Drug Glycosides: Potential Prodrugs for Colon-Specific Drug Delivery

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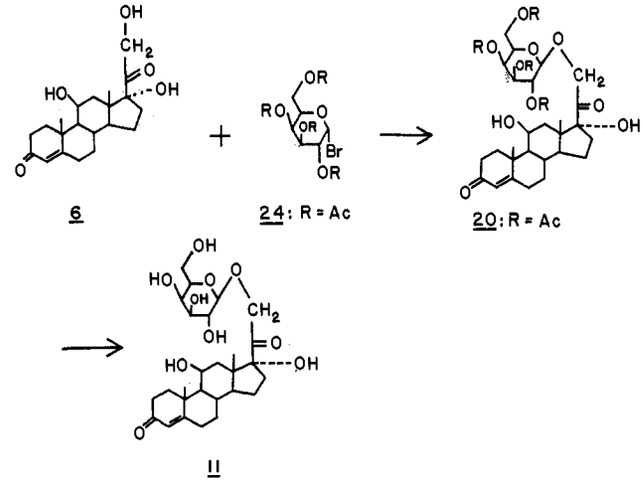
The influence of prodrug structure on specificity of glycoside/glycosidase based colon-specific drug delivery was studied by preparing nine steroid glycosides, measuring their relative lipophilicities, and hydrolyzing them with bacterial glycosidases from rat intestines. The 21-yl β-D-glucosides and galactosides of dexamethasone, prednisolone, hydrocortisone, and fludrocortisone and the 21-yl β-D-cellobioside of prednisolone were prepared by a modified Koenigs–Knorr reaction. The deacetylated glycoside prodrugs, along with the *p*-nitrophenyl derivatives of β-D-glucoside, galactoside, and cellobioside, were subjected to hydrolysis by the contents of the rat stomach, proximal small intestine (PSI), distal small intestine (DSI), and cecum. All the prodrugs were hydrolyzed slowly by PSI and stomach contents, more rapidly by contents of the DSI, and most rapidly by cecal contents. This is the basis of the site-specific drug delivery reported earlier (Friend, D. R.; Chang, G. W. *J. Med. Chem.* **1984**, *27*, 261). Furthermore, the prodrugs themselves had very different susceptibilities to hydrolysis. Hydrolysis rates catalyzed by DSI contents decreased in the following order: prednisolon-21-yl β-D-galactoside (**10**) > prednisolon-21-yl β-D-glucoside (**2**) > prednisolon-21-yl β-D-cellobioside (**13**) > dexamethason-21-yl β-D-galactoside (**9**) > dexamethason-21-yl β-D-glucoside (**1**). Hydrolysis of cellobioside **13** was only half that of glucoside **2** and one-fourth that of galactoside **10**. Hydrolysis of all the prodrugs in cecal contents was rapid, with the exceptions of hydrocortison-21-yl β-D-glucoside (**5**) and fludrocortison-21-yl β-D-glucoside (**7**), which were hydrolyzed more slowly than the other glucoside prodrugs. Eadie–Hofstee plots for hydrolysis of the glucoside compounds suggested that bacterial β-D-glucosidase activity in the colon may be more heterogeneous in nature than β-D-galactosidase activity. Relative lipophilicities of the prodrugs and free steroids were compared by measuring their octanol–buffer partition coefficients (*P*). The logarithm of the *P* of cellobioside **13** (−0.56) was considerably lower than that of the other prodrugs, which ranged from 0.11 to 0.84. Log *P* of the free steroids ranged from 1.54 to 1.73. These relative rates of hydrolysis and relative lipophilicities, along with previously reported animal experiments, enable one to estimate the site specificity of glycoside prodrugs prior to extensive animal studies.

Site-specific drug delivery offers several benefits over traditional drug therapy. Side effects can be reduced and pharmacological response increased if the active drug can be delivered specifically to its site of action. To this end, prodrugs[2] have been developed for use in a number of site-specific drug-delivery systems. A prodrug is a latent form of an active drug with certain physicochemical properties that allow it to reach the target organ or tissue. Once there, the active drug is formed chemically or enzymatically in situ.

Colon-specific drug delivery of bioactive compounds is known to occur naturally[3] in man through the liberation of aglycons from poorly absorbed plant glycosides following ingestion. Release takes place in the colon, mediated by glycosidases produced by colonic bacteria. In addition, the azo-reductase activity of colon bacteria has been utilized to deliver pharmacons to the colon: sulfasalazine[4] and a polymer-based prodrug[5] system both deliver, after reduction of their azo bonds, 5-aminosalicylate to the colon.

Scheme I



Despite the well-documented importance of the ability of the gut microflora to hydrolyze glycosides[6] (and to reduce azo bonds), only recently were drug glycosides prepared to exploit the unique glycosidase activity in the colon.[7] A glycoside/glycosidase based delivery system should derive its site specificity from the colonic location of intestinal microflora and their unique glycosidases.

Glycosides of drugs are larger and usually more hydrophilic than the drugs themselves. These properties tend to reduce penetration across biological membranes.[8,9] If

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an orally administered drug glycoside is not cleaved by digestive enzymes of the upper intestine, it should pass unabsorbed into the large intestine (i.e., the colon), where bacterial glycosidases can hydrolyze the glycoside prodrug.

Initial studies of a glycoside/glycosidase based delivery system in the laboratory rat indicated that certain anti-inflammatory steroids can be delivered to the lower intestine with varying degrees of specificity, depending on the aglycon. In order to develop a better understanding of the factors controlling specificity, the kinetics of release of aglycons from three *p*-nitrophenyl glycosides and nine steroid glycoside prodrugs were measured with use of freshly prepared homogenates from contents of the rat stomach, proximal small intestine (PSI), distal small intestine (DSI), and cecum. Gastrointestinal absorption, as predicted by octanol–buffer partition coefficients,[10] was also investigated. The results of these experiments, along with previously published animal studies,[7] enable one to estimate the degree of site specificity of glycoside prodrugs toward the large intestine.

Results

Chemistry. Glycosylations of all steroids were carried out with a modified Koenigs–Knorr reaction.[11] Bromo sugars were coupled to the appropriate steroid in dry $CHCl_3$ in the presence of freshly prepared Ag_2CO_3.[12] Yields for this step ranged from 23% for the formation of acetyl glycosides HYDTAGLU (**16**), FLUTAGLU (**17**), and FLUTAGAL (**21**) to 37% for acetyl galactoside PREDTAGAL **19**. Acetyl cellobioside PREDTACEL **22** was prepared by first brominating octa-*O*-acetyl-α-D-cellobiose in a 31% solution of HBr in acetic acid, and coupling the product, bromocellobiose **25**, to prednisolone (**4**) (25% yield). The yields obtained are typical for the Koenigs–Knorr reaction.[14] Scheme I shows the preparation of HYDGLU (**11**) from hydrocortisone (**6**) and bromo sugar **24**.

