Synthesis of Optically Pure Cyclic Lipoidal Ammonium Salts and Evaluation of Inhibition of Protein Kinase C

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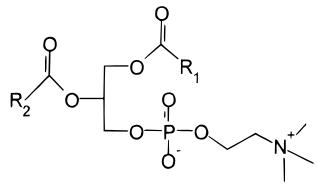
Stereoisomeric cyclic analogues of hexadecylphosphocholine (Miltefosine) and phosphatidylcholine, (2*S*,4*S*)-, (2*R*,4*S*)-, (2*R*,4*R*)-, and (2*S*,4*R*)-2,6,6-trimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane bromide (**2a**−**d**), and the enantiomers (*S*)- and (*R*)-*N*-(2-hydroxyethyl)-*N*-(2-hydroxyheptadecyl)-*N*,*N*-dimethylammonium iodide (**(*S*)-**and **(*R*)-11**) were prepared in good overall yields using optically pure glyceraldehyde surrogates as the starting materials. A four-step synthesis of the key intermediates ((*S*)- and (*R*)-1-pentadecyloxirane (**(*S*)-**and **(*R*)-3**) gave overall high optical purity and chemical yields. Approaches to the synthesis of these key intermediates utilizing asymmetric dihydroxylation on 1-pentadecene gave high chemical yields but only modest optical purity. All ammonium salts inhibited protein kinase C with the following values of IC_{50} (μM): **2a** (105), **2b** (109), **2c** (121), **2d** (113).

Introduction

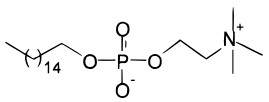
The protein kinase C (PKC) family of kinases is a prime target for most potent tumor-promoting phorbol esters.[1−3] Once activated, PKC phosphorylates other proteins, including protein kinases, leading to a cascade of abnormal cellular information modulating the proliferation, differentiation, and death of cells.[4] All members of the PKC family require binding to the phospholipid cell membrane prior to activation; thus, it is not surprising that lipid metabolites regulate the activity of PKC.[5]

In the past few years, we have synthesized lipoidal quaternary ammonium salts as PKC inhibitors.[6,7] Two promising leads are a diastereomeric pair of compounds, (2*S*,4*S*/2*R*,4*R*)- and (2*R*,4*S*/2*S*,4*R*)-2,6,6-trimethyl-2-oxo-4-hexadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane bromide, **1a,b**, which are cyclic synthetic analogues of both miltefosine,[8] a modest inhibitor of PKC in clinical use in Germany, and naturally occurring phosphatidylcholine.[9]

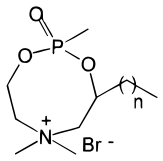
Having passed the initial cell-panel, antitumor screen at NCI, **1a** is currently under evaluation as an antitumor agent. We have begun to synthesize optically pure compounds to study inhibition of PKC with selected enantiomers and also to investigate possible selectivity in antitumor activity.



Phosphatidylcholine
R_1, R_2 = Fatty acyl chains



Miltefosine



(2*S*,4*S*)/(2*R*,4*R*) n= 15, 1a
(2*R*,4*S*)/(2*S*,4*R*) n= 15, 1b
(2*S*,4*S*) n= 14, 2a
(2*R*,4*S*) n= 14, 2b
(2*R*,4*R*) n= 14, 2c
(2*S*,4*R*) n= 14, 2d

In this report, we describe the synthesis of the four stereoisomers **2a,d**, which are one-carbon-shorter homologues of **1a,b**. Optically pure 1-pentadecyloxiranes, **(*S*)-** and **(*R*)-3**, are the key intermediates for the synthesis. We require high optical purity (>98%) to ensure the correct assignments of the biological activity. Neither enantiomer has been prepared; however, shorter homologues of **(*S*)-3**, *viz.*, (*S*)-tridecyloxirane[10] and (*S*)-undecyloxirane,[11] have been made in modest optical purity or lengthy syntheses with modest overall yields.

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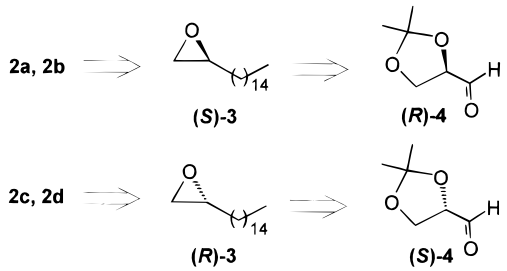
S0022-3263(96)01365-5 CCC: $12.00

Scheme 1

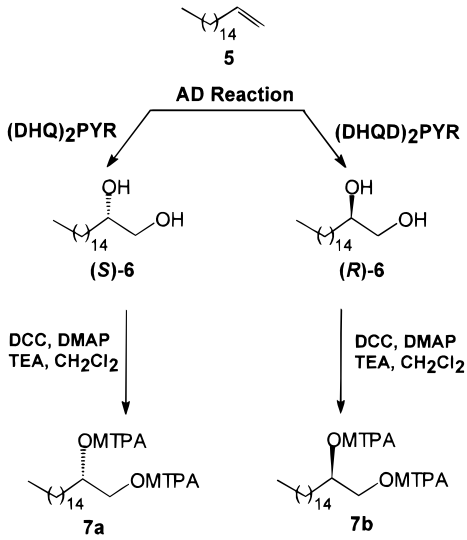


We report two approaches to the synthesis of **(*S*)-** and **(*R*)-3**. The first involves the asymmetric dihydroxylation (AD) of olefins as reported by Sharpless *et al.*[12,13] to form optically active 1,2-diols, which can be stereospecifically transformed into epoxides.[14] The second employs relatively inexpensive, optically pure glyceraldehyde surrogates (*R*)- and (*S*)-2,3-*O*-isopropylideneglyceraldehyde (**(*R*)-** and **(*S*)-4**) prepared from *d*-mannitol and *l*-ascorbic acid as reported by Schmid *et al.*[15,16] The second approach (Scheme 1) is longer but gives high optical and chemical yields.

