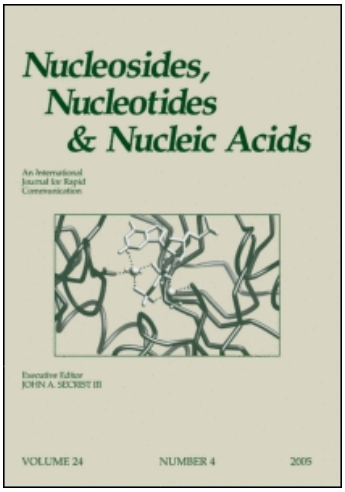
This article was downloaded by:
On: *27 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Murzne P388 Antitumor Activity of Uridine Mustard: A Preliminary Report

Abdol H. Talebian[a]; Philip S. Schein[a]; Dianna C. Green[a]
[a] Department of Chemistry and Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington, D.C.

To cite this Article Talebian, Abdol H. , Schein, Philip S. and Green, Dianna C.(1990) 'Synthesis and Murzne P388 Antitumor Activity of Uridine Mustard: A Preliminary Report', Nucleosides, Nucleotides and Nucleic Acids, 9: 5, 721 — 730
To link to this Article: DOI: 10.1080/07328319008045201
URL: http://dx.doi.org/10.1080/07328319008045201

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES & NUCLEOTIDES, 9(5), 721-730 (1990)

SYNTHESIS AND MURINE P388 ANTITUMOR ACTIVITY OF URIDINE MUSTARD: A PRELIMINARY REPORT

Abdol H. Talebian*, Philip S. Schein, and Dianna C. Green
Department of Chemistry and Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington, D.C. 20057

ABSTRACT: 5'-[Bis(2-chloroethyl)amino]-5'-deoxy uridine (uridine mustard), compound **5**, was synthesized and characterized by its ^{1}H, ^{13}C, and two-dimensional homonuclear shift correlated (COSY) and two-dimensional heteronuclear correlated NMR spectra. In comparative murine studies, uridine mustard was substantially less leukopenic than the equitherapeutic dose of uracil mustard.

Downloaded At: 08:08 27 January 2011

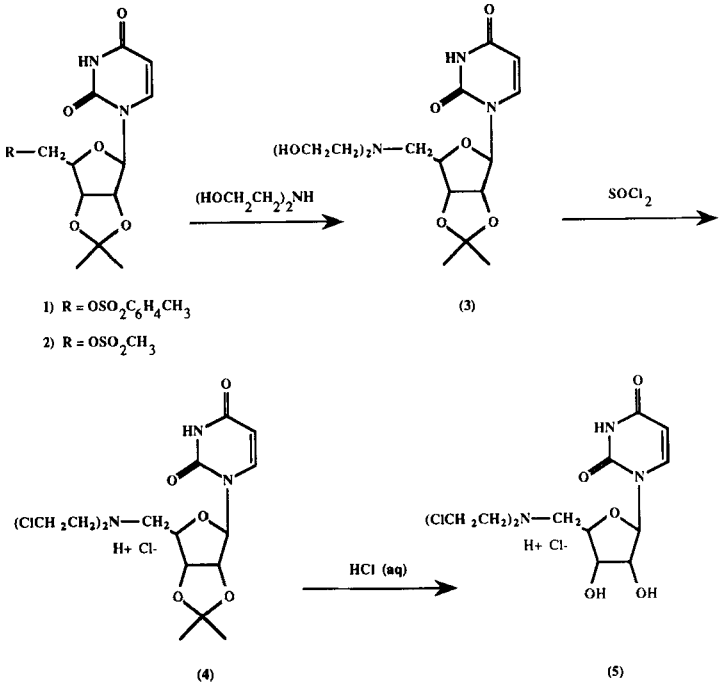
To reduce the undesirable toxic effects of antineoplastic agents, many investigators have attached the cytotoxic group to biologically important molecules such as nucleosides, sugars, and amino acids[1-7]. For example, in studies by Lin et al.[8] a single intraperitoneal (i.p.) injection of 3'-[3-(2-chloroethyl)-3-nitrosoureido]-3'-deoxythymidine (3'-CTNU), a nitrosourea analogue of thymidine, in mice bearing L1210 leukemia produced 60-day survivors. These investigators have also shown that the antitumor activity of 3'-CTNU against L1210 leukemia is enhanced when coadministered with thymidine[9]. Our laboratory has demonstrated that nitrosourea derivatives containing a sugar moiety exhibit significantly decreased bone marrow toxicity[10,11]. In recent studies, we have extended this concept to other alkylating agents. Attachment of a sugar moiety to the bis(2-chloroethyl)amine cytotoxic group results in compounds that retain full murine antitumor activity with decreased hematologic toxicity[12,13].

