Cyclic Lipids

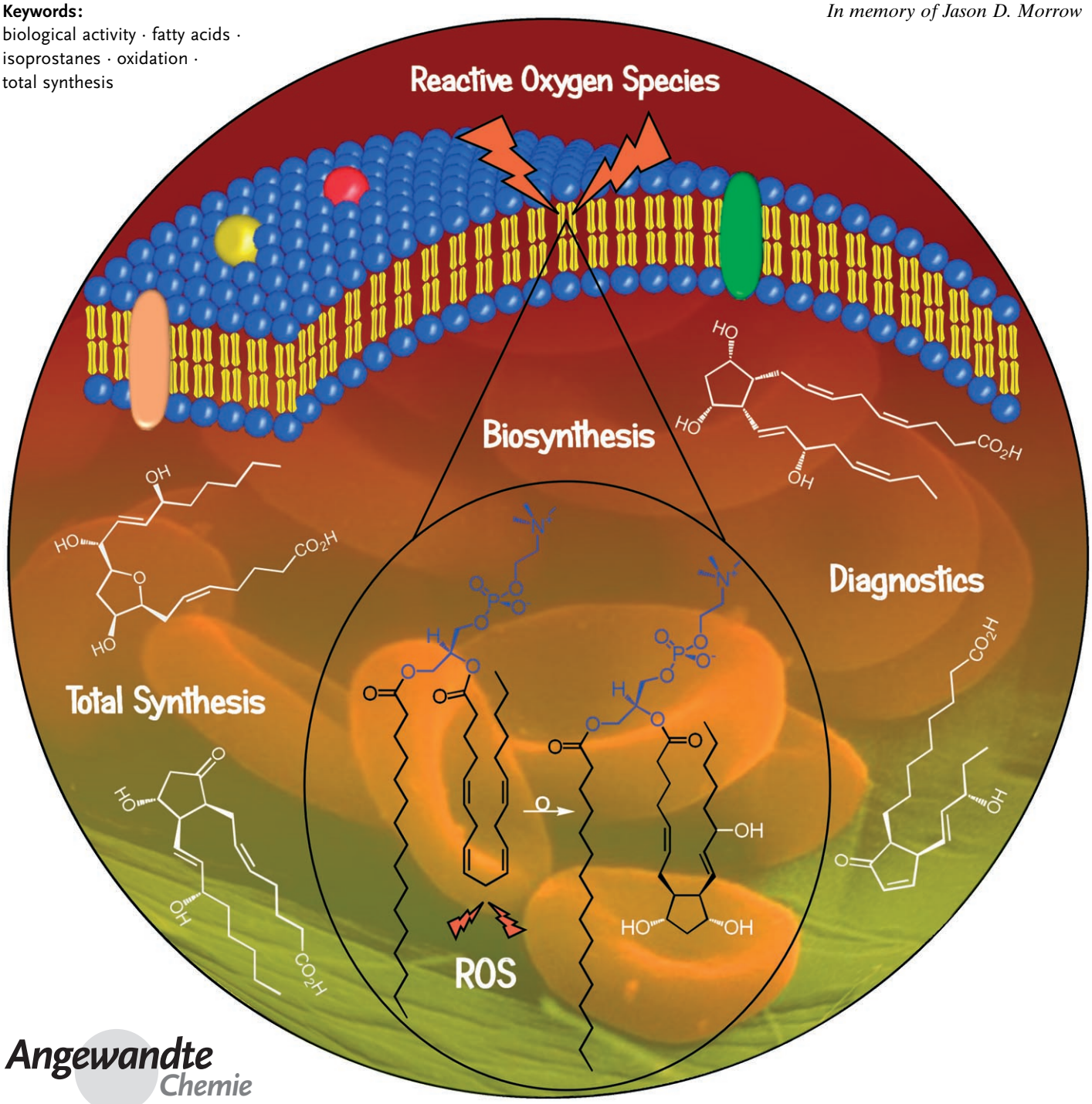
DOI: 10.1002/anie.200705122

Beyond Prostaglandins—Chemistry and Biology of Cyclic Oxygenated Metabolites Formed by Free-Radical Pathways from Polyunsaturated Fatty Acids

Ullrich Jahn, Jean-Marie Galano,* and Thierry Durand**

Keywords:
biological activity · fatty acids · isoprostanes · oxidation · total synthesis

In memory of Jason D. Morrow



Polyunsaturated fatty acids (PUFAs) are important constituents in all organisms. They fulfil many functions, ranging from modulating the structure of membranes to acting as precursors of physiologically important molecules, such as the prostaglandins, which for a long time were the most prominent cyclic PUFA metabolites. However, since the beginning of the 1990s a large variety of cyclic metabolites have been discovered that form under autoxidative conditions in vivo to a much larger extent than do prostaglandins. These compounds—isoprostanes, neuroprostanes, phytoprostanes, and isofurans—proved subsequently to be ubiquitous in nature. They display a wide range of biological activities, and isoprostanes have become the currently most reliable indicators of oxidative stress in humans. In a relatively short time, the structural variety, properties, and applications of the autoxidatively formed cyclic PUFA derivatives have been uncovered.

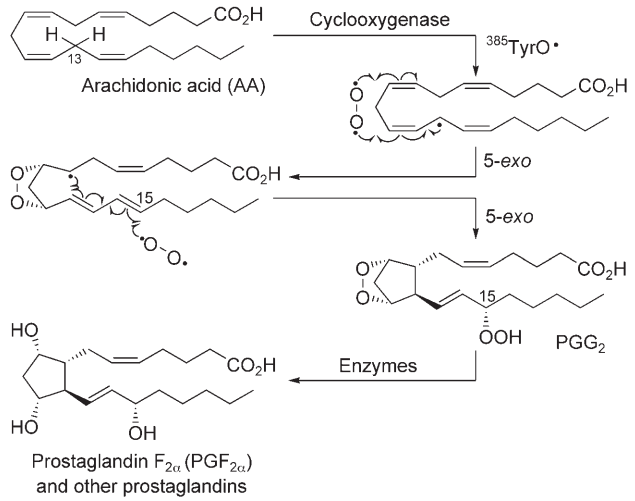
From the Contents

1. Introduction

Polyunsaturated fatty acids (PUFAs) are extremely important compounds in all organisms. In contrast to saturated and mono-unsaturated fatty acids, which are relatively inert under physiological conditions, PUFAs display a strongly increased reactivity and a large number of biological functions.[1] They are thus substrates for enzymatic and non-enzymatic transformations that give a variety of important signaling molecules, mediators, and biologically active secondary metabolites. Examples include enzymatic lipoxygenation to give hydroperoxides as well as epoxidations and alkylations.

Probably the most thoroughly studied enzymatic transformation of a PUFA is the conversion of arachidonic acid (AA) into prostaglandins (PGs; Scheme 1).[2] These natural products were first detected in the 1930s from their biological activity.[3] It took until the 1960s to isolate sufficient amounts of $PGF_{1\alpha}$ and PGE_1 to determine their structures unequivocally.[4] Subsequently, the biosynthesis of PGs was studied in much detail, and proved to consist of an unprecedented free-radical cascade of peroxidation, double 5-*exo* radical cyclization, and oxygenation to PGG_2 inside the cyclooxygenase enzymes. Subsequent enzymatic processing afforded the final PG, such as $PGF_{2\alpha}$. Figure 1 shows the stereoview of a recently published X-ray crystal structure analysis of cyclooxygenase with AA residing in the active site.[5]

Prostaglandins fulfil many important functions, such as being local hormones, inflammation and pain mediators, vasomotor regulators, and neuromodulators in animals. Similarly, the jasmonates (JAs) are central signaling molecules formed by enzymatic cyclization of α-linolenic acid (LA) in the plant kingdom.[6]



Scheme 1. Enzymatic biosynthesis of prostaglandins.

[*] Priv.-Doz. Dr. U. Jahn
Institute of Organic Chemistry and Biochemistry
Academy of Sciences of the Czech Republic
Flemingovo namesti 2, 16610 Prague 6 (Czech Republic)
Fax: (+420) 220-183-578
E-mail: jahn@uochb.cas.cz
Homepage: http://www.uochb.cz/web/structure/616.html
Dr. J.-M. Galano, Dr. T. Durand
Institut des Biomolécules Max Mousseron (IBMM)
UMR CNRS 5247—Universités de Montpellier I et II
Faculté de Pharmacie
15 Av. Charles Flahault, BP 14491
34093 Montpellier cedex 05 (France)
Fax: (+33) 4-6754-8625
E-mail: thierry.durand@univ-montp1.fr
jean.galano@univ-montp1.fr
Homepage: http://www.ibmm.univ-montp1.fr/-Lipides-Antipaludiques,34-.html

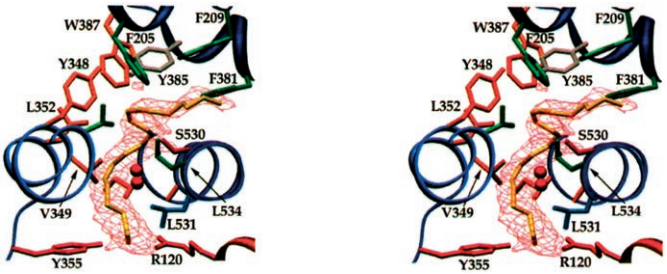


Figure 1. X-ray crystallographic stereoview of cyclooxygenase with bound AA (yellow). It shows the narrow, channel-like active site, to which AA has to enter, with the ω end first, and where it is held in place by hydrogen bonding to ^{120}Arg (red). The chain is forced into a *trans* orientation for cyclization by hydrophobic interactions with the designated amino acids Leu531, Leu534, Phe381, Phe209, Phe205, Trp387, Leu352, and Val349. The ^{385}tyrosyl radical (gray), generated by a heme group nearby, mediates highly selective abstraction of the exposed *pro*-(*13S*) hydrogen atom of AA. The two red spheres represent the most likely position of the oxygen molecule as it attacks the C11-position. The picture was taken with permission from Ref. [5].

The long-standing paradigm that only enzymatically formed compounds have important biological functions, and that metabolites forming spontaneously in vivo do not contribute significantly to the overall state of an organism, delayed the thorough investigation and the development of the understanding of the non-enzymatic lipid metabolism. The first hints on the non-enzymatic formation of isoprostanes were published by Nugteren et al. in 1967. They observed the formation of two regioisomers of "PGB_1" and "PGE_1" (now termed 8- and 15-B_1-IsoP, and -E_1-IsoP, respectively) in low yields when 8,11,14-eicosatrienoic acid was subjected to autoxidation conditions.[7] From then on, prostaglandin diastereoisomers and other cyclic PUFA metabolites were reported occasionally; however, they were considered as curiosities or as artifacts and forgotten for more than 20 years.

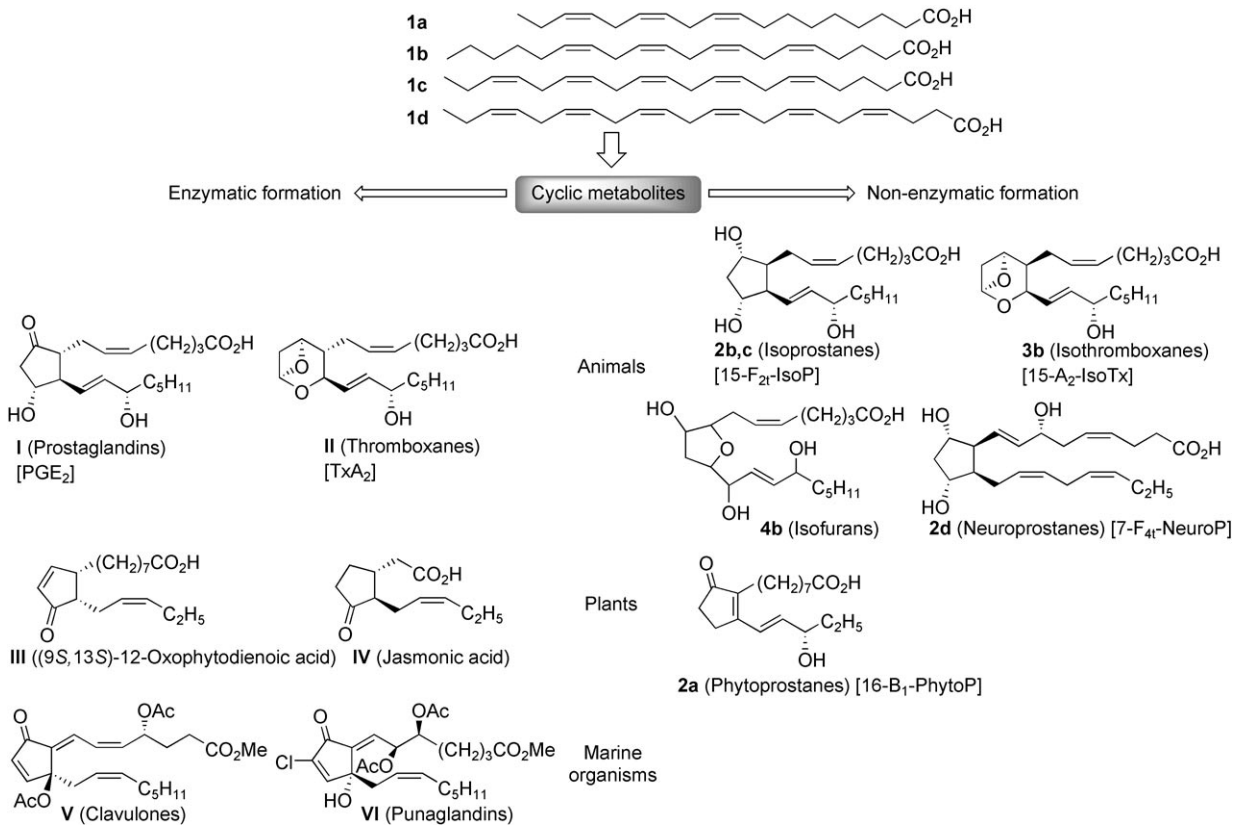
This situation changed only in 1990, when Roberts, Morrow, and co-workers discovered that racemic PG diastereoisomers, which they termed later isoprostanes (IsoPs), were produced in large amounts in vivo;[8] plasma levels amounted to $(36.3 \pm 12.9)\ pg\,mL^{-1}$ in healthy humans. In 1998, Parchmann and Mueller demonstrated that a similar nonenzymatic pathway to cyclic PUFA metabolites, which were termed phytoprostanes (PhytoPs), exists in plants.[9]

The fact that these metabolites also displayed significant biological activities triggered a strong research effort to understand the biological functions of cyclic PUFA metabolites beyond PGs and JAs. Today, there is compelling evidence that non-enzymatically formed cyclic lipids are extremely versatile natural products, which display a rich enzymatic and non-enzymatic biochemistry. In contrast to the strongly regulated enzymatic biosynthesis of PGs and JAs, which are highly regio- and stereoselective, the autoxidative in vivo conversion of PUFAs into cyclic products follows more or less conventional chemistry rules, and allows thus the generation of a much wider array of metabolites. The close constitutional similarity of cyclic PUFA metabolites on the one hand, and the diversity of their three-dimensional shape arising from the configurational differences on the other makes them an ideal "playground" to select functions in an evolutionary context. The high interest in cyclic PUFA metabolites strongly contributes to the field that is today called lipidomics, which has the aim to comprehensively understand the chemistry and biology of lipids.[10]

This Review aims to provide an overview about the chemistry and biochemistry of cyclic PUFA metabolites formed non-enzymatically. The recent development of this field is a prime example of how chemistry and biology have to work hand in hand to uncover the structures of the metabolites, their properties, and the underlying factors of their function. The major research lines in the field will be covered, including 1) the formation of cyclic PUFA metabolites, 2) the daunting task of the analytical determination, characterization, and quantification of hundreds of similar compounds formed in parallel, 3) the evaluation of their biological activities, and 4) their application as a "gold standard" diagnostic tool for the assessment of oxidative stress in tissues. The sheer number of metabolites necessitates suitable nomenclature systems, which will be presented. Clinical research on cyclic PUFA metabolites has shown a growth from a mere three publications in 1992 to more than 200 in 2007, and will be summarized concisely. The current knowledge of cyclic PUFA metabolites would not be that detailed if efficient strategies for their synthesis had not been developed. The analytical investigation and biological evaluation of cyclic PUFA metabolites would not have been

Ullrich Jahn studied at Martin-Luther Universität Halle-Wittenberg and earned his PhD in 1992. From 1993–1995 he carried out postdoctoral work with Dennis P. Curran at the University of Pittsburgh. He completed his habilitation, associated with Henning Hopf, in 2002 at the Technische Universität Braunschweig. In 2006, he was a visiting professor at the Université Montpellier I. His interests include new synthetic methods, tandem reactions involving oxidative electron transfer, and natural products synthesis. Since 2007 he has been a senior research leader at the Institute of Organic Chemistry and Biochemistry of the Academy of Sciences of the Czech Republic in Prague.

Thierry Durand studied chemistry at the Université Paris VI, then moved to Montpellier, and received his PhD at the Université Montpellier I in 1990. After postdoctoral research at the Florida Institute of Technology in Melbourne, with Joshua Rokach, he became Chargé de Recherche CNRS at the Université Montpellier I (Pharmacy Campus) in 1991. He finished his Habilitation in 1995, and became Directeur de Recherche CNRS in 2002. He is now a group leader at the Institute of Biomolecules Max Mousseron. His interests include the total synthesis of bioactive lipids, in particular iso-, neuro-, and phytoprostanes, as well as anandamide analogues.



Scheme 2. Cyclic PUFA metabolites—Sources and enzymatic or non-enzymatic formation. An example is shown for each compound type (the respective name is in some cases given in square brackets).

possible without pure synthetic reference material. Therefore, the strategies for the specific synthesis of cyclic PUFA metabolites are summarized. The current status may enable new synthetic strategic endeavors to be more easily devised.

2. Classification of PUFA Metabolites

The main PUFAs in all living organisms are α-linolenic acid (LA, **1a**), arachidonic acid (AA, **1b**), eicosapentaenoic acid (EPA, **1c**), and docosahexaenoic acid (DHA, **1d**; Scheme 2). Inspection of their structures and reactivity patterns indicates that a myriad of metabolites may be formed under physiological conditions from them by several mechanisms. In reality, their number is, however, smaller. They can be divided primarily on the basis of their basic structures into acyclic and cyclic metabolites. A secondary classification can be made by their mode of formation: either enzymatic or non-enzymatic.

Jean-Marie Galano studied chemistry at Paul Cézanne Université Marseille and obtained his PhD under the supervision of Honoré Monti in 2001. He then moved to the University of Oxford to pursue a postdoctoral fellowship with David H. Hodgson on the development of new methods for the total synthesis of natural products. In October 2005 he joined the CNRS as a Chargé de Recherche at Université Montpellier I. His research focuses on new methods and strategies towards the total synthesis of natural products.

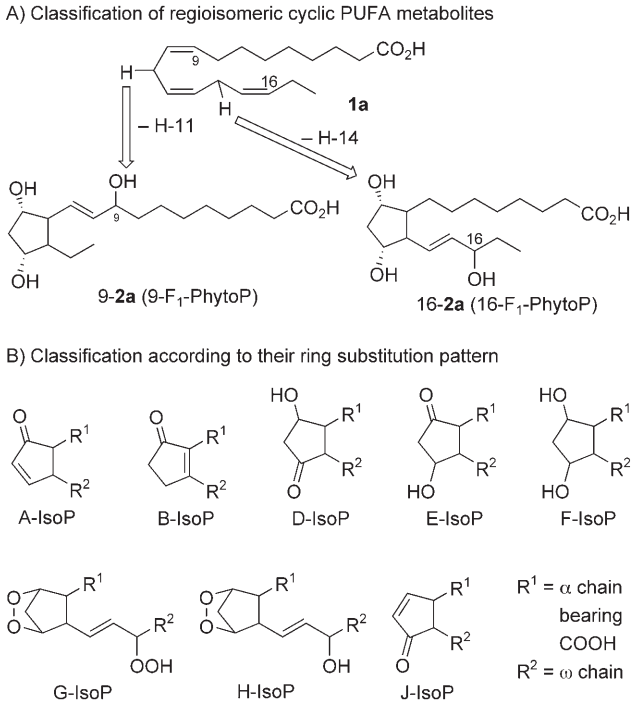
Acyclic PUFA metabolites formed with the help of enzymes are leukotrienes, PUFA-derived alcohols, and hydroperoxides.[2] Important products formed under autoxidative conditions include hydroperoxides, aldehydes, 4-hydroxyalkenals (such as 4-hydroxynonenal (HNE)), malondialdehyde (MDA), and a plethora of other metabolites.[11]

Cyclic PUFA metabolites appear to be found in all organisms (Scheme 2). There are a few that are biosynthesized enzymatically as single enantiomers such as prostaglandins (PGs, **I**) and thromboxanes (Txs, **II**) in animals, (9*S*,13*S*)-12-oxophytodienoic acid (OPDA, **III**) and jasmonic acids (JAs, **IV**) in plants,[6] and the clavulones **V** and punaglandins **VI** in marine invertebrates.[12]

Isoprostanes (IsoPs) **2b**,**c**, isothromboxanes (IsoTxs) **3b**, and isofurans (IsoFs) **4b** are the cyclic compounds formed autoxidatively from **1b** and **1c**. Neuroprostanes (NeuroPs) **2d**, which are present in human and animal brain, are derived from **1d**. Phytoprostanes (PhytoPs) **2a**, which are derived from **1a**, are the major cyclic metabolites found in plants. The common features of all autoxidatively generated PUFA

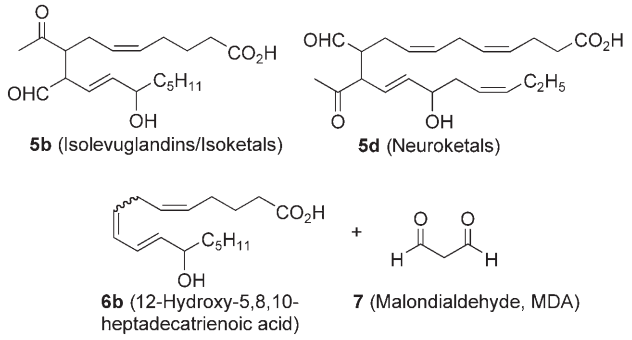
metabolites **2**–**4** are that they are racemic and that the major members of the individual classes are diastereomeric to their enzymatically formed congeners.

Autoxidatively formed PhytoPs, IsoPs, IsoTxs, IsoFs, and NeuroPs occur in regioisomeric series that can be distinguished by the site of the initial radical generation in **1a**–**d** (Scheme 3A). This is manifested by the position of the hydroxy group in the side chain, as shown for the F_1-PhytoP isomers 9-**2a** and 16-**2a**. Since assignment of the compound structure is not trivial in regards to regiochemistry and configuration, the following numbering system of the compounds is adopted in this Review: Structurally related intermediates or compound classes are assigned bold arabic numbers and a small letter that traces them to the appropriate PUFAs **1a**–**d**. A plain number denoting the position of the corresponding functional group or intermediate before the bold compound number designates individual isomers in a more general class. Another important classification of all cyclic PUFA metabolites is based on their ring-substitution pattern, since it strongly determines the chemical and biological properties (Scheme 3B). To assign the substitution pattern unequivocally, the common A-J system for PG nomenclature is adopted. Non-oxygenated cyclic products are also formed from **1c** or **1d** by heating fish oil.[13] However, these derivatives will not be covered here because they are generated exogenously under nonphysiological conditions.



Scheme 3. Classification of cyclic PUFA metabolites with respect to: A) The site of radical generation (shown for **1a**) and B) the substitution pattern of the cyclopentane ring based on the prostaglandin convention.

Some classes of acyclic PUFA metabolites can be traced to common cyclic intermediates formed along the IsoP biosynthetic pathway (Scheme 4). Isolevuglandins **5b** (IsoLGs; also termed isoketals (IsoKs) in the literature), formed from **1b**, neuroketals **5d** (NeuroKs), derived from **1d**, and 12-hydroxy-5,8,10-heptadecatrienoate (**6b**), together with **7**, for which multiple sources exist, belong to this class.[14] These lipid metabolites will be covered in this Review.



Scheme 4. Major acyclic PUFA metabolites formed on the IsoP biosynthetic pathway through cleavage of the cyclopentane ring **5b**,**d**: Shown is the name of the compound class together with an example.

3. Nomenclature of Cyclic PUFA Derivatives

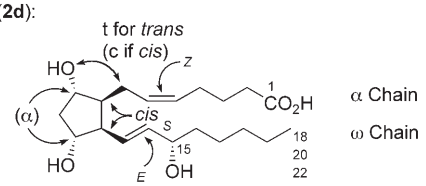
Since all cyclic PUFA metabolites **2**–**4** are formed by free-radical processes starting at different activated carbon atoms of the respective PUFA **1a**–**d**, a general nomenclature is preferred that intuitively provides information on the regioisomeric and configurational composition as well as the biosynthetic origin of a metabolite. In this section, the currently available nomenclature systems for the cyclic PUFA metabolites **2**–**4** and the isolevuglandins **5** are summarized.

3.1. Isoprostanes, Neuroprostanes, and Phytoprostanes

Since the IsoPs **2b** derived from **1b** were discovered first, and investigated quickly afterwards, there was an urgent need for a short nomenclature. Two systems were put forward almost in parallel by Rokach and co-workers[15a] (1996, revised in 1997[15b,c]) and by Taber, Roberts, and co-workers[16a] in 1997.

The Taber/Roberts nomenclature follows the normal PG convention to assign the ring-substitution pattern common to all regio- and stereoisomeric carbocyclic PUFA metabolites (Scheme 5). The two regioisomeric series of phytoprostanes **2a**, the four or six regioisomeric series of isoprostanes (**2b**,**c**), and the eight regioisomeric series of neuroprostanes **2d**[16b] are primarily distinguished by the position of the hydroxy group in the side chain. Since all isoprostanes are racemic and rather large numbers of diastereomers exist, a further assignment of the configuration of **2** is not trivial. Therefore,

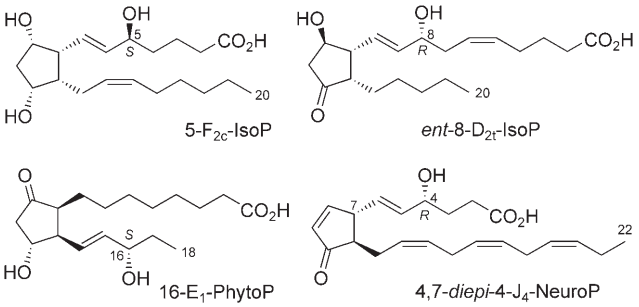
Default stereochemical relationships in PhytoPs (**2a**), IsoPs (**2b**,**c**), or NeuroPs (**2d**):



A (= 15-F_{2t}-IsoP)

Rules for naming an IsoP based on the default structure **A**:

1. Label carbon number of side chain -OH.
2. Assign ring substitution pattern with A-H, or J, based on the common PG nomenclature (see Scheme 3).
3. Note number of side-chain double bonds as a subscript.
4. Determine the relative orientation of α side chain with respect to the adjacent ring -OH group and note t or c as a subscript, if applicable.
5. Append PhytoP, IsoP, or NeuroP, based on the PUFAs **1a–d**.
6. To denote configuration inversion of the side chain -OH and/or the α chain, use "carbon number-*epi*-" as a prefix.
7. Assign enantiomers of default structures by *ent*- as a prefix.



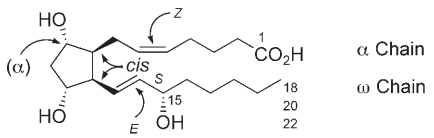
Scheme 5. Nomenclature of carbocyclic PUFA metabolites according to Taber, Roberts, and co-workers.

structure **A** was used as the default structure for further assignment, from which a name or a structure can be derived rather easily for all possible regio- and stereoisomers of all cyclic metabolites **2a–d** by applying rules 1–7.

The Rokach nomenclature is also applicable to phytoprostanes (iP_1s or PPs), isoprostanes (iPs), and neuroprostanes (iP_4s or nPs, Scheme 6). This system is more closely related to the PG nomenclature and uses the $PGF_{2\alpha}$ diastereomer **B** as the default structure. A useful feature of this nomenclature system is that the constitution and the formation pathway of the metabolites are coded by the roman type numeral following the name. The assignment of the type starts from the ω end of the PUFA chain and uses the bisallylic radicals formed by an initial hydrogen abstraction as a basis. The regioselectivity of the peroxidation of the bisallylic radicals and of the following peroxyl radical cyclizations are described by odd or even numbers. Odd numbers (A) denote radical coupling with O_2 and subsequent cyclizations in a clockwise direction from the initial radical, while even numbers (B) refer to a counterclockwise radical recombination with oxygen and subsequent radical cyclizations.

The Taber/Roberts nomenclature was approved by IUPAC, and will thus be used throughout this Review to avoid confusion. However, the Rokach nomenclature has the inherent merit that it provides information on the formation of the cyclic PUFA metabolite. Future efforts should be directed towards a unified nomenclature that reflects the advantages of both predecessors.

Default stereochemical relationships of iPs (**2a–d**):

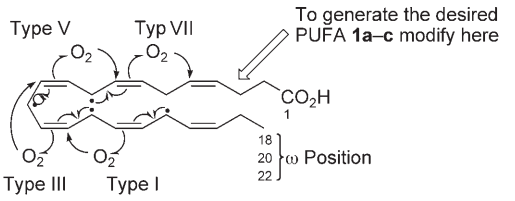


B (= $iPF_{2\alpha}$ Type-III)

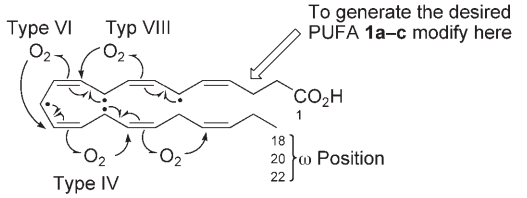
Rules for generation of a name based on structure **B**:

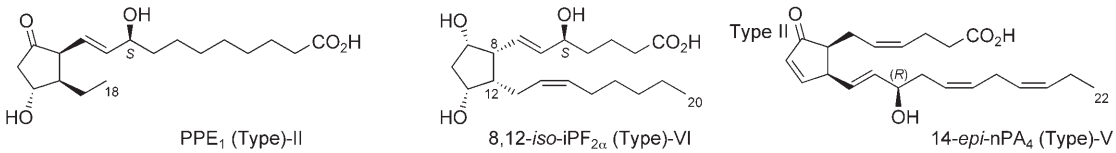
1. Name the structure as iP_1 or PP for phytoprostane, iP_2 or iP_3 for isoprostanes, iP_4 or nP for neuroprostane.
2. Assign ring substitution pattern with A-H, or J as for PG.
3. Note number of side-chain double bonds as a subscript.
4. Determine the relative orientation of the ring -OH group adjacent to the α side chain following the PG convention.
5. Use "carbon number-*epi*-" as a prefix to assign configuration inversion of the side chain -OH.
6. Use "carbon number-*iso*-" as a prefix to assign configuration inversion of the side chain carrying ring carbon atoms.
7. Assign enantiomers of default structures by *ent*- as a prefix.
8. Append a "type+roman numeral" to designate the regio-isomeric composition as well as the biosynthetic origin as follows:

A) Odd-numbered groups = Clockwise O_2 trapping of the initial PUFA radicals and subsequent radical cyclization



B) Even-numbered groups = Counterclockwise O_2 trapping of the initial PUFA radicals and subsequent radical cyclization



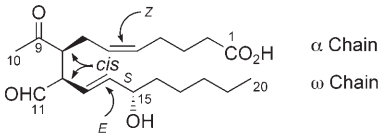


Scheme 6. Nomenclature of carbocyclic PUFA metabolites according to Rokach, and a mnemonic device to derive their formation.

3.2. Isolevuglandins (Isoketals) and Neuroketals

Two nomenclature systems were developed by Salomon et al.[17] and Roberts and co-workers[18] for the isolevuglandins **5b** (IsoLGs; also termed IsoKs later) and neuroketals **5d** (Scheme 7).

Default stereochemical relationships in IsoLGs (**5b**, IsoKs) or NeuroKs (**5d**):



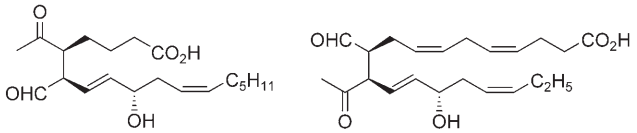
C IsoLGE$_2$ (15-E$_2$-IsoK)

Rules for generation of a name according to Salomon:

1. Isolevuglandins are abbreviated IsoLG regardless of PUFA precursor.
2. Insert number of α chain carbon atoms in square brackets (except for PG-type isomers - no number).
3. Assign the central substitution pattern as D or E by determination of the number of hydrogen atoms at the carbonyl groups and comparison with their number at the 9- or 11-positions of PGD or PGE, respectively.
4. Note number of side-chain double bonds as a subscript.

Rules for generation of a name according to Roberts:

1. Label carbon number of side chain -OH according to PUFA carbon number.
2. Assign the central substitution pattern as D or E by determination of the number of hydrogen atoms at the carbonyl groups and comparison with their number at the 9- or 11-positions of PGD or PGE, respectively.
3. Note number of double bonds as a subscript.
4. Abbreviate isoketals as IsoK and neuroketals as NeuroK.



Iso[4]LGE$_2$ (12-E$_2$-IsoK) Iso[9]LGD$_4$ (17-D$_4$-NeuroK)

Scheme 7. Nomenclature systems for IsoLGs developed by Salomon and Roberts.

The Salomon nomenclature names IsoLGs in a similar way as the enzymatically formed levuglandins (LGs). The regioisomers of IsoLGs are named by inserting the number of carbon atoms of the α chain in square brackets. An exception is the IsoLG that is diastereomeric to the enzymatically formed LG. Here, the number of carbon atoms in the α chain is not inserted.

The Roberts nomenclature uses the name isoketal (IsoK) for the γ-keto aldehyde isomers **5b** derived from AA, and neuroketals **5d** (NeuroKs) for C_{22}-keto aldehydes generated from DHA. However, the term "ketal" is misleading since ketals are commonly known as compounds where a single ketone-derived carbon atom carries two alkoxy groups. Otherwise, this nomenclature applies the rules of the IsoP nomenclature, thus establishing an easy to remember relationship of the γ-keto aldehydes to the corresponding D_2- or E_2-IsoP precursors.

3.3. Nomenclature of the Isofurans

The isofurans **4b** are formed from AA (**1b**) by two different pathways (see Section 4.2.2) and lead to the two structurally different IsoF classes A and B (Scheme 8). This leads to the possible formation of 256 isomers. To distinguish the multitude of structures, a nomenclature system based on the relative orientation of the side chains and substituents was developed by Taber, Fessel, and Roberts.[19] To relate the IsoF stereochemically to the IsoP, a defined default structure **D** was put forward which is based on the central endoperoxy hydroperoxide **8a** formed by oxygen interception of the radical that cyclizes at lower oxygen concentration to the IsoP core. Further transformation of **8a**, namely reductive endoperoxide opening followed by radical epoxidation, affords epoxyallylic radical **8b**, which is trapped by oxygen to give **8c**, which then leads to **D**.

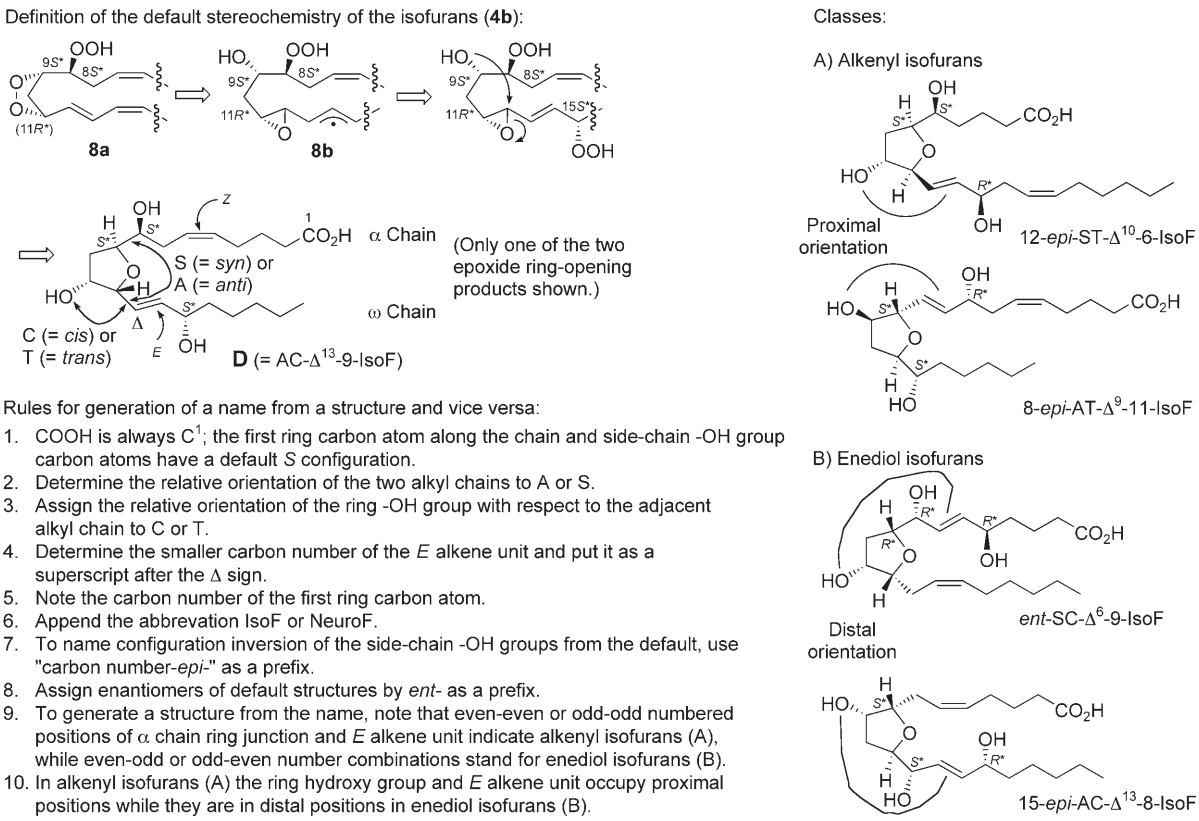
Default structure **D** thus has a relative *S* configuration (in analogy to IsoPs) at the ring junction of the α chain and at the 15-position, and also of the carbon atom with the other hydroxy group in the side chain. By this pathway, four regioisomeric IsoF classes based on a central vinyltetrahydrofuran unit are formed, which are termed alkenyl isofurans (Scheme 8A). However, matters are more complex, because IsoFs also form by another pathway (see Section 4.2.2), where the four alkenyl isofurans and four additional regioisomeric families termed enediol isofurans (Scheme 8B) are generated. Here, the *E* alkene resides between two hydroxy groups in one side chain. The same default structure **D** and the same rules are also applied to them.

4. Formation of Cyclic PUFA Metabolites In Vivo and In Vitro

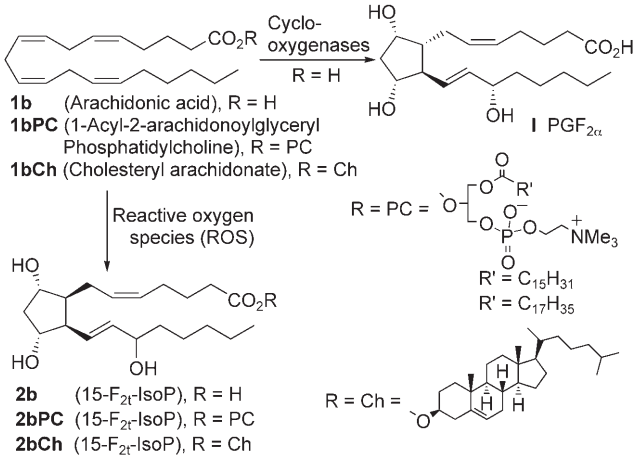
The enzymatic biosynthesis of prostaglandins **I** from free AA (**1b**, R = H in Scheme 9) was carefully investigated in the second half of the 20th century. When Morrow, Roberts, and co-workers published the isolation of prostaglandin diastereomeric compounds **2b** from human plasma and urine,[8] which they later termed isoprostanes, they recognized that prostaglandins **I** and isoprostanes **2b** must be formed by very similar pathways (Scheme 9). This was supported by the fact that racemic $PGF_{2\alpha}$ was also isolated as a minor constituent after non-enzymatic autoxidation of AA in vitro and in vivo.[20,21] Moreover, 15-F_{2t}-IsoP or 15-E_1-IsoP are also produced enzymatically as a minor side product as a single enantiomer by cyclooxygenases.[22]

Since most of the precursor fatty acids **1a–d** are in vivo bound in membranes as phospholipid esters **1PC**s,[23] or in the case of **1b** as cholesteryl arachidonate (**1bCh**),[24] which is an important constituent of low-density lipoprotein (LDL), the formation of IsoPs, PhytoPs, NeuroPs, IsoTxs, IsoLGs, and IsoFs **2a–d** to **5a–d** occurs predominately in esterified forms, from which the cyclic metabolite itself is subsequently released (see Section 4.3).

On these grounds, a plausible mechanistic explanation for the in vivo formation of cyclic PUFA metabolites **2–4** has been developed. Since **2–4** are generated by autoxidation, their formation is experimentally supported by a number of chemical investigations that were in part undertaken before their first isolation. Interestingly, these studies were mostly aimed at the validation of the biosynthesis of prostaglandins



Scheme 8. Nomenclature system to name the 256 isomeric IsoFs **4b**; there are 32 enantiomers in each of four regioisomeric series in two structural classes A and B.



Scheme 9. Formation of cyclic PUFA metabolites under different conditions.

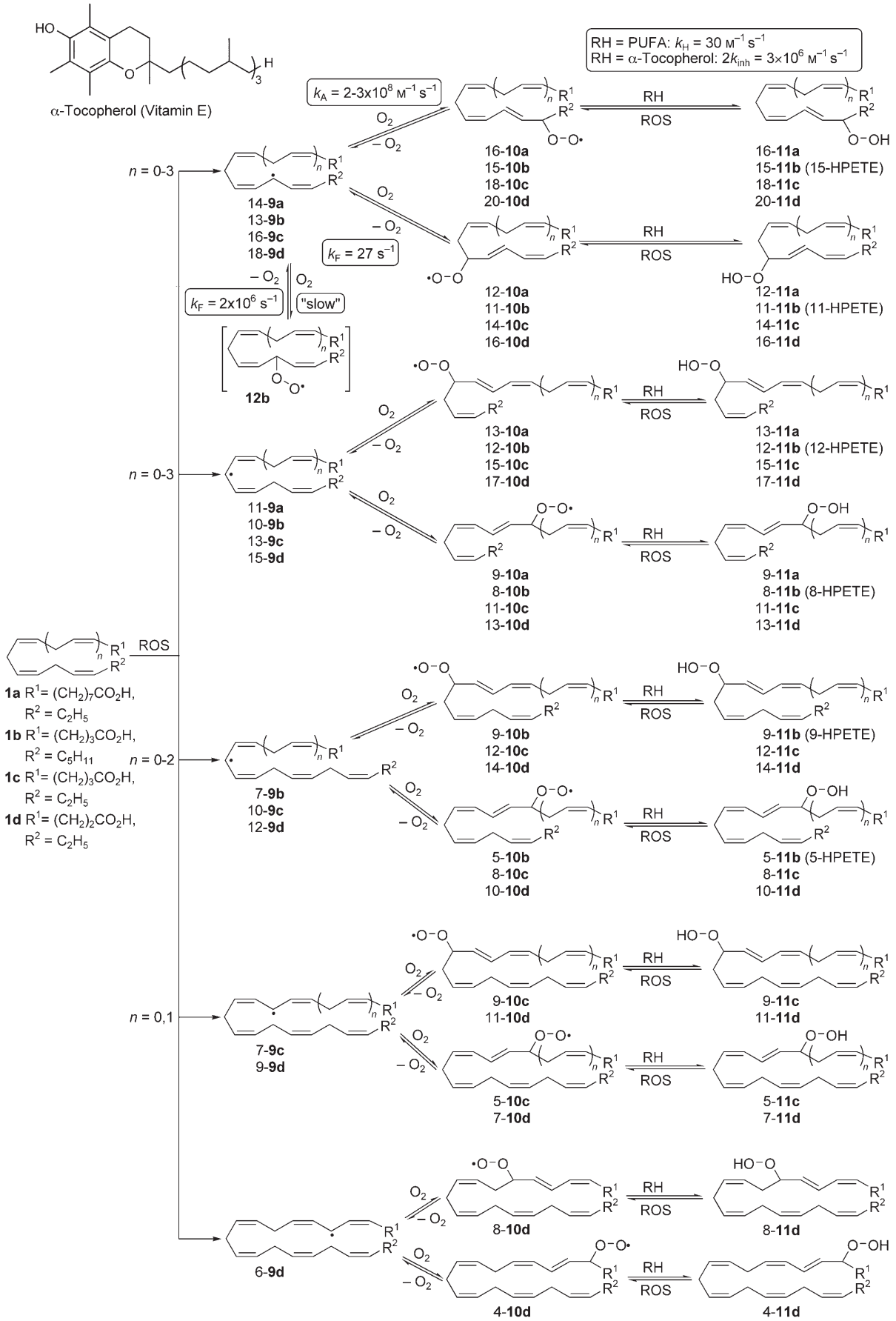
by chemical means. We know today that, instead, the major underlying factors for the autoxidative formation of **2**–**4** in vitro and in vivo were revealed.

The generation of cyclic PUFA metabolites is a multistep process that is dependent on several parameters. The most important are the redox status of the cell or the tissue (where the concentration of reactive oxygen species (ROS) is particularly important), the oxygen concentration, and the concentration of reducing agents in the surrounding tissue. Therefore, the formation of cyclic PUFA metabolites will be discussed on the basis of these factors.

4.1. Hydrogen Abstraction from PUFAs by ROS and Peroxidation

The initial event in the oxidative metabolization of all PUFAs such as **1a** ($n=0$), **1b** ($n=1$), **1c** ($n=2$), and **1d** ($n=3$) is hydrogen abstraction (Scheme 10). This occurs at an increased rate when the internal redox balance of a cell is seriously disturbed and the production of initiating species, such as ROS, cannot be sufficiently suppressed. The most common ROS are hydroxyl radicals, superoxide radical anions, alkoxyl radicals, peroxyl radicals, singlet oxygen, ozone, and hydrogen peroxide, but other reactive species such as peroxynitrite, nitrous oxide, nitric oxide, and carbon-centered radicals may also account for hydrogen abstraction from PUFAs.[25] Moreover, PUFA autoxidation may be triggered by low-valent transition-metal ions such as Cu^{I} or Fe^{II} in the presence of ascorbate or heme iron complexes under aerobic conditions.[26] Even enzymes such as myeloperoxidase in conjunction with hydrogen peroxide has been shown to induce free-radical lipid peroxidation to give mainly IsoLGs **5** under inflammation conditions (see Sections 4.2.1.2.2.2 and 5.2).[27]

It was shown in fundamental studies that only the bisallylic positions of free PUFAs **1a**–**d** and their correspond-



Scheme 10. Hydrogen abstraction and peroxidation of the major PUFA metabolites **1a–d**.

ing methyl, cholesteryl, and phospholipid-bound esters are subject to hydrogen abstraction. Two isomeric pentadienyl radicals (11-**9a** and 14-**9a**) result from **1a**, three (7-**9b**, 10-**9b**, 13-**9b**) from **1b**, four (7-**9c**, 10-**9c**, 13-**9c**, 16-**9c**) from **1c**, and five (6-**9d**, 9-**9d**, 12-**9d**, 15-**9d**, 18-**9d**) from **1d** (Scheme 10). The so-formed conjugated pentadienyl radicals **9a–d** possess a *Z*,*Z* configuration, with restricted rotation around the partial double bonds. Thus, the configuration is preserved for a reasonably long time.

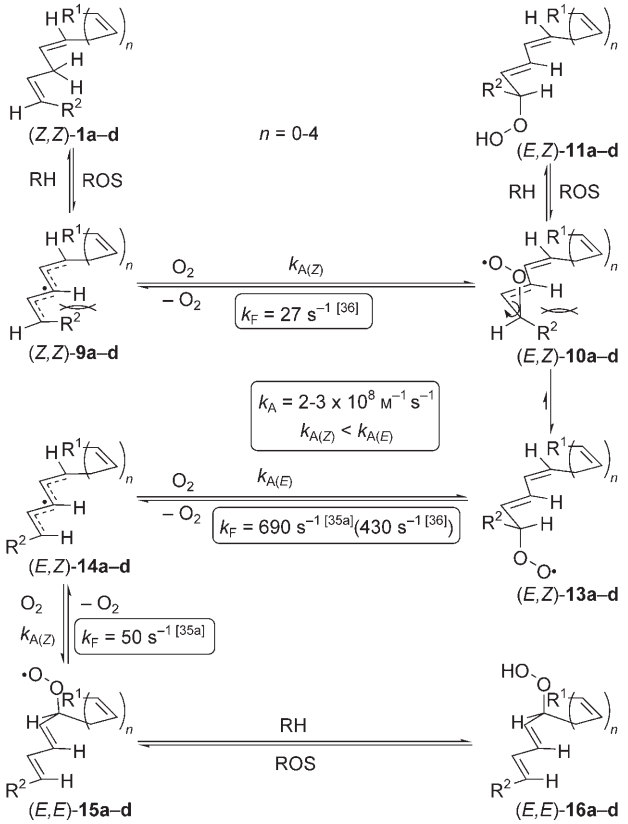
All pentadienyl radicals **9a–d** are oxygenated at their respective terminal positions with rate constants of $k_A = 2–3 \times 10^8\,M^{-1}s^{-1}$.[28] In this process, four regioisomeric (*E*,*Z*)-2,4-pentadienylperoxyl radicals (9-**10a**, 12-**10a**, 13-**10a**, and 16-**10a**) are generated from **1a**, six (5-**10b**, 8-**10b**, 9-**10b**, 11-**10b**, 12-**10b**, 15-**10b**) from **1b**, eight (5-**10c**, 8-**10c**, 9-**10c**, 11-**10c**, 12-**10c**, 14-**10c**, 15-**10c**, 18-**10c**) from **1c**, and ten (4-**10d**, 7-**10d**, 8-**10d**, 10-**10d**, 11-**10d**, 13-**10d**, 14-**10d**, 16-**10d**, 17-**10d**, 20-**10d**) from **1d**. Hydrogen transfer to radicals **10** gives four hydroperoxides **11a** derived from **1a**, six hydroperoxy-eicosatetraenoic acids (HPETEs) **11b** from **1b**,[29] eight hydroperoxides (**11c**) from **1c**, and ten (**11d**) from **1d**.[30]

Hydrogen transfer to peroxyl radicals **10** can occur by two main processes. In the presence of lipid antioxidant α-tocopherol, which reduces two peroxyl radical equivalents of **10** with a high total rate constant of $2k_{inh} \approx 3 \times 10^6\,M^{-1}s^{-1}$ in organic solvents, lipid membranes, or LDL, the autoxidation chain reaction is interrupted.[31] All hydroperoxide regioisomers **11a–d** are isolated in almost equal amounts in organic solvents.[29b,30] It is, however, interesting to note that 5-HPETE (5-**11b**) was not formed when the membrane model 1-stearyl-2-arachidonyl-*sn*-glycero-3-phosphatidylcholine was autoxidized in the presence of α-tocopherol.[32]

In the absence of antioxidants, hydrogen abstraction occurs mainly from the bisallylic positions of other PUFA molecules with a rate constant of $k_H \approx 30\,M^{-1}s^{-1}$ per abstractable hydrogen atom,[33,34] thus propagating the autoxidation chain reaction. Under these conditions, peroxidation of **1b** produces preferentially 5- and 15-HPETE (5-**11b** and 15-**11b**, respectively). Hydroperoxides **11b**, which are formed by oxidation at the 8-, 9-, 11-, and 12-positions, are found to a much lesser extent, because of facile cyclization opportunities (see Section 4.2.1.1).[32]

Hydroperoxides resulting from corresponding nonconjugated peroxyl radicals, such as **12b**, were only found as intermediates in very small amounts at extremely high antioxidant concentrations. This is mainly due to their very fast β fragmentation ($k_F \approx 1–2 \times 10^6\,s^{-1}$).[35]

The formation of pentadienyl peroxyl radicals **10a–d** can also occur from hydroperoxides **11a–d** by a reverse pathway involving hydrogen transfer to the ROS. The so-formed peroxyl radicals (*E*,*Z*)-**10a–d** can even undergo a rather slow β fragmentation ($k_F = 27\,s^{-1}$) to give the original (*Z*,*Z*)-pentadienyl radical (*Z*,*Z*)-**9a–d** and oxygen (Scheme 11).[29a,36] This small rate constant can be understood easily on the basis of the development of allylic strain during fragmentation. The conformational mobility of (*E*,*Z*)-**10a–d** means that the more favored *transoid* peroxyl radical rotamers (*E*,*Z*)-**13a–d** are easily accessible from which β fragmentation to the less strained conjugated (*E*,*Z*)-pentadienyl radicals (*E*,*Z*)-**14a–d** is much more facile, with a rate constant of $k_F = 690\,s^{-1}$.[37]



Scheme 11. Generation of 2,4-pentadienyl hydroperoxides with different double-bond configurations during autoxidation.

