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Synthesis of 5- and 6-Fluoro Derivatives of 5,8,14-Eicosatrienoic and 5,8,11,14-Eicosatetraenoic Acids. Effects of Fluorinated Arachidonic Acids on Leukotriene C_4 Production by Macrophages

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Abstract—The total syntheses of the 5-and 6-fluoro derivatives of 5,8,14-eicosatrienoic (ETA) and arachidonic (AA) acids are described. The fluorinated double bond was introduced using (*E*)-1,4-dihydroxy-2-fluoro-2-butene obtained through diisobutylaluminium hydride reduction of dimethylfluoromaleate. Recently, 5-fluoro and 6-fluoro arachidonic acids (5-F-AA and 6-F-AA) were found to be effective inhibitors of 5-lipoxygenase *in vitro* (Nave, J. F.; Jacobi, D.; Gaget, C.; Dulery, B.; Ducep, J. B., *Biochem. J.* **1991**, *278*, 549). The effect of these compounds on leukotriene C_4 (LTC_4) production by intact cells was investigated. Mouse peritoneal macrophages were cultured in the presence of 5-F-AA or 6-F-AA under conditions where AA was found to be efficiently incorporated into cellular phospholipids. Following stimulation with zymosan, macrophages treated with 20 μM 6-F-AA released 30 to 35 % less LTC_4 than control cells. In contrast, macrophages treated with 20 μM 5-F-AA released 1.5 to 1.8 times more LTC_4 than control cells. In competition experiments with [^{14}C]-AA, 5-F-AA modified the distribution profile of [^{14}C]-AA within the various classes of lipids in a way similar to AA. 6-F-AA had a distinct behaviour, producing a more important incorporation of [^{14}C]-AA into the neutral lipid fraction at the expense of the phospholipid fraction than AA and 5-F-AA. 6-F-AA is expected to be an important tool in further studies of the arachidonic acid pathway *in vivo*.

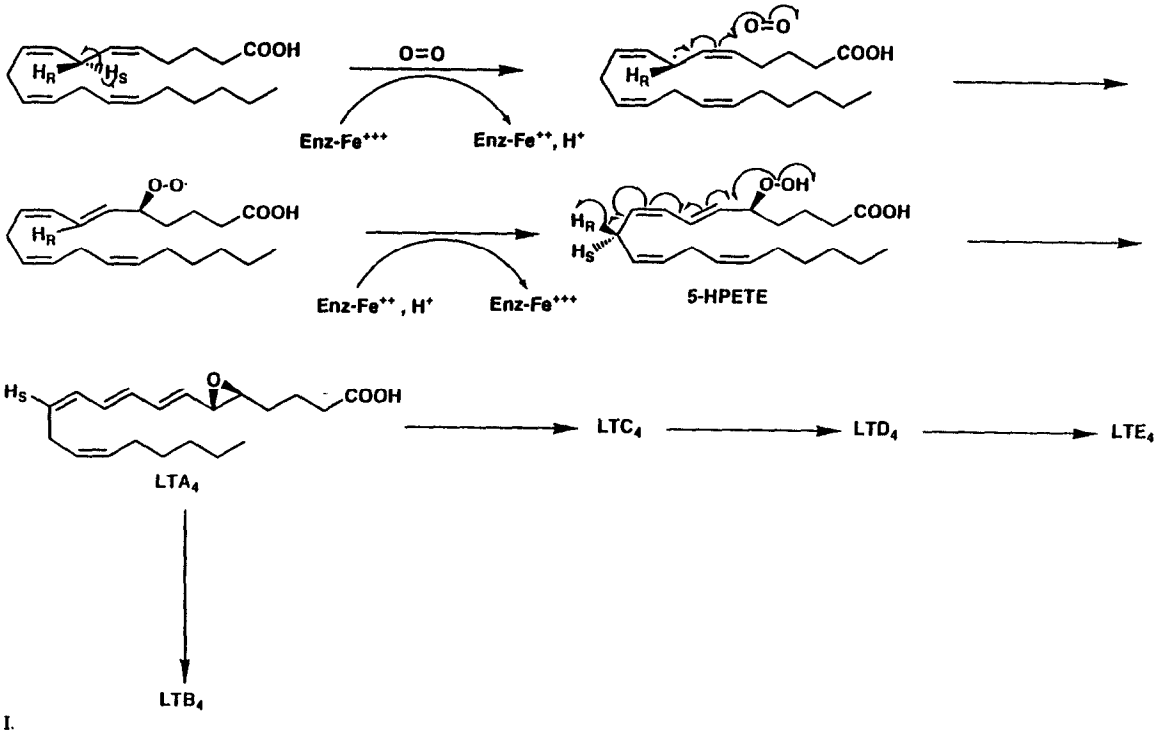
Introduction

The metabolic cascade of arachidonic acid (AA) **1** produces many metabolites[1] which are involved in various pathologies.[2,3] Therefore, suppression of some of these metabolites might have important pharmacological consequences. Thus, specific inhibition of 5-lipoxygenase (5-LO) which prevents the formation of leukotrienes (A_4, B_4, C_4, D_4, E_4) might have beneficial effects in disorders where these metabolites are involved (allergy, asthma, inflammation, psoriasis).[3] The catalytic mechanism of 5-LO has been widely studied.[4] It has been demonstrated that, in a first step, this enzyme introduces molecular oxygen on carbon 5 of AA with concomitant abstraction of the pro-*S* hydrogen at C-7[5] and formation of a C-6–C-7 double bond, producing a 5-hydroperoxide, 5-HPETE. In a second step, 5-LO catalyzes the abstraction of the pro-*R* hydrogen at C-10 of 5-HPETE with concomitant formation of two double bonds[6,7] (C-7–C-8 and C-9–C-10) and of an epoxide (C-5–C-6) with elimination of a hydroxyl group, generating a conjugated triene called leukotriene A_4 (LTA_4) (Scheme I).

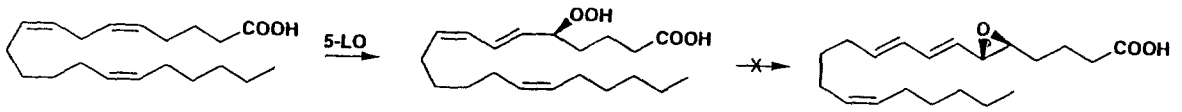
Substitution of hydrogen by fluorine in strategic positions of biologically active molecules often leads to compounds with new physiological properties.[8] Because of the similarity in Van der Waals radii between hydrogen and fluorine atoms and because of the strong electron-withdrawing effect of fluorine and its ability to behave as a good leaving group,[8] we decided to prepare fluorinated analogs of 5,8,14-eicosatrienoic (ETA) and arachidonic acids by replacing the vinylic hydrogen atoms of the double bond involved in the 5-LO reaction. Such compounds could act as inhibitors of 5-LO. Recently, we described the use of simplified substrates to study the 5-LO reaction:[9] ETA (**2**) was efficiently converted by 5-LO to 5-hydroperoxy-6,8,14-eicosatrienoic acid (5*S*-oxygenase activity). However, this 5-hydroperoxide was not recognized as a substrate of the leukotriene A_4 synthase activity of 5-LO[9] (Scheme II). These results suggested that fluorinated ETA analogs might be useful to study the effect of fluorine substitution on the 5*S*-oxygenase activity of 5-LO. In this respect, we decided to prepare the 5- and 6-monofluoro analogs of ETA (5-F-ETA and 6-F-ETA).

Study of the effect of fluorine substitution on both the 5*S*-oxygenase and the LTA_4 synthase activities of 5-LO required the use of fluorinated AA analogs. Although synthesis of 5-fluoro arachidonic acid (5-F-AA) has been reported by Taguchi *et al.*,[10] its interaction with 5-LO has never been described. Therefore, the 5- and 6-monofluoro analogs of arachidonic acid (5-F-AA and 6-F-AA) were also prepared.

In a recent paper, we described the properties of 5-F-ETA, 6-F-ETA, 5-F-AA and 6-F-AA as substrates and inhibitors of rat basophilic leukaemia cell 5-LO.[11] 5-F-AA and 6-F-AA were found to be effective and about equipotent inhibitors of 5-LO in the micromolar range. This latter result prompted us to investigate the effect of the fluorinated AA in cells involved in leukotriene formation. The structural similarity of these compounds to AA suggests that they might follow the same biochemical



Scheme I.



Scheme II.

pathways in mammals, being incorporated into phospholipids of cell membranes which serve as reservoir for AA prior to release by phospholipase A_2.[12] Upon stimulation of cells, the fluorinated arachidonic acids might be released at the same site as AA, i.e. at a site where synthesis of inflammatory mediators derived from AA takes place. Ultimately, the released fluorinated arachidonic acids might compete with AA and inhibit enzymes involved in oxidative metabolism of AA (e.g. 5-LO). To allow their possible incorporation into cellular phospholipid pools, 5-F-AA and 6-F-AA were added to primary cultures of mouse resident peritoneal macrophages under conditions where AA was efficiently incorporated into phospholipids.

We report here the effect of treatment of the mouse macrophages with AA, 5-F-AA and 6-F-AA on their subsequent ability to release leukotriene C_4 (LTC_4) when challenged with zymosan. The effect of AA, 5-F-AA and 6-F-AA on the incorporation of radiolabelled arachidonic acid into the different classes of macrophage lipids was also investigated.

Results And Discussion

Chemistry

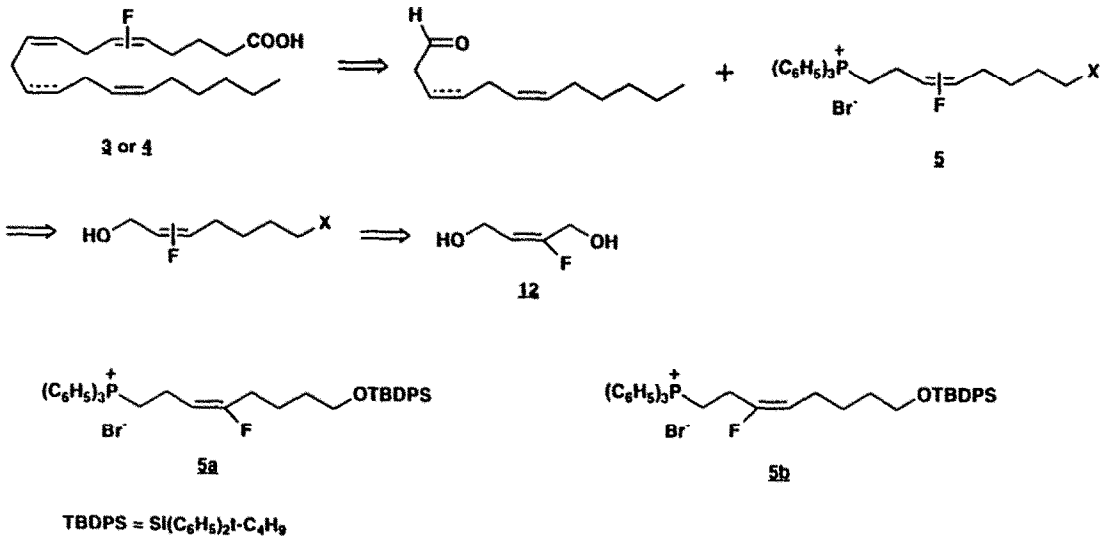
The retrosynthetic analysis for the preparation of 5-F-ETA (**3a**), 6-F-ETA (**3b**), 5-F-AA (**4a**) and 6-F-AA (**4b**) is outlined in Scheme III. The 8–9 double bond of these compounds can be introduced by Wittig type reaction between 6-dodecenal or 3,6-dodecadienal and an 8-carbon atom phosphonium salt bearing a fluorinated (*E*) double bond. Therefore, the synthesis relies on a specific synthetic method for (*E*) trisubstituted monofluorinated olefin.

The usual methods for preparation of fluorinated olefins such as Wittig type reactions,[13] opening of fluorinated cyclopropanes,[14] alkylation of *gem*-difluoro olefins,[15] monohydrofluorination of electrophilic alkynes,[16] and halofluorination of olefins followed by elimination of halohydric acid,[17] gave mixtures of *E* and *Z* isomers and were accompanied sometimes with a lack of regioselectivity. Fluoromaleic and fluorofumaric acids **10** and **8** respectively,[18] providing that reduction occurred without double bond isomerization, would be convenient synthons to prepare an (*E*) or (*Z*) trisubstituted monofluorinated olefin. Acids **8** and **10** are readily available from difluorosuccinic acid **7**, which is obtained by potassium permanganate oxidation of 2,2-difluoro-4-pentenoic acid.[19] In contrast to the acids, esters of **8** and **10** could be reduced to their corresponding diols without isomerization of the double bond. Thus, dimethylfluoromaleate **11** was reduced by an excess of diisobutylaluminium hydride to pure (*E*)-1,4-dihydroxy-2-fluoro-2-butene **12** in good yields (Scheme IV).

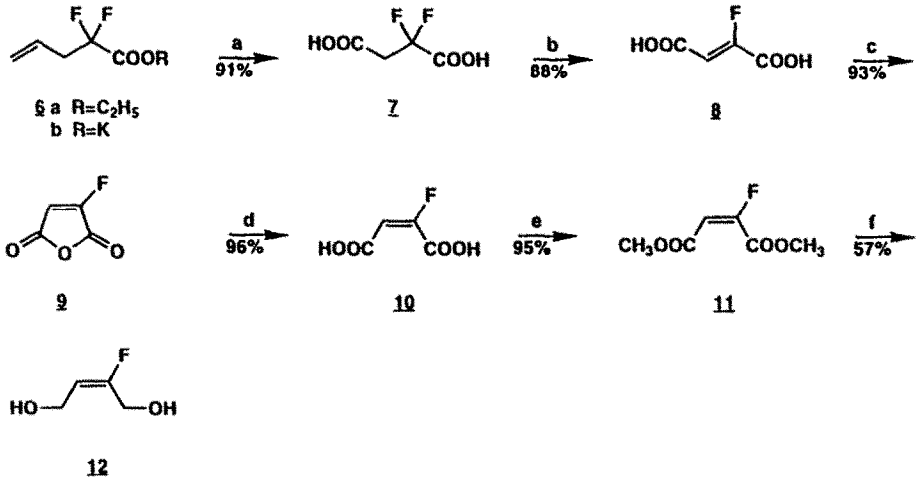
Differentiation of the two primary alcohols was obtained by treatment of **12** with *N*-chlorosuccinimide and dimethylsulfide in methylene chloride[20] to afford exclusively (*E*)-4-chloro-2-fluoro-1-hydroxy-2-butene **13**,

further converted to a tetrahydropyranyl ether derivative **14** (Scheme V).

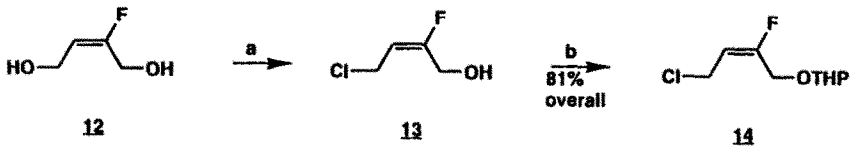
Preparation of phosphonium salts **5** requires the homologation of the chloride **14** by one carbon unit on one end and by a three carbon unit on the other end. Thus, condensation of chloride **14** with the lithium salt of 1,3-dithiane[21] afforded the tetrahydropyranyl ether **15** which was deprotected to alcohol **16**. Conversion into chloride **17** was performed using 1-chloro-*N*,*N*-2-trimethylpropenylamine.[22] Homologation by a three carbon unit was achieved through alkylation of chloride **17** by the



Scheme III.



