80% NaH (0.015 g, 0.51 mmol). The resulting mixture was then cooled to 0 °C for 1 h. After cooling it further with a mixture of dry ice and 2-propanol, trifluoromethanesulfonyl chloride (54 μL, 0.51 mmol) was added and the mixture stirred for 2 h. The reaction mixture then was reduced in volume and applied directly to a preparative TLC plate (Analtech, 2000 μ) to give a major band that corresponded to the triflate **22** (0.205 g, 74%). Part of this material (0.113 g, 0.16 mmol) was dissolved in dry THF (2 mL) and treated with tetrabutylammonium fluoride (175 μL, 1 M solution in THF) at room temperature for 30 min. Preparative silica gel TLC chromatography (Analtech, 2000 μ) developed with ethyl acetate afforded 0.072 g (78%) of the desired protected compound, **23**. This material was dissolved in 2 mL of methylene chloride and treated with trichloroacetic acid (2 mL, 3% solution in methylene chloride) at room temperature. After 5 min, the solution was loaded on a silica gel preparative TLC plate (Analtech, 2000 μ) which was developed with a methylene chloride/methanol solution (9:1). After its isolation from the plate, compound **5** was obtained as a white solid (0.03 g, 94%). It was recrystallized from methanol to give small flakes: mp 235 °C; ^{1}H NMR (D_2O) δ 8.48 (s, 1 H, H-8), 8.23 (s, 1 H, H-2), 6.31 (d, $J_{1',F}$ = 17.2 Hz, 1 H, H-1'), 5.54 (dd, $J_{2',F}$ = 52.2 Hz, $J_{2',3'a}$ = 3.4 Hz, 1 H, H-2'), 4.54 (m, 1 H, H-4'), 3.97 (dd, $J_{5'a,5'b}$ = 12.4 Hz, $J_{5'a,4'}$ = 2.6 Hz, 1 H, H-5'$_a$), 3.70 (dd, $J_{5'b,5'a}$ = 12.4 Hz, $J_{5'b,4'}$ = 3.6 Hz, 1 H, H-5'$_b$), 2.27-2.70 (m, 2 H, H-3'$_{a,b}$); accurate mass positive ion FAB MS m/z 254.1059 (MH^+, calcd 254.1052). Anal. ($C_{10}H_{12}FN_5O_2$) C, H, N.

Antiviral Activity against HIV in Vitro. Experiments on the protection of CD4+ ATH8 cells from the cytopathogenic effects of HIV-1 were carried out as previously described[24] with the exception that incorporation of [^{3}H]thymidine rather than the trypan blue exclusion method was used to quantitate protection of these susceptible cells. The target ATH8 cells were pulsed with [^{3}H]thymidine for 24 h and harvested onto glass filters and the radioactivity counted. This method is similar to that described earlier.[5a] The inhibition of the infectivity and cytopathic effect of HIV-2 was determined by exposing ATH8 cells (2×10^5) to an exceedingly potent HIV-2/LAV preparation (100 virus particles/cell). Cells were cultured in the presence of drug and/or virus. On day 7, the total viable cells were counted by the trypan dye exclusion method. In inhibition of *gag* protein expression experiments, H9 cells (10^5) were exposed to HIV-1 (500 virus particles per cell) and cultured in the absence and presence of various concentrations of 1-4. On days 7, 10, and 12 in culture, the percentage of the target H9 cells expressing p24 *gag* protein of HIV-1 was determined by indirect immunofluorescence assay using a murine monoclonal antibody (M26). Toxicity to antigen- and mitogen-driven proliferation of immunocompetent cells was conducted as previously described.[5a] The data are the average of triplicate determinations.

Supplementary Material Available: The NMR spectrum of 2'-F-dd-ara-I (4) (1 page). Ordering information is given on any current masthead.

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Synthesis and Biological Activity of Novel Quaternary Ammonium Derivatives of Alkylglycerols as Potent Inhibitors of Protein Kinase C

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Alkylglycerols such as *rac*-1-*O*-octadecyl-2-*O*-methylglycerophosphochocholine (Et-18-OMe) have shown an inhibitory effect on the metastasis and growth of various cancer cell lines. Alkyl phospholipids have been shown to accumulate at the surface in several cell lines, the selectivity of which is still not clearly understood. A consequence of this action may lead to the inhibition of cell membrane related protein kinase C (PKC). The goal of this research was to develop ether lipid inhibitors of PKC to augment antineoplastic activity. This led to the synthesis and in vitro testing of a series of novel quaternary ammonium derivatives of alkylglycerols. The biological testing of these analogues on PKC stimulated with *rac*-1-*O*-oleoyl-2-*O*-acetylglycerol showed several analogues with inhibition comparable to that of Et-18-OMe.

Since the discovery of protein kinase C (PKC),[1] the function of this ubiquitous kinase as a regulatory enzyme has been extensively investigated. Central to our interests is the interaction between PKC and naturally-occurring phospholipids and diacylglycerols.[1,2] The hydrolysis of phosphatidylinositol by phospholipase C yields diacylglycerol which acts as a stimulus for PKC.[1-3] Activation of PKC results in the translocation of the enzyme to the cell membrane, where phosphatidylserine and diacylglycerol, in the presence of calcium, are able to fully activate the enzyme.[2] Contrary to the action of diacylglycerols, endogenous alkylacylglycerols derived by phosphocholine hydrolysis[4-6] are inhibitors of PKC. In addition, synthetic dialkyl linked glycerols inhibit activation of PKC by diacylglycerols.[6]

Synthetic alkyl analogues of phosphatidylcholine such as *rac*-1-*O*-octadecyl-2-*O*-methylglycerophosphocholine (Et-18-OMe, Chart I) have also shown an inhibitory effect on this kinase.[7,8] Alkyl phospholipids are also known to

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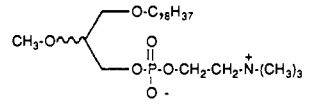
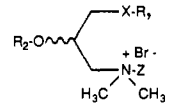
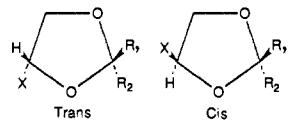
Chart I. Overview of Analogue Structures



Et-18-OMe

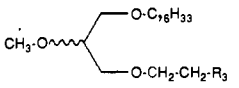


7A X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$, $Z = CH_2CH_2OH$
7B X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3CH_2$, $Z = CH_2CH_2OH$
7C X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3CH_2$, $Z = CH_2CH_2CH_2OH$
7D X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$, $Z = CH_3$
7E X = O, R_1 = Naphthyl, $R_2 = CH_3$, $Z = CH_2CH_2OH$
17[a] X = O, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$, $Z = CH_2CH_2OH$
18[a] X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$, $Z = CH_2CH_2CH_2OH$



Trans Cis

8 $R_1 = R_2 = CH_3$, $X = CH_2N(CH_3)_2CH_2CH_2OH$ Br⁻
9 Cis and Trans $R_1 = C_{17}H_{35}$, $R_2 = H$, $X = CH_2N(CH_3)_2CH_2CH_2CH_2OH$ Br⁻
9A Trans $R_1 = C_{17}H_{35}$, $R_2 = H$, $X = CH_2N(CH_3)_2CH_2CH_2CH_2OH$ Br⁻



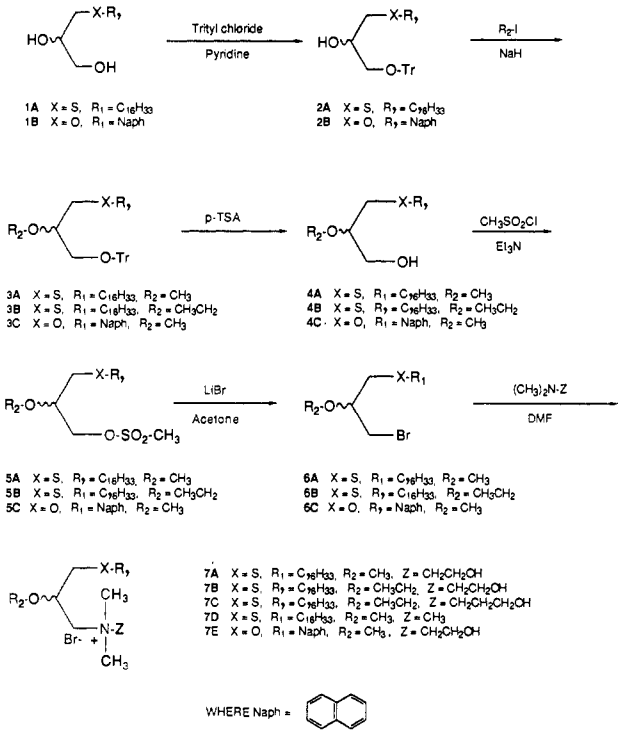
13 R_3 = OH
16 $R_3 = N(CH_3)_3$ $^+$ Br⁻

a. these analogs are as the iodo salt

possess antineoplastic activity against various tumor cell lines.[9] The efficacy and applicability of this new antineoplastic agent is supported by its current evaluation in phase 1 clinical trials.[10] Although the exact mechanism of action for this antineoplastic activity is not known, the inhibitory effect of these analogues on PKC may be involved. In support of this hypothesis, Helfman et al.[7] have shown that not only was PKC inhibited by Et-18-OMe, but also that it was preferentially inhibited over the other kinases present in the HL-60 cells. Since HL-60 cells are sensitive to the antineoplastic action of Et-18-OMe, the development of more potent inhibitors of PKC may enhance antineoplastic activity.