The overall rate constant for the recombination of **9a–d** with oxygen was determined to $k_A = 2–3 \times 10^8\,M^{-1}s^{-1}$.[28] However, trapping of O_2 at the *E* terminus of **14a–d** proved to be somewhat faster than at the *Z* end.[35,36] Thus, the (*E*,*Z*)-pentadienyl peroxyl radicals (*E*,*Z*)-**13a–d** reform preferentially. Nonetheless, recombination with O_2 at the *Z* terminus of **14a–d** can still compete. In this case, however, (*E*,*E*)-2,4-pentadienyl peroxyl radicals (*E*,*E*)-**15a–d** and finally hydroperoxides (*E*,*E*)-**16a–d** result. The latter are responsible for the formation of isoprostanes with inverse configuration at the exocyclic hydroxy group (see Section 4.2.1.1).

The labile hydroperoxides **11a–d** and **16a–d** have several opportunities to stabilize in vivo. For the most part, they decompose through a C–C β fragmentation to give a number of reactive metabolites, such as 4-hydroxynonenal and malondialdehyde.[11] Cyclization of PUFA peroxyl radicals **10a–d** or **15a–d** are far less favored than chain scission; for example, under the conditions of autoxidation in microsomes, the ratio of consumption of **1b** to the formation of F_2-IsoPs **2b** was approximately 130000:1. In the same experiment an approximately 34000 times higher amount of malondialdehyde **7** than of F_2-IsoPs **2b** was detected after autoxidation of **1b** at 21% oxygen concentration.[38] Nonetheless, cyclic compounds **2–4** are of utmost importance in PUFA metabolism because of their biological activity and diagnostic application.

4.2. Dependence of PUFA-Derived Cyclic Products on the Oxygen Concentration in Tissue

The reversibility of all reaction steps depicted in Schemes 10 and 11 increases the life span of the different radical types **9** and **10** so that several stabilization pathways, depending on the conditions in the organism, are possible. One of the most important factors in determining which intermediates and thus which class of cyclic PUFA metabolites will be formed is the partial pressure of oxygen in the corresponding tissue. At "normal" oxygen concentrations, the PUFA-derived radicals **10a–d** primarily undergo O–C/C–C bicyclizations to PhytoPs **2a**, IsoPs **2b,c**, NeuroPs **2d**, isothromboxanes **3** (IsoTxs) and IsoLGs **5b**,**d**. When the oxygen partial pressure increases, the product spectrum shifts to metabolites with higher oxygen content—namely, dioxolanes and IsoFs **4b**.

4.2.1. Radical Cyclizations at Normal Oxygen Partial Pressure

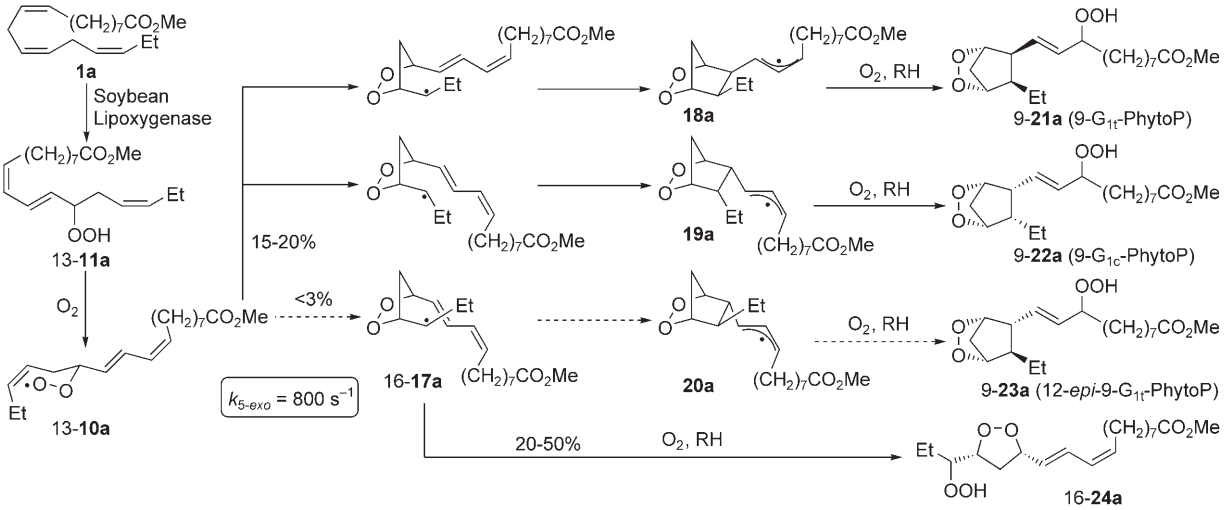
4.2.1.1. The Common Radical Bicyclization

Free and esterified PUFAs **1a–d** as well as their hydroperoxides **11a–d** may serve as precursors to initiate bicyclizations to cyclic PUFA metabolites **2a–d**, **3**, and **5**. The studies summarized in this section were performed only with derivatives of **1a** and **1b**; however, the results are, in principle, also applicable to derivatives of **1c** and **1d**.

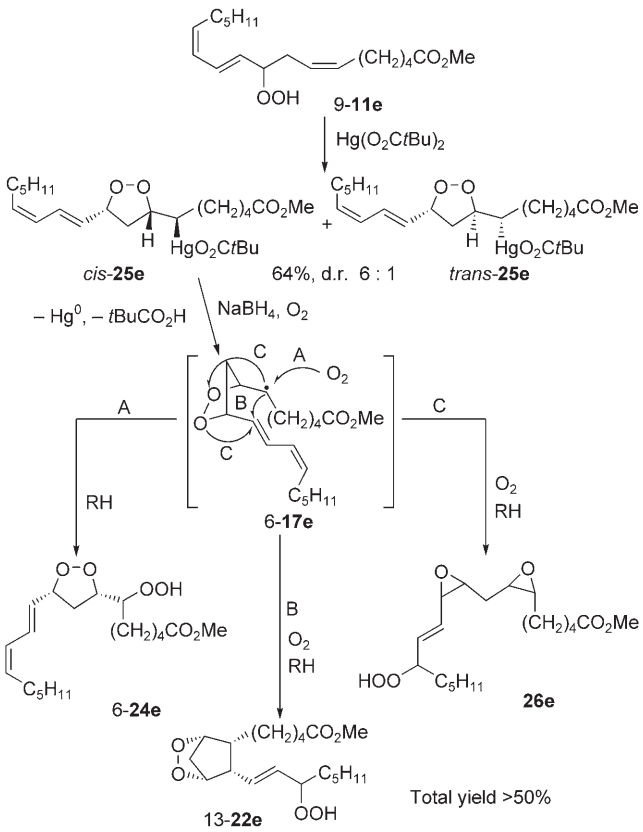
In early studies, Porter and Funk showed that reactions of methyl γ-linolenate hydroperoxides with oxygen in benzene led non-enzymatically to C18-"prostaglandin-like" structures.[39a] Methyl α-linolenate **1a** gave similar cyclic compounds,[39b,c] which should in fact be considered as F_1-PhytoPs **2a** today (see Section 3.1).[40] This finding indicated that peroxyl radicals **10a**, which are formed by autoxidation of free or esterified **1a**, undergo kinetically controlled irreversible 5-*exo* cyclizations with suitably positioned alkene units. The rate constant for this cyclization was estimated to be approximately 800 s^{-1}.[29a,c]

However, the configuration of these compounds was not established unequivocally. O'Connor et al. subsequently demonstrated in a more detailed investigation of the autoxidative cyclization behavior of α- as well as γ-linolenic acid hydroperoxides, such as 13-**11a**, that the cyclization of peroxyl radical 13-**10a** occurs with high *cis* diastereoselectivity to give 1,2-dioxolanylalkyl radical 16-**17a** (Scheme 12).[41] This reacts with oxygen to give a diastereomeric mixture of the monocyclic 3,5-*cis*-substituted 16-hydroperoxy endoperoxides 16-**24a** to an extent of 20–50%. At least 15–20% of 16-**17a** undergoes another 5-*exo* radical cyclization and subsequent peroxygenation of the allylic radicals **18a–20a** to give a mixture of 9-G_{1t}- and 9-G_{1c}-PhytoP (9-**21a** and 9-**22a**, respectively) in a 1:2–1:4 diastereomeric ratio. Compound 9-**23a**, with relative PG configuration, was found in amounts of less than 3%. The G_1-PhytoP isomers 9-**21a**–9-**23a** (and consequently also G_2-IsoPs and G_4-NeuroPs) are not particularly stable; however, they can be isolated under defined conditions. It was also firmly established that deliberate addition of antioxidants such as α-tocopherol[39b,42] or glutathione[42,43] inhibited or decreased the formation of isoprostanes, thus supporting a free-radical process.

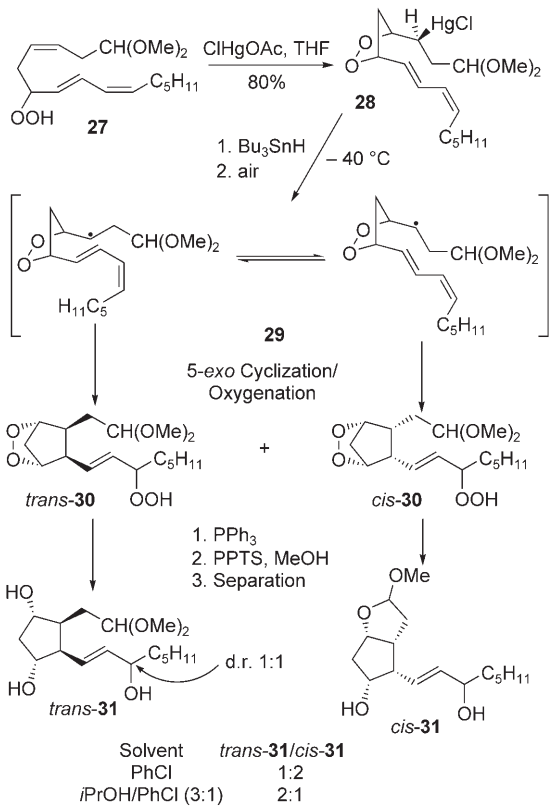
To reduce the complexity of the product spectrum and to gain a deeper understanding of the processes after formation of endoperoxide radicals, subsequent studies aimed at decoupling the two radical cyclization events. This was achieved by a facile intramolecular peroxymercuration of the hydroperoxide 9-**11e** derived from methyl γ-linolenate **1e** (Scheme 13).[44] Its reaction with Hg^{II} salts gives the required 3,5-*cis*-disubstituted mercuric endoperoxide *cis*-**25e**, as a stable precursor for radical cyclization studies, together with some of the *trans* diastereomer *trans*-**25e**. Radical 6-**17e** was generated with $NaBH_4$ in the presence of oxygen, and furnished monocyclic endoperoxide 6-**24e** (path A) and four bicyclic endoperoxide diastereomers (only the major diastereomer 13-**22e** is shown) by radical 5-*exo* cyclization (path B). 13-**22e** corresponds to the metabolite 2,3-dinor-5,6-dihydro-15-F_2-IsoP (see Section 4.3). Moreover, the diepoxide **26e** was formed, presumably by a 1,3-S_Hi/3-*exo* cyclization/oxygen



Scheme 12. Autoxidative bicyclization of 13-**11a** to endoperoxides 9-**21a** to 9-**23a**.



Scheme 13. Formation of stable products from 1,2-dioxolanylcarbinyl radicals 6-**17e**.



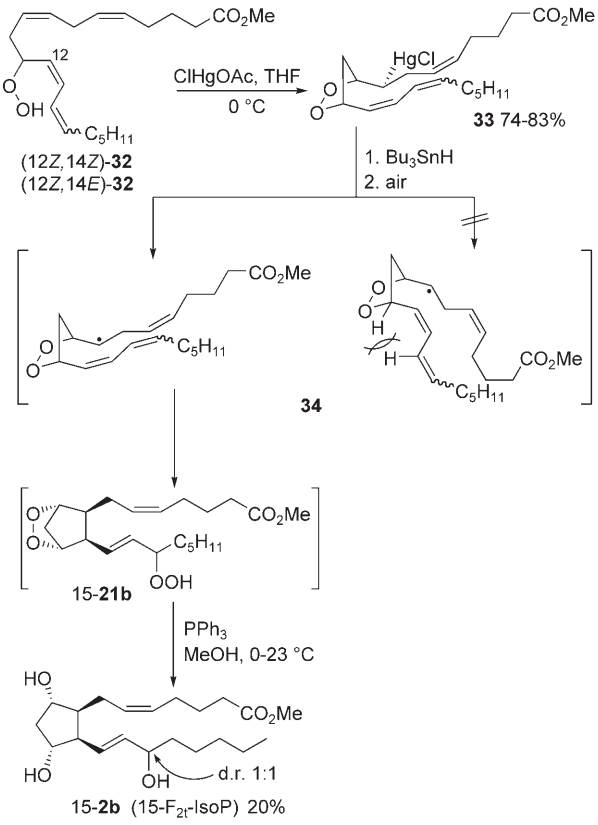
Scheme 14. Radical cyclization/oxygenation of mercurial compound **28** to F_2-IsoP precursor **31**.

trapping sequence (path C). The diepoxides could be significant intermediates in the formation of the isofurans (see Section 4.2.2).

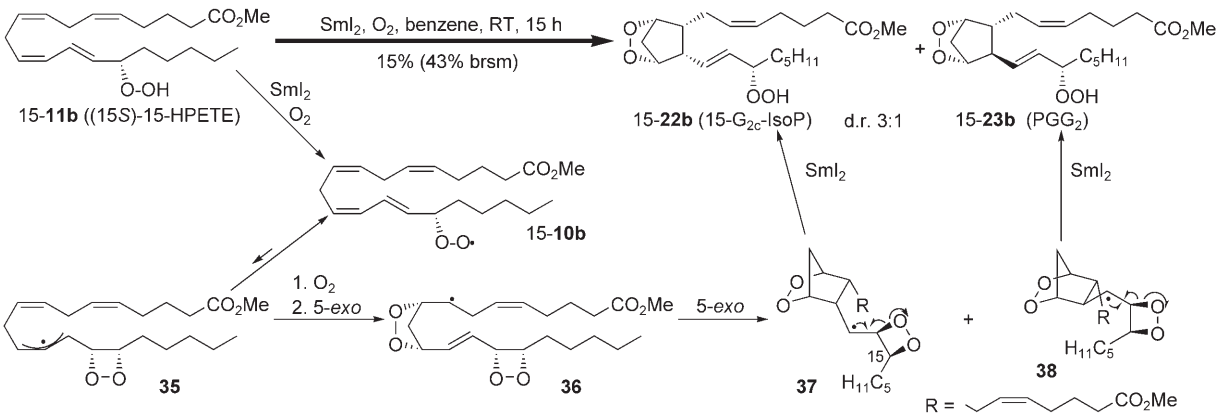
Corey et al. used the endoperoxy mercurial derivative **28** derived from hydroperoxide **27** to initiate radical 5-*exo* cyclizations of **29** to give bicyclic hydroperoxides **30** (Scheme 14).[45] Complete reduction of the peroxide provided the F_{2c}- and F_{2t}-IsoP precursors *cis*-**31** and *trans*-**31** in 60–90% yield. The *trans*-**31**/*cis*-**31** ratio was dependent on the solvent, and varied from 1:2 in nonpolar chlorobenzene to 2:1 in the more polar *i*PrOH/chlorobenzene (3:1) or H_2O/MeOH/chlorobenzene (4:3:1) mixture. Diastereomers with the PG configuration (see **23a** in Scheme 12) were found in less than 2% yield.

Only the 15-F_{2t}-IsoP diastereomers 15-**2b** were isolated (in 20% yield) from precursors **32** with non-natural (12*Z*)-diene units (Scheme 15).[46] In contrast to the natural 12*E* isomer, the ω chain is most likely forced into a β orientation in the transition state in radical **34** with a 12*Z* configuration, since an α orientation of the ω chain would be disfavored because of considerable allylic strain.[47]

Corey and Wang also investigated the radical cyclization behavior of (15*S*)-15-HPETE (15-**11b**) initiated by SmI_2 in the presence of oxygen, and isolated a PGG_2/G_2-IsoP mixture (1:3) in 15% yield (Scheme 16).[48] To account for the different stereoselectivity compared to autoxidative bicyclizations (see Scheme 12), a dioxetane-based mechanism was suggested and adopted later for in vivo generation of



Scheme 15. Biomimetic radical cyclization/oxygenation of (12*Z*)-11-HPETEs **32**.

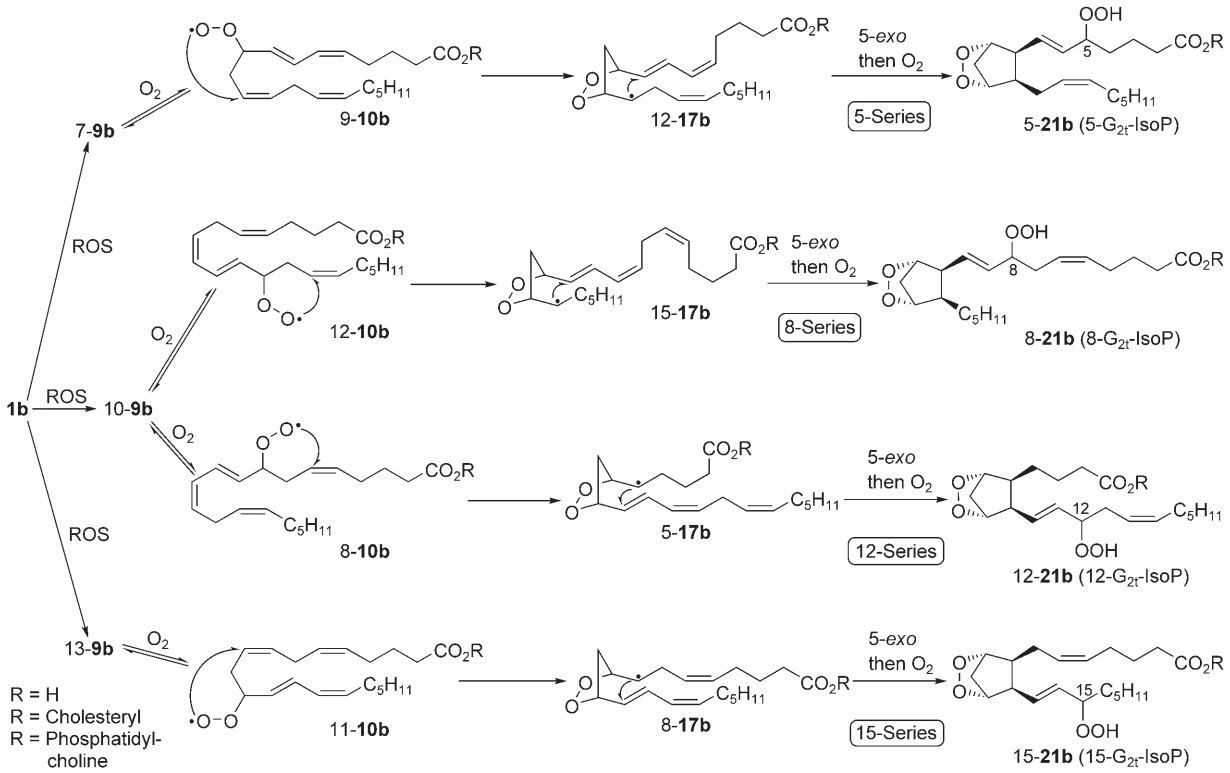


Scheme 16. Dioxetane mechanism proposed by Corey and Wang for the formation of 15-G_{2c}-IsoP **22b** and PGG_2 **23b**.

isoprostanes by Rokach and co-workers.[15c,49] Here, a 15-HPETE-derived peroxyl radical 15-**10b** cyclizes initially in a 4-*exo* mode to form an dioxetanylallylic radical **35**, which recombines with oxygen to give an (11*S*)-14,15-endoperoxy-11-HPETE radical, which undergoes the 5-*exo*/5-*exo* bicyclization via **36** to radicals **37** and **38**. These radicals finally fragment to give 15-G_{2c}-IsoP (15-**22b**) and PGG_2 (15-**23b**), respectively.

Several problematic issues are inherent to this mechanism, among them the very slow rate and the reversibility of 4-*exo* cyclizations[50] as well as the high instability of dioxetanes in general. Furthermore, the optical activity of 15-**22b** and 15-**23b** was not proven. Subsequent autoxidation studies on cholesteryl (15*S*)-15-HPETE (15-**11b**) of 98.9% *ee* disproved this mechanism, since the products 15-**22b** and 15-**23b** (shown in Scheme 16) were racemic.[24,51] Moreover, all eight possible 15-G_2-IsoP diastereomers formed from 15-**11b**. The detection of monocyclic endoperoxides **24b** (see Scheme 12) and serial endoperoxide products (see Section 4.2.2) in the reaction mixtures is also inconsistent with the dioxetane mechanism.

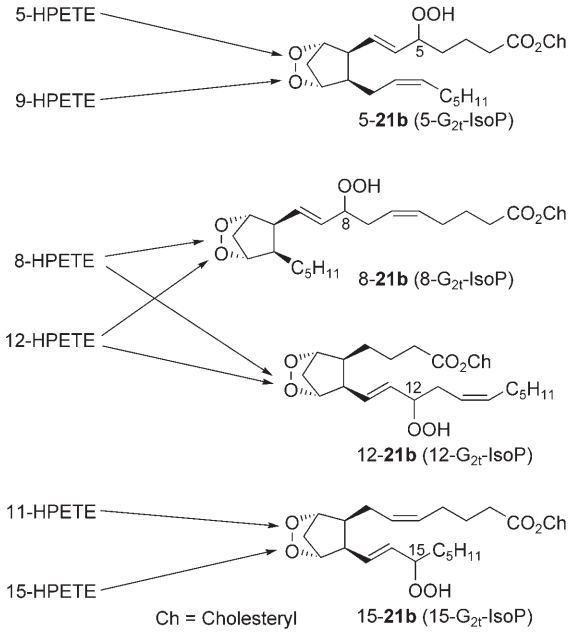
The parallel generation of all four possible arachidonate-derived peroxyl radicals **10b** on autoxidation means that four regioisomeric series of bicyclic G_2-IsoPs can be formed (Scheme 17, only ring diastereomer **21b** is shown). According to the position of the hydroxy group in the side chain, they are called 5-, 8-, 12-, and 15-G_2-IsoP, respectively. All four series have been identified unequivocally as their F_2-IsoP analogues by reduction (see Section 4.2.1.2.1.1) and were quantified.



Scheme 17. Formation of the regioisomeric G_2-IsoP series (only **21b** out of the possible four ring diastereomers is shown).

Diastereomeric mixtures of the 5- and 15-F_2-IsoPs are formed in 33 and 25% yield, respectively, while only 5% of the cyclic isomers belonged to the 8- and the 12-F_2-IsoP series.[52]

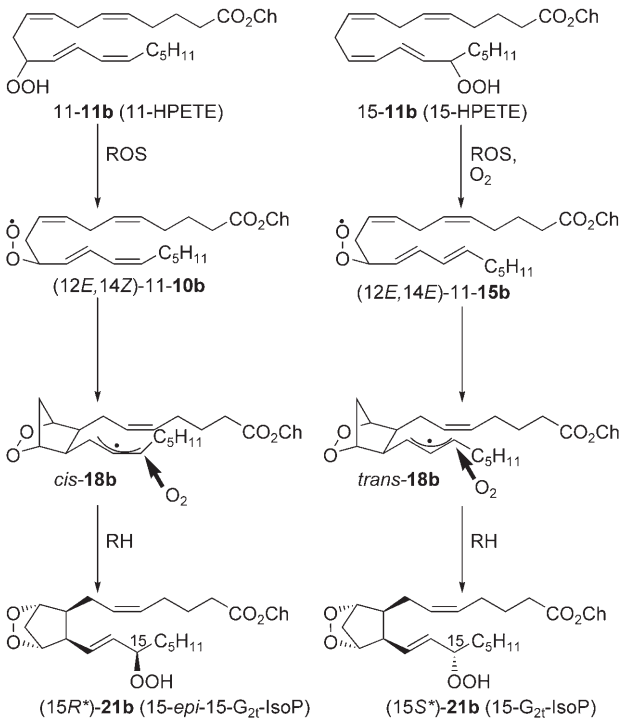
Porter and co-workers validated that all six individual HPETEs **11b** derived from cholesteryl arachidonate (**1bCh**) are competent precursors in autoxidative cyclizations (Scheme 18).[24] These hydroperoxides serve as specific pre-



Scheme 18. Cyclizations of AA hydroperoxides (HPETEs) to give G_2-IsoPs (only one ring diastereomer **21b** shown).

cursors to the 5, 8, 12, and 15 series of cholesteryl-IsoPs. Indeed, 15-HPETE and 11-HPETE produced 15-G_2-IsoP (15-**21b**) while 5-HPETE and 9-HPETE gave rise selectively to 5-G_2-IsoP (5-**21b**). On the other hand, 8-HPETE and 12-HPETE have two opportunities to cyclize, and lead to a mixture of 8- and 12-G_2-IsoP (8- and 12-**21b**), however, to a much lesser extent than expected (see Scheme 20). In accordance with previous results, the preferentially formed ring stereoisomers were G_{2t}-IsoPs **21b** and G_{2c}-IsoPs **22b**.

The configuration of the hydroperoxy group in the side chain proved to be dependent on the origin and the configuration of the pentadienyl peroxyl radicals (*E*,*Z*)-**10b** and (*E*,*E*)-**15b** (Scheme 19, see Scheme 11).[24] In the 11-HPETE-derived peroxyl radical 11-**10b**, the (12*E*,14*Z*)-diene geometry is not changed on initiation of the autoxidative bicyclization; thus a *cisoid* allylic radical *cis*-**18b** results, which is trapped by oxygen preferentially from the less hindered front face to afford predominately 15-*epi*-15-G_2-IsoP ((15*R**)-**21b**) with the relative 15β configuration. In contrast, β fragmentation of peroxyl radical 15-**10b** derived from 15-HPETE (15-**11b**) leads to (12*Z*,14*E*)-pentadienyl radical 13-**14b**, which adds oxygen to give the thermodynamically more stable (*E*,*E*)-11-**15b** (see Scheme 11). This then cyclizes to a *transoid* allylic radical *trans*-**18b**, whose recombination with oxygen leads to 15-G_2-IsoP ((*15S**)-**21b**) as the major



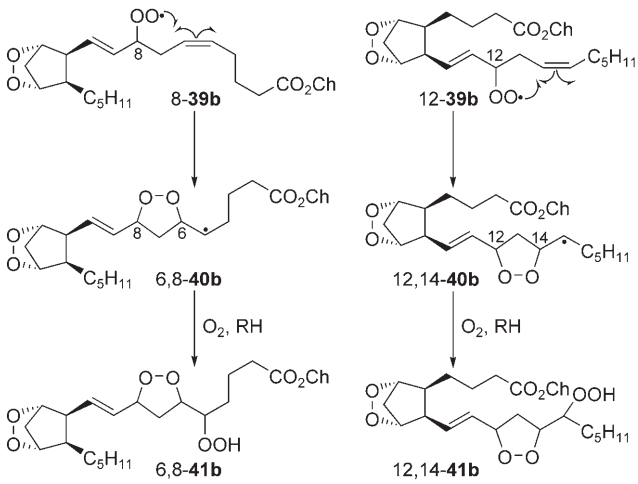
Scheme 19. Dependence of the hydroperoxide side chain configuration of G_2-IsoP **21b** on the diene configuration of pentadienyl peroxyl radicals 11-**10b** and 11-**15b** (only one ring diastereomer and the major diastereomer in the side chain hydroperoxide are shown).

product. These results also do not support the dioxetane mechanism (see Scheme 16).

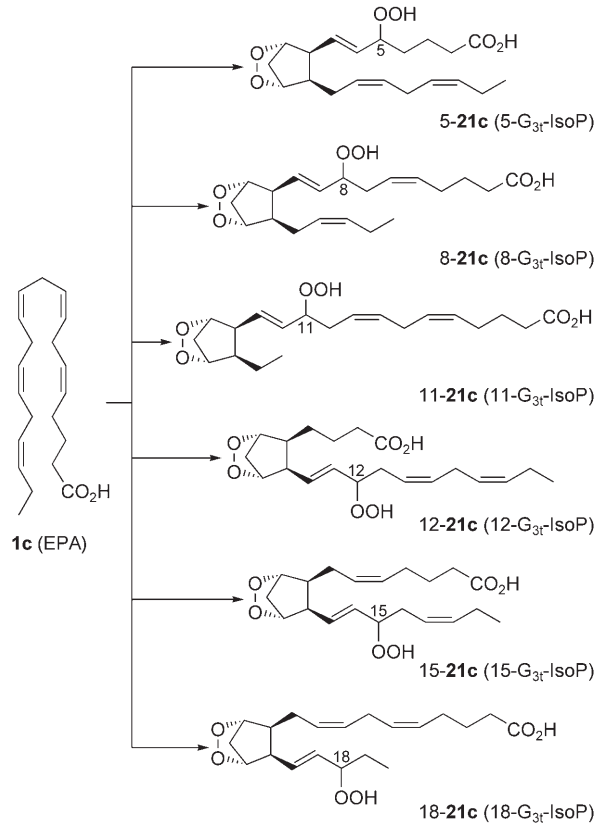
The diminished formation of the 8- and 12-F_2-IsoP series in vivo can be explained only in part by the origin of both IsoP classes from the same pentadienyl radical 10-**9b** (see Scheme 10). Recently, Porter and co-workers demonstrated that the bicyclic peroxyl radicals 8-**39b** and 12-**39b**, which are derived from 8- and 12-HPETE (8-**11b** and 12-**11b**, respectively) abstract hydrogen to afford 8- and 12-G_2-IsoP (8-**21b** and 12-**21b**, respectively) only to a minor extent.[53] They undergo a further kinetically preferred 5-*exo* cyclization of the peroxyl radical to the suitably positioned C5–C6 and C14–C15 alkene units, respectively (Scheme 20). The resulting 1,2-dioxolan-3-ylcarbinyl radicals 6,8-**40b** and 12,14-**40b** provide the 6,8-dioxolane-IsoPs 6,8-**41b** and 12,14-dioxolane-IsoPs 12,14-**41b** after trapping of oxygen and hydrogen transfer. These metabolites were also detected during the in vitro oxidation of cholesteryl arachidonate (**1bCh**) and of arachidonic phospholipids present in LDL.

Cyclization of EPA (**1c**) gives the 11- and the 18-series of isoprostane regioisomers 11-**21c** and 18-**21c** in addition to the 5-, 8-, 12-, and 15-series (Scheme 21). Similar to the product distribution in the G_2-IsoP series, 5-**21c** and 18-**21c** are the major cyclic metabolites formed from **1c** in vivo, while the 8-, 11-, 12-, and 15-series were detected to a lesser extent.[54]

To summarize, all at least triply unsaturated fatty acids **1a–d** undergo defined radical cyclizations. The initial cyclization of peroxyl radicals **10a–d** proceeds highly stereoselectively via a Beckwith–Houk transition state under enzymatic and non-enzymatic conditions to give almost exclusively 3,5-

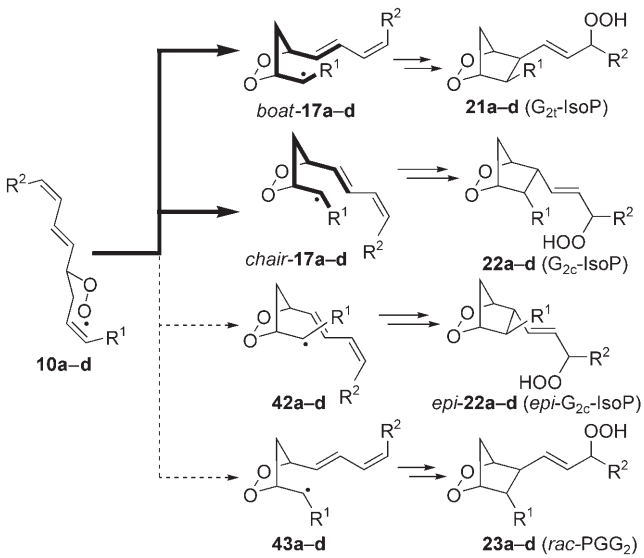


Scheme 20. Formation of dioxolane-IsoP **41b**.



Scheme 21. Formation of EPA-derived G_3-IsoPs (only one ring diastereomer **21c** shown).

cis-oriented 1,2-dioxolan-3-ylalkyl radicals **17a–d** (Scheme 22).[55] The following cyclopentane-forming 5-*exo* radical cyclization proceeds predominately via the two energetically similar chair- and boatlike transition states *boat*-**17a–d** and *chair*-**17a–d** to give G_{2t}-IsoPs **21a–d** and G_{2c}-IsoPs **22a–d**.[56] The ratio of these two cyclization products seems to be influenced somewhat by the reaction medium and the substitution pattern of the cyclizing α and ω chains.



Scheme 22. Stereochemical preferences in the radical bicyclizations to give G-type IsoPs, PhytoPs, and NeuroPs.

The alternative transition states **42a–d** and **43a–d**, which lead to the diastereomeric *epi*-G_{2c}-IsoPs *epi*-**22a–d** and racemic PGG_2 isomers **23a–d** are more disfavored because of unfavorable axial substituents and boat-type arrangements. The difference in the energies between the *chair*-**17a–d**/*boat*-**17a–d** and **42a–d**/**43a–d** transition states was calculated to be about 3.4 kcal mol^{-1}.[21] On increasing the temperature, however, the *trans* side-chain arrangement, as in **23a–d** and *epi*-**22a–d**, seems to become more favorable, but not dominating.

4.2.1.2. *Transformation of the Bicyclic G-Endoperoxide Intermediates To Cyclic PUFA Derivatives*

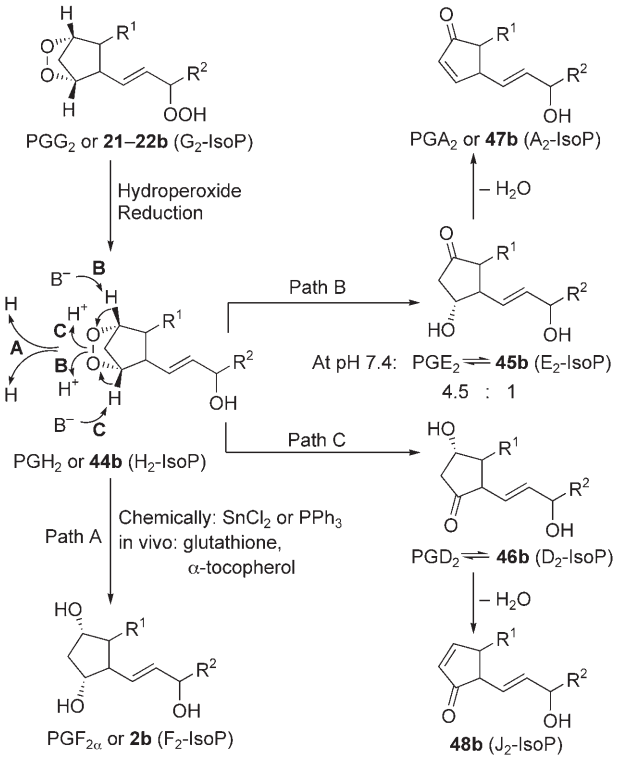
G-Type IsoPs, PhytoPs, and NeuroPs **21a–d** and **22a–d** possess two highly reactive peroxide groups that have several possibilities to stabilize. They are amenable to reduction, rearrangement, and fragmentation reactions. Most importantly, the presence of reducing equivalents in the surrounding tissues is decisive for the fate of the bicyclic G_2-IsoP, G_1-PhytoP, and G_4-NeuroP intermediates.

4.2.1.2.1. *Stabilization of Bicyclic G-IsoPs in the Presence of Reducing Agents—Formation of Isoprostanes, Neuroprostanes, Phytoprostanes, Isothromboxanes, and Isolevuglandins*

4.2.1.2.1.1. *Isoprostanes*

The central bicyclic G_2-IsoP intermediates **21b**/**22b** contain two peroxide groups. The hydroperoxide functionality is usually more easily reduced; therefore, reduction of G_2-IsoPs **21b**/**22b** to H_2-IsoPs **44b** is, in analogy to prostaglandin biosynthesis, the major process for the formation of isoprostanes. The H_2-IsoPs are the central precursors for most isolable IsoPs with different ring substitution patterns (Scheme 23).

The endoperoxide unit in PGH_2 (and naturally also in **44b**) can be reduced easily by reagents such as $SnCl_2$ or PPh_3



Scheme 23. Transformation of PGH_2 and H_2-IsoPs **44b** to metabolites with different ring-substitution patterns (the configuration of the side chains are omitted for clarity).

to generate $PGF_{2\alpha}$ (and also F_2-IsoPs; path A).[57] Several sulfur species are also capable of reducing PGH_2 to $PGF_{2\alpha}$ in competition with rearrangement.[58] In rat liver tissue of normal animals, the regioisomers **2b** are the major isolable IsoP class.[43] Their levels are more than twofold higher than the combined levels of E_2- and D_2-IsoPs **45b** and **46b**, respectively. After oxidative injury, the levels of F_2-IsoP increased even more: They exceeded those of normal animals 146-fold, while the levels of E_2-/D_2-IsoPs **45b** and **46b** increased only 39-fold. In contrast, in vitro autoxidation of **1b** in rat liver microsomes led to a (**45b** + **46b**)/**2b** ratio of more than 5:1.[59] The addition of the reductants glutathione, dithiothreitol, or cysteine to the peroxidizing microsomes resulted in the ratio decreasing to approximately 1.5:1. Antioxidants such as α-tocopherol are also effective in reducing H_2-IsoPs **44b** to **2b** at the expense of **45b** and **46b**.[42] The effect of α-tocopherol was ascribed to an action as a single-electron-transfer reductant to the endoperoxide bridge in H_2-IsoPs **44b**. These results demonstrate that reductive endoperoxide cleavage of **44b** to **2b** proceeds non-enzymatically in vivo. Thus, the formation of **2b** is preferred at high concentrations of reducing agents as well as in the nonpolar environment of cell membranes or LDL, where acid and base concentrations necessary for rearrangements are low.

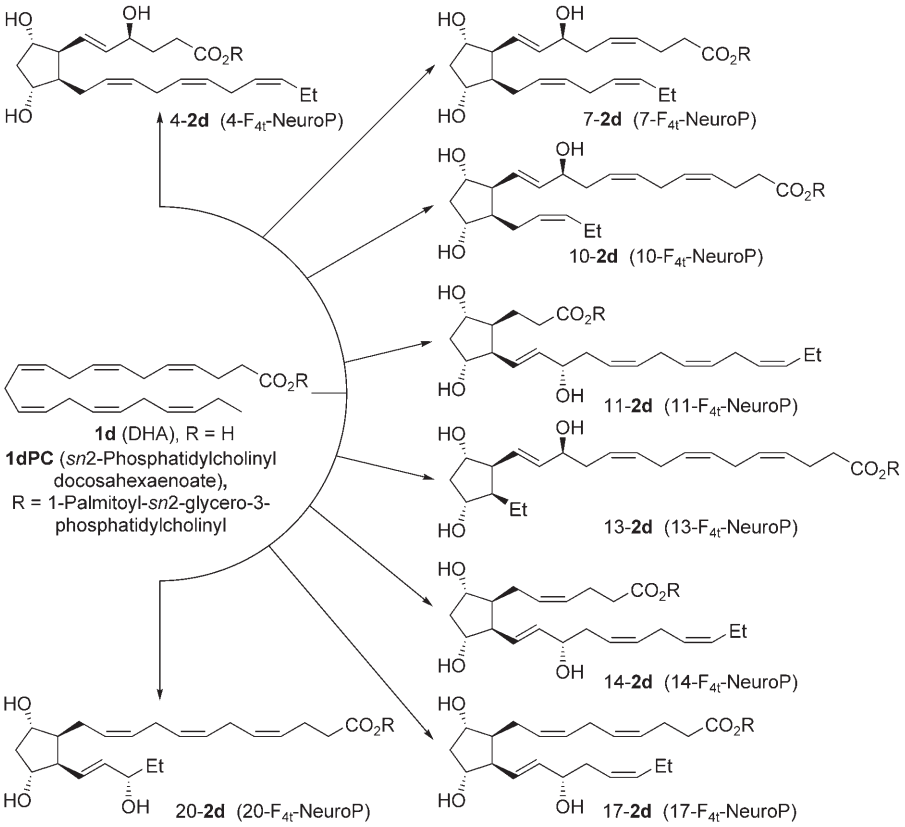
When the concentration of the reducing agents is lower and **44b** is present in aqueous environment, then rearrangement with concomitant opening of the endoperoxide to E_2- and D_2-IsoP isomers **45b** and **46b** prevails (paths B and C).[43,60] This pathway is supported by several experimental studies performed earlier with PGH_2. Since this process should be, however, independent of the side-chain configurations, it is also valid for the formation of **45b** and **46b**. Hamberg and Samuelsson observed the spontaneous formation of PGE_2 from PGH_2 by thin-layer chromatography and in aqueous solution.[57,61] Nugteren and Hazelhof demonstrated the non-enzymatic transformation of PGH_2 to PGE_2 and PGD_2 in slightly acidic aqueous solution.[58b] Subsequently, Porter et al. showed that isolated PGH_2 rearranges spontaneously in the presence of silica gel to a mixture of PGE_2 and PGD_2.[62] However, acid–base catalysis also leads to the formation of PGD_2 or PGE_2.[63]

It is noteworthy that 15-E_2- and 15-D_2-IsoP (15-**45b** and 15-**46b**, respectively) epimerize in aqueous solution at pH 7.4 to a 4.5:1 equilibrium mixture of thermodynamically more stable, but racemic *rac*-PGD_2 and *rac*-PGE_2 diastereomers.[64] The amounts of *rac*-PGD_2 and *rac*-PGE_2 formed in vivo are significant compared to the amount of enzymatically biosynthesized enantiomerically pure PGE_2. Thus, similar to $PGF_{2\alpha}$, there exists a COX-independent pathway to form biologically potent PGE_2 that has to be taken into account to evaluate biological actions of PGE_2 under oxidative stress in vivo. This result indicates also that COX-inhibiting drugs such as aspirin cannot inhibit PGE_2 formation completely.

Dehydration of membrane-bound E_2- and D_2-IsoPs **45b** and **46b**, respectively, is facile under physiological conditions and produces cyclopentenone-A_2- and -J_2-IsoPs **47b** and **48b** respectively, in vitro and in vivo.[65] The combined levels of **47b** and **48b** amounted to 5.1 ng g^{-1} in normal rat liver, but increased 23.9-fold on oxidative injury of rat livers. This value is in good agreement with the 21.2-fold increase in the levels of **45b** and **46b**. Dehydration may occur spontaneously in aqueous solution at almost neutral pH values[64] or can be catalyzed by different enzymes and albumin.[66] The A_2- and J_2-IsoPs **47b** and **48b**, respectively, are found mainly esterified to phospholipids. 15-A_2-IsoPs are especially enriched in the brain,[67] where their basal level exceeds that of F_2-IsoPs ninefold. On oxidative injury of brain tissue, a 12-fold increase in the levels of **47b** and **48b** was observed, while levels of F_2-IsoPs increased only twofold. Phospholipid-esterified **47b** and **48b** are relatively stable in vivo. Free **47b** and **48b** are, however, potent Michael acceptors and conjugate very rapidly with biological nucleophiles, such as proteins and especially glutathione.[65a,68] Levels of reducing agents are thus depleted and oxidative stress is promoted further (see Section 6).

4.2.1.2.1.2. Neuroprostanes

The common precursor for all neuroprostanes is docosahexaenoic acid (DHA, **1d**), which is found in particular in the gray matter of the brain. Esterified at the *sn*2-position of phospholipids, **1dPC** comprises approximately 25–35% of the total fatty acids in neuronal membranes. DHA is essential for normal brain function and development. Its removal through oxidative pathways is connected with the development of several neuronal disorders.



Scheme 24. Formation of F_4-NeuroPs (only one diastereomer shown). The A_4-, D_4-, E_4-, and J_4-NeuroPs **45d**–**48d** have also been detected.

The autoxidation of **1d** derivatives proceeds very similarly to that of AA (**1b**) and leads to NeuroPs **2d** (see Schemes 10, 17, and 23). The higher number of bisallylic positions results in the formation of eight classes of F_4-NeuroP **2d** by a radical peroxidation/bicyclization/oxygenation/reduction sequence (Scheme 24).[69]

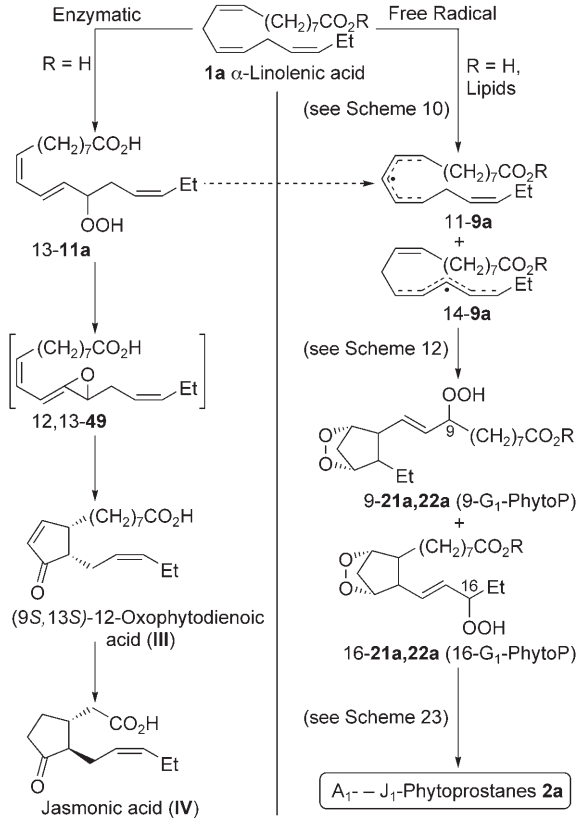
The distribution of NeuroP regioisomers was determined by mass spectrometry.[70] The regioselectivity of the peroxidation/cyclization sequence is not as pronounced as for IsoPs. The predominant regioisomers are 4- and 20-F_4-NeuroP (4-**2d** and 20-**2d**, respectively), however, only in an approximately 3:1 ratio compared to the other F_4-NeuroP isomers, whereas a much higher value of approximately 10:1 for the 5- and 15- as well as the 8- and 12-series of F_2-IsoPs was found (see Section 4.2.1.1). The assignment of the individual regioisomers of NeuroPs was based on the cyclizations of the individual DHA-derived hydroperoxides **11d** (not shown, see Scheme 18). The amounts of **2d** detected in human brain tissue were 19–33 ng g^{-1}.[71] It was found that antioxidants such as glutathione, α-tocopherol, and ascorbate display only a limited capacity to suppress the formation of NeuroPs in rat synaptosomes.[42]

The E_4- and D_4-NeuroPs **45d** and **46d**, respectively, were found in human brain tissue in amounts of 5–13 ng g^{-1}.[71] Similar to the corresponding isoprostanes, they are dehydrated readily to phospholipid-esterified A_4- and J_4-NeuroPs **47d** and **48d**, respectively (see Scheme 23).[72] Their amount of 98 ng g^{-1} in human brain tissue exceeds the amounts of **2d**, **45d**, and **46d** at least fivefold. Like **47b** and **48b**, **47d** and **48d** also add GSH and proteins rapidly, thus depleting cellular reducing equivalents.