Scheme IV. a) $KMnO_4$, CH_3COOH ; b) KOH (3eq.), H_2O, reflux 16h ; c) P_2O_5, vacuum distillation 110°C ; d) H_2O ; e) CH_2N_2, Ether ; f) DIBAL (5.5eq.), THF.



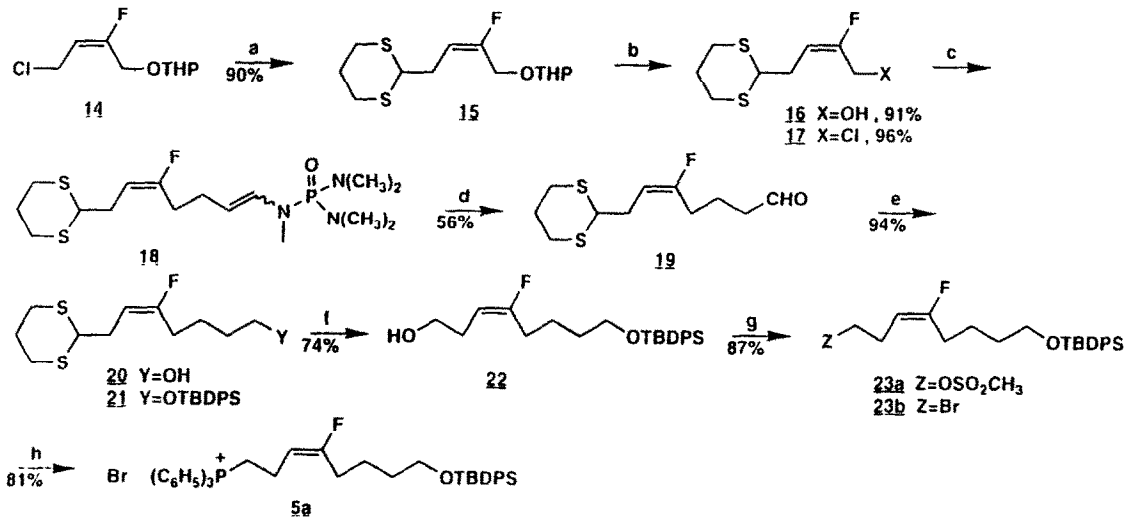
Scheme V. a) NCS (1.1eq.), CH_3SCH_3 (1.22eq.), CH_2Cl_2 ; b) DHP, PPTS (cat.), CH_2Cl_2.

anion derived from *N*-allyl-*N*,*N*',*N*"-pentamethyl phosphoramide.[23] This allylic anion reacts selectively at its γ position to generate vinyl phosphoramide **18** which is vinylogous with an enamine. Phosphoramide **18** was hydrolyzed without purification to aldehyde **19** which, after reduction and protection, gave the silyl ether **21**. Treatment of **21** with one equivalent of trimethyloxonium tetrafluoroborate followed by addition of calcium carbonate in aqueous acetone allowed the cleavage of the dithiane. The resulting β-γ unsaturated aldehyde was reduced without purification to alcohol **22**. Finally, this alcohol was converted to phosphonium salt **5a** via reaction of bromide **23b** with triphenylphosphine (Scheme VI).

Phosphonium salt **5b** was obtained by a similar sequence. Chloride **14** was homologated with a three carbon unit using the lithium salt of *N*-allyl-*N*,*N*',*N*"-pentamethyl phosphoramide. The obtained vinyl phosphoramide **24** gave, after hydrolysis of the enamine[23] and protection of the alcohol with a tetrahydropyranyl ether, aldehyde **25**. Reduction and protection of the resulting alcohol by a *t*-butyldiphenylsilyl ether afforded **27**. Serious difficulties were encountered for the selective cleavage of the tetrahydropyranyl ether of **27**. Most of the classical methods, probably because of the double bond stereochemistry, gave transfer of the silyl ether between the two hydroxyl groups. Tetrahydropyranyl ether was successfully cleaved using tetrabutyl-1,3-diisothiocyanatodistannoxane in refluxing methanol[24]. Alcohol **28** was converted to bromide **29** by treatment with one equivalent of 1-bromo-*N*,*N*-2-trimethyl-propenylamine[22] at room temperature in methylene chloride. Bromide **29** was then homologated by one carbon unit using the lithium salt of 1,3-dithiane. Dithiane **30** was converted to the desired phosphonium **5b** by a sequence, identical to the one used to prepare phosphonium salt **5a** (Scheme VII).

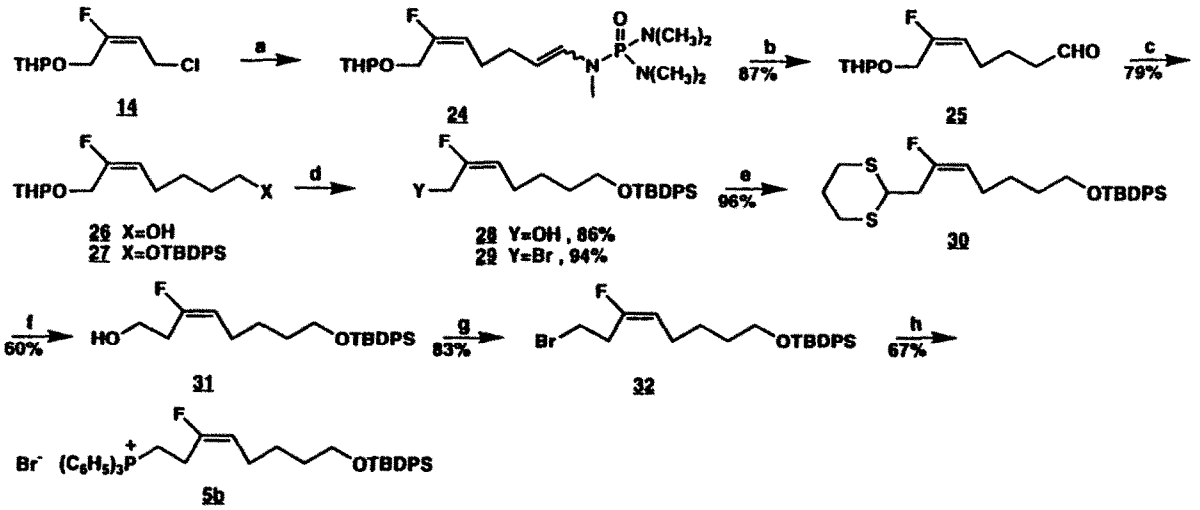
The phosphonium salts **5a** and **5b** were converted to 5- and 6-fluoro ETA by a Wittig type reaction. Treatment of **5a** and **5b** with lithium diisopropylamide in tetrahydrofuran in the presence of 10 equivalents of hexamethylphosphoramide (HMPA) afforded the corresponding anions that were reacted with (Z)-6-dodecenal.[25] Under these conditions, the bond formed was mainly (Z) (ratio Z:*E*, 96:4).[26] Finally, cleavage of the silyl ether followed by oxidation of the alcohols **34** to the corresponding acids (2.67 M Jones reagent[27] in acetone) afforded the 5- and 6-fluoro ETA, **3a** and **3b** respectively (Scheme VIII).

The 5- and 6-fluoro arachidonic acids were prepared by similar sequences using a Wittig type reaction between the anions derived from the phosphonium salts **5** and (*Z*,*Z*)-3,6-dodecedienal **41**. As in our hands most of the methods described to prepare aldehyde **41** always gave a mixture of products (catalytic hydrogenation of the corresponding diyne:use of either Lindlar catalyst,[28] or Nickel P_2[29] give **41** along with partially reduced and overreduced type compounds which are difficult to separate), we developed the following sequence, a much more convenient method for large scale preparation of **41**. Aldehyde **35**[30] was converted to alkyne **37** through dibromo olefin **36**.[31] Coupling with 1-iodo-3-octyne[32] then gave diyne **38** which was reduced in pure *Z*,*Z*-diene by hydroboration with two equivalents of dicyclohexylborane followed by treatment with acetic acid.[33] Dioxolane **39** was converted to the diol **40** by acidic treatment. Aldehyde **41** was then prepared, just before use, by sodium metaperiodate oxidation (Scheme IX).

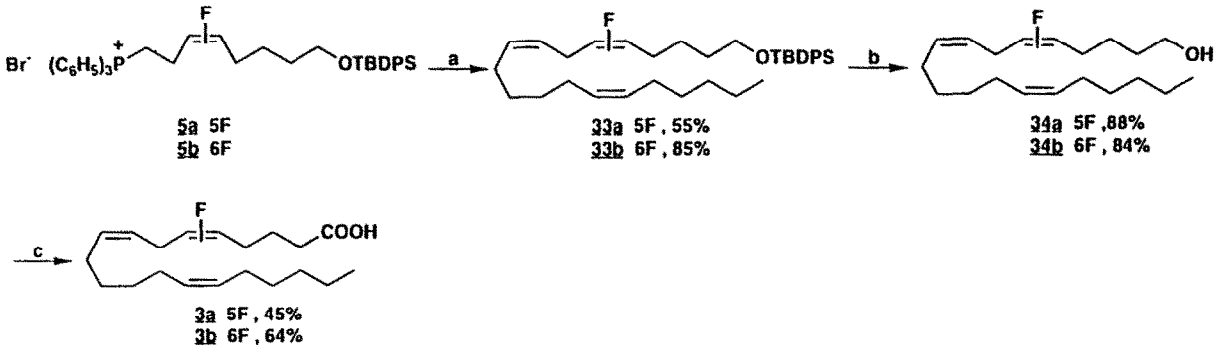


Scheme VI. a) (1eq.), THF, -30 °C to 0 °C ; b) PPTS (cat.), CH_3OH, reflux ; (1eq.), CH_2Cl_2, 0 °C ; c) (1eq.), THF, -78°C to 0 °C ; d) HCl 2N, Ether, RT ; e) $NaBH_4$, C_2H_5OH, RT ;

TBDPSCl (1.1eq.), Et_3N (1.5eq.), DMAP (cat.), CH_2Cl_2, RT ; f) $(CH_3)_3O^+BF_4^-$ (1eq.), CH_2Cl_2, RT ; $CaCO_3$ (2eq.), CH_3COCH_3:H_2O, 9:1, RT ; $NaBH_4$ (0.5eq.), C_2H_5OH, 0°C ; g) CH_3SO_2Cl (1.1eq), Et_3N (1.5eq.), CH_2Cl_2, -10 °C to RT ; Amberlyst-A-26 Br^- form (3meq.), Benzene, reflux, 16h ; h) $(C_6H_5)_3P$ (1.3eq.), CH_3CN, reflux, 48h.



Scheme VII. a) Li^+ [HMPA anion] (1eq.), THF, -78°C to 0°C ; b) HCl 2N, Ether, RT ; DHP, PPTS (cat.), CH_2Cl_2 ; c) $NaBH_4$, C_2H_5OH, RT; TBDPSCl (1.1eq.), Et_3N (1.5 eq.), DMAP (cat.), CH_2Cl_2, RT ; d) $[(Bu)_2Sn]_2O(SCN)_2$ (cat.), CH_3OH, reflux, 24h ; [2-Br-1,3-dithiane] (1eq.), CH_2Cl_2, 0°C ; e) [1,3-dithiane]$^-Li^+$ (1eq.), THF, -30°C to 0°C ; f) $(CH_3)_3O^+BF_4^-$ (1eq.), CH_2Cl_2, RT ; $CaCO_3$ (2eq.), CH_3COCH_3:H_2O 9:1, RT ; $NaBH_4$ (0.5eq.), C_2H_5OH, 0°C ; g) [2-Br-1,3-dithiane] (1eq.), CH_2Cl_2, 0°C ; h) $(C_6H_5)_3P$ (3eq.), CH_3CN, reflux, 48h.



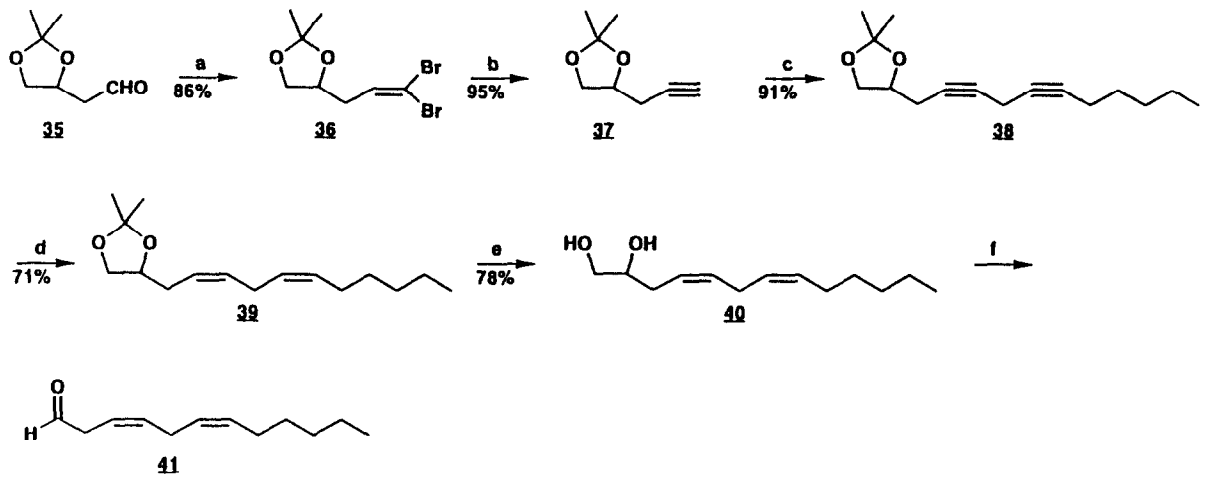
Scheme VIII. a) LDA (1eq.), THF, -78°C, 45min ; HMPA (9eq.), -78°C to -25°C ; [CHO] (0.9eq.), THF, -25°C 30min then 0°C 1hr ; b) n-$Bu_4N^+F^-$ (1.5eq.), THF, 2h, RT ; c) Jones reagent 2.67M, CH_3COCH_3, 0°C.

The Wittig reaction between phosphonium salts **5** and aldehyde **41** was carried out in the presence of 10 equivalents of HMPA in tetrahydrofuran using *n*-butyllithium instead of lithium diisopropylamide in order to avoid conjugation of the 3–4 double bond with the carbonyl group. Under such conditions, the double bond formed was mainly Z (Z:E, 97:3).[26] Silyl ethers **42** were converted to 5- and 6-fluoroarachidonic acids respectively by sequences identical to the one described for the conversion of **33** to **3** (Scheme X).

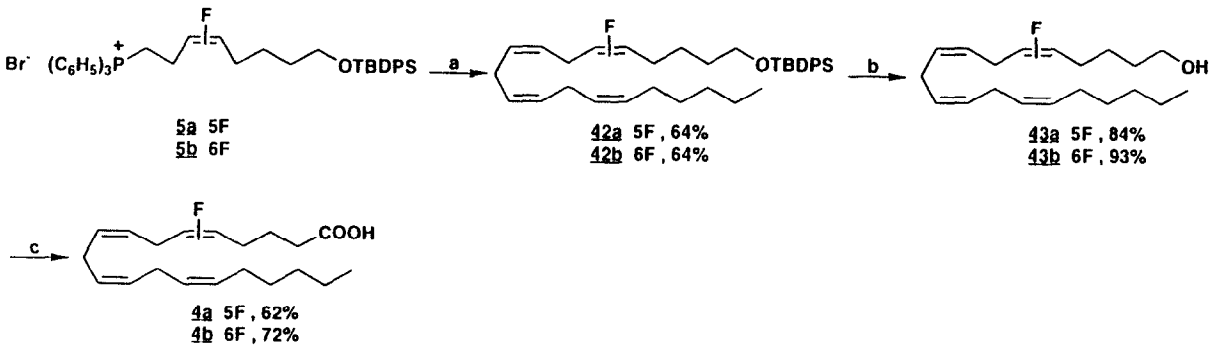
Biochemistry

Previous studies have shown that primary cultures of mouse resident peritoneal macrophages can incorporate polyunsaturated fatty acids[34] and, when treated with zymosan, release LTC_4.[35] Therefore, these cells were chosen for studying the effect of incorporation of arachidonic acid or its fluorinated analogs (5-F-AA and 6-F-AA) on the release of LTC_4 induced by zymosan.

