

# Pyrimidine Homoribonucleosides: Synthesis, Solution Conformation, and Some Biological Properties

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Conversion of uridine and cytidine to their 5'-O-tosyl derivatives, followed by cyanation with tetraethylammonium cyanide, reduction and deamination, led to isolation of the hitherto unknown homouridine (1-(5'-deoxy-β-D-allofuranosyl)uracil) and homocytidine (1-(5'-deoxy-β-D-allofuranosyl)cytosine), analogues of uridine and cytidine in which the exocyclic 5'-CH<sub>2</sub>OH chain is extended by one carbon to CH<sub>2</sub>CH<sub>2</sub>OH. Homocytidine was also phosphorylated to its 6'-phosphate and 6'-pyrophosphate analogues. In addition, it was converted, *via* its 2,2'-anhydro derivative, to arahomocytidine, an analogue of the chemotherapeutically active araC.

The structures of all the foregoing were established by various criteria, including <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, both of which were also applied to analyses of the solution conformations of the various compounds, particularly as regards the conformations of the exocyclic chains.

The behaviour of the homo analogues was examined in several enzymatic systems. Homocytidine was a feeble substrate, without inhibitory properties, of *E. coli* cytidine deaminase. Homocytidine was an excellent substrate for wheat shoot nucleoside phosphotransferase; while homouridine was a good substrate for *E. coli* uridine phosphorylase. Although homoCMP was neither a substrate, nor an inhibitor, of snake venom 5'-nucleotidase, homoCDP was a potent inhibitor of this enzyme (K<sub>i</sub> ~ 6 μM). HomoCDP was not a substrate for *M. luteus* polynucleotide phosphorylase. None of the compounds exhibited significant activity *vs* herpes simplex virus type 1, or cytotoxic activity in several mammalian cell lines.

A number of 5'-modified, chain-extended nucleoside analogues have been synthesized and examined as potential antimetabolites [1]. These include "homonucleosides", in which the 5'-CH<sub>2</sub>OH chain is extended by one carbon atom. More recently some attention has been directed to 3'-branched nucleosides, such as the 3'-hydroxymethyl congeners of 6-thio-2'-deoxy-guanosine [2] and uridine [3].

Homonucleosides were initially prepared by classical condensation of a heterocyclic base with previously synthesized homoribose. This approach, which led to the homonucleosides of adenine [4] as well as 2-chloropurine and 6-mercaptopurine [5], has not been further pursued, presumably because of the large number of steps involved and accompanying low yields.

An alternative route is extension of the 5'-exocyclic chain of nucleosides, based on conversion of the 5'-CH<sub>2</sub>OH to reactive species such as the 5'-deoxy-5'-iodo or 5'-O-tosyl, which are then dis-

placed with cyanide to give the required one-carbon extension of the chain. Reduction of the cyano group, followed by deamination, should give the desired homonucleoside. A preliminary report has appeared on such a preparation of homothymidine [6] but without experimental details, and a <sup>1</sup>H NMR spectrum of the product open to question (see below). Alternatively, homoadenosine was prepared by conversion of the cyano group of 5'-cyano-adenosine to the methyl carboxylate, followed by reduction of the latter [7] presumably to avoid the deamination step (see below).

We now describe the preparation, by modifications of the foregoing procedures, of some pyrimidine homoribonucleosides and related analogues, their conformational properties in solution, and some biological properties, including behaviour in several enzyme systems.

## Chemistry

Attempts to obtain 5'-cyanouridine by reaction of 5'-deoxy-5'-chloro-2',3'-O-isopropylideneuridine with NaCN or KCN in the presence or absence of 18-

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crown-6 in a variety of solvents (DMSO, DMF,  $\text{CH}_3\text{CN}$ , EtOH, aqueous EtOH), at elevated temperatures, were unsuccessful, and led to complex mixtures of products which included uracil and  $\text{O}^2$ :5'-cyclouridine.

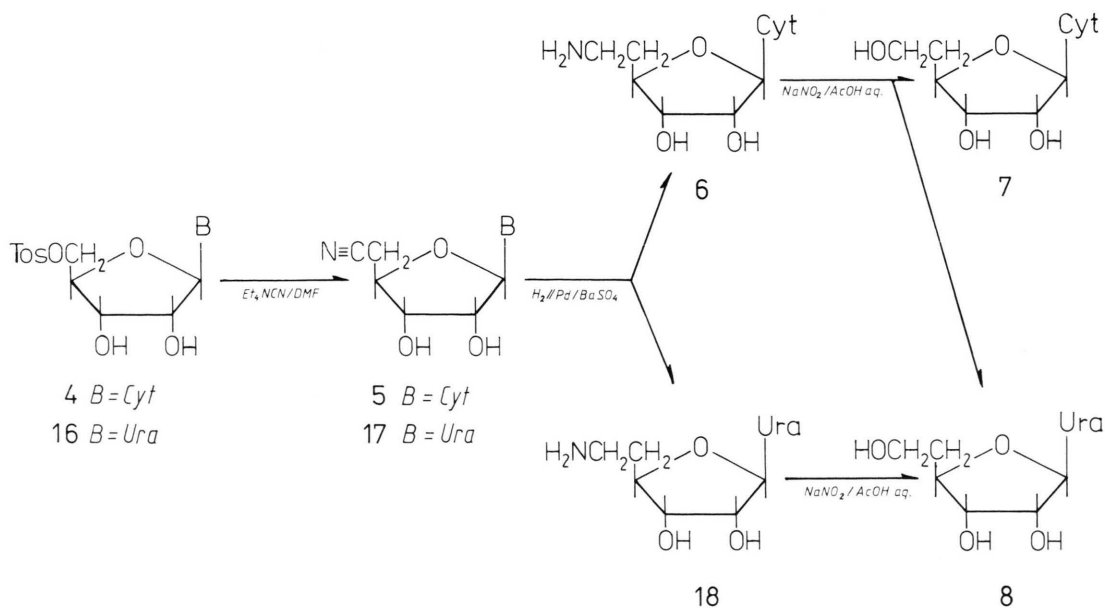
Since NaCN and KCN are only moderately soluble in aprotic solvents, even in the presence of 18-crown-6, attention was directed to the use of tetraethylammonium cyanide [8], which is very soluble in such solvents. In addition, the 5'-deoxy-5'-chloro derivatives were replaced by the more reactive 5'-deoxy-5'-iodo and 5'-O-tosyl derivatives of 2',3'-O-isopropylideneuridine, as well as 5'-O-tosyl-2',3'-O-isopropylidencytidine. Reaction of these with tetraethylammonium cyanide at room temperature led to the  $\text{O}^2$ :5'-cyclonucleosides as the major products.

