Design, Synthesis, and Pharmacological Effects of a Cyclization-Activated Steroid Prodrug for Colon Targeting in Inflammatory Bowel Disease

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Glucocorticoids are used in the treatment of inflammatory bowel disease. A limitation to their use is that they undergo absorption from the GIT before reaching the colon causing severe systemic side effects. We report here on a novel prodrug approach to targeting corticosteroids to the colon. The design involves attaching a 21-ester group that suppresses absorption during transit to the colon. The prodrug is designed to be primed by colonic microflora liberating an amino ester that cyclizes releasing the steroid. One of the prodrugs **5b** was as efficacious as prednisolone in the murine DSS model but did not cause thymic atrophy, a marker for systemic steroid effects.

Introduction

Drug targeting may be defined as the delivery of a drug to a specific organ, tissue, or cell population.[1] Chemical drug targeting involves the deliberate modification of a drug structure (usually bioreversibly), causing it to accumulate in a target tissue: site-specific release may be triggered by a chemical or enzymatic condition not present elsewhere in the body. The colon is an important challenge to the validity of the drug targeting approach, as conditions there are largely similar to those prevailing elsewhere in the gastrointestinal (GI) system, and the luminal pH gradient through the GI tract is too gradual for effective local drug release on strictly chemical grounds.[2] On the other hand, the colon is an important drug target for the treatment of pathologies of the colon itself, for the relief of the chronic constipation that accompanies opioid drug treatment, and as a potential portal site for peptide and protein drugs that are not absorbed from other regions of the gastrointestinal tract (GIT[a]).[3]

One key difference between the colon and small intestine is its luxuriant microflora. The abrupt increase in bacterial enzyme expression has been investigated as a means of targeting drugs to the colon, especially those for treating IBD. One outstandingly successful outcome of these endeavors was the development of azo-based prodrugs (e.g., **2**) of 5-ASA **1** (Figure 1a), which pass through the GI system intact before releasing their "payload" on reduction. Several drugs based on this concept, such as **2** are in clinical use for the treatment of IBD.[4] The targeting of other drug types, such as the anti-inflammatory steroids to the colon has been less successful,[5] and yet the need for appropriate systems in these cases is more pressing because steroids have multiple systemic side effects when administered chronically. Among the approaches to targeting the colon with steroids are the following: biodegradable polymers,[6] time release systems, coating with pH sensitive materials, gastrointestinal pressure controlled release,[7] and chemical drug targeting using hydrophilic carriers (e.g., sulfate esters[8]) or carbohydrate conjugates such glucuronides;[9] poly-(L-aspartic acid) derivatives;[10] α, *β*, and *γ* -cyclodextrin conjugates;[11] polymer conjugates.[12] These approaches have met with limited success, in part because of the widespread distribution of glycosidases in the GI tract (resulting in non-site-specific release) and, in some instances, because of slow release characteristics in the colon.[13] The use of the successful azoreductase approach has thus far been restricted to drugs, such as **1**, that bear a primary aromatic amine.

The purpose of this paper is to describe a prodrug strategy for targeting the colon which extends the use of azo group reduction as a vector for drug release. In the design investigated, a corticosteroid is connected via its 21-OH group to an arylazo carrier group via a primary alkyl ester (Figure 1b). In Figure 1b the azo group is used to incorporate an incipient 5-ASA such that the conjugate can act as a mutual prodrug. The design seeks to exploit the selective reduction of an azo linker in the colon, releasing a latent prodrug that subsequently undergoes lactamization liberating the steroid.

A very wide range of azo compounds have been reported to undergo cleavage in the colon including conjugates of PAF antagonists,[14] 9-aminocamphothecin,[15] as well as 5-ASA-*N*-methacrylamide, acryloyloxyethyl, and acryloylamido copolymers.[16] Meanwhile off-target drug release due to hydrolysis promises to be low because there is little luminal esterase activity

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[a] Abbreviations: AcOH, acetic acid; AP, apical side; 5-ASA, 5-aminosalicylic acid or mesalazine; BL, basolateral side; BOC, *tert*-butylcarbonyl protecting group; BOC_2O, di-*tert*-butyl dicarbonate; CD, Crohn's disease; CFU, colony forming unit; COX, cyclooxygenase; DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DCU, dicyclohexyl urea; DIAD, diisopropyl azodicarboxylate; DMAP, 4-dimethylaminopyridine; DMSO, dimethyl sulfoxide; DSS, dextran sodium sulfate; EI, electron impact; EtOAc, ethyl acetate; EtOH, ethanol; GCC, glucocorticoids; GIT, gastrointestinal tract; HBSS, Hank's balanced salt solution; HPA, hypothalamic pituitary adrenocortical axis; HRMS, high resolution mass spectroscopy; IBD, inflammatory bowel disease; IR, infrared spectroscopy; k_{obs}, observed constant of cyclization; mp, melting point; MeOH, methanol; mol wt, molecular weight; MPO, myeloperoxydase activity; MTS, methyltetrazolium salt; NaOAc, sodium acetate; NMR, nuclear magnetic resonance spectroscopy; NSAIDS, nonsteroid anti-inflammatory drugs; PBS, phosphate buffered saline; PDA UV, photodiode array ultraviolet; Pd/C, palladium on activated carbon; rpm, revolution per minutes;; TEER, trans epithelial electric resistance; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; TMS, tetramethylsilane; UC, ulcerative colitis; UV, ultraviolet spectroscopy.

