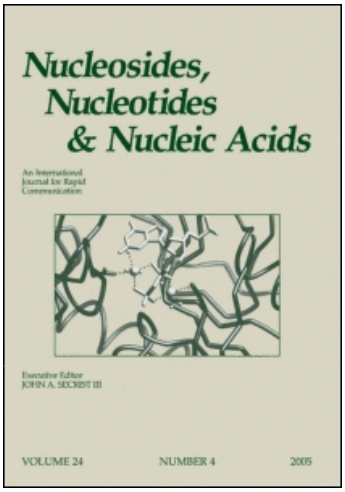

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Synthesis of 5-Ethyl-2-pyrimidinone-2'-deoxyriboside as a New Potential Antiviral Agent

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SYNTHESIS OF 5-ETHYL-2-PYRIMIDINONE-2'-DEOXYRIBOSIDE AS A NEW POTENTIAL ANTIVIRAL AGENT.

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Abstract
5-Ethyl-2-pyrimidinone (**5**) and its 2'-deoxyribosides **9** (α) and **10** (ß) have been synthesized in several steps starting from 5-ethyluracil. **10** is presented for evaluation against herpes virus infection.

Introduction and Rationale

The synthesis and anti-herpes activities of several 5-halo- and 5-alkynyl-substituted 2- pyrimidinone 2'-deoxyribosides have been previously reported[1,2]. Among these, the 5-iodo and 5-ethynyl derivatives, IPdR and EPdR, respectively, were the most potent inhibitors of both herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) *in vitro*, and the former was also significantly active *in vivo*, causing 80-100% of the HSV-2 infected mice to survive 45 days or longer on oral administration.[3] Recent studies indicated that IPdR is converted to 5-iodo-2'-deoxyuridine (IUdR) by the enzyme aldehyde oxidase in both rat and human liver, and that following oral administration of IPdR to athymic nude mice, it is incorporated to a small extent as IUdR into the DNA of intestine and bone marrow tissues.[4] Although, in contrast to IUdR, IPdR showed no toxicity as measured by weight loss of the treated animals,[4] its potential metabolic conversion to IUdR and incorporation as such into human DNA, even if limited, might be considered as a possible disadvantage when used for the purpose of antiviral therapy.

There is evidence that some other 2-pyrimidinone derivatives may also undergo enzymic oxidation to the corresponding uracil compounds.

This paper is dedicated to the memory of Roland K. Robins.

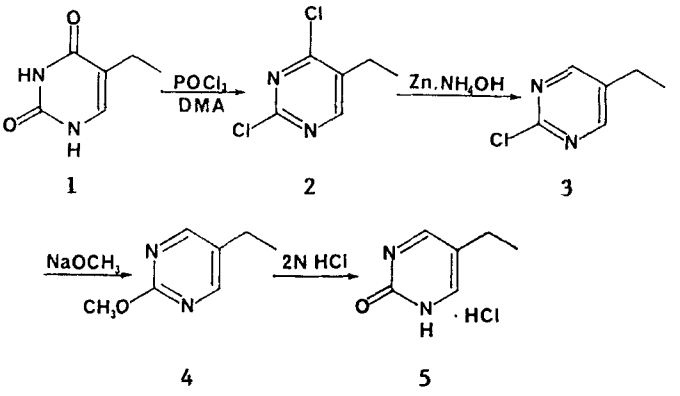
Thus, 5-fluoro-2-pyrimidinone (FP) is reportedly converted to its cytotoxic analogue, 5-fluorouracil (FU).[5,6] 5-Methyl-2-pyrimidinone-2'-deoxyriboside (MePdR), the 4-deoxo analogue of thymidine (TdR), showed wide variations in its antiherpes activity (unpublished results). This can be explained if even a small amount of the MePdR is converted to TdR, which then would reverse the inhibitory effect of the analogue. Therefore, in selecting a new 2-pyrimidinone-2'-deoxyriboside for synthesis and biological testing, it is advisable to consider whether the biological activities of the corresponding 2'-deoxyuridine derivative, if formed, would either favorably or harmfully interfere with the evaluation or therapeutic usefulness of the new drug.

