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Chemical Transformation of Prostaglandin-A₂: A Novel Series of C-10 Halogenated, C-12 Hydroxylated Prostaglandin-A₂ Analogues

Anokha S. Ratnayake, Tim S. Bugni, Charles A. Veltri, Jack J. Skalicky, and Chris M. Ireland*

Department of Medicinal Chemistry and Department of Biochemistry, University of Utah, Salt Lake City, Utah 84112

Abstract

Synthesis of a novel class of C-10 halogenated and C-12 oxygenated prostaglandin-A₂ derivatives 6(a–c) has been accomplished. (15*S*)-Prostaglandin-A₂ (1), from the gorgonian *Plexaura homomalla*, served as the starting material for the synthesis. The absolute configuration was determined using NMR.

Δ^{12} -Prostaglandin J₂ (Δ^{12} -PGJ₂), a cross-conjugated enone, is known to inhibit ubiquitin specific isopeptidase activity causing apoptosis.¹ Mechanistic studies have indicated that inhibition results from Michael addition of an isopeptidase cysteine residue to the endocyclic β -carbon of the cyclopentenone.² Punaglandins (PNGs), C-10 chlorinated and C-12 oxygenated prostanoids, are more potent inhibitors of isopeptidase activity than Δ^{12} -PGJ₂ and the PGA series (Figure 1).³ On the basis of these results, increasing the electrophilicity of the endocyclic β -carbon (C-10) should increase reactivity. To test this hypothesis, a new series of halogenated PGA₂ derivatives were synthesized (**4a–4c**) by substituting electron withdrawing groups (Cl, Br, I) at the α -position of PGA₂ (Scheme 1).

Preliminary work with these α -halogenated PGA₂ analogues (**4a–4c**) indicated that the potency of isopeptidase inhibition for the series is I >> Br > Cl.⁴ Additionally, Iodo-PGA₂ was a more potent inhibitor of ubiquitin specific isopeptidase activity compared to Δ^{12} -PGJ₂, but less potent than the PNGs. Thus it was recognized that halogenation and electrophilicity do play a role in the ability of prostaglandins to inhibit ubiquitin isopeptidase activity, but are not the only factors. These results suggested C-12 hydroxylation similar to PNGs may be necessary to achieve optimum activity. To test this hypothesis, a series of C-10 halogenated and C-12 oxygenated PGA₂ derivatives (**6a–6c**) have been synthesized (Scheme 3).⁴

The starting material for the synthesis, (15*S*)-prostaglandin-A₂ (PGA₂) was isolated in abundance (5.7% recovery)⁵ from the gorgonian *Plexaura homomalla*.⁶ The side-chain functional groups of PGA₂ (the terminal carboxylic acid at C-1 and the alcohol at C-15) were

blocked as their methyl ester and acetate respectively, using standard derivatization conditions (Scheme 1).⁷

The 15-*O*-acetyl-PGA₂ methyl ester (**2**) was treated with alkaline hydrogen peroxide at -15°C to generate a mixture of C(10,11)- α,β -epoxides (**3**).⁸ The subsequent C-10 halide substitutions⁹ were achieved by regioselective ring opening of the C(10,11)-epoxide (**3**) with halide (Cl, Br, I) salts under mildly acidic conditions (Amberlyst 15¹⁰ or silica gel support¹¹). Similar transformations of epoxy cyclopentenones into α -haloenones have been shown to occur via the halohydrin intermediates which are spontaneously dehydrated under acidic conditions to afford the vinylhalide products.¹⁰

Accordingly, the method of choice for the formation of vinylchloride (**4a**) was LiCl/Amberlyst/CH₃CN system, whereas the vinylbromide (**4b**) was formed by NaBr under otherwise identical conditions.¹² However, attempts to form the vinyl iodide (**4c**) under similar conditions (LiI or NaI/Amberlyst/CH₃CN) were unsatisfactory and resulted in the re-formation of the alkene (**2**). A subsequent literature survey of alternative methods of vinyl iodide formation¹³ from epoxides, revealed that silica gel could be employed as an efficient acid catalyst in the nucleophilic ring-opening of epoxides under solvent-free conditions.¹¹ Accordingly, the solvent-free iodination of epoxide (**3**) was carried out using LiI supported on silica gel. The reaction proceeded smoothly to give 54% of the vinyl iodide (**4c**) along with 13% of the alkene by-product (**2**).