To determine whether attachment of a nucleic acid to a cytotoxic nitrogen mustard influences the antitumor activity and myelotoxicity, we have synthesized and evaluated 5'-[bis-

Copyright © 1990 by Marcel Dekker, Inc.

(2-chloroethyl)amino]-5'-deoxy uridine (uridine mustard, compound **5**, Scheme I). In this paper, we report the synthesis and murine P388 leukemia antitumor activity of uridine mustard, as well as its acute leukopenic effects, in comparative studies with 5-[bis(2-chloroethyl)amino]uracil (uracil mustard) and nitrogen mustard (HN2).

Scheme I



EXPERIMENTAL

Material and Methods

^{1}H and ^{13}C nuclear magnetic resonance were recorded on a Brucker AM-300 WB at 300.133 and 75.469 MHz, respectively. Chemical shifts are reported in ppm units, using tetramethylsilane as internal standard for compound **3** and sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 for compound **5**. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

Downloaded At: 08:08 27 January 2011

Chemical Synthesis

5'-Deoxy-bis(2-hydroxyethyl)amino-2',3'-isopropylidene uridine 3. Compound **3** was prepared using **1**[14] or **2**[15]. Compound **1** (1.00g) in freshly distilled bis(2-hydroxyethyl)amine (10 ml) was vigorously stirred at 100 °C under a nitrogen atmosphere until TLC showed complete disappearance of tosylate ($CHCl_3$:CH_3OH, 10:1; 80°C when mesylate was used). The mixture was brought to room temperature, after which 5 ml of methanol were added. The resultant solution was passed over a silica column eluted with ethyl acetate and ethyl acetate-acetone(1:1). Solvents were removed on a rotory evaporator under reduced pressure to give 0.58 g pure **3** (68% yield) as a slightly yellow hygroscopic solid. ^{1}HNMR($CDCl_3$): δ 7.24(1H,d,H-6); 5.65(1H,d,H-5); 5.46 (1H,s,H-1'); 4.98(1H,d,H-2'); 4.72(1H,t,H-3'); 4.08(1H,q,H4'); 3.72(2H,dt,OH); 3.53(4H,bs,$HOCH_2$-); 2.85(2H,dd,H-5); 2.69(4H, m,-CH_2N-); 1.48(3H,s,CH_3); 1.27(3H,s,CH_3).