Among the known 5-substituted 2'-deoxyuridines tested, 5-ethyl-2'-deoxyuridine[7,8] (EtUdR) appears to stand out with its potent antiviral activity against both HSV-1 and HSV-2 coupled with relatively low cellular toxicity *in vitro*[9,10] as well as *in vivo*.[11,12] Although it is incorporated into the DNA of cells, it is not mutagenic or immunosuppressive and has no effect on chromosome morphology or corneal tissue regeneration.[7,13] These properties appear to make EtUdR not only a desirable "lead" compound,[14] but also an acceptable potential metabolite for a new antiherpes agent. Therefore, we decided to synthesize the title compound and present it for future evaluation.

Chemistry

Our initial attempts to synthesize the desired ß-anomer of 5-ethyl-2-pyrimidinone 2'-deoxyriboside **10** directly from a ß-nucleoside starting material, and thus avoid the necessity of separating anomeric products, were unsuccessful. A trial reaction for reducing the ethynyl moiety of the previously synthesized 1-(2-deoxy-ß-D-ribofuranosyl)-5-ethynyl-2-pyrimidinone[2] by hydrogenation revealed the susceptibility of the 2-pyrimidinone system to overreduction. Attempts to convert the known 5-ethyl-2'-deoxyuridine to **10** *via* thiation with P_2S_5 (in dioxane)[15] and subsequent reaction of the 4-thio derivative with hydrazine monohydrate[16] in absolute ethanol, yielded a mixture of several components. Therefore, these "salvage" pathways were abandoned in favor of "*de novo*" synthesis.

5-Ethyluracil (**1**) was reacted with $POCl_3$ in N,N-dimethylaniline[17] (Scheme 1) to give the 2,4-dichloro derivative (**2**) in 95% yield. The 4-

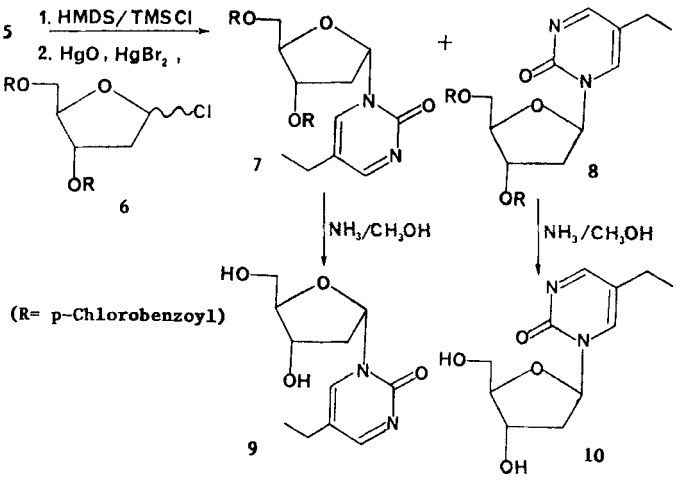


1 2 3 4 5

SCHEME 1

chloro substituent of **2** was then selectively reduced by refluxing with zinc in ammonium hydroxide and toluene for about 100 hours, following the general procedure of Brown.[18,19] The resulting 2-chloro-5-ethylpyrimidine (**3**), an oil, was purified by vacuum distillation (28% yield) and subsequently refluxed with sodium methoxide in methanol to give the 2-methoxy derivative **4** in 67% yield. The latter was hydrolyzed with dilute HCl to give an overall yield of 11% of the hitherto unknown 5-ethyl-2-pyrimidinone (hydrochloride **5**).

The pyrimidine base **5** was then silylated with hexamethyldisilazane and trimethylsilyl chloride, and the crude silyl derivative was condensed with the halogenose[20,21] **6** in the presence of HgO and $HgBr_2$ to obtain, after workup, 35% yield of an anomeric mixture of the blocked nucleosides **7** and **8** (Scheme 2). These were separated by flash column chromatography to obtain approximately equal amounts of the two anomers **7** and **8**. The anomeric proton of the blocked ß-anomer **8** appears as a pseudo-triplet with a slightly indented central band and a peak width of 14 Hz at δ 6.31, whereas that of the α-anomer **7** shows an apparent doublet of doublets, peak width 7 Hz, at δ 6.29, in agreement with the triplet-quartet peak-width rule[22]. In addition, the signals for the 4' and 5' protons showed similar patterns to those reported for the blocked anomers of other 2-pyrimidinone 2'-deoxyribosides[1,2,23], i.e., a well-separated triplet (1H) and doublet (2H) for the α-anomer, and an overlapping multiplet (3H) for the ß-anomer. Deblocking of **8** with methan-