Optimal conditions for LTC_4 release from macrophages. The time-course of LTC_4 production by macrophages stimulated with zymosan has been previously described by Rouzer *et al.*[35] A similar methodology was used here. As shown in Figure 1, zymosan-treated macrophages released LTC_4. The release was linear between 15 and 60 min after the addition of zymosan. There was no LTC_4 released from macrophages treated with medium A without zymosan (Figure 1). In all further experiments, LTC_4 release was induced with 150 μg zymosan per Petri dish and measured after an 80 min period.



Scheme IX. a) $(C_6H_5)_3P$ (4 eq), CBr_4 (2eq.), CH_2Cl_2 ; b) n-BuLi (2eq.), THF, -78°C to RT ; c) EtMgBr (1eq.), CuI (cat.), I–≡–, THF ; d) $(C_6H_{11})_2BH$ (2eq.), THF ; CH_3COOH ; e) H_2SO_4 6N, CH_3OH, RT ; f) $NaIO_4$, THF/Acetone/H_2O, 0°C.



Scheme X. a) n-BuLi (1eq.), THF, -78°C 5min then -18°C 10min ; HMPA (9eq.), -78°C ; 41 (1.1eq.), THF, -78°C 30min, 0°C 1h, RT 30min ; b) $n\text{-}Bu_4N^+F^-$ (1.5eq.), THF, 2h, RT ; c) Jones reagent 2.67M, CH_3COCH_3, 0°C.

Comparison of the effect of incorporation of AA, 5-F-AA and 6-F-AA into macrophage lipids on the subsequent ability of macrophages to produce LTC_4. In order to obtain an efficient incorporation of free fatty acids into lipids of cells in culture, it has been previously recognized that the fatty acids should be complexed to bovine serum albumin (BSA).[34,36] Therefore, in all experiments, the fatty acids were added to macrophages as solutions of fatty acid–BSA complexes in medium A. In a first experiment, the effect of increasing concentrations of AA in the culture medium on the subsequent ability of macrophages to release LTC_4 was investigated. As shown in Figure 2, cells cultured overnight with BSA alone (controls) released about 120 ng LTC_4 per dish when further challenged with zymosan for 80 min. This LTC_4 is formed from AA naturally occurring at position 2 of the glycerol backbone of membrane phospholipids (endogenous AA pool) which is liberated by the action of phospholipase A_2 after stimulation of the cell.[12] When the macrophages were cultured in the presence of increasing concentrations of AA, a progressive increase in LTC_4 release was observed (Figure 2). LTC_4 release from cells cultured with 20 μM AA represented about 3.3 times that of control cells (Figure 2). At this concentration of AA, using [^{14}C]-AA as a tracer, it was found that macrophages incorporated 42 and 53% of AA added to the culture medium, in two separate experiments. Furthermore, after extraction of lipids and separation of phospholipids from neutral lipids by thin layer chromatography (TLC), 89 and 91% of incorporated AA was found to be associated with phospholipids including phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE) and phosphatidyl inositol (PI). Less than 3.5% of incorporated AA was found to occur as the free acid (data not shown). The efficient incorporation of AA into phospholipids coupled to the 3.3-fold increase in LTC_4 release when macrophages are cultured in the presence of 20 μM AA suggested that similar conditions could be favourable for incorporation of the 5- and 6-fluorinated AA analogs. Therefore, macrophages were cultured without or with 20 μM AA or 5-F-AA or 6-F-AA and further

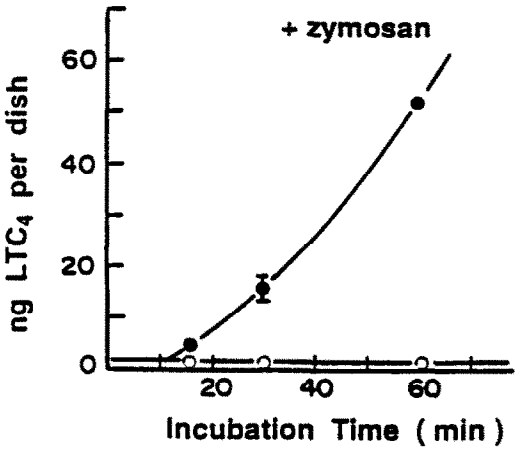


Figure 1. Time-course of LTC_4 release in response to zymosan. Macrophages were cultured overnight in medium A and further challenged with zymosan (●). Controls without zymosan (medium A alone) were performed (○). At selected time points, LTC_4 released in the medium was extracted and analyzed. Results are means of duplicates ± S.D.

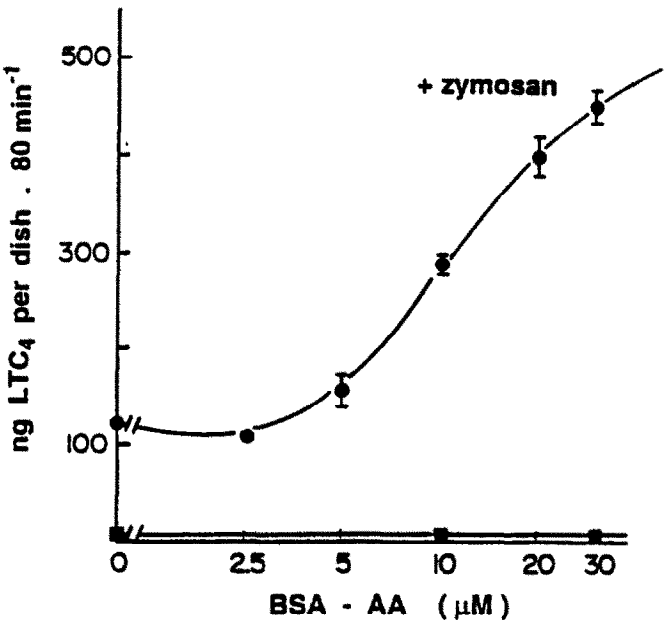


Figure 2. Effect of arachidonic acid concentration in the culture medium on the zymosan-induced release of LTC_4 from macrophages. Macrophages were cultured for 17 h in medium A containing a fixed concentration of BSA without or with arachidonic acid at various concentrations. After washing and addition of fresh medium, the cells were challenged with zymosan (●) for 80 min and the medium collected for LTC_4 analysis. Controls without zymosan (■). Results are mean of duplicates ± S.D.

challenged with zymosan. In good agreement with previous results, cells cultured with 20 μM AA released about 3 to 4 times the amount of LTC_4 released by control cells (Table 1). In this case, LTC_4 is presumably synthesized from both endogenous and exogenous (incorporated) AA. Surprisingly, cells cultured with 20 μM 5-F-AA released 1.5 to 1.8 times more LTC_4 than did the control cells (Table 1). A possible explanation for this result is that 5-

F-AA is incorporated into phospholipids and displaces endogenous AA from some phospholipid pools to others which are more susceptible to hydrolysis by phospholipase A_2. The increased LTC_4 release observed cannot be due to LTC_4 (or a LTC_4 analog) formed from 5-F-AA since we have shown that 5-F-AA is only converted to 5-oxo-6,8,11,14-eicosatetraenoic acid by 5-LO.[11] In contrast to the stimulation resulting from 5-F-AA treatment, a 30 to 35 % inhibition of LTC_4 release with respect to controls was observed in cells cultured with 20 μM 6-F-AA. The very different effects of 5-F-AA and 6-F-AA on LTC_4 release could be due either to differences in the extent of incorporation or to incorporation into different lipid classes. Ultimately, differences observed between the two fluorinated AA analogs could arise from a different ability to serve as substrates of phospholipase A_2 (as esters in phospholipids) or of the enzymes involved in the oxidative metabolism of AA (5-LO and cyclooxygenase).

Effect of AA, 5-F-AA and 6-F-AA on the incorporation of labelled AA into the different classes of macrophage lipids. Whether 5-F-AA and 6-F-AA are incorporated into phospholipids of macrophages and to what extent remains to be determined. However, to gain more information as to the behaviour of the fluorinated analogs compared with that of AA, the following experiment was designed. Mouse macrophages were cultured for 17 h in the presence of 20 μM [^{14}C]-AA without or with increasing concentrations of AA or 5-F-AA or 6-F-AA. After elimination of culture medium and washing of the cells, lipids were extracted and the associated radioactivity determined. As shown in Table 2, cells exposed to 20 μM [^{14}C]-AA incorporated 28 and 34% (experiments 1 and 2) of the radioactivity added to the culture medium in their lipids. 91 and 92% (experiments 1 and 2) of incorporated [^{14}C]-AA was associated with phospholipids (data not shown). When, in addition to [^{14}C]-AA, increasing concentrations of AA or 5-F-AA or 6-F-AA were present in the culture medium, a concentration-dependent decrease of the radioactivity incorporated into total lipids was observed (Table 2). The extent of this effect was very similar for either AA or 5-F-AA or 6-F-AA. These data suggest that 5-F-AA and 6-F-AA behave similarly to AA with regard to the rate-limiting enzyme involved in AA incorporation. Apparently, the fluorinated AA analogs would not act as inhibitors of AA incorporation into lipids.

The distribution of [^{14}C]-AA in the various classes of lipids was also studied. Figure 3 shows the radioactivity profile obtained after TLC of the total lipids of macrophages cultured in the presence of 20 μM [^{14}C]-AA. As evidenced by migration of unlabelled references, the bulk of radioactivity was associated with phospholipids. Fractions 1 and 2 contain [^{14}C]-AA associated with PI, PC, PS and PE, respectively. Fraction 3 could not be identified. Fraction 4 has a R_f value similar to that of free AA. Fraction 5 corresponds to AA incorporated into the neutral lipid fraction composed of diglycerides, triglycerides and cholesterol esters. The effect of 20 μM unlabelled AA or 5-F-AA or 6-F-AA on the distribution of [^{14}C]-AA into the different classes of lipids is summarized in Table 3. In the presence of either AA or 5-F-AA or 6-F-AA, the observed decreases in radioactivity of fraction 2 with respect to control are similar. The increases in radioactivity

Table 1. Comparison of the effects of AA, 5-F-AA or 6-F-AA added to the culture medium on the zymosan-induced LTC_4 release from macrophages

Culture conditions	LTC_4 release * (% of control)	
	Experiment 1	Experiment 2
Control (2.5 mg BSA . ml^{-1})	100	100
2 μM AA	-	102
20 μM AA	284 ± 6	414 ± 0
2 μM 5-F-AA	-	121
20 μM 5-F-AA	177 ± 12	149
2 μM 6-F-AA	-	86
20 μM 6-F-AA	65 ± 0	70 ± 1

Macrophages were cultured for 17 h in DMEM containing a fixed concentration of BSA without or with the fatty acids to be tested at the indicated concentrations. After washing and addition of fresh medium, the cells were challenged with zymosan for 80 min and the supernatants collected for LTC_4 analysis. Whatever the culture conditions, cells incubated without zymosan did not release LTC_4 in detectable amounts. Some assays were done in duplicates. Results are given as mean ± SD.

*The amounts of LTC_4 released in controls of experiments 1 and 2 were 138 ± 2 and 145 ± 1 ng, 80 min^{-1}, per dish, respectively.

Table 2. Effect of the concentration of AA, 5-F-AA and 6-F-AA on the incorporation of 20 μM [^{14}C]-AA into lipids of macrophages

17 h culture in presence of	Incorporation (% of radioactivity added per dish)	
	Experiment 1	Experiment 2
20 μM [^{14}C]-AA	28.1 ± 0.3	34.0 ± 0.1
20 μM [^{14}C]-AA + 5 μM AA	24.3 ± 0.7	31.4 ± 0.6
20 μM [^{14}C]-AA + 20 μM AA	17.8 ± 0.3	22.8 ± 1.2
20 μM [^{14}C]-AA + 5 μM 5-F-AA	24.8 ± 1.2	31.6 ± 0.5
20 μM [^{14}C]-AA + 20 μM 5-F-AA	20.4 ± 0.1	25.8 ± 0.9
20 μM [^{14}C]-AA + 5 μM 6-F-AA	27.0 ± 0.4	31.3 ± 0.1
20 μM [^{14}C]-AA + 20 μM 6-F-AA	20.3 ± 1.3	24.4 ± 1.6

Data are means of duplicates ± SD. For each experiment, a one-way ANOVA was run to compare the incorporation values of the seven experimental groups. The ANOVA overall P-values were 0.0002 and 0.0003, for experiments 1 and 2, respectively.

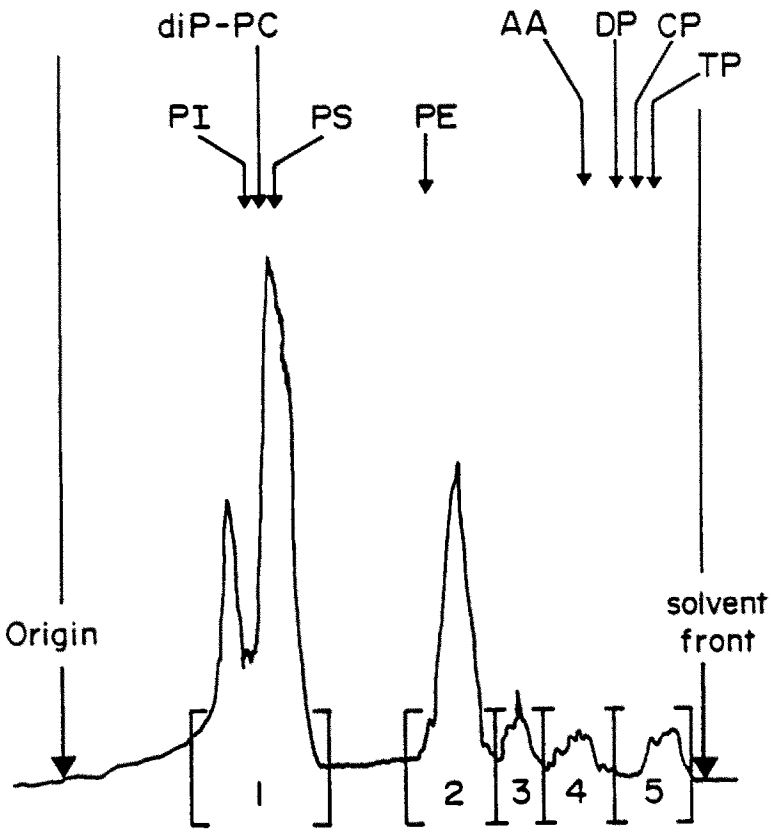


Figure 3. Separation by thin layer chromatography of the various classes of lipids from peritoneal mouse macrophages. Macrophages were cultured for 17 h in the presence of 20 μM [^{14}C]-AA–BSA complex in medium A. Extraction and separation of lipids by TLC was performed as described in the experimental section. Arrows indicate positions of reference lipids. diP-PC, DP, CP and TP are dipalmitoyl-phosphatidyl choline, 1,2-dipalmitin, cholesteryl palmitate and tripalmitin, respectively.