Results and Discussion

Asymmetric Dihydroxylation of 1-Heptadecene (5). The AD reactions on **5** were carried out under two modified procedures of the reported method. We used two ligand pairs: $(DHQ)_2PYR$ and $(DHQD)_2PYR$[12] as well as $(DHQ)_2AQN$ and $(DHQD)_2AQN$.[17] Basically, the more significant variations were a lower olefin concentration due to the low solubility of the olefin in the recommended amount of solvent and a higher reaction temperature (25 °C in lieu of 0 °C). We obtained optically enriched (*S*)- and (*R*)-1,2-heptadecanediol (**(*S*)-** and **(*R*)-6**) in 92−95% chemical yields (Scheme 2).

Scheme 2



The ee's (Table 1) were evaluated by ^1H NMR analyses of the corresponding crude bis(methoxy(trifluoromethyl)-phenyl acetates) (bis-MTPA), **7a** and **7b**. The ^1H NMR spectrum of ***rac*-7** was run for control. The ^{19}F NMR spectra of racemic and optically active **7** did not give clean base-line separation. We checked one sample by HPLC under conditions similar to those of Sharpless and coworkers[12] and found no variation from the results obtained from the ^1H NMR analysis. We assigned absolute configurations according to previous results[12] and ultimately by syntheses of **(*S*)-** and **(*R*)-6** from materials of known configuration.

As evidenced from the reaction conditions employed, variation of the reaction temperature does not affect the enantiomeric ratio. Lower temperatures and more dilute conditions increase reaction times. The 1,2-diols obtained by this method are of modest enantiomeric purity. Repeated recrystallization of **(*S*)-** and **(*R*)-6** with several solvent systems[18] does not enrich the optical purity of the compounds (67−72% ee).[19] The inability of recrystallization to enrich enantiomerically the samples is probably because packing of the lipid chains dominates crystallization rather than the packing of the polar head, which contains the chiral information.

Table 1. Comparison of the % of Enantiomeric Excess of (*R*)- and (*S*)-6 in Asymmetric Dihydroxylation of 5

compd	ligand	method	ee, %
(*R*)-6	$(DHQD)_2PYR$	A	78
(*R*)-6	$(DHQD)_2PYR$	B	74
(*R*)-6	$(DHQD)_2AQN$	B	73
(*S*)-6	$(DHQ)_2PYR$	A	63
(*S*)-6	$(DHQ)_2PYR$	B	64
(*S*)-6	$(DHQ)_2AQN$	B	69

Previous studies[13] of the AD reaction on a series of 1-alkenes show that enantioselectivity strongly depends on the chain length of the olefins. The ee % initially increases with the number of carbons atoms (from propene to 1-pentene), but then it reaches a plateau at 89% for *R* and 76% for *S* when the chain length is greater than five (1-hexene and 1-decene). Our study of 1-pentadecene indicates a small reduction in enantioselectivity from the values at the plateau.

Unable to obtain optically pure "long alkyl-chain" diols, we adopted another route as described below.

Synthesis of Stereoisomers (*S*)- and (*R*)-3. In many successful syntheses of molecules with long alkyl chains, e.g., syntheses of sphingosines,[20] Wittig olefination[21] is the key step. Wittig reactions with glyceraldehyde (**4**) have been reviewed.[22] In our case, the *E*/*Z* ratio of products presents no problem because hydrogenation of the double bond follows (*vide supra*).

With the reaction conditions employed (salt-free conditions), (*S*)-1,2-di-*O*-isopropylidene-3-(*Z*)-pentadecene (**(*S*)-8**) was the major product as expected.[23] Lower

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(18) Diols were recrystallized in toluene, MeOH:water (9:1), and hexanes:ethyl acetate (12:1).
(19) Bis-2,4-dinitrobenzoyl derivatives were also prepared for optical enrichment, but we did not find an appropriate solvent with better crystallizing properties.
(20) (a) Reist, E. J.; Christie, P. H. *J. Org. Chem.* **1980**, *45*, 4127−4130. (b) Zimmerman, P.; Schmidt, R. R. *Liebigs Ann. Chem.* **1970**, 663−667. (c) Kiso, M.; Nakamura, A.; Tomita, Y.; Hasegawa, A. *Carbohyd. Res.* **1986**, *158*, 101−112. (d) Koide, K.; Numata, M.; Sugimoto, M.; Nakahara, Y.; Ogawa, T. *Carbohyd. Res.* **1986**, *158*, 113−123.
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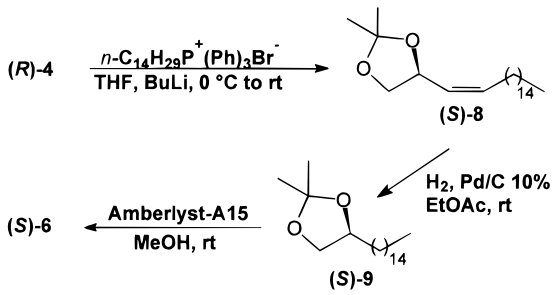
Scheme 3



homologues of **(*S*)-8**, such as (*S*)-1,2-di-*O*-isopropylidene-3(*Z*)-tetradecene and (*S*)-1,2-di-*O*-isopropylidene-3(*Z*)-undecene prepared under similar conditions, also gave the *Z*-olefin as the major product.[24] Catalytic hydrogenation of **(*S*)-8** under atmospheric pressure afforded (*S*)-1,2-di-*O*-isopropylidenepentadecane (**(*S*)-9**) in 95% yield. Deprotection of the acetonide under acidic conditions revealed the diol **(*S*)-6** in 95% yield (Scheme 3).

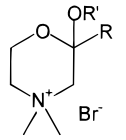
The enantiomeric purity of **(*S*)-6** obtained by this route was evaluated by ^{1}H NMR analysis of the bis-MTPA derivative as described above. No peaks from the other diastereomer were observed. Therefore, the optical purity was at least of 98% by the limits of detection. Diol **(*S*)-6** was then converted into a terminal epoxide with retention of configuration by a simple "one-pot" procedure as reported by Kolb and Sharpless.[14] The overall yield of **(*S*)-3** from **(*R*)-4** was 72%. Epoxide **(*R*)-3** was made in 68% overall yield by same method starting from **(*S*)-4**. The optical purity was confirmed by evaluation of the optical rotation, which agreed with the absolute value of **(*S*)-3**.