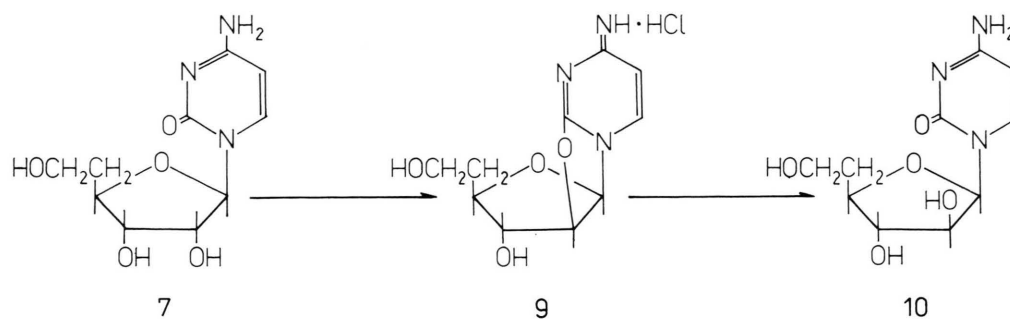
Meyer and Follman [9] reportedly obtained  $\text{O}^2$ :5'-cyclouridine in 65% yield by reaction of 5'-O-tosyl-2',3'-O-isopropylideneuridine with KCN in the presence of 18-crown-6, which was isolated and then reacted once again in the same manner to yield the 5'-cyano derivative in 32% yield. By contrast, the analogous reaction with a purine nucleoside analogue, 5'-O-tosyl-2',3'-O-isopropylideneadenosine, led directly to the 5'-cyano derivative in 73% yield, whereas Hollmann and Schlimme [7] report a yield of 36%. Under similar conditions, reaction of 5'-O-tosylthymidine resulted in a 76% yield of the 5'-cyano congener [9].

Subsequent trials were therefore conducted with the 5'-O-tosyl derivatives of cytidine and uridine (with unprotected 2' and 3' sugar hydroxyls). Treatment of these with tetraethylammonium cyanide in DMF at room temperature for 2–5 h gave the desired 5'-cyanocytidine and 5'-cyanouridine in yields of about 40%, with no detectable formation of the  $\text{O}^2$ :5'-cyclonucleosides. On the other hand, formation of the 5'-cyanonucleosides was accompanied by the appearance of the free heterocyclic base. It was therefore necessary to follow the course of the reaction by TLC, and to terminate it at the point where decomposition of the 5'-cyano analogues began to dominate. Liberation of the heterocyclic base from 5'-cyanonucleosides has been observed by others [6, 9], and Meyer and Follmann [9] examined in detail the alkali-induced cleavage of the glycosidic bond of 5'-cyanonucleosides.

The 5'-deoxy-5'-cyano derivatives of cytidine (**5**) and uridine (**17**) were subjected to reduction over Pd/BaSO<sub>4</sub> [10] to give the corresponding amino derivatives **6** and **18**, respectively.

Appropriately controlled treatment of **6** with HNO<sub>2</sub> permitted reasonably selective deamination of the 6'-NH<sub>2</sub> group to yield the desired homocytidine (**7**), accompanied by formation of only a low proportion (5%) of homouridine (**8**) as a byproduct. The latter was identical with homouridine obtained independently by the nitrous acid deamination of **18**. The





foregoing would probably be equally applicable to conversion of 5'-deoxy-5'-cyanoadenosine to homoadenosine [11], in place of the reported conversion of the 5'-cyano group to the carboxylic methyl ester and subsequent reduction of the latter, in relatively low yield [7].

Conversion of homocytidine to the corresponding arabinosyl analogue **10** proceeded smoothly *via* the 2,2'-anhydro derivative, as for the known conversion of cytidine to araC [12].

In turn, phosphorylation of homocytidine with the aid of the wheat shoot phosphotransferase system [13] led to formation of the 6'-phosphate, which was isolated in crystalline form in very good yield. This was further converted on a small scale to homoCDP (**12**) according to standard procedures [14].

Isolation from reaction mixtures of the cytosine nucleoside analogues was based largely on column chromatography with the cation exchange resin Dowex (H<sup>+</sup>), which proved very convenient for isolation of **5**, **6**, **7**, and **9**. A simple and very efficient

procedure for purification of 2',3'-O-isopropylidenecytidine, originally suggested by Dr. L. Dudycz (unpublished), was based on chromatography on Dowex (OH<sup>-</sup>), which strongly retains *p*-toluenesulfonic acid and unreacted cytidine (**1**).

All the newly synthesized derivatives exhibited UV absorption spectra in agreement with expectations, and elementary analyses were obtained for the key derivatives **5**, **7**, and **8**. Final criteria for the proposed structures were, however, based largely on <sup>1</sup>H NMR spectroscopy, as well as <sup>13</sup>C NMR and IR spectroscopy in the case of **17**.

*NMR evidence for proposed structures.* The presence of the 5'-cyano group in 5'-deoxy-5'-cyano-uridine, revealed by IR spectroscopy, was further unequivocally confirmed by the absence of a signal for the 5'-OH in the <sup>1</sup>H NMR spectrum in DMSO, and the presence in the <sup>13</sup>C spectrum of an additional signal at 51.90 ppm, close to the <sup>13</sup>C signal of the C≡N in acetonitrile, 52.60 ppm (see Tables). The chemical shifts of the H(5') and H(5'') protons are

Table I. Proton chemical shifts (in ppm ± 0.005 ppm) for uridine and cytidine analogues at 0.04 M in H<sub>2</sub>O-d<sub>2</sub> (vs internal TSP) or in DMSO-d<sub>6</sub> (vs internal Me<sub>4</sub>Si) at 30 °C.