Table 3. Effect of AA, 5-F-AA and 6-F-AA on the distribution of [^{14}C]-AA into the different classes of macrophage lipids*

17 h culture in presence of	Radioactivity in lipid fractions (% of radioactivity in total lipids)				
	Fractions				
	1	2	3	4	5
[^{14}C]-AA (control)	70.8 ± 0.5	20.8 ± 0.5	3.1 ± 0.1	2.7 ± 0.0	2.6 ± 0.0
[^{14}C]-AA + AA	67.0 ± 0.0	17.7 ± 0.1	5.3 ± 0.2	5.5 ± 0.3	4.5 ± 0.0
[^{14}C]-AA + 5-F-AA	68.8 ± 0.9	15.7 ± 1.0	4.8 ± 0.6	6.0 ± 0.2	4.9 ± 0.3
[^{14}C]-AA + 6-F-AA	59.1 ± 0.7	17.8 ± 0.3	4.1 ± 0.4	5.8 ± 0.4	12.8 ± 0.3
overall ANOVA P-value	0.0006	0.0154	0.0456	0.0017	0.0001

The concentrations of [^{14}C]-AA, AA, 5-F-AA and 6-F-AA were 20 μM. For each combination of fatty acids, the experiment was performed in duplicate. Results are means of duplicate ± SD. Fractions 1 to 5 are defined in Figure 3. Radioactivity values in fractions 1 of the four experimental groups were compared using a one-way ANOVA which allowed the determination of an overall *P*-value. Such an ANOVA was run for each type of fraction. Within the ANOVA, *t*-tests were used to make pairwise comparisons between groups. When 6-F-AA-treated samples were compared with AA-treated samples, *P*-values of *t*-tests were 0.0008, 0.95, 0.077, 0.41 and 0.0001 for fractions 1, 2, 3, 4 and 5, respectively. For the same fractions, the *P*-values were 0.0004, 0.062, 0.27, 0.58 and 0.0001, respectively, when 6-F-AA-treated samples were compared with 5-F-AA-treated samples.
*The results shown in this Table were obtained from lipids extracts of experiment 1 of Table 2. Very similar results were obtained for experiment 2.

produced by the three fatty acids in fraction 3 are also similar. The same observation is made for fraction 4. However, in fraction 1, whereas AA and 5-F-AA yield a small decrease with respect to control (4 and 2%), 6-F-AA produces a much larger decrease of 12% (Pairwise *t*-tests: $P = 0.0008$ for 6-F-AA versus AA and $P = 0.0004$ for 6-F-AA versus 5-F-AA). Besides, in fraction 5, both AA and 5-F-AA produce an increase of about 2% whereas with 6-F-AA, a 10% increase is observed (Pairwise *t*-tests: $P = 0.0001$ for 6-F-AA versus AA and $P = 0.0001$ for 6-F-AA versus 5-F-AA). Therefore, 5-F-AA modifies the distribution profile of [^{14}C]-AA within the various classes of lipids in a way similar to that of AA. 6-F-AA has a distinct behaviour, producing a more important incorporation of [^{14}C]-AA into the neutral lipid fraction at the expense of the phospholipid fraction 1 than 5-F-AA and AA.

Effects of AA and 6-F-AA on cell viability. The release of lactate dehydrogenase (LDH) from cells is generally considered as an index of cell viability. To check that the effects observed for AA and 6-F-AA in experiments of incorporation and of LTC_4 release are not due to toxic effects of the compounds, LDH activity was measured in the culture medium of cells cultured for 17 h in the presence of AA and/or 6-F-AA at various concentrations (Table 4). The release of LDH from cells cultured with 20 μM 6-F-AA (16%) was only slightly higher than that of cells cultured with 20 μM AA (13%) or BSA (12%). At high concentration of polyunsatured fatty acids (20 μM AA + 20 μM 6-F-AA), a small toxic effect was observed since the release amounted to 24% compared to 12% in control.

Conclusion

Our previous studies have revealed that 5-F-AA and 6-F-AA are effective and equipotent inhibitors of 5-LO *in vitro*.[11] In the present work, the superiority of 6-F-AA as inhibitor of LTC_4 release in intact mouse peritoneal macrophages was demonstrated. Considering the structural similarity of the fluorinated AA analogs to AA, it is likely that these compounds are incorporated into phospholipids of cell membranes which serve as reservoir for AA prior to release by phospholipase A_2. Studies where AA or the fluorinated AA analogs are in competition with [^{14}C]-AA for incorporation into cellular lipids show that 6-F-AA displaces [^{14}C]-AA from the phospholipid pool more efficiently than AA or 5-F-AA. This could mean that 6-F-AA is incorporated more efficiently in the phospholipid pool than AA or 5-F-AA. This might explain, at least in part, why 6-F-AA is an inhibitor of LTC_4 release superior to 5-F-AA.

Thus, 6-F-AA emerges as a new inhibitor of LTC_4 synthesis in cells. It will be interesting to study its potential anti-inflammatory action in animal models of diseases where leukotrienes are involved.

Table 4. Lactate dehydrogenase release from macrophages cultured in presence of fatty acid acids complexed to BSA

17 h culture in presence of	Total LDH activity per dish (nmol NADH . min^{-1})	LDH release (% of total activity in dish)
BSA (2.5 mg / ml)	960 ± 2	11.8 ± 0.1
20 μM AA	1007 ± 17	12.8 ± 0.5
20 μM AA + 5 μM 6-F-AA	983 ± 36	15.1 ± 0.6
20 μM AA + 20 μM 6-F-AA	1003 ± 19	23.6 ± 1.0
20 μM 6-F-AA	957	16.3

Data are means of 2 values ± SD, except for experiment with 6-F-AA alone.

Experimental Section

Chemistry

Infrared spectra were recorded on a Bruker IFS G6 spectrometer. ^1H NMR spectra, unless otherwise stated, were recorded on a Bruker AM 360 spectrometer at 360.134 MIIz; the data are reported as follows: chemical shift in ppm from external Me_4Si on δ scale, multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, p = quintuplet, m = multiplet) and coupling constant (Hz). ^{19}F NMR spectra, unless otherwise stated, were recorded on a Bruker AM 360 spectrometer at 338.10 MHz; the data are reported as follows: chemical shift in ppm from external C_6F_6 on δ scale, multiplicity and coupling constant (same as for ^1H). Melting points were determined on a Kofler apparatus and are uncorrected. The mass spectra were measured on a Finnigan SSQ 7000 spectrometer equipped with a thermospray ion source with mobile phase $CH_3COO^-NH_4^+$ 0.1 M:CH_3OH 40:60 and flow 1 mL/min. Elemental analysis were performed on a Carlo Erba elemental analyzer model 1106. CH_2Cl_2 was dried and distilled over P_2O_5, THF was distilled from sodium benzophenone. Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck Silica Gel 60F-254 glass plates (0.25 mm) and the products visualized with phosphomolybdic acid spray. Chromatographies were carried out with the use of Merck Silica gel 60 (230–400 Mesh) as described by Still *et al.*[37]

2,2-Difluorosuccinic acid (7)

The ester **6a**[19] (115 g, 0.7 mol) was dissolved in a 1 :1 mixture of ethanol and water (400 mL) and 2N aqueous solution of potassium hydroxide (396 mL, 0.792 mol) was added dropwise over 1 h at room temperature. The reaction mixture was stirred for an additional hour. Evaporation of the solvent under reduced pressure afforded the potassium salt of 2,2-difluoro-4-pentenoic acid **6b** which was used without purification.

To a solution of potassium permanganate (380 g, 2.4 mol) in water (3 L) was added dropwise a solution of **6b** in acetic acid (1.5 L) over 2 h under mechanical stirring whilst the reaction temperature was maintained under 9 °C with efficient external cooling (acetone–ice bath). The stirring was continued with cooling for 1 h and overnight at room temperature. Sodium bisulfite (300 g) was added with efficient cooling until complete decolourization was achieved, the reaction mixture was then stirred for an additional hour. Three quarters of the solvents were evaporated, then with efficient cooling (acetone/ice) concentrated sulfuric acid (200 mL) was added until pH = 1. The aqueous phase was extracted three times with ether and ethyl acetate. The organic layers were afforded 103.5 g of crude **7** which was crystallized in a mixture of ether and petroleum ether to give pure difluorosuccinic acid **7** (97 g, 91%), m.p. = 154 °C:[18] ^1H NMR ($CDCl_3$ + $CDCOCD_3$) δ 3.3 (t, J_{HF} = 15 Hz, 2H), 11.5 (s, 1H); ^{19}F NMR ($CDCl_3$ + CD_3COCD_3) δ 148.3 (t, J_{FH} = 15 Hz).

Fluorofumaric acid (8)

8 Was obtained as described by Raasch *et al.*,[18] m.p. = 230 °C: ^1H NMR (CD_3OD) δ 6.36 (d, J_{HF} = 28 Hz); ^{19}F NMR (CD_3OD) δ 55.76 (d, J_{FH} = 28 Hz).

Fluoromaleic anhydride (9)

9 Was obtained as described by Raasch *et al.*:[18] ^1H NMR ($CDCl_3$) δ 6.43 (d, J_{HF} = 3 Hz).

Fluoromaleic acid (10)

10 Was obtained as described by Raasch *et al.*,[18] m.p. = 116 °C: ^1H NMR (CD_3OD) δ 6.31 (d, J_{HF} = 18.2 Hz) ;^{19}F NMR (CD_3OD) δ 65.04 (d, J_{FH} = 18.2 Hz).

Dimethylfluoromaleate (11)

Fluoromaleic acid (16 g, 0.12 mol) dissolved in ether (200 mL) was treated at 0 °C with a 0.5 M ethereal solution of

diazomethane until the yellow colour was stable (500 mL). The solvent was then evaporated under reduced pressure and a kugelrohr distillation (0.05 mbar, 50 °C) afforded pure ester **12** (18.4 g, 95%): ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 3H), 3.86 (s, 3H), 6.06 (d, J_{HF} = 15.5 Hz).

(E)-1,4-Dihydroxy-2-fluoro-2-butene (12)

To a solution of diester **11** (20 g, 0.123 mol) in dry tetrahydrofuran (250 mL) cooled to -10 °C was added dropwise under argon diisobutylaluminium hydride (DIBAL) (1.2 M in hexane; 568 mL) while the temperature of the reaction mixture was maintained at 0 °C. The mixture was stirred at 0 °C for 1 h and for 1 h at room temperature. The mixture was cooled again to 0 °C and methanol (25 mL) was added dropwise to destroy the excess of DIBAL. Then the aluminium salts were precipitated with an aqueous saturated solution of ammonium chloride, added until a filterable precipitate was obtained. The white–grey solid was filtered and the cake was washed with ethyl acetate containing 10% of methanol. The filtrate was concentrated under reduced pressure. The resulting oil was purified by chromatography on silica gel using pure ethyl acetate as eluent. Diol **12** was obtained as an oil (7.36 g, 57%): ^{1}H NMR (CD_3OD) δ 2.04 (s, 2H exchangeable with D_2O), 4.2 (dd, J_{HH} = 7.7 Hz, J_{HF} = 1.8 Hz, 2H), 4.29 (d, J_{HF} = 19.6 Hz, 2H), 5.5 (dt, J_{HF} = 19.6 Hz, J_{HH} = 7.7 Hz, 1H); ^{19}F NMR, (CD_3OD) δ 54.3 (q, J_{FH} = 19.6 Hz).

(E)-1-Chloro-3-fluoro-4-(2-tetrahydropyranyloxy)-2-butene (14)

To a solution of *N*-chlorosuccinimide (24.8 g, 185 mmol) in methylene chloride (600 mL) cooled to 0 °C was added dimethylsulfide (14.9 mL, 203 mmol) and the mixture was stirred for 15 min at 0 °C. Then after cooling to -25 °C diol **12** (17.89 g, 168 mmol) in methylene chloride (200 mL) was added dropwise. The mixture was stirred successively for 30 min at -25 °C, 3 h at 0 °C and finally 30 min at room temperature. Dihydropyran (31 mL, 340 mmol) and pyridinium *para*-toluenesulfonate (2 g, 8 mmol) were added. The mixture was stirred overnight at room temperature. The reaction mixture was washed with saturated brine. The organic phase was decanted and dried over sodium sulfate. Filtration and flash chromatography on silica gel and elution with a 9:1 mixture of hexane and ethyl acetate afforded chloride **14** as an oil (28.19 g, 81%): ^{1}H NMR ($CDCl_3$) δ 1.5 to 1.9 (m, 6H), 3.52 to 3.58 (m, 1H), 3.82 to 3.88 (m, 1H), 4.15 (d, J_{HH} = 8.7 Hz, 2H), 4.23 (dd, J_{HH} = 13.5 Hz, J_{HF} = 22.5 Hz, 1H), 4.31 (dd, J_{HH} = 13.5 Hz, J_{HF} = 18.3 Hz, 1H), 4.81 (t, J_{HH} = 3.4 Hz, 1H), 5.56 (dt, J_{HH} = 8.7 Hz, J_{HF} = 17.6 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ 58.52 (dt, J_{FH} = 18.1 Hz, J_{FH} = 22.2 Hz); MNH_4^+ = 226.

(E)-1-(1,3-Dithia-2-cyclohexyl)-3-fluoro-4-(2-tetrahydropyranyloxy)-2-butene (15)

To a solution of 1,3-dithiane (1.55 g, 12.9 mmol) in tetrahydrofuran (60 mL) cooled to -30 °C was added dropwise *n*-butyllithium (1.32 M in hexane; 9.77 mL, 12.9 mmol), the mixture was stirred at -30 °C for 30 min. Then chloride **14** (2.69 g, 12.9 mmol) in tetrahydrofuran (10 mL) was added dropwise. The mixture was stirred for 30 min at -30 °C and 2 h at 0 °C. The reaction was quenched with saturated aqueous ammonium chloride and the solvent was evaporated under reduced pressure. The residue was diluted with ether and washed with water. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure. Flash chromatography on silica gel and elution with a 8:2 mixture of hexane and ethyl acetate afforded dithiane **15** as an oil (3.34 g, 90%): ^{1}H NMR ($CDCl_3$) δ 1.5 to 2.16 (m, 8H), 2.52 (t, J_{HH} = 7.2 Hz, 2H), 2.85 to 2.89 (m, 4H), 3.52 to 3.56 (m, 1H), 3.84 to 3.88 (m, 1H), 4.04 (t, J_{HH} = 6.9 Hz, 1H), 4.11 to 4.32 (m, 2H), 4.7 (t, J_{HH} = 3.3 Hz, 1H), 5.4 (dt, J_{HF} = 19.8 Hz, J_{HH} = 7.2 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ 55.43 (dt, J_{FH} = 23.5 Hz, J_{FH} = 19.7 Hz); MNH_4^+ = 310.

(E)-1-(1,3-Dithia-2-cyclohexyl)-3-fluoro-4-ol-2-butene (16)

Tetrahydropyranyl derivative **15** (3.22 g, 11 mmol) was dissolved in methanol (60 mL). Pyridinium *para*-toluenesulfonate (0.3 g, 1.2 mmol) was added and the mixture was refluxed for 2.5 h. Methanol was evaporated under reduced pressure. The residue was dissolved in ether and washed with water. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure. Flash chromatography on silica gel and elution with a 1:1 mixture of hexane and ethyl acetate afforded alcohol **16** as white crystals (2.11 g, 91%). Recrystallization in a mixture of hexane and ether afforded analytically pure sample, m.p. = 33.5–34.5 °C: ^{1}H NMR ($CDCl_3$) δ 1.65 (s, 1H exchangeable with D_2O), 1.86 to 1.96 (m, 1H), 2.09 to 2.18 (m, 1H), 2.49 (bt, J_{HH} = 7.6 Hz, 1H), 2.82 to 2.92 (m, 4H), 4.03 (t, J_{HH} = 6.7 Hz, 1H) 4.24 (d, J_{HF} = 20.9 Hz,2H), 5.30 (dt, J_{HF} = 19.9 Hz, J_{HF} = 8.4 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ 52.43 (bq, J_{FH} = 20.6 Hz); MH^+ = 209.