The next task of the synthesis was the conversion of the C-10 halogenated PGA₂ series (**4a–4c**) to the corresponding C-12 hydroxylated derivatives (**6a–6c**). This novel series of C-12 hydroxylated derivatives were believed to be accessible via allylic oxidation mediated by selenium dioxide,¹⁴ owing to the fact that the C-12 position of PGA₂ is bis-allylic to C-10 and C-13 double bonds (Scheme 2). However, the likely formation of byproducts due to multiple potential oxidation sites, were foreseen as a possible drawback of this method.

To test the feasibility of the allylic oxidation reaction, PGA₂-acetate methyl ester (**2**) was employed. As speculated, compound **2** with SeO₂ gave several oxidation by-products. One major by-product was identified as the conjugated diene (**2a**) possibly arising from allylic hydroxylation at C-7 followed by dehydration (Scheme 2). Shorter reaction times, lower reflux temperatures (95% EtOH) or reduced amounts of the oxidant did not facilitate formation of the desired tertiary alcohol product (**6**).

It was now evident that in order to selectively hydroxylate the bis-allylic position (C-12) of PGA₂ and suppress by-product formation during SeO₂ oxidation, the other allylic positions (C-4 and C-7) needed to be removed or masked in some manner. This was achieved by selective epoxidation of the C(5,6) double bond in **4** using *m*-CPBA (Scheme 3). The resulting C(5,6)- α,β -epoxide mixture (**5**) was treated as an intermediate and used without further purification for the SeO₂ oxidation. As anticipated, allylic hydroxylation at C-12 position proceeded smoothly and was accompanied by C(5,6)-epoxide ring opening to yield the novel series of prostaglandin-A₂ analogues (**6a–6c**)¹⁵ in moderate yields (Scheme 3).

The relative and absolute configurations of the five stereocenters (C-5/6/8/12/15) in **6(a–d)** were deduced as follows. The absolute stereochemistry of C-8 was assumed (*R*), based on literature precedent for coral-derived PGA₂.¹⁶ The assignment of the 15(*S*) configuration of **6** was based on the application of Mosher's method¹⁷ to (*S*)- and (*R*)-MPA derivatives of PGA₂-methyl ester.¹⁸ The *trans* (*R,R*) relationship between the two side chains (C-8/C-12) was established based on a strong NOESY cross peak observed between H-8 and H-13. Subsequent molecular modeling studies with energy-minimized conformations for each of the C-12 epimers supported this observation. The *trans*-(8*R*,12*R*) relationship was further

corroborated by heteronuclear coupling constant analysis ($^2J_{\text{H8/C12}} = \text{small}$)¹⁹, and dihedral angle measurements (-135.7°) for the 8(*R*),12(*R*) diastereomer of **6**.²⁰

The *cis* orientation of the C(5,6) epoxide mixture [(5*S*,6*R*) and (5*R*,6*S*) diastereomers] in **5** was supported by the homonuclear coupling constant value of $^3J_{\text{H5/H6}} = 4.3 \text{ Hz}$.²¹ It was presumed that the C(5,6) diol in **6**, generated during the allylic oxidation ($\text{SeO}_2/\text{dioxane}/\text{H}_2\text{O}/\text{reflux}$) of **5**, occurs via nucleophilic opening of the C(5,6) epoxide by water. Accordingly, non-regioselective attack of water on the (5*S*,6*R*) and (5*R*,6*S*) *cis*-epoxides in **5** would generate two secondary alcohol diastereomers, (5*R*,6*R*) and (5*S*,6*S*). This was supported by the $^1\text{H-NMR}$ spectra of **6(a–d)** which showed doubling of resonances due to the formation of two diastereomers in a ~1:1 ratio. Accordingly, HPLC analysis of **6d** showed two peaks eluting at 11.5 (**7**) and 13 min (**8**). The two peaks (**7** and **8**) were separated,²² and were employed for absolute stereochemical assignments of the C(5,6) centers by *J*-based analysis,¹⁹ Mosher's method¹⁷ in conjunction with molecular modeling studies (Figure 2).