On the basis of these observations, a series of novel analogues of alkyl ether lipids were synthesized. The structures of the synthesized analogues are shown in Chart I. With the exception of compounds **13** and **16**, these compounds are quaternary ammonium analogues of alkylglycerols which incorporate an "inverse choline" moiety into their structure. Although the inverse choline moiety cannot be viewed as an isosteric replacement of the phosphocholine functionality, its incorporation into these analogues offers certain advantages. First, the inverse choline analogues retain a hydrophilic moiety, but unlike a phosphocholine functionality, they should not be susceptible to degradation by phospholipases C and D. Second, the inverse choline analogues retain features present in dialkylglycerols which, as previously stated,

Scheme I. Synthesis of Racemic Quaternary Ammonium Alkylglycerols



1A X = S, $R_1 = C_{16}H_{33}$
1B X = O, R_1 = Naph
2A X = S, $R_2 = C_{16}H_{33}$
2B X = O, R_2 = Naph
3A X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$
3B X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3CH_2$
3C X = O, R_1 = Naph, $R_2 = CH_3$
4A X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$
4B X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3CH_2$
4C X = O, R_1 = Naph, $R_2 = CH_3$
5A X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$
5B X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3CH_2$
5C X = O, R_1 = Naph, $R_2 = CH_3$
6A X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$
6B X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3CH_2$
6C X = O, R_1 = Naph, $R_2 = CH_3$
7A X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$, $Z = CH_2CH_2OH$
7B X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3CH_2$, $Z = CH_2CH_2OH$
7C X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3CH_2$, $Z = CH_2CH_2CH_2OH$
7D X = S, $R_1 = C_{16}H_{33}$, $R_2 = CH_3$, $Z = CH_3$
7E X = O, R_1 = Naph, $R_2 = CH_3$, $Z = CH_2CH_2OH$

inhibit PKC.[6] These features are a free hydroxyl group and a dialkyl ether moiety. Since these analogues resemble both dialkylglycerols and alkylphosphocholines, their design may facilitate binding to the highly stereospecific diacylglycerol binding site and/or the ambiguous PS binding site.

Chemistry

Quaternary ammonium analogues **7A–E** of alkylglycerols were synthesized according to the procedure outlined in Scheme I. Starting with *rac*-1-*S*-hexadecylthioglycerol, the primary hydroxyl group was selectively protected with (triphenylmethyl)trityl chloride in pyridine to give compound **2A**.[11] The secondary hydroxyl group of compound **2A** was alkylated with either iodomethane or iodoethane in THF and sodium hydride to yield compounds **3A** and **3B**, respectively.[9] The trityl group was cleaved with *p*-toluenesulfonic acid (*p*-TSA) in chloroform/methanol (3:1) to provide alcohols **4A** and **4B**.[12] The primary hydroxyl group was then mesylated to give sulfonate esters **5A** and **5B**. Displacement of the mesylate in the presence of a 4-fold excess of lithium bromide in refluxing acetone provided compounds **6A** and **6B**. Direct amination of the halide analogues with either 2-(*N*,*N*-dimethylamino)-ethanol or 3-(*N*,*N*-dimethylamino)propanol gave the expected products **7A–C**.[13] Direct amination of compound **6A** with dry trimethylamine in CH_3CN heated in a sealed tube gave compound **7D**. In order to investigate the importance of a long alkyl chain at the 1-position, analogue **7E**, in which the alkyl chain has been replaced with a aromatic moiety, was synthesized as shown in Scheme I. The sulfonate ester of 2,2-dimethyl-4-(hydroxymethyl)-1,3-dioxolane[12] was first displaced with 1-naphthol in the

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Table I. Percent Protein Kinase C[a] Activity Retained following Incubation with Quaternary Ammonium Alkylglycerols[b]

		% protein kinase C activity retained					
compd[c]	ID_{50}	1.25 μM	2.5 μM	5.0 μM	10.0 μM	20.0 μM	40.0 μM
Et-18-OMe	12 ± 7	99 ± 8	93 ± 12	73 ± 20	60 ± 22	40 ± 13	29 ± 14
7A	27 ± 16	97 ± 14	90 ± 13	73 ± 12	60 ± 4	54 ± 11	42 ± 10
7B	37 ± 20	92 ± 5	84 ± 7	70 ± 7	70 ± 16	66 ± 25	47 ± 12
7C	26 ± 13	78 ± 9	64 ± 12	67 ± 18	60 ± 19	59 ± 15	37 ± 6
7D	25 ± 4	112 ± 23	88 ± 5	81 ± 7	74 ± 4	54 ± 9	29 ± 5
7E	≫40	99 ± 3	99 ± 5	83 ± 20	89 ± 13	87 ± 12	90 ± 6
8	≫40	88 ± 1	84 ± 1	87 ± 3	88 ± 6	96 ± 14	87 ± 8
9[d]	13 ± 5	99 ± 9	99 ± 11	61 ± 7	56 ± 6	36 ± 11	12 ± 2
9A	17 ± 8	94 ± 15	90 ± 11	70 ± 11	56 ± 11	43 ± 12	19 ± 8
13	28 ± 21	98 ± 8	86 ± 9	72 ± 12	66 ± 15	53 ± 20	43 ± 14
16	31 ± 12	92 ± 3	91 ± 5	83 ± 4	73 ± 8	57 ± 7	43 ± 13
17	17 ± 6	107 ± 20	95 ± 20	87 ± 18	64 ± 19	42 ± 15	29 ± 9
18	5 ± 4	75 ± 13	56 ± 11	49 ± 23	39 ± 21	27 ± 14	23 ± 8

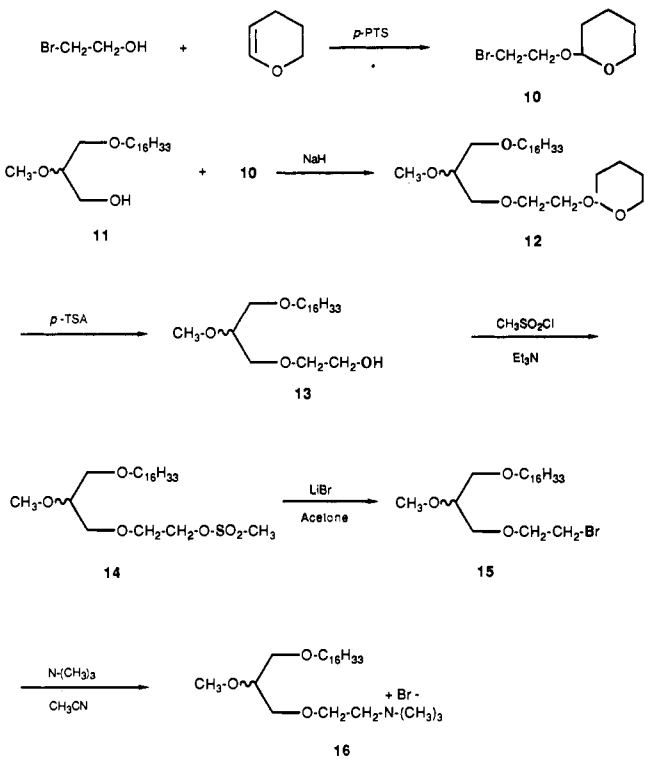
[a] PKC was isolated from HL-60 cells as described in the Experimental Section. Several analogues were tested on different preparations of PKC. [b] Each analogue and Et-18-OMe were tested in duplicate in three separate trials using a fresh preparation of PKC each time. [c] These analogues are illustrated in Chart I. [d] Mixture of 64% trans and 36% cis as determined by 400-MHz NMR.

presence of NaH, with subsequent removal of the protective ketal under acidic conditions, to produce diol derivative **1B**. The remaining intermediates and the final product **7E** were synthesized following the general reaction sequence described above for **7A–C**.

