VIP Oxidized Phospholipids

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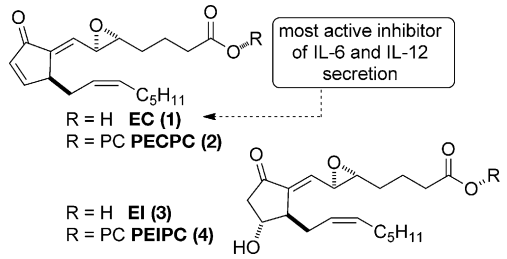
Synthesis of Epoxyisoprostanes: Effects in Reducing Secretion of Pro-inflammatory Cytokines IL-6 and IL-12**

*Julian Egger, Peter Bretscher, Stefan Freigang, Manfred Kopf, and Erick M. Carreira**

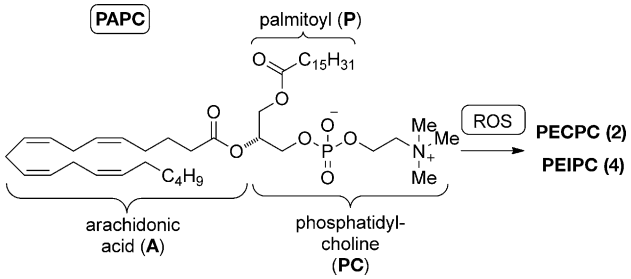
Oxidized phospholipids (OxPLs) are an important class of biomolecules generated in humans, as well as other organisms, under oxidative stress by the action of reactive oxygen species (ROS).[1] OxPLs, such as **1**–**4** (Scheme 1), have been shown to exert pro-inflammatory biological activity associated with diseases such as rheumatoid arthritis,[2] emphysema,[3] and atherosclerosis.[4] Furthermore, the cyclopentanone-containing oxidation product 1-palmitoyl-2-(5,6-epoxyisoprostane E_2)-*sn*-*glycero*-3-phosphatidylcholine (PEIPC, **4**) has been isolated from artherosclerotic lesions, implicating its prominent role in the early development of this disease.[4c,f,g] The role of the closely related cyclopentenone, 1-palmitoyl-2-(5,6-epoxyisoprostane A_2)-*sn*-*glycero*-3-phosphatidylcholine (PECPC, **2**), however, has only been marginally established.[5] Preliminary observations with mixtures of OxPLs and cells of the innate immune system suggested that preparations enriched in PECPC (**2**) and PEIPC (**4**) reduced the secretion of the pro-inflammatory cytokines IL-6 and IL-12, indicating that these could act as anti-inflammatory agents.



Scheme 1. Structures of the OxPLs synthesized for biological studies. PC = phosphatidylcholine.

This paradox highlights the fact that the role of OxPLs is poorly understood and underscores the need for more detailed investigations of these transient and elusive human-derived natural products.[6] Herein, we document an efficient synthesis of EC (**1**) and EI (**3**) along with the phosphatidylcholine esters PECPC (**2**) and PEIPC (**4**). We demonstrate the ability of these compounds to reduce the secretion of pro-inflammatory cytokines. Importantly the results indicate EC (**1**) as most active in the series.

The direct biosynthetic precursor (PAPC) of **1**–**4** is the ester of arachidonic acid with 1-palmitoyl-*glycero*-3-phosphatidylcholine (Scheme 2). Reactive oxygen species (ROS) formed under various conditions within the cell can oxidatively modify PAPC, leading to a complex heterogeneous mixture of reaction products containing cyclized, fragmented, and rearranged versions of the parent polyunsaturated fatty acid.[7]



Scheme 2. Cyclized OxPLs formed upon action of ROS on PAPC.

The synthetically more challenging structure PEIPC (**4**) has been the subject of a total synthesis, which proceeded in 20 steps and 0.19% yield.[8a] In the final step of a closely related sequence (differing in a protecting group TBDPS instead of PMB) PECPC (**2**) could be obtained as a coproduct from a 3:1 mixture with PEIPC (**4**), albeit separation by HPLC was required.[8b] An independent route to PECPC (**2**) has been reported and proceeds in 14 steps.[9] Both approaches follow a similar overarching strategy (Scheme 3), in which the cyclopentenyl ring is first constructed and the attendant C12 side chain is subsequently introduced in a stepwise manner to furnish **5**. The reported syntheses of intermediates **I** and **II** require 11 and 6 steps, respectively, with both commencing from cyclopentadiene (CpH).

