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SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF 6-VINYLURIDINE AND RELATED COMPOUNDS

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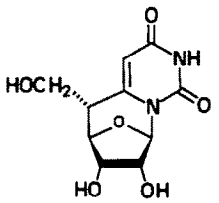
Abstract: The 6-alkenyluracil nucleosides (**7a-e**) and bases (**14a-i**) were synthesized and evaluated for their ability to inhibit the growth of mouse leukemia L1210 cells *in vitro*. While several of the free bases are cytotoxic, with IC_{50} values in the 1 - 5 μM range, the corresponding β-D-ribofuranosyl nucleosides are not significantly active.

Although the first 6-alkenyluracils were reported[2] over twenty years ago, compounds of this type have received relatively little attention, presumably because no particular type of biological activity has been associated with them. Indeed, several 6-alkenyl acyclothymidines were shown in a recent study[3] to be devoid of activity in an HIV screen. On the other hand, an observation that we made during the routine cytotoxicity testing of the 5′(*S*)- and 5′(*R*)-hydroxymethyl-5′,6′-cyclouridines [4] **1** and **2** suggested the possibility that 6-alkenyl nucleosides and bases might exhibit antitumor activity. The 5′(*S*) isomer **1** is not significantly active against mouse L1210 leukemia cells growing in culture, but in some determinations the 5′(*R*) isomer **2** did appear to have marginal activity. However, closer examination revealed that the observed activity was due to the presence of small amounts of the elimination product **3** rather than to **2** itself.[4] When alkene **3** was



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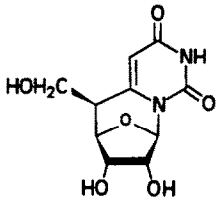

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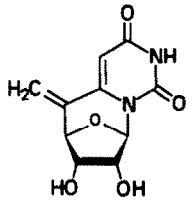

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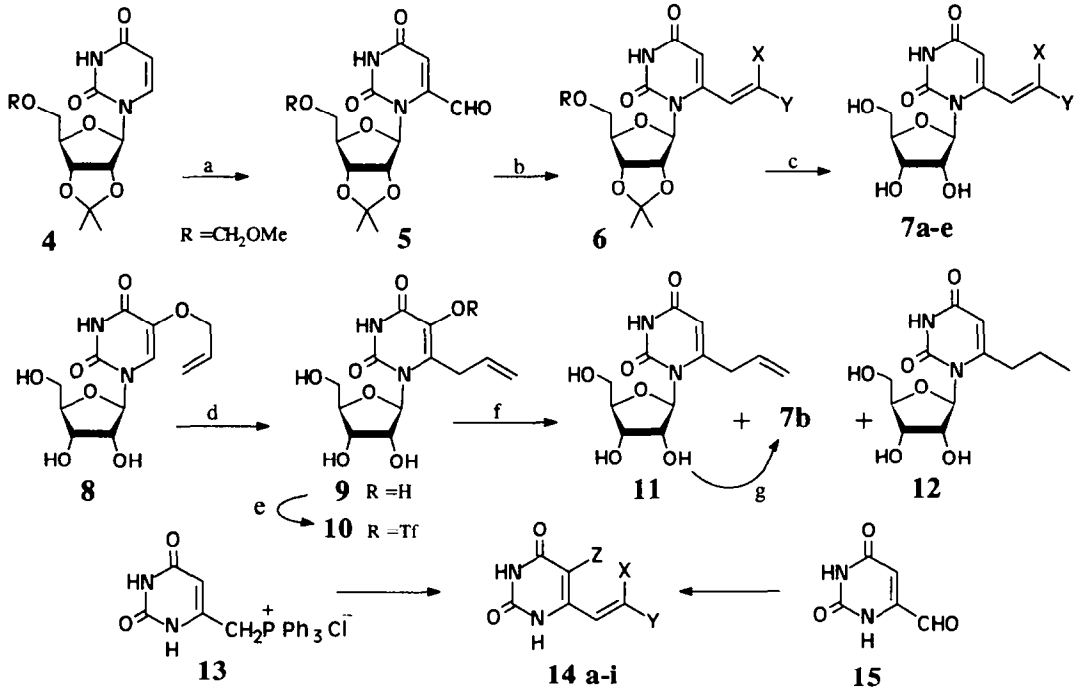
deliberately synthesized[5] and tested in the same screen it was found to be significantly active with an IC_{50} of about 5 μM, but an accurate value could not be obtained because the compound, being a rigid *cis*-diene, dimerizes in Diels-Alder fashion with remarkable ease to give a non-toxic product, and it does so under the mild aqueous conditions of the *in vitro* assay. In order to address the question as to whether more flexible and presumably more stable compounds containing the 6-vinyluracil substructure might also exhibit growth inhibitory activity, we have synthesized a series of 6-alkenyl pyrimidine nucleosides (**7**) and bases (**14**) as shown in the scheme. Furthermore, since **3** readily undergoes Michael-type addition of thiols,[5] we considered the possibility that other 6-alkenyl pyrimidines might function as alkylating agents rather than antimetabolites.