SCHEME 2

olic ammonia gave, after workup and purification using preparative TLC, 61% yield of the desired pure 1-(2-deoxy-ß-D-ribofuranosyl)-5-ethyl-2-pyrimidinone (**10**) in the form of a viscous liquid which solidified into a foam after long storage under vacuum. The NMR signal for the anomeric proton, at δ 6.17, appeared as a clear symmetrical pseudotriplet. The signals for the C^4 and C^6 protons corresponded to those of a free nucleoside, without evidence of cycloadduct formation[1,2]. The blocked α-anomer **7** was hydrolyzed in a similar manner to give 60% of the free α-nucleoside **9** as a sticky hygroscopic solid. The NMR peak for the anomeric proton appeared at δ 6.13 as a doublet of doublets.

Compound **10** has been submitted for biological evaluation.

EXPERIMENTAL

Melting points were determined in open-end capillary tubes on a MelTemp apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian T-60 or EM-390 instrument with $(CH_3)_4Si$ as a reference. The IR spectra were run on a Mattson-Polaris FT-IR spectrophotometer. The UV spectra were determined on a Cary model 118C spectrophotometer. Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA.

Thin layer chromatography (TLC) employed Analtech silica gel GHLF Uniplates, using long and short wavelength UV for visualization. Preparative scale TLC was performed on 20x20 glass plates coated with Machery-Nagel MN-Kieselgel P/UV_{254}. Flash column chromatography employed Baker's silica gel for flash chromatography.

Solvents were dried by distillation over calcium hydride or phosphorus pentoxide.

5-Ethyl-2-pyrimidinone hydrochloride(5)

Phosphorus oxychloride (29 mL, 311.15 mmol) was added dropwise to a mixture of 10 g 5-ethyluracil (**1**) (71.36 mmol) and 5.35 mL N,N-dimethylaniline (42.2 mmol). The suspension was refluxed under dry conditions for about 1.5 hour, until the disappearance of starting material as monitored by TLC (1:1 CH_2Cl_2:n-hexane). The dark solution was cooled, evaporated to half of its original volume, and poured into ice/water. The aqueous solution was extracted with 5x20 mL ether, and the ether layers washed with 5% $NaHCO_3$, dried ($MgSO_4$), concentrated and cooled to yield crystals of 2,4-dichloro-5-ethylpyrimidine (**2**) in 2 crops; 12.1 g (95.7%). NMR ($CDCl_3$) δ 1.32 (t, 3H, $-CH_2\underline{CH_3}$), 2.78 (q, 2H, $-\underline{CH_2}CH_3$), 8.44 (s, 1H, H-6).

A mixture of 12.1 g of **2** (68.26 mmol), 15.6 g zinc dust (238 mmol), 45 mL toluene and 112 mL of 3N NH_4OH saturated with NaCl was refluxed with stirring. The reaction was complete after 48-50 hr as indicated by TLC (1:1 CH_2Cl_2:n-hexane). The reaction mixture was cooled, filtered and the solids washed thoroughly with ether. The filtrates were separated and the organic layer washed with 2x25 mL water, dried ($MgSO_4$) and concentrated to an oil. Distillation at 54°C, 0.3-0.7 mm Hg, yielded 2.79 g (28.7%) of 2-chloro-5-ethylpyrimidine (**3**). NMR ($CDCl_3$) δ 1.29 (t, 3H, $-CH_2\underline{CH_3}$), 2.67 (q, 2H, $-\underline{CH_2}CH_3$), 8.48 (s, 2H, H-4, H-6).

A solution of 6.36 g **3** (50.53 mmol) in 100 mL of dry methanol was treated dropwise with 2.73 g of sodium methoxide (50.53 mmol) in 50 mL of dry methanol. After 70 minutes' reflux under anhydrous conditions, at which time the TLC (7:3 CH_2Cl_2:ethyl acetate) showed no remaining **3**, the reaction mixture was concentrated under reduced pressure to an oil which was taken up in 100 mL of CH_2Cl_2, washed with water, dried ($MgSO_4$) and concentrated to yield 5-ethyl-2-methoxypyrimidine (**4**) as an oil (4.9 g, 79.7%). NMR ($CDCl_3$) δ 1.23 (t, 3H, $-CH_2\underline{CH_3}$), 2.57 (q, 2H, $-\underline{CH_2}CH_3$), 3.96 (s, 3H, $-OCH_3$), 8.33 (s, 2H, H-4, H-6).

