Articles

Structure−Activity Studies of Cerulenin Analogues as Protein Palmitoylation Inhibitors

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Activation of *ras* oncogenes occurs in a high percentage of tumors, making the enzymes involved in the posttranslational processing of their encoded proteins (p21s) attractive targets for the development of new drugs. Although most effort has focused on farnesyl transferase, which catalyzes the first processing step, attachment of palmitate to p21 is required for optimal transformation by H-*ras* and N-*ras*. We have demonstrated that the natural product cerulenin ([2*R*,3*S*]-2,3-epoxy-4-oxo-7,10-*trans*,*trans*-dodecadienamide) inhibits the palmitoylation of H-*ras*- and N-*ras*-encoded p21s in parallel with inhibition of cell proliferation. More than 30 analogues of cerulenin, both aromatic and aliphatic, with various chain lengths and amide substitutions, have been synthesized for use in SAR studies. Studies on the inhibition of T24 cell proliferation indicate that the α-keto-epoxy moiety is critical for cytotoxicity, while alkyl chain length had only modest effects on potency. Several compounds inhibited the incorporation of [^{3}H]palmitate into p21 in intact T24 cells, with the unsubstituted carboxamides being more active than *N*,*N*-dimethyl compounds. In contrast to the effects on palmitoylation, the only compounds which inhibited fatty acid synthase contained alkyl side chains of 12 carbons or fewer. Regression analyses indicated that inhibition of palmitoylation is more closely related to inhibition of proliferation than is inhibition of fatty acid synthase. Further characterization of the molecular pharmacology of these and analogous compounds may define a new class of drugs with antitumor activity.

Introduction

The *ras* oncogenes play critical roles in the development of a high percentage of human cancers. The three human *ras* genes (H-*ras*, N-*ras*, and K-*ras*) encode low molecular weight G-proteins, termed p21s, which act as signal transducers between extracellular growth factors and the nucleus in both normal and transformed cells.[1−3] While mutations in the *ras* oncogenes are primarily responsible for activation of p21s, overexpression of the proteins can also promote uncontrolled cell growth. Consequently, much attention has been focused on the biochemical and pharmacological properties of the *ras* oncogenes and the p21s they encode with the hope that development of inhibitors of p21 function may produce clinically useful antitumor drugs.[4−6]

p21 proteins undergo a series of posttranslational modifications that result in their anchoring in the plasma membrane, which is crucial for the transforming activity of the oncoprotein.[7−10] As shown in Figure 1, the first modification of the H-*ras*-encoded p21 attaches a farnesyl moiety to cysteine 186, which is the fourth amino acid residue from the C-terminus of the protein. After farnesylation, the three C-terminal amino acid residues are cleaved by an endoprotease, and the newly formed C-terminus is esterified by a carboxymethyl transferase. H-*ras*- and N-*ras*-encoded p21s are then palmitoylated by an enzyme which forms a thioester linkage between nearby cysteine residues and the C-16 chain of palmitate, presumably via palmitoyl-CoA. This palmitoylation has been shown to be necessary for anchoring the H-Ras protein to the cell membrane and expression of the full transforming activity of the oncogene.[9,10] With the K-Ras 2B protein, the cell membrane anchoring function of the palmitate group is performed by a series of lysine residues, although the nature of this interaction is not yet defined. Because these posttranslational modification steps are crucial to the transforming activity of the p21s, much effort has gone into characterizing the enzymes involved, especially with regard to the development of inhibitors. Most of the attention has focused on the first step catalyzed by farnesyl protein transferase (FPTase), which has been isolated,[11] cloned,[12] and used in screening assays to identify inhibitors.[13,14] Recently, it has been found that geranylgeranylation of K-*ras*-encoded p21 can occur in cells treated with FPTase inhibitors, thus circumventing the effects of the inhibitor.[15−17] Efforts aimed at blocking the endoprotease enzyme involved in the second modification step have also increased.[18] Although the carboxymethyltransferase has been identified,[19] no inhibitors have been described in the literature.

The final processing step for N- and H-Ras oncoproteins involves the formation of one or two thioesters with palmitate, respectively. This palmitoylation is different

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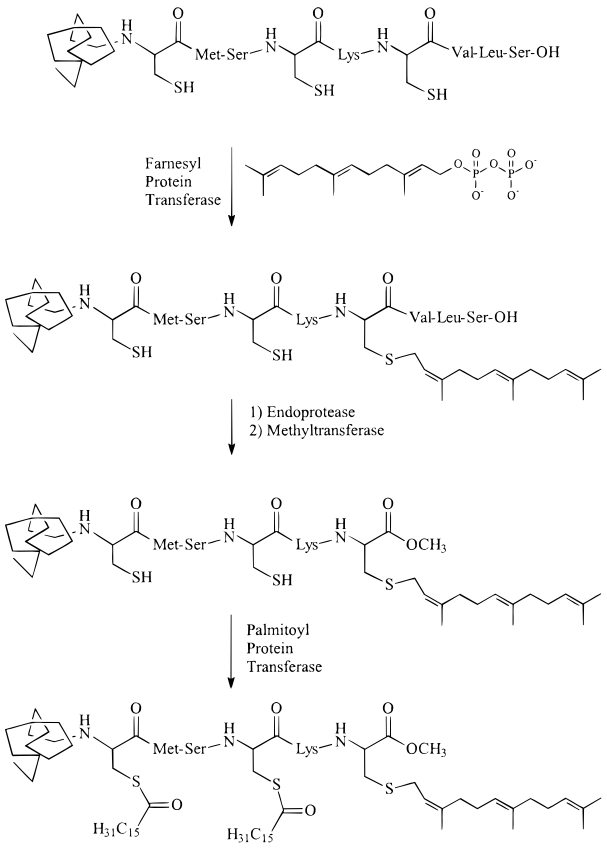


Figure 1. Posttranslational processing of Ras proteins.

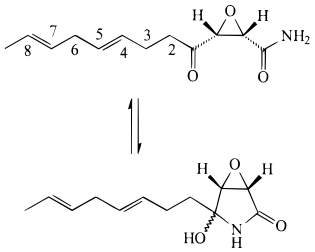
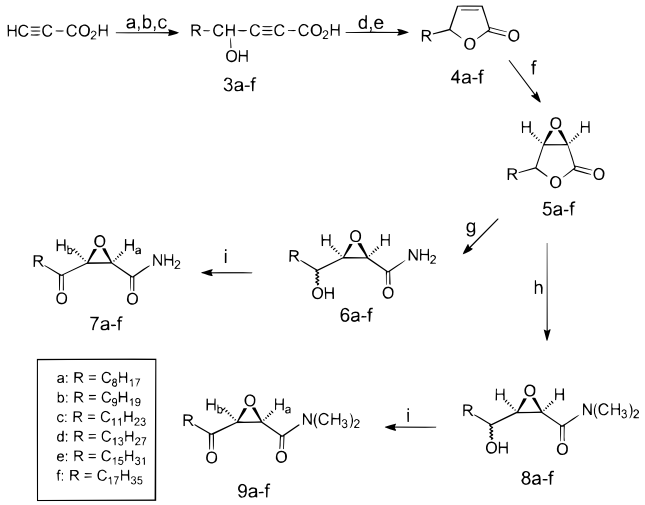


Figure 2. Open and cyclized form of cerulenin.

from the farnesylation step in that it is reversible. While the farnesyl group is permanently attached, the palmitate group is dynamically added and removed with a turnover half-life of 20 min, compared to the 24 h half-life of the p21 protein in T15 cells.[20] The cleavage of the palmitate moiety is catalyzed by thioesterases that are well-studied;[21−23] however, the enzymology of the palmitate attachment is not yet characterized. Furthermore, no selective inhibitors of p21 palmitoylation have yet been described in the literature.

The natural product cerulenin ([2*R*,3*S*]-2,3-epoxy-4-oxo-7,10-*trans*,*trans*-dodecadienamide) has long been known to inhibit fatty acid synthesis by alkylation and consequent inactivation of β-ketoacyl acyl-carrier protein synthase, a component of the fatty acid synthase complex,[24] and recent studies on its apoptic and antitumor activities have assumed this mechanism of action.[25] However, we have recently shown that cerulenin inhibits the proliferation of *ras*-transformed NIH/3T3 fibroblasts, T24 bladder carcinoma cells which overexpress H-*ras*, and MCF-7 cells which overexpress N-*ras* at doses lower than those required to inhibit the proliferation of non- or K-*ras*-transformed cells.[26,27]

Scheme 1. Synthesis of Cerulenin Analogues[a]



[a] Reagents: (a) 2 equiv of EtMgBr, THF, 0 °C; (b) RCHO, 0 °C → RT; (c) H_2SO_4(aq) or satd NH_4Cl(aq); (d) H_2(g), Lindlar's catalyst, EtOAc (or THF), RT; (e) 35 °C; (f) NaOCl(aq), *p*-dioxane (or DMF/Et_2O − 1:1), 0 °C → RT; (g) NH_3(aq), MeOH, 0 °C; (h) $HN(CH_3)_2$, THF/MeOH, 0 °C → RT; (i) $pyr_2{\cdot}Cr_2O_7$, CH_2Cl_2, RT or TPAP, NMO, CH_2Cl_2, RT.

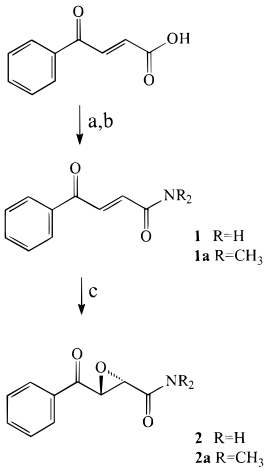
Furthermore, exogenous fatty acids did not protect against cerulenin-induced cytotoxicity, and metabolic labeling with [^{3}H]palmitate followed by immunoprecipitation of p21 demonstrated that cerulenin inhibits the acylation reaction.[26,27] This is consistent with a previous report of the ability of cerulenin to inhibit the acylation of viral surface glycoproteins expressed on chicken embryo fibroblasts.[28]

Because of the activity of cerulenin and the potential utility of selective inhibitors of p21 palmitoylation as antitumor agents, we have synthesized analogues of cerulenin for studies of the effects on both fatty acid synthase and p21 palmitoylation. Data presented here demonstrate that the antiproliferative effect of these compounds is more closely related to inhibition of p21 palmitoylation than inhibition of fatty acid synthase.

