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Studies towards the total synthesis of an epoxy isoprostane phospholipid, a potent activator of endothelial cells

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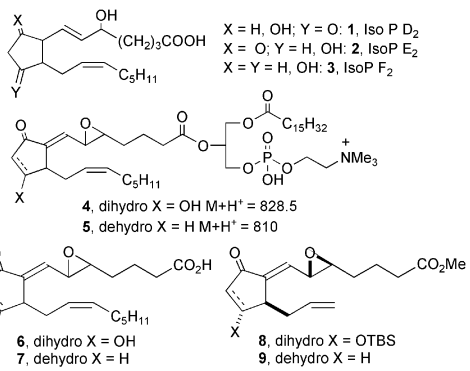
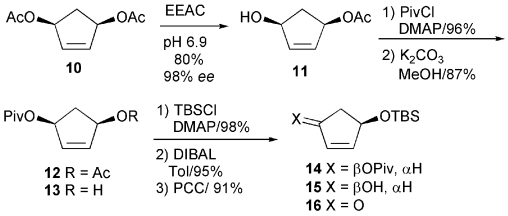
We report studies toward the total synthesis of an epoxy isoprostane, namely the preparation of compound 9 which is an analogue of the elimination product 7 of the naturally occurring epoxy isoprostane 4 by a straightforward route using a three-component coupling, and have shown by several spectroscopic criteria that it closely resembles the natural material.

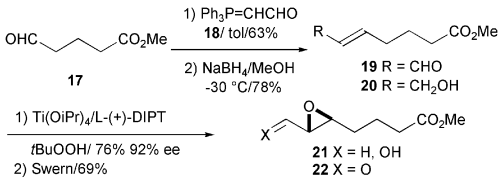
We report herein the total synthesis of the epoxy isoprostane analogues **8** and **9**. Isoprostanes,[1] isomers of the well known prostaglandins, were discovered in 1990,[2] and several substitution patterns in the cyclopentane ring are known (D, E, and F, **1**–**3**).[3] They are formed in both biological systems and *in vitro*, *via* a free-radical induced oxidation process, from arachidonic acid in a pathway independent of cyclooxygenases.[4,5] Since the formation of isoprostanes is not an enzymatic process, a large number of stereo- and regioisomers are formed with the relative ratio of the different isomers being dependent on the exact conditions under which the isoprostanes were formed. We recently reported the isolation and biological activity of an *in vitro* oxidation product of arachidonyl phosphocholine **4**, an oxidized phospholipid with the same biological properties as minimally modified low density lipoproteins (MM-LDL).[6,7] Studies indicate that this MM-LDL is involved in the development and progression of atherosclerosis. Based primarily on mass spectrometry of both the natural compound **4** and its dehydration product **5** and the isoprostane fatty acid portions **6** and **7**, and especially the proton NMR spectra of **7**, the compound was tentatively assigned the structure **4**.[7] Since the small amount of material precluded a complete structural analysis, the assignment of the relative stereochemistry, *e.g.*, the *trans* epoxide and the *E* trisubstituted alkene, is tentative. We report the synthesis of two close structural analogues of the isoprostane portion of this interesting phospholipid, namely the epoxide **8** and its dehydration product **9** and their *Z* stereoisomers, which lends evidence for the correctness of the structure of **4**.



Of the several good methods developed for the preparation of prostaglandin-like molecules, we chose a modification of Noyori's '3-component coupling.'[8] The enantiomerically enriched *R*-(+)-4-*tert*-butyldimethylsilyloxy-2-cyclopenten-1-one **16** was prepared from the prochiral diacetate **10** (made from cyclopentadiene), using deacetylation with electric eel acetylcholine esterase.[9] Replacement of the acetyl group by a silyl group was performed in four steps *via* **12**–**14** to give the monoalcohol **15** which on PCC oxidation gave **16**.[10]



The epoxy aldehyde ester **22** was prepared in five steps from the ester aldehyde **17** (prepared in one step by ozonolysis of cyclopentene).[10] Wittig reaction using (triphenylphosphoranylidene)acetaldehyde **18** gave an *E*/*Z* mixture of the aldehyde **19**.[11] Selective reduction at low temperature gave the alcohol **20**. Sharpless asymmetric epoxidation[12] of this substrate was earlier reported to be problematic.[13] Using a mild work-up allowed the desired compound **21** to be isolated in good yield and ee.[14] Although oxidation of this epoxy alcohol **21** with tetrapropyl perruthenate TPAP/NMO gave low yields and Dess-Martin periodinane oxidation gave non-reproducible yields, Swern oxidation afforded the desired epoxyaldehyde **22**.



Before attempting the required 3-component coupling[15] we first studied a simpler model, namely the addition of allylcopper[16] to cyclopentenone **23** in the presence of trimethylsilyl chloride (TMSCl)[17] to give the crude silyl enol ether **24**. Regeneration of the enolate with methyllithium[18] and trapping with the optically active aldehyde **22** afforded in 90% yield a mixture of four diastereomers **25** (two major and two minor). Elimination of the mesylate[19] and chromatographic separation gave a 1 : 1 mixture of two diastereomers of the *E*-enone **26*E*** in 19% yield and predominantly one diastereomer of the *Z*-enone **26*Z*** in 6% yield. Analogous trapping of the zinc enolate (made by the addition of 1 eq of $ZnCl_2$ to the enolate) afforded the hydroxy ketone **25** as four isomers (in an approximate 1 : 1 : 1 : 1 ratio) in lower yield (33%). Elimination of this mixture gave **26*E*** and **26*Z*** in 22% and 6% yield, respectively. The proton NMR spectrum of **26*E*** matched generally that of the isolated acids **6** and **7** but the differences in the structures are so significant that a close comparison was not expected. However, the UV and mass spectra were more informative. Compound **26*E*** had a λ_{max} of 255 nm while the λ_{max} of both **6** and **7** were 252 nm. In the ESI-MS (and the APCI-MS), compound **26*E*** showed an exact mass of 278.1518 and peaks at *m*/*z* of 131 and 159 which correspond to the breakdown of the epoxide and match the peaks of *m*/*z* of 115 and 143 seen in the mass spectra of **6** and **7**.