Synthesis of Optically Pure Quaternary Ammonium Salts (Scheme 4). The epoxides **(*S*)-3** and **(*R*)-3** were independently treated with *N*-methylethanolamine in refluxing anhydrous methanol to provide amino diols **(*S*)-** and **(*R*)-10** in 91 and 94% yields, respectively. The crude materials were sufficiently clean for the following reaction as evidenced by their ^{1}H NMR spectra. A portion of each enantiomer of **10** was used to prepare the quaternary ammonium salts **(*S*)-** and **(*R*)-11**. The quaternizations were readily accomplished with iodomethane in anhydrous ether. After two recrystallization, we obtained analytically pure samples in 78 and 74% yields, respectively.

Amino diol **(*S*)-10** was condensed with methylphosphonic dichloride to afford a diastereomeric mixture of (2*S*,4*S*)- and (2*R*,4*S*)-2,6-dimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane, **12a** and **12b**, respectively. In order to decrease the risk of polymerization, a solution of **(*S*)-10** and methylphosphonic dichloride in CH_2Cl_2 was slowly added dropwise, independently and simultaneously, to a solution of triethylamine in CH_2Cl_2.[6] The diastereomers **12a** (first fraction) and **12b** (second fraction) were readily separated by column chromatography on neutral alumina. The stereochemistries of these compounds were assigned from data obtained with their derivatives (*vide supra*). The other pair of diastereomers, (2*R*,4*R*)- and (2*S*,4*R*)-2,6-dimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane (**12c** and **12d**), were obtained similarly from the corresponding amino diol **(*R*)-10**. Finally the four stereoisomers **12a**−**d** were independently methylated with bromomethane in anhydrous ether to give **2a**−**d** in 60−65% yields.

Single-crystal X-ray analysis[7] of **2a/2c** as a racemate (first fraction from column chromatography) reveals that the methyl group on the phosphorus and the long alkyl chain are cis to each other. NOE experiments on **2a** and **2b** also confirm the stereochemistry. Upon irradiation of the methyl group on phosphorus and the methine hydrogen at C4, stereoisomer **2b** shows a weak enhancement, whereas **2a** shows no enhancement. The weak NOE is probably due to the conformational flexibility of the eight-membered ring in solution. On this basis and by comparisons of the ^{1}H NMR spectra of **2a** and **2b** with those of **2c** and **2d**, we have assigned their structures.

Inhibition of PKC. The four stereoisomers **2a**−**d**, the racemates **1a,b**, **13**, and **14** were assayed with a mixture of mouse α- and β-II PKC (Table 2). The acyclic ammonium salts **(*S*)-** and **(*R*)-11** showed a similar activity and lack of stereoselectivity (data not shown) to **2a**−**d**.



13, R' = H, R = $(CH_2)_{13}CH_3$
14, R' = CH_3, R = $(CH_2)_{15}CH_3$

Our hemicholiniums, **13** and **14**, represent quaternary ammonium salts, whose inhibition of PKC we reported[6] earlier using a different protocol for the assays. (A similar protocol was used for the assay[7] of **1a,b**.) The IC_{50} values reported here for **1a,b**, **13**, and **14** are an order of magnitude higher than those reported previously.[6,7] The assay conditions used in this study, however, have a 12-fold higher concentration of phosphatidylserine, a PKC activator,[25] than in our earlier protocols. The modest inhibition of our compounds under the current protocol suggests that they are not candidates for further development as PKC inhibitors.

Conclusion

We have developed syntheses of optically pure (*R*)- and (*S*)-1-pentadecyloxirane, which are readily transformed into lipoidal ammonium salts. These ammonium salts modestly inhibit PKC by binding at the regulatory site of the protein. The modest inhibition argues against PKC inhibition as the mechanism for the antitumor properties of these compounds.

Experimental Section

General Procedures. All nonhydrolytic reactions were carried out in an argon or N_2 atmosphere with standard techniques for the exclusion of air and moisture. The ligands for the AD reactions were provided by Professor Sharpless. All commercially available reagents were purchased from Aldrich unless otherwise specified. Reaction solvents were freshly distilled prior to use. Mass spectra (relative percentages in parentheses) were recorded on a quadrupolar instrument. Infrared (IR) spectra were recorded on an FT-IR spectrophotometer. ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and APT spectra were recorded at 400, 100, and 40 MHz, respectively. Observed coupling constants are not verified and are listed as J_{app}. Melting points were recorded on an digital melting point apparatus and are uncorrected. Optical rotations were recorded on a digital polarimeter. Elemental analyses were

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Scheme 4

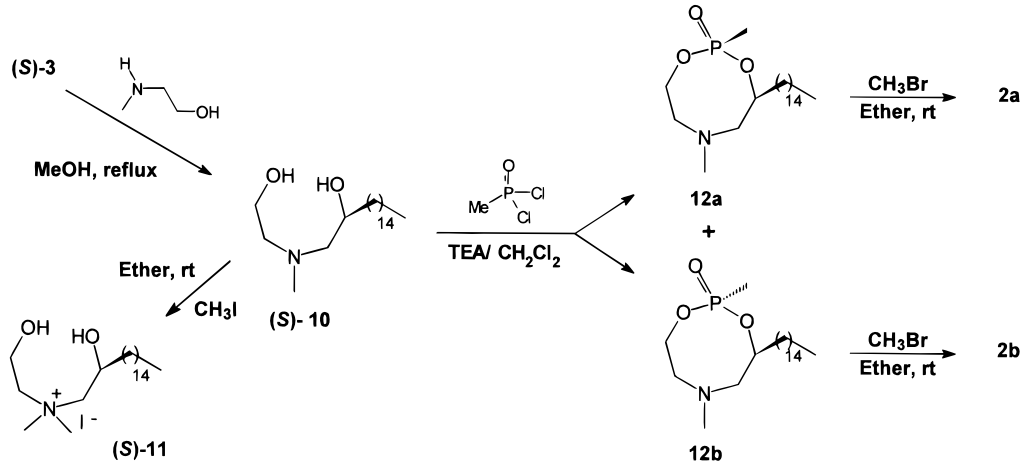

Table 2. Inhibition of Protein Kinase C by Lipoidal Ammonium Salts

compd	IC_{50}, μg/mL	IC_{50}, μM
2a	51 ± 4	105 ± 8
2b	53 ± 7	109 ± 14
2c	59 ± 12	121 ± 24
2d	55 ± 11	113 ± 22
1a	46 ± 5	92 ± 10
1b	45 ± 8	90 ± 16
13	69 ± 7	168 ± 17
14	82 ± 13	181 ± 28

performed by Atlantic Microlab at Atlanta, GA. Solutions were dried over $MgSO_4$ and concentrated in a rotary evaporator.

