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Tereza PAVLÍČKOVÁ:
Report on PhD thesis dissertation

Tereza PAVLÍČKOVÁ presents a PhD thesis manuscript entitled "Total Syntheses of Neuroprostanes" for obtaining the degree of Doctor of the Charles University (Institute of Organic Chemistry Biochemistry, Czech Academy of Sciences directed by RNDr. PhDr. Zdeněk Hostomský, CSc.) and Montpellier University (Institut des Biomolécules Max Mousseron, Faculty of Pharmacy, directed by Prof. Pascal Dumy).

The PhD work was carried out in partnership between Prague and Montpellier, under the supervision of Dr. Ullrich Jahn (IOCB Prague), Dr Jean-Marie Galano (University of Montpellier) and Dr Camille Oger (University of Montpellier).

The dissertation focuses on neuroprostanes, cyclic oxygenated metabolites elaborated *in vivo* from docosahexaenoic acid, the main polyunsaturated fatty acid of the human brain. The isolation and identification of these complex regio- and diastereoisomeric mixtures are difficult, making their synthesis imperative for investigating the biological properties of each molecule and their potential as biomarkers of oxidative stress. In this manuscript, two different synthetic approaches targeting the most abundant regioisomeric series of neuroprostanes are described.

The manuscript is structured into five chapters. The first chapter is a general presentation of the polyunsaturated fatty acids (PUFAs), isoprostanoids (IsoPs), neuroprostanes and their biosynthesis. In the second chapter, the biological background and previous approaches to isoprostanoids are described. The ambition of the work is specified in the third chapter. The following fourth chapter relies on the personal work carried out by Tereza PAVLÍČK. This part is concise, very clear and particularly pedagogic. The fifth and last chapter corresponds to the general conclusion describing the main advances of the synthetic approaches and future works. An experimental part completes the manuscript.

In the first chapter, the relation between PUFAs, IsoPs and NeuroPs is specified. PUFAs are a group of lipids essential to all living cells, which can be oxidatively transformed into oxylipins under physiological conditions. Without enzymatic assistance, PUFAs lead to isoprostanes. There are three major classes of isoprostanes: IsoPs derived from arachidonic acid (AA) in animals, phytoprostanes

(PhytoPs) from α-linolenic acid (ALA) in plants and neuroprostanes (NeuroPs) from docosohexanoic acid (DHA) in the grey matter of nervous system. The biosynthesis of NeuroPs from DHA is quite complex : in contrast to prostaglandins (PGs) whose configuration is determined by the COX active site, the configuration at the cyclopentane ring in NeuroPs corresponds to main conformers of endoperoxide radicals, which explains a larger structural diversity. Finally, a clear but relatively complex system of nomenclature of isoprostanoids is presented for unambiguously assigning a name to each molecule.

The second chapter relies on a presentation of the biological properties of NeuroPs and a description of the previous synthetic approaches relevant to this PhD work. Information concerning bioactivities of NeuroPs still remained limited due to the low number of total syntheses of these metabolites allowing access to pure material. However, it has shown anti-inflammatory and anti-arrhythmic activities for some of them. NeuroPs are also biomarkers of oxidative stress and thus find applications in diagnostics of some neurological diseases.

Previous syntheses of isoprostanoids mostly involved IsoPs but a few reports concern PhytoPs and NeuroPs. The formation of cis-disubstituted cyclopentenone moiety of some isoprostanoids is challenging due to problematic epimerization. The elaboration of A-, J-IsoPs, NeuroPs, PhytoPs and preclavulanone are reported in a first part. Then, the different synthetic approaches to (*Z*)-polyene chains of NeuroPs and their connection to the cyclopentane core are described. The Wittig homologation corresponds to the strategy used by the group. Furthermore, cross-metathesis could be considered as a valuable mild protocol for the construction of the (*E*)-hydroxyallylic chain in isoprostanoid syntheses. Finally, the pertinent Jahn's approach to isoprostanoids through radical oxidative cyclization is presented, allowing the elaboration of relevantly functionalized cyclopentanes. This strategy was later applied to the total synthesis of various IsoPs and PhytoPs.

