Synthesis of Fluoro Analogues of Unsaturated Fatty Acids and Corresponding Acyclic Metabolites

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Keywords: Fluorine / Fatty acids / Eicosanoids / Stereoselective synthesis

Polyunsaturated fatty acids, in particular arachidonic acid and its metabolites, the so-called eicosanoids, play a major role in human health. Many of them have been involved as potent mediators for various diseases such as inflammation and asthma. Therefore, numerous modifications in the structures of these natural products have been incorporated to study the structure-activity relationship, monitor the biosynthetic pathway and also to understand the biological activities as well as the mechanisms of action. The modifications include introduction of labelled elements, fluorine atoms, heteroelements, carbo- and heterocycles etc. Of all these studies, the introduction of fluorine, which has a unique role as the smallest atom in the periodic table after hydrogen and also as the most electronegative element, provided very useful information. Thus, the present microreview highlights the synthetic efforts of various research groups in the last two decades on bioactive fluorinated fatty acids and corresponding acyclic metabolites. It also presents the most relevant bioactivity data obtained from the fluorinated analogues. The data corresponding to naturally occurring fluorinated fatty acids have been added, as well as the use of fluorinated probes for some biosynthetic studies in fatty acid chemistry (desaturation and methylenation). In addition, it is important to point out that the methods and strategies which have been developed to selectively introduce a single fluorine atom, as well as CF_2 or CF_3 groups, on such very challenging molecules could also be of much use in many other areas of chemistry.

Introduction

Even though elemental fluorine was first isolated by Henri Moissan[1] way back in 1886, this atom has gained prominence only in the last decades. This importance may be attributed, at least in part, to developments in life sciences which demand new classes of molecules with differential biological properties. It has been proved beyond doubt that the incorporation of fluorine into organic molecules causes them to show exceptionally new biological profiles. The reasons for these special biological and physical properties may be attributed to the unique features of this element: (a) Fluorine has the highest electronegativity. (b) It is the second smallest element next to hydrogen. (c) It can form strong bonds with many elements, especially with carbon atoms. Therefore the exchange of strategic C–H and/or C–OH bonds by C–F bonds will induce strong modifications of the physical, chemical and biological properties of the corresponding molecules. This has been demonstrated in many cases, especially in biological and medicinal chemistry.[2] Fluorine-containing compounds are already of much use as pharmacological tools and they have appeared also in many commercial drugs. Because of increasing interest and excitement about the fluorine-containing derivatives relevant to organic and biomedical research, series of reviews and books have been published.[2a–2d] Surprisingly however, a consolidated review pertaining to the synthesis of fluorinated long chain unsaturated fatty acids and corresponding acyclic metabolites has not appeared yet, despite the fact that these molecules have attracted many research groups in the fields of both synthetic organic and medicinal chemistry.

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 DOI: 10.1002/ejoc.200400585

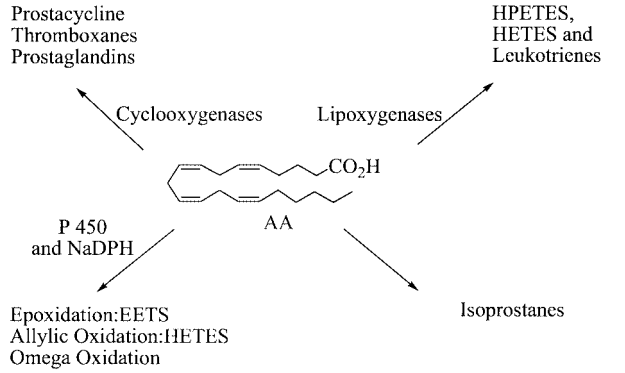
Fatty acids constitute the simplest lipids, which are characterized as long-chain hydrocarbons with a terminal carboxyl group. They are present in nature in either saturated or unsaturated state, with one or more (*Z*)-double bonds. There are four main unsaturated fatty acids: linoleic acid, α-linolenic acid, γ-linolenic acid and arachidonic acid. In the presence of suitable enzymes, linoleic acid/α-linolenic acid can be converted to arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). All these acids can then be transformed further into many important metabolic compounds. Of all the fatty acids, AA has been the most studied, since it has been clearly established that the eicosanoids arising from the arachidonic cascade play a significant role in human health.

This review will consider first the fluorinated analogues of arachidonic acid, and then in a second part, the analogues of the AA metabolites. In the third part, it will be dealing with the fluorinated analogues of linoleic acid and corresponding metabolites. The final section will consider the natural fluorinated fatty acids and the use of fluorinated compounds to establish the pathways in the biosynthesis of fatty acids.

1. Arachidonic Acid and Fluorinated Analogues

1.1. Summary of the Arachidonic Acid Metabolism: Why and Where To Introduce Fluorine Atom(s)

It is known that AA can be metabolized into several dozens of compounds. Three major pathways have been reported for the oxidative metabolism of AA, depending upon the enzymatic system involved (Scheme 1). Firstly, under the influence of cytochrome P 450 and NADPH, AA undergoes three types of transformation namely epoxidation, allylic oxidation and/or omega oxidation.[3]



Scheme 1. Principal pathways for the arachidonic acid oxidative metabolism.

Similarly under the influence of the cyclooxygenases, prostaglandin H_2 (PGH_2) is produced, which in turn results in prostacyclin, thromboxanes and various types of prostaglandins (PGs).[4] Finally, lipoxygenases afford hydroperoxy-eicosatetraenoic acids (HPETEs) and their corresponding alcohols (HETES) as well as the leukotrienes.[5] More recently, a nonenzymatic and radical-type metabolic pathway leading to the isoprostanes has been discovered.[6] A large number of these AA metabolites have demonstrated very potent biological properties. For instance, leukotrienes are known to be important mediators of respiratory diseases such as asthma, as well as inflammatory disorders such as rheumatoid arthritis. Prostacyclin has antiaggregant properties for human blood platelets, while thromboxane A_2 has the opposite effect. Prostaglandins also have very potent biological actions and several PG analogues are marketed as drugs in such diverse areas as antiulcer, antihypertensive, antiglaucoma treatment and fertility control, as well as in some veterinary uses.[4] Thus, blocking or activating a particular metabolic pathway in the AA cascade is essential in regulating many physiological phenomena. For instance, inhibition of cyclooxygenase is a key factor in the

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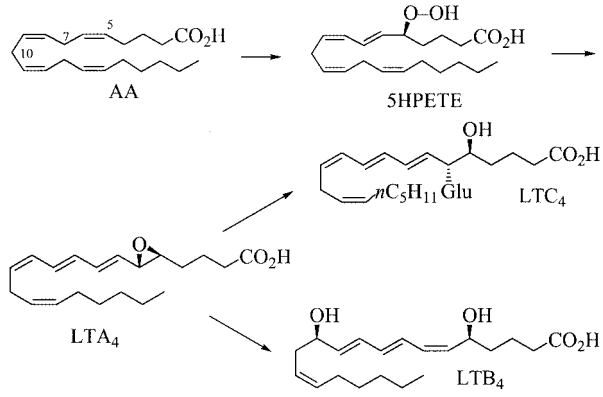
activity of many Non Steroidal Anti Inflammatory Drugs (NSAIDS).[7] Inhibition of 5-lipoxygenase (5-LO), or development of 5-LO antagonists, lead also to novel antiasthma drugs. Such inhibitions or activations necessitate an in-depth knowledge of biological events.[8] Therefore, in order to establish structure-function relationships, many analogues have to be prepared and studied with regard to their biological activity.