5'-[Bis(2-chloroethyl)amino]-5'-deoxy uridine hydrochloride 5. Compound **3** (0.5g) in chilled (0°C) dry chloroform (10 ml) was treated with thionyl chloride (2-3 ml). The mixture was gradually brought to ambient temperature, stirred for one hour and then refluxed for 20 minutes with the exclusion of moisture. This mixture was cooled to room temperature, and evaporated to dryness to remove chloroform and excess thionyl chloride. The remaining solid residue, crude 5'-[bis(2-chloroethyl) amino]-5'-deoxy-2',3'-isopropylidene uridine (**4**), was partitioned between chloroform and water. The water layer was backwashed with 2x 10 ml of chloroform. The aqueous solution was lyophilized to give 0.47 g (71% yield) pure **5** as a pale yellow hygroscopic solid which decomposed at 88-90°C (sealed tube). ^{1}HNMR(D_2O): δ 7.63(1H,d,H-6,J_{5-6}=7.85 Hz); 5.87(1H,d,H5,J_{5-6}=7.85 Hz); 5.74(1H,d,H-1'); 4.49(1H,m,H-2'); 4.39(1H,m,H-4'); 4.24 (1H,m, H-3'); 3.98(4H,m,2xN-CH_2-); 3.61(6H,mm,2x Cl-CH_2- and H-5'). ^{13}CNMR(D_2O): δ 168.76(C=O); 153.91(C=O); 146.01(C-6); 104.99 (C-5); 95.97(C-1'); 79.41(C-4'); 74.66(C-2'); 74.01(C-3'); 58.16 (C-5'); 57.66(Cl-C); 39.75(N-C). Anal. Calcd. for $C_{13}H_{19}Cl_2N_3O_5$.HCl.$5H_2O$: C,31.56; H,6.11; N,8.49. Found: C,31.80; H,6.08; N,8.07.

Downloaded At: 08:08 27 January 2011

Chemical Alkylating Activity

Comparative in vitro chemical alkylating activities for uridine mustard and nitrogen mustard were estimated by reaction with 4-(p-nitrobenzyl)pyridine (NBP), as described in a previous publication[11]. Aliquots of each compound (0.02-1.5 umol dissolved in 0.85% sodium chloride (saline) at 4°C) were added to 1.5 ml of 5% (weight/volume) NBP in acetone. Four ml of 0.025 M acetate buffer, pH 6, were quickly added and the mixture was incubated at 37°C for 2 hours; the reaction was terminated by placing the samples on ice. Two ml of acetone and three ml of ethyl acetate were then added to each tube. This mixture was made alkaline with 1.5 ml of 0.25 N NaOH, vortexed, and centrifuged at 3000 rpm for 15 sec. Absorbance of the ethyl acetate layer at 540 nm was determined, and the relative alkylating activity of uridine mustard was compared to nitrogen mustard, an alkylating agent in clinical use.

Murine Antitumor Activity and Bone Marrow Toxicity

The murine P388 leukemia was used to evaluate antitumor activity, and was maintained by intraperitoneal passage in female DBA/2F_1 mice. Male BALB/C x DBA/2F_1 (CD2F_1) mice, weighing 19-23 grams, were used for all experiments. Each test drug was administered intraperitoneally to groups of five mice on day 1 following implantation of $1x10^6$ P388 cells, as described previously[12]. Uridine mustard was dissolved in saline at 4°C immediately prior to use. Uracil mustard was dissolved in ethyl alcohol, and the solution was added to saline to give a final ethyl alcohol concentration of five percent. Nitrogen mustard, dissolved in saline at 4°C, was used as a comparative standard mustard alkylating agent. Two replicate experiments were performed. The percent increase in life span (%ILS) was calculated as follows:

$$\%ILS = (T-C)/C \times 100$$

where T is the mean survival days of the treated mice and C is the mean survival days of tumored mice receiving the drug vehicle.

Downloaded At: 08:08 27 January 2011

For studies of bone marrow toxicity, peripheral leukocyte (WBC) counts were measured using a 20-ul sample of retro-orbital sinus blood obtained from $CD2F_1$ mice (six per group) on day 4 following intraperitoneal administration of the optimal therapeutic dose of uridine mustard or uracil mustard. This 20-ul sample was added to 9.98 ml of Isoton (Curtin Matheson, Washington, DC) and counted in a Model ZBI Coulter Counter after lysis of red blood cells with Zapoglobin (Curtin Matheson). Mean WBC counts for drug-treated animals were compared with mice that received no treatment or drug vehicle only, as described previously[12].