(E)-4-Chloro-1-(1,3-dithia-2-cyclohexyl)-3-fluoro-2-butene (17)

Alcohol **16** (1.9 g, 9.13 mmol) was dissolved in dry methylene chloride (70 mL). The mixture was cooled to 0 °C and 1-chloro-*N,N'*-2-trimethylpropenylamine[22] (1.23 g, 9.2 mmol) was added. The mixture was stirred under argon for 15 min. Methylene chloride was evaporated under reduced pressure. Flash chromatography on silica gel and elution with a 9:1 mixture of hexane and ethyl acetate afforded chloride **17** as an oil (1.98 g, 96%): ^{1}H NMR ($CDCl_3$) δ 1.8 to 1.89 (m, 1H), 2.09 to 2.18 (m, 1H), 2.49 (bt, J_{HH} = 7.6 Hz, 2H), 2.82 to 2.93 (m, 4H), 4.06 (t, J_{HH} = 6.9 Hz, 1H),4.15 (d, J_{HF} = 21.2 Hz, 2H), 5.4 (dt, J_{HF} = 18.4 Hz, J_{HH} = 8.2 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ 55.85 (bq, J_{FH} = 18.6 Hz, J_{FH} = 20.9 Hz); M^+ = 226.

(E)-7-(1,3-Dithia-2-cyclohexyl)-5-fluoro-5-heptenal (19)

To a solution of *N*-allyl-*N,N',N"*-pentamethylphosphoramide[23] (1.5 g, 7.32 mmol) in tetrahydrofuran

(21 mL) cooled to -78 °C was added dropwise *n*-butyllithium (1.32 M in hexane; 5.55 mL, 7.32 mmol). The mixture was stirred under argon at -78 °C for 1 h. To the resulting red–orange solution, chloride **17** in tetrahydrofuran (10 mL) was added dropwise at -78 °C. The mixture was stirred for 1 h at -78 °C, then warmed to 0 °C within 2 h and stirred for 30 min at 0 °C. The reaction was quenched with saturated aqueous ammonium chloride and tetrahydrofuran was evaporated under reduced pressure. The resulting oil was diluted with methylene chloride and washed with water. The organic layer was dried over magnesium sulfate. Filtration and concentration under reduced pressure afforded **18** as an oil. This oil was dissolved in ether (36.5 mL) and was stirred at room temperature for 2 h with ~ 2N aqueous solution of hydrochloric acid (36.5 mL). The organic layer was washed twice with water, dried over magnesium sulfate, filtered and concentrated under reduced pressure to afford an oil (1.45 g). Flash chromatography on silica gel and elution with a 25:75 mixture of ethyl acetate and hexane afforded aldehyde **19** (1.014g, 56%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.79 to 1.9 (m, 1H), 1.88 (q, J_{HH} = 7.3 Hz, 1H), 2.08 to 2.15 (m, 1H), 2.31 (dt, J_{HF} = 22.4 Hz, J_{HH} = 7.2 Hz, 2H), 2.38 (t, J_{HH} = 7.4 Hz, 2H), 2.51 (dt, J_{HH} = 1.2 Hz, J_{HH} = 7.2 Hz, 2H), 2.79 to 2.92 (m, 4H), 4.04 (t, J_{HH} = 6.8 Hz, 1H), 5.16 (dt, J_{HF} = 21.1 Hz, J_{HH} = 7.9 Hz, 1H), 9.8 (t, J_{HH} = 1.2 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ 60.68 (bq, J_{FH} = 22.1 Hz); MNH_4^+ = 266.

(E)-7-(1,3-Dithia-2-cyclohexyl)-5-fluoro-5-heptenol (20)

Aldehyde **19** (0.937 g, 3.77 mmol) was dissolved in methanol (20 mL) and cooled to 0 °C. Sodium borohydride (0.071 g, 1.87 mmol) was added and the mixture was stirred for 30 min. Acetone was added to destroy the excess of sodium borohydride and then the reaction mixture was acidified with acetic acid. The solvents were evaporated under reduced pressure. The residue was diluted with ether and washed with water. The organic phase was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford alcohol **20** as an oil in a quantitative yield (0.94 g): ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 1H, exchangeable with D_2O), 1.6 to 1.68 (m, 4H), 1.79 to 1.91 (m, 1H), 2.08 to 2.16 (m, 1H), 2.29 (bdt, J_{HF} = 22.8 Hz, J_{HH} = 6.9 Hz, 2H), 2.79 to 2.92 (m, 4H), 3.66 (t, J_{HH} = 6.1 Hz, 2H), 4.03 (t, J_{HH} = 6.9 Hz, 1H),5.12 (dt, J_{HF} = 21.1 Hz, J_{HH} = 8 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ 61.09 (bt, J_{FH} = 22.5 Hz, J_{FH} = 24.8 Hz); MNH_4^+ = 268.

(E)-1-(t-Butyldiphenylsilyloxy)-7-(1,3-dithia-2-cyclohexyl)-5-fluoro-5-heptene (21)

To a solution of alcohol **20** (2.15 g, 9.26 mmol) in dry methylene chloride (50 mL) was added triethylamine (2 mL, 14.3 mmol), *t*-butyldiphenylchlorosilane (2.65 mL, 10.2 mmol) and 4-dimethylaminopyridine (45 mg, 0.37 mmol). The mixture was stirred overnight at room temperature. The reaction mixture was washed once with water and then dried over sodium sulfate. Filtration and evaporation under reduced pressure afforded an oil. Flash chromatography on silica gel and elution with a 8:92 mixture of ethyl acetate and hexane afforded dithiane **21** as an oil (4.12 g, 94%): ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.52 to 1.68 (m, 4H), 1.83 to 1.91 (m, 1H), 2.08 to 2.18 (m, 1H), 2.23 (dt, J_{HF} = 23 Hz J_{HH} = 7 Hz, 2H), 2.37 (t, J_{HH} = 7.4 Hz, 2H), 2.78 to 2.92 (m, 4H), 3.66 (t, J_{HH} = 5.9 Hz, 2H), 4.01 (t, J_{HH} = 6.9 Hz, 1H), 5.01 (dt, J_{HF} = 21 Hz, J_{HH} = 7.4 Hz, 1H), 7.38 to 7.69 (m, 10H); ^{19}F NMR ($CDCl_3$) δ 61.26 (dt, J_{FH} = 21.9 Hz, J_{FH} = 22.5 Hz); MNH_4^+= 506.

(E)-8-(t-Butyldiphenylsilyloxy)-4-fluoro-3-octenol (22)

To a suspension of trimethyloxonium tetrafluoroborate (0.44 g, 2.97 mmol) in dry methylene chloride (15 mL) was added at room temperature dithiane **21** (1.45 g, 2.97 mmol) and the mixture was stirred for 1 h. Then, a mixture of acetone and water (9:1; 5 mL) containing calcium carbonate (0.6 g, 5.94 mmol) was added and the mixture was stirred overnight at room temperature. The precipitate was filtered off and after dilution of the filtrate with saturated brine the mixture was extracted three times with ether. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. The resulting oil was dissolved in ethanol (10 mL) and sodium borohydride (56 mg, 1.48 mmol) was added. The mixture was stirred for 30 min at 0 °C. The excess of sodium borohydride was destroyed with acetone, the mixture was acidified with acetic acid and concentrated under reduced pressure. The residue was taken up in water and extracted three times with ether. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. Flash chromatography on silica gel and elution with a 28:72 mixture of ethyl acetate and hexane afforded alcohol **22** as an oil (0.813 g, 74%): ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 9H), 1.55 to 1.66 (m, 5H), 2.16 (q, J_{HH} = 6.6 Hz, 2H), 2.24 (dt, J_{HF} = 23 Hz, J_{HH} = 7 Hz, 2H), 3.59 (t, J_{HH} = 6.5 Hz, 2H), 3.66 (t, J_{HH} = 6 Hz, 2H), 5 (dt, J_{HF} = 21.5 Hz, J_{HH} = 8 Hz, 1H), 7.25 to 7.65 (m, 10H); ^{19}F NMR ($CDCl_3$) δ 59 (q, J_{FH} = 22.4 Hz); MNH_4^+ = 418.

(E)-8-(t-Butyldiphenylsilyloxy)-4-fluoro-1-mesyloxy-3-octene (23a)

To a solution of alcohol **22** (0.813 g, 2.03 mmol) in dry methylene chloride (10 mL) containing triethylamine (0.43 mL, 3.05 mmol) cooled to -10 °C was added dropwise mesylchloride (0.2 mL, 2.23 mmol). The mixture was stirred for 15 min at -10 °C then warmed to room temperature. The reaction mixture was washed three times with water. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford the expected mesylate **23a** as an oil (0.985 g) which was used without purification: ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.06 (s, 9H), 1.36 to 1.8 (m, 4H), 1.8 to 2.6 (m, 2H) 2.07 (q, J_{HH} = 7 Hz, 2H), 2.9 (s, 3H), 3.65 (bt, J_{HH} = 7 Hz, 2H), 4.1 (t, J_{HH} = 7 Hz, 2H), 4.96 (dt, J_{HF} = 21 Hz, J_{HH} = 8 Hz, 1H), 7.16 to 7.83 (m, 10H).

(E)-1-Bromo-8-(t-butyldiphenylsilyloxy)-4-fluoro-3-octene (23b)

To a solution of mesylate **23a** (0.985 g, 2.03 mmol) in

benzene (50 mL) was added dry Amberlyst-A-26 Br^- form (4.2 g) and the mixture was refluxed overnight with stirring. Filtration and evaporation under reduced pressure afforded an oil (0.86 g). Flash chromatography on silica gel and elution with a 98:2 mixture of hexane and ethyl acetate afforded bromide **23b** as an oil (0.825 g, 87% from **22**): ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.57 to 1.65 (m, 4H), 2.22 (dt, J_{HF} = 23 Hz, J_{HH} = 7Hz, 2H), 3.67 (t, J_{HH} = 6 Hz, 2H), 5.02 (dt, J_{HF} = 21 Hz, J_{HH} = 8 Hz, 1H), 7.21 to 7.67 (m, 10H); ^{19}F NMR ($CDCl_3$) δ 60.34 (bq, J_{FH} = 22.5 Hz); MH^+ = 465–463.

*[(E)-8-(t-Butyldiphenylsilyloxy)-4-fluoro-3-octenyl]triphenylphosphonium bromide (**5a**)*

A mixture of bromide **23b** (0.825 g, 1.78 mmol) and triphenylphosphine (0.61 g, 2.31 mmol) in dry acetonitrile (10 mL) was refluxed for 48 h. Evaporation of the solvent under reduced pressure, flash chromatography on silica gel and elution with a 9:1 mixture of methylene chloride and methanol afforded phosphonium bromide **5a** as a foam (0.982 g, 81%): ^{1}H NMR ($CDCl_3$) δ 1 (s, 9H), 1.43 to 1.52 (m, 4H), 1.97 (dt, J_{HF} = 22.8 Hz, J_{HH} = 6 Hz, 2H), 2.35 to 2.45 (m, 2H), 3.50 (t, J_{HH} = 6 Hz, 2H), 3.98 to 4.06 (m, 2H), 5.07 (dt, J_{HF} = 20.3 Hz, J_{HH} = 7.6 Hz, 1H), 7.32 to 7.92 (m, 25H); ^{19}F NMR ($CDCl_3$) δ 61.42 (dt, J_{FH} = 20.3 Hz, J_{FH} = 22.6 Hz); MH^+ = 645 ($C_{42}H_{47}FOPSi$).

*(E)-6-Fluoro-7-(2-tetrahydropyranyloxy)-5-heptenal (**25**)*

To a solution of *N*-allyl-*N,N'N"*-pentamethylphoramide[23] (44.4 g, 216 mmol) in tetrahydrofuran (490 mL) cooled to -78 °C was added dropwise *n*-butyllithium (1.58 M in hexane; 136 mL, 215 mmol). The mixture was stirred under argon at -78 °C for 1 h. To the resulting red–orange solution, chloride **14** (41 g, 196 mmol) in tetrahydrofuran (50 mL) was added dropwise at -78 °C. The mixture was stirred for 1 h at -78 °C, then warmed up to 0 °C within 2 h and stirred for 1 h at 0 °C. The reaction was quenched with saturated aqueous ammonium chloride and the tetrahydrofuran was evaporated under reduced pressure. The resulting oil was diluted with methylene chloride, washed with water and decanted. The organic layer was dried over magnesium sulfate. Filtration and concentration under reduced pressure afforded an oil. This oil was dissolved in ether (750 mL) and was stirred at room temperature for 1 h with a 2N aqueous solution of hydrochloric acid (750 mL). The organic layer was decanted, washed twice with water, dried over magnesium sulfate, filtered and concentrated under reduced pressure to afford an oil (49 g). The NMR of the crude mixture showed that the THP has been mostly cleaved. To a solution of the crude oil in methylene chloride (500 mL) was added dihydropyran (36 mL, 294 mmol) and pyridinium *para*-toluenesulfonate (4 g, 16 mmol) and the mixture was stirred overnight at room temperature. The reaction mixture was washed with water. The organic layer was decanted and dried over sodium sulfate. Filtration and concentration under reduced pressure afforded an oil (72 g). Flash chromatography on silica gel and elution with a 25:75 mixture of ethyl acetate and hexane afforded aldehyde **25** (39 g, 87%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.5 to 1.9 (m, 8H), 2.09 (q, J_{HH}= 8 Hz, 2H), 2.47 (dt, J_{HH} = 1.4 Hz, J_{HH} = 7.2 Hz, 2H), 3.49 to 3.55 (m, 1H), 3.82 to 3.88 (m, 1H), 4.18 (AB part of an ABX system $J_{H_AH_B}$ = 12.9 Hz, J_{H_AF} = 20.4 Hz, J_{H_BF} = 24 Hz, 2H), 4.68 (t, J_{HH} = 3.4 Hz, 1H), 5.25 (dt, J_{HH} = 8.1 Hz, J_{HF} = 20.4 Hz, 1H), 9.76 (t, J_{HH} = 1.4 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ 52.90 (dt, J_{FH} = 20.5 Hz, J_{FH} = 23.7 Hz); MNH_4^+ = 248. Anal. calcd for $C_{12}H_9FO_3$: C, 62.59; H, 8.32. Found: C, 62.71; H, 8.46.

*(E)-6-Fluoro-7-(2-tetrahydropyranyloxy)-5-heptenol (**26**)*

Aldehyde **25** (38.6 g, 167 mmol) was dissolved in methanol (350 mL) and cooled to 0 °C. Sodium borohydride (3.634 g, 96 mmol) was added and the mixture was stirred for 30 min. Acetone was added to destroy the excess of sodium borohydride. The solvents were evaporated under reduced pressure. The residue was diluted with ether and washed with water. The organic phase was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford alcohol **26** as an oil (38.5 g) which was used in the next step without purification: ^{1}H NMR ($CDCl_3$) δ 1.4 to 1.8 (m, 11H), 2.06 (q, J_{HH} = 7.5 Hz, 2H), 3.5 to 3.55 (m, 1H), 3.63 (t, J_{HH} = 7 Hz, 2H), 3.81 to 3.9 (m, 1H), 4.2 (AB part of an ABX system $J_{H_AH_B}$ = 12.5 Hz, J_{H_AF} = 20 Hz, J_{H_BF} = 24 Hz, 2H), 4.7 (t, J_{HH} = 35 Hz, 1H), 5.28 (dt, J_{HH} = 8 Hz, J_{HF} = 20 Hz, 1H).