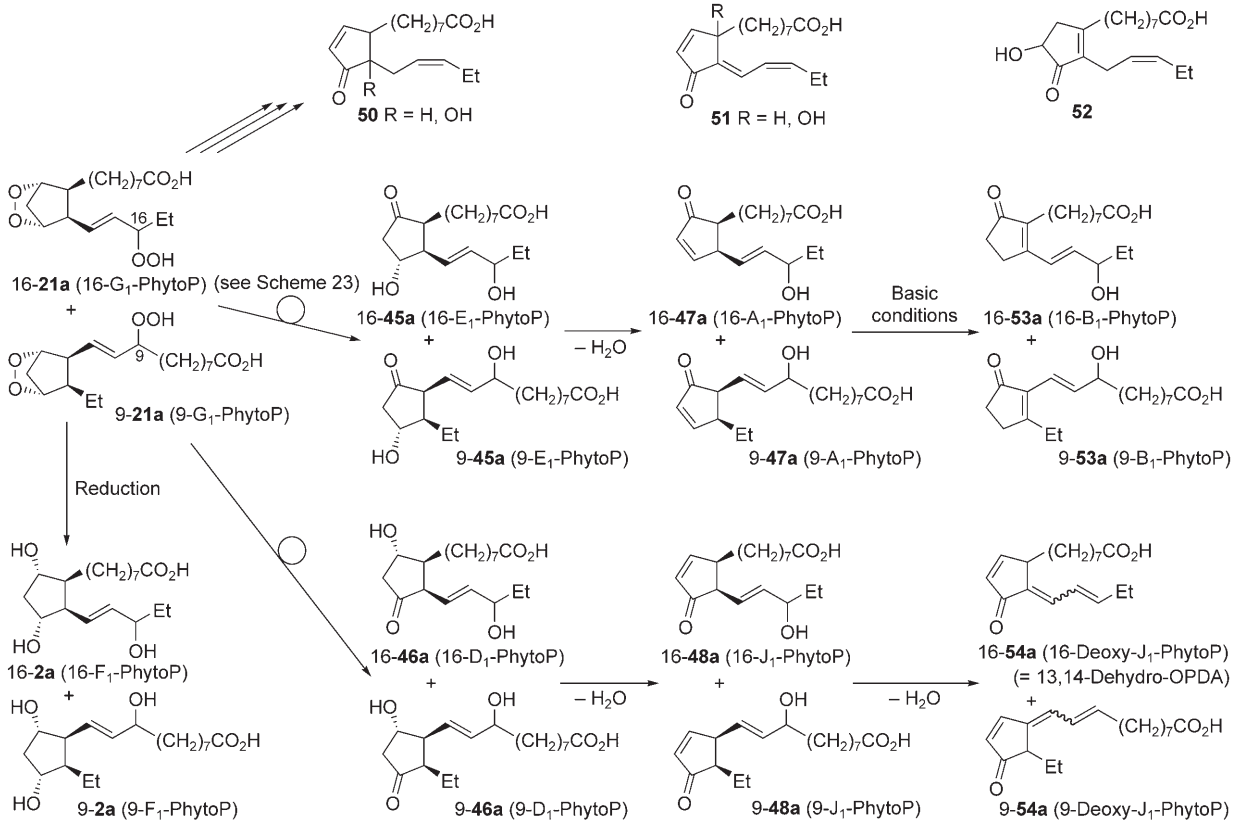
4.2.1.2.1.3. Phytoprostanes

Algae and plants do not produce large amounts of **1b**, while **1c** and **1d** are not produced at all. Nonetheless, some prostaglandin isomers have been identified in algal and plant material, although it was in most cases not established with certainty whether these metabolites are prostaglandins or isoprostanes.[73] The only PUFA present in abundant amounts in plants that is amenable to oxidative cyclization reactions is α-linolenic acid (**1a**). In general, the plant kingdom uses **1a** extensively for signaling purposes (Scheme 25).

As early as 1981, Bohlmann et al. isolated enantiomerically pure dehydrohydroxyphytoprostane metabolites **50**–**52** from *Chromolaena* species as a mixture of double-bond isomers and proposed **1a** as their biosynthetic precursor (Scheme 26).[74] It was then firmly established that the enzymatic cyclization of **1a** leads via the allene oxide 12,13-**49** to 12-oxophytodienoic acid (**III**) and



Scheme 25. Enzymatic and free-radical conversion of LA (**1a**) into cyclic metabolites by plants.



Scheme 26. Formation of major PhytoP classes (only one diastereomer is shown).

subsequently to JA (**IV**, Scheme 25), which is a central compound for the control of secondary metabolism and plant defense.[6a,75] Since plants react to wounding, pathogen infection, and other biotic or abiotic stress by the production of highly increased amounts of reactive oxygen species, **1a** has to be considered as an important compound for reducing the amounts of ROS through conversion into secondary cyclic metabolites. In 1998, Parchmann and Mueller isolated cyclic LA-derived metabolites having a structure similar to E_2-IsoPs. As a consequence of their plant origins, these metabolites were termed E_1-PhytoPs 9- and 16-**45a**.[9] The formation of PhytoP was proposed to occur in a similar manner as that of IsoP through hydrogen abstraction from **1a** to give **9a**, oxygenation, bicyclization, and a second oxygenation to give G_1-PhytoP isomers 9-**21a**/9-**22a** and 16-**21a**/16-**22a**, which are subsequently metabolized to the isolable A_1- to J_1-PhytoPs.

The proposal is firmly supported by autoxidation studies with α-linolenic esters from which mixtures of F_1-PhytoPs were isolated and characterized prior to their first detection in plants (see Scheme 12).[39b,c,41] The presence of a lipoxygenase to catalyze the initial hydrogen abstraction and oxygen trapping proved to be beneficial for the formation of E_1-PhytoPs **45a**. Two regioisomers, 9-E_1-PhytoP (9-**45a**) and 16-E_1-PhytoP (16-**45a**), were isolated from different plants as free acids in an approximately 1:1 ratio in amounts of 4–61 ng g^{-1} dry weight (Scheme 26).

The E_1-PhytoP 9-**45a** and 16-**45a** are prone to rapid dehydration in vivo to form almost equal amounts of 9- and 16-A_1-PhytoP (9-**47a** and 16-**47a**, respectively). Under basic conditions, these isomers undergo facile isomerization of the double bond to give the thermodynamically more stable 9- and 16-B_1-PhytoP (9-**53a** and 16-**53a**, respectively).[76] The combined levels of **47a** and **53a** in different plant leaves were determined to be 11–131 ng g^{-1} dry weight. The levels of **47a** and **53a** increase considerably on pathogen infection or wounding of the plant leaves.

In addition to **45a**, the corresponding free F_1-PhytoPs 9-**2a** and 16-**2a** were isolated from fresh plant material of taxonomically different species and from different plant organs in amounts of 43–1380 ng g^{-1} dry weight.[77] On drying and storage of the plant material, the amount of F_1-PhytoPs increased to 3223–20010 ng g^{-1} of dry weight without changing the regio- and stereoisomeric composition. This finding thus supports the autoxidative nature of the metabolites. Interestingly, the concentration of 9-**2a** and 16-**2a** in fresh plant material was found to be more than two orders of magnitude higher than that of F_2-IsoPs in mammalian tissues. Similar to the isoprostanes, much higher amounts of F_1-PhytoPs were found in esterified form in plant membranes than in the free form.[77] For example, 76 ng g^{-1} dry weight of free **2a** were isolated from fresh peppermint leaves, while 11240 ng g^{-1} of esterified **2aPC** were detected in the same leaves. On wounding the fresh leaves, the level of free **2a** rose to a maximum of 192 ng g^{-1} dry weight after an hour.[78] The amount of esterified E_1-PhytoPs 9-**45a** and 16-**45a** was determined to be 86-fold less than that of F_1-PhytoPs 9-**2a** and 16-**2a**. This result supports the assumption that **2a** is

formed preferentially by reduction of H_1-PhytoPs **44a** in the hydrophobic environment of the membrane, since an aqueous environment is necessary for rearrangement of **44a** to E_1-PhytoPs 9-**45a** and 16-**45a** (see Section 4.2.1.2.1.1).[78]

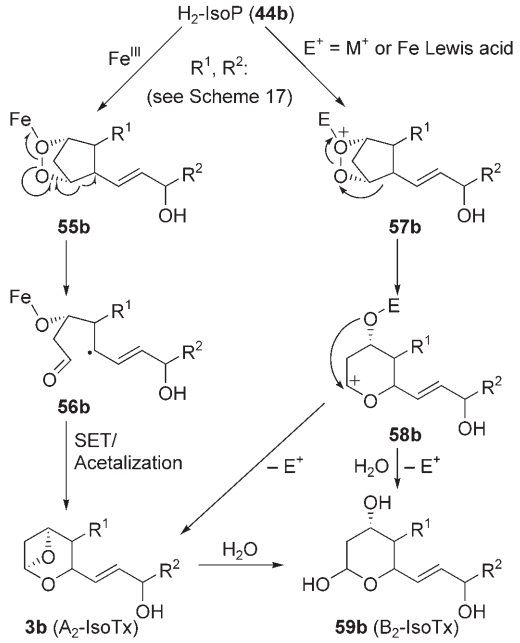
Plants were long considered to be devoid of D_1-PhytoPs 9-**46a** and 16-**46a**. Since it was shown that non-enzymatic isomerization of H_2-IsoPs **44b** leads to mixtures of E_2- and D_2-IsoPs **45b** and **46b**, respectively (see Section 4.2.1.2.1.1), it seemed to be likely that 9-**46a** and 16-**46a** are also formed in plants, but metabolize too quickly to be detected. This assumption is supported by the isolation of compounds **50**–**52** and of 13,14-dehydro-12-oxophytodienoic acid (16-**54a**), which can be biogenetically traced back to 16-J_1-PhytoP (16-**48a**) and thus to 16-**46a**. In 2003, Mueller and co-workers isolated deoxy-J_1-PhytoP 9-**54a** and 16-**54a** as a mixture of regio- and stereoisomers from plant material by conjugating them with fluorescent 7-mercapto-4-methylcoumarin through a Michael addition.[79] By using this technique, the levels of 9-**54a** and 16-**54a** were determined to be 1074–2413 and 294–932 ng g^{-1} dry weight, respectively. Moreover, the D_1-PhytoPs 9-**46a** and 16-**46a** were subsequently identified. Their quantities ranged from 840 to 6234 ng g^{-1} of the dry plant material. The amount of the autoxidatively formed deoxy-J_1-PhytoP **54a** exceeded that of the enzymatically biosynthesized OPDA (**III**) considerably.

4.2.1.2.2. Stabilization of H_2-IsoPs by Rearrangement Reactions involving the Cyclopentane Ring

4.2.1.2.2.1. Isothromboxanes (IsoTx)

Thromboxanes (TxA_2 and TxB_2; **II**) are formed enzymatically from PGH_2 by a rearrangement involving the endoperoxide linkage and the cyclopentane ring. Hecker and Ullrich demonstrated that this rearrangement also occurs non-enzymatically, albeit in low yield and only in the presence of (porphyrin)iron(III) complexes in aqueous solution (Scheme 27).[80] They ascribed the action of the iron compound to a single-electron transfer (SET) reduction of the iron-coordinated endoperoxide **55b**, followed by radical fragmentation to give **56b**. A SET oxidation of the allylic radical followed by recyclization finally afforded TxA_2 (**II**).

Morrow et al. investigated the possibility as to whether the TxA_2 diastereomers A_2-IsoTxs **3b** are formed in vitro and in vivo on a similar pathway.[81] A_2-IsoTxs **3b** could not be detected in vitro or in vivo because of their high instability. B_2-IsoTxs **59b** were also not found in vivo in plasma under normal conditions, but minute amounts of **59b** were detected in lipid extracts. After CCl_4-induced oxidative injury, however, in vivo levels increased to 102 ng g^{-1} in the lipid extract and 185 pg mL^{-1} in plasma. B_2-IsoTxs **59b** were formed as a mixture of regio- and stereoisomers. It was shown in separate experiments that the formation of B_2-IsoTxs **59b** occurs almost exclusively with membrane-bound AA (**1b**), from which it was released into plasma only subsequently. In vitro autoxidation of AA initiated by Fe/ADP/ascorbate also led to the formation of **59b**. This finding indicated that complexed iron is not essential for the rearrangement of H_2-IsoPs **44b** to **59b** and that the ring expansion may be in fact either radical-



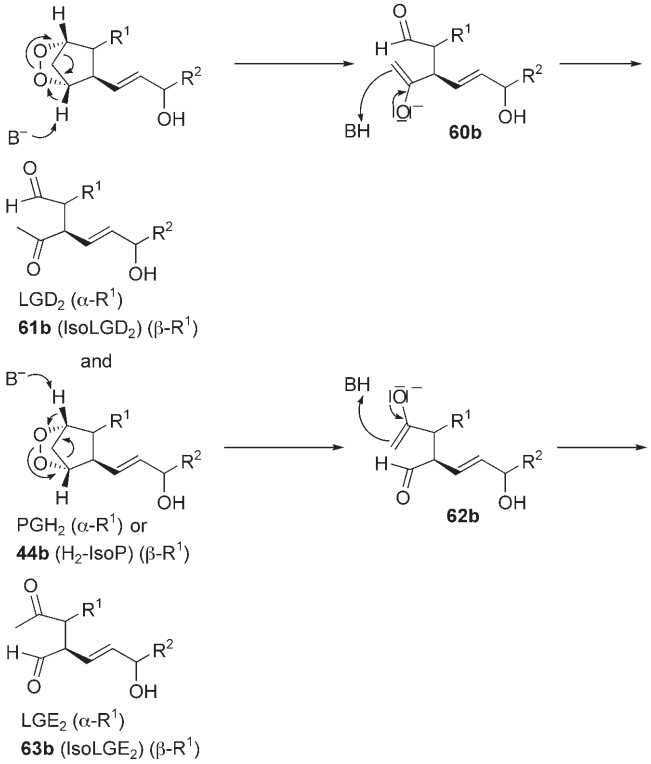
Scheme 27. Possible modes of formation of isothromboxanes **3b** and **59b**.

mediated or cationic. The latter pathway proceeds by a sequence consisting of initial coordination of an electrophile E^+ or a metal Lewis acid such as Fe^{3+} to **44b**. The activated endoperoxide **57b** undergoes a concerted 1,2-rearrangement to give six-membered carbenium ion **58b**.[82] From this intermediate, B_2-IsoTxs **59b** can form either via A_2-IsoTx **3b** or directly by hydrolysis. Clearly, more work is necessary to elucidate the mechanisms by which IsoTsx form in detail.

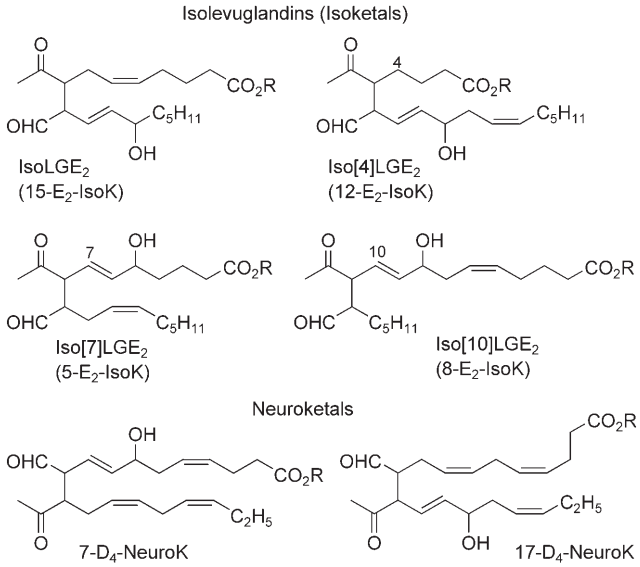
4.2.1.2.2.2. Isolevuglandins (Isoketals) and Neuroketals

It has long been known that only a part of cyclic AA metabolites are transformed to isoprostanoids. A part of PGH_2 and probably also 15-H_2-IsoP fragments to acyclic products such as 12-hydroxyheptadecatrienoate (**6b**) and MDA (**7**).[14] Another notable part of modified AA units was found conjugated to proteins and DNA.[17b,83] Early studies by Zagorski and Salomon revealed that simple dioxabicyclo-[2.2.1]heptanes rearranged in aqueous solution or organic solvents spontaneously to give highly reactive γ-keto aldehydes. PGH_2 also suffered this rearrangement to give C20-γ-keto aldehydes, which were termed levuglandins (LGs) LGD_2 and LGE_2 (Scheme 28).[84] They form competitively and to a higher extent than PGD_2 and PGE_2.[63] The ratio of LGD_2/E_2 to PGD_2/E_2 is dependent on the solvent polarity: The more nonpolar the medium, the more LGs were formed. The rearrangement was shown to be catalyzed by bases such as acetate or imidazole. Thus, proteins may catalyze the formation of LGs as well as of IsoLGs **61b** and **63b** from PGH_2 or H_2-IsoPs **44b** in vivo.

Primary and secondary kinetic isotope effects indicate a deprotonation and protonation mechanism for the rearrangement of **44b**, with deprotonation of one of the bridgehead protons triggering a concerted C–C and O–O bond cleavage of both the endoperoxide and the cyclopentane ring. The



Scheme 28. Rearrangement of PGH_2 or H_2-IsoPs **44b** to LGs and IsoLGs **61b** or **63b**.

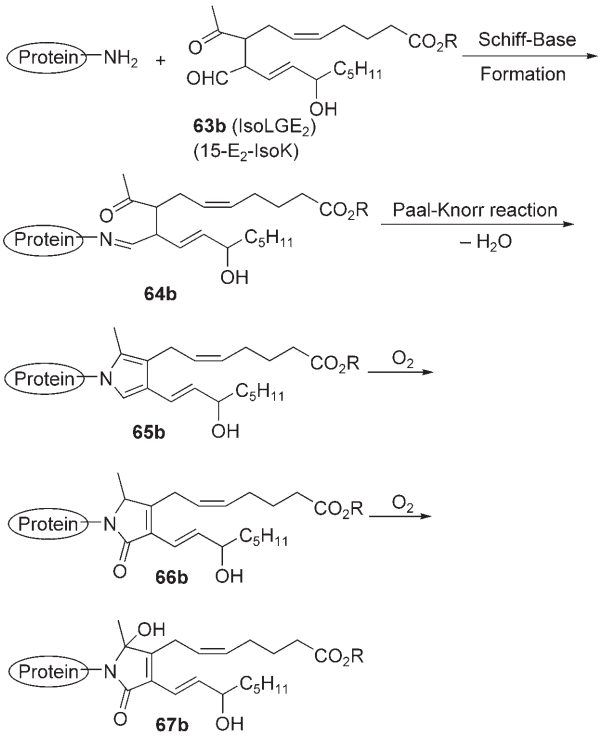


Scheme 29. Regioisomeric $IsoLGE_2$ isomers **63b** (in $IsoLGD_2$ isomers **61b**, the positions of aldehyde and acetyl groups are exchanged; not shown) and neuroketals **5d** (only two members of the D_4-series are shown).

resulting ketone enolates **60b** and **62b** are subsequently protonated to LGs or IsoLGs **61b** or **63b**, respectively. The selectivity for the formation of either E_2/D_2-IsoPs **45b**/**46b** or $IsoLGD_2/E_2$ **61b**/**63b** is determined by the pH value of the medium. Acidic co-catalysts steer the rearrangement reaction of **44b** towards the formation of **45b**/**46b** (see Scheme 23), while **61b**/**63b** are the preferred products under basic conditions. More detailed investigations revealed that membrane- as well as LDL-bound **1b** serve as the precursors for **61b**/**63b**.[17a] This process may also be triggered by enzymes such as myeloperoxidase.[27] Since **61b**/**63b** derive also from **44b**, four series of racemic regio- and stereoisomers are formed (Scheme 29).

The 4-oxoaldehyde unit in **61b**/**63b** displays a high reactivity towards primary amine functions of simple buffers such as tris(hydroxymethyl)aminomethane (Tris),[83a] phosphatidylethanolamine,[85] or lysine units present in LDL[86] and proteins. At first, Schiff bases **64b** are formed, which subsequently cyclize to pyrroles **65b** by a Paal–Knorr condensation (Scheme 30). These electron-rich heterocycles are susceptible to further oxidation to 5*H*-pyrrol-2-ones (lactam adducts) **66b** and 5-hydroxy-5*H*-pyrrol-2-ones (hydroxy lactam adducts) **67b**.[87]

Protein adduction of IsoLGs **61b**/**63b** to plasma proteins or even to membrane proteins can occur only after hydrolysis by phospholipase A_2 (PLA_2). The levels of IsoLG–protein conjugates **65b**–**67b** in plasma are approximately an order of magnitude higher than those of IsoPs **2b**. $Iso[7]LGD_2$ was determined to be the most abundant of the IsoLG–protein conjugates in blood;[88] these adducts greatly disturb normal



Scheme 30. Protein adducts from IsoLGs **61b**/**63b** (only one of the possible regio- and stereoisomers is shown).

protein function.[17b] Remarkably, the IsoLG–protein adducts **65b**–**67b** are rather resistant to protein degradation by the 20S proteasome, thus retarding clearance of the IsoLG units.[89] It must be noted that LGs and IsoLGs form the same protein conjugates. The actual precursor may be determined by analyzing the configuration of the hydroxy group in the side chain and/or by the presence of metabolites

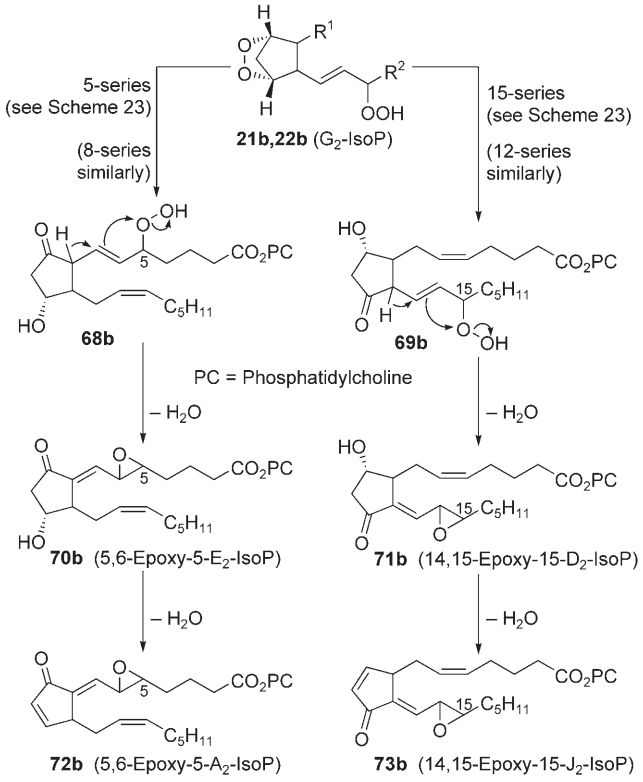
as regioisomeric and diastereomeric mixtures (see Scheme 29). In the absence of amines at pH 7.8, the levuglandins are prone to dehydration to anhydrolevuglandins.[90] Most likely, but not proven rigorously, IsoLGs should also form such products.

Neuroketals (NeuroKs, **5d**) and their protein adducts were isolated after in vitro autoxidation of DHA (**1d**) in rat brain synaptosomes initiated by an iron/ascorbate/ADP system (see Schemes 28 and 29).[18] NeuroK-derived protein adducts were also detected in vivo post-mortem at levels of 9.9 ng g^{-1} brain tissue in human brains of patients that had died under normal circumstances. Neuroketals **5d** are even more reactive than IsoLGs **61b**/**63b** in vitro and in vivo. Some of the G_4-NeuroP diastereomers can undergo oxidation at additional bisallylic positions to provide bis(hydroperoxides) and subsequently diols by reduction before conjugating to proteins in brain tissues by the IsoLG pathway (not shown). Alternatively, further hydroperoxidation of protein-bound lactams may also occur.[91]

4.2.1.2.3. *Reaction of G_2-IsoPs in the Absence of Reducing Agents to give Epoxyisoprostanes*

Phospholipid-bound epoxy-D_2-, E_2-, A_2-, and J_2-IsoPs **70b**, **71b**, **72b**, and **73b**, respectively, were detected in mildly oxidized LDL, and **71b** was shown to induce effective binding of monocytes to the endothel (Scheme 31).[92] No epoxy-F_2-IsoPs were ever found in vivo. The formation of epoxy-IsoP **70b**–**73b** is therefore best rationalized by assuming that both peroxide functionalities in G_2-IsoPs **21b** and **22b** cannot be reduced. Thus, rearrangement of the endoperoxide to hydroperoxy-E_2-IsoPs **68b** and hydroperoxy-D_2-IsoP **69b**, respectively, is the only possible pathway to form a stable compound. The acidity of the α-keto hydrogen atom and the hydroperoxy functionality of **68b** and **69b** enable a unique 1,5-dehydration reaction to occur with concomitant formation of an epoxide to give epoxy-E_2-IsoPs **70b** and epoxy-D_2-IsoPs **71b**, respectively.

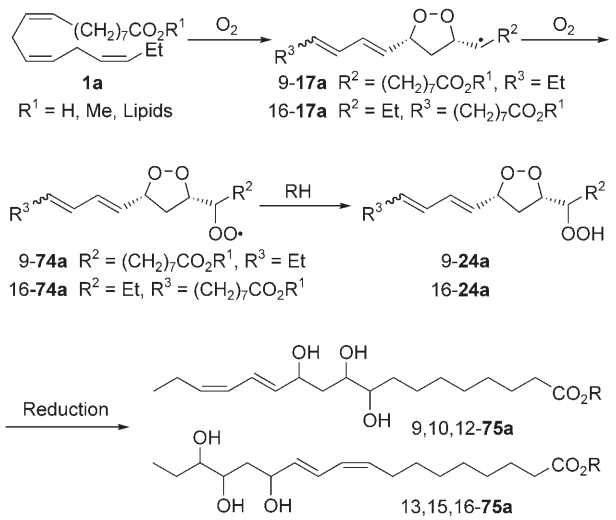
This rearrangement/dehydration sequence also accounts for the fact that, in contrast to normal IsoP generation (see Section 4.2.1.1), only a limited product spectrum with respect to the regioisomers in the 5-, 8-, 12-, and 15-series results. Specifically, 5- and 8-G_2-IsoPs (5-**21b**, 5-**22b** and 8-**21b**, 8-**22b**) rearrange to epoxy-E_2-IsoPs (**70b**) only, while 12- and 15-G_2-IsoP (12-**21b**, 12-**22b**, and 15-**21b**, 15-**22b**) lead to epoxy-D_2-IsoPs (**71b**) exclusively.[93] Epoxy-IsoPs **70b** and **71b** were shown to dehydrate spontaneously to either epoxy-A_2- or epoxy-J_2-IsoPs **72b** and **73b**, respectively.



Scheme 31. Formation of epoxy-IsoPs from G_2-IsoPs **21b** and **22b**.

4.2.2. *Autoxidative Metabolism of PUFAs at Increased Oxygen Concentration—1,2-Dioxolanes and Isofurans*

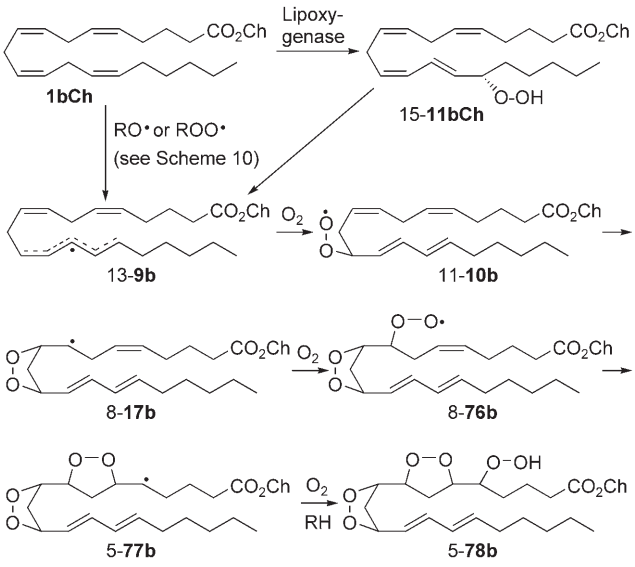
All previously discussed radical-derived cyclic PUFA metabolites are formed at rather low or normal oxygen concentrations. However, the diradical character of oxygen and its high reactivity toward carbon-centered radicals means that the oxygen concentration should play a decisive role in the fate of the PUFAs. Thus, a completely different spectrum of compounds will be formed when the oxygen concentration in the system increases. This was illustrated experimentally for free and esterified LA (**1a**) or the corresponding hydroperoxides **11a**. They undergo hydrogen abstraction, peroxidation, and radical 5-*exo* cyclization in an atmosphere of pure oxygen to generate 1,2-dioxolanylcarbinyl radicals **17a** as usual. These radicals are then trapped predominately by oxygen to give 1,2-dioxolanylperoxyl radicals **74a**, which afford monocyclic hydroperoxy endoperoxides **24a** as major products (Scheme 32).[41,94] Phytoprostanes were detected only as minor products or not at all in these experiments.



Scheme 32. Peroxidation of LA derivatives in an O_2 atmosphere.

The hydroperoxy endoperoxides **24a** undergo reduction in vitro[94d,95] and in vivo[78] to afford trihydroxyoctadienoates 9,10,12-**75a** and 13,15,16-**75a** as diastereomeric mixtures.

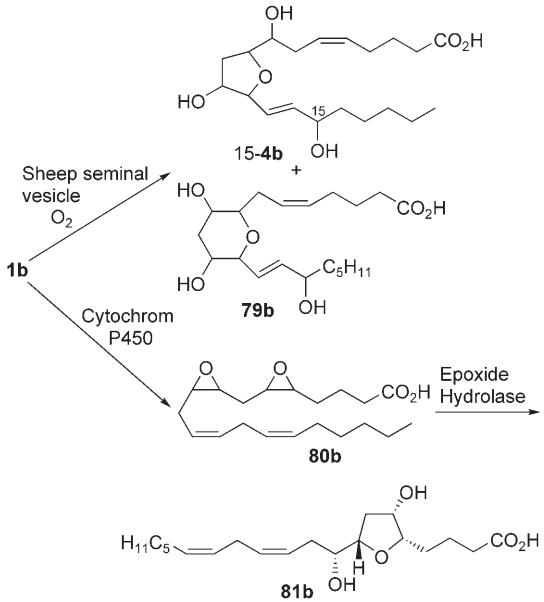
Cholesteryl arachidonate (**1bCh**) as well as the HPETE 15-**11bCh** may be used in serial peroxyl cyclization sequences under high oxygen concentration to give 1,2-dioxolane-containing PUFAs 5-**78b** (Scheme 33).[29b,94a,96] Similar serial 1,2-dioxolanes may be obtained by the in vitro photooxidation of methyl linolenate in the presence of a methylene blue sensitizer.[97]



Scheme 33. AA and 15-HPETE in serial peroxyl radical cyclizations to yield dioxolanes 5-**78b**.

In 1970, Pace-Asciak and Wolfe reported the isolation of the first AA-derived isofuran 15-**4b** and also of a tetrahydropyran **79b** in an oxygen atmosphere[98a] and made an initial attempt to explain the formation of the isofuran either enzymatically or autoxidatively from membrane-bound **1b** (Scheme 34).[98b] Moghaddam et al. reported the isolation of tetrahydrofurandiols **81b** on enzymatic diepoxidation/epoxide hydrolysis by cytochrome P450 and epoxide hydrolase action on **1b** in mouse liver microsomes.[99] The structures of the enzymatically formed THF-diols **81b** differ from autoxidatively generated 15-**4b** by one hydroxy group.

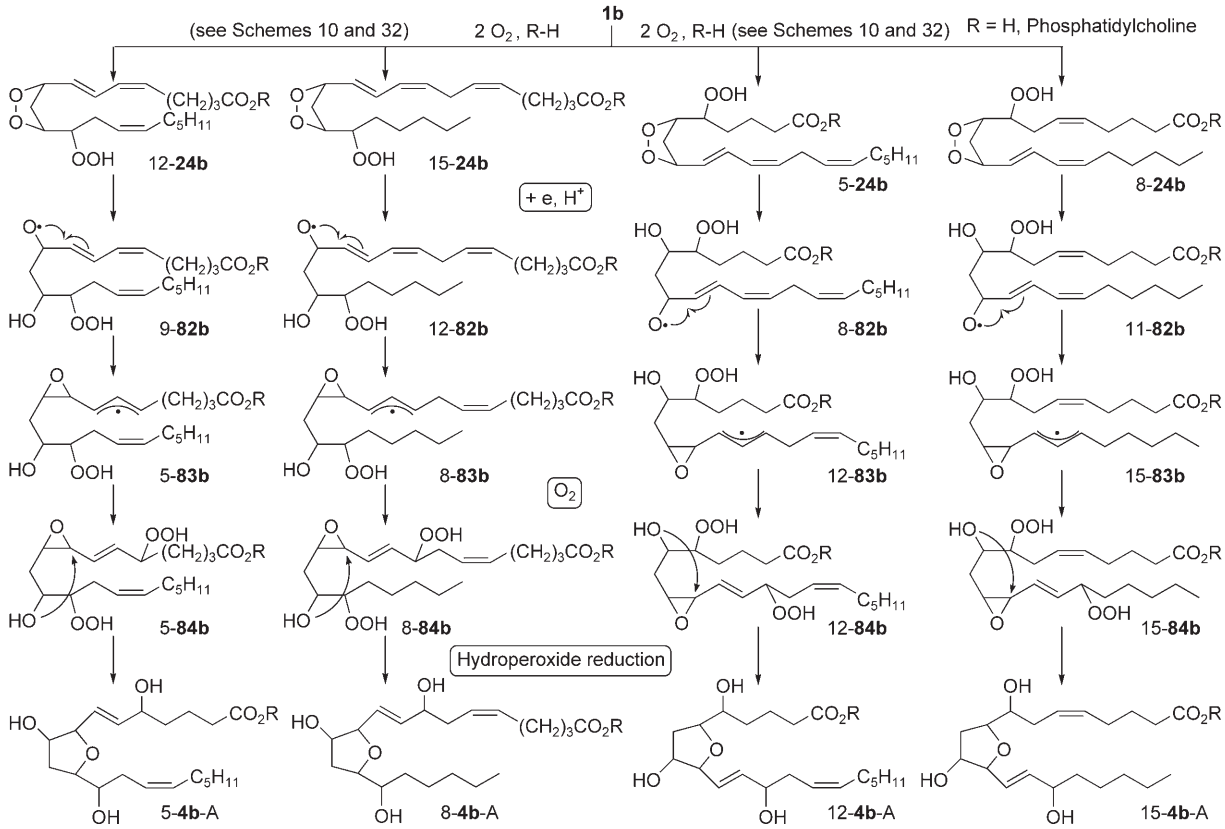
In 2002, the dependence of IsoP formation on the oxygen partial pressure was systematically investigated. It was found that only a fraction of AA (**1b**) was converted in vitro into IsoPs **2b** (see Schemes 11, 17, and 23). A significant amount of a new class of oxidative AA metabolites—termed isofurans (IsoFs, **4b**)—was isolated (Schemes 35 and 36).[100a] The ratio of **4b** to **2b** by in vitro autoxidation of **1b** was 5.6:1. The **4b**/**2b** ratio was, however, strongly dependent on the oxygen concentration. The concentration of **2b** displayed a plateau effect above 21% oxygen, while the concentration of **4b** increased further as the oxygen pressure increased in vitro. IsoFs **4b** were found to be present in all body fluids and organs under normal conditions. The detected amounts are, however, strongly dependent on the natural oxygen concentration in several organs. The **4b**/**2b** ratio in the liver was found to be 0.15:1, while it reversed to 2.3:1 in the kidneys. A fivefold increase in the concentration of **4b** was detected in the lung on hyperoxia at 100% oxygen, while the levels of **2b** did not change at all.



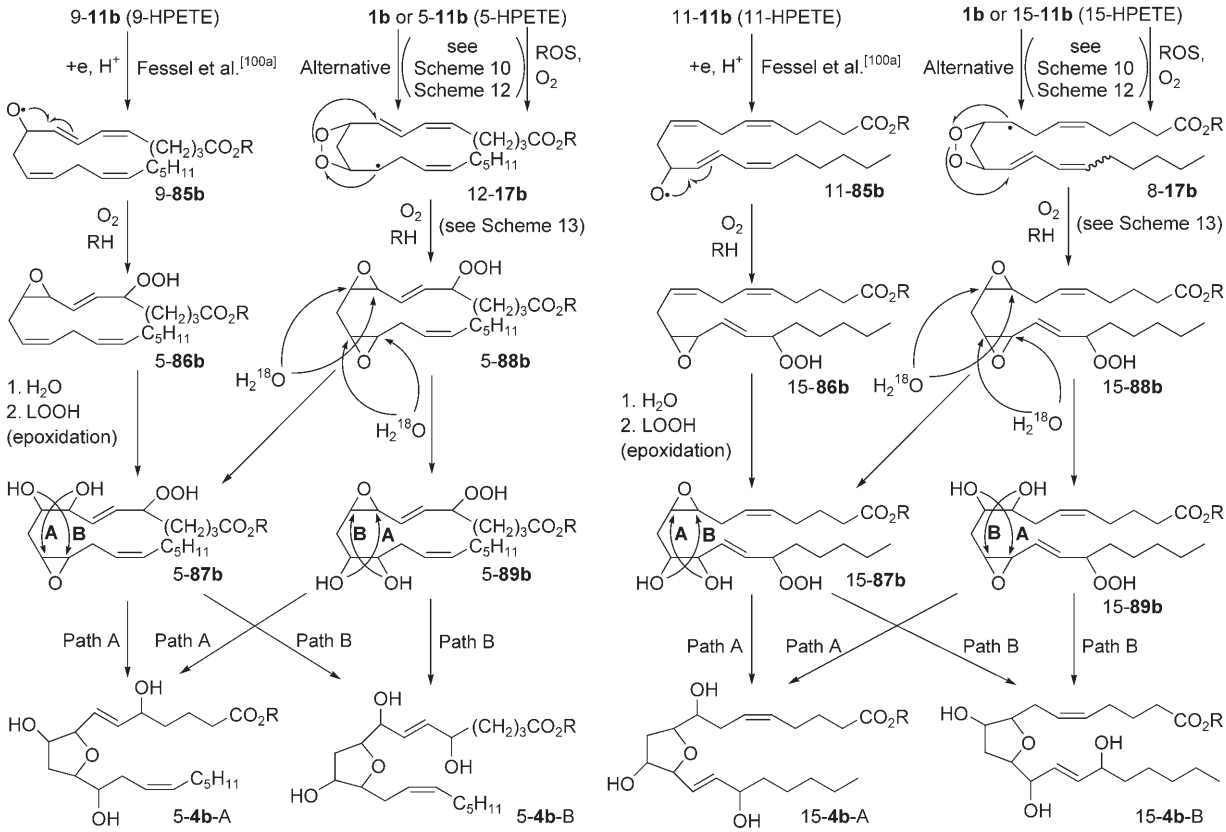
Scheme 34. Formation of oxygen heterocycles 15-**4b** and **79b** or **81b** by autoxidative or enzymatic cyclizations (only one regioisomeric product is shown for the enzymatic process).

The formation of IsoFs was rationalized on the basis of $^{18}O_2$ and $H_2{}^{18}O$ incorporation studies, and found to occur by two competing fundamental mechanisms. In a certain portion of the isofurans, namely the alkenyl isofurans **4b**-A, three molecules of $^{18}O_2$ were incorporated. This can be explained by an initial formation of hydroperoxy-1,2-dioxolanes **24b** through oxygen trapping of the dioxolanylcarbinyl radicals **17b** (see Scheme 33). A following single-electron reduction of the peroxide bridge generates an alkoxyl radical **82b**, which undergoes a 3-*exo* cyclization to the conjugated diene unit (Scheme 35). The resulting epoxyallylic radicals **83b** are then trapped by oxygen to afford the dihydroperoxy epoxides **84b**. These are susceptible to regioselective intramolecular nucleophilic ring opening of the epoxide by the hydroxy group. Final reduction of the hydroperoxide gives rise to the four regioisomeric alkenyl isofuran classes **4b**-A as mixtures of diastereomers. The formation of all the diastereomers is not unexpected, since none of the reaction steps should be highly diastereoselective. It should also be mentioned that HPETEs may serve as precursors for the reaction sequences (see Section 4.2.1.1 and Scheme 18). The order of some steps may be different from the mechanism shown, but the product distribution will not be changed.

Two oxygen molecules and one molecule of $H_2{}^{18}O$ were incorporated in the other IsoFs, namely all the enediol-IsoFs **4b**-B and a fraction of the alkenyl-IsoFs **4b**-A. On the basis of this result, Fessel et al. proposed a mechanism involving SET reduction of HPETEs **11b** to give alkoxyl radicals 9-**85b** or



Scheme 35. Formation of alkenyl IsoFs **4b**-A by reductive endoperoxide cleavage of **24b**, 3-*exo* cyclization, and epoxide ring opening of **84b**.



Scheme 36. Two alternative mechanistic rationales for the formation of **4b**-A and **4b**-B (only two of the four possible regioisomeric series are shown).

11-**85b**, which undergo a 3-*exo* cyclization to the adjacent diene unit (Scheme 36). Such processes were suggested to explain the formation of minor autoxidation products of linolenate-derived hydroperoxides.[94c] Peroxygenation of the resulting epoxyallyl radicals leads to epoxy hydroperoxides 5-**86b** and 15-**86b**. The epoxides **86b** are hydrolyzed to diols, which are subsequently epoxidized by other hydroperoxides (HPETEs) in the presence of Mn^{II} ions to give epoxy diols 5-**87b** and 15-**87b**. These epoxy diols undergo intramolecular nucleophilic ring-opening according to path A to afford alkenyl-IsoFs 5-**4b**-A and 15-**4b**-A. Enediol-IsoFs 5-**4b**-B and 15-**4b**-B are obtained by ring opening of 5-**87b** or 15-**87b** according to path B.

It seems, however, somewhat unlikely that reduction of the HPETE **11b** should be involved early in the formation of IsoFs under a high oxygen concentration. Moreover, the concentrations of Mn^{II} or other transition-metal ions and of hydroperoxides in membranes, which are the major place of IsoF formation, are rather low in vivo. We propose, therefore, that the enediol-IsoFs **4b**-B also form according to the unified isoprostane pathway via dioxolanylcarbinyl radicals 12-**17b** or 8-**17b**. This proposal is based on the studies by the research groups of Porter and Bloodworth who demonstrated convincingly that AA-derived dioxolanylcarbinyl radicals 8-**17b** or 12-**17b** undergo a facile 1,3-S_Hi reaction with a rate constant of $1.8\times10^5\ s^{-1}$, which is clearly competitive to C–C radical cyclization to IsoPs **2b**.[101] The so-formed alkoxyl radicals stabilize by 3-*exo* cyclization and oxygenation to diepoxy hydroperoxides 5-**88b** and 15-**88b** (see Scheme 13).[44] After hydrolysis to form the regioisomeric epoxy diols 5-**87b**, 15-**87b**, 5-**89b**, and 15-**89b**, an intramolecular nucleophilic ring opening of the epoxide by a suitably positioned hydroxy group leads to alkenyl-IsoFs 5- and 15-**4b**-A according to path A and to enediol-IsoFs 5- and 15-**4b**-B by path B. The last steps are also supported by the results of Porter and co-workers, who showed that structurally related epoxy alcohols recyclize in some cases spontaneously to a mixture of 2-(1-hydroxyalkyl)tetrahydrofurans and 2-alkyl-3-hydroxytetrahydropyrans analogous to **4b** and **79b** (see Scheme 34).[101a,102] This raises the question whether **79b** may also be formed in vivo. Clearly, much more work is necessary to provide a detailed understanding of the formation of the IsoFs. The neurofurans **4d** derived from DHA (**1d**) have since been identified as a novel cyclic PUFA class in mice and are present in concentrations higher than all the other cyclic PUFA metabolites.[100b]

4.3. Metabolism of Isoprostanes and Neuroprostanes

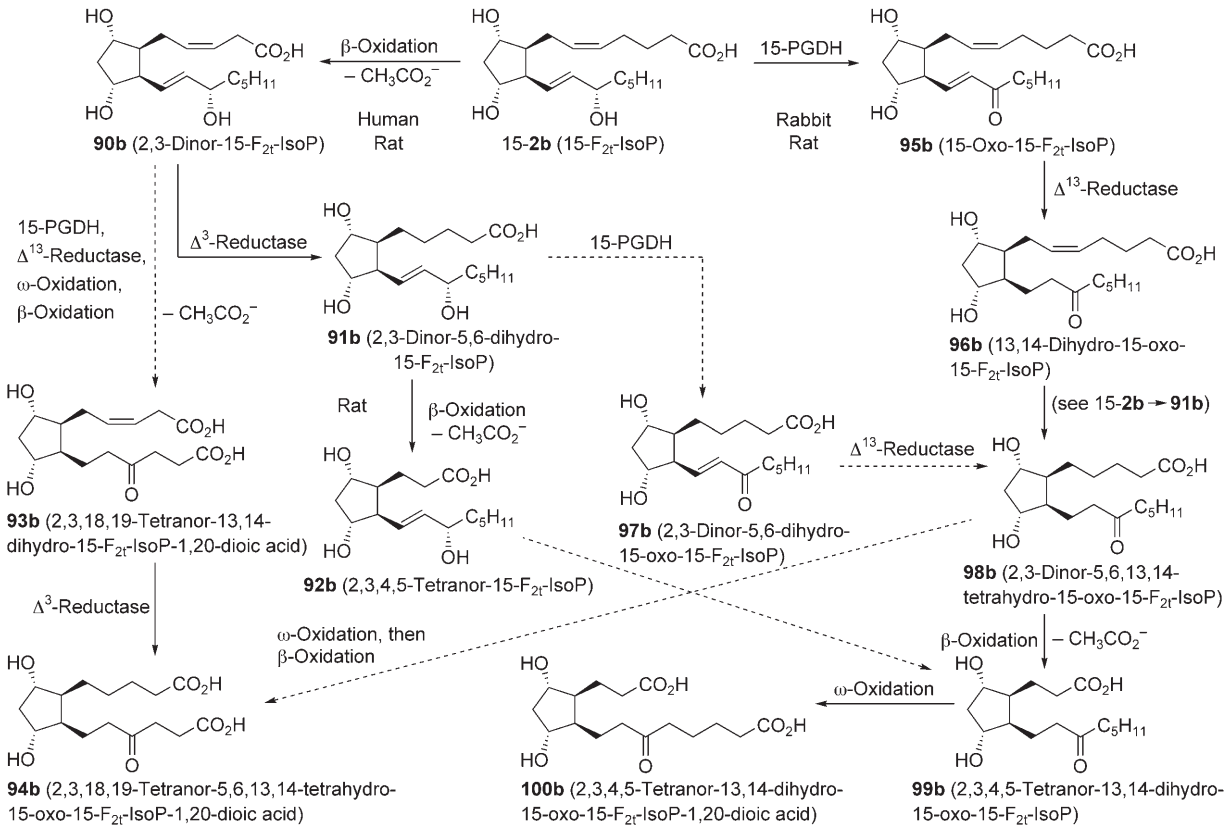
Most of the IsoPs **2b**, NeuroPs **2d**, PhytoPs **2a**, and IsoFs **4b** are formed from esterified PUFAs in lipid membranes. They are released by saponification with the aid of phospholipase A_2 (PLA_2) into plasma.[103] It was also convincingly demonstrated that esterified F_2-IsoPs are set free from membranes by both the plasma- and intracellular platelet-activating factor acetyl hydrolases.[104] A fraction of the F_2-IsoPs formed is excreted with the urine. It is noteworthy that the major amount of urinary excreted $PGF_{2\alpha}$ in man derives also from the isoprostane pathway since it is not enantiomerically pure—it is also not racemic, but exhibits an enantiomeric excess of *ent*-$PGF_{2\alpha}$.[21] Administration of ibuprofen to healthy individuals results in the level of overall excreted $PGF_{2\alpha}$ decreasing only slightly. PGE_2 levels that result from a purely cyclooxygenase-catalyzed reaction decreased dramatically in the same trial, thus providing additional support to the notion that the amounts of oxidatively generated *rac*-$PGF_{2\alpha}$ considerably exceed those biosynthesized by cyclooxygenase.

There are a few studies on the metabolism of isoprostanes **2b** in vivo. The major human urinary metabolite of 15-F_{2t}-IsoP (15-**2b**) was shown to be 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP (**91b**),[105] formed by β-oxidation via 2,3-dinor-15-F_{2t}-IsoP (**90b**) and reduction of the 5,6-double bond (Scheme 37). Metabolite **91b** is further degraded to 2,3,4,5-tetranor-15-F_{2t}-IsoP (**92b**) in rat hepatocytes.[105b] Earlier, Roberts and co-workers detected higher oxygenated tetranor-dicarboxylic metabolites **93b** and **94b** in human urine and plasma.[106] These metabolites may be formed from **90b** by oxidation of the 15-hydroxy group with 15-hydroxyprostaglandin dehydrogenase (15-PGDH), reduction of the 13,14-olefin by Δ^{13}-reductase, ω-oxidation to the carboxylic acid function, β-oxidation, and reduction of the 5,6-olefin by Δ^3-reductase.

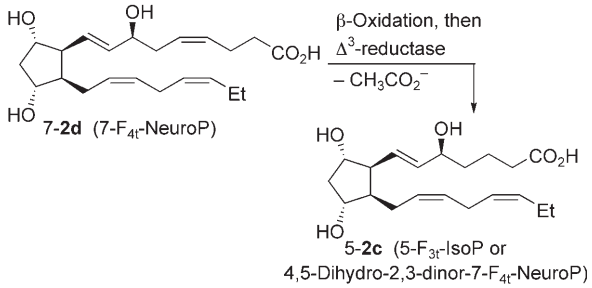
In rabbits and rats, an additional metabolic pathway for 15-F_{2t}-IsoP (15-**2b**) apparently operates in competition, but has not been observed in humans. Basu investigated the fate of 15-**2b** in rabbits and found it to be degraded in a few minutes in plasma.[107] One of the major metabolites found was 2,3,4,5-tetranor-13,14-dihydro-15-oxo-15-F_{2t}-IsoP (**99b**), whose formation can be explained in analogy to $PGF_{2\alpha}$ metabolism: Oxidation of the 15-hydroxy group by 15-PGDH to **95b**, followed by reduction of the 13,14-double bond by Δ^{13}-reductase forms 13,14-dihydro-15-oxo-15-F_{2t}-IsoP (**96b**). This metabolite is prone to further break down by β-oxidation to give **98b** and then **99b**.

Chiabrando et al. were able to detect metabolites of several diastereomers of 15-**2b** by incubation of isolated rat hepatocyte preparations with authentic compounds.[108] In this study, metabolites **90b**–**92b**, which result from initial β-oxidation, and also metabolites **95b**–**99b**, which stem from initial dehydrogenation of the 15-hydroxy group, were detected. It was also proved that the configuration of the starting isoprostane played a significant role in the metabolism: IsoPs with an 15*S* configuration were oxidized by both routes, while compounds having an 15*R* configuration and/or an *ent*-ring configuration did not form significant amounts of metabolites **95b**, **96b**, and **98b**. The tetranor metabolite **99b** was, however, formed from all the F_2-IsoP isomers, which suggests that cross-over between the two pathways from **91b** to **98b** via **97b** or from **92b** to **99b** (as well as from **98b** to **94b**) may occur (dashed arrows).

In 2006, 5-F_{3t}-IsoP (5-**2c**) was detected in human urine. There is evidence that 7-F_{4t}-NeuroP (7-**2d**), which is undetectable in urine, is metabolized in vivo through β-oxidation to give 5-**2c** (Scheme 38). Thus, 5-**2c** may be potentially used as a marker for exogenous dietary EPA as well as for endogenously formed neuroprostanes (see Section 5).[109]



Scheme 37. Detected metabolites of 15-F_{2t}-IsoP (15-**2b**) in different species. Dashed arrows indicate possible, but not confirmed, metabolic pathways (only one diastereomer is shown).



Scheme 38. Metabolism of a NeuroP.

4.4. PUFAs, Isoprostanes, and Phytoprostanes as Signaling Molecules during the Development of the Oxygen-Based Metabolism

After the start of photosynthesis and the production of toxic oxygen, living organisms then had to adapt to the increasing oxygen concentration in the atmosphere and its impact on the overall cellular redox balance.[110] Polyunsaturated fatty acids may have proved suitable as sacrificial molecules for the organisms to defend themselves from the toxic effects of oxygen by destruction of the diradical to degradable organic compounds such as alcohols and carbonyl compounds that the organism could even use to some extent for energy production. Since a combinatorial array of oxygenated fatty acid metabolites—among them the racemic isoprostanes and phytoprostanes discussed here—were formed in relative large quantities through the different processes (see Section 4.2), they may have later been selected as organisms adapted more to the aerobic conditions so as to signal more specific imbalances in the redox state of the cells. An advantageous feature of the cyclic fatty acid metabolites is that they are formed in sufficient quantity to trigger a clear signal. On the other hand, their half-lives are rather low under physiological conditions, such that the signal is quickly switched off. As this system was sufficiently efficient, most terrestrial organisms could have selected isoprostanes and phytoprostanes as local hormones.

