# Synthesis of 4-(2-Acetoxyethoxymethyl)-6-methyl-1,2,4-triazin-3(4*H*)-one 1-Oxide as Thymidine Analogue



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Alkylation of the sodium salt and the trimethyl silylated derivatives of 6-methyl-1,2,4-triazin-3(4*H*)-one 1-oxide with chloromethoxyethyl acetate, n-hexyl chloride and benzyl bromide gave the 4-substituted products. However, attempts to achieve the ring closure of $N^4$-(2-acetoxyethoxymethyl)thiosemicarbazide with bicarbonyl compounds to the corresponding *as*-triazines under different reaction conditions was not possible without disruption of the acetoxyethoxymethyl moiety. Although the *as*-triazine nucleoside analog II did not show antileukemic activity, this and other 4-alkylated *as*-triazine 1-oxides revealed good growth inhibitory effects against a representative spectrum of microorganisms.

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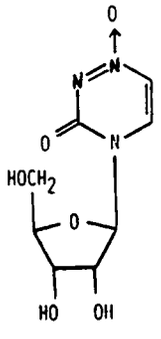
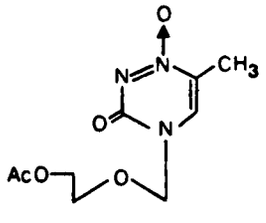
Analogs of the natural pyrimidine nucleosides have shown some success as anticancer agents. Among these, Uricytin, or 4-β-D-ribofuranosyl-1,2,4-triazin-3-one 1-oxide (I) has shown potent antileukemic activity and appears particularly promising for further modification. Uricytin was also claimed to exert its antineoplastic effect by binding preferentially to tumor cell membranes, resulting in antibody production [2]. The mode of binding was believed to be an irreversible reaction with membrane thiol groups [3].

An attempt to verify the mechanism of action of Uricytin was made by the synthesis of the fused ring benzo[*e*]- and pyrido[2,3-*e*]-1,2,4-triazin-3-one 1-oxide derivatives [4]. If thiol binding takes place by addition to $C_3$, in view of Carbon's findings of the action of hydroxyl ion on benzotriazole [5], this could provide compounds of improved binding ability by a thiol binding mechanism. The lack of ability of these fused ring analogs to inhibit P388 lymphocytic leukemia in mice suggested that Uricytin may bind to membrane thiols through its 5,6-position. To pursue this finding further, and determine whether an *as*-triazine 1-oxide analog containing an acyclic carbohydrate moiety binds irreversibly to thiols, the synthesis of 4-(2-acetoxyethoxymethyl)-6-methyl-1,2,4-triazine-3(4*H*)-one 1-oxide (II) and some other 4-substituted analogs was attemped. The acetoxyethoxymethyl moiety was selected as the glycosyl component, since the deacetylated derivative has shown marked success as a ribose simulator in antiviral compounds [6].



I

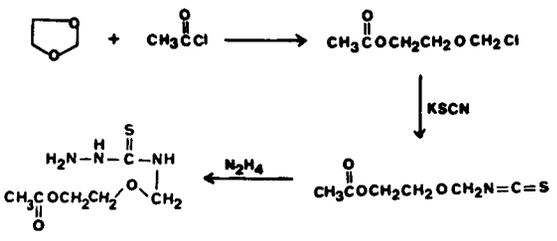


II

## Synthesis.

One method of synthesis involved the preparation of $N^4$-(2-acetoxyethoxymethyl)thiosemicarbazide, which represents the first example of a thiosemicarbazide nucleoside analog, and which should be cyclizable to *as*-triazines by reaction with 1,2-dicarbonyls. Oxidation, in analogy with the literature, should give the triazine 1-oxide. The acetoxyethoxymethyl thiosemicarbazide was prepared in three steps as shown in Scheme I. Chloromethoxyethyl acetate was prepared by modification [4] of the procedure [7] using 1,3-dioxolane and acetyl chloride. The chloro compound was converted to its isothiocyanate through reaction with potassium thiocyanate in refluxing toluene. Under these conditions 2-acetoxyethoxymethyl isothiocyanate was obtained as a marginally stable colorless liquid in fairly good yield. The formation of this compound was confirmed by the appearance of a strong band in the ir spectrum of the neat sample at 2000-2200 $cm^{-1}$, corre-