Compd	Solvent	NH NH <sub>2</sub>	H(5)	H(6)	H(1')	H(2')	H(3')	H(4')	H(5') <sup>a</sup>	H(5'') <sup>a</sup>	H(6')	H(6'')	2' OH	3' OH
Urd ( <b>13</b> )	H <sub>2</sub> O-d <sub>2</sub>		5.90	7.87	5.91	4.36	4.23	4.13	3.91	3.81	—	—		
<b>17</b>	DMSO-d <sub>6</sub>	11.41	5.69	7.66	5.78	4.18	3.87	3.98	3.02	2.96	—	—	5.42	5.55
<b>8</b>	H <sub>2</sub> O-d <sub>2</sub>		5.89	7.66	5.84	4.37	4.08	4.13	2.04	1.96	3.78	3.76		
Cyd ( <b>1</b> )	H <sub>2</sub> O-d <sub>2</sub>		6.05	7.84	5.90	4.31	4.21	4.13	3.93	3.82	—	—		
5' CMP	H <sub>2</sub> O-d <sub>2</sub>		6.14	8.12	6.01	4.36	4.36	4.25	4.05	3.98	—	—		
<b>5</b>	DMSO-d <sub>6</sub>	<sup>b</sup>	5.77	7.58	5.79	4.09	3.85	3.95	3.00	2.93	—	—	<sup>b</sup>	<sup>b</sup>
<b>7</b>	H <sub>2</sub> O-d <sub>2</sub>		6.07	7.65	5.84	4.32	4.04	4.14	2.05	1.97	3.79	3.77		
<b>11</b>	H <sub>2</sub> O-d <sub>2</sub>		6.07	7.69	5.88	4.32	4.13	4.20	2.13	2.05	4.02	3.97		
<b>10</b>	H <sub>2</sub> O-d <sub>2</sub>		6.04	7.70	6.15	4.37	4.00 <sup>c</sup>	4.00 <sup>c</sup>	2.06	2.03	3.80	3.77		

<sup>a</sup> Assignments of H(5') and H(5'') signals in 5'-cyano- and homonucleosides as for normal nucleosides.

<sup>b</sup> Spectrum run in DMSO-d<sub>6</sub>/H<sub>2</sub>O-d<sub>2</sub> demonstrated disappearance of exchangeable NH<sub>2</sub> and OH.

<sup>c</sup> Overlapping of signals from two protons.

Table II. Vicinal proton-proton coupling constants (in Hz  $\pm$  0.2 Hz) for uridine and cytidine derivatives, and resulting calculated conformer populations. Conditions as in Table I.

Compd	Solvent	<i>J</i>														Populations (%)						
		5,6	1',2'	2',3'	3',4'	4',5'	4',5''	5',5''	5',6'	5',6''	5'',6'	5'',6''	6',6''	P,5'	P,5''	S	gg	gt	tg	g'g'	g <sup>+</sup>	t
<b>13</b>	H <sub>2</sub> O-d <sub>2</sub>	8.1	4.5	5.4	5.4	2.9	4.5	-12.8	—	—	—	—	—	—	—	45	61	33	6	—	—	—
<b>17</b>	DMSO-d <sub>6</sub>	8.1	5.5	5.5	4.5	4.6	7.6	-17.1	—	—	—	—	—	—	—	55	20	55	25	—	—	—
<b>8</b>	H <sub>2</sub> O-d <sub>2</sub>	8.0	4.2	5.2	6.2	4.7	8.5	-14.5	6.8	7.3	5.7	6.3	-11.2	—	—	35	5	65	30	—	36	36
<b>1</b>	H <sub>2</sub> O-d <sub>2</sub>	7.6	4.0	5.3	6.0	2.8	4.5	-12.8	—	—	—	—	—	—	—	40	63	33	4	—	—	—
5'CMP	H <sub>2</sub> O-d <sub>2</sub>	7.6	4.5 <sup>a</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>	2.6	3.0	-11.9	—	—	—	—	—	—	3.9	50 <sup>a</sup>	80	18	2	81	—	—
<b>5</b>	DMSO-d <sub>6</sub>	7.5	4.5	5.5	5.2	4.4	7.3	-17.1	—	—	—	—	—	—	5.0	46	25	50	25	—	—	—
<b>7</b>	H <sub>2</sub> O-d <sub>2</sub>	7.5	3.5	5.2	6.5	4.6	8.6	-14.5	6.8	7.3	5.7	6.3	-11.2	—	—	35	5	65	30	—	36	36
<b>11</b>	H <sub>2</sub> O-d <sub>2</sub>	7.5	4.1	5.3	5.9	5.3	7.2	-14.5	6.5 <sup>b</sup>	6.5 <sup>b</sup>	5.8 <sup>b</sup>	5.8 <sup>b</sup>	-10.7	7.0	—	41	18	50	32	56	37	34
<b>10</b>	H <sub>2</sub> O-d <sub>2</sub>	7.6	4.8	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	-14.3	6.5	7.0	6.0	6.5	-11.2	—	—	50 <sup>a</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	—	36	36

<sup>a</sup> Values of coupling constants (and of corresponding conformer populations) only approximate because of signal overlapping.

<sup>b</sup> Mean values of  $J(5',6')$ ,  $J(5',6'')$  and  $J(5'',6')$ ,  $J(5'',6'')$ .

<sup>c</sup> Coupling constants (and corresponding conformer populations) not determined because of appreciable signal overlapping.

also displaced upfield by about 0.9 ppm relative to uridine, and the C(5') and C(4') carbons upfield by 40 and 14 ppm, respectively. The sum of the coupling constants  $J(4',5') + J(4',5'')$  is increased by 4.8 Hz, and that of  $J(5',5'')$  by 4.3 Hz (see Table II). This is largely due to replacement of the polar oxygen at C(5') by carbon. Identical modifications in the <sup>1</sup>H NMR spectrum confirm the presence of the 5'-cyano group in 5'-deoxy-5'-cyanocytidine.

