in Figure 8. Anal. Calcd for $C_{19}H_{17}ClN_3O$ [$C_{18}H_{16}ClN_3O_{0.86}$·$(C_7H_8O)_{0.14}$]: C, 67.35; H, 5.02; Cl, 10.49; N, 12.40; O, 4.72. Found: C, 67.78; H, 4.97; Cl, 10.62; N, 12.24; O (by diff), 4.39. Indicating that the sample was slightly contaminated with *m*-cresol and water.

Degradation Studies. A thick film of PANI deposited on ITO glass was cycled in 1 M HCl from −0.3 to +1.0 V/SCE at 10 mV/s for 6 h. The film was black and seemed to be partially dissolved on the edges of the glass electrode. The electrode was dried under vacuum, but the black material was oily, and no IR spectrum was recorded. Moreover, other control experiments under these conditions showed that the ITO layer was dissolved (this could explain the absence of current at the end of the experiment).

Acknowledgment. We are indebted to the Office of Naval Research and the Naval Research Laboratory for support. ESR and magnetic susceptibility studies were supported by NSF DMR85-21392. We thank Dr. Hugh Webb for mass spectrometry and Carol Koch and Jerry Wuenschell for their help with the SQUID susceptometer.

Total Synthesis of 7,7-, 10,10-, and 13,13-Difluoroarachidonic Acids

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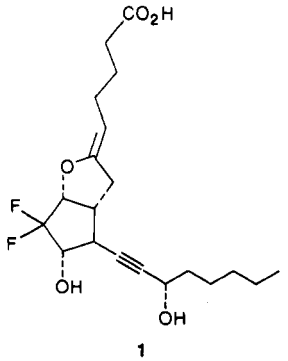
Abstract: General methodology is described for the synthesis of polyunsaturated fatty acids, in which one of the methylene groups between cis double bonds is replaced by a CF_2 group. This is exemplified by the preparation of 7,7-, 10,10-, and 13,13-difluoroarachidonic acids **22**, **18a**, and **30**, respectively. Crucial to the synthesis is the preparation of the fluorodiacetylenic system **7** by a chain reaction involving an acetylenic anion containing the substituent X and CF_2ClBr to form the bromides **4**, followed by reaction of the corresponding iodides with a second acetylenic anion bearing Y. After reduction of **7** to the diallylic system **3** the substituents X and Y are employed in the construction of the tetraunsaturated arachidonate system. In the 10,10-case two separate chain extensions are performed by sequentially converting X and Y to Br and condensing the allylic bromides with the cuprous acetylides **10** and **12a**. In the 7,7- and 13,13-cases X possesses the required carbon skeleton, and Y is provided by coupling with the cuprates of 1,4-decadiyne and methyl nona-5,8-diynoate, respectively. The synthesis is completed by semihydrogenation and enzymatic hydrolysis.

1. Introduction

During the last few years this laboratory has been engaged in the synthesis of fluorinated derivatives of the unstable prostacyclin (PGI_2) and thromboxane A_2 (TXA_2), in which fluorine is substituted for hydrogen in strategic positions of the molecule so as to destabilize, by virtue of the powerful inductive effect of fluorine, the transition states for hydrolysis. At the same time because of the similarity in the van der Waals radii between hydrogen (1.20 Å) and fluorine (1.35 Å) biological activity should be preserved. Indeed, 10,10-difluoro-13-dehydroprostacylin (**1**) was hydrolyzed



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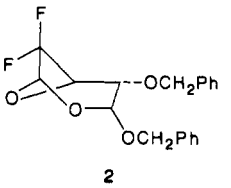
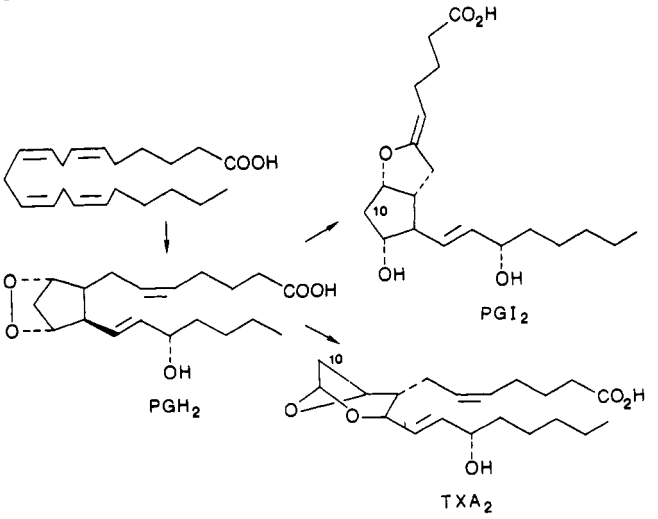


2

at 1/100 the rate and possessed biological activity of the same order as that of the natural product.[1,2] More dramatically, compound **2** containing the ring system of 10,10-difluoro TXA_2 was shown to undergo hydrolysis at 10^{-8} times the rate of TXA_2 itself.[3]

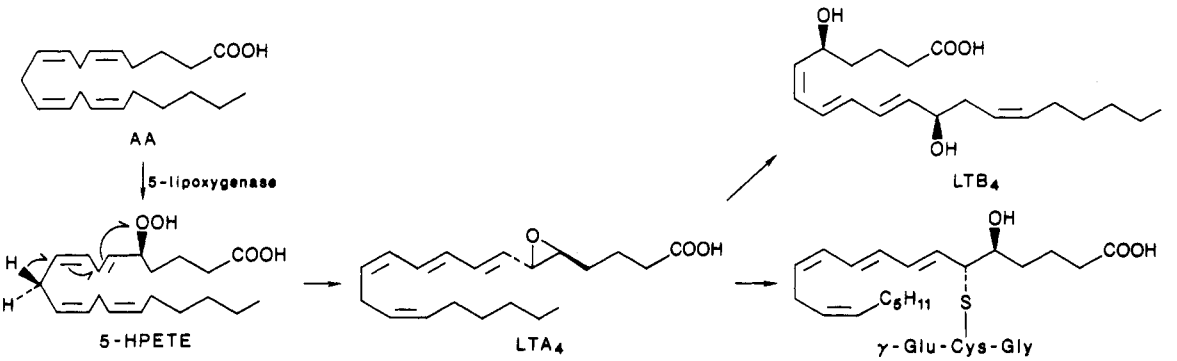
It occurred to us that if the enzymatic processes leading to PGI_2 and TXA_2 could be made to operate on the appropriately substituted fluorinated substrates,[4] the corresponding fluoro derivatives could be obtained rapidly by biosynthetic means. The precursor in the biosynthesis of all the prostaglandins and thromboxanes is the tetraunsaturated acid arachidonic acid which is converted to PGH_2 by PGH synthase.[5] PGH_2 is in turn rearranged to PGI_2 and TXA_2 by prostacyclin and thromboxane synthase, respectively.[6] For fluorine to be substituted at C-10 of PGI_2 or TXA_2 requires 10,10-difluoroarachidonic acid (10,10-DFAA) as a precursor.



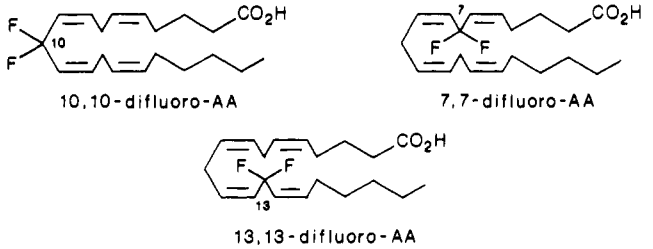
(1) Fried, J.; Mitra, D. K.; Nagarajan, M.; Mehrotra, M. M. *J. Med. Chem.* **1980**, *23*, 234.
(2) Hatano, Y.; Kohli, J. D.; Goldberg, L. I.; Fried, J.; Mehrotra, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6846.
(3) Fried, J.; Hallinan, E. A.; Szwedo, M. J., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 3871.
(4) For a comprehensive general review of the subject, see: Walsh, C. In *Advances in Enzymology*; Meister, A. Ed.; Interscience: 1983; Vol. 55.
(5) Hamberg, M.; Samuelsson, B. *J. Biol. Chem.* **1967**, *242*, 5344.
(6) Yamamoto, S. In *New Comprehensive Biochemistry*; Pace-Asciak, C., Granstrom, E., Eds.; Elsevier: Amsterdam, 1983; Vol. 5.

0002-7863/87/1509-3684$01.50/0

Scheme I



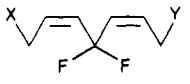
Fluorine-containing diallylic systems of this type had not been prepared before but, once available, would provide access to the corresponding 7,7- and 13,13-substituted derivatives as well. Thus, the 7,7-difluoro acid could serve as the precursor for 7,7-di-



fluoroprostacyclin, which would be expected to be highly stable to hydrolysis of its enol ether functionality. In addition, one might envision that such difluoro acids might serve as specific inhibitors of certain enzymes of the arachidonate cascade. Thus 13,13-DFAA could be a competitive inhibitor of PGH synthase by blocking the required rate-determining abstraction of the 13-*pro-S* hydrogen. Furthermore, the 7,7- and 10,10-difluoroacids might block the pathway to the leukotrienes, (Scheme I) which requires abstraction of a 7- and a 10-*pro-R* hydrogen.

2. Synthesis

Prospects appeared sufficiently intriguing to develop a general synthesis of such acids. Herein we describe the synthesis of the three arachidonic acids in which the 7-, 10-, and 13-methylenes are replaced by CF_2 groups. The classical procedure for the synthesis of arachidonic acid and other "skipped" cis polyenic acids was devised by Osbond et al.[7], which has as its primary target the corresponding tetraynoic acid, to be reduced in the final step with Lindlar catalyst. The tetraynoic acid is built up in successive condensation steps of acetylenic Grignard reagents with propargylic bromides. As it turned out, this latter methodology was not applicable in our case. What was required first was a synthesis of the as yet unknown difluorodiallylic system **3** possessing different substituents at the two termini.



3

By utilizing a literature procedure for the addition of a CF_2Br moiety to a variety of anions[8,9] including acetylenic anions[11] we were able to prepare the bromodifluoroacetylene **4**, which, surprisingly, was readily converted to the corresponding iodide **6** with sodium iodide in refluxing acetone.

$$\mathrm{THPOCH_2C{\equiv}CLi} \xrightarrow[\text{THF, } -80\ ^\circ\mathrm{C}]{CF_2ClBr} \underset{\mathbf{4}\ (52\%)}{\mathrm{THPOCH_2C{\equiv}CCF_2Br}} + \underset{\mathbf{5}\ (25\%)}{\mathrm{THPOCH_2C{\equiv}CBr}}$$

(7) Osbond, J. M.; Philpott, P. G.; Wickens, J. C. *J. Chem. Soc.* **1961**, 2779.
(8) Bey, P.; Vevert, J. P. *Tetrahedron Lett.* **1978**, 1215.
(9) Rico, I.; Cantacuzene, D.; Wakselman, C. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1063 and earlier papers.