Chemistry

Cerulenin has been studied as an antifungal and antibacterial agent for nearly 30 years. In protic media (such as a cellular environment), cerulenin exists in an equilibrium between two forms—open-chain and cyclized hydroxylactam (Figure 2). Several groups have described the total synthesis of this and related compounds, both as racemic mixtures and as stereospecific products.[29−31] Cerulenin can be considered to be comprised of an α-keto-epoxycarboxamide with an octadienyl side chain. To explore the effects of various side chain lengths, as well as the functionalities surrounding the epoxide moiety, we chose to follow the general synthetic methodology of Jakubowski et al.[32] This work describes the regioselective syntheses of not only cerulenin and tetrahydrocerulenin but also their *N*,*N*-dimethyl derivatives (Scheme 1). We sought to use these substituted amides to explore the importance of the cyclized hydroxylactam isomer of the epoxycarboxamide for inhibiting fatty acid synthase and p21 palmitoylation. Although the majority of the procedures described by Jakubowski et al. proceeded with little difficulty, the critical step of epoxide formation from the butenolide

Scheme 2. Synthesis of "BOCA" Compounds[a]



[a] Reagents: (a) isobutyl chloroformate, *N*-methyl-morpholine, DME, −13 °C; (b) NH_3(g) or $(CH_3)_2NH$, THF; (c) H_2O_2, NaOH, 55 °C.

failed to give any product in our hands. The more common method of Nozoe was used instead.[33] Although this method routinely gave only 40−50% yields, the starting material was easily recovered and could be recycled in subsequent reactions, thereby enhancing the overall yield. In addition, the classic oxidation of secondary alcohols to ketones with the Collins reagent was improved by using the more recently developed TPAP reagent.[34,35] Since preliminary studies had demonstrated that tetrahydrocerulenin possesses equivalent cytotoxic activity to its naturally occurring precursor, we reasoned that the double bonds on the octadienyl side chain of cerulenin may be unimportant to the protein acyl transferase. In fact, since the enzyme's function is to add a palmitate group to the farnesylated p21 protein, it was hypothesized that a saturated 16-carbon chain might imitate the natural substrate more effectively than cerulenin. Therefore, tetrahydrocerulenin analogues with chain lengths of C_8H_{17} to $C_{17}H_{35}$, possessing a free amide or *N*,*N*-dimethyl-substituted amide, were synthesized in nonoptimized yields (∼2% from propiolic acid). All intermediates and final compounds gave spectral data that were in agreement with the literature values for tetrahydrocerulenin and *N*,*N*-dimethyl-tetrahydrocerulenin. Samples of all intermediates with an epoxide moiety were retained for evaluation in cytotoxicity, fatty acid synthase, and p21 palmitoylation assays.

Aromatic analogues of cerulenin (e.g., 3-(benzoyl)-oxiranecarboxamides, "BOCA") were patented in 1978 as inhibitors of lipogenesis.[36] Our syntheses of BOCA (**2**) and *N*,*N*-dimethyl-BOCA (**2a**) were based on the procedures in that patent, but improved upon to some extent (Scheme 2). Notably, formation of the benzoylacrylamides from commercially available benzoylacrylic acid was done through the use of a mixed anhydride of the acid activated with isobutylchloroformate, followed by quenching with either ammonia or dimethylamine.[37] Epoxidation with hydrogen peroxide in basic EtOH gave BOCA and *N*,*N*-dimethyl-BOCA in 21% and 51% yields, respectively, with confirming spectral data. As a result of the epoxidation conditions, these aromatic products both possessed *trans* epoxides. This was confirmed by the ^{1}H NMR splitting coefficients of the epoxide protons (J_{AB} < 4 Hz). Although this synthetic strategy did not give products in high yields, it is amenable to a variety of aromatic groups by simple Friedel−Crafts acylation with maleic anhydride to give the starting benzoylacrylic acid and so may prove useful for the preparation of additional aromatic analogues of cerulenin.

Table 1. Biological Activities of Cerulenin Analogues

compound	IC_{50} for inhibition of T24 cell proliferation (μM)[a]	inhibition of fatty acid synthase (% inhibited)[b]	inhibition of p21 palmitoylation (% inhibited)[c]
cerulenin	17 ± 4	96 ± 3	48 ± 2
2	15 ± 6	85 ± 1	22
2a	29 ± 10	3 ± 2	59
5a	>240	75 ± 13	32
5b	>220	40 ± 8	33
5c	>200	4 ± 2	19
5d	>180	0 ± 0	2
5e	140 ± 16	0 ± 0	35
5f	>150	5 ± 2	45
6a	>220	4 ± 2	16
6b	>200	5 ± 2	27
6c	130 ± 0	6 ± 0	29
6d	110 ± 54	3 ± 1	34
6e	190 ± 54	10 ± 5	40
6f	>140	0 ± 0	3
7a	27 ± 11	74 ± 11	42
7b	7 ± 2	92 ± 6	63
7c	20 ± 9	44 ± 24	40
7d	13 ± 2	0 ± 0	47
7e	12 ± 0	2 ± 1	67
7f	17 ± 4	0 ± 0	28
8a	>190	0 ± 0	9
8b	220 ± 0	0 ± 0	0
8c	95 ± 19	9 ± 6	0
8d	69 ± 20	5 ± 2	16
8e	62 ± 25	0 ± 0	0
8f	52 ± 19	3 ± 1	0
9a	140 ± 23	1 ± 1	0
9b	77 ± 31	2 ± 1	17
9c	49 ± 19	5 ± 2	10
9d	40 ± 11	2 ± 1	0
9e	40 ± 16	0 ± 0	31
9f	34 ± 1	0 ± 0	21

[a] Values represent the mean ± SEM concentration required to inhibit the proliferation of T24 cells by 50% compared with untreated control cultures. At least three experiments were performed with each compound. IC_{50} values of >140 μM are considered to be essentially nontoxic. [b] Values represent the mean ± SEM percentage inhibition of fatty acid synthase activity in the presence of 150 μM of the indicated compound. At least two experiments were performed with each compound. [c] Values represent the mean percentage inhibition of p21 palmitoylation compared with untreated control samples. Cerulenin was used at 5 μg/mL and the mean ± SEM for six experiments. Other compounds were tested at 10 μg/mL, corresponding to 26−47 μM, in one or two experiments.

Results and Discussion

Cytotoxicity testing of cerulenin and its analogues was performed using concentrations up to 50 μg/mL with T24 human bladder carcinoma cells that overexpress H-[Val^{12}]-*ras*. Table 1 shows the mean IC_{50} values (in μM) obtained from at least three separate experiments using the SRB assay.[38] Similar results were obtained with the MTS assays (not shown). No inhibition of growth was observed with some compounds at the highest concentration tested, corresponding to 140−240 μM, and these compounds are considered to be essentially nontoxic. The data demonstrate that the aromatic compounds BOCA (**2**) and *N*,*N*-dimethyl-BOCA (**2a**) are nearly as cytotoxic as cerulenin, while

the aliphatic analogues display a range of toxicities depending on the chain length and functionalities surrounding the epoxide moiety. The most toxic analogues are the α-keto-epoxycarboxamides (**7a**−**f**), followed by their *N*,*N*-dimethyl derivatives (**9a**−**f**). Their reduced precursors, possessing α-hydroxyl groups, (**6a**−**f** and **8a**−**f**) are noticeably less toxic, while the parent epoxylactones (**5a**−**f**) are all essentially nontoxic. The cytotoxicities of the unsubstituted epoxycarboxamides appear to be largely independent of chain length, e.g., the IC_{50}s for **7a**−**f** vary only from 7 to 27 μM, with the analogue **7b**, with a 10-carbon chain (including the carbonyl carbon), being the most potent. The *N*,*N*-dimethyl analogues **9** show a clearer relationship between cytotoxicity and increasing chain length. Even so, the free carboxamide of each chain length was more cytotoxic than its corresponding *N*,*N*-dimethyl analogue. It is interesting to note, however, that the cytotoxicity of the cerulenin analogues is fairly structure specific. For example, the mere possession of an epoxide moiety is not sufficient to impart a toxic effect, although toxicity is significantly enhanced by oxidizing the secondary alcohol to a ketone. Furthermore, the long chain fatty acid analogues do not exert a purely physical lysogenic effect on the cells. The cytotoxicities of the epoxidized compounds were associated with typical morphological indicators of apoptosis, including cell rounding, nuclear fragmentation, and membrane blebbing (not shown). Consequently, the effects of prolonged exposure of cells to these compounds are irreversible, at least at the cellular level.

Because cerulenin and certain of its analogues have been demonstrated to inhibit fatty acid synthase, the effects of our compounds on this enzyme were determined. Results from these determinations are also indicated in Table 1. To allow comparison of the efficacies of various compounds, data indicate the percentage by which fatty acid synthesis is reduced in samples treated with 150 μM of the indicated compounds. As expected, cerulenin and compound **2** very effectively inhibited the tumor cell fatty acid synthase activity. Significant inhibition also occurred in samples incubated with compounds **5a**, **7a**, and **7b**, while modest inhibition occurred in samples incubated with compounds **5b** and **7c**. Importantly, other compounds that inhibit cell proliferation had no significant inhibitory effects on fatty acid synthesis. For example, compounds **7d**−**f** potently inhibit cell proliferation but are essentially inactive against fatty acid synthase. Similarly, Compounds **8a**−**f** and **9a**−**f** have varying inhibitory effects on tumor cell proliferation, but do not inhibit fatty acid synthase. Therefore, the mechanism for the antiproliferative activities of the cerulenin analogues is clearly distinguished from inhibition of fatty acid synthesis.

The effects of the cerulenin analogues on the palmitoylation of p21 were determined, and results are indicated in Table 1. To allow comparison of the efficacies of various compounds, data shown indicate the percentage by which incorporation of [^{3}H]palmitate into p21 is reduced in samples treated with 10 μg/mL of the indicated compounds, with the exception that cerulenin was used at a dose of 5 μg/mL, which inhibited p21 palmitoylation by approximately 50%. Since treatment of MCF-7 cells with cerulenin also inhibits cell proliferation and p21 palmitoylation, this compound reduces the palmitoylation of both H-*ras*- and N-*ras*-encoded p21. [27] Data in Table 1 also demonstrate that several of the cerulenin analogues are also able to inhibit p21 palmitoylation. For example, compounds **2** and **2a** both inhibited p21 palmitoylation, demonstrating that aromatic analogues of cerulenin are also effective. Additionally, several compounds of the *cis*-2,3-epoxyalkanoic acid γ-lactone series (compounds **5a**, **5b**, **5e**, and **5f**) inhibit p21palmitoylation nearly as effectively as cerulenin. Similarly, the longer alkyl *N*,*N*-dimethyl-*cis*-2,3-epoxy-4-oxoalkanamides (compounds **9e** and **9f**) also effectively inhibit p21 palmitoylation. The series of *cis*-2,3-epoxy-4-oxoalkanamides (compounds **7a**−**f**) comprise a family of particularly effective inhibitors of p21 palmitoylation. The greatest inhibitory activity among the compounds was demonstrated by Compound **7e**, which can be considered to be a palmitoyl analogue of cerulenin. It is important to again note that this compound, as with several other cerulenin analogues, does not inhibit fatty acid synthase. Therefore, the abilities of these compounds to inhibit tumor cell proliferation is more closely associated with inhibition of the palmitoylation of p21, and possibly other proteins, than with reduction in the biosynthesis of fatty acids. This is substantiated by regression analysis of the biological effects of the 12 compounds **7a**−**f** and **9a**−**f** that demonstrated a correlation coefficient of −0.728 ($p = 0.0048$) between the IC_{50} for cell proliferation and the inhibition of p21 palmitoylation. In contrast, a correlation coefficient of −0.381 ($p = 0.1987$) was calculated for the association between the IC_{50} for cell proliferation and the inhibition of fatty acid synthase.

