1-Alkylcarbonyloxymethyl Prodrugs of 5-Fluorouracil (5-FU): Synthesis, Physicochemical Properties, and Topical Delivery of 5-FU

H. ELIZABETH TAYLOR AND KENNETH B. SLOAN*

Contribution from the *Department of Medicinal Chemistry, University of Florida, P.O. Box 100485, Gainesville, Florida 32610.*

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Abstract □ 1-Alkylcarbonyloxymethyl (1-ACOM) prodrugs of 5-fluorouracil (5-FU) have been synthesized and characterized by their solubilities in isopropyl myristate (S_{IPM}) and pH 4.0 buffer (S_{H_2O}), by their partition coefficients between isopropyl myristate (IPM) and pH 4.0 buffer (K) and by their abilities to deliver total 5-FU species into (C_s) and through (J_i) hairless mouse skin from an IPM vehicle. All of the prodrugs were much more lipophilic (S_{IPM}) than 5-FU (>60 times), and two members of the series (alkyl = C_1 and C_2, acetyl- and propionyloxymethyl) were also more soluble in water than 5-FU. The two more water–soluble members gave larger J_i values than the other members of the series, with C_2 exhibiting the best biphasic solubility and the largest J_i value (16 times that of 5-FU). The ability of the 1-ACOM-5-FU prodrugs to deliver total 5-FU species into skin (C_s) was greater than the delivery of 5-FU by 5-FU, except for the last two members of series (alkyl = C_7 and C_9, octanoyl- and decanoyloxymethyl). However, the ratios of normalized C_s to J_i for the series was less than that exhibited by 5-FU, except for C_7 and C_9. Also, except for C_9, significant amounts of intact prodrug as percentages of total 5-FU species were found in the receptor phases during the course of the diffusion cell experiments, ranging from 55% for C_1 to 12% for C_7.

Introduction

Although 5-fluorouracil (5-FU, **1**) is useful in the topical treatment of facial actinic keratoses (AK),[1] its usefulness in the topical treatment of AK and other proliferative disease states is limited by its ability to penetrate less permeable areas of skin. For example, 5-FU is not useful in the treatment of AK of the arm where the skin is less permeable than that of the face.[2] There are two general ways to improve the ability of drugs such as 5-FU to penetrate skin. One way is by improving the formulation in which 5-FU is applied by the judicious use of excipients and possibly even penetration enhancers.[3] The other way is by transiently changing the physicochemical properties of 5-FU by using a prodrug approach.

The first report of the use of prodrugs to enhance the topical delivery of 5-FU was by Bundgaard and co-workers.[4] The delivery of 5-FU by 1-butyryloxymethyl- and by 1-pivaloyloxymethyl-5-FU from propylene glycol through human skin was reported to be five and two times more effective, respectively, than by 5-FU. However, those results were reported as mole % of 5-FU species penetrated and did not take into account the fact that the same microgram amounts of 5-FU and prodrugs, with molecular weights almost twice that of 5-FU, were applied. Thus, the 1-butyryloxymethyl- and pivaloyloxymethyl-5-FU prodrugs were actually only ~2.5-fold and onefold, respectively, as effective as 5-FU. This report was followed by the report of the use of *N*-Mannich bases of 5-FU to improve the delivery of 5-FU from isopropyl myristate (IPM) through hairless mouse skin by up to five times.[5] Only recently have improvements in delivery of 5-FU of an order of magnitude been reported using various 1-acyl types of prodrugs.[6,7] Of these, the 1-alkylcarbonyl prodrugs were the most effective, delivering almost 40-fold more 5-FU through hairless mouse skin from IPM.

Although the 1-alkylcarbonyl prodrugs of 5-FU (1-AC-5-FU) are very effective at delivering only 5-FU, they are very unstable in the presence of water or protic solvents,[8] and present obvious difficulties if a convenient topical formulation is desired for commercial development. On the other hand, the 1-alkylcarbonyloxymethyl (1-ACOM) prodrugs exhibit half-lives of 70 to 140 h (C_1 to C_3, acetyl- to butyryloxymethyl) at pH 7.4 and 37 °C[9] and hence should be reasonably stable in protic vehicles. In addition, Bundgaard and co-workers[4] observed that only 5-FU was found in the receptor phases of the diffusion cells to which the 1-butyryloxymethyl prodrug had been applied, so the straight chain 1-ACOM-5-FU prodrugs were apparently as effective as the 1-AC-5-FU prodrugs in that regard. However, only one member (C_3) of the homologous 1-ACOM series was evaluated in that report for its ability to deliver 5-FU.[9] The pivaloyloxymethyl member (C_{t4}) was also evaluated, but it is not a member of the homologous series because it is branched. Thus, the physicochemical properties of members of the series of 1-ACOM prodrugs of 5-FU with alkyl chain lengths shorter and longer than C_3 and their abilities to deliver 5-FU topically need to be determined to fully characterize this type of prodrug for use to enhance topical delivery.

In this paper, a more convenient synthesis of straight alkyl chain 1-ACOM prodrugs of 5-FU that gives better yields of the desired products without requiring chromatography is reported. In addition, the physicochemical properties of the C_1−C_9 members of the series (acetyl- to decanoyloxymethyl) have been characterized, and their abilities to deliver 5-FU into and through hairless mouse skin from IPM have been evaluated.

Methods and Materials

Melting points were determined with a Meltemp capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Ultraviolet (UV) spectra were obtained on Shimadzu UV-265 or 2501 PC spectrophotometers. The vertical, Franz-type diffusion cells were from Crown Glass, Somerville, NJ (surface area 4.9 cm^2, 20-mL receptor phase volume, 15-mL donor phase volume). The diffusion cells were maintained at 37 °C with a Fisher circulating water bath model 25. Thin-layer chromatography (TLC) analyses were run on Brinkman Polygram Sil G/UV 254 plates. IPM was

* Telephone: 352-846-1957. FAX: 352-392-9455.
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obtained from Givaudan, Clifton, NJ. 5-FU was purchased from Sigma Chemical Company, pivaloyloxymethyl chloride and all other reagent chemicals were from Aldrich Chemical Company, and all other solvents were from Fischer. The female hairless mice (SKH-hr-1) were from Charles River. The alkylcarbonyloxymethyl chlorides (**2**) were synthesized according to a modification[10] of the general procedure of Neuenschwander and co-workers.[11]

