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Improved Synthesis of the Epoxy Isoprostane Phospholipid PEIPC and its Reactivity with Amines

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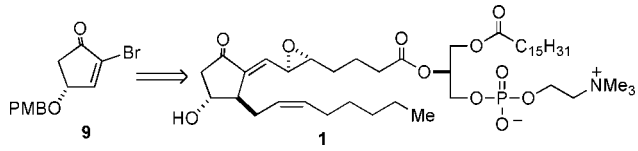
ABSTRACT



An improved synthesis of the naturally occurring hydroxy ketone 1-palmitoyl-2-(5,6)-epoxyisoprostane E_2-*sn*-glycero-3-phosphocholine (PEIPC) 1, a compound that plays a role in endothelial activation in atherosclerosis, has been carried out using a PMB ether as the key protecting group. Opening of an intermediate with pentylamine shows that the allylic epoxide is the position of attack by nucleophiles.

The in vitro oxidation product of arachidonoyl phosphatidylcholine, 1-palmitoyl-2-(5,6)-epoxyisoprostane E_2-*sn*-glycero-3-phosphocholine (PEIPC), has been previously isolated, and its biological activity described.[1] It is present in atherosclerotic lesions, oxidized lipoproteins, the membranes of cells exposed to oxidative stress, and in apoptotic and necrotic cells.[2] We have demonstrated that at least five HPLC-separable isomers of PEIPC were formed from the oxidation of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC). The most active isomer **1** was shown to activate several important inflammatory responses that contribute to atherosclerosis, including endothelial−monocyte interaction, and synthesis of monocyte activators.[3,4] On the basis of mass spectrometry of the natural compound and the proton NMR spectra of the dehydration product, the compound was tentatively assigned the structure **1**.[5] On the basis of our earlier synthetic work in this area,[6] we recently reported the development of a triply convergent[7] coupling strategy, beginning with the diacetate **3** that permitted the total synthesis of PEIPC **1** (Scheme 1).[8] Thus, conversion of **3** to the bromo alkene **4**, coupling with the epoxyaldehyde **5**, and further transformations gave the silyl-protected PEIPC **6**. However, fluoride-promoted deprotection gave significant amounts of the dehydration product PECPC **2** at the expense of **1**. We report herein an improved total synthesis of PEIPC **1** which uses a different alcohol protecting group and therefore allows a higher-yielding deprotection as the final step to give more and purer material with less of the dehydration product.

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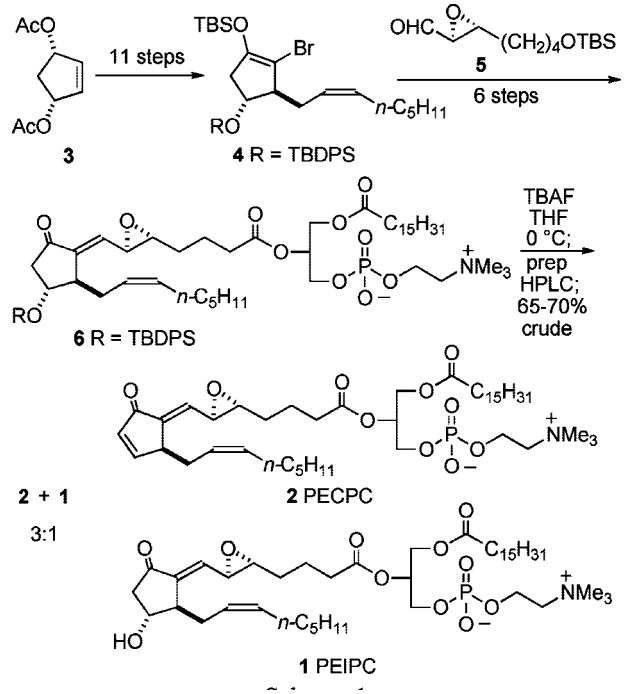
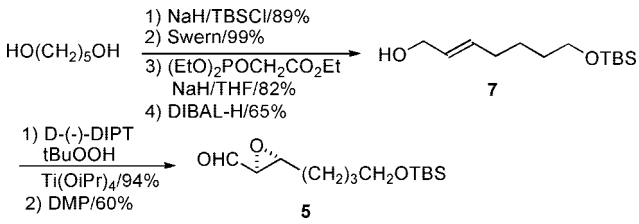
Scheme 1