The inhibitory effects of the epoxylactones (**5a**−**f**) are interesting to note. Although not as potent as the keto-epoxycarboxamides, these compounds do inhibit palmitoylation of p21 yet display very low toxicity toward the cells. Recall that the keto-epoxycarboxamide moiety can equilibrate in protic media (such as within a cell) between an open, straight chain and a closed, hydroxylactam configuration. Since the *N*,*N*-dimethyl analogues cannot form the hydroxylactam conformer and have shown reduced inhibitory behavior toward palmitoylation, it seems likely that the active form of the keto-epoxycarboxamide is the hydroxylactam. Furthermore, there is a structural similarity between the hydroxylactam and the epoxylactone moiety of compounds **5a**−**f**, which also show some inhibitory activity. We hypothesize that the palmitoylation inhibitory activity of compounds **7a**−**f** is dependent not only on chain length but also on their conformation as open straight chains or closed hydroxylactams.

Other structure−activity relationships have been explored with cerulenin analogues as inhibitors of fatty acid synthase[39] and HIV protease.[40,41] Although the studies were not systematically performed, several "desoxy" derivatives of cerulenin, lacking the α-keto group, were tested as inhibitors of HIV protease. The relative stereochemistry of the epoxide moiety was claimed to be important (2*S*-*trans*-epoxydodecenoic acid was significantly more potent than either 2*S*- or 2*R*-*cis*-epoxydodecenoic acid), while increasing the length of the hydrocarbon chain decreased activity, and the

functionality of the group adjacent to the epoxide also affected activity. Blumenstein et al.[40] also postulated that the ability to cyclize into the hydroxylactam conformation may enhance the activity against HIV protease, although the proline methyl ester-substituted amide of 2*R*-*cis*-epoxydodecenoic acid (which cannot cyclize) displayed half the activity of cerulenin. The SAR studies involving inhibition of FAS were more systematically performed, but they varied only the chain length and the presence and position of the double bonds in the chain. While cerulenin remained the most potent inhibitor of the group (IC_{50} = 4.5 μM), removal or repositioning of the double bonds or lengthening the chain (leaving the double bonds intact) resulted in less potent inhibitors (IC_{50} = 250−360 μM and 20−35 μM, respectively).

These data are consistent with that of Table 1 which clearly demonstrate that the structural requirements for cerulenin analogues are target specific. Most importantly, the size and the saturation of the aliphatic side chain have markedly different effects on the activities of the analogues toward fatty acid synthase and p21 palmitoylation. Additionally, the presence of the α-keto moiety appears to be more critical for interaction with fatty acid synthase than with p21:palmitoyltransferase.

Conclusion

We have synthesized more than 30 analogues of the natural product cerulenin, both aromatic and aliphatic, with varying chain lengths and amide substitutions. Cytotoxicity and p21 palmitoylation results indicate that the α-ketoepoxide moiety is important for activity and that increasing the length of the aliphatic chain up to 16 carbons generally increases potency. Furthermore, both cytotoxicity and palmitoylation assays show that the free ketoamide—which can cyclize to form a hydroxylactam—is more potent that the *N*,*N*-dimethylated amide which cannot cyclize. These structural requirements are clearly distinct from those that affect the abilities of these agents to inhibit fatty acid synthase, and the antiproliferative effects of these compounds are more closely related to inhibition of p21 processing. Because of the importance of p21 function in human cancer, inhibition of its posttranslational modification has become a popular therapeutic target. Although many groups have described inhibitors of the initial farnesylation step, the palmitoylation step has not been characterized from either an enzymological or a pharmacological perspective. We have now shown that cerulenin and its analogues represent a novel class of anti-Ras agents since no other compounds have yet been described as selective palmitoylation inhibitors.

It is recognized that eukaryotic cells contain several classes of palmitoylated protein.[45] While many of these acylations appear functionally inconsequential, palmitoylation of several prenylated or *N*-myristoylated proteins is required for their proper subcellular localization and function. Interestingly, most of these known palmitoylated proteins are involved in signal transduction, including several src family tyrosine kinases. Therefore, the cellular effects of protein palmitoylation inhibitors may reflect attenuation of signals generated by diverse proliferative stimuli. This may underlie the activity of these agents, at higher doses, toward K-*ras*-transformed cells that are not expected to be sensitive to palmitoylation inhibitors. Alternately, the known ability of unprocessed H-Ras to act as a dominant negative protein[46,47] would also lead to inhibition of proliferation in such cells. In any event, the present work describes the first synthetic lead compounds for development of potential therapeutic agents targeting this important cellular activity.

Experimental Section

Reagents and starting materials were obtained from Aldrich Chemical (Milwaukee, MN) and used as received unless otherwise noted. Cerulenin was obtained from Sigma at 95% purity and stored as a stock solution of 5 mg/mL in EtOH at 4 °C. No noticeable decrease in activity occurred over several months. Solvents were either purchased as "anhydrous" or "ACS grade" and stored over 4 Å molecular sieves. "Flash chromatography" refers to the method of Still et al.[42] and generally used Selecto Scientific silica gel (32−63 μm). Thin layer chromatography was performed on EM Science silica gel 60 F-254 plates (250 μm thick). Radial chromatography was performed on a Chromatotron model 7924T (Harrison Research, Palo Alto, CA) using 1, 2, or 4 mm thick silica rotors. HPLC analysis was performed on an ISCO gradient system using a Whatman Partisil ODS-2 5 μm analytical column (4.6 × 250 mm). Melting points were determined in an open capillary on a MelTemp II melting point apparatus and are uncorrected. Infrared spectra were measured on a Nicolet 250 FT-IR; values are expressed in wavenumbers (cm^{-1}). ^1H and ^{13}C NMR spectra were obtained on a Bruker 300 WB spectrometer. Chemical shifts are reported in ppm (δ) using tetramethylsilane (TMS) as reference, and coupling constants (J) are reported in hertz. Mass spectra were obtained on a Finnigan LCQ ion trap mass spectrometer with an electrospray ion source or were from Mass Consortium (San Diego, CA). Microanalyses were determined by Atlantic Microlab, Inc. (Norcross, GA). All chemical yields are unoptimized and generally represent the result of a single experiment.

Cell Culture and Cytotoxicity Assays. T24 human bladder carcinoma cells were obtained from ATCC and grown in RPMI-1640 with 10% fetal bovine serum and 50 μg/mL gentamycin sulfate. To test the effects of the compounds on cell growth, cells were seeded in 96-well tissue culture dishes at 10% confluency and were allowed to attach and recover for 24 h. Varying concentrations of cerulenin or an analogue were added to each well, and the cells were allowed to incubate for an additional 72 h. The number of surviving cells was determined by staining with sulforhodamine B as described by Skehan et al.[38] or metabolism of MTS (Promega CellTiter Aq_{ueosus} system) following the manufacturer's instructions. Similar results were obtained with both of these assays.

Fatty Acid Synthase Assay. Fatty acid synthase was partially purified from HL-60 human promyelocytic leukemia cells as described by Royer et al.[43] The HL-60 cell lysate was incubated with varying concentrations of cerulenin or one of the synthetic compounds of this invention and then assayed for fatty acid synthase according to Royer et al.[43] which monitors the rate of oxidation of NADPH in the presence of acetyl-CoA and malonyl-CoA.

p21 Palmitoylation Assay. T24 cells were seeded in 60 mm dishes at approximately 25% confluency and allowed to attach overnight. The cells were then treated with 5 μg/mL of cerulenin (or 10 μg/mL of cerulenin analogue) for 4 h followed by a 2 h incubation with [^3H]palmitate at 100−200 μCi/mL. Cell lysates were made using a PBS-TDS buffer (phosphate-buffered saline containing 1% Triton x-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 μM phenylmethylsulfonylflouride, and 2 μg/mL each of pepstatin, leupeptin, and aprotinin), and the protein concentration was determined for each sample using the Bio-Rad protein assay (Bio-Rad). Normalized samples (containing 100−300 μg of protein in different experiments) were combined with 2 μg of Y13-259 anti-Ras antibody (Santa Cruz Biotechnology) for 1−2

h, followed by the addition of 20 μL of Protein G PLUS-Agarose (Santa Cruz Biotechnology) for approximately 18 h at 40 °C. The samples were then centrifuged and washed 5 times with PBS-TDS, and radioactivity in the recovered pellets was determined using a scintillation counter.

3-Benzoylacrylamide (1) and *N,N*-Dimethyl-3-benzoylacrylamide (1a). 3-Benzoylacrylic acid (Acros Chemical, 5.50 g, 31.2 mmol) was dissolved in dimethoxyethane (DME, 55 mL) and chilled to −13 °C. After deaeration with nitrogen, isobutylchloroformate (35.9 mmol, 4.90 g, 4.65 mL) was added dropwise, followed by *N*-methylmorpholine (3.4 mL). Formation of the mixed anhydride was monitored by TLC (silica, 25% EtOAc in hexanes) and, after 1 h, was complete. Gaseous ammonia was bubbled through the reaction for 5 min and then allowed to warm to room temperature, and the ammonia bubbling continued for another 15 min. Alternatively, dimethylamine (62.4 mmol, 31.2 mL of a 2.0 M solution in THF) was added, and the reaction was stirred at −13 °C for 15 min and then allowed to warm to room temperature and stirred for 1 h. Reactions were quenched with H_2O (10 mL), followed by dilution with another 40 mL water and extraction with $CHCl_3$ (4 × 40 mL). Combined $CHCl_3$ extracts were washed with water and brine, then dried over Na_2SO_4. Evaporation and vacuum desiccation gave 4.15 g (23.7 mmol, 75.9%) of **1** as a light yellow solid, mp 121−123 °C, or 6.34 g (31.2 mmol, 100%) of **1a** as a light yellow solid, mp 57−60 °C. Products were sufficiently pure by HPLC (C_{18}, 0.5 mL/min, 75/25 MeOH/H_2O, 215 nm) to use in the next step. (**1**): IR (KBr) 3450 and 3400 (s, NH_2), 3325 (aromatic CH), 1675 (s, carbonyl); ^{1}H NMR ($CDCl_3$) δ 7.95−7.89 (m, 2H, $H_{2'}/H_{6'}$), 7.62−7.54 (m, 1H, $H_{4'}$), 7.51−7.42 (m, 2H, $H_{3'}/H_{5'}$), 7.32 (d, 2H, J_{AB} = 21 Hz, H_2/H_3), 5.78 (bs, 2H, NH_2). (**1a**): IR (KBr) 3325 (aromatic CH), 1675 (C=O); ^{1}H NMR (DMSO-d_6) δ 8.03 (d, 2H, J_{AB} = 7.6 Hz, $H_{2'}/H_{6'}$), 7.74 (d, 1H, J_{AB} = 15.2 Hz, H_2), 7.69 (d, 1H, J_{AB} = 7.3 Hz, $H_{4'}$), 7.58 (d, 2H, J_{AB} = 7.57 Hz, $H_{3'}/H_{5'}$), 7.425 (d, 1H, J_{AB} = 15.2 Hz, H_3), 3.11(s, 3H, NCH_3), 2.95 (s, 3H, NCH_3').