The skipped "*cis*" diene part of the fatty acid (AA in particular) is a central focus point for the metabolism, and most of the oxygenated metabolites are produced by modifications at around this part of the molecule. The biosynthesis of leukotrienes via the 5-LO is representative of such mechanisms (Scheme 2). In the first step, 5-LO introduces molecular oxygen at C^5 of AA with concomitant abstraction of the pro-*S*-hydrogen at C^7 and formation of a C^6–C^7 double bond producing a 5-hydroperoxide, 5-HPETE.



Scheme 2. Biosynthesis of leukotrienes by the 5-LO pathway.

In a second step, 5-LO catalyses the abstraction of the pro-*R*-hydrogen at C^{10} of 5-HPETE with concomitant formation of two double bonds (C^7–C^8 and C^9–C^{10}) and of an epoxide (C^5–C^6) with the elimination of a hydroxyl group, generating leukotriene A_4 (LTA_4).[9] Ring opening by glutathione affords the peptidoleukotriene LTC_4, which is transformed to the other members of this family, LTD_4 and LTE_4. On the other hand, enzymatic hydrolysis affords leukotriene B_4 (LTB_4).

Taking into account such a mechanism, it can be anticipated easily that the exchange of selected hydrogens and/or hydroxyl groups by fluorine atoms will induce major changes in the biological properties as well as in the metabolic pathway. Therefore, the strategic positions to be considered for such an exchange are

i) the vinylic protons, such as H^5, H^6 or H^8, H^9,

ii) the hydrogens in bis allylic positions, such as H^7, H^{10}, or H^{13},

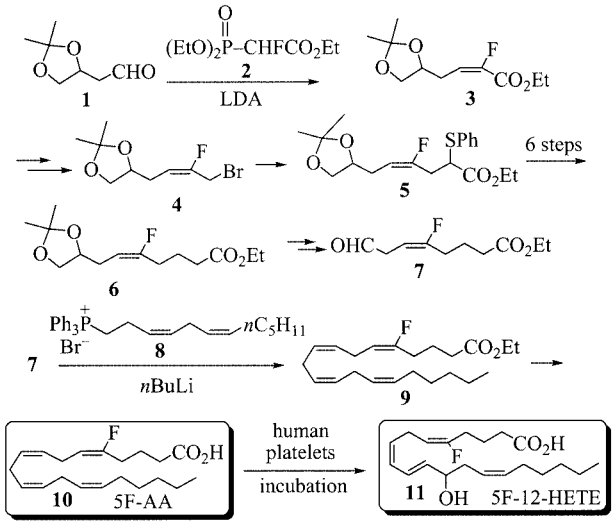
iii) the hydrogens close to the chain termini, such as H^{20}, H^{19} and H^2, which are sensitive to degradative processes,

iv) the OH groups in the various types of metabolites.

It is recognised easily that the preparation of all corresponding fluorinated analogues will be a big challenge, since the corresponding polyunsaturated molecules are usually very sensitive. Therefore, the development of novel strategies and methodologies was necessary in order to introduce selectively one fluorine atom, or CF_2 as well as CF_3 groups, in the appropriate molecules. These approaches could certainly also be of much use in other areas of chemistry.

1.2. Synthesis of 5F-AA

One of the earliest syntheses of 5-fluoroarachidonic acid (**10**) and its biotransformation into 5-fluoro-12-hydroxy-eicosatetraenoic acid (5F-12-HETE) (**11**) was achieved by Kobayashi et al. Their strategy involved Wittig type reactions both to introduce the vinylic fluoride in position 5 and also to establish the C^8–C^9 connection (Scheme 3).[10]

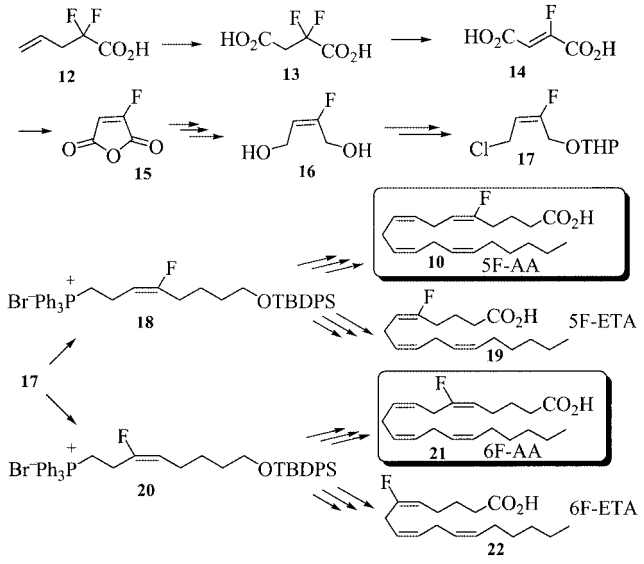


Scheme 3. 5-Fluoroarachidonic acid synthesis and metabolism.

The first key step of the synthesis involved a stereoselective Horner–Wadworth–Emmons olefination between the acetonide **1** and the fluorophosphonoacetate **2** in the presence of LDA to generate the (*E*)-fluoroenoate **3**. Reduction of the ester functionality and bromination via the mesylate to prepare the bromofluoro olefin **4** was followed by alkylation with the lithium salt of ethyl 2-(phenylthio)acetate to afford the chain-elongated intermediate **5**. Furthermore, one-carbon elongation was performed by desulfurisation, reduction and cyanation via the bromide to achieve ester **6** after cyano hydrolysis and esterification. Hydrolysis of acetonide on the other end, followed by treatment with $Pb(OAc)_4$ produced β,γ-unsaturated aldehyde **7**, which upon Wittig reaction with phosphonium salt **8** in the presence of *n*BuLi gave ethyl-5-fluoroarachidonate (**9**). The free 5-fluoro-AA (**10**), obtained after saponification of **9**, was incubated with human platelets to produce in a very convenient way the 5F-12-HETE **11**. Since it is well-established that the major product of AA metabolism by platelets is usually 12-HETE,[11] the introduction of fluorine in position 5 did not change, in this case, the metabolism by the 12-LO pathway.

1.3. Synthesis of 5F-AA, 6F-AA, 5,6-diF-AA

Ducep et al. endeavoured an elegant and more versatile synthesis of 5- and 6-fluoro derivatives of eicosatrienoic and eicosatetraenoic acids, also using fluorinated alkenes and Wittig reactions.[12] Their approach was most flexible in that a common intermediate, the fluoro olefin **17** with the two differentiated chains, allowed them to synthesise both of the proposed structures with ease, though in multistep sequences (Scheme 4).