In an attempt to reduce the rotational degrees of freedom associated with the glycerol backbone, a cyclic ketal and acetal were synthesized (Chart I, analogues **8** and **9**). 3-Bromo-1,2-propanediol was converted to a cyclic ketal upon reaction with acetone and a catalytic amount of concentrated sulfuric acid. Amination of the intermediate 2,2-dimethyl-4-(bromomethyl)-1,3-dioxolane was carried out with 2-(*N*,*N*-dimethylamino)ethanol in CH_3CN to provide compound **8**. Cyclic acetal **9** was prepared by the transacetalation of octadecanal dimethyl acetal with glycerol in the presence of sulfosalicylic acid.[14] The remaining intermediates and the final product **9**, as a diastereomeric mixture of 64% trans and 36% cis, were synthesized following the general reaction sequence described for **7A–E**. The preparation of the trans and cis isomers of analogue **9** was accomplished by Prep-HPLC separation of the 3,5-dinitrobenzoate derivative of 2-heptadecyl-4-(hydroxymethyl)-1,3-dioxolane. The trans and cis isomers of analogue **9** were then synthesized in an analogous manner to that shown in Scheme I. The cis isomer of analogue **9** can be made with relatively high diastereomeric purity; however, it undergoes rapid isomerization to a mixture which contains predominately the trans isomer, which possibly occurs due to steric factors. The separation and characterization of the trans and cis isomers of analogue **9** will be presented in a future paper.

Finally, in order to investigate the requirement of the quaternary nitrogen at the 3-position of the glycerol backbone for PKC inhibitory activity, analogues **13** and **16** were synthesized according to Scheme II. Alkylation of the dialkylglycerol **11** with bromo derivative **10**, gave intermediate **12**. Cleavage of the protective tetrahydropyran with *p*-TSA in chloroform/methanol (3:1) yielded analogue **13**. The hydroxyl group of compound **13** was then converted in consecutive steps to the corresponding trimethylammonium analogue **16** following the general reaction sequence in Scheme I.

Scheme II. Synthesis of *rac*-2-[3-(Hexadecyloxy)-2-(methoxy)propoxy]-N,N,N-trimethyl-1-ethanaminium Bromide



corporation of ^{32}P into histones in the presence of 1-*O*-oleoyl-2-*O*-acetylglycerol (OAG) and phosphatidylserine (PS). The enzyme activity in the absence of OAG was also determined. Several of the analogues reduced enzyme activity below the levels shown when OAG was not present. This may indicate that these analogues were reversing activation of PKC due not only to OAG but also to PS. In an attempt to further refine the inhibitory properties of these analogues, compound **9** was assayed in varying concentrations of PS (5 and 20 μg/mL) in the presence of OAG (5 μM) and also in varying concentrations of OAG (5 and 20 μM) in the presence of PS (5 μg/mL). As illustrated by the Dixon plot in Figure 1, this analogue competitively reversed stimulation of PKC due to PS, but not OAG. This form of inhibition was also seen with the dialkylglycerol derivative **17**. Previous literature reports[2] have shown that many structurally dissimilar compounds may antagonize PKC in this manner (i.e. sphingosine, acylcarnitine, trifluoperazine, and staurosporine). How-

Results and Discussion

Protein Kinase C Inhibition. The quaternary ammonium derivatives were tested for inhibition of PKC.[6] Enzymatic activity in this assay was defined as the in-

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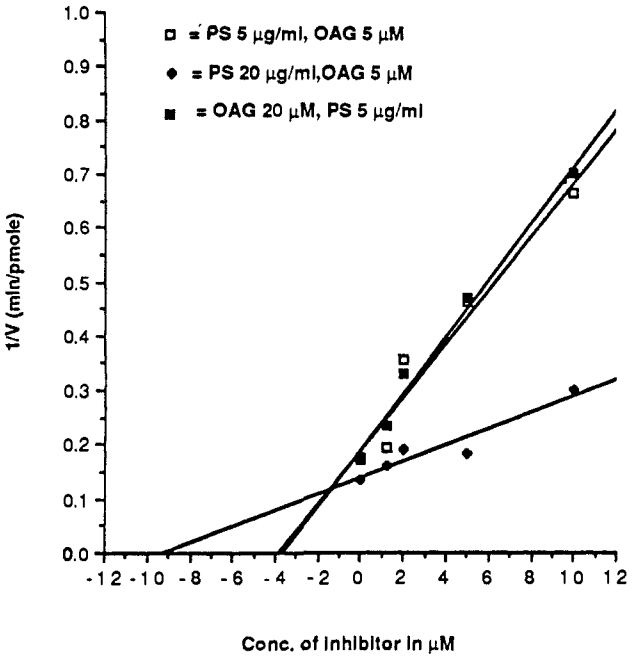

Figure 1. PKC inhibition profile of analogue **9**.

ever, all of these compounds are cationic and amphipathic in nature. Since these analogues are disrupting activation of PKC by interfering with PS and not diacylglycerol, the stereochemistry of these analogues is not critical to inhibitory activity.

In this assay, compound **18** (Table I) displayed the most pronounced inhibition of PKC activity. All of the remaining analogues, except **7E** and **8**, were inhibitors of PKC. Previous experimentation in our laboratory has indicated that there is no significant difference in potency between an 18-carbon and a 16-carbon alkyl chain in HL-60 cell growth inhibition[9] and PAF activity.[15] Studies with analogues of this type have indicated that alkyl chains shorter than 16 carbons do reduce activity.[13] Therefore, we decided to focus our studies at the 2- and 3-positions of the backbone. Evaluation of the biological data presented in Table I offered several key insights into the structure–activity relationship (SAR) of these analogues. First, replacement of the oxygen ether at position 1 with a thioether resulted in no statistically significant difference in activity, as indicated by the activity of compound **7A** versus that of **17**. Second, a long chain alkyl ether at position 1 of the three-carbon backbone was essential for activity. This is supported by the inability of analogues **7E** and **8** to significantly inhibit PKC. Third, incorporation of the methoxy and ethoxy alkyl groups at position 2 of the backbone resulted in no significant difference in activity as shown by analogues **7A** versus **7B**. Current investigations are underway to determine whether longer alkyl or acyl moieties at the 2-position of the three-carbon backbone will affect PKC inhibition. The formation of a dioxolane ring system, as observed in analogue **9**, resulted in retention of inhibitory activity. However, restricted rotation of the glycerol backbone does not singularly explain the retention of activity of analogue **9**, for when compared to analogue **8**, it is evident that a long alkyl chain is still essential for activity. It should also be noted that analogue **9**, a diastereomeric mixture of trans and cis (64% and 36% respectively), and the pure trans isomer **9A** retained the same activity. Facile isomerization of the cis isomer to a mixture of trans and cis prevented enzymatic evaluation of this pure isomer. However, this does reaffirm the assertion that stereochemistry is not a key element in cationic amphipathic inhibitors of PKC. This would indicate that the spacial presentation of the long hydrocarbon chain and the quaternary nitrogen do not affect inhibitory activity, but their incorporation into an analogue is necessary to produce inhibition of PKC. Fourth, variations in the chain length of the *N,N*-dimethylammonium alcohol affected activity. Propanol adduct **18** showed a slightly greater inhibitory activity (i.e. 2.5–40 μM) than the ethanol adduct **7A**. Fifth, the replacement of the inverse choline group with a trialkylammonium moiety, as in analogue **7D**, resulted in an inhibition of the enzyme. This inhibition was analogous to that observed with the compound Et-18-OMe, and *N,N*-dimethylammonium ethanol analogue **7A**. The inhibition produced by **7D** (i.e. 1.25–20 μM) was not as pronounced as that of *N,N*-dimethylammonium propanol analogue **18**. This indicates that a free hydroxyl group is not essential for inhibitory activity; however, in this series of analogues optimum inhibition results from the incorporation of a propanol functionality. Sixth, replacement of the quaternary nitrogen of the inverse choline with an oxygen atom did not affect PKC inhibition as seen by comparing compounds **13** and **17**. However, preliminary results from HL-60 cell growth inhibition studies have indicated that the replacement of the quaternary nitrogen with an oxygen atom completely abolishes the cytotoxicity of these analogues (ID_{50} > 10 μM). Finally, placement of the quaternary nitrogen at the end of a short alkyl chain attached to the 3-position of the backbone, did not statistically increase or decrease PKC inhibition as seen with analogue **16** versus **17**.