*(E)-1-(t-Butyldiphenylsilyloxy)-6-fluoro-7-(2-tetrahydropyranyloxy)-5-heptene (**27**)*

To a solution of crude alcohol **26** (38.5 g, 166 mmol) in dry methylene chloride (350 mL) was added triethylamine (35 mL, 250 mmol), *t*-butyldiphenylchlorosilane (47.5 mL, 183 mmol) and 4-dimethylaminopyridine (0.725 g, 6 mmol). The mixture was stirred overnight at room temperature. The reaction mixture was washed once with water and then dried over sodium sulfate. Filtration and evaporation under reduced pressure afforded an oil. Flash chromatography on silica gel and elution with a 10:90 mixture of ethyl acetate and hexane afforded the silyl ether **27** as an oil (62.23 g, 79% from **25**): ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 3H), 1.4 to 1.92 (m, 10H), 2.04 (q, J_{HH} = 8 Hz, 2H), 3.48 to 3.54 (m, 1H), 3.65 (t, J_{HH} = 6.2 Hz, 2H), 3.82 to 3.89 (m, 1H), 4.18 (AB part of an ABX system $J_{H_AH_B}$ = 12.9 Hz, J_{H_AF} = 20.4 Hz, J_{H_BF} = 24 Hz, 2H), 4.62 (t, J_{HH} = 3.4 Hz, 1H), 5.27 (dt, J_{HH} = 8 Hz, J_{HF} = 20.8 Hz, 1H), 7.39 (m, 6H), 7.68 (m, 4H); ^{19}F NMR ($CDCl_3$) δ 51.2 (dt, J_{FH} = 20.1 Hz, J_{FH} = 24 Hz); MNH_4^+ = 488. Anal. calcd for $C_{28}H_{39}FO_3Si$: C, 71.45; H, 8.35. Found: C, 71.73; H, 8.83.

*(E)-1-(t-Butyldiphenylsilyloxy)-6-fluoro-5-heptene-7-ol (**28**)*

Tetrahydropyranyl derivative **27** (42.61 g, 90 mmol) was dissolved in methanol (400 mL). Tetrabutyl-1,3-diisothiocyanatodistannoxane (0.51 g, 0.85 mmol) was added and the mixture was refluxed for 24 h. Methanol was evaporated under reduced pressure. The residue was

dissolved in ether and washed with water. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure. Flash chromatography on silica gel and elution with a 2:8 mixture of hexane and ethyl acetate afforded alcohol **28** as an oil (30 g, 86%): ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.46 to 1.55 (m, 4H), 1.75 (t, J_{HH} = 7.5 Hz, 1H exchangeable with D_2O), 2.02 (q, J_{HH} = 8 Hz, 2H), 3.66 (t, J_{HH} = 6 Hz, 2H), 4.69 (dd, J_{HH} = 7.5 Hz, J_{HF} = 21 Hz, 2H), 5.10 (dt, J_{HF} = 21 Hz, J_{HH} = 8 Hz, 1H), 7.36 to 7.7 (m, 10H); ^{19}F NMR ($CDCl_3$) δ 47.27 (q, J_{FH} = 21 Hz); MNH_4^+ = 404. Anal. calcd for $C_{23}H_{19}FO_2Si$: C, 71.46; H, 8.32. Found: C, 71.42; H, 8.10.

(E)-7-Bromo-1-(t-butyldiphenylsilyloxy)-6-fluoro-5-heptene (29)

Alcohol **28** (31.63 g, 81.94 mmol) was dissolved in dry methylene chloride (600 mL). The mixture was cooled to 0 °C and 1-bromo-*N,N*,2-trimethylpropenylamine[22] (14.6 g, 82 mmol) was added. The mixture was stirred under argon for 15 min. Methylene chloride was evaporated under reduced pressure. Flash chromatography on silica gel and elution with a 95:5 mixture of hexane and ethyl acetate afforded bromide **29** as an oil (34.68 g, 94%): ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.48 to 1.57 (m, 4H), 2.01 (q, J_{HH} = 8 Hz, 2H), 3.68 (t, J_{HH} = 6 Hz, 2H), 3.99 (d, J_{HF} = 2 Hz, 2H), 5.27 (dt, J_{HF} = 21.6 Hz, J_{HH} = 8 Hz, 1H), 7.37 to 7.73 (m, 10H); ^{19}F NMR ($CDCl_3$) δ 53.37 (q, J_{FH} = 21.7 Hz); MH^+ = 451–449. Anal. calcd for $C_{23}H_{30}BrFOSi$: C, 61.46; H, 6.73 . Found: C, 61.55; H, 6.71.

(E)-1-(t-Butyldiphenylsilyloxy)-7-(1,3-dithia-2-cyclohexyl)-6-fluoro-5-heptene (30)

To a solution of 1,3-dithiane (3.42 g, 28.5 mmol) in tetrahydrofuran (100 mL) cooled to -30 °C was added dropwise *n*-butyllithium (1.68 M in hexane; 15.4 mL, 25.8 mmol) and the mixture was stirred at -30 °C for 30 min. Bromide **29** (11.41 g, 25.52 mmol) in tetrahydrofuran (10 mL) was added dropwise. The mixture was stirred for 30 min at -40 °C and 2 h at 0 °C and then quenched with saturated aqueous ammonium chloride and tetrahydrofuran was evaporated under reduced pressure. The residue was diluted with ether and washed with water. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure. Flash chromatography on silica gel and elution with a 95:5 mixture of hexane and ethyl acetate afforded dithiane **30** as an oil (12 g, 96%): ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.44 to 1.52 (m, 4H), 1.8 to 1.88 (m, 1H), 1.96 (q, J_{HH} = 8 Hz, 2H), 2.09 to 2.17 (m, 1H), 2.67 (dd, J_{HH} = 7 Hz, J_{HF} = 21.8 Hz, 2H), 2.8 to 2.96 (m, 4H), 3.67 (t, J_{HH} = 6 Hz, 2H), 4.31 (t, J_{HH} = 7 Hz, 1H), 5.2 (dt, J_{HF} = 21.8 Hz, J_{HH} = 8 Hz, 1H), 7.37 to 7.71 (m, 10H); ^{19}F NMR ($CDCl_3$) δ 56.05 (q, J_{FH} = 21.8 Hz) MNH_4^+ = 506. Anal. calcd for $C_{27}H_{37}FOS_2Si$: C, 66.34; H, 7.63. Found: C, 66.44; H, 7.79.

(E)-8-(t-Butyldiphenylsilyloxy)-3-fluoro-3-octenol (31)

To a solution of dithiane **30** (20 g, 40.98 mmol) in dry methylene chloride (400 mL) was added trimethyloxonium tetrafluoroborate (7.5 g, 50.7 mmol) and the mixture was stirred for 1 h at room temperature. Then a mixture of acetone and water (9:1; 100 mL) containing calcium carbonate (8.2 g, 82 mmol) was added and the mixture was stirred overnight at room temperature. The precipitate was filtered off and after dilution with saturated brine the mixture was extracted three times with ether. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. The resulting oil was dissolved in ethanol (200 mL) and sodium borohydride (0.78 g, 20.6 mmol) was added. The mixture was stirred for 30 min at 0 °C. The excess of sodium borohydride was destroyed with acetone, the mixture was acidified with acetic acid and concentrated under reduced pressure. The residue was taken up in water and extracted three times with ether. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. Flash chromatography on silica gel and elution with a 25:75 mixture of ethyl acetate and hexane afforded alcohol **31** as an oil (9.69 g, 60%): ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.4 to 1.51 (m, 2H), 1.56 (s, 1H), 1.5 to 1.62 (m, 2H), 1.95 (q, J_{HH} = 8 Hz, 2H), 2.47 (dt, J_{HH} = 6.3 Hz, J_{HF} = 22.4 Hz, 2H), 3.66 (t, J_{HH} = 6.1 Hz, 2H), 3.76 (t, J_{HH} = 6.3 Hz, 2H), 5.16 (dt, J_{HH} = 8 Hz, J_{HF} = 22.4 Hz, 1H), 7.36 to 7.71 (m, 10H); ^{19}F NMR ($CDCl_3$) δ 56 (q, J_{FH} = 22.4 Hz) MNH_4^+ = 418. Anal. calcd for $C_{24}H_{33}FO_2Si$: C, 71.96; H, 8.30. Found: C, 72.37; H, 8.49.

(E)-1-Bromo-8-(t-butyldiphenylsilyloxy)-3-fluoro-3-octene (32)

Alcohol **31** (9.82 g, 24.55 mmol) was dissolved in dry methylene chloride (100 mL). The mixture was cooled to 0 °C and 1-bromo-*N,N*,2-trimethyl-propenylamine[22] (44 g, 24.7 mmol) was added. The mixture was stirred under argon for 30 min. Methylene chloride was evaporated under reduced pressure. Flash chromatography on silica gel and elution with a 95:5 mixture of hexane and ethyl acetate afforded bromide **32** as an oil (9.43 g, 83%): ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.4 to 1.48 (m, 2H), 1.53 to 1.63 (m, 2H), 1.95 (q, J_{HH} = 8 Hz, 2H), 2.77 (dt, J_{HF} = 21.6 Hz, J_{HH} = 6.3 Hz, 2H), 3.48 (t, J_{HH} = 6.3 Hz, 2H), 3.64 (t, J_{HH} = 6.1 Hz, 2H), 5.19 (dt, J_{HF} = 21.6 Hz, J_{HH} = 8 Hz, 1H), 7.38 to 7.63 (m, 10H); MH^+ = 465–463. Anal. calcd for $C_{24}H_{32}BrFOSi$: C, 62.19; H, 6.96. Found: C, 62.74; H, 7.33.

[(E)-8-(t-Butyldiphenylsilyloxy)-3-fluoro-3-octenyl]-triphenylphosphonium bromide (5b)

A mixture of bromide **32** (9.43 g, 20.36 mmol) and triphenylphosphine (16 g, 61 mmol) in dry acetonitrile (200 mL) was refluxed for 48 h. Evaporation of the solvent under reduced pressure, flash chromatography on silica gel and elution with a 9:1 mixture of methylene chloride and methanol afforded phosphonium bromide **5b** as a foam (9.82 g, 67%): ^{1}H NMR ($CDCl_3$) δ 1 (s, 9H), 1.28 to 1.32 (m, 2H), 1.42 to 1.50 (m, 2H), 1.72 to 1.80 (m, 2H), 2.78 (ddt, J_{HF} = 21 Hz, J_{HP} = 16.5 Hz, J_{HH} = 7 Hz, 2H), 3.57 (t, J_{HH} = 6 Hz, 2H), 4.06 (dt, J_{HH} = 7 Hz, J_{HP}

= 12.7 Hz, 2H), 4.94 (dt, J_{HF} = 21 Hz, J_{HH} = 7.9 Hz, 1H), 7.43 to 7.91 (m, 25 H); MH^+ = 645 ($C_{42}H_{47}FOPSi$).

*1-(t-Butyldiphenylsilyloxy)-5-fluoro-5,8,14-eicosatriene (**33a**)*

To a solution of diisopropylamine (0.15 mL, 1.08 mmol) in tetrahydrofuran (10 mL) cooled to -78 °C was added dropwise *n*-butyllithium (1.6 M in hexane; 0.68 mL, 1.08 mmol). The mixture was warmed to -10 °C and then cooled again to -78 °C. Phosphonium bromide **5a** (0.787 g, 1.08 mmol) in tetrahydrofuran (4 mL) was added dropwise and the mixture was stirred 30 min at -78 °C. Hexamethylphosphoramide (0.5 mL) was added and the reaction mixture was warmed to -30 °C. (Z)-6-Dodecenal[25] (0.187 g, 0.97 mmol) in tetrahydrofuran (2 mL) was added dropwise and the mixture was stirred for 2 h at -30 °C and 30 min at 0 °C. A saturated aqueous solution of ammonium chloride (1 mL) was added and tetrahydrofuran was evaporated under reduced pressure. The residue was taken up with water and extracted three times with ether. The organic layer was washed twice with water, decanted and dried over sodium sulfate. Filtration and evaporation of the solvent afforded an oil. Flash chromatography on silica gel and elution with a 9:1 mixture of hexane and benzene afforded the expected silyl ether **33a** as an oil (237 mg, 55%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 0.86 (t, J_{HH} = 6 Hz, 3H), 1.05 (s, 9H), 1.2 to 2.42 (m, 22H), 2.63 (t, J_{HH} = 7 Hz, 2H), 3.63 (bt, J_{HH} = 6 Hz, 2 H), 4.9 (dt, J_{HF} = 21 Hz, J_{HH} = 8 Hz, 1H), 5.2 to 5.43 (m, 4H), 7.22 to 7.89 (m, 10 H).

*1-(t-Butyldiphenylsilyloxy)-6-fluoro-5,8,14-eicosatriene (**33b**)*

To a solution of diisopropylamine (0.13 mL, 0.9 mmol) in tetrahydrofuran (5 mL) cooled to -78 °C was added dropwise *n*-butyllithium (1.5 M in hexane; 0.6 mL, 0.9 mmol). The mixture was warmed to -10 °C and then cooled again to -78 °C. The phosphonium bromide **5b** (650 mg, 0.9 mmol) in tetrahydrofuran (2 mL) was added dropwise and the mixture was stirred for 45 min at -78 °C. Hexamethylphosphoramide (1.7 mL) was added and the reaction mixture was warmed to -25 °C. (Z)-6-Dodecenal[25] (148 mg, 0.81 mmol) in tetrahydrofuran (1 mL) was added dropwise and the mixture was stirred for 30 min at -25 °C and 1 h at 0 °C. A saturated aqueous solution of ammonium chloride (0.5 mL) was added and tetrahydrofuran was evaporated under reduced pressure. The residue was taken up in water and extracted three times with ether. The organic layer was washed twice with water, decanted and dried over sodium sulfate. Filtration and evaporation of the solvent afforded an oil. Flash chromatography on silica gel and elution with a 99:1 mixture of hexane and ethyl acetate afforded triene **33b** (377 mg, 85%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.9 (t, J_{HH} = 6 Hz, 3H), 1.06 (s, 9H), 1.15 to 1.73 (m, 14H), 1.8 to 2.2 (m, 8H), 2.95 (dd, J_{HF} = 21 Hz, J_{HH} = 7.5 Hz, 2H), 3.66 (t, J_{HH} = 6 Hz, 2H), 5 (dt, J_{HF} = 21 Hz, J_{HH} = 7.5 Hz, 1H), 5.23 to 5.66 (m, 4H), 7.3 to 7.85 (m, 10H).

