Natural Product Synthesis

Total Synthesis of 2-(5,6-Epoxyisoprostane A_2)phosphorylcholine and Elucidation of the Relative Configuration of the Isoprostane Moiety**

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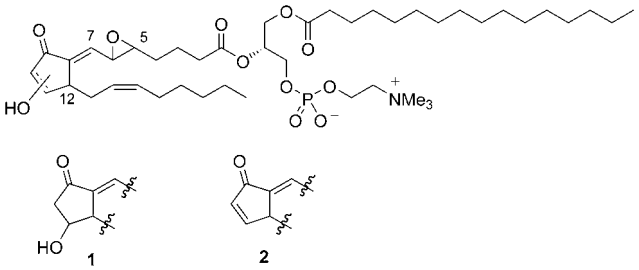
1-Palmitoyl-2-(5,6-epoxyisoprostane E_2)-*sn*-glycero-3-phosphorylcholine (**1**) and the related molecule **2** with 5,6-epoxyisoprostane A_2 at the *sn*-2 position (Scheme 1) are



Scheme 1. Aortic oxidation products of 2-arachidonoylphosphorylcholine.

oxidation products of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC)[1] that are found in interleukin-1β-stimulated human aortic endothelial cells (HAEC) and in mildly oxidized low-density lipoproteins (ox-LDL).[2] These molecules stimulate HAEC to release interleukin-8 and monocyte chemotactic protein-1. The monocytes activated by these chemokines[2,3] enter the vessel wall, where they initiate and cause the progression of atherosclerotic lesion.[3,4] The whole structures of **1** and **2**, and in particular the vinyl epoxide moiety of the isoprostane unit, are important for this activity.[5]

In contrast to the considerable progress made in the biological study of these compounds, the structures of the epoxyisoprostane parts of **1** and **2** had not been elucidated fully. Thus, although the connectivities and geometries of the epoxy and Δ^{14} olefinic moieties had been determined unambiguously by ^{1}H NMR spectroscopy and mass spectrometry,[1] the *E* geometry of the Δ^7 alkene and the relative configura-

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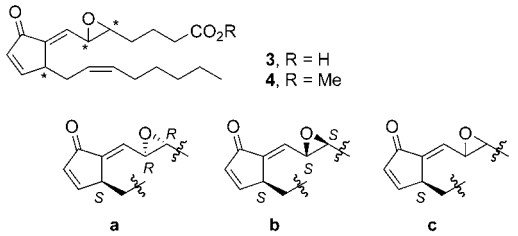
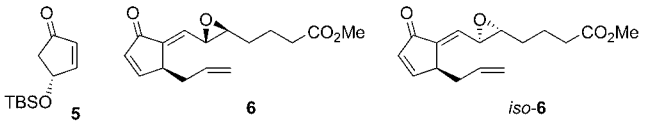
tion of the epoxy moiety and C12 were assigned for **2** by analogy with a structurally related compound.[6] Whereas it is believed that the configuration of the glycerol moiety of compounds **1** and **2** is the same as in *sn*-glycerol-3-phosphate, there was no information about the configuration of the 4 (for **1**) or 3 (for **2**) stereocenters in the respective isoprostane moieties. As **2** is the dehydration product of **1** and was isolated with **1** from the same origin, it seems reasonable to assume that the epoxyisoprostane portions of **1** and **2** share the same configuration at C5, C6, C12, and the Δ^7 olefin. Consequently, we chose to synthesize the epoxyisoprostane A_2 **3**—that is, the cyclopentenone-containing carboxylic acid constituent of compound **2**—to elucidate the relative configuration (Scheme 2).[7]



Scheme 2. 5,6-Epoxyisoprostanes A_2 that we have synthesized.