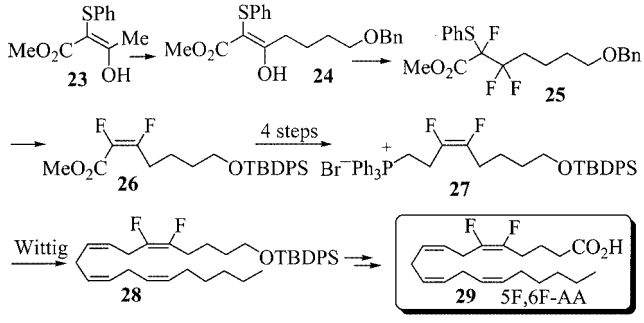
Scheme 4. Synthesis of 5-fluoro- and 6-fluoroarachidonic and eicosatrienoic acids.

The versatile building block **17** could be obtained easily on large scale, and furthermore with complete stereocontrol, in eight steps from α,α-difluoro-4-pentenoic acid (**12**). A one-carbon homologation using dithiane chemistry on one end, followed by a three-carbon homologation (using allyl phosphoramide chemistry) on the opposite end, furnished the fluoro Wittig salt **18**. Reversal of the homologation sequence, i.e. 3-carbon homologation on the chloro end, followed by 1-carbon homologation on the opposite end, furnished the regioisomeric Wittig salt **20**. Exposure of these two salts to 6-dodecen-1-al and 3,6-decadienal, respectively, followed by deprotection and oxidation produced the four fluorinated analogues 5-F-ETA (**19**), 6F-ETA (**22**), 5F-AA (**10**) and 6F-AA (**21**).

Both analogues **10** and **21** were found to be effective and about equipotent inhibitors, in the micromolar range, of the rat basophilic leukaemia cell (RBL-1) 5-LO in vitro.[13] However, they have shown a different metabolism: **10** was a poor substrate for the 5-LO, being converted to 5-oxotetraenoic acid. On the contrary, **21** was a good substrate, which was converted mainly to 5-hydroperoxy-6-fluoroeicosatetraenoic acid, with a small amount the corresponding 5-oxo acid. The effect on LTC_4 production by intact cells was also investigated. It has been found that 6F-AA was an inhibitor of LTC_4 release in intact mouse peritoneal macrophages. Furthermore, competition experiments have shown that **21** was incorporated into the phospholipids pool more efficiently than **10** and AA. These data could explain, at least in part, why 6F-AA **21** is a better inhibitor of LTC_4 release than 5F-AA (**10**).

1.4. Synthesis of 5,6-diF-AA

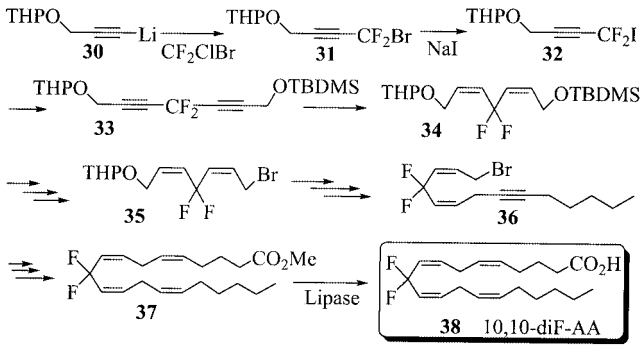
Encouraged by the results of monofluoro-AA, Ducep's group went on to synthesise, using again Wittig type reactions, the 5,6-difluoro-AA **29** as a potential inhibitor of 5-LO (Scheme 5).[14] Methyl-2-phenyl thioacetoacetate (**23**), upon alkylation with 3-benzyloxy-*n*-propyl bromide followed by MeDAST treatment, produced the trifluoro derivative **25**, which on dethiolation, protective group manipulations and β-elimination, produced methyl-2,3-difluoro-7-*O*-silyl-2-heptenoates as a (4:1) mixture of stereoisomers easily separated by chromatography. Starting from the major (*Z*) isomer **26**, the reduction of the ester group to alcohol, bromination and one-carbon homologation with concomitant salt formation were achieved by treatment with (trimethylsilyl)methylenetriphenylphosphorane. Wittig reaction between this salt, **27**, and dodecadienal followed by silyl deprotection and oxidation yielded the 5,6-difluoroarachidonic acid **29**.



Scheme 5. Synthesis of 5,6-difluoroarachidonic acid.

1.5. Synthesis of 7,7-, 10,10-, 13,13-diF-AA

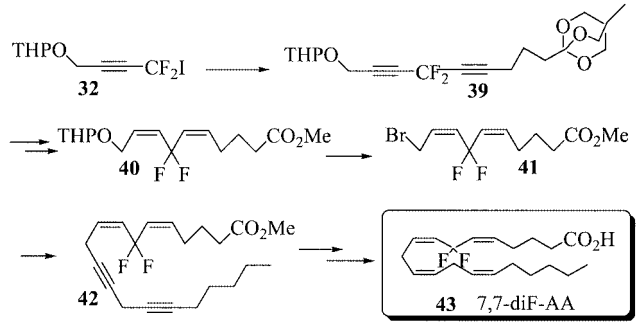
J. Fried et al. have developed elegant approaches for the synthesis of 7,7-, 10,10- and 13,13-difluoroarachidonic acids, which potentially became the starting point for the biosynthesis of fluorinated thromboxanes and prostaglandins.[15] Their strategy was different from the previous syntheses, since they used acetylenic-type chemistry followed by controlled *cis* hydrogenation with the appropriate Pd catalysts (Scheme 6).



Scheme 6. Synthesis of 10,10-difluoroarachidonic acid.

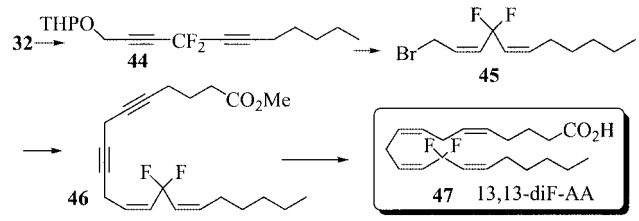
The difluoroacetylene **31** was easily prepared by addition of the lithium acetylide **30** to bromochlorodifluoromethane. After exchange to the iodo derivative **32**, the alkylation onto the lithium anion of propargyl alcohol THP ether produced the protected bis acetylenic diol **33**. The latter derivative, on reduction with Pd-$BaSO_4$ produced *cis* diene **34** and was successfully converted to bromide **35** in three steps. A second alkylation on 1-heptyne cuprate in the presence of NaCN, followed by silyl deprotection and bromination via the mesylate, produced the difluorodienyne bromide **36** in modest yield. Further coupling with the cuprate of 5-hexynoic acid methyl ester produced the C-20 unit with a difluoro group at C^{10} and partial reduction with Lindlar′s catalyst afforded ester **37**. At this stage, hydrolysis proved to be unsatisfactory under both basic and acidic conditions. Therefore, this last step could only be performed by using a lipase (*Rhizopus arrhizus*) to produce the 10,10-difluoroarachidonic acid **38**. It is of interest to note that this latter analogue proved to be more resistant to autoxidation than the arachidonic acid.