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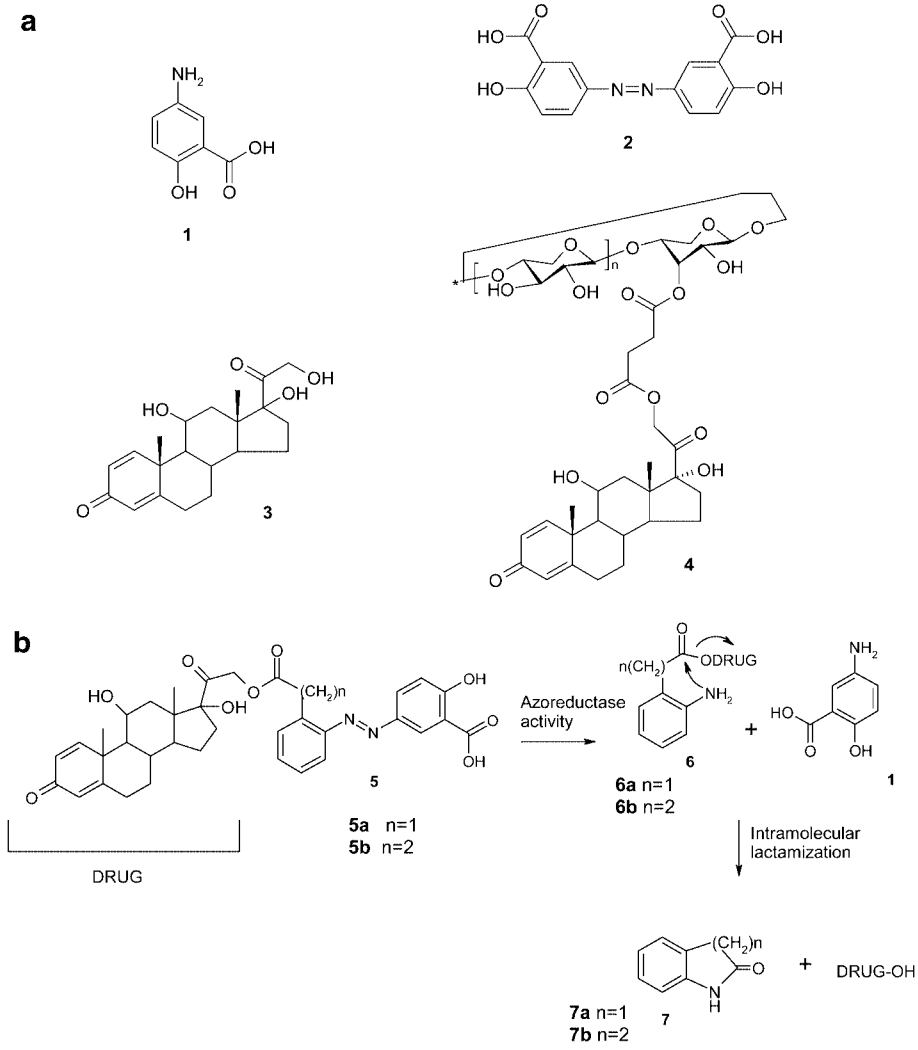


Figure 1. (a) 5-ASA (**1**), a 5-ASA prodrug (**2**), prednisolone (**3**), and prednisolone cyclodextrin conjugate (**4**). (b) Cyclization activated steroid prodrugs for the colon.

in the human GIT. Steroidal 21-esters have been shown to be robust in human and rodent simulated intestinal fluid models.[17]

The overall effect of the design is therefore to make the ester connecting the drug to the carrier group chemically vulnerable under conditions found in the colon. The prodrug is designed to have favorable physicochemical characteristics for passage through the intestine mainly because of its substantially increased mass relative to the parent.

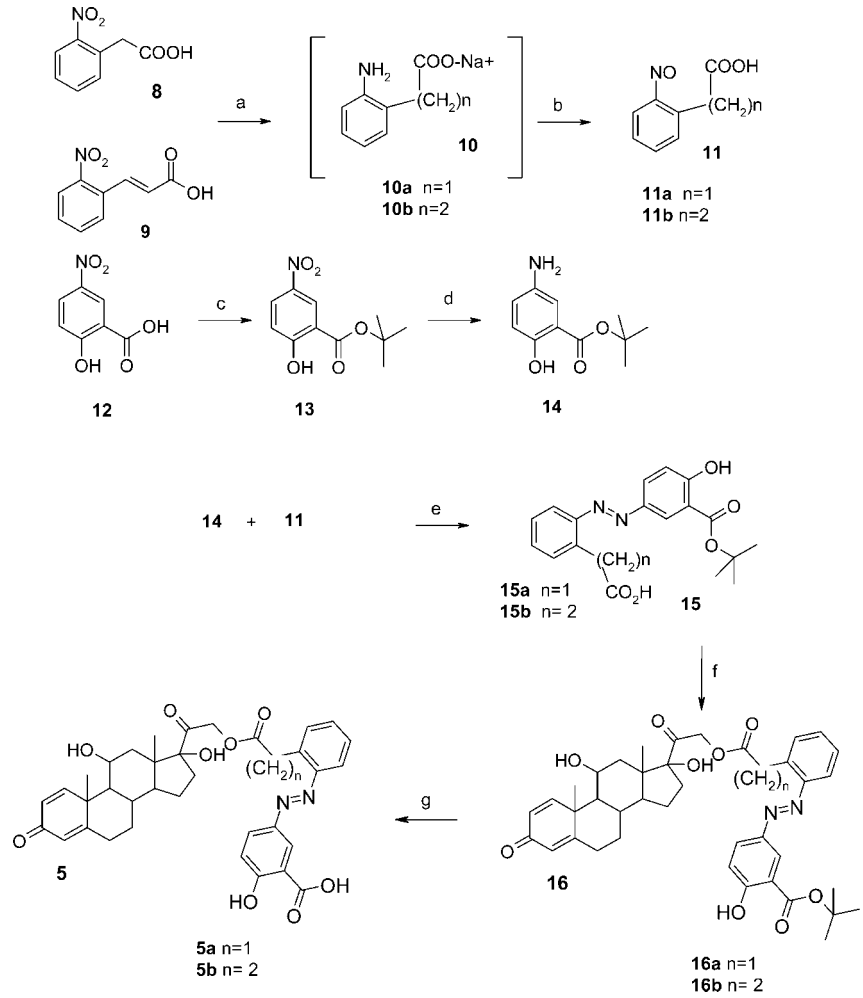
In a mouse model of UC compound **5b** exhibited similar efficacy to prednisolone, but it caused significantly less thymolysis, a sensitive marker for corticoid depression of the HPA axis.