3-Benzoyloxiranecarboxamide (2) and *N,N*-Dimethyl-3-Benzoyloxiranecarboxamide (2a). Acrylamides (**1** and **1a**, 5.708 mmol) were dissolved in 80% EtOH (50 mL). Next, 30% H_2O_2 (1 mL) was added, followed by 0.1 N NaOH (1.35 mL), and the reaction was stirred at 50 °C while monitoring by HPLC (C_{18}, 0.5 mL/min, 75/25 MeOH/H_2O, 215 nm). Additional H_2O_2 (2 mL) was added over the next 4 h period while maintaining pH at ∼8 by addition of 0.1 N NaOH. After reaction was complete, the solvents were evaporated then vacuum desiccated over P_2O_5 to remove remaining water. Crude BOCA (**2**) was recrystallized from methanol to give 300 mg of a white powder (mp 160−162 °C, 1.57 mmol, 27.5%), while crude *N,N*-dimethyl-BOCA (**2a**) was recrystallized from acetone/hexanes to give 643 mg of a white powder (mp 97−100 °C, 2.93 mmol, 51.4%). (**2**): IR (KBr) 3425 (bs, asymmetric NH), 3225 (bs, symmetric NH), 3075−3025 (aromatic CH), 1675 (ketone C=O), 1605 (amide C=O); ^{1}H NMR (DMSO-d_6) δ 8.02 (d, 2H, J_{AB} = 7.6 Hz, $H_{2'}/H_{6'}$), 7.75−7.71 (m, 1H, $H_{4'}$), 7.62−7.57 (m, 2H, $H_{3'}/H_{5'}$), 4.62 (s, 1H, H_3), 3.52 (s, 1H, H_2); ^{13}C NMR (DMSO-d_6) δ 194.46 (C_4), 168.64 (C_1), 135.30 ($C_{1'}$), 133.86 ($C_{4'}$), 128.71 ($C_{2'}/C_{6'}$), 128.01 ($C_{3'}/C_{5'}$), 56.21 (C_3), 55.17 (C_2); MS *m*/*z* 210 (M + NH_4). Anal. ($C_{10}H_9NO_3$) C: calcd, 62.82; found, 62.17; H: calcd, 4.74; found, 4.79; N: calcd, 7.33; found, 6.71. (**2a**): IR (KBr) 3060−3025 (aromatic CH), 2925 (aliphatic CH), 1680 (ketone C=O), 1660 (amide C=O); ^{1}H NMR (DMSO-d_6) δ 8.06 (d, 2H, J_{AB} = 7.6 Hz, $H_{2'}/H_{6'}$), 7.75−7.70 (m, 1H, $H_{4'}$), 7.59 (t, 2H, J = 7.6 Hz, $H_{3'}/H_{5'}$), 4.65 (s, 1H, H_3), 4.06 (s, 1H, H_2), 3.05 (s, 3H, NCH_3), 2.89 (s, 3H, NCH_3'); ^{13}C NMR (DMSO-d_6) δ 192.90 (C_4), 165.48 (C_1), 135.17 ($C_{1'}$), 134.52 ($C_{4'}$), 129.12 ($C_{2'}/C_{6'}$), 128.73 ($C_{3'}/C_{5'}$), 55.89 (C_3), 54.10 (C_2), 36.79 (NCH_3), 35.97 (NCH_3'); MS *m*/*z* 220 (M + H). Anal. ($C_{12}H_{13}NO$) C: calcd, 65.74; found, 64.99; H: calcd, 5.98; found, 5.72; N: calcd, 6.39; found, 6.35.

4-Hydroxyalk-2-ynoic Acids. Method A (3a−c). The magnesium dianion of propiolic acid and the appropriate aldehyde were condensed as in Jakubowski et al.[32]

4-Hydroxydodec-2-ynoic Acid (3a). White plates (mp 67−8 °C, 53.28%). IR (KBr): 3500−3275 cm^{-1} (OH), 2925−2850 (aliphatic CH), 2225 (C≡C), 1660 (C=O). ^{1}H NMR ($CDCl_3$): δ 5.5 (bs, 1H, OH), 4.52 (t, 1H, J = 6.6 Hz, H_4), 1.77 (t, 2H, J = 6.6 Hz, H_5), 1.27−1.47 (m, 12H, CH_2), 0.87 (t, 3H, J = 6.7 Hz, CH_3).

4-Hydroxytridec-2-ynoic Acid (3b). White plates (mp 62−63 °C, 59%). IR (KBr): 3400 cm^{-1} (OH), 2925−2850 (aliphatic CH), 2250 (C≡C), 1675 (C=O). ^{1}H NMR ($CDCl_3$): δ 6.65 (bs, 1H, OH), 4.53 (t, 1H, J = 6.6 Hz, H_4), 1.77 (m, 2H, H_5), 1.26 (bs, 14H, CH_2), 0.88 (t, 3H, J = 6.7 Hz, CH_3).

4-Hydroxypentadec-2-ynoic Acid (3c). Dodecanal was vacuum distilled and stored under nitrogen at 0 °C before use. White powder (mp 71−72 °C, 47%). IR (KBr): 3250 cm^{-1} (OH), 2925−2850 (aliphatic CH), 2325 (C≡C), 1660 (C=O), 1275 (carbonyl C−O). ^{1}H NMR ($CDCl_3$): δ 6.65 (bs, 1H, OH), 4.53 (t, 1H, J = 6.6 Hz, H_4), 1.77 (m, 2H, H_5), 1.26 (bs, 18H, CH_2), 0.88 (t, 3H, J = 6.7 Hz, CH_3).

4-Hydroxyalk-2-ynoic Acid. Method B (3d−f). Reaction was run as for **3a**, but aldehyde was dissolved in THF. After aldehyde addition, reaction was stirred at 0 °C for 3 h and then allowed to warm to room temperature overnight. When TLC (silica, 25% EtOAc/hexanes) showed no aldehyde remaining, the reaction was worked up by chilling to 0 °C, diluting with Et_2O (50 mL), and quenching with saturated NH_4Cl (36 mL). Acidification with 1 N HCl to pH ∼ 2 gave a clear, biphasic mixture which was separated, the organic phase was extracted with fresh Et_2O (2 × 50 mL), and the combined Et_2O extracts were washed with H_2O and brine (100 mL each). Drying over $MgSO_4$ and evaporation of volatiles gave the crude product as a yellow oil, which was crystallized from petroleum ether at −20 °C. Compounds **3d**−**f** were made using this method.

4-Hydroxyheptadec-2-ynoic Acid (3d). Tetradecanal was purchased from Fluka and used as received. White powder (mp 75−77 °C, 30.2%). IR (KBr): 3425 cm^{-1} (OH), 2925−2850 (aliphatic CH), 2250 (C≡C), 1720 (C=O), 1260 (carbonyl C−O). ^{1}H NMR ($CDCl_3$): δ 4.52 (t, 1H, J = 6.5 Hz, H_4), 1.77 (m, 2H, H_5), 1.26 (bs, 22H, CH_2), 0.88 (t, 3H, J = 6.7 Hz, CH_3).

4-Hydroxynonadec-2-ynoic Acid (3e). Hexadecanal was synthesized from hexadecanol.[43] White powder (mp 76−77 °C, 61.5%). IR (KBr): 3275 cm^{-1} (OH), 2950−2850 (aliphatic CH), 2250 (C≡C), 1670 (C=O), 1275 (carbonyl C−O). ^{1}H NMR ($CDCl_3$): δ 6.6 (vbs, 1H, OH), 4.52 (t, 1H, J = 6.6 Hz, H_4), 1.76 (m, 2H, H_5), 1.44 (m, 2H, H_6), 1.26 (bs, 24H, CH_2), 0.88 (t, 3H, J = 5.9 Hz, CH_3).

4-Hydroxyheneicos-2-ynoic Acid (3f). Octadecanal was synthesized from octadecanol.[43] White powder (mp 83−85 °C, 46.2%). IR (KBr): 3275 cm^{-1} (OH), 2950−2850 (aliphatic CH), 2250 (C≡C), 1670 (C=O), 1275 (carbonyl C−O). ^{1}H NMR ($CDCl_3$): δ 4.49 (t, 1H, J = 6.6 Hz, H_4), 1.70 (m, 2H, H_5), 1.49 (m, 2H, H_6), 1.29 (bs, 28H, CH_2), 0.88 (t, 3H, J = 5.9 Hz, CH_3).

Alkenoic Acid γ-Lactones (4a−f). Hydroxy-alkynoic acids were dissolved in either EtOAc (for compounds **4a**−**e**, 125 mL) or THF (compound **4f**, 125 mL) and were hydrogenated at atmospheric pressure over Lindlar catalyst as in Jakubowski et al.[32] The reaction was monitored by HPLC (C_{18}, CH_3CN/H_2O − 65/35, 0.5 mL/min, 220 nm). Starting material was consumed within 2−3 h, and the mixture was filtered through Celite to remove the catalyst. Heating the filtrate at 35 °C for 10 min closed the lactone ring. Evaporation of volatiles, followed by distillation under reduced pressure or flash chromatography (silica, 25% EtOAc/hexanes) gave pure product as a clear oil, which solidified on standing for the longer chain compounds. Compounds **4a**−**f** were made using this method.

Dodec-2-enoic Acid (4a). Clear oil (bp 113 °C @ 0.5 mmHg, 85.5%). IR ($CDCl_3$): 2975−2850 cm^{-1} (aliphatic CH), 1775 (C=O). ^{1}H NMR ($CDCl_3$): δ 7.45 (d, 1H, J_{AB} = 5.5 Hz, H_3), 6.10 (d, 1H, J_{AB} = 5.8 Hz, H_2), 5.04 (t, 1H, J_{AB} = 5.6 Hz, H_4), 1.9−1.6 (m, 2H, H_5), 1.28 (bs, 12H, CH_2), 0.88 (t, 3H, J_{AB} = 6.0 Hz, CH_3).

Tridec-2-enoic Acid γ-Lactone (4b). Clear oil (bp 115 °C @ 0.675 mmHg, 87.2%). IR (KBr): 3100 cm^{-1} (alkene CH), 2950−2850 (aliphatic CH), 1760 (C=O), 1600 (C=C), 1175 (carbonyl C−O). ^{1}H NMR ($CDCl_3$): δ 7.45 (d, 1H, J_{AB} = 5.1 Hz, H_3), 6.10 (d, 1H, J_{AB} = 5.0 Hz, H_2), 5.04 (t, 1H, J_{AB} = 5.6

Hz, H_4), 1.65 (m, 2H, H_5), 1.27 (bs, 14H, CH_2), 0.88 (t, 3H, J_{AB} = 6.0 Hz, CH_3).

Pentadec-2-enoic Acid γ-Lactone (4c). Clear oil that solidified in the condenser (bp 113 °C @ 0.05 mmHg, mp 35−37 °C, 87.0%). IR (KBr): 3100 cm^{-1} (alkene CH), 2950−2850 (aliphatic CH), 1760 (C═O), 1600 (C═C), 1175 (carbonyl C−O). ^1H NMR ($CDCl_3$): δ 7.456 (d, 1H, J_{AB} = 5.5 Hz, H_3), 6.10 (d, 1H, J_{AB} = 5.2 Hz, H_2), 5.03 (t, 1H, J_{AB} = 5.6 Hz, H_4), 1.7 (m, 2H, H_5), 1.26 (bs, 18H, CH_2), 0.88 (t, 3H, J_{AB} = 6.0 Hz, CH_3).

Heptadec-2-enoic Acid γ-Lactone (4d). Colorless crystals (mp 35−37 °C, 73.2%). IR (KBr): 3075 cm^{-1} (alkene CH), 2950−2850 (aliphatic CH), 1740 (C═O), 1600 (C═C), 1175 (carbonyl C−O). ^1H NMR ($CDCl_3$): δ 7.45 (d, 1H, J_{AB} = 5.5 Hz, H_3), 6.10 (d, 1H, J_{AB} = 5.5 Hz, H_2), 5.03 (t, 1H, J_{AB} = 5.6 Hz, H_4), 1.7 (m, 2H, H_5), 1.26 (bs, 22H, CH_2), 0.88 (t, 3H, J_{AB} = 6.0 Hz, CH_3).