It was necessary in the following evolution to decouple the formation of the most active series, the prostaglandins in the animal kingdom and OPDA and jasmonates in the plant kingdom, from random autoxidation. A truly enzymatic biosynthesis evolved over time that resulted in reliable signaling pathways inside the cells and between neighboring cells. However, when pathogenic or life-threatening conditions apply, the autoxidation of PUFA forming cyclic metabolites was, and still is, used to balance the redox state of the cell and to provide a non-enzymatic backup pathway for signaling of disastrous events.[111] Thus, phytoprostanes and isoprostanes are very likely compounds that provide the organism with archetypical self-defense functions against damaging oxidative conditions.

5. Isoprostanes as Diagnostic Tools in Biology and Medicine

One of the greatest needs in the field of modern diagnostics is the availability of reliable non-invasive approaches to assess the lipid peroxidation status in human tissue during critical illnesses. There are two viable strategies to accomplish this goal. The first consists of the quantification of reactive oxygen species (ROS), which initiate free-radical peroxidation in fluids or tissues. A major problem consists, however, in the difficulty of determining exactly the concentration of ROS and other reactive species responsible for lipid peroxidation in vivo. The second way requires reliable methods to quantify the oxidative damage products formed as a result of the action of the ROS.

Since the discovery of F_2-isoprostanes in humans, a substantial body of evidence has accumulated that quantification of F_2-IsoPs **2b** represents "the gold standard" for the assessment of oxidative damage in vivo and therefore of the oxidative stress status.[112] Before this agreement was reached, dramatic improvement in the detection and quantification of F_2-IsoPs in various biological fluids (urine, plasma, exhaled breath, cerebrospinal fluid (CSF)) and biological extracts and tissues (LDL, liver, retina, brain) had to be accomplished.

5.1. Analysis of Cyclic PUFA Metabolites

Methods currently used for the analysis of IsoPs include gas chromatography/mass spectrometry combinations (GC-MS, GC-tandem MS), liquid chromatography/tandem MS (LC-MS), and immunoassays.[113] The analytical methods for quantitative determination of F_2-IsoPs consist of a series of steps starting with the extraction from a given biological sample (Figure 2). Esterified F_2-IsoPs in biological fluids such as plasma or in tissues are hydrolyzed most often by Folch lipid extraction or by liquid–liquid extraction (LLE).[52a,114] In urine samples, isoprostanes are present in free form, and thus only acidification to pH 2–3 is needed before solid–phase extraction (SPE).[114a,115]

For GC-MS or GC-MS/MS analysis, extensive purification procedures are required. Variations of the techniques are common, which will not be fully detailed here (see the recommended review article[113]). A very typical procedure includes SPE on reverse-phase (RP C_{18}) and/or silica gel Sep-Pak cartridges (sample enrichment and purification) followed by purification by thin-layer chromatography (TLC). After derivatization of the free acids with pentafluorobenzyl bromide (PFB) the IsoP PFB esters are separated from other components by TLC. The appropriate fraction is converted into trimethylsilyl (TMS) ether derivatives ready for injection into the GC-MS combination.[116] Variations of this procedure include the use of RP-HPLC in place of TLC separation. Nourooz-Zadeh et al. developed a faster assay by using an aminopropyl (NH_2) cartridge in place of a Si cartridge and the TLC purification steps.[117] Two methods based on anion-exchange SPE cartridges (Oasis HLB or Oasis MAX) allow a simplified purification procedure.[118] Another method in which the SPE and TLC purification steps are replaced to give significantly higher selectivity is immunoaffinity chromatography (IAC).[119]

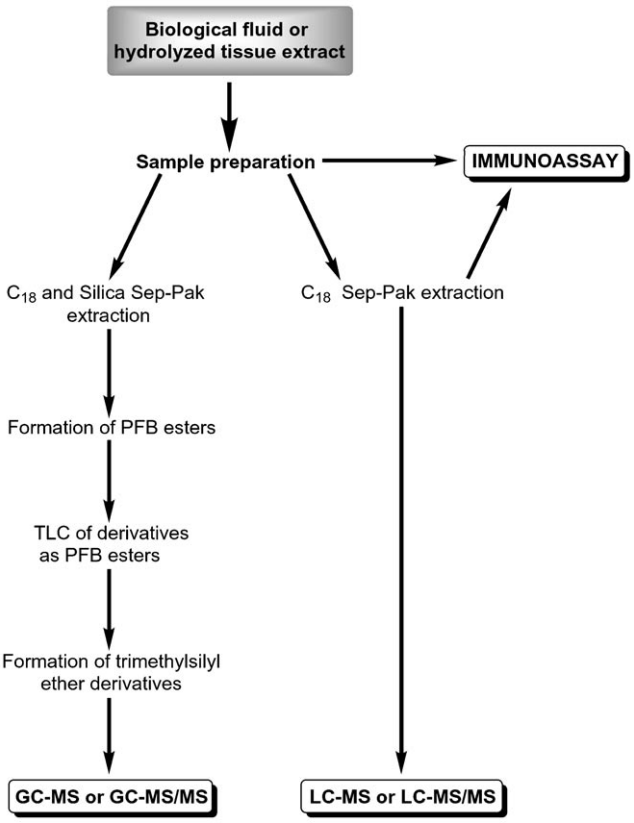


Figure 2. Typical scheme for the purification, derivatization, and analysis of IsoP derivatives.

In LC-MS analysis, the sample preparation is simpler because derivatization is not required. In most methods, free F_2-IsoPs are extracted from the biological material by using a C_{18} SPE cartridge before injection.[52a,120] Bohnstedt et al. developed a very robust and rapid method based on porous graphitic carbon HPLC columns, thus enabling a very sensitive detection of IsoPs after a single LLE extraction.[120f]

Quantification of 15-F_2-IsoP by enzyme immunosorbent assays (EIA) was introduced, but also requires extraction and purification, because structurally related metabolites may interfere to a significant extent.[112a] A simple purification using a C_{18} cartridge was proposed for application of a radioimmunoassay (RIA) technique.[121] However, Basu suggested that samples could be analyzed directly after extraction and, if necessary, hydrolysis without further purification steps.[107b]

Stable isotope dilution gas chromatography coupled to negative ion capture chemical ionization mass spectrometry (GC-NICI-MS) has been shown to be the most popular and most reliable analytical technique for the quantification of IsoPs in a purified sample. NICI mass spectrometric analysis of PFB-TMS derivatives produces mass spectra low in fragments. The spectra are dominated by an intense single ion corresponding to the carboxylate anion at m/z 569 in the selected ion monitoring (SIM) mode using $[D_4]PGF_{2\alpha}$ or $[D_4]15\text{-}F_{2t}$-IsoP as internal standards. This analytical method

permitted the quantification of F_2-IsoPs in various biological fluids[114a,117a,122] and tissue extracts.[123] To increase the selectivity and sensitivity of the detection, tandem mass spectrometric (GC-NICI-MS/MS) methods with a triple quadrupole mass spectrometer[115b,124] or an ion trap mass spectrometer were devised.[125] Although the GC-NICI-MS/MS method was developed for specific quantification of 15-F_{2t}-IsoP, it is not specific for the different regioisomeric F_2-IsoP classes, because they produce the same intense mass fragment.

In contrast, electron ionization mass spectrometry (GC-EI-MS) was applied to identify the four regioisomeric F_2-IsoP classes on the basis of the characteristic fragmentation patterns of the different PFB-TMS derivatives.[52a] On this basis, specific methods for the quantification of 15-F_2-IsoP in urine[126] and plasma[127] were developed. EI-MS is more flexible and specific than NICI-MS, but with a decrease in sensitivity because of stronger fragmentation.

Mass spectrometry coupled to liquid chromatography methods (LC-MS) were developed as alternatives to GC-MS methods. They have the advantage that sample preparation is simplified and derivatization is not required.[52b] In particular, high-performance liquid chromatography/tandem mass spectrometry equipped with an electrospray ionization interface (LC-ESI-MS/MS) allows identification of the four regioisomeric F_2-IsoP classes.[120d] A recent study using reverse-phase LC coupled to electrospray (ESI-MS) to analyze free F_2-IsoPs, and normal-phase LC coupled to atmospheric pressure chemical ionization (APCI-MS) to analyze the IsoP-PFB derivatives permitted the separation and identification of the eight F_2-IsoP diastereoisomers in all four regioisomeric series.[20] The analysis of the relative composition of the isomeric F_2-IsoPs is of great relevance and importance because their relative amounts could provide a biomarker that reflects different oxidative conditions or pathologies.[108,120c]

While mass spectrometry methods allow highly accurate, sensitive, and specific analyses, the instrumentation is not always readily available to routine investigators and practitioners. Therefore, immunoassays such as radioimmunoassays (RIA) and enzyme immunosorbent assays (EIA) were developed for 15-F_{2t}-IsoP.[121,107b] Recently, a number of enzyme-linked immunosorbent assay (ELISA) kits became commercially available. This technique is relatively low cost and easy to perform. However, several investigators have criticized the accuracy and reliability of the immunoassays.[128] This problem may be traced most likely to a cross-reactivity with other isoprostane isomers or isoprostane metabolites. Therefore, data arising from GC-MS and immunological methods should not be compared, since they do not measure the same sum of isoprostanes. So far, there is no consensus on the best methodology for the quantification of IsoPs, but chromatographic methods should currently be viewed as superior to immunoassays.

5.2. Cyclic PUFA Metabolites in Diagnostic Applications

Increased F_2-IsoP levels are associated with a wide variety of human diseases, including cardiovascular, pulmonary, neurological, renal, and liver diseases,[129] and have provided important information concerning the role of oxidative stress in the pathophysiology of those diseases (Table 1). Two of the main human diseases, in which IsoP formation has been examined in great detail, are atherosclerosis and Alzheimer's disease (AD).

Several studies have revealed insights into the exogenous factors that influence the in vivo formation of F_2-IsoPs. These factors include smoking, alcohol intake, exercise, drug treatment, various dietary antioxidant supplementations, and fruit or vegetable intake.[130]

Table 1: Human disorders and pathophysiological conditions in which oxidative stress, as implicated by F_2-IsoP levels, plays a significant role.

Cardiovascular diseases	**Liver diseases**
atherosclerosis[132]	acute and chronic alcoholic liver disease[152]
coronary artery disease[133]	acute cholestasis[153]
heart failure[134]	hepatorenal syndrome[144]
ischemia/reperfusion injury[135]	liver transplantation[154]
renovascular disease[136]	primary biliary cirrhosis[155]
risk factors for cardiovascular diseases	**neurological diseases**
diabetes[117b,137]	Alzheimer's disease[69a,156]
hypercholesterolemia[138]	Creutzfeld-Jacob's disease[157]
hyperhomocysteinemia[139]	Huntington's disease[156c]
male gender[140]	multiple sclerosis[157,158]
obesity[141]	**miscellaneous**
smoking[142]	scleroderma[159]
renal diseases	Down's syndrome[156b]
hemodialysis[143]	Crohn's disease[160]
hepatorenal syndrome[144]	osteoporosis[161]
r habdomyolysis induced renal injury[145]	autism[162]
lung diseases	chronic fatigue syndrome[132]
asthma[146]	rheumatic inflammatory response[163]
chronic obstructive pulmonary disease[147]	muscular side effects of statins[164]
cystic fibrosis[148]	obstructive sleep apnoea[165]
interstitial lung disease[149]	
acute lung injury/adult respiratory distress syndrome[150]	
acute chest syndrome of sickle cell disease[151]	

Although F_2-IsoPs are confirmed physiological markers of oxidative stress, their relationship to the pathogenesis of human disease remains to be thoroughly explored. A limited number of studies have suggested a causative link in asthma, hepatic cirrhosis, scleroderma, and AD. A recent study of chronic fatigue syndrome by Kennedy et al.[131] showed that F_2-IsoP levels are positively correlated to symptoms of the disease, including joint pain and postexertional malaise. Furthermore, the quantification of F_2-IsoPs might also represent a prognostic marker. There is a clear relationship between the F_2-IsoP levels and the severity of heart failure,[134a–b,166] and the levels also correlate with the hemodynamic response to NO in pulmonary hypertension.[167]

Furthermore, Schwedhelm et al. showed in a case-control study that the level of F_2-IsoPs in urine is an independent and cumulative risk marker of coronary heart disease.[168] Gross et al. reported in another study an association between increased concentrations of circulating F_2-IsoPs and coronary artery calcification in healthy young adults.[169] These findings confirm an association between oxidative damage and the early stages of atherosclerosis in humans, and support the hypothesis that oxidative stress is involved in the early development of atherosclerosis.

The determination of IsoP levels has also been used in clinical trials to study the effects of antioxidant supplementation on cardiovascular and neurological diseases. Although in some studies IsoP levels in humans have responded to antioxidant therapies,[170] the general accepted opinion is that lipid peroxidation is little affected by antioxidant supplements.[171] However, oxidative stress is a complex phenomenon that may be influenced by covariates, and lead to results that might reflect inappropriate antioxidants or antioxidant combinations, incorrect doses, insufficient duration of treatment, or failure to initiate treatment sufficiently early in the disease studied. Nonetheless, F_2-IsoP levels currently represent a valuable pharmacological tool for the assessment of the oxidative stress of the patient and the true efficacy of antioxidant therapies. Isoprostane quantification should therefore be included as a "surrogate end-point" in large-scale clinical trials on antioxidants.

Another class of compounds that needs to be considered are the F_2-IsoP metabolites (see Section 4.3). The analysis of F_2-IsoP metabolites in urine represents a non-invasive method for determining the systemic levels of oxidative stress in vivo. This approach has several advantages over measuring the levels of the parent IsoPs in plasma, since urine collection is simple, non-invasive, and samples do not need to be stored at −70 °C or processed immediately to prevent artifactual generation of F_2-IsoPs by autoxidation.[8] Additionally, determination of IsoP metabolites in urine circumvents the detection of F_2-IsoPs produced by the kidneys in situations of renal oxidative stress.

Until now, the only F_2-IsoP metabolites identified in human urine are those arising from 15-F_{2t}-IsoP, namely 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP (**91b**),[105a] and 2,3-dinor-15-F_{2t}-IsoP (**90b**).[105b] Therefore, they may be also applied in conjunction with available analytical techniques as biomarkers for oxidative stress (see Section 5.1). However, a recent study in which **91b** and 15-F_2-IsoP were quantified in parallel showed that values for both are comparable for the assessment of cardiovascular risk.[172]

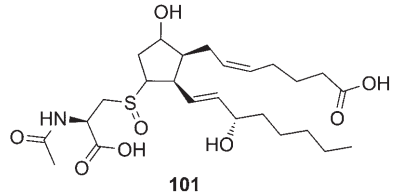
E_2- and D_2-IsoPs are formed competitively to F_2-IsoP metabolites and recent studies demonstrated that the depletion of cellular reducing agents, particularly of α-tocopherol, favors the formation of E_2/D_2-IsoPs over F_2-IsoPs.[42,173] Since E_2/D_2-IsoPs are less stable than F_2-IsoPs, they are less suitable biomarkers of oxidative stress.[112]

A_2/J_2-IsoPs are terminal products of the IsoP pathway and are formed by dehydration of E_2/D_2-IsoPs. Musiek et al.[67] confirmed that 15-A_2-IsoP is produced in higher concentrations than F_2-IsoPs in the human brain. Levels increased dramatically under oxidative and neurodegenerative conditions, thus showing that 15-A_2-IsoP isomers can be considered as potential mediators of oxidative stress in the brain. Several studies concentrated on the cellular metabolism of cyclopentenone-IsoPs, and support the contention that conjugation with GSH represents a major route of metabolic clearance,[174] thereby proving the hypothesis that free cyclopentenone-IsoPs cannot be detected in vivo because of their marked proclivity to undergo Michael additions. In contrast, 15-A_2-IsoP isomers can be detected in membrane lipids in esterified form in vivo, where they are shielded and protected from adduction to GSH in the cytosol.

However, the major urinary metabolite of 15-A_2-IsoP in rats, an *N*-acetylcysteine sulfoxide conjugate **101** in which the carbonyl group at the C9-position of the eicosanoid is reduced to the alcohol, was recently identified and quantified by LC/MS/MS (Figure 3).[52a,68] Current efforts concentrate on the identification of the major metabolites of 15-A_2-IsoP isomers in humans which could be used as novel biomarkers of oxidative stress in human disease, and especially in neurological disorders.

While AA (**1b**) is present in all cell types of brain tissue, DHA (**1d**) is very highly concentrated in neuronal membranes (25–33 % of the total of fatty acids in aminophospholipids). F_4-NeuroPs formed from DHA are detected abundantly both in vitro and in vivo. Significantly higher levels of F_4-NeuroPs were found in CSF as well as in hippocampal and temporal lobe brain tissue from patients with AD compared to normal humans.[156d,175] Studies on AD patients have since shown that F_4-NeuroPs are a more sensitive in vivo marker of neuronal oxidative damage than F_2-IsoPs.[176]

E_4- and D_4-NeuroPs can also be detected in normal brain tissue. Their level is approximately one-third of the amount of the F_4-NeuroPs. Interestingly, it has been observed that the ratio of F_4- to E_4/D_4-NeuroPs was 40–70 % lower in all brain regions of patients with AD compared to age-matched controls. The F_4-NeuroP/F_2-IsoP ratio did, however, not



101

Figure 3. Structure of the major metabolite **101** of 15-A_2-IsoP in rat urine.

change. This finding suggests that there is diminished reducing capacity in DHA-containing tissue in brains of AD patients.[71] Thus, quantification of the F_4-NeuroP to E_4/D_4-NeuroP ratio may be an indicator of AD onset.

Together with DHA, EPA (**1c**) is increasingly used as a dietary supplement as a source of ω-3-PUFA. Thus, they can both become another important and sizeable component of cellular phospholipids. It is therefore of increasing importance to assess the extent of peroxidation of these highly unsaturated fatty acids. A recent investigation has definitively confirmed the formation of F_3-IsoPs both in vitro and in vivo.[54b]

At almost the same time, Rokach and co-workers developed specific methods to analyze 5-F_{3t}-IsoP in human urine.[109] Additionally, they provided evidence that 7-F_{4t}-NeuroP is rapidly metabolized to 5-F_{3t}-IsoP by β-oxidation in rat liver homogenates. This finding suggests that endogenous 5-F_{3t}-IsoP can be formed by two pathways, namely by direct autoxidation of EPA and by β-oxidation of DHA-derived 7-F_{4t}-NeuroP. Since quantification of NeuroPs in a non-invasive way (urine or blood) has still to be uncovered, efforts should concentrate on finding further stable metabolites that are easy to quantify.

Overall, these investigations highlight the need for a better understanding of the peroxidation of ω-3-PUFA, especially the factors influencing the formation of cyclic products, their metabolism, and the biological consequences of the competitive formation of these novel compounds. The availability of a reliable assay to quantify F_3-IsoPs and NeuroPs could represent a formidable biomarker for determining the possible benefits of EPA and DHA dietary supplements in detail. Indeed, a recent study suggested that dietary ω-3-PUFA may diminish the formation of biologically active peroxidation products derived from ω-6-PUFA by channeling the free-radical pathway away from F_2-IsoPs and toward F_3-IsoPs.[34]

Preliminary studies have pinpointed that quantification of IsoFs provides a highly sensitive index of oxidative stress at elevated O_2 partial pressures, whereas that of F_2-IsoPs does not. This was later borne out in hyperoxia-mediated lung injury in mice, where significantly increased levels of esterified IsoFs were detected, whereas the amounts of F_2-IsoPs remained unchanged.[100]

Another large group of diseases where cellular oxygen partial pressure is increased are those involving mitochondrial dysfunction. Indeed, IsoF levels are significantly higher in substantia nigra in brains from patients with Parkinson's disease compared to age-matched controls.[177] This finding led to the hypothesis that analysis of both F_2-IsoPs and IsoFs might provide a more complete and reliable index of oxidative stress. Such a determination is easy to achieve since F_2-IsoPs and IsoFs can be purified on the same TLC plate and simultaneously quantified in a single GC/MS assay by including an additional ion channel for IsoFs, which is 16 Da higher than that for F_2-IsoPs.

Levuglandins are generated by rearrangement of PGH_2, and in vivo form LG–protein adducts, which can be detected by using an immunoassay.[178] Salomon et al. provided definitive proof of an in vivo IsoLG pathway by the detection of Iso[4]LGE_2–protein immunoreactivity in human plasma.[86] The initial finding of an increased production of LG–protein adducts in individuals with atherosclerosis compared to healthy controls suggested a link to oxidative injury. It was later confirmed in a clinical study that levels of IsoLG–protein adducts in the plasma of patients with atherosclerosis or end-stage renal disease are about twice those of healthy individuals. Interestingly, these elevated levels are not related to variations in age, total cholesterol, or apoB. Furthermore, IsoLG–protein adduct levels are more strongly correlated with the disease than the total cholesterol or apoB levels, thus suggesting an independent defect that results in an abnormally high level of oxidative injury associated with atherosclerosis and renal disease.

Another important study showed that even enzymes can be responsible in vivo for triggering the autoxidative formation of IsoLG–protein adducts under inflammatory conditions. Indeed, myeloperoxidase (MPO) was found to be implicated in the formation of Iso[4]LGE_2–protein adducts.[27] This study also revealed that F_2-IsoP levels were not affected, and proved that LG–protein and IsoLG–protein adducts represent a convenient dosimeter for measuring the accumulation of cyclic AA metabolites during the lifetime of proteins, thus providing a cumulative index of oxidative stress.

Non-enzymatic peroxidation of LA (**1a**) produces a large spectrum of phytoprostanes in plant tissues. Similar to IsoPs in mammals, PhytoPs have been shown to represent a reliable marker of oxidative stress in vivo.[76,78] E_1- and F_1-PhytoPs have been found to occur ubiquitously in higher plants at basal levels,[77] similar to their enzymatically biosynthesized congeners, 12-OPDA and JA. Under oxidative stress (peroxides, heavy metals, wounding), F_1-PhytoP levels increase dramatically and may exceed the levels of jasmonates in maximally elicited plant cells by more than an order of magnitude.[78,179]

Interestingly, a recent investigation showed that F_1-PhytoPs, E_1-PhytoPs, A_1-PhytoPs, and B_1-PhytoPs are found in vegetable oils and parenteral nutrition (Intralipid) in remarkably high levels (0.09–99 mg L^{-1}).[180] It was demonstrated that F_1-PhytoPs were absorbed after oral consumption, and found to circulate in plasma in an unknown conjugated form. They were excreted in free form in urine. Taking in consideration that cyclopentenone-PhytoPs display potent anti-inflammatory and apoptosis-inducing activities similar to PGA_1, deoxy-PGJ_2, A_2-IsoPs, and J_2-IsoPs (see Section 6), this study indicates that PhytoPs may contribute to the beneficial effects of the Mediterranean diet.

These findings highlight the need for a better understanding of the role of ω-3-PUFA metabolites such as PhytoPs in human diet. Efforts should concentrate on the assessment of phytoprostane levels in vivo in humans, to see if they correlate with well-known symptoms of diseases, and if these symptoms can be alleviated with a vegetarian diet rich in ω-3-PUFA.

6. Biological Activity of Cyclic PUFA Metabolites

The combined effort of analytical chemists, biochemists, biologists, and synthetic chemists culminated in the discovery

of important biological activities of PUFA metabolites. The current knowledge on the biological actions of isoprostanes mainly concerns the F_2-IsoP series (of which 15-F_{2t}-IsoP is the most studied) and 15-E_2-IsoP.[181] In initial experiments on renal function in the rat, 15-F_{2t}-IsoP proved to be an extremely potent renal vasoconstrictor in the low nanomolar range. It has since been demonstrated that 15-F_{2t}-IsoP is a vasoconstrictor in most species and vascular systems.[166,182] Its activity is not restricted to blood vessels—significant effects were also found in the lymphatic vessels, the bronchi, the gastrointestinal tract, and the uterus. In general, this compound elicits an excitatory response in a large number of tissues and vascular systems, including the aorta, carotid, coronary, cerebral, pial, and retinal vasculature,[183] largely mediated by the TP receptors. Other effects, such as activation of the peroxisome proliferator-activated receptor (PPAR), are also TP-receptor dependent (Figure 4).[184]

The effects of 15-F_{2t}-IsoP on platelets are complex, but in human whole blood it is antiaggregatory. It induces the adhesion of polymorphonuclear neutrophils and the adhesion of monocytes that is implicated in the pathophysiology of atherosclerosis. In the endothelial cell, 15-F_{2t}-IsoP-induced contraction is modulated through the release of NO and prostacyclin (PGI_2). In addition, it induces the formation of both thromboxane A_2 (TXA_2) and endothelin-1 (ET-1). Finally, contraction of smooth muscle cells is mediated by TP receptors or a still to be found specific IsoP receptor (iPR).

15-F_{2t}-IsoP possesses several activities in vitro, all of which are relevant to the pathophysiology of atherosclerosis.[185] For example, it promotes the activation of platelets[185c] and the formation of TxA_2 in brain microvasculature.[186] It also induces mitogenesis of vascular smooth muscle cells,[187] proliferation of fibroblasts[188] and endothelial cells, and increases the expression of endothelin-1 in aortic endothelial cells, therefore altering the endothelial cell biology.[189] All

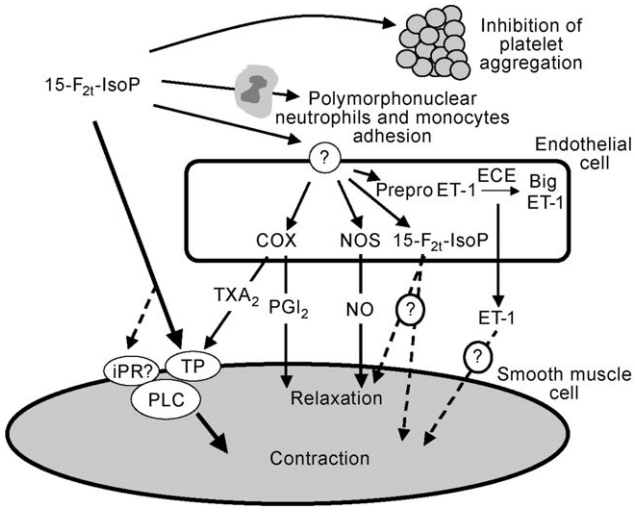


Figure 4. Schematic representation of the pharmacological activities of 15-F_{2t}-IsoP on the interface blood vessels. For a better comprehension, the thickness of the arrows is correlated to the scientific evidence supporting these mechanisms. The mechanisms of action are likely to differ within species, as well as within vessel types. Reproduced with permission from Ref. [166].

these effects are prevented by pharmacological antagonists of TP receptors, which indicates that these receptors are involved in the effects of 15-F_{2t}-IsoP. It has thus been hypothesized that 15-F_{2t}-IsoP, an agonist of TP receptors, might have a functional role in atherogenesis. Indeed, stimulation of TP receptors on endothelial cells increases the expression of adhesion molecules, such as the intercellular adhesion molecule 1 (ICAM-1),[190] thereby promoting monocyte adherence.[191] ICAM-1 is expressed in human atherosclerosis lesions,[192] and its circulating levels are associated with atherosclerosis and its progression in some studies.[193] More recently, administration of 15-F_{2t}-IsoP to ApoE knockout mice (ApoE: an apoprotein essential for the normal catabolism of triglyceride-rich lipoprotein constituents) and LDLR knockout mice was shown to promote atherogenesis directly.[194] It is not known whether the nanomolar concentrations of F_2-IsoPs in vivo are sufficient to exert biological effects, but the release of local concentrations at the site of inflammation might be sufficiently high to induce regional vasoconstriction.

Another isoprostane isomer formed in significant concentration in vivo is 15-E_2-IsoP. In contrast to cyclooxygenase-derived PGE_2 and $PGF_{2\alpha}$, which display opposite biological effects, 15-E_2-IsoP is also a vasoconstrictor and an inhibitor of TP-mediated platelet aggregation. 15-E_2-IsoP is even more potent than 15-F_{2t}-IsoP in systemic and pulmonary vessels, with its contraction effect being mediated through TP receptors. Interestingly, a contractile response was found to be exerted through EP receptors (likely of the EP_3 subtype) in porcine pulmonary vein.[195] However, 15-E_2-IsoP may also induce relaxation through EP receptors.[196]

Other F_2-IsoP isomers have been studied to a significantly lesser extent than 15-F_{2t}-IsoP. Preliminary studies by Cracowski and co-workers showed that 5-F_{2t}-IsoP and its 5-epimer do not produce vasomotor effects in the rat thoracic aorta, the human internal mammary artery, and the saphenous vein.[197] Although 5-F_{2t}-IsoP and 5-F_{2c}-IsoP are the most abundant F_2-isoprostanes in human urine and plasma, they are unlikely to be involved in the pathogenesis of vascular diseases, although 5-F_{2c}-IsoP has not been investigated so far.

Chemtob and co-workers carried out a detailed study on the effects of 5-, 12-, and 15-F_2-IsoP isomers on pig retinal and brain microvasculature.[198] They showed that 15-*epi*-15-F_{2t}-IsoP, *ent*-15-F_{2t}-IsoP, and *ent*-15-*epi*-15-F_{2t}-IsoP are also potent vasoconstrictors. The isomers 12-F_{2t}-IsoP and 12-*epi*-12-F_{2t}-IsoP also caused marked vasoconstriction. It was confirmed that 5-F_{2t}-IsoP and 5-*epi*-5-F_{2t}-IsoP indeed possessed no vasomotor properties, whereas *ent*-5-F_{2t}-IsoP caused surprisingly modest vasoconstriction. The vasoconstriction by *ent*-5-F_{2t}-IsoP, 12-F_{2t}-IsoP, and 12-*epi*-12-F_{2t}-IsoP was abolished by removal of the endothelium, by a TXA_2 synthase inhibitor, by a TXA_2 receptor blocker, as well as by receptor-mediated blocking of Ca^{2+} channels. Correspondingly, these isomers increased the formation of TXB_2 by activating Ca^{2+} influx through voltage-independent receptor-mediated Ca^{2+} channels in endothelial cells. It was demonstrated that 15-F_{2t}-IsoP, *ent*-5-F_{2t}-IsoP, 12-F_{2t}-IsoP, and 12-*epi*-12-F_{2t}-IsoP constricted both retinal and brain microvessels by inducing endothelium-dependent TXA_2 synthesis. These new

findings broaden the spectrum of biological activity of IsoPs in regard to the potential involvement of F_2-IsoPs as mediators of oxidanive injury.

In humans, the major urinary metabolite of 15-F_{2t}-IsoP is 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP (**91b**).[105a] In contrast to the loss of pharmacological activity of prostaglandin metabolites, **91b** caused marked constriction of porcine surface retinal and intraparenchymal brain microvessels at levels comparable to those of its precursor (15-F_{2t}-IsoP) in retinal and cerebral vasculature as well as on astroglial cells (both share similar mechanisms mediated by TXA_2).[199] Cracowski et al. also showed in a study on the effects of several IsoP metabolites on rat thoracic aorta that 15-keto-15-F_{2t}-IsoP (**95b**) mediates a contraction by activation of TP receptors, probably by acting as a partial agonist, and induces a weak endothelium-independent relaxation at high concentrations.[200] In contrast, 2,3-dinor-15-F_{2t}-IsoP (**90b** and **91b**) did not cause vasorelaxation or vasoconstriction on the rat thoracic aorta.

A recent study of isoprostane metabolites **95b**, both epimers of **90b** and **91b**, as well as 20-carboxy-2,3,4,5-tetranor-15-oxo-5,6,13,14-tetrahydro-15-F_{2t}-IsoP (**100b**) showed they had no pharmacological activity in human and bovine pulmonary smooth muscles.[201] These studies highlighted discrepancies in the pharmacological activities of some metabolites, thus suggesting that complex mechanisms of action are in effect and need to be examined in more detail.

Recent studies on cyclopentenone isoprostanes, such as 15-A_2-IsoP and 15-J_2-IsoP, were rendered possible by their recent total synthesis by Zanoni et al.[202] The α,β-unsaturated cyclopentenone ring structure in the A_2/J_2-IsoP isomers makes them very good electrophiles and are thus susceptible to nucleophilic addition reactions with biomolecules containing thiol functions, such as GSH or cysteine residues in cellular proteins. Levonen et al. have shown that 15-A_2-IsoP reacts with the cysteine-rich protein Keapl and activates the important cytoprotective antioxidant response elements in the cell. This finding suggests that cells use electrophilic lipids such as cyclopentenone-IsoPs to sense oxidative stress.[203] For example, 15-J_2-IsoP induces both the formation of reactive oxygen species (ROS) and cellular antioxidant defense mechanisms, such as heme oxygenase-1 (HO-1) and glutathione (GSH).[203b]

Subsequent studies on macrophages showed that 15-A_2-IsoP possesses potent biological activity, including anti-inflammatory and proangiogenic effects. For example, 15-A_2-IsoP and 15-J_2-IsoP suppress lipopolysaccharide (LPS) induced inflammatory signaling in macrophages by inhibition of the nuclear factor κ-B (NF-κB) pathway through inhibiting IκBα degradation.[204] They also abrogate inducible nitric oxide synthase (iNOS) and COX-2 expression in response to LPS, as well as the elaboration of nitric oxide, PGs, and various proinflammatory cytokines.[174] 15-J_2-IsoP possesses a similar anti-inflammatory effect, in addition to the activation of the peroxisome proliferator-activated receptor γ (PPARγ), the receptor modulating several biological processes such as inflammatory signaling and fatty acid metabolism. It was suggested that cyclopentenone–IsoPs may serve as negative feedback regulators of inflammation and have important implications for defining the role of oxidative stress in the inflammatory response. It has also been suggested that 15-A_2-IsoP can be neuroprotective in the brain; however, basal NF-κB activity in neurons is required for survival and NF-κB inhibition can promote neuronal death and enhance neurodegeneration. Once formed, 15-A_2-IsoP can in turn promote further oxidative damage by stimulating ROS production. This process could potentially initiate a feed-forward oxidation cycle that could rapidly alter the intracellular redox status of macrophages and thereby affect cellular function.

In another important study Musiek et al. showed that cyclopentenone IsoP isomers are more abundant than F_2-IsoPs in brain tissue and that their levels increased even more by oxidant injury.[67] They found that 15-A_2-IsoP is an especially potent neuronal apoptogen at submicromolar concentration (Figure 5). The thorough investigation revealed a model of how 15-A_2-IsoP induces neuronal apoptosis. It involves initial depletion of glutathione by conjugation. This causes enhanced production of reactive oxygen species, which in turn activate 12-lipoxygenase (12-LOX) and induce phosphorylation of extracellular signal-regulated kinase $^1/_2$ as well as the redox-sensitive adaptor protein $p66^{shc}$. All of them trigger caspase-3 cleavage. Moreover, the application of 15-A_2-IsoP in concentrations as low as 100 nM exacerbates neurodegeneration caused by sublethal oxidative glutamate toxicity, thus demonstrating that even low concentrations can synergize with other damaging effects to augment cell death. 15-J_2-IsoP is also highly neurotoxic,[205] and it is therefore believed that the cyclopentenone-IsoPs represent a novel class of neurotoxic lipid peroxidation products that contribute to ischemic and excitotoxic injury in the CNS, and that their actions in the brain should no longer be neglected. No significant studies have so far been

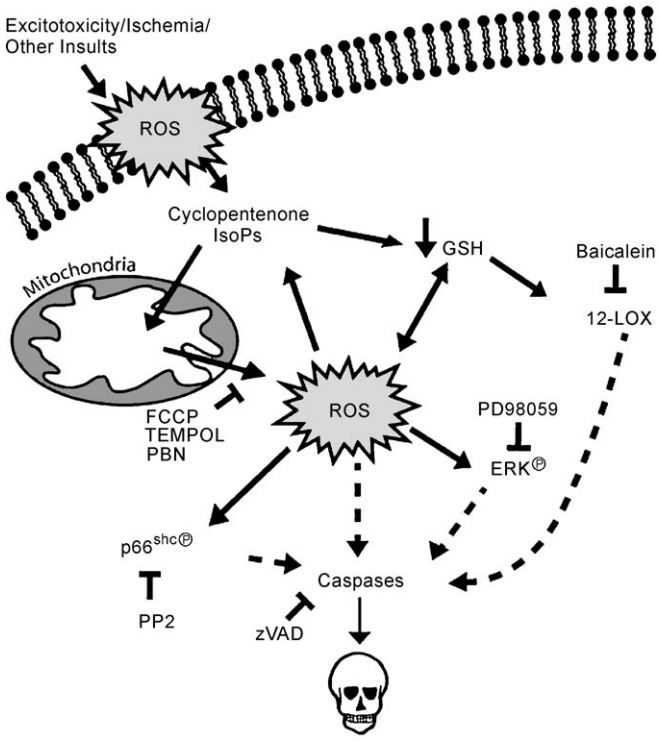


Figure 5. Model of signaling events in 15-A_2-IsoP-induced neurodegeneration. Reproduced with permission from Ref. [67].

carried out on the biological activity of NeuroPs and IsoFs, mainly because too few synthetic materials are available.

IsoLGs have an extraordinary ability to cross-link proteins and could therefore alter significantly many structural and functional aspects of proteins near the sites of lipid peroxidation.[86,87b] One of the first observations with structurally related LGs was their ability to interfere with tubulin function, thus inhibiting microtubule assembly.[206] Apparently, modification by LGs can damage the cytoskeletal machinery that is critical for cell division and thereby prevent mitosis. Furthermore, covalent modification of ovalbumin (OA) or amyloid β_{1-40} ($A\beta_{1-40}$) protein by IsoLGs strongly curtailed their subsequent processing by the 20S proteasome.[89] Furthermore, IsoLGE$_2$–OA and IsoLGE$_2$–$A\beta_{1-40}$ adducts inhibited proteasomal chymotrypsin-like activity competitively, so that formation of an adduct could potentiate intracellular accumulation of proteins. These findings potentially link the increase in amyloid β_{1-40} oligomerization to the decrease in total proteasome activity found in AD patients. Moreover, IsoLGs induce cell death at submicromolar concentrations in several cultured cell types, including lung fibroblasts, neuroblastoma, and neuroglial cells.[89,207]

In mildly oxidized LDL (MM-LDL), oxidized 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine (Ox-PAPC) activates endothelial cells to produce monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8). Both chemokines were shown to be important regulators of atherogenesis and are increased in human atherosclerotic lesions. Epoxyisoprostane phospholipids (PEIPCs) and epoxycyclopentenone isoprostane phospholipids (PECPCs),[92] major components of Ox-PAPC, are potent activators of PPARα and, therefore, play an important role in mediating the effects of these lipids in vivo. PPARα is involved in oxidized phospholipid-mediated production of chemokines, and PEIPCs and PECPCs are indeed quite effective in effecting MCP-1 and IL-8 synthesis in human aortic endothelial cells (HAECs).[93] Furthermore, the accumulation of those bioactive oxidized phospholipids in response to proinflammatory cytokines in endothelial cells could promote the synthesis of inflammatory chemokines at the sites of inflammation.[93] The epoxide portion of the molecule, rather than the cyclopentenone unit, may be responsible for the biological activity of PEIPCs and PECPCs.

In plants, phytoprostane isomers have been shown to possess a broad spectrum of biological activities. Preliminary studies indicated that cyclopentenone phytoprostanes with deoxy-J$_1$-PhytoP, A$_1$-PhytoP, and B$_1$-PhytoP ring systems activate mitogen-activated protein kinase activity in cell suspensions of tomato (*Lycopersicon esculentum*) cultures. In the same tomato cell cultures, a gene involved in primary metabolism, namely extracellular invertase, was induced by B$_1$-PhytoPs but not by A$_1$-PhytoPs.[76] There is also evidence that several classes of phytoprostanes (deoxy-J$_1$-PhytoPs, B$_1$-PhytoPs, E$_1$-PhytoPs, and F$_1$-PhytoPs) trigger antimicrobial secondary metabolites (phytoalexins) in taxonomically distant plant species.[76,208] Recent studies on B$_1$-PhytoP regioisomers showed that they induce a variety of genes and, most notably, genes involved in detoxification and secondary metabolism.[209] B$_1$-PhytoP isomers increased the expression of at least 17 glutathione *S*-transferases including GST1, which plays a role in the detoxification of reactive electrophiles, and several putative ABC transporters, which transport glutathione conjugates and chlorophyll breakdown products into the vacuole. Thus, B$_1$-PhytoP isomers can be seen as stress signals that trigger an adaptive response in plants that at least partially prevents cell death.

Those studies suggest that PhytoPs are archetypal mediators of oxidative stress: They trigger the first adaptive responses that limit the consequences of oxidative stress by inducing several plant-defense mechanisms.[111] Indeed, F$_1$-PhytoPs accumulate early—during the first five hours after pathogen infection, which coincides with the enzymatic synthesis of JA as a defense signaling compound.[210]

Early work on mammalian systems have shown that E$_1$-PhytoPs, which occur abundantly in pollen of certain species such as birch, display PGE$_2$-like activities and inhibit production of dendritic cells IL-12 and augmenting polarization of the T$_h$2 cells.[211] The interesting finding that high levels of PhytoPs are present in vegetable oils prompted more detailed investigations into their PG-like effects.[180] Indeed, Karg and co-workers showed that cyclopentenone–PhytoPs, such as A$_1$-PhytoPs and deoxy-J$_1$-PhytoPs, display anti-inflammatory activities in the same concentration range (10–50 μM) as PGA$_1$ and deoxy-PGJ$_2$ in HEK cells and RAW264.7 macrophages by down-regulating NF-κB and inhibiting NO synthesis, respectively.[180] It should be noted that the inhibitory effects observed in NF-κB-driven gene transactivation and nitrite accumulation are not due to the cytotoxicity of PhytoPs.

Furthermore, 9-A$_1$-PhytoP induces apoptosis in human leukemia Jurkat T cells in the same concentration range (10–40 μM) as PGA$_1$, while 16-A$_1$-PhytoP and B$_1$-PhytoPs are completely inactive. Deoxy-J$_1$-PhytoP and dPGJ$_2$ were equally active and triggered apoptosis in a concentration range of 20–40 μM.[180] In the same study, free F$_1$-PhytoPs were shown to be undetectable after consumption of olive or soybean oil, while an unknown esterified form of the F$_1$-PhytoPs was present in human blood. Clearly, detailed further studies on PhytoPs in mammalian systems are needed to understand their influences on the immune system, the cardiovascular system, and on the prevention of cancers that have been associated with the consumption of vegetable oils rich in ω-3 polyunsaturated fatty acids.

7. Total and Partial Syntheses of Autoxidatively Formed PUFA Metabolites

The total synthesis of cyclic PUFA metabolites is of utmost importance for the general understanding of their in vivo formation and biological functions, as well as for diagnostic applications. Since all autoxidatively formed cyclic PUFA metabolites occur in nature as regio- and stereoisomeric mixtures, in which the individual isomers have very similar chemical and physical properties, the unequivocal identification of the individual metabolites is often only successful with the help of synthetic material. Moreover, pure synthetic material is necessary as analytical standards for

quantification of the individual metabolites and also for the exact determination of potential biological functions. Without the synthetic strategies developed so far, the field of cyclic lipids would not have advanced to its current status. However, as can be seen in this section, only selected members of some cyclic PUFA families have been synthesized so far. One of the greatest needs is the development of more synthetic approaches that are more efficient with respect to the number of steps and the time needed to execute them. Since cyclopentane-containing IsoPs, NeuroPs, and PhytoPs are the most interesting metabolites, their synthesis will be presented first, followed by approaches to IsoLGs and IsoFs. However, each synthesis is not presented in every detail, but instead the general strategies and the key steps involved in the assembly of the respective skeletons are highlighted.

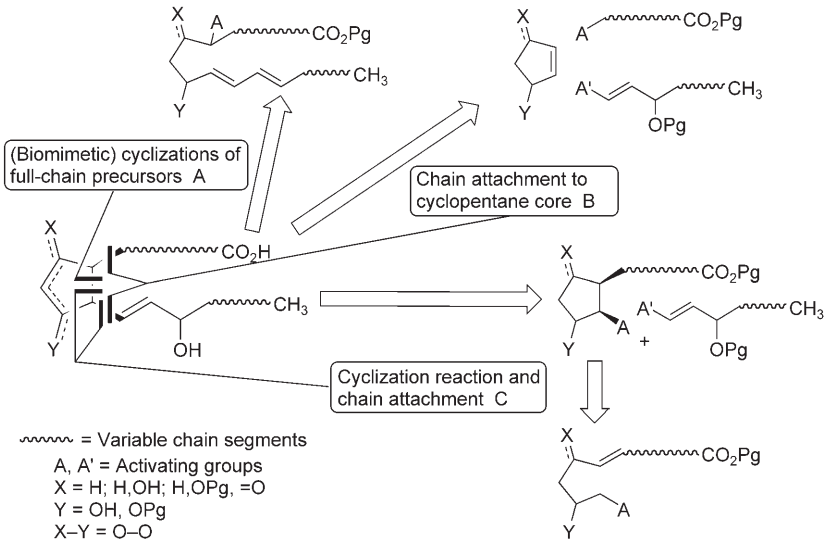
7.1. Cyclopentane-Containing PUFA Metabolites—IsoPs, NeuroPs, and PhytoPs

7.1.1. Retrosynthetic Analysis

Most of the published total syntheses use one of three basic retrosynthetic disconnections of the IsoP, NeuroP, or PhytoP skeletons (Scheme 39). According to disconnection A, precursors containing the fully assembled PUFA chain with the desired functionality were assembled first. The key step was a (sometimes biomimetic) cyclization reaction to afford the complete skeletons. The potential limiting factors of this strategy are a considerable synthetic effort to prepare the cyclization precursors and a low flexibility for the synthesis of a range of substrates.

In disconnection B, the side chains were introduced at the same time or sequentially to suitably functionalized cyclopentane cores. This strategy is very flexible for the synthesis of more than one member of different regioisomeric series of cyclic PUFA metabolites. Since the configuration of the stereocenters of the cyclopentane ring has a strong influence on the direction of the incoming side chains, a potential limitation is the accessibility of diastereomeric structures.

A number of approaches use the two-step strategy C, where one of the side chains is first disconnected. The synthesis of the cyclopentane core with the other side chain in place is possible by a number of cyclization reactions. This method can be rather easily developed and opportunities exist for divergent stereochemical control. The synthesis of the precursors may be somewhat more difficult than in disconnection B, but it is certainly much easier than pathway A.



Scheme 39. Retrosynthetic analysis of the published total and partial syntheses of cyclopentane-containing PUFA metabolites.

7.1.2. Cyclizations of Full-Chain Precursors (Path A)

7.1.2.1. Biomimetic Approaches

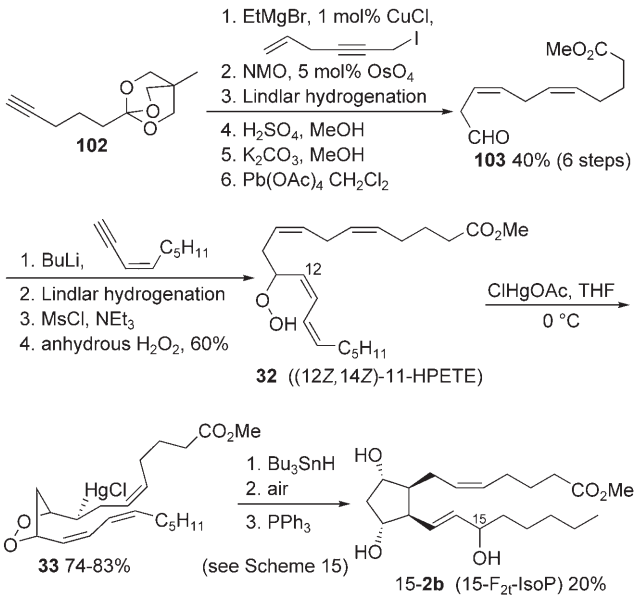
There are three total syntheses of IsoPs that can be considered biomimetic, since a fully equipped open-chain precursor was subjected to a radical 5-*exo* cyclization reaction to form the central cyclopentane ring. The first was published by Corey et al. prior to the recognition of isoprostanes as natural products and was originally aimed at the development of a biomimetic approach to $PGF_{2\alpha}$ (Scheme 40).[46]

The synthesis started from 5-hexynoic acid orthoester **102**. After deprotonation to the magnesium acetylide, the chain was extended by copper-catalyzed coupling with hex-5-en-3-ynyl iodide. An osmium tetroxide catalyzed dihydroxylation of the terminal alkene gave the the terminal diol as a masked aldehyde function. The resulting 1,4-diyne was subjected to Lindlar hydrogenation to provide the (*Z*,*Z*)-1,4-diene. A two-step transformation of the orthoester function to the methyl ester and subsequent cleavage of the glycol mediated by lead tetraacetate afforded the C1–C11 subunit **103**. After nucleophilic addition of the lithium acetylide of non-3-en-1-yne to the aldehyde function of **103** and another Lindlar semihydrogenation, the crucial hydroperoxide cyclization precursor **32** was synthesized in reasonable yield by conversion of the alcohol into the corresponding mesylate followed by substitution with anhydrous hydrogen peroxide.

Intramolecular peroxymercuration provided endoperoxide organomercurial compound **33** in good yield. Reductive demercuration induced by tributyltin hydride generated the same radical as also involved in the in vivo formation of IsoPs (see Scheme 15). The chain reaction proceeds by 5-*exo* cyclization and recombination with oxygen to give crude 15-G_{2t}-IsoP (15-**21b**). Reduction in situ with triphenylphosphine then gave 15-F_{2t}-IsoP (15-**2b**) as a single ring diastereomer, but as a 1:1 diastereomeric mixture at the 15-position, in 20% yield.

In 1994, Corey and Wang published a second biomimetic synthesis (Scheme 16).[48] Here, 15-HPETE (15-**11b**) was used as the precursor. The radical bicyclization sequence was initiated by samarium diiodide in the presence of oxygen and led to a 3:1 diastereomeric mixture of 15-G_{2t}-IsoP and PGG_2 in 15% yield (43% based on the recovered starting material).

The third biomimetic total synthesis of IsoP was reported by the research group of Durand and Rossi (Scheme 41).[212] The starting point was commer-



Scheme 40. Biomimetic total synthesis of 15-F_{2t}-IsoP (15-**2b**) according to Corey et al.

