5 h, the precipitated product was collected by filtration and recrystallized from MeOH. Physical and spectral data for the mesoionic compounds **1** are given in Table I.

Pharmacology. Male Sprague–Dawley rats (250–350 g) were anesthetized with sodium pentobarbital (35 mg/kg ip), and a femoral artery was cannulated. The animals were restrained in a supine position, and the excision area was bathed in normal saline for the duration of the experiment. A 10% heparin–saline solution (0.01 mL) was injected into the cannulated artery 30 s before recording blood pressure. Blood pressure was recorded with a Statham transducer interfaced with a Grass Model 7 polygraph. After the animals recovered from anesthesia, blood pressure was recorded continuously for 1 h. Control experiments were carried out and showed that the length of time of restraint, time of day of blood pressure recording, and administration of vehicle alone did not affect blood pressure. Compound **1b** was found by the procedure of Weil[8] to possess an approximate LD_{50} of 250 mg/kg; therefore, in the early studies of hypotensive effects, doses of up to 90 mg/kg were used. Test compounds were dissolved in corn oil (0.7–0.8 mL) and administered by gavage. Blood pressure was recorded continuously for 30 min and then intermitantly for the next several hours. Animals were sacrificed at the conclusion of each experiment.

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Ammonium 7*H*-Purin-6-yl 1-Thio-β-D-glucopyranosiduronate, a Latent, Selective Anticancer Agent

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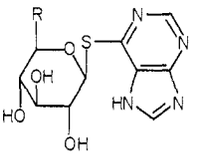
Ammonium 7*H*-purin-6-yl 1-thio-β-D-glucopyranosiduronate (**1**), a good substrate of β-glucuronidase, causes a 22% decrease in the growth of L1210 cells while not affecting the growth of Chinese hamster lung fibroblasts from a nontumor line. 7*H*-Purin-6-yl 1-thio-β-D-glucopyranosiduronamide (**2**), a poor substrate of β-glucuronidase, has no effect on the growth of either cell type.

In 1927, Armand J. Quick wrote[1] that "the significance of glycuronic [sic] acid in the economy of the organism is not fully understood, but it seems rather certain that its biological importance is underestimated," and that "little emphasis has been given to the marked ability of the organism to produce relatively large amounts of glycuronic [sic] acid without any perceptible embarrassment," during his study of the metabolism of borneol in dogs. Today we know that there exists higher than normal levels of the enzyme β-glucuronidase (EC 3.2.1.31) in human cancer tissues[2] and this may be used to advantage in devising prodrugs. Futher, the activity of β-glucuronidase is enhanced when cells become more acidic, since the pH–velocity maximum of the enzyme is between 4 and 5. The acidity of cancer cells, already known to be more acidic than normal, can specifically be increased by glucose.[3,4] Taken together, these findings indicate that glucuronides of known anticancer compounds can selectively deliver these drugs to cancer tissue. This approach is currently being explored in some laboratories[4-8] and here we report the synthesis of ammonium 7*H*-purin-6-yl 1-thio-β-D-glucopyranosiduronate (**1**) and of 7*H*-purin-6-yl 1-thio-β-D-glucopyranosiduronamide (**2**), the hydrolysis of **1** and **2** catalyzed by bovine liver β-glucuronidase, and the activity of **1** vs. **2** against L1210 cells.

Table I. Michaelis Constants for Hydrolysis of 1 and 2 Catalyzed by Bovine Liver β-Glucuronidase[a]

no.	pH	V_{max}[b]	V_{max}/K_m[c]
1	3.38	3.73×10^{-8}	6.59×10^{-4}
1	4.40	3.95×10^{-8}	4.09×10^{-4}
1	4.70	3.92×10^{-8}	2.97×10^{-4}
1	5.58	2.18×10^{-8}	9.51×10^{-5}
2	3.38	7.85×10^{-11}	8.80×10^{-9}
2	4.40	1.24×10^{-10}	1.06×10^{-8}
2	4.70	9.85×10^{-11}	1.06×10^{-8}
2	5.58	8.80×10^{-11}	1.04×10^{-8}