We set out to develop a more efficient route to targets **1**–**4**, which would furnish sufficient quantities of reference compounds for biological investigation. In crafting an approach, we were intrigued by the implementation of a strategy involving the rapid construction of a cyclopentenone core from a precursor that includes the C12 olefin side chain, by means of a stereoselective C–H insertion reaction of an acyclic intermediate, such as **6** (Scheme 3).[10] We believed

[*] J. Egger, Prof. Dr. E. M. Carreira
Laboratory of Organic Chemistry, ETH Zurich, HCI H335
Wolfgang-Pauli-Strasse 10, 8093 Zurich (Switzerland)
E-mail: carreira@org.chem.ethz.ch
Homepage: http://www.carreira.ethz.ch
P. Bretscher, Dr. S. Freigang, Prof. Dr. M. Kopf
Institute for Integrative Biology, ETH Zurich, HPL-G 12
Schafmattstrasse 27, 8093 Zurich (Switzerland)

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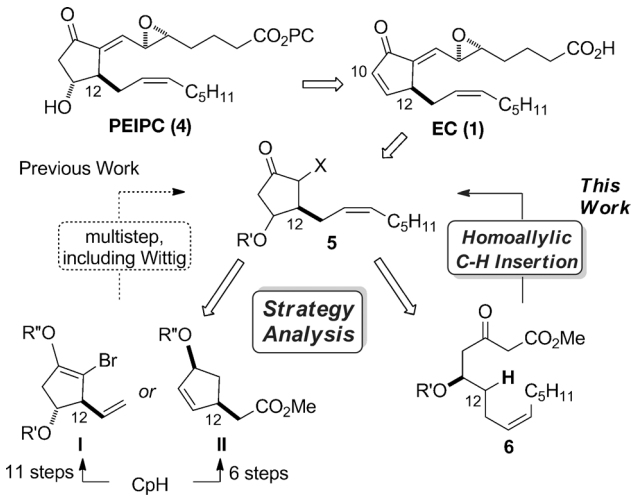


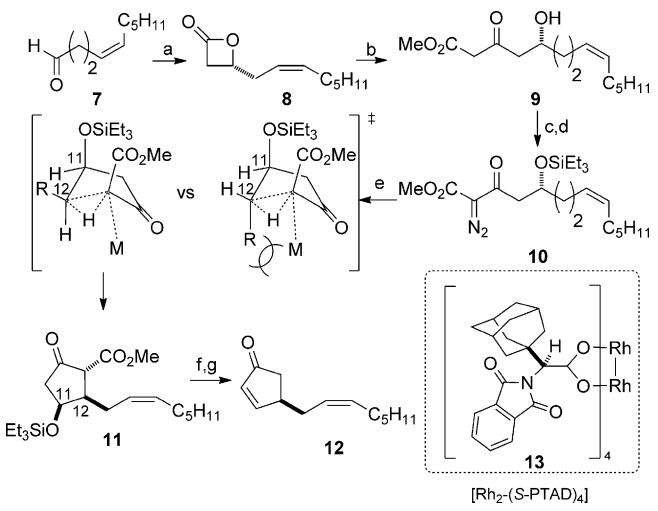
Scheme 3. Retrosynthesis of PEIPC (**4**) and strategy analysis.

that the novelty of the approach along with the efficiency gained warranted its examination in the context of the total synthesis project, especially since **6** would be accessible in fewer steps than **I** or **II**. Furthermore we envisioned EC (**1**) as a direct precursor of EI (**3**), provided hydration of the endocyclic enone in **1** (C10–C11) could be effected in a diastereo- and regioselective manner.

