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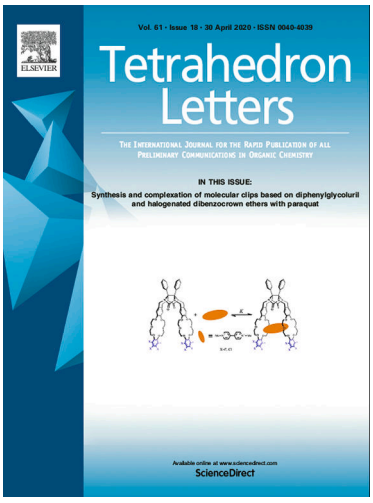

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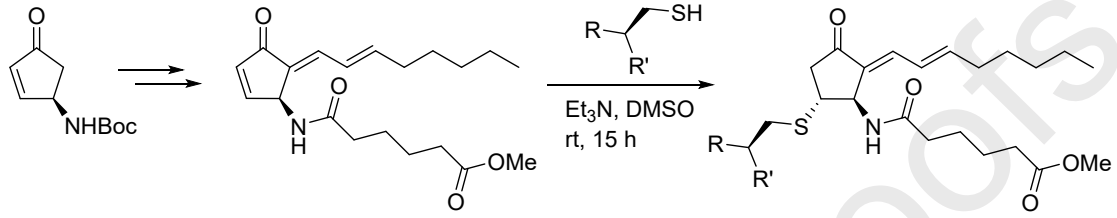

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Synthesis of the 4-aza cyclopentenone analogue of $\Delta^{12,14}$-15-deoxy-PGJ_2 and *S*-cysteine adducts

Lorna Conway,[a] Anna Riccio,[b] M. Gabriella Santoro[b] and Paul Evans[a]*

[a]*Centre for Synthesis and Chemical Biology, School of Chemistry, University College Dublin, Dublin 4, Ireland, email: paul.evans@ucd.ie*
[b]*Department of Biology, University of Rome Tor Vergata, Italy, email: santoro@uniroma2.it*

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ABSTRACT

* Corresponding author. Tel.: +353-1-716-2291; fax: +353-1-716-2501; e-mail: paul.evans@ucd.ie

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The synthesis of a series of 4-aza cross-conjugated cyclopentenones, inspired by the natural prostaglandin $\Delta^{12,14}$-15-deoxy-PGJ_2 (**5**) is described. Using the 4-aza cyclopentenone **7**, the installation of the α-side chain was performed using N-functionalisation, following a Boc-deprotection. The ω-side chain was then installed through a Baylis-Hillman type aldol reaction with *trans*-2-octenal. This afforded **11**, the aza-analogue of **5**. With this prostaglandin analogue in hand, a series of thiol adducts (**14-16**) were prepared. Included are activities for compounds **11** and **14-16** in relation to inhibition of the transcription factor NF-κB.

Prostaglandins are members of the eicosanoid family, derived from the essential fatty acid arachidonic acid.[1] Prostaglandins have a wide range of roles in the body; including regulation of the circulatory and respiratory systems as well as mediating tissue repair and the immune response.[2] Interestingly, prostaglandins can act as both pro-inflammatory modulators and anti-inflammatory modulators, depending on their structure and the receptors/systems upon which they act.[1,2]

The early members of the prostaglandin family formed by the cyclooxygenase pathway, for instance $PGF_{2\alpha}$ (**1**), can undergo further oxidation to prostanoids of the D and E series (e.g. **2**). These compounds are susceptible to elimination which produces the cyclopentenone prostaglandins (cyPGs). Examples of these include PGA_1 (**3**) and PGJ_2 (**4**), which contain a reactive α,β-unsaturated carbonyl unit (see Fig. 1).[3] Evidence demonstrates that the primary prostaglandins, e.g. **1**, promote inflammation, which the later cyPGs counteract.[4] In recent years, there has been significant interest in $\Delta^{12,14}$-15-deoxy-PGJ_2 (**5**), which was first identified in 1983 as a degradation product of PGD_2 (**2**).[5] While cyPGs are known generally to affect inflammation, cellular proliferation and differentiation, evidence indicates that the cross-conjugated cyclopentenone **5** is not only particularly active in this regard but also induces apoptosis and inhibits cellular growth. In part, this activity has been shown to be mediated through inhibition of NF-κB.[6] NF-κB (nuclear factor-kappaB) is a transcription factor responsible for the regulation of the immediate early pathogen response. It plays a key role in the promotion of inflammation, the control of cell proliferation and survival and in regulating viral gene expression.[7] Due to its frequent upregulation in many tumours, NF-κB is also an important target for anti-cancer therapies[8] and, consequently, its inhibition is of interest.[9] It has been shown that the electrophilic α,β-unsaturated cyclopentenone moiety is responsible for the inhibitory effect of PGs towards NF-κB. This allows for the binding of PGs to the β-subunit of the IKK complex, inactivating it and therefore blocking the activation of NF-κB.[10] Recently, additional members of the cross-conjugated cyclopentenone prostaglandin family have been identified and synthesised (e.g. **6**).[11]