General Method for the Synthesis of 1-Alkylcarbonyloxymethyl-5-fluorouracils (3)—1-Methylpyrrolidine (4.38 mL, 3.51 g, 0.040 mol) was added to a well-stirred solution of alkylcarbonyloxymethyl chloride (0.012 mol) and 5 mL of dry acetonitrile in a round-bottomed flask equipped with a reflux condenser and drying tube. This solution was stirred and heated at 70 °C with an oil bath for 1 h. The solution was cooled to room temperature and allowed to react with 5-FU (1.30 g, 0.010 mol) at room temperature for 1 day with stirring. The acetonitrile was evaporated at 40 °C with a rotary evaporator. The oil that resulted was diluted with 100 mL of CH_2Cl_2. The CH_2Cl_2 solution was washed with water (5 mL), aqueous acid (5 mL containing 2 mL of con HCl) and water (5 mL), and then dried over Na_2SO_4. The dried CH_2Cl_2 solution was evaporated at 40 °C, and the residue was crystallized from CH_2Cl_2 (2−5 mL) petroleum ether (25−100 mL). The initial products were one component by TLC (silica gel, ethyl acetate), and their ^1H NMR spectra were consistent with their structure. The initial products were subsequently recrystallized to constant melting points. In this way the following compounds were prepared:

*1-Acetyloxymethyl-5-Fluorouracil (**3a**, C_1)*—This compound was synthesized from acetyloxymethyl chloride (89% pure) in 83% yield: 1.68 g, mp 105−115 °C, R_f 0.47. Subsequent recrystallization from acetone−petroleum ether gave 87% recovery of **3a**: mp 123−124 °C (lit[12] mp 127−128 °C; 62% yield); UV_{max} (CH_3CN) 261 nm ($\epsilon = 8.49 \times 10^3$ L/mol), (0.05 M acetate buffer, pH = 4.0) 263 nm ($\epsilon = 8.69 \times 10^3$ L/mol).

*1-Propionyloxymethyl-5-Fluorouracil (**3b**, C_2)*—This compound was synthesized from propionyloxymethyl chloride (88% pure) in 86% yield: 1.85 g, mp 89−91 °C, R_f 0.60. Subsequent recrystallization from CH_2Cl_2 gave 88% recovery of **3b**: mp 100−102 °C (lit[12] mp 105−106 °C; 73% yield); UV_{max} (CH_3CN) 262 nm ($\epsilon = 8.71 \times 10^3$ L/mol), (0.05 M acetate buffer, pH 4.0) 264 nm ($\epsilon = 8.69 \times 10^3$ L/mol).

*1-Butyryloxymethyl-5-Fluorouracil (**3c**, C_3)*—This compound was synthesized from butyryloxymethyl chloride (87% pure) in 98% yield: 2.26 g, mp 77−80 °C, R_f 0.60. Subsequent recrystallization from CH_2Cl_2−diethyl ether gave 92% recovery of **3c**: mp 89−91 °C (lit[12] mp 96−98 °C; 87% yield); UV_{max} (CH_3CN) 262 mm ($\epsilon = 8.61 \times 10^3$ L/mol), (0.05 M acetate buffer, pH = 4.0) 264 mm ($\epsilon = 8.62 \times 10^3$ L/mol).

*1-Valeryloxymethyl-5-Fluorouracil (**3d**, C_4)*—This compound was synthesized from valeryloxymethyl chloride (85%) in 90% yield: 2.20 g, mp 84−85 °C, R_f 0.62. Subsequent recrystallization from CH_2Cl_2 gave 83% recovery of **3d**: mp 87−88 °C (lit[12] mp 91−92 °C; 77% yield); UV_{max} (CH_3CN) 261 nm ($\epsilon = 8.57 \times 10^3$ L/mol), (0.05 M acetate buffer, pH = 4.0) 264 nm ($\epsilon = 8.66 \times 10^3$ L/mol).

*1-Hexanoyloxymethyl-5-Fluorouracil (**3e**, C_5)*—This compound was synthesized from hexanoyloxymethyl chloride (93% pure) in 84% yield; 2.18 g, mp 90−92 °C, R_f 0.54. Subsequent recrystallization from CH_2Cl_2 gave 70% recovery of **3e**: mp 89−91 °C (lit[12] mp 95−96 °C; 39% yield); UV_{max} (CH_3CN) 261 nm ($\epsilon = 8.63 \times 10^3$ L/mol), (0.05 M acetate buffer, pH = 4.0) 264 nm (8.69×10^3 L/mol).

*1-Octanoyloxymethyl-5-Fluorouracil (**3f**, C_7)*—This compound was synthesized from octanoyloxymethyl chloride (91% pure) in 92% yield: 2.64 g, mp 101−102 °C, R_f 0.65. Subsequent recrystallization from CH_2Cl_2−diethyl ether gave 84% recovery of **3f**: mp 107−108 °C (lit[12] mp 112−113 °C; 74% yield); UV_{max} (CH_3CN) 262 nm ($\epsilon = 8.46 \times 10^3$ L/mol), (0.05 M acetate buffer, pH = 4.0) 263 nm ($\epsilon = 8.48 \times 10^3$ L/mol).

*1-Decanoyloxymethyl-5-Fluorouracil (**3g**, C_9)*—This compound was synthesized from decanoyloxymethyl chloride (80% pure) in 79% yield: 2.48 g, mp 112−112 0.5 °C, R_f 0.63. Subsequent recrystallization from CH_2Cl_2 gave 94% recovery of **3g**: mp 113−115 °C (lit[12] mp 115−116 °C; 68% yield); UV_{max} (CH_3CN) 261 nm ($\epsilon = 8.80 \times 10^3$ L/mol), (0.05 M acetate buffer, pH = 4.0) 262 nm ($\epsilon = 8.80 \times 10^3$ L/mol).

*1-Pivaloyloxymethyl-5-Fluorouracil (**3h**, C_{t4})*—This compound was synthesized from pivaloyloxymethyl chloride (99% pure) in 81% yield: 1.97 g, mp 161 °C, R_f 0.61. Subsequent recrystallization from CH_2Cl_2 gave 84% recovery of **3h**: mp 163−165 °C (lit[12] mp 158−160 °C; 68% yield); UV_{max} (CH_3CN) 262 nm ($\epsilon = 8.47 \times 10^3$ L/mol), (0.05 M acetate buffer, pH = 4.0) 263 nm ($\epsilon = 8.54 \times 10^3$ L/mol).