Scheme I



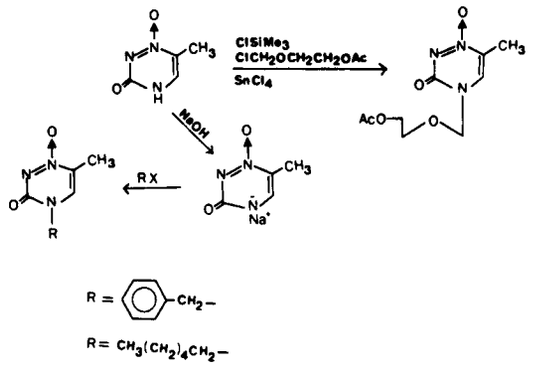
sponding to the -N=C=S stretching mode, and also by a change in the chemical shift of the methylene group adjacent to -N=C=S to about 0.50 ppm upfield relative to $-CH_2Cl$.

2-Acetoxyethoxymethyl isothiocyanate was converted to its thiosemicarbazide through a reaction with hydrazine hydrate at 0° in methanol. The formation of this compound was corroborated by ir and nmr analysis. The most significant $^1H$ nmr peaks for this compound were the acetyl peak (sharp singlet) at 2.05 ppm, the two double doublets at 3.70 and 4.10 ppm, corresponding to the two adjacent methylene groups attached to methoxy and acetoxy groups, respectively, with four non-equivalent hydrogens of the type AA'BB', and a doublet (J = 7 Hz) at 5.03 ppm corresponding to the methylene group next to NH. The peaks for the two NH's were quite distinguishable. One is coupled to its adjacent $CH_2$ and appeared as a broad triplet (J = 7 Hz) at 8.7 ppm, while the other NH showed a broad singlet at 9.25 ppm, before treatment with deuterium oxide.

The ring closure of the corresponding thiosemicarbazide with an appropriate 1,2-dicarbonyl compound, benzil, by the method of Paudler and Lee [8] for the ring closure of $N^4$-substituted thiosemicarbazides, did not result in formation of the desired product. Although the thiosemicarbazone intermediate was obtained in high yield, acid treatment of this compound in the second step resulted in disruption of the acetoxyethoxymethyl moiety, and the product obtained was the 5,6-substituted-1,2,4-triazine-3-thione. The cyclization also failed in basic media, using aqueous alkali or organic bases such as triethylamine and diethylamine. Lewis acids also failed to produce the cyclized product.

An alternative approach involved the preparation of 6-methyl-1,2,4-triazin-3(4*H*)-one 1-oxide [9] and condensation in the 4-position. Substitution of the 2-acetoxyethoxymethyl moiety and other groups such as hexyl and benzyl was then found possible (Scheme II). Since in principle the substitution can take place either on oxygen or nitrogen, two methods were tried. In the first, the 3-oxo group was protected by reaction with chlorotrimethyl silane in hexamethyldisilazine at refluxing temperature. The trimethylsilyloxy derivative was then treated with chloromethoxyethyl acetate in the presence of stannic chloride in dry methylene chloride; II was obtained in 44% yield. In the second procedure, 6-methyl-1,2,4-triazin-3(4*H*)-one 1-oxide was converted to its sodium salt by treatment with aqueous sodium hydroxide. Addition of benzyl bromide or hexyl chloride resulted in formation of substitution products. This procedure was not suitable for the hydrolytically sensitive acetoxyethoxymethyl derivative, however. In both methods the expected 4-substituted product was obtained, as shown by the nmr and ir spectra. A strong band appeared at 1680-1650 $cm^{-1}$ for the carbonyl absorption, which ruled out alkylation on the 3-oxygen.