Table I. Reaction of Alkynyllithium Reagents with CF_2BrCl

$$RLi + CF_2BrCl \rightarrow RCF_2Br$$

R	product	yield (%)
THPO	THPO, F, F, Br — **4**	52
THPO	THPO, F, F, Br — **4a**	28
	Br, F, F — **4b**	32
	F, F, Br — **4c**	46
$TBMe_2SO$	$TBMe_2SO$, F, F, Br — **4d**	>40

An ionic chain mechanism has been proposed for this reaction as follows[9]

Initiation $\quad Nu^- + BrCF_2Cl \rightarrow NuBr + CF_2Cl^-$

$$CF_2Cl^- \rightarrow :CF_2 + Cl^-$$

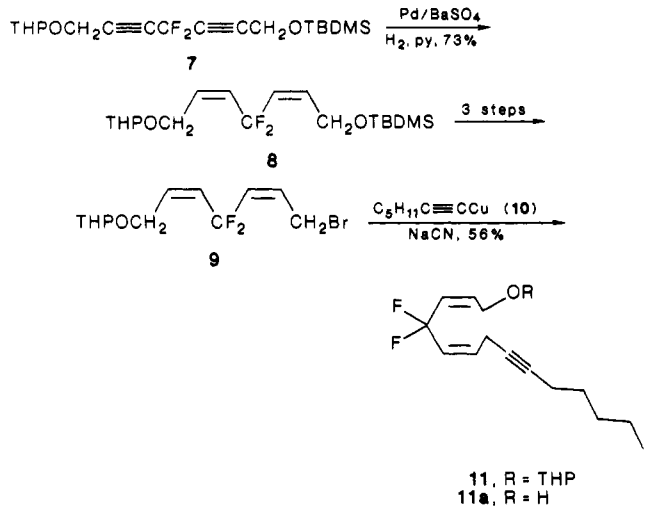
Propagation $\quad Nu^- + :CF_2 \rightarrow NuCF_2^-$

$$NuCF_2^- + BrCF_2Cl \rightarrow NuCF_2Br + CF_2Cl^-$$

This appears to be true in our case as well as demonstrated by the isolation of the bromoacetylene **5** in addition to the desired difluoro bromide **4**. This reaction has been successfully carried out with a variety of acetylenes (Table I). The iododifluoroacetylene **6**, but not the corresponding bromide **4**, reacted with a second acetylenic anion to form the unsymmetrically substituted difluorodiacetylene **7**. Again this reaction was found to be of general applicability (Table II).

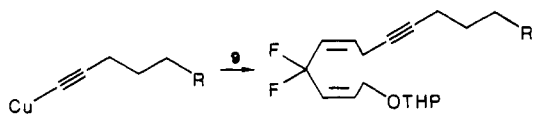
$$\underset{\mathbf{6}}{\mathrm{THPOCH_2C{\equiv}CCF_2I}} + \mathrm{LiC{\equiv}CCH_2OTBDMS} \rightarrow \underset{\mathbf{7}}{\mathrm{THPOCH_2C{\equiv}CCF_2C{\equiv}CCH_2OTBDMS}}$$

The reduction of the difluorodiyne **7** to the desired cis diene **8** required Pd–$BaSO_4$ in pyridine; Lindlar catalyst was ineffective in this case. The cis geometry of the vinyl protons was evident from the PMR spectrum of **8**, which showed four distinct vinyl signals at δ 5.92, 5.84, 5.65, and 5.57. Decoupling experiments indicated that the two high field signals were those of the vinyl protons next to the difluoromethylene moiety and that the H–H coupling constant was 12.1 Hz. Removal of the silyl protecting group with HF–Bu_4NF, followed by mesylation and substitution of the mesylate by bromide, yielded the allylic bromide **9**. The stage was now set for the addition of the remaining two double



bonds with their respective appendages. This turned out to be more difficult than expected. Only with the aid of copper chemistry could the required condensation reactions be accomplished. Thus, when the allylic bromide **9** was stirred with heptynyl cuprate **10**[10] and sodium cyanide in HMPA[11] at 25 °C the desired coupling product **11** was formed in 20% yield. A careful study of the reaction conditions raised the yield to 56%. This required the use of DMF as a solvent and a ratio of bromide/cuprate/NaCN of 1:1.5:1.5. A high concentration of the reactants (1 M in cuprate) was likewise important. Contrary to the experience of Normant[11] the corresponding diacetylenic bromide could not be used in this reaction. The PMR spectrum of the dienyne **11** showed that the molecule contained four cis vinyl protons (J_{HH} = 11.5 and 12.0 Hz) and a methylene group situated between a double bond and a triple bond (δ 3.14). The connectivity of the carbons was established by PMR decoupling experiments. The ^{19}F NMR spectrum showed a triplet at ϕ 84.04 (J_{HF} = 11.5 Hz) indicating that the CF_2 group was flanked by two double bonds.

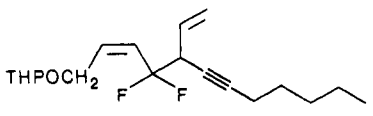
The coupling reaction between the allylic bromide **9** and alkynylcuprates was found to be broadly applicable. The cuprates of the 5-hexynoic acid derivatives **12a–c** gave the desired products **13a–c** in 50–60% yield.



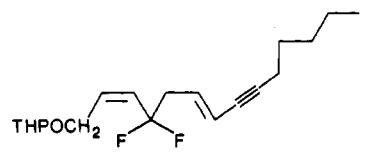
12 13

a, R = CO_2CH_3; b, R = CO_2-t-Bu; c, R = 

Two isomeric byproducts were observed in these reactions. For example, in the reaction with heptynyl cuprate **10** the branched S_N' product **14** and the conjugated enyne **15** were formed. The



14



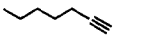
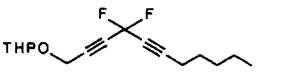
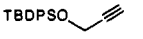
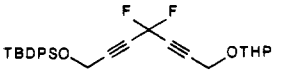
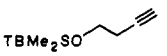
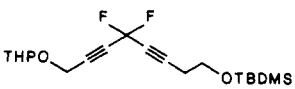
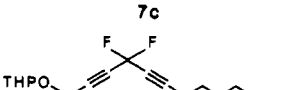
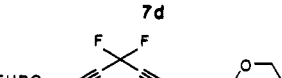
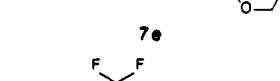
15

structural assignment for the branched dienyne **14** was based on

(10) Castro, C. E.; Ganghan, E. J.; Owsley, D. C. *J. Org. Chem.* **1966**, *31*, 4071.

(11) Normant, J. F.; Bourgain, M.; Rone, A.-M. *Compt. Rend. Ser. C* **1974**, *270*, 354.

Table II. Reaction of **6** with Alkynyllithium Reagents

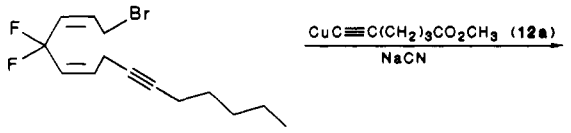
R	product	yield (%)
	7	56
	 **7a**	56
	 **7b**	59
	 **7c**	57
	 **7d**	17
	 **7e**	33
	 **7f**	36

the PMR spectrum of the allylic bromide derived from **14**. This spectrum showed well-resolved signals ranging from δ 5.3–6.1 indicating that the molecule contained five vinyl protons. Three of the vinyl protons showed the characteristic pattern for a monosubstituted vinyl group with cis and trans H–H couplings of 12 and 18 Hz, respectively. Full spectral data are presented for the analogous compounds **23**, **24**, and **25**. A low field multiplet at δ 3.68 was assigned to the methine proton attached to the tertiary carbon adjacent to a triple bond, a double bond, and a difluoromethylene group. The ^{19}F NMR spectrum exhibited an AB quartet of triplets at ϕ 93.3 and 96.4 (J_{FF} = 245 Hz, J_{HF} = 13 Hz), upfield from that of the dienyne **11** (ϕ 84), indicating that the CF_2 grouping was in a chiral environment next to one double bond and that each of the fluorine atoms was coupled to two protons. Only the branched structure **14** is consistent with all the above observations. This branched dienyne could be separated from the desired product by chromatography.

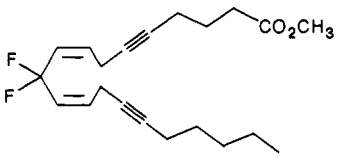
The minor byproduct was not entirely separable from the desired product even on RP-HPLC. It was identified as the conjugated dienyne **15**. The ^{19}F NMR spectrum of the alcohol derived from **15** by hydrolysis showed a quartet at ϕ 90 (J_{HF} = 15.0 Hz), indicating that there were three protons vicinal to the CF_2 group and that the CF_2 group was α to only one double bond. Its proton spectrum exhibited a doublet of triplets at δ 2.7 (J_{HF} = 15.1 Hz, J_{HH} = 5.1 Hz), indicating the presence of a methylene group situated between the difluoromethylene group and a double bond. These conclusions were confirmed by proton decoupling experiments, which fully established the structure shown.

The formation of conjugated enynes of type **15** was insignificant in the reaction between the allylic bromide **9** and the cuprates containing an ester function (**12a–c**). This observation was crucial in deciding how to proceed in constructing the full carbon chain of 10,10-DFAA from the allylic bromide **9**. Two alternatives were available, namely, to first introduce the heptynyl fragment followed by the methyl hexynoate fragment or vice versa. In view of the more favorable distribution of byproducts, the best course of action

appeared to be to introduce the heptynyl fragment first. The expected byproducts from this reaction could probably be removed almost entirely by chromatography following each of the three steps from dienyne **11** to allylic bromide **16** (deprotection, mesylation, and bromination). Since the formation of the conjugated enyne was not expected to be a problem in the reaction between the cuprate of methyl hexynoate **12a** and the allylic bromide **16**, the resulting dienediyne **17** would be largely free of this byproduct.



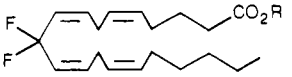
16



17

The synthesis therefore proceeded in the order indicated. The condensation product **11** was deprotected and converted to the allylic bromide **16**. The proton NMR spectrum of **16** showed that it was free of the branched or other byproducts. It was coupled with alkynyl cuprate **12a** to give the difluorodienediyne methyl ester **17**. The proton NMR spectrum of **17** showed the expected absorptions for four vinyl protons (δ 5.8 and 5.6) and two methylene groups between double and triple bonds (δ 3.1). The signals for the methylene protons next to the triple bonds (δ 2.2 for H-4 and δ 2.1 for H-16) were clearly resolved and appeared as triplets of triplets (J_{HH} = 7.0, 2.5 Hz for vicinal and through-triple bond coupling).

Semihydrogenation of the triple bonds of **17** was accomplished by using Lindlar catalyst poisoned with 5% synthetic quinoline in toluene, which furnished the desired 10,10-difluoroarachidonic acid methyl ester **18**. Depending on the amounts and the nature of the impurities present, repeated hydrogenation with fresh catalyst was often required in order to effect complete conversion. It was necessary, therefore, to follow the reaction carefully by NMR. The PMR spectrum of **18** showed that there were eight vinyl protons (signals at δ 5.66 and 5.40) and two methylene groups between double bonds (signal at δ 3.00). The downfield vinyl signal originated with the protons attached to the two double bonds adjacent to the difluoromethylene group while the upfield vinyl signal was associated with those of the remaining two double bonds. The signals for the methylene groups at C-4 and C-16 appeared as doublets of triplets (δ 2.10 for H-4 and δ 2.05 for H-16) and were upfield from those of the corresponding dienediyne **17**. When the catalytic reduction was performed with 5% palladium on barium sulfate in pyridine, hydrogenation of the double bonds adjacent to the difluoromethylene group and semihydrogenation of the triple bonds occurred simultaneously, leading to the 5,14-diene instead of the desired tetraene.

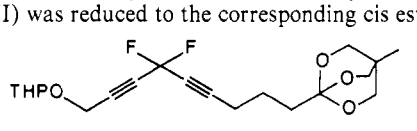


18, R = CH_3
18a, R = H

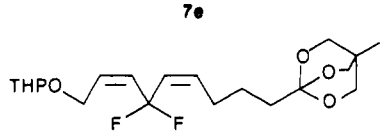
The methyl ester **18** was hydrolyzed to give 10,10-DFAA (**18a**) by using a *Rhizopus arrhizus* lipase with 1% gum arabic as an emulsifying agent,[12] after both basic and acidic hydrolysis had given unsatifactory results. Purification by RP-HPLC yielded the target molecule **18a** in over 95% purity (40% from the dienediyne **17**).

