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RESEARCH ARTICLES

Strategies in the Design of Solution-Stable, Water-Soluble Prodrugs I: A Physical–Organic Approach to Pro-Moiety Selection for 21-Esters of Corticosteroids

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Abstract □ The ideal water-soluble prodrug should exhibit sufficient aqueous solution stability to allow long-term storage of its solutions (i.e., 2 years at room temperature) and yet should be converted rapidly in vivo to the active parent drug—two severe and seemingly conflicting demands which limit the utility of many common solubilizing pro-moieties. For example, succinate esters, which are commonly utilized as water-soluble prodrugs, are unstable in solution and may undergo slow and incomplete bioconversion in vivo. In this study, the solution stability problems associated with 21-esters of corticosteroids are reviewed. It is concluded that the most important reaction limiting shelf life is ester hydrolysis. From a consideration of the influence of molecular structure on ester reactivity, a strategy for the design of solution-stable, water-soluble prodrugs of corticosteroids has been developed. Two key requirements for dilute solution stability are (*a*) high solubility at the pH of optimum stability and (*b*) appropriate design of the pH–rate profile. Several 21-esters of methylprednisolone have been synthesized, and the rates of their aqueous solution hydrolysis have been determined to test the strategy. Compounds exhibiting estimated shelf lives in dilute solution of >2 years at 25°C have been identified.

Water-soluble prodrugs employed to solubilize poorly soluble compounds for intravenous or intramuscular administration, such as succinate esters, quite often possess certain physicochemical-property-related disadvantages which limit their utility. Two such deficiencies of succinate esters are their solution instability[1–3] and their relatively slow and incomplete bioconversion in vivo.[4,5] When a rapid onset of action is desired, as in emergency therapy, it is most desirable that a water-soluble prodrug be formulated in an injection-ready solution and that the prodrug→active parent drug reconversion in vivo be rapid and predictable. Thus, the ideal prodrug in such applications should exhibit sufficient aqueous solution stability to allow long-term storage of its solutions (i.e., 2 years at room temperature) and yet should be converted rapidly in vivo, two severe and seemingly conflicting demands which limit the utility of most common solubilizing pro-moieties.

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The corticosteroids are a group of compounds having poor aqueous solubility that are frequently solubilized as prodrugs for use in emergency situations. While a variety of soluble derivatives have been synthesized and tested,[4–11] those which are commercially available are generally either 21-succinate or 21-phosphate esters. Succinate esters of corticosteroids undergo relatively rapid hydrolytic degradation in aqueous solution and are, therefore, marketed as lyophilized powders for reconstitution.[1,2] Corticosteroid 21-phosphate esters are more stable, allowing in some cases (but not all) the formulation of solutions with practical shelf lives,[12] but there is clearly a need for additional pro-moieties which afford soluble derivatives with long-term solution stability and rapid bioconversion rates in vivo.

Recent kinetic studies of the degradation of methylprednisolone 21-succinate (sodium salt)[13] have elucidated several factors which affect the aqueous solution stability of 21-carboxylic acid esters of corticosteroids.[1,2,14,15] In this study, various physical–organic considerations relating to the stability of 21-esters of corticosteroids are addressed, leading to possible strategies one might employ in developing more stable, soluble prodrugs of corticosteroids and other relatively insoluble drugs. Several new esters of methylprednisolone were synthesized, and their stabilities in aqueous solution were evaluated to test the proposed strategies. This report focuses on the stability improvement attainable in dilute solutions. A subsequent paper will treat the added stability advantages obtained in concentrated solutions due to self-association of corticosteroid derivatives.

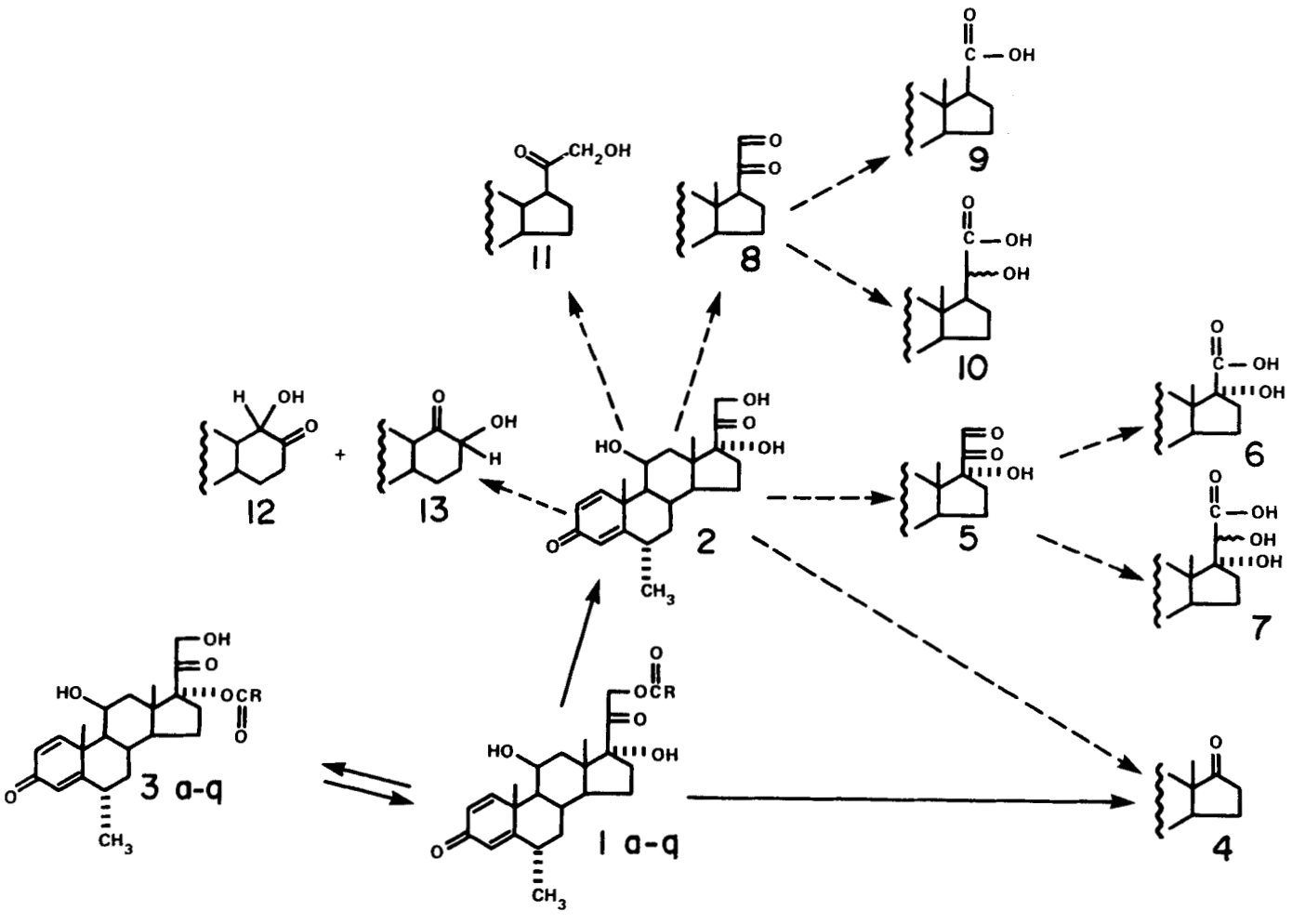
Background

Degradation Pathways—Excellent work by Bundgaard et al.[16–18] on the degradation of corticosteroids in aqueous solution combined with that of other researchers[1,14,15,19] provides a reasonable idea of the possible reactions 21-carboxylic

acid esters of corticosteroids might undergo in solution formulations stored for long periods of time. Shown in Scheme I are some of these potential primary and secondary routes of degradation for C_{21}-esters of methylprednisolone. Ester hydrolysis, 21 → 17 ester migration, and a variety of both oxidative and nonoxidative reactions of the C_{17}-dihydroxyacetone side chain can occur. Photocatalyzed A-ring degradation of corticosteroids is also known to occur, but this can be prevented by protecting solutions from light[20] and is not included in Scheme I.

As expressed in Scheme I, the overall rate of disappearance of 21-ester in solution is the sum of the rates of the primary degradative reactions (solid arrows), while the product distribution at any time may be influenced by the secondary reactions (dashed arrows). Both primary and secondary pathways may influence shelf life, but the primary reactions *must* be minimized to achieve solution stability. Therefore, the primary pathways are the focus of this discussion.