The structures of the final homo nucleosides are unequivocally established by the presence of two additional methylene protons coupled with each other, as well as with the protons on adjacent carbons, denoted as C(4') and C(6'), as shown in Tables I and II. A similar additional methylene signal at about 1.80 ppm is observed in the <sup>1</sup>H NMR spectrum of homoadenosine in DMSO-d<sub>6</sub> [7], and at about 2.5 ppm for acetylated 2,6-dichloropurine homoribofuranoside in CHCl<sub>3</sub>-d [5].

The foregoing calls for some comment regarding the NMR evidence for the structure of homothymidine, reported by Etzold *et al.* [6]. The chemical shift of one of the C(2') protons is given as 4.66 ppm, whereas it should be about 2.0–2.5 ppm for each of

the two protons at this carbon. Two different values are listed for the chemical shift of H(3'). The signal for 5'-CH<sub>2</sub> (1.91 ppm) is in the region of absorption of the 5-CH<sub>3</sub> group (1.90 ppm), and also close to the system H(2'), H(2''). Hence, in the absence of the actual spectrum, the data presented do not fully confirm the structure of the compound as homo-2'-deoxythymidine.

## Results and Discussion

### Conformational analysis

**Sugar rings.** Application of the two-state model of Altona and Sundaralingam [15] to analyses of the pentofuranose rings of the various analogues demonstrated that substitution of a cyano or homo group at the 5'-position only minimally affected the sugar conformation. Slightly larger modifications of the conformation of the sugar ring have been reported for homoadenosine [7], but with the equilibrium shifted towards the S conformer, typical of purine nucleosides in general.

**Conformations about the C(4')–C(5') bond.** These were evaluated on the assumption of a dynam-

Table III. <sup>13</sup>C chemical shifts (in ppm vs internal dioxane) for 5'-deoxy-5'-cyanouridine (**8**), 0.01 M in DMSO-d<sub>6</sub> at 30 °C.

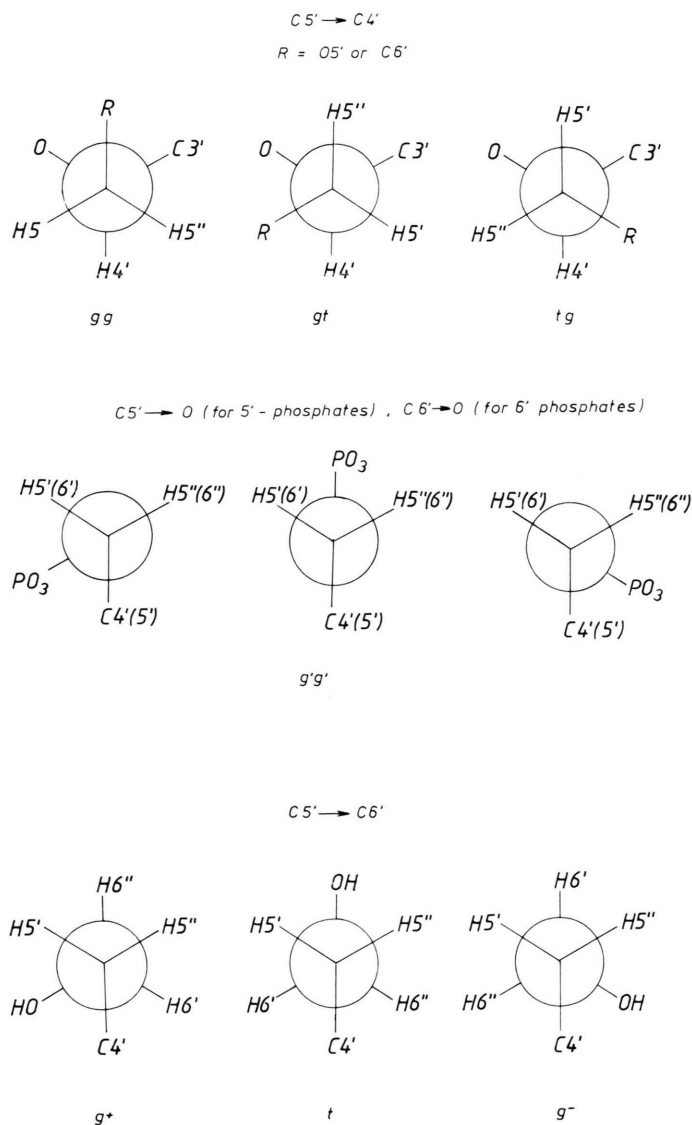
Compd	CH <sub>3</sub>	C(2)	C(4)	C(5)	C(6)	C $\equiv$ N	C(1')	C(2')	C(3')	C(4')	C(5')
<b>8</b>	—	84.20	96.54	35.89	74.72	51.90	22.51	12.20	5.78	5.43	-45.26
CH <sub>3</sub> CN	-65.72	—	—	—	—	52.60	—	—	—	—	—



ic equilibrium between the three classical conformers: *gauche-gauche*, *gauche-trans* and *trans-gauche* (see Scheme 1), with the additional assumption that these deviate  $5^\circ$  from the classical values of the dihedral angles as a result of repulsion between atoms other than hydrogen [16]. The modified Karplus relationship of Haasnoot *et al.* [17] was employed. The results demonstrated a striking decrease in the *gauche-gauche* populations of the 5'-cyano analogues (from about 60% to about 20%), homonucleosides (from about 60% to about 10%) and homonucleotides (from about 80% to about 20%), relative to the

parent nucleosides and nucleotides, where this conformation is preferred (see Table II). For both the foregoing classes of analogues there is a very marked preference for the *gauche-trans* conformation, assuming that assignments of H(5') and H(5'') are the same as in uridine and cytidine. If the reverse assignments were to hold, the predominant conformer would be *trans-gauche*, at the cost of the *gauche-gauche* population, most likely because of interaction of the chain-extended 5'-substituents with the base.

*Conformation about the C(5')–C(6') bond.* This was determined as above, assuming the existence of



Scheme 1. Newman projections along the bonds: Upper: C(5')–C(4'); middle: C(5')–O (for nucleotides), C(6')–O (for homonucleotides); lower: C(5')–C(6').

three classical conformers *gauche*<sup>+</sup>, *trans*, and *gauche*<sup>−</sup> (see Scheme 1). The observed four coupling constants lead to two independent sets of populations (Table II). These differ at most by only 10%, hence reasonably accurate, bearing in mind that the error of the experimentally determined coupling constants is higher than for the other cases because of the small separations between chemical shifts of the four protons, and the large number of couplings in both systems of protons. Rotation about the C(5′)–C(6′) bond is relatively unhindered, with no clear preference for one conformer, due to absence of steric hindrance, hence of intramolecular interactions which lead to a decrease in the *gauche-gauche* population about the C(4′)–C(5′) bond.