Nonadec-2-enoic Acid γ-Lactone (4e). White powder (mp 54−56 °C, 53.1%). IR (KBr): 2950−2850 cm^{-1} (aliphatic CH), 1775 (C═O). ^1H NMR (acetone-d_6): δ 7.73 (d, 1H, J_{AB} = 5.7 Hz, H_3), 6.09 (dd, 1H, 5.7 Hz, H_2), 5.10 (t, 1H, J_{AB} = 5.6 Hz, H_4), 1.76 (m, 2H, H_5), 1.60 (m, 2H, H_6), 1.29 (bs, 24H, CH_2), 0.88 (t, 3H, J_{AB} = 6.0 Hz, CH_3).

Heneicos-2-enoic Acid γ-Lactone (4f). White powder (mp 61−63 °C, 69.4%). IR (KBr): 2950−2850 cm^{-1} (aliphatic CH), 1740 (C═O). ^1H NMR (acetone-d_6): δ 7.74 (d, 1H, J_{AB} = 5.6 Hz, H_3), 6.09 (d, 1H, 5.6 Hz, H_2), 5.08 (m, 1H, H_4), 1.76 (m, 2H, H_5), 1.61 (m, 2H, H_6), 1.29 (bs, 28H, CH_2), 0.88 (bt, 3H, CH_3).

***cis*-2,3-Epoxyalkanoic Acid γ-Lactones (5a−f).** Butenolides (14.2 mmol) were dissolved in either *p*-dioxane (**4a, 4b,** 8.5 mL/mmol butenolide) or *N*,*N*-DMF/Et_2O (**4c−f**, 1:1, 30 mL/mmol butenolide), chilled to 0 °C, and treated with sodium hypochlorite solution (∼4% aqueous, 2 equiv) added dropwise. Reactions were stirred at 0 °C for 1 h and then allowed to warm to room temperature over 1 h, while monitoring by TLC (silica, 15% EtOAc/hexanes). When no further change in the product to starting material ratio was observed, reaction mixtures were worked up as follows: *p*-dioxane reactions were partitioned between Et_2O and 5% Na_2SO_3, the aqueous layers were extracted with fresh Et_2O (2×), and the combined Et_2O extracts were washed with brine, dried with $MgSO_4$, and evaporated to colorless oils. The DMF/Et_2O reactions were diluted with Et_2O, the layers were separated, and the organic layers were washed with 10% $NaSO_3$, 5% $NaHCO_3$, H_2O, and brine (500 mL each), followed by drying over Na_2SO_4 and evaporation of volatiles.

***cis*-2,3-Epoxydodecanoic Acid γ-Lactone (5a).** Crude product was purified by flash chromatography (silica, 15% EtOAc/hexanes) to give product as a colorless oil (47.4%) as well as recovered starting material (15.8%). IR (neat): 2975−2850 cm^{-1} (aliphatic CH), 1775 (C═O). ^1H NMR ($CDCl_3$): δ 4.57 (t, 1H, J_{AB} = 6.5 Hz, H_4), 3.97 (d, 1H, J_{AB} = 2.4 Hz, H_3), 3.77 (d, 1 H, J_{AB} = 2.0 Hz, H_2), 1.66 (t, 2H, J_{AB} = 7.6 Hz, H_5), 1.27 (bs, 12H, CH_2), 0.88 (t, 3H, J_{AB} = 5.9 Hz, CH_3). Anal. ($C_{12}H_{20}O_3$) C, H.

***cis*-2,3-Epoxytridecanoic Acid γ-Lactone (5b).** Purification by radial chromatography (silica, 25% EtOAc/hexanes) gave product as a white solid (mp 40−42 °C, 2.15 mmol, 45.2%) and recovered starting material (25.1%). IR (KBr): 3000−2800 cm^{-1} (aliphatic CH), 1780 (C═O), 1200 (carbonyl C−O). ^1H NMR ($CDCl_3$): δ 4.57 (t, 1H, J_{AB} = 6.5 Hz, H_4), 3.97 (d, 1H, J_{AB} = 2.3 Hz, H_3), 3.77 (d, 1H, J_{AB} = 2.1 Hz, H_2), 1.67 (t, 2H, J_{AB} = 7.7 Hz, H_5), 1.27 (bs, 14H, CH_2), 0.88 (t, 3H, J_{AB} = 5.9 Hz, CH_3). Anal. ($C_{13}H_{22}O_3$) C, H.

***cis*-2,3-Epoxypentadecanoic Acid γ-Lactone (5c).** Purification by radial chromatography (silica, 15% EtOAc/hexanes) gave product as a white solid (mp 46−48 °C, 40.5%) and recovered starting material (40.4%). IR (KBr): 3000−2800 cm^{-1} (aliphatic CH), 1800 (C═O), 1200 (carbonyl C−O), 850 (asym epoxide C−C). ^1H NMR ($CDCl_3$): δ 4.57 (t, 1H, J_{AB} = 6.5 Hz, H_4), 3.97(d, 1 H, J_{AB} = 2.4 Hz, H_3), 3.77 (d, 1H, J_{AB} = 2.1 Hz, H_2), 1.65 (m, 2H, H_5), 1.26 (bs, 18H, CH_2), 0.88 (t, 3H, J_{AB} = 6.0 Hz, CH_3). Anal. ($C_{15}H_{26}O_3$) C: calcd, 70.83; found, 70.26; H: calcd, 10.30; found, 10.24.

***cis*-2,3-Epoxyheptadecanoic Acid γ-Lactone (5d).** Purification by radial chromatography (silica, 15% EtOAc/hexanes) gave product as a white solid (mp 59−60 °C, 44.8%) and recovered starting material (20.8%). IR (KBr): 2950−2850 cm^{-1} (aliphatic CH), 1780 (C═O), 1200 (carbonyl C−O). ^1H NMR ($CDCl_3$): δ 4.58 (t, 1H, J_{AB} = 6.5 Hz, H_4), 3.97 (d, 1H, J_{AB} = 2.4 Hz, H_3), 3.77 (d, 1H, J_{AB} = 2.2 Hz, H_2), 1.67 (t, 2H, J_{AB} = 7.0 Hz, H_5), 1.26 (bs, 22H, CH_2), 0.88 (t, 3H, J_{AB} = 6.5 Hz, CH_3). Anal. ($C_{17}H_{30}O_3$) C, H.

***cis*-2,3-Epoxynonadecanoic Acid γ-Lactone (5e).** Purification by radial chromatography (silica, 15% EtOAc/hexanes) gave product as a white solid (mp 66−67 °C, 40.8%) and recovered starting material (30.8%). IR (KBr): 2950−2850 cm^{-1} (aliphatic CH), 1775 (C═O), 1200 (carbonyl C−O). ^1H NMR ($CDCl_3$): δ 4.57 (t, 1H, J_{AB} = 6.5 Hz, H_4), 3.95 (d, 1H, J_{AB} = 2.3 Hz, H_3), 3.77 (d, 1H, J_{AB} = 2.1 Hz, H_2), 1.67 (t, 2H, J_{AB} = 7.7 Hz, H_5), 1.26 (bs, 26H, CH_2), 0.88 (t, 3H, J_{AB} = 5.8 Hz, CH_3). Anal. ($C_{19}H_{34}O_3$) C, H.

***cis*-2,3-Epoxyheneicosanoic Acid γ-Lactone (5f).** Purification by radial chromatography (silica, 15% EtOAc/hexanes) gave product as a white solid (mp 71−73 °C, 30.8%) and recovered starting material (46.0%). IR (KBr): 2950−2850 cm^{-1} (aliphatic CH), 1775 (C═O), 1190 (carbonyl C−O). ^1H NMR ($CDCl_3$): δ 4.57 (t, 1H, J_{AB} = 6.5 Hz, H_4), 3.96 (d, 1H, J_{AB} = 2.4 Hz, H_3), 3.77 (d, 1H, J_{AB} = 2.2 Hz, H_2), 1.67 (m, 2H, H_5), 1.26 (bs, 30H, CH_2), 0.88 (t, 3H, J_{AB} = 6.8 Hz, CH_3). Anal. ($C_{21}H_{38}O_3$) C, H.

***cis*-2,3-Epoxy-4-hydroxyalkanamides (6a−f).** Epoxy lactones were dissolved in methanol (∼2 mL/mmol lactone) and chilled to 0 °C. Concentrated ammonium hydroxide (to give 10% solution) was added slowly, and the solution was stirred at 0 °C for 30 min until precipitation occurred and TLC (silica, 5% MeOH in CH_2Cl_2) showed the reaction to be complete. Dilution with CH_2Cl_2 (2× volume of MeOH), drying over Na_2SO_4, evaporation of volatiles, and vacuum desiccation gave crude products as white powders.

***cis*-2,3-Epoxy-4-hydroxydodecanamide (6a).** Purification by radial chromatography (silica, 5% MeOH in CH_2Cl_2) gave a white powder (mp 108−109 °C, 67.9%). IR (KBr): 3450−3150 cm^{-1} (NH/OH stretches), 2950−2850 (aliphatic CH), 1690 (C═O), 1460 (NH bend), 1340 (CN). ^1H NMR (acetone-d_6): δ 6.86 (bs, 1H, NH), 6.73 (bs, 1H, NH′), 4.04 (d, 1H, J_{AB} = 5.2 Hz, OH), 3.53−3.48 (m, 1H, H_4), 3.41 (d, 1H, J_{AB} = 4.5 Hz, H_2), 3.00 (dd, 1H, J_{AB} = 4.5 Hz, H_3), 1.64−1.52 (m, 2H, H_5), 1.30 (bs, 12H, CH_2), 0.88 (t, 3H, J_{AB} = 6.7 Hz, CH_3). Anal. ($C_{12}H_{23}NO$) C, H, N.

***cis*-2,3-Epoxy-4-hydroxytridecanamide (6b).** Crude white semisolid was recrystallized from $CHCl_3$/hexanes to give a white powder (mp 101−102 °C, 21.6%). IR (KBr): 3500−3150 cm^{-1} (NH/OH stretches), 3000−2800 (aliphatic CH), 1700 (C═O). ^1H NMR ($CDCl_3$): δ 6.20 (bs, 1H, NH), 6.11 (bs, 1H, NH′), 3.545 (d, 1H, J_{AB} = 4.5 Hz, H_2), 3.46 (q, 1H, H_4), 3.125 (q, 1H, H_3), 1.68 (t, 2H, H_5), 1.27 (bs, 14H, CH_2), 0.87 (t, 3H, J_{AB} = 6.8 Hz, CH_3). Anal. ($C_{13}H_{25}NO$) C: calcd, 64.16; found, 65.49; H: calcd, 10.35; found, 10.08; N: calcd, 5.76; found, 2.28.