A mixture of 7.2 g of **4** (52.12 mmol) in 108 mL (216 mmol) of 2N HCl was refluxed for about 4 hours, until TLC (7:3 CH_2Cl_2:ethyl acetate) showed the disappearance of **4**. The solution was concentrated to an oil by evaporation *in vacuo* at 40°C. Addition of a small quantity of acetone precipitated a solid, which was cooled, filtered, washed with cold CH_2Cl_2 and then with acetone, and dried under vacuum over P_2O_5 to yield 5-ethyl-2-pyrimidinone hydrochloride (**5**) as light pink crystals (5.2 g, 62.1%), which was recrystallized from methanol; mp 204°C(dec.). NMR (DMSO-d_6) δ 1.15 (t, 3H, $-CH_2\underline{CH_3}$), 2.52 (q, 2H, $-\underline{CH_2}CH_3$), 8.71 (s, 2H, H-4, H-6); IR (KBr) 2716, 2675, 1785, 1725, 1579, 1458, 1276, 1202, 1194, 891 cm^{-1}; UV (methanol) 324, 219 nm. Anal. Calc. for $C_6H_9ClN_2O$: C, 44.86; H, 5.64; N, 17.44; Cl, 22.07%. Found: C, 44.95; H, 5.66; N, 17.43; Cl, 22.01%.

1-[3',5'-Di-O-(4-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl]-5-ethyl-2-pyrimidinone (7) and 1-[3',5'-di-O-(4-chlorobenzoyl)-2-deoxy-ß-D-ribofuranosyl]-5-ethyl-2-pyrimidinone (8).

A mixture of 1.0 g (6.24 mmol) of **5**, 1.0 mL (7.8 mmol) of chlorotrimethylsilane and 10 mL (47 mmol) of hexamethyldisilazane was stirred at reflux for 70 min under dry conditions. The solution was concentrated to an oil under anhydrous conditions, then co-evaporated twice from 10 mL of 1,2-dichloroethane to remove residual hexamethyldisilazane. The oil was then dissolved in 15 mL of dry toluene and cooled in an ice-salt bath to 0°C. To this stirred, cooled solution was added 1.35 g (6.24 mmol) mercuric oxide, 2.24 g (6.24 mmol) mercuric bromide, and subsequently, 2.67 g (6.24 mmol) of 3,5-bis-O-(4-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl-1-chloride (**6**). The reaction was stirred under a drying tube at 0°C for 35 min, then at room temperature for 2.5 hr. Because the solids in the reaction formed a sticky lump about 5 min after the addition of **6**, about 0.5 eq. additional HgO and $HgBr_2$ were added over the reaction period. The reaction was monitored by TLC (15% EtOAc in CH_2Cl_2) until the disappearance of **6**. The ratio of the two product isomers did not change during the reaction, according to the appearance of their two fluorescent TLC traces. The mixture was filtered and the solids washed well with CH_2Cl_2 (100 mL). The filtrates were washed with 3x100 mL of 30% KI, dried ($MgSO_4$) and concentrated to 2.93 g of a solid, an impure mixture of the two anomeric protected nucleosides. The crude material was flash-chromatographed

using 35% ethyl acetate/CH_2Cl_2. The fractions comprising the slower-moving component were concentrated to 0.46 g (14.3%) of the α-anomer **7**; mp 178°C. NMR ($CDCl_3$) δ 1.04 (t, 3H, $-CH_2\underline{CH_3}$), 2.36 (q, 2H, $-\underline{CH_2}CH_3$), 2.61-3.24 (m, 2H, 2'-H), 4.54 (d, 2H, 5'-H), 4.91 (t, 1H, 4'-H), 5.54 (d, 1H, 3'-H), 6.29 (dd, J=6 Hz and 1 Hz, 1H, 1'-H), 7.20-8.08 (m, 9H, C^6-H, phenyl), 8.54 (d, 1H, C^4-H); IR (KBr) 2963, 1721, 1653, 1593, 1516, 1396, 1278, 1266, 1094, 1012 cm^{-1}; UV ($CHCl_3$) 325, 246 nm. Anal. Calc. for $C_{25}H_{22}Cl_2N_2O_6$: C, 58.04; H, 4.29; N, 5.41; Cl, 13.71%. Found: C, 58.09; H, 4.33; N, 5.37; Cl, 13.79%.

