Table III. Hydrogen Bonding

atoms D-H···A[a]	equiv posn of A	length, Å H···A	length, Å D···A	angle ∠D-H···A, deg
Potassium Citrate Hydrate				
O(7)-H(5)···O(6)	x, y, z	1.965	2.564	118.4
O(7)-H(5)···O(4)	$-x, 1-y, -z$	2.319	3.144	143.7
O(W)-H(W1)···O(5)	$^1/_2-x, y-^1/_2, -z$	1.740	2.709	176.6
O(W)-H(W2)···O(4)	x, y, z	1.705	2.669	171.1
Potassium Fluorodeoxycitrate Hydrate				
O(1)-HO(1)*···O(1′)	$-x, 1-y, 1-z$	1.23	2.454	(180)
O(3)-HO(3)*···O(3′)	$1-x, 2-y, -z$	1.24	2.480	(180)
O(W)-HW(1)**···O(3)	$1-x, 1-y, 1-z$	2.02	3.048	(178)
O(W)-HW(2)···O(5)	x, y, z	2.03	2.774	167

[a] Key: *, symmetrical positions; **, calculated position.

The action of 3-fluorodeoxycitrate on the enzyme aconitase, for which citrate and isocitrate are substrates, is of interest.[19,20] The fluorodeoxycitrate is acted on by this enzyme and thus appears to act somewhat like a substrate; the C-F bond is broken to yield fluoride and *cis*-aconitate. However, the C-F bond is not formed again (unlike the case of C-OH bond breaking and remaking in citrate). As a result, fluorodeoxycitrate cannot be described as a true substrate of the enzyme. These findings, it is suggested,[19] favor a carbanion mechanism for aconitase action with proton abstraction as the first step, followed by hydroxyl elimination. This is in line without our proposals on a mode of action of the enzyme[21,22] as illustrated in Figure 3. It appears that some group in the enzyme must chelate the fluorodeoxycitrate and citrate in an analogous manner. Our work on another fluorocitrate, which is a powerful inhibitor, also backs up this idea and, as illustrated in Figure 3, explains why only one of four possible stereoisomers is such a potent inhibitor.[16] Our experimental observations of tridentate chelation of fluorocitrate and fluorodeoxycitrate are crucial to these ideas.

Acknowledgment. We thank Dr. J. Kollonitsch of Merck Sharp and Dohme Research Laboratories for the dicyclohexylamine salt of 3-fluoro-3-deoxycitrate and Chao Tsai for help with table preparation. This research was supported by Grants CA-10925, CA-22780, CA-06927, and RR-05539 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania.

Supplementary Material Available: Tables of X-ray and neutron data of atomic coordinates and temperature factors and representations of bond lengths and interbond angles in potassium citrate and potassium 3-fluoro-3-deoxycitrate (23 pages); tables of observed and calculated structure factors (55 pages). Ordering information is given on any current masthead page.

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Synthesis and Evaluation of Phospholipid Analogues as Inhibitors of Cobra Venom Phospholipase A_2

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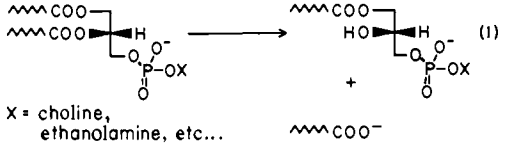
Abstract: Analogues of phospholipids that contain fluoro ketone, ketone, and alcohol replacements for the ester at the 2-position of the glycerol backbone have been prepared and analyzed as inhibitors of phospholipase A_2 from *Naja naja naja* venom. Phospholipid analogues were studied that contain two alkyl chains as well as single chain compounds that lack carbon-1 of the glycerol backbone and the attached acyl unit. Compounds that contain both long and medium length alkyl chains were studied. All of the potential inhibitors were tested in a well-defined mixed micelle system in which both the substrates and the inhibitors have been incorporated into Triton X-100 micelles. Surprisingly, the best inhibitors studied were the single chain fluoro ketones despite the fact that the enzyme has a strong preference for two-chain lipids. The most potent compound was found to have a dissociation constant some 600-3000-fold lower than the Michaelis constant for dipalmitoyl phosphatidylcholine substrate. ^{19}F NMR studies of the fluoro ketone phospholipid analogues in micelles show that whereas the single chain compounds are partially in the hydrated-ketone form, the two-chain compounds are less than 0.1% hydrated. In every case studied, potent inhibition of phospholipase A_2 was observed only with those compounds that are significantly hydrated in the micelle, and it is suggested that the hydrated fluoro ketone containing phospholipid analogues are the species responsible for the inhibition. In addition, the single chain fluoro ketones were better inhibitors than single and double chain alcohol and ketone analogues. Previous studies have shown that the cobra venom enzyme is activated by choline-containing lipids, and evidence is presented for the binding of the hydrated fluoro ketone inhibitors selectively to the activated enzyme.

Phospholipase A_2 catalyzes the hydrolysis of phospholipids at the 2-position of the glycerol backbone to produce a free fatty acid and a *lyso*-phospholipid (eq 1). The enzymes from pancreas



(1)

and snake venoms have been studied extensively[2] including the determination of the three-dimensional structure by X-ray diffraction for three enzymes.[3] All of these extracellular enzymes require millimolar concentrations of calcium for optimal activity.

There is considerable recent interest in intracellular phospholipases A_2 since these enzymes are currently thought to control

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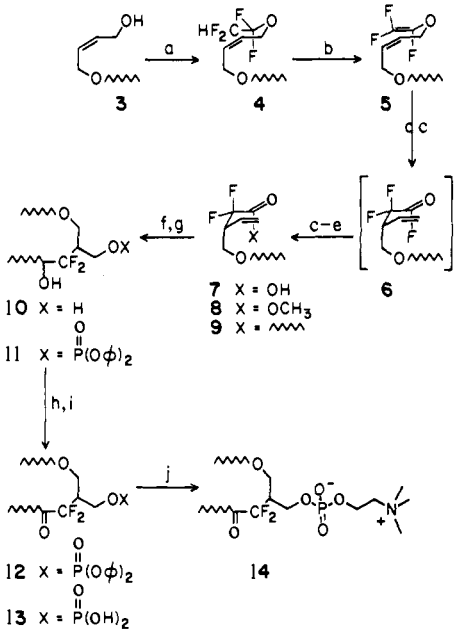
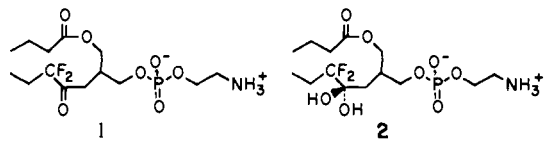
Scheme I[a]



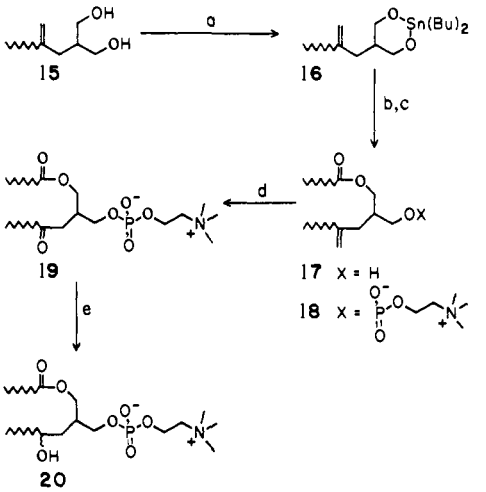
[a] (a) 5% NaH, C_2F_4, THF, −5 °C; (b) *n*-BuLi, THF, −60 °C; (c) H_2O, 25 °C; (d) CH_2N_2, ether, 25 °C; (e) R-Li, ether, −70 °C; (f) O_3 then $NaBH_4$, methanol, −70 °C; (g) $ClPO(OPh)_2$, pyridine, 25 °C; (h) CrO_3(pyridine)$_2$, CH_2Cl_2, 25 °C; (i) Pt/H_2, methanol, 25 °C; (j) choline tetraphenylborate, triisopropylbenzenesulfonyl chloride, pyridine, 35 °C. The curly lines refer to saturated alkyl chains of variable lengths.

the availability of arachidonic acid for the biosynthesis of complex lipid mediators such as the prostaglandins and the leukotrienes.[4] The purification and characterization of these enzymes is now beginning to emerge.[5] Because of the role of lipids derived from arachidonic acid in processes such as inflammation and platelet activation, there is medicinal interest in the discovery of compounds that inhibit phospholipase A_2. In addition, small molecular weight phospholipase A_2 inhibitors will be valuable tools in the elucidation of the metabolic pathways for arachidonic acid release in cells. It has been proposed that the mode of action of the anti-inflammatory steroids involves the induction of "factors" which regulate the action of intracellular phospholipases A_2.[6] Although a class of molecules termed the "lipocortins" were initially thought to act as endogenous phospholipase A_2 inhibitors, a recent study[7] has cast doubt on this role for the lipocortins and leaves open the identity of the factor that mediates the action of the anti-inflammatory steroids.

In this study, a number of phospholipid analogues were prepared as a logical step toward the rational design of phospholipase A_2 inhibitors. In addition, it was hoped that these inhibitors would shed some light on the mode of activation[8] of the cobra venom enzyme by choline-containing lipids. This work is an extension of a previous study[9] in which it was shown that **1** is a tight-binding



Scheme II[a]



[a] (a) $(Bu)_2SnO$, benzene, reflux; (b) RCOCl, $CHCl_3$, reflux; (c) ethylene chlorophosphate, Et_3N, benzene, 25 °C then Me_3N, CH_3CN, 65 °C; (d) O_3 then $(CH_3)_2S$, CH_2Cl_2/CH_3OH, −78 °C; (e) $NaBH_4$, CH_3OH, 25 °C.

inhibitor of the enzyme from *Naja naja naja* venom (K_i for **1** is 300-fold lower than the K_m for dibutyryl phosphatidylethanolamine). The hydrated form of **1** (structure **2**) was proposed as the inhibitory species since it is a structural mimic of the putative tetrahedral intermediate that forms during the enzyme-assisted attack of water onto the carbonyl group of the substrate ester.

Results and Discussion

Syntheses and Characterization of Phospholipid Analogues.[10] The preparation of phospholipid analogues in which the oxygen atom at the 2-position of the glycerol backbone is replaced with a CF_2 group is outlined in Scheme I. The synthesis begins with the addition of allylic alcohol **3** to tetrafluoroethylene in the presence of a catalytic amount of sodium hydride. The initially formed tetrafluorinated ether **4** is dehydrohalogenated with *n*-butyllithium to give the trifluorinated vinyl ether **5**. This compound undergoes a remarkably facile Claisen rearrangement at a temperature below 20 °C to generate the difluorinated acyl fluoride **6**. Related Claisen rearrangements have been reported previously.[11] Reaction of **6** with water gives difluoro acid **7** which was converted to ester **8** by reaction with diazomethane.[12] Ester **8** reacts with 1 equiv of alkyllithium to give the difluorinated ketone **9**. This reaction proceeds without the formation of any tertiary alcohol; apparently the initially formed lithio hemiketal anion resists decomposition at −30 °C.[13] Conversion of **9** to **10** by ozonolysis and reduction completes the formation of the glycerol backbone. Diol **10** could be selectively phosphorylated on the more nucleophilic nonfluorinated alcohol to give **11** which was oxidized to ketone **12** with either Jones' reagent or CrO_3(pyridine)$_2$. Ketone **12** was subjected to catalytic hydrogenation to effect the deprotection of the phosphate group, and the phosphatidic acid analogue **13** obtained was coupled to choline tetraphenylborate[14] with triisopropylbenzenesulfonyl chloride to give the final product **14**.

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(10) All new compounds were homogeneous as judged by TLC and gave high resolution NMR spectra that were fully consistent with the indicated structures. High resolution soft ionization mass spectra for selected members from each of the classes of phospholipid analogues showed the predicted molecular ion; no other high mass ions were detected.
(11) (a) Normant, J. F.; Reboul, O.; Sauvêtre, R.; Deshayes, H.; Masure, D.; Villieras, J. *Bull. Soc. Chim. Fr.* **1974**, 2072. (b) Metcalf, B. M.; Jarvi, E. T.; Burkhart, J. P. *Tetrahedron Lett.* **1985**, *26*, 2861.
(12) Although acyl fluoride **6** could be reacted with methanol to give ester **8** directly, the two-step procedure was used to facilitate the workup after the Claisen rearrangement.
(13) Reaction of the organolithium with the lithium salt of acid **7** produced the ketone **9** in low yield.
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Compounds prepared by Scheme I include single chain fluorinated ketones and two chain analogues in which the acyl unit attached to carbon-1 of the glycerol backbone is replaced with an alkyl ether.

Phospholipid analogues in which the oxygen atom at the 2-position of the glycerol backbone has been replaced with a CH_2 group and the CH_2 group on the other side of the ester carbonyl has been replaced with a CF_2 group were prepared in a manner previously described.[9] In this case, only two chain compounds were prepared.