Analytical and Physicochemical Properties—Quantitation of the 1-ACOM-5-FU prodrugs was accomplished by HPLC with a Beckman 110A pump at a flow rate of 1.0 mL/min, a Rheodyne 7125 20-μL loop injector, a Supelco 5-μm LC-8 150 × 4.6 mm column, an Upchurch Scientific guard column with Zorbax ODS packing, a Beckman model 153 fixed-wavelength detector (λ_{anal} = 254 nm), and a Hewlett-Packard 3392A integrator. The following mobile phase compositions (pH 5.0, 0.025 M acetate buffer: CH_3OH) gave the following retention times in minutes for the various prodrugs: C_1 (85:15) 4.1 or (100:0) 12.5; C_2 (85:15) 9.6 or (95:5) 11.4; C_3 (65:35) 6.9; C_4 (65:35) 14.3; C_5 (50:50) 7.8; C_7 (35:65) 5.4; C_9 (35:65) 13.1; and C_{t4} (50:50) 4.3. 5-FU was also quantitated by UV spectrophotometry from its absorbance at 265 nm ($\epsilon = 7.13 \times 10^3$ L/mol) in pH 7.1 phosphate buffer (0.11% formaldehyde).

Theophylline was quantitated by UV spectrophotometry from its absorbance at 270 nm ($\epsilon = 1.02 \times 10^4$ L/mol) in pH 7.1 phosphate buffer (0.11% formaldehyde).

The hydrolysis of 2.5×10^{-4} M 1-ACOM-5-FU prodrugs at pH 8.9 (0.01 M borate) and 46.7 °C containing 5% CH_3CN was followed by UV spectrophotometry at 300 nm according to the general procedure of Buur et al.[9]

Lipid solubilities were determined in IPM according to a previously described procedure.[6] Three suspensions of each prodrug were stirred at 22 ± 1 °C for 48 h. The suspensions were filtered through 0.45-μm nylon filters, and then the saturated solutions were diluted with dry acetonitrile and quantitated by UV spectrophotometry using molar absorptivities previously determined in triplicate at 261−262 nm.

Partition coefficients (K) were determined at 22 ± 1 °C using the saturated IPM solutions (n = 3) from the lipid solubility determinations.[6] For most compounds, equal volumes (1 mL) of saturated IPM and 0.05 M acetate buffer (pH 4.0) were partitioned. The two phases were shaken vigorously for 10 s, then allowed to separate for 60 s. The IPM layer was diluted with acetonitrile and the UV absorbance was determined. The K values were calculated as follows:

$$K = [A_a/(A_b - A_a)]\ V_{H_2O}/V_{IPM} \quad (1)$$

where A_b and A_a are the absorbances from the IPM layer before and after partitioning, respectively, and V_{H_2O} and V_{IPM} are the volumes of the buffer and IPM phases, respectively. For those compounds exhibiting large solubility differences in the two phases, volume ratios (IPM:buffer) other than 1:1 were necessary to achieve accurate results, but the ratio never exceeded 10:1 or 1:10 (for C_1 and C_2 the volume ratio = 6:1, for C_7 and C_9 the volume ratio = 1:10).

For aqueous solubilities, three suspensions of each prodrug were vigorously stirred in 0.05 M acetate buffer (pH 4.0) at 22 ± 1 °C for 24 h. The suspensions were filtered through 0.45-μm nylon filters, and then the saturated solutions were diluted with HPLC mobile phase and quantitated by HPLC.

Diffusion Cell Experiments—The diffusion cell experiments were run in essentially the same way as previously described.[6] Briefly, female hairless mice were sacrificed by cervical dislocation. Their skins were removed by blunt dissection and then placed epidermal side up in contact with pH 7.1 phosphate buffer (0.05 M, I = 0.11 M, 32 °C) containing 0.11% formaldehyde (2.7 mL of 36% aqueous formaldehyde/L) to prevent microbial growth and to ensure the integrity of the mouse skins during the course of the experiment.[13] The skins were kept in contact with the buffer for at least 48 h to condition the skins and to allow UV absorbing materials to leach out of the skins; the receptor phases were changed at least three times during this time to facilitate the leaching process. In control experiments, there were no significant differences among the fluxes of a standard solute (theophylline) applied in a standard vehicle (propylene glycol) after 4, 24, 48, or 120 h of contact between the skins and the buffer containing 0.11% formaldehyde.[13]

Aliquots (0.5 mL, 0.25 M) of suspensions of each prodrug in IPM were applied to the donor side of each of three diffusion cells. The donor phase was removed and fresh donor phase (0.5 mL) was applied whenever the suspensions started to clear: for C_1 at 24 and 34 h; for C_2 at 24, 34, and 43 h; for C_3 at 24 and 34 h; and for

C_4 at 45 h. For C_1, C_2, and C_3, the donor phases were saved and the solid residues were separated from the solutions and analyzed by ^{1}H NMR in DMSO-d_6 (1-ACOM C^6-**H** at δ 8.10, 5-FU C^6-**H** at δ 7.67) while the solutions were analyzed by UV as already described. After the suspensions were applied, 6-mL samples of the receptor phases were removed, generally at 8, 19, 22, 25, 28, 31, 45, and 48 h. The entire receptor phase was replaced with fresh receptor fluid each time a sample was removed. The amount of prodrug in each sample at 22, 25, 28, and 31 h (the initial steady-state portion of the experiment) was determined immediately by HPLC using the conditions just described. For each prodrug, 5-FU as well as prodrug could be observed in the HPLC, but in all cases, 5-FU could not be quantitated at the same time because the conditions that allowed for convenient quantitation of prodrug caused 5-FU to elute in the solvent front. Aliquots (2 mL) of all samples were then adjusted to pH 9 with NaOH (2 mL), and the prodrugs were allowed to hydrolyze until intact prodrug was not detected by HPLC. 5-FU in the samples was then quantitated from its UV absorption at 266 nm ($\epsilon = 4.5 \times 10^3$ L/mol). At the same time, control experiments ($n = 3$) in which receptor phase solutions of 5-FU were also adjusted to pH 9 with aqueous NaOH were run to determine if changes in the UV spectra of 5-FU occurred with time, which would indicate decomposition of 5-FU, and to determine ϵ values for 5-FU at that pH (see previous discussion).