Initial rate studies of the degradation of methylprednisolone 21-succinate (**1a**) and methylprednisolone 21-acetate (**1b**) have shown that both ester hydrolysis to form **2** and 21 → 17 ester migration to form **3a** and **3b**, respectively, are important primary reactions in the decomposition of these compounds.[2,15] Both reactions are base catalyzed and in neutral or basic solutions generally proceed at similar initial rates. Acid catal-



a : R = $-(CH_2)_2\,COO^-\ Na^+$
b : R = $-CH_3$
c : R = $-(CH_2)_2\,CONH(CH_2)_2N(CH_3)_2\cdot HCl$
d : R = $-(CH_2)_2\,CONCH_3(CH_2)_2\,N(CH_3)_2$
e : R = $-(CH_2)_4\,CONCH_3(CH_2)_2\,N(CH_3)_2\cdot HCl$
f : R = $-(CH_2)_4\,CONEt\,(CH_2)_2\,N(Et)_2\cdot HCl$
g : R = $-(CH_2)_6\,CONH(CH_2)_2\,N(Et)_2\cdot HCl$
h : R = $-(CH_2)_6\,CONCH_3(CH_2)_2\,N(CH_3)_2\cdot HCl$
i : R = $-(CH_2)_6\,CONEt(CH_2)_2\,N(Et)_2\cdot HCl$
j : R = $-(CH_2)_2\,CONH\,(CH_2)_2\,SO_3^-\ Na^+$
k : R = $-(CH_2)_2\,CONCH_3(CH_2)_2\,SO_3^-\ Na^+$
l : R = $-(CH_2)_6\,CONH(CH_2)_2\,SO_3^-\ Na^+$
m : R = $-(CH_2)_6\,CONCH_3(CH_2)_2\,SO_3^-\ Na^+$
n : R = $-(CH_2)_5\,N(Et)_2\cdot HCl$
o : R = $-(CH_2)_2\,SO_3^-\ Na^+$
p : R = $-(CH_2)_5\,SO_3^-\ Na^+$
q : R = $-(CH_2)_{10}SO_3^-\ Na^+$

Scheme I

ysis promotes hydrolysis over acyl migration, however, so that in moderately acidic solutions (below pH 4–5), hydrolysis proceeds more rapidly. The shelf life of formulations of soluble corticosteroid derivatives is limited most severely by the extent to which hydrolysis can occur before the concentration of free corticosteroid in solution exceeds its saturation solubility. Commercially marketed solution formulations of methylprednisolone 21-succinate exhibit precipitate formation within a few hours to several days (depending on the ester concentration), due at least in part to the formation of free methylprednisolone.[21] The 17-ester reaction product formed from a soluble 21-carboxylic acid ester would in all likelihood also be water soluble. Consequently, ester hydrolysis appears to be more important than migration in determining the shelf life of soluble corticosteroid C_{21}-esters.

Evidence in the literature suggests that the 17-ketosteroid **4** may form either via a base-catalyzed retroaldol reaction which requires a free 21-hydroxyl or via direct attack of hydroxide ion at C_{17}[22] which can occur on either the free corticosteroid or the 21-ester. Existing data in the literature indicate that ester hydrolysis and migration are more important reactions in the initial decomposition of corticosteroid esters, but 17-ketosteroid formation may nevertheless be a factor due to the low water solubility of the hydrophobic 17-keto compounds. Precipitation of 17-ketosteroid has been observed in aqueous formulations of dexamethasone 21-phosphate.[23]

Reactions involving the degradation of the C_{17}-dihydroxyacetone side chain are thought to occur only on the free corticosteroid.[23,24] The degradation of fluprednisolone 21-acetate in aqueous solution, for example, follows a consecutive first-order reaction sequence involving first ester hydrolysis to fluprednisolone and subsequent decomposition to other products.[25] This supports the hypothesis that the 17-dihydroxyacetone side chain is, in effect, protected by substitution at the 21-hydroxyl.

While other reactions may also play a role, clearly ester hydrolysis must be inhibited to achieve significant improvements in solution stability. Therefore, the strategies outlined below focus on minimizing 21-ester hydrolysis. Fortunately, factors (such as pH adjustment) which minimize 21-ester hydrolysis also reduce acyl migration and 17-ketosteroid formation.

Physical–Organic Considerations—A serious disadvantage of succinate esters and other dicarboxylic acid hemiesters as water-soluble prodrugs is their limited solubility at the pH of optimum ester stability. Shown in Fig. 1 is a plot of the logarithm of the solubility of methylprednisolone 21-succinate (**1a**) versus pH at 25°C. The logarithm of the solubility is constant below the pK_a of the carboxylic acid and increases, with a slope approaching 1, above the pK_a. Deviation from the theoretical slope of 1 at higher pH is evidence for self-association of the ester at higher concentrations. This plot is superimposed on a plot of the logarithm of the hydrolysis rate of the same ester versus pH at 25°C.[2] The optimum stability of this 21-ester lies in the pH range of 3–4, while dicarboxylic acid hemiesters are highly soluble only in neutral or basic solutions. Commercial formulations of succinate esters of corticosteroids, for example, are usually adjusted to pH 7–8.[26] Significant improvement in shelf life could be realized if such esters were soluble at the pH of optimum stability. Since this pH is generally ~3–5 for carboxylic esters, ester prodrugs should be designed to exhibit high solubility in this pH range.

To achieve this objective, solubilizing moieties which are largely ionized at pH 3–5, such as tertiary amine or sulfonic acid-containing pro-moieties rather than carboxylic acid-containing moieties, are preferred.

In addition to solubilization at the pH of optimum stability, the pH–rate profile must be carefully designed. Shown in Fig. 2 are the pH–rate profiles for the hydrolysis in aqueous solution at 25°C of methylprednisolone 21-succinate (**1a**),[2] methylprednisolone 21-acetate (**1b**),[14] and the simple aliphatic ester ethyl acetate.[27] The horizontal dashed line in Fig. 2 represents the maximum rate constant allowable for 2-year stability assuming that 10% degradation can occur.

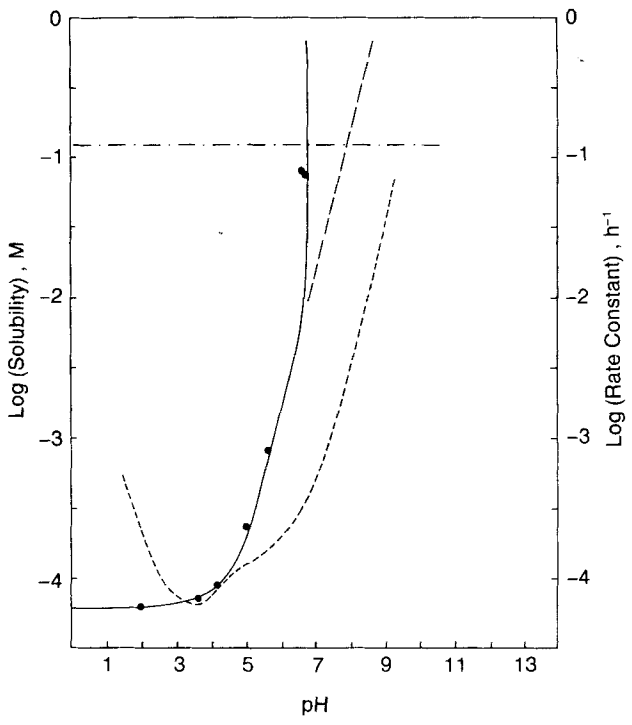


Figure 1—*Logarithm of the solubility of methylprednisolone 21-hemisuccinate versus pH superimposed on the pH–hydrolysis rate profile (– – –) of the same compound (both plots at 25°C). Key: (— · —) formula concentration.*

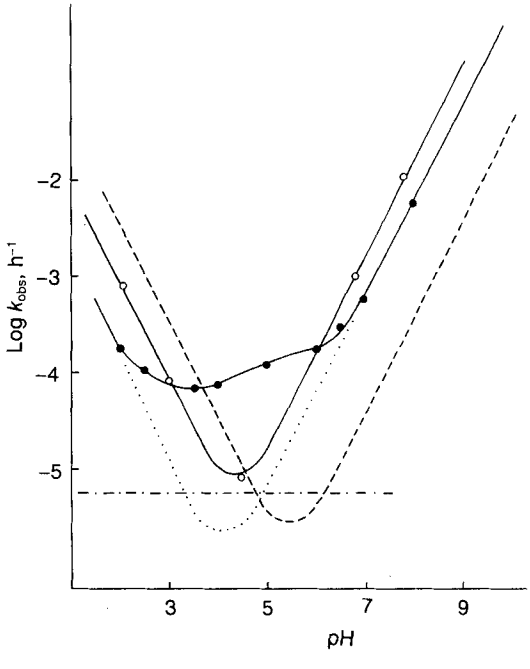


Figure 2—pH–hydrolysis rate profiles for methylprednisolone 21-succinate (●), *methylprednisolone 21-acetate* (○), *and ethyl acetate* (– – –). *The horizontal dashed line* (· — ·) *represents the maximum rate constant allowable for 2-year shelf life assuming that 10% degradation can occur.*