Results and Discussion

Chemistry

The synthesis of 5'-[bis(2-chloroethyl)amino]-5'-deoxy uridine (**5**) is outlined in Scheme **I**. Treatment of 5'-uridine tosylate (**1**) or 5'-uridine mesylate (**2**) with bis(2-hydroxyethyl)amine at 80-100°C for 2-4 hours produced 5'-[bis(2-hydroxyethyl)amino]-5'-deoxy-2',3'-isopropylidene uridine (**3**). Reaction of compound **3** with thionyl chloride in dry chloroform (0°C, and then at room temperature for 1 hr and refluxed for 20 minutes), yielded 5'-[bis(2-chloroethyl)amino]-5'-deoxy-2',3'-isopropylidene uridine, compound **4**. Exposure of compound **4** to aqueous acid, during the work-up, removed the isopropylidene protecting group and gave the final product, 5'-[bis(2-chloroethyl)amino]-5'-deoxy uridine in 71% yield (see experimental section).

Homonuclear shift correlated 2D NMR (COSY)[16] and heteronuclear correlated 2D NMR[17] spectroscopy on compound **5** established all C-H connectivities and confirmed the proposed structure for uridine mustard (Figure **1**). For complete assignments of the protons and carbons of uridine mustard see experimental section.

Chemical Alkylating Activity

Alkylating activities for uridine mustard and nitrogen mustard were measured by the extent of alkylation of NBP that occurred in 2 hours at 37°C and pH 6. On a molar basis, chemical alkylating activity for uridine mustard was approximately 20% that of nitrogen mustard.