The column fractions comprising the faster-moving anomer were concentrated to 0.5 g (15.5%) of the ß-anomer **8** as a solid which was recrystallized from CH_2Cl_2:ethyl acetate; mp 180-182°C. NMR ($CDCl_3$) δ 1.03 (t, 3H, $-CH_2\underline{CH_3}$), 2.28 (q, 2H, $-\underline{CH_2}CH_3$), 2.00-2.48 (m, 1H, 2'-H), 3.02-3.3 (m, 1H, 2'-H), 4.52-4.83 (m, 3H, 4'H, 5'-H), 5.58 (d, 1H, 3'-H), 6.31 (pseudo-t, J=6 Hz and 8 Hz, 1H, 1'-H), 7.32-7.51 (m, 4H, phenyl), 7.74-8.05 (m, 5H, C^6-H, phenyl), 8.45 (d, 1H, C^4-H); IR (KBr) 3069, 2972, 1722, 1666, 1633, 1592, 1521, 1404, 1281, 1274, 1123, 1107, 1090, 994 cm^{-1}; UV ($CHCl_3$) 325, 246 nm. Anal. Calc. for $C_{25}H_{22}Cl_2N_2O_6$: C, 58.04; H, 4.29; N, 5.41; Cl, 13.71%. Found: C, 57.96; H, 4.31; N, 5.39; Cl, 13.79%.

1-(2-deoxy-α-D-ribofuranosyl)-5-ethyl-2-pyrimidinone (9)

Anhydrous methanol (25 mL) was saturated with ammonia at 0-5°C, 0.35 g (0.67 mmol) of **7** was added to it and the flask was sealed and stirred at 4°C for 10 h. The solution was concentrated under reduced pressure without heating, and the residue triturated with ether to yield a soft solid which was filtered and washed repeatedly with ether and CH_2Cl_2. The crude product was purified using preparative TLC (10% methanol/CH_2Cl_2) to give 0.08 g (49.4%) of **9** as a hygroscopic solid which was identified by NMR. NMR (acetone-d_6) δ 1.15 (t, 3H, $-CH_2\underline{CH_3}$), 2.48 (q, 2H, $-\underline{CH_2}CH_3$), 2.22-2.98 (m, 2H, 2'-H), 3.67 (d, 2H, 5'-H), 4.55 (m, 2H, 3'-H, 4'-H), 6.13 (dd, J=7 Hz and 2 Hz, 1H, 1'-H), 8.15 (d, $J_{6,4}$=3 Hz, 1H, C^6-H), 8.46 (d, $J_{4,6}$=3 Hz, 1H, C^4-H).

1-(2-deoxy-ß-D-ribofuranosyl)-5-ethyl-2-pyrimidinone (10)

A solution of 0.215 g **8** (0.41 mmol) in 25 mL of anhydrous methanol presaturated with ammonia was stirred at 4°C for 6 h. The solution was concentrated *in vacuo* without heating and triturated with ether to obtain a semisolid precipitate, which was washed with ether and further

purified by preparative TLC (1:1 CH_2Cl_2/ethyl acetate), developing the plate several (2-3) times to fully separate the product from an impurity with similar retention characteristics, and eluting the appropriate band using 30% methanol/CH_2Cl_2. The extracts were concentrated to an oil which eventually solidified on drying under vacuum, yielding 0.060 g (61.2%) of **10**; mp 101°C. NMR (acetone-d_6) δ 1.19 (t, J=7 Hz, 3H, $-CH_2\underline{CH_3}$), 2.20-2.71 (m (including q of J=7 Hz), 4H, $-\underline{CH_2}CH_3$, 2'-H), 3.91 (d, 2H, 5'-H), 3.99-4.29 (m, 1H, 4'-H), 4.41-4.63 (m, 1H, 3'-H), 6.17 (t, $J_{1'2'}=J_{1'2''}=6$ Hz, 1H, 1'-H), 8.21 (bs, 1H, C^6-H), 8.50 (bs, 1H, C^4-H) ; IR (KBr) 2990, 2671, 1785, 1725, 1696, 1580, 1458, 1276, 1202, 1193, 888 cm^{-1}; UV (methanol) 323, 219 nm. Anal. Calc. for $C_{11}H_{16}N_2O_4 \cdot 0.3\ H_2O$: C, 53.78; H, 6.81; N, 11.40. Found: C, 53.73; H, 6.71; N, 11.51.

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