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Registry No. 1, 114200-20-3; 1-HCl, 114200-30-5; 1 (2,4-dinitrobenzenesulfonate salt), 114200-29-2; 2, 114200-21-4; 3, 694-43-9; 4, 1755-04-0; 5, 114200-22-5; 6, 71194-15-5; 7 (isomer 1), 114200-23-6; 7 (isomer 2), 114200-26-9; 8, 114200-24-7; 8·HCl, 114200-28-1; 9, 114200-25-8; 9·HCl, 114200-27-0; PCP, 77-10-1; piperidine, 110-89-4; phenyllithium, 591-51-5; 1,5-dibromopentane, 111-24-0.

Nucleosides of 1,4-Thiazin-3-one and Derivatives as Tetrahedral Intermediate Analogues of Enzymes in Pyrimidine Nucleoside Metabolism

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Reaction of the trimethylsilylated derivative of 1,4-thiazin-3-one with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of $SnCl_4$ gave, after deblocking, 4-β-D-ribofuranosyl-1,4-thiazin-3-one (**8**). Treatment of 1,4-thiazin-3-one with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose in the presence of sodium hydride provided, after deblocking, the corresponding 2-deoxy-β-D-ribofuranosyl derivatives (**19**). Oxidation of 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazin-3-one (**7**) with 1 equiv of *m*-chloroperbenzoic acid resulted in 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazine-2,3-dione (**9**) and 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazin-3-one 1-oxide (**10**). Evidence is presented that indicates that the oxidation of the thiazine at the 2-position is due to a Pummerer rearrangement. The new compounds failed to show significant activity against tumor cell lines in culture, L1210 cells in vivo, virus cytotoxicity in cell culture, or cytidine deaminase.

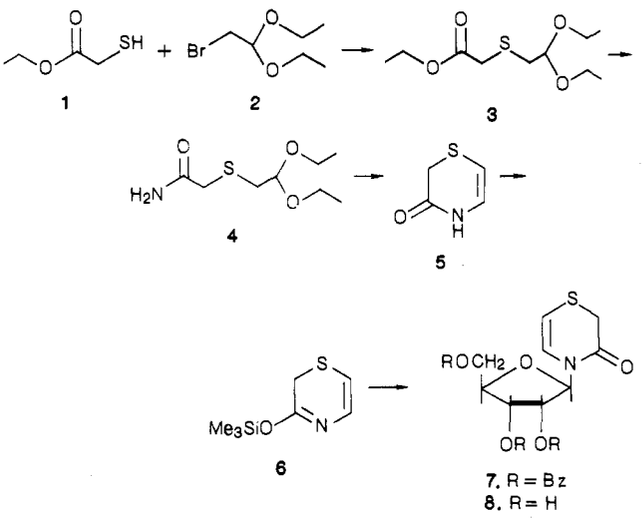
Stable analogues of tetrahedral intermediates formed in the course of enzymic reactions can be potent inhibitors of those enzymes. Classes of enzymes susceptible to inhibition by these analogues are those hydrolases and ligases that catalyze an $sp^2 \rightarrow sp^3 \rightarrow sp^2$ conversion. Among those enzymes in these classes that catalyze this type of transformation at the 4-position of the pyrimidine nucleosides and nucleotides are cytidine deaminase and CTP synthetase. Bartlett and co-workers[1] have shown that a tetrahedral intermediate analogue based on a 4-phosphapyrimidine nucleoside is a potent inhibitor of the former enzyme. The latter enzyme, thought to involve a phosphorylated tetrahedral intermediate in the transition state,[2] should be susceptible to inhibition by a suitable tetrahedral intermediate analogue, in analogy with the inhibition of the ligase glutamine synthetase by the tetrahedral sulfur compound methionine sulfoximine.[3]

As part of a program to prepare pyrimidine nucleoside derivatives with tetrahedral atoms in the 4-position, we have prepared several nucleosides of 1,4-thiazin-3-one, its oxides, and its 5-methyl and 5-carboxy derivatives. Such analogues may inhibit nucleoside-utilizing enzymes (such as cytidine deaminase) directly, or, after in vivo conversion to their triphosphates, inhibit CTP synthetase. Inhibition of this enzyme, known to occur in highly elevated levels in cancer cells,[4] could provide agents of interest in cancer chemotherapy. Additionally, the 5-carboxy congeners are analogues of orotidine.