Downloaded At: 08:08 27 January 2011

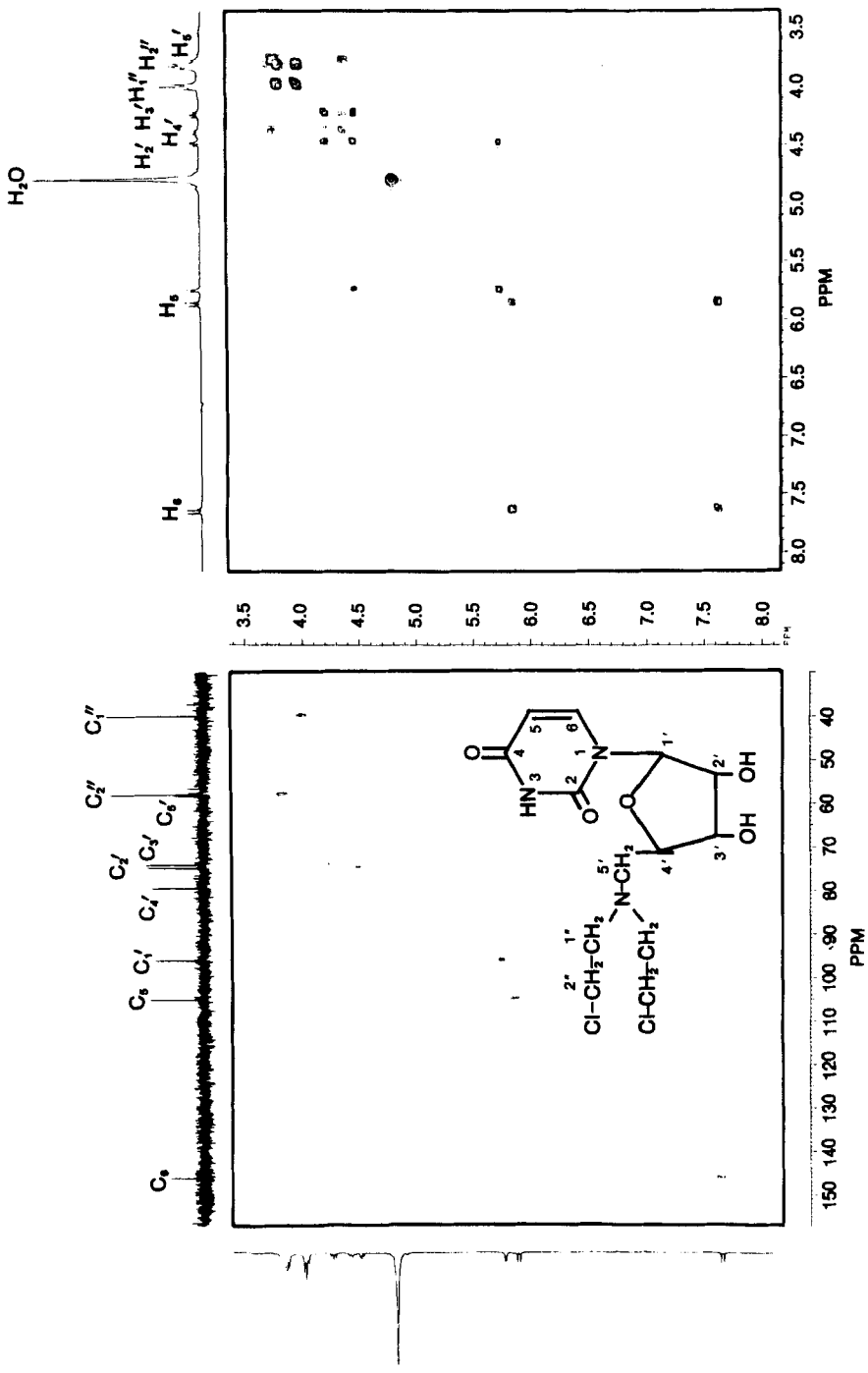


FIGURE 1. Two-dimensional ^{1}H-^{13}C chemical shift correlated and ^{1}H-^{1}H chemical shift correlated (COSY) NMR spectra of uridine mustard, **5**.

Downloaded At: 08:08 27 January 2011

Murine Antitumor Activity and Bone Marrow Toxicity

The murine P388 antitumor activity of uridine mustard was evaluated in comparative studies with uracil mustard and nitrogen mustard, and the results are summarized in Table I. Two doses of uridine mustard, 15 mg/kg and 30 mg/kg, were evaluated. For uracil mustard, Lane et al.[18] reported that a single 4.5 mg/kg dose demonstrated significant antitumor activity in the murine L1210 leukemia system. In dose ranging studies, we have previously determined that the optimal single i.p. dose of nitrogen mustard for P388 leukemia is 2.9 mg/kg[12,13]. Uridine mustard, 15 mg/kg i.p., increased the mean survival time of P388 leukemic mice to 18.0 days, compared to 9.0 days for the vehicle treated tumor bearing mice, a 100% increase in life span (ILS). At the 30 mg/kg dose of uridine mustard, twenty percent of the mice died of drug toxicity, and the mean survival time was 16.0 days (78% ILS). Uracil mustard, 4.5 mg/kg, increased the mean survival time to 17.4 days (93% ILS), while nitrogen mustard, 2.9 mg/kg, produced a mean survival of 14.4 days (60% ILS).

In a structure-activity analysis of chemical and biological parameters of chloroethylnitrosourea alkylating agents in mice, our laboratory has demonstrated a linear relationship between the molar LD_{10} dose and chemical NBP alkylating activity: the greater the alkylating activity, the lower the molar LD_{10}[11]. For the mustard alkylating agents, uridine mustard has approximately 20% of the NBP alkylating activity of nitrogen mustard. In vivo, nitrogen mustard at a dose of 2.9 mg/kg (15.1 umol/kg) is toxic to ten percent of normal $CD2F_1$ mice. In comparison, uridine mustard at 15 mg/kg (30 umol/kg) produced no deaths due to drug toxicity, while a dose of 30 mg/kg (60 umol/kg) is toxic to twenty percent of the mice.

The bone marrow toxic potential of uridine mustard was compared to uracil mustard (Table I). Equitherapeutic doses of uridine mustard (15 mg/kg) and uracil mustard (4.5 mg/kg) were administered intraperitoneally to normal $CD2F_1$ mice, and peripheral leukocyte (WBC) counts were determined on day 4.