Prior experimentation with ethanediol and three carbon backbone derivatives with a quaternary ammonium functionality have been shown to significantly inhibit KB cell growth in vitro.[13] This study involving structural modifications on the compound Et-18-OMe has identified a series of analogues with improved PKC inhibition. Preliminary enzymatic studies have shown that these analogues act as competitive inhibitors of the PKC activator PS. As previously stated, the inhibition of PKC by Et-18-OMe has been linked to its antineoplastic activity.[7] In vitro and in vivo studies with these quaternary ammonium analogues of alkylglycerols are in progress in order to evaluate their antineoplastic activity and to determine if increased PKC inhibition will continue to parallel antineoplastic activity.

Experimental Section

Chemicals. Proton nuclear magnetic resonance spectra were recorded in $CDCl_3$ on either a JEOLCO 60-MHz or a Varian 80-MHz and 400-MHz spectrometer. Chemical shifts are reported in parts per million relative to internal tetramethylsilane. Infrared spectra were recorded on a Perkin-Elmer 1320 spectrometer as thin films. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab Inc. All reactions were performed under a positive pressure of dry nitrogen with dry solvents. Tetrahydrofuran (THF) was distilled from Na and benzophenone, dichloromethane (DCM) was distilled from phosphorus pentoxide, triethylamine (Et_3N) was distilled from KOH, acetonitrile (CH_3CN) was distilled from calcium hydride, and dimethylformamide (DMF) was placed over potassium hydroxide 1 week before use. Chromatographic purification was performed with silica gel 60 (230–400 mesh). Thin-layer chromatographic plates were visualized either by iodine vapor or charring following sulfuric acid spray. To determine the purity of all final analogues a typical TLC would first be developed in $CHCl_3$, followed by $CHCl_3$/MeOH (9:1), and finally $CHCl_3$/MeOH (6:1) with iodine vapor visualization. The final analogues are

(15) Surles, J. R. Ph.D. Dissertation, University of North Carolina at Chapel Hill, 1987.

stored over calcium sulfate in a desiccator at room temperature. After a period of 6 months TLC shows no decomposition.

(±)-1-*S*-Hexadecylthioglycerol (1A). To a stirred solution of 3-mercapto-1,2-propanediol (40.0 g, 0.037 mol) in 300 mL of 95% ethanol was added KOH (24.9 g, 0.44 mol) under an inert atmosphere of nitrogen. Then, a solution of bromohexadecane (135.0 g, 0.44 mol) in 100 mL of 95% ethanol was added dropwise over a 1-h period, and the reaction was allowed to proceed at room temperature with vigorous stirring for 24 h. The precipitate was filtered, taken up in 1 L of boiling methanol, and cooled to 0 °C for 12 h. The precipitate was filtered and allowed to air-dry, producing 117 g (95%) of product [mp 72–74 °C (lit.[9] mp 76–77 °C)].

(±)-1-*O*-1′-Naphthylglycerol (1B). Into a three-neck 500-mL round-bottom flask equipped with a reflux condenser, stir bar, and a nitrogen inlet was added a slurry of 60 mL of DMF and 2.5 g (0.083 mol) of 80% NaH (oil dispersion). A solution of 6.0 g (0.042 mol) of 1-naphthol in 70 mL of DMF was added to the slurry over a period of 30 min. The reaction mixture was then heated at a gentle reflux for 1 h, followed by the slow addition of 10.7 g (0.051 mol) of 2,2-dimethyl-4-(mesylmethyl)-1,3-dioxolane in 45 mL of DMF. Heating and stirring was continued for 5 h. The reaction mixture was then cooled to 0 °C before 1 mL of water was added and the reaction mixture was filtered. The filtrate was diluted with 200 mL of ether and extracted once with 150 mL of water. The nonaqueous layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexane/ethyl acetate 10:1 as eluent) provided 7.8 g of a clear oil. This oil was transferred to a 250-mL round-bottom flask equipped with a reflux condenser and stir bar, and a solution of 3 mL of concentrated HCl in 100 mL of methanol was added. The reaction mixture was refluxed for 12 h, the solvent was removed in vacuo, and the residue was dissolved in 100 mL of ether. The organic layer was extracted once with 100 mL of water, and the water layer was back-extracted twice with 50-mL portions of ether. The ether layers were combined, dried over anhydrous sodium sulfate, filtered, and removed in vacuo to yield a solid. Recrystallization from hexanes and ethyl acetate provided 5.5 g (60.5% based on 1-naphthol) of pure diol **1B**, (mp 93–95.5 °C). ^{1}H NMR ($CDCl_3$): δ 2.5 (b s, 2 H, 2 OH), 3.7–4.25 (m, 5 H, OCH_2CHCH_2O), 6.75–9.95 (m, 1 H, naph), 7.35–7.6 (m, 4 H, naph), 7.7–7.95 (m, 1 H, naph), 8.1–8.3 (m, 1 H, naph).

(±)-1-*S*-Hexadecyl-3-*O*-tritylthioglycerol (2A). To a round-bottom flask equipped with a magnetic stir bar and drying tube was added a solution of compound **1A** (70 g, 0.2 mol) in 300 mL of pyridine, followed by the addition of triphenylmethyl chloride (76.0 g, 0.27 mol). The reaction mixture was stirred at room temperature. After 36 h, the pyridine was removed in vacuo and the resulting oil was dissolved in 450 mL of $CHCl_3$. The solution was extracted twice with 500-mL portions of 5% HCl and twice with 500-mL portions of water and the nonaqueous solution was dried over anhydrous sodium sulfate. The drying agent was filtered, the solvent was removed in vacuo, the residue was dissolved in 500 mL of hexanes, and the solution was placed at −5 °C for 24 h. The resulting precipitate was redissolved in 500 mL of acetone and reprecipitated as before. The final precipitate was filtered and dried under vacuum to give 113 g (94%) of product, [mp 61–62 °C (lit.[9] 60.5–61.5 °C)]. ^{1}H NMR ($CDCl_3$): δ 0.7–1.7 (m, 31 H, $(CH_2)_{14}CH_3$), 2.3–2.8 (m, 4 H, CH_2SCH_2), 3.25 (d, 2 H, $CH_2OC(C_6H_5)_3$), 3.6–4.0 (m, 1 H, CH_2CHCH_2), 7.0–7.6 (m, 15 H, $C(C_6H_5)_3$).

(±)-1-*O*-1′-Naphthyl-3-*O*-tritylglycerol (2B). Into a 200-mL round-bottom flask equipped with a reflux condenser, stir bar, and nitrogen inlet was added a solution of compound **1b** (5.4 g, 0.025 mol), 50 mL of DCM, and 10 mL of triethylamine. A solution of triphenylmethyl chloride (8.3 g, 0.029 mol) was added followed by 0.1 g of 4-(dimethylamino)pyridine. The reaction mixture was stirred overnight at room temperature. The reaction mixture was extracted with two 50-mL portions of water, 5% HCl (until slightly acidic), and finally with water. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to yield a viscous oil. Purification by silica gel chromatography eluting with a discontinuous gradient of 12:1 to 5:1 hexanes/ethyl acetate provided 8.5 g (75%) of ether **2B**. ^{1}H NMR ($CDCl_3$): δ 2.5 (b s, 2 H, 2 OH), 3.5 (d, 2 H, $CH_2O(C_6H_5)_3$), 4.3 (m, 3 H, OCH_2CHCH_2O), 6.8–6.9 (m, 1 H, naph), 7.15–7.65 (m, 19 H, naph, $C(C_6H_5)_3$), 7.7–7.95 (m, 1 H, naph), 8.1–8.3 (m, 1 H, naph).

(±)-1-*S*-Hexadecyl-2-*O*-methyl-3-*O*-tritylthioglycerol (3A). A solution of compound **2A** (6.0 g, 0.01 mol) in 250 mL of THF was added to a slurry of 80% NaH (oil dispersion) (0.4 g, 0.14 mol) in 50 mL of THF and the reaction mixture stirred at room temperature for 1 h. Then CH_3I (1.4 g, 0.13 mol) was added and the reaction was allowed to proceed at room temperature for 24 h. The reaction mixture was then diluted with 300 mL of distilled water and extracted twice with 200-mL portions of ether. The nonaqueous extracts were combined, dried over anhydrous sodium sulfate, filtered, and removed in vacuo to provide 6.0 g of crude product as an oil. ^{1}H NMR ($CDCl_3$): δ 0.7–1.7 (m, 31 H, $(CH_2)_{14}CH_3$), 2.3–2.8 (m, 4 H, CH_2SCH_2), 3.25 (d, 5 H, CH_3O, $CHCH_2OC(C_6H_5)_3$), 3.6–4.0 (m, 1 H, CH), 7.0–7.6 (m, 15 H, $C(C_6H_5)_3$).