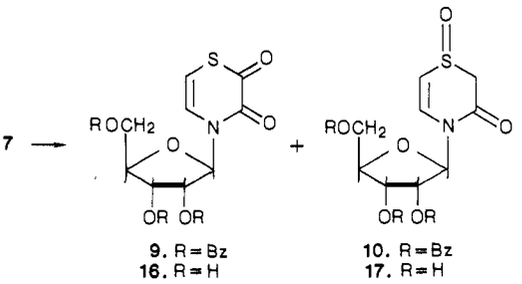
Chemistry

The nucleoside 4-β-D-ribofuranosyl-1,4-thiazin-3-one (**8**) was prepared in good yield in six steps as shown in Scheme I. Treatment of ethyl thioglycolate (**1**) with bromoacetaldehyde diethyl acetal (**2**) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave ethyl 2-[(2,2-diethoxyethyl)thio]acetate (**3**). Ammonolysis of the ester **3** gave

Scheme I



Scheme II



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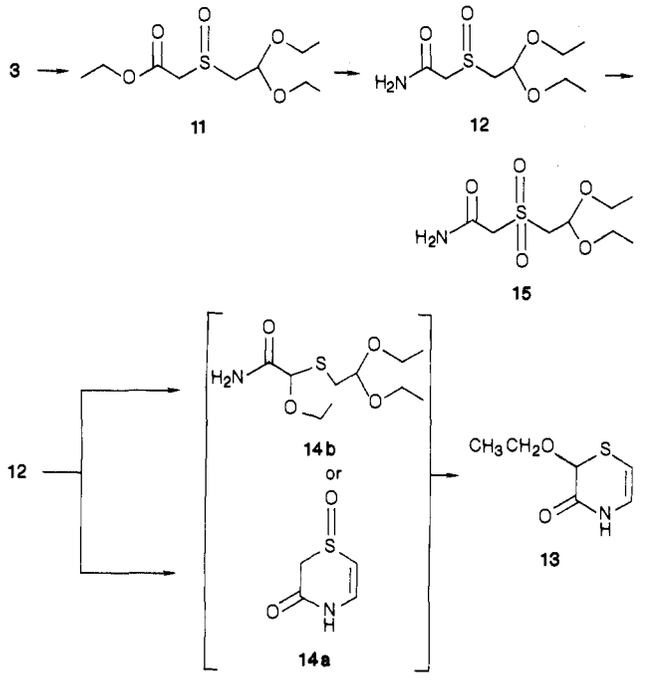
Scheme III



2-[(2,2-diethoxyethyl)thio]acetamide (4).[5] Ring closure of the amide 4 in the presence of a catalytic amount of *p*-toluenesulfonic acid gave 1,4-thiazin-3-one (**5**).[5] Treatment of the thiazine **5** with hexamethyldisilazane in the presence of trimethylchlorosilane (catalytic amount) gave the 3-[(trimethylsilyl)oxy]-1,4-thiazine (**6**).

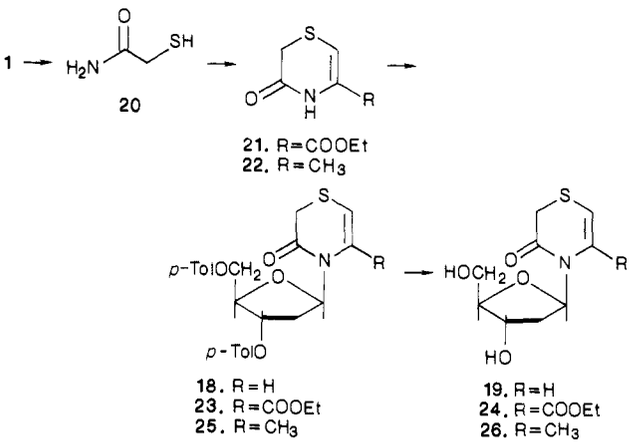
Condensation of **6** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of $SnCl_4$ gave the blocked nucleoside 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazin-3-one (**7**), which was deblocked by treatment with NaOMe in MeOH to give 4-β-D-ribofuranosyl-1,4-thiazin-3-one (**8**). A possible O-glycosylation for compounds **7** and **8** is ruled out because of the carbonyl (C=O stretch) at 1660 cm^{-1} of IR spectrum, as well as the ^{13}C peak at 187.57 ppm for C=O.

Blocked thiazine nucleoside **7** was treated with *m*-chloroperbenzoic acid to obtain a mixture of 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazine-2,3-dione (**9**) in 32% yield and 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazin-3-one 1-oxide (**10**) in 16% yield as shown in Scheme II. The formation of compound **9** was unexpected but was rationalized as the result of two consecutive Pummerer reactions, as discussed below.

An alternate route to the thiazine 1-oxides via 2-[(2,2-diethoxyethyl)sulfinyl]acetamide (**12**) or 2-[(2,2-diethoxyethyl)sulfonyl]acetamide (**15**) was investigated. The ester **3** was treated with sodium periodate[6] in aqueous EtOH to give ethyl 2-[(2,2-diethoxyethyl)sulfinyl]acetate (**11**). Treatment of the ester **11** with alcoholic ammonia gave the amide **12**, which was converted to the sulfone **15** by treatment with 1.1 equiv of *m*-chloroperbenzoic acid (Scheme III). Attempts to cyclize **15** with acid catalysis at elevated temperature gave decomposition, while milder treatment gave no reaction.