Scheme II outlines the syntheses of a phospholipid analogue in which the ester at C-2 has been replaced by either a ketone or a secondary alcohol. The synthesis begins with the preparation of diol **15** which was monoacylated to ester **17** by formation of the stanoxane **16** followed by reaction with an acid chloride.[15] Ester **17** was converted into the phosphatidylcholine analogue **18** by reaction with ethylene chlorophosphate followed by treatment with trimethylamine.[16] The ketone **19** is generated in the final step by ozonolysis of the alkene. Routes which involved the formation of the ketone early on were complicated by the tendency of γ-hydroxy ketones to undergo partial cyclization to form a mixture of hemiketals. Alcohol-containing phospholipid analogue **20** was prepared as a mixture of diastereomers by reduction of **19** with sodium borohydride.

Critical micelle concentrations (CMC) values for ketones **14** and **19** were measured as described in the Experimental Section and found to have values of 48 and 62 μM, respectively. These CMC values are lower than the value of 200 μM for dioctanoyl phosphatidylcholine[17] presumably as a result of the replacement of the ester with the more hydrophobic ketone group.

Initial Inhibition Studies. The phospholipid analogues prepared in this study were tested as inhibitors of the phospholipase A_2 from *Naja naja naja* venom. In studies of phospholipase A_2 inhibition it is crucial that a well-defined enzymatic assay system be used since the substrates and often the inhibitors are usually water insoluble and the activity of the enzyme depends critically on the physical structure of the substrate–inhibitor aggregate. The inhibitors prepared in this study were tested in mixed micelles consisting of a mixture of substrate phospholipid and detergent (Triton X-100). This system has been well characterized[18] and consists of Triton X-100 micelles of well-defined size (approximately 140 Triton X-100 molecules per micelle) containing small amounts of phospholipid substrate and inhibitor. These mixed micelles are composed predominantly of detergent, and the presence of small quantities of substrate and inhibitor have little effect on the overall physical structure of the aggregate. Thus, any inhibition observed using this system is likely the result of the inhibitor directly interacting with the enzyme rather than altering the structure of the substrate aggregate. Furthermore, the mixed micelles are assumed to be uniform in the distribution of substrate and inhibitor among the ensemble of micelles.[18] This enables the kinetics of the inhibition to be understood in a way that is similar to the kinetics of enzymes that operate on water soluble substrates.

All of the inhibitors were initially tested in mixed micelles containing constant amounts of Triton X-100 (40 mM), dipalmitoyl phosphatidylcholine (DPPC) substrate (5 mM), and $CaCl_2$ (10 mM). The enzymatic activity was detected by titration of the liberated fatty acid in a pH-stat (see Experimental Section). Initial inhibitor evaluation was carried out by varying the concentration of inhibitor and measuring the enzymatic activity relative to the activity in the absence of the inhibitor. In this way, the concentration of inhibitor required to give 50% inhibition (IC_{50} values) was determined. As discussed below, the relative affinity of the enzyme for inhibitors versus substrates is best considered in terms of surface concentration; however, the use of IC_{50} values

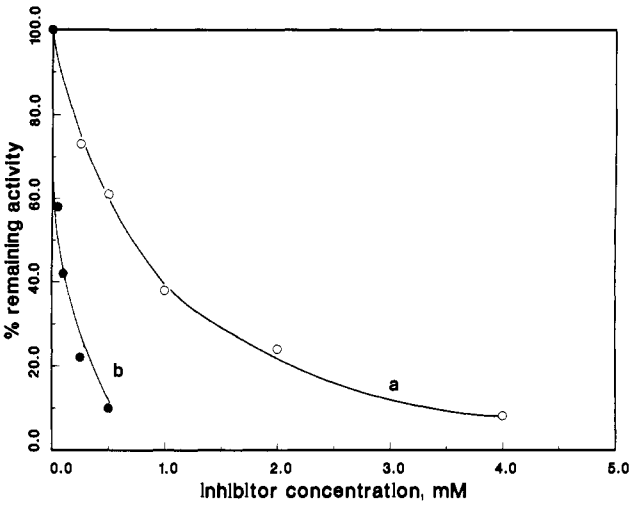

Figure 1. (a) Inhibition of phospholipase A_2 by increasing concentrations of inhibitor **21**. The concentrations of Triton X-100 (40 mM), DPPC (5 mM), and $CaCl_2$ (10 mM) were kept constant, and the amount of enzyme used was 0.1 units. (b) Inhibition by **29** under the same conditions as in (a).

is adequate for the ranking of inhibitors according to potency. For example, Figure 1 (curve a) shows that the reaction velocity for the hydrolysis of DPPC decreases to zero with increasing amounts of inhibitor **21**. The IC_{50} value for this compound was found to have a value of 0.7 mM. Table I lists the IC_{50} values for all of the inhibitors prepared in this study. The shorter single chain compound **22** was found to be almost as potent as **21** (see below). Compounds **23** and **14**, which are similar to **21** and **22** except that they contain an upper alkyl chain in an ether linkage to the glycerol backbone, were found to be much less potent inhibitors. This result was unexpected since phospholipase A_2 is known to hydrolyze two-chain substrates much more readily than single-chain substrates.[19] Furthermore, a study of phospholipase A_2 inhibitors that contain an amide as a replacement for the enzyme-susceptible ester showed that inhibitors with an ether-linked alkyl chain at the 1-position were much more potent than those with an ester-linked chain.[20] For these reasons, it is difficult to understand why ether-containing two-chain compounds **14** and **23** are much poorer inhibitors than the single-chain compounds **21** and **22**. An explanation for this result is given below.

Reduction of the difluorinated ketone of **21** to the difluorinated alcohol **24** results in a dramatic drop in the potency of inhibition. In addition, nonfluorinated ketone **25** is a much less active inhibitor than difluorinated ketone **22**. Together, these results show that the difluorinated ketone unit of **21** and **22** is required for potent inhibition. Compounds **26** and **27** contain a methylene replacement for the ester oxygen at the 2-position and a difluoromethylene group on the other side of the ketone. The results in Table I show that, surprisingly, these compounds are also significantly less potent than **21** and **22**. No significant improvement in the potency of inhibition was seen with the fluorinated alcohol **28**, the nonfluorinated ketone **19**, or the nonfluorinated alcohol **20**.

The results in Table I show that phosphatidylethanolamine analogue **29** is an extremely potent inhibitor of phospholipase A_2 being some 10-fold more potent than phosphatidylcholine analogue **21**. The reason for this increase in potency is discussed further below. Again, the addition of a second alkyl chain (compound **30**) results in a dramatic decrease in potency.

NMR Studies of Fluoro Ketone Phospholipid Analogues in Micelles. The above results demonstrate that single chain fluoro ketones are much better inhibitors than two-chain compounds and that a fluoro ketone functional group is required for potent in-

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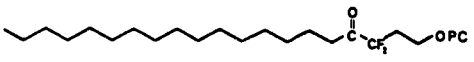
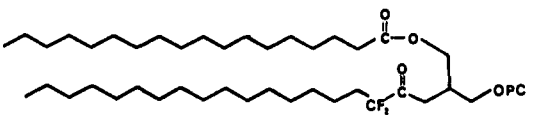
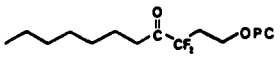
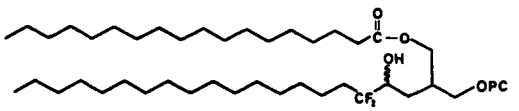
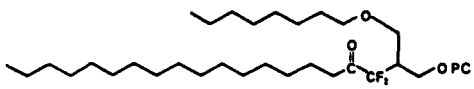
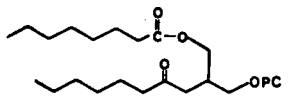
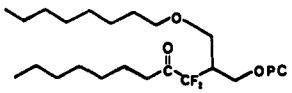
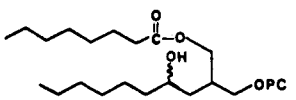
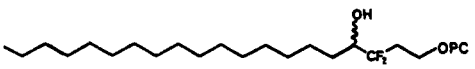
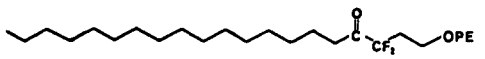
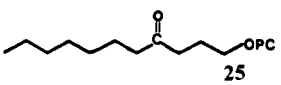
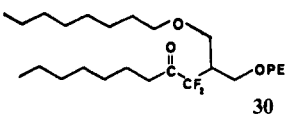
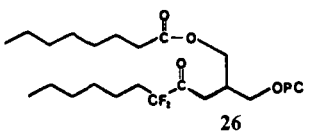
Table I. Inhibition of Phospholipase A_2 by Phospholipid Analogues

compd no. and structure	IC_{50} (mM)	compd no. and structure	IC_{50} (mM)
 **21**	0.7 [0.18][b]	 **27**	~4
 **22**	1.6 [0.16]	 **28**	1.4
 **23**	≫4[c]	 **19**	3.3
 **14**	~4	 **20**	1.5
 **24**	≫4	 **29**	0.07 [0.013]
 **25**	≫4	 **30**	>2
 **26**	1.7		

[a] All inhibition studies were carried out with fixed concentrations of Triton X-100 (40 mM), DPPC (5 mM), and $CaCl_2$ (10 mM). [b] Numbers in parentheses are the estimated IC_{50} values based on the actual concentration of hydrated ketone. [c] Less than 5% inhibition was seen with an inhibitor concentration of 4 mM. PC and PE refer to phosphorylcholine and phosphorylethanolamine, respectively.

hibition. In order to better understand these results, the hydration state of the fluoro ketone analogues bound in the detergent micelles was studied by ^{19}F NMR. The ^{19}F NMR spectrum of **21** in $CHCl_3$ (Figure 2b) shows a single triplet near −32.5 ppm (relative to a sodium trifluoroacetate reference). This signal is assigned to the difluoro ketone since the ^1H NMR of **21** in $CHCl_3$ was consistent only with a ketone structure. The observed splitting is due to coupling of the fluorine with the hydrogens of the adjacent methylene group. The ^{19}F NMR spectrum of **21** in Triton X-100 micelles in water (Figure 2a) shows two triplets near −32 and −39 ppm. The upfield resonance is assigned to the hydrated fluoro ketone based on analogous chemical shift changes seen with other fluoro ketones.[21] It is clear that **21** exists as a mixture of ketone and hydrate in Triton X-100 micelles. A similar result was obtained with the short chain compound **22** (Figure 2c). In this case four resonances were observed. The sharper triplets at −32.2 and −38.8 ppm are assigned to the hydrated and nonhydrated compound in the aqueous phase (these values are identical with the shifts seen with **22** in pure water) and the broader signals at −31.8 and −37.8 ppm to the micellar species. Integration of the signals in Figure 2c shows that **22** in water (46% hydrated) becomes slightly more dehydrated in the micelle (29% hydrated). Similar results were seen with phosphatidylethanolamine analogue **29**. Figure 2d shows that **21** is also partially hydrated in small unilamellar vesicles[22] made from egg phosphatidylcholine.

NMR studies with the two chain fluoro ketones showed that, in all cases, only the ketone was present in the detergent micelle. The ^{19}F NMR of **14** in micelles (Figure 2e) shows only signals from the two diastereotopic fluorines of the nonhydrated compound. These assignments are based on a comparison to the spectrum of the same compound in $CHCl_3$ (Figure 2f). Only ketone signals were seen for **23**, **26**, **27**, and **30** in micelles as well. In the case of **14**, less than 0.1% hydrated ketone could have been detected since an internal reference compound was detected at this low level in a spectrum that was acquired over a prolonged period. It is not clear from the present studies why the two-chain compounds are not hydrated in the micelle. It could be that the extra alkyl chain pulls the fluoro ketone deeper into the hydrophobic core of the micelle. Alternatively, the two-chain compounds could adopt a structure in the micelle that enforces a trigonal geometry at the carbonyl carbon.

These NMR results taken together with the inhibition studies demonstrates that only those fluoro ketones that are hydrated in the micelle are potent inhibitors of phospholipase A_2. This suggests that the hydrate is the true inhibitory species. The chemical modification studies described above are also consistent with the requirement for a *gem*-diol species. The IC_{50} values for **21**, **22**, and **29** in Table I reflect the concentration of total inhibitor species that give 50% inhibition. The IC_{50} values for the hydrate species are likely to be about fourfold lower since only about 25% of the inhibitors are hydrated in the micelle. The IC_{50} values for **21** and **22**, after correction for the degree of hydration and the fact that **22** is only partially in the micelle, are quite similar (0.18 versus 0.16, respectively). This indicates that increasing the chain length from eight to seventeen carbons has little effect on the interaction with the enzyme.

