

Communications to the Editor

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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF 5-SUBSTITUTED
6-PHENYLTHIO AND 6-IODOURIDINES, A NEW CLASS OF ANTILEUKEMIC NUCLEOSIDES

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A new class of 5,6-disubstituted uridines, in which the C-6 position was occupied by phenylthio group or iodine, were synthesized via lithiation of the corresponding 5-substituted 2',3'-O-isopropylidene-5'-O-methoxymethyluridines and subsequent electrophilic reactions. These newly-synthesized uridine derivatives exhibited antileukemic activities against mouse leukemia L5178Y cells in culture.

KEYWORDS— lithiation; LDA; 6-phenylthiouridine derivative; 6-iodouridine derivative; 5,6-disubstituted uridine derivative; mouse leukemia L5178Y; antileukemic activity

Recently, we reported the synthesis of various types of 6-substituted uridines based on the regiospecific lithiation of 2',3'-O-isopropylidene-5'-O-methoxymethyluridine (1).¹⁾ Among these 6-substituted uridines, 6-phenylthiouridine (2) exhibited weak activity against mouse leukemia L5178Y cells in culture (ED₅₀ 70 µg/ml). On the other hand, we found that 2 and its 5'-phosphate were highly susceptible to nucleophilic addition-elimination reactions with sulfur nucleophiles even under very mild conditions.²⁾ Given 2's chemical and biological behaviors, one would predict that uridine derivatives bearing a leaving group at the C-6 position would react irreversibly with biological nucleophiles, and thus would manifest cytotoxic activity. In fact, the result of assay of 6-iodouridine³⁾ (3: ED₅₀ 8 µg/ml) was promising for further investigations. In this communication, as an extension of our preliminary study, synthesis and biological evaluation of some 5-substituted 6-phenylthio and 6-iodouridines are described.

Examination of the literature revealed that the classical condensation method was unsuccessful in synthesizing such a type of nucleoside, presumably due to the steric hindrance imposed by the C-6 substituent.⁴⁾ The only exception is the preparation of 6-fluorothymine glycosides.⁵⁾ Thus, in the synthesis reported here, we utilized our recent methodology¹⁾ to overcome this problem.

5-Substituted 2',3'-O-isopropylidene-5'-O-methoxymethyluridine (4ave)⁶⁾ were prepared in high yields by the acetal exchange of dimethoxymethane with the corresponding 2',3'-O-isopropylidene derivatives in the presence of acetone and a catalytic amount of methanesulfonic acid. Lithiation of 4 was carried out with 2.5 eq of LDA in THF below -70°C to give a clear solution of the dilithio derivative.

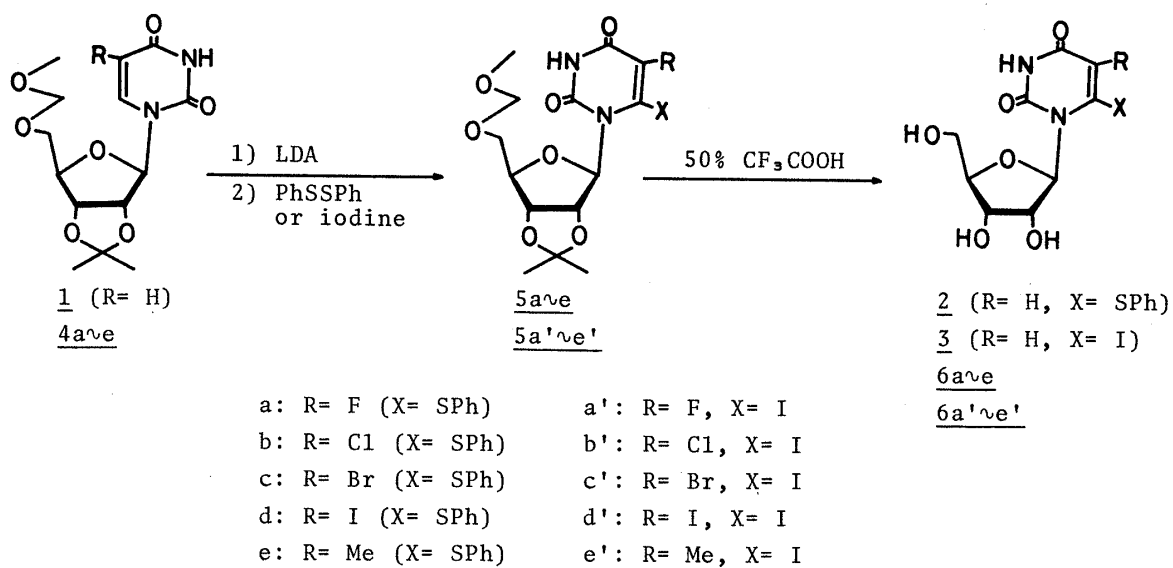


Table I

	yield(%) of <u>5</u>		yield(%) of <u>6</u>
<u>5a</u>	100	<u>6a</u>	85
<u>5a'</u>	92	<u>6a'</u>	86
<u>5b</u>	91	<u>6b</u>	79
<u>5b'</u>	94	<u>6b'</u>	81
<u>5c</u>	97	<u>6c</u>	83
<u>5c'</u>	87	<u>6c'</u>	51
<u>5d</u>	85	<u>6d</u>	76
<u>5d'</u>	88	<u>6d'</u>	46
<u>5e</u>	38	<u>6e</u>	99
<u>5e'</u>	28	<u>6e'</u>	100

Yields were not optimized, and those of 6c' and 6d' can certainly be improved.