Treatment of the amide **12** with a trace of *p*-toluenesulfonic acid in EtOH did not give the expected thiazine oxide **14a** but instead gave in good yield 2-ethoxy-1,4-thiazin-3-one (**13**) as white fibers (Scheme III). It is likely that this product arises from a Pummerer rearrangement of cyclized oxide **14a** or from cyclization of rearrangement product **14b**. A similar product has been found by Hojo and co-workers,[7] who observed that 4-alkyl-1,4-thiazin-3-ones react with *m*-chloroperbenzoic acid to yield the corresponding 2-[(*m*-chlorobenzoyl)oxy]-1,4-thiazin-3-ones. Additionally, they found that benzoyl peroxide reacts with the same starting material and in MeOH gave the 2-methoxy derivatives, while excess benzoyl peroxide in MeOH gives the 2,2-dimethoxy derivatives.[8] These authors do not comment on the possible mechanism of this reaction but imply[8] that the reaction of the peroxide takes place directly at the 2-position. We feel that the direct conversion of compound **12** to **13** must indicate that this product is formed by an acid-catalyzed Pummerer reaction,[9] with the cationic intermediate being trapped by the EtOH solvent. By extension, it seems likely that the 2-acyloxy and 2-alkoxy substituents introduced during oxidation into the thiazine ring in the earlier work[7,8] cited above is due to an initial S-oxidation, followed by a Pummerer rearrangement. The formation of the 2-oxo derivative **9** noted above is probably due to two consecutive Pummerer reactions, with the intermediate product of the first being the 2-hydroxy derivative, and the 2,2-dihydroxy product of the second quickly dehydrating to the ketone.

Scheme IV



The deblocked nucleosides 4-β-D-ribofuranosyl-1,4-thiazine-2,3-dione (**16**) and 4-β-D-ribofuranosyl-1,4-thiazin-3-one 1-oxide (**17**) were obtained by treatment of nucleosides **9** and **10**, respectively, with NaOMe in MeOH. The fact that the methylene protons of the thiazine moiety were not found in the ^1H NMR spectrum, in addition to the appearance of a new carbonyl band at 1710 cm^{-1} in the IR spectrum, provided evidence for compound **16**. Similarly, the dominant singlet integrating for 2 H at 3.56 ppm in ^1H NMR spectrum and an S=O band[10] at 1080 cm^{-1} provided evidence for compound **17**. With use of the sodium salt procedure described by Robins and co-workers,[11,12] 1,4-thiazin-3-one was treated with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-*erythro*-pentofuranose in the

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presence of sodium hydride to give the 2'-deoxy-blocked nucleoside **18**. This was deblocked by treatment with methanolic ammonia to give 4-(2-deoxy-β-D-ribofuranosyl)-1,4-thiazin-3-one (**19**) (Scheme IV).

The synthetic scheme outlined above also gives entry into nucleosides of the orotic acid analogue 3-oxo-1,4-thiazine-5-carboxylic acid. Accordingly, condensation of thioglycolamide[13] with ethyl bromopyruvate gave ethyl 3-oxo-1,4-thiazine-5-carboxylate (**21**). Condensation of **21** with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose in the presence of sodium hydride gave the nucleoside ethyl 4-(2-deoxy-3,5-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3-oxo-1,4-thiazine-5-carboxylate (**23**). This was deblocked to the nucleoside **24** upon treatment with NaOMe in MeOH (Scheme IV) without any observed transesterification. 5-Methyl-1,4-thiazin-3-one[13] (**22**) was condensed with this same glycosyl halide under the same conditions to give the nucleoside **25**, which then was deblocked by NaOMe in MeOH to 4-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-1,4-thiazin-3-one (**26**) (Scheme IV). The anomeric configuration of the 2'-deoxy nucleosides was assigned by ^{1}H NMR spectroscopy. The β anomers exhibited the characteristic triplet for the anomeric proton, which has been observed for the anomeric proton of other 2'-deoxy β-nucleosides.[14,15] Attempts to prepare the ribosides of **21** and **22**, by using $SnCl_4$-catalyzed ribosylation of the trimethylsilylated thiazine as for compound **7**, were unsuccessful.

Biological Activity

The compounds were tested for their ability to inhibit the growth of three tumor cell lines in vitro: L1210 murine lymphocytic leukemia, WIL-2 human B lymphoblastic leukemia, and CCRF-CEM human T lymphoblastic leukemia. All but one compound were devoid of growth inhibitory activity at concentrations of ≤10 μM. Compounds **18**, **19**, **23**, and **25** were dissolved in DMSO at 4 mM and tested at concentrations of ≤10 μM to avoid problems of DMSO cytotoxicity. Compound **22** was somewhat effective in inhibiting leukemia cell growth with ID_{50} values (the micromolar concentration of compound that inhibits cell growth by 50%) of 3.0, 41.7, and 35.5 against L1210, WIL-2, and CCRF-CEM, respectively.