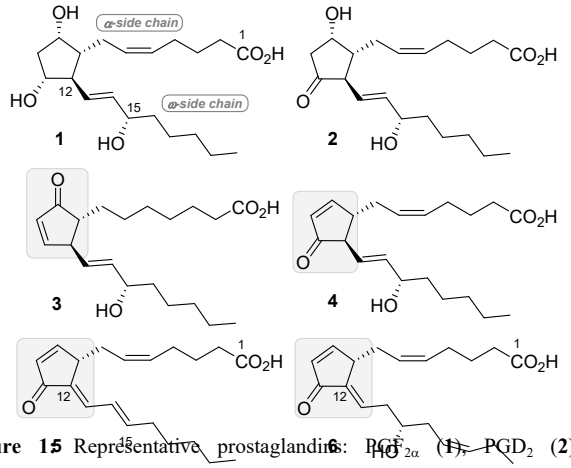

Figure 1 Representative prostaglandins: $PGF_{2\alpha}$ (**1**), PGD_2 (**2**), and cyclopentenones PGA_1 (**3**), PGJ_2 (**4**) and cross-conjugated cyclopentenones $\Delta^{12,14}$-15-deoxy-PGJ_2 (**5**), and Δ^{12}-PGJ_3 (**6**).

Since their discovery by von Euler in the 1930s, much work has been devoted to the synthesis of natural prostaglandins. The ground-breaking work by Corey in the 1960s and 70s paved the way for many elegant syntheses of such PGs exploring the challenging installation of the two side chains as well as 3, or 4

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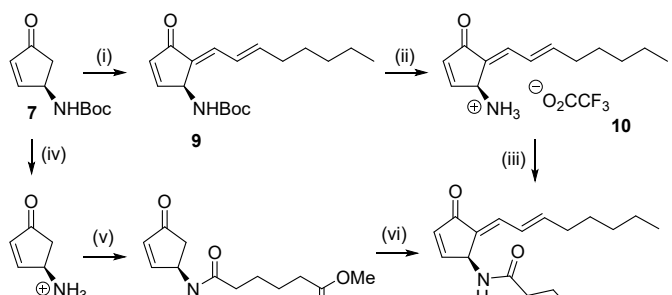
described a three-component coupling strategy towards the synthesis of primary prostaglandins.[2] An efficient two-step method for the preparation of cross-conjugated cyPGs *via* a conjugate addition-Peterson olefination reaction was developed by Iqbal and co-workers.[4] In recent years there has been significant interest in the synthesis of $\Delta^{12,14}$-15-deoxy-PGJ_2. [11,12] However, these routes involve multi-step synthetic sequences and do not allow for the straightforward synthesis of analogues.[11,12] As a result of the range of interesting biological activities coupled with the poor physiochemical properties of these natural prostaglandins, the preparation of synthetically simpler and more stable analogues have been reported.[13-15] With this in mind, we explored the presence of a suitably placed nitrogen atom to allow for the facile derivatisation of analogues and, based on preliminary work,[16] in this fashion we aimed to develop a direct analogue of $\Delta^{12,14}$-15-deoxy-PGJ_2 (see Scheme 1).

Scheme 1: Planned 4-aza cross-conjugated cyclopentenone analogues (**8**) of $\Delta^{12,14}$-15-deoxy-PGJ_2 (**5**) and their S-conjugate addition.

While the susceptibility of the electrophilic, endocyclic alkene to attack by "soft" nucleophilic thiol groups allows for the desirable biological activities it can also lead to the undesirable attack of circulating thiols such as glutathione.[3,17] With this in mind, masking of the thiol reactive endocyclic Michael acceptor was also explored.