Results and Discussion

Synthesis. Our synthetic approach to the target prodrugs (**5**) was to generate the appropriate carrier groups (**15**) and then link by esterification to prednisolone (**3**) (Scheme 1). Two components were needed to form the carriers by condensation: nitrosoacid **11** and amino ester **14** (we were unable to generate **15** using conventional diazotization chemistry). Reduction of 2-nitrocinnamic acid (**9**) under hydrogen atmosphere with Pd/C (10%) was carried out in 2% aqueous sodium hydroxide to yield the intermediate **10b** which was oxidized without purification using Oxone to 2-nitrosophenylpropionic acid (**11b**). A similar sequence was used for the production of **11a** from nitrophenylacetic acid (**8**). In each case, but especially series **b**, it was necessary to perform the reduction under basic conditions in order to inhibit cyclization of intermediate **10**. *tert*-Butyl protection was chosen for the acid of the amino component, mindful that there would be competition between the steroid and the phenol group −OH during esterification later in the sequence. Protection of 5-nitrosalicylic acid (**12**) was performed in *tert*-butanol with DMAP as catalyst; DCC was introduced to the reaction mixture dropwise in a THF solution. Solubility problems with 5-aminosalicylic acid under these reaction conditions made it unsuitable as starting material. *tert*-Butyl 5-aminosalicylate (**14**) was obtained by reducing *tert*-butyl 5-nitrosalicylate (**13**). The condensation between compounds **11** and **14** to form the carrier **15** was carried out in acetic acid. Mitsunobu conditions were used to attach **15** to prednisolone at the 21-OH. Although the steroid has three hydroxyl groups, secondary groups at positions 11 and 17 are much less reactive than the primary alcohol at C-21.[18] The position of attachment in each case was confirmed by ^{1}H COSY/HMBC (SI). Finally, rapid removal of the *tert*-butyl group of **16** using TFA afforded prodrugs **5a** and **5b**. Both were obtained as ethyl acetate solvates that resisted attempts at removal under heat or vacuum, as evidenced by ^{1}H NMR.

Pharmaceutical Characterization. Drug release as envisaged in Figure 1b hinges on intramolecular lactamization of

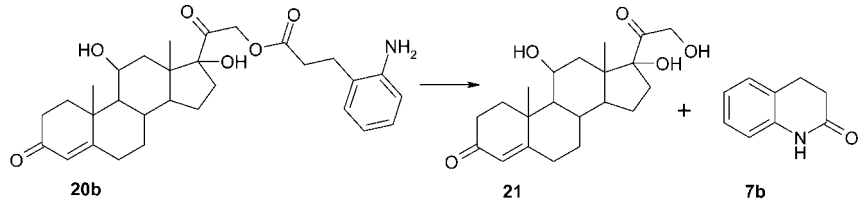
Scheme 1. Synthetic Route to Prodrug **5**[a]



[a] Conditions: (a) Pd/C, H_2 in 2 N NaOH; (b) Oxone; (c) DCC in THF, DMAP in *tert*-butanol; (d) Pd/C, H_2; (e) AcOH; (f) **3**, Ph_3P, DIAD in THF; (g) TFA, DCM.

the amino-ester intermediate of type **6**. Although anilines possess low nucleophilicity (compared to aliphatic amines), the high effective molarity of the intramolecular arrangement was expected to promote rapid drug release. Reports on the intramolecular aminolysis of aminophenyl esters under roughly comparable conditions were encouraging. The spontaneous cyclization of methyl 3-(2-aminophenyl)propionate at neutrality (roughly the pH of the colon) and 37 °C was reported to proceed with an apparent first-order rate constant of 2×10^{-4} s^{-1}, corresponding to a half-life of 57 min.[19] The ring closure of methyl- and trifluoroethyl (2-aminophenyl)acetate (yielding the dihydroindolone) was reported to follow similar kinetics under mildly basic conditions (pH 7−8).[20] These half-lives are ideal for drug release in the colon, where residence time tends be extended. However, it was not obvious that cyclization of the more complex steroid ester would follow similar kinetics given the possibilities for steric congestion or competing intramolecular arrangements, for example, H-bonding with the anilide, which might prevent its access to the ester. We therefore decided to study a model amino steroid ester in order to demonstrate the feasibility of lactamization before proceeding to biological testing.

Figure 2 illustrates the model test system we selected for evaluation of cyclization kinetics: compound **20b**, the aminophenylpropionic acid ester of hydrocortisone (**21**). The synthesis and isolation of **20b** were complicated by competing aminolysis intra- and intermolecularly, and it had to be made in a roundabout way (Scheme 2). Esterification of **9** in methanol with DCC and DMAP at room temperature yielded the methyl ester of 2-nitrocinnamic acid. The nitro group was selectively reduced with zinc and ammonium chloride to afford **17**, leaving the trans-alkene group intact. BOC protection of the amino group before reducing the alkene group was required in order to prevent lactamization. Once the amino group was protected, the alkene was reduced (H_2, Pd/C), yielding **18**. Base hydrolysis afforded the BOC-amino acid (**19**) (Scheme 2), which was coupled to hydrocortisone (**21**) by esterification to yield **20**. Lactamization was generally found to be very rapid in organic solutions; therefore, the deprotection of **20** was carried out in DCM at 0 °C with TFA for several minutes, the volatiles were removed under a stream of nitrogen, and the residue was dissolved immediately in the appropriate buffer solution before starting kinetic studies. The lactamization of compound **20b** was studied in PBS at pH 2.6, 4, 7.4, and 8 (37 °C) using HPLC to measure the disappearance of the parent and the appearance of hydrocortisone **21** and dihydroquinolone **7b**. The disappearance followed first-order kinetics with half-lives in the region 0.45−1.5 h, similar to those reported previously for simple methyl esters of aminophenylacetic acid and propionic acid.[19,20] Intramolecular aminolysis of alkyl (2-aminophenyl)propionates is unusual in being susceptible to general base and acid catalysis. The low pK_a of the anilide results in substantial amounts being