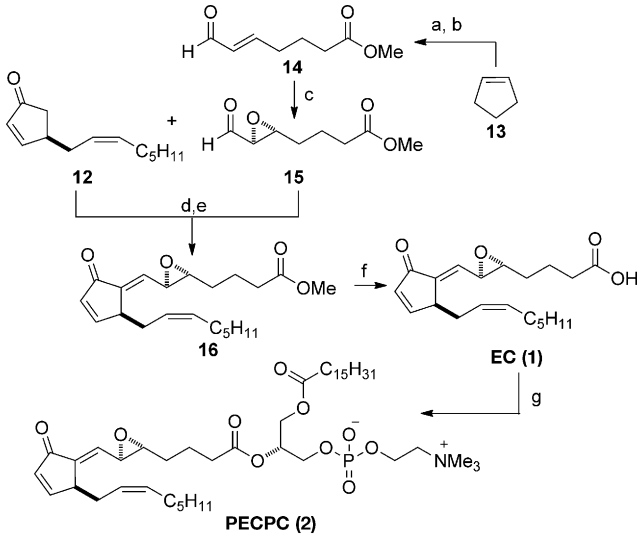
The synthesis commenced with exposure of commercially available (*Z*)-decenal (**7**)[11] to ketene following the conditions disclosed by Nelson et al. (Scheme 4).[12] β-Lactone **8** was formed in 62% yield and 92% *ee*, as determined by SFC analysis. Opening of the β–lactone with methyl acetate enolate gave β-ketoester **9** in 77% yield. This ester was subjected to sequential diazotization and protection as the corresponding triethylsilyl ether. An initial foray into the Rh-catalyzed C–H insertion reaction using $[Rh_2(OAc)_4]$ gave the cyclized product resulting from insertion into the homoallylic C–H bond; the product was obtained as a 4:1 mixture of diastereomers as determined by analysis of the crude NMR spectra,[13] which revealed a preference for the desired C11–C12 *cis* product (81%).

Analysis of putative competing transition states[13] for the reaction prompted us to screen sterically more demanding Rh catalysts in order to obtain better *cis*/*trans* selectivity. In agreement with this hypothesis, with $[Rh_2(esp)_2]$ (esp = α,α,α′,α′-tetramethyl-1,3-benzenedipropionate) as catalyst the reaction afforded an improved 6:1 ratio of products, whereas the use of $[Rh_2(S\text{-PTAD})_4]$ (**13**) furnished **11** in 71% yield with an improved diastereomer ratio of 9:1 *cis*/*trans*. Krapcho decarboxylation of **11** resulted in the triethylsilyl-protected β-hydroxyketone, allowing facile separation of the major *cis*-diastereomer from the minor *trans*-cyclization product by chromatography on silica gel. Subsequent elimination of triethylsilanol promoted by DBU in CH_2Cl_2 at 0°C gave cyclopentenone **12** in 60% yield over two steps.

Installation of the C8 side chain of the cyclopentenyl ring was conducted using a modification of a procedure by Kobayashi,[9a] involving the aldol addition of cyclopentenone **12** and epoxyaldehyde **15**, followed by a *trans*-selective elimination to obtain dienone **16** (Scheme 5). Epoxyaldehyde



Scheme 4. Reagents and conditions: a) $LiClO_4$ (3 equiv), Me_3Si-quinidine (12 mol%), *i*Pr_2NEt (2.5 equiv), AcCl (2.5 equiv, addition by syringe pump over 4 h), Et_2O/CH_2Cl_2, −78°C, 62%, 92% *ee* (determined by SFC analysis); b) *i*Pr_2NLi (3.8 equiv), methyl acetate (3.8 equiv), THF, −78°C, 77%; c) *p*-ABSA (1.3 equiv), Et_3N (2 equiv), MeCN, 0°C to RT, 97%; d) Et_3SiCl (1.5 equiv), imidazole (2 equiv), DMF, 0°C to RT, 98%; e) $[Rh_2(S\text{-PTAD})_4]$ (**13**; 1 mol%), CH_2Cl_2, reflux, d.r. = 9:1, 71%; f) NaCl (30 equiv), Me_2SO, 140°C, 65%; g) DBU (10 equiv), CH_2Cl_2, 0°C, 93%. *p*-ABSA = *para*-acetamidobenzenesulfonyl azide, *S*-PTAD = *S*-(1-adamantyl)-(*N*-phthalimido)acetato, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