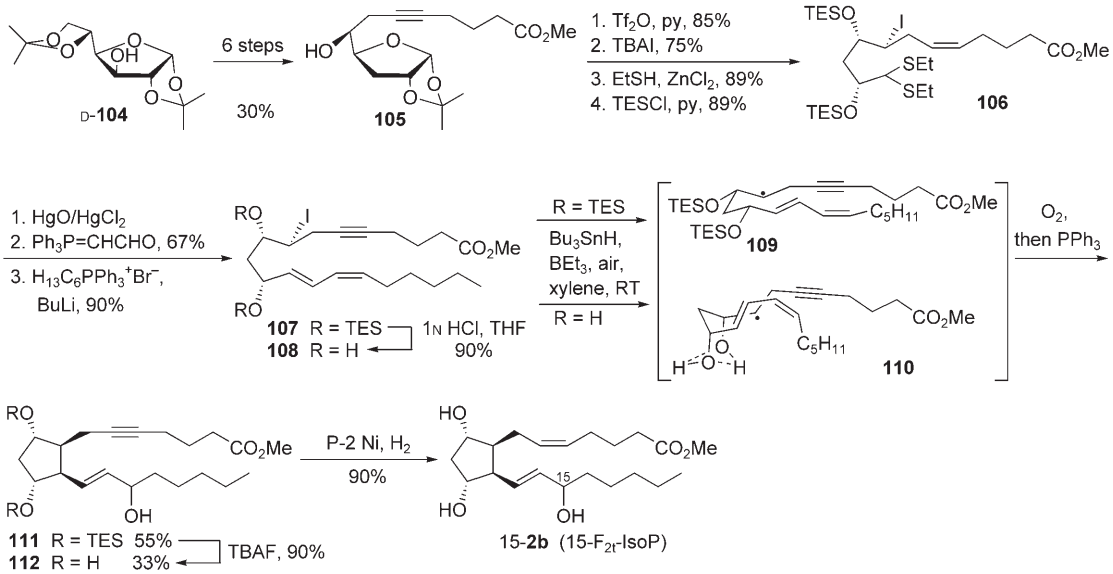
cially available D-diacetone glucose (D-**104**), which was converted in six steps into homopropargylic alcohol **105**.[213] The alcohol function in **105** was converted into the iodide in a Finkelstein reaction, followed by ring opening of the modified furanose to the dithioacetal. Protection of the remaining hydroxy group as a TES ether delivered the C1–C12 subunit **106** in good yield. Cleavage of the dithioacetal and two subsequent Wittig reactions, which proceeded smoothly in the presence of the iodide function, furnished the full C_{20} precursor **107**.

To check the influence of the silyl protecting groups on the efficiency of the following steps, a fraction of **107** was deprotected to the free diol **108**. Both, **107** and **108** were subjected to a radical 5-*exo* cyclization/oxygenation sequence induced by tributyltin hydride in the presence of oxygen under ambient conditions. A single diastereomer **111** or **112**, respectively, having the F_{2t}-IsoP configuration was formed in the two reactions. The stereochemical course of the radical cyclization of **107** can be rationalized by assuming a Beckwith–Houk chair transition state **109**, in which all substituents are positioned pseudoequatorially. However, for the cyclization of the free diol **108**, an alternative hydrogen-bonded transition state **110**, which closely resembles the endoperoxide cyclization transition state during the formation of natural IsoPs, may also be viable (see Scheme 22). Oxygen trapping occurred exclusively at the 15-position to give a 1:1 diastereomeric mixture of 15*S*- and 15*R*-hydroperoxides. Final reduction of the crude hydroperoxide mixture with triphenylphosphine, and P-2-nickel-catalyzed *Z*-selective semihydrogenation of the alkyne gave F_{2t}-IsoP methyl ester 15-**2b** in 15 steps from D-**104**.

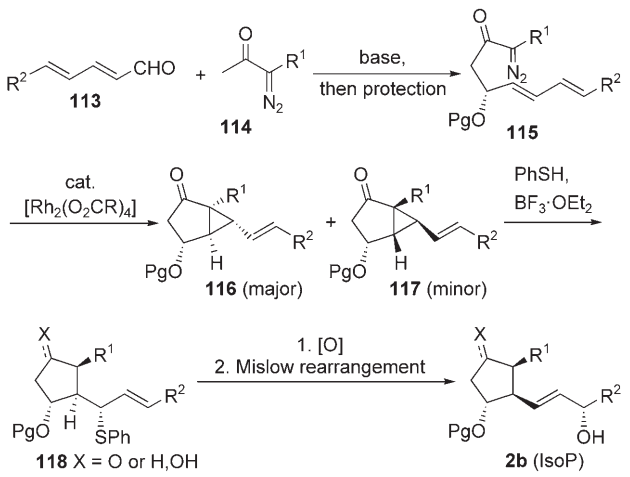
7.1.2.2. Taber's Full-Chain Diazoketone Approach for the Synthesis of IsoPs

Starting in the early 1990s, Taber et al. developed a flexible strategy to synthesize different IsoP classes by applying fully assembled C_{20} chains as precursors (Scheme 42).[214] Central to the approach is a synthetic sequence of an aldol addition of diazoketones **114** to suitable unsaturated aldehydes **113** for assembly of the C_{20} cyclization precursor **115**, followed by a rhodium- or copper-catalyzed intramolecular cyclopropanation to give bicyclo-[3.1.0]hexanone units **116** and **117**. Subsequent $BF_3{\cdot}OEt_2$-mediated ring opening of **116** by thiophenol gave rise to the cyclopentane **118** with an allylic sulfide side chain,[215] which was readily transformed to the desired IsoP systems **2b** by a Mislow rearrangement.

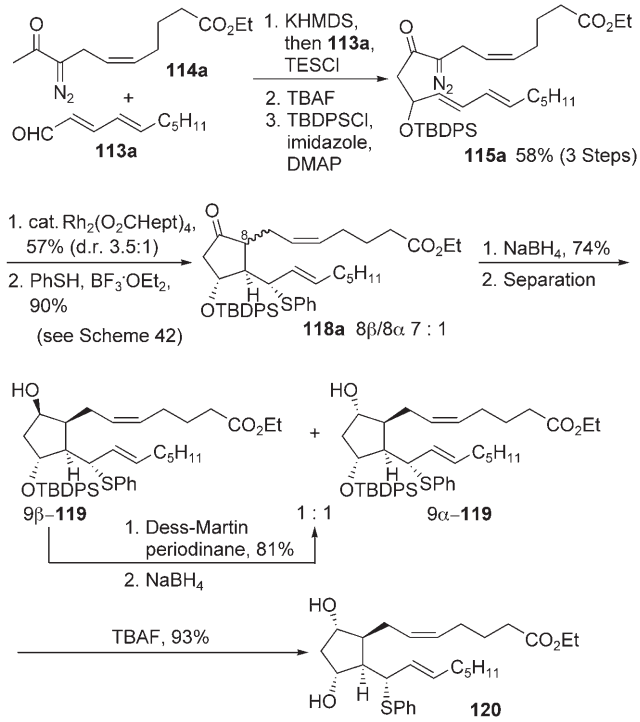
A nice illustration of the concept was the total synthesis of all the stereoisomers of 15-F_{2t}-IsoP (Schemes 43 and 44).[216]



Scheme 41. Asymmetric biomimetic total synthesis of 15-F_{2t}-IsoP (15-**2b**) according to Durand, Rossi, and co-workers.



Scheme 42. General strategy for the synthesis of IsoP isomers according to Taber et al.



Scheme 43. Synthesis of the 15-F_{2t}-IsoP intermediate **120** by the diazoketone approach.

The C_{20} chain was assembled in 61% yield by an aldol addition of the potassium enolate of diazoketone **114a** to commercially available 2,4-decadienal (**113a**) in the presence of chlorotriethylsilane. A change of the protecting group from the Lewis acid labile TES to the more stable TBDPS group gave the cyclopropanation precursor **115a** in good yield. The Rh-catalyzed cyclopropanation to give the bicyclo-[3.1.0]hexanone system proceeded with a reasonable diastereoselectivity of 3.5:1.

A subsequent BF_3-mediated ring opening by thiophenol afforded cyclic ketone **118a** in excellent yield and good diastereoselectivity (see Scheme 42). As expected, no diastereoselectivity was observed in the reduction of the keto function of **118a** by $NaBH_4$ to give 9α-**119** and 9β-**119**. However, the configuration of the undesired diastereomer 9β-**119** could be corrected to 9α-**119** by a sequence of Dess–Martin oxidation and sodium borohydride reduction. For the synthesis of the individual 15-F_{2t}-IsoP enantiomers, thioether 9α-**119** was deprotected to give racemic diol **120**.

The pseudosymmetric compound **120** proved very convenient for enzymatic desymmetrization by Amano Lipase AK in neat vinyl acetate over five days (Scheme 44). The separable monoacetates 9α-**121** and 9β-**121** were obtained in 42% and 48% yield, respectively, and with excellent enantiomeric excess. They were subjected individually to standard Mislow rearrangement conditions and the resulting allylic alcohols were selectively oxidized by DDQ to enones 9α-**122** and 9β-**122** in good yields. Reduction by sodium borohydride was, as expected, unselective and afforded the separable alcohol diastereomers, which gave rise to the individual 15-F_{2t}-IsoP stereoisomers after saponification of the acetate and ester functions.

This strategy has allowed many IsoP regio- and stereoisomers to be synthesized (Scheme 45). Among them are the four 5-F_{2t}-IsoP stereoisomers, such as 5-**2b**,[217a] all four 8-F_{2t}-IsoP stereoisomers, such as *ent*-8-**2b**,[217b] all eight stereoisomers of 12-F_2-IsoP, such as 12-**2b**,[217c] 15-E_2-IsoP (15-**45b**),[217d] 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP (**91b**),[217e] as well as the potential 15-E_2-IsoP ketodicarboxylic acid metabolite **123**.[217f]

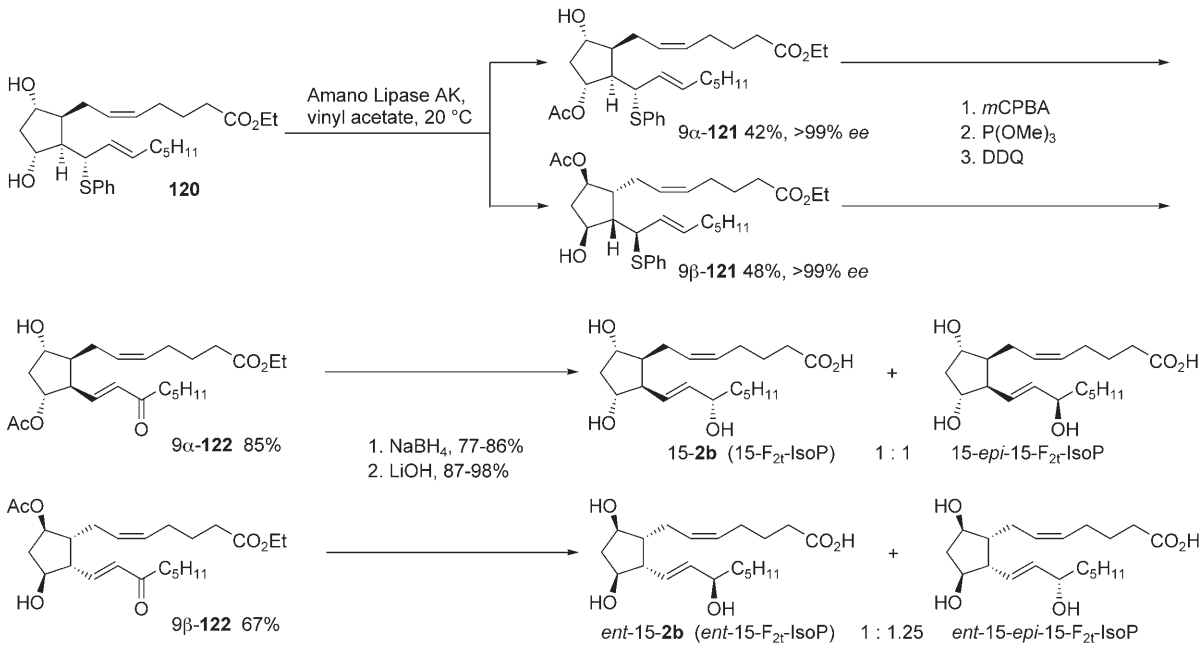
The β-alcohol **124** resulting from reduction of enone 9β-**122** (see Scheme 44) served as a starting material for the synthesis of *ent*-15-**45b** and *ent*-PGE_2 **125** (Scheme 46).[218] A change in the protecting group pattern followed by Dess–Martin oxidation afforded *ent*-15-E_2-IsoP (*ent*-15-**45b**), which epimerized smoothly at the 8-position in the presence of potassium acetate in methanol to give the thermodynamically more stable **125**.

Alcohol **126** proved to be a suitable starting material for a simple synthesis of 8,15-*diepi*-15-D_2-IsoP (**128**, Scheme 47).[219] After a change in the protecting group pattern in **126** the way was paved for a Dess–Martin oxidation to give ketone **127**, which was transformed to **128** by deprotection of the 1-ethoxyethyl ether and subsequent Mislow rearrangement. A quantitative epimerization of the configurationally very labile 12-position of IsoP derivative **127** was observed after oxidation of the sulfide by *m*CPBA so that an isomer with a relative PG ring configuration but inverted configuration at the 15-position was obtained.

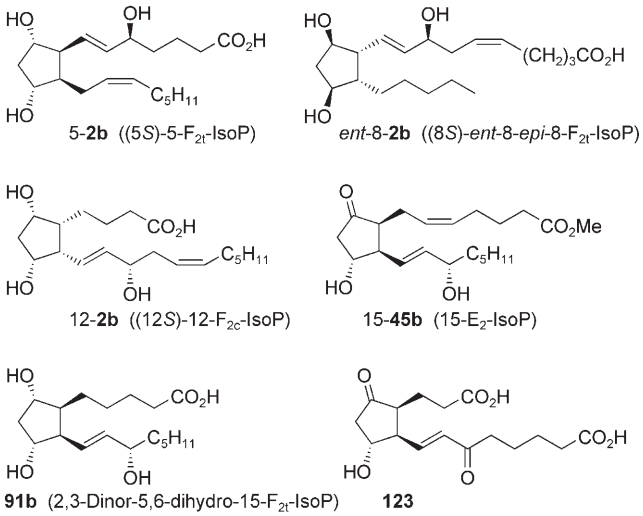
7.1.3. Synthesis of IsoPs, NeuroPs, and PhytoPs by Attachment of the Side Chains to a Suitably Functionalized Cyclopentane Core (Path B)

7.1.3.1. From Functionalized Cyclopentene Derivatives

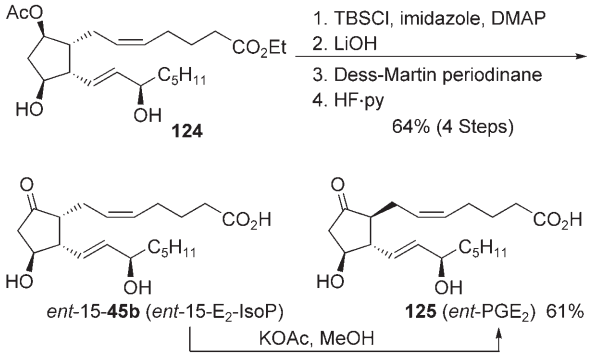
Only one year after the in vivo discovery of IsoPs, Larock and Lee developed a highly stereoselective synthesis of 15-F_{2c}-IsoP based on a one-pot, palladium-promoted three-component coupling (Scheme 48).[220] This is perhaps the shortest synthesis of an IsoP so far. It started with an



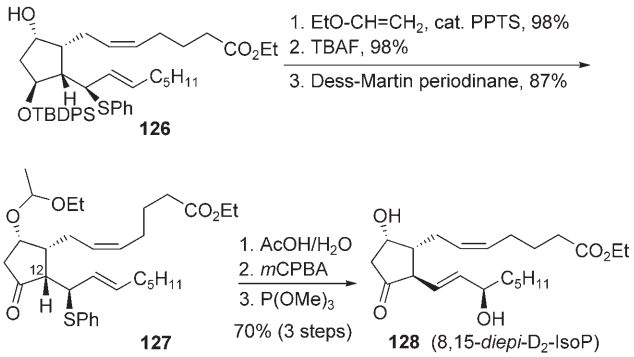
Scheme 44. Synthetic access to all four 15-F_{2t}-IsoP stereoisomers from **120**.



Scheme 45. IsoP classes synthesized by Taber et al.[217]



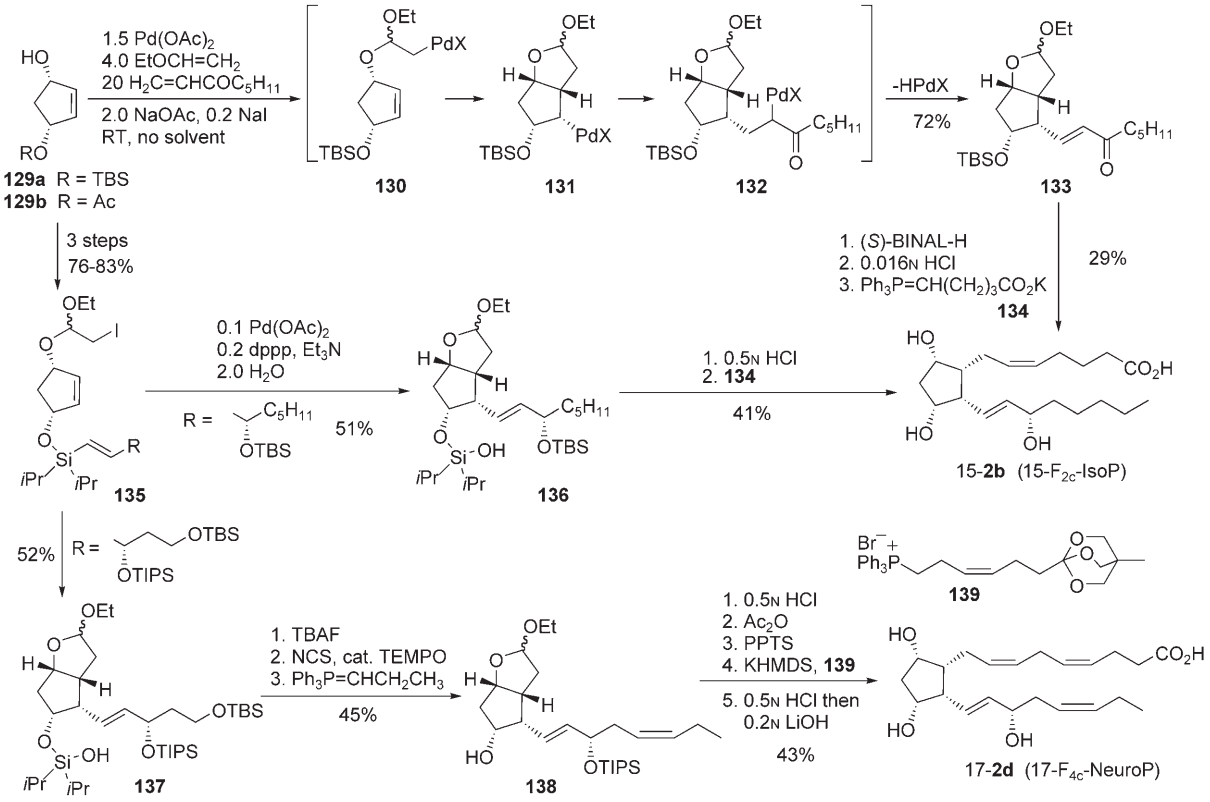
Scheme 46. Synthesis of *ent*-15-E_2-IsoP and *ent*-PGE_2.



Scheme 47. Approach to the 15-D_2-IsoP diastereomer **128** by functional-group interconversion.

alkoxypalladation of ethyl vinyl ether by enantiopure 2-cyclopentene-1,4-diol derivative **129a** to generate **130**, followed by two sequential carbopalladation steps via **131** and **132** to afford bicycle **133**. Three standard steps, consisting of asymmetric reduction of the ketone by BINAL-H, deprotection, and Wittig reaction with commonly used phosphorane **134** completed the synthesis of 15-F_{2c}-IsoP (15-**2b**). A drawback of this method is the necessary use of rather large excesses of $Pd(OAc)_2$ and the coupling components.

Cha and co-workers extended this approach by developing an intramolecular version, thus enabling the use of catalytic amounts of the Pd catalyst, minimizing reagent excesses, and improving the flexibility of introducing the α and ω side chain.[221a] Starting from enantiopure **129b**, iodoacetal formation and silylation with appropriate enantiopure chlorodiisopropyl vinylsilanes gave compounds **135**. The key double cyclization with $Pd(OAc)_2$ used as the catalyst, dppp as a ligand, and Et_3N as a base resulted in medium to good



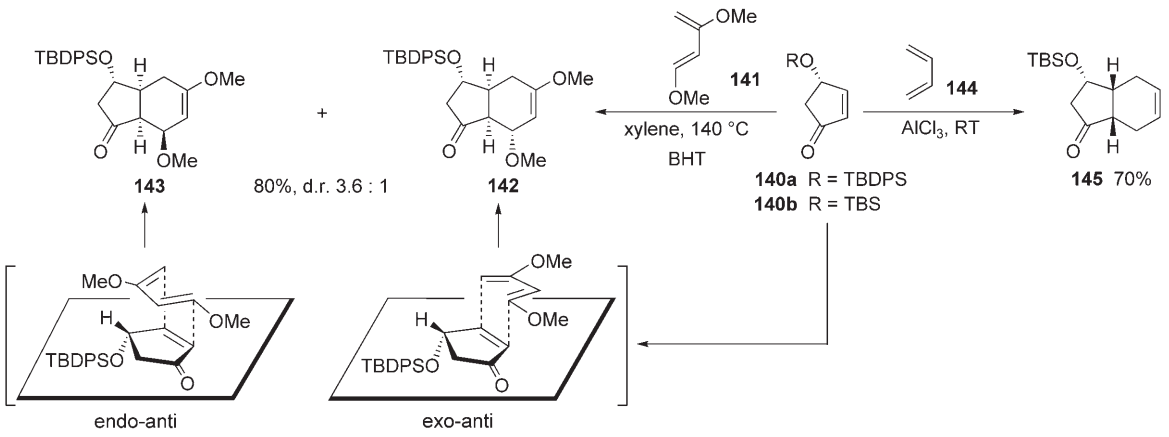
Scheme 48. Original total synthesis of 15-F_{2c}-IsoP according to Larock and Lee and the intramolecular version according to Quan and Cha.[220,221]

yields of the corresponding coupled silanols **136** and **137**. Thus, a wide range of ω side chains could be potentially installed stereoselectively by appropriate choice of the alkenylsiloxane. The synthesis of 15-F_{2c}-IsoP (15-**2b**) was completed from **136** after global deprotection with HCl and introduction of the α side chain by a Wittig reaction with phosphorane **134**. The high flexibility of this strategy is further highlighted by the successful synthesis of 17-F_{4c}-NeuroP (17-**2d**).[221b] After the coupling reaction to give **137**, selective cleavage of the TBS group mediated by TBAF, followed by selective oxidation of the primary alcohol and Wittig reaction with propylidenetriphenylphosphorane furnished **138**. Subsequent removal of the TIPS group, O acetylation of both alcohol units, and PPTS-mediated deprotection of the acetal led to a bicyclic lactol ready for another Wittig reaction with orthoester phosphonium salt **139**. 17-F_{4c}-NeuroP (17-**2d**) was obtained after ester hydrolysis and cleavage of the acetate protecting group.

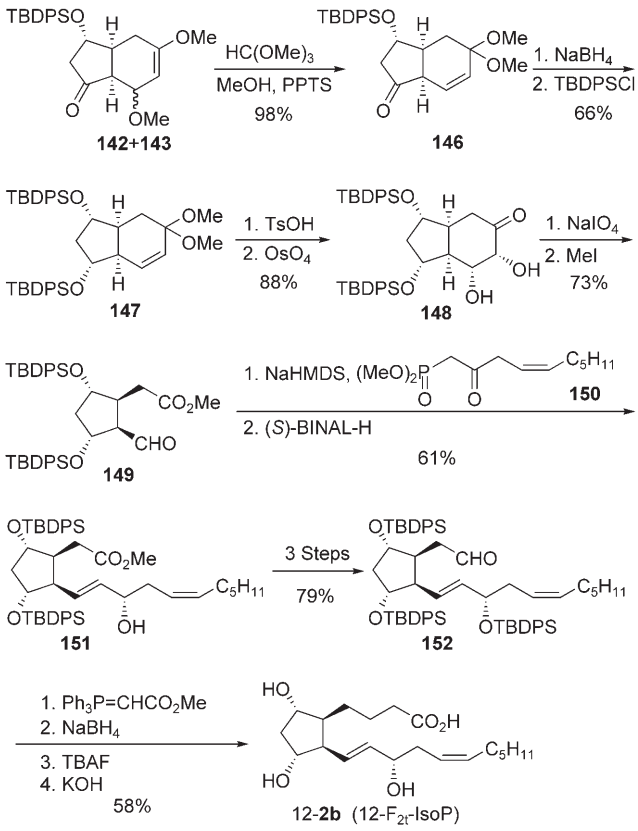
The Rokach research group developed a strategy for IsoPs based on a Diels–Alder reaction, which guarantees that the two side chains at the cyclopentane ring will be arranged *cis* to each other.[222] They reported that the reaction of dienophiles **140a** and **140b** with dienes **141** and **144**, respectively, led to the hydroxybicyclo[4.3.0]nonenones **142** and **143**, and **145**, respectively, as the major products (Scheme 49). A further minor bicyclic isomer (not shown) was also formed in 7% yield in the reaction with **141**. The authors explained the formation of the major product **143** by an *endo-anti* attack by **141** on the less-hindered face of **140a**. Similarly, the *syn-anti-syn* product **142** forms by an *exo-anti* attack at the less-hindered face. In the Lewis acid mediated reaction, the diene **144** approaches the sterically more congested face of **140b** with a selectivity of 96:4 to give cycloadduct **145** in 70% yield.

The synthesis of 12-F_{2t}-IsoP (12-**2b**) was carried out in 16 steps from a mixture of bicyclic ketones **142** and **143** (Scheme 50). The first step was an allylic rearrangement with trimethyl orthoformate in the presence of PPTS to give a common ketal derivative **146**. The following reduction of the carbonyl group with $NaBH_4$ occurred preferentially from the β face in a 5:1 ratio and led to the alcohol derivative with a F_{2t} configuration, which was transformed to the TBDPS derivative **147** in good yield. Hydrolysis of the dimethyl ketal with *p*-TsOH and subsequent dihydroxylation with OsO_4 afforded the 1,2-*cis*-diol **148** in 88% yield. Cleavage of the glycol and decarboxylation of **148** with sodium periodate, followed by methylation of the resulting carboxyl group with MeI gave methyl ester aldehyde **149** in 72% yield. The ω side chain was then appended by a Horner–Wadsworth–Emmons (HWE) reaction with β-ketophosphonate **150**. The resulting enone was then reduced by BINAL-H to give alcohol **151**, which was transformed to aldehyde **152** in three standard steps. The introduction of the α chain was achieved by a Wittig reaction with methyl phosphoranylidene acetate. The total synthesis 12-F_{2t}-IsoP (12-**2b**) was completed by three deprotection steps.

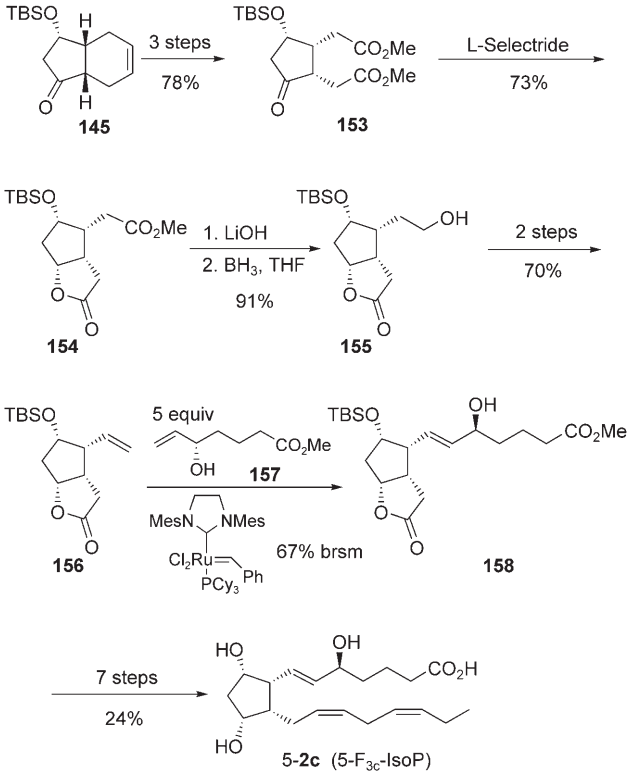
In 2006, the same research group published the first synthesis of 5-F_{3c}-IsoP (Scheme 51) from cycloadduct **145** (see Scheme 49).[223] This compound was converted into diester



Scheme 49. The Diels–Alder approach to IsoPs according to Rokach and co-workers.[222]



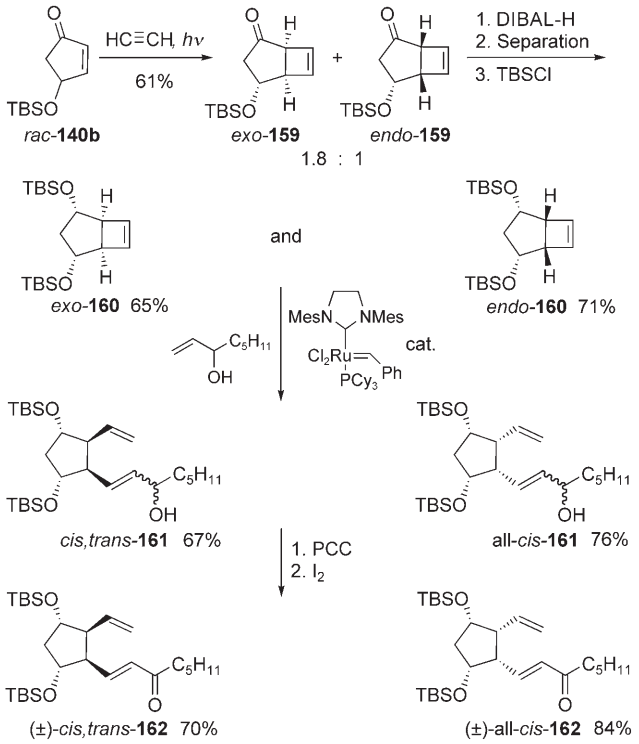
Scheme 50. Total synthesis of 12-F_{2t}-IsoP according to Rokach and co-workers.



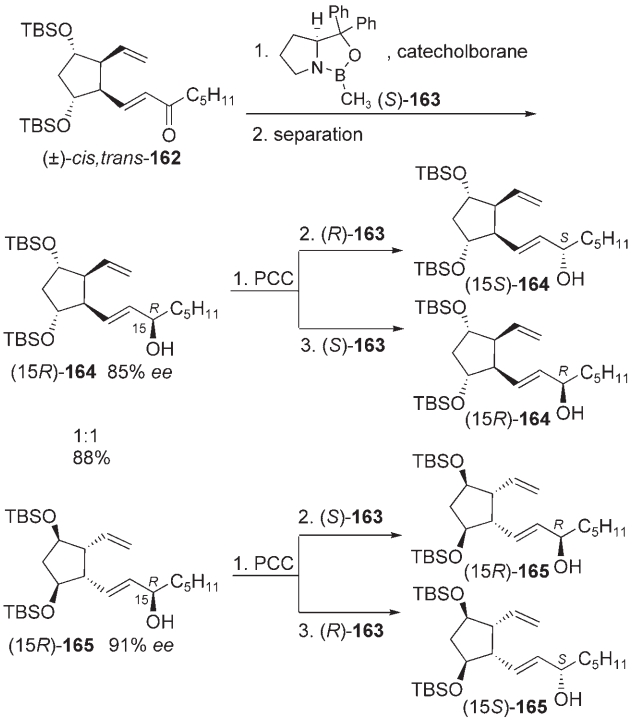
Scheme 51. Total synthesis of 5-F_{3c}-IsoP accoding to Rokach and co-workers.[223]

153 in three standard steps in 78% overall yield. The two methyl ester groups were differentiated by a stereocontrolled reduction of the keto group using L-Selectride, which attacked **153** selectively from the β face and triggered lactonization to bicyclic lactone **154** in 73% yield. The selective reduction of the carboxylic acid function generated after hydrolysis of the methyl ester with LiOH was carried out with BH_3·THF and afforded **155** in 91% yield. This bicyclic alcohol was transformed to the vinyllactone **156** in 70% yield by transformation to the corresponding *o*-nitrophenyl seleno ether and subsequent oxidative elimination with H_2O_2. The α chain was introduced by an olefin cross-metathesis of **156** with allylic alcohol **157**, which was prepared from D-arabinose in 11 steps. The desired product **158**, which was obtained in reasonable yield, was transformed to 5-F_{3c}-IsoP (5-**2c**) in seven steps in 24% overall yield by using straightforward reactions.

Snapper and co-workers published a stereodivergent synthesis of the eight possible 15-F_2-IsoP isomers that was based on an imaginative ring-opening/cross-metathesis strategy (Scheme 52).[224] The starting point was the known [2+2] photocycloaddition of racemic 4-silyloxy-2-cyclopentenone **140b** with acetylene that gave an inseparable 1.8:1 mixture of cycloadducts *exo*-**159** and *endo*-**159**. This method



Scheme 52. Ring-opening metathesis approach to 15-F_2-IsoP isomers according to Snapper and co-workers.[224]



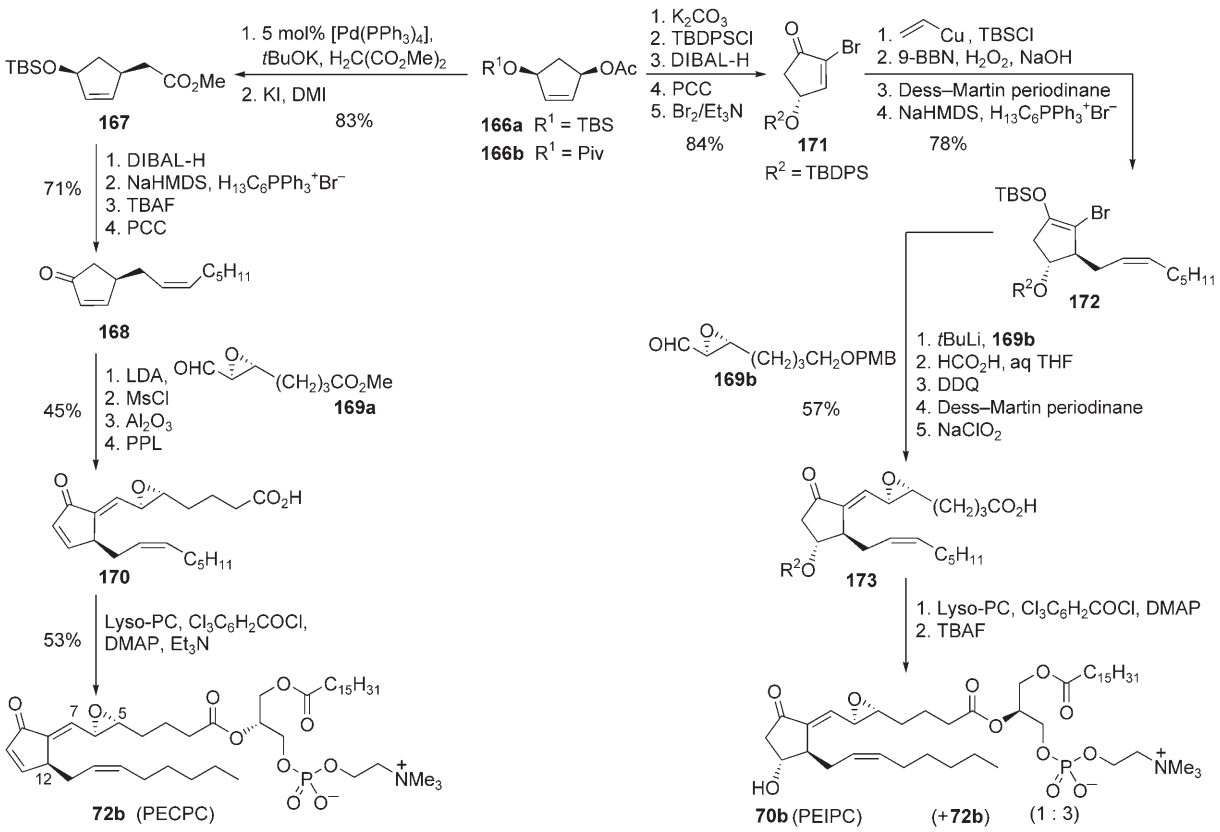
Scheme 53. Stereodivergent approach to the 15-F_{2t}-IsoP stereoisomers according to Snapper and co-workers.[224]

secured the relative *cis* orientation of the side chains for the 15-F_{2t}- and 15-F_{2c}-IsoP stereoisomers. Reduction with DIBAL-H led to a diastereomeric mixture of alcohols, which were separated and protected with TBSCl to give the desired *meso*-cyclobutenes *exo*-**160** and *endo*-**160**. The metathesis sequence carried out with an excess of 1-octen-3-ol in the presence of the Grubbs second generation catalyst produced *cis*,*trans*-**161** and all-*cis*-**161**, respectively, with the ω side chain in place. The obtained mixture of *E*/*Z* and alcohol isomers was simplified to the racemic *E*-enones *cis*,*trans*-**162** and all-*cis*-**162**, respectively, by PCC oxidation of the alcohol and iodine-catalyzed olefin isomerization.

Enantiopure compounds were synthesized by two strategies. The first is demonstrated by the synthesis of the 15-F_{2t}-IsoP series (Scheme 53). Asymmetric catalytic reduction of (±)-*cis*,*trans*-**162** using the (*S*)-2-methyl-CBS-oxazaborolidine catalyst (*S*)-**163** and catecholborane produced enantiomerically enriched diastereomeric alcohols **164** and **165** with the *R* configuration at C15. Their separation, individual reoxidation into enantioenriched enones **162**, and subsequent asymmetric reduction with both enantiomers of the CBS catalyst **163** gave the four enantiopure diastereoisomers **164** and **165** in greater than 98% ee for each product. The total synthesis of all individual 15-F_{2t}-IsoP enantiomers from **164** and **165** was accomplished by hydroboration of the terminal alkene and oxidation to the corresponding alcohol, selective oxidation to the aldehyde, Wittig reaction with **134** and TBS cleavage (not shown, see Scheme 48). This synthetic strategy is also suited to access specific compounds, such as *ent*-15-*epi*-F_{2t}-IsoP, starting from (*R*)-**140b**.[224c]

In the 15-F_{2c}-IsoP series (not shown), reduction of enone (±)-all-*cis*-**162** with $NaBH_4$ yielded the corresponding two diastereomeric allylic alcohols. Classical resolution of each diastereomer using (*R*)-*O*-acetylmandelic acid chloride gave access to four different enantiomerically pure alcohols. The total synthesis of the four 15-F_{2c}-IsoP enantiomers was completed as described for the 15-F_{2t}-IsoP isomers. This synthesis permitted the biological activity of previously not accessible IsoP stereoisomers to be explored (see Section 6). Moreover, 15-F_{2t}-IsoP-phosphatidylethanolamine and *ent*-15-*epi*-F_{2t}-IsoP-phosphatidylcholine were synthesized by esterification of the corresponding TBS-protected isoprostanes with the corresponding phosphatidyl lipid fragments.[224d]

In 2005, two research groups reported the total synthesis of epoxy isoprostane phospholipids, namely 1-palmitoyl-2-(5,6-epoxy-15-E_2-IsoP)-*sn*-glycero-3-phosphatidylcholine (PEIPC, **70b**) and the related 1-palmitoyl-2-(5,6-epoxy-15-A_2-IsoP)-*sn*-glycero-3-phosphatidylcholines (PECPC, **72b**) by using 1,4-bis(acetoxy)-2-cyclopentene as the starting material. With their synthesis of **72b**, Acharya and Kobayashi established the correct relative configuration of the epoxy group and C12 as well as the *E* configuration of the Δ^7 alkene unit (Scheme 54).[225,226] The synthesis began with the palladium-catalyzed allylic alkylation of the potassium enolate of dimethyl malonate with enantiopure monoacetate **166a**. Subsequent decarboxylation afforded ester **167**, which was converted stereoselectively into *Z*-olefin **168** by a Wittig reaction after reduction to the aldehyde. A base-mediated aldol reaction of **168** with epoxyaldehyde **169a** yielded a mixture of *E*- and *Z*-aldol condensation products, which were isomerized cleanly by Al_2O_3 to produce the 7*E*-enone methyl



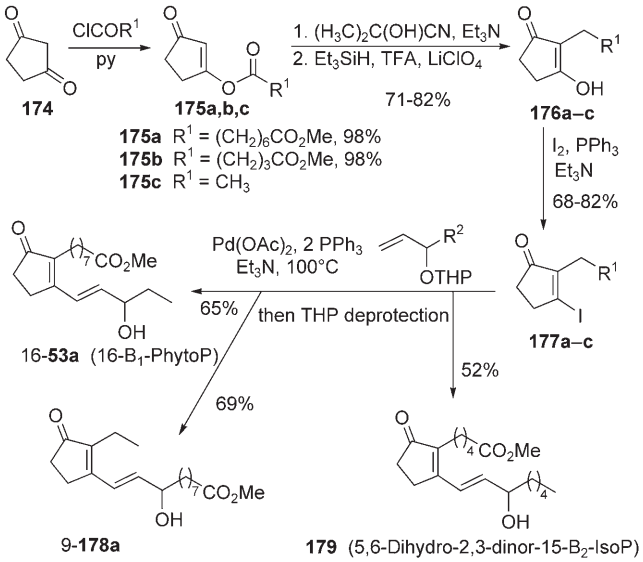
Scheme 54. Total syntheses of PEIPC (**70b**) and PECPC (**72b**).

ester. Finally, enzymatic ester hydrolysis with porcine pancreas lipase (PPL) furnished carboxylic acid **170**, which was esterified with 1-palmitoyl-2-lyso-PC using Yamaguchi's reagent to afford **72b**. In subsequent work, Acharya and Kobayashi used a similar approach to gain access to **72b** and its 14,15-epoxyisoprostane isomer.[225b,c]

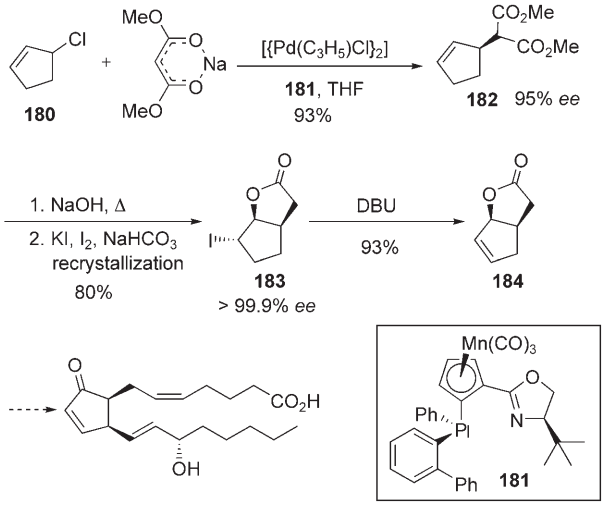
Jung et al. focussed on the synthesis of **70b**. They converted enantiopure **166b** in five steps into 2-bromo-4-(silyloxy)cyclopentenone **171** (Scheme 54).[227] Introduction of the ω side chain was achieved by an initial conjugate addition of vinylcopper in the presence of TBSCl. The vinyl group served as the basis for completing the ω chain of the allylic cyclopentanone silyl enol ether **172** through a sequence of hydroboration, oxidation, and Wittig reactions. Generation of the vinyllithium species from **172** by lithium–bromine exchange followed by treatment with epoxy aldehyde **169b** furnished the corresponding aldol adducts. Cleavage of the TBS group and dehydration with formic acid afforded a vinylic epoxide. The primary PMB ether was cleaved with DDQ and the resulting alcohol was subsequently transformed to carboxylic acid **173** by a two-step Dess–Martin and Pinnick oxidation. Yamaguchi esterification and deprotection gave PEIPC (**70b**) and PECPC (**72b**) as a 1:3 mixture, with **72b** generated by β elimination during the deprotection stage with TBAF.

In 2007, Schmidt and Boland developed a very short and general strategy for the synthesis of B_1-PhytoPs, dinor-IsoPs, and several analogues (Scheme 55).[228] Their approach is based on five key operations to introduce the two side chains. Starting from 1,3-cyclopentanedione (**174**), an O acylation with a variety of acyl chlorides was first carried out to give **175**. A subsequent 1,3-O,C-acyl shift effected by acetone cyanohydrin/NEt_3 and reduction of the exocyclic keto function by triethylsilane yielded the desired C-alkylated 3-hydroxyenones **176a–c** with the α chain in place. Reaction with the iodine-triphenylphosphine complex gave access to 2-alkyl-3-iodocyclopentenones **177a–c**, which served as precursors for the introduction of the ω side chains by Heck cross-coupling reactions catalyzed by a 2:1 mixture of PPh_3 and $Pd(OAc)_2$ in the presence of Et_3N. The THP-protected 1-alken-3-ols proved to be the coupling substrates of choice. Cleavage of the THP group completed the total synthesis of 16-B_1-PhytoP (16-**53a**), 9-**178a**, and 5,6-dihydro-2,3-dinor-15-B_2-IsoP (**179**) in good overall yield. The flexibility of this approach was also demonstrated by the synthesis of acetylenic and O-alkylated phytoprostane analogues.

In 2004, Helmchen et al. published an asymmetric route to *cis*-disubstituted lactones **184** (Scheme 56).[229] The diester **182** was obtained in almost quantitative yield by an asymmetric Pd-catalyzed allylic substitution of cyclopentenyl chloride **180** by the sodium enolate of methyl malonate in the presence of a chiral manganese tricarbonyl ligand **181**. Interestingly, the authors noted that the allylic chloride substituent was a more efficient leaving group than the more commonly used acetate or carbonate. Two subsequent steps, namely saponification/decarboxylation and iodolactonization, gave **183** in high yield.



Scheme 55. Highly divergent synthesis of B_1-PhytoP (16-**53a**) and analogues according to Schmidt and Boland.[228]

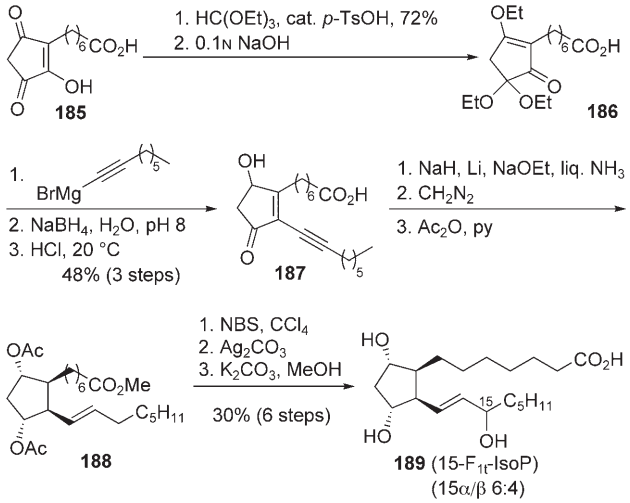


Scheme 56. Synthesis of IsoP synthon **184** according to Helmchen et al.[229]

Dehydroiodination with DBU furnished unsaturated lactone **184** in 93% yield. The synthesis of jasmonoids, such as 12-OPDA, was then possible from **184**. The isoprostane series may also be accessible by similar routes.

7.1.3.2. From Cyclopentane Precursors bearing Additional Carbon Atoms

3-Alkylcyclopentan-1,2,4-trione **185** proved to be a suitable precursor in an early synthesis of IsoPs (Scheme 57).[230] Its simultaneous acetalization and esterification with triethyl orthoformate afforded triethoxycyclopentenone **186**. Nucleophilic addition of the magnesium acetylide of 1-octyne, dehydration, subsequent reduction of the resulting ketone by sodium borohydride, and acidic cleavage of the acetal

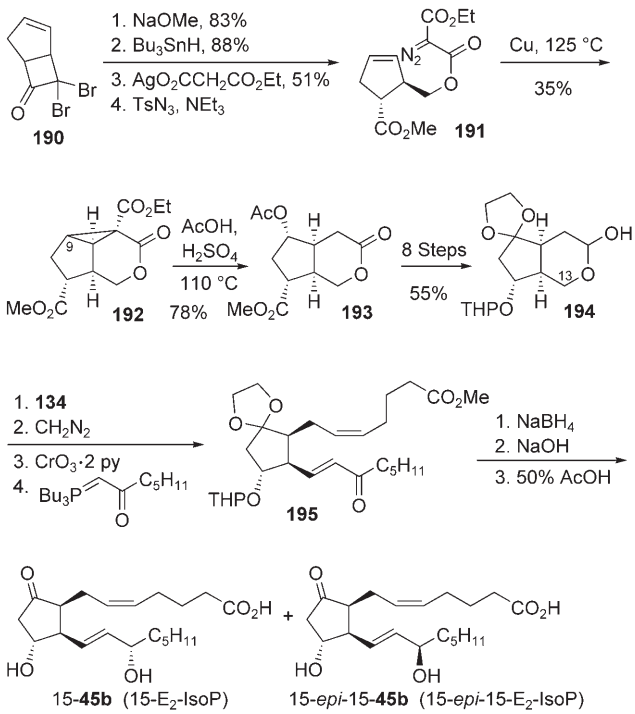


Scheme 57. Total synthesis of 15-F_{1t}-IsoP from a cyclopentanetrione precursor.

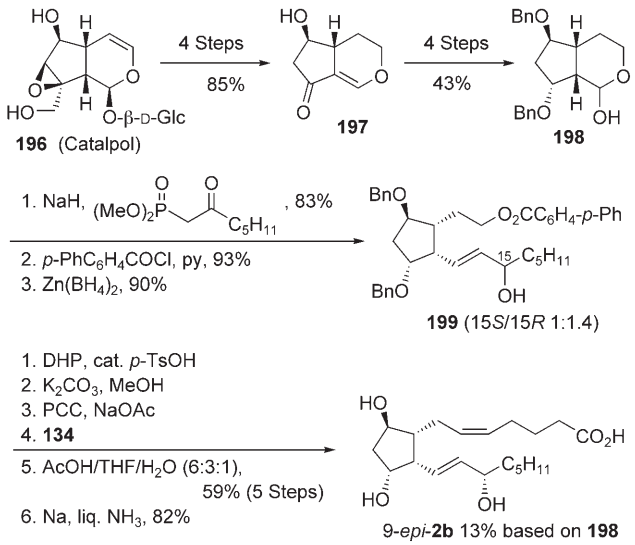
furnished the hydroxycyclopentenone **187** in 48% yield. After exhaustive reduction of the enone and the alkyne units with lithium in liquid ammonia, the 15-deoxy-15-F_{1t}-IsoP core was formed with good diastereoselectivity; it was successfully isolated as its diacetate **188**. Interestingly, the introduction of the 15-hydroxy group in **188** was accomplished by a Wohl–Ziegler bromination followed by a nucleophilic substitution of the allylic bromide with silver carbonate. Saponification of the acetate and ester functions provided 15-F_{1t}-IsoP (**189**) in 30% yield over six steps.

An intramolecular Cu-catalyzed cyclopropanation of a 2-cyclopentenylester was used as a keystep in the synthesis of 15-E_1- and 15-E_2-IsoP (Scheme 58).[231] The starting point was the bicyclic ketone **190**, which was prepared by [2+2] cycloaddition of dibromoketene and cyclopentadiene. Its nucleophilic ring opening by sodium methoxide and selective monoreduction of the dibromide under radical conditions (tributyltin hydride) gave a cyclopentenylmethyl bromide, which was esterified with the silver salt of monoethyl malonate. A diazo transfer reaction with tosyl azide afforded the diazomalonate **191**. A copper-mediated intramolecular cyclopropanation in xylene under reflux gave the tricyclic lactone **192** in moderate yield. The oxygen atom at the 9-position of the IsoP was introduced by solvolytic ring opening of the cyclopropane unit by acetic acid to give bicyclic lactone **193**.