In contrast to arachidonic acid, 10,10-difluoroarachidonic acid was found to be resistant to autoxidation. When the acid **18a** was left as a film in air at room temperature for 2 days, no polar products were observed when the sample was analyzed by RP-HPLC and the pure difluoroarachidonic acid was the only compound present. In contrast to its stability in air, the difluorotetraene moiety proved to be unstable under basic conditions and when exposed to silica gel. Thus, when the crude methyl arachidonate from the semihydrogenation of **17** was passed through a column of silica gel, a mixture of compounds was formed showing ^{19}F NMR signals indicating vinylic fluorine. The UV spectrum of the mixture showed peaks at 319.5, 304.8, and 291.8 nm and a shoulder at 280 nm, characteristic of conjugated tetraenes. Conjugated tetraenic structures formed by 1,4-elimination of HF are proposed for the UV absorbing components. This facile elimination of HF is presumably a consequence of the strength of the Si–F bond (135 kcal/mol) coupled with the stability of the tetraene system.

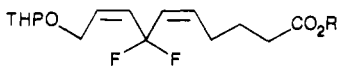
The synthesis of 7,7-difluoroarachidonic acid proceeded along a similar path. The very acid sensitive diacetylenic OBO-ester **7e** (Table II) was reduced to the corresponding cis ester **19** with Pd–$BaSO_4$ in pyridine/ethanol. The addition of ethanol resulted in a cleaner product. Hydrolysis to the neopentyl ester **20** with acetic acid in THF/water followed by transesterification with K_2CO_3 in anhydrous methanol furnished the methyl ester **20a**. In view of the extreme acid sensitivity of the OBO-ester **7e**, we consider it more prudent to perform the hydrolysis and transesterification prior to the catalytic hydrogenation. Conversion of **20a** to the bromide **21a** afforded the opportunity to attach the remaining half of the molecule by using the cuprate of the commercially available 1,4-decadiyne in 42% yield. Reduction of the crude dienediyne methyl ester **21b** with Lindlar catalyst followed by hydrolysis with the fungal lipase and RP-HPLC completed the synthesis of 7,7-difluoroarachidonic acid **22**. As in previous cuprate coupling reactions approximately 10% of the product was present as the branched S_N2' product, as evidenced by the isolation after reduction and HPLC of **23**, **24**, and **25**. The structure of these compounds is based on their NMR spectra, most notably the signals for the methine proton at C-8, the three protons of the 8-vinyl substituent, and the 11-methylene group.



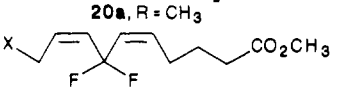
7e



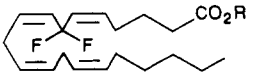
19



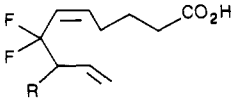
20, R = CH_2CCH_2OH (CH_3, CH_2OH)
20a, R = CH_3



21, X = OH
21a, X = Br
21b, X = $C{\equiv}CCH_2C{\equiv}CC_5H_{11}$
21c, X = $C{\equiv}CCH_2CH{=}CHC_5H_{11}$



22, R = H
22a, R = CH_3



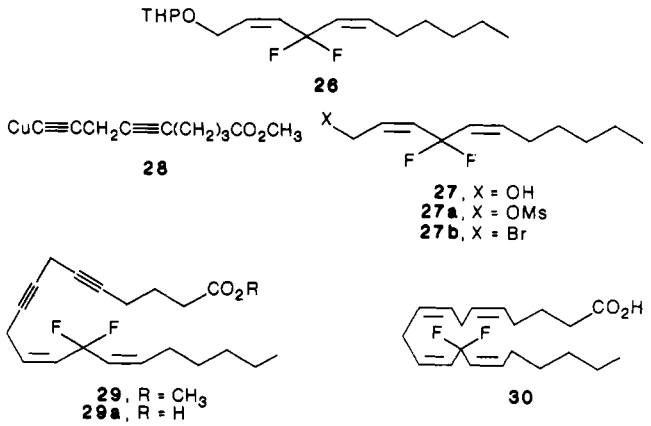
23, R = $C{\equiv}CCH_2C{\equiv}CC_5H_{11}$
24, R = $C{\equiv}CCH_2CH{=}CHC_5H_{11}$
25, R = $CH{=}CHCH_2CH{=}CHC_5H_{11}$

Three additional minor products were isolated in the lipase reaction. These are the 5- and 9-hydroxy-7-fluoroeicosatetraenoic

(12) Brockerhoff, H.; Jensen, R. G. *Lipolytic Enzymes* Academic Press: New York, 1974; p 146.

acids and the 1,5-lactone of the former. They became the major products when the reaction was allowed to proceed overnight. The evidence for the structure of these products and their mechanism of formation will be discussed in the accompanying paper.

For the synthesis of the 13,13-difluoroarachidonic acid the difluorodiyne THP ether **7a** (Table II) was converted by semihydrogenation into the corresponding diene **26** and then into the



bromide **27b**. The coupling reaction of the latter with the cuprate **28** of the methyl ester of nona-5,8-diynoic acid[7] proceeded uneventfully and furnished the difluorodienediyne methyl ester **29** in 29% yield. As in previous cases hydrolysis with the fungal lipase furnished after RP-HPLC 59% of the pure acid **29a**, which on semihydrogenation with Lindlar catalyst gave 62% of the desired 13,13-difluoroarachidonic acid **30**.

3. Experimental Section

Methods and Instrumentation. Thin-layer chromatograms were run on 0.25 mm EM precoated plates of silica gel 60 F254. Spots of compounds containing iodine or conjugated double bonds were observed by using UV light at 254 nm; spots of all compounds were visualized with 10% phosphomolybdic acid in ethanol by using heat to develop. The routine workup of the organic phase, after the appropriate washes, consisted of drying of the organic layer over anhydrous sodium sulfate, filtering of the organic phase, removal of the solvent at room temperature under house vacuum or under a stream of nitrogen, and purification of the product by column chromatography according to Still et al.[13] or by reverse-phase high performance liquid chromatography (RP-HPLC). Reagent or HPLC grade solvents were used as is upon initial opening and then stored over 3Å, 4Å, or 13X molecular sieves according to Burfield et al.[14] Gas liquid chromatography was performed on a Varian Aerograph Series 1700 equipped with a flame ionization detector, a linear temperature programmer, and a Model A-20 recorder by using a 6-ft glass column containing 1.5% OV-1 on Chromasorb-W. Melting points were obtained on a Thomas Hoover melting point apparatus and were uncorrected. The ^{1}H NMR spectra were obtained in $CDCl_3$ on a University of Chicago DS-1000 spectrometer at 500 MHz and were processed by Fourier transformation by using a Nicolet Instrument Corporation (now G.E. Magnetics) 1180 or 1280 Data Acquisition system. The ^{19}F NMR spectra were obtained on a Bruker HX-90-E instrument at 84.7 MHz (in C_6D_6 or $CDCl_3$) with use of a 1080 Data Acquisition system and on a Nicolet Instrument Corporation NTC-200 at 188.2 MHz (in $CDCl_3$) with use of a 1280 Data Acquisition system. The chemical shifts of the ^{1}H NMR signals were reported in δ parts per million (ppm) with chloroform as internal standard (δ 7.25). The chemical shifts of the ^{19}F NMR signals were reported in ϕ ppm upfield from the internal standard $CFCl_3$. Coupling constants, J, are reported in Hz. The abbreviations s, d, t, q, p, m, and br signify singlet, doublet, triplet, quartet, quintet, multiplet, and broad, respectively. Carbon skeletons are numbered so as to maintain consistency in NMR assignments. Low resolution mass spectra were determined at 70 eV by using a Finigan 1015 quadrupole mass spectrometer or a VG 70-250HF mass spectrometer equipped with GLC, gas, and solid probe inlets. High resolution mass spectra were determined at 70 eV by using a VG 70-250HF mass spectrometer equipped with GLC, gas, and solid probe inlets. Microanalyses were performed by Baron, Orange, CN and MicroTech, Skokie, IL. Purification of 10,10-DFAA, 7,7-DFAA, and 13,13-DFAA was performed on a Waters Associates RCM-100 radial compression module fitted with an analytical 5-mm × 10-cm Radial Pak-A (C-18, 5 μm) cartridge or on a Rainin Instruments semipreparative 10-mm × 25-cm Dynamax Macro-HPLC (C-18, 8 μm) column by using a Waters Associates Model 590 Programmable Solvent Delivery Module, a U6K injector, and a 0.2 μm prefilter. Elution rates were 2 or 3 mL/min with varying ratios of water (double deionized, adjusted to pH 3.5 with acetic acid) and HPLC grade acetonitrile. Elution was followed by the measurement of the absorbance with a Lambda-Max Model 481 LD spectrophotometer and a Hewlett Packard HP-3390A integrator. The pH value of aqueous buffers was determined by the use of a Fisher Accumet Model 815MP pH meter. IR spectra were obtained on a Perkin-Elmer Model 283 infrared spectrophotometer. The lipase from *Rhizopus arrhizus* was purchased from Boehringer Mannheim Biochemicals as a suspension in ammonium sulfate solution, 3.2 M, and potassium phosphate 0.01 M, pH approximately 6.

4-Bromo-4,4-difluorobut-2-ynyl Tetrahydropyranyl Ether 4. To the THP ether of propargyl alcohol (1 g, 7.13 mmol) in dry THF (20 mL) was added *n*-butyllithium (5.5 mL of 1.44 M solution in hexane, 1.1 equiv) at 0 °C. The mixture was allowed to stir at room temperature for 0.5 h. CF_2BrCl (3.5 g, 3 equiv) was condensed into a flask maintained at −78 °C and then added quickly to the reaction mixture at −78 °C. It was stirred at −78 °C for 2 h before being warmed to room temperature and worked up with saturated aqueous ammonium chloride and hexane. Purification by column chromatography (67% CH_2Cl_2/hexane) gave **4** (R_f 0.42, 0.96 g, 50%) and the bromoacetylene **5** (R_f 0.32, 0.39 g, 25%).

4: yellow oil; IR ($CDCl_3$) 2230 cm^{-1}, alkyne stretch; ^{1}H NMR δ 4.78 (t, 1 H, J = 3.3, H-2′), 4.39 (t, 2 H, J = 4.0, H-1), 3.83 (m, 1 H, H-6′), 3.57 (m, 1 H, H-6′), 1.85–1.54 (4 m, 6 H, H-3′, H-4′, H-5′); ^{19}F NMR (C_6D_6, 84.7 MHz) ϕ 32.4 (t, J = 4.1); MS (70 eV, m/z), 269, 267 (M^+ − 1, 0.5%). Anal. Calcd for $C_9H_{11}O_2F_2Br$: C, 40.17; H, 4.12; F, 14.12. Found: C, 40.42; H, 4.40; F, 14.37.

5: yellow oil; ^{1}H NMR δ 4.79 (t, 1 H, J = 3.2, H-2′), 4.27 (q, 2 H, J = 15.5, H-1), 3.82 (m, 1 H, H-6′), 3.55 (m, 1 H, H-6′), 1.88–1.54 (4 m, 6 H, H-3′, H-4′, H-5′); MS (70 eV, m/z), 219, 217 (M^+ − 1, 0.2%).

General Method for Preparing Bromodifluoromethylalkynes. To the alkyne (10 mmol) in dry THF (30 mL) was added *n*-butyllithium (as a hexane solution, 1.1 equiv) at 0 °C. The solution was stirred at room temperature for 1 h before being cooled to −78 °C. Bromochlorodifluoromethane (3–7 equiv) was condensed into a flask at −78 °C and then added quickly to the reaction vessel. After having been stirred for 2 h at −78 °C, the reaction mixture was worked up with saturated aqueous ammonium chloride and hexane followed by column chromatography to give the desired product.