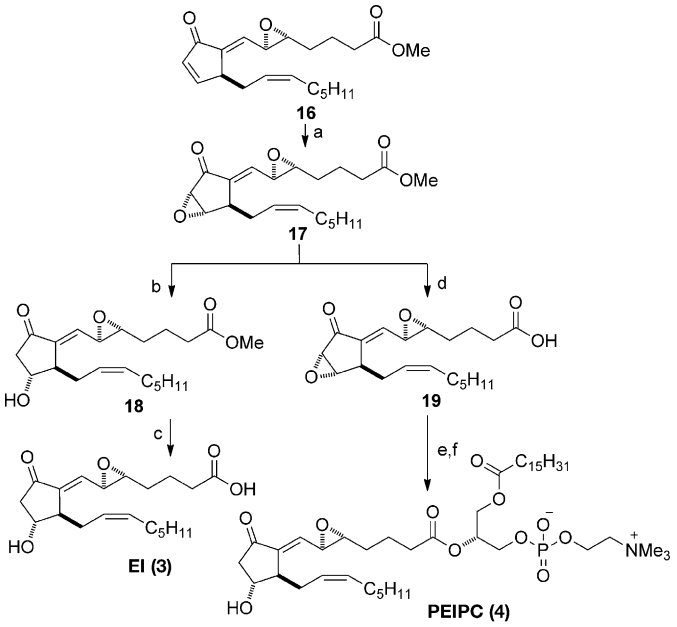


Scheme 5. Reagents and conditions: a) O_3, $NaHCO_3$ (0.3 equiv), $MeOH/CH_2Cl_2$, −78°C then Et_3N (2 equiv), Ac_2O (2 equiv), PhH, 0°C; b) $Ph_3PCHCHO$ (2.1 equiv), toluene, 70°C, d.r. = 5:1, 55%; c) (*S*)-2-(diphenyl[(trimethylsilyl)oxy]methyl)pyrrolidine (10 mol%), H_2O_2 (1.3 equiv), CH_2Cl_2, RT, d.r. = 10:1, 51%, 92% *ee*; d) $LiN(SiMe_3)_2$ (1.2 equiv), then **15** (2 equiv), THF, −78°C; e) $MeSO_2Cl$ (3 equiv), Et_3N (6 equiv), CH_2Cl_2, −78°C then Al_2O_3, CH_2Cl_2, RT, 64% over two steps; f) Novozyme, buffer pH 7/THF, 70%; g) 2,4,6-$Cl_3C_6H_2COCl$ (10 equiv), 4-$Me_2NC_5H_4N$ (10 equiv), lyso-PC (3 equiv), $CHCl_3$, 69%. Novozyme = lipase from *Candida antarctica* on acrylic resin, lyso-PC = 1-palmitoyl-*sn-glycero*-3-phosphatidylcholine.

15 was readily synthesized by use of Jørgensen's organocatalytic epoxidation of α,β-unsaturated aldehydes with (*S*)-2-(diphenyl[(trimethylsilyl)oxy)methyl]pyrrolidine as a catalyst.[14] The presence of an ester unit in enal **14** renders it a challenging substrate for epoxidation,[15] resulting in epoxyaldehyde **15** in 51% yield and with an enantioselectivity of 92% *ee* according to SFC analysis. Dienone **16** proved highly sensitive to saponification under both acidic and alkaline conditions. Thus, it was subjected to enzymatic hydrolysis under neutral conditions with a phosphate buffer/THF mixture to obtain EC (**1**), which could then be further coupled to lyso-PC using Yamaguchi's conditions to yield PECPC (**2**).[8a]

Previous analysis and experimental work with prostaglandin Δ^7-PGA_1 by Noyori and co-workers[16] suggested to us that the endocyclic enone in isoprostanoid **16** could be more electrophilic than its exocyclic counterpart. Consequently, we examined the possibility of introducing the β-hydroxy group through nucleophilic epoxidation and reductive opening of the epoxide ring. This, of course, raises the specter of subsequent complications in the preferential opening of the endocyclic epoxide in the presence of an exocyclic enone/epoxide.[17] In the experiment (Scheme 6), treatment of **16** at 0°C with *tert*-butylhydroperoxide and DBU gave epoxide **17** as a single diastereomer. Various reagents to effect opening of α,β-epoxy ketones were examined.[17,18] However, we found that treatment of **17** with SmI_2 at −90°C was the only method that did not result in elimination to the enone or cause product decomposition.[19] We observed that the conditions employing SmI_2 reliably provided **18** in an average yield of 54%. Enzymatic hydrolysis of the methyl ester required careful control of the pH (7.0–7.2) to avoid elimination. Under optimal conditions employing Novozyme, isolation of EI (**3**) in 60% yield was achieved. Enzymatic hydrolysis of the methyl ester in **17** prior to epoxide opening gave carboxylic acid **19**, which could be coupled to lyso-PC employing Yamaguchi's protocol. Treatment of the coupled product with SmI_2 in THF/MeOH afforded PEIPC (**4**) in 43% yield.