[a] Solvent = 0.1 M potassium acetate buffer; μ = 0.1 M (KCl); t = 30 °C. [b] Millimoles per minute per Fishman unit of enzyme activity. [c] Milliliters per minute per Fishman unit of enzyme activity. Here, 1 Fishman unit (FU) of enzyme activity is that amount of enzyme necessary to produce 1 μg of phenolphthalein from phenolphthalein glucuronide per hour at pH 5 at 30 °C. Spontaneous hydrolysis of 1 and 2 was negligible as determined by stable absorbance values for 1 and 2 in acetate buffer at the assay pH. For 1 at pH 4.7, $V_{max} = 3.92 \times 10^{-7}$ M min^{-1} when enzyme activity = 10 FU/mL; this corresponds to 0.49 absorbance unit/h. For 2, at pH 4.7, $V_{max} = 2 \times 10^{-6}$ M min^{-1} when enzyme activity = 20 278 FU/mL; this corresponds to 2.5 absorbance units/h.



1, R = CO_2NH_4
2, R = $CONH_2$

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Results and Discussion

Methyl 7*H*-purin-6-yl 1-thio-2,3,4-tri-*O*-acetyl-β-D-glucopyranosiduronate (**3**) was synthesized from methyl 1-deoxy-1-bromo-2,3,4-tri-*O*-acetyl-α-D-glucopyronosiduronate (**4**)[9] and 6-mercaptopurine in DMF/K_2CO_3. Glucopyranosiduronate **1** was obtained by hydrolysis of **3** in dilute KOH solution, followed by ion exchange on DOWEX 50W-X8 (H^+ form) and treatment of the syrupy glucopyranosiduronic acid with aqueous NH_3. Glucopyranosiduronamide **2** was obtained from **3** and methanolic NH_3.[10]

As expected, **1** is a substrate for β-glucuronidase (Table I). Unexpectedly, **2** is also a substrate (Table I): *p*-nitrophenyl β-D-glucopyranosiduronamide is reported to be neither a substrate nor an inhibitor of liver lysosomal enzyme.[11] To the best of our knowledge this is the first report of a glycopyranosiduronamide serving as a substrate, albeit a poor one, for a β-glucuronidase. We believe that the substrate behavior of **2** is not due to a nonspecific protein effect and that hydrolysis of **2** occurs in the enzyme active site.[12] Thus, **2** was not hydrolyzed in the presence of 1% bovine serum albumin, β-glucuronidase absent, nor was **2** hydrolyzed in the presence of β-glucuronidase and 10^{-5} M saccharo-1,4-lactone, a potent competitive inhibitor of the enzyme.

Glucopyranosiduronide **1** (10^{-5}–10^{-3} M) had no effect on the growth of Chinese hamster lung fibroblasts, V79 line, a cell line not of tumor origin. Thus, the number of cells increased during 24 h from 5×10^5 to 3.44×10^6 in both the control and test systems. In contrast, **1** (10^{-4} M) caused a 22% decrease in the growth of L1210 cells in 48 h. At 10^{-7}–10^{-5} M **1**, no inhibition of cell growth was observed in 48 h. Glucopyranosiduronamide **2** had no effect on the growth of either Chinese hamster lung fibroblasts or on the growth of L1210 cells. These preliminary results show that glucuronides possess selectivity and that β-glucuronidase is most likely an obligate partner in drug delivery. In connection with this latter point, **2** is not cytotoxic probably because it is too poor a substrate of β-glucuronidase to release an adequate amount of 6-mercaptopurine to inhibit L1210 cell growth.

We are presently arranging to have both **1** and its C-6 methyl ester tested in animals over a longer period of time, since there is evidence that longer test times with antimetabolite glucuronides give more dramatic results against cancer cells.[7]

Experimental Section

Apparatus. A Gilford Model 2400 spectrophotometer was used for the collection of rate data. A Cary 118C spectrophotometer was used to obtain UV spectra. Temperature was maintained in the cuvettes by circulating water of constant temperature from a Tamson T9 bath through thermospacers. A Radiometer PHM26 pH meter with a GK 2301 B electrode was used to measure pH. NMR spectra were taken on a Varian T-60A or FT-80 spectrometer with Me_4Si as internal standard; proton signals are reported in δ values. Melting points were taken in open capillary tubes with a Mel-Temp apparatus, or on slides with a Fisher-Johns apparatus, and are uncorrected. IR spectra were taken on a Nicolet FT-IR 7000 series Model 293A. Calculations were performed with a Hewlett-Packard 9820A with a 9862A plotter, with a linear-regression program. Microanalyses were done by Atlantic Microlabs, Inc. An Electrozone Celloscope or a Coulter electronic particle counter was used to count cells.