Proton NMR spectroscopy of the acetyl glycosides indicated a β-linkage at their anomeric carbons. All the compounds exhibited a doublet at approximately 4.2 ppm for the anomeric proton with coupling constants ranging from 7.2 to 8.0 Hz. These resonance signals indicate a trans-diaxial relationship between the C-1′ and C-2′ protons.[15]

Removal of the acetyl protecting groups on the sugar residues was accomplished by base-catalyzed hydrolysis using 0.01 N NaOH in MeOH. Yields ranged from 60% to 83% for this step. ^1H NMR spectroscopy confirmed that the free glycosides were still β-linked.

In Vitro Metabolism. Total activities (μmol/min) and specific activities (nmol min^{-1} g^{-1}) were measured in stomach, PSI, DSI, and cecal content homogenates with use of three *p*-nitrophenyl glycoside substrates. This was accomplished by following the release of *p*-nitrophenol from *p*-NP-glc (via β-D-glucosidase), *p*-NP-gal (via β-D-galactosidase), and *p*-NP-cel (via β-D-cellobiosidase and β-D-glucosidase). Results of these measurements are shown in Table I. Release of *p*-nitrophenol from all three substrates in all four segments tested indicated the presence of glycosidase activity all along the rat gastrointestinal tract. Total activities of each glycosidase were generally lowest in the stomach and PSI, higher in the DSI, and higher still in the cecum. Specific activities of the three glycosidases followed the same general pattern, with highest activities found in the cecum. In addition to the gradient of glycosidase activity along the gastrointestinal tract, there were differences in enzyme levels. Specific activity of cecal β-D-galactosidase was about 4 times that of cecal β-D-glucosidase and about 16 times that of cecal β-D-cellobiosidase.

Table I. Hydrolysis of *p*-NP-glc, *p*-NP-gal, and *p*-NP-cel by the Contents of Different Segments of Rat Intestine[a]

	substrate					
	p-NP-glc		*p*-NP-gal		*p*-NP-cel	
intestinal segment	tot. act.	sp act.	tot. act.	sp act.	tot. act.	sp act.
stomach[b]	0.08	72.2	0.70	57.8	0.35	34.2
PSI[c]	0.15	43.2	0.20	58.3	0.05	7.2
DSI	0.65	86.6	3.6	425	0.25	26.0
cecum	9.1	454	30.8	1620	2.6	96.5

[a] Tot. act.: activity of contents of the entire intestinal segment, expressed as μmol/min. Sp act.: specific activity of contents of the intestinal segment, expressed as nmol min^{-1} g wet weight. [b] pH of stomach homogenates was ca. 5.0. [c] pH of PSI, DSI, and cecal homogenates was 7.0.

Total activities and specific activities for hydrolysis of all nine glycoside prodrugs were also measured. Results of those measurements are given in Table II (glucosides DEXGLU (**1**), PREDGLU (**2**), HYDGLU (**5**), and FLUGLU (**7**)) and Table III (galactosides DEXGAL (**9**), PREDGAL (**10**), HYDGAL (**11**), FLUGAL (**12**), and cellobioside PREDCEL (**13**)). Total and specific activities for the hydrolysis of all prodrugs were relatively low in the stomach and PSI contents. They increased in the DSI and were highest in the cecal content homogenates. This pattern follows that found for the hydrolysis of the *p*-NP-glycosides; however, in all homogenates tested, total and specific activities for hydrolysis of each type of prodrug (glucoside, galactoside, and cellobioside) were considerably lower than were those of the corresponding *p*-NP-glycosides.

The galactoside prodrugs were usually hydrolyzed more rapidly than were the glucosides or cellobioside prodrugs in each intestinal segment. This was probably due to higher levels of β-D-galactosidase activity in the rat gastrointestinal tract. Hydrolysis of cellobioside **13** (PREDCEL) was considerably slower than that of glucoside **2** (PREDGLU) and galactoside **10** (PREDGAL), all of which are derivatives of prednisolone. This result, as well as that obtained with *p*-NP-cel as a substrate, indicates that β-D-cellobiosidase activity in the rat intestinal tract is relatively low. HPLC analysis of the hydrolysis products of cellobioside **13**, following 15 min of incubation with the various homogenates, showed that formation of prednisolone glucoside was almost twice that of free prednisolone, indicating that much of cellobioside **13** was converted to glucoside **2**, which was then further hydrolyzed to release free steroid **4**.

$K_{M(app)}$ and V_{max} Determinations. Eadie–Hofstee plots (V vs. $V/[S]$) for hydrolysis of *p*-NP-glc and *p*-NP-gal in homogenates of pooled cecal contents from four animals are presented in Figures 1 and 2. At lower substrate concentrations, the plot of the velocity–substrate rela-

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Table II. Hydrolysis of Glucoside Prodrugs DEXGLU (1), PREDGLU (2), HYDGLU (5), and FLUGLU (7) by the Contents of Different Segments of Rat Intestine[a]

	prodrug							
	DEXGLU		PREDGLU		HYDGLU		FLUGLU	
intestinal segment	tot. act.	sp act.	tot. act.	sp act.	tot. act.	sp act.	tot. act.	sp act.
stomach	0.12	6.8	0.12	6.8	0.09	5.5	0.14	7.7
PSI	0.07	11.0	0.07	12.2	0.10	15.9	0.08	12.3
DSI	0.34	19.3	1.0	56.8	0.70	39.2	0.43	33.0
cecum	3.5	141	6.6	265	0.76	30.5	1.4	42.0

[a] Measured by following the release of the free steroid by HPLC. Abbreviations and units are the same as those in Table I.

Table III. Hydrolysis of Galactoside Prodrugs DEXGAL (9), PREDGAL (10), HYDGAL (11), FLUGAL (12), and Cellobioside Prodrug PREDCEL (13) in Contents of Different Segments of Rat Intestine[a]

	prodrug									
	DEXGAL		PREDGAL		HYDGAL		FLUGAL		PREDCEL[b]	
intestinal segment	tot. act.	sp act.	tot. act.	sp act.	tot. act.	sp act.	tot. act.	sp act.	tot. act.	sp act.
stomach	0.004	2.0	0.19	12.1	0.02	12.5	0.01	7.0	0.01	7.5
PSI	0.004	11.7	0.23	49.3	0.02	42.5	0.01	12.5	0.11	26.0
DSI	0.41	29.3	1.72	122	1.6	110	0.80	56.7	0.64	32.5
cecum	6.5	322	13	665	11.9	592	12.9	642	2.2	109

[a] Measured by following the release of the free steroid by HPLC. Abbreviations and units are the same as in Table I. [b] Expressed as release of free steroid 4.

