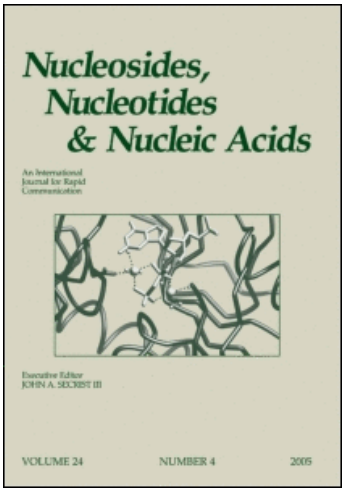


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

AS Triazine Acyclonucleosides: Potential Inhibitors of Pyrimidine Enzymes[1]

Subhasish Purkayastha[a]; Bihi H. Lazrek[a]; Raymond P. Panzica[a]; Fardos N. M. Naguib[b]; Mahmoud H. el Kouni[b]
[a] Departmentss of Chemistry and Medicinal Chemistry, University of Rhode Island, Kingston, Rhode, Island [b] Division of Biology and Medicine Brown University, Providence, Rhode, Island

To cite this Article Purkayastha, Subhasish , Lazrek, Bihi H. , Panzica, Raymond P. , Naguib, Fardos N. M. and Kouni, Mahmoud H. el(1989) 'AS Triazine Acyclonucleosides: Potential Inhibitors of Pyrimidine Enzymes[1]', Nucleosides, Nucleotides and Nucleic Acids, 8: 3, 349 — 356
To link to this Article: DOI: 10.1080/07328318908054180
URL: http://dx.doi.org/10.1080/07328318908054180

NUCLEOSIDES & NUCLEOTIDES, 8(3), 349-356 (1989)

AS TRIAZINE ACYCLONUCLEOSIDES: POTENTIAL INHIBITORS OF PYRIMIDINE ENZYMES[1]

Subhasish Purkayastha, Bihi H. Lazrek[2], and Raymond P. Panzica*

Departments of Chemistry and Medicinal Chemistry
University of Rhode Island, Kingston, Rhode Island, 02881

and

Fardos N. M. Naguib and Mahmoud H. el Kouni

Division of Biology and Medicine
Brown University, Providence, Rhode Island, 02912

Abstract: Seven as-triazine-3,5-dione acyclonucleosides were synthesized and evaluated as inhibitors of orotate phosphoribosyltransferase (OPRTase, EC 2.4.2.10), orotidine 5'-monophosphate decarboxylase (ODCase, EC 4.1.2.23), uridine phosphorylase (UrdPase, EC 2.4.2.3), and thymidine phosphorylase (dThdPase, EC 2.4.2.4).

The preparation of acyclonucleosides has commanded the world-wide attention of many research groups because of their high potential to exhibit chemotherapeutic activity.[3] Recently, benzylacyclouridines, e.g., 5-benzyl-1-[2-hydroxyethoxy)methyl]uracil (BAU) and 5-(3'-benzyloxybenzyl)-1-(2-hydroxyethoxy)methyl]uracil (BBAU), were shown to be potent inhibitors of uridine phosphorylase (UrdPase)[4] and to enhance the efficacy and selective toxicity of 5-fluoro-2'-deoxyuridine (FdUrd).[5] Inhibitors of orotidine 5'-monophosphate decarboxylase (ODCase) have been shown to be useful chemotherapeutic agents in the treatment of neoplastic and certain non-neoplastic diseases.[6] None of these inhibitors are acyclonucleosides, yet two of the most notable that exhibit well-defined clinical utility possess the as-triazine aglycone, i.e., 2-(β-D-ribofuranosyl)-as-triazine-3,5-dione (6-azauridine) and its

2',3',5'-triacetyl derivative, azaribine.[7] With this in mind, we explored the synthesis of certain as-triazine-3,5-dione acyclonucleosides. A desired target of this project was 6-benzylthio-2-[(2-hydroxyethoxy)methyl]-as-triazine-3,5-dione (3a) due to the close structural resemblance to BAU and BBAU.

