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An Evaluation of a C-Glucuronide as a Liver Targeting Group: Conjugate of a Glucocorticoid Antagonist

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Abstract—A β-C-glucuronide conjugate of the glucocorticoid receptor antagonist, Mifepristone **1**, was prepared which maintained binding affinity, had modest in vitro activity, and was metabolically more stable than the parent. Pharmacokinetic studies suggest that the conjugate is recognized by the liver like *O*-glucuronides and may undergo a portion of the enterohepatic recirculation loop.

Cortisol is a hormone synthesized in the adrenal gland which has anti-inflammatory effects and influences hepatic glucose production (HGP).[1] Other glucocorticoids are widely employed as anti-inflammatory agents which unfortunately can aggravate established diabetes. The underlying mechanism is glucocorticoid-induced upregulation of hepatic enzymes required for gluconeogenesis mediated by the glucocorticoid receptor (GR).[2] The primary modulated enzymes are phosphoenolpyruvate carboxykinase and glucose-6-phosphatase which catalyze two key transformations required to convert oxaloacetate into glucose.[3] GR is a nuclear hormone receptor that resides in the cytoplasm in an inactive state until bound to a ligand.[4] Upon binding, the ligand bound GR complex translocates to the cell nucleus where it can either initiate, or repress, specific gene transcription. We sought to identify a GR antagonist that could be used to prevent upregulation of gluconeogenesis by cortisol as a treatment for diabetes.[5]

The glucocorticoid receptor antagonist mifepristone (RU-486) **1** reduces levels of PEPCK and glucose-6-phosphatase and causes a 50% reduction of plasma glucose levels in obese diabetic *db*/*db* mice.[6] However, the utility of this agent is limited by cross-reactivity with other steroid receptors, such as the progesterone and mineralcorticoid receptor, and by systemic glucocorticoid antagonism. Systemic antagonism could potentially lead to problems of glucocorticoid insufficiency, such as hypotension, inflammation, and symptoms of Addison's disease.[7] In addition, systemic blockade of the glucocorticoid receptor by mifepristone **1** produces compensatory responses mediated through the hypothalamic-pituitary-adrenal axis.[8] Activation of this axis leads to secretion of adrenocorticotrophic hormone, which stimulates production of endogenous glucocorticoid, potentially reducing the effectiveness of a GR antagonist (Fig. 1).

Selectively targeting a GR antagonist to the liver should lower HGP while avoiding the effects resulting from systemic exposure. To achieve liver targeting, we sought to design compounds that undergo enterohepatic recirculation.[9] Glucuronidation is one potential strategy to induce a GR antagonst to undergo enterohepatic recirculation. After oral administration, absorption, and passage into the liver via the portal vein an agent can undergo glucuronidation. Glucuronidation is a conjugation reaction of an activated form of glucuronic acid **2** that takes place in the liver, and is an important pathway of drug metabolism and clearance.[10] These reactions, catalyzed by glucuronyl transferases, result in the formation of structurally varied glucuronide conjugates **3**. Polar, high molecular weight (>300),

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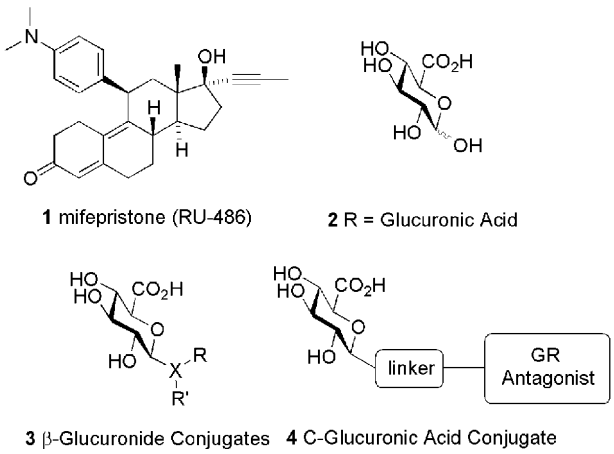