Kinetics of the Inhibition. The kinetics of inhibition of some of the phospholipid analogues were analyzed in more detail by varying the amount of DPPC substrate in the mixed micelle at different fixed inhibitor concentrations. The data were analyzed

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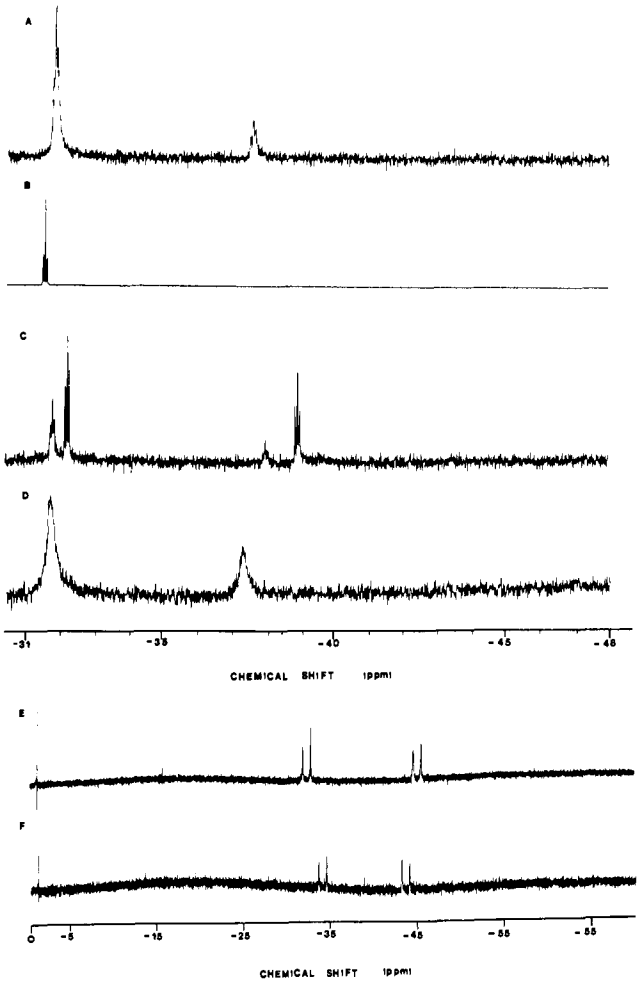

Figure 2. ^{19}F NMR (282 MHz) spectra of phospholipid analogues: (a) compound **21** in Triton X-100 micelles in water; (b) compound **21** in pure chloroform; (c) compound **22** in Triton X-100 micelles in water; (d) compound **21** in small unilamillar vesicles made from egg phosphatidylcholine; (e) compound **14** in micelles in water; (f) compound **14** in pure chloroform. Spectra of mixed micelles were obtained with 2 mM inhibitor, 5 mM DPPC, 40 mM Triton X-100, and 10 mM $CaCl_2$ at 40 °C.

in terms of the "dual phospholipid" model for the action of the venom enzyme on mixed micelles.[23]

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad (2)$$

$$ES + S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ESS \qquad (3)$$

$$ESS \xrightarrow{k_3} ES + P \qquad (4)$$

In this model, the enzyme first binds to a phospholipid molecule in the mixed micelle (eq 1). This process is dependent on the bulk concentration of DPPC in units of mol/vol. The binding of the enzyme to the micelle must involve an interaction with micellar phospholipid since the enzyme has no detectable affinity for pure Triton X-100 micelles.[23b] Once bound to the mixed micelle, the enzyme binds a second phospholipid in a process that depends on the surface concentration of phospholipid in units of mol/(surface area). The dual phospholipid model is the simplest scheme that

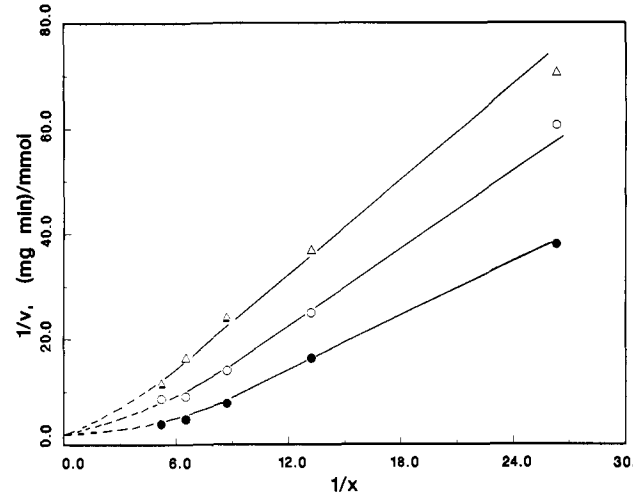

Figure 3. Double reciprocal plot for the inhibition of phospholipase A_2 by 0 mM (●), 1.0 mM (○), and 1.6 mM (△) **21**. The DPPC substrate was varied from 2.0 to 10.0 mM (0.038–0.19 mole fraction), and the Triton X-100 concentration was adjusted so that the concentration of inhibitor, substrate, and detergent totaled 52 mM. The amount of enzyme used was 0.1 units for all experiments.

accounts for the observed dependency of the enzymatic velocity on both the bulk and surface substrate concentrations. For example, if the bulk substrate concentration is held constant but its surface concentration is decreased by increasing the amount of detergent, the reaction velocity decreases. The assumptions are usually made that the size of the mixed micelles remain constant as the ratio of phospholipid to detergent is changed and that the phospholipid and the detergent have the same surface area per molecule.[23] With these assumptions, the surface concentration of phospholipid can simply be expressed as the mole fraction of phospholipid in the micelle. An equation relating the velocity of substrate hydrolysis to the amount of substrate present and kinetic quantities has been derived for eq 2–4 by using the steady-state approximation (eq 5).[23] Here K_s is the dissociation

$$\frac{1}{v} = \left[\left(\frac{K_m}{V_m}\right)\left(1 + \frac{K_s}{S_0}\right)\right]\frac{1}{X_s} + \frac{1}{V_m} \qquad (5)$$

constant $(k_{-1})/k_1$ for the interaction of the water soluble enzyme with the phospholipid in the micelle (in units of mol/vol), and K_m is the Michaelis constant $(k_{-2} + k_3)/k_2$ for the interaction of the micellar enzyme with phospholipid substrate (expressed as mole fraction). S_0 is the bulk phospholipid concentration (mol/vol), and X_s is the mole fraction of phospholipid in the micelle. S_0 and X_s can be varied independently by controlling the bulk concentration of both the phospholipid and detergent. For the substrate DPPC, K_s has a value is about 0.4–1.5 mM, and K_m is about 0.9 mole fraction.[23a,24] The reaction velocity is expressed by v, and V_m is the maximal velocity. The K_s-dependent term in eq 5 describes the initial binding of the enzyme to the mixed micelle, and the K_m-dependent term describes the interaction of the phospholipid with the enzyme in the surface of the mixed micelle. V_m is obtained if both S_0 is large compared to K_s (all of the enzyme is bound to the micelle) and X_s is large compared to K_m (all of the micellar enzyme is saturated with phospholipid).

Figure 3 shows a plot of the reciprocal values of the enzymatic reaction velocities versus the reciprocal values of the DPPC mole fraction in the absence of inhibitor and with two different fixed inhibitor mole fraction values. In this series of experiments, the values of S_0 ranged from 2–10 mM and were all well above K_s. Thus, essentially all of the enzyme is bound to the micelle in all experiments, and the K_s-dependent term in eq 5 is near unity.

(23) Some of the curvature seen in Figure 3 may arise from the fact that the lower substrate concentrations used may not be well above the K_s for DPPC. However, previous studies revealed that this curvature persists even when the DPPC concentration is held constant and well above K_s, and the mole fraction of substrate is varied by changing the detergent concentration. See: (a) Hendrickson, H. S.; Dennis, E. A. *J. Biol. Chem.* **1984**, *259*, 5734. (b) Roberts, M. F.; Deems, R. A.; Dennis, E. A. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1950. (c) Deems, R. A.; Eaton, B. R.; Dennis, E. A. *J. Biol. Chem.* **1975**, *250*, 9013.

(24) This value of 0.9 mole fraction for K_m is only an approximate value since mixed micelles cannot be made with such a large percentage of phospholipid. It is clear that K_m is significantly higher than 0.2–0.3 since the enzyme is still far from saturated at these phospholipid mole fractions.

According to eq 5, with $S_0 \gg K_s$, the change in $^1/_v$ with $^1/_{X_s}$ should be linear yet the lines in Figure 3 appear slightly curved. This behavior has been seen in earlier studies[23] and is probably due to the fact that the surface areas of the phospholipid and Triton X-100 molecules are not equal, and the use of mole fraction to express surface concentration may not be completely valid.

The equation describing the reaction velocity in the presence of the inhibitor can, in general, be quite complex since the interactions involve two different lipid species with two sites on the enzyme. In this case, a total of nine enzyme species may be present. A simplified approach to this problem is to consider the behavior of the system at values of $S_0 \gg K_s$ so that all of the enzyme is in the micelle. In this case, a decrease in the reaction velocity will be the result of the inhibitor competing with the substrate for the binding to the enzyme in the micellar surface (however, see below). Equation 5 can easily be modified to give eq 6 by including an X_i-dependent term for competitive inhibition

$$\frac{1}{v} = \left[\left(\frac{K_m}{V_m}\right)\left(1 + \frac{X_i}{K_i}\right)\right]\frac{1}{X_s} + \frac{1}{V_m} \qquad (6)$$

and again setting the K_s-dependent term to unity. Here, K_i is the dissociation constant for the interaction of the micellar enzyme with the inhibitor (expressed in mole fraction), and X_i is the mole fraction of inhibitor in the mixed micelle. The data in Figure 3, despite the curvature, clearly show that the presence of inhibitor affects the slope of the curves but not the ordinate intercept. Thus it is concluded that V_m is invariant to the concentration of inhibitor and that the substrate and inhibitor compete for the interaction with the enzyme in the surface of the micelle.

The K_i value for the inhibitors can be estimated from the IC_{50} values in Table I. In these experiments, the X_s values for DPPC ranged from 0–0.1 mole fraction and are much less than K_m (0.9 mole fraction). Evaluation of IC_{50} using eq 6, with $X_s \ll K_m$, shows that the value of X_i that produces 50% inhibition is equal to K_i. Thus, approximate values of K_i for the inhibitors in Table I are obtained simply by converting the IC_{50} values from bulk concentration to mole fraction. The K_i for inhibitor **29**, the most potent compound prepared, is approximately 600 times lower than the K_m for DPPC.[25] This degree of potency is gratifying since this phospholipid analogue contains only a single chain. As discussed above, the results in Table I and the ^{19}F NMR studies suggest that the hydrated fluoro ketone is required for tight binding to the enzyme.

Studies with Phosphatidylethanolamine Substrate. Elegant studies by Dennis[8] have shown that the cobra venom phospholipase A_2 hydrolyses choline-containing lipids about ten times faster than lipids containing ethanolamine when only one class of lipids is present in the mixed micelle. Yet if mixed micelles containing both types of lipids are made, the ethanolamine-containing material is often preferentially hydrolyzed. Further analysis has shown that the enzyme is activated by choline-containing lipids but not by ethanolamine-containing compounds. The binding of the activator to the enzyme does not significantly alter the K_m for the hydrolysis of ethanolamine lipids nor does it alter the affinity of the enzyme to the surface of the micelle. Rather, the activator dramatically increases the V_m of the enzyme.[8a] Since activation depends on the surface rather than the bulk concentration of activator,[8a] it must involve the interaction of the activator with the enzyme in the micelle. The activation appears to be due to an enzyme/lipid interaction rather than a lipid/lipid interaction.[8b] The molecular details of the activator-induced conformation change in the enzyme are not yet understood. It is clear that the activation site on the enzyme is distinct from the catalytic site since both the activator and substrate can be bound simultaneously to the enzyme. These results suggested that ethanolamine-containing inhibitors might be more potent than analogous choline compounds since the former would be expected to bind only to the catalytic site and the latter could simultaneously activate and inhibit the enzyme. Consistent with this idea is the fact that ethanolamine-containing fluoro ketone **29** is a significantly better inhibitor than the choline-containing **21** (Table I and Figure 1, curve b).

(25) Since **29** is only about one-fifth hydrated in the micelle, the actual value of the K_i for the hydrated lipid is probably some 3000-fold below the K_m for DPPC.

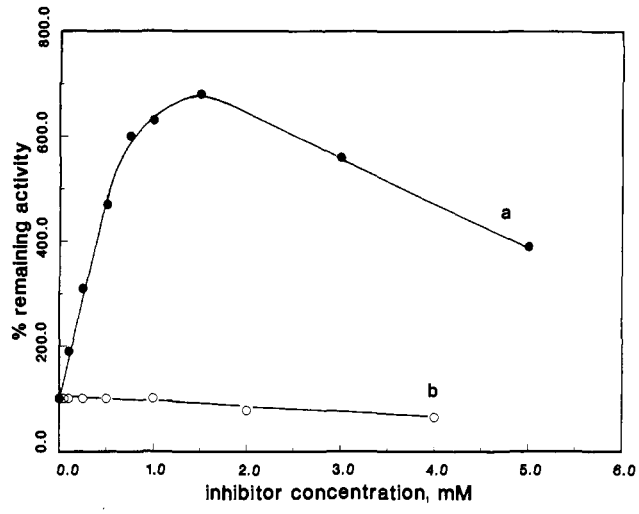

Figure 4. (a) Activity of phospholipase A_2 toward egg phosphatidylethanolamine as a function of the concentration of **21** with fixed concentrations of substrate (5 mM), Triton X-100 (40 mM), and $CaCl_2$ (10 mM). (b) Same as (a) except the inhibitor was **29**. The amount of enzyme used was 0.1 units in (a) and 0.7 units in (b).