**Conformation of phosphate group.** The *gauche'-gauche'* population about the C(5′)–O and C(6′)–O bonds was determined from the coupling constants <sup>31</sup>P–<sup>1</sup>H, with the aid of the newly reported Karplus relationship [18]. The *gauche'-gauche'* population, preferred to the extent of 80% in 5′-CMP, decreases to about 60% in the homonucleotides, and is still the preferred conformation.

### Biological Results

Under conditions where 5′-CMP was rapidly dephosphorylated by purified snake venom 5′-nucleotidase, homoCMP was fully resistant, even with a 3-fold prolongation of incubation time. It was only a feeble inhibitor of the enzyme, with *K*<sub>i</sub> ~ 200 μM at pH 9. By contrast, homoCDP was a relatively effective inhibitor, with *K*<sub>i</sub> ~ 6 μM, hence almost as potent as ADP [19].

Homocytidine was a poor substrate for *E. coli* cytidine deaminase, being deaminated at about 1% the rate for cytidine. When added to a solution of cytidine at 0.3 equimolar concentration, the rate of deamination of the cytidine was not detectably affected.

Homouridine proved to be a good substrate for *E. coli* uridine phosphorylase [20] (*K*<sub>m</sub> = 0.6 mM, *V*<sub>max</sub> = 190 μM/min/mg protein) as compared to uridine, *K*<sub>m</sub> = 0.13 mM, *V*<sub>max</sub> = 250 μM/min/mg protein. Relevant to this is the suggestion, based on indirect evidence, that the homoribonucleoside of 6-mercaptopurine is cleaved by mammalian purine nucleoside phosphorylase [5].

Homocytidine was an excellent substrate for wheat shoot phosphotransferase, from which the 6′-phosphate could be isolated on a preparative scale in

crystalline form in 55% yield (see Experimental Section).

Under conditions where CDP was readily polymerized to poly(C) by *M. luteus* polynucleotide phosphorylase [21], homoCDP was inactive as a substrate, either alone, or when used with an equimolar concentration of UDP in attempts to synthesize a copolymer.

**Cytotoxicities** of homocytidine, homouridine, homoCMP and homoaraC were tested against the following cell lines: HL-60, K-562, U-937 and human LY-PHA. None of the foregoing compounds exhibited detectable activity at concentrations up to 500 μg/ml. By contrast, the 5′-deoxy-5′-cyano derivatives of cytidine and uridine exhibited feeble activity (ID<sub>50</sub> = 50–100 μg/ml), probably due to the inherent instability of these compounds in slightly alkaline medium, leading to cleavage of the glycosidic bond and formation of a reactive sugar species [9], most likely responsible for the low observed cytotoxicity.

**Antiviral activity.** The potential activities of homocytidine, homouridine and homoaraC were examined against herpes simplex virus type 1. In KB-infected cells, none of the foregoing significantly inhibited viral replication at concentrations up to 20 μM.

### Experimental Section

Melting points (uncorr.) were measured on a Boetius (Leipzig, GDR) microscope hot stage. Elemental analyses were performed by the Institute of Organic Chemistry, Polish Academy of Sciences.

<sup>1</sup>H NMR spectra were run on a Bruker 270 AM spectrometer; chemical shifts in H<sub>2</sub>O-d<sub>2</sub> were measured *vs* internal TSP (2,2,3,3-tetradeutero-3-trimethylsilyl-propionate), and in DMSO-d<sub>6</sub> *vs* internal TMS (tetramethylsilane). <sup>13</sup>C spectra were recorded on a Bruker 500 AM instrument operating at 125.77 MHz, with chemical shifts *vs* internal dioxane (see Tables for further details).

UV absorption spectra were recorded on a Zeiss (Leipzig, GDR) Specord UV-VIS, and IR absorption spectra on a Specord 75 IR (1% in KBr pellet).

Dowex resins (200–400 mesh) were from BioRad (Richmond, CA, USA) and Serva (Heidelberg, GFR), and silica gel 60 for column chromatography from Macherey & Nagle (GFR). An LKB 2070 Ultrac II fraction collector, recording at 254 nm, was employed for column chromatography.

Preparative silica gel plates were prepared using Merck (Darmstadt, GFR) silica gel 60 F<sub>254</sub>. Thin-layer chromatography made use of Merck silica gel 60 F<sub>254</sub> and cellulose F<sub>254</sub> plates. The following solvent systems were employed (v/v): (A) MeOH:CHCl<sub>3</sub>, 1:9, (B) MeOH:CHCl<sub>3</sub>, 1:4, (C) MeOH:CHCl<sub>3</sub>, 1:5, (D) 1 M ammonium acetate:96% EtOH, 2:5, (E) *n*-BuOH:96% EtOH:H<sub>2</sub>O, 80:10:25, (F) *n*-PrOH:H<sub>2</sub>O:EtOAc, 1:2:4, upper phase, (G) satur. ammonium sulphate:H<sub>2</sub>O:isoPrOH, 80:20:1, (H) isobutyric acid:25% NH<sub>4</sub>OH:H<sub>2</sub>O, 66:1:33.

Pd/BaSO<sub>4</sub> (10% Pd) was from Merck-Schuchardt (GFR), and *p*-nitrophenylphosphate, *p*-toluenesulphonic acid and *p*-toluenesulphonylchloride from Merck. Anhydrous pyridine was prepared by distillation and stored over 4 Å molecular sieves. DMF was purified by distillation with H<sub>2</sub>O and benzene, followed by distillation under reduced pressure and stored over 4 Å molecular sieves. All other reagents and solvents were of analytical grade unless otherwise specified.

Tetraethylammonium cyanide was prepared from the corresponding bromide and potassium cyanide, essentially according to Kobler *et al.* [8] in 62% yield, with use of boron trifluoride ethyl etherate in place of the recommended methyl etherate.