Table IV. $K_{m(app)}$ (μM) and V_{max} (μmol min^{-1} g^{-1}) for Hydrolysis of *p*-NP-glc, *p*-NP-gal, and Prodrugs 1, 2, 5, 7, and 9–12[a]

substrate	$K_{m(app)}$	V_{max}	substrate	$K_{m(app)}$	V_{max}
p-NP-glc[b]	360	697	FLUGLU (7)	4.4	2.4
p-NP-gal[b]	166	1233	DEXGAL (9)	13.3	3.9
DEXGLU (1)[c]	2.5	2.2	PREDGAL (10)	20.0	10.3
PREDGLU (2)	4.5	2.6	HYDGAL (11)	16.7	10.2
HYDGLU (5)	5.7	2.6	FLUGAL (12)	33.3	20.6

[a] Measurements made in pooled cecal content homogenates. [b] Reaction followed by measuring the release of *p*-nitrophenol spectrophotometrically at 403 nm. [c] Measured by following the release of free steroid using HPLC.

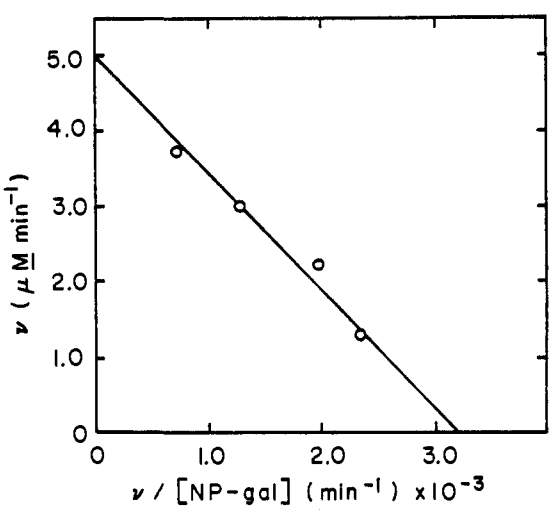

Figure 1. Eadie–Hofstee plot for hydrolysis of *p*-NP-gal by cecal contents. Hydrolysis was followed by measuring the release of *p*-nitrophenol spectrophotometrically at 403 nm.

tionship for hydrolysis of *p*-NP-glc deviated from linearity. Hydrolysis of *p*-NP-gal was, however, essentially linear. The K_M–V_{max} data for hydrolysis of the *p*-NP-glc, *p*-NP-gal, and the glucoside and galactoside prodrugs are given in Table IV. The galactoside prodrugs all showed very good linearity in the Eadie–Hofstee plots. Like that of *p*-NP-glc, the plots for DEXGLU (1) and HYDGLU (5) deviated from linearity at lower substrate concentrations; however, the deviation was not as great as that observed in plots for *p*-NP-glc.

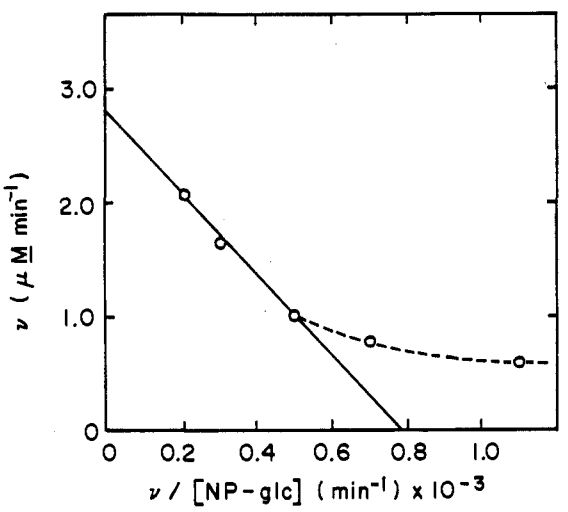

Figure 2. Eadie–Hofstee plot for hydrolysis of *p*-NP-glc by cecal contents. Hydrolysis was followed by measuring the release of *p*-nitrophenol spectrophotometrically at 403 nm.

Table V. Apparent 1-Octanol–Buffer Partition Coefficients (Log *P*)[a]

compd	log *P*	compd	log *P*
DEXGLU (1)	0.59	FLUGAL (12)	0.25
PREDGLU (2)	0.27	PREDCEL (13)	−0.56
HYDGLU (5)	0.44	dexamethasone (3)	1.72[b]
FLUGLU (7)	0.84	prednisolone (4)	1.55[c]
DEXGAL (9)	0.49	hydrocortisone (6)	1.54[d]
PREDGAL (10)	0.11	fludrocortisone (8)	1.73[e]
HYDGAL (11)	0.15		

[a] Agitated for 30 min at 37 °C with concentration determined in the aqueous phase. [b] Lit.[10] values: 1.90, 1.59 (both using diethyl ether as organic phase). [c] Lit.[10] value: 1.42. [d] Lit.[10] values: 1.53, 0.96 (diethyl ether), 0.89 (benzene), 1.93 (isobutyl alcohol). [e] Lit.[10] value: 1.68.

Partition Coefficients. The apparent 1-octanol–buffer (0.01 M phosphate buffer, pH 7.0) partition coefficients (*P*) were measured at 37 °C. The results of these measurements, expressed as log *P*, are given in Table V. Cellobioside 13 (PREDCEL) had the lowest log *P* (−0.56) of any of the prodrugs tested. The galactoside prodrugs all had lower log *P* values than those of the corresponding glucoside prodrugs. As expected, the log *P* values for the

free steroids were much greater than those of the glycosides.

Discussion

Delivering drugs specifically to the lower intestine through the use of polar, poorly absorbed glycoside prodrugs has recently been reported.[7] Following oral administration, such prodrugs pass through the stomach and small intestine without appreciable loss due to absorption. Once a prodrug reaches the lower intestine (i.e., the colon), the polar sugar residue is released by the hydrolytic activity of bacterial glycosidases, thus liberating the pharmacon in the large intestine. Many other studies have pointed to the importance of gut microflora in transforming a wide variety of naturally occurring plant glycosides, often into mutagenic substances.[3] Fecalase,[16] a cell-free extract of human feces, has been found to contain glycosidases capable of hydrolyzing a wide variety of glycosides.

This report presents a more thorough study of the glycoside/glycosidase based delivery system. Seven glycoside prodrugs were prepared and characterized for this purpose; two prodrugs previously tested in vivo were also studied in these in vitro investigations.

