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Improved Synthesis and in Vitro Antiviral Activities of 5-Cyanouridine and 5-Cyano-2′-deoxyuridine

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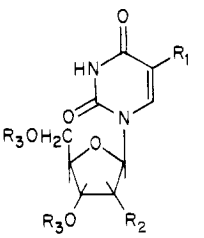
In order to evaluate the influence of the cyano group on the antiviral activity of pyrimidine deoxyribonucleosides, a moderate yield, unified approach to the synthesis of both 5-cyanouridine and 5-cyano-2′-deoxyuridine was developed. Thus, treatment of the appropriate acetylated 5-bromouracil nucleoside with NaCN or KCN in Me_2SO at 90–110 °C gave, after deblocking, 35–45% yields of the corresponding 5-cyanouracil nucleosides. 5-Cyanouridine was devoid of significant activity against vaccinia virus, herpes simplex-1, and vesicular stomatitis virus, but 5-cyano-2′-deoxyuridine, while lacking activity against herpes simplex, showed significant inhibition of vaccinia virus; for instance, 5-cyano-2′-deoxyuridine inhibited vaccinia virus replication at concentrations 10–20 times that required for inhibition by the known antivirals, 5-iodo-2′-deoxyuridine and 1-(β-D-arabinofuranosyl)adenine. Replacement of the 5-halogeno substituents of pyrimidine deoxyribonucleosides thus decreases, but does not abolish, antiviral activity.

A number of 5-substituted 2′-deoxyuridines possess significant in vivo and/or in vitro antiviral activity against a variety of DNA viruses. The most well known of these, 5-iodo-2′-deoxyuridine,[1,2] 5-bromo-2′-deoxyuridine,[1,2] and 5-trifluoromethyl-2′-deoxyuridine,[3] express their inhibitory action primarily after they have been incorporated into viral DNA. These nucleosides have pioneered our understanding of viral replication and have demonstrated the promise of nucleosides as antiviral agents; nonetheless, they possess several important limitations. All are incorporated into viral DNA and host cell DNA with resultant mutagenic events in phage, bacteria, and eukaryotic cells as well as oncornavirus activation in mammlian cell culture.[1,2] Additionally, 5-iodo- and 5-bromo-2′-deoxyuridine are embryotoxic and may also lead to immunosuppression, at least in vitro.[1,2]

For these reasons, we felt it would be interesting to expand the variety of substituents available at the pyrimidine C-5 position of the pyrimidine deoxyribonucleosides to take advantage of the multitude of steric and electronic alterations which could be thus obtained. Such alterations would surely influence the biochemistry of the pyrimidine deoxyribonucleoside analogues, thereby providing additional information for the design of useful nucleoside antivirals.

One such alteration of interest is the cyano group which is somewhat unique with respect to other substituents that endow 2′-deoxyuridine with antiviral activity. For steric purposes, the C–CN moiety can be considered as a cylinder with a diameter of 3.6 Å whereas the C–Cl, C–Br, and C–I groups may be considered roughly as spheres with diameters of 3.6, 3.9, and 4.2 Å, respectively.[4] The trifluoromethyl group can be regarded as a hemisphere with an overall diameter of 5.0 Å.[4] Thus sterically, the CN group most closely approximates either a chloro or bromo substituent. The cyano group, however, shows the greatest electrical effect of any common substituent; e.g., the dipole moments of CH_3X increase in the order Cl, CF_3, NO_2, and CN.[4] The inductive and resonance σ values for cyano are comparable to the values for NO_2 but exceed the values for F, Cl, CF_3, etc.[4] It can be reasonably expected that substitution of CN for H, I, Br, F, or CF_3 will bring about a marked difference in biological behavior compared to the parent compounds.

In fact, both 5-cyanouridine (I) and 5-cyano-2′-deoxyuridine (II) have been reported earlier in the literature.