5'-nucleotidase (EC 3.1.3.5) from snake venom was obtained from Sigma (St. Louis, MO., USA). Enzyme activity vs homoCMP and CMP was followed by TLC, and the inhibition constants by a continuous fluorimetric assay procedure, with formycin-5'-phosphate as substrate, as elsewhere described [19].

Uridine phosphorylase (EC 2.4.2.3) was a highly purified, homogenous preparation from *E. coli* BB [20]. Enzyme activity was followed spectrophotometrically [20].

Cytidine deaminase (EC 3.5.4.5). The source of enzyme was a partially purified extract from *E. coli* BB, totally free of uridine phosphorylase. Deamination was followed spectrophotometrically according to standard procedures [22].

Nucleoside phosphotransferase (EC 2.7.1.77) was isolated from wheat shoots and employed for phosphorylation of nucleosides as elsewhere described [13].

Cytotoxicities of compounds were evaluated by Dr. J. A. Vilpo, Dept. of Clinical Chemistry, University

of Oulu, Finland, according to published procedures [23].

Activities vs *herpes simplex type 1* were evaluated by Dr. Y.-C. Cheng, University of North Carolina, according to published procedures [24].

2',3'-*O*-Isopropylidenecytidine (**2**). To a suspension of 12.2 g (50 mmol) of **1** in a mixture of 100 ml acetone and 100 ml 2,2-dimethoxypropane was added 500 ml acetone, followed by 11.4 g (60 mmol) of *p*-toluenesulphonic acid monohydrate. The mixture was stirred overnight at room temperature, 200 ml water added and, after 15 min, the pH was brought to neutrality with conc. NH<sub>4</sub>OH. The reaction mixture was concentrated under reduced pressure to 1/4 volume and deposited on a 3.5 × 25 cm column of Dowex 1 × 4 (OH<sup>-</sup>). The column was washed with 500 ml water, and the product then eluted with 50% MeOH (750 ml). The eluate was brought to dryness, and the resulting oil dissolved in anhydrous EtOH and brought to dryness twice from anhydrous EtOH. The resulting white amorphous solid, dried over P<sub>2</sub>O<sub>5</sub>, amounted to 14.2 g (100%) and was chromatographically homogenous with *R*<sub>f</sub> on silica gel in solvent B 0.49 as compared to 0.07 for **1**.

2',3'-*O*-Isopropylidene-5'-*O*-tosylcytidine (**3**). This was prepared from **2** essentially as described for the corresponding adenosine analogue by Hollmann and Schlimme [7]. The product (*R*<sub>f</sub> on silica gel with solvent A 0.35, as compared to 0.15 for **2**), containing traces of impurities, was used in the next step without further purification.

5'-*O*-Tosylcytidine (**4**). The product from the preceding step (**3**) was treated with 200 ml of 95% HCOOH at room temperature. The reaction, monitored by TLC on silica gel with solvent B, was complete in 4 h. The mixture was brought to dryness under reduced pressure and successively brought to dryness from H<sub>2</sub>O, aqueous EtOH and MeOH. The resulting oil was taken up in 25 ml MeOH and deposited on 21 silica gel PLC plates. The plates were developed twice with solvent C, and the major UV-absorbing band eluted with anhydrous MeOH. The eluate was brought to dryness to yield 6.5 g (32% relative to **2**) of **4**, chromatographically homogeneous (*R*<sub>f</sub> = 0.12 with solvent A and 0.36 with solvent F).

5'-Deoxy-5'-cyanocytidine (**5**). To 4.11 g (26.3 mmol) tetraethylammonium cyanide (dried at 110 °C, and then under vacuum over P<sub>2</sub>O<sub>5</sub>) in a tightly closed flask was rapidly added 35 ml DMF and the

mixture stirred for 5 min at room temperature, followed by addition of 3.5 g (8.75 mmol) of **4**. Stirring was continued and progress of the reaction monitored by TLC with solvents B and F. The reaction was terminated after 2 h by addition of 25 ml of 20% AcOH, and the mixture purged with air overnight to remove HCN (AgNO<sub>3</sub> test negative: *caution*, under a good hood) and deposited on a 2.5 × 50 cm of column of Dowex 50W × 8 (H<sup>+</sup>). Washing with water removed two poorly separated peaks (2500 OD<sub>262</sub><sup>pH2</sup> and 550 OD<sub>262</sub><sup>pH2</sup> units, respectively, not identified). The column was then eluted with a linear gradient of 0–1 M HCl (2.5 × 2.5 l), with collection of 19-ml fractions. The first peak (fractions 178–195, 3250 OD<sub>280</sub><sup>pH2</sup> units, not identified) was discarded. The second (fractions 200–231, 55,500 OD<sub>280</sub><sup>pH2</sup> units, 49% relative to **4**) was brought to dryness thrice from H<sub>2</sub>O and twice from MeOH to yield **5** as a white, amorphous powder, contaminated with 5–10% unidentified UV-absorbing material, and used in the subsequent step as such. Final elution with 2.5 M HCl in 50% i-PrOH gave 30,000 OD<sub>279</sub><sup>pH2</sup> units of unreacted **4** (26% of starting amount).

An analytical sample of **5** was prepared as follows: 30,000 OD<sub>280</sub><sup>pH2</sup> units of **5** from another run was deposited on a 4.4 × 22 cm column of Dowex 1 × 4 (HCO<sub>3</sub><sup>−</sup>) and eluted with water, with collection of 18-ml fractions. The first, small, peak was discarded. The second (fractions 31–57) was brought to dryness under reduced pressure and crystallized from 96% EtOH to yield 390 mg (70%, dried over P<sub>2</sub>O<sub>5</sub>) of **5** in the form of small needles, m.p. 188–190 °C (dec.), *R*<sub>f</sub> on silica gel 0.05 with solvent A and 0.24 with solvent F, *R*<sub>f</sub> on cellulose 0.65 with solvent D and 0.30 with solvent E. Elem. anal.: Calculated for C<sub>10</sub>H<sub>12</sub>N<sub>4</sub>O<sub>4</sub>: C, 47.62%; H, 4.79%; N, 22.22%. Found: C, 47.68%; H, 4.76%; N, 22.13%.