Methyl 7*H*-Purin-6-yl 1-Thio-2,3,4-tri-*O*-acetyl-β-D-glucopyranosiduronate (3). 6-Mercaptopurine monohydrate, 7.71 g (0.045 mol), was dissolved in 125 mL of hot (steam bath) dry DMF. C_6H_6, 75 mL, was added, and the C_6H_6–water was distilled off at atmospheric pressure. Dry K_2CO_3, 7.0 g (0.051 mol), was added to the cooled (60 °C) DMF solution, and the mixture was stirred as it cooled to room temperature. Compound **4**,[9,13] 18.12 g (0.046 mol), dissolved in 20 mL of dry DMF was added. The mixture became dark and was stirred at room temperature until no solid was visible (ca. 8 h). The dark-brown mixture was kept at room temperature overnight. The reaction mixture was added to 1 L of $CHCl_3$, washed three times with cold saturated $NaHCO_3$, and twice with cold water, and then dried with Na_2SO_4. The volume of the filtrate was reduced on a rotary evaporator. The extract was then carbon treated and dried with Na_2SO_4. The volume was reduced to approximately 45 mL, and the extract was applied to the "Fritte" prepared from 130 g of silica gel H (for TLC according to Stahl) in $CHCl_3$, which was poured into a large sintered glass funnel with a stopcock, diameter ca. 3.5 in. so that the layer of silica gel was ca. 2.5-in. thick. The column was first eluted with $CHCl_3$ (200-mL fractions) until no solid was eluted from the column upon concentration of the fractions. The solvent was changed to $CHCl_3/Me_2CO$ (4:1), and concentration of the yellow eluates resulted in precipitation of a white material that had the appearance of polystyrene (Styrofoam) beads. Elution was continued until the eluate became pale yellow, and precipitation no longer occurred upon concentration. Use of longer columns with a height to width ratio of ca. 10 and a more gradual shift in solvent polarity, or a more polar solvent mixture, $CHCl_3/Me_2CO$ 3:1 or lower, did not improve the separation; in fact, the separation was poorer. White solid, from the $CHCl_3/Me_2CO$ (4:1) fractions, was pooled, and recrystallization from hot $CHCl_3$ gave 6.4 g (0.011 mol, 24% yield), mp 128 °C. During the use of the silica gel "Fritte" to purify compound **3**, the ultraviolet spectra of the fractions were taken before deciding which fractions to pool. The starting material, 6-mercaptopurine, exhibits a strong absorption at 324 nm (log ε 4.3); the absorption maximum of the desired product is shifted to 277 nm. The $CHCl_3$ effluent had a spectrum similar to starting material, but it has been suggested that these fractions may also contain small amounts of the diglucuronide (on S and N) of 6-mercaptopurine.[14] Fractions of the $CHCl_3/Me_2CO$ (4:1) effluent all had spectra indicating desired product; upon pooling and concentrating these fractions, we obtained solid material with the properties of **3**: TLC (silica; C_6H_6/MeOH, 9:1) R_f 0.28; UV λ_{max} (EtOH) 277 nm (log ε 4.15); NMR ($CDCl_3$) δ 8.6 (s, 1 H, purine), 8.1 (s, 1 H, purine), 7.3 (s, $CHCl_3$), 6.4 (d, 1 H, H-1′, J = 8 Hz), 5.5–5.2 (m, 3 H), 4.4–4.2 (m, 1 H), 3.7 (s, 3 H, Me), 2.06–2.01 (s with shoulder, 9 H, OAc). Anal. ($C_{18}H_{20}N_4O_9S \cdot CHCl_3$) C, H.