*5-Fluoro-5,8,14-eicosatrienol (**34a**)*

To a solution of silyl ether **33a** (237 mg, 0.43 mmol) in tetrahydrofuran (5 mL) was added tetrabutylammonium fluoride trihydrate (205 mg, 0.65 mmol). The mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure. The residue was dissolved in methylene chloride, washed with water, decanted and dried over sodium sulfate. Filtration and concentration under reduced pressure afforded an oil. Flash chromatography on silica gel and elution with a 15:85 mixture of ethyl acetate and benzene afforded alcohol **34a** as an oil (119 mg, 88%): ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J_{HH} = 6 Hz, 3H), 1.23 to 1.52 (m, 15H), 1.98 to 2.07 (m, 6H), 2.32 (dm, J_{HF} = 21.6 Hz, 2H), 2.72 (t, J_{HH} = 7 Hz, 2H), 3.73 (t, J_{HH} = 6 Hz, 2H), 5.09 (dt, J_{HF} = 21.6 Hz, J_{HH} = 8 Hz, 1H), 5.31 to 5.4 (m, 4H).

*6-Fluoro-5,8,14-eicosatrienol (**34b**)*

To a solution of silyl ether **33b** (379 mg, 0.63 mmol) in tetrahydrofuran (13 mL) was added tetrabutylammonium fluoride trihydrate (326 mg, 0.96 mmol). The mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure. The residue was dissolved in methylene chloride, washed with water, decanted and dried over sodium sulfate. Filtration and concentration under reduced pressure afforded an oil. Flash chromatography on silica gel and elution with a 2:8 mixture of ethyl acetate and hexane afforded alcohol **34b** as an oil (176 mg, 84%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.9 (t, J_{HH} = 6 Hz, 3H), 1.16 to 1.66 (m, 14H), 1.8 (s, 1H, exchangeable with D_2O), 1.9 to 2.36 (m, 14H), 3 (dd, J_{HH} = 6 Hz, J_{HF} = 22.5 Hz, 2H),3.66 (t, J_{HH} = 6 Hz, 2H), 5.05 (dt, J_{HF} = 21 Hz, J_{HH} = 7 Hz, 1H), 5.30 to 5.75 (m, 4H).

*5-Fluoro-5,8,14-eicosatrienoic acid (**3a**)*

To a solution of alcohol **34a** (119 mg, 0.38 mmol) in acetone (3 mL) cooled to 0 °C was added dropwise 2.67 M Jones reagent[27] over 15 min until the orange colour was stable. The mixture was stirred for 15 min at 0 °C. The excess of Jones reagent was consumed with isopropanol. Acetone was evaporated under reduced pressure without heating. The residue was taken up in water and extracted three times with ethyl acetate. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to leave an oil (90 mg). Flash chromatography on silica gel and elution with a 15:85 mixture of ethyl acetate and benzene gave pure acid **3a** as an oil (55 mg, 45%): ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J_{HH} = 6.9 Hz, 3H),1.24 to 1.45 (m, 20H), 1.86 (P, J_{HH} = 7.2 Hz, 2H), 2.01 (m, 6H), 2.16 (dt, J_{HF} = 22 Hz, J_{HH} = 7.3 Hz, 2H), 2.4 (t, J_{HH} = 7.4 Hz, 2H), 2.64 (t, J_{HH} = 7.5 Hz, 2H), 5.06 (dt, J_{HF} = 21.5 Hz, J_{HH} = 8 Hz, 1H), 5.24 to 5.44 (m, 4H); ^{19}F NMR ($CDCl_3$) δ 56.39 (q, J_{FH} = 22 Hz).

*6-Fluoro-5,8,14-eicosatrienoic acid (**3b**)*

To a solution of the alcohol **34b** (21 mg, 0.067 mmol) in acetone (2 mL) cooled to 0 °C was added dropwise 2.67 M Jones[27] reagent until the orange colour was stable. The mixture was stirred for 15 min at 0 °C. The excess of

Jones reagent was consumed with isopropanol. Acetone was evaporated under reduced pressure without heating. The residue was taken up in water and extracted three times with ethyl acetate. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to leave an oil (20 mg). Flash chromatography on silica gel and elution with a 25:75 mixture of ethyl acetate and hexane gave pure acid **3b** (14 mg, 64%): ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J_{HH} = 7 Hz, 3H), 1.21 to 1.45 (m, 10H), 1.51 (p, J_{HH} = 7 Hz, 2H), 1.98 to 2.12 (m, 8H), 2.37 (t, J_{HH} = 7 Hz, 2H), 2.96 (dd, J_{HF} = 22.7 Hz, J_{HH} = 6.8 Hz, 2H), 4.98 (dt, J_{HH} = 21.2 Hz, J_{HH} = 7.9, 1H), 5.3 to 5.5 (m, 4H); ^{19}F NMR ($CDCl_3$) δ 59.49 (dt, J_{FH} = 22 Hz, J_{FH} = 22.4 Hz); MNH_4^+ = 342. TLC: R_f = 0.15 (silica gel, hexane:ethyl acetate, 7:3).

4-(3,3-Dibromo-2-propenyl)-2,2-dimethyl-1,3-dioxolane (36)

A solution of triphenylphosphine (15.8 g, 60.2 mmol) in dry methylene chloride (10 mL) was added dropwise to a solution of carbon tetrabromide (10 g, 30.1 mmol) in dry methylene chloride (10 mL) at 0 °C. After 10 min a solution of aldehyde **35**[30] (2.17 g, 15.05 mmol) in methylene chloride (10 mL) was added and was stirred for an additional 15 min. Then the mixture was cooled to -25 °C and triethylamine (8.39 mL, 60.2 mmol) was added dropwise. The mixture was stirred for 15 min. Then water (15 mL) was continuously added while the reaction temperature was maintained at 0 °C. The mixture was diluted with methylene chloride and washed with water. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was taken up in ether to precipitate triphenylphosphine oxide which was filtered off. The filtrate was concentrated under reduced pressure. Flash chromatography on silica gel and elution with a 9:1 mixture of petroleum ether and ethyl acetate afforded dibromide **36** as a colourless oil (3.87 g, 86%): ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H), 1.44 (s, 3H), 2.35 to 2.39 (m, 2H), 3.59 (dd, J_{HH} = 6.5 Hz, J_{HH} = 8.2 Hz, 1H), 4.05 (dd, J_{HH} = 6 Hz, J_{HH} = 8 Hz, 1H), 4.2 (p, J_{HH} = 6 Hz, J_{HH} = 6.2 Hz, 1H),6.48 (t, J_{HH} = 7.15 Hz, 1H); MH^+ = 301.

2,2-Dimethyl-4-propynyl-1,3-dioxolane (37)

n-Butyllithium (1.6 M in hexane; 21.4 mL, 34.2 mmol) was added to a solution of bromide **36** (5.13 g, 17.1 mmol) in tetrahydrofuran (50 mL) at -78 °C. The reaction was stirred during 1 h at -78 °C, then warmed up to room temperature and stirred for 1.5 h. Saturated aqueous ammonium chloride (2 mL) was added and tetrahydrofuran was evaporated under reduced pressure. The residue was taken up in ether and washed with water. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure. Short path distillation (b.p. = 60 °C, 15 mmHg) afforded pure alkyne **37** (2.35 g, 95%): ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3H), 1.47 (s, 3H), 2 (t, J_{HH} = 2.7 Hz, 1H), 2.47 (AB part of a ABXX', $J_{H_AH_X}$ = 2.7 Hz, $J_{H_BH_X}$ = 2.7 Hz, $J_{H_AH_X}$ = 7.3 Hz, $J_{H_BH_X'}$ = 5.3 Hz, J_{AB} = 16.6 Hz, 2H), 3.77 (dd, J_{HH} = 6.1 Hz, J_{HH} = 8.3 Hz, 1H), 4.11 (dd, J_{HH} = 6 Hz, J_{HH} = 8.3 Hz, 1H), 4.24 (p, J_{HH} = 6 Hz, J_{HH} = 7.2 Hz, 1H).

1-(2,2-Dimethyl-1,3-dioxolane-4-yl)-2,5-undecadiyne (38)

Ethylmagnesium bromide (1.6 M in tetrahydrofuran; 24.5 mL, 39.3 mmol) was added dropwise at room temperature to a solution of alkyne **37** (5 g, 35.7 mmol) in tetrahydrofuran (50 mL). The reaction mixture was stirred during 2 h at room temperature and then cuprous iodide (273 mg, 1.43 mmol) was added. Stirring was continued for 15 min. The solution was cooled to 0 °C and 1-iodo-2-octyne[32] (8.8 g, 37.3 mmol) in tetrahydrofuran was added dropwise. The reaction mixture was stirred overnight at room temperature. Saturated aqueous ammonium sulfate was added and tetrahydrofuran (10 mL) was evaporated under reduced pressure. The residue was dissolved in ether, washed with water, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. Flash chromatography on silica gel and elution with a 9:1 mixture of petroleum ether and ethyl acetate afforded diyne **38** as an oil (8.08 g, 91%) which was slightly unstable (needed to be stored at -25 °C): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, J_{HH} = 6 Hz, 3H), 1.35 (s, 3H), 1.41 (s, 3H), 1.06 to 1.6 (m, 6H), 1.9 to 2.23 (m, 2H), 2.33 to 2.56 (m, 2H), 3.08 (p, J_{HH} = 2.21 Hz, 2H), 3.6 to 4.33 (m, 3H).

(Z,Z)-1-(2.2-Dimethyl-1,3-dioxolane-4-yl)-2,5-undecadiene (39)

Cyclohexene (17.16 mL, 169.4 mmol; freshly distilled on calcium hydride) was added dropwise at 0 °C to a 10 M solution of borane in dimethyl sulfide (8.47 mL, 84.7 mmol) diluted with tetrahydrofuran (150 mL). The mixture was warmed to room temperature and stirred during 2 h. This mixture was cooled to 0 °C and the diyne **38** (8.08 g, 32.6 mmol) in tetrahydrofuran (10 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred during 2 h. Acetic acid (52.2 mL, 912 mmol) was added dropwise and the mixture was stirred overnight. Finally at 0 °C 5N aqueous sodium hyroxide (197 mL) was added followed by 30% aqueous hydrogen peroxide (50 mL). The mixture was poured into ice cold water (200 mL) and extracted with hexane. The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. Flash chromatography on silica gel and elution with a 9:1 mixture of petroleum ether and ethyl acetate afforded pure diene **39** as a colourless oil (5.87 g, 71%): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, J_{HH} = 6.5 Hz, 3H), 1.36 (s, 3H), 1.4 to 1.48 (m, 6H), 1.44 (s, 3H), 2.55 (q, J_{HH} = 6.9 Hz, 2H), 2.30 (q, J_{HH} = 6.8, J_{HH} = 7.1 Hz, J_{HH} = 7.3 Hz, 1H), 2.45 (q, J_{HH} = 6.4 Hz, J_{HH} = 7.1 Hz, J_{HH} = 7.2 Hz, 1H), 2.79 (t, J_{HH} = 7.1 Hz, 2H), 3.56 (dd, J_{HH} = 7.1 Hz, J_{HH} = 8.1 Hz, 1H), 4.02 (dd, J_{HH} = 6 Hz, J_{HH} = 7.9 Hz, 1H), 4.13 (qp, J_{HH} = 6.1 Hz, J_{HH} = 6.2 Hz, J_{HH} = 6.7 Hz, J_{HH} = 6.7 Hz, 1H), 5.28 to 5.52 (m, J_{HH} = 11 Hz,4H).

(Z,Z)-1,2-Diol-4,7-tridecadiene (40)

A mixture of dioxolane **39** (9.88 g, 39.1 mmol) and 6N sulfuric acid (5 mL) in methanol (150 mL) was stirred at

room temperature during 6 h. The mixture is neutralized with saturated aqueous sodium bicarbonate. Methanol was evaporated under reduced pressure. The residue was extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered and evaporated under reduced pressure. Finally flash chromatography on silica gel and elution with a 1:1 mixture of petroleum ether and hexane afforded pure diol **40** as a colourless oil (6.48 g, 78%): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, J_{HH} = 6.5 Hz, 3H),1.23 to 1.4 (m, 6H), 2.08 (q, J_{HH} = 7 Hz, 2H), 2.1 (s, 2H, exchangeable with D_2O), 2.23 to 2.38 (m, 2H), 2.8 (t, J_{HH} = 7 Hz, 2H), 2.5 (dd, J_{HH} = 6.5 Hz, J_{HH} = 10 Hz, 1H), 3.29 (dd, J_{HH} = 2.5 Hz, J_{HH} = 10.5 Hz, 1H), 3.71 to 3.82 (m, 1H), 5.28 to 5.37 (m, 1H), 5.37 to 5.59 (m, 2H), 5.54 to 5.63 (m, 1H); coupling constants of 10.8 Hz and 10.7 Hz for the olefinic proton on the decoupled spectrum of the dibenzoate of **40** are in agreement with *Z* double bonds.

(Z,Z)-3,6-Dodecadienal (41)

Diol **40** (5.45 g, 25.7 mmol) in an 8:4:13 mixture tetrahydrofuran, acetone and water (230 mL) was stirred at 0 °C during 15 min with a suspension of sodium metaperiodate (19.24 g, 90 mmol). The solution was filtered and the filtrate successively washed with water and brine. Kugelrohr distillation (100 °C, 0.05 mbar) afforded aldehyde **41** (3.3 g, 67%): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, J_{HH} = 6.5 Hz, 3H), 1.3 to 1.48 (m, 6H), 2.04 (q, J_{HH} = 6.5 Hz, 2H), 2.8 (t, J_{HH} = 6 Hz, 2H), 3.22 (dd, J_{HH} = 2 Hz, J_{HH} = 6 Hz, 2H), 5.16 to 5.82 (m, 4H), 9.68 (t, J_{HH} = 2 Hz, 1H); MH^+ = 181; IR νC=O = 1725 cm^{-1}.

1-(t-Butyldiphenylsilyloxy)-5-fluoro-5,8,11,14-eicosatetraene (42a)

To a solution of phosphonium **5a** (1.1 g, 1.51 mmol) in tetrahydrofuran (10 mL) cooled to -78 °C was added dropwise *n*-butyllithium (1.5 M in hexane; 1.01 mL, 1.51 mmol). The reaction mixture was stirred for 5 min at -78 °C and 10 min at -18 °C. The mixture was cooled to -78 °C and hexamethylphosphoramide (1.1 mL) was added. The reaction mixture was stirred for 20 min. Aldehyde **41** (500 mg, 1.66 mmol) in tetrahydrofuran (2 mL) was added dropwise. The mixture was successively stirred for 30 min at -78 °C, 1 h at 0 °C and 30 min at room temperature. A saturated aqueous solution of ammonium chloride (0.5 mL) was added and the mixture was extracted three times with diethyl ether. The organic layer was dried over sodium sulfate concentrated under reduced pressure to afford an oil. Flash chromatography on silica gel and elution with a 5:95 mixture of methylene chloride and hexane afforded silyl ether **42a** as an oil (528 mg, 64%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.88 (t, J_{HH} = 6 Hz, 3H), 1.06 (s, 9H), 1.16 to 1.48 (m, 6H),1.48 to 1.78 (m, 4H), 1.93 to 2.5 (m, 4H), 2.6 to 2.88 (m, 6H), 3.8 (t, J_{HH} = 6 Hz, 2H), 5.03 (dt, J_{HF} = 21.5 Hz, J_{HH} = 8 Hz, 1H), 5.26 to 5.6 (m, 7H), 7.36 to 7.9 (m, 10H);^{19}F NMR (84.67 MHz, $CDCl_3$) δ 56.3 (q, J_{FH} = 21.4 Hz).