The effect of compounds **21** and **29** on the hydrolysis of phosphatidylethanolamine substrate (PE-substrate) was then studied. The results are shown in Figure 4. The hydrolysis of the PE-substrate (5 mM) in the absence of inhibitor is only about 10% of the rate of DPPC (5 mM) hydrolysis. Small quantities of **21** were found to activate the enzyme (Figure 4, curve a). The maximum activation of 6.5-fold occurred with 1.5 mM **21**. Increasing the concentration of **21** further resulted in a decrease in the rate of hydrolysis of the PE-substrate. This result is fully consistent with the "dual phospholipid" model and suggests that inhibitor **21** is binding to both the activator and catalytic sites on the enzyme. Activation is more important than inhibition at low concentrations of **21** since the enzyme is highly sensitive to activation of the otherwise slowly hydrolyzed PE-substrate. At higher concentrations of **21**, both the activator and catalytic sites become saturated and the enzymatic activity decreases. This biphasic inhibition pattern has been observed previously with alkyl phosphorylcholines[8] and an amide analogue of phosphatidylcholine.[20] Activation is not seen for the inhibition of DPPC by **21** (Figure 1, curve a) presumably because the enzyme is already fully activated by DPPC which functions as both a substrate and an activator. One possible explanation for the inhibition of DPPC hydrolyses by **21** is that the inhibitor binds to the activator site without causing activation and thus competes with DPPC for the activation of the enzyme. Since **21** can function as an activator, the inhibition seen in Figure 1 must be mainly due to the binding of **21** at the catalytic site.

Figure 4 (curve b) shows that whereas the PE-inhibitor **29** is a potent inhibitor of DPPC hydrolysis, it shows almost no effect on the rate of hydrolysis of the PE-substrate at concentrations up to 4 mM. Figure 1 (curve b) shows that 4 mM **29** is more than sufficient to completely inhibit the hydrolysis of DPPC. Since the K_m values for the hydrolysis of DPPC and egg PE are similar,[23a] this result strongly suggests that the PE-inhibitor **29** binds tightly only to the activated enzyme. This hypothesis was confirmed when the inhibition of PE-substrate hydrolysis by **29** was determined in the presence of the PC-inhibitor/activator **21** at a concentration that partially activates the enzyme (0.5 mM). Under these conditions, **29** (1 mM) produced 42% inhibition, whereas the same concentration of **29** in the absence of the activator **21** produced no detectable inhibition (Figure 4, curve b).

It is tempting to speculate on the reasons for the selective tight binding of **29** and presumably **21** only to the activated enzyme.

Activation by choline-containing lipids changes the catalytic efficiency of the enzyme (V_m) rather than the interaction of the phospholipid substrate with the enzyme (K_m).[8a] It is logical to propose that the process of activation increases the affinity of the enzyme toward the high energy reaction intermediate in which the substrate ester has become distorted toward a tetrahedral geometry and that activation does not significantly alter the binding of the substrate in its ground-state geometry (this interpretation assumes that the V_m reflects a chemical step rather than a physical step such as product dissociation). Perhaps the selectivity in binding observed with the hydrated fluoro ketones is due to their closer structural resemblance to a tetrahedral intermediate rather than the ground state.

In summary, the results of this study demonstrate that potent phospholipase A_2 inhibitors can be prepared by incorporating tetrahedral structures into phospholipids at the 2-position of the glycerol backbone. The best inhibitors were found to be those fluoro ketones which are partially hydrated in the micelle. It is anticipated that the incorporation of "enforced" tetrahedral structures such as phosphorus or sulfur derivatives into two-chain phospholipids will produce phospholipase A_2 inhibitors of even higher affinity.

Experimental Section

Materials. Phospholipase A_2 from *Naja naja naja* venom (200–600 units per mg) was from Sigma. One unit of enzyme will hydrolyze 1 μmol of phosphatidylcholine per minute at pH 8.9 and 25 °C. DPPC and phosphatidylethanolamine (prepared by transesterification of egg phosphatidylcholine) were from Avanti Polar Lipids, Birmingham, AL. Tetrafluoroethylene was purchased from SCM Specialty Chemicals, Gainesville, FL. Mixed-bed ion exchanger (Rexyn I-300) was from Fisher. Triton X-100 was from Aldrich.

General Synthetic Methods. Flash chromatography was carried out as described[26] on silica gel 60 (230–400 mesh, Merck). Thin-layer chromatography was performed on silica gel plates (0.25 mm, Merck) by using the following detection methods: A, dipped into a solution containing 1% potassium permanganate and 0.08% sodium hydroxide; B, dipped into a solution containing *p*-anisaldehyde (37 mL), concentrated H_2SO_4 (50 mL), acetic acid (15 mL), and 95% ethanol (1.35 L) and heated on a hot plate until the spots were seen. Solvents for chromatography were ethyl acetate (E), petroleum ether (bp 30–60 °C) (P), hexane (H), methanol (M), water (W), chloroform (C), and methylene chloride (MC). All NMR chemical shift values are reported in δ units (ppm) referenced to tetramethylsilane for proton spectra and to sodium trifluoroacetate for fluorine spectra. Gas chromatography was carried out with a megabore DB-5+ column (J & W Scientific) with He carrier gas (approximately 10 mL/min). Liquid secondary ion mass spectra (LSIMS) were obtained at the University of California San Francisco mass spectrometry facility. Freshly dried ether and THF were obtained by distillation from sodium benzophenone under argon. Pyridine, benzene, and triethylamine were dried by distillation from CaH under argon, and CH_2Cl_2 was dried by distillation from P_2O_5 under argon. Solutions of organolithiums were standardized as described.[27] All organic extracts were dried over $MgSO_4$ prior to concentration.

2,2-Difluoro-4-pentenoic Acid, Methyl Ester (31). The following procedure is essentially as described previously.[11a] A 250-mL, three-necked flask equipped with a magnetic stir bar, a low-temperature thermometer, a condenser filled with dry ice/acetone, and a balloon was charged with dry THF (100 mL). The flask was cooled to approximately −50 °C, and tetrafluoroethylene was passed through a tube of silica and then through a needle into the THF until saturation was reached (as judged by inflation of the balloon). The flask was allowed to warm to −5 to −10 °C which caused the balloon to inflate slightly (most of the gas will be trapped by the condenser). To a separate 50-mL flask was added NaH (100 mg, 4.2 mmol, 80% suspension in oil), and the oil was removed by repeated washing with dry THF under argon. A solution of previously distilled allyl alcohol (3.0 g, 52 mmol) in dry THF (2 mL) was slowly added by syringe with stirring, and the mixture was allowed to stir for an additional 10 min. This solution was then transferred dropwise by syringe to the three-necked flask with stirring at −5 to −10 °C. After approximately 3 h, the reaction was checked by GC (60 °C) of a small aliquot, and, if needed, additional tetrafluoroethylene was bubbled into the solution for about 5 min. The product ether eluted just after the allyl alcohol. Further additions of tetrafluoroethylene were usually required. The addition reaction usually required 6 h. The reaction was placed under argon and cooled to −60 °C, and *n*-butyllithium (1 equiv in hexane) was added dropwise over 2 h from an addition funnel with the temperature kept constant at −60 °C. The mixture was cooled to −78 °C, water (2 mL) was added, the cooling bath was removed, and the solution was stirred overnight. The solution was basified with aqueous NaOH, and the solvent was removed in vacuo. The residue was acidified with 1 N HCl and extracted 3 times with ether. The organic layers were combined and extracted with saturated $NaHCO_3$. The aqueous extract was acidified to pH 1 with HCl and extracted 3 times with ether. The presence of the difluoro acid was confirmed by NMR of a small aliquot. The ether solution was treated directly with diazomethane in ether until the yellow color persisted. The solution was concentrated in vacuo, and the residue was purified on silica (2.5 % E in P) to obtain ester **31** (3.29 g, 42%) as a faint yellow liquid: TLC (2.5 % E in H, A) R_f 0.88; NMR ($CDCl_3$, 80 MHz) δ 2.6–3.1 (dt, 2 H), 3.7 (s, 3 H), 5.0–6.0 (m, 3 H).

4,4-Difluoro-1-dodecen-5-one (32). Ester **31** (2.17 g, 14.6 mmol) was dissolved in dry ether (30 mL) and cooled to −70 °C under argon. Heptyllithium, prepared as described,[28] (14.6 mmol in ether) was added by syringe with stirring, and the mixture was stirred an additional hour at the same temperature. The solution was warmed to −30 °C, saturated NH_4Cl (2 mL) was added, and the mixture was warmed to room temperature. The mixture was transferred to a separatory funnel containing water (30 mL). The layers were separated, and the aqueous layer was extracted once with ether. The combined organic layers were concentrated in vacuo. The oil obtained was purified on silica (H) to give the pure ketone (1.54 g, 48.6%): TLC (H, A) R_f 0.25; NMR ($CDCl_3$, 80 MHz) δ 0.9 (br t, 3 H), 1.3 (br s, 8 H), 1.6 (br d, 2 H), 2.45–3.0 (one t and three d, 4 H), 5.0–6.0 (m, 3 H).

(±)-3,3-Difluoro-1,4-undecanediol (33). Compound **32** (1.52 g, 7.0 mmol) in methanol (20 mL) was placed in a 100-mL flask cooled to −70 °C. Ozone was bubbled into the solution until the solution turned blue followed by the addition of $NaBH_4$ (1.59 g, 41.8 mmol). The mixture was stirred for 1 h at low temperature, allowed to warm to room temperature, and treated with water (2 mL). The solvent was removed in vacuo, and 0.1 N HCl (40 mL) was added. The solution was extracted 3 times with ether and concentrated in vacuo, and the residue was purified on silica (H:E, 2:1) to give a white solid (1.4 g, 90 %): TLC (H:E, 1:1, B) R_f 0.60; NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, 3 H), 1.10–1.45 (m, 8 H), 1.45–1.72 (m, 2 H), 2.05–2.46 (m, 2 H), 2.42–2.70 (br s, 2 H), 3.68–3.78 (m, 1 H), 3.79–3.90 (m, 2 H).

Phosphoric Acid, (±)-3,3-Difluoro-4-hydroxyundecyl Diphenyl Ester (34). Compound **33** (0.46 g, 2.1 mmol) was dissolved in dry pyridine (10 mL) in a 50-mL, tightly stoppered flask with a stir bar. Diphenyl chlorophosphate (1.6 g, 6 mmol, Aldrich) in pyridine (4 mL) was added, and the mixture was stirred at room temperature for about 1 h. The reaction was followed by TLC. Water (2 mL) was added, and the mixture was concentrated to a small volume in vacuo. The residue was added to a mixture of water and ether, the layers were separated, and the aqueous layer was extracted twice with ether. The ether layers were combined and concentrated in vacuo. The residue was purified on silica (25% E in H) to give 0.94 g (100%) of product: TLC (E:H, 1:1, B) R_f 0.78; NMR ($CDCl_3$, 80 MHz) δ 0.80 (br t, 3 H), 1.0–1.7 (m, 12 H), 2.0–2.8 (m, 2 H), 3.4–4.0 (m, 1 H), 4.3–4.7 (dd, 2 H), 7.25 (br m, 10 H).

Phosphoric Acid, 3,3-Difluoro-4-oxoundecyl Diphenyl Ester (35). Compound **34** (1.74 g, 3.8 mmol) was added to dry CH_2Cl_2 in a 100-mL flask with a stir bar. Freshly prepared $CrO_3(pyridine)_2$ (15.8 g, 58 mmol)[29] was added, and the reaction was stirred for 3–4 h under argon. The reaction was followed by TLC and stopped when no further formation of product was observed. The solution was filtered, and the filtrate was concentrated in vacuo. The residue was purified on silica (20% E in H) to yield 1.04 g (60%) of product: TLC (E:H, 1:1, B) R_f 0.80; NMR ($CDCl_3$, 80 MHz) δ 0.85 (br t, 3 H), 1.25 (br s, 8 H), 1.41–1.75 (br s, 2 H), 2.10–2.5 (m, 2 H), 2.52–2.72 (t, 2 H), δ 4.26–4.55 (dd, 2 H), 7.05–7.50 (m, 10 H).

Phosphoric Acid, 3,3-Difluoro-4-oxoundecyl Ester (36). Compound **35** (1.04 g, 2.3 mmol) in methanol (15 mL) was mixed with PtO_2 (250 mg) in a 100-mL flask attached to a balloon filled with H_2. The reaction was stirred at room temperature. The reaction progress was carefully checked by TLC (E:H, 1:1) to detect the loss of the starting material and with methanol to detect the much slower loss of the monophenyl ester. The reaction time between separate runs was highly variable but averaged about 8–10 h. Prolonged hydrogenation may result in reduction of the difluoro ketone to the alcohol. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The

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white product was used in the next step without further purification. TLC (M, B) R_f 0.75; NMR ($CDCl_3$, 500 MHz) δ 0.85 (t, 3 H), 1.20–1.35 (br s, 8 H), 1.64 (m, 2 H), 2.38–2.52 (m, 2 H), 2.70 (t, 2 H), 4.18–4.28 (br d, 2 H), 5.56–5.88 (br s, 2 H).