After the initial application period of 48 h, the donor phases were removed and the donor surfaces were quickly washed with 3×5-mL portions of methanol to remove any residual prodrug or 5-FU. After the methanol wash, the skins were kept in contact with fresh receptor fluid for 23−24 h to allow any 5-FU or 5-FU prodrugs to leach out. Samples of the receptor phase were removed, the receptor phases were replaced with fresh receptor fluid, and 0.5-mL aliquots of a standard drug vehicle (theophylline/propylene glycol, Th/PG) were applied. Samples of the receptor phases (3 mL) from this second application were removed at 1, 2, 3, and 4 h, and the amounts of theophylline in the receptor phases were quantitated from its UV absorption. Each time a sample was removed, the entire receptor phase was replaced with fresh receptor fluid. At the same time, the samples from the 23−24 h leaching process were adjusted to pH 9 with aqueous NaOH, and any prodrug in the samples was allowed to hydrolyze to 5-FU until no intact prodrug was detected by HPLC. 5-FU in the sample was then quantitated from its UV absorption (see previous discussion).

In all cases, the rates of delivery of total 5-FU (J_i), intact 5-FU prodrug (J_p), or theophylline (J_j) through skin were determined by plotting the cumulative amount (μmol) of 5-FU, intact 5-FU prodrug, or theophylline measured in the receptor phase against time and dividing the steady-state portions of those plots by the surface area of the diffusion cells. Permeability coefficients (P_i) were determined by dividing the J_i values by the solubilities (S_{IPM}) of the prodrugs in IPM.

Solubility Parameters—The solubility parameters were obtained by the method of Fedors[14] as illustrated by Martin et al.[15] and Sloan et al.[16]

Statistical Analyses—Statistical analysis was accomplished using Student's t test. Unless otherwise indicated, statistical significance is for $p < 0.05$.

Results and Discussions

Synthesis—There are numerous synthetic approaches that have been taken to obtain 1-ACOM prodrugs of 5-FU (1-ACOM-5-FU).[12,17,18] However, the base-catalyzed alkylation of 5-FU by ACOM chlorides has probably been used most frequently. The requisite ACOM chlorides can be synthesized from a wide range of acid chlorides and aldehydes,[11,19,20] so there is a great range of flexibility in the physicochemical properties that can be designed into the promoiety. However, because 5-FU has two sites available for alkylation, alkylation usually gives mixtures of two possible mono-derivatives (1- and 3-) and the bis-derivative, which requires chromatography to isolate the desired 1-ACOM-5-FU.

Previously, Kamata et al.[21] had shown that for the alkylation of 5-FU with a cyclic ACOM halide (phthalidyl halide), greater selectivity for alkylation of the 1-position could be achieved by converting the halide-leaving group to a poorer leaving group, a quaternary ammonium ion, by reacting the halide with an appropriate tertiary amine. Subsequent reaction of the quaternary salt with 5-FU required a catalytic amount of base for alkylation to take place. When this approach was tried with acyclic ACOM halides in this investigation, two observations were made. First, that the time for conversion of the halide to the quaternary salt and completeness of the reaction were more dependent on the steric requirements of the amine than on its basicity. Thus, *N*-methylpyrrolidine ($pK_a = 10.46$)[22] reacted much faster with ACOM chlorides than *N*-methylimidazole ($pK_a = 6.95$),[22] but both reactions were complete in 1 h at 60 °C. On the other hand, it took ~3 h for the same reaction to proceed to completion with triethylamine ($pK_a = 10.65$).[22] Second, the reaction between the quaternary salt and 5-FU proceeded to only a limited extent and gave only a trace of the bis-derivative if no extra base was present, proceeded slowly if one equivalent of tertiary amine base was present, but proceeded rapidly to give almost exclusively 1-mono-derivative if three equivalents of tertiary amine base were present initially. Thus, the reaction of 1.2 equivalents of 1-ACOM chloride with 4 equivalents of *N*-methylpyrrolidine at 60−80 °C in dry acetonitrile for 1 h gave quantitative conversion of the chloride to the quaternary ammonium salt by ^{1}H NMR spectroscopy (OC**H**$_2$Cl at δ 5.68 to OC**H**$_2$N$\oplus$, at δ 5.73 in $CDCl_3$). In the subsequent reactions with 5-FU, 90−100% conversions of 5-FU to the corresponding 1-ACOM-5-FU prodrugs were observed by ^{1}H NMR spectroscopy (C^6-**H** doublet centered at δ 7.42 to C^6-**H** centered at δ 7.67 in acetonitrile, C**H**$_3$CN at δ 1.95). Although trace amounts of the *bis*-derivatives were observed by TLC analyses of the reaction mixtures, 80−98% yields of the 1-ACOM-5-FU prodrugs were initially isolated upon workup, which were one component free of *bis*-derivative by TLC and which gave reasonably sharp melting points except for C_1.

The yields of the initially isolated 1-ACOM-5-FU were generally significantly better than those previously reported, and no chromatography was necessary to isolate them. Their melting points and ^{1}H NMR spectra (C^6-**H** doublet centered at δ 7.57 to 7.62 and N-C**H**$_2$O singlet at δ 5.60 to 5.65 in $CDCl_3$) were consistent with those previously reported.[12] Their UV spectra were also consistent with those of a previous report[9] for three members of the series. The initially isolated prodrugs were subsequently recrystallized to give 83−94% recovery of the prodrugs, which exhibited constant melting points and which were then used for the remaining experiments. Those constant melting points are listed in Table 1.

Physicochemical Properties—The solubilities in IPM (S_{IPM}) and in pH 4.0 buffer (S_{H_2O}), and the partition coefficients of the 1-ACOM-5-FU prodrugs between IPM and pH 4.0 buffer (K) are listed in Table 1. Although all of the prodrugs were at least 60-fold more soluble in IPM than 5-FU, there was less than a fivefold range in the IPM solubilities for the entire series and IPM solubilities decreased after the C_4 member. This sort of behavior has been previously observed for ACOM-type prodrugs of 6-mercaptopurine (6-MP),[23] and for 1-alkylcarbonyl type prodrugs of 5-FU.[7,24] The causes of this behavior have been discussed by Yalkowsky.[25] Generally, the initial increases in lipid solubility exhibited by members of a homologous series occur because the promoiety masks a hydrogen bond donor (N-H) and the short alkyl chains decrease crystal packing efficiency. As the alkyl chains become longer, van der Waals interaction between the chains become stronger, leading to increased crystal lattice energy and decreased solubility.