In vivo most of these compounds produced no increase in life span and also produced no drug-induced weight loss or morbidity when given ip on day 1, one time, 24 h after ip inoculation with 1×10^6 L1210/0 at a level of 800 mg/kg. However, compound **22**, when given ip on day 1, one time, 24 h after ip inoculation with 1×10^6 L1210/0 at a level of 288 mg/kg, was lethally toxic for 3/3 mice. In a follow-up dosage ranging test, **22** was given at 173, 104, 62, and 37 mg/kg. The 173 mg/kg dosage was lethally toxic for 3/3 mice. The 104 mg/kg dosage produced transient morbidity, but the mice recovered from the drug effects. These nonlethal toxic dosages produced no significant weight loss and produced no increase in life span.

The compounds were tested for antiviral activity in cell culture against herpes simplex, adeno, rhino, influenza, and para-influenza viruses at concentrations as high as 1 mM. The only activity observed was with compound **8**, which inhibited rhinovirus type 1-A cytotoxicity in HeLa cells by 50% at 320 um.

Compound **17**, which is most likely to mimic the tetrahedral transition state in the hydrolytic enzyme cytidine deaminase, was tested as an inhibitor of that enzyme from mouse kidney. Under conditions where the K_m of cytidine was 0.05 mM, the K_i of **17** was 0.33 mM. Apparently this sulfoxide does not resemble the binding points of the hypothetical transition state, or, however unlikely, our product has been isolated as a single diastereomer that has the wrong geometry at the tetrahedral center.

Experimental Section

General Methods. Melting points were determined in a Thomas-Hoover Uni-Melt apparatus with capillary tubes and were uncorrected. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were run on either an IBM NR-300 AF spectrometer at 300 and 75 MHz, respectively, or a JEOL FX-90Q at 90 and 22.5 MHz, respectively. Chemical shifts are reported downfield from internal Me_4Si. Infrared spectra (IR in KBr) were obtained on a Perkin-Elmer 1420 spectrophotometer. Elemental analysis were performed either by MHW Laboratories in Phoenix, AZ, or by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (TLC) was performed on Bakerflex (J. T. Baker Co.) silica gel TB2-F plates (2.5 × 7.5 cm) containing fluorescent indicator and an inert binder. Spots were visualized either by UV light or by spraying the plates with EtOH/*p*-anisaldehyde/sulfuric acid (100:1:1) spray. After heating, dark green spots were obtained for nucleosides. Evaporations were conducted under vacuum at room temperature on a rotary evaporator, unless otherwise specified. Chemicals (reagents and starting materials) that were commercially available were obtained either from Aldrich Chemical Co. or Sigma Chemical Co.

2-[(2,2-Diethoxyethyl)thio]acetamide (4). Anhydrous ammonia gas was bubbled for 20 min into a stirred solution of ethyl 2-[(2,2-diethoxyethyl)thio]acetate (**3**, 1.8 g, 7.63 mmol) in 30 mL MeOH in an ice bath. The solution was then stirred in the ice bath for about 2 h and then at room temperature for another 18 h. Upon completion of the reaction, as followed by TLC (90:10 hexane/EtOAc), evaporation gave a thick oil (1.5 g, 95% yield): ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 6 H, 2 CH_3), 2.60 (d, 2 H, C_3H), 3.15 (s, 2 H, C_2H), 3.50 (q, 4 H, 2 CH_2), 4.50 (t, 1 H, C_4H), 6.9 (s, 2 H, NH).

1,4-Thiazin-3-one (5). A mixture of the amide **4** (1 g, 4.8 mmol) and a trace of *p*-toluenesulfonic acid in toluene was refluxed for 20 h. The solution was then evaporated at reduced pressure to give a thick oil. The oil was recrystallized from hot benzene to afford yellowish crystals (0.45 g, 89.6% yield) of 1,4-thiazin-3-one (**5**): mp 75–76 °C (lit.[5] mp 74.5–75 °C; UV (H_2O) 297 nm; ^{1}H NMR ($CDCl_3$) δ 3.31 (s, 2 H, C_2H), 5.85 (d, 1 H, C_6H), 6.3 (dd, 1 H, C_5H), 8.25 (s, 1 H, NH).

3-[(Trimethylsilyl)oxy]-1,4-thiazine (6). A mixture of 1,4-thiazin-3-one (**5**, 1 g, 8.695 mmol) and hexamethyldisilazane (2.468 mL, 11.7 mmol) was brought to a reflux with exclusion of humidity. Ammonia was vigorously evolved, and ammonium chloride was deposited on the lower part of the reflux condenser; after about 4 h, the white solid had dissolved and the solution had turned dark. The excess HMDS was evaporated. The oil was distilled under vacuum to give a yellow oil distillate of 3-[(trimethylsilyl)oxy]-1,4-thiazine (**6**, 1.3 g, 69.5%): bp 95–98 °C (0.1 mmHg); ^{1}H NMR ($CDCl_3$) δ 0.1 (s, 9 H, 3 CH_3), 5.9 (d, 1 H, C_6H), 6.3 (d, 1 H C_6H).