Both ethyl acetate and methylprednisolone 21-acetate exhibit V-shaped pH–rate profiles, typically seen for neutral aliphatic esters. Three degradation pathways are operative in

their hydrolysis (acid-catalyzed hydrolysis, base-catalyzed hydrolysis, and neutral hydrolysis) as expressed by:

$$k_{obs} = k_{H^+}[H^+] + k_{H_2O} + k_{OH^-}[OH^-] \quad (1)$$

The hydrolysis rate of methylprednisolone 21-succinate deviates from the simple U-shape (dotted line in Fig. 2) due to intramolecular catalysis of hydrolysis by the terminal succinate carboxyl group.[15]

Ethyl acetate exhibits 2-year solution stability between pH 4 and 6, while methylprednisolone 21-acetate approaches 2-year stability at pH 4–4.5. Barring intramolecular catalysis, the 21-succinate would also exhibit 2-year stability in this region. These profiles indicate that shelf lives of 2 years are not out of the question for 21-esters of corticosteroids.

At low pH, ester hydrolysis is acid catalyzed, as indicated in Fig. 2 by the linear increase in log k_{obs} with decreasing pH (with a slope of 1). Under acidic conditions, the order of reactivity is ethyl acetate > methylprednisolone 21-acetate > methylprednisolone 21-succinate.

The effect of substituents on the reactivity of aliphatic esters can be expressed by the modified Taft equation:[28]

$$\log (k/k_0) = \rho^*\sigma^* + \delta E_s \quad (2)$$

where ρ^* represents the sensitivity of the reaction rate constant, k, to polar substituent effects, σ^*, and δ is a measure of the sensitivity of the reaction to steric effects; E_s is the steric substituent constant. k_0 is the reaction rate constant for a reference compound in which the substituent of interest is absent.

Polar effects are small in acid-catalyzed hydrolytic reactions[29] so the difference in acid stability of these esters may be attributable to differences in steric hindrance. Using ethyl acetate as the reference ester with rate constant k_0, the steric effect of the steroid molecule compared to that of ethanol is seen to be quite large, resulting in a reduction in rate constant of fourfold. Changing from methylprednisolone 21-acetate to the 21-succinate results in a further reduction in the acid-catalyzed rate constant of fourfold. This may be due to an additional steric effect of the succinate side chain.

An earlier study to improve the solution stability of dicarboxylic acid hemiesters of hydrocortisone utilizing steric hindrance in the pro-moiety has been reported by Garrett.[30] More than 10-fold improvement in stability was reported in sterically hindered hemiesters, but 2-year solution stability was not approached due to the solubility limitations of these compounds.

In addition, since steric effects alter nonenzymatic and enzymatic ester hydrolysis rates in the same direction,[31] it is unlikely that steric modifications can be utilized to great advantage in further retarding ester hydrolysis in vitro without a trade-off in the in vivo reconversion rate. For this reason, increased steric bulk in the pro-moiety near the ester linkage was avoided in this study.

While acid-catalyzed hydrolysis is assumed to be sensitive only to steric effects, both steric and electronic substituent effects are important in alkaline hydrolysis. As shown previously, steroid 21-esters are more sterically hindered than the simple aliphatic ester (ethyl acetate) and are therefore more stable in acid; but in base, the steroid 21-esters in Fig. 2 are more reactive than ethyl acetate by more than an order of magnitude. This change in order of reactivity can be readily rationalized from a consideration of electronic substituent effects. Above pH 6–7, the rate-determining step in the hydrolysis of most esters is hydroxide-ion attack.[32] Thus, hydrolysis should be accelerated by electron-withdrawing substituents near the site of OH^- attack.

Assuming a σ^* of 1.65[33] for the carbonyl substituent at C_{20} and a ρ^* of approximately 1.5,[34] a rate enhancement of 2.5 log units is predicted for an ester containing an α-carbonyl group. Correcting for steric differences between ethyl acetate and methylprednisolone determined from relative rates of acid-catalyzed hydrolysis, the acceleration in rate due to polar substituents in methylprednisolone acetate is 160-fold or 2.2 log units, quite close to that predicted.

Since few substituents are significantly deactivating (electron donating) through field effects, it appears that the degree of stabilization that can be achieved through the incorporation of deactivating substituents into the pro-moiety is minimal. On the other hand, additional electronic activation of the already activated 21-ester linkage must be avoided. Amino functional groups in their ionic state, for example, are strongly electron withdrawing and, hence, activating. Polar substituent effects are known to decrease with distance,[35] so activation of the ester linkage by electron-withdrawing groups in the pro-moiety should be minimized by increasing the alkyl chain length between the ester linkage and the solubilizing moiety.

Intramolecular catalysis is also quite sensitive to the distance separating the interactive groups,[36] so increasing the distance of catalytic functional groups from the ester linkage would also tend to eliminate intramolecular catalysis as a factor in the hydrolysis.

Experimental Section

All melting points were obtained with a Thomas-Hoover Capillary Melting Point Apparatus. The ^1H NMR spectra were recorded on a Varian T-60 (60 MHz) or a Varian XL-200 (200 MHz) spectrometer with tetramethylsilane as an internal standard. UV spectra were obtained in methanol on a Zeiss DMR 21 Recording Spectrophotometer. Microanalysis was performed on a Perkin-Elmer 240 CHN Analyzer. Sulfur and chlorine analyses were accomplished by Schöniger combustion followed by a Fritz titration (for sulfur) or a coulometric titration (for chlorine). Sodium was determined by residue on ignition as a sulfated ash. All elemental analyses were within ± 0.4% (except as noted)[37] after correcting for water, as determined using a Mitsubishi Moisture Meter CA-02.

HPLC analyses with UV detection at 243 nm were utilized to detect UV-absorbing impurities possibly remaining from the synthesis. UV absorption of the compounds at 243 nm served as a measure of purity in terms of intact A-ring since methylprednisolone exhibits a molar extinction coefficient at 243 nm of $\epsilon_{243} = 1.46 \times 10^4$ and esterification at C_{21} had no perceptible influence on ϵ_{243}.

General Procedures for the Synthesis of Methylprednisolone 21-Hemiester Amides (1c–1m)—Taurine, *N,N*-dialkyl or *N,N,N'*-trialkyl ethylenediamines (Aldrich Chemical Company, Milwaukee, WI), or *N*-methyltaurine (Lachat Chemicals, Inc., Mequon, WI) was treated with a mixed anhydride of an appropriate dicarboxylic acid 21-hemiester of methylprednisolone. Methylprednisolone 21-hemisuccinate was used as supplied (The Upjohn Company, Kalamazoo, MI), while methylprednisolone 21-hemiadipate and 21-hemisuberate were prepared as described previously.[13]

Solutions of methylprednisolone 21-hemiester in tetrahydrofuran (THF) (generally ~5 mmol in 70 mL) containing 10–15% molar excess of triethylamine were cooled under a nitrogen atmosphere to −15°C. To these solutions was added a 10–15% molar excess of isobutyl chloroformate. The mixtures were allowed to warm to room temperature over a 15-min period resulting in precipitation of triethylamine hydrochloride, after which a 10–15% molar excess of the appropriate *N,N*-disubstituted or *N,N,N'*-trisubstituted ethylenediamine, taurine, or *N*-methyltaurine was added. (*N*-Methyltaurine and taurine were dissolved in 10% H_2O:90% dimethylformamide (DMF) together with an equimolar amount of triethylamine before adding to the mixture.) Product formation was monitored by HPLC (see *Liquid Chromatographic Analyses*).

The products were purified using classical pH-controlled extraction techniques with ethyl acetate or isobutyl alcohol as

solvents. Further purification, when necessary, was accomplished using preparative reversed-phase liquid chromatography. Generally two size "B" RP-8 Lobar columns (E. Merck, Darmstadt, F.R.G.) were employed. As a final step, the compounds were generally converted to an appropriate salt form by titration and were recrystallized or reprecipitated from a suitable solvent.