7*H*-Purin-6-yl 1-Thio-β-D-glucopyranosiduronamide (2). In a 25-mL pear-shaped flask, with a sidearm, equipped with a drying tube was dissolved 0.300 g (0.51 mmol) of **3** in ca. 10 mL of MeOH at room temperature. The solution was cooled in an ice–water bath, and anhydrous NH_3 was bubbled in via a tube fitted in the sidearm, until the solution was saturated. The reaction mixture was allowed to warm to room temperature and stand overnight. The solution, exhibiting a strong odor of NH_3, was degassed and concentrated with a rotary evaporator (bath 35 °C) until precipitation of a while solid occurred. The product (0.121 g, 3.37 mmol, 72% yield) was collected by gravity filtration, rinsed with water and EtOH, and stored in a desiccator. The dried compound decomposed at 215 °C, with previous softening at 210 °C, with darkening and effervescence: lit.[10] mp 205–207 °C; UV λ_{max} (EtOH) 279 nm (log ε 4.14); NMR (Me_2SO-d_6) δ 8.69 (s, 1 H, purine), 8.46 (s, 1 H, purine), 7.19 (d, 1 H, H-1′, J = 12 Hz), 5.17–5.96 (m, 2 H, H-2′,5′); TLC (cellulose; *n*-BuOH/H_2O/MeOH, 3:1:0.4) R_f 0.35. Anal. ($C_{11}H_{13}N_5O_5S$) C, H.

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Ammonium 7*H*-Purin-6-yl 1-Thio-β-D-glucopyranosiduronate (1). Compound 3 (1.17 g, 1.99 mmol) was dissolved in 35 mL of MeOH and 10 mL of water to which was added 10 mL of 1.0 M KOH, pH ~10. The mixture warmed intially. After 3 h, Dowex 50W-X8 resin, H^+ form, was added to the solution, and the mixture was swirled. The pH decreased gradually to a minimum pH 2.45, whereupon the mixture was filtered and the resin was rinsed extensively, first with methanol and then with water. The methanol was removed by rotary evaporation, and the water solution was concentrated. NH_4OH (28%, 0.55 mL) was added (pH ~9.5), the solution was kept for 2 h and concentrated, and the salt was precipitated by addition of Me_2CO to the water solution. The salt was removed by filtration, redissolved in water, and again precipitated by the addition of Me_2CO. The white solid was collected and dissolved in water. The solution was frozen and lyophilized. The product (0.480 g, 1.38 mmol, 69% yield) began to darken at 150 °C, softened at 178 °C, and by 185 °C had completely decomposed: pK_a = 3.08 ± 0.02 (25 °C); UV λ_{max} (water) 281.5 nm (log ε 4.12); TLC (cellulose; *n*-BuOH/H_2O/MeOH, 3:1:0.4) R_f 0.18; NMR (D_2O) δ 8.1 (s, 1 H, purine), 7.95 (s, 1 H, purine), 5.6 (m, 1 H, H-1′), 5.0–4.4 (m, H-2′,5′), 4.0–3.4 (m, H_2O). Anal. ($C_{11}H_{15}N_5O_6S \cdot H_2O$) C, H.

Kinetics of Hydrolysis of 1 and 2 Catalyzed by Bovine Liver β-Glucuronidase. Three milliliters of 0.1 M acetate buffer (μ = 0.1 M, KCl) at each of several pH values was pipetted into 1-cm cuvettes and placed in Gilford spectrophotometer cell holder for 20 min at 30 °C. For all pH studies, except that at pH 3.38, 10 μL of the substrate in water was also added at this point. At pH 3.38, spontaneous substrate hydrolysis was appreciable, and the substrate was added after 20 min. The final substrate concentration in each cuvette ranged from 4.0×10^{-4} to 4.8×10^{-3} M. Fifteen microliters of bovine β-glucuronidase (30 FU) in 0.1 M, pH 5.0, acetate buffer was added at the end of 20 min. The change in absorbance per minute at 324 nm was recorded, and initial velocities were computed with $\Delta\epsilon = 2 \times 10^4$ M^{-1} cm^{-1}. At each substrate concentration, duplicate experiments were performed. For hydrolysis of **2**, the protocol was similar except 20 μL of **2** in Me_2SO was added to 2 mL of acetate buffer and 50 μL of bovine β-glucuronidase (42 000 FU) per reaction was used.