A drawback of this synthesis is that eight steps were necessary to adjust the functional group pattern from that in **193** to that in bicyclic lactol **194** for the further elaboration of the IsoP skeleton. A sequence of a *Z*-selective Wittig reaction, in which **134** was used to install the α-chain, followed by esterification, Collins oxidation of the 13-alcohol to the aldehyde, and a second Wittig reaction with a ketophoshorane furnished the C_{20} carbon skeleton **195**. Interestingly, although many synthetic approaches to prostaglandins relied on cyclopentane precursors with the IsoP configuration that epimerized at the C13-aldehyde stage during installation of the ω chain, epimerization did not occur



Scheme 58. Total synthesis of 15-E_2-IsoP (15-**45b**) and its 15 epimer according to Nakamura and Sakai.[231]

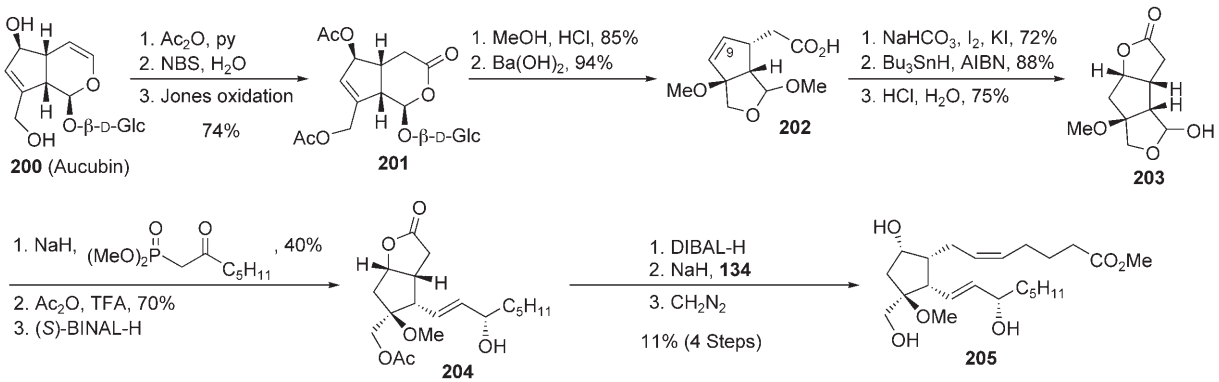


Scheme 59. Total synthesis of (15*S*)-9-*epi*-15-F_{2c}-IsoP from the iridoid catalpol **196**.

in this case on application of the tributyl-β-ketophosphorane reagent. The total synthesis of 15-**45b** and 15-*epi*-15-**45b** was completed by an unselective reduction of the 15-keto function, saponification, and cleavage of the cyclic ketal.

Iridoid glucosides are abundantly occurring plant metabolites that have been used frequently as enantiomerically pure chiral pool starting materials for the synthesis of prostaglandins and also of some isoprostane analogues. Weinges et al. used catalpol **196** as a starting material for the synthesis of (15*R*)- and (15*S*)-9-*epi*-15-F_{2c}-IsoP (9-*epi*-**2b**, Scheme 59).[232a] Catalpol was converted on a large scale into lactol **198** via keto enol ether **197**. This compound possesses two conveniently differentiated functionalities for introduction of the side chains. The ω side chain was introduced first by a HWE reaction by using 2-oxoheptylphosphonate in the presence of NaH. After temporary protection of the primary alcohol, the keto function was reduced with zinc borohydride (albeit with low diastereoselectivity). After separation of the 15*R* and 15*S* diastereomers of **199**, a sequence consisting of protection of the 15-position as a THP ether, saponification of the benzoate, and oxidation of the resulting primary alcohol to the aldehyde, a highly *Z*-selective Wittig reaction with 5-phosphoniovaleric acid **134**, and two final deprotection steps gave the target isoprostane 9-*epi*-**2b**. An unnatural branched 15-methyl analogue was also synthesized by a similar strategy.[232b]

A related synthetic strategy was used for the synthesis of isoprostanes modified at the 11-position (Scheme 60).[233] The iridoid glucoside aucubin (**200**) was used as the starting material. Lactone **201** was obtained after three functional group modification steps. Hydrolytic removal of glucose, saponification of the acetate groups, and ring opening afforded the bicyclic carboxylic acid **202**. The oxygen atom at the 9-position was introduced by an iodolactonization. Radical reduction of the iodide followed by acidic deprotection gave the lactol **203**. Similarly to the syntheses described by Weinges et al., the ω chain was first appended

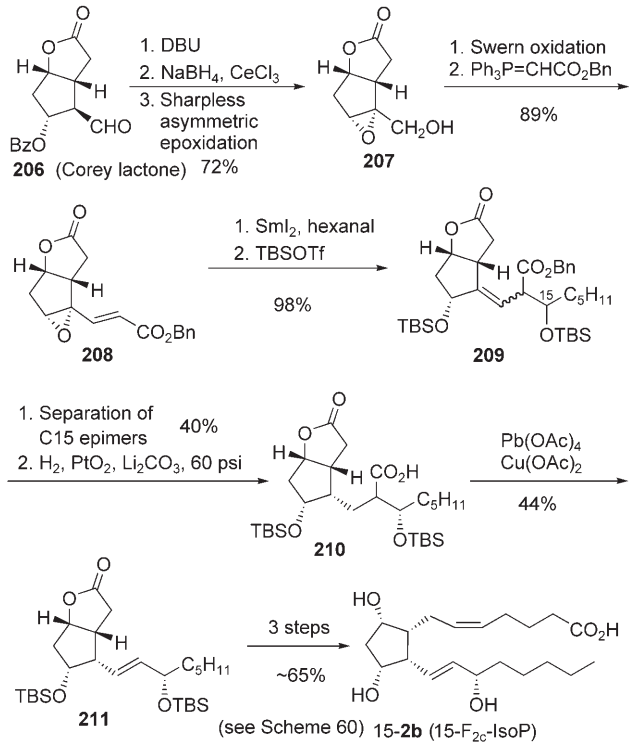


Scheme 60. Synthesis of IsoP analogues from the iridoid glucoside aucubin (**200**).

to **203** by a HWE reaction with 2-oxoheptylphosphonate in the presence of NaH as a base. After acetylation of the newly created alcohol, the enone was reduced stereoselectively using Noyori's (*S*)-BINAL-H to give (15*S*)-hydroxylactone **204**. The synthesis of **205** was completed by standard reduction of the lactone to the lactol with DIBAL-H, introduction of the α chain by a Wittig reaction with **134**, and esterification of the carboxylic acid with diazomethane.

This synthetic strategy is similar to that of Ohno and co-workers, who prepared a variety of IsoP analogues, mostly modified at the 11-position, from aucubin (**200**).[234] On the basis of these results, a number of cyclopentane derivatives were prepared that can potentially be used to synthesize isoprostanes.[235]

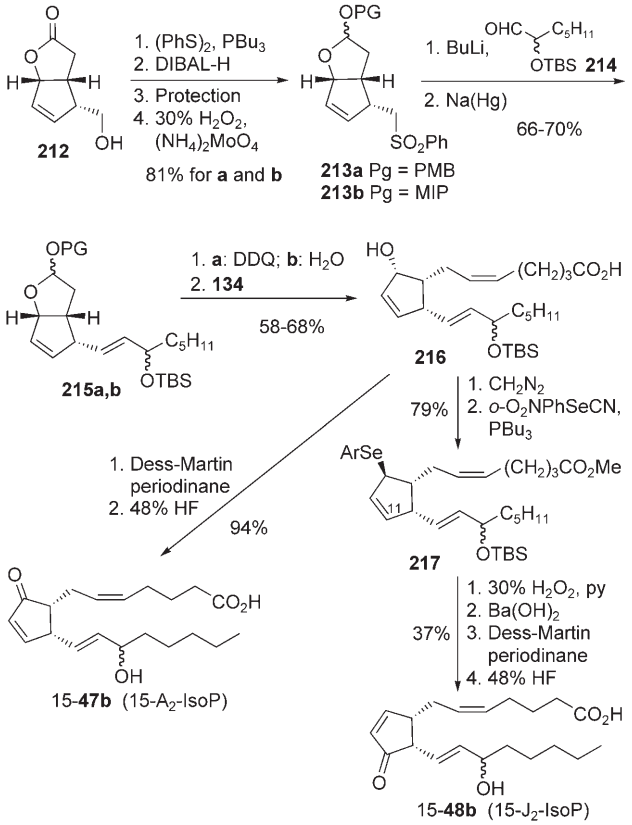
In 1999, Cha and co-workers developed an approach to 15-F_{2c}-IsoP (15-**2b**) starting from commercially available benzoyl-protected Corey lactone (**206**, Scheme 61).[236] An



Scheme 61. Total synthesis of 15-F_{2c}-IsoP from the Corey lactone (**206**).

E1cB elimination of the benzoyl group in **206** with DBU and a subsequent Luche reduction gave the corresponding allylic alcohol, which was subjected to Sharpless epoxidation conditions to afford epoxy alcohol **207** in 72% yield. The γ,δ-epoxy-α,β-unsaturated ester unit **208** was introduced by a Swern oxidation and a Wittig reaction. A SmI_2-mediated reductive ring opening of the epoxide and in situ trapping of the resulting dienolate with hexanal yielded a mixture of six diastereomeric aldol coupling products, which were protected with TBSOTf to give silyl ethers **209**. At this stage, the 15*R* and 15*S* epimers could be successfully separated. The olefin was hydrogenated stereoselectively and the benzyl ester cleaved to furnish the *cis*-dialkyl-substituted cyclopentane **210** cleanly (only the 15*S* diastereomer is shown). Oxidative decarboxylation afforded bicyclic lactone **211** with a 13,14-*E*-olefin unit exclusively. Classical lactone reduction, α-chain introduction by a Wittig reaction with **134**, and deprotection provided both 15-F_{2c}-IsoP (15-**2b**) and 15-*epi*-F_{2c}-IsoP.

Cyclopentenone-IsoP derivatives are the focus of the synthetic efforts by Zanoni et al. Their approach to A_2- and J_2-IsoPs is based on a common precursor **212** obtained by a palladium(II)-catalyzed translactonization reaction of bicyclic lactone isomers (Scheme 62).[237a] Bicyclic sulfones **213a** or **213b** were obtained in good yield in four steps involving manipulation of the hydroxy group in **212** to a sulfide, reduction of the lactone to a lactol, protection as a PMB ether or MIP acetal, and oxidation of the sulfide.[202a] The sulfones underwent a Julia–Lythgoe olefination with α-silyloxyaldehyde **214** to give bicyclic compounds **215a**,**b** which have the ω side chain with the desired *E* configuration. Deprotection of the lactol group and a Wittig reaction with **134** gave the C_{20}-cyclopentenol skeleton **216**. A Dess–Martin oxidation of **216** and cleavage of the remaining TBS ether completed the total synthesis of 15-A_2-IsoP (15-**47b**) as a diastereomeric mixture at C15.



Scheme 62. Total synthesis of 15-A_2-IsoP and 15-J_2-IsoP according to Zanoni et al.[237a]

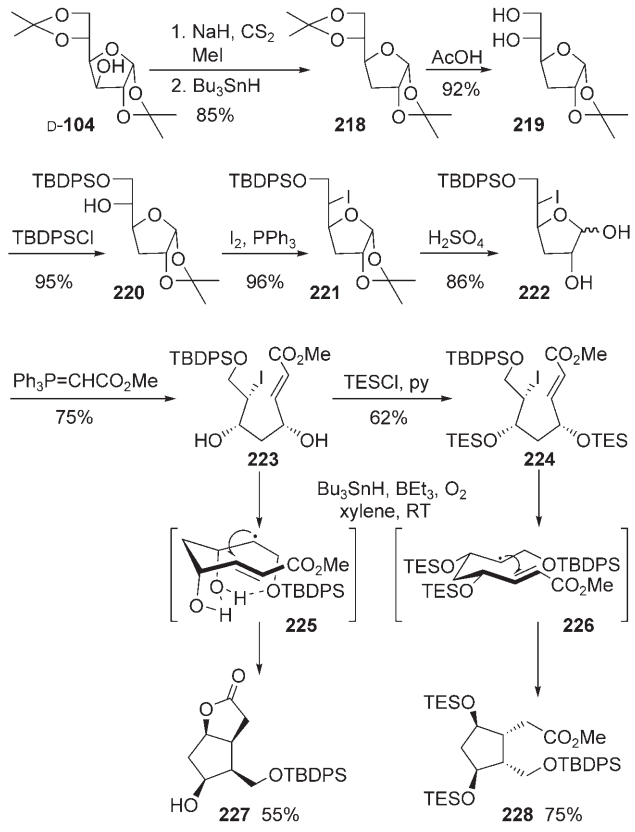
The synthesis of 15-J_2-IsoP (15-**48b**) was also achieved from **216** by a six-step sequence starting with esterification by

diazomethane and conversion of the cyclopentenol into the corresponding selenide **217**.[202b] Treatment with hydrogen peroxide in pyridine effected a [2,3]-sigmatropic rearrangement to the corresponding allylic alcohol at the 11-position. Ester hydrolysis, oxidation of the cyclopentenol to the cyclopentenone unit, and removal of the TBS group gave 15-J_2-IsoP as a diastereomeric mixture at C15. Zanoni et al. very recently published the first synthesis of 14-A_4-NeuroP by using a similar strategy.[237b]

7.1.4. Cyclization Reactions Followed by Attachment of the Remaining Side Chain (Path C)

7.1.4.1. Radical Cyclizations

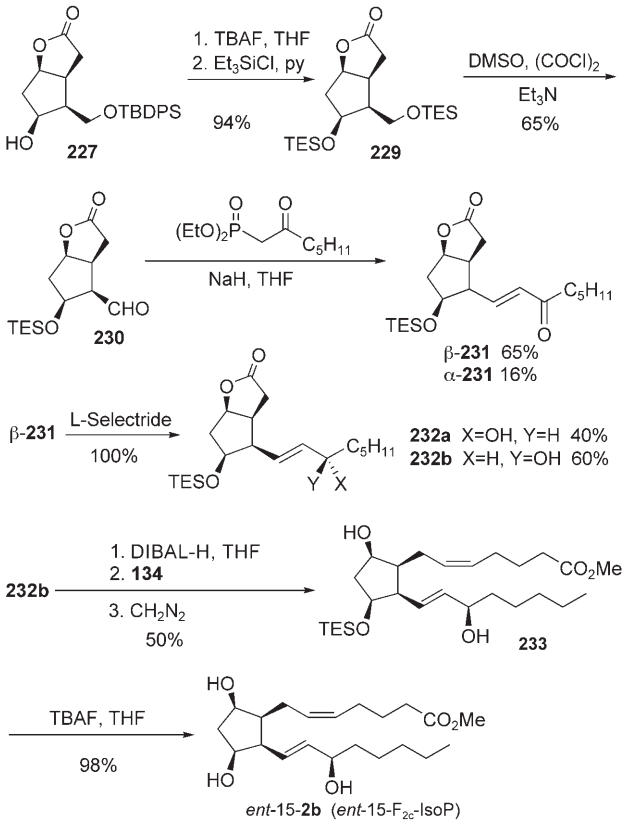
Since radical cyclizations are well-suited for the synthesis of cyclopentane rings with *cis*-oriented substituents,[55] they are attractive for the synthesis of IsoP intermediates. At the beginning of the 1990s, the Durand/Rossi research group as well as Rokach and co-workers together reported the synthesis of key intermediate **227**, a diastereomer of Corey's formyllactone, via an acyclic thionocarbonate.[238] Durand, Rossi, and co-workers subsequently developed radical carbocyclizations of functionalized iodo precursors **223** and **224** (Scheme 63), which replace the thionocarbonate precursors initially proposed.[238] The synthesis of **227** started with commercially available 1,2,5,6-di-*O*-isopropylidene-α-D-glucofuranose (D-**104**), which was transformed to the corresponding 3-deoxy sugar **218** in 85% yield by using the Barton–McCombie procedure. A selective deprotection of the isopropylidene group at the 5,6-position to give diol **219** was accomplished in good yield in the presence of 70% aqueous acetic acid. Protection of the primary alcohol using 1.1 equivalents of *tert*-butyldiphenylsilyl chloride in DMF and imidazole led to the pure monosilyl ether **220**. Introduction of iodine at C5 to give **221** was accomplished in 96% yield by using I_2, Ph_3P, and imidazole in xylene in a procedure developed earlier by Durand, Rossi, and co-workers.[239]



Scheme 63. Radical cyclization approach to give the central IsoP intermediates **227** and **228** according to Durand, Rossi, and co-workers.

Hydrolysis of the isopropylidene group at the 1,2-position was achieved in the presence of 10% aqueous sulfuric acid in THF/dioxane (3:1) to afford the diol **222** in 86% yield. The next step was a Wittig reaction with methyl triphenylphosphoranylideneacetate, which afforded the cyclization precursor **223** in 75% yield. Protection of the two hydroxy groups in **223** with triethylsilyl chloride in pyridine gave **224** in 62% yield. The crucial 5-*exo* radical cyclizations were conducted in the presence of tributyltin hydride and initiated by triethylborane/oxygen at room temperature. The factors controlling the diastereoselectivity of the cyclization of these polyhydroxylated hex-5-enyl radicals were carefully examined: It was shown to be strongly dependent on the protecting-group pattern of the two secondary hydroxy groups in **223** and **224**.[240] Compound **223**, with free hydroxy groups, cyclizes preferentially via hydrogen-bonded transition state **225** to give all-*cis*-**227**. In contrast, the bis(TES) derivative **224** cyclizes via a Beckwith–Houk transition state **226** to give *syn-anti-syn*-**228**. All the reactions shown for D-glucose were also performed with L-glucose to make all the stereoisomers of the *syn-anti-syn*- and all-*cis*-cyclopentane families accessible.

Compounds **227** and **228** were converted into a large set of enantiomerically pure IsoPs, NeuroPs, and PhytoPs by the following standard methods: a) Sequential appendage of the side chains by Wittig and/or HWE reactions; b) protection/deprotection reactions; c) regioselective oxidation; and d) enantioselective reduction. This is illustrated by the first total synthesis of *ent*-15-F_{2c}-IsoP (*ent*-15-**2b**), which was achieved in 1996 starting from lactone **227** (Scheme 64).[241] After deprotection of the TBDPS group and protection of both hydroxy groups with triethylsilyl chloride in pyridine, lactone **229** was obtained in quantitative yield. One-pot deprotection and Swern oxidation of **229** gave the rather unstable formyl lactone **230** in 65% yield. Introduction of the ω chain in all-*cis*-**230** was achieved by a HWE reaction with diethyl 2-oxoheptylphosphonate/NaH, and afforded a mixture of enone β-**231** in 65% yield as well as 16% of the epimerized derivative α-**231**. Conversion of β-**231** into *ent*-15-F_{2c}-IsoP methyl ester was accomplished in a similar manner as the original method reported by Vionnet and Renaud.[242] Reduction of the keto function with L-selectride afforded the epimeric hydroxy derivatives **232a** and **232b** quantitatively as a readily separable 2:3 mixture. The major lactone **232b** was reduced in 71% yield to the corresponding lactol with DIBAL-H in THF. The α chain was subsequently introduced by a Wittig reaction with **134** to afford **233** in 70% yield after esterification with diazomethane. Finally, cleavage of the silyl ether with nBu_4NF in THF yielded *ent*-15-F_{2c}-IsoP methyl ester (*ent*-15-**2b**) quantitatively. A number of IsoPs, NeuroPs,

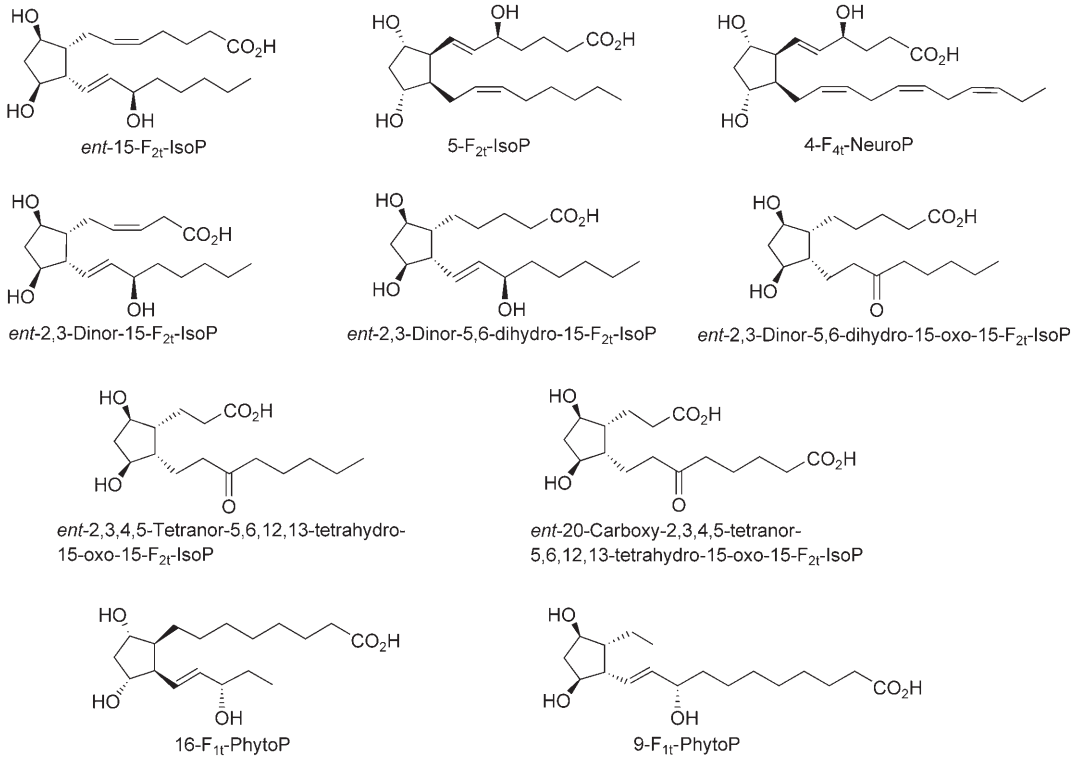


Scheme 64. Total synthesis of *ent*-15-F_{2c}-IsoP by Durand, Rossi, and co-workers.[238]

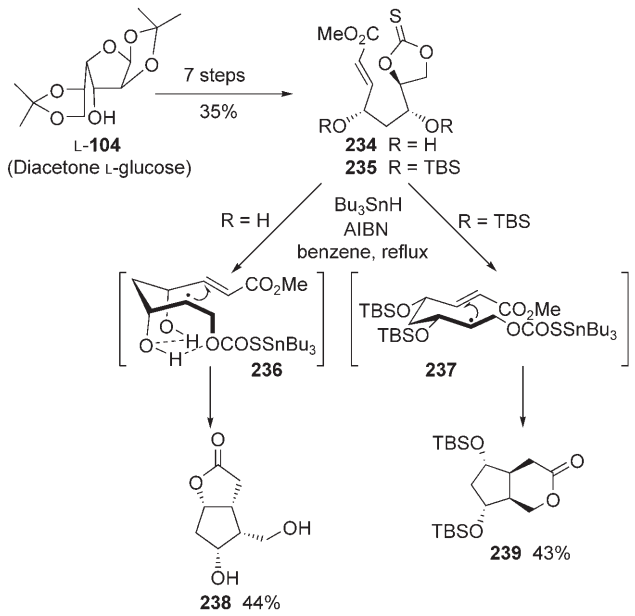
PhytoPs, and IsoPs metabolites in Scheme 65 were synthesized by this radical cyclization.[243]

Rokach and co-workers selected cyclic thionocarbonates **234** and **235** derived from diacetone L-glucose (L-**104**) as radical cyclization precursors, and demonstrated that they very efficiently generated a secondary radical for the cyclization step (Scheme 66).[244] Additionally, the authors suggested that the introduction of the thionocarbonate group avoids prior protection and later deprotection of the primary hydroxy group in the synthetic sequence. The diastereoselectivity of the 5-*exo* radical cyclizations is also sensitive to the protecting group pattern, and proceeds from **234** to the oxabicyclo[3.3.0]octanone ring system **238** via hydrogen-bonded transition state **236**, while the oxabicyclo-[4.3.0]nonanone ring **239** is formed from **235** via Beckwith–Houk transition state **237**. This strategy permitted the total synthesis of the 5-, 8-, 12-, and 15-F_{2t}-IsoP classes, of 15-F_{2t}-IsoP metabolites, of the 7-series of F_4-NeuroP, and of isotopic markers of the four series of F_2-IsoPs (Scheme 67).[15c,105c,109]

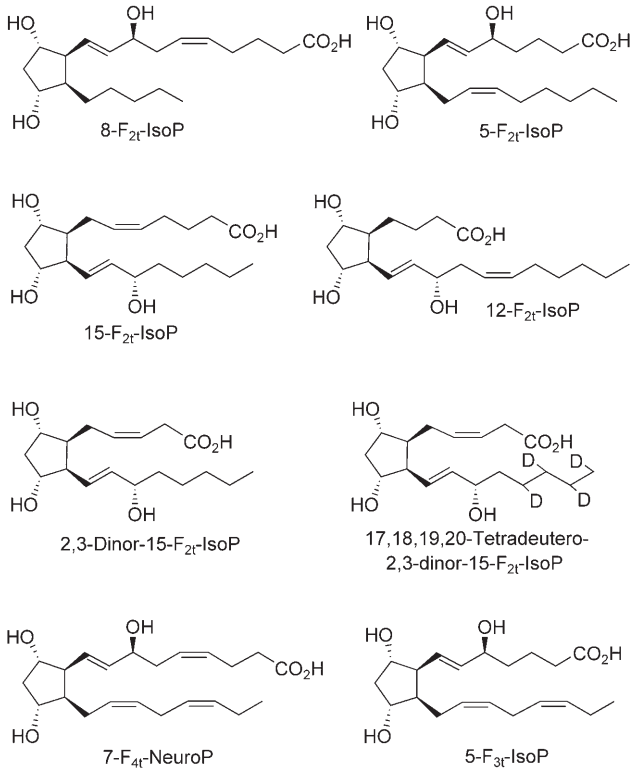
In 1994 Renaud and Vionnet reported the total synthesis of 15-F_{2c}-IsoP (15-**2b**) in which they used a convergent pathway starting from (±)-7-oxabicyclo[2.2.1]hept-5-en-2-one (**240**).[242] A sequence of group-transfer radical addition of phenylselenylmalonate and acyl migration under irradiation led to the rearranged product **241** (Scheme 68). The following steps involve reduction of the ketone, decarboxylation, and protection of the secondary alcohol to give the *endo*-silyloxy bicycle **242**. Oxidative hydrolysis of the acetal and acidic lactonization under mild conditions provided the all-*cis*-formyllactone **243**. The introduction of the ω chain was



Scheme 65. IsoP classes synthesized by the Durand/Rossi radical approach.

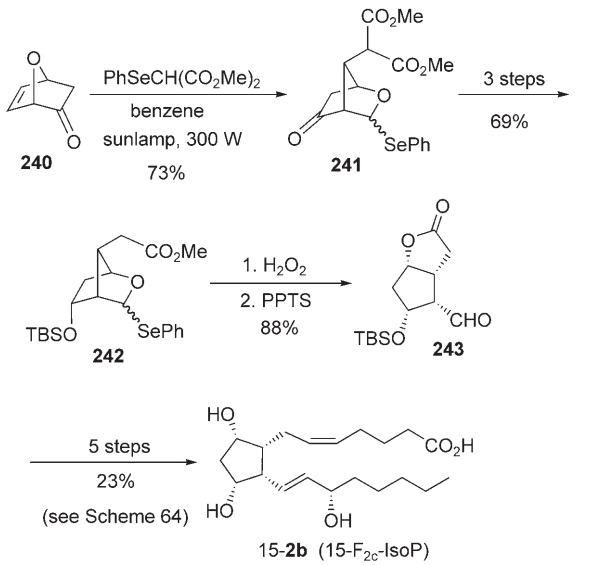


Scheme 66. Radical cyclization approach to lactones **238** and **239** according to Rokach and co-workers.[244]



Scheme 67. IsoP classes synthesized by Rokach and co-workers.[15c,105c,109]

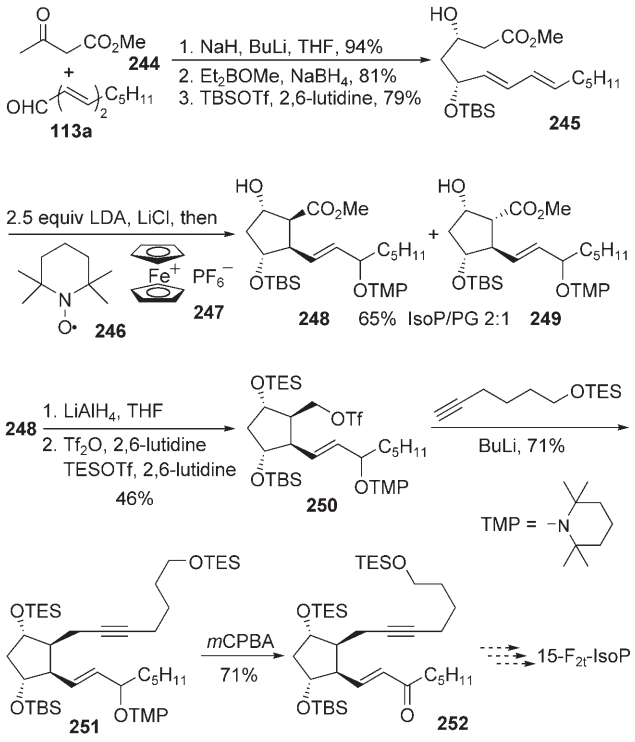
carried out by a HWE reaction with dimethyl 2-oxoheptylphosphonate/NaH. It is important to note that the choice of the silyl protecting group in this step was crucial to avoid competitive elimination and epimerization. The final steps of



Scheme 68. Total synthesis of 15-F_{2c}-IsoP according to Vionnet and Renaud.[242]

the synthesis of 15-F_{2c}-IsoP were accomplished in a similar way as used by Corey et al. for the synthesis of $PGF_{2\alpha}$ (see Scheme 64).[245]

Jahn's research group developed an approach to the F_{2t}-IsoP skeleton that was based on oxidative electron-transfer-induced cyclization of 3-hydroxy ester dianions (Scheme 69).[246] The cyclization precursor **245** was synthesized in three steps by a vinylogous aldol addition of the dianion of methyl acetoacetate (**244**) to 2,4-decadienal



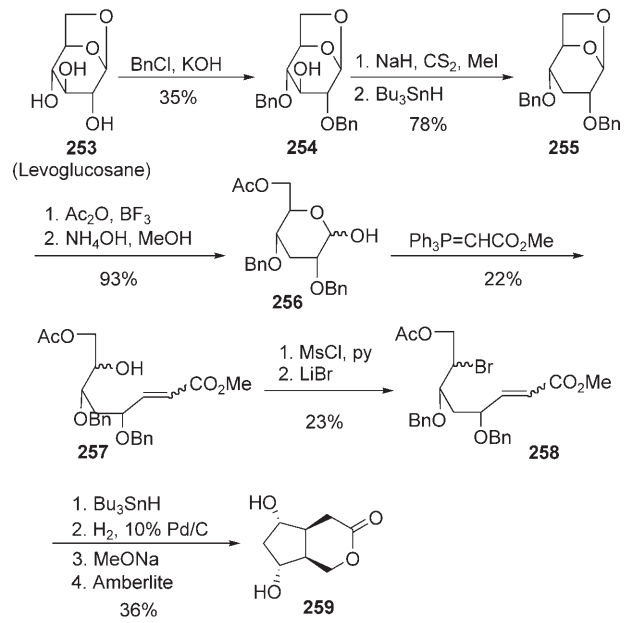
Scheme 69. Synthesis of IsoP precursors by oxidative cyclization according to Jahn et al.[246]

(**113a**), followed by a *syn*-selective reduction of the ketone mediated by diethyl(methoxy)borane and sodium borohydride, and a highly selective monoprotection of the allylic alcohol function as a TBS ether. The key step of the synthesis was the oxidative cyclization of the dianion of **245**, which was generated by deprotonation of the alcohol and ester groups with an excess of LDA. Single-electron oxidation of the enolate unit with ferrocenium hexafluorophosphate (**247**) generated a radical anion, which underwent a 5-*exo* radical cyclization to the diene unit. The resulting allylic radical was oxygenated with high regioselectivity at the 15-position by the stable free-radical TEMPO (**246**). The diastereoselectivity of the cyclization for the IsoP skeleton **248** was moderate. The oxgenation by TEMPO (**246**) occurred, not unexpectedly, without diastereoselectivity.

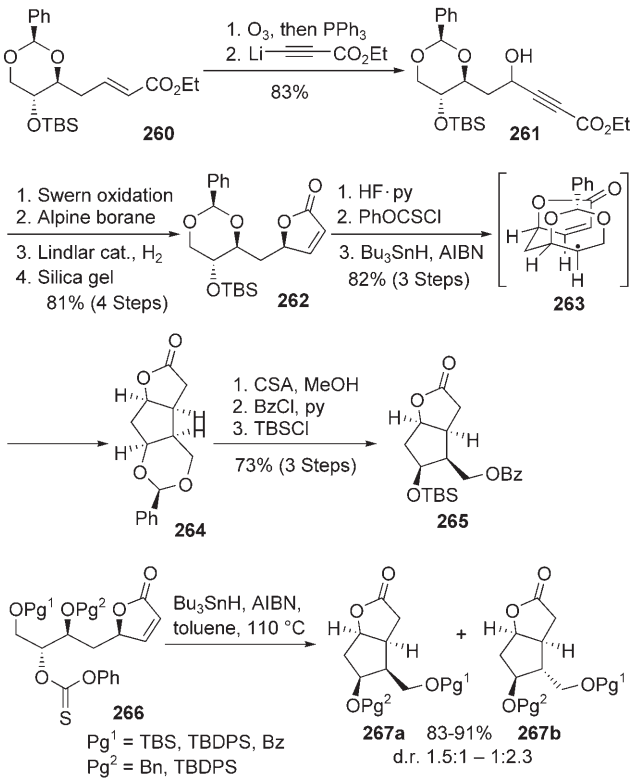
Reduction of the cyclopentanecarboxylate **248** by $LiAlH_4$ gave the alcohol, from which the corresponding primary triflate **250** was subsequently generated by a one-pot O triflation/O′-TES protection sequence. The introduction of the C1–C6 chain to the C_{20} skeleton **251** was achieved in good yield by an alkylation of the lithium acetylide of the TES ether of 5-hexynol. It was important that the hydroxy function at the 9-position was protected in this step. At this point, the TMP protecting group was oxidatively removed by *m*CPBA to give the enone **252**. The synthesis of 15-F_{2t}-IsoP can be accomplished from this compound in a few steps.

Other research groups developed radical cyclization strategies for the synthesis of cyclic IsoP precursors. In 1992, Tolstikov et al. reported the synthesis of useful PG and IsoP intermediates, particularly that of bicyclic *syn-anti-syn* derivative **259** (Scheme 70).[247] The synthesis began with levoglucosane (**253**), which was first dibenzylated. The dibenzyl ether **254** was then deoxygenated by a Barton–McCombie reaction. A transacetalization and acetylation of **255** led to **256**, which was converted into α,β-unsaturated ester **257** by a Wittig reaction. Conversion of the secondary alcohol in **257** into bromide **258** was achieved in 23% yield by using a two-step Finkelstein protocol. Its radical cyclization in the presence of Bu_3SnH led to the desired cyclopentane ring. Cleavage of the benzyl and acetate groups followed by lactonization under acidic conditions afforded **259** in 36% yield.

Mulzer et al. reported a formal synthesis of enantiomerically pure *ent*-15-F_{2c}-IsoP (*ent*-15-**2b**) through 5-*exo* radical cyclizations to butenolides (Scheme 71).[248] Starting from α,β-unsaturated ester **260**, the side chain was lengthened by ozonolysis and nucleophilic addition of the lithium acetylide of ethyl propiolate to give 4-hydroxyynoate **261** as a diastereomeric mixture.[248a] The configuration of the alcohol was fixed by a sequence of Swern oxidation and reduction with Alpine borane. The butenolide unit in **262** was readily synthesized by a *Z*-selective semihydrogenation with Lindlar's catalyst and a subsequent lactonization in the presence of silica gel. Cleavage of the TBS group in **262** and acylation of the secondary alcohol with phenyl chlorothionoformate set the stage for the cyclization. The crucial 5-*exo* radical cyclization in the presence of tributyltin hydride/AIBN led to the single tricyclic diastereomer **264** in high yield with the required all-*cis*-configuration at the central cyclopentane ring. The stereoselectivity of the cyclization can be explained



Scheme 70. Approach to F_{2t}-IsoP precursor **259** according to Tolstikov et al.[247]



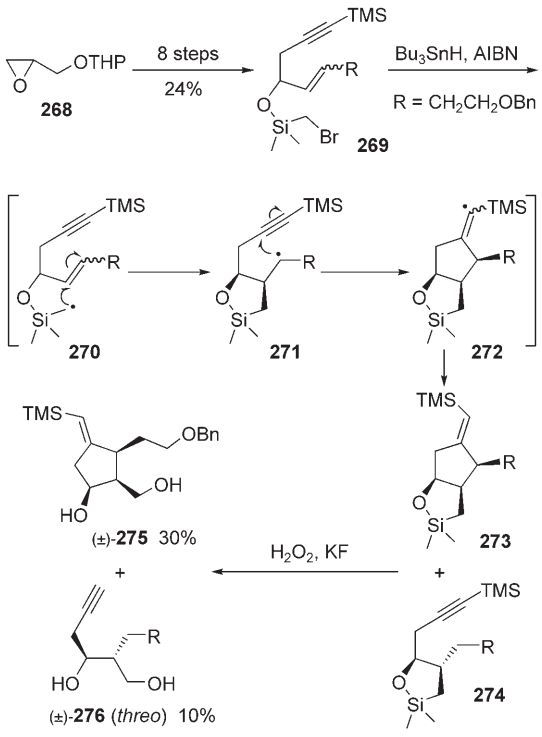
Scheme 71. Synthesis of 15-F_{2c}-IsoP intermediates **265**–**267** according to Mulzer et al.[248]

through a *cis*-decaline-type transition state **263**.[249] The protecting group pattern was exchanged in three steps to give **265**.

The use of a range of differently protected monocyclic butenolides **266** under similar radical cyclizations gave the

bicyclic lactones **267a** and **267b** in high yields, although the diastereoselectivity was only moderate.[248b] The introduction of the ω chain was accomplished by deprotection of the benzoate group in **265**, Swern oxidation, and a HWE reaction under application of the mild Masamune–Roush modification, without detectable epimerization (see Scheme 64).

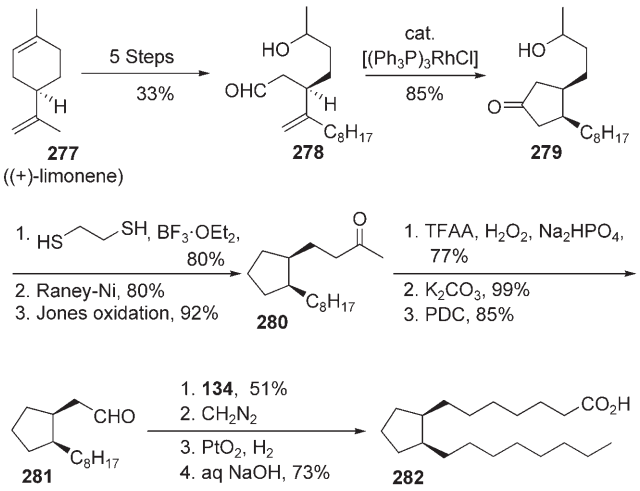
In 1999, Lucas and co-workers reported a tandem radical cyclization of α-functionalized allyl (bromomethyl)dimethylsilyl ether **269** that led to all-*cis*-substituted isoprostanoid precursor **275** together with the desilylated acyclic diol **276** (Scheme 72).[250] The key intermediate, homopropargylic silyl ether derivative **269**, was prepared from tetrahydropyranylglycidol **268** in eight steps in 24% yield. This ether was subjected to a tandem 5-*exo*-*trig*/5-*exo*-*dig* radical cyclization via radicals **270**–**272** to give a mixture of the expected oxasilabicyclo[3.3.0]octane **273** and the monocyclic oxasilacyclopentane **274**. A Tamao–Fleming oxidation of this mixture gave cyclopentane **275** and the acyclic diol **276** in yields of 30 and 10%, respectively. The cyclopentanediol **275** may serve as a precursor to IsoPs; however, their synthesis still remains to be completed.



Scheme 72. Synthesis of IsoP building block **275** according to Lucas and co-workers.[250]

7.1.4.2. *Transition-Metal-Catalyzed Cyclization Reactions for the Synthesis of Isoprostanes*

The monoterpene limonene **277** served as a starting material from the chiral pool for the synthesis of 8-isoprostanoic acid (**282**) by using a rhodium-catalyzed hydroacylation as the key step (Scheme 73).[251] Thus, hydroxy aldehyde **278** was prepared in five steps from **277**. An intramolecular

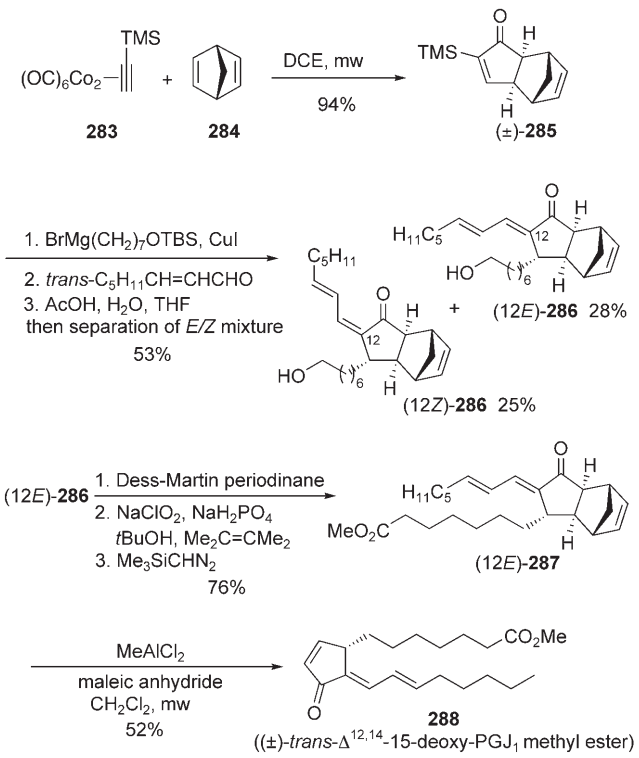


Scheme 73. Synthesis of isoprostanoic acid (**282**) according to Sakai and co-workers.[251]

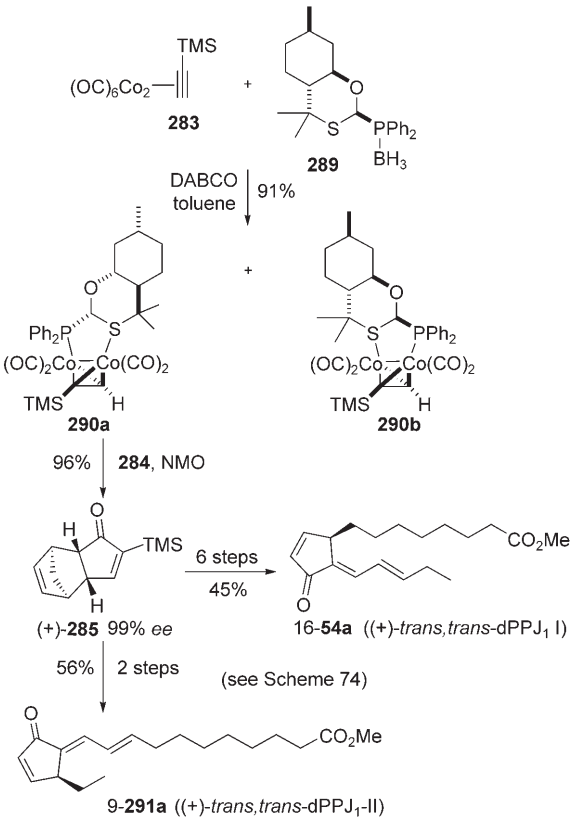
hydroacylation of **278** catalyzed by the Wilkinson complex afforded *cis*-3,4-dialkylcyclopentanone **279** as a single diastereomer in high yield. The removal of the keto function was accomplished by dithioacetalization and reductive desulfuration. A subsequent Jones oxidation gave the acyclic ketone **280**. The synthetic sequence continued with a Baeyer–Villiger oxidation mediated by trifluoroperacetic acid, cleavage of the resulting acetate, and PDC oxidation of the alcohol to the aldehyde **281**. The α chain was introduced by a Wittig reaction with **134**, and the synthesis of **282** was completed by esterification, platinum-catalyzed hydrogenation, and saponification of the methyl ester.

Evans and co-workers reported an interesting Pauson–Khand strategy for the synthesis of J-type IsoP or PhytoP isomers (Scheme 74).[252] The reaction between $\{Co_2(CO)_6\}$-complexed trimethylsilylacetylene (**283**) and norbornadiene (**284**) led to the racemic key intermediate **285**. The yield of this cyclization step was optimized by using microwave (mw) heating. The following key step for the construction of the cross-conjugated dienone unit **286** represents an adaption of the well-known three-component coupling reaction for the synthesis of prostaglandins. The side chains were introduced sequentially by a copper(I)-catalyzed conjugate addition of 7-silyloxyheptylmagnesium bromide to **285** followed by an aldol addition of *trans*-2-octenal. A subsequent Peterson olefination under acidic conditions gave an initial 12*E*/12*Z*-diene mixture (1:3).

After removal of the silyl protecting group and separation, the individual diene isomers (12*Z*)- and (12*E*)-**286** were isolated in yields of 25 and 28%, respectively. The authors named this sequence a "conjugate addition-Peterson olefination reaction". Conversion of the primary alcohol into the methyl ester **287** was achieved by using a three-step protocol. Alcohol (12*E*)-**286** was oxidized with Dess–Martin periodinane and then subsequently with sodium chlorite to give the corresponding carboxylic acid, which was esterified with trimethylsilyldiazomethane in good yield. The total synthesis was completed by a retro-Diels–Alder reaction of the cyclopentadienyl-protected methyl ester **287** with $MeAlCl_2$ and



Scheme 74. Total synthesis of (±)-*trans*-$\Delta^{12,14}$-15-deoxy-PGJ$_1$ methyl ester **288** according to Evans and co-workers.[252]



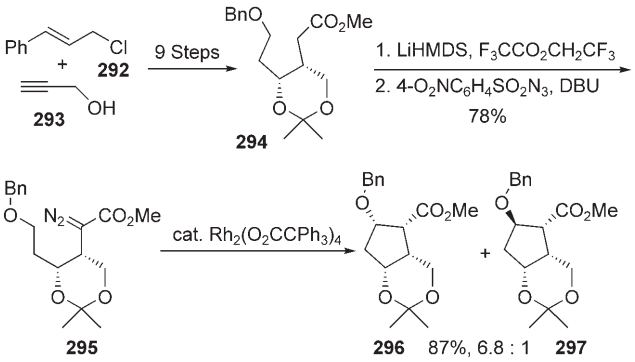
Scheme 75. Total synthesis of (+)-*trans,trans*-dPPJ$_1$ **54a** and **291a** according to Evans, Riera, and co-workers.[208b]

maleic anhydride under microwave conditions to give a 52% yield of (±)-*trans*-$\Delta^{12,14}$-15-deoxy-PGJ$_1$ methyl ester (**288**) and 14% of its *cis* isomer.

In 2005, the Evans and Riera research groups together published the synthesis of optically pure 13,14-dehydro-12-oxophytodienoic acids (deoxy-J$_1$-phytoprostanes) 16-**54a** and 9-**291a** by using an asymmetric version of their earlier approach (Scheme 75).[208b] This synthesis was achieved by ligand exchange of **283** with bidentate chiral ligand **289** in the presence of DABCO in toluene to give the two diastereomeric cobalt complexes **290a** and **290b** in good yield, which were separated by crystallization and/or chromatography. The Pauson–Khand reaction of **290a** with norbornadiene (**284**) in the presence of NMO in dichloromethane furnished (+)-**285** in excellent yield and enantiomeric excess.

Under similar conditions as used for the synthesis of racemic compound **288** (see Scheme 74), the synthesis of (+)-*trans,trans*-dPPJ$_1$-I (16-**54a**) as a single isomer was accomplished from (+)-**285** in a six-step sequence, which included a 1,4-conjugate addition with 7-(silyloxy)heptylmagnesium bromide, a Peterson olefination with *trans*-pent-2-enal, and removal of the cyclopentadienyl protecting group with $MeAlCl_2$ and maleic anhydride under microwave irradiation. A similar sequence with lithium diethylcuprate and the appropriately functionalized α,β-unsaturated aldehyde provided (+)-*trans,trans*-dPPJ$_1$-II (9-**291a**) in good yield.

Finally, Taber et al. devised an approach to a central F$_{2c}$-IsoP precursor **296** that was based on a C–H insertion (Scheme 76).[253] The protected ester **294**, which is accessible in nine steps from cinnamyl chloride (**292**) and propargyl



Scheme 76. Synthesis of the F$_{2c}$-IsoP intermediate **296** by an intramolecular C–H insertion according to Taber et al.[253]

alcohol (**293**) was subjected to a diazo transfer reaction to give diazoester **295**. This material underwent an intramolecular C–H insertion catalyzed by the sterically demanding dirhodium tetrakis(triphenylacetate) to give the all-*cis*-cyclopentanecarboxylate **296** in good yield and reasonable diastereoselectivity.