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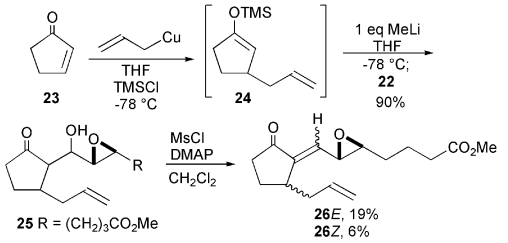
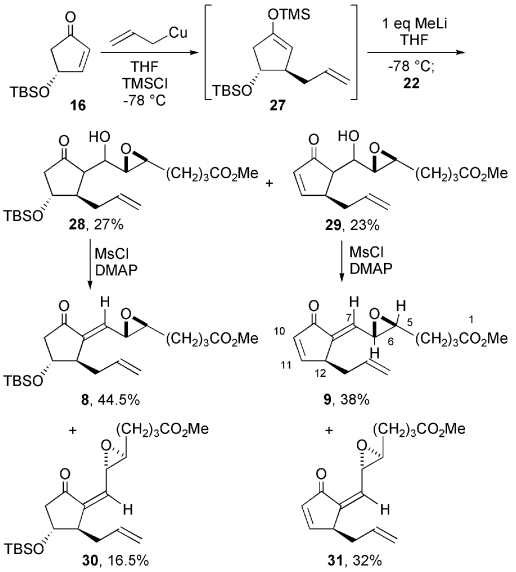


Having established that the 3-component coupling and elimination worked even with the sensitive epoxide functionality, we then carried out the process on the chiral cyclopentenone **16**. 1,4-Addition of allylcopper to **16** in the presence of TMSCl afforded the crude silyl enol ether **27**. Regeneration of the enolate and trapping with the aldehyde **22** gave the desired product **28** in 27% yield together with the enone **29** in 23% yield, in which elimination of the silyloxy group had occurred. Elimination of the hydroxy group was carried out as before with the silyl ether **28** giving the desired analogue of **6**, the enone **8**, in 44.5% yield along with the *Z* isomer **30** in 16.5% yield. Similar treatment of the enone **29** afforded the analogue of **7**, the dienone **9** in 38% yield along with the *Z* isomer **31** in 32% yield. The structural assignment of **8** and **9** was based largely on NOE data, *e.g.*, NOESY spectra for both compounds showed



interactions between H_5 and H_7 and, more importantly, between H_6 and H_{12}. It is instructive to compare the spectral data—NMR, MS, and UV—of the analogues **8** and **9** with the naturally derived materials **6** and **7**. In particular, the proton NMR spectrum of **9** matched very closely the relevant regions of the proton NMR spectrum of **7**. As shown in Table 1, the peaks for H_{11}, H_{10}, H_7, H_{12}, H_6, and H_5 were at nearly identical chemical shift and had virtually identical coupling constants in the two compounds. The only significant differences were observed in the olefinic protons of the allyl group and the *cis*-2-octenyl unit, as would be expected. This adds compelling evidence to the assignment of the structure of **7** to the compound derived from the natural material. By contrast, the proton NMR spectrum of the stereoisomer **31** does not match the spectral pattern of **7**, especially with regard to protons H_7, H_6, and H_5, all of which are quite different. In addition, the mass spectrum of **9** underwent an analogous fragmentation to that of **7**, showing mass ions at *m*/*z* 147 and 131, which represent cleavages at the epoxy group that were also seen in **7** (*m*/*z* 217 and 115). Furthermore, the major cleavage in **9** is at the trisubstituted alkene (*m*/*z* 159), which matches a similar peak in **7** (*m*/*z* 143). In addition, the silyl ether **8** showed the expected mass spectral fragmentation as seen with other compounds in this series. Finally the ultraviolet spectrum of **9** (λ_{max} of 255) matched very closely that of **6** and **7** (λ_{max} of 252).

Table 1 Comparison of ^{1}H NMR Spectra of **7**, **9**, and **31**

Proton	**7**	Mult.	**9**	Mult.	**31**	Mult.
H_{11}	7.53	dd	7.57	dd	7.47	dd
H_{10}	6.34	dd	6.37	dd	6.32	dd
H_7	6.16	d	6.14	d	5.58	d
H_{12}	3.65	m	3.67	m	3.40	m
H_6	3.39	dd	3.36	dd	4.64	dd
H_5	2.99	m	3.01	app td	2.86	app td
$J_{10,11}$	6.0		6.0		6.0	
$J_{11,12}$	2.0		2.4		2.4	
$J_{10,12}$	1.7		1.8		1.8	
$J_{6,7}$	8.4		9.3		8.4	
$J_{5,6}$	1.9		1.9		2.0	

Thus our studies strongly support the unambiguous structural assignment of the naturally occurring epoxy isoprostane formed by free radical-induced oxidation of arachindonyl phosphatidylcholine. Furthermore the strategy and methods reported herein should pave the way for a total synthesis of the epoxy isoprostane phospholipid **7**. We are currently carrying out biological investigations of the analogues **8**, **9**, **26*EZ***, **30** and **31** and the synthesis of the epoxy isoprostanes **6** and **7** and their derived phospholipids **4** and **5** to further study the endothelial activation by this novel class of compounds.

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