A reaction mixture containing 20 μL of **2** (1.1 μmol) in Me_2SO in 2 mL of 0.01 M acetate buffer (pH 4.96) and 14520 FU of bovine β-glucuronidase was lyophilized after 48 h, and the residue was treated with 50 μL of H_2O. Water-soluble material was spotted on a cellulose plate (Eastman 13254), which was developed in BuOH–pyridine–H_2O (6:4:3), subsequently sprayed with a solution of 1.23% *p*-anisidine and 1.66% phthalic acid in MeOH and dried with a heater–blower gun. A peach-colored spot with R_f 0.22 ± 0.01 corresponded to that of authentic D-glucuronomide. D-Glucuronic acid, which does not migrate in this system, was not detected. Parenthetically, there is no lag phase for hydrolysis of **2**, and purin-6-yl 1-thio-β-D-glucopyranoside is also a poor substrate.

Biological Testing. Ammonium 7*H*-purin-6-yl 1-thio-β-D-glucopyranosiduronate (1) was investigated with respect to its effect on Chinese hamster lung fibroblast (V-79) growth.[15] The V-79 cell line is not of tumor origin. It has a doubling time of 9.0 h under normal culture conditions (RPMI-1640, 10% FCS, 1% antibiotics penicillin and streptomycin, 5% CO_2 humidified incubator). This cell line has been actively cultured in Dr. Bardos' laboratory for over 2 years. New cells are acquired from frozen stock approximately every 6 months.

Exponentially growing cultures of asynchronous cells plated 24 h prior to the experiment were treated, as is, with various doses ranging from 10^{-3} to 10^{-5} M compound for 1 h. Following treatment, duplicate plates for each dose were rinsed free of drug-containing media, refed with fresh media, and placed back into the incubator for the short term (24 h) viability study. Twenty–four hours posttreatment, control plates approached confluency. At this time, all plates were trypsinized, and the cells from each plate were counted with an electrozone particle counter.

Compounds 1 and **2** were investigated with respect to their effect on L1210 cell growth.[16] The L1210 line is of tumor origin. One milliliter [RPMI-1640, 20% FCS (HI)] of 20 mM Hepes buffer containing 10^5 L1210 cells was added to 1 mL of RPMI-1640, 20 mM Hepes buffer containing 2×10^{-3} to 2×10^{-6} M **1** or **2** in a tube. Triplicate experiments were done at each drug concentration. The tubes were incubated at 37 °C for 48 h. Forty-eight hours posttreatment, the cells from each tube were counted with a Coulter counter.

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(15) Testing was done by Dr. Joseph A. Dunn at SUNY/Buffalo, Department of Biochemical Pharmacology, under Dr. Thomas J. Bardos' Direction.

(16) Testing was done by Ms. Patricia Dixon at Roswell Park Memorial Institute.

Crystal Structure and Anti Herpes Simplex Virus Activity of 2,2′-Anhydro-1-β-D-arabinofuranosylthymine

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1-β-D-Arabinofuranosylthymine (aThy; *ara*-T) is a potent selective anti herpes simplex virus drug. Its anhydro analogue, 2,2′-anhydro-aThy, was shown to be 9-fold less active and at least 3-fold less toxic than aThy. This compound was relatively stable at physiological pH and in strong acid but was rapidly hydrolyzed in base with a half-life of 18.3 min. The three-dimensional crystal structure of 2,2′-anhydro-aThy revealed a rigid structure with the arabinose ring in the unusual O1′ endo, pucker, conformation. The trans–gauche conformation along the C4′–C5′ bond permits only intermolecular hydrogen bonding of the 5′-hydroxy and O3′.

Several β-D-arabinofuranosyl nucleosides are potent inhibitors of viral and mammalian cell replication. Prominent examples of this diverse group of compounds, which inhibit herpes simplex viruses (HSV), are the clinically approved drug 9-β-D-arabinofuranosyladenine (*ara*-A; Vidarabine; Vira-A) and the experimental drug 1-β-D-arabinofuranosylthymine (*ara*-T; aThy).[1,2]

We have recently described a rapid and simple method for preparing pyrimidine arabinosyl nucleosides via 2,2′-

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