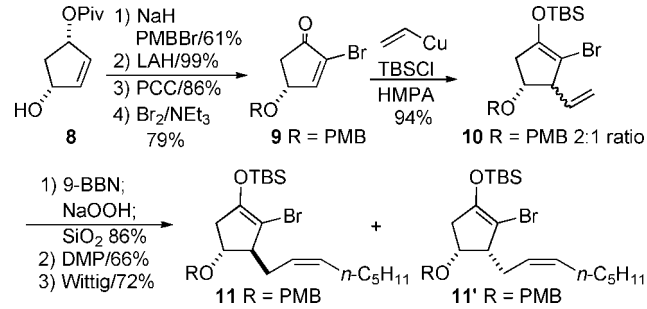
The components for the key coupling were prepared as follows. The epoxy aldehyde **5** was prepared by a slight modification of our earlier route,[5] namely, initial silyl protection of pentane-1,5-diol followed by Swern oxidation, Horner−Emmons reaction, and reduction to give the *E*-allylic alcohol **7** which was subjected to Sharpless asymmetric epoxidation and finally oxidized (Dess−Martin periodinane) to give the epoxide **5** (Scheme 2). The second key component was prepared in the

Scheme 2



same manner as described earlier,[6,8] namely, conversion of the achiral diacetate **3** via three steps into the monopivalate **8** using a lipase resolution as the key step. Protection of the alcohol as the *p*-methoxybenzyl (PMB) ether followed by reductive removal of the pivalate, oxidation, and bromination gave the 2-bromo-4-arylmethoxycyclopentenone **9** (Scheme 3). 1,4-Addition of vinylcopper to the enone **9** in the presence of *tert*-butyldimethylsilyl (TBS) chloride[9] gave an inseparable 2:1 mixture of the trans and cis disubstituted bromoenol ethers **10** in 94% yield. Although these compounds could not be separated at this stage, they were easily separated at the next. As we had done in our earlier synthesis, selective

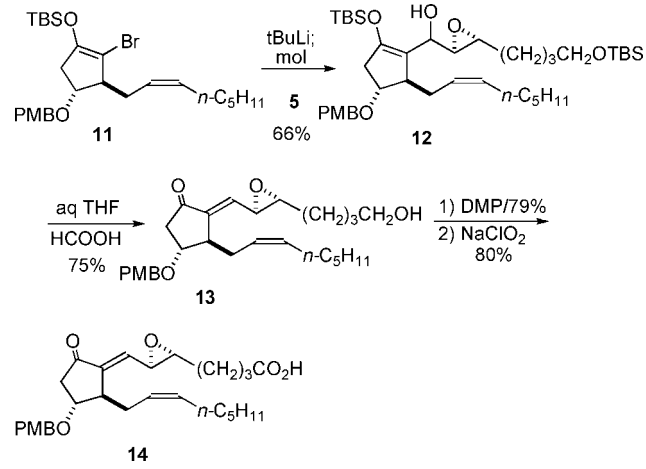
Scheme 3



hydroboration−oxidation of the vinyl group at −78 °C gave a 2:1 mixture of the separable primary alcohols in 86% yield (57% of the trans and 29% of the cis). Dess−Martin periodinane oxidation gave in 66% yield the aldehyde which on Wittig reaction with the ylide from hexyl bromide afforded the desired *Z*-alkene **11** in 72% yield. In a like manner, the *cis*-alcohol could be converted into the epimer **11′**.

The coupling of the bromide **11** with the aldehyde **5** was carried out by first forming the vinyllithium species by treatment of **11** (dried with molecular sieves) with *tert*-butyllithium followed by addition of the epoxyaldehyde **5** (also dried with molecular sieves) to give the allylic alcohol **12** in 66% yield (Scheme 4). Hydrolysis of the silyl enol ether, dehydration, and

Scheme 4



deprotection of the silyl ether were effected in 75% yield with formic acid in aq. THF to give the enone **13**. The final two steps (Dess−Martin periodinane and then chlorite oxidation) converted the primary alcohol into the desired acid **14**, namely, the PMB ether of EI in 63% yield over the two steps. To test the ability to oxidatively remove the PMB ether in the real system, we decided to convert this protected EI into EI itself.

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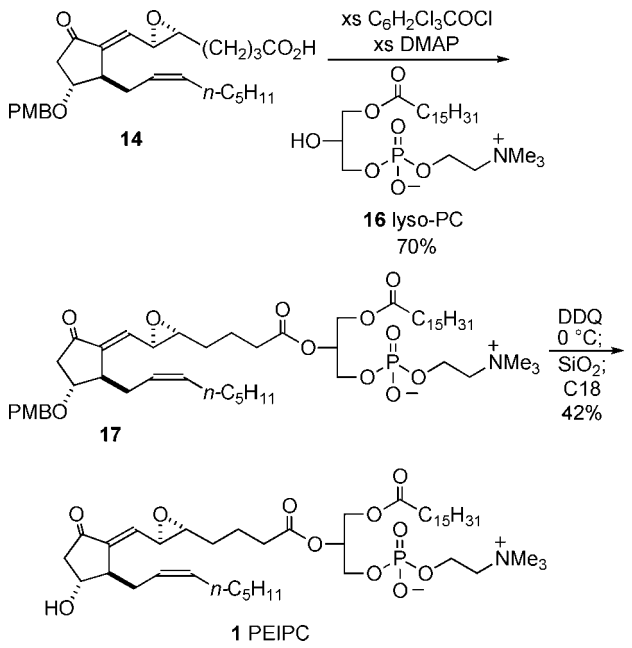
This was done by the oxidation of **14** with DDQ in dichloromethane/pH 7 buffer followed by filtration through Celite and then silica gel chromatography to give EI, **15**, in 54% yield. Thus, the oxidative removal of the PMB ether does not result in dehydration to give EC as did our earlier attempts at desilylation of the corresponding silyl ethers.[8]