1-(t-Butyldiphenylsilyloxy)-6-fluoro-5,8,11,14-eicosatetraene (42b)

To a solution of phosphonium **5b** (11.58 g, 15.967 mmol) in tetrahydrofuran (250 mL) cooled at -78 °C was added dropwise *n*-butyllithium (1.66 M in hexane; 9.62 mL, 15.97 mmol). The reaction mixture was stirred during 10 min at -78 °C and then warmed to -10 °C. The mixture was cooled again to -78 °C and hexamethylphosphoramide (43 mL) was added and the resulting mixture was stirred for 1 h. Freshly prepared aldehyde **41** (3.2 g, 17.7 mmol) in tetrahydrofuran (50 mL) was added dropwise. The mixture was stirred for 1 h at -78 °C, warmed over 3 h to 0 °C and finally stirred for 1 h at 0 °C. Saturated aqueous ammonium chloride was added and the mixture was concentrated under reduced pressure. The residue was taken up in water, extracted once with ether and twice with ethyl acetate. The organic phase was washed twice with water, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. Flash chromatography on silica gel and elution with an 8:2 mixture of petroleum ether and toluene afforded silyl ether **42b** as a colourless oil (5.57 g, 64%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J_{HH} = 6.8 Hz, 3H), 1.04 (s, 9H), 1.23 to 1.61 (m, 10H), 1.94 (q, J_{HH} = 7.4 Hz, 2H), 2.05 (q, J_{HH} = 7 Hz, 2H) 2.81 (t, J_{HH} = 6.3 Hz, 2H), 2.85 (t, J_{HH} = 6.3 Hz, 2H), 2.97 (dd, J_{HF} = 22.7 Hz, J_{HH} = 6.6 Hz, 2H), 3.65 (t, J_{HH} = 6.3 Hz, 2H), 2.97 (dd, J_{HF} = 22.7 Hz, J_{HH} = 6.6 Hz, 2H), 3.65 (t, J_{HH} = 6.3 Hz, 2H), 4.99 (dt, J_{HF} = 21.6 Hz, J_{HH} = 7.9 Hz, 1H), 5.29 to 5.54 (m, 6H), 7.43 to 7.68 (m, 10H); ^{19}F NMR ($CDCl_3$) δ 57.88 (bq, J_{FH} = 22.4 Hz); MNH_4^+ = 564.

5-Fluoro-5,8,11,14-eicosotetraenol (43a)

Silyl ether **42a** (528 mg, 0.96 mmol) was dissolved in tetrahydrofuran (20 mL) and tetrabutylammonium fluoride trihydrate (460 mg, 1.44 nmol) was added. The mixture was stirred during 1 h at room temperature. The reaction was diluted with methylene chloride and washed twice with water. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. Flash chromatography on silica gel and elution with a 15:85 mixture of ethyl acetate and hexane afforded alcohol **43a** as an oil (248 mg, 84%): ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J_{HH} = 6.6 Hz, 3H), 1.22 to 1.40 (m, 6H), 1.55 (bs, 1H), 1.58 to 1.67 (m, 4H), 2.05 (q, J_{HH} = 7 Hz, 2H), 2.28 (dt, J_{HF} = 23 Hz, J_{HH} = 6.5 Hz, 2H), 2.80 (t, J_{HH} = 6.3 Hz, 2H), 2.82 (t, J_{HH} = 6.2 Hz, 2H), 3.66 (t, J_{HH} = 6 Hz, 2H), 5.02 (dt, J_{HF} = 21.5 Hz, J_{HH} = 8 Hz, 1H), 5.28 to 5.44 (m, 6H); ^{19}F NMR ($CDCl_3$) δ 57.88 (q, J_{FH} = 22.3 Hz); MNH_4^+ = 326.

6-Fluoro-5,8,11,14-eicosotetraenol (43b)

Silyl ether **42b** (5.82 g, 10.65 mmol) was dissolved in tetrahydrofuran (60 mL) and tetrabutylammonium fluoride trihydrate (5 g, 10 mmol) was added. The mixture was stirred during 1.5 h at room temperature. The reaction

mixture was concentrated under reduced pressure, diluted with ethyl acetate and washed with water. The organic layer dried over sodium sulfate, filtered and concentrated under reduced pressure to afford an oil. Flash chromatography on silica gel and elution with a 25:75 mixture of ethyl acetate and cyclohexane afforded alcohol **43b** as an oil (3.04 g, 93%) ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J_{HH} = 6.8 Hz, 3H), 1.25 to 1.63 (m, 11H), 1.96 to 2.08 (m, 4H), 2.81 (t, J_{HH} = 6.4 Hz, 2H), 2.86 (t, J_{HH} = 6.4 Hz, 2H), 3.00 (dd, J_{HH} = 6.6 Hz, J_{HF} = 22.8 Hz, 2H), 3.64 (t, J_{HH} = 6.5 Hz, 2H), 5.02 (dt, J_{HF} = 21.5 Hz, J_{HH} = 8 Hz, 1H), 5.29 to 5.54 (m, 6H); ^{19}F NMR ($CDCl_3$) δ 58.2 (dt, J_{FH} = 22 Hz, J_{FH} = 22.5 Hz); MNH_4^+ = 326. Anal. calcd for $C_{20}H_{33}FO$: C, 77.87; H,10.78. Found: C, 77.38; H,10.97.

*5-Fluoro-5,8,11,14-eicosatetraenoic acid (**4a**)*

Alcohol **43a** (248 mg, 0.8 mmol) was dissolved in acetone (20 mL) and cooled to 0 °C Jones reagent[27] 2.67 M (0.6 mL) was added until the orange colour was stable. The mixture was stirred for 1 h at 0 °C and isopropanol was added to consume the excess of Jones reagent. The reaction mixture was concentrated under reduced pressure, diluted with water and extracted four times with ethyl acetate. The organic phase was dried over sodium sulfate, filtered and concentrated under reduced pressure. Flash chromatography on silica gel and elution with a 20:80 mixture of ethyl acetate and hexane afforded 5-fluoroarachidonic acid **4a**[10] as an oil (160 mg, 62%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J_{HH} = 6.9 Hz, 3H), 1.39 to 1.48 (m, 6H), 1.86 (p, J_{HH} = 7.3 Hz, 2H), 2.05 (q, J_{HH} = 7 Hz, 2H), 2.33 (dt, J_{HF} = 22.6 Hz, J_{HH} = 7.2 Hz, 2H) 2.41 (t, J_{HH} = 7.4 Hz, 2H), 2.69 (t, J_{HH} = 7.3 Hz, 2H), 2.81 (q, J_{HH} = 6 Hz, 4H) 5.06 (dt, J_{HF} = 21.4 Hz, J_{HH} = 8.1 Hz, 1H), 5.27 to 5.44 (m, 7H); ^{19}F NMR ($CDCl_3$) δ 56.7 (dt, J_{FH} = 22.5 Hz, J_{FH} = 21.8 Hz).

*6-Fluoro-5,8,11,14-eicosatetraenoic acid (**4b**)*

Alcohol **43b** (2.8 g, 9.09 mmol) was dissolved in acetone (40 mL) and cooled to 0°C. 2.67 M Jones reagent[27] (6 mL) was added until the orange colour was stable. The mixture was stirred for 1.25 h at 0 °C and isopropanol was added to consume the excess of Jones reagent. The mixture was concentrated under reduced pressure, diluted with water and extracted four times with ethyl acetate. The organic phase was dried over sodium sulfate, filtered and concentrated under reduced pressure. Flash chromatography on silica gel and elution with a 30:70 mixture of ethyl acetate and hexane afforded 6-fluoroarachidonic acid **4b** (2.11 g, 72%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J_{HH} = 6.8 Hz, 3H), 1.26 to 1.38 (m, 6H), 1.71 (p, J_{HH} = 7.3 Hz, 2H), 2 to 2.08 (m, 4H), 2.36 (t, J_{HH} = 7.4 Hz, 2H), 2.81 (t, J_{HH} = 6.4 Hz, 2H), 2.85 (t, J_{HH} = 6.6 Hz, 2H), 3.00 (dd, J_{HH} = 6.7 Hz, J_{HF} = 22.7 Hz, 2H), 5.00 (dt, J_{HF} = 21.1 Hz, J_{HH} = 8 Hz, 1H), 5.26 to 5.55 (m, 6H), 7.28 (s, 1H); ^{19}F NMR ($CDCl_3$) δ 59.52 (q, J_{FH} = 22.4 Hz); MNH_4^+ = 340. Anal. calcd for $C_{20}H_{31}FO_2$: C, 74.50; H, 9.69. Found: C, 74.04; H 9.76.

Biochemistry

Materials. Media for purification and culture of macrophages were obtained from Gibco BRL (France). [1-^{14}C]-arachidonic acid and LTC_4 radioimmunoassay kit were bought from Dupont-NEN, France. LTC_4 was from Paesel and Lorei (Germany). Fatty acid free BSA (Ref. A7511), Zymosan A, NADH, pyruvate, lactate dehydrogenase and lipids used as references in TLC were obtained from Sigma (St Louis, MO, U.S.A.).

Macrophage cultures. The method used was derived from the work of Lokesh *et al.*[34] Primary cultures of peritoneal macrophages were established from resident cells of male Albinos mice weighing 30–35 g. Mice were killed by cervical dislocation, the skin was cut and pulled apart, muscles were washed with ethanol and 4 mL of Ca^{++}- and Mg^{++}-free phosphate-buffered saline containing 10 I.U. heparin/mL were injected intraperitoneally. The abdomen was massaged, cut on a small length and the fluid pipetted under sterile conditions. The fluid collected from 35–45 mice was pooled and centrifuged. The cells were washed once with Dulbecco's modified Eagle medium containing 1 mM sodium pyruvate, 1g/L glucose and 50 I.U.-μg penicillin–streptomycin/mL (medium A) to which was added 10% foetal calf serum (FCS). Then, cells were resuspended in the same medium at a concentration of 2.5 x 10^6 cells/mL.

5 x 10^6 Macrophages were plated per 35 mm diameter Petri dish and cultured (37 °C, 5% CO_2, H_2O sat.) for 2 h to allow adherence of macrophages to the plastic.

Incorporation of fatty acids into macrophage lipids. Fatty acid–bovine serum albumin (BSA) complexes were prepared as described by Lokesh and Wrann[36] except that incubation at 50 °C was omitted. Stock solutions of the fatty acids (82 μM) complexed with BSA (2.5 mg/mL) in medium A were diluted under sterile conditions to the desired concentrations with a solution of BSA (2.5 mg/mL) in medium A. The macrophage layer was washed twice with 2 mL of medium A. 2 mL of the diluted fatty acid solution was added and the cells were cultured for 17 h. The next day, the cell population consisted of a dense monolayer of macrophages. At this stage, the culture medium was collected for measurement of lactate dehydrogenase (LDH) activity or discarded. In some experiments, the cell layer was frozen–thawed three times, sonicated (probe with mini tip) for 30 s at 0 °C in 0.5 mL of 0.1 M potassium phosphate buffer (pH = 7.0), the homogenate was centrifuged at 10,000 *g* for 3 min (Eppendorf centrifuge) and the supernatant collected for measurement of cell LDH activity. In other experiments, the cell layer was washed twice with 2 mL medium A and immediately used for studies of LTC_4 release.

Stimulation of macrophages with zymosan, extraction and analysis of leukotriene C_4. To the cells which had been cultured without or with the fatty acids complexed to BSA, 0.9 mL of medium A was added. Cells were preincubated

for 10 min at 37 °C and LTC_4 synthesis was initiated by addition of 0.1 mL of a suspension of zymosan in medium A (1.5 mg/mL) prepared as described by Bonney *et al.*[38] After an 80 min incubation under culture conditions, the supernatants were transferred into ice-cold polypropylene tubes containing four times the volume of ethanol. The ethanol samples were kept frozen at this stage (-80 °C). Then, they were thawed, centrifuged for 15 min at 8,000*g* and the supernatants collected. The supernatants were made up to a volume of 30 mL with water and their pHs brought to 3.2 with 1 M citric acid. These were passed onto C_{18} Sep Pak cartridges (Millipore–Waters) which had been conditioned with 20 mL ethanol and 20 mL water. The cartridges were then washed with 20 mL water. Air was forced through the cartridges to expel the remaining water and LTC_4 was then eluted with 5 mL methanol. The methanol extracts were evaporated at room temperature under a stream of nitrogen. The dry residues were reconstituted in the buffer used for the radioimmunoassay. Polypropylene tubes were used throughout extraction. Recovery of authentic LTC_4 as determined by HPLC analysis[39] and U.V. detection was 91 ± 3% (mean ± SD; n = 2). The samples were either frozen under argon or used directly for titration of their LTC_4 content with the Dupont–NEN radio-immunoassay kit. According to the experiments, a 50 to 200-fold dilution of the sample was necessary. Controls (without zymosan) were diluted similarly.

Incorporation of labelled arachidonic acid and analysis of radioactivity in the different lipid classes. After incubating macrophages for 17 h with 2 mL of a solution of [^{14}C]-arachidonic acid (20 μM; 12.2μCi/μmol)–BSA complex in medium A, the cells were washed twice with 2 mL medium A, frozen–thawed three times and sonicated in 0.5 mL water as described above. The lipids were extracted according to the Bligh and Dyer's method[40] except that 25 μL glacial acetic acid were added (total volume of mixture = 3.75 mL) and that a second extraction with 1.25 mL chloroform was performed. The chloroform phases were pooled, evaporated under nitrogen and the dry residue dissolved in 80 μL of a mixture of chloroform:methanol (1:1). Aliquots were counted in 10 mL of Fluorolloy (composed of 6 g of 2,5-diphenyl-oxazole, 0.2 g of 1,4-bis-(4-methyl-5-phenyl-oxazol-2-yl) benzene in 1 L of toluene:methanol (4:1) for determination of arachidonic acid incorporation into total lipids. Neutral lipids and phospholipids were separated by TLC on silica gel 60 (0.25mm; Merck) using a solvent system of chloroform:methanol:water:acetic acid (140:60:10:5, by vol.). Radioactive peaks were detected with a Berthold Scanner. The silica gel containing the lipids was scraped and transferred into scintillation vials containing 10 mL Fluorolloy and the radioactivity determined. Individual phospholipid and neutral lipid classes were identified by the R_f values of known standards run simultaneously and visualized after exposing the plates to iodine vapour.

Effect of AA, 5-F-AA and 6-F-AA on the incorporation of labelled arachidonic acid into macrophage lipids. Macrophages were incubated for 17 h with 2 mL of a solution containing [^{14}C] arachidonic acid (20 μM; 12.2 μCi/μmol)–BSA complex in the absence or in the presence of AA–BSA or 5-F-AA–BSA or 6-F-AA–BSA complexes at various concentrations. Washing, disruption of cells as well as extraction and separation of cell lipids was conducted as described above.

Measure of lactate dehydrogenase activity. The rate of reaction was monitored by the continuous measurement of NADH consumption with a DU-7 Beckman spectrophotometer at 340 nm. The assay contained 2800 μL of 0.1 M potassium phosphate buffer (pH = 7.0), 100μL of 23 mM sodium pyruvate in water and 50 μL of 12 mM NADH in water. The mixture was preincubated for 2 min at 30 °C. The reaction was started by addition of 50 μL of the enzyme solution to be tested. Using a commercial sample of LDH, it was checked that the rate of reaction was proportional to the enzyme concentration under assay conditions.

Miscellaneous methods. To eliminate traces of hydroperoxides, AA, 5-F-AA and 6-F-AA were purified by silicic acid chromatography (Silicar CC4, Mallinckrodt, KY, U.S.A.). Batches (10 mg each) of the fatty acids loaded on 4 g columns were eluted with hexane:diethyl ether (9:1, v:v). Stock solutions (50 mM) in ethanol were then stored at -80 °C under argon atmosphere and titrated by gas chromatography as described previously.[9]

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