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Registry No. 1, 143-62-4; **2**, 18404-43-8; **2** (acetonide), 88253-68-3; α-**3a**, 88253-72-9; β-**3a**, 6814-78-4; α-**3b**, 88253-70-7; β-**3b**, 88253-71-8; **4a**, 73986-99-9; **4b**, 88253-69-4; **5**, 17059-16-4; **6**, 33275-60-4; **7**, 88293-57-6; **8**, 69932-81-6; Na^+,K^+-ATPase, 9000-83-3.

A Colon-Specific Drug-Delivery System Based on Drug Glycosides and the Glycosidases of Colonic Bacteria[1]

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Steroid glycosides and the unique glycosidase activity of the colonic microflora form the basis of a new colon-specific drug-delivery system. Drug glycosides are hydrophilic and, thus, poorly absorbed from the small intestine. Once such a glycoside reaches the colon it can be cleaved by bacterial glycosidases, releasing the free drug to be absorbed by the colonic mucosa. This concept was illustrated with dexamethasone 21-β-D-glucoside (1) and prednisolone 21-β-D-glucoside (2), two prodrugs that may be useful in treating inflammatory bowel disease. Hydrolysis of the prodrugs by β-glucosidase and fecal homogenates in vitro released the free steroids. Glucosides 1 and 2 were administered to rats intragastrically to determine when and where the free steroids were released. Unmodified dexamethasone (3) and prednisolone (4) were also given to rats intragastrically to compare absorption of the glucosides with the free steroids. Both glucosides were found to reach the rat lower intestine in 4–5 h, where they were rapidly hydrolyzed, releasing the free steroids. Delivery of steroid 3 (via glucoside 1) was more specific than that of steroid 4 (via glucoside 2): nearly 60% of an oral dose of glucoside 1 reached the cecum, whereas less than 15% of glucoside 2 reached the cecum. When free steroids 3 and 4 were administered orally, they were almost exclusively absorbed in the small intestine: less than 1% of an oral dose of each reached the cecum.

The delivery or activation of drugs at specific sites to reduce side effects and increase pharmacological response has received increased emphasis recently. Implantable pumps,[2,3] adhesive patches impregnated with drugs,[3] vesicle-enclosed drugs,[4,5] and drug carriers[6] have all been proposed to achieve site-specific drug delivery. Prodrugs[7,8] have also been used in drug-delivery systems. A prodrug is inactive as administered, but its physicochemical properties permit its activation in vivo once it reaches its target. Prodrugs have been used to deliver drugs systemically to the kidneys,[9,10] brain,[11,12] breasts,[13] and central nervous system[14,15] and topically to the eyes[16] and skin.[17,18] Prodrugs are also currently being designed for use in cancer chemotherapy.[19–21] In all these cases, the prodrug is converted to the parent drug chemically or by specific enzyme(s) at the target site.

Colon-specific delivery of bioactive compounds is known to occur in man. In the plant kingdom, a great many compounds are found as glycosides.[22–32] Upon ingestion, many of these glycosides pass through the upper intestine and into the colon. Once there, the glycosidases of the colonic microflora liberate aglycons, which can then act on the colon. Certain sulfa drugs[33] are now known to be

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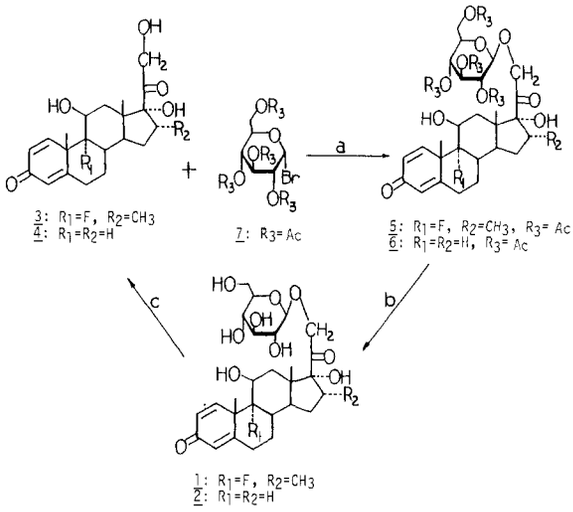
Scheme I. Preparation and Enzymatic Hydrolysis of Compounds 1 and 2[a]



[a] a = Ag_2CO_3, molecular sieve, CCl_4. b = 0.01 N NaOH, MeOH. [c] β-Glucosidase.

activated by the azo-reductase activity of the colonic microflora. A prodrug system based on polymers has also been developed[34,35] whereby certain aromatic amines are released by reduction of an azo link between the drug and polymeric carrier.