Hydrolysis of all prodrugs was relatively slow when incubated with homogenates of contents from the stomach and PSI, faster in the DSI homogenates, and fastest in the cecum. Loss of prodrugs through hydrolysis in the stomach and PSI is probably negligible. Transit through this portion of the rat gastrointestinal tract is quite rapid: transit times can be as low as 40 min. This combination of low enzyme activity and rapid transit in the rat stomach and PSI means that specificity of delivery is probably a function of glycosidase activity and residence time in the DSI. Transit slows considerably in the DSI, increasing the possibility of premature hydrolysis and less specific drug delivery to the rat cecum.

Differences in total and specific activities became apparent when each prodrug was incubated with the homogenates of the DSI contents. Dexamethasone prodrugs 1 and 9 (DEXGLU and DEXGAL, respectively) were more resistant to hydrolysis than the other prodrugs. Of the two glucosides tested in vivo, DEXGLU (1) was found to be hydrolyzed about 3 times slower in the homogenates of the DSI contents than was PREDGLU (2). In animal tests, DEXGLU (1) was delivered to the rat lower intestine about 4 times more specifically than was PREDGLU (2) (59% for 1 and 14% for 2).[7] Therefore, there was a rough correlation between total and specific activities in homogenates of DSI contents and specificity of delivery observed in vivo. Because the other prodrugs were all hydrolyzed to a much greater extent in DSI homogenates, they probably would not be delivered to the lower intestine as specifically as was glucoside 1, DEXGLU.

All the prodrugs except HYDGLU (5) and FLUGLU (7) were hydrolyzed much faster by cecal contents than by DSI contents. The galactosides were hydrolyzed more rapidly by cecal homogenates than were their glucoside counterparts. Thus, any prodrug reaching the cecum following oral administration would be expected to rapidly liberate the pharmacon as desired, with the possible exception of glucosides 5 and 7. However, the galactoside prodrugs would probably release significant amounts of their free steroids in the DSI, prior to reaching the lower intestine. Therefore, the galactoside prodrugs described here would probably be poor candidates for drug delivery to the rat cecum. It should be noted that the human intestine has a much sharper gradient of bacterial colonization; compunds that are not delivered specifically in the rat may be in the human intestine. Furthermore, there may be applications in which it is desirable to deliver a drug to the DSI.

The rate of release of prednisolone (4) from prednisolone cellobioside (13) was much slower than that of the steroid from either PREDGLU (2) or PREDGAL (10), when incubated in DSI or cecal homogenates. Delivery of steroid 4, via cellobioside 13, to the rat cecum would probably still not be as specific as that of dexamethasone (3), via DEXGLU (1) or DEXGAL (9). However, the prednisolone glycosides were the most enzymatically labile prodrugs investigated. A cellobioside derivative of any of the other three steroids would probably improve their specificity of delivery. Release of prednisolone (4) from cellobioside 13 in the rat cecum would probably be rapid, approximately the same as was observed in vivo for release of dexamethasone (3) from DEXGLU (1). Slow release of prednisolone (4) from cellobioside 13 is probably due to the fact that the rate of hydrolysis by β-D-cellobiosidase is slower than the two-step hydrolysis by β-D-glucosidase.

In calculating the K_M and V_{max} values for hydrolysis of *p*-NP-glc, *p*-NP-gal, and the glucoside and galactoside prodrugs, it was found that the β-D-glucosidase activity may be more heterogeneous in nature than β-D-galactosidase activity. This was seen in the Eadie–Hofstee plots, as shown in Figures 1 and 2, and may reflect the production of different β-D-glucosidases by the many bacterial species living in the large intestine.

The physicochemical properties of the prodrug also influence the specificity of this delivery system. Lipophilicity is very important in determining rates of penetration across biological membranes,[8] including the intestinal mucosa. Partition coefficients,[10a] often measured between 1-octanol and an aqueous phase, have proved very useful in correlating lipophilicity with absorption patterns of compounds absorbed by passive diffusion from the gastrointestinal tract. Therefore, partition coefficients of the prodrugs and the free steroids were measured and are given in Table V. The effect of incorporating a hydrophilic moiety (i.e., glucose) into dexamethasone was assessed previously: following oral administration of DEXGLU (1), 78% of the dose was recovered intact from the animals' intestinal lumen 3 h later; in contrast, only 3.9% of an oral dose of free dexamethasone (3) was recovered 3 h later.[7] Thus, attaching a hydrophilic glucose moiety to steroid 3 drastically impeded its absorption. This is reflected in the difference in partition coefficients (log *P*) of glucoside 1 (0.59) and steroid 3 (1.72). Because the log *P* of PREDGLU (2) was even lower than that of DEXGLU (1), it appears that its poorer specificity of delivery in the rat model is due primarily to premature release of the prednisolone in the upper intestine rather than absorption from the gastrointestinal lumen prior to reaching the cecum.

Specificity of delivery of a glycoside prodrug to the lower intestine can be estimated from its rate of hydrolysis by DSI contents and its octanol–buffer partition coefficient. These parameters limit the amount of prodrug that can survive premature hydrolysis or absorption in the DSI. For example, under the assay conditions used in this study, DEXGLU (1) supported a specific activity of hydrolysis of 19 nmol min^{-1} g^{-1} in DSI contents and had a log *P* value of 0.59. When this prodrug was administered orally to rats, about half of the dose reached the cecum.[7] Even more effective delivery could be expected of any prodrug that supports less hydrolysis by DSI contents and that has a lower log *P* value.

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Use of a disaccharide such as cellobiose, or even a trisaccharide, as the hydrophilic moiety will probably produce a superior prodrug. Such oligosaccharide carriers may be essential if larger or more lipophilic drugs are to be delivered to the large intestine. Further in vivo experiments are called for, especially in laboratory animals other than the rat. The rat is convenient, but it has a much larger bacterial population in its upper gastrointestinal tract than man does. Animals such as rabbits or guinea pigs will probably give a better indication of site specificity that could be expected in man. These latter two animals resemble man in having relatively few bacteria residing in their stomach and small intestine.[17] Furthermore, testing these antiinflammatory prodrugs in a carrageenan-induced ulcerative colitis guinea pig model[19] would be of immense value in assessing the actual efficacy of this glycoside/glycosidase based delivery system.

Experimental Section

All solvents were redistilled and dried over molecular sieves, 4 Å, 4–8 mesh (Aldrich Chemical Co.). All solvent evaporations were performed with a rotary evaporator with water aspirator reduced pressure. Melting 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 either a UCB 200 or a UCB 250 (homemade 200- and 250-MHz Fourier transform devices 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/programmer, and a stainless steel column (4.6 × 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 MeOH/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 × 22 cm column of 40 μm RP-18 with MeOH/water (68:32) as eluent or a 3.0 × 18 cm column of 40 μm silica gel with $CHCl_3$/95% EtOH (65:35) as 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 (20:80 w/v) and heating for 10 min at 110 °C.