Scheme II




Biological Activities.

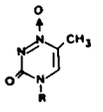
Antimicrobial activities were obtained by the *in vitro* serial tube dilution procedure [10] to give minimal inhibitory concentrations (MIC). The organisms used include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. These organisms represent a Gram-positive and two Gram-negative bacteria, a yeast, and a mold, respectively.

For these triazines, substitution at position 4 is required for antimicrobial activity. The most active compound was found to have the acetoxyethoxymethyl moiety at position 4. This compound inhibited the growth of *E. coli* at $10^{-4}$ *M*. It also showed good activity against *P. aeruginosa* and *A. niger* at $10^{-3}$ *M* (Table I). On comparison of the overall antimicrobial activities of II with that of the standard, 5-fluorodeoxyuridine, a lower MIC was observed for II.

Tests for anticancer activity against P388 leukemia in mice did not show any activity for II at dose levels of 60-240 mg/kg. The test was carried out at the National Cancer Institute in accordance with NCI protocol [11].

Table 1

The Minimum Inhibitory Concentrations of 4-Substituted-6-methyl-1,2,4-triazin-3(4*H*)-one 1-Oxides



| R | Minimum Inhibitory Concentration, M | | | | |
|---|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *P. aeruginosa* | *C. albicans* | *A. niger* |
| (1) Na | – – | – – | – – | – – | – – |
| (2) $CH_3CO_2CH_2CH_2OCH_2$- | – – | $10^{-4}$ | $10^{-3}$ | $10^{-2}$ | $10^{-3}$ |
| (3) $C_6H_5CH_2$- | $10^{-2}$ | – – | – – | $10^{-2}$ | – – |
| (4) $CH_3(CH_2)_4CH_2$- | $10^{-3}$ | – – | – – | $10^{-3}$ | – – |
| (+)5-Fluorodeoxyuridine | $10^{-2}$ | $10^{-3}$ | – – | $10^{-3}$ | $10^{-2}$ |

## EXPERIMENTAL

Melting points were determined in capillaries using a Mel-Temp apparatus and required no correction. The ir spectra were measured with a Perkin-Elmer model 457-A spectrometer. Proton nmr spectra were obtained with a Varian T-60 spectrometer using tetramethylsilane as internal standard. Tlc was carried out using silica gel plates with fluorescent indicator. Elemental analyses were done by MultiChem Laboratories, Lowell, MA.

2-Acetoxyethoxymethyl Isothiocyanate.

To a solution of chloromethoxyethyl acetate (152.5 g, 1 mole) [7] in 300 ml of toluene was added potassium thiocyanate (106.4 g, 1.2 moles). The temperature was raised to reflux for 10 hours. The pale yellow solution was filtered and the filtrate was fractionally distilled to give 106.5 g (61%) of colorless liquid at 93°/0.65 torr; $^1$H nmr (deuteriochloroform): δ 2.05 (s, 3H, $CH_3CO$), 3.80 (m, 2H, $CH_2O$), 4.20 (m, 2H, $AcOCH_2$), 5.0 (s, 2H, $OCH_2N$); ir (neat): 2200-2000 (N=C=S), 1735 (C=O), 1050 (C-O-C) $cm^{-1}$.

*N*$^4$-(2-Acetoxyethoxymethyl)thiosemicarbazide.