5-Bromo-5,5-difluoropent-3-ynyl Tetrahydropyranyl Ether, 4a. But-3-ynyl tetrahydropyranyl ether (2 g, 13.0 mmol) and *n*-BuLi (9.9 mL of a 1.44 M hexane solution, 1.1 equiv) reacted with CF_2BrCl (10.7 g, 5 equiv) to give **4a** (1.04 g, 28%): colorless oil; ^{1}H NMR δ 4.66 (t, 1 H, J = 3.5, H-2′), 3.85 (m, 2 H, H-1, H-6′), 3.61 (m, 1 H, H-1), 3.54 (m, 1 H, H-6′), 2.66 (m, 2 H, H-2), 1.85–1.53 (4 m, 6 H, H-3′, H-4′, H-5′); ^{19}F NMR ϕ 31.26 (t, J = 4.5); MS (70 eV, m/z), 262, 264 (M^+, 0.5%), 183 (M^+ − Br, 2%). Anal. Calcd for $C_{10}H_{15}F_2Br$: C, 42.42; H, 4.63; F, 13.42. Found: C, 42.76; H, 4.93; F, 13.75.

1-Bromo-1,1-difluoroundeca-2,5-diyne 4b. 1,4-Decadiyne (746.7 mg, 5.6 mmol) and *n*-BuLi (4 mL of a 1.4 M solution in hexane, 1 equiv) reacted with CF_2BrCl (6.2 g, 6.7 equiv) to give **4b** (462.3 mg, 32%): yellow oil; ^{1}H NMR δ 3.31 (tt, 2 H, J = 2.3, 4.2, H-4), 2.16 (tt, 2 H, J = 2.3, 7.1, H-7), 1.55 (p, 2 H, J = 7, H-8), 1.35 (m, 4 H, H-9, H-10), 0.90 (t, 3 H, J = 7.0, H-11); ^{19}F NMR ϕ 32.20 (br s).

1-Bromo-1,1-difluoro-2-octyne 4c. 1-Heptyne (2 g, 20.8 mmol) and *n*-BuLi (14.3 mL of a 1.6 M hexane solution, 1.1 equiv) reacted with CF_2BrCl (6 equiv) to give the product **4c** (2.14 g, 46%): yellow oil; ^{1}H NMR δ 2.35 (tt, 2 H, J = 4.8, 2.4, H-4), 1.60 (p, 2 H, J = 7.1, H-5), 1.37 (m, 4 H, H-6, H-7), 0.93 (t, 3 H, J = 7.4, H-8); MS (70 eV, m/z), 170, 168 (M − C_4H_8, 2%), 145 (M − Br, 10%), 125 (M − BrF, 90%).

4-Bromo-4,4-difluorobut-2-ynyl *tert*-Butyldimethylsilyl Ether, 4d. Propargyl *tert*-butyldimethylsilyl ether (2.5 g, 15 mmol) and *n*-BuLi (11.46 mL of 1.44 M hexane solution, 1.1 equiv) reacted with CF_2BrCl (7.4 g, 3 equiv) to give **4d** (1.81 g, 40%): colorless oil; R_f 0.28 (11% CH_2Cl_2/hexane); ^{1}H NMR δ 4.45 (t, 2 H, J = 4, H-2), 0.926 (s, 9 H, *t*-Bu), 0.15 (s, 6 H, 2Me); ^{19}F NMR ϕ 33.09 (t, J = 4).

4-Iodo-4,4-difluorobut-2-ynyl Tetrahydropyranyl Ether, 6. 4-Bromo-4,4-difluorobut-2-ynyl tetrahydropyranyl ether (**4**) (236.2 mg, 0.88 mmol) in 5 mL of 1 M sodium iodide in acetone was refluxed for 2 h. Gas chromatography was used to monitor the reaction. The reaction mixture was poured into a mixture of hexane and water (40 mL) and was extracted with hexane (3 × 20 mL). Workup and column chromatography (R_f 0.42, 67% CH_2Cl_2/hexane) gave pure **6** (211.1 mg, 0.67 mmol, 76%): yellow oil; IR ($CDCl_3$) 2235 cm^{-1}, alkyne stretch; ^{1}H NMR δ 4.78

(13) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(14) Burfield, D. R.; Smithers, R. H. *J. Org. Chem.* **1983**, *48*, 2420.

(t, 1 H, J = 3, H-2'), 4.38 (m, 2 H, H-1), 3.83 (m, 1 H, H-6'), 3.57 (m, 1 H, H-6'), 1.86-1.47 (4 m, 6 H, H-3', H-4', H-5'); ^{19}F NMR (C_6D_6, 84.7 MHz) ϕ 28.53 (t, J = 4.3); MS (70 eV, m/z), 315 ($M^+ - 1$, 1%).

General Method for Preparing Difluorodiynes. To the 1-alkyne (6 mmol, 2 equiv) in dry THF (40 mL) was added n-BuLi (as a hexane solution, 6 mmol, 2 equiv) under nitrogen at 0 °C. The mixture was stirred for 1 h at room temperature before being cooled to −15 °C. The difluoroiodomethylalkyne (3 mmol, 1 equiv) was added in 10 mL of THF. After 2 h, the reaction mixture was worked up with water and hexane to give the desired product. In most of the reactions, the crude iodide prepared from the corresponding bromide was used without purification.

7-(*tert*-Butyldimethylsilyl)oxy-4,4-difluorohepta-2,5-diynyl Tetrahydropyranyl Ether, 7. Method 1. *tert*-Butyldimethylsilyl propargyl ether (4.3 g, 25.30 mmol) and n-BuLi (17 mL of a 1.5 M hexane solution, 25.30 mmol) reacted with crude iodide **6** (from 12.7 mmol of **4**) to give **7** (2.829 g, 62.3% from **4**) after column chromatography (R_f 0.25, 67% CH_2Cl_2/hexane).

Method 2. Propargyl tetrahydropyranyl ether (1.27 g, 9.1 mmol) and n-BuLi (5.7 mL of a 1.6 M hexane solution, 9.0 mmol) reacted with 1-iodo-1,1-difluorobut-2-ynyl *tert*-butyldimethylsilyl ether (from 6.1 mmol of bromo difluoro ether) to give **7** (1.122 g, 51.7% from **4d**) after column chromatography (R_f 0.25, 67% CH_2Cl_2/hexane): colorless oil; ^{1}H NMR δ 4.78 (t, 1 H, J = 3.3, H-2'), 4.41 (t, 2 H, J = 4.3, H-7), 4.35 (t, 2 H, J = 4.3, H-1), 3.83 (m, 1 H, H-6'), 3.55 (m, 1 H, H-6'), 1.84–1.50 (4 m, 6 H, H-3', H-4', H-5'), 0.92 (s, 9 H, t-Bu), 0.14 (s, 6 H, 2Me); ^{19}F NMR ϕ 66.74 (p, J = 4.3, F-4); MS (70 eV, m/z), 357 ($M^+ - 1$, 0.5%); 301 ($M - t$-Bu, 8%). Anal. Calcd for $C_{18}H_{28}O_2F_2Si$: C, 60.31; H, 7.87; F, 10.60. Found: C, 59.90; H, 7.93; F, 9.66.

4,4-Difluoroundeca-2,5-diynyl Tetrahydropyranyl Ether, 7a. 1-Heptyne (1.22 g, 12.7 mmole) and n-BuLi (8.8 mL of a 1.44 M hexane solution, 12.7 mmol) reacted with iodide **6** (2 g, 6.3 mmol) to give **7a** (1.00 g, 56%): Colorless oil; R_f 0.27 (67% CH_2Cl_2/hexane); IR ($CDCl_3$) 2237 cm^{-1}, alkyne stretch; ^{1}H NMR δ 4.79 (t, 1 H, J = 3.3, H-2'), 4.35 (t, 2 H, J = 4.1, H-1), 3.83 (m, 1 H, H-6'), 3.55 (m, 1 H, H-6'), 2.29 (m, 2 H, H-7), 1.86–1.50 (4 m, 8 H, H-3', H-4', H-5', H-8), 1.40–1.30 (m, 4 H, H-9, H-10), 0.90 (t, 3 H, J = 7.2, H-11); ^{19}F NMR (C_6D_6, 84.7 MHz) ϕ 63.42 (m); MS (70 eV, m/z), 283 ($M^+ - 1$, 1.2%). Anal. Calcd for $C_{16}H_{24}O_2F_2$: C, 67.58; H, 7.80; F, 13.36. Found: C, 67.85; H, 7.86; F, 13.87.

7-(*tert*-Butyldiphenylsilyl)oxy-4,4-difluorohepta-2,5-diynyl Tetrahydropyranyl Ether, 7b. *tert*-Butyldiphenylsilyl propargyl ether (3.87 g, 13.2 mmol) and n-BuLi (9.2 mL of a 1.44 M hexane solution, 13.2 mmol) reacted with **6** (crude iodide from 6.6 mmol of **4**) to give **7b** (1.42 g, 44.8% from **4**) after column chromatography (R_f 0.49, 67% CH_2Cl_2/hexane): colorless oil; ^{1}H NMR δ 7.69–7.39 (2 m, 10 H, 2 Ph), 4.78 (t, 1 H, J = 3.3, H-2'), 4.38 (t, 2 H, J = 4, H-7), 4.35 (t, 2 H, J = 4, H-1), 3.83 (m, 1 H, H-6'), 3.54 (m, 1 H, H-6'), 1.84–1.50 (4 m, 6 H, H-3', H-4', H-5'), 1.07 (s, 9 H, t-Bu).

8-(*tert*-Butyldimethylsilyl)oxy-4,4-difluoroocta-2,5-diynyl Tetrahydropyranyl Ether, 7c. *tert*-Butyldimethylsilyl but-2-ynyl ether (1 g, 5.4 mmol) and n-BuLi (3.8 mL of a 1.42 M hexane solution, 5.4 mmol) reacted with the crude iodide **6** (from 3.75 mmol of **4**) to yield the product **7c** (600.2 mg, 43% from **4**): yellow oil; R_f 0.20 (67% CH_2Cl_2/hexane); ^{1}H NMR δ 4.79 (t, 1 H, J = 3, H-2'), 4.35 (t, 2 H, J = 4, H-1), 3.83 (m, 1 H, H-6'), 3.77 (t, 2 H, J = 7, H-8), 3.56 (m, 1 H, H-6'), 2.52 (tt, 2 H, J = 7, 5, H-7), 1.85–1.53 (4 m, 6 H, H-3', H-4', H-5'), 0.91 (s, 9 H, t-Bu), 0.09 (s, 6 H, 2 Me); MS (70 eV, m/z), 372 (M^+, 0.2%), 315 ($M - t$-Bu, 1%).

10-(*tert*-Butyldimethylsilyl)oxy-4,4-difluorodeca-2,5-diynyl Tetrahydropyranyl Ether, 7d. *tert*-Butyldimethylsilyl hex-5-ynyl ether (100 mg, 0.47 mmol) and n-BuLi (0.3 mL of 1.5 M hexane solution, 0.47 mmol) reacted with **6** (87.8 mg, 28 mmol) to yield **7d** (31.3 mg, 17%): colorless oil; R_f 0.18 (50% CH_2Cl_2/hexane; ^{1}H NMR δ 4.79 (t, 1 H, J = 3.2, H-2'), 4.35 (t, 2 H, J = 4.0, H-1), 3.83 (m, 1 H, H-6'), 3.64 (tt, 2 H, J = 6.3, 5.0, H-7), 1.85–1.53 (4 m, 10 H, H-3', H-4', H-5', H-8, H-9), 0.90 (s, 9 H, t-Bu), 0.08 (s, 6 H, 2 Me); ^{19}F NMR ($CDCl_3$, 84.7 MHz) ϕ 64.50 (p, J_{HF} = 4.5).

1-(9-Tetrahydropyranyloxy-6,6-difluorononona-4,7-diynyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, 7e. 1-(4-Hexynyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane[15] (OBO-ortho ester of hexynoic acid, 3.154 g, 16.1 mmol) and n-BuLi (10.7 mL of a 1.5 M hexane solution, 16.1 mmol) reacted with crude **6** (from 10.6 mmol of **4**) to give **7e** (1.03 g, 25.4% from **4**): ^{1}H NMR δ 4.79 (t, 1 H, J = 3, H-2'), 4.35 (t, 2 H, J = 4, H-9), 3.87 (s, 6 H, $-CH_2O-$ in OBO), 3.85 (m, 1 H, H-6'), 3.55 (m, 1 H, H-6'), 2.33 (tt, 2 H, J = 7, 4, H-3), 1.74 (m, 4 H, H-1, H-2), 1.85–1.53 (4 m, 6 H, H-3', H-4', H-5'), 0.80 (s, 3 H, Me); ^{19}F NMR ϕ 64.53 (p, J = 4.5).