Table 1—Melting Points (mp), Lipid Solubilities (S_{IPM}), pH 4.0 Buffer Solubilities (S_{H_2O}), and Partition Coefficients between IPM and pH 4.0 Buffer (K) for 1-Alkylcarbonyloxymethyl Prodrugs of 5-FU (1-ACOM-5-FU)

alkyl =	mp °C	S_{IPM} (±SD), mM	S_{H_2O}(±SD), mM	K (±SD)
5-FU, **1**	280–284	0.049[a]	96[a]	0.00058[a]
CH_3, **3a**	123–124	3.29 (0.13)	204 (14)	0.018 (0.00069)[b]
C_2H_5, **3b**	100–102	9.83 (0.25)	151 (4.0)	0.059 (0.0031)[b]
C_3H_7, **3c**	89–91	14.4 (0.30)	52.3 (0.60)	0.34 (0.017)
C_4H_9, **3d**	87–88	14.8 (0.18)	14.3 (0.40)	1.2 (0.090)
C_5H_{11}, **3e**	89–91	14.7 (0.53)	3.37 (0.050)	6.6 (0.73)
C_7H_{15}, **3f**	107–108	9.99 (0.22)	0.16 (0.015)	59 (3.2)[c]
C_9H_{19}, **3g**	113–115	4.28 (0.10)	0.0031 (0.0010)	—[d]
$(CH_3)_3C$, **3h**	163–165	7.78 (0.35)	9.95 (0.14)	0.99 (0.053)

[a] From ref 7. [b] IPM:buffer = 6:1. [c] IPM:buffer = 1:10. [d] Could not be determined.

Table 2—Values for Log Partition Coefficients between IPM and pH 4.0 Buffer (log K), Log Ratio of Solubilities in IPM and pH 4.0 Buffer (log SR), Rate Constants for Hydrolyses at pH 8.9 and 46.7 °C, and Charton's Steric Parameters (ν) for 1-ACOM-5-FU

alkyl =	log K	π[a]	log SR[b]	π[a]	k_{OBS} (±SD), $min^{-1} \times 10^3$	ν[c]
5-FU	–3.24	—	–3.29	—	—	—
CH_3	–1.73	—	–1.79	—	6.6 (0.24)	0.52
C_2H_5	–1.23	0.50	–1.19	0.60	5.33 (0.036)	0.56
C_3H_7	–0.47	0.76	–0.56	0.63	3.49 (0.062)	0.68
C_4H_9	0.08	0.55	0.01	0.57	3.21 (0.012)	0.68
C_5H_{11}	0.82	0.74	0.64	0.63	3.20 (0.059)	0.68
C_7H_{15}	1.77	0.44	1.80	0.58	2.96 (0.038)	0.73
C_9H_{19}	—[d]	—	3.14	0.67	2.86 (0.014)	—[e]
$(CH_3)_3C$	–0.01	—	0.78	—	0.652 (0.0047)	1.24

[a] $\pi = (\log K_{n+m} - \log K_n)/m$, where n is the number of methylene units in the promoiety of one prodrug and m is the number of additional methylene units in the promoiety of the prodrug with which it is compared and where log SR can be substituted for log K. [b] Solubility ratio, S_{IPM}/S_{H_2O}. [c] Charton's steric parameters from ref 27. [d] Could not be determined. [e] Not available.

The solubilities of the first three members of the homologous series and C_{t4} (**3h**) in pH 4.0 buffer (S_{H_2O}) had been previously reported.[4] The S_{H_2O} values reported here are not significantly different from those previously reported for the C_1 and C_2 members, but are 25 and 5% greater than those reported for the C_3 and C_{t4} members, respectively. The first two members of the series are also more soluble in pH 4.0 buffer than 5-FU. This sort of behavior has also been previously observed for ACOM-type prodrugs of 6-MP[23] and for 1-alkylcarbonyl- and 1-alkyloxycarbonyl-type prodrugs of 5-FU.[6,7] The phenomena of increased lipid solubility and increased water solubility for members of the ACOM-type prodrugs of 5-FU have been discussed previously by Bundgaard and co-workers[9] in the context of decreased crystal lattice energy.[26]

The partition coefficient (K) could be determined for all of the prodrugs except C_9 (Table 1). Although the mean of the methylene π values calculated from log K values (Table 2) for the 1-ACOM-5-FU prodrugs (0.60 ± 0.14) was consistent with mean methylene π values for the three series of 1-acyl prodrugs of 5-FU (alkylcarbonyl, $\pi = 0.59 \pm 0.05$; alkyloxycarbonyl, $\pi = 0.61 \pm 0.05$; alkylaminocarbonyl, $\pi = 0.60 \pm 0.05$)[7] also determined from partitioning between IPM and pH 4.0 buffer, the standard deviation was almost threefold larger. However, if the log solubility ratios ($SR = S_{IPM}/S_{H_2O}$, Table 2) were used instead of log K, a mean methylene π value also consistent with those of the 1-acyl series but with a much smaller standard deviation was obtained ($\pi = 0.61 \pm 0.04$). The mean methylene

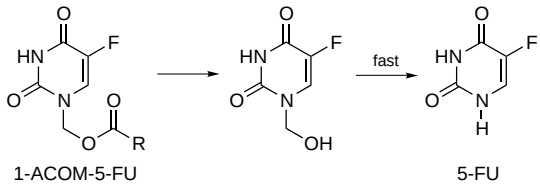

Figure 1—The two-step hydrolysis of 1-ACOM-5-FU prodrugs to 5-FU.

π value obtained from partitioning between IPM and buffer is somewhat larger than that obtained from partitioning between 1-octanol and buffer ($\pi = 0.57$, range = 0.01).[9]

Bundgaard and co-workers[9] reported the pH–rate profile for the 1-ACOM-5-FU prodrugs in detail and showed that they quantitatively revert to 5-FU. They also showed that the rates of hydrolyses were solely dependent on steric differences among the four members of the series that they studied: the linear regression equation for the plot of log k_{OH} versus Charton's steric parameter[27] gave $r = 0.999$. Charton's steric parameters are derived from correlations between van der Waals radii of the substituents on the carboxylic acid (steric effect) and the rates of acid-catalyzed hydrolyses and esterifications. Assuming that steric effects will be the same whether the hydrolysis is acid or base catalyzed, this dependence of log k_{OH} on the steric properties of the alkylcarbonyl group supports the conclusion that the 1-ACOM-5-FU are hydrolyzing by an addition–elimination reaction typical for ester hydrolyses. Subsequent loss of formaldehyde from the intermediate 1-hydroxymethyl-5-FU to give 5-FU is an extremely fast process with an estimated $t_{1/2}$ of 0.4 s[9] (Figure 1). It was therefore of interest to see if the rate constants for the hydrolyses of the additional members of the series that were synthesized here would fit the same equation. The pseudo-first-order rates constants (k_{obs}) were determined at pH 8.9 and at 46.7 °C to facilitate acquisition of data. The regression equation for the plot of log k_{obs} versus Charton's steric parameter (ν, Table 2) for the same four members (C_1, C_2, C_3, and C_{t4}) of the series previously studied gave: log $k_{obs} = -1.36\ \nu - 1.50$ ($r = 0.998$). Inclusion of the log k_{obs} for the remaining members of the series (C_4, C_5, and C_7) in the regression equation gave: log $k_{obs} = -1.35\ \nu - 1.53$ ($r = 0.994$). Thus, the regression of the plot of log k_{obs} for all the prodrugs versus Charton's steric parameters indicates a reasonably good fit.