All steroids, 2,3,4,6-tetra-*O*-acetyl-1-bromo-α-D-glucopyranose, 2,3,4,6-tetra-*O*-acetyl-1-bromo-α-D-galactopyranose, *p*-nitrophenyl substrates, and *p*-nitrophenol were purchased from Sigma Chemical Co. Octa-*O*-acetyl-α-D-cellobiose was obtained from Aldrich and the 31% hydrobromic acid in acetic acid was purchased from Eastman Kodak Co. Glucosides 1 and 2 (DEXGLU and PREDGLU, respectively) were prepared as described previously.[7]

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-galactopyranoside (DEXTAGAL, 18). Dexamethasone (3; 1.2 g, 3.1 mmol) was dissolved in dry $CHCl_3$ (200 mL) over 4-Å molecular sieves in a 500-mL round-bottom flask. After 10-20 mL had been distilled, freshly prepared Ag_2CO_3[12] (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-galactopyranoside (24; 3.5 g, 8.5 mmol) in dry $CHCl_3$ (100 mL) was added dropwise from an addition funnel. The reaction mixture was protected from light and stirred continuously. The addition of bromo sugar took approximately 1 h, and the solvent was distilled continuously during that time. Distillation was continued an additional hour after all the bromo sugar had been added; volume was maintained by the addition of dry $CHCl_3$. Then the solution was filtered, washed with cold saturated NaCl solution, and dried (Na_2SO_4) and the solvent 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 galactoside 18 was crystallized from MeOH/water to yield 0.56 g (26%): mp 133–135 °C; TLC R_f 0.39 (ethyl acetate/isooctane, 9:1); UV λ_{max} 239 nm (ϵ 15 400); IR (KBr) 3450 (OH), 1750 (OAc), 1660 (C=O), 1620 (C=C), 1240 (OAc), 985, 960, 895 cm^{-1}; ^{1}H NMR δ 0.78 (d, 3 H, C-16α CH_3), 0.88 (s, 3 H, C-18), 1.49 (s, 3 H, C-19), 1.93 (s, 3 H, C-4′ OAc), 2.00 (s, 3 H, C-3′ OAc), 2.06 (s, 3 H, C-2′ OAc), 2.12 (s, 3 H, C-6′ OAc), 4.18 (d, 1 H, C-1′, J = 7.2 Hz), 4.52 (AB q, 2 H, C-21, J = 18.3 Hz), 6.01 (s, 1 H, C-2, J = 11 Hz), 7.32 (d, 1 H, C-1, J = 10 Hz). Anal. ($C_{36}H_{47}O_{14}F$) C, H.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (PREDTAGAL; 19). Acetyl galactoside 19 was prepared from prednisolone (4) as described for 18 from steroid 3. Crystallization of 19 from MeOH/water yielded 0.82 g (37%): mp 134–136 °C; TLC R_f 0.39 (ethyl acetate/isooctane, 9:1); UV λ_{max} 242 nm (ϵ 14 700); IR (KBr) 3500 (OH), 1760 (OAc), 1650 (C=O), 1620 (C=C), 1240 (OAc), 900 cm^{-1}; ^{1}H NMR δ 0.78 (s, 3 H, C-18), 1.38 (s, 3 H, C-19), 1.91 (s, 3 H, C-4′ OAc), 1.99 (s, 3 H, C-3′ OAc), 2.03 (s, 3 H, C-2′ OAc), 2.09 (s, 3 H, C-6′ OAc), 4.23 (d, 1 H, C-1′, J = 7.2 Hz), 4.49 (AB q, 2 H, C-21, J = 18 Hz), 5.92 (s, 1 H, C-4), 6.15 (d, 1 H, C-1, J = 11 Hz), 7.40 (d, 1 H, C-2, J = 11 Hz). Anal. Calcd for $C_{35}H_{46}O_{14}$: C, 60.78; H, 6.66. Found: C, 59.83; H, 6.89.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-4-en-21-yl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (HYDTAGLU; 16). Acetyl glucoside 16 was prepared from hydrocortisone (6) and bromo sugar 23 as described for galactoside 18 from steroid 3 and bromo sugar 24. Crystallization of 16 from MeOH/water yielded 0.52 g (23%): mp 120.5–122 °C; TLC R_f 0.39 (ethyl acetate/isooctane, 9:1); UV λ_{max} 242 nm (ϵ 15 700); IR (KBr) 3450 (OH), 1760 (OAc), 1645 (C=O), 1610 (C=C), 1240 (OAc), 950, 908, 875 cm^{-1}; ^{1}H NMR δ 0.80 (s, 3 H, C-18, 1.40 (s, 3 H, C-19), 1.96 (s, 3 H, C-4′ OAc), 1.99 (s, 3 H, C-3′ OAc), 2.04 (s, 3 H, C-2′ OAc), 2.05 (s, 3 H, C-6′ OAc), 4.20 (d, 1 H, C-1′, J = 7.5 Hz), 4.60 (AB q, 2 H, C-21, J = 18 Hz), 5.60 (s, 1 H, C-4). Anal. ($C_{35}H_{48}O_{14}$) H; C: calcd, 60.69; found, 60.02.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-4-en-21-yl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (HYDTAGAL, 20). Acetyl galactoside 20 was prepared from hydrocortisone (6) and bromo sugar 24 as described for galactoside 18 from steroid 3. Crystallization of 20 from MeOH/water yielded 0.57 g (26%): mp 122–124 °C; TLC R_f 0.42 (ethyl acetate/isooctane); UV λ_{max} 242 nm (ϵ 16 700); IR (KBr) 3450 (OH), 1760 (OAc), 1660 (C=O), 1620 (C=C), 1230 (OAc), 950, 908, 896 cm^{-1}; ^{1}H NMR δ 0.80 (s, 3 H, C-18), 1.42 (s, 3 H, C-19, 1.92 (s, 3 H, C-4′ OAc), 1.98 (s, 3 H, C-3′ OAc), 2.04 (s, 3 H, C-2′ OAc), 2.09 (s, 3 H, C-6′ OAc), 4.24 (d, 1 H, C-1′, J = 7.6 Hz), 4.58 (AB q, 2 H; C-21, J = 18 Hz), 5.60 (s, 1 H, C-4). Anal. ($C_{35}H_{48}O_{14}$) H, C: calcd, 60.69; found, 60.27.