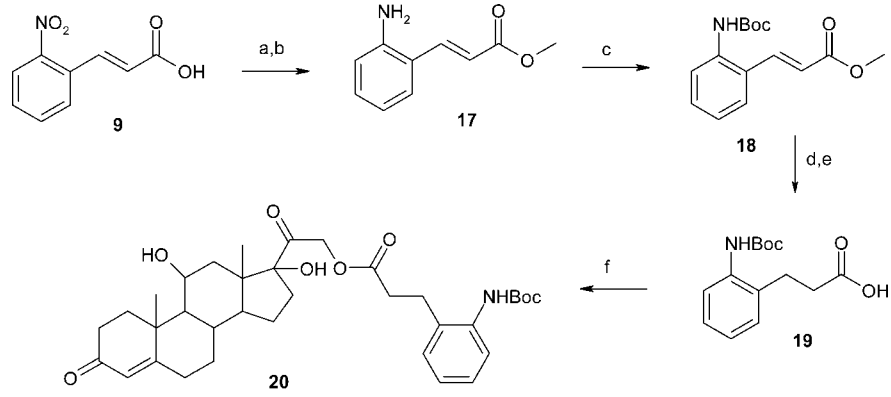


pH	8	7.4	4	2.6
†$k_{obs}\times10^3$ (min^{-1})	15.5–13.5	9.4–7.6	22.6-14.9	12.8-11.4
$t_{1/2}$ (min)	44-51	73-91	30-46	54-60

†$r^2 > 0.99$, 95% confidence interval

Figure 2. Intramolecular lactamization of cortisone aminophenyl propionate: disappearance kinetics for **20b** during lactamization.

Scheme 2. Synthetic Route to BOC-Protected Model Compound **20**[a]



[a] Conditions: (a) DCC, DMAP, MeOH, and DCM; (b) Zn, NH_4OH_{aq}; (c) $(BOC)_2O$, DMAP, EtOAc, 12 h; (d) Pd/C, H_2, EtOAc, 2 h; (e) 2 M NaOH, MeOH, 24 h; (f) hydrocortisone (**21**), DCC, DMAP, DCM, room temp.

neutral at pH values where there is significant protonation of the ester group. This presumably explains the rapid cyclization observed here at pH 4. The (2-aminophenyl)propionic acid hydrolysis product of **20b** was not observed in chromatograms at any pH value, though it might have been cyclizing itself as it formed.

Another factor that could influence the potential utility of the prodrugs is their stability to conditions in the lumen of the GIT during transit to the colon. The aqueous stability of **5b** was tested in the pH range 1.5−12 and in freshly aspirated human gastric juice. There was no change in the concentration following incubation in gastric juice for 24 h. There was no significant change in drug concentration in borate buffers in the pH range 3.4−7.4 over 24 h, but there was evidence of base-catalyzed hydrolysis in the range pH 8−9.

The transport of **3** and compounds **5** through a CACO-2 cell line was evaluated in order to determine if the prodrugs were transported by passive transcellular diffusion in the GIT. There was no evidence of absorptive transport for either **5a** or **5b** in the CACO-2 model. Secretory transport (BL > AP) was observed when **5b** was applied to the basolateral side ($P_{app} = 1.4 \times 10^{-5}$). In contrast, absorptive and secretory transport was observed for **3** ($P_{app} = 7.5 \times 10^{-6}$ cm/s and $P_{app} = 2.0 \times 10^{-5}$, respectively). An efflux mechanism for prednisolone has previously been reported.[21] The data indicated that compounds **5a** and **5b** have suitable physicochemical characteristics for gastrointestinal transit to the colon.

Murine DSS Colitis Study. The in vivo efficacy of the prodrugs was investigated using the DSS-induced model of ulcerative colitis (UC), which exhibits many of the symptoms observed in human IBD.[22−27] As oral treatment of prednisolone attenuates the severity of disease in DSS-induced colitis,[22] the efficacy of **5a** and **5b** was tested relative to prednisolone in the model. Following addition of DSS to the drinking water of mice, animals develop progressive weight loss, diarrhea, rectal bleeding, and colon inflammation with marked reduction in the length of the colon.[23,24] Following DSS treatment, mice treated with vehicle developed progressive weight loss (Figure 3) and an increase in clinical scores of disease symptoms (disease activity index (DAI, Figure 4) and at autopsy had significant reduction in the length of the colon (Figure 5). Oral treatment with **3** or **5b** attenuated weight loss and DAI scores relative to the vehicle-treated group (Figures 4 and 5). However, with respect to DSS-induced shortening of the colon, compound **5b** was significantly more effective than prednisolone (Figure 5). These data demonstrate that oral delivery of **5b** conferred protection from colitis that was at least comparable to the parent compound in the mouse model. In contrast, compound **5a** did not exhibit anti-inflammatory activity in any measure.

Glucocorticoids cause thymolysis by suppression of the HPA axis resulting in a reduction in the ratio of thymus weight to body weight (TW/BW) in mice treated with prednisolone orally. Thymic atrophy is therefore a sensitive marker of corticosteroid exposure in the systemic circulation.[28] In the present study there was significantly reduced TW/BW ratio in the prednisolone and DSS-treated group relative to vehicle and DSS-treated mice ($P < 0.001$; Figure 6), an indication of systemic effects in the mice and thymolysis. In contrast, the TW/BW ratio for the **5b**

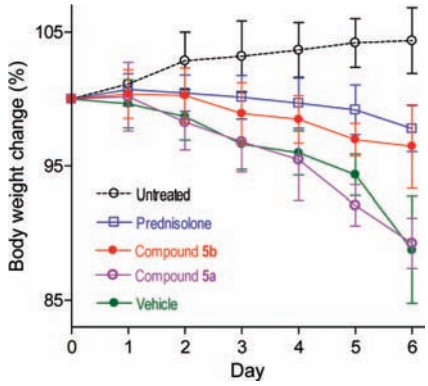


Figure 3. Body weight loss percentage profile of healthy mice (untreated) and DSS-induced colitis mice treated at 5 mg/kg: change in body weight, as percentage of starting weight, in healthy mice (untreated) mice and mice treated with DSS for 6 days and administered vehicle, **3**, or **5**. Data are the mean ± SD from 6 mice per group.

