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**To be cited as:** Eur. J. Org. Chem. 10.1002/ejoc.201601301

**Link to VoR:** <http://dx.doi.org/10.1002/ejoc.201601301>

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# Total synthesis of isoketal 5-D<sub>2</sub>-IsoK natural product based on organocatalysis

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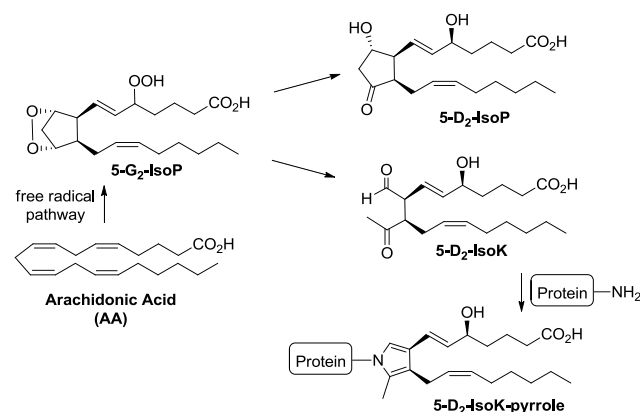
**Abstract:** The enantioselective total synthesis of the highly reactive isoketal 5-D<sub>2</sub>-IsoK has been accomplished. The synthesis features construction of the isoketals core via an organocatalyzed Michael addition between ideally functionalized aldehyde and nitroolefin partners in good enantioselectivity. The lateral chains are branched through Horner-Wadsworth-Emmons and Wittig olefinations in order to provide flexibility while the final deprotections and oxidation steps are run under mild conditions to avoid known racemization and/or degradation of the very sensitive isoketal.

Isoprostanes (IsoPs) are prostaglandin-like compounds discovered in the nineties as oxidative stress-induced non-enzymatic peroxidation products of arachidonic acid (C20:4 n-6).<sup>[1]</sup> They are formed in human body from phospholipid through bicyclic endoperoxide intermediates (G-IsoPs) which can be partially or completely reduced to produce the well known D-, E- or F-type of IsoPs (Scheme 1).<sup>[2]</sup> The same endoperoxides may also undergo rearrangement *in vivo* to produce isoketal derivatives (IsoKs), also called isolevuglandins.<sup>[3]</sup> IsoKs react with primary amine of proteins (lysine residues) or phosphatidylethanolamine to form corresponding pyrroles that are readily oxidized over time into highly stable lactam and hydroxylactam adducts.<sup>[4]</sup> Much more than just a dosimeter of oxidative stress,<sup>[5]</sup> IsoKs appear to be disease biomarkers in pathological processes as exogenous IsoKs on cultured cells include induction of inflammatory pathways, immune responses, and cell death, as well as inhibiting ion channel function.<sup>[6]</sup>

From a structural point of view, the IsoKs possess two aliphatic side chains containing two double bonds (*E* and *Z*), fixed onto a methylic  $\gamma$ -ketoaldehyde. However, despite its relatively simple chemical structures, this class of compounds represents a challenging target for the synthetic community. First, the endoperoxide rearrangements could give rise to the E- and D-isomers that bear three stereogenic centers, resulting in 64 possible IsoK isomers. Secondly, the construction of the highly reactive  $\gamma$ -ketoaldehyde motif is not a trivial task since the C8 and C12 stereogenic centers can readily racemize under acidic or basic conditions, and third, the  $\beta,\gamma$ -unsaturated aldehyde may easily go through conjugation or conjugation/dehydration of the hydroxyl group at C5.

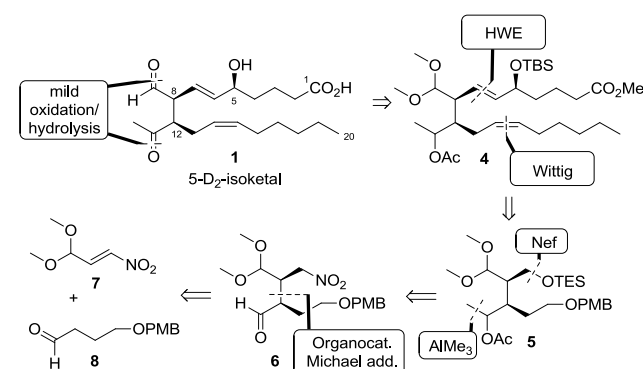
To the best of our knowledge, only the group of Salomon attempts this synthetic challenge. More particularly, they

