mp 238-240°; ir: ν 3200-3500 (OH, NH), 1700 (C=0) cm^{-1}; uv (*p*H 1): λ max 206 nm (ε 13,900), 241 (ε 5900), 283 (ε 2100); (*p*H 7): λ max 206 nm (ε 10,500), 242 (ε 5200), 281 (ε 2860); (*p*H 11): λ max 206 nm (ε 24,000), 238 (ε 7900); ^1H nmr (dimethyl sulfoxide-d_6): δ 3.4-3.6 (m, 2H, H-5'), 3.8-3.9 (m, 1H, H-4'), 4.2 (m, 1H, H-3'), 4.65 (m, H, OH), 4.90 (m, 1H, H-2'), 5.3-5.4 (m, 2H, OH), 6.5 (d, J = 7.2 Hz, 1H, H-1'), 9.10 (s, 1H, H-5), 9.15 (s, 1H, H-7), 10.8 (br s, 1H, NH).

Anal. Calcd. for $C_{11}H_{12}N_4O_6 \cdot 0.1H_2O$: C, 44.33; H, 4.12; N, 18.90. Found: C, 44.59; H, 4.08; N, 18.91.

1(2',3',5'-Tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,4(1*H*,3*H*)-pteridinedione (**13**).

In a manner similar to that described for **11**, the reaction of **2** (0.33 g, 2.0 mmoles) with **10** (1.12 g, 2.2 mmoles) gave the title compound 0.83 g (68%), mp 172-174°; ir: ν 2900-3470 (NH), 1729 (C=O) cm^{-1}; uv (*p*H 1): λ max 202 nm (ε 19,000), 229 (ε 31,000), 314 (ε 4600); (*p*H 7): λ max 202 nm (ε 19,000), 229 (ε 31,000), 314 (ε 4500); (*p*H 11): λ max 206 nm (ε 60,000), 227 (ε 29,000), 320 (ε 4600); ^1H nmr (dimethyl sulfoxide-d_6): δ 4.55-4.70 (m, 3H, H-4',H-5'), 5.15 (m, 1H, H-3'), 5.90-6.05 (m, 1H, H-2'), 6.45 (m, 1H, H-1'), 8.65 (d, J = 2.2 Hz, 1H, H-6), 8.75 (d, J = 2.2 Hz, 1H, H-7), 12.05 (s, 1H, NH); ^{13}C nmr (deuteriochloroform): δ 64.8, 77.9, 80.8, 82.3, 88.1, 128.8, 128.9, 130.3, 130.4, 133.6, 134.0, 134.1, 141.7, 147.3, 148.8, 149.7, 159.9, 166.3, 166.5.

1-α-D-Arabinofuranosyl-2,4(1*H*,3*H*)pteridinedione (**14**).

Debenzoylation of **13** (0.61 g, 1.0 mmole) and purification by hplc gave 0.23 g (77%) of **14**, mp 208-210°; ir: ν 3100-3450 (NH), 1708 (C=O) cm^{-1}; uv (*p*H 1): λ max 204 nm (ε 10,600), 233 (ε 15,700), 318 (ε 7800); (*p*H 7): λ max 204 nm (ε 12,700), 237 (ε 15,200), 318 (ε 7800); (*p*H 11): λ max 211 nm (ε 46,000), 240 (ε 17,000), 323 (ε 8000); ^1H nmr (dimethyl sulfoxide-d_6): δ 3.50-3.90 (m, 3H, H-4', H-5'), 4.20 (m, 1H, H-3'), 4.60 (m, 1H, OH), 4.95 (m, 1H, H-2'), 5.30-5.50 (m, 2H, OH), 6.52 (d, J = 6.7 Hz, 1H, H-1'), 8.6 (d ,J = 2.2 Hz, 1H, H-6), 8.7(d, J = 2.2 Hz, 1H, H-7) and 11.8 (br s, 1H, NH).

Anal. Calcd. for $C_{11}H_{12}N_4O_6$•0.4$CHCl_3$: C, 39.78; H, 3.63; N, 16.29. Found: C, 39.92; H, 4.13; N, 16.60.