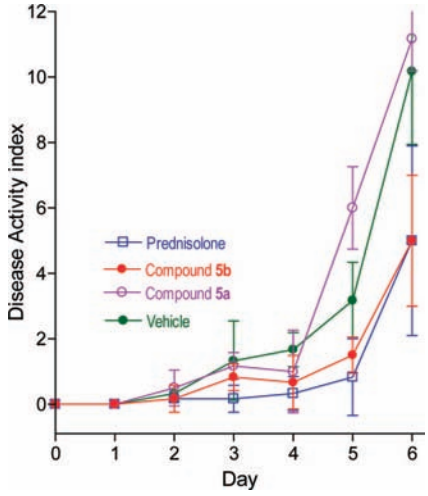


Figure 4. Drug activity index score profile of DSS-induced colitis mice treated at 5 mg/kg: disease activity index (DAI) in mice treated with DSS for 6 days and administered vehicle, prednisolone, or **5**. Data are the mean ± SD from 6 mice per group.

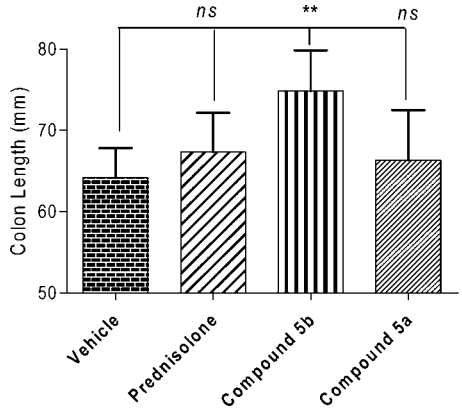


Figure 5. Colon length profile DSS-induce colitis mice treated at 5 mg/kg dosage: colon lengths of mice treated for 6 days with DSS and administered vehicle, prednisolone, or compounds **5**. Colon lengths were measured at autopsy on day 6. Data are the mean ± SD from 6 mice per group. Student's *t* test was used to test for statistical difference between groups: (**) $P < 0.01$ significant difference between groups; ns, nonsignificant.

treatment group was not significantly different to vehicle group. The TW/BW for **5b** was also significantly higher ($P < 0.001$)

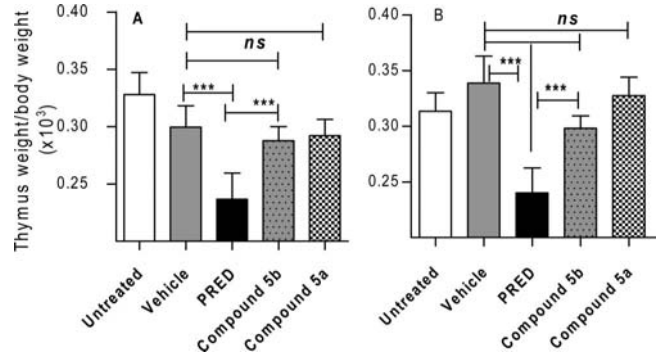


Figure 6. Thymus weight after treatment body weight ratios profile of healthy mice (untreated) and DSS-induce colitis mice treated at 5 mg/kg dosage: thymus weight and body weight ratios in healthy mice (untreated) mice and mice treated with DSS for 6 days and administered vehicle, prednisolone, or **5**. Graph A is for starting weight, and graph B is the ratio to final weight. Data are the mean ± SEM from 6 mice per group. Student's *t*-test was used to test for statistical difference between vehicle, prednisolone, and **5** groups: (***) $P < 0.001$ significant difference between groups; ns, nonsignificant.

than mice receiving oral prednisolone, consistent with reduced systemic prednisolone (Figure 6). Therefore, the data show that while **5b** acted as an anti-inflammatory agent as effectively as prednisolone, it caused significantly lower systemic side effects as reflected in the TW/BW ratio analysis. This is consistent with the CACO-2 data which indicate that **5** is unlikely to be absorbed from the GIT. Compound **5a** did not induce thymolysis, but there was no evidence of drug release in the anti-inflammatory assays.

Another possible interpretation of the thymus weight data is that compounds **5a** and **5b** were absorbed but did not release **3** in blood leading to reduced systemic exposure. In order to exclude this possibility, both compounds were incubated in mouse plasma diluted with pH 7.4 buffer and the resultant mixture was analyzed by HPLC. Rapid disappearance of both esters was observed with equimolar evolution of **3** resulting from 21-ester cleavage (**5b** $k_{obs} = 0.534 \pm 0.027$, $t_{1/2} = 25.1$ s), indicating that prednisolone would have been produced if either compound had been absorbed from the GIT in the mouse study.

Conclusion

The cyclization−activation prodrug strategy is usually adopted in cases where there is a desire to increase passive diffusion and oral biovailability.[29] In this paper we have shown that it can be adopted to depress steroid absorption. Compound **5b** is not transported in the CACO-2 cell line, suppresses DSS induced inflammation as potently as prednisolone, but has significantly reduced steroid related side effects. Compound **5a** based on a phenylacetic acid linker unit did not exhibit anti-inflammatory activity or induce thymolysis, indicating that it did not release prednisolone in vivo. The difference in behavior between the two compounds seems likely to arise from a difference in activation, which could be due to rates of azoreduction, cyclization, or intestinal hydrolysis.

The design of the successful compound **5b** incorporates 5-ASA and produces a dihydroquinolone byproduct, about which we were unable to find toxicity data. However, systemic exposure to the cyclization byproduct is likely to be low if the colon is targeted successfully. The cyclization byproduct could in future designs be used to modulate pharmacological or pharmaceutical characteristics such as polarity or cellular transport. Compound **5b** has significant potential in the treatment

of IBD with reduced steroid side effects. The design could be applied to target other hydroxyl-bearing therapeutics to the colon.