Despite the well-documented importance of the ability of the gut microflora to hydrolyze glycosides,[36] it appears that the deliberate synthesis of drug glycosides to utilize the unique glycosidase activity in the colon has not been reported. Such a system should derive its site specificity from release of the drug in the colon by the appropriate bacterial enzymes.

Glycosides of drugs are larger and usually more hydrophilic than the drugs themselves. These properties tend to reduce penetration across biological membranes.[37-40] If an orally administered drug glycoside is not cleaved by the digestive enzymes of the upper intestine, it will pass unabsorbed into the colon, where bacterial glycosidases can hydrolyze it, resulting in the liberation of the lipophilic drug to be absorbed by the colonic mucosa. This prodrug delivery system is unlike most other prodrug systems in that release of the parent drug is mediated by bacterial glycosidases rather than a specific enzyme present in the target tissue.

A colon-specific (or in the case of the laboratory rat, *cecum-specific*) drug-delivery system has been tested with two steroid prodrugs, dexamethasone 21-β-D-glucoside (1) and prednisolone 21-β-D-glucoside (2). These prodrugs may be useful in treating inflammatory bowel disease.

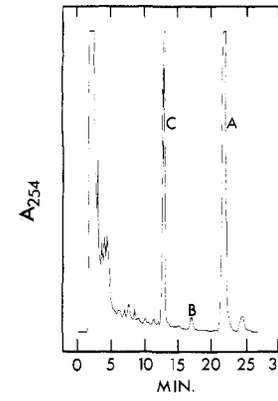


Figure 1. HPLC chromatogram of the cecal contents of a rat given 7.5 mg of 1 and sacrificed 6 h later. Compound 1, 3, or 4 was added as an internal standard prior to homogenization: peak A, 1; peak B, 3; peak C, 4. The Altex 5-μm Ultrasphere, C-18 column was eluted with MeOH/0.01 M KH_2PO_4 (56.5:43.5) at a flow rate of 1.2 mL/min.

Table I. Recovery of Glucoside 1 and Steroid 3 from the Small Intestine and Cecum at Various Times After Administration of 7.5 mg of Glucoside 1[a]

	small intestine		cecum	
time, h	1 (glucoside), mg	3 (steroid), mg	1 (glucoside), mg	3 (steroid), mg
3	5.61	0.13	0.21	0.09
4	5.00	0.11	0.02	0.05
5	0.24	0.12	0.23	2.24
6	0.94	0.18	0.04	1.66

[a] Values represent the average of four animals.

When these glucosides were administered to the stomachs of rats, they reached the cecum in 4–5 h, where they were rapidly hydrolyzed. Delivery of dexamethasone (3) (via glucoside 1) to the rat lower intestine was more specific than that of prednisolone (4) (via glucoside 2): Nearly 60% of an oral dose of glucoside 1 reached the cecum, whereas less than 15% of glucoside 2 reached the cecum. In contrast, when free steroids 3 and 4 were administered orally, they were absorbed almost exclusively from the small intestine; less than 1% of either reached the cecum.

Results

Chemistry. Glycosylations of steroids 3 and 4 were carried out with a modified Koenigs–Knorr reaction.[43,44] Bromo sugar 7 was coupled to the appropriate steroid in CCl_4 in the presence of silver carbonate (Scheme I). Yields were 25% for acetyl glucoside 5 and 38% for 6. These yields are typical for this reaction.[45]

Proton NMR confirmed that the acetyl glucosides formed were β-linked. The anomeric proton (C-1′) exhibited a doublet at 4.18 ppm for acetyl glucoside 5 and 4.20 ppm for 6. The coupling constants were 8 Hz for both compounds. These resonance signals indicate a trans-diaxial relationship between the vicinal C-1′ and C-2′ protons.[46]

The acetyl protecting groups on the sugar residues of 5 and 6 were removed by treatment with 0.01 N NaOH. The ^1H NMR of these compounds again provided evidence of the stereochemistry at their anomeric carbons (β-linked).