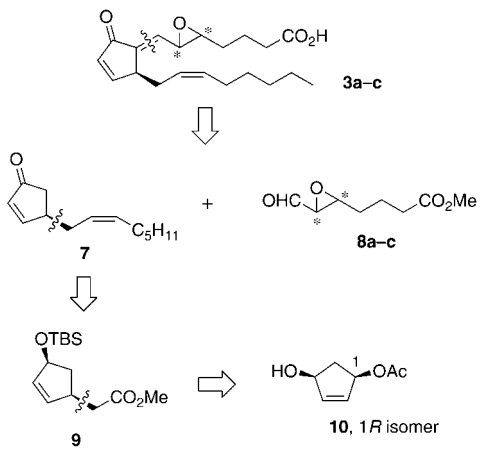
Methyl ester **6**, with a similar structure to acid **3**, was synthesized by Jung et al. by a three-component coupling strategy[8] starting from enone **5** (TBS = *tert*-butyldimethylsilyl). The same relative configuration as in **6** was assigned to **2**



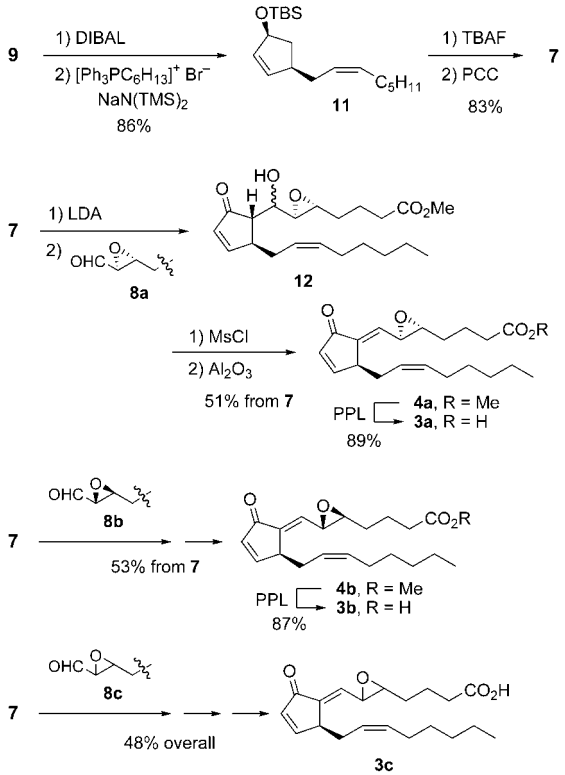
(i.e., **2b**) by comparison of their ^{1}H NMR spectra.[6] The results reported in the following prove that this assignment was wrong—the error having occurred mostly because Jung et al. did not include the isomer *iso*-**6** in their study—and that the natural product **2** either has the stereostructure **2a** or is the enantiomer of **14**.

Our approach to the epoxyisoprostane A_2 stereoisomers **3a–c**, which involves the combination of enone **7** and epoxyaldehydes **8a–c** in an aldol reaction, is summarized in Scheme 3. First, ester **9** was synthesized on a multigram scale from the TBS ether of **10**[9] (> 95% *ee*) through a palladium-catalyzed reaction with dimethyl malonate/*t*BuOK followed by decarboxylation,[10] and was converted into olefin **11** stereoselectively by reduction with DIBAL and subsequent Wittig reaction of the resulting aldehyde in 86% yield (Scheme 4). The silyl group was removed with TBAF, and oxidation of the resulting alcohol with PCC afforded **7** in 83% yield.

The aldol reaction of **7** with the epoxyaldehyde **8a**[11] afforded **12** as a mixture of the *anti* and *syn* aldol products. Without separation, the mixture was converted into the corresponding mesylate, which was exposed to Al_2O_3[12] to



Scheme 3. Retrosynthetic analysis of **3**.



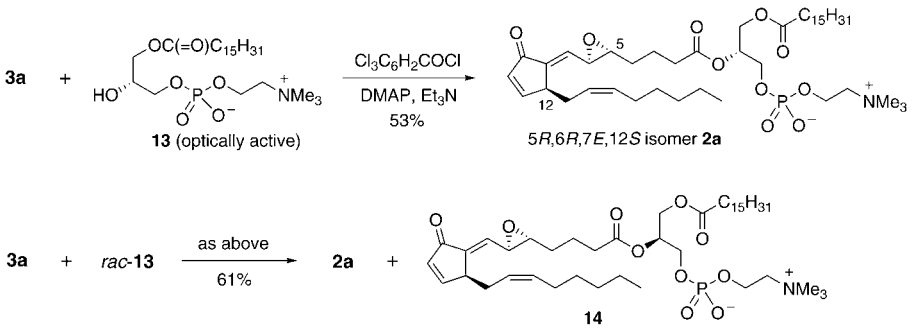
Scheme 4. Stereoselective synthesis of **3a**, **3b**, and **3c** via enone **7**. DIBAL = diisobutylaluminum hydride, LDA = lithium diisopropylamide, Ms = methanesulfonyl, TBAF = tetrabutylammonium fluoride, TMS = trimethylsilyl, PCC = pyridinium chlorochromate.