Experimental Section

Chemistry. Melting points were obtained using a Stuart melting point SMP11 melting point apparatus. Spectra were obtained using a Perkin-Elmer 205 FT infrared Paragon 1000 spectrometer. Band positions are given in cm^{-1}. Solid samples were obtained by KBr disk; oils were analyzed as neat films on NaCl plates. ^1H and ^{13}C spectra were recorded at 27 °C on a Bruker Advance II 600 MHz spectrometer (600.13 MHz for ^1H, 150.91 MHz for ^{13}C) and Bruker DPX 400 MHz FT NMR spectrometer (400.13 MHz for ^1H, 100.16 MHz for ^{13}C), in either $CDCl_3$ or CD_3OD (tetramethylsilane as internal standard). For $CDCl_3$, ^1H NMR spectra were assigned relative to the TMS peak at 0.00 δ, and ^{13}C NMR spectra were assigned relative to the middle $CDCl_3$ triplet at 77.00 ppm. For CD_3OD, ^1H and ^{13}C NMR spectra were assigned relative to the center peaks of the CD_3OD multiplets at 3.30 δ and 49.00 ppm, respectively. Coupling constants were reported in hertz (Hz). For ^1H NMR assignments, chemical shifts are reported: shift values (number of protons, description of absorption (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) where applicable, proton assignment). High resolution mass spectrometry (HRMS) was performed on a Micromass mass spectrophotometer (EI mode) at the Department of Chemistry, Trinity College. HPLC was performed on a reverse phase 250 mm × 4.6 mm Waters Spherisorb ODS-2, 5 μm column using a Waters Alliance 2695 chromatograph equipped with an autosampler, column oven, and dual wavelength detector. The flow rate was 1 mL/min with a mobile phase consisting of 40% phosphate buffer, pH 2.5, and 60% acetonitrile at time 0 and grading to 85% acetonitrile at 4 min. Injection volume was 20 μL, and areas were determined at 254 nm. The isocratic HPLC method was aqueous phosphate buffer solution, pH 2.5, 40% and acetonitrile 60%. Flow rate was 1 mL/min. Another HPLC method using gradient mobile phase allowed better resolution at lower concentration and better peak shapes: column, Xbridge 48 4.6 mm × 250 mm, 5 μm. The mobile phase was (A) 0.5% NH_4HCOO^- pH 10.0 or (B) acetonitrile, with gradient 10−90% B over 20 min. Flow rate was 1.5 mL/min, and the column temperature was room temperature. Flash chromatography was performed on Merck Kieselgel 60 particle size 0.040−0.063 mm. Thin layer chromatography (TLC) was performed on silica gel Merck F-254 plates. Compounds were visually detected by absorbance at 254 nm and/or vanillin staining. Test compounds **20**, **5a**, and **5b** were >98% by HPLC.

5-(2-{2-[2-(11,17-Dihydroxy-10,13-dimethyl-3-oxo-6,7,8,9,10,11,-12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[α]phenanthren-17-yl)-2-oxoethoxycarbonyl]methyl}phenylazo)-2-hydroxybenzoic Acid *tert*-Butyl Ester (16a). Prednisolone **3** (1.1 equiv, 1.18 g, 0.0013 mol) and triphenylphosphine (3 equiv, 0.88 g, 0.0033 mol) in dry THF (50 mL) were added to a solution of **15a** (0.40 g, 0.0011 mol) followed by DIAD (3 equiv, 0.66 mL, 0.0033 mol) dropwise over 12 min at 40 °C. The mixture was stirred at 40 °C for an hour and left overnight at room temperature under nitrogen atmosphere. Solvents were removed under reduced pressure to afford the product as an orange oil. This was purified by flash chromatography using dichloromethane/ethyl acetate (50:50). After this column the triphenylphosphine oxide was removed and a second flash column was performed to yield the product as orange crystals (0.33 g, 43%): mp 125 ± 1 °C. ^1H NMR δ ($CDCl_3$): 11.49 (1H, s), 8.44 (1H, s), 8.05 (1H, d, J = 9 Hz), 7.75 (1H, d, J = 7.52 Hz), 7.40 (3H, m), 7.24 (1H, d, J = 10.04), 7.06 (1H, d, J = 9 Hz), 6.25 (1H, s), 5.98 (1H, s), 4.27 (2H, s), 2.68−0.84 (19H), 1.67 (9H, s). ^{13}C NMR δ ($CDCl_3$): 204.40, 186.26, 171.36, 170.10, 169.09, 163.85, 156.25, 149.53, 145.07, 133.25, 130.98, 130.53, 129.89, 127.85, 127.17, 125.52, 121.79, 118.14, 115.14, 113.26, 89.17, 83.30, 69.51, 67.98, 54.86, 50.86, 47.26, 43.68, 38.85, 36.44, 33.92, 33.58, 31.56, 30.71, 27.78, 23.36, 20.56, 16.37.

5-(2-{2-[2-(11,17-Dihydroxy-10,13-dimethyl-3-oxo-6,7,8,9,10,11,-12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[α]phenanthren-17-yl)-2-oxoethoxycarbonyl]methyl}phenylazo)-2-hydroxybenzoic Acid (5a). To a solution of *tert*-butyl ester **15a** 2 (0.1 g, 0.000 155 mol) in dichloromethane (1 mL), trifluoroacetic acid (1 mL) was added. The mixture was left at room temperature for 4 h. After completion the solvents and trifluoroacetic acid were evaporated using a stream of nitrogen to afford the product as a orange oil. The crude product was purified by flash chromatography using dichloromethane/ethyl acetate (50:50) to yield the product as orange crystals (0.078 g, 78%): mp 177 ± 1 °C. MS: 665.2503 calculated mass 665.2475. ^1H NMR δ ($CDCl_3$): 8.48 (1H, s), 8.10 (1H, d, J = 9.04 Hz), 7.75 (1H, d, J = 7.52 Hz), 7.46 (4H, m), 7.08 (1H, d, J = 9 Hz), 6.24 (1H, d, J = 10 Hz), 6.00 (1H, s), 5.03 (2H, d, J = 17.56 Hz), 4.35 (1H, s), 4.31 (2H, d, J = 8.52 Hz), 2.68−0.8 (19H). ^{13}C NMR δ (MeOD): 205.18, 187.15, 172.99, 171.82, 164.11, 158.38, 156.35, 149.62, 144.93,133.49, 133.36, 130.90, 129.97, 127.39, 127.0, 126.20, 125.88, 120.57, 117.01, 114.64, 88.75, 68.91, 67.95, 55.40, 50.91, 44.19, 38.19, 36.09, 33.70, 32.75, 31.31, 27.66, 22.95, 19.72, 15.36. Contained ∼5% ethyl acetate with signals at 1.26 (t), 2.05 (s), 4.12 (q). This could not be removed by heating to 150 °C and/or vacuum.