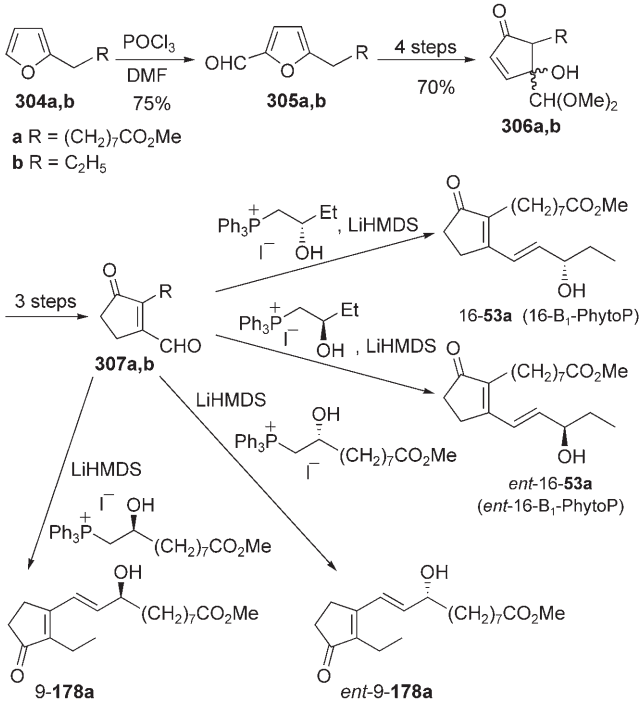
7.1.4.3. IsoP Syntheses Based on Furan Ring Transformations

Rodríguez and Spur developed very efficient syntheses, particularly of the E-IsoP and -PhytoP series, by a two-component coupling process typically used for the synthesis

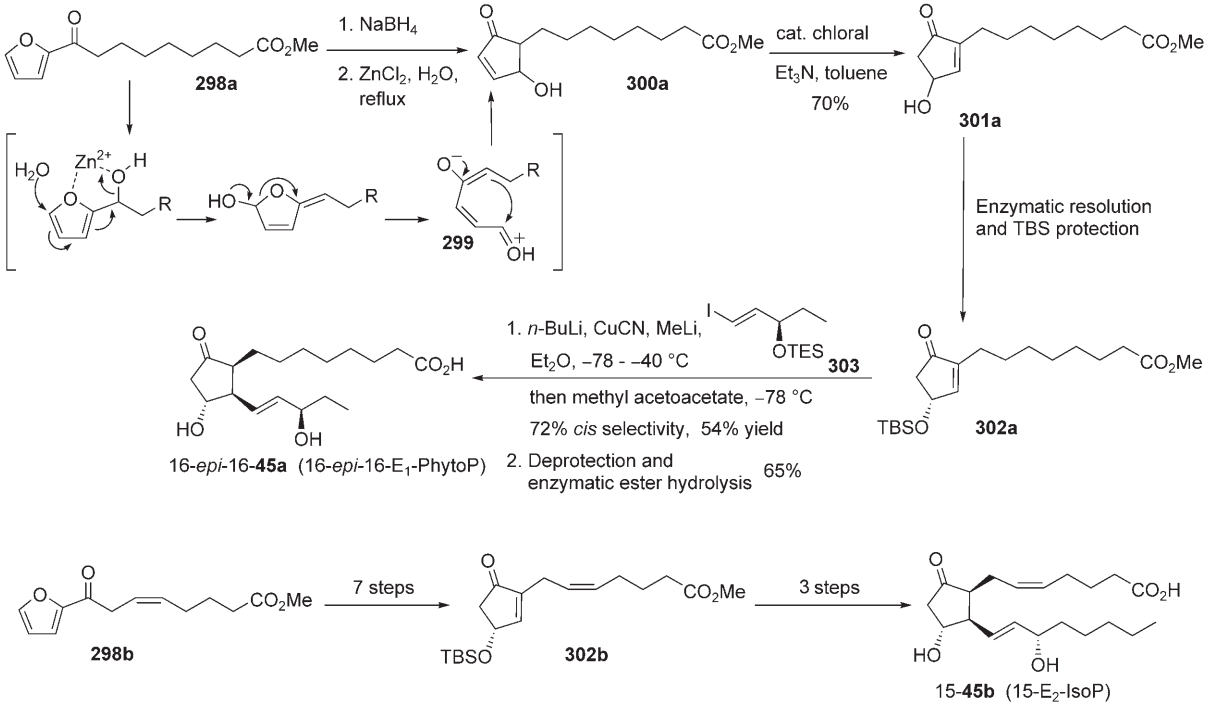
of PGs and analogues.[254] By simply modifying the work-up conditions, by using chelating proton sources, a switch from a *trans*-dialkyl PG configuration to a *cis*-dialkyl IsoP configuration was accomplished.[255] The synthesis of chiral cyclopentenone component **302a** started with a Friedel–Crafts acylation of furan and the mixed anhydride of azelaic acid monoester to give furoyl ester **298a** (Scheme 77).[256a] $NaBH_4$ reduction of the ketone followed by a rearrangement induced by $ZnCl_2$ in dioxane/water at reflux afforded 4-hydroxycyclopentenone **300a**. The outcome of the reaction can be explained by a ring opening to intermediate **299** and a subsequent intramolecular aldol addition. Treatment of **300a** with chloral yielded the more stable 4-hydroxycyclopentenone **301a** in 68% overall yield.

Enzymatic resolution and protection of the alcohol as a TBS ether gave **302a**, which was subjected to conjugate addition with a chiral component, obtained by lithium–iodine exchange of the corresponding vinyl iodide **303**. The resulting 2,3-dialkylcyclopentanone enolate intermediate was then added to a cold solution of methyl acetoacetate to provide a *cis*-2,3-dialkylcyclopentanone with 72% selectivity. Deprotection and ester hydrolysis gave 16-*epi*-16-E_1-PhytoP (16-*epi*-**45a**); *ent*-16-E_1-PhytoP was also synthesized, but starting from the enantiomer of **302a**. Following the same strategy, but applying **298b** as the starting material and different coupling partners, other isoprostanes, such as 15-E_2-IsoP (15-**45b**), were obtained.[256b,c]

In 2004, the Durand research group became interested in developing new and flexible routes to B-, D-, and E-IsoPs as well as B-, D-, and E-PhytoP isomers starting from two common intermediates, namely the 4-hydroxy-2-cyclopentenone precursors **306a**,**b**, reported by Freimanis and coworkers (Scheme 78).[257] The synthesis of **306a**,**b** started with a Vilsmeier formylation reaction at the 5-position of the furans **304a**,**b**. A selective rearrangement of **305a**,**b** yielded the 4-hydroxy-2-cyclopentenones **306a**,**b** after four steps (see Scheme 77). These two compounds were transformed to 3-oxocyclopentenecarbaldehydes **307a**,**b** in three steps and served as precursors for the synthesis of both enantiomers of



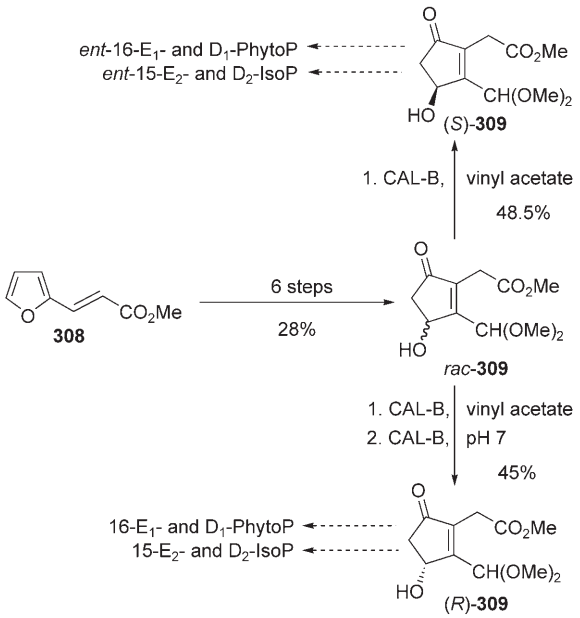
Scheme 78. Total synthesis of B-PhytoP isomers by a furan-based approach according to Durand and co-workers.[258]



Scheme 77. Total synthesis of E-IsoPs and E-PhytoPs by conjugate addition and *cis*-selective protonation.

16-B_1-PhytoP (16-**53a**) as well as of 9-**178a** by Wittig reactions with chiral β-hydroxyphosphonium salts.[258]

In another synthetic venture, methyl *trans*-3-(2-furyl)-acrylate (**308**) was transformed in six steps (as shown in Scheme 78) to racemic 4-hydroxycyclopentenone **309**.[259] An efficient enzymatic resolution of **309** into the two enantiomerically pure hydroxycyclopentenones (*R*)-**309** and (*S*)-**309** was developed by using CAL-B (Scheme 79). The application of this methodology towards the synthesis of E- and D-IsoPs as well as of D- and E-PhytoPs is in progress.

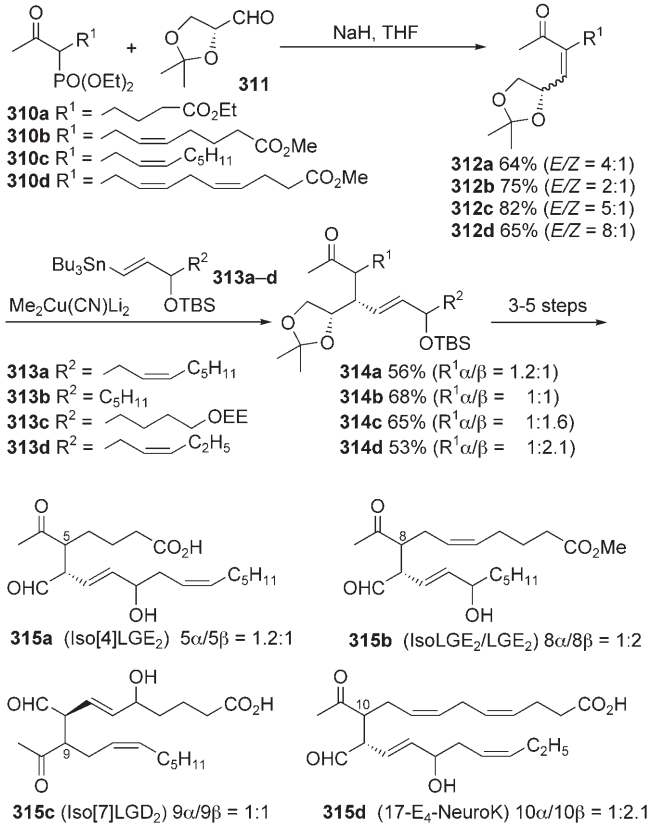


Scheme 79. Enzymatic resolution of 4-hydroxycyclopentenone **309** according to Durand and co-workers.[259]

7.2. Total Syntheses of Isolevuglandins

Much less effort has been devoted to the synthesis of IsoLGs. The only general asymmetric approach to several IsoLG classes was developed by Salomon and co-workers, and was based on an early synthesis of levuglandins (Scheme 80).[260a] The synthesis began with a HWE reaction of β-ketophosphonates **310a–d** with glyceraldehyde acetonide (**311**) as the source of chirality. The 4,5-(isopropylidenedioxy)enones **312a–d** were isolated in good yield with moderate to good *E*/*Z* selectivity. The full C_{20} or C_{22} chain was subsequently synthesized by a conjugate addition of vinylcuprates to **312**. The organocuprate was generated in situ by transmetalation of the corresponding (*E*)-vinylstannane **313a–d** with the higher order cuprate $Me_2Cu(CN)Li_2$, followed by addition of **312a–d**. The IsoLG intermediates **314a–d** were formed in good yield and with excellent diastereoselectivity in the conjugate addition step. The following protonation proceeded, however, with no or moderate diastereoselectivity. The *syn* and *anti* diastereomers were separable in most cases.

A few straightforward adjustments of the functional groups by saponification of the C1 ester function, cleavage

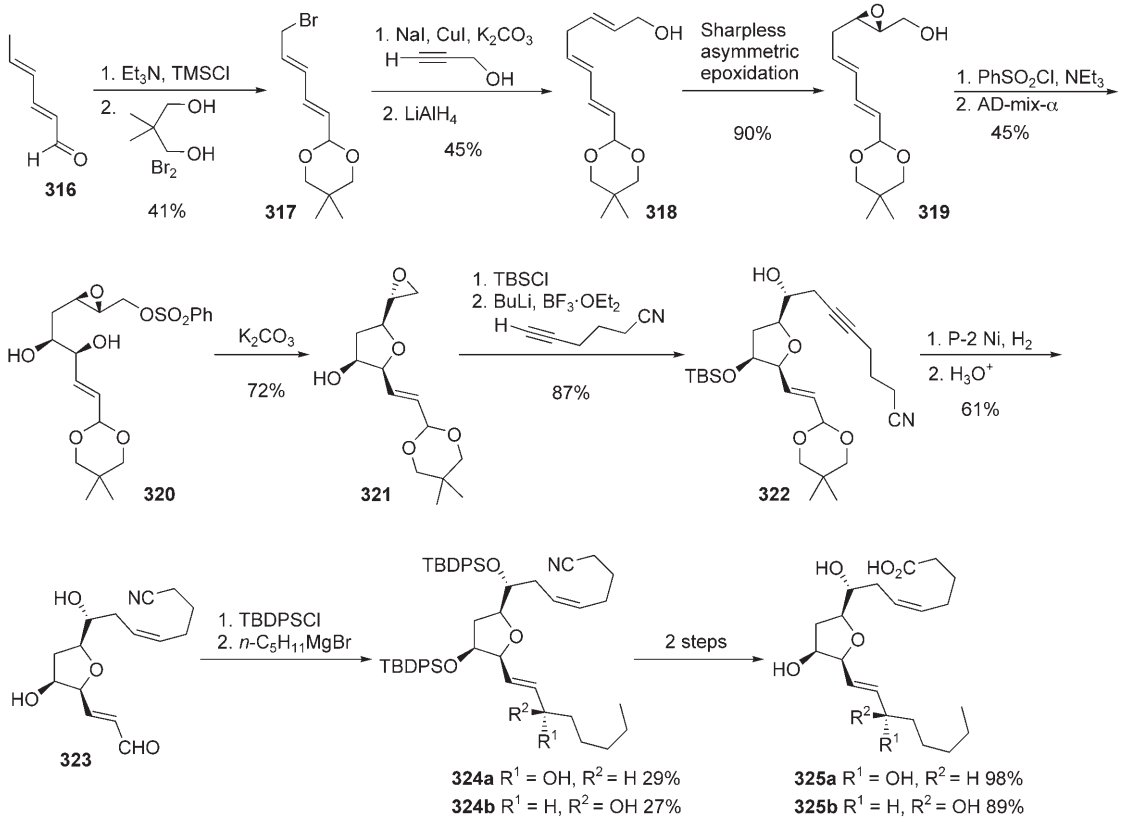


Scheme 80. Total synthesis of IsoLGs according to Salomon and co-workers.[260]

of the TBS protecting group, and unmasking of the aldehyde group by cleavage of the glycol allowed the synthesis of Iso[4]LGE_2 (**315a**),[260a] IsoLGE_2 (**315b**),[260b] Iso[7]LGD_2 (**315c**),[260c] and 17-E_4-NeuroK (**315d**) as *anti*/*syn* mixtures.[260d] Epimerization takes place during the final steps, and the isolated *syn*/*anti* mixtures of the IsoLGs **315a–d** probably represent the thermodynamic equilibrium since both individual diastereomers of the conjugate addition product **314** afforded the same diastereomeric mixtures of the corresponding IsoLG. Recently, Roberts and co-workers reported a very similar synthesis of racemic IsoLGs by using glyoxal dimethyl acetal instead of the glyceraldehyde derivative.[261]

7.3. Total Syntheses of Isofurans

In 2004, Taber et al. described a flexible approach to the total synthesis of 8-*epi*-SC-Δ^{13}-9-IsoF (**325a**) and its 15 epimer **325b** via a versatile epoxide intermediate **320** (Scheme 81).[262] The synthesis started with conversion of (*E*,*E*)-sorbic aldehyde (**316**) into its silyl enol ether followed by bromination and acetalization with 2,2-dimethylpropane-1,3-diol to give ω-bromoacetal **317**. Chain extension by alkylation with propargylic alcohol, reduction to **318**, and Sharpless asymmetric epoxidation gave the epoxide **319**. The derived benzenesulfonate was then subjected to Sharpless asymmetric dihydroxylation using AD-mix-α to provide a



Scheme 81. Total synthesis of 8-*epi*-SC-Δ^{13}-9-IsoF (**325**) according to Taber et al.[262]

1.3:1 mixture of regioisomeric diols (only the desired **320** is shown). A clean sequence of 5-*exo-tet* cyclization induced by potassium carbonate in methanol followed by epoxide formation delivered epoxide intermediate **321**. At this point the configuration of the ring hydroxy group may be changed by a Mitsunobu inversion to provide access to the ST series (not shown).

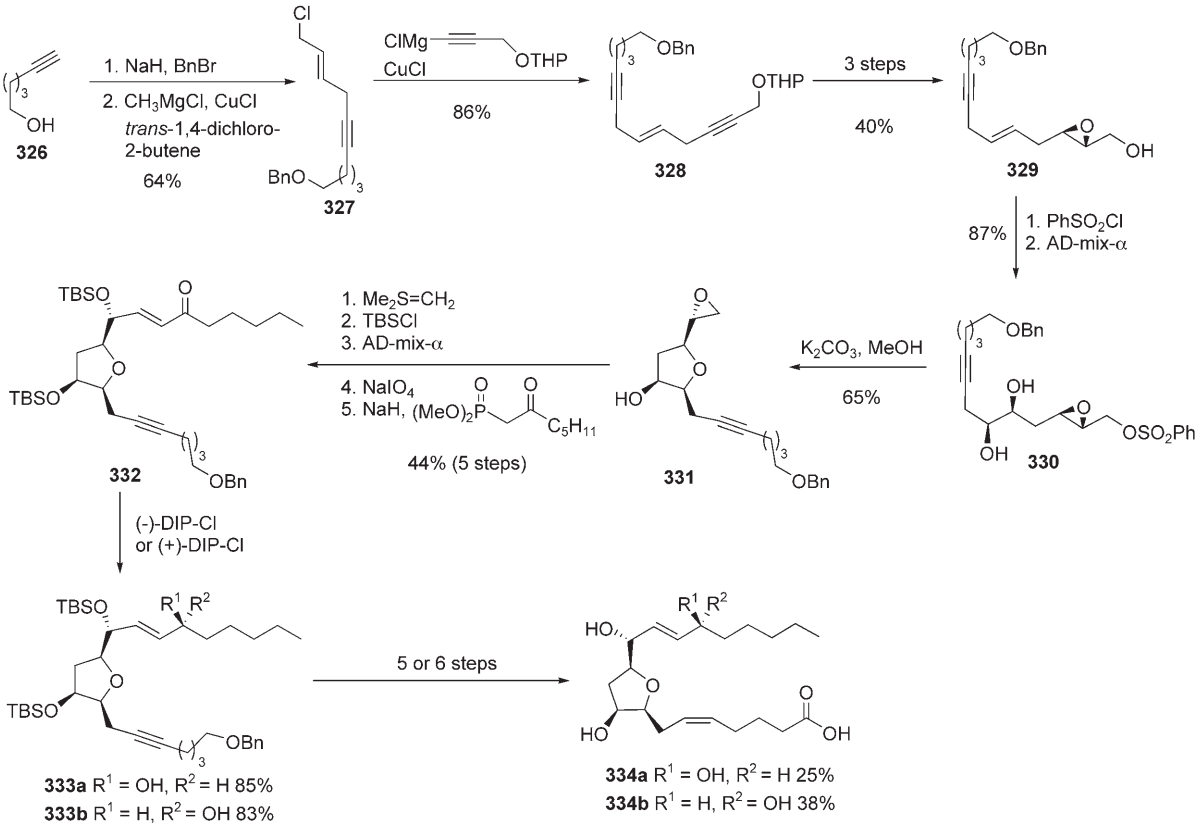
Assembly of the C1–C15 skeleton was accomplished from **321** by protection of the secondary alcohol as a TBS ether and subsequent Lewis acid assisted opening of the epoxide with the lithium anion of 5-hexynenitrile. The resulting alkyne **322** was semihydrogenated with P-2 Ni/H_2 to give a *Z* alkene. Global deprotection afforded dihydroxy aldehyde **323**. The C16–C20 side chain was introduced by addition of pentylmagnesium bromide after protection of the two alcohol functions with TBDPSCl to give a separable mixture of alcohol diastereoisomers **324a** and **324b**. The synthesis of the IsoF isomers **325a** and **325b** was completed by desilylation and nitrile hydrolysis.

In 2006, the same research group reported a general route to the other major class of IsoFs, the enediol-IsoFs represented by 12-*epi*-SC-Δ^{13}-8-IsoF (**334a**) and 12,15-*diepi*-SC-Δ^{13}-8-IsoF (**334b**, Scheme 82).[263] This approach is based on a similar epoxide cyclization cascade. The key intermediate, diol epoxide **330**, was efficiently obtained from 5-hexyn-1-ol (**326**). After protection as a benzyl ether and transformation into a magnesium acetylide, a coupling reaction with *trans*-1,4-dichloro-2-butene gave monoalkylation product **327**. A second copper(I)-mediated chain extension with a Grignard reagent derived from propargylic alcohol yielded 2,5,8-enediyne **328**. Cleavage of the THP group, partial reduction with $LiAlH_4$, and Sharpless epoxidation furnished epoxy alcohol **329**, which was transformed to epoxy sulfonate **330** by sulfonylation and Sharpless dihydroxylation.

The key cyclization was again carried out with potassium carbonate in methanol to provide the desired epoxytetrahydrofuran **331**. Construction of the ω side chain was secured by a C_1 homologation of the epoxide with dimethylsulfonium ylide. Protection of the allylic alcohol with TBSCl, Sharpless dihydroxylation, gentle oxidative cleavage of the glycol with sodium periodate, and a HWE reaction gave enone **332**. Reduction to the C15-allylic alcohol functionality **333a** or **333b** was accomplished either by using the Luche protocol to give a separable diastereomeric mixture of allylic alcohols or by asymmetric reduction using the appropriate enantiomer of chlorodiisopinocampheylborane (DIP-Cl). The functional group pattern of the α side chain was readily adjusted by partial reduction of the alkyne with P-2 Ni, reductive cleavage of the benzyl ether with Li/naphthalene, oxidation of the resulting alcohol to the acid, and global deprotection to accomplish the first synthesis of 12-*epi*-SC-Δ^{13}-8-IsoF (**334a**) and 12,15-*diepi*-SC-Δ^{13}-8-IsoF (**334b**).

8. Conclusions and Outlook

Polyunsaturated fatty acids are extremely important compounds in all organisms. Since the first hints by Nugteren



Scheme 82. Approach to enediol isofurans **334** according to Taber and Zhang.[263]

et al. in the 1960s on the nonenzymatic formation of PG derivatives, and the discovery by Morrow, Roberts et al. in the 1990s that IsoPs were produced in extraordinary amounts in vivo, it has become evident that cyclic PUFA metabolites are much more important than initially imagined. During the last 15 years, the chemistry, analysis, biology, and application of IsoPs has experienced significant developments. It was convincingly demonstrated that probably all living organisms use autoxidation of all at least triply unsaturated fatty acids to produce biologically active cyclic metabolites.

The explosive growth in the numbers of different cyclic metabolites necessitated the establishment of general nomenclature systems. Two nomenclatures were proposed in 1997, which allow an easy differentiation of the IsoP isomers. Future efforts should be directed towards a unique nomenclature that incorporates the advantages of their predecessors.

A large number of studies have been directed at the elucidation of pathways for the formation of such PUFA derivatives. A unified mechanism for the autoxidation of all PUFA compounds in vitro and in vivo evolved from these investigations, and it was shown that IsoPs are major products of this pathway. Racemic prostaglandins are also formed to a minor extent, and it may be concluded that nature selected them to be biosynthesized enzymatically because of their favorable bioactivity profile. On the basis of the present mechanistic framework, further interesting cyclic metabolites are expected to be isolated over the next few years.

Immunoassays and mass spectrometry in combination with gas chromatography (GC-MS, GC-tandem MS) and liquid chromatography (LC-MS, LC-tandem MS) have been developed for the exact analytical determination of such cyclic PUFA derivatives. As a result of their reliability, the quantification of F_2-IsoPs has to date become the "gold standard" for the assessment of the oxidative stress status and of oxidative damage in vivo. This finding confirms the usefulness of these molecules as biomarkers. The amount of data must be, however, considerably increased, and thus a large amount of research is necessary to explore the relation between oxidative stress, the formation of IsoPs and other bioactive PUFA metabolites, and the pathogenesis of human disease in more detail.

Pure synthetic material is necessary both for use as an analytical standard for unequivocal structure determination as well as for the quantification and the exact elucidation of the potential biological functions of the individual metabolites. Without the synthetic strategies developed so far, the field of cyclic lipids, and particularly the field of IsoPs, would not have advanced to its current status. Despite the development of diverse strategies for the specific synthesis of cyclic PUFA metabolites, only a few selected members of some cyclic PUFA families and metabolites have so far been synthesized. Thus, one of the greatest needs is the development of synthetic approaches that are more efficient with respect to the number of steps and the time needed to execute them.

Important progress has also been made in biology: The amount of clinical research on cyclic PUFA metabolites showed an explosive growth from a mere 3 publications in

1992 to more than 200 in 2007. Also here, the current knowledge of cyclic PUFA metabolites would not be nearly that detailed if pure synthetic material and analytical standards were not available. A large body of clinical and experimental evidence supports the hypothesis that lipid peroxidation products, including F_2-IsoP isomers, are important transducers of the effects of metabolic and hemodynamic abnormalities in patients with increased cardiovascular risk and diabetes. Recently, there has been growing interest in studying the role of IsoPs as pathologically relevant mediators, which should even be considered as a novel class of inflammatory mediators. It is clear, that there is much to learn about this in the next few years. 15-F_2-IsoP and some 15-E_2-IsoP isomers mediate vasoconstriction in different vascular beds and species, at least in part through interaction with the TXA_2 receptor. Whether distinct IsoP receptors exist remains a matter of debate.

The recent total synthesis of cyclopentenone-IsoPs such as 15-A_2-IsoP enabled a number of studies aimed at exploring the biological activity of these highly reactive electrophilic metabolites. They inhibit nuclear factors (NF-κB and PPARγ) and enzymes (COX-2 and iNOS), which play important roles in the pathogenesis of many diseases. In the coming years, the biology of NeuroPs and IsoFs need to be comprehensively investigated.

Available data indicate that PhytoPs display similar biological activities as OPDA and JA with respect to phytoalexin biosynthesis in plants. Cyclopentenone-PhytoP derivatives rapidly activate mitogen-activated protein kinase (MAPK) and triggers the activation of genes involved in primary and secondary metabolism. The B_1-PhytoP series triggers plant defense and detoxification responses. Work is in progress to elucidate the biological activities of PhytoPs in humans and animals in more detail.

It is certainly predicted, that ongoing and future studies will generate important knowledge on the formation, metabolism, and biological activities of cyclic PUFA metabolites through the availability of synthetically pure materials. Taking into account the current role of IsoPs in human biology, it is clearly expected that the importance of cyclic PUFA metabolites in chemistry and biology will continue to grow strongly in the future.

Addendum

Four important total syntheses of IsoPs were published during the production of the Review. Pandya and Snapper reported a total synthesis of all 5-F_2-IsoP stereoisomers (5-**2b**) by adapting their metathesis approach (see Schemes 52 and 53).[264a] Taber et al. synthesized *ent*-13-*epi*-13-F_{4t}-NeuroP (13-**2d**) starting from a 2-cyclopentene-1,4-diol derivative (see Section 7.1.3.1) by employing a thermal ene-cyclization as the key step to assemble the cyclopentane core.[264b] Durand and co-workers accomplished the total synthesis of E_1-PhytoP and 15-E_2-IsoP stereoisomers (**45a** and 15-**45b**) based on furan derivative **309** (see Scheme 79) by using Wittig and HWE reactions to introduce the side chains.[264c] Finally, Helmchen and co-workers. published a synthesis of *ent*-5-F_{2c}-IsoP starting from nortricyclanone, which was elaborated to an all-*cis*-diastereomer of the Corey lactone (similar to **227** in Scheme 64), which was transformed to the IsoP by HWE and Wittig reactions.[264d]

Abbreviations

AA	Arachidonic acid
ABC	ATP-binding cassette
Ac	Acetyl
AD	Alzheimer's disease
AD-mix	Asymmetric dihydroxylation mix
AE	Asymmetric epoxidation
AIBN	Azobis(isobutyronitrile)
apoB	Apolipoprotein B
9-BBN	9-Borabicyclo[3.3.1]nonane
BHT	2,6-Di-*tert*-butyl-4-methylphenol
BINAL-H	Lithium (1,1′-binaphthyl-2,2′-dioxy)aluminum dihydride
Bn	Benzyl
Bz	Benzoyl
CAL-B	*Candida antarctica* lipase B
CBS	Corey–Bakshi–Shibata catalyst
Ch	Cholesteryl
CNS	Central nervous system
COX	Cyclooxygenase
CSA	Camphorsulfonic acid
CSF	Cerebrospinal fluid
DABCO	Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-Dichloroethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DHA	4,7,10,13,16,19-Docosahexaenoic acid
DHP	Dihydropyran
DIBAL-H	Diisobutylaluminum hydride
DMAP	4-(Dimethylamino)pyridine
DMF	Dimethylformamide
DMI	*N*,*N*′-Dimethylimidazolin-2-one
DMSO	Dimethylsulfoxide
dppp	1,3-Bis(diphenylphosphanyl)propane
ECE	Endothelin conversion enzyme
EI	Electron ionization
EIA	Enzyme immunosorbent assay
ELISA	Enzyme-linked immunosorbent assay
EP receptor	Prostaglandin E receptor
EPA	5,8,11,14,17-Eicosapentaenoic acid
ET-1	Endothelin-1
GC	Gas chromatography
Glc	Glucose
GSH	Glutathione
HEK cell	Human embryonic kidney cell
HNE	4-Hydroxynonenal
HPETE	Hydroperoxyeicosatetraenoic acid(s)
HWE	Horner–Wadsworth–Emmons reaction
ICAM-1	Intercellular adhesion molecule 1
IL	Interleukin
iNOS	Inducible nitric oxide synthase

IsoF	Isofuran
IsoK	Isoketal
IsoLG	Isolevuglandin
IsoP	Isoprostane
IsoTx	Isothromboxane
JA	Jasmonic acid
KHMDS	Potassium hexamethyldisilazide
LA	α-Linolenic acid
LC	Liquid chromatography
LDA	Lithium diisopropylamide
LDL	Low-density lipoprotein
LDLR	Low-density lipoprotein receptor
LiHMDS	Lithium hexamethyldisilazide
LLE	Liquid–liquid extraction
12-LOX	12-Lipoxygenase
LPS	Lipopolysaccharide
L-Selectride	Lithium tri-*sec*-butylborohydride
lyso-PC	lyso-Phosphatidylcholine
MCP	Monocyte chemotactic protein
*m*CPBA	*m*-Chloroperbenzoic acid
MDA	Malondialdehyde
Mes	Mesityl
MIP	2-Methoxyprop-2-yl (2-Methoxyisopropyl)
MPO	Myeloperoxidase
MS	Mass spectrometry
Ms	Mesyl
NaHMDS	Sodium hexamethyldisilazide
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
NeuroK	Neuroketal
NeuroP	Neuroprostane
NF-κB	Nuclear factor-kappa B
NICI	Negative ion capture chemical ionization
NMO	*N*-Methylmorpholine *N*-oxide
NOS	Nitrous oxide system
OA	Ovalbumin
OAc	Acetate
OPDA	12-Oxophytodienoic acid
PC	Phosphatidylcholine
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
PECPC	Epoxycyclopentenone isoprostane phospholipid
PEIPC	Epoxyisoprostane phospholipid
PFB	Pentafluorobenzyl
PG	Prostaglandin
Pg	Protecting group (not specified)
15-PGDH	15-Prostaglandin dehydrogenase
PhytoP	Phytoprostane
Piv	Pivaloyl
PLA_2	Phospholipase A_2
PLC	Phospholipase C
PMB	*p*-Methoxybenzyl
PPAR	Peroxisome-proliferator-activated receptor
PPL	Porcine pancreatic lipase
PPTS	Pyridinium *p*-toluenesulfonate
PUFA	Polyunsaturated fatty acid
py	Pyridine
RIA	Radioimmunoassay
ROS	Reactive oxygen species
SET	Single-electron transfer
SIM	Selected ion monitoring
SPE	Solid-phase extraction
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBDPS	*tert*-Butyldiphenylsilyl
TBS	*tert*-Butyldimethylsilyl
TEMPO	2,2,6,6-Tetramethylpiperidine *N*-oxyl
TES	Triethylsilyl
Tf	Triflyl, Triflic
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THP	Tetrahydropyran-2-yl
TIPS	Triisopropylsilyl
TMS	Trimethylsilyl
TP receptor	Thromboxane A2 receptor
Ts	Tosyl
p-TsOH	*p*-Toluenesulfonic acid
Tx	Thromboxane

We thank our co-workers, who are cited in the references, for their dedicated work in the field of cyclic lipids. We are also grateful for their comments during the preparation of this Review. U.J. thanks the Region Languedoc-Roussillon, Université Montpellier 1, and Professor Jean-Claude Rossi for a fellowship as a Visiting Professor at the Faculté de Pharmacie of Université Montpellier 1, as well as Thierry Durand and his group for the stimulating atmosphere, their support, and hospitality during this stay. We thank the CNRS, the French Ministery of Education and Research, Deutsche Forschungsgemeinschaft, and Fonds der Chemischen Industrie for their continuous support of our research in this field. J.-M.G. and T.D. are deeply grateful to Professor Jean-Yves Lallemand and the ICSN for their generous financial support.

Received: November 6, 2007

[1] a) U. N. Das, *Biotechnol. J.* **2006**, *1*, 420–439; b) for a review on the chemistry and biology of lipids at the time of the discovery of non-enzymatic formation of cyclic PUFA metabolites, see: K. C. Nicolaou, J. Y. Ramphal, N. A. Petasis, C. N. Serhan, *Angew. Chem.* **1991**, *103*, 1119–1136; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1100–1116.

[2] a) *Prostaglandins, Leukotrienes and Essential Fatty Acids* (Eds.: D. F. Horrobin, M. S. Manku, P. Sirois, P. Borgeat), Churchill Livingstone, Edinburgh, **2002**; b) F. Marks, G. Fürstenberger, *Prostaglandins, Leukotrienes, and Other Eicosanoids*, Wiley-VCH, Weinheim, **1999**; c) *CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids*, *Vol. 1+2* (Ed.: A. L. Willis), CRC, Boca Raton, FL, **1987**.

[3] a) R. Kurzrock, C. C. Lieb, *Proc. Soc. Exp. Biol. Med.* **1930**, *28*, 268–272; b) U. S. von Euler, *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **1934**, *175*, 78–84.

[4] a) S. Bergström, J. Sjövall, *Acta Chem. Scand.* **1957**, *11*, 1086; b) S. Bergström, J. Sjövall, *Acta Chem. Scand.* **1960**, *14*, 1693–1700; c) S. Bergström, J. Sjövall, *Acta Chem. Scand.* **1960**, *14*, 1701–1705; d) S. Bergström, R. Ryhage, B. Samuelsson, J. Sjövall, *Acta Chem. Scand.* **1962**, *16*, 501–502; e) D. H.

Nugteren, D. A. van Dorp, S. Bergström, M. Hamberg, B. Samuelsson, *Nature* **1966**, *212*, 38 – 39.
[5] M. G. Malkowski, S. L. Ginell, W. L. Smith, R. M. Garavito, *Science* **2000**, *289*, 1933 – 1937.
[6] a) F. Schaller, A. Schaller, A. Stintzi, *J. Plant Growth Regul.* **2005**, *23*, 179 – 199; b) *J. Plant Growth Regul.* **2005**, *23*(3).
[7] D. H. Nugteren, H. Vonkeman, D. A. van Dorp, *Recl. Trav. Chim. Pays-Bas* **1967**, *86*, 1237 – 1245.
[8] a) J. D. Morrow, T. M. Harris, L. J. Roberts II, *Anal. Biochem.* **1990**, *184*, 1 – 10; b) J. D. Morrow, K. E. Hill, R. F. Burk, T. M. Nammour, K. F. Badr, L. J. Roberts II, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9383 – 9387.
[9] S. Parchmann, M. J. Mueller, *J. Biol. Chem.* **1998**, *273*, 32650 – 32655.
[10] O. G. Mouritsen, *Life as a Matter of Fat. The Emerging Science of Lipidomics*, Springer, Berlin, **2005**.
[11] a) K. Uchida, *Prog. Lipid Res.* **2003**, *42*, 318 – 343; b) M. Parola, G. Bellomo, G. Robino, G. Barrera, M. U. Dianzani, *Antioxid. Redox Signaling* **1999**, *1*, 255 – 284; c) H. Esterbauer, R. J. Schaur, H. Zollner, *Free Radical Biol. Med.* **1991**, *11*, 81 – 128.
[12] A. N. Grechkin, *J. Lipid Mediators Cell Signalling* **1995**, *11*, 205 – 218.
[13] O. Berdeaux, V. Fournier, P. Lambelet, F. Dionisi, J. L. Sebedio, F. Destaillats, *J. Chromatogr. A* **2007**, *1138*, 216 – 224.
[14] a) H. John, W. Schlegel, *Anal. Chim. Acta* **2002**, *465*, 441 – 450; b) H. John, K. Cammann, W. Schlegel, *Prostaglandins Other Lipid Mediators* **1998**, *56*, 53 – 76.
[15] a) M. Adiyaman, J. A. Lawson, S.-W. Hwang, S. P. Khanapure, G. A. FitzGerald, J. Rokach, *Tetrahedron Lett.* **1996**, *37*, 4849 – 4852; b) J. Rokach, S. P. Khanapure, S. W. Hwang, M. Adiyaman, J. A. Lawson, G. A. FitzGerald, *Prostaglandins* **1997**, *54*, 853 – 873; c) J. Rokach, S. Kim, S. Bellone, J. A. Lawson, D. Pratico, W. S. Powell, G. A. FitzGerald, *Chem. Phys. Lipids* **2004**, *128*, 35 – 56.
[16] a) D. F. Taber, J. D. Morrow, L. J. Roberts II, *Prostaglandins* **1997**, *53*, 63 – 67; b) D. F. Taber, L. J. Roberts II, *Prostaglandins Other Lipid Mediators* **2005**, *78*, 14 – 18.
[17] a) R. G. Salomon, G. Subbanagounder, U. Singh, J. O'Neil, H. F. Hoff, *Curr. Res. Toxicol.* **1997**, *10*, 750 – 759; b) R. G. Salomon, *Chem. Phys. Lipids* **2005**, *134*, 1 – 20.
[18] N. Bernoud-Hubac, S. S. Davies, O. Boutaud, T. J. Montine, L. J. Roberts II, *J. Biol. Chem.* **2001**, *276*, 30964 – 30970.
[19] D. F. Taber, J. P. Fessel, L. J. Roberts II, *Prostaglandins Other Lipid Mediators* **2004**, *73*, 47 – 50.
[20] H. Yin, N. A. Porter, J. D. Morrow, *J. Chromatogr. B* **2005**, *827*, 157 – 164.
[21] H. Yin, L. Gao, H.-H. Tai, L. J. Murphey, N. A. Porter, J. D. Morrow, *J. Biol. Chem.* **2006**, *282*, 329 – 336.
[22] a) M. Hecker, V. Ullrich, D. Fischer, C. O. Meese, *Eur. J. Biochem.* **1987**, *169*, 113 – 123; b) D. Pratico, G. A. FitzGerald, *J. Biol. Chem.* **1996**, *271*, 8919 – 8924; c) D. Pratico, J. A. Lawson, G. A. FitzGerald, *J. Biol. Chem.* **1995**, *270*, 9800 – 9808; d) E. G. Daniels, W. C. Krueger, F. P. Kupiecki, J. E. Pike, W. P. Schneider, *J. Am. Chem. Soc.* **1968**, *90*, 5894 – 5895; e) J. E. Pike, F. H. Lincoln, W. P. Schneider, *J. Org. Chem.* **1969**, *34*, 3552 – 3557.
[23] J. D. Morrow, J. A. Awad, H. J. Boss, I. A. Blair, L. J. Roberts II, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 10721 – 10725.
[24] H. Yin, C. M. Havrilla, J. D. Morrow, N. A. Porter, *J. Am. Chem. Soc.* **2002**, *124*, 7745 – 7754.
[25] Review: B. Halliwell, M. Whiteman, *Br. J. Pharmacol.* **2004**, *142*, 231 – 255.
[26] Review: Z. Cheng, Y. Li, *Chem. Rev.* **2007**, *107*, 748 – 766.
[27] E. Poliakov, M.-L. Brennan, J. MacPherson, R. Zhang, W. Sha, L. Narine, R. G. Salomon, S. L. Hazen, *FASEB J.* **2003**, *17*, 2209 – 2220.
[28] a) A. B. Ross, P. Neta, *Natl. Stand. Ref. Data Ser.* (U.S., Natl. Bur. Stand.) **1982**, pp. 1–103; b) K. Hasegawa, L. K. Patterson, *Photochem. Photobiol.* **1978**, *28*, 817 – 823.
[29] a) N. A. Porter, *Acc. Chem. Res.* **1986**, *19*, 262 – 268; b) C. M. Havrilla, D. L. Hachey, N. A. Porter, *J. Am. Chem. Soc.* **2000**, *122*, 8042 – 8055; c) N. A. Porter, L. S. Lehman, B. A. Weber, K. J. Smith, *J. Am. Chem. Soc.* **1981**, *103*, 6447 – 6455; d) N. A. Porter, R. A. Wolf, E. M. Yarbro, H. Weenen, *Biochem. Biophys. Res. Commun.* **1979**, *89*, 1058 – 1064.
[30] J. R. Seal, N. A. Porter, *Anal. Bioanal. Chem.* **2004**, *378*, 1007 – 1013; *Anal. Bioanal. Chem.* **2004**, *380*, 356.
[31] L. Bedard, M. J. Young, D. Hall, T. Paul, K. U. Ingold, *J. Am. Chem. Soc.* **2001**, *123*, 12439 – 12448, and references therein.
[32] H. Weenen, N. A. Porter, *J. Am. Chem. Soc.* **1982**, *104*, 5216 – 5221.
[33] M. Alessi, T. Paul, J. C. Scaiano, K. U. Ingold, *J. Am. Chem. Soc.* **2002**, *124*, 6957 – 6965, and references therein.
[34] T. A. Davis, L. Gao, H. Y. Yin, J. D. Morrow, N. A. Porter, *J. Am. Chem. Soc.* **2006**, *128*, 14897 – 14904.
[35] a) B. Roschek, K. A. Tallman, C. L. Rector, J. G. Gillmore, D. A. Pratt, C. Punta, N. A. Porter, *J. Org. Chem.* **2006**, *71*, 3527 – 3532, and references therein; b) K. A. Tallman, B. Roschek, N. A. Porter, *J. Am. Chem. Soc.* **2004**, *126*, 9240 – 9247, and references therein.
[36] N. A. Porter, B. G. Wujek, *J. Am. Chem. Soc.* **1984**, *106*, 2626 – 2629. All rate constants cited here were determined for methyl linoleate, which is not able to cyclize. The rate constants for the other PUFAs should be, however, very similar.
[37] The older literature cites $430\ s^{-1}$ as the fragmentation rate constant.[36] This value should be corrected to $690\ s^{-1}$.[35a]
[38] A. W. Longmire, L. L. Swift, L. J. Roberts II, J. A. Awad, R. F. Burk, J. D. Morrow, *Biochem. Pharmacol.* **1994**, *47*, 1173 – 1177.
[39] a) N. A. Porter, M. O. Funk, *J. Org. Chem.* **1975**, *40*, 3614 – 3615; b) W. A. Pryor, J. P. Stanley, *J. Org. Chem.* **1975**, *40*, 3615 – 3617; c) W. A. Pryor, J. P. Stanley, E. Blair, *Lipids* **1976**, *11*, 370 – 379.
[40] The detected prostaglandin isomers formed in a lipoxygenase-triggered reaction should be considered as isoprostanes rather than prostaglandins, since the reaction occurred under aerobic conditions: G. S. Bild, S. G. Bhat, C. S. Ramadoss, B. Axelrod, *J. Biol. Chem.* **1978**, *253*, 21 – 23.
[41] a) D. E. O'Connor, E. D. Mihelich, M. C. Coleman, *J. Am. Chem. Soc.* **1981**, *103*, 223 – 224; b) D. E. O'Connor, E. D. Mihelich, M. C. Coleman, *J. Am. Chem. Soc.* **1984**, *106*, 3577 – 3584.
[42] T. J. Montine, K. S. Montine, E. E. Reich, E. S. Terry, N. A. Porter, J. D. Morrow, *Biochem. Pharmacol.* **2003**, *65*, 611 – 617.
[43] J. D. Morrow, L. J. Roberts, V. C. Daniel, J. A. Awad, O. Mirochnitchenko, L. L. Swift, R. F. Burk, *Arch. Biochem. Biophys.* **1998**, *353*, 160 – 171.
[44] N. A. Porter, P. J. Zuraw, J. A. Sullivan, *Tetrahedron Lett.* **1984**, *25*, 807 – 810.
[45] E. J. Corey, K. Shimoji, C. Shih, *J. Am. Chem. Soc.* **1984**, *106*, 6425 – 6427.
[46] E. J. Corey, C. Shih, N.-Y. Shih, K. Shimoji, *Tetrahedron Lett.* **1984**, *25*, 5013 – 5016.
[47] R. W. Hoffmann, *Chem. Rev.* **1989**, *89*, 1841 – 1860.
[48] E. J. Corey, Z. Wang, *Tetrahedron Lett.* **1994**, *35*, 539 – 542.
[49] a) J. A. Lawson, J. Rokach, G. A. FitzGerald, *J. Biol. Chem.* **1999**, *274*, 24441 – 24444; b) J. Rokach, S. P. Khanapure, S. W. Hwang, M. Adiyaman, J. A. Lawson, G. A. FitzGerald, *Prostaglandins* **1997**, *54*, 823 – 851.
[50] Data for the cyclization/ring opening of dioxetanylcarbinyl radicals are not available. Comparison with the well known 4-pentenyl radical cyclization/cyclobutylcarbinyl radical ring opening suggests that 4-*exo* cyclization should not be favored:

J. Fossey, D. Lefort, J. Sorba, *Free Radicals in Organic Chemistry*, Wiley, New York, **1995**, pp. 158 – 160.
[51] H. Yin, C. M. Havrilla, L. Gao, J. D. Morrow, N. A. Porter, *J. Biol. Chem.* **2003**, *278*, 16720 – 16725.
[52] a) R. J. Waugh, J. D. Morrow, L. J. Roberts II, R. C. Murphy, *Free Radical Biol. Med.* **1997**, *23*, 943 – 954; b) R. J. Waugh, R. C. Murphy, *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 490 – 499.
[53] H. Yin, J. D. Morrow, N. A. Porter, *J. Biol. Chem.* **2003**, *279*, 3766 – 3776.
[54] a) J. Nourooz-Zadeh, B. Halliwell, E. E. Anggard, *Biochem. Biophys. Res. Commun.* **1997**, *236*, 467 – 472; b) L. Gao, H. Yin, G. L. Milne, N. A. Porter, J. D. Morrow, *J. Biol. Chem.* **2006**, *281*, 14092 – 14099.
[55] a) A. L. J. Beckwith, C. H. Schiesser, *Tetrahedron* **1985**, *41*, 3925 – 3941; b) D. C. Spellmeyer, K. N. Houk, *J. Org. Chem.* **1987**, *52*, 959 – 974.
[56] D. P. Curran, N. A. Porter, B. Giese, *Stereochemistry of Radical Reactions*, VCH, Weinheim, **1996**, pp. 67 – 68.
[57] M. Hamberg, J. Svensson, T. Wakabayashi, B. Samuelsson, *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 345 – 349.
[58] a) A. Raz, R. Kenig-Wakshal, M. Schwartzman, *Biochim. Biophys. Acta Lipids Lipid Metab.* **1977**, *488*, 322 – 329; b) D. H. Nugteren, E. Hazelhof, *Biochim. Biophys. Acta Lipids Lipid Metab.* **1973**, *326*, 448 – 461.
[59] In microsomes the structure and polarity differences present in intact cells are reduced to a great extent. Thus, acid-base catalysis to give D_2-/E_2-IsoPs may dominate over reduction to F_2-IsoP.
[60] J. D. Morrow, T. A. Minton, C. R. Mukundan, M. D. Campbell, W. E. Zackert, V. C. Daniel, K. F. Badr, I. A. Blair, L. J. Roberts II, *J. Biol. Chem.* **1994**, *269*, 4317 – 4326.
[61] M. Hamberg, B. Samuelsson, *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 899 – 903.
[62] a) N. A. Porter, J. D. Byers, R. C. Mebane, D. W. Gilmore, J. R. Nixon, *J. Org. Chem.* **1978**, *43*, 2088 – 2090; b) N. A. Porter, J. D. Byers, K. M. Holden, D. B. Menzel, *J. Am. Chem. Soc.* **1979**, *101*, 4319 – 4322.
[63] R. G. Salomon, D. B. Miller, M. G. Zagorski, D. J. Coughlin, *J. Am. Chem. Soc.* **1984**, *106*, 6049 – 6060.
[64] L. Gao, W. E. Zackert, J. J. Hasford, M. E. Danekis, G. L. Milne, C. Remmert, J. Reese, H. Yin, H.-H. Tai, S. K. Dey, N. A. Porter, J. D. Morrow, *J. Biol. Chem.* **2003**, *278*, 28479 – 28489.
[65] a) Y. Chen, J. D. Morrow, L. J. Roberts II, *J. Biol. Chem.* **1999**, *274*, 10863 – 10868; b) Y. Chen, W. E. Zackert, L. J. Roberts II, J. D. Morrow, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1999**, *1436*, 550 – 556.
[66] F. A. Fitzpatrick, M. A. Wynalda, *J. Biol. Chem.* **1983**, *258*, 11713 – 11718.
[67] E. S. Musiek, R. S. Breeding, G. L. Milne, G. Zanoni, J. D. Morrow, B. McLaughlin, *J. Neurochem.* **2006**, *97*, 1301 – 1313.
[68] G. L. Milne, L. Gao, A. Porta, G. Zanoni, G. Vidari, J. D. Morrow, *J. Biol. Chem.* **2005**, *280*, 25178 – 25184.
[69] a) L. J. Roberts II, T. J. Montine, W. R. Markesbery, A. R. Tapper, P. Hardy, S. Chemtob, W. D. Dettbarn, J. D. Morrow, *J. Biol. Chem.* **1998**, *273*, 13605 – 13612; b) J. Nourooz-Zadeh, E. H. C. Liu, E. E. Anggard, B. Halliwell, *Biochem. Biophys. Res. Commun.* **1998**, *242*, 338 – 344.
[70] H. Yin, E. E. Musiek, L. Gao, N. A. Porter, J. D. Morrow, *J. Biol. Chem.* **2005**, *280*, 26600 – 26611.
[71] E. E. Reich, W. R. Zackert, C. J. Brame, Y. Chen, L. J. Roberts II, D. L. Hachey, T. J. Montine, J. D. Morrow, *Biochemistry* **2000**, *39*, 2376 – 2383.
[72] S. S. Fam, L. J. Murphey, E. S. Terry, W. E. Zackert, Y. Chen, L. Gao, S. Pandalai, G. L. Milne, L. J. Roberts, N. A. Porter, T. J. Montine, J. D. Morrow, *J. Biol. Chem.* **2002**, *277*, 36076 – 36084.
[73] Review: M. Hamberg, H. W. Gardner, *Biochim. Biophys. Acta Lipids Lipid Metab.* **1992**, *1165*, 1 – 18.
[74] a) F. Bohlmann, R. K. Gupta, R. M. King, H. Robinson, *Phytochemistry* **1981**, *20*, 1417 – 1418; b) F. Bohlmann, N. Borthakur, R. M. King, H. Robinson, *Phytochemistry* **1982**, *21*, 125 – 127; c) F. Bohlmann, P. Singh, J. Jakupovic, R. M. King, H. Robinson, *Phytochemistry* **1982**, *21*, 371 – 374.
[75] For a recent theoretical investigation in the biosynthetic pathway, see: C. S. Lopez, O. N. Faza, D. M. York, A. R. de Lera, *J. Org. Chem.* **2004**, *69*, 3635 – 3644.
[76] I. Thoma, C. Loeffler, A. K. Sinha, M. Gupta, M. Krischke, B. Steffan, T. Roitsch, M. J. Mueller, *Plant J.* **2003**, *34*, 363 – 375. Mueller and co-workers do not distinguish the orientation of the carbonyl group relative to the α side chain in the regioisomeric 9- and 16-series of A_1-, B_1-, D_1-, E_1-, and J_1-PhytoPs correctly in this and all later publications on this topic. This leads to a clear conflict of structure and accepted prostaglandin ring nomenclature for PhytoPs (see Scheme 3). For the correct representation, see Scheme 26.
[77] R. Imbusch, M. J. Mueller, *Free Radical Biol. Med.* **2000**, *28*, 720 – 726.
[78] R. Imbusch, M. J. Mueller, *Plant Physiol.* **2000**, *124*, 1293 – 1303.
[79] M. Krischke, C. Loeffler, M. J. Mueller, *Phytochemistry* **2003**, *62*, 351 – 358.
[80] M. Hecker, V. Ullrich, *J. Biol. Chem.* **1989**, *264*, 141 – 150.
[81] J. D. Morrow, J. A. Awad, A. Wu, W. E. Zackert, V. C. Daniel, L. J. Roberts II, *J. Biol. Chem.* **1996**, *271*, 23185 – 23190.
[82] There is also precedence of an endoperoxide opening with concomitant polar 1,2-rearrangement, where iron(II) acts as a Lewis acid: M. Kamata, M. Ohta, K. Komatsu, H. S. Kim, Y. Wataya, *Tetrahedron Lett.* **2002**, *43*, 2063 – 2067.
[83] a) R. G. Salomon, *Acc. Chem. Res.* **1985**, *18*, 294 – 301; b) O. Boutaud, C. J. Brame, P. Chaurand, J. Li, S. W. Rowlinson, B. C. Crews, C. Ji, L. J. Marnett, R. M. Caprioli, L. J. Roberts II, J. A. Oates, *Biochemistry* **2001**, *40*, 6948 – 6955.
[84] M. G. Zagorski, R. G. Salomon, *J. Am. Chem. Soc.* **1982**, *104*, 3498 – 3503.
[85] N. Bernoud-Hubac, L. B. Fay, V. Armarnath, M. Guichardant, S. Bacot, S. S. Davies, L. J. Roberts II, M. Lagarde, *Free Radical Biol. Med.* **2004**, *37*, 1604 – 1611.
[86] R. G. Salomon, W. Sha, C. Brame, K. Kaur, G. Subbanagounder, J. O'Neil, H. F. Hoff, L. J. Roberts II, *J. Biol. Chem.* **1999**, *274*, 20271 – 20280.
[87] a) C. J. Brame, O. Boutaud, S. S. Davies, T. Yang, J. A. Oates, D. Roden, L. J. Roberts II, *J. Biol. Chem.* **2004**, *279*, 13447 – 13451; b) C. J. Brame, R. G. Salomon, J. D. Morrow, L. J. Roberts II, *J. Biol. Chem.* **1999**, *274*, 13139 – 13146.
[88] E. Poliakov, S. G. Meer, S. C. Roy, C. Mesaros, R. G. Salomon, *Curr. Res. Toxicol.* **2004**, *17*, 613 – 622.
[89] S. S. Davies, V. Amarnath, K. S. Montine, N. Bernoud-Houbac, O. Boutaud, T. J. Montine, L. J. Roberts II, *FASEB J.* **2002**, *16*, 715 – 717.
[90] R. S. Iyer, D. B. Miller, R. G. Salomon, *J. Org. Chem.* **1990**, *55*, 3175 – 3180.
[91] N. Bernoud-Hubac, L. J. Roberts II, *Biochemistry* **2002**, *41*, 11466 – 11471.
[92] A. D. Watson, G. Subbanagounder, D. S. Welsbie, K. F. Faull, M. Navab, M. E. Jung, A. M. Fogelman, J. A. Berliner, *J. Biol. Chem.* **1999**, *274*, 24787 – 24798.
[93] G. Subbanagounder, J. W. Wong, H. Lee, K. F. Faull, E. Miller, J. L. Witztum, J. A. Berliner, *J. Biol. Chem.* **2002**, *277*, 7271 – 7281.
[94] a) R. K. Haynes, S. C. Vonwiller, *J. Chem. Soc. Chem. Commun.* **1990**, 1102 – 1104; b) E. N. Frankel, W. E. Neff, K. Miyashita, *Lipids* **1990**, *25*, 40 – 47; c) W. E. Neff, E. N. Frankel, D. Weisleder, *Lipids* **1981**, *16*, 439 – 448; d) I. Toyoda, J. Terao, S. Matsubashita, *Lipids* **1982**, *17*, 84 – 90.
[95] a) H. W.-S. Chan, J. A. Mathew, D. T. Coxon, *J. Chem. Soc. Chem. Commun.* **1980**, 235 – 236; b) D. T. Coxon, K. R. Price,

H. W.-S. Chan, *Chem. Phys. Lipids* **1981**, *28*, 365 – 378; c) M. Roza, A. Francke, *Biochim. Biophys. Acta Lipids Lipid Metab.* **1978**, *528*, 119 – 126.