Synthesis

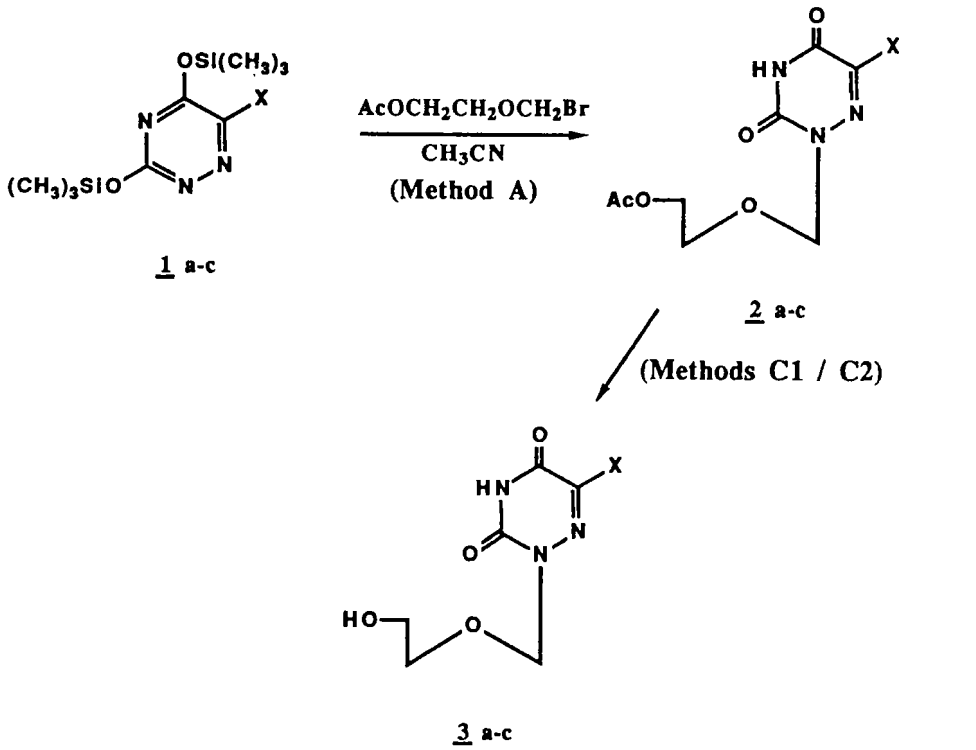
The synthesis of 3a was accomplished by two different routes. The first pathway involved alkylation of 1a, which was prepared by silylating[8] 6-benzylthio-as-triazine-3,5-dione[9] with hexamethyldisilazane, with (2-acetoxyethoxy)methyl bromide[10] in dry acetonitrile to furnish 6-benzylthio-2-[(2-acetoxyethoxy)methyl]-as-triazine-3,5-dione (2a) in 84% yield. The other route leading to 3a entailed nucleophilic displacement of the 6-bromo substituent on 2b with benzyl mercaptan in pyridine. This procedure provided 2a in 51% yield. Deprotection of 2a with methanolic ammonia at room temperature afforded 3a.

With the exception of 4, the remaining two acyclonucleosides, 3b and 3c, were prepared by the first pathway. 6-Amino-2-[(2-hydroxymethoxy)methyl]-as-triazine-3,5-dione (4) was synthesized directly from 2b. Treatment of 2b with liquid ammonia and a catalytic amount of copper powder at 80°C in a steel reaction vessel provided 4 in moderate yield.

The site of alkylation of the acyclonucleosides prepared in this study was established as N2 by UV spectroscopy. The UV spectra of 3c and 4 were identical to their reported ribosylated counterparts.[11-13] It is worth mentioning, that 2c was converted to 2b in the presence of bromine water (1 mL of bromine in 100 mL of distilled water) at 50°C. The reaction was carefully monitored by tlc (chloroform-methanol; 9:1, v/v) and stopped after 20 minutes. After work-up, the product was shown to be identical to 2b (tlc and UV).