A similar cuprate-coupling and semihydrogenation strategy was extended for the synthesis of 7,7-difluoro- and 13,13-difluoroarachidonic acids (**43** and **47**). Both started from the same intermediate (**32**), but involved the sequential introduction of the appropriate side chains (Scheme 7 and Scheme 8).

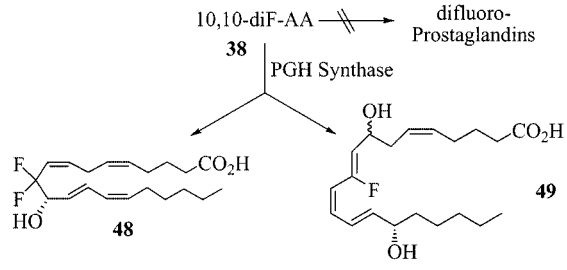


Scheme 7. Synthesis of 7,7-difluoroarachidonic acid.

Then, the 10,10-difluorinated AA (**38**) was subjected to enzymatic conversion[16] with PGH synthase to produce 10,10-difluoro-11(*S*)-HETE (**48**) and 10-fluoro-8,15-di-HETE (**49**) (Scheme 9). It is important to note that, under those conditions, no fluorinated prostaglandins could be obtained, in contrast to the normal biochemical pathway starting from AA. The same mixture of **48** and **49** was also obtained by using the soybean lipoxygenase (a 15-LO), followed by $NaBH_4$ reduction. These results again clearly indicate major modifications of the biochemical pathways by introduction of the *gem*-difluoromethylene group in this position.



Scheme 8. Synthesis of 13,13-difluoroarachidonic acid.

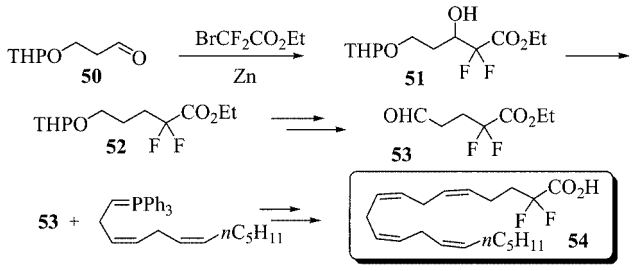


Scheme 9. Bioconversion of 10,10-difluoroarachidonic acid.

It is worth mentioning that the same authors have prepared, by total synthesis, both the 10,10-difluoro-dehydroprostacyclin[17] and the difluorothromboxane A_2.[18] These fluorinated analogues of cyclic AA metabolites have demonstrated not only potent biological properties, but also an impressive increase in chemical and biological stability.

1.6. Synthesis of 2,2-diF-AA

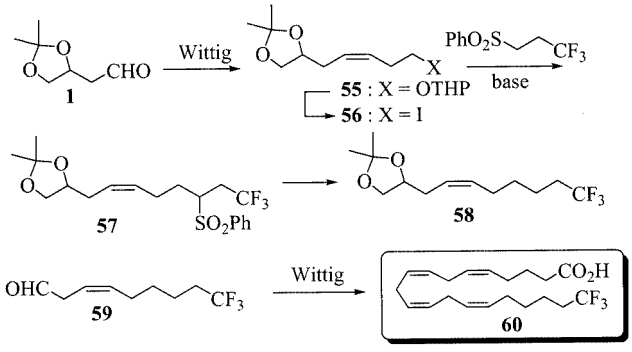
2,2-Difluoroarachidonic acid (**54**) appeared as another attractive target molecule since the two fluorine atoms in position 2 should enhance, by electronic factors, the acidity of the carboxyl group and also the stability of the molecule towards degradation by β-oxidation. The synthesis of this compound is represented in Scheme 10. The CF_2 group was introduced by a Reformatsky type reaction on aldehyde **50**. A dehydration, followed by hydrogenation to **52** and functional group transformation, afforded the aldehyde **53**, which, after the Wittig reaction and saponification, gave the desired 2,2-diF-AA (**54**).[19] The corresponding difluoro analogues of linoleic and linolenic acids were also prepared by the same route.



Scheme 10. Synthesis of 2,2-difluoroarachidonic acid.

1.7. Synthesis of 20-CF_3-AA

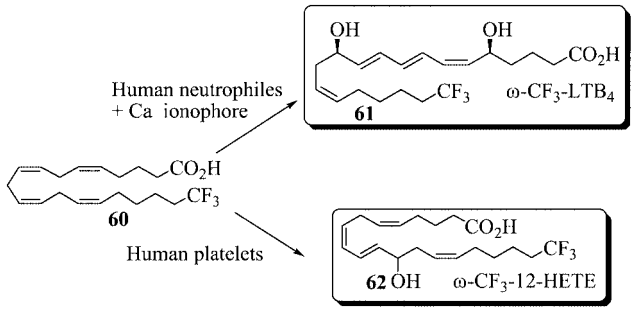
The C^{20}-trifluorinated arachidonic acid **60** was prepared from 3,4-(isopropylidenedioxy)butyraldehyde (**1**) by Kobayashi et al. (Scheme 11).[20] A Wittig olefination with 3-(tetrahydropyranyloxy)propylphosphorane, followed by a deprotection of –OTHP ether and iodination, generated the (isopropylidenedioxy)heptene iodide **56**. Alkylation with 2-trifluoromethyl-1-(phenylsulfonyl)ethane produced the ω-CF_3 derivative **57**.



Scheme 11. Synthesis of 20-CF3 arachidonic acid.

After reduction to **58**, isopropylidene group cleavage, followed by periodate oxidation, produced aldehyde **59** uneventfully. A second Wittig olefination with the appropriate phosphorane resulted in 20-CF_3-AA (**60**), after purification by chromatography.

It has to be mentioned that incubation of **60** with human neutrophiles, in the presence of a calcium ionophore, afforded the ω-CF_3-LTB_4**61** (Scheme 12). This analogue demonstrated the same chemotactic activity as the natural LTB_4, however with a much higher stability, probably because of the CF_3 group blocking the ω-oxidation metabolism. In a similar manner, incubation with human platelets afforded the ω-CF_3-12-HETE **62**. Compared to natural 12-HETE, the latter derivative had the same chemotactic activity on human neutrophiles and the same binding activity to the 12-HETE receptor, but it had again a very high metabolic stability.[21]

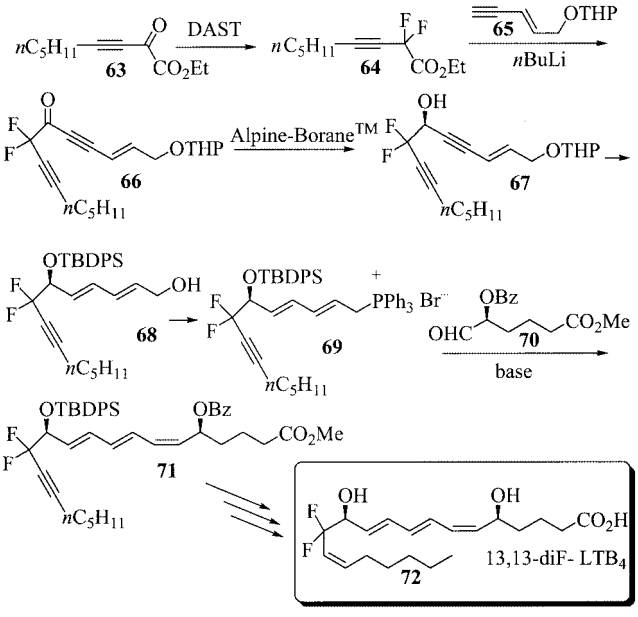


Scheme 12. Biotransformations from 20-CF3 arachidonic acid.