Asymmetric Dihydroxylation of 1-heptadecene (5). The optical rotation data for the diols obtained by the AD reaction were below the detection limit of our instrument and are not presented. The absolute configurations were assigned on the basis of published data.[12] The enantiomeric excesses (Table 1) were measured by ^{1}H NMR analyses of the bis-MTPA esters.

Method A: for 1 mmol of **5**, $(DHQ)_2PYR$ or $(DHQD)_2PYR$, 14 mg (1.7% mol), $K_3Fe(CN)_6$, 1000 mg (3.03 equiv); K_2CO_3, 430 mg (3.12 equiv); $K_2OsO_2(OH)_4$, 10 mg (0.25% mol); *t*-BuOH: water (1:1, v:v), 14 mL. [**5**] = 0.007 M. Temperature: 0 °C to rt.

Method B: same as method A, but the temperature was 25 °C throughout the reaction.

Preparation of the AD mixes. The reagents $K_3Fe(CN)_6$ (1000 mg, 3.030 equiv), K_2CO_3 (430 mg, 3.12 equiv), $(DHQ)_2$-PYR or $(DHQD)_2PYR$ (14 mg, 1.7% mol), and $K_2OsO_2(OH)_4$ (10 mg, 0.25% mol) were placed together in a vial. The mixtures were denoted as $(DHQ)_2PYR$-ADmix and $(DHQD)_2PYR$-ADmix, respectively, according to the chiral ligand employed for their preparation. The $(DHQ)_2AQN$- and $(DHQD)_2AQN$-AD mixes were prepared similarly.

(*R*)-1,2-Heptadecanediol ((*R*)-6): Method A. $(DHQD)_2$-PYR-ADmix for 1 mmol of olefin was poured into *t*-BuOH: water 1:1 (14 mL) and was vigorously stirred at rt until two clear phases were obtained (*ca*. 15 min). At this stage the temperature was lowered to 0 °C and **5** (0.24 g, 1.0 mmol) was added while maintaining constant stirring. An orange precipitate formed upon addition of the olefin. The temperature was mantained at 0 °C for 1 d; the reaction was monitored by TLC with (CH_2Cl_2:MeOH 15:1). Mostly starting material R_f = 0.94 was observed, as well as traces of product R_f = 0.36. The reaction was allowed to warm to rt and was stirred for 2 d until disappearance of the olefin. The reaction was quenched by addition of $NaHSO_3$ (1.5 g) over 20 min. Stirring was continued for 2.5 h until complete separation of phases (clear top organic phase and blue bottom aqueous phase) was observed. The phases were separated, and the aqueous layer was further extracted with CH_2Cl_2 (3 × 8 mL). The combined organic layers were dried and concentrated to yield a white residue, which was purified by column chromatography with (CH_2Cl_2:MeOH 16:1): yield (0.26 g, 95%) of **(*R*)-6**; mp = 77.8−78.2 °C. Alternatively the product can be purified with (hexanes:EtOAc 8:1): IR (KBr, cm^{-1}) 3483, 3400−3200b, 2916, 1470; ^{1}H NMR (400 MHz, $CDCl_3$) 3.71−3.66 (m, 1H), 3.67−3.63 (m, 1H), 3.46−3.40 (m, 1H), 2.25 (d, J_{app} = 4.3 Hz, 1H), 2.17 (t, J_{app} = 5.7 Hz, 1H), 1.43 (m, 2H), 1.26 (s, 26H), 0.88 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 72.32, 66.83, 33.2, 31.91, 29.68, 29.65, 29.64, 29.58, 29.53, 29.34, 25.53, 22.67, 14.09.

Method B. The reaction was run with $(DHQD)_2PYR$-ADmix scale-up for **5** (0.72 g, (3.0 mmol). The reaction was totally run at rt for 30 h: yield after purification (0.80 g, 98%); mp 78−78.5 °C. Spectra were the same as above.

Method B. The reaction was run with $(DHQD)_2AQN$ AD-Mix scaled up for 0.48 g (2.0 mmol) of **5**. The yield after purification was 0.43 g (79%). Spectra were the same above.

(*S*)-1,2-Heptadecanediol ((*S*)-6): Method A. The procedure is the same as that for **(*R*)-6** with method A. A $(DHQ)_2PYR$-AD mix was employed instead. The yield recovered from 1 mmol of olefin after purification was 0.26 g (95%): mp 77.8−78.2 °C. Spectra were same as for **(*R*)-6**.

Method B. The reaction was run with $(DHQ)_2PYR$-AD mix scale-up for **5** (0.72 g, 3.0 mmol). The reaction was run at rt for 30 h. The yield after purification was 0.80 g (98%): mp 78 −78.9 °C. Spectra were the same as above.

Method B. The reaction was run with $(DHQ)_2AQN$ AD-Mix scaled up for **5** (0.48 g, 2.0 mmol). The yield after purification was 0.45 g (82%). Spectra were the same as above.