1-(3',5'-Di-*O*-acetyl-2'-deoxy-D-ribofuranosyl)pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**18**).

A mixture of dry **1** (0.33 g, 2.0 mmoles), ammonium sulfate (5 mg), 1,1,1,3,3,3-hexamethyldisilazane (3 ml), and chlorotrimethylsilane (2 ml) was heated under reflux for 4 hours. Excess 1,1,1,3,3,3-hexamethyldisilazane and chlorotrimethylsilane were removed by distillation and the residue was dissolved in dry toluene (30 ml). Mercuric oxide (0.25 g, 1.2 mmoles) and mercuric bromide (0.25 g, 0.74 mmoles) were added and the mixture refluxed for 3 hours. 1-Chloro-3,5-di-*O*-acetyl-2-deoxy-D-ribofuranose (**17**) (0.48 g, 2.0 mmoles) in toluene (10 ml) was added and the mixture refluxed for an additional 2 hours. The solvent was removed, chloroform (75 ml) was added and the mixture filtered. The filtrate was washed with 10% potassium iodide solution (2 x 25 ml), water (50 ml), dried (sodium sulfate), the solvent removed, and the residue chromatographed (chloroform:acetone, 9:1) to yield the title compound 0.10 g (14%) as an anomeric mixture (9α:1β); ir: ν 3440 (NH), 1742 (C=O) cm^{-1}; uv (*p*H 1): λ max 206 nm (ε 20,000), 239 (ε 6200), 276 (ε 3900); (*p*H 7): λ max 212 nm (ε 22,000), 238 (ε 14,500), 279 (ε 8400); (*p*H 11): λ max 208 nm (ε 31,000), 236 (ε 13,700), 277 (ε 5600); ^1H nmr (deuteriochloroform): (α anomer) δ 2.1-2.3 (2s, 6H, CH_3CO), 2.0-2.5 (m, 2H, H-2'), 4.0-4.1 (m, 2H, H-5'), 5.10 (m, 1H, H-4'), 5.60 (m, 1H, H-3'), 6.70 (d, J = 9.8 Hz, 1H, H-1'), 9.10 (s, 1H, H-5), 9.17 (s, 1H, H-7), 10.05 (br s, 1H, NH).

1(2'-Deoxy-α-D-ribofuranosyl)pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**19**).

A solution of **18** (0.55 g, 1.5 mmoles) in methanolic ammonia (75 ml) was deacetylated in a manner similar to that described for **5**. A crude product was obtained, which was purified by hplc to give 0.34 g of the title compound, mp >300°; ir: ν 3100-3450 (OH, NH), 1700 (C=O) cm^{-1}; uv (*p*H 1): λ max 206 nm (ε 18,500), 241 (ε 8,500), 273 (ε 5,400); (*p*H 7): λ max 206 nm (ε 21,800), 241 (ε 8000), 273 (ε 5400); (*p*H 11): λ max 210 nm (ε 29,200), 236 (ε 14,000), 274 (ε 5700); ^1H nmr (dimethyl sulfoxide-d_6): δ 1.80-3.20 (m, 2H, H-2'), 3.6 (m, 3H, H-5',OH), 4.0 (m, 1H, H-4'), 4.8 (m, 2H, H-3',OH), 6.6 (d, J = 10.8 Hz, 1H, H-1'), 9.10 (d, 1H, H-6), 9.17 (d, 1H, H-7), 11.9 (br s, 1H, NH).

Anal. Calcd. for $C_{11}H_{12}N_4O_5$: C, 47.14; H, 4.29; N, 20.00. Found: C, 47.13; H, 4.29; N, 19.59.

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[22] Although spectroscopic data was consistent with the assigned structure, satisfactory elemental analysis of this compound was not obtained. Conversion to the characterized free nucleoside substantiates this assignment.

[23] Difficulty in obtaining this compound in an analytically pure state, as noted for **11**, led us to convert this compound to the free nucleoside, **14**, which has been characterized.

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