[96] J. A. Khan, N. A. Porter, *Angew. Chem.* **1982**, *94*, 220 – 221; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 217 – 218.

[97] W. E. Neff, E. N. Frankel, D. Weisleder, *Lipids* **1982**, *17*, 780 – 790.

[98] a) C. Pace-Asciak, L. S. Wolfe, *Chem. Commun.* **1970**, 1235 – 1236; b) C. Pace-Asciak, *Biochemistry* **1971**, *10*, 3664 – 3669.

[99] M. F. Moghaddam, K. Motoba, B. Borhan, F. Pinot, B. D. Hammock, *Biochim. Biophys. Acta Gen. Subj.* **1996**, *1290*, 327 – 339.

[100] a) J. P. Fessel, N. A. Porter, K. P. Moore, J. R. Sheller, L. J. Roberts II, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16713 – 16718; b) W.-L. Song, J. A. Lawson, D. Reilly, J. Rokach, C.-T. Chang, B. Giasson, G. A. FitzGerald, *J. Biol. Chem.* **2008**, *283*, 6 – 16.

[101] a) N. A. Porter, J. R. Nixon, *J. Am. Chem. Soc.* **1978**, *100*, 7116 – 7117; b) A. J. Bloodworth, J. L. Courtneidge, A. G. Davies, *J. Chem. Soc. Perkin Trans. 2* **1984**, 523 – 527.

[102] J. R. Nixon, M. A. Cudd, N. A. Porter, *J. Org. Chem.* **1978**, *43*, 4048 – 4052.

[103] W. Sametz, K. Hummer, M. Butter, R. Wintersteiger, H. Juan, *Br. J. Pharmacol.* **2000**, *131*, 145 – 151.

[104] D. M. Stafforini, J. R. Sheller, T. S. Blackwell, A. Sapirstein, F. E. Yull, T. M. McIntyre, J. V. Bonventre, S. M. Prescott, L. J. Roberts II, *J. Biol. Chem.* **2005**, *281*, 4616 – 4623.

[105] a) L. J. Roberts II, K. P. Moore, W. E. Zackert, J. A. Oates, J. D. Morrow, *J. Biol. Chem.* **1996**, *271*, 20617 – 20620; b) C. Chiabrando, A. Valagussa, C. Rivalta, T. Durand, A. Guy, E. Zuccato, P. Villa, J.-C. Rossi, R. Fanelli, *J. Biol. Chem.* **1999**, *274*, 1313 – 1319, and references therein; c) S. Kim, W. S. Powell, J. A. Lawson, S. H. Jacobo, D. Pratico, G. A. FitzGerald, K. Maxey, J. Rokach, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1613 – 1617.

[106] J. A. Awad, J. D. Morrow, K. Takahashi, L. J. Roberts II, *J. Biol. Chem.* **1993**, *268*, 4161 – 4169.

[107] a) S. Basu, *FEBS Lett.* **1998**, *428*, 32 – 36; b) S. Basu, *Prostaglandins Leukotrienes Essent. Fatty Acids* **1998**, *58*, 319 – 325.

[108] C. Chiabrando, C. Rivalta, R. Bagnati, A. Valagussa, T. Durand, A. Guy, P. Villa, J.-C. Rossi, R. Fanelli, *J. Lipid Res.* **2002**, *43*, 495 – 509.

[109] J. A. Lawson, S. Kim, W. S. Powell, G. A. FitzGerald, J. Rokach, *J. Lipid Res.* **2006**, *47*, 2515 – 2524.

[110] a) R. J. P. Williams, *Dalton Trans.* **2007**, 991 – 1001; b) R. J. P. Williams, J. J. R. Frausto da Silva, *The Chemistry of Evolution. The Development of our Ecosystem*, Elsevier, Amsterdam, **2006**.

[111] M. J. Mueller, *Curr. Opin. Plant Biol.* **2004**, *7*, 441 – 448.

[112] a) L. J. Roberts II, J. D. Morrow, *Free Radical Biol. Med.* **2000**, *28*, 505 – 513; b) M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plastaras, L. J. Roberts II, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett, *Free Radical Biol. Med.* **2005**, *38*, 698 – 710.

[113] a) O. Berdeaux, O. Scruel, J.-L. Cracowski, T. Durand, *Curr. Pharm. Anal.* **2006**, *2*, 69 – 78; b) D. Tsikas, *J. Chromatogr. B* **1998**, *717*, 201 – 245.

[114] a) J. D. Morrow, L. J. Roberts II, *Methods Enzymol.* **1999**, *300*, 3 – 12; b) M. F. Walter, J. B. Blumberg, G. G. Dolnikowski, G. J. Handelman, *Anal. Biochem.* **2000**, *280*, 73 – 79; c) J. Nourooz-Zadeh, E. H. Liu, B. Yhlen, E. E. Anggard, B. Halliwell, *J. Neurochem.* **1999**, *72*, 734 – 740.

[115] a) T. A. Mori, K. D. Croft, I. B. Puddey, L. J. Beilin, *Anal. Biochem.* **1999**, *268*, 117 – 125; b) M. Richelle, M. E. Turini, R. Guidoux, I. Tavazzi, S. Métairon, L. B. Fay, *Eur. J. Mass Spectrom.* **2001**, *7*, 427 – 432.

[116] J. D. Morrow, L. J. Roberts II, *Methods Enzymol.* **1994**, *233*, 163 – 174.

[117] a) J. Nourooz-Zadeh, N. K. Gopaul, S. Barrow, A. I. Mallet, E. E. Anggard, *J. Chromatogr. B* **1995**, *667*, 199 – 208; b) N. K. Gopaul, E. E. Anggard, A. I. Mallet, D. J. Betteridge, S. P. Wolff, J. Nourooz-Zadeh, *FEBS Lett.* **1995**, *368*, 225 – 229.

[118] a) Z. Zhao, N. M. Hjelm, C. W. Lam, C. S. Ho, *Clin. Chem.* **2001**, *47*, 1306 – 1308; b) C. Y. Lee, A. M. Jenner, B. Halliwell, *Biochem. Biophys. Res. Commun.* **2004**, *320*, 696 – 702.

[119] A. Bachi, E. Zuccato, M. Baraldi, R. Fanelli, C. Chiabrando, *Free Radical Biol. Med.* **1996**, *20*, 619 – 624.

[120] a) Y. Liang, P. Wei, R. W. Duke, P. D. Reaven, S. M. Harman, R. G. Cutler, C. B. Heward, *Free Radical Biol. Med.* **2003**, *34*, 409 – 418; b) Y. Murai, T. Hishinuma, N. Suzuki, J. Satoh, T. Toyota, M. Mizugaki, *Prostaglandins Other Lipid Mediators* **2000**, *62*, 173 – 181; c) H. Li, J. A. Lawson, M. Reilly, M. Adiyaman, S.-W. Hwang, J. Rokach, G. A. FitzGerald, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 13381 – 13386; d) J. A. Lawson, H. Li, J. Rokach, M. Adiyaman, S. W. Hwang, S. P. Khanapure, G. A. FitzGerald, *J. Biol. Chem.* **1998**, *273*, 29295 – 29301; e) N. Ohashi, M. Yoshikawa, *J. Chromatogr. B* **2000**, *746*, 17 – 24; f) K. C. Bohnstedt, B. Karlberg, L.-O. Wahlund, M. E. Jonhagen, H. Basun, S. Schmidt, *J. Chromatogr. B* **2003**, *796*, 11 – 19.

[121] Z. Wang, G. Ciabattoni, C. Creminon, J. Lawson, G. A. Fitzgerald, C. Patrono, J. Maclouf, *J. Pharmacol. Exp. Ther.* **1995**, *251*, 94 – 100.

[122] a) J. D. Morrow, W. E. Zackert, J. P. Yang, E. H. Kurhts, D. Callewaert, R. Dworski, K. Kanai, D. Taber, K. Moore, J. A. Oates, L. J. Roberts, *Anal. Biochem.* **1999**, *269*, 326 – 331; b) J. Nourooz-Zadeh, *Methods Enzymol.* **1999**, *300*, 13 – 17.

[123] a) J. Nourooz-Zadeh, P. Pereira, *Ophthalmic Res.* **2000**, *32*, 133 – 137; b) I. N. Guha, K. Moore, *Prostaglandins Other Lipid Mediators* **2003**, *72*, 73 – 84.

[124] a) D. Tsikas, E. Schwedhelm, J. Fauler, F. M. Gutzki, E. Mayatepek, J. C. Frolich, *J. Chromatogr. B* **1998**, *716*, 7 – 17; b) H. Schweer, B. Watzer, H. W. Seyberth, R. M. Nüsing, *J. Mass Spectrom.* **1997**, *32*, 1362 – 1370.

[125] C. Signorini, M. Comporti, G. Giorgi, *J. Mass Spectrom.* **2003**, *38*, 1067 – 1074.

[126] a) J. Bessard, J.-L. Cracowski, F. Stanke-Labesque, G. Bessard, *J. Chromatogr. B* **2001**, *754*, 333 – 343; b) T. Obata, K. Tomaru, T. Nagakura, Y. Izumi, T. Kawamoto, *J. Chromatogr. B* **2000**, *746*, 11 – 15.

[127] T. Obata, Y. Sakurai, Y. Kase, Y. Tanifuji, T. Horiguchi, *J. Chromatogr. B* **2003**, *792*, 131 – 140.

[128] a) D. Il'yasova, J. D. Morrow, A. Ivanova, L. E. Wagenknecht, *Ann. Epidemiol.* **2004**, *14*, 793 – 797; b) J. Proudfoot, A. Barden, T. A. Mori, V. Burke, K. D. Croft, L. J. Beilin, I. B. Puddey, *Anal. Biochem.* **1999**, *272*, 209 – 215; c) T. P. Stein, M. J. Leskiw, *Am. J. Physiol. Endocrinol. Metab.* **2000**, *278*, 375 – 382.

[129] a) J. D. Morrow, L. J. Roberts II, *Prog. Lipid Res.* **1997**, *36*, 1 – 21; b) D. Pratico, J. A. Lawson, J. Rokach, G. A. FitzGerald, *Trends Endocrinol. Metab.* **2001**, *12*, 243 – 247; c) P. Montuschi, P. J. Barnes, L. J. Roberts II, *FASEB J.* **2004**, *18*, 1791 – 1800; d) G. L. Milne, E. S. Musiek, J. D. Morrow, *Biomarkers* **2005**, *10 Suppl. 1*, 10 – 23.

[130] S. Basu, J. Helmersson, *Antioxid. Redox Signaling* **2005**, *7*, 221 – 235.

[131] G. Kennedy, V. A. Spence, M. McLaren, A. Hill, C. Underwood, J. J. F. Belch, *Free Radical Biol. Med.* **2005**, *39*, 584 – 589.

[132] D. Pratico, L. Iuliano, A. Mauriello, L. Spagnoli, J. A. Lawson, J. Rokach, J. Maclouf, F. Violi, G. A. FitzGerald, *J. Clin. Invest.* **1997**, *100*, 2028 – 2034.

[133] C. Vassalle, N. Botto, M. G. Andreassi, S. Berti, A. Biagini, *Coron. Artery Dis.* **2003**, *14*, 213 – 218.
[134] a) M. Nonaka-Sarukawa, K. Yamamoto, H. Aoki, H. Takano, T. Katsuki, U. Ikeda, K. Shimada, *Heart* **2003**, *89*, 871 – 874; b) Z. Mallat, I. Philip, M. Lebret, D. Chatel, J. Maclouf, A. Tedgui, *Circulation* **1998**, *97*, 1536 – 1539.
[135] a) N. Delanty, M. P. Reilly, D. Pratico, J. A. Lawson, J. F. McCarthy, A. E. Wood, S. T. Ohnishi, D. J. Fitzgerald, G. A. FitzGerald, *Circulation* **1997**, *95*, 2492 – 2499; b) M. P. Reilly, N. Delanty, L. Roy, J. Rokach, P. O. Callaghan, P. Crean, J. A. Lawson, G. A. FitzGerald, *Circulation* **1997**, *96*, 3314 – 3320.
[136] P. Minuz, P. Patrignani, S. Gaino, M. Degan, L. Menapace, R. Tommasoli, F. Seta, M. L. Capone, S. Tacconelli, S. Palatresi, C. Bencini, C. Del Vecchio, G. Mansueto, E. Arosio, C. L. Santonastaso, A. Lechi, A. Morganti, C. Patrono, *Circulation* **2002**, *106*, 2800 – 2805.
[137] G. Davi, G. Ciabattoni, A. Consoli, A. Mezzetti, A. Falco, S. Santarone, E. Pennese, E. Vitacolonna, T. Bucciarelli, F. Costantini, F. Capani, C. Patrono, *Circulation* **1999**, *99*, 224 – 229.
[138] G. Davi, S. Basili, M. Vieri, F. Cipollone, S. Santarone, C. Alessandri, P. Gazzaniga, C. Cordova, F. Violi, *Am. J. Respir. Crit. Care Med.* **1997**, *156*, 1794 – 1799.
[139] a) G. Davi, G. Di Minno, A. Coppola, G. Andria, A. M. Cerbone, P. Madonna, A. Tufano, A. Falco, P. Marchesani, G. Ciabattoni, C. Patrono, *Circulation* **2001**, *104*, 1124 – 1128; b) S. Voutilainen, J. D. Morrow, L. J. Roberts II, G. Alfthan, H. Alho, K. Nyyssonen, J. T. Salonen, *Arterioscler. Thromb. Vasc. Biol.* **1999**, *19*, 1263 – 1266.
[140] T. Ide, H. Tsutsui, N. Ohashi, S. Hayashidani, N. Suematsu, M. Tsuchihashi, H. Tamai, A. Takeshita, *Arterioscler. Thromb. Vasc. Biol.* **2002**, *22*, 438 – 442.
[141] J. F. Keaney Jr. , M. G. Larson, R. S. Vasan, P. W. Wilson, I. Lipinska, D. Corey, J. M. Massaro, P. Sutherland, J. A. Vita, E. J. Benjamin, *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 434 – 439.
[142] a) M. Reilly, N. Delanty, J. A. Lawson, G. A. FitzGerald, *Circulation* **1996**, *94*, 19 – 25; b) J. D. Morrow, B. Frei, A. W. Longmire, J. M. Gaziano, S. M. Lynch, Y. Shyr, W. E. Strauss, J. A. Oates, L. J. Roberts II, *N. Engl. J. Med.* **1995**, *332*, 1198 – 1203.
[143] a) T. A. Ikizler, J. D. Morrow, L. J. Roberts II, J. A. Evanson, B. Becker, R. M. Hakim, Y. Shyr, J. Himmelfarb, *Clin. Nephrol.* **2002**, *58*, 190 – 197; b) M. A. Spittle, N. A. Hoenich, G. J. Handelman, R. Adhikarla, P. Homel, N. W. Levin, *Am. J. Kidney Dis.* **2001**, *38*, 1408 – 1413.
[144] J. D. Morrow, K. P. Moore, J. A. Awad, M. D. Ravenscraft, G. Marini, K. F. Badr, R. Williams, L. J. Roberts II, *J. Lipid Mediators* **1993**, *6*, 417 – 420.
[145] a) S. Holt, B. Reeder, M. Wilson, S. Harvey, J. D. Morrow, L. J. Roberts II, K. Moore, *Lancet* **1999**, *353*, 1241; b) K. P. Moore, S. G. Holt, R. P. Patel, D. A. Svistunenko, W. Zackert, D. Goodier, B. J. Reeder, M. Clozel, R. Anand, C. E. Cooper, J. D. Morrow, M. T. Wilson, V. Darley-Usmar, L. J. Roberts II, *J. Biol. Chem.* **1998**, *273*, 31731 – 31737.
[146] a) P. Montuschi, M. Corradi, G. Ciabattoni, J. Nightingale, S. A. Kharitonov, P. J. Barnes, *Am. J. Respir. Crit. Care Med.* **1999**, *160*, 216 – 220; b) R. Dworski, J. J. Murray, L. J. Roberts II, J. A. Oates, J. D. Morrow, L. Fisher, J. R. Sheller, *Am. J. Respir. Crit. Care Med.* **1999**, *160*, 1947 – 1951.
[147] a) P. Montuschi, J. V. Collins, G. Ciabattoni, N. Lazzeri, M. Corradi, S. A. Kharitonov, P. J. Barnes, *Am. J. Respir. Crit. Care Med.* **2000**, *162*, 1175 – 1177; b) D. Pratico, S. Basili, M. Vieri, C. Cordova, F. Violi, G. A. Fitzgerald, *Am. J. Respir. Crit. Care Med.* **1998**, *158*, 1709 – 1714.
[148] a) P. Montuschi, S. A. Kharitonov, G. Ciabattoni, M. Corradi, L. van Rensen, D. M. Geddes, M. E. Hodson, P. J. Barnes, *Thorax* **2000**, *55*, 205 – 209; b) G. Ciabattoni, G. Davi, M. Collura, L. Iapichino, F. Pardo, A. Ganci, R. Romagnoli, J. Maclouf, C. Patrono, *Am. J. Respir. Crit. Care Med.* **2000**, *162*, 1195 – 1201.
[149] P. Montuschi, G. Ciabattoni, P. Paredi, P. Pantelidis, R. M. du Bois, S. A. Kharitonov, P. J. Barnes, *Am. J. Respir. Crit. Care Med.* **1998**, *158*, 1524 – 1527.
[150] C. T. Carpenter, P. V. Price, B. W. Christman, *Chest* **1998**, *114*, 1653 – 1659.
[151] E. S. Klings, B. W. Christman, J. McClung, A. F. Stucchi, L. McMahon, M. Brauer, H. W. Farber, *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 1248 – 1252.
[152] a) E. A. Meagher, O. P. Barry, A. Burke, M. R. Lucey, J. A. Lawson, J. Rokach, G. A. FitzGerald, *J. Clin. Invest.* **1999**, *104*, 805 – 813; b) S. I. Aleynik, M. A. Leo, M. K. Aleynik, C. S. Lieber, *Alcohol. Clin. Exp. Res.* **1998**, *22*, 192 – 196; c) D. Pratico, L. Iuliano, S. Basili, D. Ferro, C. Camastra, C. Cordova, G. A. FitzGerald, F. Violi, *J. Invest. Med.* **1998**, *46*, 51 – 57.
[153] M. A. Leo, S. I. Aleynik, J. H. Siegel, F. E. Kasmin, M. K. Aleynik, C. S. Lieber, *Am. J. Gastroenterol.* **1997**, *92*, 2069 – 2072.
[154] A. Burke, G. A. FitzGerald, M. R. Lucey, *Transplantation* **2002**, *74*, 217 – 221.
[155] A. Aboutwerat, P. W. Pemberton, A. Smith, P. C. Burrows, R. F. McMahon, S. K. Jain, T. W. Warnes, *Biochim. Biophys. Acta Mol. Basis Dis.* **2003**, *1637*, 142 – 150.
[156] a) T. J. Montine, M. D. Neely, J. F. Quinn, M. F. Beal, W. R. Markesbery, L. J. Roberts II, J. D. Morrow, *Free Radical Biol. Med.* **2002**, *33*, 620 – 626; b) D. Praticò, C. M. Clark, V. M. Lee, J. Q. Trojanowski, J. Rokach, G. A. FitzGerald, *Ann. Neurol.* **2000**, *48*, 809 – 812; c) T. J. Montine, M. F. Beal, M. E. Cudkowicz, H. O'Donnell, R. A. Margolin, L. McFarland, A. F. Bachrach, W. E. Zackert, L. J. Roberts II, J. D. Morrow, *Neurology* **1999**, *52*, 562 – 565; d) T. J. Montine, W. R. Markesbery, W. Zackert, S. C. Sanchez, L. J. Roberts II, J. D. Morrow, *Am. J. Pathol.* **1999**, *155*, 863 – 868; e) D. Pratico, V. M.-Y. Lee, J. Q. Trojanowski, J. Rokach, G. A. Fitzgerald, *FASEB J.* **1998**, *12*, 1777 – 1783.
[157] A. Greco, L. Minghetti, G. Levi, *Neurochem. Res.* **2000**, *25*, 1357 – 1364.
[158] A. Greco, L. Minghetti, *Curr. Neurovasc. Res.* **2004**, *1*, 341 – 354.
[159] a) J. L. Cracowski, C. Marpeau, P. H. Carpentier, B. Imbert, M. Hunt, F. Stanke-Labesque, G. Bessard, *Arthritis Rheum.* **2001**, *44*, 1143 – 1148; b) C. M. Stein, S. B. Tanner, J. A. Awad, L. J. Roberts II, J. D. Morrow, *Arthritis Rheum.* **1996**, *39*, 1146 – 1150.
[160] a) J. L. Cracowski, B. Bonaz, G. Bessard, J. Bessard, C. Anglade, J. Fournet, *Am. J. Gastroenterol.* **2002**, *97*, 99 – 103; b) B. E. Wendland, E. Aghdassi, C. Tam, J. Carrrier, A. H. Steinhart, S. L. Wolman, D. Baron, J. P. Allard, *Am. J. Clin. Nutr.* **2001**, *74*, 259 – 264.
[161] S. Basu, K. Michaelsson, H. Olofsson, S. Johansson, H. Melhus, *Biochem. Biophys. Res. Commun.* **2001**, *288*, 275 – 279.
[162] X. Ming, T. P. Stein, M. Brimacombe, W. G. Johnson, G. H. Lambert, G. C. Wagner, *Prostaglandins Leukotrienes Essent. Fatty Acids* **2005**, *73*, 379 – 384.
[163] S. Basu, M. Whiteman, D. L. Mattey, B. Halliwell, *Ann. Rheum. Dis.* **2001**, *60*, 627 – 631.
[164] H. Sinzinger, G. Lupattelli, F. Chehne, A. Oguogho, C. D. Furberg, *J. Clin. Pharm. Ther.* **2001**, *26*, 303 – 310.
[165] K. Minoguchi, T. Yokoe, A. Tanaka, S. Ohta, T. Hirano, G. Yoshino, C. P. O'Donnell, M. Adachi, *Eur. Respir. J.* **2006**, *28*, 378 – 385.
[166] J. L. Cracowski, T. Durand, *Fundam. Clin. Pharmacol.* **2006**, *20*, 417 – 427.
[167] J. L. Cracowski, C. Cracowski, G. Bessard, J. L. Pepin, J. Bessard, C. Schwebel, F. Stanke-Labesque, C. Pison, *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 1038 – 1042.

[168] E. Schwedhelm, A. Bartling, H. Lenzen, D. Tsikas, R. Maas, J. Brummer, F. M. Gutzki, J. Berger, J. C. Frolich, R. H. Boger, *Circulation* **2004**, *109*, 843 – 848.
[169] M. Gross, M. Steffes, D. R. Jacobs Jr. , X. Yu, L. Lewis, C. E. Lewis, C. M. Loria, *Clin. Chem.* **2004**, *51*, 125 – 131.
[170] a) B. Halliwell, *Cardiovasc. Res.* **2000**, *47*, 410 – 418; b) L. J. Roberts II, J. D. Morrow, *Cell. Mol. Life Sci.* **2002**, *59*, 808 – 820.
[171] L. Kritharides, R. Stocker, *Atherosclerosis* **2002**, *164*, 211 – 219.
[172] J. Helmersson, S. Basu, *Prostaglandins Leukotrienes Essent. Fatty Acids* **1999**, *61*, 203 – 205.
[173] E. E. Reich, K. S. Montine, M. D. Gross, L. J. Roberts II, L. L. Swift, J. D. Morrow, T. J. Montine, *J. Neurosci.* **2001**, *21*, 5993 – 5999.
[174] G. L. Milne, E. S. Musiek, J. D. Morrow, *Antioxid. Redox Signaling* **2005**, *7*, 210 – 220.
[175] E. S. Musiek, J. K. Cha, H. Yin, W. E. Zackert, E. S. Terry, N. A. Porter, T. J. Montine, J. D. Morrow, *J. Chromatogr. B* **2004**, *799*, 95 – 102.
[176] E. E. Reich, W. R. Markesbery, L. J. Roberts II, L. L. Swift, J. D. Morrow, T. J. Montine, *Am. J. Pathol.* **2001**, *158*, 293 – 297.
[177] J. P. Fessel, C. Hulette, S. Powell, L. J. Roberts II, J. Zhang, *J. Neurochem.* **2003**, *85*, 645 – 650.
[178] R. G. Salomon, G. Subbanagounder, J. O'Neil, K. Kaur, M. A. Smith, H. F. Hoff, G. Perry, V. M. Monnier, *Chem. Res. Toxicol.* **1997**, *10*, 536 – 545.
[179] S. Parchmann, H. Gundlach, M. J. Mueller, *Plant Physiol.* **1997**, *115*, 1057 – 1064.
[180] K. Karg, V. M. Dirsch, A. M. Vollmar, J. L. Cracowski, F. Laporte, M. J. Mueller, *Free Radical Res.* **2007**, *41*, 25 – 37.
[181] a) E. S. Musiek, H. Yin, G. L. Milne, J. D. Morrow, *Lipids* **2005**, *40*, 987 – 994; b) J. D. Morrow, *Curr. Pharm. Des.* **2006**, *12*, 895 – 902.
[182] J. L. Cracowski, P. Devillier, T. Durand, F. Stanke-Labesque, G. Bessard, *J. Vasc. Res.* **2001**, *38*, 93 – 103.
[183] L. J. Janssen, *Chem. Phys. Lipids* **2004**, *128*, 101 – 116.
[184] P. McNamara, J. A. Lawson, J. Rokach, G. A. FitzGerald, *Adv. Exp. Med. Biol.* **2002**, *507*, 351 – 355.
[185] For further discussions, see: a) J. M. Dogne, J. Hanson, D. Pratico, *Trends Pharmacol. Sci.* **2005**, *26*, 639 – 644; b) G. Davì, A. Falco, *Lupus* **2005**, *14*, 760 – 764; c) C. Patrono, G. A. FitzGerald, *Arterioscler. Thromb. Vasc. Biol.* **1997**, *17*, 2309 – 2315.
[186] X. Hou, F. Gobeil Jr. , K. Peri, G. Speranza, A. M. Marrache, P. Lachapelle, J. Roberts II, D. R. Varma, S. Chemtob, *Stroke* **2000**, *31*, 516 – 525.
[187] K. Takahashi, T. M. Nammour, M. Fukunaga, J. Ebert, J. D. Morrow, L. J. Roberts II, R. L. Hoover, K. F. Badr, *J. Clin. Invest.* **1992**, *90*, 136 – 141.
[188] P. Kunapuli, J. A. Lawson, J. Rokach, G. A. FitzGerald, *J. Biol. Chem.* **1997**, *272*, 27147 – 27154.
[189] T. Yura, M. Fukunaga, R. Khan, G. N. Nassar, K. F. Badr, A. Montero, *Kidney Int.* **1999**, *56*, 471 – 478.
[190] T. Ishizuka, K. Suzuki, M. Kawakami, T. Hidaka, Y. Matsuki, H. Nakamura, *Eur. J. Pharmacol.* **1996**, *312*, 367 – 377.
[191] P. J. Spagnuolo, J. J. Ellner, A. Hassid, M. J. Dunn, *Inflammation* **1988**, *12*, 1 – 9.
[192] I. Tzoulaki, G. D. Murray, A. J. Lee, A. Rumley, G. D. Lowe, F. G. Fowkes, *Circulation* **2005**, *112*, 976 – 983.
[193] L. Lind, *Atherosclerosis* **2003**, *169*, 203 – 214.
[194] M. Tang, T. Cyrus, Y. Yao, L. Vocun, D. Pratico, *Circulation* **2005**, *112*, 2867 – 2874.
[195] a) L. J. Janssen, A. Catalli, P. Helli, *Antioxid. Redox Signaling* **2005**, *7*, 244 – 255; b) L. J. Janssen, T. Tazzeo, *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1060 – 1066; c) J.-L. Cracowski, P. Devillier, O. Chavanon, F.-A. Sietchiping-Nzepa, F. Stanke-Labesque, G. Bessard, *Life Sci.* **2001**, *68*, 2405 – 2413.
[196] A. E. Catalli, L. J. Janssen, *Am. J. Physiol. Lung Cell Mol. Physiol.* **2004**, *287*, L1035 – 1041.
[197] S. Marlière, J. L. Cracowski, T. Durand, O. Chavanon, J. Bessard, A. Guy, F. Stanke-Labesque, J. C. Rossi, G. Bessard, *Br. J. Pharmacol.* **2002**, *135*, 1276 – 1280.
[198] X. Hou, L. J. Roberts II, F. Gobeil Jr. , D. F. Taber, K. Kanai, D. Abran, S. Brault, D. Checchin, F. Sennlaub, P. Lachapelle, D. R. Varma, S. Chemtob, *Free Radical Biol. Med.* **2004**, *36*, 163 – 172.
[199] X. Hou, L. J. Roberts II, D. F. Taber, J. D. Morrow, K. Kanai, F. Gobeil Jr. , M. H. Beauchamp, S. G. Bernier, G. Lepage, D. R. Varma, S. Chemtob, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2001**, *281*, 391 – 400.
[200] J.-L. Cracowski, L. Camus, T. Durand, P. Devillier, A. Guy, G. Hardy, F. Stanke-Labesque, J.-C. Rossi, G. Bessard, *J. Cardiovasc. Pharmacol.* **2002**, *39*, 396 – 403.
[201] C. Liu, T. Tazzeo, A. Guy, T. Durand, L. J. Janssen, *Prostaglandins Leukotrienes Essent. Fatty Acids* **2007**, *76*, 57 – 64.
[202] a) G. Zanoni, A. Porta, G. Vidari, *J. Org. Chem.* **2002**, *67*, 4346 – 4351; b) G. Zanoni, A. Porta, F. Castronovo, G. Vidari, *J. Org. Chem.* **2003**, *68*, 6005 – 6010.
[203] a) A. L. Levonen, A. Landar, A. Ramachandran, E. K. Ceaser, D. A. Dickinson, G. Zanoni, J. D. Morrow, V. M. Darley-Usmar, *Biochem. J.* **2004**, *378*, 373 – 382; b) A. L. Levonen, D. A. Dickinson, D. R. Moellering, R. T. Mulcahy, H. J. Forman, V. M. Darley-Usmar, *Arterioscler. Thromb. Vasc. Biol.* **2001**, *21*, 1846 – 1851.
[204] E. S. Musiek, L. Gao, G. L. Milne, W. Han, M. B. Everhart, D. Wang, M. G. Backlund, R. N. DuBois, G. Zanoni, G. Vidari, T. S. Blackwell, J. D. Morrow, *J. Biol. Chem.* **2005**, *280*, 35562 – 35570.
[205] E. S. Musiek, G. L. Milne, B. McLaughlin, J. D. Morrow, *Brain Pathol.* **2005**, *15*, 149 – 158.
[206] K. K. Murthi, R. G. Salomon, H. Sternlicht, *Prostaglandins* **1990**, *39*, 611 – 622.
[207] K. K. Murthi, L. R. Friedman, N. L. Oleinick, R. G. Salomon, *Biochemistry* **1993**, *32*, 4090 – 4097.
[208] a) I. Thoma, M. Krischke, C. Loeffler, M. J. Mueller, *Chem. Phys. Lipids* **2004**, *128*, 135 – 148; b) M. Iqbal, P. Evans, A. Lledo, X. Verdaguer, M. A. Pericas, A. Riera, C. Loeffler, A. K. Sinha, M. J. Mueller, *Chembiochem* **2005**, *6*, 276 – 280.
[209] C. Loeffler, S. Berger, A. Guy, T. Durand, G. Bringmann, M. Dreyer, U. von Rad, J. Durner, M. J. Mueller, *Plant Physiol.* **2005**, *137*, 328 – 340.
[210] C. Grun, S. Berger, D. Matthes, M. J. Mueller, *Funct. Plant Biol.* **2007**, *34*, 65 – 71.
[211] C. Traidl-Hoffmann, V. Mariani, H. Hochrein, K. Karg, H. Wagner, J. Ring, M. J. Mueller, T. Jakob, H. Behrendt, *J. Exp. Med.* **2005**, *201*, 627 – 636.
[212] a) A. Guy, T. Durand, J.-P. Vidal, J.-C. Rossi, *Tetrahedron Lett.* **1997**, *38*, 1543 – 1546; b) T. Durand, A. Guy, J.-P. Vidal, J.-C. Rossi, *J. Org. Chem.* **2002**, *67*, 3615 – 3624.
[213] G. Just, C. Luthe, *Can. J. Chem.* **1980**, *58*, 1799 – 1805.
[214] D. F. Taber, R. S. Hoerrner, R. J. Herr, D. M. Gleave, K. Kanai, R. Pina, Q. Jiang, M. Xu, *Chem. Phys. Lipids* **2004**, *128*, 57 – 67.
[215] In very early synthetic investigations in the prostaglandin field, related C20 derivatives with oxobicyclo[3.1.0]hexylcarbinol structures were solvolyzed to give 15-E_1-IsoP and 15-F_1-IsoP. Their yields were, however, below 10%; see: a) W. P. Schneider, U. Axen, F. H. Lincoln, J. E. Pike, J. L. Thompson, *J. Am. Chem. Soc.* **1968**, *90*, 5895 – 5896; b) G. Just, C. Simonovitch, F. H. Lincoln, W. P. Schneider, U. Axen, G. B. Spero, J. E. Pike, *J. Am. Chem. Soc.* **1969**, *91*, 5364 – 5371; c) W. P. Schneider, U. Axen, F. H. Lincoln, J. E. Pike, J. L. Thompson, *J. Am. Chem. Soc.* **1969**, *91*, 5372 – 5378.
[216] a) D. F. Taber, R. J. Herr, D. M. Gleave, *J. Org. Chem.* **1997**, *62*, 194 – 198; b) D. F. Taber, K. Kanai, *Tetrahedron* **1998**, *54*, 11767 – 11782.

[217] a) D. F. Taber, K. Kanai, R. Pina, *J. Am. Chem. Soc.* **1999**, *121*, 7773 – 7777; b) D. F. Taber, Q. Jiang, *J. Org. Chem.* **2001**, *66*, 1876 – 1884; c) D. F. Taber, M. Xu, J. C. Hartnett, *J. Am. Chem. Soc.* **2002**, *124*, 13121 – 13126; Correction: *J. Am. Chem. Soc.* **2002**, *124*, 15400; d) D. F. Taber, R. S. Hoerrner, *J. Org. Chem.* **1992**, *57*, 441 – 447; e) D. F. Taber, K. Kanai, *J. Org. Chem.* **1999**, *64*, 7983 – 7987; f) D. F. Taber, D. Teng, *J. Org. Chem.* **2002**, *67*, 1607 – 1612.
[218] D. F. Taber, Q. Jiang, *Tetrahedron* **2000**, *56*, 5991 – 5994.
[219] D. F. Taber, K. Kanai, *J. Org. Chem.* **1998**, *63*, 6607 – 6609.
[220] R. C. Larock, N. H. Lee, *J. Am. Chem. Soc.* **1991**, *113*, 7815 – 7816.
[221] a) L. G. Quan, J. K. Cha, *J. Am. Chem. Soc.* **2002**, *124*, 12424 – 12425; b) L. G. Quan, J. K. Cha, *Chem. Phys. Lipids* **2004**, *128*, 3 – 14.
[222] Z. Pudukulathan, S. Manna, S.-W. Hwang, S. P. Khanapure, J. A. Lawson, G. A. FitzGerald, J. Rokach, *J. Am. Chem. Soc.* **1998**, *120*, 11953 – 11961.
[223] S. H. Jacobo, C.-T. Chang, G.-J. Lee, J. A. Lawson, W. S. Powell, D. Pratico, G. A. FitzGerald, J. Rokach, *J. Org. Chem.* **2006**, *71*, 1370 – 1379.
[224] a) T. O. Schrader, M. L. Snapper, *Tetrahedron Lett.* **2000**, *41*, 9685 – 9689; b) T. O. Schrader, M. L. Snapper, *J. Am. Chem. Soc.* **2002**, *124*, 10998 – 11000; c) M. Shizuka, M. L. Snapper, *Synthesis* **2007**, 2397 – 2403; d) M. Shizuka, T. O. Schrader, M. L. Snapper, *J. Org. Chem.* **2006**, *71*, 1330 – 1334.
[225] a) H. P. Acharya, Y. Kobayashi, *Angew. Chem.* **2005**, *117*, 3547 – 3550; *Angew. Chem. Int. Ed.* **2005**, *44*, 3481 – 3484; b) H. P. Acharya, Y. Kobayashi, *Tetrahedron Lett.* **2005**, *46*, 8435 – 8438; c) H. P. Acharya, K. Miyoshi, Y. Kobayashi, *Org. Lett.* **2007**, *9*, 3535 – 3538.
[226] In an earlier investigation by Jung et al. the relative configuration of the epoxy moiety and of C12 was missassigned, see: M. E. Jung, A. Kers, G. Subbanagounder, J. A. Berliner, *Chem. Commun.* **2003**, 196 – 197.
[227] M. E. Jung, J. A. Berliner, D. Angst, D. Yue, L. Koroniak, A. D. Watson, R. Li, *Org. Lett.* **2005**, *7*, 3933 – 3935.
[228] A. Schmidt, W. Boland, *J. Org. Chem.* **2007**, *72*, 1699 – 1706.
[229] G. Helmchen, M. Ernst, G. Paradies, *Pure Appl. Chem.* **2004**, *76*, 495 – 506.
[230] a) F. Van Hulle, V. Sipido, M. Vandewalle, *Tetrahedron Lett.* **1973**, *14*, 2213 – 2216; b) P. De Clercq, M. De Smet, K. Legein, F. Vanhulle, M. Vandewalle, *Bull. Soc. Chim. Belg.* **1976**, *85*, 503 – 522.
[231] N. Nakamura, K. Sakai, *Tetrahedron Lett.* **1978**, *19*, 1549 – 1552.
[232] a) K. Weinges, G. Braun, B. Oster, *Liebigs Ann. Chem.* **1983**, 2197 – 2214; b) K. Weinges, W. Huber, U. Huber-Patz, H. Irngartinger, M. Nixdorf, H. Rodewald, *Liebigs Ann. Chem.* **1984**, 761 – 772.
[233] R. Bernini, E. Davini, C. Iavarone, C. Trogolo, *J. Org. Chem.* **1986**, *51*, 4600 – 4603.
[234] a) M. Naruto, K. Ohno, N. Naruse, H. Takeuchi, *Chem. Lett.* **1978**, 1423 – 1424; b) K. Ohno, M. Naruto, *Chem. Lett.* **1979**, 1015 – 1016; c) K. Ohno, M. Naruto, *Chem. Lett.* **1980**, 175 – 176.
[235] a) W. F. Berkowitz, I. Sasson, P. S. Sampathkumar, J. Hrabie, S. Choudhry, D. Pierce, *Tetrahedron Lett.* **1979**, *20*, 1641 – 1644; b) C. Bonini, C. Iavarone, C. Trogolo, R. Di Fabio, *J. Org. Chem.* **1985**, *50*, 958 – 961; c) K. Weinges, D. Brunner, *Liebigs Ann. Chem.* **1986**, 54 – 68.
[236] S. Lai, D. Lee, J. S. U, J. K. Cha, *J. Org. Chem.* **1999**, *64*, 7213 – 7217.
[237] a) G. Zanoni, A. Porta, A. Meriggi, M. Franzini, G. Vidari, *J. Org. Chem.* **2002**, *67*, 6064 – 6069; b) G. Zanoni, E. M. Brunoldi, A. Porta, G. Vidari, *J. Org. Chem.* **2007**, *72*, 9698 – 9703.
[238] B. Rondot, T. Durand, J. P. Girard, J. C. Rossi, L. Schio, S. P. Khanapure, J. Rokach, *Tetrahedron Lett.* **1993**, *34*, 8245 – 8248.
[239] B. Rondot, T. Durand, J.-C. Rossi, P. Rollin, *Carbohydr. Res.* **1994**, *261*, 149 – 156.
[240] a) B. Rondot, T. Durand, J.-P. Vidal, J.-P. Girard, J.-C. Rossi, *J. Chem. Soc. Perkin Trans. 2* **1995**, 1589 – 1594; b) A. Roland, T. Durand, D. Egron, J.-P. Vidal, J.-C. Rossi, *J. Chem. Soc. Perkin Trans. 1* **2000**, 245 – 251.
[241] A. Roland, T. Durand, B. Rondot, J.-P. Vidal, J.-C. Rossi, *Bull. Soc. Chim. Fr.* **1996**, *133*, 1149 – 1154.
[242] J.-P. Vionnet, P. Renaud, *Helv. Chim. Acta* **1994**, *77*, 1781 – 1790.
[243] a) T. Durand, A. Guy, O. Henry, A. Roland, S. Bernad, S. El Fangour, J.-P. Vidal, J.-C. Rossi, *Chem. Phys. Lipids* **2004**, *128*, 15 – 33; b) S. El Fangour, A. Guy, V. Despres, J.-P. Vidal, J.-C. Rossi, T. Durand, *J. Org. Chem.* **2004**, *69*, 2498 – 2503.
[244] a) S. W. Hwang, M. Adiyaman, S. Khanapure, L. Schio, J. Rokach, *J. Am. Chem. Soc.* **1994**, *116*, 10829 – 10830; b) J. Rokach, S. P. Khanapure, S. W. Hwang, M. Adiyaman, L. Schio, G. A. FitzGerald, *Synthesis* **1998**, 569 – 580.
[245] E. J. Corey, N. M. Weinshenker, T. K. Schaaf, W. Huber, *J. Am. Chem. Soc.* **1969**, *91*, 5675 – 5677.
[246] a) U. Jahn, E. Dinca, unpublished results; for an outline of the strategy, see: b) U. Jahn, P. Hartmann, I. Dix, P. G. Jones, *Eur. J. Org. Chem.* **2002**, 718 – 735.
[247] G. A. Tolstikov, F. A. Valeev, I. P. Ibragimova, I. N. Gaisina, L. V. Spirikhin, M. S. Miftakhov, *Zh. Org. Khim.* **1992**, *28*, 1875 – 1882.
[248] a) J. Mulzer, M. Czybowski, J. W. Bats, *Tetrahedron Lett.* **2001**, *42*, 2961 – 2964; b) J. Mulzer, A. K. Kermanchahi, J. Buschmann, P. Luger, *Liebigs Ann. Chem.* **1994**, 531 – 539.
[249] D. P. Curran, N. A. Porter, B. Giese, *Stereochemistry of Radical Reactions*, VCH, Weinheim, **1996**, pp. 59 – 61.
[250] F. Belval, A. Fruchier, C. Chavis, J.-L. Montero, M. Lucas, *J. Chem. Soc. Perkin Trans. 1* **1999**, 697 – 704.
[251] H. Suemune, T. Kawahara, K. Sakai, *Chem. Pharm. Bull.* **1986**, *34*, 550 – 557.
[252] a) M. Iqbal, P. Evans, *Tetrahedron Lett.* **2003**, *44*, 5741 – 5745; b) M. Iqbal, Y. Li, P. Evans, *Tetrahedron* **2004**, *60*, 2531 – 2538.
[253] D. F. Taber, J. H. Green, W. Zhang, R. Song, *J. Org. Chem.* **2000**, *65*, 5436 – 5439.
[254] a) C. J. Sih, P. Price, R. Sood, R. G. Salomon, G. Peruzzotti, M. Casey, *J. Am. Chem. Soc.* **1972**, *94*, 3643 – 3644; b) F. S. Alvarez, D. Wren, A. Prince, *J. Am. Chem. Soc.* **1972**, *94*, 7823 – 7827; c) A. F. Kluge, K. G. Untch, J. H. Fried, *J. Am. Chem. Soc.* **1972**, *94*, 7827 – 7832.
[255] For a review on directed protonation of enolates, see: N. Krause, S. Ebert, A. Haubrich, *Liebigs Ann./Recueil* **1997**, 2409 – 2418.
[256] a) A. R. Rodríguez, B. W. Spur, *Tetrahedron Lett.* **2003**, *44*, 7411 – 7415; b) A. R. Rodríguez, B. W. Spur, *Tetrahedron Lett.* **2002**, *43*, 9249 – 9253; c) A. R. Rodríguez, B. W. Spur, *Tetrahedron Lett.* **2002**, *43*, 4575 – 4579.
[257] E. Loza, D. Lola, J. Freimanis, I. Turovskis, M. Gavars, A. Liepipa, *Latv. PSR Zinat. Akad. Vestis Kim. Ser.* **1985**, *4*, 465 – 472.
[258] S. El Fangour, A. Guy, J.-P. Vidal, J.-C. Rossi, T. Durand, *J. Org. Chem.* **2005**, *70*, 989 – 997.
[259] E. Pinot, A. Guy, A.-L. Guyon, J.-C. Rossi, T. Durand, *Tetrahedron: Asymmetry* **2005**, *16*, 1893 – 1895.
[260] a) D. B. Miller, S. R. Raychaudhuri, K. Avasthi, K. Lal, B. Levison, R. G. Salomon, *J. Org. Chem.* **1990**, *55*, 3164 – 3175; b) G. Subbanagounder, R. G. Salomon, K. K. Murthi, C. Brame, L. J. Roberts II, *J. Org. Chem.* **1997**, *62*, 7658 – 7666; c) S. C. Roy, L. Nagarajan, R. G. Salomon, *J. Org. Chem.* **1999**, *64*, 1218 – 1224; d) W. Sha, R. G. Salomon, *J. Org. Chem.* **2000**, *65*, 5315 – 5326.
[261] V. Amarnath, K. Amarnath, T. Masterson, S. Davies, L. J. Roberts II, *Synth. Commun.* **2005**, *35*, 397 – 408.

[262] D. F. Taber, Y. Pan, X. Zhao, *J. Org. Chem.* **2004**, *69*, 7234 – 7240.
[263] D. F. Taber, Z. Zhang, *J. Org. Chem.* **2006**, *71*, 926 – 933.
[264] a) B. A. Pandya, M. L. Snapper, *J. Org. Chem.* **2008**, *73*, 3754 – 3758; b) D. F. Taber, P. G. Reddy, K. O. Arneson, *J. Org. Chem.* **2008**, *73*, 3467 – 3474; c) E. Pinot, A. Guy, A. Fournial, L. Balas, J.-C. Rossi, T. Durand, *J. Org. Chem.* **2008**, *73*, 3063 – 3069; d) P. Elsner, P. Jetter, K. Brodner, G. Helmchen, *Eur. J. Org. Chem.* **2008**, 2551 – 2563.