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(*Z*)-8-Methoxy-4,4-difluoroocta-7-en-2,5-diynyl Tetrahydropyranyl Ether, 7f. (*Z*)-1-Methoxybut-1-en-3-yne (774.7 mg, 9.44 mmol) and n-BuLi (6.6 mL of a 1.43 M hexane solution, 9.44 mmol) reacted with crude **6** (from 1.86 mmole of **4**) to give **7f** (137.5 mg, 27.4% from **4**): yellow oil, R_f 0.24 (83% CH_2Cl_2/hexane); ^{1}H NMR δ 6.45 (d, 1 H, J = 6.5, H-8), 4.78 (t, 1 H, J = 3.2, H-2'), 4.55 (dt, 1 H, J = 6.4, 4, H-7), 4.35 (t, 2 H, J = 4, H-1), 3.82 (s, 3 H, OMe), 3.81 (m, 1 H, H-6'), 3.54 (m, 1 H, H-6'), 1.85–1.50 (4 m, 6 H, H-3', H-4', H-5'); MS (70 eV, m/z), 270 (M^+, 0.2%); 214 ($M - [CH{=}CHOCH_2]$, 8%).

7-(*tert*-Butyldimethylsilyl)oxy-4,4-difluorohepta-2,5-dienyl Tetrahydropyranyl Ether, 8. The difluorodiyne **7** (1.31 g, 3.66 mmol) was stirred with the catalyst (5% Pd–$BaSO_4$, 450 mg) in pyridine (20 mL) under H_2 at room temperature for 8 h, by which time 120% of theoretical H_2 uptake was observed. The mixture was then diluted with ethyl acetate and filtered through a short column of Celite. After removal of the solvents the residue was purified by column chromatography (R_f 0.55, 25% EtOAc/hexane) to yield the diene **8** (970 mg, 73.2%): yellow oil; ^{1}H NMR δ 5.92 (m, 1 H, H-2), 5.84 (m, 1 H, H-6), 5.65 (q, 1 H, J = 13, H-3), 5.57 (q, 1 H, J = 13, H-5), 4.62 (t, 1 H, J = 3.5, H-2'), 4.44 (m, 1 H, H-1), 4.41 (m, 2 H, H-7), 4.27 (m, 1 H, H-1), 3.85 (m, 1 H, H-6'), 3.51 (m, 1 H, H-6'), 1.88–1.53 (4 m, 6 H, H-3', H-4', H-5'), 0.91 (s, 9 H, t-Bu), 0.08 (s, 6 H, 2Me); ^{19}F NMR ϕ 84.57 (t, J = 13.1).

4,4-Difluoro-1,7-dihydroxyhepta-2,5-dienyl Tetrahydropyranyl Ether, 8a. To tetrabutylammonium fluoride (9.6 mmol) in dry pyridine (10 mL) was added hydrofluoric acid (700 μL of 48% aqueous solution, 19.2 mmol).[16] The solvent was removed under reduced pressure. An additional 10 mL of dry pyridine was added, and the solvent was again removed, azeotroping off the water and the acid. The diene **8** (870 mg, 2.4 mmol) was added to the HF–TBAF reagent in 10 mL of dry pyridine. The mixture was stirred at room temperature for 4 h. After checking by TLC, the reaction mixture was quenched by the addition of 0.01 M HCl and extracted with ether. Workup followed by column chromatography (R_f 0.27, 50% EtOAc/hexane) gave **8a** (541.8 mg, 91%): yellow oil; ^{1}H NMR δ 5 92 (m, 2 H, H-2, H-6), 5.74 (m, 2 H, H-3, H-5), 4.63 (t, 1 H, J = 3.5, H-2'), 4.35 (br s, 2 H, H-7), 4.34 (m, 2 H, H-2), 3.85 (m, 1 H, H-6'), 3.51 (m, 1 H, H-6'), 1.85–1.53 (4 m, 6 H, H-3', H-4', H-5'); ^{19}F NMR ϕ 82.07 (t, J = 11.7).

4,4-Difluoro-1-(tetrahydropyranyloxy)hepta-2,5-dien-7-yl Mesylate, 8b. To the alcohol **8a** (56 mg, 0.23 mmol) and triethylamine (7.6 equiv) in dichloromethane (15 mL) at 0 °C was added mesyl chloride (6.6 equiv). The mixture was stirred for 1 h at 0 °C. Workup with water and dichloromethane followed by column chromatography (R_f 0.37, 50% EtOAc/hexane) gave the pure product **8b** (61.8 mg, 84%): colorless oil; ^{1}H NMR δ 5.98 (m, 1 H, H-6), 5.89 (m, 1 H, H-2), 5.84 (q, 1 H, J = 13, H-5), 5.68 (q, J = 13, H-3), 4.96 (m, 2 H, H-7), 4.61 (t, 3 H, J = 3.3, H-2'), 4.42 (m, 1 H, H-1), 4.28 (m, 1 H, H-1), 3.85 (m, 1 H, H-6'), 3.51 (m, 1 H, H-6'), 3.03 (s, 3 H, $MeSO_3$), 1.85–1.53 (4 m, 6 H, H-3', H-4', H-5'); ^{19}F NMR ϕ 85.16 (t, J = 11).

7-Bromo-4,4-difluorohepta-2,5-dienyl Tetrahydropyranyl Ether, 9. The mesylate **8b** (198.7 mg, 0.61 mmol) was stirred with lithium bromide (283.2 mg, 3.3 mmole)[17] in THF (3 mL) at room temperature for 14 h. Water was added, and the mixture was extracted with hexane. Workup followed by column chromatography (R_f 0.25, 67% CH_2Cl_2/hexane) yielded the pure bromide **9** (151.6 mg, 80%): pale yellow oil; ^{1}H NMR δ 5.70 (q, 2 H, J = 13, H-3, H-5), 5.98 (m, 2 H, H-2, H-6), 4.63 (t, 1 H, J = 3.5, H-2'), 4.45 (m, 1 H, H-1), 4.28 (m, 1 H, H-1), 4.11 (br d, 2 H, J = 7), 3.86 (m, 1 H, H-6'), 3.52 (m, 1 H, H-6'), 1.85–1.55 (3 m, 6 H, H-3', H-4', H-5'); ^{19}F NMR ϕ 84.11 (t, J = 13.1).

General Method for the Preparation of Alkynyl Cuprates.[18] Copper(II) sulfate pentahydrate (5 g, 20 mmol) was placed in a 500-mL Erlenmeyer flask, and 20 mL of concentrated ammonium hydroxide was added. The deep blue solution was stirred under nitrogen for a short time. After the addition of 80 mL of water, solid hydroxylamine hydrochloride (2.78 g, 40 mmol) was added. The dark blue solution turned lighter in color. After about 5 min, the 1-alkyne (20 mmol) in ethanol (100 mL) was added. A yellow precipitate was formed instantly. The mixture was stirred with cooling for about 5 min before it was filtered and washed successively with water (5 × 20 mL), absolute ethanol (5 × 20 mL), and ether (5 × 20 mL). The yellow solid was dried for 4 h at 65 °C in vacuo. (Note different procedure for preparation of deca-1,4-diynyl cuprate).

1-Heptynyl Cuprate, 10. 1-Heptyne (1.92 g, 20 mmol) reacted with copper(II) sulfate pentahydrate (5 g, 20 mmol), hydroxylamine hydrochloride (2.78 g, 40 mmol), and ammonium hydroxide in water–ethanol

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(18) Castro, C. E.; Gaughan, E. J.; Owsley, D. C. *J. Org. Chem.* **1966**, *31*, 4071.

to produce the canary yellow cuprate (2.24 g, 70%): mp 122–125 °C dec.

Cuprate of Methyl 5-Hexynoate 12a. Methyl 1-hexynoate (578.9 mg, 4.6 mmol) reacted with copper(II) sulfate pentahydrate (1.15 g, 4.6 mmol), hydroxylamine hydrochloride (639 mg, 9.2 mmol), and ammonium hydroxide in water–ethanol to yield the yellow cuprate (630.7 mg, 72.8%): mp 184–250 °C dec.

4,4-Difluorotetradeca-2,5-dien-8-yn-1-yl Tetrahydropyranyl Ether, 11. 1-Heptynyl cuprate (**10**) (173.9 mg, 1.08 mmol), the bromide **9** (224.5 mg, 0.72 mmol), and sodium cyanide (53.1 mg, 1.08 mmol) were placed in a test tube. The solvent, DMF (1.1 mL, to make a 1 M solution in the cuprate and the cyanide), was added, and the mixture was stirred at room temperature for 5 h. Workup with dichloromethane–water followed by column chromatography (R_f 0.26, 86% CH_2Cl_2/hexane) yielded the desired product **11** (131.0 mg, 55.7%). Small amounts of the branched dienyne **14** and the conjugated dienyne **15** were still evident in the NMR spectrum of this product: colorless oil; ^1H NMR δ 5.92 (m, 1 H, H-2), 5.76 (m, 1 H, H-6), 5.65 (m, 2 H, H-3, H-5), 4.61 (t, 1 H, J = 3.5, H-1′), 4.45 (m, 1 H, H-1), 4.28 (m, 1 H, H-1), 3.86 (m, 1 H, H-5′), 3.52 (m, 1 H, H-5′), 3.14 (m, 2 H, H-7), 2.14 (tt, 2 H, J = 2.3, 7.0, H-10), 1.85–1.53 (4 m, 6 H, H-2′, H-3′, H-4′), 1.31 (m, 6 H, H-11, H-12, H-13), 0.91 (t, 3 H, J = 7, H-14); ^{19}F NMR ϕ 84.04 (t, J = 11.5).

4,4-Difluorotetradeca-2,5-dien-8-yn-1-ol, 11a. The tetrahydropyranyl ether **11** (40.9 mg, 0.13 mmol) was stirred at room temperature in 0.01 M TsOH in MeOH (30 mL) for 4 h. Workup with CH_2Cl_2/H_2O followed by column chromatography (R_f 0.27, 25% EtOAc/hexane) yielded the pure alcohol **11a** (21.8 gm, 72%): colorless oil; ^1H NMR δ 5.93 (dtt, 1 H, J = 10.1, 2.1, 7.2, H-2), 5.79 (dtt, 1 H, J = 11.5, 2.3, 7.3, H-6), 5.70 (q, 1 H, J = 13.1, H-3), 5.65 (q, 1 H, J = 12.5, H-5), 4.38 (m, 2 H, H-1), 3.125 (m, 2 H, H-7), 2.14 (tt, 2 H, J = 7, 2.5, H-10), 1.49 (p, 2 H, J = 7, H-11), 1.04 (m, 4 H, H-12, H-13), 0.91 (t, J = 7, H-14).

1-Bromo-4,4-difluorotetradeca-2,5-dien-8-yne, 16. To the alcohol **11a** (21.8 mg, 90.1 μmol) in dichloromethane (10 mL) at 0 °C was added triethylamine (15 equiv) followed by mesyl chloride (10 equiv). After 1 h at 0 °C, the reaction mixture was worked up with CH_2Cl_2/H_2O, followed by column chromatography to give a crude mesylate. This material was stirred with lithium bromide (10 equiv) in THF (10 mL) at room temperature overnight. The reaction mixture was worked up with CH_2Cl_2/H_2O followed by column chromatography (R_f 0.46, 33% CH_2Cl_2/hexane) to yield the pure bromide **16** (25.3 mg, 92.1%): colorless oil; ^1H NMR δ 5.98 (dtt, 1 H, J = 10, 9, 2, H-2), 5.84 (dtt, 1 H, J = 11, 7, 2, H-6), 5.70 (q, 1 H, J = 12, H-3), 5.65 (qt, 1 H, J = 12, 2, H-5), 4.11 (d, 2 H, J = 9, H-1), 3.15 (m, 2 H, H-7), 2.14 (tt, 2 H, J = 7, 3, H-10), 1.49 (p, 2 H, J = 7, H-11), 1.34 (m, 4 H, H-12, H-13), 0.91 (t, 3 H, J = 7, H-11); ^{19}F NMR ϕ 84.48 (t, J = 12.4).