4-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazin-3-one (7). To a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.268 g, 4.5 mmol) in 1,2-dichloroethane (27 mL) was added a solution of **6** (0.9 g, 4.8 mmol) in dichloroethane (2.25 mL). The mixture was cooled with ice, and redistilled $SnCl_4$ (0.42 mL, 3.6 mmol) in 2 mL of dichloroethane was added with vigorous stirring and exclusion of humidity. The yellowish solution was kept for 6 h at room temperature. The reaction was followed by TLC (85:15 hexane/EtOAc). Upon completion, the reaction mixture was shaken with saturated sodium bicarbonate (20 mL), and the resulting emulsion was filtered over a layer of sand/Celite. The filter cake was washed with 1,2-dichloroethane. The combined organic phases were dried over magnesium sulfate and evaporated. The slightly yellowish gum material was crystallized from acetone/EtOH to give 1.96 g (78% yield): mp 126–127 °C; ^{1}H NMR ($CDCl_3$) δ 3.35 (s, 2 H, C_2H), 5.9 (d, 1 H, C_1H), 6.12 (d, 1 H, C_6H), 6.36 (d, 1 H, C_5H), 7.4 (m, 9 H, aromatic protons), 7.95

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(m, 6 H, aromatic protons), and other sugar protons. Anal. ($C_{30}H_{25}NO_8S$) C, H, N.

4-β-D-Ribofuranosyl-1,4-thiazin-3-one (8). To **7** (0.1 g, 0.178 mmol) in MeOH (4 mL) was added a NaOMe solution (0.3 mL, 0.27 mmol). After being stirred for 3 h at room temperature, the solution was passed through a 1 × 10 cm column of Dowex-50 (H^+) resin. The column was washed with 10 mL of MeOH/H_2O (2:1). The elute was then evaporated. The residue was dissolved in a little water and extracted three times with ether. The water solution was then evaporated to give a gum, which crystallized from acetone/ether; recrystallization from acetone afforded 0.033 g (75% yield) of pure **8** as white needles: mp 108–109 °C; UV (H_2O) 294 nm; IR 3400, 3070, 2978, 1660, 1450, 1370, 1230, 1200, 1000, 750, 650 cm^{-1}; ^{13}C NMR (D_2O) δ 42.40 (C_2), 75.54 ($C_{5'}$), 84.94 ($C_{3'}$), 87.41 ($C_{2'}$), 99.83 ($C_{4'}$), 102.92 ($C_{1'}$), 123.49 (C_6), 142.20 (C_5), 187.57 (C_3); ^1H NMR (D_2) δ 3.36 (s, 2 H, C_2H), 6.00 (d, 1 H, $C_{1'}H$), 6.10 (d, 1 H, C_6H), 6.6 (d, 1 H, C_5H), and other sugar protons. Anal. ($C_9H_{13}NO_5S$) C, H, N.

4-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazine-2,3-dione (9) and 4-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazin-3-one 1-Oxide (10). To a solution of **7** (1 g, 1.78 mmol) in 1,2-dichloromethane (30 mL) stirred at −10 °C was added *m*-chloroperbenzoic acid (0.337 g, 1.958 mmol). The mixture was allowed to stir for an additional 2 h at −10 °C and then brought to room temperature to stand overnight. The precipitate that formed was filtered off to give 0.3 g of the dione **9**, mp 105–107 °C dec. The dichloromethane solution was evaporated to dryness and passed through a short silica gel column (ethyl acetate/hexane, 1:4). Elution gave an additional 0.03 g of dione **9** (total yield, 32%) and 0.157 g of the sulfoxide **10** (16%), mp 74–76 °C.

Dione 9: IR 3100, 3021, 2980, 1772, 1620, 1649, 1610, 1450, 1100, 1063, 900, 800, 600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.40 (s, 2 H, C_2H), 5.81 (d, 1 H, $C_{1'}H$), 6.30 (d, 1 H, C_6H), 6.66 (d, 1 H, C_6H), 7.37 (m, 9 H), 7.75 (m, 6 H), and other sugar protons. Anal. ($C_{30}H_{23}NO_9S$) C, H, N.

Sulfoxide 10: IR 3100, 3090, 2950, 1780, 1686, 1630, 1415, 1310, 1290, 1150, 900, 800, 715, 672 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.40 (s, 2 H, C_2H), 5.81 (d, 1 H, $C_{1'}H$); 6, 30 (d, 1 H, C_6H), 6.66 (d, 1 H, C_5H), 7.35 (m, 9 H, aromatic protons), 7.70 (m, 6 H, aromatic protons), and other sugar protons. Anal. ($C_{30}H_{25}NO_9S$) C, H, N.