***cis*-2,3-Epoxy-4-hydroxypentadecanamide (6c).** Purification by radial chromatography (silica, 5% MeOH in CH_2Cl_2) gave a white powder (mp 103−105 °C, 29.7%). IR (KBr): 3500−3100 cm^{-1} (NH/OH stretches), 3000−2800 (aliphatic CH), 1700 (C═O). ^1H NMR ($CDCl_3$): δ 6.18 (bs, 1H, NH), 5.99 (bs, 1H, NH′), 3.56 (d, 1H, J_{AB} = 4.5 Hz, H_2), 3.45 (q, 1H, H_4), 3.13 (m, 1H, H_3), 1.68 (m, 2H, H_5), 1.26 (bs, 18H, CH_2), 0.88 (t, 3H, J_{AB} = 6.8 Hz, CH_3). Anal. ($C_{15}H_{29}NO$) C: calcd, 66.38; found, 65.95; H: calcd, 10.77; found, 10.35; N: calcd, 5.16; found, 4.72.

***cis*-2,3-Epoxy-4-hydroxyheptadecanamide (6d).** Purification by radial chromatography (silica, 5% MeOH in CH_2Cl_2) gave a white powder (mp 106−108 °C, 29.1%). IR (KBr): 3450−3175 cm^{-1} (NH/OH stretches), 2950−2850 (aliphatic CH), 1680 (C═O). ^1H NMR ($CDCl_3$): δ 6.15 (bs, 1H, NH), 5.78 (bs, 1H, NH′), 4.93 (bs, 1H, OH), 3.56 (d, 1H, J_{AB} = 4.8 Hz, H_2), 3.47 (m, 1H, H_4), 3.14 (m, 1 H, H_3), 1.67 (m, 2H, H_5), 1.26 (bs, 22H, CH_2), 0.88 (t, 3H, J_{AB} = 6.7 Hz, CH_3). Anal. ($C_{17}H_{33}NO{\cdot}C_3H_6O$) C, H, N.

***cis*-2,3-Epoxy-4-hydroxynonadecanamide (6e).** Purification by radial chromatography (silica, 5% MeOH in CH_2Cl_2) gave a white powder (mp 110−112 °C, 73.8%). IR (KBr): 3450−3400 cm^{-1} (NH/OH stretches), 2950−2850 (aliphatic CH), 1700 (C═O). ^1H NMR ($CDCl_3$): δ 6.75 (bs, 1H, NH), 6.55 (bs, 1H, NH′), 3.86 (bs, 1H, OH), 3.53 (m, 1H, H_4), 3.40 (d, 1H, J_{AB} = 4.3 Hz, H_2), 3.00 (m, 1H, H_3), 1.56 (m, 2H, H_5), 1.30 (bs, 26H, CH_2), 0.88 (t, 3H, J_{AB} = 6.7 Hz, CH_3). Anal. ($C_{19}H_{37}NO$) C: calcd, 69.68; found, 67.57; H: calcd, 11.39; found, 11.08; N: calcd, 4.28; found, 3.96.

***cis*-2,3-Epoxy-4-hydroxyheneicosanamide (6f).** Purification by radial chromatography (silica, 5% MeOH in CH_2Cl_2) gave a white powder (mp 111−113 °C, 30.9%). IR (KBr): 3450−3175 cm^{-1} (NH/OH stretches), 2950−2850 (aliphatic CH), 1680 (C═O), 1470 (carbonyl C−N). ^1H NMR ($CDCl_3$): δ 6.76 (bs, 1H, NH), 6.58 (bs, 1H, NH′), 3.88 (bs, 1H, OH), 3.53 (m, 1H, H_4), 3.40 (d, 1H, J_{AB} = 4.3 Hz, H_2), 3.01 (m, 1H, H_3), 1.55 (m, 2H, H_5), 1.30 (bs, 30H, CH_2), 0.88 (t, 3H, J_{AB} = 6.7 Hz, CH_3). Anal. ($C_{21}H_{41}NO$) C, H, N.

***cis*-2,3-Epoxy-4-oxoalkanamides (7a−f).** Hydroxyamides were oxidized with pyridinium dichromate according to Jakubowski et al.[32]

***cis*-2,3-Epoxy-4-oxododecanamide (7a, Tetrahydrocerulenin).** Purification by radial chromatography (silica, CH_2Cl_2/Et_2O − 5:1) gave a white solid (37.4%, 1.9% from propiolic acid). IR (KBr): 3400 cm^{-1} (asym NH stretch), 3200 (sym NH), 3000−2800 (aliphatic CH), 1725 (ketone C═O), 1660 (amide C═O). ^1H NMR ($CDCl_3$): δ 6.3 (bs, 1H, NH), 5.55 (bs, 1H, NH′), 3.79 (AB q, 2H, J_{AB} = 5.3 Hz, H_2, H_3), 2.56 (m, 2H, H_5), 1.6 (m, 2H, H_6), 1.27 (bs, 10H, CH_2), 0.87 (t, 3H, J_{AB} = 6.8 Hz, CH_3). Anal. ($C_{12}H_{21}NO$) C: calcd, 63.41; found, 63.82; H: calcd, 9.31; found, 8.21; N: calcd, 6.16; found, 5.62.

***cis*-2,3-Epoxy-4-oxotridecanamide (7b).** White solid (6.0%). IR (KBr): 3400 cm^{-1} (asym NH stretch), 3100 (sym NH), 3000−2800 (aliphatic CH), 1725 (ketone C═O), 1675 (amide C═O). ^1H NMR ($CDCl_3$): δ 6.31 (bs, 1H, NH), 5.50 (bs, 1H, NH′), 3.875 (d, 1H, J_{AB} = 5.2 Hz, H_3), 3.73 (d, 1H, J_{AB} = 5.2 Hz, H_2), 2.57 (m, 2H, H_5), 1.61 (m, 2H, H_6), 1.26 (bs, 12H, CH_2), 0.88 (t, 3H, J_{AB} = 5.9 Hz, CH_3). Anal. ($C_{13}H_{23}NO$) C, H, N.

***cis*-2,3-Epoxy-4-oxopentadecanamide (7c).** White solid (mp 75−80 °C, 29%). IR (KBr): 3500 cm^{-1} (asym NH stretch), 3150 (sym NH), 2925−2850 (aliphatic CH), 1725 (ketone C═O), 1660 (amide C═O). ^1H NMR ($CDCl_3$): δ 6.33 (bs, 1H, NH), 5.65 (bs, 1H, NH′), 3.80 (AB q, 2H, J_{AB} = 5.2 Hz, H_2, H_3), 2.58 (m, 2H, H_5), 1.60 (m, 2H, H_6), 1.26 (bs, 16H, CH_2), 0.88 (t, 3H, J_{AB} = 5.9 Hz, CH_3). Anal. ($C_{17}H_{27}NO$) C: calcd, 66.88; found, 66.46; H: calcd, 10.10; found, 9.81; N: calcd, 5.20; found, 4.85.

***cis*-2,3-Epoxy-4-oxoheptadecanamide (7d).** A crude sample of **6d** (235 mg, 0.785 mmol) was oxidized according to Corey and Schmidt[44] with 1.5 equiv of $pyr_2{\cdot}Cr_2O_7$. After 51 h of stirring at room temperature, TLC (silica, 5% MeOH in CH_2Cl_2) showed the reaction to be complete. Filtration through Celite with CH_2Cl_2, followed by filtration through a plug of silica with CH_2Cl_2/Et_2O (1:1), gave 127 mg of a white solid. Partially purified product was radially chromatographed (silica, CH_2Cl_2/Et_2O − 1:1) to give 6 mg of a white solid (0.02 mmol, 3%). IR (KBr): 3400 cm^{-1} (asym NH stretch), 2950−2850 (aliphatic CH), 1720 (ketone C═O), 1660 (amide C═O), 1375 (carbonyl CN). ^1H NMR ($CDCl_3$): δ 6.33 (bs, 1H, NH), 5.54 (bs, 1H, NH′), 3.74 (AB q, 2H, J_{AB} = 5.2 Hz, H_2, H_3), 2.57 (m, 2H, H_5), 1.60 (m, 2H, H_6), 1.25 (bs, 22H, CH_2), 0.88 (t, 3H, J_{AB} = 6.0 Hz, CH_3). Anal. ($C_{17}H_{31}NO$) C: calcd, 68.65; found, 68.41; H: calcd, 10.51; found, 9.99; N: calcd, 4.71; found, 4.19.

***cis*-2,3-Epoxy-4-oxononadecanamide (7e).** Hydroxyamide **6e** was oxidized as for **7d** above. Purification by flash chromatography (silica, CH_2Cl_2/Et_2O − 1:1) gave 74 mg of an off-white solid (mp 98 °C (dec), 0.227 mmol, 48.7%). IR (KBr): 3390 cm^{-1} (asym NH stretch), 3150 (sym NH), 2950−2850 (aliphatic CH), 1720 (ketone C═O), 1660 (amide C═O). ^1H NMR ($CDCl_3$): δ 6.33 (bs, 1H, NH), 5.63 (bs, 1H, NH′), 3.87 (bs, 1H, H_3), 3.72 (bs, 1H, H_2), 2.57 (bm, 2H, H_5), 1.60 (bm, 2H, H_6), 1.25 (bs, 24H, CH_2), 0.88 (bt, 3H, CH_3). MS: *m/e* 326.3 (M + 1). Anal. ($C_{19}H_{35}NO$) C: calcd, 70.11; found, 67.64; H: calcd, 10.84; found, 10.64; N: calcd, 4.30; found, 3.99.