In the third chapter, the objectives of the PhD work were presented. The group envisages the elaboration of A- and J-NeuroPs through the synthesis of accurately functionalized cyclopentenones by RCM and further introduction of the side chains. They also intend to develop a strategy based on Jahn's oxidative dianion cyclization from an enantiomerically enriched precursor, for an application to the total synthesis of 18-F_{3t}-IsoP and 20-NeuroPs.

The fourth chapter involves personal works of Tereza PAVLÍČKOVÁ.

The first part describes the results toward the total synthesis of cyclopentenone 4-NeuroPs. Three strategies were investigated. The first one involved asymmetric organocatalytic Michael addition of a malonate to an enal, through iminium catalysis. However, this route was discarded due to various difficulties. The second strategy also relies on asymmetric organocatalytic Michael addition as a key step, but employing a nitroolefin and an aldehyde derived from butanediol as starting materials. The yield is good (85%), the low observed diastereoselectivity (rd: *syn*/*anti* 1:3) could be overcome hereafter and the enantioselectivity is sufficiently high (86 and 90% ees) to study the following steps of the synthesis. Indeed thermodynamic equilibration allows the formation of the desired *trans*-lactone. Finally, the required metathesis precursor was obtained by an appropriate and particularly subtle functionalization of different carbonyles. The third strategy involves the racemic formation of this precursor, in a more direct sequence including β-vinylation of commercial 5,6-dihydro-2*H*-pyran-2-one and subsequent diastereoselective α-vinylation. The successful second asymmetric strategy was then applied to the synthesis of 4-A_4-NeuroP. HG-II catalyst added in small portions allowed a double olefin metathesis (RCM and CM) providing desired cyclopentenes with the α-chain installed, in 55% yield as a 2:1 diastereomeric mixture. Further reduction of the ketone with CBS did not proceed in high

selectivity. For connecting the ω-chain, a Wittig reaction was employed to efficiently provide a skeleton with the skipped (*Z*)-triene. Final oxidation and deprotection steps were quite problematic due to the instability of these systems; however, two potential metabolites of 4-A4-NeuroP were elaborated in 1.7 and 1.2% isolated yield over 21 and 22 steps.

The second part reports the results about the total synthesis of isoprostanoids with 3-hydroxypentenyl ω-chain and skipped (Z)-polyene α-chain. A tandem ferrocenium-mediated SET oxidation/radical cyclization/TEMPO oxygenation of an enantiomerically enriched β-hydroxy-ester followed by an alkynylation with propargyl chloride allowed the synthesis of a central precursor. Submitted to Cu(I)-mediated C(sp3)-C(sp) bond coupling conditions and then to Lindlar semi-hydrogenation of the formed diynes, this framework leads to *rac*-18(*RS*)-18-F_{3t}-IsoP, synthesized for the first time, in 5% yield over 14 steps. For an application to the synthesis of 20-NeuroPs, the conditions of hydrogenation of a diyne were optimized thus providing a skipped diene as a key precursor in 14% yield over 10 steps.

The general conclusion discussed in **Chapter Five** summarizes the important points of this PhD work which allowed the synthesis of two potential metabolites of 4-A4-NeuroP and to *rac*-18(*RS*)-18-F_{3t}-IsoP along with a general access to a precursor of 20-NeuroPs.

The experimental part is important, rigorous and particularly precise; the experimental protocols are very accurately described. The compounds are characterized without ambiguity.

The **summary at the end of the manuscript** is entirely welcome.

A particularly original and well adapted synthetic strategy of NeuroPs and IsoPs has been designed and developed; many problems have been solved.

Tereza PAVLÍČK performed a remarkable PhD work, of excellent scientific quality and impressive in quantity.

This is a very ambitious and particularly difficult work, both in synthetic methodology and in multi-step synthesis of targets with very complex and unstable structures. Through very well thought out and original strategies, Tereza PAVLÍČK was able to practically achieve all the objectives.

The obtained results are impressive; they confirm the relevance of the developed projects and arise from methodical and finely analyzed studies. Reading the manuscript highlights the interest but also the difficulty of the various covered topics.

The bibliography is important and well documented.

The manuscript is very well written, concise, which makes it pleasant to read.

It reveals Tereza PAVLÍČK's excellent training, her competence in organic chemistry and total synthesis as well as her qualities as a researcher.

Consequently, Tereza PAVLÍČK has my total agreement to defend her thesis.

Janick Ardisson