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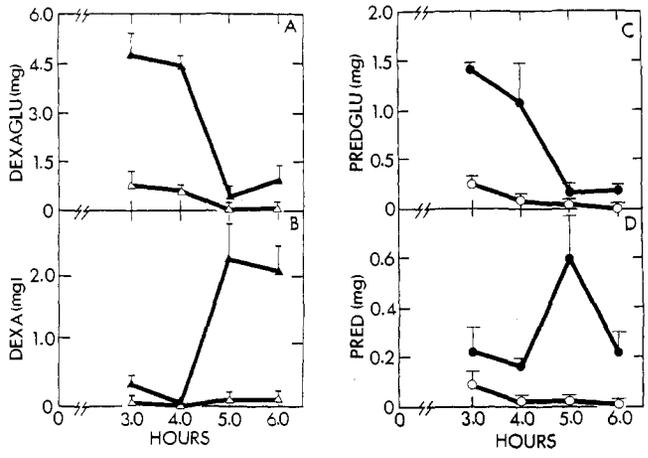

Figure 2. Recovery of steroid glycoside and free steroid (DEXAGLU = 1; DEXA = 3; PREDGLU = 2; PRED = 4) at various times after intragastric administration of 7.5 mg of the glycosides 1 (panels A and B) and 2 (panels C and D). Data are given as means plus or minus SEM ($n = 4$). Solid symbols (●, ▲) indicate recovery from intestinal contents and open symbols (○, △) indicate recovery from intestinal tissues.

Also, treatment of glucosides 1 and 2 with commercial β-glucosidase led to the removal of the glucose moiety in each case. Glucoside 2 was hydrolyzed several orders of magnitude faster than was glucoside 1. In addition, incubating the glucosides with homogenized rat feces resulted in extensive hydrolysis of both.

Separation of the glucosides from the free steroids was performed by HPLC. A typical chromatogram of the cecal contents of a rat given glucoside 1 intragastrically and sacrificed 6 h later is shown in Figure 1. Peak C is steroid 4, which was added prior to homogenization as an internal standard.

In Vivo Testing. The recovery of glucoside 1 and free steroid 3 from the small intestine and cecum at various times following oral administration of glucoside 1 is given in Table I. After 3 and 4 h, glucoside 1 was recovered primarily in the lower small intestine. By 5 h, very little of 1 was observed in either the small intestine or cecum. At the same time, large amounts of steroid 3 were recovered from the cecum. The recovery of glucoside 1 and steroid 3 from the intestinal contents and tissues is also shown graphically (Figure 2, panels A and B). As glucoside 1 passed from the lower small intestine into the cecum, the free drug was rapidly released. Some free steroid was detected in the small intestine at the times tested, indicating that some hydrolysis occurred before the prodrug reached the cecum or colon.

Overall, the delivery of glucoside 1 and subsequent release of steroid 3 in the rat cecum were quite specific. At 4 h, 59% of the administered dose of glucoside 1 was recovered from the lower small intestine contents unhydrolyzed. If all this glucoside passed into the cecum, then nearly 60% of the dose would have been delivered specifically to the cecum. At 5 h, an average of 2.24 mg (or an equivalent of 44% of the administered dose) was recovered in the cecum as free steroid. The difference (59 vs. 44%) is probably due to absorption of free drug by the cecal mucosa following hydrolysis of the prodrug in the cecum.

The recovery of glucoside 2 and free steroid 4 from the small intestine and cecum at various times following oral administration of glucoside 2 is given in Table II. Recovery of glucoside 2 after 3 and 4 h from the small intestine was much lower than that for glucoside 1. By 5 h, some free steroid was found in the cecum; however, the specificity was quite low. Free steroid 4 were detected in the small intestine at all time points tested, again indicating the presence of glycosidases in the rat small intestine. The recovery of glucoside 2 and steroid 4 from the intestinal contents and tissues is also presented graphically (Figure 2, panels C and D).

Table II. Recovery of Glucoside 2 and Free Steroid 4 from the Small Intestine and Cecum at Various Times After Administration of 7.5 mg of Glucoside 2[a]

	small intestine		cecum	
time, h	2 (glucoside), mg	4 (steroid), mg	2 (glucoside), mg	4 (steroid), mg
3	1.57	0.30	0.0	0.0
4	1.73	0.18	0.0	0.0
5	0.19	0.09	0.0	0.57
6	0.18	0.03	0.06	0.29

[a] Values represent the average of four animals.

Delivery of glucoside 2 was less efficient than that of glucoside 1. Only 14.8% of the administered dose of glucoside 2 could be recovered as such from the lower small intestine after 4 h. Therefore, only about 15% of the dose could have been delivered to the cecum. By 5 h, an average of 0.57 mg of steroid 4 (or an equivalent of 11% of the administered dose) was recovered in ceca. Again, the difference (14.8 vs. 11%) is probably due to absorption of steroid 4 into the systemic circulation following hydrolysis in the cecum.