Phosphoric Acid, 3,3-Difluoro-4-oxoundecyl 2-(Trimethylamino)ethyl Ester (22). Compound **36** was dissolved in dry pyridine (40 mL) and 2,4,6-triisopropylbenzenesulfonyl chloride (2.17 g, 7.1 mmol, freshly recrystallized from pentane containing 1% thionyl chloride, Aldrich) was added followed by chlorine tetraphenylborate[14] (2.0 g, 4.72 mmol). The mixture was stirred for 4 h at 35 °C with the flask tightly stoppered. Water (1 mL) was added, and the solution was concentrated in vacuo. The residue was purified twice on silica (M:MC:W, 10:10:1, B). The product containing fractions was concentrated, and the residue was lyophilized from benzene to give the product as a pale yellow solid (0.56 g, 63% from **35**): TLC (same solvent, B); NMR (DMSO-d_6, 300 MHz) δ 0.95 (t, 3 H), 1.30 (br s, 8 H), 1.58 (br t, 2 H), 2.30–2.48 (m, 2 H), 2.78 (t, 2 H), 3.20 (s, 9 H), 3.58 (m, 2 H), 3.80 (dd, 2 H), 4.02 (br s, 2 H); LSIMS-MS (positive ion), 388 (M + 1).

4,4-Difluoro-1-heneicosen-5-one (37). This compound was prepared as described for **32** starting with **31** (3.2 g, 21.3 mmol) and *n*-hexadecyllithium[28] (25.5 mmol in ether) in dry ether (100 mL). The yield of **37** after chromatography on silica (P) was 3.2 g (44%): TLC (P, A) R_f 0.28; NMR ($CDCl_3$, 80 MHz) δ 0.92 (br t, 3 H), 1.0–1.4 (br s, 26 H), 1.64 (br m, 2 H), 2.2–2.82 (m, 4 H), 4.8–5.8 (m, 3 H).

(±)-3,3-Difluoro-1,4-eicosanediol (38). This compound was prepared as described for **33** except that the solvent was methanol:methylene chloride (1:1) instead of pure methanol in order to dissolve **37** at low temperature. The ozonolysis of **37** (3.2 g) was carried out at −50 °C to give 2.01 g (62%) of **38** as a white solid after chromatography on silica (25% E in P): TLC (20% E in P, B) R_f 0.20; NMR (acetone-d_6, 300 MHz) δ 0.89 (t, 3 H), 1.22 (br s, 28 H), 1.60 (br t, 2 H), 2.05–2.35 (m, 2 H), 3.6–3.8 (m, 1 H), 3.75 (t, 2 H).

Phosphoric Acid, (±)-3,3-Difluoro-4-hydroxyeicosyl Diphenyl Ester (39). Compound **38** (0.83 g) was reacted as described for **34** to afford **39** (1.37 g, 99%) after purification on silica (E:P, 2:1): TLC (E:H, 1:1, B) R_f 0.80; NMR (acetone-d_6, 300 MHz) δ 0.89 (t, 3 H), 1.20–1.35 (br s, 28 H), 1.58 (br s, 2 H), 2.35–2.55 (m, 2 H), 3.62–3.75 (m, 1 H), 4.50 (dd, 2 H), 7.25 (m, 6 H), 7.40 (m, 4 H).

Phosphoric Acid, 3,3-Difluoro-4-oxoeicosyl Diphenyl Ester (40). CrO_3 (2.3 g) was dried overnight over P_2O_5 in vacuo and added to a mixture of Celite (5 g) in dry pyridine (3.7 mL) and dry CH_2Cl_2 (45 mL) at 0 °C. Stirring was continued for 2 h followed by the addition of **39** (1.37 g, 2.36 mmol) in dry CH_2Cl_2 (10 mL). The reaction was followed by TLC and stopped after 1.5 h by the addition of ether (50 mL). The mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was purified on silica (P:E, 5:1) to give **40** (1.19 g, 88%): TLC (25% E in H, B) R_f 0.60; NMR (acetone-d_6, 300 MHz) δ 0.87 (t, 3 H), 1.25–1.40 (br s, 26 H), 1.57 (br t, 2 H), 2.48–2.65 (m, 2 H), 2.72 (t, 2 H), 4.48 (dd, 2 H), 7.24–7.42 (m, 10 H).

Phosphoric Acid, 3,3-Difluoro-4-oxoeicosyl Ester (41). The procedure is the same as that used for **36** starting from **40** (0.74 g) to yield **41** (0.55 g, 100%) as a white solid: NMR (DMSO-d_6, 300 MHz) δ 0.89 (t, 3 H), 1.28 (br s, 26 H), 1.54 (br t, 2 H), 2.35–2.55 (m, 2 H), 2.75 (t, 2 H), 3.97 (dd, 2 H), 4.20–5.50 (br s, 2 H).

Phosphoric Acid, 3,3-Difluoro-4-oxoeicosyl 2-(Trimethylamino)ethyl Ester (21). By using a procedure described for **22**, **41** (0.49 g) was converted into **21** (0.32 g, 54%) after two chromatographic purifications (M:MC:W, 10:10:1): TLC (same solvent, B) R_f 0.15; NMR (CD_3OD:$CDCl_3$:D_2O, 10:10:1, 300 MHz) δ 0.89 (t, 3 H), 1.28 (br s, 26 H), 1.60 (br t, 2 H), 2.30–2.50, (m, 2 H), 2.70 (t, 2 H), 4.02 (dd, 2 H), 4.20 (br s, 2 H); LSIMS (positive ion), 514 (M + 1).

Phosphoric Acid, (±)-3,3-Difluoro-4-hydroxyeicosyl 2-(Trimethylamino)ethyl Ester (24). Compound **21** (37 mg, 0.072 mmol) in methanol/methylene chloride (1:1) (10 mL) and $NaBH_4$ (10 mg) and the reaction was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue was purified on silica (M:MC:W, 10:10:1) and lyophilized from benzene to give **24** (37 mg, 99%) as a pale yellow solid: TLC (same solvent, B) R_f 0.15; NMR (CD_3OD/$CDCl_3$, 1:1, 300 MHz) δ 0.89 (t, 3 H), 1.27 (s, 28 H), 1.60 (br s, 2 H), 2.10–2.50 (br m, 2 H), 3.21 (s, 9 H), 3.55–3.70 (m and t, 3 H), 4.10 (dd, 2 H), 4.25 (br s, 2 H).

(±)-Methyl 2,2-Difluoro-3-octyloxymethyl-4-pentenoate (8). The preparation of **8** was essentially the same as described for **31** starting with *cis*-4-octoxyl-2-buten-1-ol (5.0 g, 25 mmol) in dry THF (80 mL). The starting material was prepared as follows: *cis*-butenediol (88 g, 1 mol) in dry THF (200 mL) was treated with a solution of washed NaH (7.7 g, 0.32 mol, 80% suspension in oil) in dry THF (20 mL) at 0 °C, and stirring was continued for 0.5 h at room temperature. A solution of octyl methylsulfonate (53.5 g, 0.26 mol) was added at 0 °C, and stirring was continued for 1 h at room temperature followed by refluxing for 20 h and stirring at room temperature for 10 h. Ice (50 g), water (100 mL) and ether (100 mL) were added, and the ether layer was washed with brine until TLC of the aqueous extract failed to contain butenediol. Concentration and distillation (107–115 °C, 0.2–0.25 mm) afforded the product (37 g, 72%) as a clear liquid. The addition of tetrafluoroethylene was monitored by GC (180 °C) and required about 2 h. The retention times for the alcohol and ether were 2.05 and 1.75 min, respectively. Pure **8** (3.1 g, 43%) was obtained as a pale yellow liquid after chromatography on silica (5% E in P): TLC (10% E in P, A) R_f 0.60; NMR ($CDCl_3$, 60 MHz) δ 0.85 (br t, 3 H), 1.0–1.4 (br s, 10 H), 1.65–1.85 (br m, 2 H), 2.7–4.0 (m, 5 H), 3.66 (s, 3 H), 4.9–6.0 (m, 3 H).

(±)-4,4-Difluoro-3-octyloxymethyl-1-dodecen-5-one (9). Compound **8** (2.94 g) was reacted with *n*-heptyllithium as described for **32** to give **9** (2.5 g, 69%) as a pale yellow liquid after purification on silica (2% E in P): TLC (25% E in P, B) R_f 0.88; NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 6 H), 1.22–1.35 (br s, 18 H), 1.48 (br t, 2 H), 1.60 (br m, 2 H), 2.62 (t, 2 H), 3.15–3.25 (m, 1 H), 3.32 (t, 2 H), 3.40–3.62 (m, 2 H), 5.23–5.35 (m, 2 H), 5.66–5.82 (m, 1 H).

(*R,*R**)- and (*R**,*S**)-3,3-Difluoro-2-octyloxymethyl-1,4-undecanediol (10).** Compound **9** (2.4 g) was converted to the pale yellow liquid **10** (1.4 g, 57%) as described for **33** after purification on silica (E:H, 1:2.5). Compound **10** consists of a equal mixture of two diastereomers which could be separated on silica. The mass spectra for the two compounds were similar but the NMR were different: TLC (E:H, 1:2.5, B) R_f 0.38 and 0.5; NMR ($CDCl_3$, 500 MHz, mixture) δ 0.89 (t, 6 H), 1.22–1.40 (br s, 18 H), 1.52–1.70 (m, 4 H), 2.52 (br d, 1 H), 3.45–3.75 (m, 5 H), 3.75–3.95 (dm, 2 H).

Phosphoric Acid, (*R,*R**)- and (*R**,*S**)-3,3-Difluoro-4-hydroxyundecyl Diphenyl Ester (11).** Starting with **10** (1.2 g), **11** (2.0 g, 100%) was obtained as a pale yellow liquid following chromatography on silica (20% E in P) as described for **34**: TLC (E:P, 1:2.5, B) R_f 0.68; NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, 6 H), 1.20–1.40 (br s, 18 H), 1.50–1.70 (m, 4 H), 2.60–2.80 (br d, 0.5 H), 2.98 (d, 0.5 H), 3.30–3.75 (m, 5 H), 4.25 (m, 0.5 H), 4.55 (m, 1.5 H), 7.10–7.25 (m, 6 H), 7.25–7.40 (m, 4 H).

Phosphoric Acid, (±)-3,3-Difluoro-2-octyloxymethyl-4-oxoundecyl Diphenyl Ester (12). This compound was prepared as described for **35** starting with **11** (2.0 g) to give **12** (0.85 g, 43%) after chromatography on silica (20% E in P): TLC (25% E in P, B) R_f 0.58; NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 6 H), 1.22–1.35 (br s, 18 H), 1.45 (t, 2 H), 1.58 (t, 2 H), 2.62 (t, 2 H), 2.88 (br s, 1 H), 3.28 (t, 2 H), 3.52 (m, 2 H), 4.38–4.58 (dm, 2 H), 7.22 (m, 6 H), 7.35 (m, 4 H).

Phosphoric Acid, (±)-3,3-Difluoro-2-octyloxymethyl-4-oxoundecyl Ester (13). In a manner similar to the preparation of **36**, **12** (0.73 g) afforded **13** (0.54 g, 99%) as a white gelatinous solid which was used in the next step without further purification: NMR ($CDCl_3$, 300 MHz) δ 0.88 (br t, 6 H), 1.30 (br s, 18 H), 1.48 (br s, 2 H), 1.58 (br s, 2 H), 2.65 (br t, 2 H), δ 2.90 (br s, 1 H), 3.35 (br t, 2 H), 3.61 (m, 2 H), 4.10–4.35 (br d, 2 H), 8.20 (br s, 2 H).

Phosphoric Acid, (±)-3,3-Difluoro-2-octyloxymethyl-4-oxoundecyl 2-(Trimethylamino)ethyl Ester (14). Compound **13** (0.5 g) was converted to **14** as described for **22** giving product (0.36 g, 60%) after two chromatographic purifications on silica (M:MC:W, 20:20:1) and lyophilization from benzene. TLC (same solvent, B) R_f 0.15; NMR ($CDCl_3$, 300 MHz), δ 0.90 (t, 6 H), 1.21–1.38 (br d, 18 H), 1.45 (br t, 2 H), 1.58 (br t, 2 H), 2.65 (t, 2 H), 2.80–2.98 (br m, 1 H), 3.30 (br t, 2 H), 3.40 (s, 9 H), 3.45–3.66 (m, 2 H), 3.84 (br s, 2 H), 3.90–4.18 (dm, 2 H), 4.31 (br s, 2 H).

