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Prostaglandin phospholipid conjugates with unusual biophysical and cytotoxic properties

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ABSTRACT

The synthesis of two secretory phospholipase A_2 IIA sensitive 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 phospholipid conjugates is described and their biophysical and biological properties are reported. The conjugates spontaneously form particles in the liposome size region upon dispersion in an aqueous buffer and both phospholipids are hydrolyzed by phospholipase A_2, but with different conversion rates and extent of hydrolysis. The cytotoxicity was evaluated in HT-29 and Colo205 cells and the conjugates induced cell death in the presence of phospholipase A_2 and surprisingly also in the absence of the enzyme.

Since von Euler[1] and Goldblatt[2] independently isolated and studied prostaglandins for the first time, these fatty acids have attracted attention due to their involvement in many important biological functions.[3–5] The biosynthetic precursor for prostaglandins is arachidonic acid, which by a number of enzyme catalyzed reactions is converted into the diverse selections of prostaglandins known today.[3–5] Our interest in prostaglandins arose when we started to develop a new generation of liposomal drug delivery system consisting of secretory phospholipase A_2 ($sPLA_2$) IIA degradable liposomes with potential in cancer treatment.[6,7]

The anticancer agents are covalently attached to the *sn*-2-position of the lipid backbone and thereby the drug is an integral part of the lipophilic liposome membrane. The design uses bioactive compounds with a carboxylic acid moiety, and therefore, we decided to investigate prostaglandins. Many prostaglandins have shown antiproliferative activity in tumor cells, but among the most studied and active prostaglandins are the dienone prostaglandins like Δ^{12}-PGJ_2 and Δ^7-PGA_1 (see Fig. 1).[8] Likewise, 15-deoxy-$\Delta^{12,14}$-PGJ_2, a metabolic derivative of Δ^{12}-PGJ_2, has demonstrated high antitumor activity against L1210 leukemia cells[9] and for the investigation of the liposomal drug delivery system that compound is more suitable than the former prostaglandins since 15-deoxy-$\Delta^{12,14}$-PGJ_2 has a higher lipophilicity. The phospholipid conjugates are designed to have phosphatidylglycerol headgroups and C_{18} chains in the *sn*-1-position linked through either an ether or an ester functionality (see Fig. 2).

The *sn*-1-ether conjugate **1** was synthesized by a tetrazole mediated coupling of the known primary alcohol **3** and the phosphoamidite **5**[6] (Scheme 1) followed by oxidation to the phosphate with ^{t}BuOOH. The *p*-methoxybenzyl group was removed with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in moist CH_2Cl_2 affording the secondary alcohol that was coupled to 15-deoxy-$\Delta^{12,14}$-PGJ_2 applying a dicyclohexylcarbodiimide (DCC) mediated ester coupling. The conjugate **1** was procured after removal of the cyanoethyl group with 1,8-diazabicyclo[5.4.0]undec-7-ene

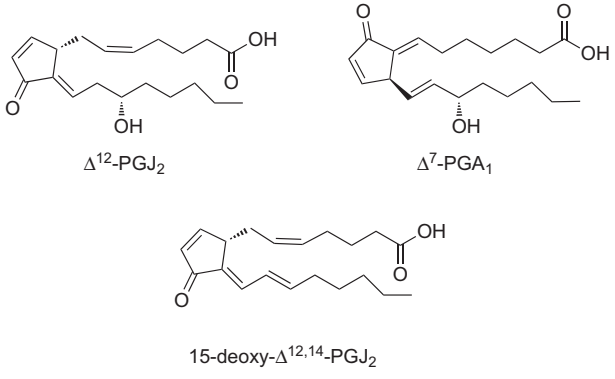

Figure 1. Potent antiproliferative prostaglandins.

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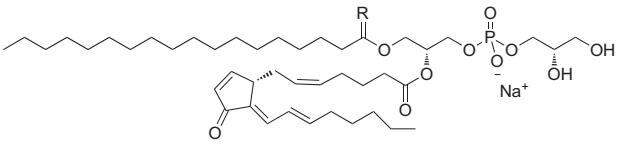
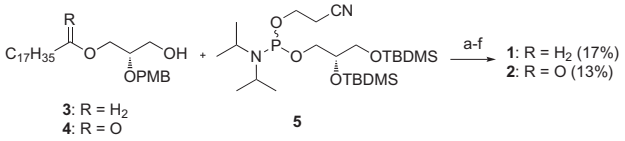


15-deoxy-$\Delta^{12,14}$-PGJ_2 phospholipid conjugates
1: R = H_2
2: R = O

Figure 2. Targeted 15-deoxy-$\Delta^{12,14}$-PGJ_2 phospholipid conjugates.



3: R = H_2
4: R = O
5

Scheme 1. Synthesis of the 15-deoxy-$\Delta^{12,14}$-PGJ_2 lipid conjugates **1** and **2**. Reagents: (a) tetrazole, CH_2Cl_2, MeCN; (b) ^{t}BuOOH; (c) DDQ, H_2O, CH_2Cl_2; (d) 15-deoxy-$\Delta^{12,14}$-PGJ_2, DCC, DMAP, CH_2Cl_2; (e) DBU, CH_2Cl_2; (f) HF, H_2O, CH_2Cl_2, MeCN.