Methyl 10,10-Difluoroeicosa-8,11-diene-5,14-diynoate, 17. The bromide **16** (60.4 mg, 0.2 mmol), the cuprate of methyl 5-hexynoate **12a** (1.5 equiv), and sodium cyanide (3 equiv) was stirred in DMF (300 μL) under nitrogen at room temperature for 5 h. Workup with CH_2Cl_2/H_2O, followed by column chromatography (R_f 0.11, 50% CH_2Cl_2/hexane) gave the pure ester **17** (39.8 mg, 57.4%): colorless oil; ^1H NMR δ 5.77 (m, 2 H, H-8, H-12), 5.63 (q, 2 H, J = 12, H-9, H-11), 3.68 (s, 3 H, OMe), 3.14 (m, 4 H, H-7, H-13), 2.43 (t, 2 H, J = 7, H-2), 2.23 (tt, 2 H, J = 7, 2.5, H-4), 2.14 (tt, 2 H, J = 2.5, 7, H-16), 1.82 (p, 2 H, J = 7, H-3), 1.49 (p, 2 H, J = 7, H-17), 1.34 (m, 4 H, H-18, H-19), 0.92 (t, 3 H, J = 7, H-20); ^{19}F NMR ϕ 83.59 (t, J = 11.6); high resolution MS (70 eV, m/z), calcd for $C_{21}H_{27}O_2F$ (M − HF) 330.1995, found 330.1987 (4%); calcd for $C_{17}H_{18}F$ (M − HFC_3H_6COOMe) 229.1392, found 229.1335 (5%).

Methyl 10,10-Difluoroarachidonate, 18. The diendiyne **17** (10.2 mg, 29.1 μmol) was hydrogenated over 5% palladium on calcium carbonate and poisoned with lead (Lindlar catalyst, 5 mg) in toluene (400 μL) containing 5 μL of 5% synthetic quinoline in toluene. The reaction was terminated in 2 h at room temperature; during which time 1.5 mL of hydrogen was taken up (theory: 1.3 mL). The reaction mixture was filtered through Celite, and the filtrate was concentrated under a stream of nitrogen to give the crude product to be used without purification in the next step. Depending on the nature of the impurities present, repeated hydrogenations were often required. In such cases, the crude product was resuspended in toluene containing quinoline, fresh catalyst was added (same conditions as before), and the mixture was then stirred under H_2. This process was repeated until NMR showed the total disappearance of starting alkyne: yellow oil; ^1H NMR δ 5.66 (m, 4 H, H-8, H-9, H-11, H-12), 5.40 (m, 4 H, H-5, H-6, H-14, H-15), 3.67 (s, 3 H, OMe), 3.00 (br, 4 H, H-7, H-13), 2.32 (t, 2 H, J = 7.5, H-2), 2.10 (dt, 2 H, J = 7.2, 6.7, H-4), 2.05 (dt, 2 H, J = 7.1, 7.1, H-16), 1.71 (p, 2 H, J = 7.4, H-3), 1.36 (m, 2 H, H-17), 1.31 (m, 4 H, H-18, H-19), 0.89 (t, 3 H, J = 6.9, H-20); ^{19}F NMR ϕ 81.94 (p, J = 5); high resolution MS (70 eV, m/z), calcd for $C_{21}H_{31}O_2F$ (M − HF) 334.2308, found 334.2289 (25%); calcd for $C_7H_9F_2$ ($CH_2CH{=}CHCF_2CH{=}CHCH_2$ + 1) 131.0673, found 131.0638 (37%).

10,10-Difluoroarachidonic Acid, 18a. The methyl ester **18** (from 18.3 mg of **17**) was hydrolyzed with *Rhizopus arrhizus* lipase (400 μL, 20 000 units) in potassium phosphate buffer (6 mL, 0.1 M, pH 7.02) containing 3 mg of NaCl (0.01 M solution in NaCl) and 60 mg of gum arabic (1% solution). The mixture was agitated on the Vortex Junior mixer for 4 min, sonicated for 1 min, and then stirred at 25 °C for 2 h. The reation was terminated by acidification with 1 N HCl to pH 3.0 and extraction of the product with CH_2Cl_2. Purification by RP-HPLC gave 6.8 mg of pure 10,10-DFAA, **18a**, (40% from **16**). RP-HPLC conditions: Rainin Instruments Dynamax macro-HPLC column (C-18, 10 mm × 25 cm, 8 μm) eluted with 80% CH_3CN/20% H_2O (pH 3.5) at 3 mL/min, absorbance monitored at 192 nm. The retention times for 10,10-DFAA and its recovered methyl ester (ca. 1 mg) were 21.5 and 62.1 min, respectively. Two additional fractions were obtained (<0.5 mg) with retention times of 9.8 and 10.4 min, respectively. They will be described in detail in connection with the enzymatic conversions of **18a**.

The fractions containing these compounds were collected, and the acetonitrile was removed under a stream of nitrogen. The residual aqueous solutions were then extracted 3 times with equal volumes of dichloromethane, and the extracts were dried by passing through a small column of anhydrous sodium sulfate in a disposable pasteur pipette and concentrated under a stream of nitrogen. The residues were placed under high vacuum, and the isolated products were kept frozen in benzene at −20 °C under nitrogen until use. **18a**: colorless oil; ^1H NMR δ 5.66 (m, 4 H, H-8, H-9, H-11, H-12), 5.38 (m, 4 H, H-5, H-6, H-14, H-15), 3.00 (br, 4 H, H-7, H-13), 2.37 (t, 2 H, J = 7.5, H-2), 2.13 (q, 2 H, J = 7.1, H-4), 2.05 (q, 2 H, J = 7.2, H-16), 1.73 (p, 2 H, J = 7.4, H-3), 1.36 (p, 2 H, J = 7.1, H-17), 1.31 (m, 4 H, H-18, H-19), 0.90 (t, 3 H, J = 6.8, H-20); ^{19}F NMR ϕ 81.97 (t, J = 5); high resolution MS (70 eV, m/z), calcd for $C_{20}H_{25}O_2F$ (M − HF) 320.2151; found 320.2128 (25%).

1-(6,6-Difluoro-9-(tetrahydropyranyloxy)nona-4,7-dienyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, 19. To a suspension of 5% palladium on barium sulfate (202 mg) in 19 mL of 25% pyridine in absolute ethanol was added the difluorodiyne **7e** (348 mg, 0.91 mmol), and after evacuation hydrogen was admitted. After 0.5 h of stirring, 125% of the stoichiometric amount of hydrogen was consumed. Passage through a short column of Celite, followed by flash chromatography on silica gel (1:2 ethyl acetate/hexane, R_f 0.31), provided the pure diene **19** (269 mg, 77%): ^1H NMR δ 5.87 (m, 1 H, H-8), 5.64 (m, 3 H, vinyl), 4.61 (t, 1 H, J = 3.5, H-2 (THP)), 4.44 (m, 1 H, H-9), 4.27 (m, 1 H, H-9), 3.88 (s, 6 H, H-2, H-6, H-7), 3.85 (m, 1 H, H-6 (THP)), 3.51 (m, 1 H, H-6 (THP)), 2.25 (m, 2 H, H-3), 1.88–1.52 (m, 10 H, H-3, H-5 (THP), H-1, H-2); ^{19}F NMR ϕ 83.24 (t, J_{FH} = 11.2 Hz).

3-Hydroxy-2-(hydroxymethyl)-2-methylpropyl 7,7-Difluoro-10-(tetrahydropyranyloxy)deca-5,8-dienoate, 20. A solution of the OBO ester **19** (952 mg, 2.46 mmol) was stirred at room temperature for 1.5 h in 48 mL of a mixture of acetic acid, tetrahydrofuran, and water (4:2:1). Removal of the solvents in vacuo provided 997 mg of the neopentyl ester **20** (100%) (R_f 0.40, ethyl acetate), which was used without purification in the next step: yellow oil; ^1H NMR δ 5.89 (m, 1 H, H-9), 5.74–5.61 (m, 3 H, H-5, H-6, H-8), 4.61 (t, 1 H, J = 3.5, H-2 (THP)), 4.44 (m, 1 H, H-10), 4.27 (m, 1 H, H-10), 4.18 (s, 2 H, H-1 (THP)), 3.85 (m, 1 H, H-6 (THP)), 3.51 (m, 1 H, H-6 (THP obscured by 3.59 q), 3.59 (AB quartet, 4 H, J = 7.5, J = 15, H-3 (neopentyl)), 2.42 (t, J = 7, 2 H, H-2), 2.32 (q, 2 H, H-4), 1.90–1.52 (m, 8 H, H-3, H-4, H-5 (THP), H-3), 0.89 (s, 3 H, CH_3).

Methyl 7,7-Difluoro-10-(tetrahydropyranyloxy)deca-5,8-dienoate, 20a. To a solution of neopentyl ester **20** (997 mg, 2.46 mmol) in dry methanol (19 mL) was added potassium carbonate (715 mg, 5.18 mmol). The mixture was stirred at room temperature for 1.5 h. Workup with methylene chloride provided the methyl ester **20** (664 mg, 85%) which was used in the next step without furhter purification (R_f 0.38, 1:3 ethyl acetate/hexane): yellow oil; ^1H NMR δ 5.87 (m, 1 H, H-9), 5.73–5.60 (m, 3 H, H-5, H-6, H-8), 4.61 (t, 1 H, J = 3.5, H-2 (THP)), 4.45 (m, 1 H, H-10), 4.27 (m, 1 H, H-10), 3.88 (m, 1 H, H-6 (THP)), 3.51 (m, 1 H, H-6 (THP)), 3.68 (s, 1 H, OCH_3), 2.35 (t, 2 H, J = 7.5 Hz, H-2), 2.30 (m, 2 H, H-4), 1.88–1.52 (m, 8 H, H-3 (THP), H-4 (THP), H-5 (THP), H-3); ^{19}F NMR ϕ 83.35 (t, 9.1 Hz).

Methyl 7,7-Difluoro-10-hydroxydeca-5,8-dienoate, 21. The tetrahydropyranyloxy methyl ester **20a** (289 mg, 0.91 mmol) was stirred at room temperature for 3 h in 218 mL of 0.01 M *p*-toluenesulfonic acid monohyrate in methanol, under nitrogen. Aqueous workup and extraction with methylene chloride followed by flash chromatography on silica gel (1:1 ethyl acetate/hexane, R_f 0.33) provided the pure alcohol **21** (127 mg, 60%): ^1H NMR δ 5.91 (m, 1 H, H-9), 5.77–5.61 (m, 3 H, H-5, H-6, H-8), 4.39 (br s, 2 H, H-10), 3.68 (s, 3 H, OCH_3), 2.36 (t, 2 H, J = 7.5, H-2), 2.30 (m, 2 H, H-4), 1.76 (p, 2 H, J = 7.5, H-3).