Prior to the present work, only a single 6-alkenyluridine — the *E*-ethoxycarbonyl nucleoside **7c** (X = H, Y = COOEt) — had been reported,[2] but very recently Palmisano and Santagostino[6] have described the synthesis of some additional blocked derivatives, including compound **6** (X = Y = H), *via* an Sn-Pd transmetalation coupling process. We had already prepared **6** *via* the blocked 6-formyluridine **5**, a compound that Tanaka *et al*[7] prepared by quenching the 3,6-bis(lithio) species of **4** with ethyl formate. Aldehyde **5** was not isolated in the original work[7] because of its presumed instability, but we have found that the compound can be obtained in pure form in 72% yield.[8,9] A Wittig reaction between **5** and $H_2C{=}PPh_3$ afforded **6** (X = Y = H) in 40% yield, and removal of the protecting groups gave 6-vinyluridine[12] (**7a**) in 74% yield. Similar reactions of **5** with the appropriate resonance-stabilized ylides and deblocking led to *E/Z* mixtures of **7d** (X = Me, Y = COOEt) and **7e** (X = Br, Y = COOMe) in comparable yields, but the reaction between **5** and $H_3CHC{=}PPh_3$ did not proceed satisfactorily. We have therefore prepared the 6-(1-propenyl) nucleoside **7b** (X = H, Y = Me) *via* the alternative route from 5-allyloxyuridine[13] (**8**) as shown in the scheme. The key step in this sequence is the Pd(0)-catalyzed reduction of the triflate **10** to give an approximately 3:1:1 mixture of **11**, **7b** and **12**, which can be readily separated by preparative reversed-phase HPLC. Isomerization of **11** to **7b** can also be effected in essentially quantitative yield by treatment with refluxing sodium methoxide.

Some of the 6-alkenyluracil bases corresponding to those of the nucleosides **7** were already known from an earlier study,[2] and the others were prepared using analogous Wittig reactions. For example, reaction of the phosphonium salt **13** with sodium ethoxide and acetaldehyde affords the HPLC-separable **14e** and **14f**,



Reagents: **a)** LDA, HCOOEt, -70°; **b)** Wittig reagents, MeCN, 0°; **c)** 50% TFA, rt; **d)** H_2O, reflux; **e)** K_2CO_3, $(CF_3SO_2)_2NPh$, dioxane-water, rt (ref 14); **f)** $[(Ph)_3P]_4Pd$, LiCl, Bu_3SnH, THF, reflux (ref 15); **g)** NaOMe-MeOH, reflux.

whereas reaction of aldehyde **15** with the appropriate ylides affords **14h** and **14i** as *E/Z* mixtures. Also, since 6-vinyluracil (**14a**) is known[2] to undergo smooth bromination at C-5, we prepared the 5-chloro (**14b**) and 5-iodo (**14d**) compounds, using *N*-chlorosuccinimide in acetic acid and ICl in water, respectively.

As shown in the following table, the parent compound, 6-vinyluracil (**14a**), is moderately effective at inhibiting the growth of mouse L1210 leukemia cells *in vitro*.[16] Incorporation of a halogen substituent at the 5-position (**14b-d**) markedly increases the rate at which these compounds react with thiols, but does not produce a corresponding increase in activity. Extending the chain length (**14e,f**) or incorporating a terminal ethoxycarbonyl group (**14g**) renders the compounds essentially inactive, but growth inhibitory activity can be restored by introducing a bromo or chloro substituent at the 2-position of the alkenyl chain (**14h-i**). It is interesting to note that whereas **14a-d** undergo nucleophilic attack by thiols at their terminal carbon atoms, **14h-i** probably undergo attack at the carbon atom adjacent to the pyrimidine ring.

***In Vitro* Growth Inhibition of Mouse L1210 Leukemia Cells by Certain 6-Vinyluridines (7) and 6-Vinyluracils (14)**

Cmpd.	X	Y	IC_{50} (μM)	Cmpd.	Z	X	Y	IC_{50} (μM)
7a	H	H	12	**14a**•	H	H	H	3
				14b	Cl	H	H	4.5
				14c•	Br	H	H	11
				14d	I	H	H	2
7b	H	Me	>35	**14e**	H	H	Me	>90
				14f	H	Me	H	46
7c•	H	COOEt	>35	**14g**•	H	H	COOEt	38
7d$^+$	Me	COOEt	>30					
7e$^+$	Br	COOMe	>25	**14h**$^+$	H	Br	COOEt	1
				14i$^+$	H	Cl	COOEt	3

• ref 2, $^+$ *E/Z* mixture, with one isomer predominating (eg **7e** is 4.5:1), but since they are non-separable by TLC and HPLC, assignments have not been made. Ara-C IC_{50} is 0.05 μM.