21-[[4-[[2-(Dimethylamino)ethyl]amino]-1,4-dioxobutyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Hydrochloride (1c)—The free amine was precipitated as a fine white solid by adding an ethyl acetate solution of the amine in a dropwise manner into hexane. This solid was then collected, dissolved in THF, and precipitated as the hydrochloride salt by bubbling dry HCl into the solution. A 41% yield of fine white crystalline solid was obtained, mp 196.6–201.3°C; UV: λ_{max} 243 nm (ϵ 14,500); ^{1}H NMR (Me_2SO-d_6): δ 0.81 (s, 3, 18-CH_3), 1.08 (d, 3, 6-CH_3), 1.40 (s, 3, 19-CH_3), 2.79 [s, 6, —$N(CH_3)_2$], 4.3 (br s, 1, 11-H), 4.9 (AB, 2, 21-CH_2), 5.8 (s, 1, 4-H), 6.1 (d, 1, 1-H), and 7.34 ppm (d, 1, 2-H). Anal. ($C_{30}H_{44}N_2O_7 \cdot HCl$) C, H, N, Cl.

21-[[4-[[2-(Dimethylamino)ethyl]methylamino]-1,4-dioxobutyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione (1d)—The free amine was crystallized from ethyl acetate in two crops for an overall yield of 87%. The product was a colorless solid, mp 180.1–181.6°C; UV: λ_{max} 243 nm (ϵ 14,700); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.81 (s, 3, 18-CH_3), 1.08 (d, 3, 6-CH_3), 1.40 (s, 3, 19-CH_3), 2.17 [d, 6, —$N(CH_3)_2$], 4.3 (br s, 1, 11-H), 4.9 (AB, 2, 21-CH_2), 5.8 (s, 1, 4-H), 6.1 (d, 1, 1-H), and 7.25 ppm (d, 1, 2-H). Anal. ($C_{31}H_{46}N_2O_7$) C, H, N.

21-[[6-[[2-(Dimethylamino)ethyl]methylamino]-1,6-dioxohexyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Hydrochloride (1e)—The free amine, isolated as an oil, was dissolved in THF and titrated to an apparent end point with 1 M HCl. The solvent was removed, leaving a yellowish oil which was triturated with ether for several days to yield a white amorphous solid; UV: λ_{max} 243 nm (ϵ 14,100); purity >99% by HPLC; no detectable impurities by TLC [silica gel GF, ethyl acetate:methanol:NH_4OH (20:4:1); R_f = 0.52, visualized by charring with ammonium sulfate]; ^{1}H NMR (Me_2SO-d_6): δ 0.81 (s, 3, 18-CH_3), 1.08 (d, 3, 6-CH_3), 1.40 (s, 3, 19-CH_3), 2.75 [s, 6, —$N(CH_3)_2$], 3.0 (s, 3, —$CONCH_3$), 4.32 (br s, 1, 11-H), 4.95 (AB, 2, 21-CH_2), 5.83 (s, 1, 4-H), 6.17 (d, 1, 1-H), and 7.42 ppm (d, 1, 2-H). Anal. ($C_{33}H_{50}N_2O_7 \cdot HCl$) C, H, N, Cl.

21-[[6-[[2-(Diethylamino)ethyl]ethylamino]-1,6-dioxohexyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Hydrochloride (1f)—The reaction product was isolated as the free base, redissolved in THF, and titrated to an endpoint with 0.1 M HCl. On removing the solvent, the hydrochloride salt was obtained as an oil which resisted crystallization. After standing for several months at room temperature, the oil solidified. The yellowish solid was then triturated with ether to yield a colorless crystalline product (~90%), mp 121–130°C; UV: λ_{max} 243 nm (ϵ 14,700); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.81 (s, 3, 18-CH_3), 1.40 (s, 3, 19-CH_3), 4.32 (br s, 1, 11-H), 4.95 (AB, 2, 21-CH_2), 5.82 (s, 1, 4-H), 6.17 (d, 1, 1-H), and 7.42 ppm (d, 1, 2-H). Anal. ($C_{36}H_{56}N_2O_7 \cdot HCl$) C, H, N, Cl.

21-[[8-[[2-(Diethylamino)ethyl]amino]-1,8-dioxooctyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Hydrochloride (1g)—The product, isolated as the free base, was dissolved in THF and titrated with 1 M HCl to an endpoint. On solvent removal and trituration of the residue with ether, a white solid was obtained in 81% yield. Recrystallization from acetonitrile removed minor impurities detected by TLC and reduced the yield to 70%, mp 160.0–161.5°C; UV: λ_{max} 243 nm (ϵ 14,600); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.82 (s, 3, 18-CH_3), 4.33 (br s, 1, 11-H), 4.95 (AB, 2, 21-CH_2), 5.82 (s, 1, 4-H), 6.17 (d, 1, 1-H), and 7.38 ppm (d, 1, 2-H). Anal. ($C_{36}H_{56}N_2O_7 \cdot HCl$) C, H, N, Cl.

21-[[8-[[2-(Dimethylamino)ethyl]methylamino]-1,8-dioxooctyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Hydrochloride (1h)—The free base was titrated in THF with 1 M HCl to an endpoint. The solvent was removed, and the residue was triturated with butyl chloride to give a yield of ~80% of a white amorphous solid. Recrystallization from acetonitrile gave a crystalline product, mp 168.5–170.0°C; UV: λ_{max} 243 nm (ϵ 14,600); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.80 (s, 3, 18-CH_3), 1.07 (d, 3, 6-CH_3), 1.41 (s, 3, 19-CH_3), 2.77 [s, 6, —$N(CH_3)_2$], 3.0 (s, 3, —$CONCH_3$), 4.32 (br s, 1, 11-H), 4.95 (AB, 2, 21-CH_2), 5.83 (s, 1, 4-H), 6.18 (d, 1, 1-H), and 7.38 ppm (d, 1, 2-H). Anal. ($C_{35}H_{54}N_2O_7 \cdot HCl$) C, H, N, Cl.

21-[[8-[[2-(Diethylamino)ethyl]ethylamino]-1,8-dioxooctyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Hydrochloride (1i)—The free base was titrated in aqueous THF with 1 M HCl to an endpoint. The solution was concentrated under reduced pressure and then lyophilized to yield a flaky amorphous solid. A portion of this material was recrystallized from acetone, mp 120–125°C; UV: λ_{max} 243 nm (ϵ 14,800); purity ≈99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.82 (s, 3, 18-CH_3), 4.35 (br s, 1, 11-H), 4.97 (AB, 2, 21-CH_2), 5.83 (s, 1, 4-H), 6.18 (d, 1, 1-H), and 7.42 ppm (d, 1, 2-H). Anal. ($C_{38}H_{60}N_2O_7 \cdot HCl$) C, H, N, Cl.

21-[[4-[(2-Sulfoethyl)amino]-1,4-dioxobutyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Sodium Salt (1j)—The product was purified by preparative reversed-phase chromatography using acetonitrile:water (25:75) with 0.07% trifluoroacetic acid as mobile phase. The pure fractions were concentrated and passed through a Dowex column, which was in the Na^+ form. The pooled product fractions were concentrated to an oil which was then taken up in methanol and precipitated as a solid by dripping into acetone. A 36% yield of amorphous white solid was obtained; UV: λ_{max} 243 nm (ϵ 14,100); purity ≈99% by HPLC. Anal. ($C_{28}H_{38}NO_{10}SNa$) C, H, N, S, Na.

21-[[4-[(2-Sulfoethyl)methylamino]-1,4-dioxobutyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Sodium Salt (1k)—The product was initially isolated as the free acid and was converted to the sodium salt by passage through a carefully prepared Dowex column which was in the Na^+ form. The pooled column fractions were concentrated to an oil and the residue was taken up in methanol. Addition of acetonitrile to the solution caused the product to precipitate as an amorphous solid, with 87% overall yield; UV: λ_{max} 243 nm (ϵ 14,000); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.82 (s, 3, 18-CH_3), 1.08 (d, 3, 6-CH_3), 1.42 (s, 3, 18-CH_3), 4.33 (br s, 1, 11-H), 4.95 (AB, 2, 21-CH_2), 5.83 (s, 1, 4-H), 6.18 (d, 1, 1-H), and 7.43 ppm (d, 1, 2-H). Anal. ($C_{29}H_{40}NO_{10}SNa$) C, H, N.

21-[[8-[(2-Sulfoethyl)amino]-1,8-dioxooctyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Sodium Salt (1l)—The free acid was isolated as a white solid in 80% yield by adding a butanolic solution of the compound into butyl chloride in a dropwise manner. A portion of the product was further purified by preparative reversed-phase chromatography using acetonitrile:water:trifluoroacetic acid (33:67:0.7) as mobile phase. The compound was then extracted into butanol, washed with dilute HCl, and titrated to an endpoint with sodium bicarbonate solution. Finally, the solution was concentrated to dryness, the residue was taken up in methanol, and the product was precipitated with isopropyl alcohol as an amorphous solid; UV: λ_{max} 243 nm (ϵ 14,700); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.82 (s, 3, 18-CH_3), 1.07 (d, 3, 6-CH_3), 1.40 (s, 3, 19-CH_3), 4.30 (br s, 1, 11-H), 4.92 (AB, 2, 21-CH_2), 5.82 (s, 1, 4-H), 6.15 (d, 1, 1-H), and 7.33 ppm (d, 1, 2-H). Anal. ($C_{32}H_{46}NO_{10}SNa$) C, H, N.