produce the 7*E* alkene–methyl ester **4a** in 51% yield from **7**.[13] No signal was observed for the hydrogen atom at C7 of the *Z* isomer in the expected 0.5–1-ppm upfield region.[14] Finally, the hydrolysis of **4a** with porcine pancreatic lipase (PPL) furnished epoxyisoprostane **3a** in 89% yield. Similarly, the aldol reaction of **7** with epoxyaldehyde **8b**[11] followed by hydrolysis with PPL afforded **3b** in 46% yield from **7**. The diastereomeric mixture **3c** (a 1:1 mixture of **3a** and **3b**) was synthesized from **7** and the racemic epoxide **8c**.

Expanded ^{1}H NMR spectra of **3a**, **3b**, **3c** (a 2:3 mixture of **3a** and **3b**), [15] and natural **3** that include the signals for the diagnostic 7-H atom ($\delta = 6.1$–6.2 ppm) and the less variable 10-H atom ($\delta = 6.3$–6.4 ppm) are presented in Figure 1.[16,17] For comparison, the spectrum of the analogue **6** is also sketched, but in two possible ways, since the chemical shift of residual $CHCl_3$ ($\delta = 7.24$ or 7.26 ppm) in $CDCl_3$, which was used as the reference, is not reported. The observed baseline separation of the signals for the 7-H atom in **3a** and **3b**, and the negligible drift in the chemical shifts for 7-H and 10-H of **3a** and **3b** in the mixture **3c**, show the high reliability of this method for the elucidation. Clearly, the spectrum of **3a** is coincident with that of natural **3**, thus allowing the determination of the 5*R**,6*R**,12*S** relative configuration and the 7*E* olefin geometry for natural **3**. This configuration is different from that assigned by analogy with the analogue **6**.[6]

Finally, the synthesis of the isoprostane phosphorylcholine **2a** was investigated. From the methods available for the synthesis of phospholipids,[18,19] we chose the direct esterification of **3a** with 1-palmitoyl-2-lyso-PC (**13**; PC = phosphorylcholine) since the reagents or conditions established for the other methods seemed incompatible with **3a**. Unfortunately, an attempted esterification of **3a** and the lyso-PC **13** with the standard reagent (DCC/DMAP)[19] did not take place even upon treatment at elevated temperatures for several days.[20] However, the treatment of **3a** and **13** with the Yamaguchi reagent[21,22] in CH_2Cl_2[23] at room temperature for 36 h afforded **2a** in 53% yield after chromatography on silica gel to obtain a mixture of **2a** and DMAP, and then reversed-phase chromatography to separate the mixture (Scheme 5).

According to the literature, the formation of **2** in vivo from 1-palmitoyl-2-arachidonoyl-PC may be a non-enzymatic process;[2,5] therefore, **2** is probably a diastereomeric mixture



Scheme 5. Synthesis of two diastereomers of **2**. DMAP = 4-dimethylaminopyridine.