(±)-4,4-Difluoro-3-octyloxymethyl-1-heneicosen-5-one (42). As described for **9**, **31** (1.0 g) was converted to **42** (0.96 g, 58%, pale yellow liquid) after chromatography on silica (2% E in P): TLC (same solvent) R_f 0.19; NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 6 H), 1.15–1.40 (br s, 36 H), 1.45 (br s, 2 H), 1.58 (br s, 2 H), 2.61 (t, 2 H), 3.15–3.35 (m, 1 H, t, 3 H), 3.40–3.60 (m, 2 H), 5.24–5.34 (m, 2 H), 5.65–5.70 (m, 1 H).

(*R,*R**)- and (*R**,*S**)-3,3-Difluoro-2-octyloxymethyl-1,4-eicosanediol (43).** Compound **42** (0.96 g) was converted into the pale yellow liquid **43** (0.44 g, 45%) as described for **10**. The product was purified on silica (P/E, 7:1): TLC (20% E in P, B) R_f 0.18 and 0.26.

Phosphoric Acid, (*R,*R**)- and (*R**,*S**)-3,3-Difluoro-4-hydroxyeicosyl Diphenyl Ester (44).** As described for **11**, **43** (0.44 g) was converted to the white solid **44** (0.64 g, 98%) after purification on silica (20% E in P): TLC (E:P, 3.5:1, B) R_f 0.84; NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, 6 H), 1.15–1.45 (br s, 36 H), 1.50–1.70 (br s, 4 H), 2.60–2.90 (br s, 0.5 H), 3.08 (d, 0.5 H), 3.45 (t, 2 H), 3.55–3.85 (m, 3 H), 4.30 (m, 0.5 H), 4.58 (m, 1.5 H).

Phosphoric Acid, (±)-3,3-Difluoro-2-octyloxmethyl-4-oxoeicosyl Diphenyl Ester (45). Compound **44** (0.63 g) was converted into the white solid **45** (0.48 g, 76%) as described for **12**. Purification was on silica (P:E, 8:1): TLC (P:E, 7:1, B) R_f 0.40; NMR ($CDCl_3$, 500 MHz) δ 0.9 (t, 6 H), 1.15–1.35 (m, 36 H), 1.42 (br s, 2 H), 1.55 (br m, 2 H), 2.6

(t, 2 H), 2.78–2.90 (br s, 1 H), 3.28 (t, 2 H), 3.52 (d, 2 H), 4.35–4.55 (br d, 2 H), 7.2–7.38 (m, 10 H).

Phosphoric Acid, (±)-3,3-Difluoro-2-octyloxymethyl-4-oxoeicosyl Ester (46). The white solid **46** (0.38 g, 100%) was obtained from **45** (0.63 g) as described for **13**: NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, 6 H), 1.22 (br s, 36 H), 1.45 (br s, 2 H), 1.58 (br s, 2 H), 2.61 (t, 2 H), 2.9–3.0 (br m, 1 H), 3.33 (t, 2 H), 3.5–3.65 (m, 2 H), 4.1–4.35 (br d, 2 H), 5.1–5.5 (br s, 2 H).

Phosphoric Acid, (±)-2-(trimethylamino)ethyl 2-Octyloxymethyl-3,3-difluoro-4-oxoeicosyl Ester (23). Starting with **46** (0.38 g), **23** (0.26 g, 59%) was obtained as a white solid as described for **14**. The product was purified by passage through a mixed-bed ion exchanger (50 g) (M:MC, 1:1) followed by chromatography on silica (M:MC:W, 20:20:1) and finally lyophilization from benzene: TLC (same solvent, B) R_f 0.15; NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 6 H), 1.28 (br s, 36 H), 2.72 (t, 3 H), 2.85–2.95 (m, 1 H), 3.28 (t, 2 H), 3.38 (s, 9 H), 3.5–3.65 (m, 2 H), 3.82 (br s, 2 H), 3.90–4.15 (dm, 2 H), 4.31 (br s, 2 H).

Phosphoric Acid, 3,3-Difluoro-4-oxoeicosyl 2-Aminoethyl Ester (29). Compound **41** (0.34 g) was reacted with *N*-tritylethanolamine[34] (0.47 g) and 2,4,6-triisopropylbenzenesulfonyl chloride (0.71 g) in dry pyridine (20 mL) as described for **22** to give **29**. The crude product was partially purified on silica (25% E in P) to give material which still contained some *N*-tritylethanolamine: TLC (same solvent) R_f 0.32. The trityl compound was dried in vacuo and dissolved at 0 °C under argon in trifluoroacetic acid (about 2 mL).[35] After 20 min, the trifluoroacetic acid was removed in vacuo. The product was purified on silica (M:C, 1:1) and then lyophilized from benzene to give pure **29** (0.2 g, 54%): TLC (same solvent, B) R_f 0.23; NMR ($CDCl_3/CD_3OD$, 1:2, 300 MHz) δ 0.89 (t, 3 H), 1.3 (s, 26 H), 1.62 (t, 2 H), 2.32–2.50 (br m, 2 H), 2.72 (t, 2 H), 3.12 (t, 2 H), 4.06 (m, 4 H), 4.54 (s, water and amino group).

Phosphoric Acid, 2-Aminoethyl 3,3-Difluoro-2-octyloxymethyl-4-oxoundecyl Ester (30). As described for **29**, **13** (48 mg) was converted into *N*-trityl lipid and then to **30** (37 mg, 70%): TLC (M:C, 1:1, B) R_f 0.30; NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 6 H), 1.18–1.35 (two br s, 18 H), 1.4–1.5 (br s, 2 H), 1.5–1.62 (br t, 2 H), 2.61 (t, 2 H), 2.75–2.92 (br s, 1 H), 3.18 (br s, 2 H), 3.28 (t, 2 H), 3.52 (br m, 2 H), 3.75–4.0 (dm, 2 H), 4.1 (br s, 2 H), 8.30 (br s, 3 H).

2,2-Dimethyl-5,5-bis(ethoxycarbonyl)-1,3-dioxane (47). Diethyl bis(hydroxymethyl)malonate (100 g, 0.45 mol, Aldrich) and acetone (53 g, 0.91 mol) were added to benzene (700 mL) containing *p*-methyl toluenesulfonic acid (4.3 g), and the mixture was refluxed for 6 h with azeotropic removal of water. The solution was washed twice with saturated $NaHCO_3$ (2 × 100 mL) and twice with brine (2 × 100 mL). The organic layer was concentrated in vacuo, and the residue was distilled (132 °C, 7 mm) to furnish diester **47** (91.4 g, 77.4%) as a clear liquid: NMR ($CDCl_3$, 80 MHz) δ 1.25 (t, 3 H), δ 1.40 (s, 6 H), 4.25 (q, 2 H), 4.30 (s, 4 H).

2,2-Dimethyl-5-(ethoxycarbonyl)-1,3-dioxane (48). Diester **47** (81.1 g, 0.312 mol) was decarbethoxylated as described[30] except that the reaction was refluxed for only 4 h. The crude mixture was distilled with a kugelrohr (75 °C, 0.4 mm) to give the clear liquid **48** (42.1 g, 72%): NMR ($CDCl_3$, 80 MHz) δ 1.28 (t, 3 H), 1.40 (s, 3 H), 1.42 (s, 3 H), 2.82 (m, 1 H), 4.20 (m, 6 H).

2,2-Dimethyl-5-(hydroxymethyl)-1,3-dioxane (49). Ester **48** (27.1 g, 0.144 mol) was reduced with $LiAlH_4$ in the usual manner to give **49** (17.2 g, 82%) as a clear liquid: NMR ($CDCl_3$, 80 MHz) δ 1.40 (s, 3 H), 1.44 (s, 3 H), 1.75 (m, 1 H), 2.04 (br s, 1 H), 3.95 (m, 6 H).

5-(Bromomethyl)-2,2-dimethyl-1,3-dioxane (50). To a stirred solution of alcohol **49** (7.8 g, 53 mmol) in dry CH_2Cl_2 (10.8 mL) and dry pyridine (4.8 mL) was added carbon tetrabromide (26.83 g, 81 mmol, Aldrich). To this solution was added triphenylphosphine (14.2 g, 54 mmol, Aldrich) in dry CH_2Cl_2 (15.4 mL) over 1.5 h under argon. After an additional hour, the mixture was poured into cold petroleum ether (290 mL), the suspension was filtered, and the filtrate was concentrated in vacuo. Purification by distillation (58–60 °C, 0.7 mm) afforded **50** (7.9 g, 71%) as a clear liquid: NMR ($CDCl_3$, 80 MHz) δ 1.40 (s, 3 H), 1.43 (s, 3 H), 2.02 (m, 1 H), 3.50 (d, 2 H), 3.75 (dd, 2 H), 4.05 (dd, 2 H).

2-Oxooctanoic Acid, Ethyl Ester (51). The 1,3-dithiane of ethyl glyoxylate (10.0 g, 0.052 mol) was alkylated with hexyl bromide as described[31] to give the dithiane (12.3 g, 86%) as a clear oil. The compound was used in the next step without further purification: TLC (10% E in P, B) R_f 0.87; NMR ($CDCl_3$, 80 MHz) δ 0.85 (t, 3 H), 1.30 (m, 13 H), 1.95 (m, 2 H), 2.62 (m, 2 H), 3.27 (m, 2 H), 4.25 (q, 2 H). The dithiane was desulfurized with *N*-bromosuccinimide as described[31] to give **51** (5.5 g, 68%) as a clear oil. The product was distilled with a kugelrohr (85–90 °C, 2.5 mm): NMR ($CDCl_3$, 80 MHz) δ 0.90 (t, 3 H), 1.35 (m, 11 H), 2.80 (t, 2 H), 4.33 (q, 2 H).

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2,2-Difluorooctanoic Acid, Ethyl Ester (52). To a stirred solution of (diethylamino)sulfur trifluoride (3.8 mL, 31 mmol, Aldrich) in dry CH_2Cl_2 (15.1 mL) was added **51** (5.5 g, 30 mmol) over 5 min at room temperature. The solution was stirred for an additional hour under a $CaSO_4$-filled dry tube and then treated cautiously with chilled water (19 mL). Additional CH_2Cl_2 was added, the layers were separated, and the organic layer was washed with water and concentrated in vacuo followed by distillation (75–80 °C, 5 mm) afforded **52** (4.5 g, 73%) as a yellow oil: TLC (5% E in P, B) R_f 0.72; NMR ($CDCl_3$, 80 MHz) δ 0.89 (t, 3 H), 1.35 (m, 11 H), 2.10 (m, 2 H), 4.25 (q, 2 H).

3,3-Difluoro-1-(2,2-dimethyl-1,3-dioxan-5-yl)-2-nonone (53). To a −78 °C solution of bromide **50** (5.708 g, 27 mmol) in dry ether (78 mL) under argon was added *tert*-butyllithium (28.3 mL, 55 mmol in ether) dropwise by syringe with stirring over 10 min. Ester **52** (5.68 g, 27 mmol) in dry ether (6.9 mL) was added by syringe over 2 min. The bath was removed, and the solution was stirred for 15 min followed by the addition of aqueous K_2HPO_4 (124 mL). The layers were separated, and the aqueous layer was extracted twice with ether. The organic layers were combined and concentrated in vacuo, and the residue was purified on silica (10% E in P) to give ketone **53** (2.16 g, 27%) as a clear oil: TLC (same solvent, B) R_f 0.57; NMR ($CDCl_3$, 80 MHz) δ 0.89 (m, 3 H), 1.33 (br s, 8 H), 1.40 (s, 3 H), 1.42 (s, 3 H), 2.07 (m, 3 H), 2.84 (d, 2 H), 3.55 (dd, 2 H), 4.03 (dd, 2 H).

***cis*-(±)- and *trans*-(±)-5-(1,1-Difluoroheptyl)tetrahydro-5-hydroxy-3-furanmethanol (54).** To a stirring solution of **53** (2.16 g, 7.4 mmol) in THF (14 mL) was added 1 N HCl (30 mL). After having been stirred overnight at room temperature the THF was removed in vacuo, and the crude oil was extracted 3 times with ether. The ether solution was concentrated in vacuo, and the residue was purified on silica (45% E in P) to furnish diol **54** (1.62 g, 87%) as a clear oil: TLC (30% E in P, B) R_f 0.24; NMR ($CDCl_3$, 80 MHz) δ 0.90 (br t, 3 H), 1.25 (m, 8 H), 1.6–2.6 (m, 6 H), 3.67 (m, 3 H), 4.05 (m, 2 H).

Octanoic Acid, *cis*-(±)- and *trans*-(±)-[5-(1,1-Difluoroheptyl)tetrahydro-5-hydroxy-3-furanyl]methyl Ester (55). To a 0 °C solution of diol **54** (0.41 g, 1.61 mmol) in $CHCl_3$ (2 mL) and pyridine (0.13 mL, 1.61 mmol) was added octanoyl chloride (0.27 mL, 1.61 mmol) dropwise over 30 min. The solution was stirred overnight at room temperature in a tightly stoppered flask. A mixture of water and ether were added, and the water layer was extracted once more with ether. The organic layers were combined and concentrated in vacuo, and the residue was purified on silica (10% E in P) to furnish alcohol **55** (0.48 g, 80%) as a clear oil: TLC (30% E in P, B) R_f 0.64; NMR ($CDCl_3$, 80 MHz) δ 0.89 (m, 6 H), 1.30 (m, 16 H), 1.63 (m, 2 H), 1.95 (m, 3 H), 2.30 (t, 2 H), 4.10 (m, 4 H).