(DBU) and the TBDMS-groups with aqueous HF in MeCN and CH_2Cl_2. The corresponding *sn*-1-ester conjugate **2** (Scheme 1) was accessed in a similar way starting from the acylated glycerol **4** (see Supplementary data).

The prostaglandin conjugates **1** and **2** were hydrated in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.5) at 20 °C yielding clear solutions. Dynamic light scattering (DLS) analysis of the lipid suspension of **2** revealed that particles with an average diameter of around 100 nm were formed, indicating that the prostaglandin conjugates spontaneously form small unilamellar vesicles (SUVs) at 20 °C. Self-aggregation into SUVs upon dispersion in a buffer is very rare for phospholipids and has only been reported in the literature once before.[10] Extrusion through a 100 nm filter narrowed the average diameter and the polydispersity of the vesicles (Table 1 and Supplementary data), presumably because filtration removed the minor population of flocculated particles. Twelve days after formulation, the particle distribution of the lipid suspensions was investigated with DLS again. As evident from Table 1, neither the diameter nor the polydispersity changed significantly, showing that the vesicles formed maintain their size and does not aggregate into larger particles.

The formulated lipid suspensions of **1** and **2** were investigated for their susceptibility to undergo $sPLA_2$-mediated hydrolysis. Purified snake venom $sPLA_2$ from *Agkistrodon piscivorus piscivorus* and *Naja mossambica mossambica* were used. Previously, these enzymes have shown activity towards a broad range of phospholipids and the substrate specificity is comparable to human $sPLA_2$ IIA.[11] The enzyme activity was investigated with matrix-assisted laser desorption–ionization time of flight (MALDI-TOF) MS, which we and others have demonstrated to be a reliable and valid tool for detection of phospholipids.[6,7,12,13] For the measurements, 2,5-dihydroxybenzoic acid (DHB) and CF_3COONa in methanol containing 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG) as an internal standard was used as the matrix, which did not interfere with the regions of interest in the mass spectrometry spectra for the 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugates.

Table 1
DLS analysis of the 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugates formulated by extrusion

Compound	0 days		12 days[b]	
	Diameter	PdI[c]	Diameter	PdI[c]
1	51	0.26	61	0.26
2[a]	112	0.45	98	0.39
2	81	0.35	76	0.41

Data was obtained immediately after the formulation and 12 days later
[a] Not extruded.
[b] The lipid suspensions were stored at 4 °C for 12 days.
[c] PdI = polydispersity index.

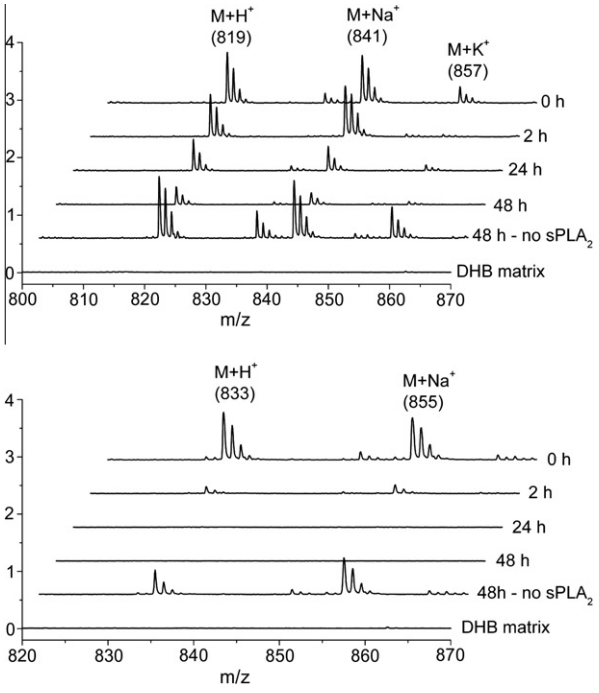


Figure 3. MALDI-TOF MS monitoring of snake (*Naja mossambica mossambica*) venom $sPLA_2$ activity on the 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugates **1** (top) and **2** (bottom).

The formulated lipids **1** and **2** were subjected to $sPLA_2$ and stirred at 37 °C and samples for MALDI-TOF analysis were taken after 2, 24 and 48 h. As evident from the MS spectra in Figure 3 the *sn*-1-ester 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugate (**2**) was completely consumed by $sPLA_2$ after 24 h. However, the *sn*-1-ether 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugate (**1**) was not completely degraded after 48 h, the conversion was estimated to be 70%. This difference in hydrolysis rate and extent for the *sn*-1-ester versus the *sn*-1-ether phospholipids is remarkable and to our knowledge has not been observed before, but the observation verifies that *sn*-1-ester phospholipids are better substrates for $sPLA_2$ than *sn*-1-ether phospholipids.[14] For both conjugates 15-deoxy-$\Delta^{12,14}$-PGJ_2 and the lysophospholipids were detected as the released products (data not shown). Also evident from Figure 3 is that $sPLA_2$ is required for hydrolysis of both **1** and **2**.