(*S*)-1,2-Di-*O*-isopropylidene-3(*Z*)-heptadecene ((*S*)-8). To a cold solution (0 °C, ice bath) of tetradecyltriphenylphosphonium bromide (42.5 g, 78.8 mmol) in THF (145 mL) vigorously stirred was added BuLi (37 mL, 2.2 M in hexanes, 80 mmol) dropwise over a period of 15 min to yield a dark red solution. This solution was stirred at 0 °C for an additional 15 min. Aldehyde **(*R*)-4** (9.0 g, 68 mmol), prepared by following a published procedure,[15] was dissolved in 50 mL of THF and added to the red solution via cannula. The reaction was stirred at 0 °C for 20 min and then at rt for additional 16 h. **Work-up:** the precipitate formed was filtered and washed with ether (*ca*. 300 mL). The filtrate was quenched with saturated aqueous $NH_4^+Cl^-$ (200 mL) and then water (200 mL), and the phases were separated. The aqueous layer was further extracted with ether (2 × 75 mL). The combined organic solution was dried, filtered, and concentrated to yield a wet amber residue. The residue was purified by silica gel column chromatography, eluting with (hexanes:EtOAc 25:1) to yield a pale yellow oil (20 g, 94%). The olefin was obtained mainly in the (*Z*) configuration, 98:2 (*Z*:*E*), after purification, by integration of the ^{1}H NMR olefinic peaks, 4.9−4.8 and 4.5−4.4 ppm: IR (neat, cm^{-1}) 3018, 2983, 1796 (*trans* CH=CH), 1659 (*cis* CH=CH); ^{1}H NMR (400 MHz, $CDCl_3$) 5.61 (m, 2H), 5.39 (m, 1H), 4.9 (m, 1H), 4.05 (dd, 1H, J_{app} = 6.8 Hz), 3.5 (m, 1H), 2.1 (m, 2H), 1.41 (s, 3H), 1.37 (s, 3H), 1.25 (s, 22H), 0.87 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 135.18, 126, 108.97, 71.98, 69.44, 31.89 29.65, 29.63, 29.60, 29.56, 29.43, 29.33, 29.16,

27.73, 26.76, 25.98, 22.66, 14.08; MS 310 (M^+), 295 (22), 289 (5), 265 (6), 252 (14), 208 (12), 108 (48), 97 (89), 83 (95), 72 (99), 55 (100). Anal. Calcd for $C_{20}H_{38}O_2$: C, 77.36; H, 12.34. Found: C, 77.62; H, 12.35.

(*R*)-1,2-Di-*O*-isopropylidene-3(*Z*)-heptadecene ((*R*)-8). This compound was prepared using the procedure for **(*S*)-8**, scaled for **(*S*)-4** (4.70 g, 35.8 mmol), to afford the entitled compound in 9.7 g (87%) after purification by column chromatography, mainly in the (*Z*) configuration, 94:6 (*Z*/*E*). Spectral data were the same as those for **(*S*)-8**.

(*S*)-1,2-Di-*O*-isopropylideneheptadecane ((*S*)-9). To a solution of **(*S*)-8** (19.8 g, 63.7 mmol) in 800 mL of degassed EtOAc was added 3 g of 10% Pd/C. The flask was capped with a rubber septum and equipped with a balloon at the end of a needle. Argon and vacuum were applied consecutively three times. Hydrogen was flushed until the balloon was inflated. The reaction was run at rt with constant stirring for 2−3 d. The reaction was monitored by TLC, co-running both the starting material and product. Both materials have the same R_f values (hexanes:EtOAc 9:1, R_f = 0.6). However, when stained with a solution of $KMnO_4$, only the starting material gives a yellow spot at rt; subsequent heating develops the product as another single yellow spot. Upon completion of the reaction, the solid was filtered over a short path of Celite under vacuum and washed with EtOAc. The filtrate was concentrated to give a thick cloudy oil 18.28 g (92%), which started crystallizing at rt: mp 34.1−34.4 °C; $[\alpha]^{22}_D = +11.8°$ (*c* =2.6, $CHCl_3$); IR (KBr, cm^{-1}) 2981, 1473, 1245, 1107; ^{1}H NMR (400 MHz, $CDCl_3$) 4.03 (m, 2H), 3.49 (m, 1H), 1.64 (m, 2H), 1.51−1.44 (m, 2H), 1.4 (s, 3H), 1.34 (s, 3H), 1.24 (s, 24H), 0.87 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 108.54, 76.16, 69.53, 33.57, 26.93, 25.75, 31.90, 29.66, 29.65, 29.63, 29.62, 29.55, 29.49, 29.34, 22.67, 14.09; MS (EI^+) 299 (7), 298 (57), 297 (100, $M^+ - 15$), 125 (19), 111 (78), 101 (83), 97 (94), 83 (98), 69 (95), 55 (94). Anal. Calcd for $C_{20}H_{40}O_2$: C, 76.86; H, 12.90. Found: C, 76.82; H, 12.92.

(*R*)-1,2-Di-*O*-isopropylideneheptadecane ((*R*)-9). The procedure was the same as for the preparation of **(*S*)-9**, adapted for **(*R*)-8** (9.50 g, 3.06 mmol). The yield was 8.6 g (95%) of **(*R*)-9** after purification: mp 34.3−34.6 °C; $[\alpha]^{22}_D = -12.2°$ (*c* = 1.4 in $CHCl_3$). Anal. Calcd for $C_{20}H_{40}O_2$: C, 76.86; H, 12.90. Found: C, 76.73; H, 12.84. Spectral data were the same as those for **(*S*)-9**.

(*S*)-1,2-Heptadecanediol ((*S*)-6). To a solution of **(*S*)-9** (17.5 g, 55.9 mmol) in 1.2 L of MeOH was added Amberlyst-A15 (6.5 g). The mixture was stirred for 2 d at rt; during that time, the solution became cloudy. The reaction was monitored by TLC (CH_2Cl_2:MeOH 16:1) until disappearance of starting material. Diol gives a single spot, R_f = 0.24. The product was purified by column chromatography on silica gel (hexanes: EtOAc 12:1, 5:1, 1:2) to yield a white crystalline product (14.4 g, 95.0%): mp 80.9−81.3 °C; $[\alpha]^{22}_D = -7.85°$ (*c* = 0.8, MeOH). Anal. Calcd for $C_{17}H_{36}O_2$: C, 74.94; H, 13.32. Found: C, 74.98; H, 13.24. Spectra were the same as those generated in the AD experiments.