reported the synthesis and biological investigations of two IsoK derivatives, namely, the 5-D<sub>2</sub>-IsoK and the 12-E<sub>2</sub>-IsoK, and the 17-E<sub>4</sub>-NeuroK derived from docosaheptaenoic acid.<sup>[7]</sup> The key features of their synthetic route include: 1) a diastereoselective 1,4-addition of an elaborated vinylcyanocuprate to a chiral  $\gamma$ -alkoxyenone to build the central core, 2) deprotection of the sensitive aldehyde function (protected as acetonide) in the last step under acidic conditions (AcOH, H<sub>2</sub>O) followed by a mild oxidative cleavage of a vicinal diol. However, this elegant strategy has some disadvantages that limits its practicality such as the low control of the stereochemical outcome of the 1,4-addition (dr 1.5/1), combined with epimerization of the acidic centers next to the keto and aldehyde functionalities.



**Scheme 1.** Biosynthesis of IsoKs and pyrrole derivatives.

Our interest in lipid peroxidation prompted us to develop a new strategy allowing the synthesis of IsoK derivatives by taking into account the inherent epimerization problems and also a more flexible approach for side chains introduction. Herein, we report our efforts to the enantioselective synthesis of 5-D<sub>2</sub>-IsoK-1 using as a key step an organocatalyzed Michael addition between a nitroolefin and an aldehyde.

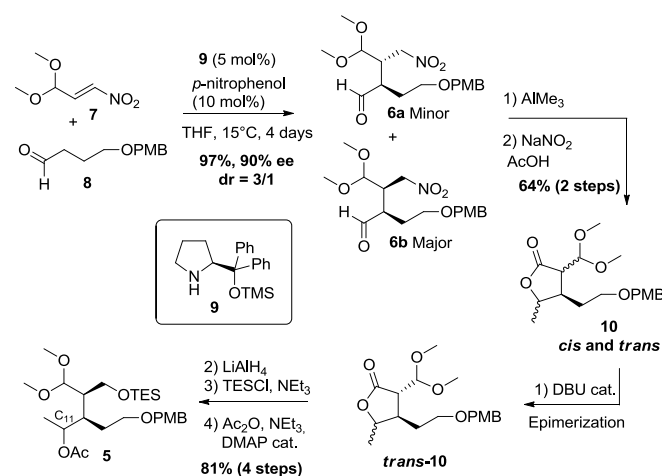


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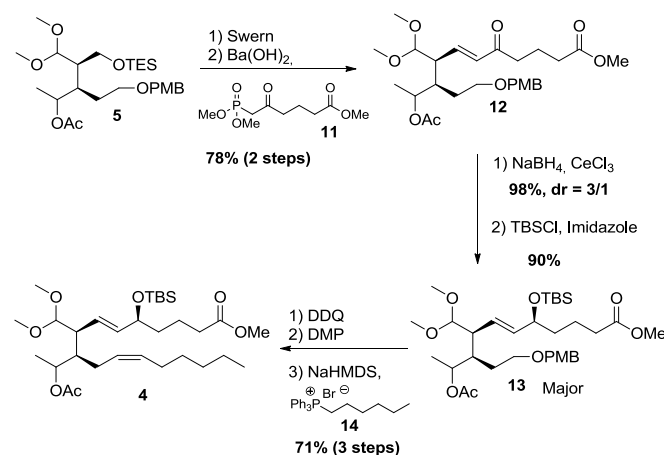
**Scheme 2.** Retrosynthetic approach.

Scheme 2 reveals the essential features of our retrosynthetic disconnections. First of all, we planned to uncover the sensitive aldehyde function of 5-D<sub>2</sub>-IsoK-1 at the last stage under mild conditions for dimethyl acetal hydrolysis of compound **4**. More classical and therefore versatile introduction of the two lateral chains would be installed by Wittig and Horner-Wadsworth-Emmons olefinations from **5**. Interconversion of the OTES and OAc groups of **5** by Nef reaction and simple methyl alkylation revealed intermediated **6** speaks for an organocatalyzed Michael addition between simple aldehyde **8** and nitroolefin **7**.