I, R_1 = CN; R_2 = OH; R_3 = H
II, R_1 = CN; R_2 = H; R_3 = H
III, R_1 = Br; R_2 = OAc; R_3 = Ac
IV, R_1 = Br; R_2 = H; R_3 = Ac

Watanabe and Fox[5] used the $Hg(CN)_2$–nitromethane procedure to prepare the tribenzoate of I in 70% yield from the polyacetylated glycosyl halide and 5-cyanouracil. Inoue and Ueda[6] and Ueda et al.[7] reported a 70% yield of the 5′-acetyl-2′,3′-isopropylidene derivative of I when the corresponding 5-bromo derivative was treated with NaCN according to methods developed for the simpler uracil derivatives.[8-11] Prystas and Sorm[12] reported deblocking such an intermediate, obtained via a Hilbert–Johnson type synthesis, to give a 29% yield of I. It is noteworthy that the condensation type reactions required preparation of 5-cyanouracil in two steps from ureidomethylene malonitrile. Shaw et al.[13] condensed tri-O-benzoylated D-ribosylamine with α-cyano-β-ethoxy-*N*-ethoxycarbonylacrylamide to give, after deblocking, a 20% yield of I which was also obtained by a similar condensation using the isopropylidene derivative of the furanosylamine to give an

Table I. Evaluation of the Antiviral Activity of 5-Cyanouracil Nucleosides

Nucleoside	ID_{50},[a] M			
	Human skin fibroblasts		Primary rabbit kidney cells	
	Vaccinia	Herpes simplex-1	Vaccinia	Herpes simplex-1
5-Cyanouridine[b] (I)	$>7 \times 10^{-4}$	$>7 \times 10^{-4}$	$>3.7 \times 10^{-4}$	3.7×10^{-4}
5-Cyano-2'-deoxyuridine (II)	2.7×10^{-5}	$>7 \times 10^{-4}$	1.6×10^{-5}	1.6×10^{-4}
5-Iodo-2'-deoxyuridine	1.1×10^{-6}	5.7×10^{-7}	1.1×10^{-6}	5.7×10^{-7}
5-Bromo-2'-deoxyuridine	6.5×10^{-7}	3.2×10^{-7}	3.2×10^{-7}	3.2×10^{-7}
5-Chloro-2'-deoxyuridine	1.5×10^{-6}	7.6×10^{-7}	7.6×10^{-7}	7.6×10^{-7}
5-Fluoro-2'-deoxyuridine	4×10^{-7}	4×10^{-7}	4×10^{-7}	1.6×10^{-7}
5-Trifluoromethyl-2'-deoxyuridine	1.3×10^{-6}	1.3×10^{-6}	3.3×10^{-7}	6.6×10^{-7}
Adenine arabinoside	2.6×10^{-5}	3.7×10^{-5}	1.4×10^{-6}	1.5×10^{-5}

[a] ID_{50} ≡ dose inhibiting virus induced cytopathogenicity by 50%. [b] 5-Cyanouridine was also inactive ($>7 \times 10^{-4}$ M) against an RNA virus (vesicular stomatitis virus in primary rabbit kidney cells).

unspecified yield of I.[14] By reacting a persilylated 5-iodo-2'-deoxyuridine with CuCN, followed by column chromatography and countercurrent distribution purification of this product, Bleakley et al. obtained a 4% yield of the deoxy analogue II.[15]

Herein we report an improved and unified approach to the syntheses of the ribonucleoside I and the deoxy analogue II. This has permitted an in vitro evaluation of the antiviral activity of these nucleosides.

Chemistry. Both 5-cyanouridine (I) and 5-cyano-2'-deoxyuridine (II) could be obtained in moderate yield (35–45%) by the reaction of the acetylated bromouracil nucleosides III and IV with KCN or NaCN in Me_2SO. A significant improvement in yield (45 vs. 30%) was noted for the deoxyuridine analogue II when the reaction was carried out in the presence of an equivalent of potassium acetate. The mechanism for this yield enhancement is unclear. The cyanouracil derivatives prepared in this manner gave satisfactory elemental analyses, had spectral properties consistent with the assigned structures, and possessed properties (melting point, UV, and ^1H NMR) agreeing well with those reported in the literature for I and II prepared by other synthetic routes.