2. Leukotriene Analogues

2.1. Synthesis of 13,13-diF-LTB_4

Kobayashi et al. were instrumental in developing a different strategy for the synthesis of 13,13-difluoroleukotriene B_4 (**72**) (Scheme 13).[22] The two fluorine atoms were efficiently introduced by the DAST reaction on the propargylic ketone **63** to obtain **64**, which, upon further reaction with the lithiated acetylenic derivative **65**, produced prochiral ketone **66**, ready for asymmetric reduction with Alpine-Borane™.



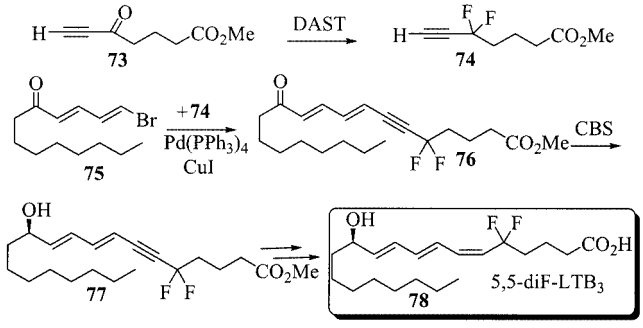
Scheme 13. Synthesis of 13,13-difluoroleukotriene B4.

Subsequent reduction of the C^4–C^5 triple bond (Red-Al), silyl protection and THP-deprotection produced the dienol **68**. Bromination and exposure to PPh_3 produced the Wittig salt **69**, which, upon reaction with the known chiral hydroxy aldehyde **70** furnished the C-20 skeleton **71**. The next two steps, silyl deprotection and reduction of the triple bond to the *cis* double bond, were rather poor in yield. After potassium carbonate-mediated ester hydrolysis, purification by

reverse-phase HPLC provided the 13,13-difluoro-LTB$_4$**72**. This analogue, because of its C^{12}-hydroxyl group and also because of its enhanced polarity by virtue of the strong electron-withdrawing nature of fluorine, may show enhanced biological activity and also provide more information about the nature of the binding or receptor site of the enzyme, or the receptor site of the target tissues.

2.2. Synthesis of 5,5-diF-LTB$_3$

The hydroxyl group and fluorine atom have close electronic and steric properties, but they have very different hydrogen-bonding patterns. Therefore, the exchange of OH by F could give useful information about the importance of the hydrogen bonds in the binding of bioactive molecules to their receptors. As part of a programme dealing with the preparation and biological evaluation of different mono- and difluoro-LTB$_3$′s, the 5,5-difluoro-LTB$_3$**78** was prepared as indicated in Scheme 14.

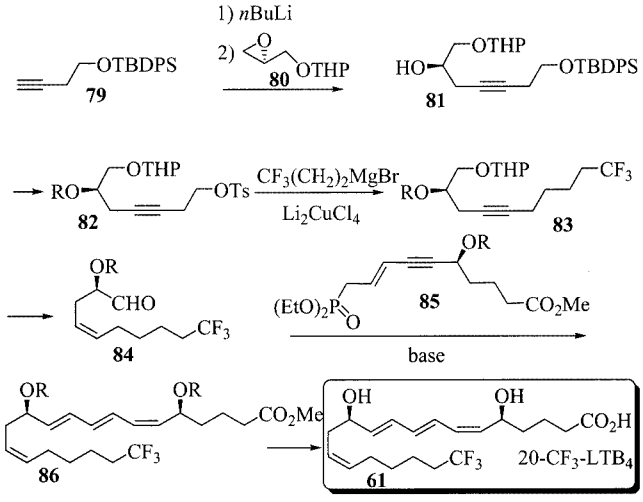


Scheme 14. Synthesis of 5,5-difluoroleukotriene B3.

The *gem*-difluoro intermediate **74** was obtained by the DAST reaction with ynone **73**. Then, Sonogashira coupling with bromodienone **75** afforded **76**. Alcohol **77** was obtained in high *ee* (>88%) by a CBS type asymmetric reduction of **76**. A semihydrogenation by using Lindlar's catalyst followed by saponification afforded the target molecule **78**. A similar strategy has been developed for the synthesis of the 5-fluoro-LTB$_3$.[23]

2.3. Synthesis of 20-CF$_3$-LTB$_4$

In parallel to their bioconversion studies of the 20-CF$_3$-AA, the Kobayashi group went on to prepare by total synthesis the 20-CF$_3$-LTB$_4$**61** in order to study the metabolism and binding properties of the latter derivative (Scheme 15).[20] Lithiated homopropargyl-*tert*-butyl diphenyl silyl ether (**79**) was treated with (*R*)-glycidyl THP ether (**80**) to produce heptynol derivative **81**. Silyl deprotection, primary-alcohol-selective tosylation and silylation of the secondary hydroxyl group produced **82**.

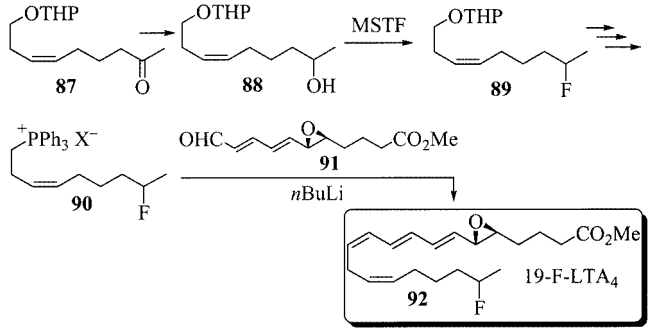


Scheme 15. Synthesis of 20-CF3 leukotriene B4.

Exposure of this tosylate to 2-(trifluoromethyl)ethylmagnesium bromide in the presence of dilithium tetrachlorocuprate furnished the ω-trifluoromethyl alkyne **83**. Reduction of the triple bond (Pd-BaSO$_4$) and THP deprotection followed by PDC oxidation resulted in the aldehyde **84**. Horner–Wadworth–Emmons olefination with phosphonate **85** in the presence of LDA, followed by exposure to Pd-BaSO$_4$ yielded 20-trifluoromethyl disilyloxy LTB$_4$**86**. First, the silyl groups were knocked down. Subsequent stirring in saturated NaCl solution and HPLC purification resulted in the sought 20-CF$_3$-LTB$_4$ (**61**).