Preparation of 9α-Fluoro-11β,17α-dihydroxy-3,20-dioxopregna-4-en-21-yl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (FLUTAGLU, 17). Acetyl glucoside 17 was prepared from fludrocortisone (8) as described for glucoside 16 from steroid 6. Crystallization of 17 from MeOH/water yielded 0.48 g (23%): mp 124–125 °C; TLC R_f 0.39 (ethyl acetate/isooctane); UV λ_{max} 239 nm (ϵ 17 500); IR (KBr) 3450 (OH), 1750 (OAc), 1660 (C=O), 1625 (C=C), 1250 (OAc), 898 cm^{-1}; ^{1}H NMR δ 0.76 (s, 3 H, C-18), 1.49 (s, 3 H, C-19), 1.95 (s, 3 H, C-4′ OAc), 2.00 (s, 6 H, C-2′,3′ OAc), 2.04 (s, 3 H, C-6′ OAc), 4.25 (d, 1 H, C-1′, J = 7.8 Hz), 4.45 (AB q, 2 H, C-21, J = 18), 5.75 (s, 1 H, C-4). Anal. ($C_{35}H_{47}O_{14}F$) C, H.

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Preparation of 9α-Fluoro-11β,17α-dihydroxy-3,20-dioxapregna-4-en-21-yl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (FLUTAGAL, 21). Acetyl galactoside **21** was prepared from steroid **8** as described for galactoside **18** from steroid **2**. Crystallization of **21** from MeOH/water yielded 0.45 g (23%): mp 130–132 °C; TLC R_f 0.40 (ethyl acetate/isooctane); UV λ_{max} 239 nm (ε 17 100); IR (KBr) 3500 (OH), 1780 (OAc), 1660 (C=O), 1620 (C=C), 1250 (OAc) cm^{-1}; ^{1}H NMR δ 0.78 (s, 3 H, C-18), 1.48 (s, 3 H, C-19), 1.92 (s, 3 H, C-4′ OAc), 1.99 (s, 3 H, C-3′ OAc), 2.03 (s, 3 H, C-2′ OAc), 2.09 (s, 3 H, C-6′ OAc), 4.25 (d, 1 H, C-1′, J = 8 Hz), 4.53 (AB q, 2 H, C-21, J = 18 Hz), 5.73 (s, 1 H, C-4). Anal. ($C_{35}H_{47}O_{14}F$) C, H.

Preparation of Hepta-*O*-acetyl-1-bromo-α-D-cellobiose (25). Bromo sugar **25** was prepared according to published procedures.[13] Octa-*O*-acetyl-α-D-cellobiose (7.5 g, 11.1 mmol) was dissolved in 31% HBr in acetic acid (35 mL). The mixture was stirred at 4 °C for 24 h. Then ice-cold water (5 mL) was added, followed by $CHCl_3$ (10 mL). The organic phase was then washed several times with cold saturated NaCl solution and dried (Na_2SO_4). Bromo sugar **25** was crystallized by addition of dry ethyl ether to yield 4.68 g (61%): mp 181–182 °C (lit.[13b] mp 183 °C); $[\alpha]_D$ +93.5° (*c* 5.4, $CHCl_3$) (lit.[20] $[\alpha]_D$ +95.8°).

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl Hepta-*O*-acetyl-β-D-cellobioside (PREDTACEL, 22). Acetyl cellobioside **22** was prepared from steroid **4** (0.6 g, 1.6 mmol) and bromo sugar **25** (3.7 g, 5.2 mmol) as described for the preparation of galactoside **18** from steroid **3** and bromo sugar **24**. Crystallization of **22** from MeOH/water yielded 0.42 g (25%): mp 135–136 °C; TLC R_f 0.39 (ethyl acetate/isooctane); UV λ_{max} 242 nm (ε 16 900); IR (KBr) 3500 (OH), 1750 (OAc), 1660 (C=O), 1620 (C=C), 1230 (OAc), 912, 870, 782 cm^{-1}; ^{1}H NMR δ 0.78 (s, 3 H, C-18), 1.42 (s, 3 H, C-19), 1.91 (s, 3 H, OAc), 1.97 (s, 6 H, OAc), 1.99 (s, 3 H, OAc), 2.01 (s, 6 H, OAc), 2.07 (s, 3 H, OAc), 4.28 (d, 1 H, C-1′, J = 7.6 Hz), 4.49 (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 = 10 Hz). Anal. ($C_{47}H_{62}O_{22}$) C, H.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-4-en-21-yl β-D-Glucopyranoside (HYDGLU, 5). Acetyl glucoside **16** (0.2 g, 0.32 mmol) was dissolved in MeOH (10 mL) and benzene (5 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 was added to neutralize the solution. The solvent was removed and the residue was purified by flash chromatography on silica gel. Purified glucoside **16** was then dissolved in *tert*-butyl alcohol/water (15 mL, 1:1). This solution was frozen, and the solvent was removed by lyophilization to yield 0.12 mg (77%): R_f 0.49 ($CHCl_3$/95% EtOH, 65:35); UV λ_{max} 242 nm (ε 15 800); IR (KBr) 3450 (OH), 1650 (C=O), 1620 (C=C), 945, 910, 868 cm^{-1}; ^{1}H NMR δ 0.76 (s, 3 H, C-18), 1.36 (s, 3 H, C-19), 4.17 (d, 1 H, C-1′, J = 7.7 Hz), 4.57 (AB q, 2 H, C-21, J = 18.2 Hz), 5.56 (s, 1 H, C-4). Anal. ($C_{27}H_{40}O_{10}\cdot H_2O$) C, H.

Preparation of 9α-Fluoro-11,17α-dihydroxy-3,20-dioxopregna-4-en-21-yl β-D-Glucopyranoside (FLUGLU, 7). Glucoside **7** was prepared from acetyl glucoside **17** as described for glucoside **5** from acetyl glycoside **16**. After lyophilization, 0.11 g (71%) of glucoside **7** was obtained: TLC R_f 0.50 ($CHCl_3$/95% EtOH, 7:3); UV λ_{max} 239 nm (ε 17 800); IR (KBr) 3450 (OH), 1650 (C=O), 1620 (C=C), 938, 895 cm^{-1}; ^{1}H NMR δ 0.78 (s, 3 H, C-18), 1.49 (s, 3 H, C-19), 4.19 (d, 1 H, C-1′, J = 7.6 Hz), 4.57 (AB q, 2 H, C-21, J = 18 Hz), 5.70 (s, 1 H, C-4). Anal. ($C_{27}H_{40}O_{10}F\cdot H_2O$) H; C: calcd, 57.96; found, 58.53.