Octanoic Acid, (*R,*R**)- and (*R**,*S**)-5,5-Difluoro-4-hydroxy-2-(hydroxymethyl)undecyl Ester (56).** Hemiketal **55** (1.69 g, 4.5 mmol) in absolute ethanol (100 mL) was reduced with $NaBH_4$ (0.17 g, 4.5 mmol) for 0.5 h at room temperature. Most of the solvent was removed in vacuo, and the solution was acidified with cooling to pH 7 with 0.1 N HCl. Ether and water were added, and the water layer was extracted with ether. The organic layers were concentrated in vacuo to give a mixture of diastereomeric diols **56** (1.7 g, 100%) as a clear oil which was used in the next step without further purification: TLC (30% E in P, B) R_f 0.26 and 0.37; NMR ($CDCl_3$, 80 MHz, mixture) δ 0.88 (m, 6 H), 1.27 (br s, 16 H), 1.66 (m, 6 H), 2.0 (m, 1 H), 2.30 (t, 2 H), 3.17 (br s, 2 H), 3.65 (m, 3 H), 4.13 (t, 2 H).

Octanoic Acid, (*R,*R**)- and (*R**,*S**)-5,5-Difluoro-4-hydroxy-2-[[[diphenoxyphosphinyl]oxy]methyl]undecyl Ester (57).** As described for the preparation of **34**, diol **56** (0.39 g, 1.03 mmol) was converted to **57** (0.58 g, 87%) followed by purification on silica (22% E in P): TLC (30% E in P, B) R_f 0.76; NMR ($CDCl_3$, 80 MHz) δ 0.88 (m, 6 H), 1.28 (br s, 16 H), 1.58 (m, 6 H), 2.0 (m, 1 H), 2.29 (t, 2 H), 3.75 (m, 1 H), 4.10 (m, 2 H), 4.31 (m, 2 H), 7.20 (m, 10 H).

Octanoic Acid, (±)-5,5-Difluoro-4-oxo-2-[[[diphenoxyphosphinyl]-oxy]methyl]undecyl Ester (58). Alcohol **57** (0.58 g, 0.95 mmol) was oxidized with Collins' reagent as described for **35** to give **58** (0.45 g, 74%) as a clear oil after purification on silica (20% E in P): TLC (10% E in P, A) R_f 0.44; NMR ($CDCl_3$, 80 MHz) δ 0.88 (m, 6 H), 1.27 (br s, 16 H), 1.55 (m, 2 H), 1.90 (m, 2 H), 2.25 (t, 2 H), 2.72 (br s, 2 H), 4.05 (m, 2 H), 4.27 (m, 2 H), 7.20 (m, 10 H).

Octanoic Acid, (±)-5,5-Difluoro-4-oxo-2-(phosphonooxymethyl)undecyl Ester (59). Ketone **58** (0.2 g, 0.33 mmol) in methanol (23 mL) was carefully hydrogenated as described for **36** to give **59** (0.11 g, 74%) and used in the next step without further purification: TLC (30% M in C, B) R_f 0.28; NMR ($CDCl_3$, 500 MHz) δ 0.89 (br t, 6 H), 1.30 (br s, 14 H), 1.45 (m, 2 H), 1.60 (br s, 2 H), 1.98 (m, 2 H), 2.31 (m, 2 H), 2.69 (br s, 1 H), 2.82 (br s, 2 H), 4.08 (br s, 2 H), 4.21 (br s, 2 H), 6.70 (br s, 2 H).

Octanoic Acid, (±)-2-[[[(2-(Trimethylamino)ethoxy)hydroxyphosphinyl]oxy]methyl]-5,5-difluoro-4-oxoundecyl Ester (26). Compound

59 (0.24 g) was converted to **26** (0.13 g, 38%) as described for **22**. The product was purified by passage through a mixed-bed ion exchanger (59 g) (M:MC, 1:1). The material was then purified on silica (M:MC:W, 10:10:1): TLC (same solvent, B) R_f 0.22; NMR ($CDCl_3$, 300 MHz) δ 0.90 (m, 6 H), 1.30 (br s, 16 H), 1.55 (m, 2 H), 1.92 (m, 2 H), 2.24 (t, 2 H), 2.59 (m, 1 H), 2.80 (d, 2 H), 3.37 (s, 9 H), 3.82 (br s, 4 H), 3.95 (dd, 1 H), 4.22 (dd, 1 H), 4.31 (br s, 2 H); LSIMS (positive ion) 544 (M + 1).

2-Oxooctadecanoic Acid, Ethyl Ester (60). This compound was prepared as described for **51** by alkylation of the dithiane (12.0 g) with hexadecyl bromide to give the initial product (25.0 g, 96%) which was desulfurized to give **60** (10.1 g, 52%) as a white solid after purification on silica (2% E in P): TLC (5% E in P, B) R_f 0.44; NMR ($CDCl_3$, 60 MHz) δ 0.9 (m, 3 H), 1.3 (m, 31 H), 2.8 (t, 2 H), 4.3 (q, 2 H).

2,2-Difluorooctadecanoic Acid, Ethyl Ester (61). As described for **52**, ester **60** (0.5 g) was fluorinated to give **61** (0.43 g, 78%) as a yellow solid after purification on silica (1% E in P): TLC (30% E in P, B) R_f 0.82; NMR ($CDCl_3$, 60 MHz) δ 0.9 (br t, 3 H), 1.3 (br s, 31 H), 2.1 (m, 2 H), 4.3 (q, 2 H).

3,3-Difluoro-1-(2,2-dimethyl-1,3-dioxan-5-yl)-2-nonadecanone (62). As described for **53**, ester **61** (2.04 g) was condensed with bromide **50** (1.23 g) to give **62** (0.48 g, 19%) as a white solid after purification on silica (1.5% E in P): TLC (10% E in P, B) R_f 0.48; NMR ($CDCl_3$, 60 MHz) δ 0.9 (m, 3 H), 1.3 (br m, 28 H), 1.4 (s, 6 H), 2.1 (m, 3 H), 2.8 (d, 2 H), 3.6 (dd, 2 H), 4.0 (dd, 2 H).

***cis*-(±)- and *trans*-(±)-5-(1,1-Difluoroheptadecyl)tetrahydro-5-hydroxy-3-furanmethanol (63).** As described for **54**, fluoro ketone **62** (0.48 g) was converted in cyclic hemiketal **63** (0.34 g, 77%) as a mixture of diastereomers after purification on silica (45% E in P): NMR ($CDCl_3$, 60 MHz, mixture) δ 0.9 (br t, 3 H), 1.3–1.5 (br m, 28 H), 1.6–2.8 (m, 5 H), 3.8–4.3 (m, 4 H).

Octadecanoic Acid, *cis*-(±)- and *trans*-(±)-[5-(1,1-Difluoroheptadecyl)tetrahydro-5-hydroxy-3-furanyl]methyl Ester (64). Diol **63** (0.34 g) was reacted with stearoyl chloride (Aldrich) as described for **55** to produce **64** (0.49, 86%) after purification on silica (2% M in C): TLC (30% E in P, B) R_f 0.63; NMR ($CDCl_3$, 60 MHz, mixture) δ 0.9 (br t, 6 H), 1.2–1.6 (br m, 56 H), 1.7 (br m, 4 H), 2.0–2.5 (m, 5 H), 3.6–4.3 (m, 4 H).

Octadecanoic Acid, (*R,*R**)- and (*R**,*S**)-5,5-Difluoro-4-hydroxy-2-(hydroxymethyl)heneicosanyl Ester (65).** Hemiketal **64** (0.28 g, 0.43 mmol) in absolute ethanol/chloroform (1:1) (9.7 mL) was reduced with $NaBH_4$ (16.2 mg, 0.43 mmol) with stirring for 0.5 h at 32 °C. The solvent was removed in vacuo, and chloroform and water were added. The solution was acidified to pH 7 with 0.1 N HCl while cooling on ice. The organic layer was concentrated in vacuo, and the residue was purified on silica (10% M in C) to furnish **65** (0.17 g, 60%) as a white solid: TLC (30% E in H, B) R_f 0.37; NMR ($CDCl_3$, 300 MHz, mixture) δ 0.88 (br t, 6 H), 1.26 (br s, 56 H), 1.50–2.0 (m, 6 H), 2.32 (m and t, 3 H), 3.63–3.85 (m, 2 H), 4.13 (m, 3 H).

Octadecanoic Acid, (*R,*R**)- and (*R**,*S**)-5,5-Difluoro-4-hydroxy-2-[[[diphenoxyphosphinyl]oxy]methyl]heneicosanyl Ester (66).** Diol **65** (0.17 g) was phosphorylated as described for **34** except that chloroform was substituted for ether during the extraction. Purification on silica (2% M in C) afforded **66** (0.16 g, 87%) as a white solid: TLC (30% E in H, B) R_f 0.61; NMR ($CDCl_3$, 300 MHz, mixture) δ 0.88 (br t, 6 H), 1.26 (br s, 56 H), 1.48–1.60 (m, 4 H), 1.84 (m, 2 H), 2.26 (t, 2 H), 2.34 (m, 1 H), 3.77 (m, 1 H), 4.08 (m, 2 H), 4.32 (m, 2 H), 7.0–7.38 (m, 10 H).

Octadecanoic Acid, (±)-5,5-Difluoro-4-oxo-2-[[[diphenoxyphosphinyl]oxy]methyl]heneicosanyl Ester (67). Alcohol **66** (0.16 g) in acetone (10 mL) was oxidized with Jones' reagent (12 equiv) by stirring for 3.5 h at room temperature. Methylene chloride and 5% aqueous $NaHCO_3$ were added, the organic layer was concentrated in vacuo, and the residue was purified on silica (20% E in P) to give **67** (0.095 g, 60%) as a white solid: TLC (30% E in H, B) R_f 0.82; NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, 6 H), 1.26 (br s, 56 H), 1.40–1.56 (m, 2 H), 1.90 (m, 2 H), 2.26 (t, 2 H), 2.68 (m, 1 H), 2.72 (m, 2 H), 4.05 (m, 2 H), 4.28 (m, 2 H), 7.20–7.37 (m, 10 H).

Octadecanoic Acid, (±)-5,5-Difluoro-4-oxo-2-[phosphonoxymethyl]-heneicosanyl Ester (68). Ketone **67** (95 mg) in methanol/chloroform (4:1) (7.5 mL) plus PtO_2 (10 mg) was carefully hydrogenated as described for **36**. The crude product (60 mg, 76%) was used in the next step without further purification: TLC (30% M in C, B) R_f 0.20; NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 6 H), 1.25–1.65 (br m, 58 H), 1.98 (m, 2 H), 2.30 (t, 2 H), 2.72 (m, 1 H), 2.84 (m, 2 H), 4.03 (m, 2 H), 4.18 (m, 2 H).

Octadecanoic Acid, (±)-2-[[[(2-(Trimethylamino)ethoxy)hydroxyphosphinyl]oxy]methyl]-5,5-difluoro-4-oxoheneicosanyl Ester (27). Phosphatidic acid analogue **68** (73 mg) was converted to product (62 mg, 77%) as described for **22**. The product was purified by passing the crude material through a mixed-bed ion exchanger (4 g) (M:MC, 1:1) followed by chromatography on silica (M:MC:W, 10:10:1): TLC (same solvent, B) R_f 0.20; NMR ($CDCl_3$, 300 MHz) δ 0.88 (br t, 6 H), 1.28 (br s, 56 H), 1.57 (m, 2 H), 1.94 (m, 2 H), 2.26 (t, 2 H), 2.61 (m, 2 H), 2.81 (t, 2 H), 3.37 (s, 9 H), 3.82 (m, 4 H), 4.0 (dd, 1 H), 4.21 (dd, 1 H), 4.31 (m, 2 H); LSIMS (positive ion) 824 (M + 1).

Octadecanoic Acid, (*R,*R**)- and (*R**,*S**)-[[[(2-(Trimethylamino)-ethoxy)hydroxyphosphinyl]oxy]methyl]-5,5-difluoro-4-hydroxyheneicosyl Ester (28).** Ketone **27** (30 mg, 0.037 mmol) in ethanol/chloroform (1:1) was reduced with $NaBH_4$ (1.5 mg, 0.035 mmol) with stirring for 0.5 h at room temperature. The solvent was removed in vacuo, and the residue was passed through a mixed-bed ion exchanger (3.7 g) (M:MC, 1:1) and through a column of silica (M:MC:W, 10:10:1) to give alcohol **28** (30 mg, 100%) as a white solid: TLC (M:MC:W, 10:10:1) R_f 0.2; NMR ($CDCl_3$, 300 MHz, mixture) δ 0.89 (br t, 6 H), 1.26 (br s, 56 H), 1.4–1.64 (m, 6 H), 2.32 (m, 2 H), 3.29 (s, 9 H), 3.71 (m, 4 H), 3.90 (m, 1 H), 4.06 (m, 1 H), 4.18 (m, 1 H), 4.28 (m, 2 H).