No glucoside or free steroid was recovered from the colon of those animals tested. This was probably due to slow transit times and the fact that the time points tested following administration did not allow for any released steroid to pass into the rat colon. Transit times in this experiment were slower, but still close to the value of 6.6 ± 0.4 h reported for passage through the alimentary canal of rats fed a stock diet.[47]

The specificity of drug release was evaluated further by comparing the difference in free steroid recovered in the small intestine and in the cecum. A paired t test indicated that the preferential release of free steroid 3 in the cecum over that in the small intestine was statistically significant ($t = 2.32$; $p < 0.025$). A similar analysis of recoveries of steroid 4 showed that the preferential release of 4 in the cecum was not quite statistically significant ($t = 1.72$; $0.05 < p < 0.10$).

Intestinal transit times varied greatly, and in many cases the administered dose did not reach the cecum by the time of sacrifice. When these animals were excluded from the statistical calculations, the specificity of release was greater for both steroids 2 and 4. Measurable amounts of glucoside 1 reached the cecum in 11 of the 16 animals tested. Analysis of data from only these 11 animals showed that the preferential release of steroid 3 had high statistical significance ($t = 3.17$; $p < 0.01$). For glucoside 2, it was found that the administered dose reached the cecum in 8 of 16 animals tested. Data from these eight animals indicated that the preferential release of steroid 4 was statistically significant ($t = 3.94$; $p < 0.005$). However, the combined total recoveries of glucoside 2 and steroid 4 at 4 and 5 h was very low. Therefore, the efficiency of drug delivery to the cecum was very low, despite the calculated significance.

Control experiments in which unmodified steroids 3 and 4 were administered showed that they were absorbed almost completely from the small intestine (Figure 3).

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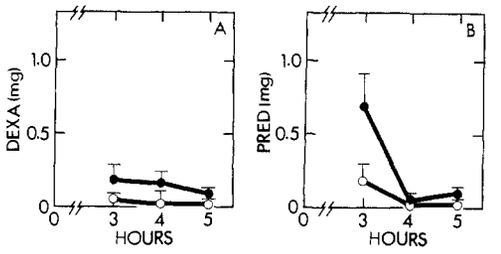

Figure 3. Recovery of steroid at various times after intrastric administration of the free steroids **3** (5.25 mg) and **4** (5.10 mg). Data points are means plus or minus SEM ($n = 3$) from intestinal contents (●) and tissues (○).

Discussion

Many recent studies have pointed to the importance of gut microflora in transforming a wide variety of naturally occurring glycosides, often into mutagenic compounds.[22-29,31] However, the use of glycosides to carry therapeutics to the large intestine, where they can be activated by gut bacteria, apparently has not been exploited.

Fecalase,[48] a cell-free extract of human feces, has been shown to contain various glycosidases capable of hydrolyzing a wide range of glycosides. This indicates that hydrolysis of a large variety of drug glycosides in the colon should be possible.

The system developed in this work is based on the release of antiinflammatory steroids from poorly absorbed steroid glycosides in the rat cecum. Despite the obvious anatomical differences between the laboratory rat and man, the rat cecum can be considered to be a satisfactory model for the proximal colon of man because both organs are recipients of digesta from the small intestine and both are sites of large bacterial populations and extensive microbial activity. Therefore, we have used the term "lower intestine" for either the combined rat cecum and colon or the human colon with its poorly defined cecal area.

The rat model suffers from the problem of a relatively high bacterial population and, subsequently, high level of glycosidase activity present in its stomach, upper small intestine, and lower small intestine. There are an average of $10^{7.7}$, $10^{6.9}$, and $10^{7.7}$ microorganisms/g wet weight in the rat stomach, upper small intestine, and lower small intestine, respectively. In contrast, the bacterial population in man's stomach and small intestine is much lower. There are only an average of 10^{0}, $10^{2.6}$, and $10^{4.2}$ microorganisms/g wet weight residing in the human stomach, upper small intestine, and lower small intestine, respectively.[36,50] Bacterial population of the large intestines of rat and man are more nearly comparable ($\sim 10^{8.3}$ microorganisms/g wet weight).[50] *Bacteroides* and *Bifidobacteria* are the bacterial species comprising the majority of microorganisms in the gastrointestinal system of both the laboratory rat and man. Both species have been shown to produce measurable quantities of β-glucosidase in vivo.[50]

Despite the high level of microbial activity in the rat upper intestine, glucoside **1** showed remarkable specificity toward the lower intestine. This prodrug should be even more specific when used in man because the microbial activity in human stomach and small intestine is much lower than that of the rat. Sulfasalazine, a prodrug used successfully in man, also requires activation by colonic microflora.[52,53] Similarities between these two prodrug delivery systems and the degree of specificity of glucoside **1** demonstrated in the rat model make it likely that certain drugs can be effectively delivered to the colon of man via glycoside prodrugs.