Preparation of 9α-Fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl β-D-Galactopyranoside (DEXGAL, 9). Galactoside **9** was prepared from acetyl galactoside **18** as described for glucoside **5** from acetyl glucoside **16**. After lyophilization, 95 mg (60%) was obtained: TLC R_f 0.46 ($CHCl_3$/95% EtOH, 7:3); UV λ_{max} 239 nm (ε 14 400); IR (KBr) 3450 (OH), 1680 (C=O), 1625 (C=C), 990, 891 cm^{-1}; ^{1}H NMR δ 0.76 (d, 3 H, C-16α CH_3, J = 7 Hz), 0.85 (s, 3 H, C-18), 1.50 (s, 3 H, C-19), 4.19 (d, 1 H, C-1′, J = 7.8 Hz), 4.58 (AB q, 2 H, C-21, J = 18 Hz), 6.01 (s, 1 H, C-4), 6.24 (d, 1 H, C-1, J = 10.1 Hz), 7.39 (d, 1 H, C-2, J = 10.2 Hz). Anal. Calcd for $C_{28}H_{39}O_{10}F\cdot H_2O$: C, 60.65; H, 7.17. Found: C, 60.44; H, 6.98.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl β-D-Galactopyranoside (PREDGAL, 10). Galactoside **10** was prepared from acetyl galactoside **19** as described for glucoside **5** from acetyl glucoside **16**. After lyophilization, 0.13 g (83%) was obtained: TLC R_f 0.39 ($CHCl_3$/95% EtOH, 7:3); UV λ_{max} 242 nm (ε 14 500); IR (KBr) 3450 (OH), 1650 (C=O), 1615 (C=C), 899 cm^{-1}; ^{1}H NMR δ 0.78 (s, 3 H, C-18), 1.39 (s, 3 H, C-19), 4.13 (d, 1 H, C-1′, J = 7.3 Hz), 4.53 (AB q, 2 H, C-21, J = 18.1 Hz), 6.16 (d, 1 H, C-1, J = 10.1 Hz), 7.33 (d, 1 H, C-2, J = 10.1 Hz). Anal. ($C_{27}H_{38}O_{10}\cdot H_2O$) C, H.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-4-en-21-yl β-D-Galactopyranoside (HYDGAL, 11). Galactoside **11** was prepared from acetyl galactoside **20** as described for glucoside **5** from acetyl glucoside **16**. After lyophilization, 95 mg (60%) was obtained: TLC R_f 0.42 ($CHCl_3$/95% EtOH, 7:3); UV λ_{max} 242 nm (ε 15 700); IR (KBr) 3450 (OH), 1660 (C=O), 1620 (C=C), 1080, 1033, 930, 896, 870 cm^{-1}; ^{1}H NMR δ 0.79 (s, 3 H, C-18), 1.42 (s, 3 H, C-19), 4.17 (d, 1 H, C-1′, J = 7.6 Hz), 4.54 (AB q, 2 H, C-21, J = 18 Hz), 5.58 (s, 1 H, C-4). Anal. ($C_{27}H_{40}O_{10}\cdot H_2O$) C, H.

Preparation of 9α-Fluoro-11β,17α-dihydroxy-3,20-dioxopregna-4-en-21-yl β-D-Galactopyranoside (FLUGAL, 12). Galactoside **12** was prepared from acetyl galactoside **21** as described for glucoside **5** from acetyl glucoside **16**. After lyophilization, 0.11 g (71%) was obtained: TLC R_f 0.49 ($CHCl_3$/95% EtOH, 7:3); UV λ_{max} 239 nm (ε 17 700); IR (KBr) 3450 (OH), 1660 (C=O), 1620 (C=C), 1080, 1033, 930, 896, 870 cm^{-1}; ^{1}H NMR δ 0.78 (s, 3 H, C-18), 1.50 (s, 3 H, C-19), 4.20 (d, 1 H, C-1′, J = 7.8), 4.55 (AB q, 2 H, C-21, J = 18 Hz), 5.70 (s, 1 H, C-4). Anal. ($C_{27}H_{39}O_{10}F\cdot H_2O$) C, H.

Preparation of 11β,17α-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl β-D-Cellobioside (PREDCEL, 13). Cellobioside **13** was prepared from acetyl cellobioside **22** as described for glucoside **5** from acetyl glucoside **16**. After lyophilization, 86 mg (60%) was obtained: TLC R_f 0.33 ($CHCl_3$/95% EtOH, 7:3); UV λ_{max} 242 nm (ε 15 500); IR (KBr) 3450 (OH), 1680 (C=O), 1620 (C=C), 1170, 950, 898, 875 cm^{-1}; ^{1}H NMR δ 0.75 (s, 3 H, C-18), 1.28 (s, 3 H, C-19), 4.15 (d, 1 H, C-1′, J = 7.6 Hz), 4.51 (AB q, 2 H, C-21, J = 18 Hz), 5.91 (s, 1 H, C-4), 6.20 (d, 1 H, C-1, J = 10.2 Hz), 7.30 (d, 1 H, C-2, J = 10.1 Hz). Anal. ($C_{33}H_{48}O_{15}\cdot H_2O$) C, H.

In Vitro Metabolism. Rates of hydrolysis of *p*-nitrophenyl-β-D-glucoside (*p*-NP-glc), *p*-nitrophenyl-β-D-galactoside (*p*-NP-gal), and *p*-nitrophenyl-β-D-cellobioside (*p*-NP-cel) were measured in homogenized contents of the rat stomach, proximal small intestine (PSI), distal small intestine (DSI), and cecum. The entire small intestine was divided into two, equal length segments to give PSI and DSI. Gastrointestinal contents were obtained from male, Sprague–Dawley rats (300–400 g) which had been maintained on a stock diet (Purina rat chow) and water ad libitum. The rats were fasted for 4 h prior to sacrifice. Contents of each section of the gastrointestinal tracts of four animals were pooled. Pooling the gut contents would be expected to lower the SD by a factor of 2. Following removal, the contents were quickly weighed and then diluted to 100 mL (stomach, PSI, and DSI) or 200 mL (cecum) with cold 0.01 M phosphate buffer, pH 7.0. The diluted contents were homogenized with a Polytron homogenizer (Brinkman Instrument Co.) at medium speed for 1–2 min, and the solution pH was measured. Homogenates were then stored on ice (ca. 30 min). Homogenates (0.8 mL of stomach and PSI, 0.2 mL of DSI, and 0.04 mL of cecum) were added to the appropriate substrate solution (0.01 M phosphate buffer, pH 7.0) to give 1.0 mM substrate (total volume: 2.25 mL). The reaction was run at 37 °C in a shaking water bath. The reaction was stopped after 10, 20, or 30 min by addition of 0.2 N NaOH (0.25 mL). The amount of *p*-nitrophenol released was measured spectrophotometrically at 403 nm.