The final steps in the synthesis of PEIPC involved coupling of the PMB protected EI **14** to commercially available lyso-PC **16** and final deprotection (Scheme 5).

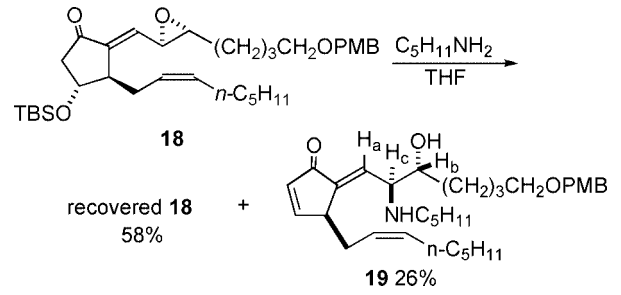
Scheme 5



We again used the method we had developed in our earlier work,[8] namely, reaction of **14** with 10 equiv of the Yamaguchi reagent (2,4,6-trichlorobenzoyl chloride) and DMAP at 23 °C for 1 h in the presence of fresh lyso-PC **16**, to afford a 70% yield of the coupled product **17**.[10] The key removal of the PMB ether protecting group was effected as above using DDQ in dichloromethane/pH 7 buffer at 22 °C for 2 h followed by filtration through Celite to give the crude PEIPC. This material could be purified by silica gel chromatography followed by passage through a short C18 column to give the desired compound PEIPC **1** as a pure material in 42% isolated yield. Thus, the change of the protecting group strategy from the base-labile TBDPS group, the removal of which caused extensive dehydration of the β-hydroxyketone to give PECPC **2**, to the oxidatively removable PMB group allowed for the easy purification and isolation of PEIPC **1**. The synthetic PEIPC **1** had an HPLC retention time and mass spectrometric profile identical to the naturally occurring compound[1,3,5] and also exhibited the biological activity expected of PEIPC.[11]

Finally, it is likely that PEIPC may form a covalent adduct with various cellular proteins since treatment of proteins with PEIPC and then extensive washing does not free the material from the peptide.[12] Therefore, it is very important to know what part of the PEIPC molecule is responsible for its covalent attachment to proteins, since then one may be better able to develop inhibitors of this covalent linking. A priori, either the enone or the allylic epoxide could be the required electrophile (or perhaps the cyclopentenone PECPC could be formed in cells and serve as the electrophile). To obtain some information on the reactivity of this system, we treated the protected enone epoxide **18** (prepared by a route similar to that described here for **13**)[8] with pentyl amine as a surrogate for lysine-containing peptides in cells (Scheme 6). We isolated a large amount of

Scheme 6



the starting material **18** but also the product **19** in which the allylic epoxide has been opened by the amine.[13] The structure was determined by extensive NMR experiments which showed that the proton α to the amine (H_c: δ 3.37) was a dd and coupled to the proton (H_a: δ 6.17) on the exocyclic double bond of the enone while the proton α to the oxygen (H_b: δ 3.66) was a multiplet and did not couple to H_a. Thus, it is likely that other biological nucleophiles may also react with PEIPC in a similar fashion.

Thus, we have synthesized the important naturally occurring material PEIPC **1** by a modified route that allowed for the production of larger quantities and purer material. We have also determined that the allylic epoxide functionality is the most likely center for the attack of nucleophiles on this system; e.g., **18** gives **19**. Further work in this area is underway.

Acknowledgment. We thank the National Institutes of Health (HL64731) for generous support.

Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Other methods for such couplings have been developed. See: (a) Gu, X.; Sun, M.; Gugiu, B.; Hazen, S.; Crabb, J. W.; Salomon, R. G. *J. Org. Chem.* **2003**, *68*, 3749. (b) Acharya, H. P.; Kobayashi, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 3481.

(11) See Supporting Information for more details about the biological activity.

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(13) The silyloxy group in the cyclopentanone ring underwent β-elimination under these mildly basic conditions to give the cyclopentenone product as we had seen earlier in the synthesis of PECPC.[8]