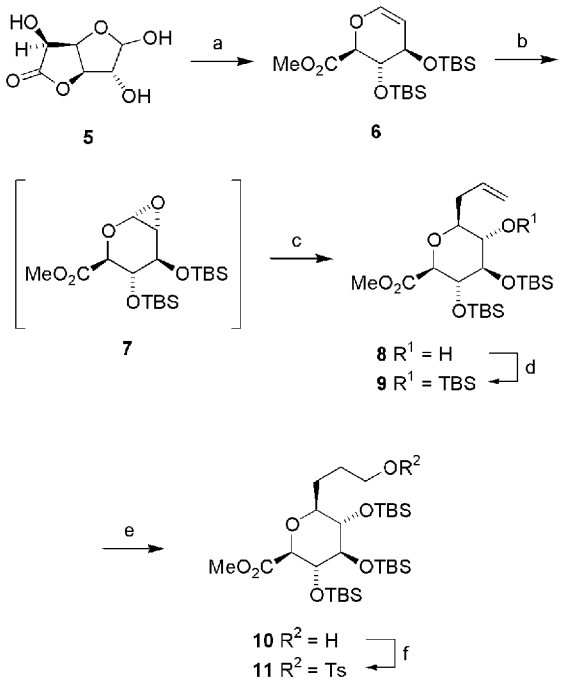
Figure 1. Steroidal glucocorticoid receptor antagonist, mifepristone **1**, glucuronic acid **2**, β-glucuronide conjugate **3**, and C-glucuronide GR antagonist conjugate **4**.

glucuronides often pass from the hepatocyte to the biliary fluid *en route* to the intestine. Once in the intestine, the conjugate may be reabsorbed, pass through the colon due to low membrane permeability and be excreted, or, as is the case with *O*-linked glucuronide conjugates, be enzymatically hydrolyzed by β-glucuronidases. This liberates the parent molecule for reabsorption. Through enterohepatic recirculation a drug may have multiple passes through the liver and have low systemic exposure. Alternatively, low molecular weight (<300) glucuronides are frequently exported from the hepatocyte into the bloodstream where they are filtered by the kidney and undergo urinary excretion.

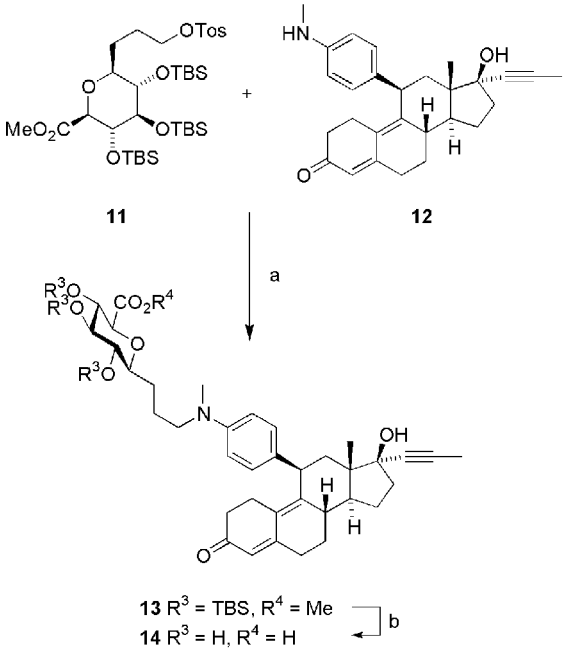
With these considerations in mind, we sought to determine whether glucuronic acid GR antagonist conjugates **4** are liver targeted and undergo enterohepatic ciruculation. Curley and co-workers reported β-C-glucuronic acid conjugates of retinoids that are stable to acid and β-glucuronidase hydrolysis.[11] Furthermore, they show in vitro and in vivo (oral administration) effects in cancer models.[12] These results suggested C-glucuronic acid conjugates as a starting point.