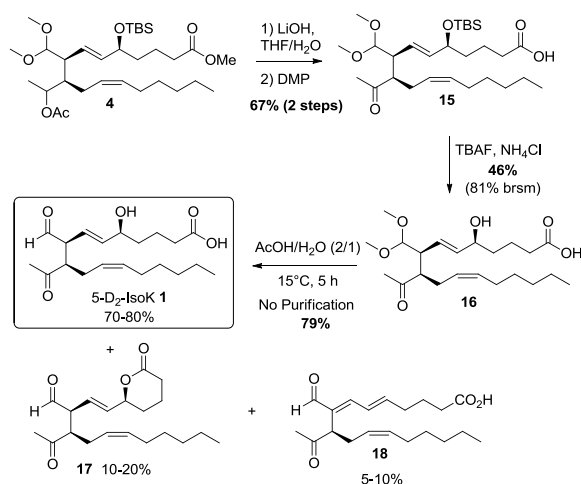
**Scheme 3.** Synthesis of key intermediate **5**.

Synthesis of common skeleton **5** of all possible types of IsoK started with the organocatalytic Michael addition (Scheme 3) of aldehyde **8**<sup>[8]</sup> and nitroolefin **7** (1.15/1 ratio aldehyde/olefin) using Jørgensen-Hayashi's catalyst **9** (5 mol%) and *p*-nitrophenol as co-catalyst (10 mol%) to afford the expected 1,4-nitro aldehyde in excellent yield (97%), good enantiomeric excess (90%) and diastereomeric ratio (3:1 dr), clearly in the range of previous report by Ruiz *et al.*<sup>[9]</sup> The C10 methyl group was then introduced by a mild alkylation of aldehydes **6a/b** using trimethyl aluminum, avoiding epimerization of the base sensitive aldehyde. The resulting nitro compound was subjected to the Nef reaction<sup>[10]</sup> which proved to be delicate and unsuccessful following standards protocols. Milder conditions developed by McMurtry<sup>[11]</sup> led also to degradation of the expected aldehyde. Finally, the use of sodium nitrite and acetic acid as reported by Mioskowski's group proceed smoothly.<sup>[12]</sup> These reaction conditions, known to convert primary nitro groups into carboxylic acids led in our case to lactone **10** *cis* and *trans* in 64% yield (over two steps) as a mixture of four diastereoisomers, probably via intermolecular trapping of the known nitrile oxide intermediate formed.<sup>[13]</sup> Gratifyingly, the treatment of the mixture of *cis/trans* **10** with a catalytic amount of DBU induced a full epimerization of the *cis*-lactone **10** to the thermodynamically more stable and desired *trans*-lactone **10**. By this way, the unwanted diastereoisomer could be easily converted into the

desired one. This strategy fully overcame the low diastereoselectivity of the first Michael addition thus avoiding loss of material. Next, the *trans*-lactone **10** was reduced with lithium aluminum hydride to the corresponding diol prior to the selective protection of primary alcohol as a triethylsilyl ether and secondary hydroxyl group as its acetate, to furnish intermediate **5** in excellent yield (81% yield over four steps) and a unique purification. At this stage, flash column chromatography allowed the separation of the two C11 diastereomers of **5** which is not necessary for the completion of the synthesis but quite handy. Therefore, the synthesis proceeded with the major diastereomer of compound **5** (configuration of C11 not determined), for NMR simplification and the attribution of the absolute configuration of the allylic alcohol that remained to be introduced.

**Scheme 4.** Introduction of the lateral chains.

With the requisite intermediate **5** in hands, we next focused on the construction of the lateral chains (Scheme 4). Exposing TES protected primary alcohol **5** to Swern conditions cleanly furnished, without purification, the corresponding aldehyde. The  $\alpha$ -chain (bearing the acid moiety) was then introduced using a HWE olefination with phosphonate **11**<sup>[14]</sup> and barium hydroxide as base to prevent epimerization at C8<sup>[15]</sup> and the desired ester **12** was obtained in 78% yield. Luche reduction of the  $\alpha,\beta$ -unsaturated ketone gave a 3/1 diastereomeric mixture of the corresponding allylic alcohol in 98% yield. The C5 epimers were separated by column chromatography and the configuration of the major epimer was unambiguously attributed as (5*S*) by NMR analysis of the corresponding mandelate derivatives.<sup>[16]</sup> The  $\omega$ -chain was introduced from the (5*S*) diastereoisomer by protection as its TBS ether **13** prior to the cleavage of PMB ether using DDQ to release the primary alcohol. Dess-Martin periodinane oxidation and a Wittig olefination using phosphonium salt **14** and NaHMDS as a base furnished desired *E,Z*-diene **4** in 71% yield over 3 steps.