Downloaded At: 08:08 27 January 2011

TABLE I. Biological Parameters

Compound	Dose (mg/kg)	P388 %ILS*	WBC Nadir (day 4): % of Control	Deaths due to Drug Toxicity
Uridine M	15	100	74.6	
	30	78		20%
Uracil M	4.5	93	44.1	
Nitrogen M	2.9	60	ND**	10%

*Single dose of drug injected intraperitoneally on day 1 following i.p. implantation of $1X10^6$ P388 cells; ILS= Increase in life span.
**Not Done

Downloaded At: 08:08 27 January 2011

Uridine mustard, 15 mg/kg, reduced the WBC count to 74.6% of control, while the 4.5 mg/kg dose of uracil mustard was significantly more myelotoxic, reducing the WBC count to 44.1% of control. These results with uracil mustard are in agreement with previously published studies of Lane et al.[18].

In summary, the antitumor activity of uridine mustard, 15 mg/kg, for intraperitoneal P388 leukemia (100% ILS) was equivalent to that achieved with a 4.5 mg/kg dose of uracil mustard (93% ILS). Moreover, at these equitherapeutic doses, uridine mustard was substantially less toxic to normal murine bone marrow, as measured by depression of peripheral leukocyte (WBC) counts. Uridine mustard maintained antitumor activity equivalent to uracil mustard, with a concomitant decrease in leukopenia.

ACKNOWLEDGMENT: This investigation was supported in part by NIH Grant 5RO1CA17583.

REFERENCES

1. Lin, T. S.; Fisher, P. H.; Prusoff, W. H. J. Med. Chem. 1980, **23**, 1235-37.

2. Okruszek, A.; Verkade, J. G. J. Med. Chem. 1979, **22**, 882-85.

3. Lin, T. S.; Brubaker, W. F.; Wang, Z. H.; Park, S.; Prusoff, W.H. J. Med. Chem. 1986, **29**, 862-65.

4. Suami, T.; Kato, T.; Takino, H.; Hisamatsu, T. J. Med. Chem. 1982, **25**, 829-32.

5. Tsujihara, K.; Ozeki, M.; Morikawa, T.; Taga, N.; Miyazaki, M.; Kawamori, M.; Arai, Y. Chem. Pharm. Bull. 1981, **29**(11), 3262-73.

6. Gram, H. F.; Mosher, C. W.; Baker, B. R. J. Am. Chem. Soc. 1959, **81**, 3103-08.

7. Rodriguez, M.; Imbach, J. L.; Martinez, J. J. Med. Chem. 1984, **27**, 1222-25.

8. Lin, T. S.; Fisher, P. H.; Marsh, J. C.; Prusoff, W. H. Cancer Res. 1982, **42**, 1624-29.

9. Lin, T. S.; Prusoff, W. H. Cancer Res. 1987, **47**, 394-97.

10. Schein, P.S.; McMenamin, M.G.; Anderson, T. Cancer Res. 1973, **33**, 2005-9.

11. Panasci, L. C.; Green, D.; Nagourney, R.; Fox, P.; Schein, P.S. Cancer Res. 1977, **37**, 2615-18.

12. Cantrell, J. E.; Green, D.; Schein, P. S. Cancer Res. 1986, **46**, 2340-44.

13. Schein, P.S.; Green, D.; Dean, S. W.; McPherson, E. Cancer Res. 1987, **47**, 696-99.

14. Levene, P. A.; Tipson, R. S. J. Biol. Chem. 1934, **106**, 116.

15. Skaric, V.; Katalenic, D.; Skaric, D.; Salaj, I. J. Chem. Soc. Perkin. Trans. **I**. 1982, 2091-97.

Downloaded At: 08:08 27 January 2011

16. Bax, A.; Freeman, R. J. Magn. Reson. 1981, **44**, 542.

17. Wilde, F. A.; Bolton, P. H. J. Magn. Reson. 1984, **59**, 343.

18. Lane, M.; Kelly, M. G. Cancer Res. 1960, **20**, 511-17.

Received January 22, 1990.

Downloaded At: 08:08 27 January 2011