5-(2-{2-[2-(11,17-Dihydroxy-10,13-dimethyl-3-oxo-6,7,8,9,10,11,-12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[α]phenanthren-17-yl)-2-oxoethoxycarbonyl]ethyl}phenylazo)-2-hydroxybenzoic Acid *tert*-Butyl Ester (16b). To a solution of azo carrier (**15b**) (1.21 g, 3.3 mmol), prednisolone (**3**) (1 equiv, 1.18 g, 3.3 mmol), and PPh_3 (3 equiv, 2.59 g, 9.9 mmol) in dry tetrahydrofuran (50 mL), DIAD (3 equiv, 1.9 mL, 9.9 mmol) was added dropwise over 12 min after the reaction temperature reached 40 °C. The mixture was stirred at 40 °C for an hour and then left overnight at room temperature under a nitrogen atmosphere. TLC (DCM) analysis showed completion. The solvent was removed under reduced pressure to afford the product as an orange oil. This was flash-columned using DCM/ethyl acetate (50:50). The triphenylphosphine oxide was removed using a second flash chromatography [hexane (200 mL), hexane/ethyl acetate (70:30)] to yield the product as orange crystals (0.92 g, 40%): mp 117 ± 1 °C. ^1H NMR δ ($CDCl_3$): 8.40 (1H, d, J = 2 Hz), 8.10 (1H, dd, J = 8.52 and 2 Hz), 7.68 (1H, d, J = 8.04 Hz), 7.44 (3H, m), 7.33 (1H, t, J = 6.52 Hz), 7.09 (1H, d, J = 8.52 Hz), 6.27 (1H, d, J = 10.04 Hz), 6.01 (1H, s), 5.00 (1H, d, J = 17.56 Hz), 4.88 (1H, d, J = 17.56 Hz), 4.39 (1H, s), 3.49 (2H, t, J = 9.52), 2.82 (2H, t, J = 8.04 Hz), 2.66−0.9 (19H), 1.69 (9H, s). ^{13}C NMR δ ($CDCl_3$): 204.8, 186.5, 172.5, 170.7, 169, 163.7, 156.7, 149.5, 145.1, 139.3, 130.5, 130.0, 129.26, 127.0, 126.9, 125.7, 121.6, 118.9, 114.9, 113.3, 89.20, 83.2, 69.6, 67.7, 54.9, 50.8, 47.2, 43.8, 38.9, 35.5, 33.8, 33.5, 31.6, 30.7, 27.7, 26.7, 23.4, 20.5, 16.4. HRMS: found $(M - Na)^+$ = 735.3283, required $(M - Na)^+$ = 735.3258.

5-(2-{2-[2-(11,17-Dihydroxy-10,13-dimethyl-3-oxo-6,7,8,9,10,11,-12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[α]phenanthren-17-yl)-2-oxoethoxycarbonyl]ethyl}phenylazo)-2-hydroxybenzoic Acid (5b). To a solution of **16b** (0.9 g, 1.26 mmol) in DCM (3 mL) was added trifluoroacetic acid (3 mL), and the mixture was left at room temperature for 4 h. The solvents and trifluoroacetic acid were evaporated using nitrogen to afford the product as an orange oil. The crude product was purified by flash chromatography using dichloromethane/ethyl acetate (50:50), (100 mL), ethyl acetate (100 mL), and acetone/ethyl acetate (70:30) to yield the product as orange crystals (0.7 g, 84%): mp 177 ± 1 °C. ^1H NMR δ (MeOD): 8.44 (1H, s), 8.07 (1H, d, J = 9.03 Hz), 7.65 (1H, d, J = 7.53 Hz), 7.43 (3H, m), 7.31 (1H, t, J = 7.53 Hz), 7.07 (1H, d, J = 9.04 Hz), 6.26 (1H, d, J = 10.04 Hz), 5.99 (1H, s), 4.88 (2H, d, J = 17.56 Hz), 4.36 (1H, s), 3.44 (2H, t, J = 7.53 Hz), 2.80 (2H, t, J = 8.03 Hz), 2.64−0.89 (19H). ^{13}C NMR δ (MeOD): 205.4, 187.2, 173.1, 172.1, 164.0, 161.3, 158.4, 149.6, 144.9, 139.1, 129.8, 128.6, 126.5, 125.9, 124.5, 120.6, 117.6, 116.8, 114.5, 88.7, 68.9, 67.5, 55.4, 50.9, 44.2, 38.4, 35.2, 33.7, 32.8, 31.3, 30.8, 26.3, 22.9, 19.7, 15.45. MS: found $(M - H)^+$ = 657.2814, requires $(M - H)^+$ = 657.2812; found $(M)^-$ = 655.2662, requires $(M)^-$ = 655.2656. Contained

~5% ethyl acetate with signals at 1.26 (t), 2.05 (s), 4.12 (q). This could not be removed by heating to 150 °C and/or vacuum.