Biochemical Evaluation

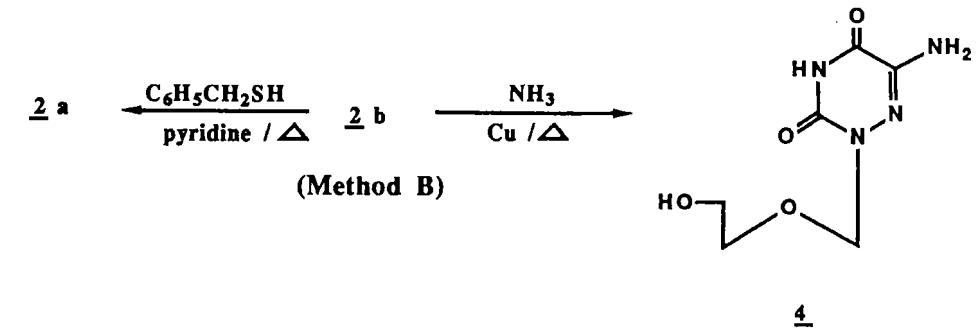
UrdPase, dThdPase, OPRTase, and ODCase were prepared from the 105,000 x g supernatant of mouse liver homogenate and assayed as previously described.[4,14] Unlike BAU, 3a did not inhibit UrdPase. Similarly, no inhibition was observed with either OPRTase, ODCase, or dThdPase. None of the other acyclonucleosides, i.e., 2a-c, 3b, 3c, and 4, inhibit any of these enzymes.



1 a-c

2 a-c

3 a-c



4

a, X = $SCH_2C_6H_5$; b, X = Br; c, X = H

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover melting apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian EM-390 spectrometer and the ultraviolet absorption spectra were recorded with a Beckman DU-7 spectrometer (Table 2). Thin Layer chromatography was run on precoated (0.2 mm) silica gel 60 F-254 plates manufactured by EM Laboratories, Inc., and short-wave ultraviolet light (254 nm) was used to detect the UV-absorbing spots. Silica gel (Merck, 230–400 mesh, 60A) suitable for chromatographic use was employed for column chromatography. All solvent proportions are by volume unless otherwise stated. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

General Procedure for Silyl Alkylation (Method A)

The as-triazine-3,5-diones were silylated using hexamethyldisilazane (HMDS) in the presence of a catalytic amount of trimethylsilyl chloride. The stirred mixture was heated at reflux with the exclusion of moisture for 24 h. Excess HMDS was removed under diminished pressure and the individual oily, silylated heterocycles (1a-c) were dissolved in dry acetronitrile and cooled to 0°C. To this stirred solution was slowly added a solution of (2-acetoxyethoxy)methyl bromide in dry acetonitrile. The stirred reaction mixture was allowed to warm to room temperature and the course of the reaction was monitored by tlc. Alkylation was usually completed in ca. 4h. At this point, the reaction mixture was concentrated in vacuo and the resulting gum was dissolved in a minimal amount of chloroform-methanol (49:1) and applied to a silica gel column. The column was eluted with chloroform-methanol (49:1) and the pure N2-alkylated heterocycles (2a-c) were crystallized from the solvent specified in Table 1.

Nucleophilic displacements (Method B)

6-Benzylthio-2-[2-acetoxyethoxy)methyl]-as-triazine-3,5-dione (2a)

A solution of 2b (292 mg, 0.95 mmol) in pyridine (2 mL) and benzyl mercaptan (2.02 mL, 17.2 mmol) was placed in a glass-lined stainless steel reaction vessel and heated at 120°C for 24 h. After cooling, the vessel was vented and the reaction mixture poured into ethyl acetate (30 mL) and washed (3 x 10 mL) with water. The organic layer was dried