Diffusion Cell Experiments—The steady-state fluxes of total 5-FU species (J_i; 5-FU and intact prodrug) in Table 3 exhibit the same trend observed previously for S[6]-ACOM-6-MP prodrugs[23]: the more water-soluble members of a series of more lipid-soluble prodrugs give the higher flux values (see Figure 2 for a plot of cumulative total 5-FU and intact prodrug versus time for C_1 and C_2). Although there is no correlation between lipid solubility and J_i, it is clear that lipid solubility is important because the C_2 prodrug and not the more water-soluble C_1 prodrug gives the highest J_i value. This fact may be because C_2 is almost three times as lipid soluble as C_1 yet retains 75% of the water solubility of C_1. Thus, the C_2 prodrug exhibits the better biphasic solubility properties and so gives the larger J_i value.

The steady-state fluxes for intact prodrug (J_p) follow the same trend as found for J_i. The ratio of J_p to J_i (as a percentage) decreases as the alkyl chain becomes longer, starting with 55% for C_1 in the homologous series and ending with 0% for C_9, whereas the percentage of intact 1-pivaloyloxymethyl-5-FU (C_{t4}) was 67%, as expected, due

Table 3—Flux of Total 5-FU Species (J_i) and Flux of Intact 1-ACOM-5-FU Prodrug (J_p) During Steady State from Application of 1-ACOM-5-FU/IPM, Flux of Theophylline (Th) from Application of Th/Propylene Glycol (PG) (J_j), and Concentration of 5-FU Species in Skin (C_s) after 1-ACOM-5-FU/IPM Donor Phase Removed

alkyl =	J_i (±SD),[a] μmol/cm²h	J_p (±SD),[b] μmol/cm²h	J_j (±SD),[c] μmol/cm²h	C_s (±SD),[d] μmol
5-FU	0.24 (0.09)[e]	—	1.2 (0.20)[e]	3.7 (0.90)[e]
CH_3	2.88 (0.17)	1.57 (0.29)	1.2 (0.17)	5.2 (0.81)
C_2H_5	3.82 (0.30)	1.91 (0.21)	1.1 (0.090)	9.7 (1.0)
C_3H_7	2.57 (0.04)	1.23 (0.033)	1.0 (0.026)	8.8 (2.1)
C_4H_9	1.29 (0.16)	0.37 (0.096)	0.97 (0.17)	6.8 (0.26)
C_5H_{11}	0.56 (0.097)	0.11 (0.020)	1.3 (0.17)	6.5 (3.3)
C_7H_{15}	0.12 (0.027)	0.014 (0.0058)	1.3 (0.21)	2.8 (0.50)
C_9H_{19}	0.015 (0.0019)	—[f]	1.1 (0.032)	1.6 (0.34)
$(CH_3)_3C$	0.30 (0.087)	0.20 (0.096)	0.97 (0.26)	6.1 (0.16)

[a] J_i = flux of total 5-FU species. [b] J_p = flux of intact 1-ACOM-5-FU. [c] J_j = flux of theophylline from propylene glycol in a second application. [d] C_s = concentration of total 5-FU species in receptor phase after keeping the skin in contact for 24 h after donor phase from first application was removed to allow 5-FU and intact prodrug to leach out. [e] From ref 7. [f] Could not be detected.

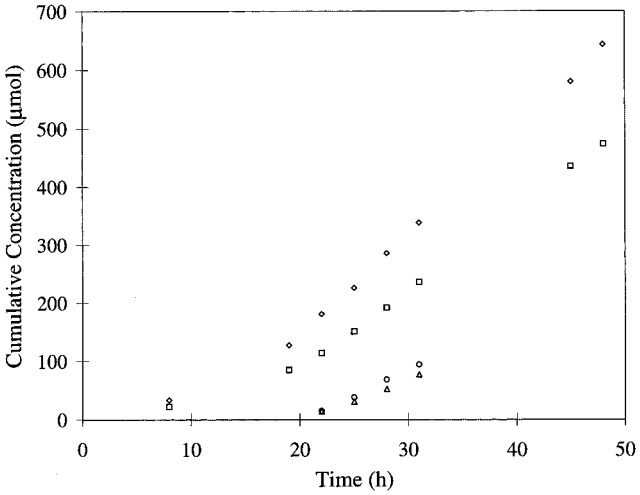

Figure 2—Plot of cumulative concentrations of total 5-FU or of intact prodrug, respectively, in the receptor phases of diffusion cells where the acetyl- (□, △) or propionyloxymethyl (◇, ○) prodrugs (C_1 and C_2) had been applied as suspensions in IPM. Values are averages of $n = 3$.

to steric hindrance to hydrolysis. In the previous study[4] of the use of ACOM prodrugs to deliver 5-FU, no intact prodrug was observed in the receptor phase after the application of 1-butyryloxymethyl-5-FU (C_3) in propylene glycol/ethanol to human skin, but ~44% of intact C_{t4} was observed in the receptor phase after application of C_{t4}. There are two possible explanations for this discrepancy in the behavior of the C_3 prodrug. First, J_i for the C_3 prodrug in these experiments is 200-fold greater than previously reported. Hairless mouse skin has been reported to be as much as 10-fold more permeable than human skin,[28] but that still leaves a factor of 20-fold greater J_i that would lead to much higher skin concentrations of C_3, which could saturate esterase enzymes. On the other hand, the lower flux of C_{t4} (one-eighth C_3) in these experiments would lead to lower skin concentrations and less chance of saturating esterase enzymes. Second, the mouse skins in these experiments were kept in contact with the receptor phase for 48 h before application of the prodrugs so that conditions would be comparable to those that have been used for the study of other prodrugs.[5−7] During that 48 h, esterase enzymes responsible for hydrolyzing the prodrugs leach out of the skin[4,23] and decrease the ability of the skin to hydrolyze ester substrates.