Key enantioenriched (4*R*)-aza-cyclopentenone **7** was available from a previously reported cycloaddition-enzymatic resolution process.[18] With the cyclopentenone core (**7**) in hand, we initially focused on the installation of the α-side chain of the natural prostaglandin (*i.e.* **7** → **9**). Attempts at this transformation using typical aldol conditions, as described for the synthesis of Δ^{12}-PGJ_3 (**6**)[11] and $\Delta^{12,14}$-15-deoxy-PGJ_2 (**5**),[12] resulted in no discernable product. This observation is likely due to the instability of the enolate derived from **7**. With this in mind, a Baylis-Hillman type aldol reaction, originally investigated by Takanami and co-workers,[19] was employed for the α-alkylidenation (Scheme 2). Although these conditions afforded a low yield of **9** (13%), this is arguably still an appealing approach to achieve the direct α-alkylidenation step in the synthesis of this type of prostaglandin. In this reaction a 54% recovery of starting material **7** was also achieved. The desired *E,E*-stereochemistry of **9** was evident from proton NMR spectroscopy.[4a] Additionally, small amounts of the *Z,E*-isomer were isolated (3% - for details see ESI). Next, the value of the key nitrogen substituent was demonstrated. The Boc protecting group was cleaved using trifluoroacetic acid to give **10**. Due to its unstable nature the intermediate was swiftly subjected to a coupling reaction with methyl 6-chloro-6-oxohexanoate[20] and triethylamine. In the event the amide formation phase of this sequenceproved unsuccessful. Since this result indicated the poor compatibility of the acid chloride and **10** a carbodiimide coupling strategy was explored. The reaction of the ammonium salt **10** with mono-methyl adipate (0.9 equiv.), EDCI·HCl (1.3 equiv.), triethylamine (3.5 equiv.) and catalytic DMAP resulted in a 24% yield of the target **11**.



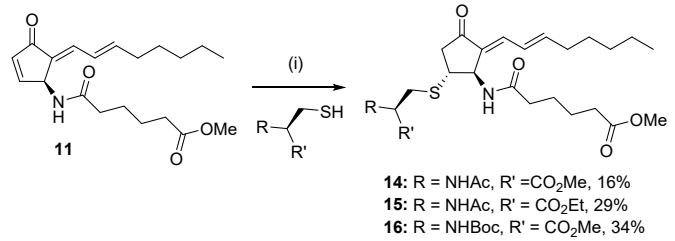
Scheme 2: Synthesis of 4-aza analogue (11) of $\Delta^{12,14}$-15-deoxy-PGJ_2. Reagents and conditions: (i) *trans*-2-Octenal, $TiCl_4$, Ti(O*i*-Pr)$_4$, PPh_3, CH_2Cl_2, -50 °C to rt; then $K_2CO_{3(aq)}$, 13% (54% recovery of **7**); (ii) TFA, CH_2Cl_2, rt; (iii) mono-methyl adipate, EDCI (1.3 equiv.), Et_3N (3.5 equiv.), DMAP (cat.), CH_2Cl_2, rt, 24%; (iv) TFA, CH_2Cl_2, rt, 86%; (v) methyl-6-chloro-6-oxohexnoate, Et_3N (2 equiv.), CH_2Cl_2, 0 °C to rt, 72%; (vi) *trans*-2-octenal, $TiCl_4$, Ti(O*i*-Pr)$_4$, PPh_3, CH_2Cl_2, -50 °C to rt; then $K_2CO_{3(aq)}$, 12-22% (44% recovery of **13**).

In an attempt to improve the overall yield of the target while also showing the versatility of the Ti-mediated aldol reaction, we decided to install the ω-side chain of the prostaglandin mimic first. This involved the deprotection of the Boc protecting group on the starting cyclopentenone **7**. Ammonium salt **12** was isolated with a yield of 86%. The coupling reaction with the previously used acid chloride, and triethylamine (2 equiv.) at 0 °C successfully resulted in amide **13** with a yield of 72%. A reaction time of 3 hours was used to avoid the formation of the β-keto amide (for more details see ESI) observed upon leaving the reaction for 15 hours. With the novel amide **13** in hand this was subjected to Takanami's α-alkylidenation reaction[19] using freshly distilled *trans*-2-octenal. Satisfyingly, the aldol-type reaction successfully achieved the synthesis of the natural product mimic **11** with an improved yield of up to 22%.[21] Importantly, recovery of the valuable amide **13** was also achievable (44%).

Although the overall yields for the formation of **11** by the Ti-aldol reaction are modest, due to its one-pot nature, its tolerance for the aza-functional group, the recovery of starting material and the failure of the direct aldol approach to this class of compound means that this synthetic approach is a viable method to access this compound.