Rates of hydrolysis of glycoside prodrugs **1**, **2**, **5**, **7**, and **9–13** were measured in the same manner except that a higher concentration of each homogenate was used. Stomach and PSI contents were diluted to 50 mL with 0.01 M phosphate buffer, pH 7.0, DSI contents to 25 mL, and cecal contents to 200 mL prior to homogenization. The homogenates (2.1 mL of stomach, PSI, and DSI and 1.7 mL of cecum) were added to the appropriate substrate solution (0.01 M phosphate buffer, pH 7.0) to give 1.0

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mM substrate in a total volume of 2.5 mL. At various times up to 30 min, aliquots (0.3 mL) were removed and quenched with MeOH (4.7 mL). After centrifugation (5000g, 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.

$K_{M(app)}$ and V_{max} Determinations. The $K_{M(app)}$–V_{max} for the hydrolysis of *p*-NP-glc and *p*-NP-gal were determined with use of pooled cecal homogenates (200 mL) as described above. A range of substrate concentrations (56–1000 μM, final volume 2.25 mL), spanning their apparent K_M, was used for each reaction. The amount of cecal homogenate used was 0.04 mL. Reaction mixtures were incubated, in duplicate at 37 °C in a shaking water bath, and the reaction was stopped by addition of 0.2 N NaOH (0.25 mL) after 15 min. Release of *p*-nitrophenol was measured spectrophotometrically at 403 nm. Eadie–Hofstee plots were used to determine the $K_{M(app)}$ (μM) and V_{max} (μmol min^{-1} g^{-1}) of both reactions. The wet weight (g), measured immediately after removal and pooling, was used throughout.

The $K_{M(app)}$ and V_{max} were also measured for the hydrolysis of glycoside prodrugs **1**, **2**, **5**, **7**, and **9**–**12**. Again, cecal contents from four rats were pooled, weighed, diluted (100 mL, 0.01 μM phosphate buffer, pH 7.0), and homogenized. A range of substrate concentrations (0.5–48 μM, final volume 2.5 mL) spanning the apparent K_M was used for each reaction. The amount of cecal homogenate used was 0.8 mL. Reactions were run, in duplicate, at 37 °C in a shaking water bath. After 15 min, the reactions were stopped by removing aliquots (0.3 mL) and quenching them with MeOH (4.7 mL). Following centrifugation (5000g, 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. Eadie–Hofstee plots were used to determine the $K_{M(app)}$ and V_{max}.

Determination of Apparent Partition Coefficients. The partitioning of prodrugs and free steroids between 1-octanol and an aqueous phase (0.01 M phosphate buffer, pH 7.0) were determined at 37 °C. Both octanol and buffer were saturated with the relevant aqueous or organic phase before use. Equal volumes (1.0 mL) of both phases were used and agitated for 30 min. The initial concentration of glycoside was 10 mM, dissolved in the aqueous phase. The initial concentration of steroid was 10 mM dissolved in the organic phase. The amount of glycoside and free steroid in the aqueous phase at equilibrium was measured spectrophotometrically at 239 nm for the dexamethasone and fludrocortisone compounds and 242 nm for the prednisolone and hydrocortisone compounds. The concentration of glycoside or free steroid in the octanol phase was determined by difference.

Note Added in Proof: After this manuscript was accepted, the authors learned of an earlier publication describing the synthesis of steroid glycoside prodrugs for release in the synovial fluid of arthritis victims (Hirschmann, R., Strachan, R. G.; Buchschacher, P.; Sarett, L. H.; Steelman, S. L.; Silber, R. *J. Am. Chem. Soc.* **1964**, *86*, 3903).

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Registry No. 1, 88158-43-4; 2, 88158-44-5; 3, 50-02-2; 4, 50-24-8; 5, 92901-21-8; 6, 50-23-7; 7, 92901-22-9; 8, 127-31-1; 9, 92901-23-0; 10, 92901-24-1; 11, 92901-25-2; 12, 92901-26-3; 13, 92901-27-4; 16, 92901-28-5; 17, 92901-29-6; 18, 92901-30-9; 19, 92901-31-0; 20, 92937-53-6; 21, 92901-32-1; 22, 92901-33-2; 23, 572-09-8; 24, 3068-32-4; 25, 14227-66-8; β-D-glucosidase, 9001-22-3; β-D-galactosidase, 9031-11-2.

Angiotensin-Converting Enzyme Inhibitors. New Orally Active Antihypertensive (Mercaptoalkanoyl)- and [(Acylthio)alkanoyl]glycine Derivatives[1]

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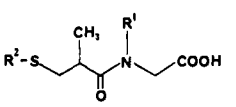
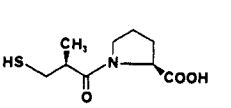
A variety of N-substituted (mercaptoalkanoyl)- and [(acylthio)alkanoyl]glycine derivatives was synthesized and their ability in inhibiting the activity of angiotensin-converting enzyme (ACE) was examined in vitro and in vivo. The acylthio derivatives prepared are assumed to act as prodrugs since they are much less active than the corresponding free SH compounds in vitro and can be expected to act in vivo only after conversion to the free sulfhydryl compounds. A number of these compounds are potent ACE inhibitors that lowered blood pressure in Na-deficient, conscious spontaneously hypertensive rats (SHR), a high renin model. One of the most active members of the series was (*S*)-*N*-cyclopentyl-*N*-[3-[(2,2-dimethyl-1-oxopropyl)thio]-2-methyl-1-oxopropyl]glycine (REV 3659-(S), pivopril). Structure–activity relationships are discussed.

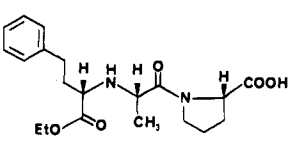
The renin–angiotensin–aldosterone system is an important humoral mechanism involved in the regulation of blood pressure[2-4] and renal function.[5] In particular, the development of antihypertensive drugs that act selectively by inhibiting angiotensin-converting enzyme[6,7] (ACE) has received much attention in recent years. Recently orally active ACE inhibitors have been reported to show promising clinical antihypertensive properties.[8-14] We now report the design and synthesis[15] of an orally active novel series of substituted (mercaptoalkanoyl)glycines of generic formula **1**. Unlike the known inhibitors such as captopril (**2**)[6a,b] and enalapril (**3**),[7e] which embody a C-terminal proline, this series of compounds contains exclusively the nonchiral amino acid glycine.



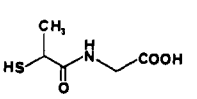
1



2 (captopril)



3 (enalapril)



4 (tiopronin)

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