Next we studied the effects of isoprostanoids **1** and **3** along with the corresponding phosphatidylcholine derivatives **2** and **4** on the secretion of pro-inflammatory cytokines IL-6 and IL-12 in vitro (Figure 1).[20] Bone-marrow-derived dendritic cells (BMDCs) were treated with the synthetic **1**–**4** in Fetal Bovine Serum (FBS)-supplemented RPMI (Roswell Park Memorial Institute) medium for 60 min. Subsequently, the cells were washed and stimulated for 18 h with Toll-like receptor ligand R837 (5 $\mu g\,mL^{-1}$) to induce cytokine secretion. In the experiment, we observed a dose-dependent decrease in the secretion of the pro-inflammatory cytokines IL-6 and IL-12. Interestingly the free acids EC (**1**) and EI (**3**) elicited a stronger effect than their esterified counterparts PECPC (**2**) and PEIPC (**4**). Comparison of the effects induced by hydroxylated isoprostanoid EI (**3**) with the cross-conjugated counterpart EC (**1**) indicates a stronger decrease in cytokine



Scheme 6. Reagents and conditions: a) *tert*-BuOOH (1 equiv), DBU (1 equiv), THF, 0°C, 74%; b) SmI_2 (2 equiv), THF/MeOH, −90°C, 54%; c) Novozyme, buffer pH 7.1/THF, 60%; d) Novozyme, buffer pH 7/THF, 74%, e) 2,4,6-$Cl_3C_6H_2COCl$ (10 equiv), 4-$Me_2NC_5H_4N$ (10 equiv), lyso-PC (3 equiv), $CHCl_3$, 69%; f) SmI_2 (2 equiv), THF/MeOH, −90°C, 43%.

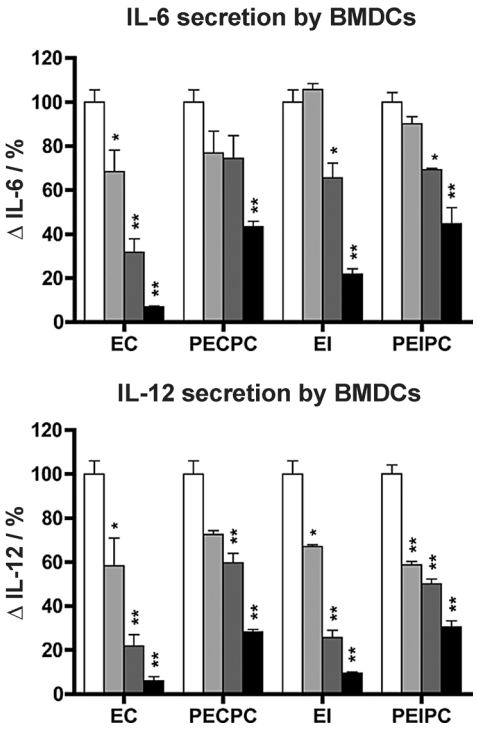


Figure 1. IL-6 and IL-12 production by BMDCs exposed to synthetic EC (**1**), PECPC (**2**), EI (**3**), and PEIPC (**4**) prior to TLR7-stimulation with R837. Concentrations (from left to right) for EC, PECPC, and EI: 0, 0.37, 1.11, and 3.33 μM; for PEIPC: 0, 1.52, 3.04, and 6.07 μM. White bars represent cytokine responses observed in the absence of synthetic compounds ("negative/solvent control"). Data are normalized to the negative control. IL-6 and IL-12 levels in the supernatants were determined by ELISA.

secretion for the latter, highlighting its role as a highly potent mediator of anti-inflammatory effects.

In summary, we have designed and implemented an efficient and general synthetic route to the cyclized oxidized phospholipids PECPC (**2**) and PEIPC (**4**) and the respective free carboxylic acids EC (**1**) and EI (**3**). The route we report provides PECPC (**2**) and PEIPC (**4**) in 11 steps (5.4% yield) and 13 steps (1.8% yield), respectively. EC (**1**) and EI (**3**) could be conveniently accessed in 10 steps (7.8% yield) and 12 steps (2.7% yield), respectively. We have shown the activity of these isoprostanoids in reducing the secretion of pro-inflammatory cytokines IL-6 and IL-12. An intriguing result from our study is the identification of the free carboxylic acid EC (**1**) as the most active compound. Further studies designed to elucidate the origin of the different activities and biological investigations are in progress.

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