(±)-1-*S*-Hexadecyl-2-*O*-ethyl-3-*O*-tritylthioglycerol (3B). This compound was prepared in a manner analogous to that of compound **3A** from compound **2A** (10.0 g, 0.02 mol) and CH_3CH_2I (3.3 g, 0.03 mol) to provide 10.0 g of crude product as an oil. ^{1}H NMR ($CDCl_3$): δ 0.7–1.7 (m, 34 H, $(CH_2)_{14}CH_3$, CH_3CH_2O), 2.3–2.8 (m, 4 H, CH_2SCH_2), 3.5–3.9 (m, 5 H, CH_2OCH, $CH_2OC(C_6H_5)_3$), 7.1–7.5 (m, 15 H, $C(C_6H_5)_3$).

(±)-1-*S*-Hexadecyl-2-*O*-methylthioglycerol (4A). To a solution of compound **3A** (6.0 g, 0.01 mol) in 100 mL of chloroform/methanol (3:1) was added 0.2 g of *p*-toluenesulfonic acid and the reaction was stirred at room temperature for 24 h. The reaction mixture was neutralized with 1.0 g of sodium bicarbonate and then extracted three times with 200-mL portions of water. The nonaqueous layer was separated and the solvent was removed in vacuo to give the crude product as an oil. Silica gel chromatography ($CHCl_3$ eluent) provided 3.1 g (89%) of pure product as a semiwaxy solid. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 28 H, $(CH_2)_{14}$), 2.58 (m, 2 H, SCH_2), 2.62 (m, 2 H, $CHCH_2S$), 3.40 (m, 1 H, CH), 3.44 (s, 3 H, CH_3O), 3.75 (m, 2 H, CH_2OH).

(±)-1-*S*-Hexadecyl-2-*O*-ethylthioglycerol (4B). This compound was prepared in a manner analogous to that of compound **4A** from compound **3B** (10.0 g, 0.02 mol) and 0.2 g of *p*-toluenesulfonic acid. Silica gel chromatography ($CHCl_3$ eluent) provided 5.8 g (95%) of a semiwaxy solid. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 31 H, $(CH_2)_{14}$, CH_3CH_2O), 2.58 (m, 2 H, SCH_2), 2.62 (m, 2 H, $CHCH_2S$), 3.40–3.50 (m, 3 H, CH, CH_3CH_2O), 3.75 (m, 2 H, CH_2OH).

(±)-1-*O*-1′-Naphthyl-2-*O*-methylglycerol (4C). Alkylation of compound **2B** was performed in a manner analogous to that of compound **3A** from compound **2B** (8.1 g, 0.018 mol), 80% NaH (oil dispersion) (0.90 g, 0.300 mol), and 2.5 mL (0.040 mol) of iodomethane to provide 8.7 g of compound **3C** as a glassy oil. Compound **3C** without further purification was directly deprotected by using a procedure similar to that utilized for compound **4A**. Trityl ether **3C** was reacted with 1.0 g of *p*-toluenesulfonic acid in 70 mL of a 5:2 mixture of chloroform and methanol. Purification by silica gel chromatography (elution with a discontinuous gradient of 4:1 to 1:1 of hexanes/ethyl acetate) provided 3.8 g (92.7% based on starting compound **2B**) of compound **4C**. ^{1}H NMR ($CDCl_3$): δ 2.35 (b s, 1 H, OH), 3.5 (b s, 3 H, OCH_3), 3.65–3.9 (m, 3 H, $CHCH_2OH$), 4.13 (d, 2 H, CH_2O-naph), 6.65–6.85 (m, 1 H, naph), 7.25–7.5 (m, 4 H, naph), 7.6–7.85 (m, 1 H, naph), 8.05–8.3 (m, 1 H, naph).

(±)-1-*S*-Hexadecyl-2-*O*-methyl-3-*O*-mesylthioglycerol (5A). To a solution of compound **4A** (3.0 g, 0.009 mol) in 200 mL of DCM and 1.0 mL (0.010 mol) of triethylamine was added methanesulfonyl chloride (1.2 g, 0.012 mol) and the reaction was stirred at room temperature for 24 h. The reaction mixture was then extracted twice with 200-mL portions of water, twice with 200-mL portions of 5% HCl, once with 200 mL of saturated $NaHCO_3$, and once with 200 mL of brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to yield 3.4 g (89%) of a colorless oily residue. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 28 H, $(CH_2)_{14}$), 2.58 (m, 2 H, SCH_2), 2.62 (m, 2 H, $CHCH_2S$), 3.08 (s, 3 H, SO_2CH_3), 3.40–3.50 (m, 4 H, CH, CH_3O), 4.35 (m, 2 H, CH_2OSO_2).

(±)-1-*S*-Hexadecyl-2-*O*-ethyl-3-*O*-mesylthioglycerol (5B). This compound was prepared in a manner analogous to that of compound **5A** from compound **4B** (5.5 g, 0.016 mol) and 2.2 g (0.019 mol) of methanesulfonyl chloride. This resulted in 6.2 g of product (94%) as an oil. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 31 H, $(CH_2)_{14}$, CH_3CH_2O), 2.58 (m, 2 H, SCH_2), 2.62 (m, 2 H, $CHCH_2S$), 3.08 (s, 3 H, SO_2CH_3), 3.40–3.50 (m, 3 H, CH, CH_3CH_2O), 4.35 (m, 2 H, CH_2OSO_2).

(±)-1-*O*-1′-Naphthyl-2-*O*-methyl-3-*O*-mesylglycerol (5C). This compound was synthesized in a manner analogous to that of compound **5A** from alcohol **4C** (2.85 g, 0.012 mol) and 2.1 mL (0.016 mol) of methanesulfonyl chloride. Purification by silica gel chromatography using a discontinuous gradient of 5:1 to 2:1 hexanes/ethyl acetate yielded 3.4 g (89.5%) of sulfonate ester **5C**. ^{1}H NMR ($CDCl_3$): δ 2.9 (s, 3 H, SO_2CH_3), 3.5 (s, 3 H, OCH_3), 3.8–4.0 (m, 1 H, $CHOCH_3$), 4.1–4.25 (m, 2 H, CH_2O-naph), 4.35–4.5 (m, 2 H, CH_2OSO_2), 6.65–6.8 (m, 1 H, naph), 7.25–7.5 (m, 4 H, naph), 7.65–7.8 (m, 1 H, naph), 8.1–8.25 (m, 1 H, naph).

(±)-1-(Hexadecylthio)-2-methoxy-3-bromopropane (6A). In a flask equipped with a magnetic stir bar, drying tube, and a reflux condenser, a solution of compound **5A** (3.0 g, 0.007 mol) and lithium bromide (2.5 g, 0.029 mol) in 150 mL of acetone was refluxed for 24 h with continuous stirring. The reaction was cooled to room temperature, the lithium mesylate was filtered, and the solvent was evaporated in vacuo. The residue was dissolved in 300 mL of ether and extracted three times with 500-mL portions of sodium thiosulfate, twice with 500-mL portions of water, and the organic layer was dried over anhydrous sodium sulfate. Filtration of the drying agent, evaporation of the ether in vacuo, and subsequent silica gel chromatography (hexane/ether 9:1 as eluent) afforded 2.5 g (87%) of an oily product. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 28 H, $(CH_2)_{14}$), 2.58 (m, 2 H, SCH_2), 2.62 (m, 2 H, $CHCH_2S$), 3.38 (s, 3 H, OCH_3), 3.48 (m, 3 H, CH_2Br, OCH).

(±)-1-(Hexadecylthio)-2-ethoxy-3-bromopropane (6B). This compound was prepared in a manner analogous to that of compound **6A** from 6.0 g (0.014 mol) of compound **5B** and lithium bromide (20.0 g, 0.07 mol). Silica gel column chromatography as before gave 5.5 g (93%) of product as an oil. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 31 H, $(CH_2)_{14}$, CH_3CH_2O), 2.58 (m, 2 H, SCH_2), 2.62 (m, 2 H, $CHCH_2S$), 3.3–3.5 (m, 5 H, CH, CH_3CH_2O, CH_2Br).