Table II

	ED ₅₀ (μg/ml) against L5178Y
<u>6a</u>	30
<u>6a'</u>	0.02
<u>6b</u>	8
<u>6b'</u>	55
<u>6c</u>	20
<u>6c'</u>	52
<u>6d</u>	40
<u>6d'</u>	55
<u>6e</u>	60
<u>6e'</u>	4
<u>2</u>	70
<u>3</u>	8
FUR	0.01
FU	0.1

Subsequent addition of diphenyl disulfide or iodine (2.0 eq) to the anion solution furnished the 6-phenylthio (5a_{ve}) or 6-iodo (5a'_{ve}') derivatives after quenching with AcOH, followed by chromatographic purification on a silica gel column. In the cases of 5e and 5e', where the electron-donating effect of the methyl group can participate in decreasing the acidity of H-6, the extent of metalation through an "acid-base mechanism"⁷⁾ seems to be reduced, as shown in Table I. The structures of 5a_{ve}⁸⁾ and 5a'_{ve}'⁹⁾ were confirmed by MS and PMR spectra. The by-products derived from aryne formation, nucleophilic attack by the lithiating agent, or halogen-lithium exchange were hardly detected during the lithiation and subsequent electrophilic reactions. As pointed out by Cushley and his co-workers,¹⁰⁾ the anomeric proton of the 5-fluorouridine derivative 4a appeared as a double doublet due to a long-range coupling between it and the fluorine atom. In contrast to 4a, 6-substituted 5-fluorouridine analogues 5a and 5a' were void of such an extra splitting in the anomeric signals. This observation could be attributed to the glycosidic "syn-conformation" of these compounds vs. "anti-conformation" of 4a. That is, while H-1' of 4a lies in a favorable geometrical arrangement (zig-zag pattern), those of 5a and 5a' do not.

Concurrent deprotection of the isopropylidene and methoxymethyl groups in 5a_{ve} 5a'_{ve}' was successfully accomplished with 50% aqueous trifluoroacetic acid at room temperature to give the requisite free nucleosides 6a_{ve} and 6a'_{ve}'.¹¹⁾

The newly-synthesized compounds were tested for their cytotoxic effectiveness against mouse leukemia L5178Y cells in culture, and the results are shown in Table II in terms of their ED₅₀ (μg/ml). 5-Fluorouracil (FU) and 5-fluorouridine (FUR) are also included for comparison. As we intended, all the compounds are found to be more or less toxic to mouse leukemia cells. Above all, 5-fluoro-6-iodouridine (6a') is five times more potent than 5-fluorouracil and two times less potent than 5-fluorouridine. Since 6-iodouridine (3) itself showed significant effectiveness, it seems likely that the mechanism of action of 6a' is quite different from those of 5-fluorouracil and 5-fluorouridine. Thus, although no well-defined relationships between structures and activities have been obtained at present, it must be emphasized that our studies have disclosed a new class of antileukemic substances in the nucleoside field. Further work is in progress to elucidate the mechanism of action of the compounds involved in the present investigation.

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- 2) H. Tanaka, S. Iijima, A. Matsuda, H. Hayakawa, T. Miyasaka, and T. Ueda, *Chem. Pharm. Bull.*, **31**, 1222 (1983).
- 3) Compound 3 was obtained as crystals (mp 130~132°C) from 1 by the same reaction sequences as the preparation of 2 and gave physical data consistent with its structure.

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- 6) Compounds 4a_{ve} were obtained as crystals and gave physical data consistent with their structures. Melting points of 4a_{ve}: 4a mp 126~127°C; 4b mp 109~111°C; 4c mp 134~135°C; 4d mp 146~148°C; 4e mp 109~110°C.
- 7) H. W. Gschwend and H. R. Rodriguez, "Organic Reactions," Vol. 26, ed. by W. G. Dauben, John Wiley and Sons, Inc., New York, Chichester, Brisbane, and Toronto, 1979, pp. 1~360.
- 8) Compounds 5a_{ve} were obtained as syrups and gave physical data consistent with their structures.
- 9) Compounds 5a'_{ve} were obtained as crystals and gave physical data consistent with their structures. Melting points of 5a'_{ve}: 5a' mp 204~205°C; 5b' mp 189~190°C; 5c' mp 198~199°C; 5d' mp 167.5~169.5°C; 5e' mp 197.5~198.5°C.
- 10) R. J. Cushley, I. Wempen, and J. J. Fox, *J. Am. Chem. Soc.*, 90, 709 (1968).
- 11) Compounds 6a_{ve} and 6a'_{ve} were obtained as crystals and gave physical data consistent with their structures. Melting points of 6a_{ve} and 6a'_{ve}: 6a mp 88~95°C; 6b mp >300°C; 6c mp 255~260°C (dec.); 6d mp 104~107°C; 6e mp 162.5~164.5°C; 6a' mp 163~166°C (resolidified at 170°C, decomp. at 208°C); 6b' mp 160~162°C (resolidified at 165°C, decomp. at 255°C); 6c' mp 164~166°C (resolidified at 168°C, decomp. at 175°C); 6d' mp 144~146°C (resolidified at 150°C, decomp. at 180°C); 6e' mp 133~136°C.

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