(*R*)-1,2-Heptadecanediol ((*R*)-6). The procedure was the same as that for the deprotection of **(*S*)-6**, adapted for **(*R*)-9** (8.40 g, 27.2 mmol). The yield was 7.04 g (95%): mp 81−81.3 °C; $[\alpha]^{22}_D = +8.15°$ (*c* = 0.8 in MeOH). Anal. Calcd for $C_{17}H_{36}O_2$: C, 74.94; H, 13.32. Found: C, 75.02; H, 13.25. Spectra were the same as those generated in the AD experiments.

(*S*)-1-Pentadecyloxirane ((*S*)-3). Diol **(*S*)-6** (5.0 g, 18 mmol) and pyridinium *p*-toluenesulfonate (0.46 g, 1.8 mmol) were placed in a vessel. Dichloromethane (300 mL) was added (not all the solid dissolved) and the mixture stirred at rt under argon for 10 min. Trimethyl orthoacetate (2.8 mL, 22 mmol) was added via syringe, and the solution was stirred for 1 h. TLC of the product was checked (hexanes:EtOAc 8:1). Solvent was concentrated to yield a wet white solid, which was taken up in CH_2Cl_2 (180 mL). Acetyl bromide (1.70 mL, 22.9 mmol) was added via syringe. The reaction was stirred at rt under argon for 1.5 h. The solvent was concentrated to give a pale amber oil, which was taken up in 100 mL of MeOH. K_2CO_3 (4.05 g, 29.2 mmol) was added and the mixture stirred at rt for 1 d. The reaction was quenched with saturated aqueous $NH_4^+Cl^-$ (140 mL) and extracted with CH_2Cl_2. The combined organic layer was dried, and the solvent was concentrated to yield a white solid. The product was purified by column chromatography on silica gel (hexanes:EtOAc 25:1) to give pure epoxide (4.1 g, 87%): $[\alpha]^{22}_D = -5.4°$ (*c* = 3.5, $CHCl_3$); IR (KBr, cm^{-1}) 2923, 2853 1466, 1467; ^{1}H NMR (400 MHz, $CDCl_3$) 2.89 (m, 1H), 2.74 (m, 1H), 2.46 (dd, 1H), 2.46 (dd, 1H, J_{app} = 2.3, 2.7 Hz), 1.51 (m, 2H), 1.44 (m, 2H), 1.26 (m, 24H), 0.87 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 52.38, 47.10, 32.48, 31.91, 29.69, 29.67, 29.66, 29.63, 29.55, 29.44, 22.35, 25.95, 25.93, 22.67, 14.09; MS (CI) 255 ($M^+ + 1$), 235 (20), 125 (30), 111 (60), 97 (99), 83 (100). Anal. Calcd for $C_{17}H_{34}O$: C, 80.24; H, 13.47. Found: C, 80.23; H, 13.42.

(*R*)-1-Pentadecyloxirane ((*R*)-3). The procedure was the same as that for the preparation of **(*S*)-3** adapted for a smaller amount of **(*R*)-6** (4.60 g, 16.8 mmol). The yield was 3.7 g (86%): $[\alpha]^{22}_D = +5.43°$ (*c* =1.4, $CHCl_3$). Anal. Calcd for $C_{17}H_{34}O$: C, 80.24; H, 13.47. Found: C, 80.38; H, 13.38. Spectral data were the same as those for **(*S*)-3**.

(*S*)-*N*-(2-Hydroxyethyl)-*N*-(2-hydroxyheptadecyl)methylamine ((*S*)-10). To a solution of **(*S*)-3** (4.32 g, 16.9 mmol) in MeOH (250 mL) was added 2-(methylamino)ethanol (1.40 mL, 16.9 mmol). The reaction was refluxed for 2 d under N_2. TLC of the product was checked on neutral alumina and developed with I_2 (hexanes:EtOAc 2:3, R_f = 0.46). Evaporation of the solvent gave a white solid (5.4 g, 98%), which was used without further purification: IR (KBr, cm^{-1}) 3600−3150, 2917,1473, 1085; ^{1}H NMR (400 MHz, $CDCl_3$) 3.66 (m, 3H), 2.70−2.48 (m, 2H), 2.40−2.36 (s, 3H), 1.50−1.36 (m, 2H), 1.26 (s, 26H), 0.88 (t, J_{app} = 7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 67.6, 64.15, 59.66, 59.40, 42.42, 34.92, 31.92, 29.77, 29.69, 29.66, 29.61, 29.35, 25.63, 22.60, 14.11; $[\alpha]^{22}_D = +27.69°$ (*c* = 0.8, $CHCl_3$); mp 42.4−43.5 °C.

(*R*)-*N*-(2-Hydroxyethyl)-*N*-(2-hydroxyheptadecyl)methylamine ((*R*)-10). The procedure was the same as that for the preparation of **(*S*)-10** adapted for **(*R*)-3** (5.00 g, 18.1 mmol). The crude yield was 5.2 g (91%). Spectra were the same as for **(*S*)-10**. **(*R*)-10:** $[\alpha]^{22}_D = -26.56°$ (*c*= 0.9, $CHCl_3$); mp 41.7−42.6 °C.