Antiviral Activity. The antiviral activity of the cyanouracil nucleosides I and II was assessed against two DNA viruses (vaccinia and herpes simplex-1) in two different cell lines [primary rabbit kidney (PRK) and human skin fibroblasts (HSF)]. The results are expressed as minimum inhibitory concentrations; that is, the concentration of the nucleoside that inhibited viral-induced cytopathogenicity by 50%. The cyano nucleosides were compared with other 5-substituted pyrimidine deoxyribonucleosides and with adenine arabinoside, all of which have been previously shown to possess in vitro and/or in vivo antiviral activity (Table I). It is clear from Table I that while II does not achieve the same high level of activity as seen with the halogeno deoxyribonucleosides, its activity is comparable to other substances which show in vivo activity.[17]

We conclude that replacement of halogeno substituents by the cyano group in pyrimidine deoxyribonucleosides leads to a significant decrease in in vitro antiviral activity but by no means abolishes it. The reason for this decrease in potency of antiviral activity upon substitution of cyano for halogen is not at all clear. The steric bulk of the CN group is comparable to that of Cl or Br.[4] There is a significant difference in the pK_a (6.8) of II as compared to, e.g., 5-iodo-2'-deoxyuridine ($pK_a = 8.2$[16]), but whether or not this is sufficient to account for the lowered activity of II remains to be determined. It is clear, however, that the cyano group significantly alters the antiviral activity compared to halogeno nucleosides and it may also alter other biological effects associated with such analogues, e.g., immunosuppression, inhibition of host cell DNA biosynthesis, mutagenicity, oncornavirus activation, etc. With the aid of this improved synthesis of II, studies are in progress to examine the effect of cyano substitution on the above biological properties as well as to evaluate the in vivo antiviral activity of 5-cyano-2'-deoxyuridine.

Experimental Section

The following chemicals were from commercial sources: uridine and 5-fluoro-2'-deoxyuridine (Aldrich, Milwaukee, Wis.), 5-iodo-2'-deoxyuridine, 5-bromo-2'-deoxyuridine, and 5-trifluoromethyl-2'-deoxyuridine (Sigma, St. Louis, Mo.), 2'-deoxyuridine (Calbiochem., Los Angeles, Calif.), and 1-(β-D-arabinofuranosyl)adenine (P-L Biochemicals, Milwaukee, Wis.). The acetates of both 5-bromouridine and 5-bromo-2'-deoxyuridine were prepared from uridine and 2'-deoxyuridine, respectively, according to the literature methods.[18] Melting points (uncorrected) were determined on a Thomas-Hoover apparatus and the following spectra as indicated: UV on a Cary 15, ^1H NMR on a Varian HA-100, and infrared on a Perkin-Elmer Infracord. pK_a values were determined spectrophotometrically using Teorell-Stenhagen buffers. Methodology used for assay of antiviral activity has been described earlier.[19]

1-(β-D-*erythro*-Pentofuranosyl)-5-cyanouracil (5-Cyanouridine, I). 1-(2',3',5'-Tri-*O*-acetyl)-5-bromouridine (III, 11.13 g, 25 mmol) and NaCN (1.53 g, 30 mmol) were dissolved in Me_2SO (25 mL, dried over molecular sieves) and the resulting solution was warmed at 110 °C for 1 h. After cooling, the mixture was diluted with MeOH and neutralized with HOAc to pH 6–7. After evaporation in vacuo, the residue was chromatographed on a silica gel column with CH_2Cl_2–EtOAc (3:1). Two fractions were eluted. The second and major fraction was evaporated to give 4 g of solid which was the intermediate triacetylated 5-cyanouridine. The solid was dissolved in MeOH (100 mL) that had been saturated at 0 °C with NH_3, and the solution held at 4 °C overnight. After in vacuo evaporation, the residue was crystallized from EtOH to give 2.2 g (35%) of 5-cyanouridine: mp 190–192 °C (lit. mp 185 °C[13,14] and 191–192 °C[12]); IR ν_{max} 2230 cm^{-1} (CN); UV λ_{max}^{pH6} 278 nm (ε 12 900); UV λ_{max}^{pH2} 276 nm (ε 13 100); UV λ_{max}^{pH11} 275 nm (ε 9600).

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-cyanouracil (5-Cyano-2'-deoxyuridine, II). A mixture of 3',5'-di-*O*-acetyl-5-bromo-2'-deoxyuridine (IV, 11.7 g, 30 mmol), KCN (1.8 g, 36 mmol), potassium acetate (3.6 g, 36 mmol, anhydrous), and Me_2SO (dried over molecular sieves, 20 mL) was heated under a nitrogen atmosphere at 90–100 °C in an oil bath for 30 min. The Me_2SO was then distilled off at the same temperature in vacuo (time ~20 min). The residue was cooled, dissolved in benzene (50 mL), and washed with water (3 × 50 mL) to remove salts. The benzene solution was evaporated to dryness in vacuo, and the residue was dissolved in 100 mL of MeOH saturated with NH_3 at 0 °C. After 24 h at ambient temperature, the solution was evaporated to dryness in vacuo. Crystallization of the solid mass from CH_3OH gave II as colorless crystals (3.42 g, 45%): mp 177–178 °C (lit.[15] mp 161 °C); UV λ_{max}^{EtOH} 278 nm (ε 13 000); IR ν_{max} 2230 cm^{-1} (CN).