21- [[8-[(2-Sulfoethyl)methylamino]-1,8-dioxooctyl]-oxy] - 11β,17 - dihydroxy - 6α - methylpregna - 1,4-diene 3,20-dione Sodium Salt (1m)—The compound was purified by chromatography on silica gel with a mobile phase consisting of ethyl acetate:methanol:acetic acid (80:20:0.5). The product fractions were concentrated to an oil, taken up in THF, and titrated with sodium bicarbonate to convert to the sodium salt. The final product was obtained by concentrating the solution to an oil, dissolving the residue in methanol, and adding the methanolic solution in a dropwise manner into 10 volumes of ethyl acetate. A white amorphous solid was obtained in 47% overall yield; UV: λ_{max} 243 nm (ϵ 14,200); purity >99% by HPLC; ^{1}H NMR (CD_3OD): δ 0.91 (s, 3, 18-CH_3), 1.13 (d, 3, 6-CH_3), 1.49 (s, 3, 19-CH_3), 3.0 (m, 2, CH_2S), 3.12 (s, 3, —NCH_3), 3.74 (m, 2, CH_2—N), 4.42 (br, 1, 11-H), 4.97 (AB, 2, 21-CH_2), 6.0 (s, 1, 4-H), 6.26 (d, 1, 1-H), and 7.49 ppm (d, 1, 2-H). Anal. ($C_{33}H_{48}NO_{10}SNa$) C, H, N, S, Na.

General Procedures for the Preparation of Methylprednisolone 21-(ω-Sulfo)- and -(ω-Amino)-alkanoates (1n–1q)—These compounds were prepared in two reaction steps involving (*a*) the synthesis of an ω-substituted sulfo or dialkylamino alkanoic acid and (*b*) reaction of the intermediate prepared in step *a* with methylprednisolone 21-mesylate or methylprednisolone 21-iodide in the presence of a sterically hindered tertiary amine. These two steroids were obtained from the Upjohn Company, Kalamazoo, MI; however, they may be readily prepared from methylprednisolone. The 21-mesylate is synthesized in over 95% yield by treating methylprednisolone with a twofold excess of methanesulfonyl chloride in pyridine at 0–10°C. The 21-iodide is readily prepared from the 21-mesylate through displacement of methanesulfonate by sodium iodide in acetone.

21-[[6-(Diethylamino)-1-oxohexyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Hydrochloride (1n)—*Step a*—6-(Diethylamino)hexanoic acid was prepared by adding in a dropwise manner 15 mL (0.1 mol) of 6-bromohexanoic acid to 100 mL (1 mol) of diethylamine. The mixture was stirred for 2 h at room temperature, during which time some diethylamine hydrobromide precipitated as a white solid. After an additional 1.5 h at 45°C, the mixture was filtered and concentrated to an oil. The residual amine was removed by purification of the compound on an anion-exchange column (BioRad AG 21K) in the hydroxide form, eluting with water followed by 1 M HCl. The product-containing fractions were adjusted with NaOH to pH 7.7, water was removed on a rotary evaporator, and the residual oil was dissolved in acetonitrile, resulting in precipitation of NaCl. The supernatant was concentrated to an oil which was used in step *b* without further purification.

Step b—The amino acid prepared above (2.5 g, 12 mmol) was combined with 4.84 g (10 mmol) of methylprednisolone 21-iodide in 30 mL of dry DMF and stirred at 75°C for 1 h under nitrogen. The product was purified by standard pH-controlled extraction procedures, recrystallized from isopropyl alcohol:butyl chloride and dried at 60°C under reduced pressure. The overall yield for step *b* was 55%, mp 161.5–163.5°C; UV: λ_{max} 243 nm (ϵ 14,700); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.80 (s, 3, 18-CH_3), 1.40 (s, 3, 19-CH_3), 4.32 (br s, 1, 11-H), 4.97 (AB, 2, 21-CH_2), 5.83 (s, 1, 4-H), 6.17 (d, 1, 1-H), and 7.37 ppm (d, 1, 2-H). Anal. ($C_{32}H_{49}NO_6 \cdot HCl$) C, H, N, Cl.

21 - [[3-Sulfo-1-oxopropyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Sodium Salt (1o)—*Step a*—3-Sulfopropionic acid was prepared by treating 11 g (0.1 mol) of sodium bisulfite with 9 mL (0.1 mol) of methyl acrylate in aqueous isopropyl alcohol for 1 h at 60°C. The solution was concentrated to dryness, dissolved in concentrated HCl, allowed to stand 20 min, and filtered. The dissolved product was then converted to the pyridinium salt, concentrated, and used without further purification in the subsequent reaction.

Step b—The product of step *a* was dissolved in 30 mL of pyridine and combined with 15 g (0.04 mol) of methylprednisolone and 13 g (0.06 mol) of dicyclohexylcarbodiimide. An exothermic reaction ensued. After ~16 h, the mixture was concentrated to an oil and then diluted with 300 mL of dilute HCl plus 50 mL of ethyl acetate. The dicyclohexylurea was removed by filtration, and the product in the aqueous acid phase was washed with ethyl acetate. Finally, the aqueous phase was adjusted to pH 2.4, and the product was extracted into isoamyl alcohol. On concentration and trituration with ethyl acetate, 14.4 g (70% yield) of white solid was obtained. This material was further purified by passage through a Dowex 50W-X8 column (H^+ form) and by trituration of the recovered solid in ethyl acetate. Conversion to the sodium salt was accomplished by titration with sodium bicarbonate. Water was removed after the titration as an azeotrope with acetonitrile. The residue was triturated with THF to yield 10 g of white amorphous solid; UV: λ_{max} 243 nm (ϵ 13,700); purity ≈98% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.82 (s, 3, 18-CH_3), 1.07 (d, 3, 6-CH_3), 1.42 (s, 3, 19-CH_3), 2.75 [s, 4, —$CO(CH_2)_2SO_3^-$], 4.32 (br s, 1, 11-H), 4.97 (AB, 2, 21-CH_2), 5.88 (s, 1, 4-H,), 6.20 (d, 1, 1-H), and 7.37 ppm (d, 1, 2-H). Anal. ($C_{25}H_{33}O_9SNa$) C, H, S, Na.

21-[[6-Sulfo-1-oxohexyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Sodium Salt (1p)—*Step a*—6-Sulfohexanoic acid was prepared by combining 12.6 g (0.1 mol) of sodium sulfite, 9.75 g (0.05 mol) of 6-bromohexanoic acid, and 1.92 g (0.05 mol) of sodium hydroxide in 75 mL of water. The mixture was refluxed 24 h, acidified, and concentrated to a thick slurry. The slurry was triturated with hot ethanol and filtered. The filtrate was concentrated to an oil, dissolved in water, and passed through a Dowex cation-exchange column (H^+ form). Product fractions were concentrated to an oil and used without further purification in the subsequent reaction. A portion of the acid was converted to the monosodium salt, mp 229–234°C.

Step b—The ester was prepared by treating 3.3 g (0.017 mol) of the sulfohexanoic acid with 3.62 g (0.008 mol) of methylprednisolone 21-mesylate in 35 mL of DMF in the presence of 5.9 mL (0.034 mol) of *N,N*-diisopropylethylamine. After heating for 48 h at 80–90°C, the mixture was diluted with 0.1 M HCl and washed with ethyl acetate. The aqueous phase was then adjusted to pH 2 and extracted with isobutyl alcohol. The isobutyl alcohol extract was concentrated and purified by preparative reversed-phase chromatography [RP-8 Lobar columns; mobile phase, acetonitrile:water:sodium bisulfate buffer (30:70:0.1). The product fractions were extracted with isobutyl alcohol, the extract was concentrated, and the residue was titrated with 1 M NaOH to form the sodium salt. This salt was isolated by concentrating, dissolving the residue in methanol, and precipitating with acetone. A 39% overall yield of colorless solid was obtained, mp 200–210°C; UV: λ_{max} 243 nm (ϵ 14,200); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.82 (s, 3, 18-CH_3), 1.07 (d, 3, 6-CH_3), 4.32 (br s, 1, 11-H), 4.93 (AB, 2, 21-CH_2), 5.83 (s, 1, 4-H), 6.18 (d, 1, 1-H), and 7.35 ppm (d, 1, 2-H). Anal. ($C_{28}H_{39}O_9SNa$) C, H, S, Na.