2-Methyleneoctanal (69). The aldehyde was prepared in 87% yield as described for related compounds.[32] The material was steam distilled.

2-Methyleneoctanol (70). Aldehyde **69** (21.6 g) was reduced with $LiAlH_4$ in the usual manner to furnish the product (16.6 g, 75%) as a clear oil after distillation (98 °C, 12 mm): TLC (10% E in P, B) R_f 0.49.

2-(Bromomethyl)octene (71). Alcohol **70** (16.8 g) was brominated with PBr_3 in the usual manner. The crude material was distilled (86 °C, 8 mm) to give the product (18.4 g, 76%) as a clear oil: NMR ($CDCl_3$, 80 MHz) δ 0.89 (t, 3 H), 1.32 (br m, 6 H), 1.80 (m, 2 H), 2.18 (t, 2 H), 3.94 (s, 2 H), 5.02 (d, 2 H).

2-(Methyleneoctyl)propanedioic Acid, Dimethyl Ester (72). Bromide **71** (17.0 g, 0.083 mol) was dried with anhydrous K_2CO_3 and dissolved in methanol (32 mL), and dimethyl malonate (55.1 g, 0.42 mol, Aldrich) was added. The solution was added to a stirring solution of sodium methoxide (4.5 g, 0.083 mol) in methanol (64 mL) dropwise over 30 minutes. The solution was refluxed for 3 h, and water and methylene chloride were added. The organic layer was concentrated in vacuo, and the residue was distilled (108–111 °C, 0.8 mm) giving diester **72** (11.1 g, 52%) as a clear oil: TLC (10% E in P, B) R_f 0.66; NMR ($CDCl_3$, 80 MHz) δ 0.88 (t, 3 H), 1.27 (br s, 8 H), 2.0 (t, 2 H), 2.6 (d, 2 H), 3.6 (m, 1 H), 3.72 (s, 6 H), 4.74 (d, 2 H).

2-(2-Methyleneoctyl)-1,3-propanediol (15). Diester **72** (7.47 g, 0.029 mol) was reduced with $LiAlH_4$ in the usual manner, and the crude product was purified on silica (40% E in P) to give the product (2.78 g, 48%) as a clear oil: TLC (30% E in P, B) R_f 0.19; NMR ($CDCl_3$, 80 MHz) δ 0.88 (t, 3 H), 1.30 (br s, 8 H), 1.66 (m, 1 H), 1.96 (br s, 4 H), 2.82 (br s, 2 H), 3.68 (br s, 4 H), 4.75–5.15 (m, 2 H).

Octanoic Acid, (±)-2-(Hydroxymethyl)-4-methylenedecyl Ester (17). Diol **15** was monoacylated as described.[15] The diol (1.7 g, 8.5 mmol), dry benzene (21 mL), and dibutyltin oxide (2.12 g, 8.5 mmol, Aldrich) were refluxed for 8 h with azeotropic removal of water. The benzene was removed in vacuo, the residue was taken up in chloroform (previously passed through a column of basic alumina) (38 mL) and brought to reflux under argon, and octanoyl chloride (1.38 g, 8.5 mmol, Aldrich) was added slowly via syringe. The solution was refluxed for 2 h, then 6% dioxane in water (38.5 mL) was added, and the solution was refluxed an additional 2 h. An equal volume of chloroform was added, and the solution was washed with aqueous $NaHCO_3$. The organic layer was concentrated in vacuo, and the residue was purified on silica (14% E in P) to give **17** (1.6 g, 58%) as a clear oil: TLC (10% E in P, B) R_f 0.20; NMR ($CDCl_3$, 80 MHz) δ 0.88 (br t, 6 H), 1.30 (br s, 16 H), 1.65 (m, 2 H), 2.02 (m, 5 H), 2.31 (t, 2 H), 3.45 (m, 2 H), 4.12 (m, 2 H), 4.77–5.15 (m, 2 H).

Octanoic Acid, (±)-2-[[[(2-(Trimethylamino)ethoxy)hydroxyphosphinyl]oxy]methyl]-4-methylenedecyl Ester (18). Alcohol **17** (0.45 g, 1.39 mmol) was phosphorylated with 2-chloro-2-oxo-1,3,2-dioxaphosphalane (0.22 g, 1.53 mmol) as described[16] to furnish the phosphate triester (0.48 g, 79%) which was used without further purification since it decomposes on silica. This compound (1.94 g, 4.5 mmol) was treated with trimethylamine in acetonitrile in a sealed tube as described.[16] After the reaction, the solution was concentrated in vacuo, and the residue was passed through a mixed-bed ion exchanger (23 g) (M:MC, 1:1). The solvent was removed in vacuo, and the residue was purified on silica (M:MC:W, 10:10:1) to give **18** (1.4 g, 64%) as a white solid: TLC (same solvent, B) R_f 0.24; NMR ($CDCl_3$, 300 MHz) δ 0.89 (br t, 6 H), 1.29 (br s, 16 H), 1.58 (m, 2 H), 1.95–2.15 (m, 5 H), 2.27 (t, 2 H), 3.40 (s, 9 H), 3.71 (m, 1 H), 3.82–3.92 (m, 4 H), 4.02 (m, 1 H), 4.15 (m, 1 H), 4.28 (br m, 2 H), 4.73–5.11 (m, 2 H).

Octanoic Acid, (±)-2-[[[(2-(Trimethylamino)ethoxy)hydroxyphosphinyl]oxy]methyl]-4-oxodecyl Ester (19). Olefin **18** (1.4 g, 2.85 mmol) in CH_2Cl_2/CH_3OH (5:1) at −78 °C was treated with ozone until

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the solution became blue. Dimethyl sulfide (4.2 mL) was immediately added, and the solution was stirred for 2 h at room temperature. The solvent was removed in vacuo, and the residue was passed through a mixed-bed ion exchanger (40 g) (M:MC, 1:1). The product obtained was purified further on silica (M:MC:W, 10:10:0.5) to furnish ketone **19** (0.84 g, 60%) as a white solid: TLC (M:MC:W, 10:10:1, B) R_f 0.22; NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, 6 H), 1.29 (br s, 14 H), 1.57 (m, 4 H), 2.27 (t, 3 H), 2.39 (t, 2 H), 2.51 (m, 3 H), 3.38 (s, 9 H), 3.80 (m, 4 H), 3.97 (dd, 1 H), 4.19 (dd, 1 H), 4.30 (br s, 2 H).

Octanoic Acid, (*R,*R**)- and (*R**,*S**)-2-[[[(2-Trimethylamino)ethoxy)hydroxyphosphinyl]oxy]methyl]-4-hydroxydecyl Ester (20).** Ketone **19** (41.9 mg, 0.81 mmol) in absolute ethanol (4 mL) was reduced with $NaBH_4$ (5.0 mg, 0.13 mmol) by stirring 1.5 h at room temperature. The ethanol was removed in vacuo, and the residue was purified as described for **41** with use of 4 g of ion exchanger to give **20** (35 mg, 83%) as a white solid: TLC (same as **41**) R_f 0.20; NMR ($CDCl_3$, 500 MHz) δ 0.88 (br t, 6 H), 1.27 (br s, 16 H), 1.40–1.68 (m, 6 H), 2.25 (m, 3 H), 3.37 (s, 9 H), 3.80 (m, 4 H), 4.02 (m, 1 H), 4.16 (m, 1 H), 4.31 (br m, 2 H).

2-(Methylenenonyl)propanedioic Acid, Dimethyl Ester (73). This compound was made in an identical manner as **72**: bp (105–110 °C, 0.3 mm); TLC (10% E in P, B) R_f 0.38.

4-Methyleneundecanol (74). Diester **73** was partially saponified and decarboxylated as described.[33] The ester was reduced with $LiAlH_4$ in ether by using the standard procedure to give alcohol **74** (60%, overall): TLC (20% E in P, B) R_f 0.20.

Phosphoric Acid, 4-Methyleneundecyl 2-(Trimethylamino)ethyl Ester (75). Alcohol **74** (0.87 g) was phosphorylated and reacted with trimethylamine as described for **18** to give **75** (69%). The product was purified on silica (M:MC:W, 20:20:1): TLC (same solvent, B) R_f 0.24; NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, 3 H), 1.25 (s, 8 H), 1.48 (br t, 2 H), 1.60–1.75 (m, 2 H), 1.85–2.08 (m, 4 H), 3.30 (s, 9 H), 3.75 (br m, 2 H), 3.95 (br s, 2 H), 4.25 (br m, 2 H), 4.68 (s, 1 H), 5.10 (br t, 1 H).

Phosphoric Acid, 4-Oxoundecyl 2-(Trimethylamino)ethyl Ester (25). Olefin **75** (0.5 g) was reacted with ozone as described for **19** to give the ketone (0.25 g, 50%) after purification on a mixed-bed ion exchanger (M:MC, 1:1) and on silica (M:MC:W, 20:20:1): TLC (same solvent, B) R_f 0.24; NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, 3 H), 1.25 (s, 8 H), 1.53 (t, 2 H), 1.85 (t, 2 H), 2.40 (t, 2 H), 2.50 (t, 2 H), 3.39 (s, 9 H), 3.80–3.90 (m, 4 H), 4.32 (br s, 2 H).

Biological Methods. The phospholipase assays were carried out in a pH-stat as described previously.[34] The required amounts of DPPC and inhibitor were transferred to glass tubes from stock solutions in methanol/chloroform (1:1). The solvent was removed with a stream of argon, and 1 mL of a solution containing Triton X-100 (40 mM) and $CaCl_2$ (10 mM) in quartz-distilled water was added. The tubes were sonicated at 40 °C for several minutes to solubilize the lipids. Assays were conducted in a pH-stat by placing the reaction mixture into a small glass tube containing a magnetic stir bar and a side arm fitted with a rubber septum. A small diameter pH electrode was inserted through the top, and a stream of argon was passed over the solution to prevent CO_2 absorption. The tube was immersed in a water bath at 40 ± 0.2 °C, and the solution was continuously stirred. The pH of the reaction mixture was adjusted to approximately 8.5 with 0.1 N NaOH, and enzyme (amounts given in figure legends) was added. The pH was allowed to drop to 8.0 and held near this value (±0.1 pH units) by continuous addition of 0.01 N NaOH from a Hamilton syringe inserted directly into the solution through the rubber septum. The amount of base added over 2–3 min was recorded. Measurements were repeated two to three times and were found to be reproducible within 15%. CMC values for the phospholipid analogues were determined as described.[35]

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Communications to the Editor

Oxygenation of Polychloro Aromatic Hydrocarbons by a Superoxide Ion in Aprotic Media

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Although primary and secondary haloalkanes and polychloroethenes are readily oxidized by superoxide ion ($O_2^{\bullet-}$) in aprotic media,[1,2] chlorobenzene and monohalogenated aromatic hydrocarbons do not react at significant rates.[3] This has prompted the belief that all halogenated aromatic hydrocarbons are unreactive. However, we now report that hexachlorobenzene, pentachlorobenzene, tetrachlorobenzene, and trichlorobenzene as well as decachlorobiphenyl and other "heavy" polychlorobiphenyls (PCB's) are rapidly oxygenated by $O_2^{\bullet-}$ in dimethylformamide, acetonitrile, or dimethyl sulfoxide.

The extent of the reactions for electrogenerated $O_2^{\bullet-}$ and $(Me_4N)O_2$[4] with polychloro aromatics has been determined by cyclic voltammetric assay of $O_2^{\bullet-}$ concentrations and their decrease in the presence of excess polychloroaromatic substrates. The overall reaction and product stoichiometries for the degradation of various polychloro aromatics by $O_2^{\bullet-}$ in dimethylformamide (DMF) are summarized in Table I.[5] Within the limits of a reaction time of 60 min or less, chlorobenzene and dichlorobenzenes are not oxidized. Although the trichlorobenzenes react, the rates are too slow to ascertain the stoichiometries and the products.

The reactivities of the various substrates have been determined by cyclic voltammetry via measurement of the decay rate of superoxide ion concentration,[6] and the apparent rate constants are summarized in Table I. The latter correlate with the reduction potentials for the substrates; the more positive the potential the greater the reactivity with $O_2^{\bullet-}$, which is in accord with the oxygenation of alkyl chlorohydrocarbons.[3]

Although the substrates are degraded by $O_2^{\bullet-}$ in acetonitrile and dimethyl sulfoxide, the rates of reaction are about one-tenth as great in MeCN and 20 times slower in Me_2SO. A reasonable initial step for these oxygenations is nucleophilic addition of $O_2^{\bullet-}$ to the polyhalobenzene (e.g., C_6Cl_6; Scheme I). Subsequent loss

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