Ethyl 2-[(2,2-Diethoxyethyl)sulfinyl]acetate (11). To a stirred ice-cold suspension of sodium *m*-periodate (17.082 g, 79.86 mmol) in 160 mL of 48% aqueous EtOH was added the ester **3** (17.975 g, 76.06 mmol). The mixture was stirred overnight. The precipitate was removed by filtration and washed with dichloromethane, and the combined washings and filtrates were transferred to a separatory funnel. Following the removal of the organic phase, the aqueous phase was extracted with dichloromethane (4 × 100 mL). The combined organic extracts were washed with water (150 mL), treated with activated charcoal (1 g), dried over magnesium sulfate, and taken to dryness under reduced pressure. The residue, a light yellow syrup (19.63 g), was chromatographed on a silica gel column (6 × 20 cm) with EtOAc/hexane (1:1). The column gave an oily material, which crystallized from ether to give white needles of **11** (14.80 g, 73.1%): mp 39–39.5 °C; ^1H NMR ($CDCl_3$) δ 1.20 (td, 6 H, 2 CH_3), 1.44 (t, 3 H, CH_3), 3.15 (d, 2 H, C_3H), 3.65 (q, 4 H, 2 CH_2), 3.82 (s, 2 H, C_2H), 4.25 (q, 2 H, CH_2), 5.00 (t, 1 H, C_4H). Anal. ($C_{10}H_{20}O_5S$) C, H, O.

2-[(2,2-Diethoxyethyl)sulfinyl]acetamide (12). Into a stirred solution of **11** (5.0 g, 19.8 mmol) in 65 mL of MeOH was bubbled anhydrous ammonia gas for 20 min at 0 °C. The solution was first stirred at 0 °C for about 2 h, warmed to room temperature for another 18 h, and evaporated to give a thick oil, which was recrystallized from ether to afford 4.1 g (93%, mp 93–94 °C) of the amide **12**: ^1H NMR ($CDCl_3$) δ 1.25 (td, 6 H, 2 CH_3), 3.15 (d, 2 H, C_3H), 3.7 (m, 6 H), 4.95 (t, 1 H, C_4H), 6.20 (s, 1 H, N*H*), 7.10 (s, 1 H, N*H*). Anal. ($C_8H_{17}NO_4S$) C, H, N.

2-Ethoxy-1,4-thiazin-3-one (13). A mixture of the amide oxide **12** (1 g, 4.48 mmol) and a trace of toluene-*p*-sulfonic acid in EtOH was refluxed for 24 h. The solution was then evaporated to give a thick oil. The oil was dissolved in a small amount of MeOH and passed through a short column of silica gel (MeOH/$CHCl_3$, 3:4). The appropriate fractions were evaporated, and the residue was crystallized from pentane to afford **13** (0.435 g, 61%): mp 69–70 °C; ^1H NMR ($CDCl_3$) δ 1.25 (t, 3 H, CH_3), 3.7 (dq, 2 H, CH_2), 4.95 (d, 1 H, C_2H), 5.67 (d, 1 H, C_6H), 6.33 (dd, 1 H, C_5H), 8.45 (s, 1 H, N*H*). Anal. ($C_6H_9NO_2S$) C, H, N.

2-[(2,2-Diethoxyethyl)sulfonyl]acetamide (15). To a stirred solution of **12** (0.5 g, 2.24 mmol) in 30 mL of $CHCl_3$ at 0 °C was added *m*-chloroperbenzoic acid (0.922 g, 5.34 mmol). The solution was warmed to room temperature, stirred for another hour, and then evaporated. The residue was washed with ether three to four times, dissolved in water, and extracted with ether. The water solution was evaporated to dryness; the solid material obtained was crystallized from EtOH to give **15** (0.327 g, 61%): mp 94–95 °C; ^1H NMR (D_2O) δ 1.30 (d, 6 H, 2 CH_3), 3.21 (d, 2 H, C_3H), 4.05 (m, 6 H), 6.17 (s, 1 H, N*H*), 7.15 (s, 1 H, H*N*). Anal. ($C_8H_{17}NO_5S$) C, H, N.

4-β-D-Ribofuranosyl-1,4-thiazine-2,3-dione (16). To a suspension of **9** (0.1 g, 0.174 mmol) in MeOH (4 mL) was added a 0.9 M NaOMe solution (0.3 mL, 0.27 mmol). After being stirred for 3 h at room temperature, the solution was passed through a 1 × 10 cm column of Dowex-50 (H^+) resin. The column was washed with 10 mL of MeOH/water (2:1). The elute was evaporated, and the residue was dissolved in water and extracted three times with ether. The water was then evaporated to give a gum, which crystallized on standing at 0 °C to give **16** (25 mg, 55%): mp 107 °C; UV (H_2O) 294 nm; IR 3081, 2970, 1710, 1610, 1450, 1280, 1240, 1100, 1063, 950, 900, 800, 700, 600 cm^{-1}; ^1H NMR (D_2O) δ 5.77 (d, 1 H, $C_{1'}H$), 5.96 (d, 1 H, C_6H), 6.25 (d, 1 H, C_5H), and other sugar protons. Anal. ($C_9H_{11}NO_6S \cdot {}^1/_2H_2O$) C, N; H: calcd. 4.48; found, 5.00.