The synthesis of a C-glucuronide is outlined in Scheme 1. We sought to build β-C-glucuronide precursors capable of coupling to GR antagonists. The synthesis started with D-glucuronolactone **5**, and through known procedures, produced methyl (3,4-di-*O*-*t*-butyldimethylsilyl-D-glucuronate) glycal **6.**[13,14] Treatment of glycal **6** with dimethyldioxirane provided epoxide **7** diastereoselectively.[13] The crude epoxide underwent Lewis acid mediated reaction with allyl tri-*n*-butylstannane to provide β-C-glycoside **8**.[15] Protection of the alcohol with a TBS group provided the fully protected allyl β-C-glycoside **9.** Hydroboration of the alkene using 9-BBN followed by oxidation with sodium borate yielded alcohol **10** which was converted to the tosylate **11**. Alkene **9** and tosylate **10** could be coupled to GR antagonists by Suzuki and alkylation reactions, respectively.

The formation of the C-glucuronide mifepristone conjugate is outlined in Scheme 2. *N*-desmethyl-mifepristone **12** was alkylated with the C-glycoside tosylate **11** to produce the protected mifepristone C-glucuronide **13**. The protecting groups, including the methyl ester, were removed using tetrabutylammonium fluoride (TBAF) to provide the desired conjugate **14**. The deprotection conditions gave a clean conversion. However, separaton of the polar conjugate **14** from the TBAF by reverse phase HPLC was of modest efficiency (unoptimized). It



Scheme 1. Formation of β-C-glucuronide precursors. (a) See refs [13,14] (6 steps-22%); (b) dimethyldioxirane, CH_2Cl_2, rt; (c) allyl tri-*n*-butylstannane, *n*-Bu_3SnOTf, CH_2Cl_2, −78 °C, 57%; (d) TBSCl, imidazole, DMF, 120 °C, 54%; (e) 9-BBN, THF; $NaBO_4$, H_2O, rt, 89%; (f) TsCl, pyridine, $CHCl_3$, rt, 76%.



Scheme 2. Formation of β-C-glucuronide mifepristone conjugate. (a) $(i\text{-Pr})_2NEt$, NaI, CH_3CN, 90 °C, 61%; (b) TBAF, THF, H_2O, rt, 20%.

was hoped conjugate **14** would maintain the potency of mifepristone **1** since groups at this position project outside the ligand binding domain of the GR.[16]

Table 1 compares the biological activity of mifepristone **1** and conjugate **14**. In a competitive binding assay using the radiolabeled agonist dexamethasone and human GR, conjugate **14** gave an IC_{50} of 16 nM which was weaker then mifepristone **1**.[17] The second assay in column 3 uses a glucocorticoid hormone reporter CHO cell line genetically engineered to express the glucocorticoid response element and a reporter gene encoding a secreted form of alkaline phosphatase (ALP). Compounds are evaluated for antagonism of dexamethasone induced ALP expression. The conjugate **14** has diminished in vitro potency. Low cell permeability may account for the weaker activity. The assay in column 4 measures the inhibition of the tyrosine aminotransferase (TAT) activity induced by a dose of the GR agonist prednisolone by a GR antagonist in rat hepatocytes. Given the difference in potency in the binding assay, the compound shows excellent functional potency in hepatocytes, suggesting the possibility of active uptake by hepatocytes.

One desirable property of a liver targeting group is protection from metabolism. Mifepristone **1** is known to undergo *N*-demethylation, alkyne metabolism, and its metabolites inhibit its own metabolism.[18] Figure 2 shows the results from a metabolism study using rat liver microsomes incubated with **1** and **14**. The C-glucuronide conjugate exhibits greater stability and does not undergo *N*-dealkylation, which is the major metabolic pathway for mifepristone **1**. Thus the C-glucuronide group protects the GR antagonist **14** from metabolism.

The oral and iv pharmacokenetic profile of conjugate **14** at 5 mg/kg in Sprague–Dawley rats is shown in Figure 3. The conjugate has a high plasma clearance (5.3 L/h kg) and a short half life ($t_{1/2} = 1.8$ h). Plasma concentrations at 7 h after iv dosing are low (0.003 μg/mL). No parent drug was detected in the plasma after oral dosing. No trace of the C-glucuronide **14** was detected in the livers regardless of the mode of dosing (7 h).