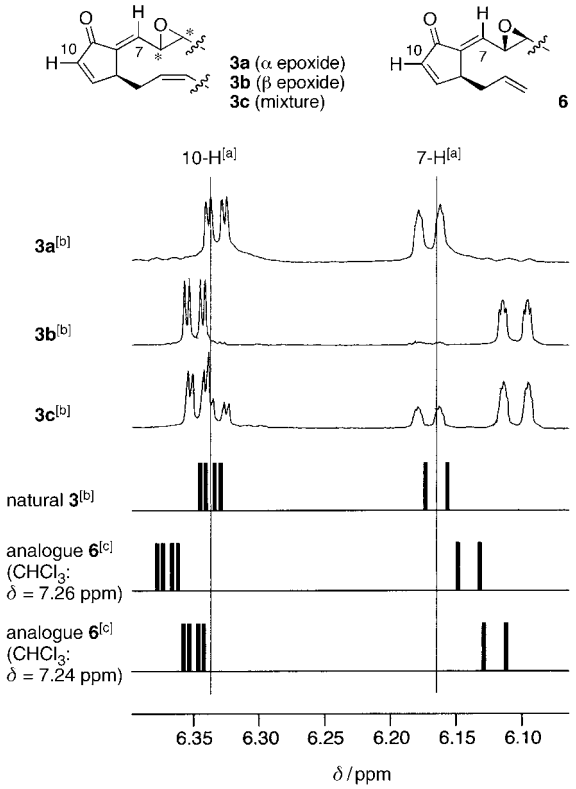


Figure 1. Expanded ^{1}H NMR spectra (500 MHz, $CDCl_3$) of **3a–c**, natural **3**, and the analogue **6**. [a] Reference lines that cross the center of the signals for natural **3**. [b] As residual $CHCl_3$, used as a reference in the ^{1}H NMR spectrum (500 MHz) of natural **3**, is set at $\delta = 7.24$ ppm, the spectra of **3a**, **3b**, and **3c** were referenced in the same way. [c] As the chemical shift of $CHCl_3$ is not reported, two spectra of **6** are represented in which it is assumed that the signal for $CHCl_3$ is observed at $\delta = 7.26$ or 7.24 ppm in the original spectrum.

in which both possible substituents derived from the carboxylic acids **3a** and *ent*-**3a** are present at the *sn*-2 position. To obtain spectroscopic and chromatographic information about the diastereomer derived from *ent*-**3a**, the condensation of **3a** and *rac*-**13**[24] was carried out under the above conditions to synthesize **2a** and **14**, the latter of which is the enantiomer of the compound in question. The mixture (**2a** and **14**), produced in 61% yield, was inseparable by TLC and column chromatography. Furthermore, the ^{1}H NMR (500 MHz) spectrum of the mixture was superimposable on that of pure **2a**.

In conclusion, we have established the relative configuration of the epoxyisoprostane component of **2** as **3a**. Moreover, **2a** was successfully constructed from **3a** and 1-palmitoyl lyso-PC (**13**) by using the Yamaguchi reagent. The present method would also be applicable to the synthesis of the regioisomers of **2** that were described recently.[5] We believe that the biological study of atherosclerosis will be spurred by the stereodefinition of 2-(5,6-epoxyisoprostane A_2)phosphorylcholine.

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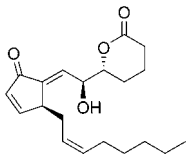
[15] This 2:3 mixture of diastereomers was synthesized by another sequence of reactions: 1) aldol reaction of **7** with $MeO_2C(CH_2)_3CH{=}CHCHO$; 2) epoxidation of the resulting *anti* aldol product with *t*BuOOH/$Ti(O\mathit{i}Pr)_4$; 3) mesylation; 4) elimination of the mesylate group with Al_2O_3.

[16] The ^{1}H NMR spectra of **3a**, **3b**, and **3c** were recorded under identical conditions to those reported[1] for natural **3** (500 MHz, $CDCl_3$, residual $CHCl_3$ set at $\delta = 7.24$ instead of 7.26 ppm).

[17] The exposure of **3a** to silica gel afforded lactone **I**, whose signals in the ^{1}H NMR spectrum overlap with another diagnostic multiplet at $\delta = 2.5$–2.7 ppm; therefore we used the clearly resolved region $\delta = 6.0$–6.2 ppm for the assignment. Furthermore, the signals for the lactone can be seen in the reported spectrum of **3**[1] at $\delta \approx 4.40$ and 4.80 ppm as the isolated signals and at $\delta = 6.35$ and 7.55 ppm as shoulders. Compound **I** is probably formed by epoxide-ring opening by H_2O under weakly acidic conditions (silica gel and/or DCl in $CDCl_3$) and subsequent lactonization of the resulting diol.



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