4-β-D-Ribofuranosyl-1,4-thiazin-3-one 1-Oxide (17). To a suspension of **10** (0.1 g, 0.173 mmol) in MeOH (5 mL) was added a 0.9 M NaOMe solution (0.3 mL, 0.27 mmol). After being stirred for 3 h at room temperature, the solution was passed through a 1 × 10 cm column of Dowex-50 (H^+) resin. The column was washed with 10 mL of MeOH/water (2:1). The elute was evaporated. The residue was dissolved in water and extracted three times with ether. The water solution was then evaporated, and the residue was dissolved in MeOH and crystallized on standing, giving 20 mg (43%) of **17**: mp 105 °C; IR 3100, 2950, 1630, 1416, 1310, 1290, 1251, 1149, 1110, 1080, 1000, 965, 900, 850, 712, 670 cm^{-1}; ^1H NMR (D_2O) δ 3.56 (s, 2 H, C_2H), 5.81 (d, 1 H, $C_{1'}H$), 5.95 (d, 1 H, C_6H), 6.12 (d, 1 H, C_5H), and other sugar protons. Anal. ($C_9H_{13}NO_6S \cdot {}^1/_2H_2O$) C, H, N.

4-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-1,4-thiazin-3-one (18). To a suspension of **5** (2.0 g, 17.4 mmol) in dry MeCN (100 mL) was added NaH (60% in oil, 0.775 g, 19.2 mmol) in portions, and the mixture was stirred at room temperature for 30 min. 1-Chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranose (6.76 g, 17.4 mmol) was added portionwise with stirring. After 3 h the solution was filtered, evaporated to dryness, and passed through a 5 × 30 cm silica gel column (toluene/acetone, 9:1) to give 6.4 g (79%) of **18**: mp 157–157.5 °C; ^1H NMR (Me_2SO-d_6) δ 2.25 (s, 6 H, 2 CH_3), 3.16 (s, 2 H, C_2H), 5.46 (d, 1 H, $C_{1'}H$), 5.83 (d, 1 H, C_6H), 6.14 (d, 1 H, C_6H), 6.96–7.81 (4 d, 8 H), and other sugar protons. Anal. ($C_{25}H_{25}NO_8S$) C, H, N.

4-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-1,4-thiazin-3-one (19). To **18** (0.1 g, 0.21 mmol) in dry methanol (6 mL), was added a 0.81 M NaOMe solution (0.4 mL, 0.324 mmol). After 3 h at room temperature, the solution was passed through a 1 × 10 cm column of Dowex-50 (H^+) resin. The column was washed with 10 mL of MeOH/water (2:1). The elute was evaporated, and the residue was crystallized from MeOH to give 0.32 g (65%) of **19**: mp 102–103 °C; ^1H NMR (D_2O) δ 3.32 (s, 2 H, C_2H), 5.51 (d, 1 H, $C_{1'}H$), 5.61 (d, 1 H, C_6H), 5.99 (d, 1 H, C_5H), and other sugar protons. Anal. ($C_9H_{13}NO_4S \cdot H_2O$) C; H: calcd, 5.90; found, 5.40. N: calcd, 5.50; found, 5.05.

Ethyl 3-Oxo-1,4-thiazine-5-carboxylate (21). To 2 g (21.9 mmol) of thioglycolamide was added ethyl bromopyruvate (4.8 g, 24.6 mmol). A vigorous evolution of hydrogen bromide occurred spontaneously. The reaction mixture was warmed to about 45 °C for approximately 1 h. After 20 g of ice and water were added to the warm reaction mixture, the precipitate was filtered and recrystallized from MeOH to give 2.0 g of **21** (50%): mp 64.5–65.5 °C; IR 3250, 3100, 2950, 1760, 1630, 1420, 1300, 1200, 1100, 1000, 750, 700 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.25 (t, 3 H, CH_3), 3.22 (s,

2 H, C_2H), 4.25 (q, 2 H, CH_2), 6.8 (s, 1 H, C_6H), 9.5 (s, 1 H, NH). Anal. ($C_7H_9NO_3S \cdot {}^1/_4H_2O$), C, H, N.

Ethyl 4-(2-Deoxy-3,5-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3-oxo-1,4-thiazine-5-carboxylate (23). To a solution of **21** (1 g, 5.34 mmol) in dry MeCN (60 mL) was added NaH (60% in oil, 0.238 g, 5.9 mmol) in portions, and the mixture was stirred at room temperature for 30 min. 1-Chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-*erythro*-pentofuranose (2.07 g, 5.34 mmol) was added in portion with stirring. After 3 h the solution was filtered, evaporated to dryness, and passed through a silica gel column (toluene/acetone, 9:1) to give **23** (1 g, 35%): mp 120 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.26 (t, 3 H, CH_3), 2.30 (s, 6 H, 2 CH_3), 3.24 (s, 2 H, C_2H), 4.16 (q, 2 H, CH_2), 5.55 (t, 1 H, $C_1'H$), 6.58 (s, 1 H, C_6H), 7.01–7.69 (4 d, 8 H), and other sugar protons. Anal. ($C_{28}H_{29}NO_8S$) C, H, N.