To a cold solution of 2-acetoxyethoxymethyl isothiocyanate (17.5 g, 0.1 mole) in 100 ml of methanol was added anhydrous hydrazine (3.2 g, 0.1 mole). The mixture was stirred in an ice bath for 10 minutes. The white precipitate was filtered and dried at 60° *in vacuo*, yielding 1.80 g (85%) of white crystals; mp 110-111°; $^1$H nmr (deuteriochloroform): δ 2.05 (s, 3H, $CH_3CO$), 3.70 (m, 2H, $CH_2O$), 4.15 (m, 2H, $AcOCH_2$), 4.55 (bs, 2H, $NH_2$), 5.03 (d, 2H, $OCH_2N$), 8.73 (bm, 1H, NH), 9.25 (bs, 1H, NH); ir (potassium bromide): 3350-3150 (N-H), 1735 (C=O), 1540 (CNH), 1260 (C=S), 1060 (C-O-C) $cm^{-1}$.

*Anal.* Calcd. for $C_6H_{13}N_3O_3S$: C, 34.77; H, 6.32; N, 20.27. Found: C, 34.62; H, 6.25; N, 20.48.

5,6-Diphenyl-1,2,4-triazin-3(4*H*)-thione.

*N*$^4$-(2-Acetoxyethoxymethyl)thiosemicarbazide (1.04 g, 5.0 mmoles) was treated at reflux temperature with benzil (0.53 g, 2.5 mmoles) dissolved in 20 ml of ethanol. The *N*$^4$-(2-acetoxyethoxymethyl)benzil thiosemicarbazone which formed was collected after 30 minutes and dissolved in 15 ml of acetic acid. The solution was heated under reflux for 2 hours and evaporated to dryness. Recrystallization from acetone gave 0.92 g (70%) of the title compound as yellow crystals, mp 218° (lit [12] mp 220°).

6-Methyl-1,2,4-triazin-3(4*H*)-one 1-Oxide.

A mixture of 3-methoxy-6-methyl-1,2,4-triazine 1-oxide [9] (1.05 g, 5 mmoles) and sodium carbonate (600 mg) in 1:1 methanol-water (16 ml) was stirred at 70-75° in a closed flask for 24 hours. The solution was cooled to room temperature, diluted with water (16 ml), and neutralized with Dowex 50[$H^+$]. The mixture was heated and filtered, and the resin was washed with hot water (5 ml). The combined filtrates were evaporated on a rotary evaporator at 50°, giving 430 mg (96%), mp 250° dec (lit [9] 222°); $^1$H nmr (deuterium oxide): δ 2.30 (s, 3H, $CH_3$), 8.30 (s, 1H, CH).

6-Methyl-1,2,4-triazin-3(4*H*)-one 1-Oxide Sodium Salt Hydrate.

To a solution of sodium hydroxide (0.16 g, 4.0 mmoles) in water (8 ml) was added 6-methyl-1,2,4-triazin-3(4*H*)-one 1-oxide (0.5 g, 4.0 mmoles) with stirring. The yellow solution was evaporated at room temperature at 0.5 torr over sulfuric acid, and the yellow residue was extracted in a Soxhlet with 50 ml of 95% ethanol for 5 hours. The extract was cooled, and the white precipitate which formed was filtered and dried at 80° *in vacuo* for 10 hours, yielding 0.5 g (75%), mp 273° dec; $^1$H nmr (deuteriochloroform): δ 2.30 (s, 3H, $CH_3$), 4.70 (s, 2H, $H_2O$), 8.30 (s, 1H, CH).

4-(2-Acetoxyethoxymethyl)-6-methyl-1,2,4-triazin-3(4*H*)-one 1-Oxide (II).