*1-(5',6'-Dideoxy-6'-amino-β-D-allofuranosyl)cytosine (6)*. To a solution of 55,000 OD<sub>280</sub><sup>pH2</sup> units of **5** in 100 ml MeOH was added a suspension of 1.2 g freshly reduced 10% Pd/BaSO<sub>4</sub> in 100 ml MeOH. The stirred mixture was acidified with 10 ml conc. HCl and purged with hydrogen at atmospheric pressure, while monitoring progress of the reaction on cellulose with solvent D. After 24 h catalyst was removed by filtration, the filtrate supplemented with 100 ml water, and MeOH removed under reduced pressure below 30 °C. Water was added to a total volume of 200 ml and the mixture applied to a 1 × 20 cm column of Dowex 50W × 8 (H<sup>+</sup>). The col-

umn was washed with 1.2 l 1 M HCl to remove unreacted **5** and several minor peaks. Subsequent elution with 2.5 M HCl in 50% i-PrOH gave a single peak (420 ml, 25,000 OD<sub>280</sub><sup>pH2</sup> units, 45% yield) of **6**. Two evaporations from H<sub>2</sub>O and one from EtOH, led to a pale yellow foam, chromatographically homogeneous (*R*<sub>f</sub> = 0.40 on cellulose with solvent D). Attempts at crystallization were unsuccessful, and the product was used as such in the subsequent step.

*1-(5'-Deoxy-β-D-allofuranosyl)cytosine (7, homocytidine)*. To a solution of 25,000 OD<sub>280</sub><sup>pH2</sup> units of **6**, from the preceding step, in 20 ml H<sub>2</sub>O was added 6 ml AcOH, followed by 12 ml 1 N NaNO<sub>2</sub>. After 1 h at 37 °C the mixture was brought to dryness under reduced pressure. The residue was taken up in 20 ml H<sub>2</sub>O, the pH adjusted to 6 with NH<sub>4</sub>OH, and the solution applied to a 1 × 20 cm column of Dowex 50W × 8 (H<sup>+</sup>). The column was washed with H<sub>2</sub>O to remove 2300 OD<sub>263</sub><sup>pH2</sup> units (unidentified). Elution with a linear gradient of 0–1 M HCl (2.5 l), with collection of 20-ml fractions at a flow rate of 4 ml/min, gave a peak at 0.55 M HCl (fractions 75–97, 12,500 OD<sub>280</sub><sup>pH2</sup> units). These fractions were pooled, brought to dryness, and dried twice from H<sub>2</sub>O and twice again from MeOH. The final residue was crystallized from 96% EtOH to yield 215 mg (38%) of **7** · HCl in the form of small rods, m.p. 182–184 °C. On cellulose, *R*<sub>f</sub> = 0.60 with solvent D and 0.23 with solvent E. UV: λ<sub>max</sub><sup>pH 2</sup> 280 nm (ε<sub>max</sub> = 13.5 × 10<sup>3</sup>), p*K*<sub>a</sub> 4.2. NMR data, see Tables I and II. Elem. anal.: Calc. for C<sub>10</sub>H<sub>15</sub>N<sub>3</sub>O<sub>5</sub>: C, 40.89%; H, 5.49%; N, 14.31%. Found: C, 41.03%; H, 5.54%; N, 14.39%.

*1-(5'-Deoxy-β-D-allofuranosyl)uracil (8, homouridine)*. Water-eluted fractions from the column in the preceding step (combined from several runs) were pooled, brought to dryness, the residue taken up in the minimal quantity of MeOH and applied to PLC silica gel plates. Development twice with 15% MeOH in CHCl<sub>3</sub> gave two major bands. The less mobile one was eluted with MeOH, the eluate brought to dryness, the residue taken up in anhydrous MeOH and passed through a G4 glass filter. The filtrate was brought to dryness and the residue crystallized from anhydrous EtOH to give pure **8** in the form of needles, m.p. 154–157 °C, *R*<sub>f</sub> on cellulose 0.35 with solvent E and 0.59 with solvent G; on silica gel, 0.26 with solvent B and 0.40 with solvent F (uridine = 0.48). UV: λ<sub>max</sub><sup>pH 2</sup> 262 nm. NMR data, see Tables I and II. Elem. anal.: Calc. for C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>6</sub>: C,



46.51%; H, 5.43%; N, 10.86%. Found: C, 46.66%; H, 5.48%; N, 10.86%.

**2,2'-Anhydrohomocytidine (9).** To a suspension of 150 mg (0.5 mmol) of **7** in 12 ml EtOAc was added 1.5 ml partially hydrolyzed POCl<sub>3</sub> (POCl<sub>3</sub>:H<sub>2</sub>O, 5:1; v/v). The mixture was stirred at 60 °C for 2.3 h, during which  $\lambda_{\text{max}}$  at pH 2 shifted to 267 nm. The solution was cooled, crushed ice added and stirring continued for 30 min. EtOAc was then removed under reduced pressure, water added to a total volume of 100 ml, and the solution applied to a 3 × 26 cm column of Dowex 50W × 8 (H<sup>+</sup>). The column was washed with water, then eluted with a linear gradient of 0–1.5 M HCl (4 l). Subsequent elution with 2 M HCl (0.5 l) gave a single peak (2850 OD<sub>263.5</sub><sup>pH2</sup> units), which was brought to dryness and evaporated 3 × from H<sub>2</sub>O to yield a pale yellow amorphous film, used as such for the next step. It was chromatographically homogeneous (on cellulose with solvent D  $R_f$  = 0.70), with  $\lambda_{\text{max}}^{\text{pH } 2}$  263.5 nm, characteristic for 2,2'-anhydrocytidine.