Initial attempts focused on utilizing *J*-based analysis¹⁹ to relate the configuration of the vicinal diol segment C(5,6) to that of the known absolute configuration at C-8. However, diastereomer **7** proved undesirable for *J*-analysis due to overlap of resonances for H-5 and H-6 ($\delta 3.51\text{--}3.57$, m, 2H) as well as for H-7_h and H-7_l ($\delta 1.98$, m, 2H). Therefore, the viable alternative was to attempt the *J*-based analysis on the diastereomer **8**.

For diastereomer **8**, sufficient chemical shift dispersion of all proton resonances were observed in CDCl_3 at 0°C . While all of the couplings required for *J*-analysis were determined successfully (Table 1), the information failed to fit any of the typical rotamers with certainty.¹⁹

The reason for the occurrence of atypical coupling values in **8** could be explained based on a molecular modeling study, which revealed that the cyclopentenone carbonyl (C-9) is involved in an intra-molecular H-bonding with C-6(OH) adopting a seven-membered cyclic conformation (Figure 3). The measured *J*-values and NOESY data were in accordance with such a seven-membered conformation.

Having failed to unequivocally determine the C(5,6) stereochemistry by *J*-based analysis, Mosher's method was attempted on **8**. Accordingly, reaction of **8** with (*R*)- and (*S*)-MPA acids²³ was expected to yield the corresponding C(5,6)-*bis*-MPA esters. However, $^1\text{H-NMR}$ data confirmed that the C-5 MPA derivative was the sole product. Comparison of the chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) in the $^1\text{H-NMR}$ spectra of the C-5 (*R*)- and (*S*)-MPA derivatives of **8** at 0°C indicated the absolute configuration was (*R*) (Figure 4).

Thus the absolute configuration of all five stereo-centers (C-5/6/8/12/15) in **8** was established as (5*R*,6*R*,8*R*,12*R*) and 15(*S*). Consequently, the absolute configuration of the diol diastereomer **7** was assigned as (5*S*,6*S*,8*R*,12*R*) and 15(*S*). The absolute configuration assignments of **7** and **8** were in good agreement with their $^1\text{H NMR}$ data, coupling constant analysis and molecular modeling studies employing MM2 and AM1.²⁴

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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21. Due to the overlap of H-5 and H-6, a combination of ¹H-NMR, HOMODEC and 1D-TOCSY experiments were employed to extract the coupling constants with certainty.
22. See Experimental section for details of the separation procedure.
23. 2 eqvi. of MPA per OH was employed. Reaction was carried out at room temperature for ~16 h, until complete conversion to the product, as indicated by TLC and NMR.
24. CS Chem3D Pro; ChembridgeSoft Corporation: Cambridge, MA.

Figure 1.
Electrophilic prostanoids

Figure 2.
C(5,6)-diol diastereomers of **6d**

Figure 3.
The MM2/AM1-optimized model for **8**

Figure 4.

$\Delta\delta$ values for 8 ($\Delta\delta = \delta_R - \delta_S$, 500 MHz, CDCl_3 , 0 °C)

Scheme 1.
Synthesis of α -halogenated PGA₂ analogues

Scheme 2.
SeO₂ oxidation of **2**

Scheme 3.
Synthesis of C-12 oxygenated PGA₂ derivatives

Table 1^{2,3}*J* values of **8** for the C-6/C-7 segment measured in CDCl₃

^{2,3} <i>J</i> values ^a	<i>J</i> (Hz)	classification ^b
³ <i>J</i> (H-6, H-7 _h)	+ 6.3	medium
³ <i>J</i> (H-6, H-7 _l)	+ 6.3	medium
³ <i>J</i> (H-6, C-8)	+ 2.2	small
³ <i>J</i> (H-5, H-7 _h)	+4.4	medium
³ <i>J</i> (H-5, H-7 _l)	+2.2	small
² <i>J</i> (C-6, H-7 _h)	−4.3	medium
² <i>J</i> (C-6, H-7 _l)	−1.1	small

^a H-7_h and H-7_l represent the high- and low-field H-7 protons respectively.^b Classification for magnitude of the coupling constants: values between large and small are regarded as medium¹⁹