Table 2
IC_{50} values for 15-deoxy-$\Delta^{12,14}$-PGJ_2, lysolipids and the conjugates **1** and **2** in HT-29 and Colo205 colon cancer cell lines[a]

Compound	IC_{50} (μM)		
	HT-29	HT-29 + $sPLA_2$[b]	Colo205
15-Deoxy-$\Delta^{12,14}$-PGJ_2	1.6 ± 0.3	*nd*	4 ± 2
sn-1-Ether lysolipid	11 ± 6	*nd*	25 ± 2
sn-1-Ester lysolipid	7 ± 1	*nd*	22 ± 3
1	6 ± 1	2.2 ± 0.2	9 ± 2
2	32 ± 2	6.4 ± 0.4	17 ± 5

[a] Cytotoxicity was measured using the MTT assay as cell viability 48 h after incubation with the indicated substances for 24 h and shown by mean ± SD ($n \geqslant 3$); nd = not determined; Snake (*Agkistrodon piscivorus piscivorus*) venom $sPLA_2$ caused no change in cell viability within 24 h.
[b] $sPLA_2$ was added to the cell media to a final concentration of 5 nm.

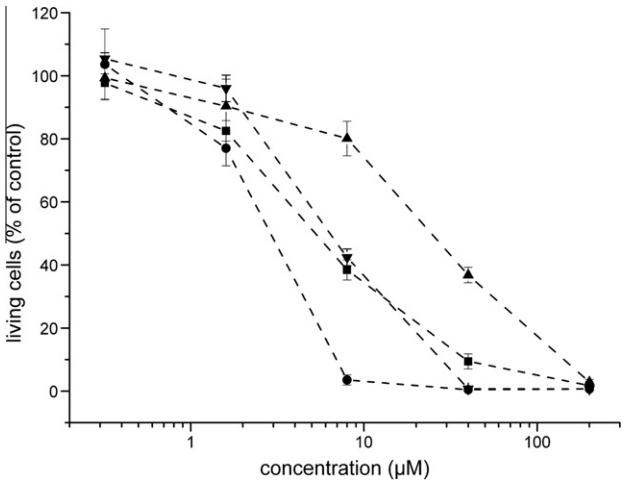


Figure 4. Dose–response curves for the treatment of HT-29 cells with the 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugates; **1** (■), **1** + $sPLA_2$ (●), **2** (▲) and **2** + $sPLA_2$ (▼).

The cytotoxicity of the conjugates was evaluated in two colon cancer cell lines, HT-29 and Colo205. HT-29 cells do not secrete $sPLA_2$ whereas Colo205 cells do. Therefore, the activity of the conjugates towards HT-29 cells was investigated in the presence and in the absence of $sPLA_2$, which enabled us to evaluate the importance of enzymatic activity for cytotoxicity. Surprisingly, we observed that the conjugates induced significant cell death (see Table 2 and Fig. 4) also in the absence of the enzyme, albeit to a lesser degree than when $sPLA_2$ was present. Despite having studied a number of other phospholipid prodrugs,[6,7] this is the first time we have observed cytotoxicity of the phospholipid conjugates in the absence of $sPLA_2$. We speculate that this behavior can arise through spontaneous cellular uptake of the 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugates followed by metabolic breakdown in the cytosol. This facile uptake is likely a consequence of the dynamic behavior of 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugates, which also manifests itself during formulation, where self-aggregation into SUVs were observed (vide supra).

The cytotoxicity of the release compounds 15-deoxy-$\Delta^{12,14}$-PGJ_2 (see Table 2) and the lysophospholipids[7] was also obtained. As evident, the IC_{50} values for the released compounds are in the same range as for the conjugates **1** and **2** indicating that the activity originate from 15-deoxy-$\Delta^{12,14}$-PGJ_2 and the lysophospholipids, released either extra- or intracellularly. The conjugates were also able to induce cell death in Colo205 cells, with IC_{50} values below 20 μM (Table 2) and complete cell death was observed when higher concentrations were used (see Supplementary data). However, since Colo205 cells secrete $sPLA_2$ it is not possible to conclude whether the hydrolysis of the conjugates was brought about through the action of $sPLA_2$, but it is promising that the 15-deoxy-$\Delta^{12,14}$-PGJ_2 conjugates show activity in this cancer cell line as well.

In conclusion, we have synthesized a novel class of phospholipid conjugates with 15-deoxy-$\Delta^{12,14}$-PGJ_2 attached to the *sn*-2-position. The conjugates spontaneously form SUVs upon dispersion in HEPES buffer, which is rare for phospholipids. Furthermore, we observed a significant difference in rate and extend of $sPLA_2$ hydrolysis between the *sn*-1-ester and the *sn*-1-ether conjugates. In the cytotoxicity studies we observed that the **1** and **2** induced cell death in Colo205 cells and in HT-29 cells both in the presence and absence of $sPLA_2$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.054.

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