The oral results indicate the conjugate **14** is not absorbed. The iv results are consistent with several elimination possibilities. Once in the liver, the conjugate may reenter the bloodstream where it is filtered by the kidney and excreted in the urine. The conjugate may also be actively transported from the liver to the biliary fluid. From there, the conjugate could enter the intestine and be eliminated in the feces. To check on excretion, three

Table 1. Comparison of mifepristone **1** and the conjugate **14** in binding and functional assays

Compd	GR binding IC_{50} (nM)	GR-ALP (nM)	TAT[a] inhibition IC_{50} (μM)
1	1.1	5.0	0.41
14	16.7	550	2.8

[a]Liver enzyme upregulated by GR agonists.

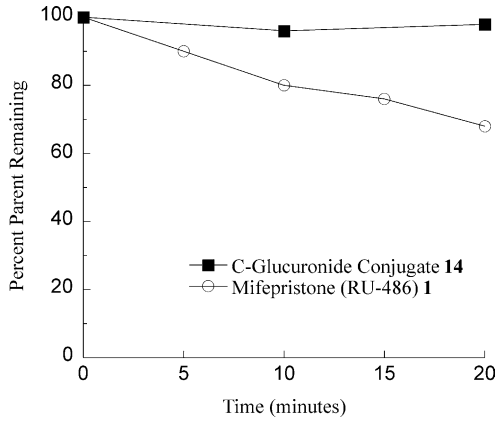


Figure 2. Metabolism of mifepristone **1** and the conjugate **14** both at 10 μM in rat liver microsomes (0.25 mg microsomal protein/mL) at 37 °C.

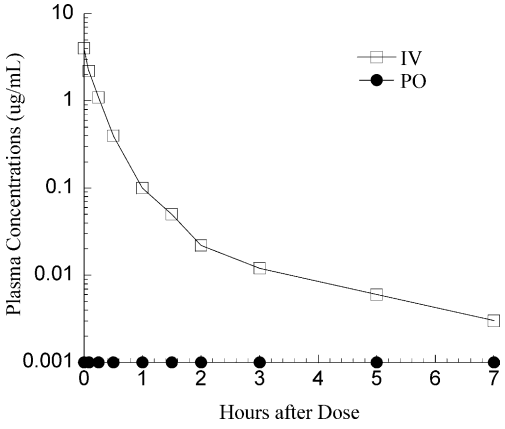


Figure 3. Pharmacokinetic profile of the conjugate **14** at 5 mg/kg iv and oral.

rats were iv dosed (5 mg/kg) and, as before, plasma drug levels were low (1–2 μg/mL). This time, there were low, but measureable, liver levels (0.22 μg/g). Most importantly, no parent drug was found in the urine or kidney. These results suggest active transport from the liver to the bile, indicating the conjugate may be undergoing a portion of the enterohepatic recirculation loop. A final study incubating efflux pump substrates 17β-estradiol-D-glucuronide, a cMOAT/MRP2 inhibitor, and verapamil, a MRP2 inhibitor, with the conjugate in the rat hepatocyte/TAT assay was conducted.[19,20] The apparent efficacy of the conjugate was increased by as much as 10-fold using these efflux pump inhibitors, supporting the hypothesis.

β-C-glucuronides were successfully prepared and conjugated to the GR antagonist, mifepristone **1**. The conjugate **14** maintained GR binding and had modest in vitro effects. Furthermore, it was metabolically more stable then the parent. Pharmacokinetic studies suggest that the conjugate may be treated by the liver like *O*-glucuronides and may undergo part of the enterohepatic recirculation loop. Unfortunately, the conjugate had low bioavailability. Decreasing the hydrophilicity of this compound may increase membrane permeability and absorption. Such modifications may realize the goal of these studies: the discovery of a liver targeting group.

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