21-[[11-Sulfo-1-oxoundecyl]oxy]-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione Sodium Salt (1q)—*Step a*—11-Sulfoundecanoic acid was prepared by treating 10.6 g (0.04 mol) of 11-bromoundecanoic acid with 7.6 g (0.06 mol) of sodium sulfite in 76 mL of 0.5 M NaOH and 10 mL of *n*-propyl alcohol. After refluxing 8 h, the solution was cooled and acidified, resulting in precipitation of 9.2 g of the monosodium salt of 11-sulfoundecanoic acid (80% yield), mp 206–213°C. A portion of this salt was dissolved in hot aqueous THF and converted to the diacid by batchwise treatment with Dowex resin in the H^+ form.

Step b—The sulfoundecanoic acid (diacid form) (3.2 g, 0.012 mol) was treated with 5.43 g (0.012 mol) of methylprednisolone

21-mesylate in the presence of 4.2 mL (0.024 mol) of *N,N*-diisopropylethylamine in DMF. After stirring for 6 h at 75°C, the mixture was cooled, diluted with water (adjusted to pH 6), and washed with ethyl acetate. The aqueous phase was then acidified and extracted with isobutyl alcohol. The isobutyl alcohol phase was washed several times with 0.4 M sodium phosphate buffer and then concentrated to an oil. This residue was triturated with acetone and then with ether to yield (after drying) 2.4 g of an off-white solid (31% yield), mp 203–205°C; UV: λ_{max} 243 nm (ϵ 14,300); purity >99% by HPLC; ^{1}H NMR (Me_2SO-d_6): δ 0.8 (s, 3, 18-CH_3), 1.05 (d, 3, 6-CH_3), 4.30 (br s, 1, 11-H), 4.92 (AB, 2, 21-CH_2), 5.80 (s, 1, 4-H), 6.13 (d, 1, 1-H), and 7.33 ppm (d, 1, 2-H). Anal. ($C_{33}H_{49}O_9SNa$) C, H, S, Na.

Solubility Determinations—Solubilities of methylprednisolone 21-hemisuccinate were determined at various pH values between 2 and 7 by suspending an amount of solid well in excess of the saturation solubility in 0.01 M ionic strength buffers[38] and shaking at 25°C for at least 24 h. In some cases, dilute NaOH was added to neutralize the acid. The pH was measured after equilibration. The suspensions were filtered through 0.45- or 0.6-μm Millipore filters, and appropriate dilutions of the filtrate were made for HPLC analysis. Chromatographic conditions for solubility determinations were similar to those employed in the kinetic studies (see below).

Initial Rate Kinetic Studies—Hydrolysis rates of methylprednisolone 21-esters were determined by monitoring the initial rates of product formation. Reactant solutions were prepared at concentrations of 5×10^{-4} M at various pH values using 0.01 M ionic strength buffers.[38] Samples were maintained at 25°C in a constant-temperature water bath. Methylpredni solone concentrations were monitored versus time up to a total percentage of no more than 1–2% of the initial reactant concentration. Sample concentrations were determined by HPLC.

Liquid Chromatographic Analyses—A modular high-performance liquid chromatographic system consisting of an automated sample injector (Wisp model 710A; Waters Associates, Milford, MA), a constant-flow pump (model 110A; Altex Scientific, Berkeley, CA) operated at 2 mL/min, a reversed-phase column packed with 5-μm Spheri-5 RP-18 (Brownlee MPLC Cartridge System; Rainin Instruments Co., Woburn, MA), a variable-wavelength UV detector (Altex/Hitachi Model 153-00; Altex Scientific) operated at 243 nm, and a digital integrator (model 3380A, Hewlett-Packard, Avondale, PA) was used for all analyses.

The mobile phase was altered depending on the compound analyzed, but the mobile phase composition was generally 30–45% acetonitrile in water buffered with acetic acid:sodium acetate (0.1%) at pH 4.5–5.5. *N,N*-Dimethyloctylamine (0.02–0.06%) was added as a polar modifier for the separation of compounds containing tertiary amino groups to reduce tailing or as an ion-pairing reagent for sulfonate-containing compounds.

Standard solutions of methylprednisolone were prepared at concentrations ranging from 1×10^{-6} to 1×10^{-5} M and stored at 4°C. Product concentrations were determined using peak height ratios. The peak height response versus concentration of standards was linear throughout the above concentration range.

Results and Discussion

Pro-Moiety Design and Prodrug Synthesis—Compounds having the general structures shown in Scheme I were synthesized as water-soluble prodrugs of methylprednisolone with potentially improved solution stability in comparison to the currently marketed succinate ester. Solubilization in the pH region of optimum stability was achieved by using pro-moieties containing sulfonate or tertiary amino groups, both of which are largely ionized at a pH of 3–5. Solubilities of amine compounds **1c–i** and **1n** appeared to have no practical solubility limitations below a pH of 7. Solubilities of the sulfonate compounds **1j–m**, **1o**, and **1p** also appeared to have no practical limit. The solubility of **1q** was limited by formation of a gel phase above 20 mg/mL.

Since both the amine and sulfonate groups are inductively electron-withdrawing in their ionized form, with σ^* values of 0.81 and 4.36, respectively,[38] the insertion of additional methylene groups between the activating substituent and the reactive center was explored as a means of further reducing the lability of the ester linkage. The σ^* constant is reduced by a factor of about 2.8 per methylene group increment in distance.[35]

Variation in the distance between the reactive center and the ionic substituent was achieved most conveniently by utilizing commercially available dicarboxylic acids varying in chain length as spacer linkages. The sulfonate- or tertiary amine-containing moieties were introduced at one end of the dicarboxylic acid via amide formation with taurine, *N*-methyltaurine, *N,N*-disubstituted ethylenediamine, or *N,N,N'*-trisubstituted ethylenediamine, while the other terminus of the dicarboxylic acid was attached to methylprednisolone at C_{21}. Variation in distance between the ester linkage and electron-withdrawing ionic substituents was achieved in ω-substituted alkanoate esters by simple increases in chain length.

Hydrolysis Studies—Kinetic studies in dilute solutions (5×10^{-4} M) were conducted at various pH values to obtain estimates of the acid-catalyzed, neutral, and base-catalyzed rates of ester hydrolysis for these compounds. These studies were carried out at 25°C by monitoring the initial rates of free methylprednisolone formation. In Fig. 3 are shown typical plots of the formation of methylprednisolone versus time obtained for **1m**. Pseudo-first-order hydrolysis rate constants for each compound were obtained from the slopes of such plots at various pH values. The 95% confidence limits of these slopes averaged ±10.8%.

Estimates of k_{H^+}, k_{H_2O}, and k_{OH^-} were obtained by fitting eq. 1 to the observed rate constants versus pH using a least-squares curve-fitting technique.[39] These estimates are listed in Table I.

pH–hydrolysis rate profiles for some of the compounds in

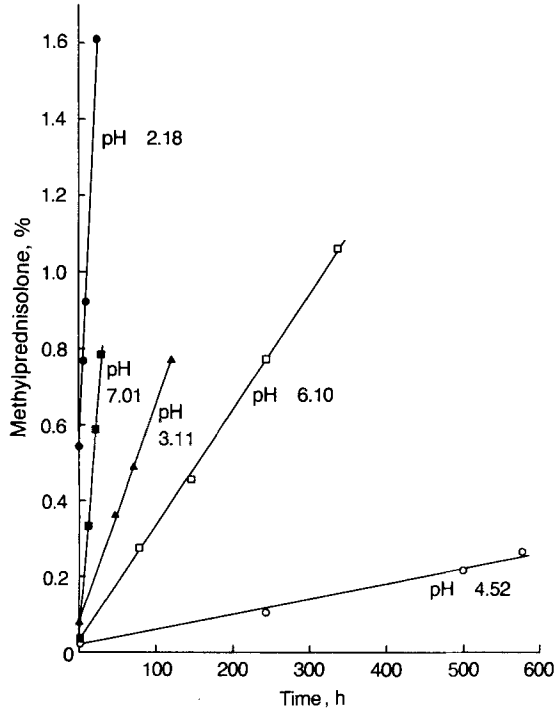


Figure 3—*Typical plot of the formation of methylprednisolone from* **1m** *versus time in various buffers at 25°C.*

Table I—Estimates of Acid-Catalyzed, Neutral, and Base-Catalyzed Hydrolysis Rate Constants for Various 21-Esters of Methylprednisolone at 25°C[a]