With a stock of the direct analogue of the $\Delta^{12,14}$-15-deoxy-PGJ_2 - analogue **11** - now in hand, the conjugation of various cysteine adducts was investigated. A similar strategy for the preparation of cross-conjugated cyclopentenone *S*-cysteine adducts has been investigated previously and promising biological activities were uncovered.[22] Thus, reaction of **11** (1.2 equiv.) with *N*-acetyl-L-cysteine methyl ester (1.0 equiv.) and triethylamine (1.0 equiv.) in DMSO yielded the Michael-addition product **14** in a 16% yield. Small quantities of the *cis*-addition product were also isolated as a mixture with the *trans*-addition product and a 30% recovery of the starting material (**11**) was achieved. ^{1}H-NMR spectroscopy of the crude reaction mixture indicated the favourable addition to the less hindered face of the cyclopentenone ring resulting in the *trans*-product being the major diastereomer (see ESI). With mammalian compatibility in mind the conjugate addition of (*R*)-ethyl 2-acetamido-3-mercaptopropanoate to the cyclo-pentenone **11** was also considered. Following the synthesis of (*R*)-ethyl 2-acetamido-3-mercaptopropanoate using literature conditions,[23] the conjugate addition resulted in **15** with a yield of 29%. Finally, the conjugate addition of *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester resulted in the formation of **16** with a yield of 34%. The reasonably low yields for these conjugate addition reactions are

the reaction conditions. Furthermore, this type of S-adduct proved to be only partially stable during silica-gel chromatographic purification and variable levels of retro-conjugate addition (reforming **11**), and decomposition, were observed. It should be mentioned that no adducts resulting from S-addition at the exocyclic enone were detected.



Scheme 3: The synthesis of S-Michael-type adducts **14-16**. Reagents and conditions: (i) RR'CHCH$_2$SH (1 equiv.), Et$_3$N (1.0 equiv.), DMSO, rt

The ability of synthetic compounds: **11** and **14-16**, to inhibit the transcription factor NF-κB was investigated using a gene reporter, HeLa cell-based assay system (Table 1). The data generated was compared with that measured for $\Delta^{12,14}$-15-deoxy-PGJ$_2$ (**5**). It was found that at concentrations between 3-12 μM compounds **11** and **14-16** all blocked TPA (12-*O*-tetradecanoylphorbol-13-acetate) challenged NF-κB activation by half (ED$_{50}$). Furthermore, an Alamar blue® cell viability assay demonstrated that toxicity (measured as an LD$_{50}$ value in HeLa cells) was only observed at doses significantly higher than the ED$_{50}$ of the individual compounds. As a trend, the S-adducts, **14-16**, proved slightly less active than the cyclopentenone **11**, which proved more active in this assay than the natural prostanoid, **5**. However, the S-adducts were also markedly less toxic.

Table 1: NF-κB inhibition and toxicity of compounds **5**, **11** and **14-16**.

Entry	Compound	NF-κB ED$_{50}$	Alamar blue® LD$_{50}$
1	**5** ($\Delta^{12,14}$-15-deoxyPGJ$_2$)	7 μM	400 μM
2	**11**	3 μM	210 μM
3	**14**	10 μM	400 μM
4	**15**	12 μM	800 μM
5	**16**	7.5 μM	600 μM

In conclusion, this manuscript describes a flexible synthesis of **11**, the methyl ester analogue of the cross-conjugated natural product $\Delta^{12,14}$-15-deoxy-PGJ$_2$ (**5**). In addition, the more reactive endocyclic double bond of **11** was reacted with three protected forms of cysteine forming S-adducts **14-16**. Compounds **11** and **14-16** were shown to inhibit NF-κB at comparable levels to the natural prostanoid **5**. The S-adducts (particularly **15**) demonstrated diminished toxicity in comparison to **5** and **11**. The emergence of the irreversible kinase inhibitors,[24] amongst other examples, has renewed interest in the biological possibilities for compounds that can (selectively) react covalently with a range of disease relevant biomolecules.[25]

Acknowledgments

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Supplementary Material

Supplementary material (experimental procedures, including the determination of biological activity, proton and carbon NMR spectra) are available free-of-charge *via* the internet.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Paul Evans

TETLD_20_00463

of $\Delta^{12,14}$-15 -deoxy-PGJ$_2$ and *S*-cysteine adducts

Lorna Conway, Anna Riccio, M. Gabriella Santoro and Paul Evans

• Preparation of a new type of cross-conjugated cyclopentenone prostaglandin analogue
• Masking of the endocyclic enone by S-cysteine conjugate addition
• Comparable inhibition of nuclear factor kappa B (NF-kB) to natural prostanoid $\Delta^{12,14}$-15-deoxy-PGJ$_2$

Synthesis of the 4-aza cyclope

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