2.4. Synthesis of 19-F-LTA$_4$

The introduction of fluorine in the ω-1 position is also a suitable option to inhibit the degradation of polyunsaturated fatty acids and corresponding metabolites. Therefore, Rossi et al. have explored the synthesis of 19(*R*,*S*)-F-LTA$_4$ methyl ester (**92**) by using a flexible strategy (Scheme 16).[24] The synthesis involved initial preparation of **89** in two steps from the easily accessible derivative **87**. After conversion to the Wittig salt **90** and reaction with the epoxydienal ester **91** in the presence of *n*BuLi, the required ω-(19-*R*,*S*)-LTA$_4$ methyl ester (**92**) was obtained.



Scheme 16. Synthesis of 19-fluoroleukotriene A4 methyl ester.

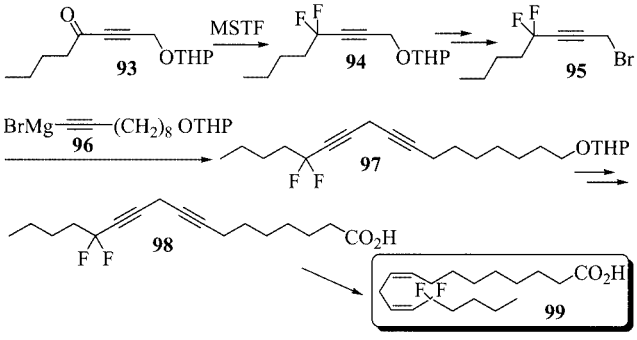
It is worth mentioning that the same strategy was used to prepare the corresponding acetylenic analogues: such

14,15-dehydroleukotrienes can be of interest, for instance, with regard to labelling experiments. Furthermore, compounds such as **87** and **88** are also useful intermediates for the introduction of various amino substituents in position 19. All these analogues appear as new molecular probes for the characterisation of a potential LTC_4 receptor.

3. Fluorinated Analogues of Linoleic Acid and Corresponding Metabolites

3.1. Synthesis of 14,14-diF Linoleic Acid

The group of Kornilov was instrumental in developing the versatile strategy for the incorporation of a CF_2 group adjacent to the acetylenic group and for the further transformation into 14,14-difluorolinoleic acid (**99**) (Scheme 17).[25]



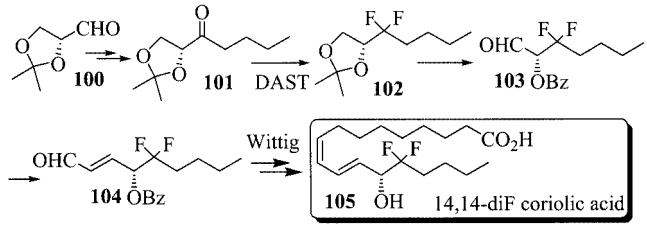
Scheme 17. Synthesis of 14,14-difluorolinoleic acid.

The *gem*-difluorination of propargylic ketone **93**, affording **94**, was performed by using morpholino DAST. After deprotection and transformation to the bromide **95**, a copper-mediated coupling with Grignard **96** led to bis propargylic derivative **97**. Deprotection, followed by oxidation and semihydrogenation of the two triple bonds, afforded the target molecule **99**. It was reported that 4,4-difluoroarachidonic acid could be prepared using a similar approach.

3.2. Synthesis of 14,14-diF Coriolic Acid

Coriolic acid (also called 13-HODE) is yet another metabolite of linoleic acid isolated from rice; it acts as a self-defence substance against rice blast disease[26a] and has attracted much attention from organic chemists. This substance is also present in heart mitochondria[26b] as well as in the sera of patients with familial Mediterranean fever[26c] and possesses cation-specific ionophoric activity. Some reports have also shown its role in controlling thrombosis.[26d]

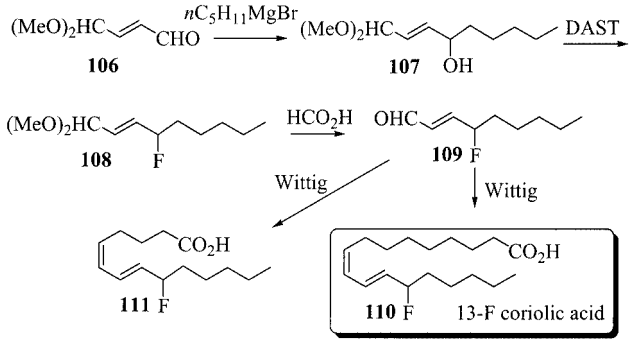
Kornilov et al. proposed that the presence of two fluorine atoms adjacent to the hydroxy carbon should exhibit enhanced biological activity because of increase in polarity. The total synthesis of (*R*)-14,14-difluorocoriolic acid **105** began from (*D*)-glyceraldehyde acetonide **100**, which, upon Grignard reaction with butylmagnesium bromide followed by Swern oxidation, furnished ketone **101**. Exposure of this compound to DAST furnished the difluoroacetonide **102** as a major product. This, upon hydrolysis of the acetonide group followed by protection-deprotection-oxidation, furnished the α-benzoyloxy aldehyde **103**. The latter derivative, on Wittig homologation with (formylmethylene)-triphenyl phosphorane yielded the α,β-unsaturated aldehyde **104**. The ester side chain was introduced by yet another Wittig olefination with [8-(methoxycarbonyl)octyl]triphenylphosphonium bromide. A final ester hydrolysis furnished optically pure difluorocoriolic acid **105** (Scheme 18).[27]



Scheme 18. Synthesis of 14,14-difluorocoriolic acid.

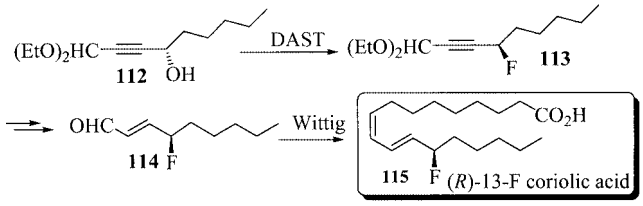
3.3. Synthesis of 13-F Coriolic Acid

Our group has a long-term interest in the synthesis of fluorinated bioactive molecules, and as part of a programme aimed at the synthesis of fluorinated analogues of polyunsaturated fatty acid metabolites, 4-hydroxy nonenal and coriolic acid were taken up (Scheme 19).[28] The key reaction was the regioselective fluorination of an allyl alcohol by using DAST at low temperature. The easily accessible alcohol **107**, upon exposure to DAST, produced exclusively **108**, which, after formolysis afforded fluorononaldehyde **109**. The latter derivative is the fluoro analogue of 4-hydroxy-nonenal (4HNE), another well known fatty-acid metabolite with potent biological properties.[29] Wittig olefination of **109** with the appropriate phosphonium salt, followed by saponification, yielded 13F-coriolic acid (**110**) in racemic form. The tetranor derivative **111** was obtained in a similar way.