Compound	k_{H^+}, L·mol^{-1}·h^{-1}	k_{H_2O}, h^{-1}	k_{OH^-}, L·mol^{-1}·h^{-1}
1b	8.52×10^{-2}	1.88×10^{-6}	1.45×10^{4}
1c	1.15×10^{-2}	3.55×10^{-5}	3.28×10^{6}
1d	1.06×10^{-2}	3.98×10^{-6}	2.63×10^{4}
1e	2.90×10^{-2}	1.66×10^{-6}	1.13×10^{4}
1f	2.92×10^{-2}	1.73×10^{-6}	9.25×10^{3}
1g	3.42×10^{-2}	1.10×10^{-6}	7.41×10^{3}
1h	3.39×10^{-2}	1.69×10^{-6}	6.68×10^{3}
1i	3.00×10^{-2}	2.52×10^{-6}	6.51×10^{3}
1j	4.00×10^{-2}	1.41×10^{-5}	7.08×10^{4}
1k	3.89×10^{-2}	2.28×10^{-6}	4.82×10^{3}
1l	6.90×10^{-2}	1.60×10^{-6}	3.62×10^{3}
1m	7.14×10^{-2}	1.13×10^{-6}	2.57×10^{3}
1n	2.63×10^{-2}	2.39×10^{-6}	1.24×10^{4}
1o	3.90×10^{-2}	1.21×10^{-5}	2.12×10^{4}
1p	8.49×10^{-2}	1.20×10^{-6}	2.96×10^{3}
1q	7.97×10^{-2}	1.08×10^{-6}	2.56×10^{3}

[a] Concentration ≅ 5×10^{-4} M.

Scheme I are given in Figs. 4 and 5, respectively. The pH–rate profile of methylprednisolone 21-acetate (**1b**) is also shown for reference purposes. As evident in these figures, the hydrolysis of all of the compounds studied exhibited the typical V-shaped pH–rate profiles described by eq. 1.

Acid-Catalyzed Hydrolysis—In Table II are the relative k_{H^+} estimates for several of the compounds of Scheme I divided into two groups according to the solubilizing moiety (sulfonate or amine) and listed according to distance between the ester linkage and the solubilizing group. Compounds having an amide linkage appear to be slightly more hindered than the ω-substituted alkanoates. This amide steric effect decreases with distance and for sulfonates, appears to be small when the amide bond is six methylene groups removed from the ester linkage.

The most striking differences in Table II, however, are between the tertiary amine-containing compounds and their sulfonate analogues. Apparently there is a long-range electronic

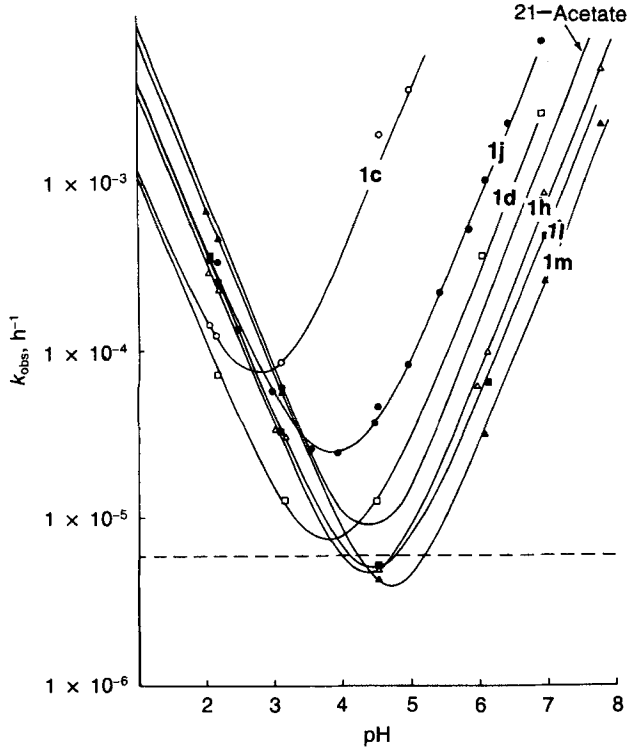


Figure 4—*pH–hydrolysis rate profiles for compounds having dicarboxylic acid spacers in the pro-moiety (see Scheme I).*

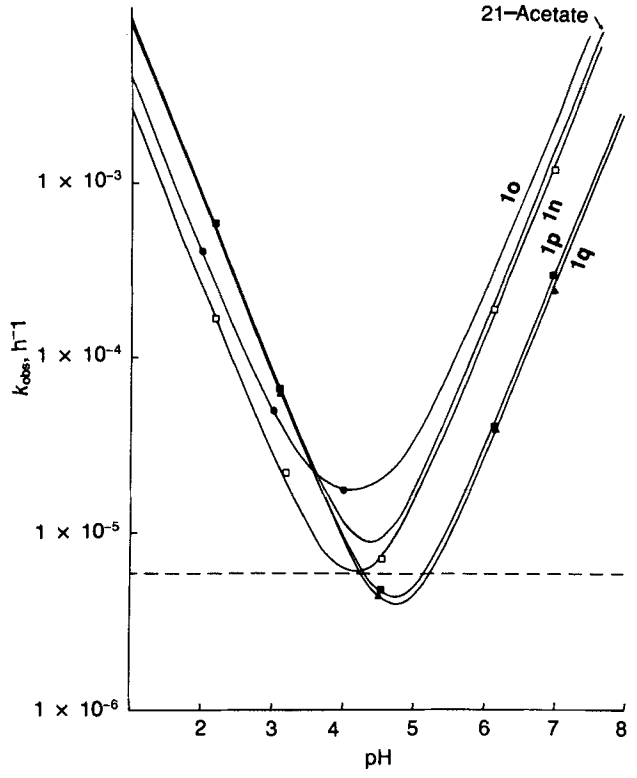


Figure 5—*pH–hydrolysis rate profiles for ω-substituted alkanoates (see Scheme I).*

Table II—Relative Estimates of Acid-Catalysis Rate Constants, k_{H^+}, for the Hydrolysis of Various 21-Esters of Methylprednisolone[a]

Compound	Pro-Moiety	Relative k_{H^+}
1n	$-CO(CH_2)_5NH(Et)_2^+$	0.31
1c	$-CO(CH_2)_2CONH(CH_2)_2NH(CH_3)_2^+$	0.13
1d	$-CO(CH_2)_2CONCH_3(CH_2)_2NH(CH_3)_2^+$	0.12
1e	$-CO(CH_2)_4CONCH_3(CH_2)_2NH(CH_3)_2^+$	0.34
1h	$-CO(CH_2)_6CONCH_3(CH_2)_2NH(CH_3)_2^+$	0.40
1p	$-CO(CH_2)_5SO_3^-$	1.0
1j	$-CO(CH_2)_2CONH(CH_2)_2SO_3^-$	0.47
1k	$-CO(CH_2)_2CONCH_3(CH_2)_2SO_3^-$	0.46
1m	$-CO(CH_2)_6CONCH_3(CH_2)_2SO_3^-$	0.84

[a] For the 21-acetate, $k_{H^+} = 1.0$.

effect exerted by the protonated amine group on the acid-catalyzed ester hydrolysis, causing an additional suppression in rate of two- to threefold even when 11 bonds separate the ester bond and the positive charge. While previous investigators have shown that electron-withdrawing substituents do consistently reduce k_{H^+}, although by relatively small amounts,[40] the long-range effects found in this study were far greater than expected. These results suggest that for structurally similar compounds both steric and electronic factors may be important in determining k_{H^+}.

If it is assumed that only steric effects are operative in determining the relative k_{H^+} values of sulfonates, then steric effects account for a factor of only two in k_{H^+} throughout the series. If it is further assumed that the differences in k_{H^+} between sulfonate- and tertiary amine-containing analogues reflect a decrease in the ease of protonation of the ester due to the positively charged amino group, then this electronic effect is at least as great or, for shorter chain lengths, greater than the steric effect in this series.