The relatively poor performance of glucoside **2** in the rat model could be due to several factors. Although it is possible that glucoside **2** was absorbed more extensively from the stomach and small intestine than was glucoside **1**, it was more likely that glucoside **2** was hydrolyzed to a greater extent in the stomach and small intestine than was glucoside **1**. Commercial β-glucosidase was much more active toward glucoside **2** than toward glucoside **1**. Similar factors may be functioning in the rat gastrointestinal tract. This point is currently under investigation with these and additional prodrugs.

Modification of the enzymatic activity of the gut microflora to improve delivery is a significant feature in this system. It has been shown that certain enzymes produced by gut bacteria are inducible with diet.[42,51] For instance, bean diets dramatically increase α-galactosidase activity in human subjects, while bran diets increase β-glucosidase activity. Manipulation of glycosidase activity by diet may be very useful in standardizing glycosidase activity and also in raising enzyme activity in patients with a diseased colon where enzyme levels may be depressed.

Modification of the glycon, aglycon, and glycosidic linkage can probably all be used to improve or alter the rate and location of drug release. Altering the sugar residue by modifying functionalities[49] or using an oligosaccharide carrier might hinder the rate of hydrolysis in vivo. Slow hydrolysis of refractory prodrugs in the colon might prove to be an effective mechanism for sustained release. Changing the aglycon, as was demonstrated in this work, can also alter delivery. Furthermore, the stereochemistry of the glycosidic link might be utilized to vary rates and sites of release. A sustained-release system for the small intestine could be based on α-glycosides cleaved by digestive enzymes. Absorption would then be moderated by the rate of release in the small intestine, as well as the physicochemical properties of the parent drug.

The steroids tested may be useful in treating inflammatory bowel disease. Other situations where this system might be useful can be imagined. The delivery of certain antineoplastic agents to the colon might be beneficial in controlling colon cancer. Antibiotics might be delivered specifically to the colon via glycoside carriers. In each of these cases, colon-specific delivery would allow the use of higher doses of more potent agents with fewer systemic side effects.

In summary, a colon-specific drug-delivery system has been developed based on drug glycosides and the unique glycosidase activity of the gut microflora. Of the two compounds tested, dexamethasone glucoside appeared to be a very good candidate to deliver dexamethasone specifically to the colon.

Experimental Section

All solvents were redistilled and dried over molecular sieves, 4Å, 4–8 mesh (Aldrich Chemical Co.) Dioxane was distilled from CaH_2 prior to use. All solvent evaporations were performed with a rotary evaporator with water aspirator reduced pressure. Melting

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points were obtained on a Buchi melting point apparatus and are uncorrected. UV spectra were determined on a Cary 210 spectrometer. IR spectra were determined on a Perkin-Elmer Model 137 spectrometer. ^{1}H NMR spectra were determined on a UCB 200 spectrometer (a home-made Fourier transform 200-MHz device located in the College of Chemistry, University of California, Berkeley) and were recorded in dimethyl-d_6 sulfoxide; they are expressed in parts per million (δ) downfield from Me_4Si, with coupling constants (J) expressed in hertz. Elemental analyses were performed by the Analytical Laboratory, College of Chemistry, University of California, Berkeley. Analyses were within ±0.4% of theoretical values, except where noted.

Chromatographic Analysis. High-pressure liquid chromatography (HPLC) was performed on an Altex analytical system consisting of two model 110A pumps, a model 160 UV detector, a model 420 microprocessor controller/programmer and a stainless-steel column (4.6 mm × 25 cm, 5 μm Ultrasphere C-18). A flow rate of 1.2 mL/min was used, with absorbance monitoring at 254 nm. The solvent system for all separations was methanol/0.01 M KH_2PO_4 (56.5:43.5). Low-pressure preparative chromatography (flash chromatography, J. T. Baker Chemical Co.) was performed with either a 3.7 × 20 cm column of 40 μm RP-18 with methanol/water (7:3) as eluent or a 3.0 × 18 cm column of 40 μm silica gel with $CHCl_3$/95% EtOH (65:35) as the eluent. TLC was performed on aluminum-backed plates of silica gel 60 (E. Merck). Steroids and their glycosides were identified by spraying the developed plates with *p*-toluenesulfonic acid/95% EtOH (2:8, w/v) and heating for 10 min at 110 °C. Dexamethasone, prednisolone, 2,3,4,6-tetra-*O*-acetyl-1-bromo-α-D-glucopyranose and β-glucosidase were purchased from Sigma Chemical Co.