(±)-1-(1′-Naphthyloxy)-2-methoxy-3-bromopropane (6C). This intermediate was synthesized in a manner analogous to that of compound **6A** from compound **5C** (3.15 g, 0.010 mol) and 4.3 g (0.041 mol) of lithium bromide in 30 mL of acetone, resulting in 2.7 g (90%) of the product as an oil. ^{1}H NMR ($CDCl_3$): δ 3.5 (s, 3 H, OCH_3), 3.6–3.9 (m, 3 H, $CHCH_2Br$), 4.25 (d, 2 H, CH_2O-naph), 6.7–6.85 (m, 1 H, naph), 7.3–7.6 (m, 4 H, naph), 7.7–7.9 (m, 1 H, naph), 8.2–8.35 (m, 1 H, naph).

(±)-*N*-[3-(Hexadecylthio)-2-methoxyprop-1-yl]-*N*,*N*-dimethyl-*N*-(β-hydroxyethyl)ammonium Bromide (7A). Into a two-neck 25-mL round-bottom flask equipped with an air condenser, thermometer, and stir bar was placed compound **6A** (2.0 g, 0.05 mol), 0.5 mL (0.006 mol) of 2-(*N*,*N*-dimethylamino)ethanol, and 15 mL of DMF. The solution was maintained at 45–50 °C for 72 h with continuous stirring. The reaction mixture was then cooled to room temperature, 125 mL of ether was added, and the solution was kept at 0 °C for 24 h. The resulting precipitate (800 mg) was filtered and swirled with five 50-mL portions of ether to give **7A** (32%) (mp 107–109 °C). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 28 H, $(CH_2)_{14}$), 2.45–3.0 (m, 4 H, CH_2SCH_2), 3.48 (s, 9 H, CH_3O, $N(CH_3)_2$), 3.9–4.3 (m, 7 H, $CHCH_2NCH_2CH_2OH$). Anal. ($C_{24}H_{52}NO_2SBr$) C, H, N.

(±)-*N*-[3-(Hexadecylthio)-2-ethoxyprop-1-yl]-*N*,*N*-dimethyl-*N*-(β-hydroxyethyl)ammonium Bromide (7B). This product was prepared in a manner analogous to that of compound **7A** from compound **6B** (2.2 g, 0.005 mol) and 0.5 mL (0.006 mol) of 2-(*N*,*N*-dimethylamino)ethanol to give 775 mg (30%) of product (mp 112–113 °C). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 31 H, $(CH_2)_{14}$, CH_3CH_2O), 2.58 (m, 2 H, SCH_2), 2.7–2.9 (m, 2 H, CH_2S), 3.48 (s, 6 H, $N(CH_3)_2$), 3.9–4.3 (m, 9 H, $CHCH_2NCH_2CH_2OH$, CH_3CH_2O). Anal. ($C_{25}H_{54}NO_2SBr$) C, H, N.

(±)-*N*-[3-(Hexadecylthio)-2-ethoxyprop-1-yl]-*N*,*N*-dimethyl-*N*-(γ-hydroxypropyl)ammonium Bromide (7C). This product was prepared in a manner analogous to that of compound **7A** from compound **6B** (2.2 g, 0.005 mol) and 0.6 mL (0.006 mol) of 3-(*N*,*N*-dimethylamino)propanol to give 575 mg (22%) of product (mp 96–98 °C). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 31 H, $(CH_2)_{14}$, CH_3CH_2O), 2.2 (m, 2 H, $NCH_2CH_2CH_2$), 2.58 (m, 2 H, SCH_2), 2.7–2.9 (m, 2 H, CH_2S), 3.48 (s, 6 H, $N(CH_3)_2$), 3.9–4.3 (m, 9 H, CH_3CH_2OCH, $CH_2NCH_2CH_2CH_2OH$). Anal. ($C_{26}H_{56}NO_2SBr$) C, H, N.

(±)-*N*-[3-(Hexadecylthio)-2-methoxyprop-1-yl]-*N*,*N*,*N*-trimethylammonium Bromide (7D). To a 100-mL heavy-wall glass tube equipped with a magnetic stir bar was added a solution of compound **6A** (2.0 g, 0.005 mol) dissolved in 50 mL of CH_3CN and the vessel was cooled to −10 °C. An excess of condensed trimethylamine was added, the tube was sealed, and the vessel was heated to 60 °C for 24 h. The reaction mixture was then cooled to room temperature, resulting in a precipitate which was filtered. The precipitate was swirled with five 50-mL portions of ether; the ether was decanted off and dried under vacuum, resulting in 2.1 g (92%) of pure product (mp 197–199 °C). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.7 (m, 28 H, $(CH_2)_{14}$), 2.45–3.0 (m, 4 H, CH_2SCH_2), 3.48 (s, 12 H, CH_3O, $N(CH_3)_3$), 4.0–4.35 (m, 3 H, $CHCH_2N$). Anal. ($C_{23}H_{50}NOSBr$) C, H, N.

(±)-*N*-[3-(1′-Naphthyl)-2-methoxyprop-1-yl]-*N*,*N*-dimethyl-*N*-(β-hydroxyethyl)ammonium Bromide (7E). This analogue was prepared in a manner similar to that of compound **7A** from compound **6C** (1.0 g, 0.003 mol) and 0.374 mL (0.003 mol) of 2-(*N*,*N*-dimethylamino)ethanol to give 450 mg (35%) of the product (dec >150 °C). ^{1}H NMR ($CDCl_3$): δ 3.37 (d, 6 H, $N(CH_3)_2$), 3.51 (s, 3 H, OCH_3), 3.66–3.84 (m, 3 H, $CHOCH_3$, CH_2OH), 4.02–4.4 (m, 6 H, CH_2NCH_2, CH_2O-naph), 6.82 (1 H, naph), 7.3–7.52 (m, 4 H, naph), 7.74–7.79 (m, 1 H, naph), 8.14–8.2 (m, 1 H, naph). Anal. ($C_{18}H_{26}NO_3Br$) C, H, N.

(±)-*N*-[(2,2-Dimethyldioxol-4-yl)methyl]-*N*,*N*-dimethyl-*N*-(β-hydroxyethyl)ammonium Bromide (8). To a 500-mL round-bottom flask equipped with a magnetic stir bar and drying tube was added a solution of (28.0 g, 0.181 mol) of 3-bromopropane-1,2-diol in 200 mL of acetone. Then, 0.5 mL of concentrated sulfuric acid was added and the reaction was allowed to proceed at room temperature for 24 h with continuous stirring. The reaction mixture was neutralized with 13.0 g of K_2CO_3 for 30 min, the solution was filtered, and the solvent was evaporated in vacuo. The resulting oil was dissolved in 150 mL of ether, extracted three times with 150-mL portions of water, and the organic layer was dried over anhydrous sodium sulfate. The filtered ether was evaporated in vacuo and 100 mL of hexanes was added, resulting in two layers. The upper layer was decanted and evaporated in vacuo to provide 19.1 g (53.5%) of 2,2-dimethyl-4-(bromomethyl)-1,3-dixolane. The desired analogue was then prepared in a manner analogous to that of compound **7A** from 2,2-dimethyl-4-(bromomethyl)-1,3-dioxolane (2.5 g, 0.013 mol), *N*,*N*-dimethylaminoethanol (1.3 g, 0.014 mol), and 15 mL of CH_3CN. Chromatography with Florisil ($CHCl_3$/MeOH 3:1 as eluent) provided 860 mg (23%) of **8** as a thick oil. ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3), 3.4–3.48 (s, 6 H, $CH_2N(CH_3)_2$), 3.75–4.25 (m, 9 H, CH, CH_2O, $CH_2NCH_2CH_2OH$). Anal. ($C_{10}H_{22}NO_3Br$) C, H, N.

***trans*/*cis*-*N*-[(2-Heptadecyldioxol-4-yl)methyl]-*N*,*N*-dimethyl-*N*-(γ-hydroxypropyl)ammonium Bromide (9).** 2-Heptadecyl-4-(hydroxymethyl)-1,3-dioxolane was synthesized according to the method of Piantadosi et al.[14] The final product (**9**) was prepared in a manner analogous to that of compound **7A** from 2-heptadecyl-4-(bromomethyl)-1,3-dioxolane (2.0 g, 0.005 mol) and 3-(*N*,*N*-dimethylamino)propanol (0.6 mL, 0.006 mol) in 10 mL of DMF (62–65 °C for 72 h) to yield 725 mg (30%) of product as an isomeric mixture of 64% trans and 36% cis (mp 93–95 °C). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal methyl), 1.2–1.4 (m, 32 H, $CH(CH_2)_{16}$), 2.10 (m, 2 H, $NCH_2CH_2CH_2OH$), 3.40 (s, 6 H, $N(CH_3)_2$), 3.7–4.4 (m, 9 H, $CH_2NCH_2CH_2CH_2OH$, $CHCH_2O$), 4.95 (t, 0.36 H, OCHO), 5.05 (t, 0.64 H, OCHO). Anal. ($C_{26}H_{54}NO_3Br$) C, H, N.