TABLE 1. Preparative Methods for Certain As-Triazine-3,5-dione Acyclonucleosides

Starting Material				Product					
Compound	Amount[a] (g)	Mmol	Method[b]	Compound	Amount (g)	Yield (%)	Mp (°C)	Crystallizing Solvent	Elemental Analyses (±0.4 %)
1a	1.20	5.10	A	2a	1.500	83.7	96-97	95% EtOH	C,H,N, and S
2b	0.292	0.94	B	2a	0.170	51.0	96-97	95% EtOH	
1b	1.00	5.21	A	2b	1.220	76.3	97-99	MeOH	C,H, and N
1c	2.10	18.50	A	2c	2.900	84.0	100-102	$CHCl_3$	C,H, and N
2a	1.00	2.85	C1/C2	3a	0.695	78.9	122-124	CH_2Cl_2	C,H, and N
2b	1.70	5.52	C1/C2	3b	1.400	95.0	135-136	95% EtOH	C,H, and N
2c	1.70	7.41	C1	3c	1.300	93.7	142-143	MeOH	C,H, and N
2b	1.20	3.89	B	4	0.460	58.4	204-205	95% EtOH	C,H, and N

[a]For compounds 1a-c, the weight is that of the heterocycle prior to silylation. [b]A, silyl alkylation; B, nucleophilic displacement; C1, deprotection-methanolic ammonia; C2, deprotection-NaOMe/methanol.

TABLE 2. ^{1}H NMR and Ultraviolet Spectral Data

Compound	^{1}H NMR[a] (δ, ppm)	Ultraviolet Data [λ_{max}, nm (ε)] pH 1	H_2O	pH 11
2a	1.97 (s,3.,$\underline{CH}_3$COO), 3.53-3.80 (m,2,AcOCH$_2\underline{CH}_2$), 3.93-4.13 (m,2,AcO$\underline{CH}_2$-CH$_2$), 4.16(s,2,$\underline{CH}_2C_6H_5$), 5.20 (s,2,O$\underline{CH}_2$N), 7.03-7.47 (m,5,CH$_2\underline{C_6H_5}$)			
2b	2.00 (s,3), 3.66-3.86 (m,2), 4.00-4.23 (m,2), 5.16 (s,2)			
2c	1.96 (s,3), 3.63-3.83 (m,2), 4.00-4.16 (m,2), 5.16 (s,2), 7.46 (s,1,H6)			
3a	3.26[b] (s,1,OH), 3.5 (br s,4,O$\underline{CH}_2\underline{CH}_2$O), 4.16 (s,2,$\underline{CH}_2C_6H_5$), 5.16 (s,2,O$\underline{CH}_2$N), 7.16-7.46 (m,5,O$\underline{CH}_2\underline{C_6H_5}$)	309.5(6342)	309(6311)	315(3527)
3b	3.63 (br s,4), 4.0[b] (br s,1), 5.16 (s,2), 12.46[b] (br s,1,NH)	275(7024)	274(6811)	263.5(6279)
3c[c]	3.36-3.63 (m,4), 3.76[b] (br s,1), 5.20 (s,2), 7.46 (s,1)	260(6494)	260(6644)	253(6551)
4[d]	3.53 (br s,4), 5.03 (s,2), 6.16[b] (s,2,NH$_2$)	296.5(5358)	296.5(5459)	315(1759) 269.5(4326)

[a]All samples were run in DMSO-d_6 and the chemical shifts are expressed in δ units downfield from TMS; br s = broad singlet, s = singlet, m = multiplet. [b]D_2O exchangeable. [c]6-Azauridine (ref.11): UV (λmax) pH1, 262 nm (6761); pH 7, 259 nm (6918); pH 11, 253 nm (7413). 1-(2-deoxy-β-D-glucopyranosyl)-6-azauracil (ref. 12): UV (λmax) pH 1, 259 nm (5495), 95% EtOH, 260 nm (7413), pH 11, 252 nm (6166). [d]5-Amino-6-azauridine (ref. 13): UV (λmax) pH 1-7, 298 nm (5170), pH 13, 290 nm (4300).

over anhydrous sodium sulfate, filtered, and then taken to near-dryness. The resulting residue was coevaporated with toluene (3 x 10 mL) and then applied to a silica gel column (10 g). The column was eluted with chloroform-methanol (99:1) and 25 mL fractions taken. Fractions 6-10 contained the title compound and were pooled and evaporated to furnish pure 2a. This acyclonucleoside was identical (tlc, ^{1}H NMR, and mixture melting point) to 2a prepared by direct alkylation (see Table 1 and 2).