***cis*-2,3-Epoxy-4-oxoheneicosanamide (7f).** Hydroxyamide **6f** (50 mg, 0.141 mmol) was oxidized according to the procedures of Griffith et al.[34,35] by dissolving in 10% CH_3CN in CH_2Cl_2 (40 mL). After addition of 4 Å molecular sieves (powdered, 70.5 mg) and *N*-methyl morpholine oxide (0.212 mmol, 24.8 mg), the mixture was stirred for 10 min at room temperature. The reaction was initiated by addition of [tetra(propyl)ammonium]perruthenate (5 mol %, 7.05 μmol, 2.5 mg). After the mixture was stirred for 23 h at room temperature, TLC (silica, 5% MeOH/CH_2Cl_2) showed reaction to be ∼50% complete. Another 70 mg of powdered molecular sieves were added, and the reaction stirred another 71 h. TLC showed that reaction did not proceed to completion (ca. 60%). Filtration through a plug of silica with CH_2Cl_2 → CH_2Cl_2/Et_2O (1:1), followed by flash chromatography (silica, CH_2Cl_2/Et_2O − 1:1), gave 29 mg of white solid (mp 99−100 °C, 0.082 mmol, 58%). IR (KBr): 3400 cm^{-1} (asym NH stretch), 3150 (sym NH), 2950−2850 (aliphatic CH), 1725 (ketone C═O), 1675 (amide C═O). ^1H NMR ($CDCl_3$): δ 6.31 (bs, 1H, NH), 5.45 (bs, 1H, NH′), 3.88 (d, 1H, J_{AB} = 5.2 Hz, H_3), 3.73 (d, 1H, J_{AB} = 5.2 Hz, H_2), 2.57 (m, 2H, H_5), 1.59 (bs, 2H, H_6), 1.25 (bs, 28H, CH_2), 0.88 (t, 3H, J_{AB} = 6.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 202.91 (C4), 167.56 (C1), 58.55 (C3), 55.56 (C2), 41.37 (C5), 32.15 (C19), 29.91, 29.80, 29.58, 29.49, 29.26 (C7−C18), 23.31 (C6), 22.91 (C20), 14.34 (C21). Anal. ($C_{21}H_{39}NO$) C: calcd, 71.34; found, 70.54; H: calcd, 11.12; found, 10.79; N: calcd, 3.96; found, 2.95.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-hydroxyalkanamides (8a−f).** Epoxy lactones (2.36 mmol) were dissolved in MeOH (10 mL) and chilled to 0 °C. Dimethylamine (2 equiv, 2.36 mL of a 2.0 M solution in THF) was added dropwise, and the mixture was stirred at 0 °C for 2 h and then allowed to warm to room temperature overnight. When TLC (silica, 3% MeOH in CH_2Cl_2) indicated that the reaction was complete, all volatiles were removed by rotary evaporation, followed by vacuum desiccation, to give the crude product as a yellow semicrystalline solid.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-hydroxydodecanamide (8a).** Recrystallization from hexanes gave pure product as colorless needles (mp 64−65 °C, 89.4%). IR (KBr): 3475 cm^{-1} (OH stretch), 2950−2850 (aliphatic CH), 1640 (C═O). ^1H NMR ($CDCl_3$): δ 3.85 (bs, 1H, OH), 3.575 (d, 1H, J_{AB} = 4.1 Hz, H_2), 3.19 (s, 3H, NCH_3), 3.17 (m, 1H, H_4), 3.08 (dd, 1H, J_{AB} = 4.1 Hz, H_3), 2.99 (s, 3H, NCH_3'), 1.67 (t, 2H, J_{AB} = 7.3 Hz, H_5), 1.27 (bs, 12H, CH_2), 0.88 (t, 3H, J_{AB} = 5.9 Hz, CH_3). Anal. ($C_{14}H_{27}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-hydroxytridecanamide (8b).** Purification by radial chromatography (silica, 3% MeOH in CH_2Cl_2) gave a white solid (mp 46−48 °C, 66.0%). IR (KBr): 3475 cm^{-1} (OH stretch), 2950−2850 (aliphatic CH), 1640 (C═O). ^1H NMR ($CDCl_3$): δ 3.575 (d, 1H, J_{AB} = 4.1 Hz, H_2), 3.195 (s, 3H, NCH_3), 3.17 (m, 1H, H_4), 3.08 (dd, 1H, J_{AB} = 4.1 Hz, H_3), 2.99 (s, 3H, NCH_3'), 1.67 (m, 2H, H_5), 1.26 (bs, 14H, CH_2), 0.88 (t, 3H, J_{AB} = 6.35 Hz, CH_3). Anal. ($C_{15}H_{29}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-hydroxypentadecanamide (8c).** White solid (mp 54−56 °C, 75.4%). IR (KBr): 3475 cm^{-1} (OH stretch), 2950−2850 (aliphatic CH), 1640 (C═O). ^1H NMR ($CDCl_3$): δ 3.575 (d, 1H, J_{AB} = 4.0 Hz, H_2), 3.195 (s, 3H, NCH_3), 3.17 (m, 1H, H_4), 3.104 (dd, 1H, J_{AB} = 4.1 Hz, H_3), 2.99 (s, 3H, NCH_3'), 1.67 (m, 2H, H_5), 1.26 (s, 18H, CH_2), 0.88 (t, 3H, J_{AB} = 6.5 Hz, CH_3). Anal. ($C_{17}H_{33}NO$) C: calcd, 68.19; found, 68.32; H: calcd, 11.11; found, 10.81; N: calcd, 4.68; found, 4.21.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-hydroxyheptadecanamide (8d).** White solid (mp 61−63 °C, 85.2%). IR (KBr): 3500 cm^{-1} (OH stretch), 2925−2850 (aliphatic CH), 1640 (C═O). ^1H NMR ($CDCl_3$): δ 3.575 (d, 1H, J_{AB} = 4.1 Hz, H_2), 3.19 (s, 3H, NCH_3), 3.16 (m, 1H, H_4), 3.08 (m, 1H, H_3), 2.99 (s, 3H, NCH_3'), 1.67 (m, 2H, H_5), 1.25 (s, 22H, CH_2), 0.88 (t, 3H, J_{AB} = 6.4 Hz, CH_3). Anal. ($C_{19}H_{37}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-hydroxynonadecana-**

mide (8e). White solid (mp 67−69 °C, 90.2%). IR (KBr): 3475 cm^{-1} (OH stretch), 2950−2850 (aliphatic C−H), 1640 (C═O), 1380 (C−N). ^1H NMR ($CDCl_3$): δ 3.87 (bs, 1H, OH), 3.575 (d, 1H, J_{AB} = 3.8 Hz, H_2), 3.19 (s, 3H, NCH_3), 3.15 (m, 1H, H_4), 3.08 (m, 1H, H_3), 2.99 (s, 3H, NCH_3'), 1.67 (t, 2H, J_{AB} = 7.1 Hz, H_5), 1.26 (bs, 26H, CH_2), 0.88 (t, 3H, J_{AB} = 5.5 Hz, CH_3). Anal. ($C_{21}H_{41}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-hydroxyheneicosanamide (8f).** White solid (mp 73−75 °C, 85.4%). IR (KBr): 3475 cm^{-1} (OH stretch), 2950−2850 (aliphatic C−H), 1640 (C═O). ^1H NMR ($CDCl_3$): δ 3.86 (bs, 1H, OH), 3.57 (d, 1H, J_{AB} = 4.1 Hz, H_2), 3.19 (s, 4H, NCH_3 + H_4), 3.08 (dd, 1H, J_{AB} = 4.1 Hz, H_3), 2.99 (s, 3H, NCH_3'), 1.67 (m, 2H, H_5), 1.25 (bs, 30H, CH_2), 0.88 (t, 3H, J_{AB} = 6.8 Hz, CH_3). Anal. ($C_{23}H_{45}NO$) C: calcd, 72.01; found, 71.87; H: calcd, 11.82; found, 11.14; N: calcd, 3.65; found, 3.35.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-oxoalkanamides (9a−f).** *N*,*N*-Dimethyl-hydroxyamides were oxidized as for **7a** above. After the mixture was stirred overnight at room temperature, TLC (silica, CH_2Cl_2/Et_2O − 1:1) showed reaction to be complete. Workup as for **7a** gave a brown oil which was filtered through Florisil and radially chromatographed to give a pale yellow oil (36.6%, 2.4% from propiolic acid).

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-oxododecanamide (9a, *N,N*-Dimethyl-tetrahydrocerulenin).** IR (neat): 2950−2850 cm^{-1} (aliphatic CH), 1710 (ketone C═O), 1660 (amide C═O). ^1H NMR ($CDCl_3$): δ 3.82 (d, 1H, J_{AB} = 4.8 Hz, H_3), 3.69 (d, 1H, J_{AB} = 4.9 Hz, H_2), 3.13 (s, 3H, NCH_3), 2.93 (s, 3H, NCH_3'), 2.535 (m, 2H, H_5), 1.56 (m, 2H, H_6), 1.26 (bs, 10H, CH_2), 0.88 (t, 3H, J_{AB} = 5.9 Hz, CH_3). Anal. ($C_{14}H_{25}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-Epoxy-4-oxotridecanamide (9b).** Pale yellow oil (52.6%). IR (neat): 2950−2850 cm^{-1} (aliphatic CH), 1710 (ketone C═O), 1650 (amide C═O). ^1H NMR ($CDCl_3$): δ 3.76 (AB q, 2H, J_{AB} = 5.0 Hz, H_2/H_3), 3.13 (s, 3H, NCH_3), 2.93 (s, 3H, NCH_3'), 2.53 (m, 2H, H_5), 1.56 (m, 2H, H_6), 1.25 (bs, 12H, CH_2), 0.87 (t, 3H, J_{AB} = 6.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 205.67 (C4), 164.43 (C1), 58.28 (C3), 54.82 (C2), 39.91 (C5), 36.37, 35.35 (NCH_3), 31.92 (C11), 29.48, 29.46, 29.32, 29.11 (C7−C10), 22.83, 22.72 (C6, C12), 14.16 (C13). Anal. ($C_{15}H_{27}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-oxopentadecanamide (9c).** Clear oil (52.0%). IR (neat): 2950−2850 cm^{-1} (aliphatic CH), 1710 (ketone C═O), 1660 (amide C═O). ^1H NMR ($CDCl_3$): δ 3.75 (AB q, 2H, J_{AB} = 4.9 Hz, H_2/H_3), 3.13 (s, 3H, NCH_3), 2.93 (s, 3H, NCH_3'), 2.53 (m, 2H, H_5), 1.56 (m, 2H, H_6), 1.25 (bs, 16H, CH_2), 0.88 (t, 3H, J_{AB} = 5.6 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 205.65 (C4), 164.44 (C1), 58.26 (C3), 54.81 (C2), 39.91 (C5), 36.36, 35.35 (NCH_3), 31.96 (C13), 29.66, 29.52, 29.45, 29.39, 29.11 (C7−C12), 22.83, 22.74 (C6, C14), 14.17 (C15). Anal. ($C_{17}H_{31}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-oxoheptadecanamide (9d).** Clear oil (44.0%). IR (neat): 2950−2850 cm^{-1} (aliphatic CH), 1710 (ketone C═O), 1660 (amide C═O). ^1H NMR ($CDCl_3$): δ 3.76 (AB q, 2H, J_{AB} = 4.9 Hz, H_2/H_3), 3.13 (s, 3H, NCH_3), 2.93 (s, 3H, NCH_3'), 2.53 (m, 2H, H_5), 1.56 (m, 2H, H_6), 1.25 (bs, 20H, CH_2), 0.88 (t, 3H, J_{AB} = 6.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 205.78 (C4), 164.53 (C1), 58.36 (C3), 54.93 (C2), 40.07 (C5), 36.49, 35.50 (NCH_3), 32.09 (C15), 29.81, 29.78, 29.64, 29.56, 29.52, 29.22 (C7−C14), 22.95, 22.86 (C6, C16), 14.29 (C17). Anal. ($C_{19}H_{35}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-oxononadecanamide (9e).** Off-white solid (11%). IR (KBr): 2950−2850 cm^{-1} (aliphatic CH), 1720 (ketone C═O), 1645 (amide C═O), 1375 (carbonyl CN). ^1H NMR ($CDCl_3$): δ 3.75 (AB q, 2H, J_{AB} = 4.5 Hz, H_2/H_3), 3.13 (s, 3H, NCH_3), 2.93 (s, 3H, NCH_3'), 2.53 (m, 2H, H_5), 1.56 (m, 2H, H_6), 1.25 (bs, 24H, CH_2), 0.88 (t, 3H, J_{AB} = 6.6 Hz, CH_3). MS: *m/e* 354.4 (M + 1). Anal. ($C_{21}H_{39}NO$) C, H, N.