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Supporting Information Available: Synthesis, characterization data, and purity data for all compounds; cyclization data for **20b**; stability data for compound **5b** in aqueous buffer solution and in mouse plasma. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Scheier, H. In *Drug Targeting Technology: A Critical Analysis of Physical, Chemical and Biological Methods*; Schreier, H., Ed.; Marcel Dekker Inc.: New York, 2001.
(2) Bauer, K. H. Colon-Specific Drug Delivery. In *Drug Targeting Technology: A Critical Analysis of Physical, Chemical and Biological Methods*; Schreier, H., Ed.; Marcel Dekker Inc.: New York, 2001.
(3) Bai, J. P.; Chsang, L. L.; Guo, J. H. Targeting of peptide and protein drugs to specific sites in the oral route. *Crit. Rev. Ther. Drug Carrier Syst.* **1995**, *12*, 339–371.
(4) Green, J. R. B.; Lobo, A. L.; Holdsworth, C. D. Balsalazide is more effective and better tolerated than mesalazine in the treatment of acute ulcerative colitis. *Gastroenterology* **1998**, *114*, 15–22.
(5) Sinha, V. R.; Kumria, R. Colonic drug delivery: prodrug approach. *Pharm. Res.* **2001**, *18* (5), 557–564.
(6) Basit, A. W. Oral colon-specific drug delivery using amylose-based film coatings. *Pharm. Technol. Eur.* **2000**, *12*, 30–36.
(7) Hu, Z.; Kimura, G.; Mawatari, S.; Shimokawa, T.; Yoshikawa, Y.; Takada, K. New preparation method of intestinal pressure-controlled colon delivery capsules coating machine and evaluation in beagle dogs. *J. Controlled Release* **1998**, *56*, 293–302.
(8) Jung, Y. J.; Doh, M. J.; Kim, I. H.; Kong, H. S.; Lee, J. S.; Kim, Y. M. Prednisolone 21-sulfate sodium: a colon-specific pro-drug of prednisolone. *J. Pharm. Pharmacol.* **2003**, *55*, 1075–1082.
(9) Haeberlin, B.; Rubas, W.; Nolen, H. W.; Friend, D. R. In vitro evaluation of dexamethasone-beta-D-glucuronide for colon-specific drug delivery. *Pharm. Res.* **1993**, *11*, 1553–1562.
(10) Leopold, C. S.; Friend, D. R. In vivo pharmacokinetic study for the assessment of poly(L-aspartic acid) as a drug carrier for colon-specific drug delivery. *J. Pharmacokinet. Biopharm.* **1995**, *23*, 397–406.
(11) Yano, H.; Hirayama, F.; Kamada, M.; Arima, H.; Uekama, K. Colon-specific delivery of prednisolone-appended alfa-cyclodextrin conjugate: alleviation of systemic side effect after oral administration. *J. Controlled Release* **2002**, *79*, 103–112.
(12) McLeod, A. D.; Fedorak, R. N.; Friend, D. R.; Tozer, T. N.; Cui, N. A glucocorticoid prodrug facilitates normal mucosal function in rat colitis without adrenal suppression. *Gastroenterology* **1994**, *106*, 405–413.
(13) Wilding, I. R.; Davis, S. S.; O'Hagan, D. T. Targeting of drugs and vaccines to the gut. *Pharmacol. Ther.* **1994**, *62*, 97–124.
(14) Carceller, E.; Salas, J.; Merlos, M.; Giral, M.; Ferrando, R.; Escamilla, I.; Ramis, J.; García-Rafanell, J.; Forn, J. Novel azo derivatives as prodrugs of 5-aminosalicylic acid and amino derivatives with potent platelet activating factor antagonist activity. *J. Med. Chem.* **2001**, *44*, 3001–3013.
(15) Sakuma, S.; Lu, Z. R.; Kopecková, P.; Kopecek, J. Biorecognizable HPMA copolymer−drug conjugates for colon-specific delivery of 9-aminocamptothecin. *J. Controlled Release* **2001**, *75*, 365–379.
(16) Van den Mooter, G.; Samyn, C.; Kinget, R. The relation between swelling properties and enzymatic degradation of azo polymers designed for colon-specific drug delivery. *Pharm. Res.* **1994**, *11*, 1737–1741.
(17) Fleisher, D.; Johnson, K. C.; Stewart, B. H.; Amidon, G. L. Oral absorption of 21-corticosteroid esters: a function of aqueous stability and intestinal enzyme activity and distribution. *J. Pharm. Sci.* **1986**, *75*, 934–939.
(18) Fang, L.; Zhang, Y.; Lehmann, J.; Wang, Y.; Ji, H.; Ding, D. Design and synthesis of furoxan-based nitric oxide-releasing glucocorticoid derivatives with potent anti-inflammatory activity and improved safety. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1062–1066.
(19) Kirby, A. J.; Mujahid, T. G. Anilide formation from an aliphatic ester. The mechanism of cyclisation of methyl 3-(2-aminophenyl)propionate. *J. Chem. Soc., Perkin Trans. 2* **1979**, 1610–1616.
(20) Fife, T. H.; Duddy, N. W. Intramolecular aminolysis of esters. Cyclization of esters of (*o*-aminophenyl)acetic acid. *J. Am. Chem. Soc.* **1983**, *105*, 74–79.
(21) Dilger, K.; Schwab, M.; Fromm, M. Identification of budesodine and prednisone as substrates of the intestinal drug efflux pump P-glycoprotein. *Inflammatory Bowel Dis.* **2006**, *10*, 578–583.
(22) Verdu, E. F.; Bercik, P.; Cukrowska, B.; Farre-Castany, M. A.; Bouzourene, H.; Saraga, E.; Blum, A. L.; Corthesy-Theulaz, I.; Tlaskalora-Hogenova, H.; Michetti, P. Oral administration of antigens from intestinal flora anaerobic bacteria reduces the severity of experimental acute colitis in BALB/c mice. *Clin. Exp. Immunol.* **2000**, *120*, 46–50.
(23) Okayasu, I.; Hatakeyama, S.; Yamada, M.; Ohkusa, T.; Inagaki, Y.; Nakaya, R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* **1990**, *98*, 694–702.
(24) Elson, C. O.; Sartor, R. B.; Tennyson, G. S.; Riddell, R. H. Experimental models of inflammatory bowel disease. *Gastroenterology* **1995**, *109*, 1344–1367.
(25) Smith, P.; Mangan, N. E.; Walsh, C. M.; Fallon, R. E.; McKenzie, A. N.; van Rooijen, N.; Fallon, P. G. Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism. *J. Immunol.* **2007**, *178*, 4557–4566.
(26) Cummins, E. P.; Seeballuck, F.; Keely, S. J.; Mangan, N. E.; Callanan, J. J.; Fallon, P. G.; Taylor, C. T. The hydroxylase inhibitor dimethyloxalylglycine is protective in a murine model of colitis. *Gastroenterology* **2008**, *134*, 156–165.
(27) Feighery, L. M.; Smith, P.; O'Mahony, L.; Fallon, P. G.; Brayden, D. J. Effects of *Lactobacillus salivarius* 433118 on intestinal inflammation, immunity status and in vitro colon function in two mouse models of inflammatory bowel disease. *Dig. Dis. Sci.* **2008**, *53*, 2495–2506.
(28) Yano, H.; Hirayama, F.; Kamada, M.; Arima, H.; Uekama, K. Colon-specific delivery of prednisolone-appended alpha-cyclodextrin conjugate: alleviation of systemic side effect after oral administration. *J. Controlled Release* **2002**, *79*, 103–112.
(29) Gomes, P.; Vale, N.; Moreira, R. Cyclization-activated prodrugs. *Molecules* **2007**, *12*, 2484–2506.

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