The much higher J_i values obtained from the present application of 5-FU, C_3, and C_{t4} in IPM (44-, 198-, and 51-fold, respectively) than obtained in the previous study[4] can be attributed to three factors. First, the J_i values obtained here were from the application of suspensions of the prodrugs in IPM where the suspensions were maintained by applying fresh suspensions whenever significant depletion of the solid in the suspension was observed. In the cases of the C_1, C_2, and C_3 prodrugs, the donor suspensions that were removed so that fresh suspensions could be applied were analyzed by UV and ^{1}H NMR for concentration of prodrug in the solution phase and for intact prodrug in the solid phase of the suspension, respectively. Only intact prodrug was observed in the solid phase and the concentration of prodrug in the solution phase was 97 ± 2% of the measured solubility in IPM. Thus, saturation of the prodrug in IPM in the donor phase was maintained until the donor phase was removed. These conditions lead to maximum flux values because the thermodynamic activity of the solute equals one.[16] On the other hand, in the previous study the prodrugs were applied as solutions in ethanol/propylene glycol (PG) (88:12). After the ethanol evaporated, it was not clear whether the donor phases were suspensions, supersaturated solutions, or simple solutions because the solubilities of the solutes in PG were not given and no visual observations were recorded. Second, IPM has a penetration-enhancing effect on the flux of 5-FU,[29] which amounts to an 18-fold greater flux of 5-FU from IPM than PG. However, IPM was used as the vehicle in these experiments so that the conditions would be comparable to those that have been used for the study of other prodrugs that are unstable in PG.[5−7,28] Third, hairless mouse skin is generally considered to be as much as 10-fold more permeable than human skin.[28] Thus, any combination of two or more of these factors could account for the differences in the J_i values from the two studies.

After the donor phases from the initial application of prodrug/IPM were removed, the skins were maintained in contact with receptor phase for 23−24 h to allow any 5-FU or intact prodrug in the skin to leach out. The concentrations of total 5-FU species leached from the skin (C_s, Table 3) were at most only 3.6-fold greater than the amounts that leached from the skin after the application of 5-FU/IPM. Using these C_s values as an indication of the relative ability of the members of the series to deliver 5-FU species into the skin, the ratios of C_s for the members of the series (normalized for skin area and time during which the samples were collected) to J_i (defined as D/T delivery ratios, Table 4) show that the prodrugs that are more effective than 5-FU at delivering total 5-FU species through the skin are less effective at delivering total 5-FU species into the skin. Thus, if increased local delivery to more effectively treat a local condition without causing increased systemic drug burden is desired, the 1-ACOM-5-FU prodrugs are not the prodrugs of choice. However, the fact that the highly lipophilic C_7 and C_9 prodrugs give relatively high C_s values compared with J_i, and D/T delivery ratios greater than that for 5-FU suggests that if the C_7 and C_9 prodrugs were more effectively delivered into the skin, they might form an effective reservoir for the release of 5-FU in the skin.

To determine if the differences in the J_i values were due to differences in damage caused by the application of the prodrugs/IPM, the fluxes of a standard solute/vehicle [theophylline (Th)/PG] were measured 24 h after the prodrug/IPM donor phases were removed. The flux values of Th/PG (J_j) given in Table 3 show that there are no

Table 4—Dermal/Transdermal Delivery Ratios (D/T) and Log Permeability Coefficients (log P_i) for 1-ACOM-5-FU Prodrugs, and Log P_i, J_i, S_{IPM}, and S_{H_2O} Values for 1-AC-5-FU Prodrugs

alkyl =	D/T[a]	log P_i,[b] cm/h	log P_i,[b,c] cm/h	J_i,[c] μmol/cm^{2}h	S_{IPM},[c] mM	S_{H_2O},[c] mM
5-FU	0.13	0.69	—	—	—	—
CH_3	0.015	–0.06	–0.38	9.3	22.1	120.
C_2H_5	0.022	–0.41	–0.93	4.4	36.4	47.6
C_3H_7	0.029	–0.75	–1.13	1.3	17.4	6.50
C_4H_9	0.045	–1.06	–1.59	1.0	39.2	3.48
C_5H_{11}	0.099	–1.42	–2.01	1.1	112.7	2.94
C_7H_{15}	0.20	–1.92	–2.27	0.60	110.7	0.15
C_9H_{19}	0.91	–2.46	–2.66[d]	0.13[d]	59.0[d]	—[d,e]
$(CH_3)_3C$	0.17	–1.41	—	—	—	—

[a] Calculated from $[C_S/(4.9\ cm^2\ 24\ h)]/J_i$ to give dimensionless ratio. [b] Calculated from J_i/S_{IPM}. [c] From ref 7. [d] From ref 24. [e] Could not be estimated.

significant differences between the J_j values obtained after the application of the prodrugs/IPM and after the application of 5-FU/IPM. Thus, if J_j values are used as measure of relative damage,[16] there are no differences in the damage caused by the application of the prodrugs/IPM, and the differences in J_i are due to differences in the abilities of the prodrugs to deliver 5-FU.

A plot of the log permeability constants (log P_i, Table 4) for the delivery of total 5-FU species through hairless mouse skin against the calculated solubility parameters for the corresponding 1-ACOM-5-FU prodrugs (δ_i, data not shown) gives a curve (plot not shown) that is similar to that observed for the ACOM prodrugs of 6-MP.[23] The log P_i values for the delivery of 5-FU by the 1-alkylcarbonyl–5-FU prodrugs (1-AC-5-FU)[7] have been included in Table 4 to allow for convenient comparison of the 1-ACOM-5-FU series with the best series of the 1-acyl-type prodrugs of 5-FU. Also included are J_i, S_{IPM}, and S_{H_2O} values for the 1-AC-5-FU series. In comparisons of log P_i for equal-length alkyl chain prodrugs from the two series, the 1-AC-5-FU series always gives smaller log P_i values. This result is because 1-AC-5-FU prodrugs exhibit larger S_{IPM} values, because, except for the C_3 and C_4 members, the J_i values for the 1-AC-5-FU series are larger.[7] The C_3 and C_4 members of the 1-AC-5-FU series are anomalies because they exhibit smaller S_{H_2O} and S_{IPM} values than expected for their positions in the series and their poorer biphasic solubilities lead to smaller J_i values than expected.[7] Thus, for members of equal-length alkyl chains, the 1-AC-5-FU series is generally more effective than the 1-ACOM-5-FU series, despite exhibiting smaller P_i values.