To a solution of 6-methyl-1,2,4-triazin-3(4*H*)-one 1-oxide (254 mg, 2.0 mmoles) in 10 ml of hexamethyldisilazine was added 0.5 ml of chlorotrimethylsilane with stirring. The mixture was heated at reflux with protection from atmospheric moisture for 14 hours. Dry toluene (10 ml) was added and the solution was filtered and evaporated to dryness in a rotary evaporator. The silylated residue was dissolved in 10 ml of dry methylene chloride and cooled to 0°. A solution of chloromethoxyethyl acetate (182 mg, 1.2 mmoles) in 10 ml of dry methylene chloride was added in small portions and the mixture was stirred at 0° for 1 hour. It was then warmed to room temperature and stirred for 5 hours in which the tlc in chloroform-methanol (9:1) showed the completion of the reaction. The clear solution was cooled to 0° and stannic chloride (0.2 ml, 2.0 mmoles) was added. The mixture was warmed to room temperature, and stirred for 16 hours. The pale yellow solution was diluted with 10 ml of methylene chloride and poured into cold saturated sodium bicarbonate. The resulting emulsion was separated by filtering through Celite, and the filtering aid was washed with 5 ml of methylene chloride. The organic phase was separated and the aqueous phase was extracted further with 20 ml of ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate and evaporated in a rotary evaporator. The residue obtained was triturated with diethyl ether, and the white precipitate was filtered and dried at room temperature *in vacuo*, yielding 160 mg (44%), mp 110-111°; $^1$H nmr (deuteriochloroform): δ 2.05 (s, 3H, $CH_3CO$), 2.25 (s, 3H, $CH_3$), 3.85 (m, 2H, $OCH_2$) 4.20 (m, 2H, $AcOCH_2$), 5.30 (s, 2H, $OCH_2N$), 7.70 (s, 1H, CH); ir (potassium bromide): 3040 (=CH), 2900 (CH), 1740 (C=O), 1660 (C=O amide), 1050 (C-O-C) $cm^{-1}$.

*Anal.* Calcd. for $C_9H_{13}N_3O_5$: C, 44.41; H, 5.38; N, 17.34. Found: C, 44.23; H, 5.39; N, 16.93.

6-Methyl-4-benzyl-1,2,4-triazin-3(4*H*)-one 1-Oxide.

To a solution of 6-methyl-1,2,4-triazin-3(4*H*)-one 1-oxide sodium salt (167 mg, 1.0 mmole) in dimethylformamide (6 ml) was added benzyl bromide (171 mg, 1.0 mmole). The mixture was stirred at 30° overnight in a flask protected from atmospheric moisture. Addition of water (4 ml) gave a precipitate which was filtered and recrystallized from methanol and 1,2-dimethoxyethane. It was dried at 60° *in vacuo*, yielding 170 mg (77%) of white crystals, mp 211-213°; $^1$H nmr: δ 2.20 (s, 3H, $CH_3$), 5.10 (s, 2H, $CH_2$), 7.40 (s, 5H, phenyl), 8.90 (s, 1H, CH); ir (potassium bromide): 3050 (=CH), 1660 (C=O amide), 1430 (CNH) $cm^{-1}$.

*Anal.* Calcd. for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.11; N, 19.34. Found: C, 60.90; H, 5.20; N, 19.23.

6-Methyl-4-hexyl-1,2,4-triazin-3(4*H*)-one 1-Oxide.

To a solution of 6-methyl-1,2,4-triazin-3(4*H*)-one 1-oxide sodium salt (63 mg, 0.5 mmole) in dimethylformamide (3 ml) was added hexyl chloride (60 mg, 0.5 mmole). The mixture was stirred at 30° for 24 hours, tlc on silica gel with ethyl acetate as mobile phase showed a single spot (Rf = 0.34). Addition of cold water (3 ml) gave a white precipitate which was filtered, washed with water, and dried at 60° *in vacuo*, yielding 65 mg (62%), mp 142-144°; $^1$H nmr (deuteriochloroform): δ 0.8-2.0 (m, 11H, $C_5H_{11}$), 2.25 (s, 3H, $CH_3$), 3.85 (t, 2H, $CH_2N$), 7.70 (s, 1H, CH); ir (potassium bromide): 3050 (=CH), 2960-2860 (CH), 1660 (C=O amide), 1430 (CNH), 1380 ($CH_3$) $cm^{-1}$.

*Anal.* Calcd. for $C_{10}H_{17}N_3O_2$: C, 56.85; H, 8.11; N, 19.89. Found: C, 56.65; H, 8.13; N, 19.80.

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