***N,N*-Dimethyl-*cis*-2,3-epoxy-4-oxoheneicosanamide (9f).** Produced by TPAP oxidation (as for **7f**) as a white solid (mp 53−55 °C, 93.4%). IR (KBr): 2950−2850 cm^{-1} (aliphatic CH), 1725 (ketone C═O), 1650 (amide C═O). ^1H NMR ($CDCl_3$): δ 3.82 (d, 1H, J_{AB} = 4.9 Hz, H_3), 3.70 (d, 1H, J_{AB} = 4.9 Hz, H_2), 3.13 (s, 3H, NCH_3), 2.93 (s, 3H, NCH_3'), 2.53 (m, 2H, H_5), 1.56 (m, 2H, H_6), 1.25 (bs, 28H, CH_2), 0.88 (t, 3H, J_{AB} = 6.6 Hz, CH_3). ^{13}C NMR ($CHCl_3$): δ 205.58 (C4), 164.34 (C1), 58.14 (C3), 54.72 (C2), 39.87 (C5), 36.29, 35.30 (NCH_3), 31.89 (C19), 29.65, 29.43, 29.35, 29.02, (C7−C18), 22.74, 22.66 (C6, C20), 14.09 (C21). Anal. ($C_{23}H_{43}NO$) C, H, N.

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References

(1) Barbacid, M. *Ras* genes. *Annu. Rev. Biochem.* **1987**, *56*, 779−827.
(2) Lowy, D. R.; Willumsen, B. M. Function and regulation of *ras*. *Annu. Rev. Biochem.* **1993**, *62*, 851−891.
(3) Khosravi-Far, R.; Der, C. J. The *ras* signal transduction pathway. *Cancer Metastasis Rev.* **1994**, *13*, 67−89.
(4) Sigal, I. S.; Smith, G. M.; Jurnak, F.; Marsico-Ahern, J. D.; D'Alonzo, J. S.; Scholnick, E. M.; Gibbs, J. B. Molecular approaches towards an anti-ras drug. *Anti-Cancer Drug Des.* 2 **1987**, 107−115.
(5) Gibbs, J. B.; Oliff, A.; Kohl, N. E. Farnesyltransferase inhibitors: ras research yields a potential cancer therapeutic. *Cell* **1994**, *77*, 175−178.
(6) Bolton, G. L.; Sebolt-Leopold, J. S.; Hodges, J. C. Ras oncogene directed approaches in cancer chemotherapy. *Ann. Rep. Med. Chem.* **1994**, *29*, 165−174.
(7) Willumsen, B. M.; Christensen, A. The p21 ras C-terminus is required for transformation and membrane association. *Nature* **1984**, *310*, 583−586.
(8) Hancock, J. F.; Magee, A. I.; Childs, J. E.; Marshall, C. J. All ras proteins are polyisoprenylated but only some are palmitoylated. *Cell* **1989**, *57*, 1167−1177.
(9) Hancock, J. F.; Paterson, H.; Marshall, C. J. A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize p21 ras to the plasma membrane. *Cell* **1990**, *6*, 133−139.
(10) Cadwallader, K. A.; Paterson, H.; MacDonald, S. G.; Hancock, J. F. N-terminally myristoylated ras proteins require palmitoylation or a polybasic domain for plasma membrane localization. *Mol. Cell. Biol.* **1994**, *14*, 4722−4730.
(11) Pompliano, D. L.; Rands, E.; Schaber, M. D.; Mosser, S. D.; Anthony, N. J.; Gibbs, J. B. Steady-state kinetic mechanism of Ras farnesyl:protein transferase. *Biochemistry* **1992**, *31*, 3800−3807.
(12) Chen, W.-J.; Andres, D. A.; Goldstein, J. L.; Russell, D. W.; Brown, M. S. cDNA cloning and expression of the peptide binding subunit of rat p21 ras farnesyltransferase, the counterpart of yeast RAM1/DPR1. *Cell* **1991**, *66*, 327−334.
(13) Kohl, N. E.; Mosser, S. D.; deSolms, S. J.; Giuliani E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scholnick, E. M.; Oliff, A.; Gibbs, J. B. Selective inhibition of ras-dependent transformation by a farnesyltransferase inhibitor. *Science* **1993**, *260*, 1934−1937.
(14) James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C., Jr. Benzodiazepine peptidomimetics: potent inhibitors of ras farnesylation in animal cells. *Science* **1993**, *260*, 1937−1942.
(15) James, G. L.; Goldstein, J. L.; Brown, M. S. Polylysine and CVIM sequences of K−RasB dictate specificity of prenylation and confer resistance to benzodiazepine peptidomimetic in vitro. *J. Biol. Chem.* **1995**, *270*, 6221−6226.
(16) Rowell, C. A.; Kowalczyk, J. J.; Lewis, M. D.; Garcia, A. M. Direct demonstration of geranylgeranylation and farnesylation of Kiras in vivo. *J. Biol. Chem.* **1997**, *272*, 14093−14097.
(17) Whyte, D. B.; Kirschmeier, P.; Hockenberry, T. N.; Nunez-Oliva, I.; James, L.; Catino, J. J.; Bishop, W. R.; Pai, J. K. K- and N-ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. *J. Biol. Chem.* **1997**, *272*, 14459−14464.
(18) Castro, A. C.; Adams, J.; Behnke, M. L.; Chen, S.; Grenier, L.; Klunder, J. M.; McCormack, T. A.; Oalmann, C. J.; Plamondon, L.; Soucy, F.; Wong, H. *Abstracts of Papers*. 216th National Meeting of the American Chemical Society, Boston, MA; American Chemical Society: Washington, DC, 1998; MEDI 215.
(19) Clarke, S.; Vogel, J. P.; Deschenes, R. J.; Stock, J. Posttranslational modification of the Ha-ras oncogene protein: Evidence for a third class of protein carboxy methyltransferases. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4643−4647.
(20) Magee, A. I.; Gutierrez, L.; McKay, I. A.; Marchall, C. J.; Hall, A. Dynamic fatty acylation of p21 N-ras. *EMBO J.* **1987**, *6*, 3353−3357.
(21) Camp, L. A.; Hoffmann, S. L. Purification and properties of a palmitoyl-protein thioesterase that cleaves palmitate from H-ras. *J. Biol. Chem.* **1993**, *268*, 22566−22574.

(22) Camp, L. A.; Verkruyse, L. A.; Afendis, S. J.; Slaughter, C. A.; Hoffmann, S. L. Molecular cloning and expression of palmitoyl-protein thioesterase. *J. Biol. Chem.* **1994**, *269*, 23212−23219.
(23) Duncan, J. A.; Gilman, A. G. A cytoplasmic acyl-protein thioesterase that removes palmitate from G protein α subunits and $p21^{ras}$. *J. Biol. Chem.* **1998**, *273*, 15830−15837.
(24) Omura, S. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. *Bacteriol. Rev.* **1976**, *40*, 681−697.
(25) Pizer, E. S.; Wood, F. D.; Heine, H. S.; Romantsev, F. E.; Pasternick, G. R.; Kuhajda, F. P. Inhibition of fatty acid synthesis delays disease progression in a xenograft model of ovarian cancer. *Cancer Res.* **1996**, *56*, 1189−1193.
(26) Smith, C. D.; Zilfou, J. T.; Laing, N.; Lawrence, D. S.; Dexter, D. W. Inhibition of palmitoylation of ras oncoproteins by cerulenin. *Proc. Am. Assoc. Cancer Res.* **1980**, *40*, 118.
(27) Smith, C. D.; Zilfou, J. T.; Laing, N.; Varner, A. S. Cerulenin inhibits protein palmitoylation and ras function. Submitted, *Cancer Res.*
(28) Schlessinger, M. J.; Malfer, C. Cerulenin blocks fatty acid acylation of glycoproteins and inhibits vesicular stomatitis and Sinbis virus particle formation. *J. Biol. Chem.* **1982**, *257*, 9887−9890.
(29) Boekman, R. K., Jr.; Thomas, E. W. A total synthesis of DL-cerulenin: a novel fatty acid antibiotic and lipid synthesis inhibitor. *J. Am. Chem. Soc.* **1977**, *99*, 2805−2806.
(30) Mani, N. S.; Townsend, C. A. A concise synthesis of (+)-cerulenin from a chiral oxiranyllithium. *J. Org. Chem.* **1997**, *62*, 636−640.
(31) Ohri, H.; Emoto, S. Stereoselective synthesis of (+)- and (−)-tetrahydrocerulenin from D-glucose. The absolute configuration of cerulenin. *Tetrahedron Lett.* **1978**, *24*, 2095−2098.
(32) Jakubowski, A. A.; Guziec, F. S., Jr.; Sugiera, M.; Tam, C. C.; Tischler, M. Total synthesis of (±)-cerulenin, (±)-tetrahydrocerulenin, and related compounds. *J. Org. Chem.* **1982**, *47*, 1221−1228.
(33) Ohta, T.; Tsuchiyama, H.; Nozoe, S. Total synthesis of (±)-cerulenin and (±)-tetrahydrocerulenin. *Heterocycles* **1986**, *24*, 1137−1143.
(34) Griffith, W. P. Preparation and use of tetra-*n*-butylammonium per-ruthenate (TBAP reagent) and tetra-*n*-propylammonium per-ruthenate (TPAP reagent) as new catalytic oxidants for alcohols *J. Chem. Soc., Chem. Commun.* **1987**, 1625−1627.
(35) Griffith, W. P.; Ley, S. V. TPAP: Tetra-*n*-propylammonium perruthenate, a mild and convenient oxidant for alcohols. *Aldrichim. Acta* **1990**, *23*, 13−19.
(36) Carr, J. B.; Durham, H. G. 3-(Benzoyl)oxiranecarboxamides. U.S. Patent 4,091,221, 1978.
(37) Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: New York, 1994; pp 89−90.
(38) Skehan, P.; Stoneng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesh, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst. U.S.A.* **1990**, *82*, 1107−1112.
(39) Morisaki, N.; Funabashi, H.; Shimazawa, R.; Furukawa, J.; Kawaguchi, A.; Okuda, S.; Iwsaki, S. Effect of side-chain structure on inhibition of yeast fatty-acid synthase by cerulenin analogues. *Int. J. Biochem.* **1993**, *211*, 111−115.
(40) Blumenstein, J. J.; Copeland, T. D.; Oroszlan, S.; Michejda, C. J. Synthetic non-peptide inhibitors of HIV protease. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 980−987.
(41) Blumenstein, J. J.; Michejda, C. J.; Oroszlan, S.; Copeland, T. D. U.S. Patent 5,190,969, 1993.
(42) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic techniques for preparative separation with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923−2924.
(43) Royer, G. P.; Townsend, C. A. Cerulenin compounds for fatty acid synthesis inhibition. U.S. Patent 5,539,132, 1996.
(44) Corey, E. J.; Schmidt, G. Useful procedures for the oxidation of alcohols involving pyridinium dichromate in aprotic media. *Tetrahedron Lett.* **1979**, 399−402.
(45) Resh, M. D. Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. *Biochim. Biophys. Acta* **1999**, *1451*, 1−16.
(46) Gibbs, J. B.; Schaber, M. D.; Schofield, T. L.; Scolnick, E. M.; Sigal, I. S. *Xenopus* oocyte germinal-vesicle breakdown induced by [Val^{12}]Ras is inhibited by a cytosols-localized Ras mutant. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6630−6634.
(47) Lerner, E. C.; Qian, Y.; Blaskovich, M. A.; Fossum, R. D.; Vogt, A.; Sun, J.; Cox, A. D.; Der, C. J.; Hamilton, A. D.; Sebti, S. M. Ras CAAX peptidomimetic FTI-277 selectively blocks oncogenic ras signaling by inducing cytoplasmic accumulation of inactive ras-raf complexes. *J. Biol. Chem.* **1995**, *260*, 26802−26806.

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