Preparation of 9α-Fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (5). Dexamethasone (**3**; 1.20 g, 3.1 mmol) was dissolved in hot dioxane (30 mL) and added to dry, boiling CCl_4 (200 mL) over 4Å molecular sieves in a 500-mL round-bottom flask. After 10–20 mL had been distilled, freshly prepared[41] Ag_2CO_3 (3.9 g, 14.1 mmol) was added to the flask. Then a solution of 2,3,4,6-tetra-*O*-acetyl-1-bromo-α-D-glucopyranose (**7**; 3.5 g, 8.5 mmol) in dry CCl_4 (100 mL) was added dropwise from an additional funnel. The reaction mixture was protected from light and stirred throughout. The addition of bromo sugar took approximately 1 h, and the solvent was distilled continuously during that time. Distillation was continued for an additional hour after all the bromo sugar had been added; the volume was maintained by the addition of dry CCl_4. The solution was filtered, washed with cold saturated NaCl solution, and dried with Na_2SO_4, and the solvent was removed. The oily residue was dissolved in several milliliters of MeOH and purified by flash chromatography on RP-18. The appropriate fractions were collected, and the solvent was removed. Acetyl glucoside **5** was crystallized from MeOH/water to yield 0.55 g (25%): mp 119.5–121 °C; TLC R_f 0.45 (ethyl acetate/isooctane, 9:1); UV λ_{max} 239 nm (ε 14 300); IR (KBr) 3450 (OH), 1760 (OAc), 1650 (C=O), 1190 (OAc), 896 cm^{-1}; ^{1}H NMR δ 0.76 (d, 3 H, C-16 α-CH_3, J = 6 Hz), 0.88 (s, 3 H, C-18), 1.49 (s, 3 H, C-19), 2.00 (s, 9 H, C-2′, C-3′, and C-4′ OAc), 2.09 (s, 3 H, C-6′ OAc), 4.18 (d, 1 H, C-1′, J = 8 Hz), 4.57 (AB q, 2 H, C-21, J = 18 Hz), 6.02 (s, 1 H, C-4), 6.23 (d, 1 H, C-1, J = 11 Hz), 7.33 (d, C-2, J = 11 Hz). Anal. Calcd for $C_{36}H_{47}O_{14}F$: C, 59.75; H, 6.63. Found: C, 59.08; H, 6.54.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (6). Acetyl glucoside **6** was prepared from prednisolone (**4**) as described for acetyl glucoside **5** from steroid **3** and bromo sugar **7**. Acetyl glucoside **6** was crystallized from MeOH/water to yield 0.87 g (38%): mp 119–121 °C; TLC R_f 0.36 (ethyl acetate/isooctane 9:1); UV λ_{max} 242 nm (ε 13 500); IR (KBr) 3450 (OH), 1760 (OAc), 1650 (C=O), 1190 (OAc), 891 cm^{-1}; ^{1}H NMR: δ 0.77 (s, 3 H, C-18), 1.45 (s, 3 H, C-19), 1.99 (s, 9 H, C-2′, C-3′, and C-4′ OAc), 2.08 (s, 3 H, C-6′ OAc), 4.20 (d, 1 H, C-1′, J = 8 Hz), 4.58 (AB q, 2 H, C-21, J = 18 Hz), 5.92 (s, 1 H, C-4), 6.15 (d, 1 H, C-2, J = 11 Hz), 7.40 (d, 1 H, C-1, J = 11 Hz). Anal. ($C_{35}H_{46}O_{14}$) C, H.