***trans*-*N*-[(2-Heptadecyldioxol-4-yl)methyl]-*N*,*N*-dimethyl-*N*-(γ-hydroxypropyl)ammonium Bromide (9A).** The final product (**9A**) was prepared in a manner analogous to that

of compound **7A** from *trans*-2-heptadecyl-4-(bromomethyl)-1,3-dioxolane (0.130 g, 0.00032 mol) and 3-(*N*,*N*-dimethylamino)-propanol (0.04 mL, 0.00035 mol) in 4 mL of DMF (62–65 °C for 72 h). The reaction mixture was cooled to room temperature and diluted with 25 mL of ether. Approximately a third of the resulting precipitate was then purified by silica gel chromatography (chloroform/methanol 5:1 as eluent) to yield 11 mg of product. ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal methyl), 1.2 (s, 28 H, $(CH_2)_{14}$), 1.6 (m, 2 H, $CHCH_2CH_2$), 1.7 (m, 2 H, $CHCH_2CH_2$), 2.1 (m, 2 H, $NCH_2CH_2CH_2OH$), 3.35 (d, 6 H, $N(CH_3)_2$, 3.5 (dd, 2 H, dioxolane C-5 protons), 3.75 (m, 2 H, NCH_2CH_2), 3.9 (dd, 2 H, $CHCH_2N$), 4.4 (m, 2 H, $NCH_2CH_2CH_2OH$), 4.6 (m, 1 H, dioxolane C-4 proton), 5.05 (t, 1 H, $CH(CH_2)_{16}CH_3$).

Tetrahydro-2-(2-bromoethoxy)-2*H*-pyran (10). In a round-bottom flask equipped with a nitrogen inlet, 7.0 mL (0.08 mol) of dihydropyran was added to a solution of 5.0 mL (0.04 mol) of 2-bromoethanol and 0.1 g of pyridinium *p*-toluenesulfonate (*p*-PTS) in 75 mL of DCM. After 24 h the reaction mixture was extracted twice with 100-mL portions of water, dried over anhydrous sodium sulfate, filtered and evaporated in vacuo to a dark oil. Purification by silica gel chromatography (hexane/ethyl acetate 8:1 as eluent) yielded 5.3 g of a pale yellow oil (63%). ^{1}H NMR ($CDCl_3$): δ 1.25 (b s, 6 H, protons on pyran C3–5), 3.34–4.0 (m, 6 H, $BrCH_2CH_2O$, protons on pyran C6), 4.5 (s, 1 H, OCHO).

(±)-1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[β-(2-tetrahydropyranyloxy)ethyl]glycerol (12). *rac*-1-*O*-Hexadecyl-2-*O*-methylglycerol (**11**) was prepared as described previously.[6] A solution of compound **11** (2.0 g, 0.006 mol), 80% NaH (oil dispersion) (0.3 g, 0.010 mol) in 100 mL of THF was placed in a round-bottom flask and stirred for 1 h. To this solution, 4.2 g (0.020 mol) of compound **10** was added and stirred for 24 h at room temperature. The reaction mixture was diluted with 250 mL of water and extracted three times with 125-mL portions of ether, and the organic extractions were combined. The organic extract was dried over anhydrous sodium sulfate and filtered, and the solvents were evaporated in vacuo. Purification by silica gel chromatography (hexane/ethyl acetate as eluent) afforded 1.7 g (62%) of product as a semisolid. ^{1}H NMR ($CDCl_3$): δ 0.8 (m, 3 H, terminal methyl), 1.25 (b s, 34 H, $(CH_2)_{14}$, protons on pyran C3–5), 3.2–3.7 (m, 16 H, CH_2OCH_2, $CH_3OCHCH_2OCH_2CH_2OH$, protons on pyran C6), 4.5 (s, 1 H, proton on pyran C1).

(±)-1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(β-hydroxyethyl)-glycerol (13). This intermediate was prepared in a manner analogous to that of compound **4A** from compound **12** (1.7 g, 0.004 mol) and 0.3 g of *p*-TSA in 100 mL of $CHCl_3$/MeOH (3:1). Purification by silica gel chromatography (hexane/ethyl acetate 4:1 as eluent) yielded 1.1 g (82%) of product as a semisolid. ^{1}H NMR ($CDCl_3$): δ 0.8 (t, 3 H, terminal methyl), 1.2 (s, 28 H, $(CH_2)_{14}$), 3.3–3.6 (m, 14 H, CH_2OCH_2, CH_3OCH, $CH_2OCH_2CH_2OH$). Anal. ($C_{22}H_{46}O_4$) C, H.

(±)-1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(β-mesylethyl)-glycerol (14). This intermediate was prepared in a manner analogous to that of compound **5A** from compound **13** (0.5 g, 0.001 mol), 0.15 mL (0.002 mol) of methanesulfonyl chloride, and 0.3 mL (0.002 mol) of triethylamine in 50 mL of DCM. Without further purification, 700 mg of crude product was obtained. ^{1}H NMR ($CDCl_3$): δ 0.8 (t, 3 H, terminal methyl), 1.2–1.4 (b s, 28 H, $(CH_2)_{14}$), 3.0 (s, 3 H, CH_3SO_3), 3.2–3.8 (overlapping m, 12 H, CH_2OCH_2, CH_3OCH, $CH_2OCH_2CH_2O$), 4.2 (t, 2 H, $CH_2OSO_2CH_3$).

(±)-1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(β-bromoethyl)-glycerol (15). This intermediate was prepared in a manner analogous to that of compound **6A** from compound **14** (0.7 g, 0.008 mol) and 3.9 g (0.040 mol) of LiBr in 50 mL of acetone 36 h of reflux. Without further purification 0.740 g of product was obtained. ^{1}H NMR ($CDCl_3$): δ 0.8 (t, 3 H, terminal methyl), 1.2–1.4 (b s, 28 H, $(CH_2)_{14}$), 3.2–3.8 (m, 14 H, CH_2OCH_2, CH_3OCH, $CH_2OCH_2CH_2Br$).

(±)-2-[3-(Hexadecyloxy)-2-methoxypropoxy]-*N*,*N*,*N*-trimethyl-1-ethanaminium Bromide (16). This analogue was prepared in a manner analogous to that of compound **7D** from compound **15** (0.740 g, 0.008 mol) and an excess of anhydrous trimethylamine. Precipitation from $CHCl_3$/ether (1:25) afforded 362 mg (91%) of product as a white solid (dec >200 °C). ^{1}H NMR ($CDCl_3$): δ 0.8 (t, 3 H, terminal methyl), 1.1–1.3 (b s, 26 H, $(CH_2)_{13}$), 1.45 (t, 2 H, OCH_2CH_2), 3.3 (s, 3 H, CH_3O), 3.3–3.6 (m, 7 H, CH_2O, CH_2OCH_2, OCH), 3.4 (s, 9 H, $N(CH_3)_3$), 3.85 (m, 4 H, OCH_2CH_2N). Anal. ($C_{25}H_{54}NO_3Br$) C, H, N.

(±)-*N*-[3-(Hexadecyloxy)-2-methoxyprop-1-yl]-*N*,*N*-dimethyl-*N*-(β-hydroxyethyl)ammonium Iodide (17) and (±)-*N*-[3-(Octadecylthio)-2-methoxyprop-1-yl]-*N*,*N*-dimethyl-*N*-(γ-hydroxypropyl)ammonium Iodide (18). These analogues were prepared in the usual manner according to Scheme I. Anal. for **17** ($C_{24}H_{52}O_3NI$) C, H, N. Anal. for **18** ($C_{27}H_{58}O_2NSI$) C, H, N.