6-Amino-2-[(2-hydroxyethoxy)methyl]-as-triazine-3,5-dione (4)

6-Bromo-2-[(2-acetoxyethoxy)methyl]-as-triazine-3,5-dione (2b, 1.2 g, 3.89 mmol), copper powder (2 mg), and liquid ammonia (10 mL) were heated in a glass-lined steel reaction vessel at 80°C for 24 h. After cooling, the excess ammonia gas was vented off and the residual solid dissolved in methanol-water (1:1, 25 mL). The blue solution was acidified to pH 4 with Amberlite IR-120 (H^+) resin. The resin was removed by filtration and washed with hot methanol-water (1:1, 3 x 10 mL). The combined filtrate and wash was evaporated *in vacuo* to provide a white solid. This material was recrystallized from 95% ethanol to provide pure 4 (see Table 1 and 2).

Deprotection:Method C1

Solutions of 2a and 2c in methanolic ammonia (previously saturated at -5°C) were allowed to stand at room temperature for 24 h in sealed flasks. After removal of the solvent, the resulting gums (3a,c) were recrystallized from the solvents specified in Table 1.

In the case of 2b, the residual gum was covered with ethyl acetate (10 mL) and kept at 4°C for 12 h. The resulting crystalline, ammonium salt was collected by filtration and dissolved in water. The solution was carefully neutralized to pH 6.5 with Amberlite IR-120 (H^+). The resin was filtered off and washed with methanol (3 x 10 mL). The filtrate and wash were combined and evaporated *in vacuo* to dryness. The residual solid was recrystallized from 95% ethanol.

Deprotection:Method C2

This method used sodium methoxide and was identical to the procedure reported by Robins and Hatfield.[10] The yields were comparable to those of Method C1.

ACKNOWLEDGEMENTS

One of us (B.H.L.) would like to thank the Council for International Exchange of Scholars for the opportunity to study in the U.S.A. This work was supported in part by Grants CH-136 of the American Cancer Society and CA 20892 awarded by the National Cancer Institute, N.I.H., DHHS, to the Roger Williams Cancer Center.

REFERENCES

1. Presented in part at the 6th International Round Table on Nucleosides, Nucleotides and Their Biological Applications, La Grand Motte, France, 1984.

2. Fullbright Scholar from the Faculty of Science, University of Cadi Ayyad, Marrakech, Morocco.

3. (a) C. K. Chu and S. J. Cutler, J. Heterocyclic Chem., 23, 289, (1986). (b) R. J. Remy and J. A. Secrist III, Nucleosides Nucleotides, 4, 411 (1985).

4. F. N. M. Naguib, M. H. el Kouni, S. H. Chu, and S. Cha, Biochem. Pharmacol., 36, 2195 (1987) and references cited therein.

5. M. Y. Chu, F. N. M. Naguib, M. H. Iltzsch, M. H. el Kouni, S. H. Chu, S. Cha, and P. Calabresi, Cancer Res., 44, 1852 (1984).

6. T. W. Kensler and D. A. Cooney, Adv. Pharm. Chemother., 18, 274 (1981) and references cited therein.

7. P. Calabresi, C. H. Doolittle, G. H. Heppner, and C. J. McDonald, Ann. N.Y. Acad. Sci., 255, 190 (1975).

8. T. Nishimura, Methods Carbohyd. Chem., 6, 436 (1972).

9. C. Cristescu and T. Panaitescu, Pharmazie, 17, 209 (1962).

10. M. J. Robins and P. W. Hatfield, Can. J. Chem., 60, 547 (1982).

11. K. Y. Zee-Cheng and C. C. Cheng, J. Org. Chem., 27, 976 (1962).

12. G. J. Durr, Syn. Proc. Nucleic Acid Chem., 1, 347 (1968).

13. I. V. Alekseeva, A. S. Shalamai, E. G. Sidorenko, and V. P. Chernetskii, Ukr. Khim. Zh., 45, 48 (1979).

14. M. H. Iltzsch, J. G. Niedzwicki, A. W. Senft, S. Cha, and M. H. el Kouni, Molec. Biochem. Parasitol., 12, 153 (1984).

Received March 29, 1988.