Methyl 10-Bromo-7,7-difluorodeca-5,8-dienoate, 21a. Triethylamine (0.76 mL, 5.51 mmol) was added to a methylene chloride (29 mL) solution of the alcohol **21** (86 mg, 0.37 mmol). The solution was stirred

under nitrogen at 0 °C for 10 min, and methanesulfonyl chloride (0.28 mL, 3.67 mmol) was added. After 1.5 h at 0 °C the reaction was worked up with water and methylene chloride. This crude mesylate was used without further purification. A solution of lithium bromide (327 mg, 3.67 mmol) and the above mesylate in 30 mL of tetrahydrofuran was stirred at 25 °C under nitrogen for 16 h. Aqueous workup followed by extraction with methylene chloride and flash chromatography on silica gel (2:1 methylene chloride/hexane, R_f 0.26) yielded the pure bromide **21a** (91 mg, 84%): ^{1}H NMR δ 5.96 (m, 1 H, H-9), 5.80–5.63 (m, 3 H, H-5, H-6, H-8), 4.11 (d, 2 H, J = 8, H-10), 3.68 (s, 3 H, OCH_3), 2.36 (t, 2 H, J = 7.5, H-2), 2.31 (m, 2 H, H-4), 1.76 (p, 2 H, J = 7.5, H-3); ^{19}F NMR ϕ 82.77 (t, J = 10.8).

1,4-Decadiynyl Cuprate. To a stirred solution of ammonium hydroxide (3.8 mL) and copper sulfate pentahydrate (0.95 g, 3.8 mmol) was added water (15.2 mL) and hydroxylamine hydrochloride (0.53 g, 7.6 mmol). The initially dark blue solution turned green and colorless after 30 s. The solution was cooled to 0 °C, and 1,4-decadiyne (0.51 g, 3.8 mmol) in absolute ethanol (18.8 mL) was added. The resulting bright yellow suspension was stirred at 0 °C for 45 min. The solid was then filtered and washed with water, ethanol, and benzene, taking care that the filtercake was not allowed to dry. When allowed to dry the material turned dark brown. The cuprate was stored as a suspension in benzene and was transferred to the reaction vessel in this form. Removal of most of the benzene by centrifugation followed by trituration with the reaction solvent, DMF, and additional centrifugation provided the cuprate as a bright yellow solid.

Methyl 7,7-Difluoroeicosa-5,8-diene-11,14-diynoate, 21b. To a suspension of 1,4-decadiynyl cuprate (44.2 mg, 6.23 mmol) in 0.4 mL of DMF was added the bromide **21a** (47.8 mg, 0.16 mmol) and sodium cyanide (12.1 mg, 0.25 mmol). The greenish yellow suspension was stirred at 25 °C for 8 h. The color of the suspension did not change, and the reaction was worked up with water and methylene chloride. Flash chromatography on silica gel (2:1 methylene chloride/hexane, R_f 0.28) yielded 23.5 mg of crude **21b** (42%): colorless oil; ^{1}H NMR δ 5.79–5.61 (m, 4 H, H-5, H-6, H-8, H-9), 3.68 (s, 3 H, OCH_3), 3.17 (m, 2 H, H-10), 3.14 (m, 2 H, H-13), 2,37–2.27 (m, 4 H, H-2, H-4), 2.16 (m, 2 H, H-16), 1.77 (m, 2 H, H-3), 1.50 (m, 2 H, H-17), 1.37 (m, 4 H, H-18, H-19), 0.91 (t, 3 H, J = 7, H-20); ^{19}F NMR ϕ 82.85 (t, J = 10.8).

Methyl 7,7-Difluoroarachidonate, 22a. Catalytic semihydrogenation of the dienediyne methyl ester **21b** (3.8 mg, 0.01 mmol) was performed as described for the preparation of the 10,10-difluoro ester **18**. As in that case more than one reduction was often required, depending on the amount of impurities present in the dienediyne: yellow oil (3.7 mg); ^{1}H NMR δ 5.68 (m, 4 H, H-5, H-6, H-8, H-9), 5.42 (m, 2 H, vinyl), 5.34 (m, 2 H, vinyl), 3.67 (s, 3 H, OCH_3), 3.03 (m, 2 H, H-10), 2.80 (t, 2 H, J = 7.0, H-13), 2.33 (t, 2 H, J = 7.6, H-2), 2.30 (m, 2 H, H-4), 2.05 (q, 2 H, J = 7.0, H-16), 1.75 (p, 2 H, J = 7.5, H-3), 1.40–1.27 (m, 6 H, H-17, H-18, H-19), 0.90 (t, 3 H, J = 6.9, H-20); ^{19}F NMR ϕ 82.06 (m).

Enzymatic Hydrolysis of Methyl 7,7-Difluoroarachidonate, 22a. Methyl 7,7-difluoroarachidonate (12.3 mg, 0.035 mmol) was hydrolyzed as described for methyl 10,10-difluoroarachidonate (**18**). Purification was accomplished by RP-HPLC (65% CH_3CN/H_2O, pH 3.5, monitored at 205 nm), yielding 1.6 mg of pure **22** (17%).

7,7-Difluoroarachidonic Acid, 22: (74.0 min, 43.5%); ^{1}H NMR δ 5.69 (m, 4 H, H-5, H-6, H-8, H-9), 5.41 (m, 2 H, vinyl), 5.34 (m, 2 H, vinyl), 3.03 (m, 2 H, H-10), 2.80 (t, 2 H, J = 6.9, H-13), 2.38 (t, 2 H, J = 7.5, H-2), 2.33 (m, 2 H, H-4), 2.05 (q, 2 H, J = 7.1, H-16), 1.77 (p, 2 H, J = 7.3, H-3), 1.34-1.27 (m, 6 H, H-17, H-18, H-19), 0.90 (t, 3 H, J = 6.7, H-20); ^{19}F NMR ϕ 82.00 (m); high resolution MS (70 eV, m/z), calcd for $C_{20}H_{29}O_2F$ (M – HF) 320.2152, found 320.2142 (6.6%).

The crude material subjected to RP-HPLC contained the accumulated impurities from the cuprate coupling reaction, the catalytic reduction, and the enzymatic hydrolysis. The efficiency of the column allowed for the isolation of several of these byproducts and their characterization by NMR and mass spectrometry. The products are listed in the order of their elution from the column.

7-Fluoro-9-hydroxyeicosa-5,7,11,14-tetraenoic Acid: (23.1 min, 3.6%).

7-Fluoro-5-hydroxyeicosa-6,8,11,14-tetraenoic Acid: (25.3 min, 4.9%).

(Z)-8-Vinyl-7,7-difluorooctadec-5-ene-9,12-diynoic Acid, 23: (27.8 min, 3.0%); ^{1}H NMR δ 5.81 (m, 2 H, H-1′, H-5), 5.59 (m, 1 H, H-6), 5.54 (d, 1 H, J = 17.0, H-2′trans), 5.34 (dd, 1 H, J = 0.9, J = 9.7, H-2′cis), 3.62 (m, 1 H, H-8), 3.19 (m, 2 H, H-11), 2.39 (m, 4 H, H-2, H-4), 2.19 (m, 2 H, H-14), 1.78 (p, 2 H, J = 7.5, H-3), 1.50 (m, 2 H, H-15), 1.38 (m, 4 H, H-16, H-17), 0.91 (t, 3 H, J = 7.3, H-18); ^{19}F NMR (376.3 MHz) ϕ 92.24 (dt, J_{FF} = 247.1, J_{F_1H} = 12.8, F_1), 95.85 (dt, J_{F_2H} = 15.4, F_2).

(Z,Z)-8-Vinyl-7,7-difluorooctadeca-5,12-dien-9-ynoic Acid, 24: (46.7 min, 4.7%); ^{1}H NMR δ 5.80 (m, 2 H, H-1′, H-5), 5.57 (m, 1 H, H-6), 5.52 (d, 1 H, J = 16.7, H-2′trans), 5.44 (m, 2 H, H-12, H-13), 5.32 (d, 1 H, J = 9.6, H-2′cis), 3.60 (m, 1 H, H-8), 2.98 (d, 2 H, J = 6.1, H-11), 2.38 (m, 4 H, H-2, H-4), 2.05 (q, 2 H, J = 6.9, H-14), 1.77 (p, 2 H, H-3), 1.44 (p, 2 H, H-15), 1.32 (m, 4 H, H-16, H-17), 0.89 (t, 3 H, J = 6.6, H-18); ^{19}F NMR ϕ 92.3 (dt, J_{FF} = 246.0, J_{F_1H} = 10.8, F_1), 95.9 (dt, J_{F_2H} = 13.2, F_2); high resolution MS (70 eV, m/z), calcd for $C_{20}H_{27}O_2F$ (M – HF) 318.1995, found 318.1976 (3.0%).

7,7-Difluoroeicosa-5,8,14-trien-11-ynoate, 21c: (51.8 min, 5.7%); ^{1}H NMR δ 5.70 (m, 4 H, H-5, H-6, H-8, H-9), 5.39 (m, 2 H, H-14, H-15), 3.14 (m, 2 H, H-10), 2.91 (m, 2 H, H-13), 2.39 (t, 2 H, J = 7.6, H-2), 2.31 (m, 2 H, H-4), 2.04 (m, 2 H, H-16), 1.77 (p, 2 H, J = 7.5, H-3), 1.40–1.20 (m, 6 H, H-17, H-18, H-19), 0.90 (t, 3 H, J = 6.6, H-20); ^{19}F NMR ϕ 82.71 (m).

(Z,Z,Z)-8-Vinyl-7,7-difluorooctadeca-5,9,12-trienoic Acid, 25: (69.6 min, 4.5%); ^{1}H NMR δ 5.84 (m, 1 H, H-1′), 5.72 (m, 1 H, H-5), 5.64 (m, 1 H, H-6), 5.46 (m, 5 H, H-2′trans, H-9, H-10, H-12, H-13), 3.59 (m, 1 H, H-8), 2.81 (m, 2 H, H-11), 2.39 (m, 4 H, H-2, H-4), 2.03 (q, J = 6.7, 2 H, H-14), 1.77 (p, J = 7.9, 2 H, H-3), 1.38 (m, 2 H, H-15), 1.32 (m, 4 H, H-16, H-17), 0.90 (t, 3 H, J = 6.7, H-18); ^{19}F NMR ϕ 95.67 (m); high resolution MS (70 eV, m/z, calcd for $C_{20}H_{29}O_2F$ (M – HF) 320.2152, found 320.2103 (3.6%); calcd for $C_{13}H_{21}$ (M – $C_7H_9O_2F_2$) 177.1643; found 177.1630 (12.9%).

7-Fluoro-5-hydroxyeicosa-6,8,11,14-tetraenoic Acid 1,5-Lactone: (97.3 min, 2.6%).

After 113 min the eluting solvent was changed to 80% CH_3CN/H_2O. **Methyl 7,7-difluoroarachidonate** (27.4%) was eluted at 37.9 min.

4,4-Difluoroundeca-2,5-dien-1-yl Tetrahydropyranyl Ether, 26. To the difluorodiynl tetrahydropyranyl ether **7a** (284 mg, 1 mmol) and 5% palladium on barium sulfate (142 mg) was added 25% pyridine in ethanol (8 mL). The mixture was purged with H_2 and stirred under hydrogen (780 mm Hg) until the theoretical uptake was reached (about 1 h). It was then filtered through a short column of Celite by using CH_2Cl_2 as eluent. The solvent was removed, and the product was purified by flash chromatography (silica gel, 40–63 mμ, CH_2Cl_2/hexane (1:1) as eluent): yield 285 mg (99%); ^{1}H NMR δ 5.86 (m, 1 H, H-2), 5.72 (m, 2 H, H-3, H-6), 5.59 (dt, 1 H, $J_{4,5}$ = 12.4, $J_{5,6}$ = 12.0, H-5), 4.62 (t, J = 3.6, 1 H, H-2′), 4.39 and 4.22 (2 dm, $J_{1,1}$ = 14.5, 1 H and 1 H, H-1), 3.86 and 3.51 (2 m, 2 H, H-6′), 2.23 (m, $J_{6,7}$ = 12, $J_{7,8}$ = 7, 2 H, H-7), 1.86–1.50 (m, 6 H, H-3′, H-4′, H-5′), 1.40 (p, J = 7, 2 H, H-8), 1.35–1.25 (m, 4 H, H-9, 10), 0.90 (t, 2 H, J = 7.0, H-11); ^{19}F NMR ($CDCl_3$, 376.3 MHz) ϕ 83.15 (t, J = 12), 83.11 (t, J = 12).