(*S*)-*N*-(2-Hydroxyethyl)-*N*-(2-hydroxyheptadecyl)-*N*,*N*-dimethylammonium Iodide, ((*S*)-11). Iodomethane (0.76 mL, 12 mmol) was added in one portion to a solution of **(*S*)-10** (0.80 g, 2.4 mmol) in ether (35 mL). The reaction was run in the dark and stirred for 3 d at rt giving an insoluble white powder. The solvent was concentrated to yield crude product (1.1 g, 96%) which was recrystallized from EtOAc:CH_2Cl_2: MeOH (20:8:0.5) with moderate heating (*ca.* 45 °C). The yield after two recrystallizations was 0.90 g (78%): mp 73.7−74.0 °C; $[\alpha]^{21}_D = +11.55°$ (*c* = 0.9, $CHCl_3$); IR (KBr, cm^{-1}) 3326, 2917, 1469; ^{1}H NMR (400 MHz, $CDCl_3$) 4.4−4.3 (m, 1H, C*H*), 4.2−4.1 (m, 2H), 3.9−3,8 (m, 3H), 3.70−3.6 (m, 1H), 3.46 (s, 6H), 1.6−1.4 (m, 2H), 1.26 (s, 26H), 0.87 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 70.1, 66.7, 65.69, 55.98, 53.98, 53.74, 35.88, 31.91, 29.72, 29.66, 29.62, 29.55, 29.37, 25.17, 22.67, 14.10; MS (CI^+) 331 ($M^+ - CH_3$, (45)), 299 (30), 142 (25), 88 (100), 58 (30). Anal. Calcd for $C_{21}H_{46}INO_2$: C, 53.49; H, 9.83; N, 2.97. Found: C, 53.57; H, 9.77; N, 2.98.

(*R*)-*N*-(2-Hydroxyethyl)-*N*-(2-hydroxyheptadecyl)-*N*,*N*-dimethylammonium Iodide ((*R*)-11). The procedure was the same as that for the preparation of **(*S*)-11** scaled for **(*R*)-10** (0.38 g, 1.2 mmol). The yield after two recrystallizations was 0.4 g (74%): mp 73.7−74.3 °C; $[\alpha]^{21}_D = -11.89°$ (*c* =0.37, $CHCl_3$). Spectral data were the same as for **(*S*)-11**. Anal. Calcd for $C_{21}H_{46}INO_2$: C, 53.49; H, 9.83; N, 2.97. Found: C, 53.22; H, 9.75; N, 2.93.

Preparation of the Cyclic Phosphorus Compounds. To Et_3N (8.96 mL, 64.3 mmol) in CH_2Cl_2 (300 mL) were added simultaneously **(*S*)-10** (5.30 g, 16.1 mmol) in CH_2Cl_2 (125 mL) and $MeP(O)Cl_2$ (3.8 g, 23 mmol) in CH_2Cl_2 (125 mL) over a period of 6 h. Then, the reaction was stirred overnight at rt. The solvent was evaporated; the residue was taken up in ether (200 mL) and filtered. The filtrate was concentrated, and the residue was purified by column chromatography on neutral alumina with EtOAc:hexanes (1:1). Two diastereomers were collected: first fraction, diastereomer **(12a)** 2.04 g; second fraction, diastereomer **(12b)** 1.18 g. The total yield was 51%.

Diastereomers **12c** (first fraction) and **12d** (second fraction) were prepared similarly from **(*R*)-10** (5.00 g, 15.2 mmol) to yield **12c** (2.00 g) and **12d** (1.45 g). The total yield was 57%.

(2*S*,4*S*)-2,6-Dimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane (12a). $[\alpha]^{22}_D = +6.12°$ ($c = 1.3$, $CHCl_3$); IR (KBr, cm^{-1}) 2915, 1455, 1231 (P═O), 1083; ^{1}H NMR (400 MHz, $CDCl_3$) 4.42 (m, 1H), 4.25 (m, 1H), 3.75 (m, 1H), 2.9 (m, 1H), 2.78 (m, 2H), 2.76−2.51 (m, 1H), 2.55 (s, 3H, *N*-C*H*$_3$), 1.42 (d, 3H, $J_{app} = 18$ Hz, PC*H*$_3$), 1.26 (s, 26H), 0.87 (t, 3H, $J_{app} = 7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 76.69 (d, $J_{app} = 7.5$ Hz), 65.59 (d, $J_{app} = 8.4$ Hz), 62.71, 58.68, 42.14, 32.88 (d, $J_{app} = 9.1$ Hz), 31.89, 29.66, 29.62, 29.53, 29.47, 29.43, 29.33, 25.43, 22.66, 14.09, 12.36 (d, $J_{app} = 155.5$ Hz, P*C*H$_3$; ^{31}P NMR (40 MHZ, $CDCl_3$) 26.81; MS (CI^+) 390 (100, M^+), 294 (15), 96 (12), 85 (12).

(2*R*,4*S*)-2,6-Dimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane (12b): mp 45.1−48.2 °C; $[\alpha]^{21}_D = +12.10°$ ($c = 1.5$, $CHCl_3$); IR (KBr, cm^{-1}) 2918, 1465, 1251 (P═O), 1083; ^{1} H NMR (400 MHz, $CDCl_3$) 4.39−432 (m, 1H), 4.16−4.08 (m, 1H), 3.91−3.87 (m, 1H), 2.95−2.89 (m, 1H), 2.76−2.61 (m, 3H), 2.53, 1.48 (d, 3H, $J_{app} = 16.4$ Hz, PCH$_3$), 1.6−1.4 (m, 2H), 1.25 (s, 26H), 0.88 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 77.96 (d, $J_{app} = 8.39$ Hz), 65.33 (d, $J_{app} = 7.63$ Hz), 62.53, 55.78, 45.55, 33.72 (d, $J_{app} = 6.9$ Hz), 31.9, 29.67, 29.63, 29.60, 29.54, 29.45, 29.40, 29.34, 25.71, 22.67, 14.13, 11.41 (d, $J_{app} = 148.02$ Hz, PCH$_3$); ^{31}P NMR (40 MHz, $CDCl_3$) 27.96. Anal. Calcd for C $_{21}$H $_{44}$NO $_3$P: C, 64.75; H, 11.38; N, 3.59. Found: C, 64.63; H, 11.29; N, 3.66.

(2*R*,4*R*)-2,6-Dimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane (12c). Preparation and spectral data are the same as those for **12a**. **12c:** $[\alpha]^{21}_D = -5.75°$ ($c = 1.2$, $CHCl_3$).

(2*S*,4*R*)-2,6-Dimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane (12d). Preparation and spectral data were the same as those for **12b**. **12d:** $[\alpha]^{21}_D = -12.54°$ ($c = 1.10$, $CHCl_3$). Anal. Calcd for $C_{21}H_{44}NO_3P$: C, 64.75; H, 11.38; N, 3.59. Found: C, 64.84; H, 11.35; N, 3.65.