Preparation of 9α-Fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl β-D-Glucopyranoside (1). Acetyl glucoside **5** (0.2 g, 0.36 mmol) was dissolved in MeOH (5.0 mL) and benzene (5.0 mL). NaOH in MeOH (0.04 N, 5.0 mL) was then added. The reaction was run under N_2 at room temperature with stirring. After 45 min, several drops of acetic acid were added to neutralize the solution. The solvent was removed, and the residue was purified by flash chromatography on silica gel. The appropriate fractions were combined, and the residue was dissolved in a *tert*-butyl alcohol/water (15 mL, 1:1) solution. This solution was frozen, and the solvent was removed by lyophilization to give 0.12 g (75%) of glucoside **1**: TLC R_f 0.51 ($CHCl_3$/95% EtOH, 7:3); UV λ_{max} 239 nm (ε 14 500); IR (KBr) 3450 (OH), 1650 (C=O), 896 cm^{-1}; ^{1}H NMR δ 0.78 (d, 3 H, C-16 α-CH_3, J = 7 Hz), 0.88 (s, 3 H, C-18), 1.49 (s, 3 H, C-19), 4.17 (d, 1 H, C-1′, J = 18 Hz), 4.57 (AB q, 2 H, C-21, J = 18 Hz), 6.03 (s, 1 H, C-4), 6.23 (d, 1 H, C-1, J = 11 Hz), 7.35 (d, 1 H, C-2, J = 11 Hz). Anal. ($C_{28}H_{39}O_{10}F\cdot H_2O$) C, H.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl β-D-Glucopyranoside (2). Glucoside **2** was prepared from acetyl glucoside **6** as described for glucoside **1** from acetyl glucoside **5**. Glucoside **2** was recovered by removing the solvent by lyophilization to yield 0.11 g (73%): TLC R_f 0.58 ($CHCl_3$/95% EtOH, 7:3); UV λ_{max} 242 nm (ε 13 200); IR (KBr) 3450 (OH), 1650 (C=O), 896 cm^{-1}; ^{1}H NMR: δ 0.77 (s, 3 H, C-18), 1.45 (s, 3 H, C-19), 4.20 (d, 1 H, C-1′, J = 8 Hz), 4.58 (AB q, 2 H, C-21, J = 18 Hz), 5.92 (s, 1 H, C-4), 6.15 (d, 2 H, C-1, J = 11 Hz), 7.40 (d, 1 H, C-2, J = 11 Hz). Anal. ($C_{27}H_{38}O_{10}\cdot H_2O$) C, H.

In Vitro Testing. Glucoside **1** (5.0 mg) of **2** (5.0 mg) was incubated at 37 °C in a 0.05 M acetate buffer, pH 5.0 (10 mL), with β-glucosidase (EC 3.2.1.21, from almonds, 1 unit liberates 1.0 μmol of glucose from salicin per minute at pH 5.0 and 37 °C). Glucosides **1** and **2** were treated with 500 and 50 units of enzyme, respectively. At various times, aliquots (0.1 mL) were removed and quenched with MeOH (9.9 mL). After centrifugation (5000*g*, 10 min), the samples were diluted (1:1) with 0.01 M KH_2PO_4, and 20 μL of the resulting solution was injected directly onto the HPLC column for analysis.

Glucosides **1** and **2** (5.0 mg) were also incubated at 37 °C with homogenized rat feces (0.5 g/10 mL 0.01 M phosphate buffer, pH 7.5). The feces were obtained from rats maintained on a high cellulose diet.[42] Aliquots (0.2 mL) were removed and quenched with MeOH (3.66 mL). After centrifugation (5000*g*, 10 min), the samples were passed through membrane filters (0.45 μm pore, Versapor 450, Gelman Sciences, Inc.). They were then diluted (1:1) with 0.01 M KH_2PO_4. Twenty microliters of the resulting solution was injected directly onto the HPLC column for analysis.

In Vivo Testing. Male, Sprague–Dawley rats (ca. 250 g) were maintained on a stock diet (Purina rat chow) and water ad libitum. These animals were fasted overnight (16 h) prior to administration of the glucoside or free steroid. Water bottles were removed from the cages at least 30 min prior to drug administration, to assure that the rats' stomach would be empty. Glucoside **1** (7.5 mg) or **2** (7.5 mg) was administered by gastric intubation as a solution (0.5 mL) in water/95% EtOH (3:1). Steroid **3** (5.1 mg) or **4** (5.25 mg) was administered as a solution (0.5 mL) of water/95% EtOH (1:1). After an appropriate time interval (3, 4, 5, or 6 h), the animals were sacrificed by CO_2 anesthesia, followed by thoracotomy. The small intestine and the cecum were removed and cut into segments. Because postmortem peristalsis might have pushed some of the cecal contents into the proximal colon, 0.5–1.0 cm of the colon was removed along with the cecum. The colon (8–10 cm) proximal to the cecum of some of the animals was also analyzed for steroid in the same manner. Intestinal contents were separated from the tissues by rinsing the segments with cold, 0.9% saline (5.0 mL). The contents were immediately diluted to 30 mL with MeOH. The tissues were suspended in 0.01 M KH_2PO_4 (5.0 mL). Then an internal standard, either steroid **4** or **3**, depending on the experimental steroid, was added to all the samples. Contents and tissues were then homogenized with a Polytron homogenizer (Brinkman Instrument Co.) at medium speed for 1–2 min. The contents were then diluted to 40 mL total volume with MeOH. The tissues were diluted to 25 mL total volume with MeOH. All the samples were centrifuged (5000*g*, 15 min), and then the supernatant solutions were passed through membrane filters (0.45 μm, Versapor 450). The samples (1.4 mL) were then diluted with 0.01 M KH_2PO_4 (0.6 mL), and 20 μL of the resulting solution was injected directly onto the HPLC column for analysis.