Biological Evaluation. Preparation of Protein Kinase C. PKC was prepared and assayed as described previously.[6] In brief, HL-60 cells were grown in 75-mL flasks, harvested, and washed with ice-cold normal saline. After centrifugation (600*g*, 5 min, 4 °C), the cell pellet was resuspended (20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 10 mM EGTA, 50 mM 2-mercaptoethanol, 2 mM phenylmethanesulfonyl fluoride) and sonicated for 20 s with a stepped microprobe sonicator. Unbroken cells were removed by centrifugation as above and the supernatant was centrifuged (65000*g*, 90 min, 4 °C). The supernatant from this step (cytosol) was then fractionated on a 1 × 8 cm DEAE-Sephacel column after addition of sucrose to a final concentration of 10%. After equilibrating the column (20 mM Tris, pH 7.5, 0.2 mM EDTA, 0.2 mM EGTA, 50 mM 2-mercaptoethanol, 10% sucrose), the sample was loaded and unbound material was washed through with 40 mL of the equilibrating buffer. Then PKC was eluted by a gradient from 0 to 0.5 M NaCl in the buffer described above. Fractions of 1 mL were collected at 25 mL/h and 0.05-mL aliquots were assayed for PKC activity as described below. The fractions with the highest activity were pooled and used in further experiments.

Assay of Protein Kinase C Activity. The assays were done at pH 7.5 in a total volume of 0.25 mL and all tubes contained 25 mM Tris, 10 mM $MgCl_2$, 40 μg/mL histone, 10 μM ATP (including 1 μCi of [γ-^{32}P]ATP), 0.1 mM $CaCl_2$, 20 μg/mL phosphatidylserine, 0.05 mL of the PKC preparation, and 2.5 μM of OAG. Enzymatic activity was determined as the incorporation of ^{32}P from [γ-^{32}P]ATP into histone in the presence of Ca^{2+}, phosphatidylserine, and OAG. Reactions were initiated by the addition of the enzyme preparation and halted after 20 min at 30 °C by the addition of 0.05 mL of bovine serum albumin (10 mg/mL) and 1 mL 25% ice-cold trichloroacetic acid. The tubes were kept on ice and then filtered in a millipore vacuum box using Millipore HA filters and washed with 25% trichloroacetic acid. The radioactivity bound to the filters was determined by scintillation spectrometry in 5 mL of Budget Solve. The amount of enzyme used was shown to result in linear activity for at least 20 min and the assay was linearly dependent on the amount of enzyme used. The analogue to be tested was dissolved in ethanol and added directly into the reaction mixture before the addition of PKC. As a control, 0.1% ethanol was included in the samples with the enzyme but with no inhibitor.

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Registry No. **1A**, 25666-00-6; **1B**, 88547-42-6; **2A**, 103321-06-8; **2B**, 124581-69-7; **3A**, 103304-66-1; **3B**, 103304-67-2; **3C**, 124581-70-0; **4A**, 103304-68-3; **4B**, 103304-69-4; **4C**, 124581-71-1; **5A**, 124581-72-2; **5B**, 124581-73-3; **5C**, 124581-74-4; **6A**, 124581-75-5; **6B**, 124581-76-6; **6C**, 124581-77-7; **7A**, 124581-78-8; **7B**, 124581-79-9; **7C**, 124581-80-2; **7D**, 124581-81-3; **7E**, 124581-82-4; **8**, 124581-83-5; *cis*-**9**, 124581-86-8; *trans*-**9**, 124581-85-7; **10**, 59146-56-4; **11**, 111188-59-1; **12**, 124581-88-0; **13**, 124581-89-1; **14**, 124581-90-4; **15**, 124581-91-5; **16**, 124581-92-6; **17**, 124581-93-7; **18**, 124581-94-8; PKC, 89800-68-0; $HOCH_2CH(OH)CH_2SH$,

53023-42-0; $C_{16}H_{33}Br$, 112-82-3; $(CH_3)_2N(CH_2)_2OH$, 108-01-0; $(CH_3)_2N(CH_2)_3OH$, 3179-63-3; $(CH_3)_3N$, 75-50-3; $HOCH_2CH(OH)CH_2Br$, 34637-21-3; $Br(CH_2)_2OH$, 540-51-2; 1-naphthol, 90-15-3; (±)-1,2-*O*,*O*-isopropylidene-3-*O*-mesylglycerol, 34331-40-3; 1,2-(isopropylidenedioxy)-3-bromopropane, 34637-20-2; 2-heptadecyl-4-(bromomethyl)-1,3-dioxolane, 124581-84-6; *trans*-2-heptadecyl-4-(bromomethyl)-1,3-dioxolane, 124581-87-9; dihydropyran, 110-87-2.

Hydroxamic Acid Inhibitors of 5-Lipoxygenase: Quantitative Structure–Activity Relationships

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An evaluation of the quantitative structure–activity relationships (QSAR) for more than 100 hydroxamic acids revealed that the primary physicochemical feature influencing the in vitro 5-lipoxygenase inhibitory potencies of these compounds is the hydrophobicity of the molecule. A significant correlation was observed between the octanol–water partition coefficient of the substituent attached to the carbonyl of the hydroxamate and in vitro inhibitory activity. This correlation held for hydroxamic acids of diverse structure and with potencies spanning 4 orders of magnitude. Although the hydrophobicity may be packaged in a variety of structural ways and still correlate with potency, the QSAR study revealed two major exceptions. Specifically, the hydrophobicity of portions of compounds in the immediate vicinity of the hydroxamic acid functionality does not appear to contribute to increased inhibition and the hydrophobicity of fragments beyond approximately 12 Å from the hydroxamate do not influence potency. The QSAR study also demonstrated that inhibitory activity was enhanced when there was an alkyl group on the hydroxamate nitrogen, when electron-withdrawing substituents were present and when the hydroxamate was conjugated to an aromatic system. These observations provide a simple description of the lipoxygenase–hydroxamic acid binding site.

The enzyme 5-lipoxygenase is the first dedicated enzyme in the biosynthetic pathway leading to the leukotrienes. Since leukotrienes have been implicated as important mediators in several diseases including asthma, arthritis, and psoriasis, inhibition of 5-lipoxygenase represents a potential new approach for therapeutic intervention in these diseases. Simple stable molecules containing the hydroxamic acid functionality have been shown to inhibit 5-lipoxygenase.[1-5] In fact, several hydroxamates are orally active inhibitors of the enzyme as determined by their ability to block the biosynthesis of leukotrienes in vivo.[2-4] The hydroxamic acid moiety is essential for the inhibition observed with these compounds. Molecules in which the hydroxamate has been replaced by related functional groups exhibit little or no 5-lipoxygenase inhibitory activity in vitro.[1]

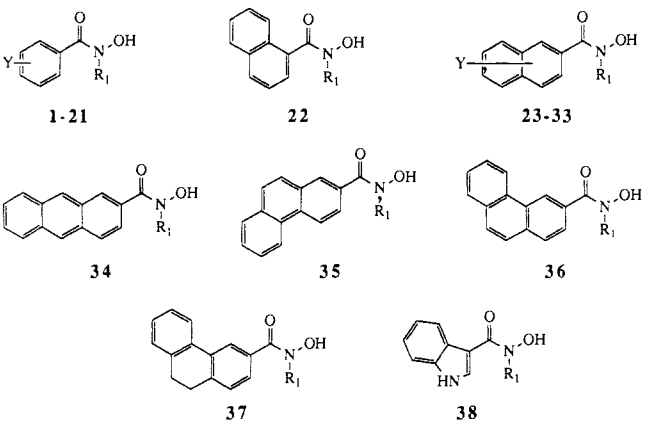
In this paper we report the in vitro 5-lipoxygenase inhibitory activities of many new "type A"[6] hydroxamic acids. We have combined these new compounds with those previously reported to study the influence of the structural features on their in vitro inhibitory potency. The results of a quantitative evaluation of structure–activity relationships (QSAR)[7] involving more than 100 hydroxamic acid of diverse structure are described.

Results and Discussion

Table I lists the 111 hydroxamic acids used in this QSAR study. The compounds have been classified into four groups on the basis of common structural features. The structure–activity relationships of each group are discussed below.

Group A: Arylhydroxamic Acids (Chart I). We have previously described the 5-lipoxygenase inhibitory properties of a series of para-substituted benzohydroxamic acids, 1–10.[1] A highly significant correlation was noted between the hydrophobicity and electronic nature of the para substituent and the inhibitory potency of these compounds. This correlation is described in eq 1. The term

Chart I. Group A. Arylhydroxamic Acids



$$\log (1/IC_{50}) = 0.49(\pm 0.08)\pi + 0.45(\pm 0.17)\sigma_p + 3.10(\pm 0.20) \quad (1)$$

$$n = 10,\ s = 0.220,\ r = 0.945,\ F_{2,7} = 28.9,\ p < 0.0001$$

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