Ethyl 4-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-oxo-1,4-thiazine-5-carboxylate (24). To **23** (0.1 g, 0.18 mmol) in MeOH (6 mL) was added a 0.81 M NaOMe solution (0.4 mL, 0.324 mmol). After 3 h at room temperature, the solution was passed through a 1 × 10 cm column of Dowex-50 (H^+) resin. The column was washed with 10 mL of MeOH/H_2O (2:1). The eluate was then evaporated, and the residue was dissolved in water. Lyophilization gave **24** (0.03 g, 52% yield): mp 121–122 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.21 (t, 3 H, CH_3), 3.26 (s, 2 H, C_2H), 4.17 (q, 2 H, CH_2), 5.54 (t, 1 H, $C_1'H$), 6.57 (s, 1 H, C_6H), and other sugar protons. Anal. ($C_{12}H_{17}NO_6S \cdot 2H_2O$) C, H, N.

4-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-methyl-1,4-thiazin-3-one (25). To a suspension of 5-methyl-1,4-thiazin-3-one[13] (22, 0.5 g, 3.87 mmol) in dry MeCN (40 mL) was added NaH (60% in oil, 0.169 g, 4.2 mmol) in portions, and the mixture was stirred at room temperature for 30 min. 1-Chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (1.5 g, 3.87 mmol) was added in portions with stirring. After 3 h, the solution was filtered, evaporated to dryness, and passed through a silica gel column (toluene/acetone, 9:1) to give **25** (0.7 g, 38%): mp 125–126 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.15 (s, 3 H, CH_3), 2.31 (s, 6 H, 2 CH_3), 3.15 (s, 2 H, C_2H), 5.46 (t, 1 H, $C_1'H$), 5.91 (s, 1 H, C_6H), 6.98–7.65 (4 d, 8 H), and other sugar protons. Anal. ($C_{26}H_{27}NO_6S$) C, H, N.

4-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-methyl-1,4-thiazin-3-one (26). To **25** (0.1 g, 0.2 mmol) in MeOH was added a 0.81 M NaOMe solution (0.4 mL, 0.324 mmol). After 3 h at room temperature, the solution was passed through a 1 × 10 cm column of Dowex-50 (H^+) resin. The column was washed with approximately 10 mL of MeOH/water (2:1). The elute was evaporated. The residue was dissolved in a small amount of water/MeOH. The deblocked nucleoside **26** was obtained as a white solid on standing (28.5 mg, 57% yield): mp 114–115 °C; UV (H_2O) 298 nm; ^{1}H NMR (Me_2SO-d_6) δ 2.17 (s, 3 H, CH_3), 3.14 (s, 2 H, C_2H), 5.50 (t, 1 H, $C_1'H$), 5.93 (s, 1 H, C_6H), and other sugar protons. Anal. ($C_{10}H_{15}NO_4S \cdot 2H_2O$) C, H, N.

Cytidine Deaminase Inhibition. Mouse kidney cytidine deaminase was isolated and partially purified from mouse kidney acetone powder (prepared with acetone only) obtained from Sigma Chemical Co., St. Louis, MO. The powder was extracted as described by Liu et al.[16] and fractionated with ammonium sulfate as described by Wentworth and Wolfenden.[17] We found the K_m for cytidine with this preparation (0.10 M HEPES buffer, pH 7.0) to be 0.025 mM (lit.[16] 0.05 mM). Compound **17** gave a K_i of 0.33 mM.

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Nucleotide Derivatives of 2,7-Diaminomitosene

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Treatment of mitomycin C with pyrimidine nucleotides in acidic media produced derivatives of 2,7-diaminomitosene in which C-1 was covalently bound to the phosphate group of the nucleotides. On reduction, these derivatives liberated the nucleotides and a mitomycin intermediate that alkylated DNA. Their reduction in the presence of 2′-deoxyguanosine produced some bifunctional alkylation as did mitomycin C. They were readily taken up by L1210 leukemia cells, in which they showed potent cytotoxicity. These properties suggest that they are acting as prodrugs capable of conversion into two active species. The uridylate derivative showed activity comparable to that of mitomycin C against P-388 leukemia in mice.

The design of prodrugs that are readily converted into one or more cytotoxic species by bioactivating mechanisms is an important goal in cancer chemotherapy. This design strategy may afford reduced toxicity to patients when the bioactivating mechanism of the prodrug is primarily a property of the tumor. The reduction of quinones to hydroquinones or semiquinone radicals by cellular enzymes is one such mechanism, and it is a significant in vivo route of activation in the mitomycin family of compounds. A related approach has been taken in the investigation of nitrobenzyloxycarbonyl derivatives of 5-fluorouracil as potential conjugated bioreductive alkylating agents.[1] In vitro activation of mitomycins by acidification has also been described.[2-5] We have used this acid-catalyzed activation mechanism to form a 1-substituted mitosene co-

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