Scheme 19. Synthesis of 13-fluorocoriolic acid.

More recently, the first asymmetric synthesis of these monofluorinated analogues was also reported (Scheme 20). It takes advantage of the regio- and stereoselective dehydroxyfluorination of propargylic alcohols, such as **112**, affording **113**.

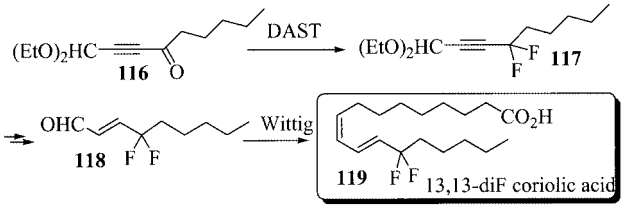


Scheme 20. Asymmetric synthesis of (*R*)-4-fluoro-HNE and (*R*)-13-fluorocoriolic acid.

After hydrogenation and acid-catalyzed *Z*-*E* isomerization, **114** was isolated. As previously, a Wittig reaction afforded the desired coriolic acid analogue **115**. Both compounds were obtained in very high *ee*'s (96%).[30] The fluorinated analogue was found to be more potent than coriolic acid in the inhibition of human platelet aggregation. Furthermore, it was also an activator of the PPARα nuclear receptor.

3.4. Synthesis of 13,13-diF Coriolic Acid

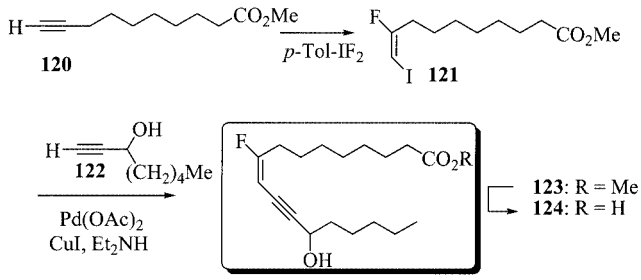
By using a similar propargylic-type approach, it was possible to prepare efficiently the corresponding difluorinated analogue **119** (Scheme 21).[31] Here again the strategy using difluorination of propargylic ketones, such as **116**, proved to be important since no reaction was observed by the action of DAST or Deoxofluor™ on the corresponding enones. These results could be rationalized by taking into account the stability of the intermediate carbenium ions.[32]



Scheme 21. Synthesis of 13,13-difluorocoriolic acid.

3.5. Synthesis of 9-Fluoro-11,12-dehydrocoriolic Acid

In these series, the 11,12-dehydrocoriolic acid was reported to have a stronger inhibitory activity than coriolic acid against rice blast fungus.[33] Therefore, the 9-fluoro analogue **124** was also synthesised by Hara et al. (Scheme 22).



Scheme 22. Synthesis of 9-fluorodehydrocoriolic acid.

The key intermediate, the fluorovinyl iodide **121** was obtained in a regio- and stereoselective manner by addition of iodotoluenedifluoride to alkyne **120**. The palladium-copper catalyzed cross-coupling of this intermediate with propargyl alcohol **122**, followed by a saponification step afforded in excellent yield the target molecule **124**.[34]

4. Natural Fluorinated Fatty Acids and Biosynthetic Studies

There are very few fluorine-containing natural products: only thirteen have been isolated to date from plants and microorganisms, of which eight are ω-fluorinated fatty acids from C^{16} to C^{20} with 0, 1, or 2 double bonds (Figure 1).[35a] They have been isolated from the seeds of a west African shrub, *Dichapelatum toxicarium*.[36] This plant is responsible for livestock losses and its toxicity is attributed to its degradation by β-oxidation to the fluoroacetate, which is a well-known toxic agent.[37] The main fluorinated component (75–80%) from the extract was the ω-fluorooleic acid **125**; the other, minor components were ω-fluoro acids **126**, **128** and **130**. The remaining compounds were obtained only in trace quantities. The threo-18-fluoro-dihydroxy stearic acid **127** is probably a metabolite of **125** via the corresponding epoxide. Taking into account the similarity of patterns, in this plant, between fluorinated and non-fluorinated fatty acids, it was considered that they should have similar precursors for biosynthesis. Therefore, fluoroacetyl-CoA should be the starting point for the synthesis of all derivatives indicated in Figure 1. At this point, it is important to note that the first fluorinase enzyme, involved in the biosynthesis of fluoroacetate, has been isolated and characterised only very recently. This was the starting point for elegant studies regarding the mechanism of this enzymatic fluorination.[38]

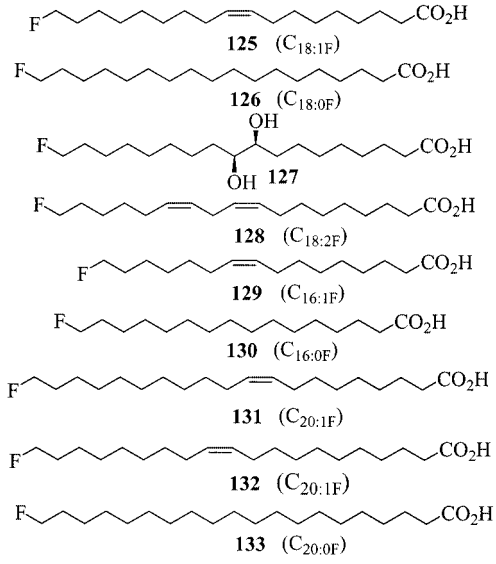
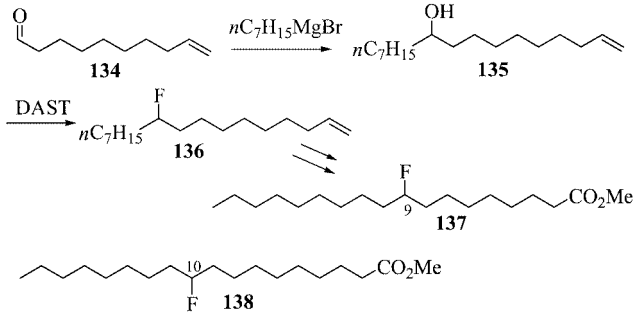


Figure 1. Natural products with the ω-fluorinated fatty acid structure.