4,4-Difluoroundeca-2,5-dien-1-ol, 27. To the THP ether **26** (576 mg, 2 mmol) in 15 mL of CH_3OH was added 139.4 mg (0.6 mmol) of camphorsulfonic acid, and the mixture was stirred at 25 °C for 2 h. Workup with EtOAc/H_2O and purification by flash chromatography, CH_2Cl_2/hexane (1:1), eluted the unreacted starting material (103.7 mg, 18%). The desired alcohol **27** was eluted with EtOAc/hexane (3:7) (298 mg, 74%): ^{1}H NMR δ 5.89 (m, 1 H, H-2), 5.72 (m, 2 H, H-3, H-6); 5.60 (m, 1 H, H-5), 4.39 (m, 2 H, H-1), 2.23 (m, 2 H, H-7), 1.40 (p, J = 7.2, 2 H, H-8), 1.31 (m, 4 H, H-9, H-10), 0.90 (t, 3 H, J = 7, H-11); ^{19}F NMR ($CDCl_3$, 376.3 MHz) ϕ 82.74 (t, J = 12.4).

4,4-Difluoroundeca-2,5-dien-1-yl Mesylate, 27a. The diene alcohol **27** (239 mg, 1.17 mmol) in 17 mL of CH_2Cl_2 was cooled to 0 °C. Triethylamine (711 mg, 7.03 mmol) was added followed by MsCl (671 mg, 5.86 mmol). The mixture was stirred at 0 °C for 1 h and then worked up with CH_2Cl_2/H_2O. The mesylate **27a** was used in the next reaction without further purification: ^{1}H NMR δ 5.75–5.87 (m, 3 H, H-2, H-3, H-6), 5.59 (dt, $J_{4,5}$ = 12.4, $J_{5,6}$ = 12.0, 1 H, H-5), 4.99 (m, 2 H, H-1), 3.03 (s, 3 H, CH_3SO_3–), 2.23 (m, 2 H, H-7), 1.40 (p, J = 7, 2 H, H-8), 1.31 (m, 4 H, H-9, H-10), 0.90 (t, J = 7, 3 H, H-11); ^{19}F NMR ($CDCl_3$, 376.3 MHz) ϕ 83.76 (t, J = 11.5).

1-Bromo-4,4-difluoroundeca-2,5-diene, 27b. The mesylate **27a** (330 mg, 1.17 mmol) and LiBr (1.02 g, 11.7 mmol) in 11.7 mL of dry THF was stirred at 25 °C for 16 h. The mixture was worked up with hexane/H_2O and purified by flash chromatography (CH_2Cl_2/hexane (1:4)): yield 237 mg (76% from alcohol **27**); ^{1}H NMR δ 5.95 (m, 1 H, H-2), 5.79 (m, 1 H, H-3), 5.72 (dt, $J_{5,6}$ = 12, $J_{6,7}$ = 7, 1 H, H-6), 5.63 (dt, $J_{4,5}$ = 12.4, $J_{5,6}$ = 12, 1 H, H-5), 4.12 (d, J = 8.4, 2 H, H-1), 2.25 (m, 2 H, H-7), 1.42 (p, J = 7, 2 H, H-8), 1.32 (m, 4 H, H-9, H-10), 0.90 (t, J = 7, 3 H, H-11); ^{19}F NMR ($CDCl_3$, 376.3 MHz) ϕ 82.47 (t, J = 12.4).

Methyl Nona-5,8-diynoate. A solution of nona-5,8-diynoic acid (410 mg) in 20 mL of diethyl ether was added at 0 °C to excess diazomethane in ethyl ether and allowed to remain at 0 °C for 15 min. It was then concentrated and chromatographed on silica gel (CH_2Cl_2/hexane (9:1)): ^{1}H NMR δ 3.68 (s, 3 H, $-OCH_3$), 3.14 (m, 2 H, H-7), 2.44 (t, J = 7, 2 H, H-2), 2.25 (m, 2 H, H-4), 2.06 (t, J = 1.2, 1 H, H-9), 1.83 (p, J = 7, 2 H, H-3).

Cuprate of Methyl Nona-5,8-diynoate, 28. To a solution of copper(II) sulfate pentahydrate (450 mg) in 1.8 mL of 28% ammonium hydroxide was added 250 mg of NH_2OH·HCl in 7.2 mL of water. After about 5 min, 295 mg of methyl nona-5,8-diynoate in 14 mL of absolute ethanol

was added. A yellow-orange precipitate was formed instantly. After stirring for another 15 min, the precipitate was filtered off and washed successively with H_2O (5 × 10 mL), EtOH (3 × 10 mL), ethyl ether (5 × 10 mL), and benzene (2 × 5 mL). It was then suspended in benzene (15 mL) and stored at −20 °C.

Methyl 13,13-Difluoroeicosa-11,14-dien-5,8-diynoate, 29. The dienecuprate (140 mg) suspension in benzene (7 mL) was centrifuged, the solvent was decanted, and the solid was further washed 4–6 times with ether followed by centrifugation and decanting the ether. It was then dried over N_2, mixed with 26.5 mg (0.54 mmol) of sodium cyanide and dissolved in 1.0 mL of dry DMF. The bromide **27b** (103 mg, 0.36 mmol) in 200 μL of dry DMF was then added, and the mixture was stirred at 25 °C for 2 h. It was worked up by quenching with H_2O and extracting with EtOAc. The ethyl acetate extract was concentrated and passed through a short silica gel column by using CH_2Cl_2 as eluent to remove most of the DMF. The methylene chloride extracts were concentrated and purified by flash chromatography by using CH_2Cl_2/hexane (1:1) as eluent. The crude methyl ester weighed 36 mg (29%): ^{1}H NMR δ 5.5–5.8 (m, 4 H, H-11, H-12, H-14, H-15), 3.68 (s, 3 H, $-OCH_3$), 3.16 (m, 2 H, H-10), 3.11 (m, 2 H, H-7), 2.44 (t, J = 6.8, 2, H, H-2), 2.24 (m, 4 H, H-4), H-16), 1.83 (p, J = 7.0, 2 H, H-3), 1.40 (p, J = 7.2, 2 H, H-17), 1.31 (m, 4 H, H-18, H-19); 0.90 (t, J = 7, 3 H, H-20); ^{19}F NMR ($CDCl_3$, 376.3 MHz) φ 82.62 (t, J = 11.4).

13,13-Difluoroeicosa-11,14-dien-5,8-diynoic Acid, 29a. The methyl ester **29** (36 mg) was stirred vigorously with *Rhizopus arrhizus* lipase (540 μL of suspension in 3.2 M ammonium sulfate, 0.01 M pH 6.0 potassium phosphate, 27 000 units) in potassium phosphate buffer (14.4 mL, 0.1 M pH 7.00) containing 14.4 mg of NaCl and 21.6 mg of gum arabic. After 50 min, over 90% hydrolysis was observed (according to NMR integration). The mixture was acidified with 1.4 mL of 0.1 N HCl to pH 3 and then extracted with ethyl acetate. The ethyl acetate extract was dried over Na_2SO_4, filtered, and concentrated. It was passed through a short silica gel column by using EtOAc/hexane (1:4) as eluent to remove the unreacted methyl ester, followed by EtOAc/hexane (1:1) + 0.5% AcOH as eluent to obtain the acid. The acid was further purified by reversed phase HPLC (65% CH_3CN/H_2O) to give 21.1 mg (59%) of the pure diene diyne acid **29a**: ^{1}H NMR δ 5.55–5.78 (m, 4 H, H-11, H-12, H-14, H-15), 3.17 (m, 2 H, H-10), 3.12 (m, 2 H, H-7), 2.50 (t, J = 7.2, 2 H, H-2), 2.27 (m, 2 H, H-4), 2.23 (m, 2 H, H-16), 1.84 (p, J = 7.1, 2 H, H-3), 1.40 (p, J = 7.1, 2 H, H-17), 1.31 (m, 4 H, H-18, H-19), 0.90 (t, J = 6.7, 3 H, H-20); ^{19}F NMR ($CDCl_3$, 376.3 MHz) φ 82.61 (t, J = 12).

13,13-Difluoroarachidonic Acid 30. The diene diyne acid **29a** (10.4 mg, 0.031 mmol) was hydrogenated over 5% palladium on calcium carbonate poisoned with lead (Lindlar's catalyst, 5.2 mg) in toluene (500 μL) containing 5 μL of 5% synthetic quinoline in toluene. The reaction was terminated after 30 min at 25 °C, during which time 1.6 mL of hydrogen was taken up (theory: 1.5 mL). The reaction mixture was filtered through Celite, and the filtrate was concentrated under a stream of nitrogen to give the crude product. This was purified by reversed phase HPLC (65% CH_3CN/H_2O) to give 6.4 mg (62%) of pure 13,13-difluoroarachidonic acid (**30**): ^{1}H NMR δ 5.6–5.78 (m, 4 H, H-11, H-12, H-14, H-15), 5.34–5.43 (m, 4 H, H-5, H-6, H-8, H-9), 3.03 (m, 2 H, H-10), 2.80 (t, J = 5.8, 2 H, H-7), 2.36 (t, J = 7.2, 2 H, H-2), 2.24 (m, 2 H, H-16), 2.13 (dt, $J_{3,4}$ = 7.2, $J_{4,5}$ = 6.8, 2 H, H-4), 1.72 (p, J = 7.2, 2 H, H-3), 1.40 (p, J = 7.2, 2 H, H-17), 1.31 (m, 4 H, H-18, H-19), 0.90 (t, J = 7.0, 3 H, H-20); ^{19}F NMR ($CDCl_3$, 376.3 MHz) φ 81.80 (t, J = 12.4).

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Enzymatic Conversions of 10,10-Difluoroarachidonic Acid with PGH Synthase and Soybean Lipoxygenase

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Abstract: 10,10-Difluoroarachidonic acid (**1**) was found to be a substrate for PGH synthase and soybean lipoxidase. PGH synthase catalyzed the conversion of this substrate to (11*S*)-10,10-difluoro-11-hydroxyeicosa-5(*Z*),8(*Z*),12(*E*),14(*Z*)-tetraenoic acid (10,10-difluoro-11*S*-HETE, **4**) and (8,15*S*)-10-fluoro-8,15-dihydroxyeicosa-5(*Z*),9(*Z*),11(*Z*),13(*E*)-tetraenoic acid (10-fluoro-8,15-diHETE, **5**), the latter as a mixture of 8-epimers. Cyclization to prostaglandins was not observed. The same epimeric mixture **5** was also obtained on incubation of **1** with soybean lipoxidase, a 15-lipoxygenase, followed by reduction with sodium borohydride. When exposed to aqueous buffer solutions between pH 7 and 9 diallylic difluorides such as **1** or 7,7-difluoroarachidonic acid (**6**) underwent S_N' substitution of fluoride by water with the formation of the fluoroHETES **2**, **3**, **7**, and **9**. In the case of the 7,7-acid, attack by carboxylate anion furnished the 1,5-lactone **8** in addition to **7**. The formation of diHETE **5** is the result of both enzymatic oxygenation and S_N' substitution.

In the accompanying paper we have described a general synthesis of polyunsaturated fatty acids, in which a methylene group residing between two double bonds is replaced by a CF_2 group.[1] Polyunsaturated acids are substrates for a variety of oxygenase enzymes of both plant and animal origin. Our interest in these fluorinated acids was to examine their ability to serve as substrates of such enzymes, most notably of PGH synthase,[2] the enzyme responsible for the biosynthesis of PGH_2, which in turn is the precursor for all the prostaglandins. If successful, fluorinated prostaglandins could be prepared rapidly for biological studies by such a procedure.

We describe here our results of incubations of 10,10-difluoroarachidonic acid (10,10-DFAA) with PGH synthase derived from ram seminal vesicle microsomes (RSVM) and with soybean lipoxygenase. Prior to this work several investigators have examined the substrate specificity of PGH synthease as measured by prostaglandin formation by varying the chain length and number or position of double bonds[3-7] of the fatty acid and by

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