In contrast to the results obtained with the free bases, none of the nucleosides **7a-e** is markedly cytotoxic towards L1210 cells. Whether this lack of activity means that the nucleosides are not transported into cells efficiently, or whether the free bases simply interact with targets that are not available to the nucleosides is not known at the present time. As expected, compounds **7** and **14** appear to be much more stable than cyclonucleoside **3**. However, 6-vinyluridine (**7a**) does undergo a partial loss of the vinyl group on prolonged storage in aqueous solution, as shown by a shift of the UV absorption of diluted samples from 276 nm to shorter wavelengths. This could reflect an internal addition of the 5′- or 2′-hydroxyl group to the double bond, or it could mean that the compound undergoes a Diels-Alder dimerization. Molecular modeling (HyperChem™, MM^+ force field) suggests that **7a** conformers in which the vinyl group is orientated *cis* with

respect to the C5-C6 double bond, and which can potentially dimerize, are considerably lower in energy than the *trans* conformers. For 6-vinyluracil (**14a**), which shows no tendency to dimerize, the *trans* form is the most stable. Studies of the structure of the **7a** decomposition product are currently underway, as are attempts to maximize the cytotoxic activity of 6-alkenyluracils by determining the SAR of some new derivatives.

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References and Notes.

(1) Present address: American Health Foundation, Valhalla, NY., 10595.

(2) Klein, R. S.; Fox, J. J. *J. Org. Chem.* **1972**, *37*, 4381.

(3) Tanaka, H.; Baba, M.; Hayakawa, H.; Sakamaki, T.; Miyasaka, T.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R.; Balzarini, J.; De Clerq, E. *J. Med. Chem.* **1991**, *34*, 349.

(4) Sasson, I. M.; Otter, B. A. *J. Org. Chem.* **1981**, *46*, 1114. Alkene **3** is formed as a minor side product during the deblocking of the 2',3'-*O*-isopropylidene precursor of **2** with MeOH-HCl. The 5'(*S*) isomer **1** was prepared by base-catalyzed epimerization of **2**, and was not contaminated with **3**.

(5) Sasson, I. M.; Otter, B. A. Unpublished results.

(6) Palmisano, G.; Santagostino, M. *Tetrahedron* **1993**, *49*, 2533.

(7) Tanaka, H.; Hayakawa, H.; Miyasaka, T. *Tetrahedron* **1982**, *38*, 2635. Groziak et al[10] have shown recently that the supposed "instability" of 6-formyluridines is really a facile hydration reaction.

(8) All new compounds gave satisfactory elemental analyses and were fully characterized by UV and NMR spectroscopy.

(9) ^{1}H-NMR ($CDCl_3$, 200Mz): δ 9.62 (1H, s, CHO), 8.69 (1H, br s, NH), 6.52 (1H, d, H1'), 6.23 (1H, d, H5), 5.15 (1H, dd, H2'), 4.85 (1H, dd, H3'), 4.65 (2H, s, O-CH_2-O), 4.27 (1H, 6-line m, H4'), 3.76 and 3.70 (2H, 7-line m, H5', H5"), 3.36 (3H, s, OMe), 1.58 and 1.36 (two 3H s, Ip); $J_{1',2'}$ = 2.0, $J_{2',3'}$ = 6.7, $J_{3',4'}$ = 4.7, $J_{4',5'}$ = 4.3, $J_{4',5''}$ = 7.4, $J_{5',5''}$ = 10.5, $J_{5,NH}$ = 2.3 Hz. Like other 6-formyluridines[10], and 6-formyluracil (**15**) itself,[11] the aldehyde group of **5** readily undergoes hydration, so that in DMSO-d_6 solution the *gem*-diol structure (δ 7.10, 2H, d, $CH(OH)_2$; δ 5.58, 1H, t, $CH(OH)_2$, J = 5.4 Hz.) and the non-hydrated form are present in an approximately 1:1 ratio. UV (MeOH) λ_{max} 261 nm (hemiacetal); UV (MeCN) λ_{max} 295nm (aldehyde).

(10) Groziak, M.P; Koohang, A. *J. Org. Chem.* **1992**, *57*, 940.

(11) Otter, B. A.; Falco, E. A; Fox, J. J. *J. Org. Chem.* **1978**, *43*, 481.

(12) ^{1}H-NMR (D_2O, 40 °C): δ 6.72 (1H, dd, C**H**=CH_2), 5.98 (1H, dd, *trans* =CH_2), 5.91 (1H, s, H5), 5.76 (1H, dd, *cis* =CH_2), 5.70 (1H, d, H1'), 4.74 (1H, dd, H2'), 4.30 (1H, t, H3'), 3.97 and 3.89 (2H, m, H4' overlapping H5'), 3.77 (1H, dd, H5"); J_{cis} = 11.0, J_{trans} = 17.0, J_{gem} = 0.8, $J_{1',2'}$ = 3.8, $J_{2',3'}$ = $J_{3',4'}$ = 6.2, $J_{4',5'}$ = 2.7, $J_{4',5''}$ = 6.3, $J_{5',5''}$ = 12.2 Hz.

(13) Otter, B. A.; Taube, A.; Fox, J. J. *J. Org. Chem.* **1971**, *36*, 1251.

(14) Crisp, G. T.; Flynn, B. L. *Tetrahedron Lett.* **1990**, *31*, 1347.

(15) Scott, W. J.; Stille, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 3033.

(16) *In vitro* cytotoxicities were determined in triplicate relative to untreated controls using a standard XTT assay, with known compounds as positive controls: see Scudieri, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827.

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