Base-Catalyzed Hydrolysis—Estimated relative k_{OH^-} values are listed in Table III for compounds having a dicarboxylic acid spacer, again grouped according to solubilizing moiety and

Table III—Relative Estimates of Hydroxide-Ion-Catalyzed Hydrolysis Rate Constants, k_{OH^-}, for Various 21-Esters of Methylprednisolone Having a Dicarboxylic Acid Spacer in the Pro-Moiety[a]

Compound	Pro-Moiety	Relative k_{OH^-}
1c	$-CO(CH_2)_2CONH(CH_2)_2NH(CH_3)_2^+$	226
1d	$-CO(CH_2)_2CONCH_3(CH_2)_2NH(CH_3)_2^+$	1.81
1e	$-CO(CH_2)_4CONCH_3(CH_2)_2NH(CH_3)_2^+$	0.78
1g	$-CO(CH_2)_6CONH(CH_2)NH(Et)_2^+$	0.51
1h	$-CO(CH_2)_6CONCH_3(CH_2)_2NH(CH_3)_2^+$	0.46
1j	$-CO(CH_2)_2CONH(CH_2)_2SO_3^-$	4.9
1k	$-CO(CH_2)_2CONCH_3(CH_2)_2SO_3^-$	0.33
1l	$-CO(CH_2)_6CONH(CH_2)_2SO_3^-$	0.25
1m	$-CO(CH_2)_6CONCH_3(CH_2)_2SO_3^-$	0.18

[a] For the 21-acetate, $k_{OH^-} = 1.0$.

Table IV—Relative Estimates of Hydroxide-Ion-Catalyzed Hydrolysis Rate Constants, k_{OH^-}, for Various ω-Substituted Alkanoates of Corticosteroids[a]

Compound	Pro-Moiety	Relative k_{OH^-}
—	$-COCH_2NH(CH_3)_2^+$	1376[b]
—	$-CO(CH_2)_2NH(CH_3)_2^+$	350[b]
1n	$-CO(CH_2)_5NH(CH_3)_2^+$	0.86
1o	$-CO(CH_2)_2SO_3^-$	1.5
1p	$-CO(CH_2)_5SO_3^-$	0.20
1q	$-CO(CH_2)_{10}SO_3^-$	0.18

[a] For the 21-acetate, $k_{OH^-} = 1.0$. [b] Estimates obtained from hydrocortisone ester hydrolysis data (ref. 9).

ranked by chain length. Similar comparisons for the ω-substituted alkanoates are listed in Table IV.

In Table III the most dramatic observations are the rate accelerations of the succinamide derivatives of 226-fold and 4.9-fold. The relative k_{OH^-} values of the *N*-methylsuccinamides are much lower and closer to the expected values [σ^* for $-(CH_2)_2CONH_2$ is 0.19[38]]. While conclusive evidence is not available, it is suggested that intramolecular nucleophilic attack by the amide anion, as depicted in Scheme II, occurs. This mechanism has been reported to occur in the hydrolysis of a β-benzyl ester of an aspartyl peptide.[41] The acceleration of k_{OH^-} is significantly greater when the solubilizing group is a tertiary amino rather than a sulfonate. This is consistent with the hypothesis that the rate acceleration is due to intramolecular nucleophilic attack by the amide anion. The pK_a of the amide would be greatly decreased by electron withdrawal of a protonated amine on the carbon β to the amido nitrogen, resulting in a higher concentration of anion at a given pH below the pK_a. Apart from the dramatic acceleration in rate due to amide anion catalysis, the relative k_{OH^-} values are similar to that of methylprednisolone 21-acetate, decreasing gradually with increasing chain length.

The powerful activation by the protonated amino group on ester hydrolysis when it is in close proximity to the ester is demonstrated in Table IV. This effect falls off with an increase in the number of methylene groups, as expected,[35] and activation by the protonated amino group is small (but not negligible) when the chain length is five. The sulfonate anion, even though negatively charged, is somewhat activating ($\sigma^* = 0.81$),[38] but again this activation diminishes with increasing chain length.

Neutral Hydrolysis—At the pH-hydrolysis rate minimum, the contribution of k_{H_2O}, the neutral hydrolysis rate constant, to the observed rate is significant for the compounds in this study. A consideration of the effects of substituents on k_{H_2O} may therefore be very important.

Shown in Fig. 6 is a plot of the logarithms of k_{H_2O} versus the corresponding log k_{OH^-} values for the compounds reported in this study. There is a significant correlation between the neutral and hydroxide-ion-catalyzed rate constants (slope = 0.51, r = 0.94), indicating that both steric and electronic effects are important in determining k_{H_2O}. Similar studies in the literature support this conclusion.[42,43]

Shelf Life Estimates—The horizontal dashed lines in Figs. 4 and 5 represent the k_{obs} required for 10% hydrolysis in 2

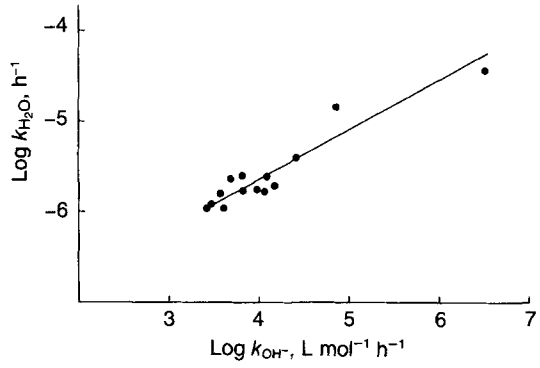
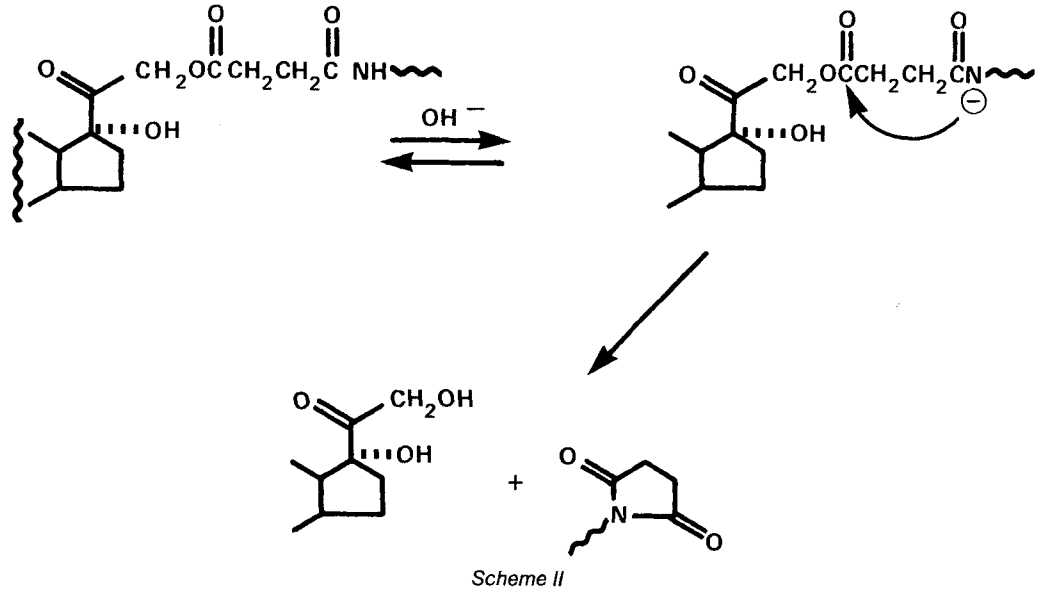


Figure 6—*Plot of the logarithms of k_{H_2O} versus the corresponding log k_{OH^-} values at 25°C for the compounds reported in this study.*



Scheme II

years. As shown, some of the compounds reported in this study exhibit hydrolysis rates below this 2-year line at their pH–rate minima. Because of their lower k_{OH^-}, long-chain sulfonate-containing derivatives are the most stable in dilute solution. Compounds **1m** and **1q**, for example, are estimated to undergo 7% hydrolysis in 2 years at 25°C.

The development of solution-stable formulations of such compounds, however, may be a more complex problem. Other degradative processes, such as 17-ester formation and 17-ketosteroid formation, undoubtedly contribute to the overall decomposition as discussed earlier. In addition, the effect of temperature on these reactions may be pronounced. Since "controlled room temperature" is defined as between 15–30°C,[44] a demonstrated 2-year shelf life at 30°C would be desirable. Preliminary studies of the effect of temperature on the hydrolysis of the compounds reported herein suggest that a 5°C increase in temperature may increase reaction rates by a factor of 1.6–1.8.

An added consideration in more concentrated solution formulations is the degree to which ester hydrolysis can occur before the solution becomes saturated with respect to the hydrolysis product, the free corticosteroid. The aqueous solubility of methylprednisolone is 0.09 mg/mL.[45] Therefore, in a formulation containing the equivalent of 40 mg/mL of methylprednisolone, only 0.25% hydrolysis would lead to a concentration of methylprednisolone in excess of its aqueous solubility. A subsequent paper will discuss this problem in more detail with an emphasis on the advantageous influence of prodrug self-micellization on the solubility of the free corticosteroid hydrolysis product and on the rates of prodrug hydrolysis in concentrated solutions.

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