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B/C-*cis*- and -*trans*-1,3,4,9,10,10a-Hexahydro-2*H*-10,4a-methanoiminoethanophenanthrene (Homo- and Homoisomorphinan) Derivatives as Analgesics

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N-Alkyl derivatives of B/C-*cis*- and B/C-*trans*-6-hydroxy-1,3,4,9,10,10a-hexahydro-2*H*-10,4a-methanoiminoethanophenanthrene have been prepared. The analgesic potency and physical dependence capacity of these compounds were determined. The *N*-methyl derivatives were analgesically equipotent with morphine. None of these compounds except the *N*-methyl-B/C-trans isomer **2b** suppressed or precipitated the abstinence syndrome. Compound **2b** was a narcotic agonist. The *N*-methyl-B/C-cis compound **2a** appears to warrant further examination for its potential as a potent analgesic having no physical dependence liability.

Chemical modifications of 6,7-benzomorphan and morphinan have produced many compounds possessing interesting profiles with respect to narcotic antagonist and analgesic activities.[1] The structure–activity effects of substituents at positions 2, 5, 9, and 2′ of 6,7-benzomorphan and positions 3, 4, and 17 of morphinan have been intensively studied.

In order to investigate further the structure–activity relationships of 6,7-benzomorphans and morphinans, we have designed compounds where ring C of 6,7-benzomorphan or ring D of morphinan has been modified by introducing an extra methylene group between the nitrogen and bridgehead carbon (homobenzomorphan and homomorphinan).[2] This modification creates a somewhat conformationally flexible nitrogen-containing ring and should affect the steric environment around the nitrogen and above the aromatic ring. The importance of this steric environment for binding to the receptor site has been proposed by Lewis,[3] Belleau,[4] and Cochran.[5]

On the other hand, it has been reported that replacement of the *N*-methyl group of 6,7-benzomorphan and morphinan by an allyl or a cyclopropylmethyl group furnishes good narcotic antagonist activity.[6] It was, therefore, of interest to introduce these substituents into the homobenzomorphan or homomorphinan skeleton and to evaluate the effect of these modifications on the antagonistic and other pharmacological properties.

In this paper we report the synthesis of *N*-allyl (**5a,b**) and *N*-cyclopropylmethyl (**7a,b**) derivatives of B/C-*cis*- (homo-) and -*trans*-6-hydroxy-1,3,4,9,10,10a-hexahydro-2*H*-10,4a-methanoiminoethanophenanthrene (homoisomorphinan) and the analgesic potency, antagonistic activity, and physical dependence capacity of these compounds as well as the *N*-methyl derivatives **2a,b**.

Chemistry. The synthesis of *N*-methyl derivatives of homo- and homoisomorphinan **1a**, **1b**, **2a**, and **2b** was reported in our previous paper.[2a] Treatment of the *N*-methyl derivative **1a** with $ClCO_2Et$ in refluxing benzene gave carbamate **3a**.[7] When refluxed with 12 M HCl, the 6-methoxy carbamate **3a** was converted to the 6-hydroxy-*N*-nor compound **4a**. Alkylation of **4a** with allyl bromide gave the *N*-allyl compound **5a**.[6b,8] Compound **4a** was acylated with cyclopropylcarbonyl chloride followed by lithium aluminum hydride reduction to give 6-hydroxy-*N*-cyclopropylmethyl derivative **7a**.[6b,8] Homoiso counterparts **4b**, **5b**, and **7b** were obtained from **1b** by similar procedures.

Pharmacology. In Table I are given analgesic activities (Eddy hot-plate test[9,10]) and physical dependence capacities [monkey, single-dose suppresion (SDS)[10]] of compounds **1a**, **1b**, **2a**, **2b**, **5a**, **5b**, **7a**, and **7b**. These compounds, except **5a**, exhibit good analgesic potencies ranging from the morphine to codeine level, maximum activity being shown by compound **2a**. The 6-methoxy