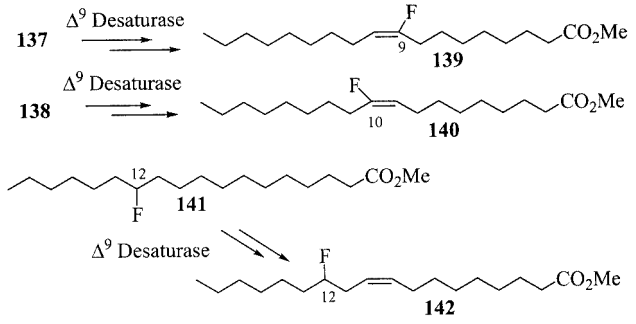
The desaturation of fatty acids is a ubiquitous biotransformation, playing a key role in the biosynthesis of many

fatty acids and their metabolites. However, the studies on corresponding enzymes are extremely difficult since the desaturases are membrane-bound proteins.[39] In order to probe the active site of the soluble plant Δ^9-desaturases, Buist et al. have prepared and submitted to biological tests various fluorinated analogues of its natural substrate (stearic acid). Among these derivatives, the 9-fluorostearate **137** has been synthesized in racemic form by a classical route, as indicated in Scheme 23. The 10-fluoro analogue **138** has been obtained similarly starting from the appropriate aldehyde.[40]



Scheme 23. Synthesis of 9- or 10-fluorostearates.

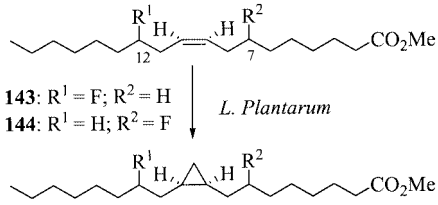
After incubation of these substrates with cells of *Saccharomyces cerevisiae* and esterification during the work-up process, only the desaturated fatty acid methyl esters **139** and **140** were isolated and fully characterised (Scheme 24).



Scheme 24. Desaturation of fluorooctadecanoates.

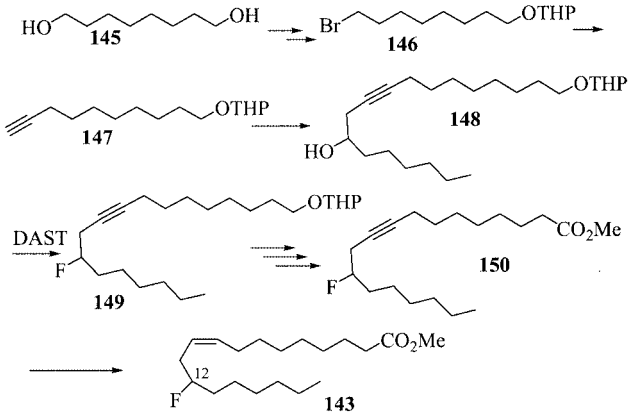
Furthermore, for the desaturation process, the retardation effect of fluorine (as compared to hydrogen) was found to be three times larger in position 9 (compound **137**) than in position 10 (derivative **138**). A strong retardation effect was observed also with the fluorine in position 12 (compound **141**). By using the latter derivative, it was established that the desaturation was occurring at comparable rates, either on the racemic compound or the pure (*S*) enantiomer, indicating that there was no stereocontrol in this biotransformation.

The same group has synthesised two oleic acid analogues, **143** and **144**, with fluorine atoms in homoallylic positions (7 and 12) as internal fluorinated substances for biomethylenation by the microorganism *Lactobacillus plantarum* (Scheme 25).[41]



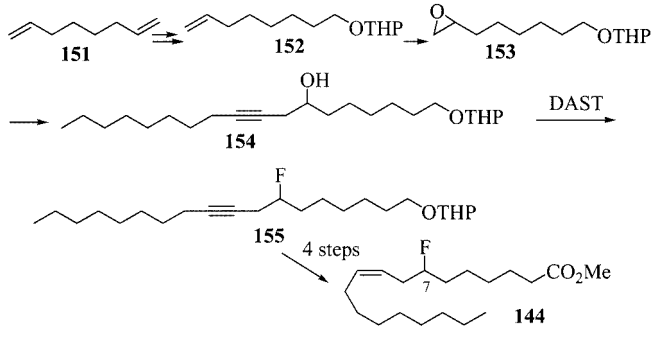
Scheme 25. Biomethylenation of fluorinated analogues of oleic acid.

The synthetic route followed for 12-fluorooleate began from 1,8-octanediol, which, on monobromination and tetrahydropyranation, yielded **146**. Alkylation with sodium acetylide furnished **147**, which, on opening with octene oxide, yielded the required carbon skeleton **148**. Conversion of the hydroxy function to the fluoro derivative **149**, deprotection of the THP ether, oxidation-esterification and partial hydrogenation were other routine steps to yield the 12-fluorooleate **143** (Scheme 26).



Scheme 26. Synthesis of methyl 12-fluorooleate.

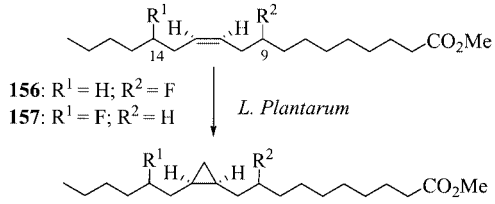
Methyl 7-fluorooleate (**144**) was synthesised from 1,7-octadiene (**151**), which, on selective hydroboration and tetrahydropyranation followed by exposure to mCPBA, furnished epoxide **153**. Lithium decylide treatment, fluorination and other steps were identical to those in the previous synthesis (Scheme 27).



Scheme 27. Synthesis of methyl 7-fluorooleate.

These two fluoro analogues were administered to growing cultures of *L. plantarum*. The 7-fluorooleate **144** (with the fluorine closer to the acid chain) was methylenated to a greater extent than the 12-fluoro isomer **143**, indicating that

the retardation effect of fluorine was stronger in position 12 than in 7. Such a product distribution of cyclopropanation suggested a nonsymmetrical positive-charge distribution in the putative carbocationic intermediate, as imposed by the presence of a closely situated, negatively charged group on the enzyme surface.[41] In a complementary study, the same group prepared the two similar homoallylic fluorides, **156** and **157**, derived from *cis*-vaccenic acid (the double bond is now in position 11–12). Using the same bacterium, they discovered that the pattern of the fluorine substituent effect was reversed: the retardation effect of fluorine was much more important for **156** (closer to the ester end) than for **157** (Scheme 28), contrary to what was observed in the case of **143** and **144**.[42]



Scheme 28. Biomethylenation of fluorinated analogues of *cis* vaccenic acid.

Finally, it has to be further mentioned that ^{19}F NMR proved to be a very useful tool to monitor the relative efficiency of desaturase-mediated sulfoxidation of thia fatty acid analogues.[43]

Conclusion

A large number of fluorinated analogues of unsaturated fatty acids and corresponding metabolites have been prepared to date. Versatile strategies have been developed in order to introduce selectively a single fluorine atom, as well as CF_2 or CF_3 groups, in the desired positions of these highly sensitive compounds. The biological tests performed on these fluorinated analogues afforded useful contributions to the structure-function relationships in this family of lipids.

Acknowledgments

Fruitful discussions with Drs. J. B. Ducep, J. C. Rossi, M. Lagarde, J. P. Salaun, C. Mioskowski and F. Bellamy are gratefully acknowledged. We thank the CNRS and the French Ministry of Education and Research for financial support. S. C. thanks the CEFIPRA/IFCPAR (2305–1) for supporting the visits to Rennes.

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Received: August 17, 2004