(2*S*,4*S*)-2,6,6-Trimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctyl Bromide (2a). Compound **12a** (0.48g 1.2 mmol) was dissolved in ether (30 mL). The reaction flask was protected from light, and MeBr was bubbled through for 30 min. The mixture was then stirred for 5 d at rt. Additional MeBr was bubbled through on the third day. A white precipitate formed. The solvent was evaporated, and the crude product was washed thoroughly with ether to yield 0.39 g (65%) of the product: mp 91.7−159.3 °C (liquid crystal formation and decomposition); $[\alpha]^{22}_D = -7.96°$ ($c = 0.59$, $CHCl_3$); IR (KBr, cm^{-1}) 2918, 1472, 1245 (P═O), 1077; ^{1}H NMR (400 MHz, $CDCl_3$) 4.84 (m, 1H), 4.64 (m, 1H), 4.55−4.35 (m, 2H), 4.25−4.12 (m, 2H), 3.93−3.80 (m, 1H), 3.89 (s, 3H), 1.71 (m, 2H), 1.61 (d, $J_{app} = 18$ Hz, 3H, PC*H*$_3$), 1.44 (m, 2H), 1.25 (s, 24 H), 0.87 (t, $J_{app} = 7$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$)- 71.14, 69.23 (d, $J_{app} = 7.63$ Hz), 64.22, 60.09 (d, $J_{app} = 7.79$ Hz), 56.55, 50.93, 34.94 (d, $J_{app} = 6.9$ Hz), 31.98, 29.66, 29.62, 29.59, 29.53, 29.42, 29.32, 29.98, 24,88, 22.65, 14.08, 10.89 (d, $J_{app} = 145.73$, P*C*H$_3$); ^{31}P NMR (40 MHz, $CDCl_3$) 33.62; MS (EI^+) 389 ($M^+ - 15$), 375 (10), 279 (45), 192 (30), 152 (15), 96 (50), 70 (100), 58 (99). Anal. Calcd for $C_{22}H_{47}NO_3PBr$: C, 54.54; H, 9.77; N, 2.89. Found: C, 54.39; H, 9.72; N, 2.85.

(2*R*,4*S*)-2,6,6-Trimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctyl Bromide (2b). Compound **12b** (0.33 g, 0.85 mmol) was dissolved in ether (25 mL). The reaction flask was protected from light, and MeBr was bubbled through for 30 min. Reaction was stirred for 5 d at rt. Additional MeBr was bubbled through on the third day. The solvent was evaporated; the white solid was washed thoroughly with cold ether to yield 0.26 g (63%) of the product: $[\alpha]^{22}_D = -12.57°$ ($c = 0.81$, $CHCl_3$); IR (KBr, cm^{-1}) 2918, 1464, 1252 (P═O), 1082; ^{1}H NMR (400 MHz, $CDCl_3$) 4.70−4.59 (m, 1H), 4.60−4.40 (m, 2H), 4.35−4.20 (m, 3H) 3.68−3.65 (m, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 1.85−1.65 (m, 2H), 1.64 (d, $J_{app} = 17.6$ Hz, 3H, PCH$_3$), 1.5−1.13 (m, 2H), 1.25 (s, 24H), 0.88 (t, $J_{app} = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 73.06 (d, $J_{app} = 7.6$ Hz, 71.10, 63.51, 59.70 (d, $J_{app} = 7.6$ Hz), 58.23 , 49.42 , 33.79 (d, $J_{app} = 2.9$ Hz), 31.90, 29.68, 29.66, 29.59, 29.53, 29.41, 29.34, 29.22, 25.17, 22.66, 14.09, 10.79 (d, $J_{app} = 141.2$ Hz, PCH$_3$); ^{31}P NMR (40 MHz, $CDCl_3$) 31.8. Anal. Calcd for $C_{22}H_{47}BrNO_3P$: C, 54.54; H, 9.77; N, 2.89. Found: C, 54.58; H, 9.82, N, 2.98.

(2*R*,4*R*)-2,6,6-Trimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctyl Bromide (2c). Preparation and spectral data were the same as those for **2a**. **2c:** $[\alpha]^{22}_D = +8.46°$ ($c = 0.52$, $CHCl_3$). Anal. Calcd for $C_{22}H_{47}BrNO_3P$: C, 54.54; H, 9.77; N, 2.89. Found: C, 54.43; H, 9.75; N, 2.82.

(2*S*,4*R*)-2,6,6-Trimethyl-2-oxo-4-pentadecyl-1,3-dioxa-6-aza-2-phosphacyclooctane Bromide (2d). Preparation and spectral data were same as those for **2b**. **2d:** mp 94.8−162 °C (liquid crystal formation and decomposition); $[\alpha]^{22}_D = +12.95°$ ($c = 0.78$, $CHCl_3$). Anal. Calcd for $C_{22}H_{47}BrNO_3P$: C, 54.54; H, 9.77; N, 2.89. Found: C, 54.38; H, 9.75; N, 2.80.

PKC Assays. The activity of PKC was assayed in a 50 μL reaction in the presence of 20 mM Tris-Cl buffer (pH = 7.4), 0.1 mg/mL phosphatidylserine, 0.1 mM $CaCl_2$, 10 nM tetradecylphorbol acetate, 10 mM $MgCl_2$, 10 μM/μL leupeptin, 5 μM *p*-nitrophenylphosphate, 0.24 mg/mL lysine-rich histone, 0.2 mg/mL bovine serum albumin, and 20 μM [γ-^{32} P]ATP (*ca.* 1000 cpm/pmol). The PKC used was a 1:1 mixture of mouse α and mouse β-II prepared separately by expressing in Sf9 cells and partial purification on DEAE-cellulose. The reactions were started by simultaneous addition of histone and ATP, allowed to proceed for 30 min at rt and stopped by transferring 5 μL to phosphocellulose paper. The papers were washed in water, dried, and cut into scintillation vials. The reactivity bound to histone was determined by scintillation counting. Results of duplicate assays were averaged and the assays lacking PKC activity subtracted.

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