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Synthesis and Biological Activity of Nucleosides and Nucleotides Related to the Antitumor Agent 2-β-D-Ribofuranosylthiazole-4-carboxamide

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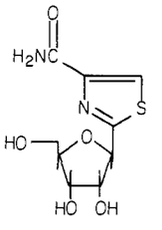
Phosphorylation of 2-β-D-ribofuranosylthiazole-4-carboxamide (1) provided the 5′-phosphate derivative **2**, which was converted to the corresponding 5′-triphosphate **4** and the cyclic 3′,5′-phosphate **5**. Treatment of 2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)thiazole-4-carbonitrile (**6**) with NH_3–NH_4Cl provided 2-β-D-ribofuranosylthiazole-4-carboxamidine hydrochloride (**7**), and treatment with H_2S–pyridine provided the corresponding 4-thiocarboxamide **9**. Compound **9** was treated with ethyl bromopyruvate, followed by treatment with methanolic ammonia, to yield 2′-(2-β-D-ribofuranosylthiazol-4-yl)thiazole-4′-carboxamide (**11**). 5′-Phosphate **2** was cytotoxic to L1210 cells in culture and significantly effective against the intraperitoneally implanted murine leukemias in mice. Amidine **7** was slightly toxic to L1210 in culture and inhibitory to purine nucleoside phosphorolysis. The cyclic 3′,5′-phosphate **5** was less effective than the corresponding 5′-phosphate **2** or the parent nucleoside **1** as an antitumor agent.

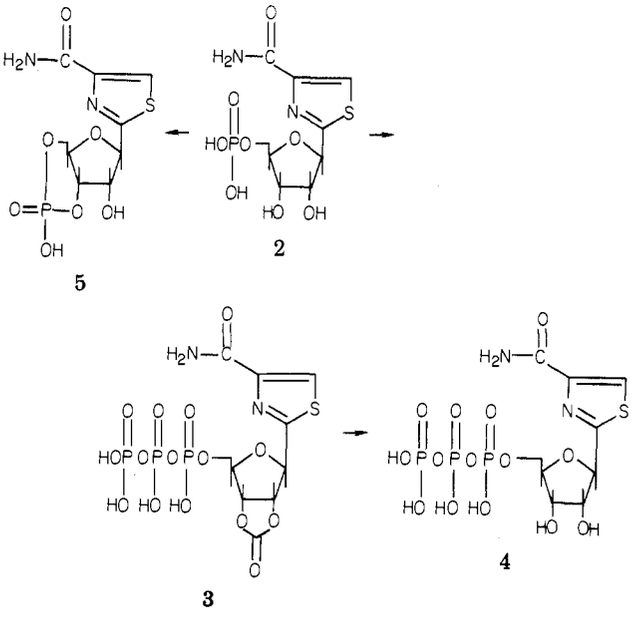
Tiazofurin,[1] 2-β-D-ribofuranosylthiazole-4-carboxamide (**1**), is a C-nucleoside previously reported by us.[2] Tiazofurin has shown potent antitumor activity in animals and is currently being pursued by the National Cancer Institute as a high-priority candidate for clinical trials with potential importance for treatment of lung tumors and metastases.[3]



1

Studies of the mechanism of action and metabolism of this new oncolytic nucleoside were subsequently initiated and have recently been published.[4] The nucleoside **1** would be expected to be metabolized in vivo via the 5′-monophosphate to the corresponding 5′-triphosphate. The syntheses of these and other analogues of **1** were therefore initiated to make these compounds available for biological evaluation. The metabolic formation of 2-β-D-ribofuranosylthiazole-4-carboxamide 5′-phosphate (**2**) was first observed[4] when the parent nucleoside **1** was incubated with P-388 cells in culture and the nucleoside pools were examined by HPLC. The 5′-phosphate **2**, isolated by HPLC, was found to be identical with an authentic sample[4] of **2**, first synthesized by us and supplied to Dr. David G. Johns for comparison and to Dr. Ven L. Narayanan for antitumor screening. Compound **2** was synthesized in our laboratory by phosphorylation of **1** with trichloropyrophosphopyridinium chloride[5] generated in situ via the treatment of phosphoryl chloride with pyridine and water in acetonitrile. This method of 5′-phosphorylation of carboxamide-bearing nucleosides was found superior[5] to the method utilizing trimethyl phosphate–phosphoryl chloride.

Scheme I



5 **2** **3** **4**

The latter reagent also reacts with the carboxamide group, resulting in the formation of unidentified side products. The synthesis of **2** has also been recently reported by Johns and co-workers.[6]

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(1) Generic name given to compound 1.
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