However, the ACOM-type promoiety contains an extra CH_2 and an extra O group, each of which extend the alkyl chain and removes it farther from the 5-FU ring than the AC-type promoiety of equal alkyl chain length. These extra groups allow the effect of the van der Waals attraction between the alkyl chains to become predominant sooner in the 1-ACOM homologous series. Thus, the S_{IPM} values decrease significantly after the C_7 member of the 1-AC series, whereas S_{IPM} values decrease after the C_5 member of the 1-ACOM series. If a similar displacement of the comparison of J_i values for the members of each series is made, then the 1-ACOM type prodrug does give larger J_i values than the 1-AC type.

Conclusions—For the 1-ACOM-5-FU series of prodrugs, all of which are more lipophilic than 5-FU, the more water-soluble members are the more effective ones at delivering total 5-FU species from IPM through hairless mouse skin. The differences in delivery are not due to differences in damage caused by the different prodrugs/IPM because fluxes from the subsequent application of a standard solute/solvent (Th/PG) were not different from the Th/PG flux after application of 5-FU/IPM. The 1-ACOM-5-FU series was relatively more effective at delivering total 5-FU species through the skin than at delivering total 5-FU species into the skin compared with 5-FU based on the ratios of C_s values to J_i values (D/T delivery ratios). The 1-ACOM-5-FU prodrugs were generally less effective than the 1-AC-5-FU prodrugs when members from each series of equal alkyl chain length were compared based on J_i values. On the other hand, if the CH_2O spacer was taken into account, the 1-ACOM-5-FU prodrugs were generally more effective than the 1-AC-5-FU prodrugs.

References and Notes

1. Dillaha, C. J.; Jansen, G. T.; Honeycutt, W. M.; Holt, C. A. *Arch. Dermatol.* **1965**, *92*, 410–417.
2. Robinson, T. A.; Kligman, A. M. *Br. J. Dermatol.* **1975**, *92*, 703–706.
3. Goodman, M.; Barry, B. W. *J. Invest. Dermatol.* **1988**, *91*, 323–327.
4. Mollgaard, B.; Hoelgaard, A.; Bundgaard, H. *Int. J. Pharm.* **1982**, *12*, 153–162.
5. Sloan, K. B.; Koch, S. A. M.; Siver, K. G. *Int. J. Pharm.* **1984**, *21*, 251–264.
6. Beall, H.; Prankerd, R.; Sloan, K. B. *Int. J. Pharm.* **1994**, *111*, 223–233.
7. Beall, H.; Sloan, K. B. *Int. J. Pharm.* **1996**, *129*, 203-210.
8. Beall, H. D.; Prankerd, R. J.; Sloan, K. B. *Drug Dev. Ind. Pharm.* **1996**, *22*, 85–90.
9. Buur, A.; Bundgaard, H.; Falch, E. *Int. J. Pharm.* **1985**, *24*, 43–60.
10. Taylor, H. E. Ph.D. Dissertation, University of Florida, **1997**.
11. Bigler, P.; Schonholzer, S.; Neuenschwander, M. *Helv. Chim. Acta* **1978**, *61*, 2059–2080.
12. Ozaki, S.; Watanabe, Y.; Hoshiko, T.; Mizuno, H.; Ishikawa, K.; Mori, H. *Chem. Pharm. Bull.* **1984**, *32*, 733–738.
13. Sloan, K. B.; Beall, H. D.; Weimar, W. R.; Valleneuva, R. *Int. J. Pharm.* **1991**, *73*, 97–104.
14. Fedors, R. F. *Polym. Eng. Sci.* **1974**, *14*, 147–154.
15. Martin, A.; Wu, P. L.; Velasquez, T. *J. Pharm. Sci.* **1985**, *74*, 277–282.
16. Sloan, K. B.; Koch, S. A. M.; Siver, K. G.; Flowers, F. P. *J. Invest. Dermatol.* **1986**, *87*, 244–252.
17. Ahmad, S.; Ozaki, S.; Nagase, T.; Iigo, M.; Tokazen, R.; Hoshi, A. *Chem. Pharm. Bull.* **1987**, *35*, 4137–4143.
18. Ozaki, S.; Nagase, T.; Tamai, H.; Mori, H.; Hoshi, A.; Iigo, M. *Chem. Pharm. Bull.* **1987**, *35*, 3894–3897.
19. Ulich, L. H.; Adams, R. *J. Am. Chem. Soc.* **1921**, *43*, 660–667.
20. Davidson, S. K.; Summers, J. B.; Albert, D. H.; Holms, J. H.; Heyman, H. R.; Magoc, T. J.; Conway, K. G.; Rhein, D. A.; Carter, G. W. *J. Med. Chem.* **1994**, *37*, 4423–4429.
21. Kamata, S.; Haga, N.; Matsui, T.; Nagata, W. *Chem. Pharm. Bull.* **1985**, *33*, 3160–3175.
22. Jencks., W. P.; Regenstein, J. In *Handbook of Biochemistry and Molecular Biology; Physical and Chemical Data*, 2nd ed.; Sober, H. A., Ed.; CRC: Cleveland, OH, 1970; pp J188–J226.
23. Waranis, R. P.; Sloan, K. B. *J. Pharm. Sci.* **1988**, *77*, 210–215.
24. Patrick, A. I.; Beall, H. D.; Gilroy, P.; Sloan, K. B. *Int. J. Pharm.* **1997**, *154*, 39–48.
25. Yalkowsky, S. H. In *Design of Biopharmaceutical Properties through Prodrugs and Analogues*; Roche, E. B., Ed.; American Pharmaceutical Association Academy of Pharmaceutical Sciences: Washington, D. C., 1977; pp 392–408.
26. Yalkowsky, S. H.; Flynn, G. L.; Slunick, T. G. *J. Pharm. Sci.* **1972**, *61*, 852–857.
27. Charton, M. *J. Am. Chem. Soc.* **1975**, *97*, 1552–1556.
28. Sheretz, E. F.; Sloan, K. B.; McTiernan, R. G. *Arch. Dermatol. Res.* **1990**, *282*, 463–468.
29. Sheretz, E. F.; Sloan, K. B.; McTiernan, R. G. *J. Invest. Dermatol.* **1987**, *89*, 147–151.

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