**Scheme 5.** Completion of the synthesis.

Having succeeded in the stereoselective installation of the olefin lateral chains, we next conducted compound **4** through deprotection and oxidation steps to achieve the synthesis of desired 5-D<sub>2</sub>-IsoK-**1** (Scheme 5). Ester and acetate functions were simultaneously cleaved using lithium hydroxide but while the methyl ester reacted rapidly, the acetate group was found to be surprisingly more stable and five days were required to achieve full cleavage. As for a note, the classical use of potassium carbonate in methanol was totally ineffective to remove the acetate group. The resulting secondary alcohol was readily oxidized using Dess-Martin periodinane to give ketone **15** in 67% yield over two steps. At this stage, our plan was to convert intermediate **15** into isoketal-**1** by a concomitant acidic deprotection of both TBS ether and dimethylacetal groups. Despite our efforts, under all conditions tested (Amberlyst 15, BiBr<sub>3</sub>, AcOH/H<sub>2</sub>O) the elimination product **18** was formed in a large amount along with desired product **1** and other undetermined by-products. We thus decided to remove the TBS group as corresponding alcohol **16** would be less prompt to elimination. The use of a large excess of TBAF buffered with ammonium chloride,<sup>[17]</sup> afforded alcohol **16** in 46% yield (81% brsm) without epimerization at C12 and traces of lactonization product (separable if desired). Only one step far from the desired and sensitive IsoK, we had further problems to deal with, as the right balance between conversion into **1**, minimum formation of lactone **17** and elimination product **18** and other potential double epimerization. Furthermore target **1** was not found to be suitable for silica gel purification. Finally, subjecting acetal **16** to a mixture of acetic acid and water at 15 °C for 5 h gave the best possible outcome by avoiding any purification and limiting the formation of side products. As a result the 5-D<sub>2</sub>-IsoK-**1** was thus obtained in 79% yield reproducibly with up to 10-20% of lactonization product **17** and 5-10% of compound **18**. However, despite this fact, the purity of our IsoK-**1** is significantly improved compared to the first synthesis reported.<sup>7b</sup> Finally, because IsoK-**1** is a bioactive compound of high interest but clearly quite unstable, we decided to provide a sub-μmol scale access to IsoK-**1** from a storable solution of compound **16**.<sup>[18]</sup>

Thus, a simple treatment of a methanolic solution of **16** with acetic acid and water at 15 °C provided the desired compound **1** in good purity suitable for direct biological use.

In conclusion, we achieved a novel enantioselective synthesis of 5-D<sub>2</sub>-isoketal-**1** (5-D<sub>2</sub>-IsoK) using a unified strategy for the construction of the isoketal core. Multigram access to this core intermediate **5** and easiness of side chains introduction and last step deprotection sequence may be employed for the synthesis of other polyunsaturated fatty acids isoketal derivatives. Furthermore, a sub-μmol scale for the last step synthesis of **1** has been described. This represents an easy access to reliable quantities of highly reactive **1** suitable for biological applications.

## Experimental Section

Full details of experimental procedures, characterization data, and NMR spectra can be found in the Supporting Information.

## Acknowledgements

We gratefully thank the University of Montpellier (M.C. postdoctoral fellowship), the CNRS and Dr. Stellios Arseniyadis ESPCI ParisTech, France) for the measurement of enantiomeric excesses by chiral Super Fluidic Chromatography.

**Keywords:** Total Synthesis • Isoketal • Organocatalysis • Polyunsaturated Fatty Acids • Isolevuglandins

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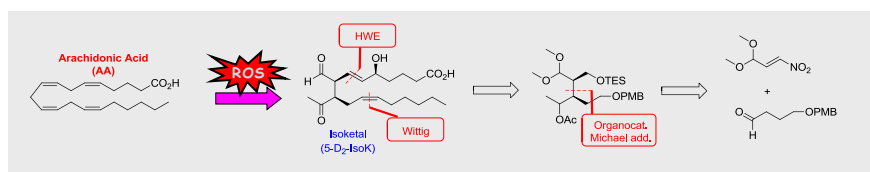
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- [18] See supporting information.

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## COMMUNICATION



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