**Arahomocytidine (10).** The product **9**, from the preceding step, was taken up in 2 ml H<sub>2</sub>O and slowly passed through a small column (14 ml) of Dowex 1 × 4 (HCO<sub>3</sub><sup>−</sup>). Elution with water gave a mixture of **9** and **10**, the pooled fractions of which were reduced to small volume, brought to pH ~ 11 with NH<sub>4</sub>OH, and stored at room temperature for 1 h. The mixture was brought to dryness, taken up in water and insoluble matter filtered off. The filtrate was brought to dryness, the residue taken up in MeOH and filtered, the filtrate brought to dryness, and the residue crystallized from MeOH to give 37 mg of white, irregular plates, m.p. 222–224 °C (dec.). A second crop yielded 11 mg (total yield 37% with respect to **7**). The product was chromatographically homogeneous on cellulose,  $R_f$  0.32 with solvent E, and 0.64 with solvent D ( $R_f$  0.60 for **7**). NMR data, see Tables I and II.

**Homocytidine-6'-phosphate (11).** To 6900 OD<sub>280</sub><sup>pH2</sup> units (0.53 mmol) of **7** in 15 ml of wheat shoot extract was added 2.78 g (7.5 mmol) *p*-nitrophenylphosphate, and the mixture stirred for 4 h at 37 °C. The mixture was brought to room temperature, extracted with Et<sub>2</sub>O, and the aqueous phase freed of Et<sub>2</sub>O under reduced pressure. It was then applied to a 3 × 23 cm column of Dowex 1 × 4 (HCOO<sup>−</sup>). A water wash removed unreacted nucleoside. Elution with a linear gradient of 0–1.5 M HCOOH (3 l) led to appearance of the product at about 0.5 M

HCOOH. The appropriate fractions were pooled, brought to dryness 3 × from H<sub>2</sub>O, the residue dissolved in hot water, and EtOH added to turbidity. Cooling to room temperature afforded an oil, which crystallized overnight in the refrigerator to yield 100 mg (56%) of **11** in the form of fine needles, m.p. 212–213 °C (dec.). On cellulose,  $R_f$  was 0.06 with solvent D and 0.73 with solvent F. NMR data, see Tables I and II. On treatment with alkaline phosphatase, the product was converted quantitatively to **7**.

**Homocytidine-6'-pyrophosphate (12).** A small sample (17 mg, 0.05 mmol) of **11** was converted to the triethylammonium salt, and treated with 1,1'-carbonyldiimidazole and triethylammonium phosphate under anhydrous conditions, as described by Hoard and Ott [14]. The product was isolated on a 2 × 20 cm column of DEAE-Sephadex A-25 (HCO<sub>3</sub><sup>−</sup>), which was first washed with H<sub>2</sub>O, and then eluted with a linear gradient of 0–0.6 M Et<sub>3</sub>N bicarbonate (1 l). The third, symmetrical, peak (230 OD<sub>280</sub><sup>pH2</sup> units, 34% yield), eluted at 0.45 m. The fractions were pooled and evaporated several times from H<sub>2</sub>O and aqueous EtOH to remove the carbonate salt. The product was dissolved in 1.5 ml MeOH, converted to the sodium salt with NaI, precipitated with acetone, washed several times with acetone and dried under reduced pressure over P<sub>2</sub>O<sub>5</sub> to give 7 mg of **12** as a white powder. On cellulose, with solvent H,  $R_f$  was identical with that of CDP (0.08).

**2',3'-O-Isopropylideneuridine (14).** This was prepared from uridine in 82% yield by the procedure of Hampton [25].

**2',3'-O-Isopropylidene-5'-O-tosyluridine (15).** Prepared in 77% yield from **14** according to the procedure of Kuhn [26].

**5'-O-Tosyluridine (16).** A solution of 15 g (34.2 mmol) of **15** in 300 ml 97% HCOOH was stored for 2.5 h at room temperature. The solution was brought to dryness, and the residual colorless oil taken up in EtOH/*i*-PrOH/H<sub>2</sub>O (1:1:2, v/v) and brought to dryness. The white residue was crystallized from 96% EtOH to yield 10.4 g (76%) of **16** in the form of small rods, m.p. 166–168 °C. On silica gel,  $R_f$  0.42 with solvent B and 0.80 with solvent F.

**5'-Deoxy-5'-cyanouridine (17).** Following treatment of 660 mg (1.65 mmol) of **16** with 770 mg (5 mmol) tetraethylammonium cyanide (as described for **5**, above) for 5 h, the reaction was terminated by

addition of 10 ml of 10% AcOH. HCN was removed *in vacuo* (caution: use good hood) and water and DMF under reduced pressure (oil pump). The residual oil was taken up in 2 ml MeOH and deposited on two PLC plates, which were developed twice with solvent B. The product was eluted with anhydrous MeOH to yield 6000 OD<sub>262</sub><sup>PH<sub>2</sub></sup> units (36%). An analytical sample was obtained by two crystallizations from MeOH, in the form of needles, m.p. 184–188 °C. On silica gel, *R<sub>f</sub>* 0.65 with solvent F; on cellulose, *R<sub>f</sub>* 0.59 with solvent D. IR (KBr matrix), 2250 cm<sup>-1</sup> (C≡N). NMR data, see Tables I, II and III.

*1-(5',6'-Dideoxy-6'-amino-β-D-allofuranosyl)uracil (18)*. As described for **6**, above, 150 mg (0.58 mmol) of **17** was reduced with hydrogen for 28 h. The catalyst was removed by centrifugation, H<sub>2</sub>O added to the supernatant, and MeOH removed *in vacuo*. Water was added to a total volume of 100 ml and the mixture applied to a 1.4 × 29 cm column of Dowex 50W × 8 (H<sup>+</sup>). The column was washed with H<sub>2</sub>O, leading to removal of 3100 OD<sub>261</sub><sup>PH<sub>2</sub></sup> units, followed by a linear gradient of 0–1 M HCl. Fractions containing the product (at ~0.6 M HCl) were pooled, brought to dryness 2 × from H<sub>2</sub>O and 2 × from aqueous EtOH to yield 2600 OD<sub>262</sub><sup>PH<sub>2</sub></sup> units

(43%) of the desired product as a colorless amorphous solid. On cellulose, *R<sub>f</sub>* 0.44 with solvent D and 0.80 with solvent G.

*1-(5'-Deoxy-β-D-allofuranosyl)uracil (8, homouridine)*. Deamination of **18** with NaNO<sub>2</sub> in AcOH, followed by PLC on silica gel plates with solvent B, gave **8** in 62% yield, with analytical properties identical to those of the product obtained by complete deamination of **6**, above.

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